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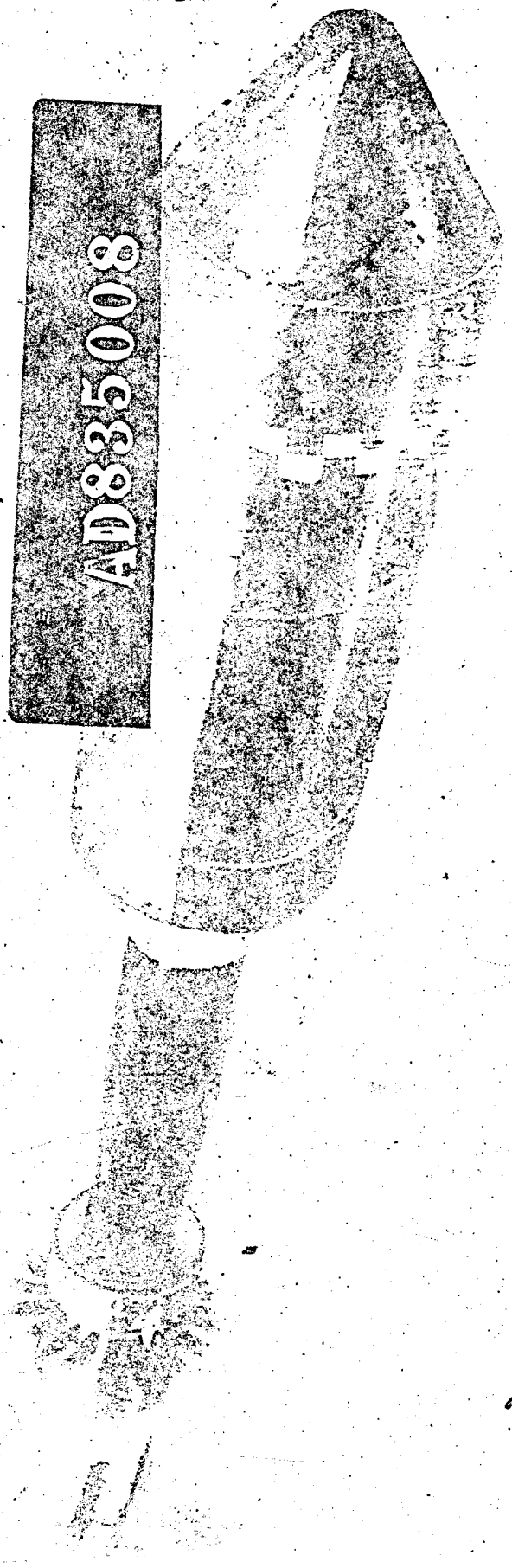

PROCEEDINGS OF THE 3RD ANNUAL CONFERENCE ON ATMOSPHERIC CONTAMINATION IN CONFINED SPACES

9-11 MAY 1967

DECEMBER 1967

AEROSPACE MEDICAL RESEARCH LABORATORIES
AEROSPACE MEDICAL DIVISION
AIR FORCE SYSTEMS COMMAND
WRIGHT-PATTERSON AIR FORCE BASE, OHIO

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The experiments reported herein were conducted according to the "Guide for Laboratory Animal Facilities and Care," 1965 prepared by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences--National Research Council; the regulations and standards prepared by the Department of Agriculture; and Public Law 89-544, "Laboratory Animal Welfare Act," August 24, 1967.

The voluntary informed consent of the subjects used in this research was obtained as required by Air Force Regulation 169-8.

1200 - June 1968 - CO455 - 38-823

AMRL-TR-67-200

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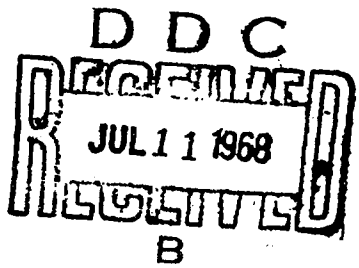

FOREWORD

The 3rd Annual Conference on Atmospheric Contamination in Confined Spaces was held in Dayton, Ohio on 9, 10, and 11 May 1967. Sponsor was the Aerospace Medical Research Laboratories, Aerospace Medical Division, Air Force Systems Command. Arrangements were made by the Toxic Hazards Research Unit of Aerojet-General Corporation under the terms of Contract F33615-67-C-1025. The Toxic Hazards Research Unit is located at the Toxic Hazards Division, Biomedical Laboratory, Wright-Patterson Air Force Base, Ohio. Dr. Anthony A. Thomas, Director, Toxic Hazards Division, and Dr. Kenneth C. Back, Chief, Toxicology Branch, served as Co-Chairmen. Mrs. Mildred Pinkerton served as Conference Coordinator for the Air Force, and Mr. Edmond Vernot for Aerojet-General Corporation.

Acknowledgment is made on behalf of the Aerospace Medical Research Laboratories to Colonel George E. Schafer, Vice Commander, Aerospace Medical Division, Brooks Air Force Base, Texas, for his Introductory Remarks and support, to the session Chairmen and speakers, to the panel members of the Open Forum, and to all those who actively participated in the discussions. Special thanks are due to TSgt Edgar Hagan, TSgt James G. King III, Sgt James Valentine, Sgt B. N. Holcombe, and SMSgt John L. Naylor, of the Toxic Hazards Division, and to Mrs. Lois Doncaster and Mrs. Marilyn Collins of Aerojet-General Corporation.

ABSTRACT

This report is a complete compilation of the papers presented and the Proceedings of the 3rd Annual Conference on Atmospheric Contamination in Confined Spaces, sponsored by the Aerospace Medical Research Laboratories and held in Dayton, Ohio on 9, 10, and 11 May 1967. Major technical areas discussed by the invited speakers, members of the Open Forum and Conference attendees included toxicological evaluation of atmospheres and contaminants, histopathological evidences of toxicity, evaluation of cabin materials, instruments and detection techniques, measurement of behavioral responses, and life support systems. Included as an Appendix, but not presented at the Conference, is a status report on oxygen toxicity prepared by Dr. Harold Kaplan, which is considered sufficiently pertinent to the Conference proceedings that it should be of interest to all participants.

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INTRODUCTION

The 3rd Annual Conference on Atmospheric Contamination in Confined Spaces continued to focus its attention on refined tools of research for the evaluation of changes in subcellular biochemistry and morphology resulting from long-term continuous exposure to basic cabin atmospheres and contaminants. While such changes are observed to a greater or lesser degree in most of our studies, the clinical significance of these is uncertain and questionable in many instances. Correlation of these changes is the only meaningful way to distinguish adaptive processes from destructive changes due to true toxic effects. The real philosophical question, then, is: are we going to accept a certain amount of adaptive response as normal and harmless? I think that as long as adaptation is not a progressive process leading to irreversible biochemical and histological changes which would prevent readaptation to an earthly environment, we should accept it as a normal physiological response. Moreover, we should accept it as a desirable physiological response which prevents the development of toxic damage. In my mind, adaptation is complete only if it is a self-limiting phenomenon. To prove that it is self-limiting, longer postexposure observations must be made to ascertain completeness of readaptation.

In many instances in the past we did not have the tools or the facilities to extend and improve the postexposure observation. We are enlarging our facilities at the present time and are continually improving the sensitivity of our tools. In the new facilities we will be able to run concurrent controls in the dome environment. We will have electron microscopy at our disposal. Our mitochondrial and microsomal techniques will be improved and expanded. Automation of routine clinical chemistries will be completed within this year.

While all this is desirable, it will certainly result in increased need for speedy and efficient data handling and analysis. Without these, we might not "see the forest for the trees".

In reading the Proceedings you will become aware of the emphasis on improved methodology and instrumentation techniques in the detection area and in the behavioral responses measurement field. Also, you will appreciate the increasing concern over safety of the operators entering high oxygen concentration environments. The recent tragic events fully justify this concern. Although our operations are shut down for an indefinite period, the length of which will depend on the development of an adequate fire extinguishing system and attendant scrutiny of combustible materials and ignition sources within the chambers, we still will be able to operate at ambient pressure and reduced pressure atmospheres using air to perform continuous exposure studies. We will at least have the luxury of running a true control study with animals in the dome environment for several months. We will also be able to study contaminants in ambient air to obtain important baseline data for carbon monoxide and other contaminants of major significance. Therefore, the outlook is not bleak at all as far as productivity of the facility is concerned, and we are confident that we will have interesting data to report again next year.

Dr. Anthony A. Thomas

WELCOMING REMARKS

Raymond A. Yerg, Colonel, USAF, MC

Commander
Aerospace Medical Research Laboratories

Ladies and gentlemen, welcome to the 3rd Annual Conference on Atmospheric Contamination in Confined Spaces. As you may have noted from the program, we have a busy and demanding schedule. Over the next three days, you will be hearing presentations from scientists in industry and from our in-house facilities on the results of their investigations on atmospheric contamination. We have again broadened the scope of the Conference. In addition to the usual topics of comparative toxicology and the pathology of basic cabin atmospheres and trace contaminants, we have added three new sessions. One is on instrumentation and detection techniques; one is on the measurement of behavioral responses; and one is on life support systems. We think these are valid and valuable additions to the Conference.

We've had quite a busy year in the Toxic Hazards Division. Dr. Thomas and his group have gotten the Apollo Materials Screening Program well under way and you will hear some of these results; we have learned more about the toxicological properties of mixed gas atmospheres; and we have definitely advanced our knowledge on the toxicity of oxygen.

Those of you who attended last year's Conference should have received your copy of the proceedings. I think it's appropriate to mention this because in many instances the work that is being reported during this Conference is a continuation of or a logical extension of the work that was reported last year.

The prospects for the future are quite bright, and, as you may know, we are in the process of doubling our facilities to accelerate the toxicology research program. We are adding four additional Thomas Domes which will, in effect, allow us to put more emphasis on all aspects of toxicity and to materially enhance the screening program. We are adding an interconnecting surgical operating suite to the air lock system which will be interconnecting the four new Thomas Domes. This will allow the researchers to perform surgical procedures or to obtain clinical or pharmacological preparations within the actual exposure environment without returning to ambient pressures following some duration of exposure. We believe that with this facility and this technique, we'll be able to answer some of the questions about whether or not the ultrastructural changes are exposure-related or whether they are artifacts brought about by the rapid change in environments prior to sacrifice of animals.

I extend a warm and cordial welcome to each of you participating in the Conference, and I want to express my sincere appreciation to the real contributors, the chairmen of the sessions, the speakers and presenters, and, finally, you, the attendees, who really are the heart of the program. If we can be of any assistance to you over the next few days, please let us know and we'll be very pleased to help you in any way we can. Again, welcome, and I wish you every good wish for a successful Conference.

INTRODUCTORY REMARKS

George E. Schafer, Colonel, USAF, MC

Vice Commander
Aerospace Medical Division

It is a real privilege to be able to welcome you on behalf of General Roadman and the Aerospace Medical Division to this third Conference. Last year I had the same privilege, and I think those of you who attended will remember that I mentioned there would be increasing emphasis placed on toxicology research as it influenced Air Force research and development programs. I think you will also remember that many of the papers last year and the work prior to that time involved the Apollo Project. I think you will note this year that in addition to applying the work to the benefit of the Apollo Project, you will notice increasing application to the Air Force's Manned Orbital Laboratory Program, and this will be a significant one.

As we move further along in space, of course, we are going to be experiencing much greater periods of time in confined atmospheres, and because of this increased exposure to confined atmospheres, it becomes apparent that we must understand some of the basic underlying mechanisms of toxicity, more than we have before. We must exploit, if possible, some of the processes of adaptation and I guess what I am really saying is that we must look more carefully at some of the fundamental aspects of toxicology if we are to understand some of the toxic problems in confined atmospheres. Consequently, it becomes apparent that we must intensify our research. We must also try to catch up from the delays that we experienced, especially in the Aerospace Medical Division as the result of the Apollo tragedy and the space cabin simulator fire we had at Brooks Air Force Base some months ago. As you know, the Aerospace Medical Division has been the lead division for the investigation of chambers and the review of chamber procedures for those operations that involve hyperoxic atmospheres. The team that has been charged with representation by the Division includes not only the members of the original investigating team that were reappointed, but also the consultants who served on that team. It was felt that by utilizing these people, we could take advantage of the experience gained from their investigation of the accident. These people have looked into all of the chamber operations within the Division in considerable detail and have been in close consort with the National Aeronautics and Space Administration; they have reviewed the operations of some 29 chambers within the Aerospace Medical Division; and I think that they can say that there have been a number of soft spots discovered, not only in the design of chambers, but in some of the procedures utilized in the

operation of experimental protocol within these chambers. I don't think there is any question but that there can and should be fire detection and fire extinguishment systems in any hyperoxic chamber operation involving human exposure. I don't believe there is any doubt but that we should use noncombustible clothing which is available and is being designed and is within the state-of-the-art and being tested at the present time. I think we in the Aerospace Medical Division will do everything possible to hurry up putting these chambers back into operation and I think we are always mindful of the fact that we can go overboard if we are not careful. We must prevent going overboard and, of course, stopping any research. This could be very easily done if we want to respond to the past tragedies on an overwhelming basis. The impact of such an accident, of course, has an influence not only as far as the tragic significance of the accident is concerned, but on future research and this is what we must remember at all times.

I don't believe it is necessary to emphasize to this group, but I would urge you to emphasize to those that surround you and especially to those not in this type of business, the possible fallouts and spinoffs that can occur from this type of research to society in general. I don't think there's any question that we should emphasize to our lay public the effects that this type of research can have on environmental pollution and its national significance. Some of the fallouts of the fundamental research will have considerable impact on clinical medicine in the future. I think we must emphasize these as well as the benefits that must be derived before satisfactory space flight can be performed for long periods of time.

I wish you a very successful meeting. I want to congratulate the Aerospace Medical Research Laboratories for sponsoring this meeting, and I want to thank them for allowing me to be able to participate.

SESSION I

TOXICOLOGICAL EVALUATION OF ATMOSPHERES AND CONTAMINANTS

Chairman

Dr. Emanuel M. Roth
Department of Aerospace Medicine
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Albuquerque, New Mexico

EIGHT-MONTH CONTINUOUS EXPOSURE OF ANIMALS TO AN OXYGEN-NITROGEN ATMOSPHERE AT 5 PSIA

E. J. Fairchild II, Ph. D.

Aerojet-General Corporation
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INTRODUCTION

It is generally known that one of the stumbling blocks in the development of our manned spacecraft program has been, from time to time, the environmental control system and its associated problems. Chief among these was the question of whether cabin air and pressure should imitate conditions of a sea level air mixture of nitrogen (N_2) and oxygen (O_2), or whether systems development should abide by the experience of aviation and use at the highest altitude whatever would guarantee a minimum O_2 need. The majority of the experts stood behind the latter opinion and agreed that the most feasible systems should involve use of an environment of pure O_2 with a total pressure of approximately 260 mm Hg. This seemed more practical from the standpoint of weight reduction, controlling leak rate, and avoiding the difficult problems of providing reliable partial pressure sensors. Furthermore, a single gas system of 100% O_2 at reduced pressure would reduce the risk of bends during walks and/or work in a suit on the outside of a spacecraft.

There is agreement, even now following the disastrous Apollo accident of 27 January 1967, that the all-O_2 system is reliable and safe under the correct conditions of pressure. The Soviet choice of a near sea level environment is good in many respects, but is fraught with the danger of decompression sickness for space work and maneuvers upon leaving the craft. Thus, the original choice by the United States of 100% O_2 at a reduced pressure of five pounds per square inch absolute (5 PSIA) has proven successful, both toxicologically and engineering-wise, for the relatively short duration flights. There has been found, however, some histopathological evidence from animal studies which indicates that prolonged, continuous exposure to O_2 tensions of the above magnitude might ultimately result in slight, yet irreversible damage (Hagebusch, 1966; Schaffner, 1966; Klion, et al, 1967; Lewerenz, 1967).

Although the single-gas system (100% O_2 at 5 PSIA) has been judged innocuous for flight durations as long as 135 days, e.g., the contemplated Apollo program, and for even longer periods up to 235 days (Kaplan, et al, 1967), there still remains a genuine interest in the two-gas system; the Air Force has interest in use of systems

employing O_2-N_2, as well as O_2-Helium atmospheres, for manned orbital laboratories. Accordingly, the data reported here concern a study designed to determine the effects, if any, of an eight-month continuous exposure of animals to an environmental atmosphere of 68% O_2 and 32% N_2 at a reduced pressure of 5 PSIA.

MATERIALS AND METHODS

The exposure was conducted in a Thomas Dome (Thomas, 1965) under the test conditions listed in table I. As seen, the experimental atmosphere was maintained at 68% O_2 and 32% N_2 at a total pressure of 260 mm Hg. The temperature ranged from 72 to 77 F, and relative humidity from 44 to 74%. Carbon dioxide occasionally rose to insignificant high values (0.8%) but generally remained below 0.15%. Other operating conditions, including those for control, are indicated.

TABLE I

EIGHT-MONTH MIXED GAS STUDY OPERATING PARAMETERS

Parameters	Experimental Approx. Values	Control Approx. Values
Atmosphere		
Oxygen	68%	Ambient
Nitrogen	32%	Ambient
Carbon Dioxide	0.18%	Ambient
Total Pressure	260 mm Hg	Atmospheric
Flow Rate	25 CFM	NA
Temperature	75 F	77 F
Relative Humidity	52%	46%
Continuous Days	246	248

Animal species, sex, and strain, as well as numbers of each used as experimentals and controls, are shown in table II. All animals were fed and watered ad libitum. Uninterrupted maintenance of the experimental dome atmosphere was accomplished by use of an air lock through which entry could be made for performance of routine feeding, cage cleaning, and blood sampling.

Blood analyses of all dogs and monkeys were initiated prior to the beginning of the experiment in order to have at least two baseline values, two weeks apart. Thereafter, sampling was conducted biweekly during the entire period of study.

Routine clinical laboratory tests are listed in table III, and were performed on controls as well as exposed animals. Periodic weight measurements, noteworthy

TABLE II

EIGHT-MONTH MIXED GAS STUDY ANIMAL COMPLEMENT

Species	Experimental		Control	
	M	F	M	F
Mice - Harlan ICR	40		40	
Rats - Sprague-Dawley	25	25	25	25
Dogs - Beagle	4	4	2	2
Monkeys - Rhesus	4		2	

symptomatology, mortality, etc. were recorded during the course of the experimental period; following which all species or representative numbers, in the case of mice, of both experimental and controls were sacrificed (euthanasia) and necropsied. Tissue preparation for histopathologic evaluation by light microscopy was routine; however, additional special preparations of lung, liver, and kidney from dogs, monkeys, and rats were processed for electron microscopy.

TABLE III

CLINICAL LABORATORY TESTS PERFORMED BIWEEKLY ON BEAGLE DOGS AND RHESUS MONKEYS

Hematology	Chemistry
Total WBC (cells/mm^3)	Sodium (meq/l)
Differential (per 100 cells)	Potassium (meq/l)
Total RBC (million cells/mm^3)	Calcium (meq/l)
Hemoglobin (Gm%)	Total Protein (Gm%)
Hematocrit (vol. %)	Albumin (Gm%)
	S-GPT (RF Units)
	S-GOT (RF Units)
	Alkaline P-tase (RKB Units)
	Total Phosphorus (mg%)
	LDH (CW Units)

Pertinent information concerning detailed aspects of chamber technology and biological applications in this type of continuous exposure at simulated altitude has been documented (MacEwen, 1965; Kaplan, et al, 1967; Back, 1966; and McNerney and MacEwen, 1965) and requires no further elaboration.

RESULTS

The effects upon animals of eight-months continuous living in an environmental atmosphere of 68% O_2 and 32% N_2 at a simulated altitude pressure one-third of atmospheric were few; there were, however, some apparent effects when the exposed animal populations were compared with counterpart controls. Some findings were significant, but the majority were not; others, although not significant, were indicative of trends toward toxic response and/or an imbalance of homeostasis.

Growth Data

The most meaningful growth data were obtained from rats, inasmuch as the greater numbers lend themselves to better statistical interpretation. Figure 1 compares growth of exposed rats with controls by continuous plot of mean body weights and their respective standard deviations (S. D.) over the period of study. A difference in growth rate is seen, in that exposed rats showed consistent retardation. It is readily apparent, however, that the difference is not one of significant proportion, inasmuch as the S. D. bars (one sigma limit) show considerable overlap. Growth curves for dogs and monkeys are shown in figures 2 and 3, respectively, but without the S. D.; these were omitted because they were proportionately too large for graphic inclusion. It follows, then, that the weight differences between exposed and controls of these species were insignificant.

Mortality Data

Resulting animal mortality during the course of study is presented in table IV. None of the differences between experimental and controls was significant. The discrepancy between total numbers of animals used and those numbers shown in table II is due to some deaths known not to be associated with experimental conditions, or because an occasional animal was deliberately removed from the study.

TABLE IV

EIGHT-MONTH MIXED GAS STUDY MORTALITY
(DEATHS/TOTAL USED)

	Experimental	Control
Mice, Male	9/39 - 23%	5/37 - 14%
Rats, Male	2/24 - 8%	1/24 - 4%
Female	3/25 - 12%	1/24 - 4%
Dogs, Male	0/4	0/2
Female	0/4	0/2
Monkeys, Male	0/4	0/2

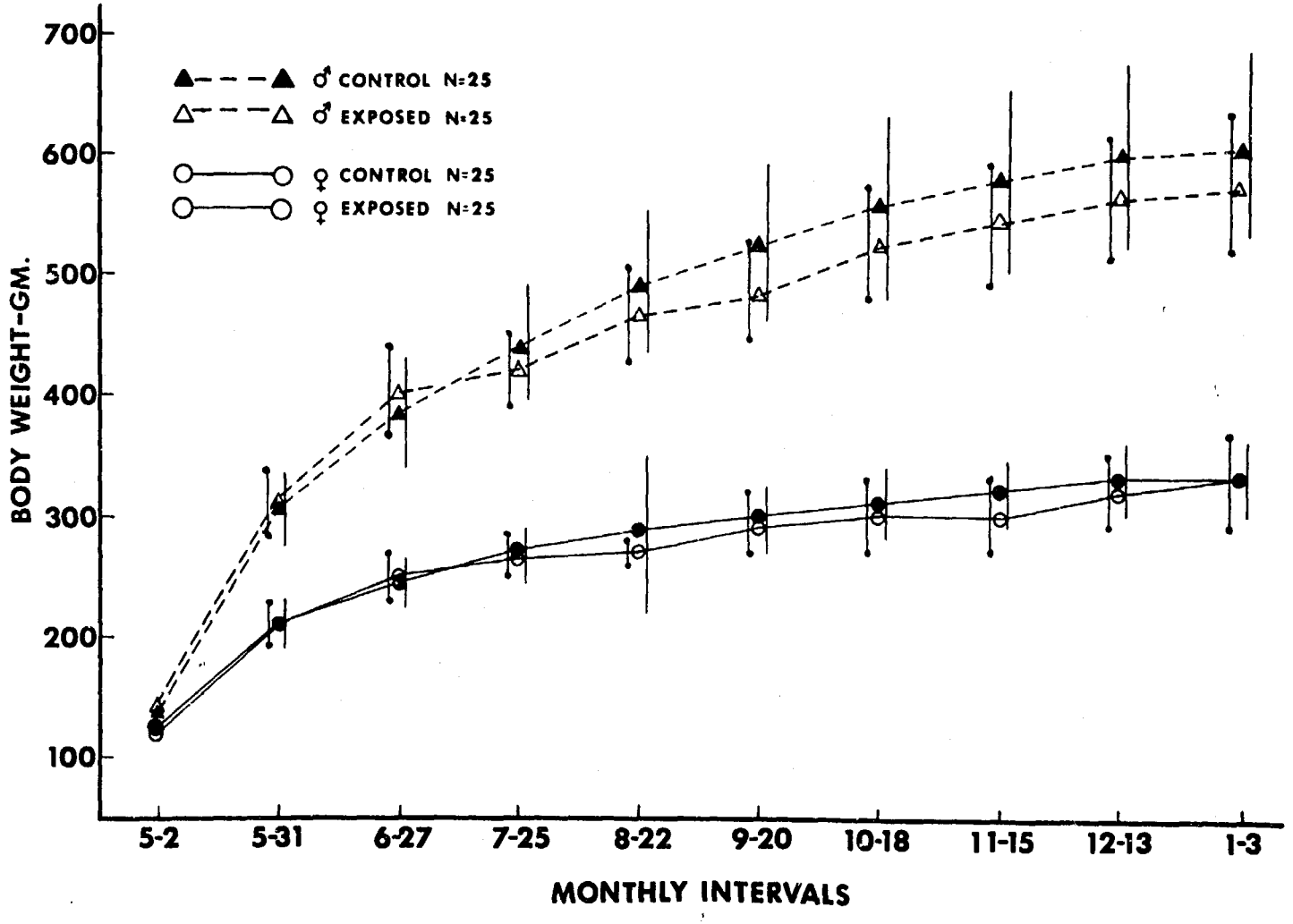

Figure 1. BODY WEIGHT GAIN OF EXPOSED AND CONTROL RATS.
Plot points represent mean gram weight $\pm$ 1 S. D.

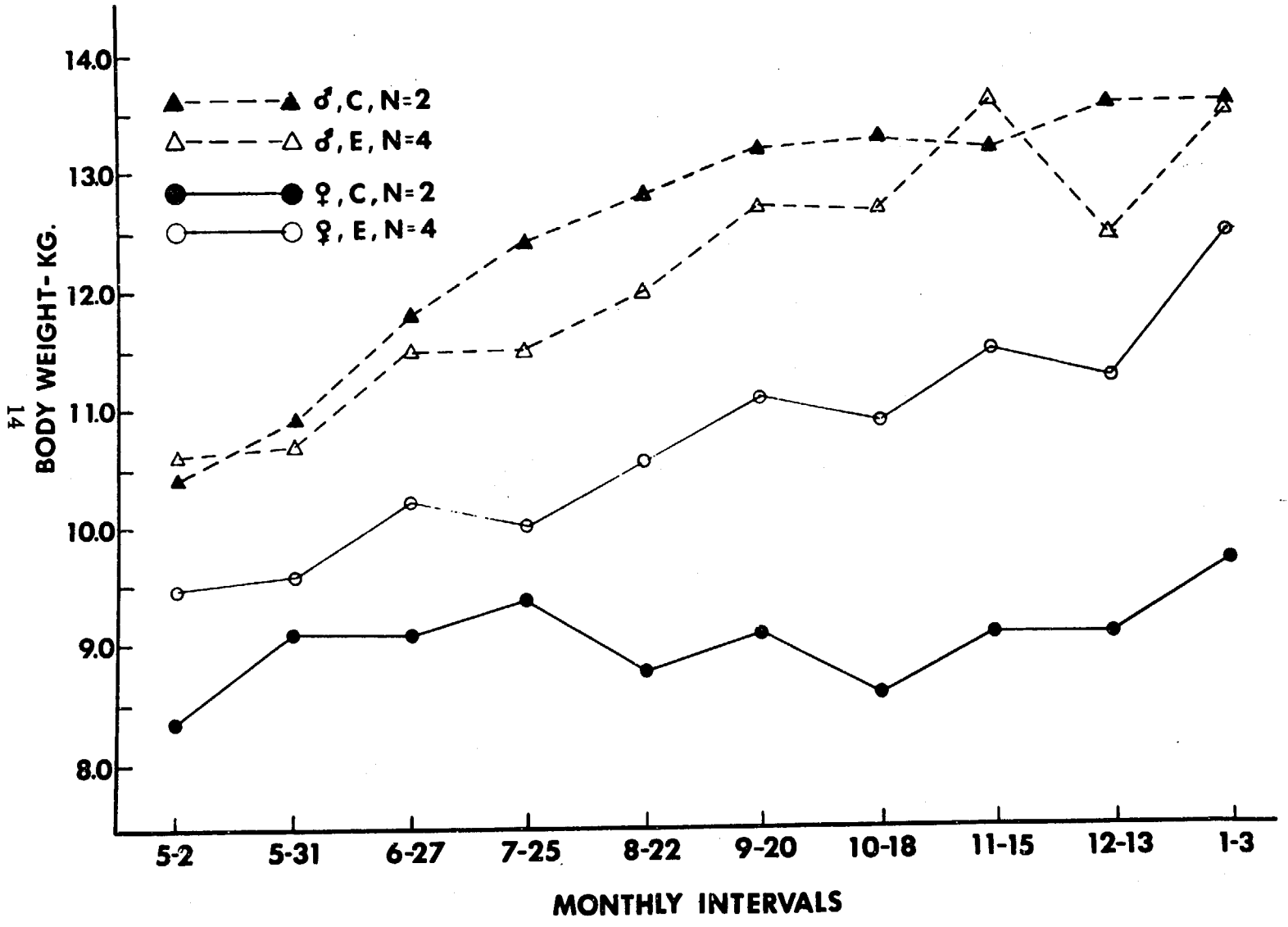

Figure 2. BODY WEIGHT GAIN OF EXPOSED (E) AND CONTROL (C) BEAGLE DOGS. Plot points represent mean kilogram weight.

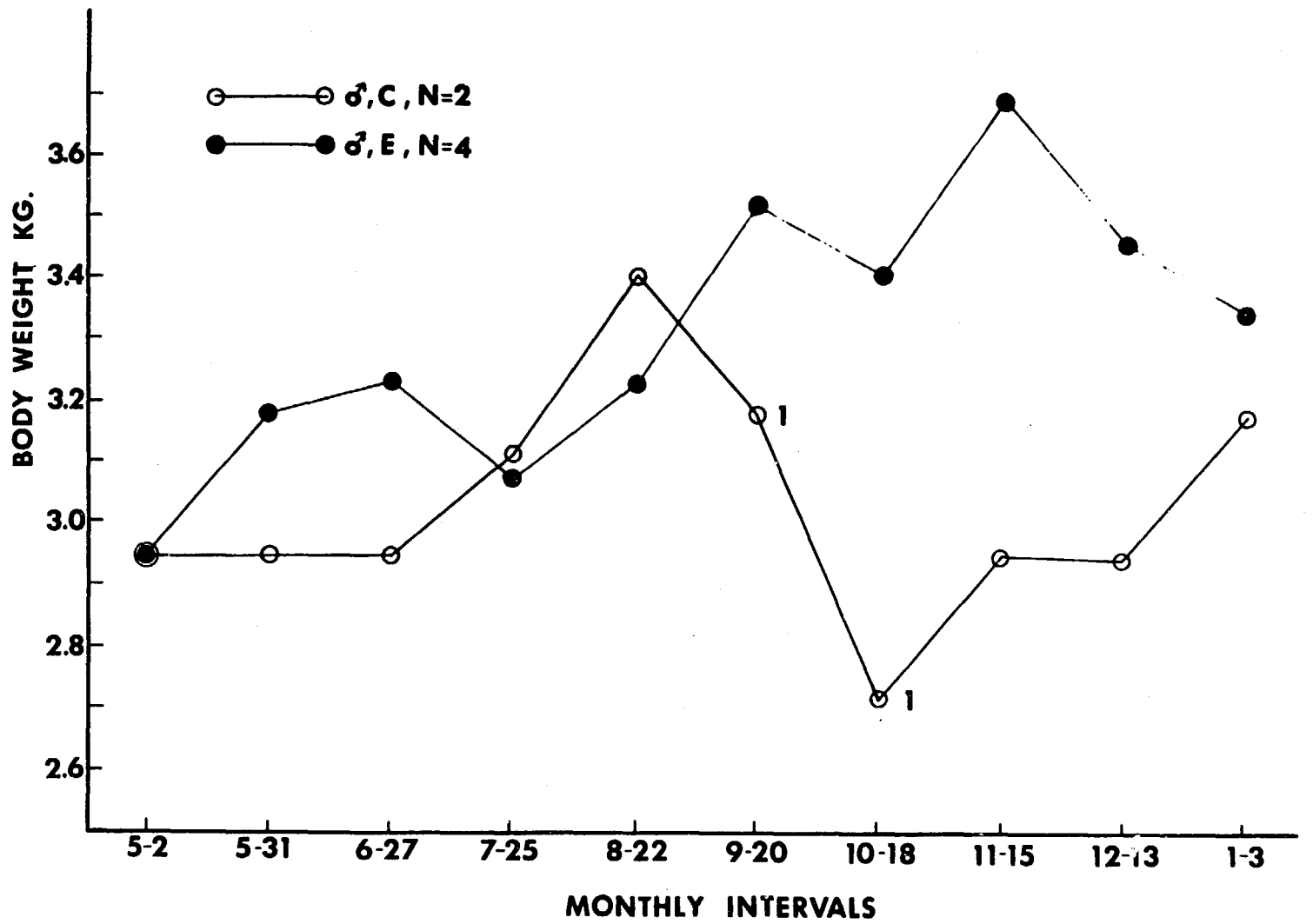

Figure 3. BODY WEIGHT GAIN OF EXPOSED (E) AND CONTROL (C) RHESUS MONKEYS. Plot points represent mean kilogram weight. Points designated "1" represent a single animal.

Necropsy Data

Some of the data collected at necropsy, e.g., individual body weights and corresponding organ weights, were utilized to calculate organ-to-body weight ratios which are represented in figure 4. The histogram compares ratio values for three of the four species under study. Interpretation of the data revealed but one statistically significant comparison, i.e., liver/body weight ratios between exposed and control dogs. Small numbers (indicated under N), in some instances, rendered statistical treatment without real meaning, and in the case of monkeys, no comparison could be made on lungs and hearts since all were required for special fixation techniques. The trend for lower liver/body weight ratio value was also found for exposed monkeys, but the difference between controls was insignificant. The only other finding of significance for organ/body weight ratios (not shown in figure 4) was that of increased size of hearts and lungs of male rats ($P<0.05$). Values for females, or when the sexes were combined (figure 4), did not differ significantly between exposed and controls.

Histopathologic findings revealed by both light and electron microscopy are presented in other papers of these proceedings (Patrick, 1967; Klion, 1967), and are only briefly alluded to below under the section on Discussion.

Clinical Laboratory Data

The clinical laboratory measurements (table III) gave voluminous amounts of data, some of which have yet to be thoroughly analyzed. Computer analysis may eventually indicate subtle alterations in hematologic and chemical data which have eluded our initial examination and evaluation for significant differences between the exposed dogs and monkeys and their counterpart controls.

Hematology and clinical chemistry data were screened for trends of significance, and those showing values indicative of consistent differences between exposed and control animals were subjected to stringent examination. For example, in the case of exposed dogs that showed marked changes in certain blood determinations, comparison was made not only with contemporary controls, but also with control values from a prior study by Robinson and Ziegler (1966) of a large population of "normal" beagles; these investigators reported means and S.D.'s calculated from approximately 800 determinations of each of the blood parameters in table III. The samples represented a six-month study of 202 dogs. Application of their study, in relation to this, has been to utilize their three sigma (3 S.D.) limits of blood values for comparison with those calculated from data on the four control dogs for the mixed gas study. This historical approach is considered justified for comparative purposes, inasmuch as all samples (including those from exposed dogs) were taken, analyzed, and reported by personnel from Wright-Patterson Air Force Base.

An illustration of statistical treatment of selected data is seen in figure 5; hematocrit determinations are used to give an example of an ideal normal pattern. The broad crosshatch band with stippling defines the upper and lower control limits (UCL and LCL), as determined by calculating the mean $\pm$ three S.D. for hematocrit values for each of the four controls sampled at biweekly intervals. The second, overlapping band (crosshatching from left to right) was similarly determined from approximately 806 hematocrit values reported in the Robinson and Ziegler study.

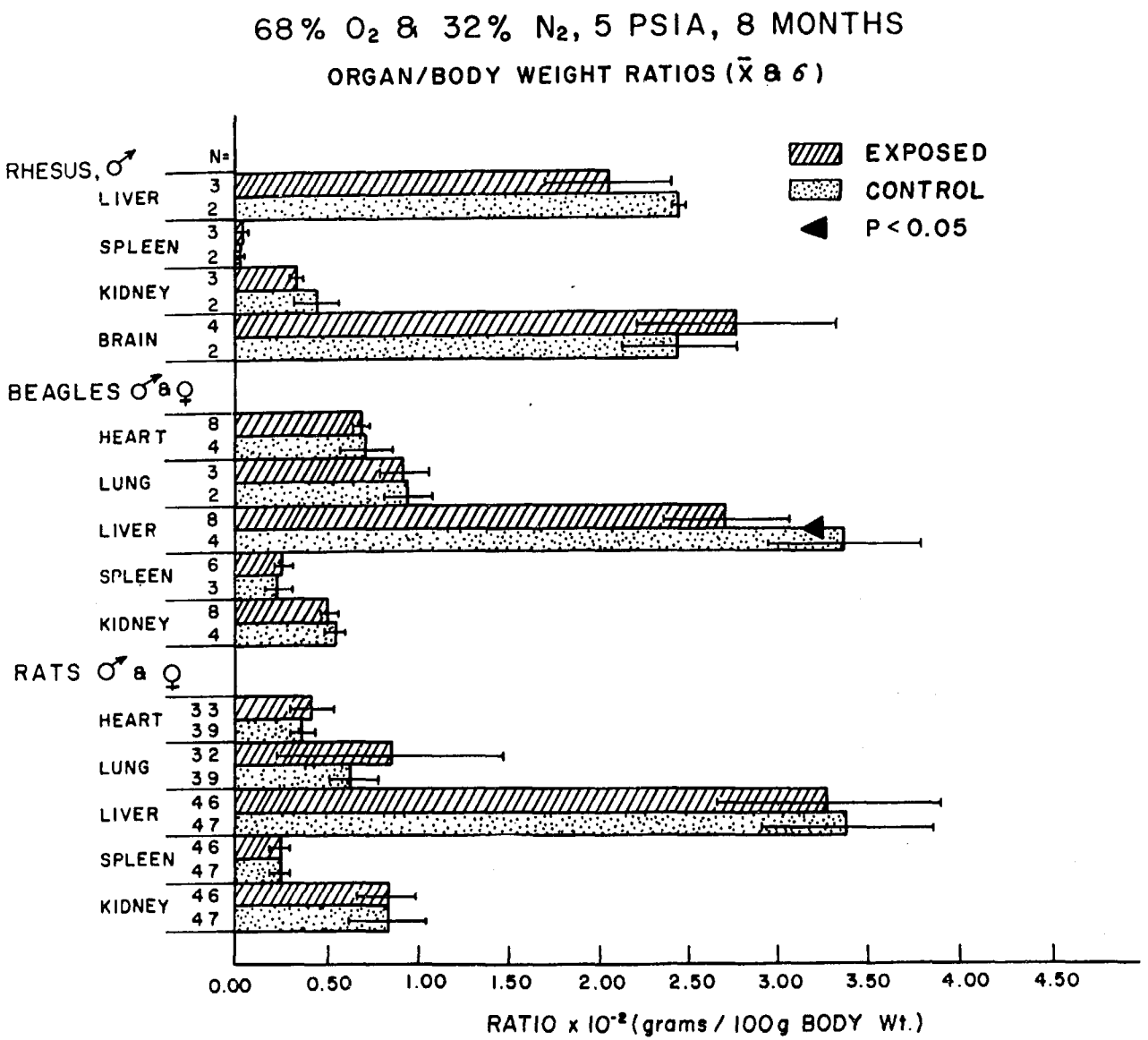

Figure 4. ORGAN/BODY WEIGHT RATIOS FOR EXPOSED AND CONTROL SPECIES. Bars represent calculated mean ratios with indicated ± 1 S. D.

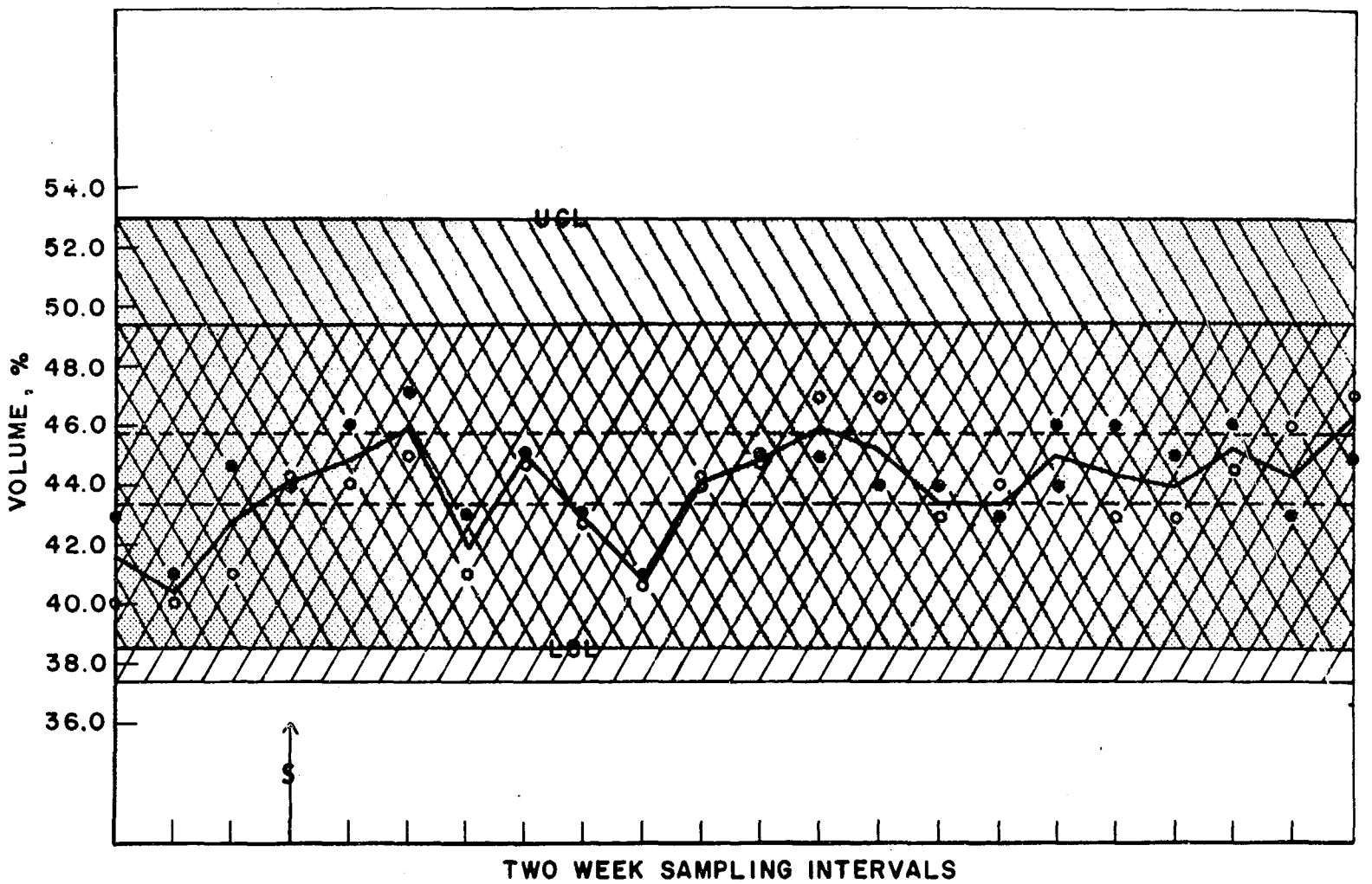

Figure 5. STATISTICAL REPRESENTATION OF HEMATOCRIT VALUES OF EXPOSED AND CONTROL BEAGLE DOGS. See text for complete explanation of data treatment.

Values from exposed dogs are shown as point plots superimposed upon the control bands. Each sampling interval is represented by two plots, solid and open circles, each of which was derived by random selection of hematocrit value means for four dogs (equal sex representation); thus, each point represents mean values for two male and two female dogs in a group. In this manner, experimental and control groups of the same size are compared. Values shown to the left of the vertical arrow (with "S" indicating start) were derived from measurements prior to the start of exposure.

Interpretation of the statistical data demonstrated in figure 5 indicates that the hematocrit values for the eight exposed dogs (four by four plot) did not differ from the four control dogs, nor did either of these significantly differ from hematocrit values derived from the large population of dogs used by Robinson and Ziegler.

One measurement on exposed dogs which showed a highly significant difference from that for controls (both the small and large population groups) was total serum protein; this is demonstrated in figure 6. The upper and lower limits (UCL and LCL) for the contemporary controls, as well as the three sigma ($\pm$ 3 S. D.) limits for the 202 dogs, were comparable until the fourth month of exposure. After this time, the values for total protein of exposed dogs were high and remained so through the eighth month; the change was highly significant ($P < 0.01$). Thus, there is indication that some occurrence altered the total protein content of serum. This finding did not occur in similarly exposed monkeys.

The sudden rise in total protein content of serum from exposed dogs cannot be accounted for by a corresponding rise in serum albumin. As a matter of fact, figure 7 shows that serum albumin determinations for exposed dogs were considerably lower than for control dogs; the majority of the time the difference was highly significant. The important aspect, however, was that the values for the exposed dogs remained consistently lower than those for the controls, and, thus, were noncontributory in the rise in total protein noted above. Had the rise been due to the albumin constituent of protein, it would have become evident and would be seen in the graphic representation of the data.

The serum albumin values as shown in figure 7 for exposed dogs do not fall outside the limits established from samples of dogs from the above mentioned large population. Thus, it appears that the seemingly different values determined for exposed and controls may only reflect the inherent error of small population sampling.

Inasmuch as albumin was not found to be the cause of increased protein, the globulin fractions appeared to be the causative factor. Globulins were not measured directly, on a routine basis, but were calculated indirectly by calculating the difference between total protein and albumin. In this manner then, a globulin value was obtained and the albumin/globulin (A/G) ratios were calculated.

Plots of A/G ratios are demonstrated graphically in figures 8 and 9. The former represents ratios calculated for dogs; for comparative purposes the curves of A/G ratio plots are given for dogs exposed for eight months continuously to 100% O_2 at 5 PSIA, as well as the exposed and controls of the O_2-N_2 study reported here. Figure 9 shows the same data for monkeys. It is readily apparent that the one real difference between A/G ratios of the groups of exposed and control animals was that of the dogs that lived for eight months in 68% O_2 and 32% N_2 at 5 PSIA.

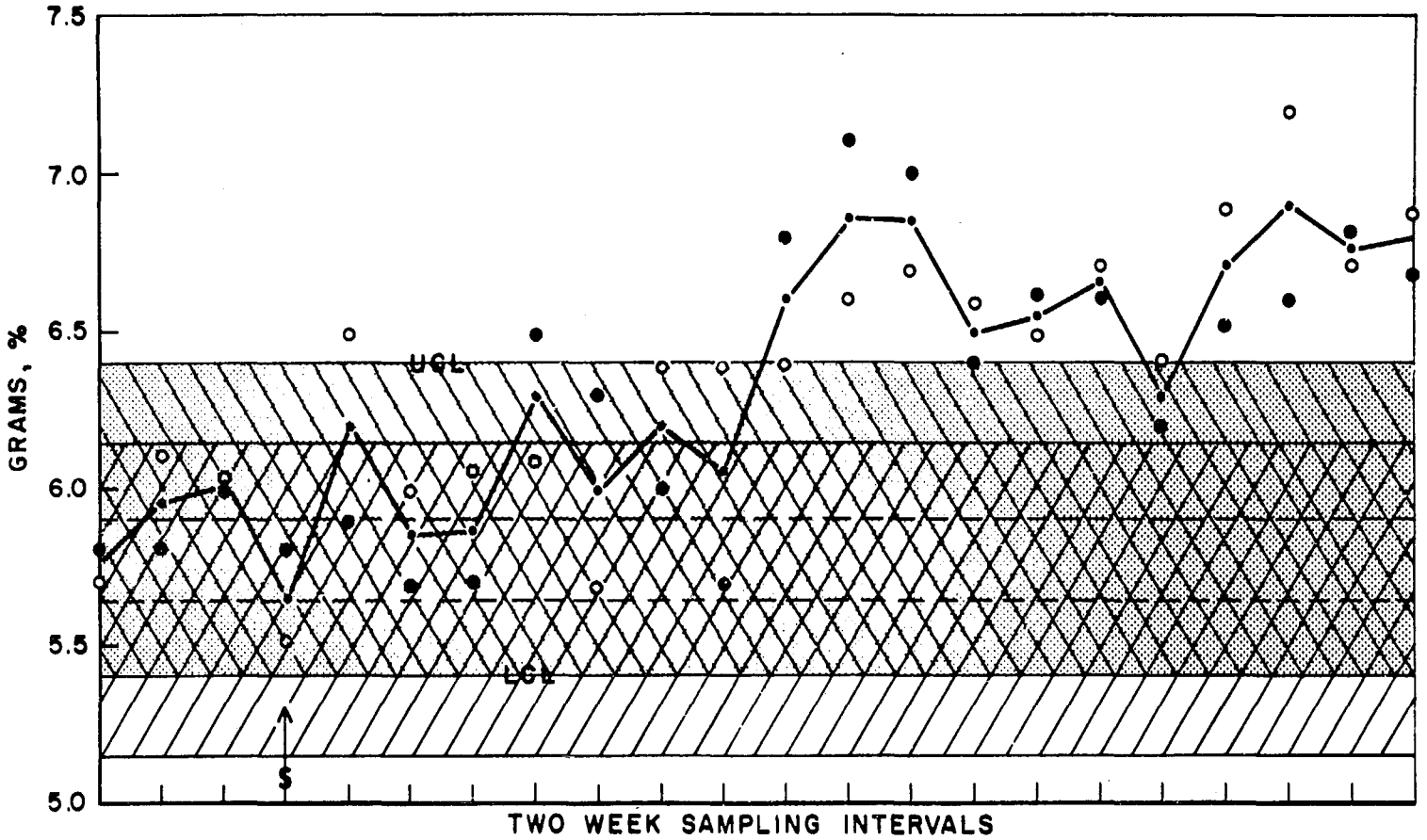

Figure 6. STATISTICAL REPRESENTATION OF TOTAL SERUM PROTEIN VALUES OF EXPOSED AND CONTROL BEAGLE DOGS.
See text for complete explanation of data treatment.

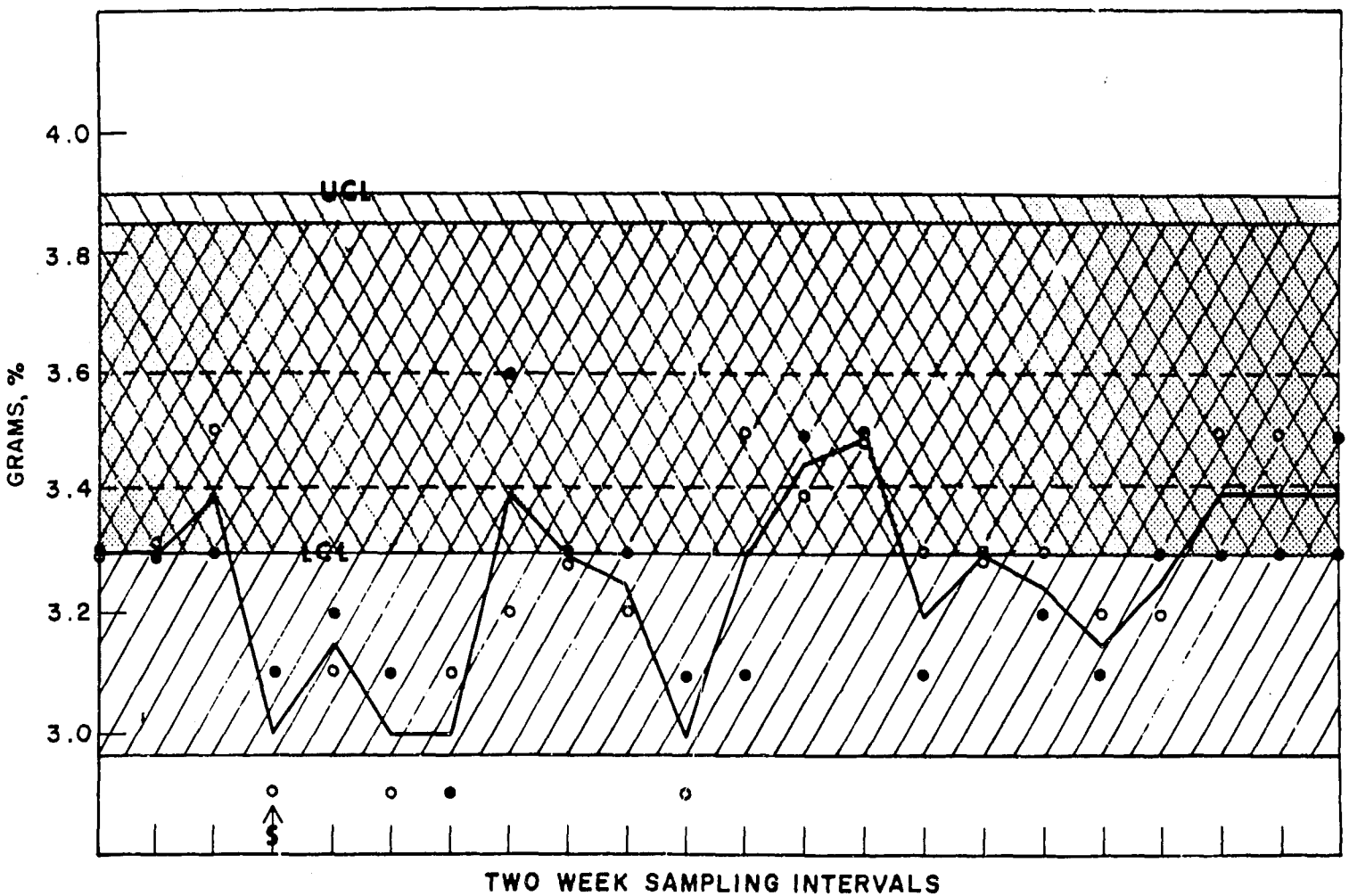

Figure 7. STATISTICAL REPRESENTATION OF SERUM ALBUMIN VALUES OF EXPOSED AND CONTROL BEAGLE DOGS. See text for complete explanation of data treatment.

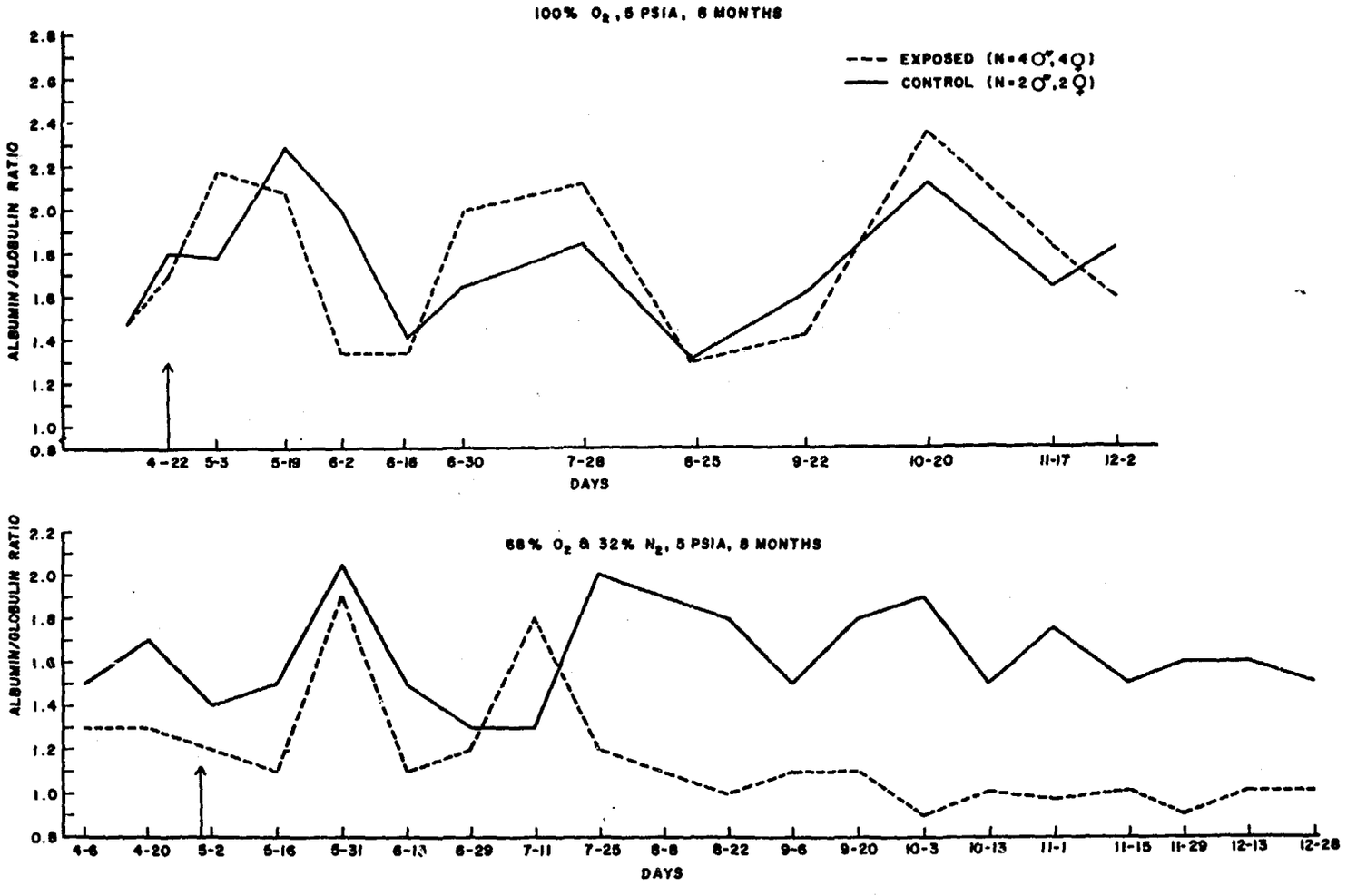

Figure 8. A/G RATIO PLOTS FOR EXPOSED AND CONTROL BEAGLE DOGS USED IN TWO SEPARATE EIGHT-MONTH STUDIES.

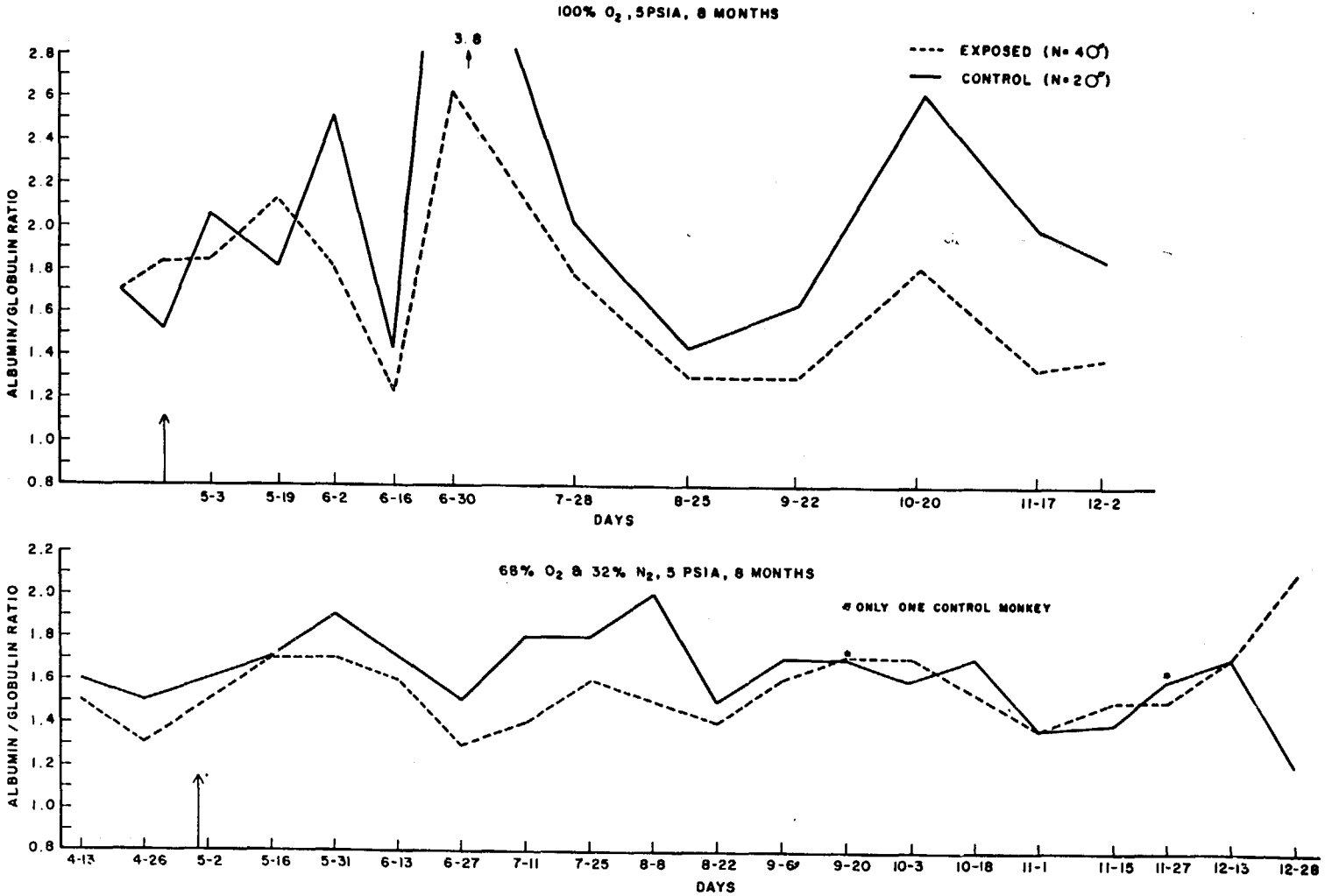

Figure 9. A/G RATIO PLOTS FOR EXPOSED AND CONTROL RHESUS MONKEYS USED IN TWO SEPARATE EIGHT-MONTH STUDIES.

Once it became apparent that total serum protein values were rising, and since it seemed to be due to globulin, an attempt was made to study protein fractionation by direct measurement of the albumin-globulin components; disc electrophoresis was used to accomplish this. Electrophoretic studies were begun in the fifth month of the experiment; from this time forward, until completed, all biweekly blood samples of exposed and control dogs were subjected to analysis of total serum protein by electrophoresis as well as by the routine salt precipitation method (sodium sulfate). Thus, A/G ratios were determined by two methods, the former which measures directly both albumin and globulins, and the latter (wet chemistry) which measures only the albumin directly.

Comparative results of the two methods of protein fractionation of dog serum are presented in figure 10, as A/G ratios. Statistical expression is similar to that previously detailed, with one exception; here, A/G ratio values from the Robinson and Ziegler study of a large beagle population are not utilized since their work did not include any electrophoresis determinations. Rather, in order to demonstrate the inherent difference between the two methods of fractionation, samples from 21 apparently "normal" beagles were compared, each sample by both methods. It is apparent in figure 10 that the determination of mean ($\pm$ one S. D.) A/G ratios for the 21 dogs is lower by the wet chemistry method (indicated by circle-dot symbol) than by electrophoresis (diamond-dot symbol). This same trend was found for the biweekly samples from the eight exposed dogs over the three-month comparative period, as seen by examination of the four by four plots of mean A/G ratios for the two methods. It should also be noted, however, that while A/G ratio values for exposed dogs were higher by the method of electrophoresis, the values for the controls (expressed and shown as stippled bands representing the mean $\pm$ three S. D. in figure 10) were higher by the wet chemistry method. This in itself created an incongruity insofar as comparison of the methods was concerned. Other aspects are mentioned below under the next section.

DISCUSSION

Some of the results of this study of exposure to a mixed gas environment indicate trends of toxic response, although their real meaning is somewhat obscure. Findings from electron microscopy (Klion, 1967) would seem to correlate with those from necropsy data. The liver ultrastructure of the exposed dogs showed alterations not seen in controls; hence, a finding of reciprocal relation to the liver/body weight ratios of exposed dogs which differed significantly from controls. Other than this, histopathologic studies revealed little difference between experimental and control animal groups (Patrick, 1967). It is possible, however, that current work concerning electron microscopic and morphometric evaluation of lungs from this study may reveal some unobtrusive results related to exposure. Such studies proved rewarding in delineation of the effects of 100% O_2 inhalation under various conditions of exposure (Kistler, 1966; Lewerenz, 1967).

The question of the meaning of rise in total serum protein in exposed dogs poses a quandry. It is generally recognized that there are many disease conditions which will alter the albumin and globulin fractions of serum; the albumin, when altered quantitatively, is almost invariably low while the globulin tends to increase reciprocally - usually the result of these two changes is a lowered total protein (Henry, 1964; Damm and King, 1965). Exceptions to this general rule do exist,

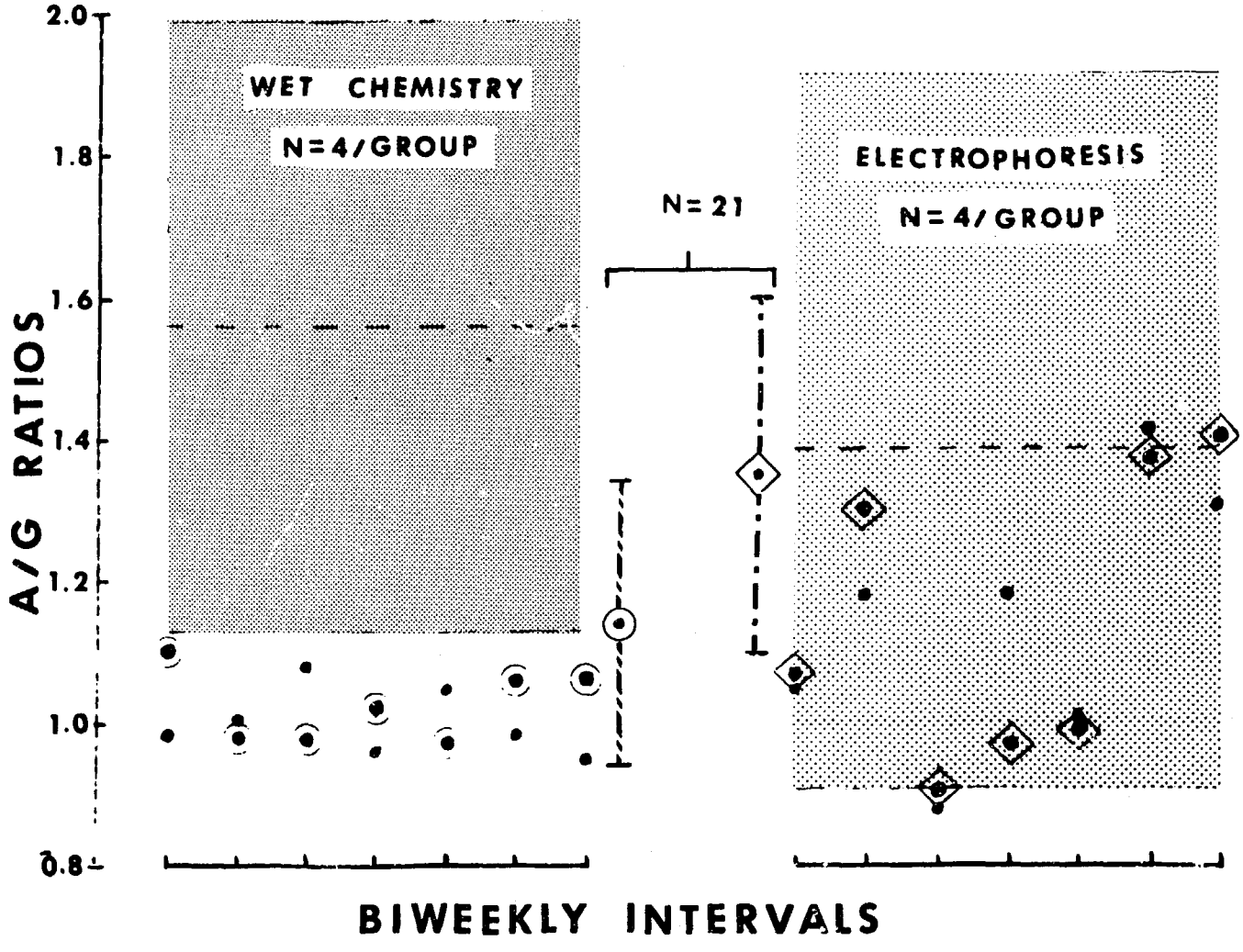

Figure 10. STATISTICAL REPRESENTATION OF A/G RATIOS DETERMINED BY EACH OF THE TWO METHODS INDICATED. See text for complete explanation of data treatment.

however, and the findings of this study seem to represent such an exception, inasmuch as total protein did show definite rise.

There would appear to be some causal relationship between the above mentioned findings concerning necropsy and histopathologic data on the livers of exposed dogs and the fact that biosynthesis of albumin and a large portion of the globulins takes place in the liver.

Interpretative analysis of the results was not helped by the fact that albumin values for the exposed dogs were quite different, statistically speaking, from control values (figure 7). This, in itself, is suggestive at first glance of a subnormal state of health; further consideration, however, would seem to indicate that the difference merely reflects the inherent error of using small numbers of animals. Otherwise, the seemingly low albumin values of exposed dogs would probably have shown a steady decline (progressive illness). These assumptions are also borne out by the statistical comparison (figure 7) with albumin values from the greater numbers of dogs used by Robinson and Ziegler, results of which were pointed out above.

Interpretation of the results concerning A/G ratios is also restricted because of the seemingly poor agreement between the methods (electrophoresis and salt precipitation) for collection of data from which the ratios were calculated. Mention has already been made of the general agreement of greater reliability of data from electrophoresis fractionation than from salt precipitation methods for calculation of A/G ratios. Yet, for the limited time of use of both methods in this study, the salt precipitation method showed greater consistency of A/G ratios than did electrophoresis; this was demonstrated by the close proximity of the four by four plots shown for wet chemistry, as opposed to the scatter of plots shown for electrophoresis (figure 10). Thus, because of the variability of the latter, some of the inverted A/G ratios calculated from electrophoresis data were masked by high values during the same sampling period; the mean values shown as four by four plots, therefore, were higher than those found by salt precipitation.

SUMMARY

In brief summary, it can be said that very few differences between experimental and control animals were detected. The differences noted were:

1. A slower mean growth rate of exposed rats, but the difference was not significant.

2. A slight difference between experimental and control group mortalities; these also were insignificant.

3. The exposed dogs, after several months, in contrast to controls, began to show inversion of the A/G ratios. This response appeared to be a function of increased total protein content, more specifically, the globulins. The latter implication, however, was not completely borne out by limited studies of serum protein fractionation with electrophoresis.

4. Serum albumin of exposed dogs appeared to differ significantly when compared with their counterpart controls, yet the difference did not fall outside of limits of significance derived from values representing a large sample of normal dogs.

5. Necropsy data revealed that the liver/body weight ratios of exposed dogs differed significantly from controls. This finding indicated correlation with changes reported in ultrastructure of the livers of exposed dogs.

Based upon findings reported here, there is indication that the mixed gas system of 68% O_2 and 32% N_2 at a reduced pressure of 5 PSIA was not totally without effect. Specificity of effect cannot be delineated, but most certainly the results indicate a less than optimal physiologic function for at least one species tested. Further experimentation with mixed gas systems is most emphatically indicated, with particular emphasis being placed upon monitoring the changes in serum proteins. Methodology consisting of various wet chemistry determinations and electrophoresis should be evaluated. In addition, the question of liver involvement and its possible influence upon serum protein constituents requires more concentrated study.

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PROBLEMS AND PITFALLS IN AUTOMATED MULTICHANNEL CLINICAL CHEMICAL ANALYSES

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For nearly three years we have been conducting batteries of clinical chemical tests on hospitalized patients, large groups of healthy subjects, and animals which have been on chronic toxicity studies for new drugs and feed or food additives. It is not my purpose today to discuss the clinical findings on either humans or animals. It is enough to say that these findings corroborate those of others in disclosing unsuspected disease conditions in human subjects as well as some rather widespread changes in animals, many of which we have not yet been able to interpret.

Today I want to talk about some of the problems in multichannel simultaneous analyses, particularly as related to continuous flow systems. We are now at the stage at which we feel each week that we have met every obstacle and experienced every mechanical and human error possible, and every week we learn that we have become overconfident and overly optimistic because something new, strange, and troublesome occurs.

In spite of all the problems, however, we are pleased with our automated system and are convinced that it is worth the effort. Refinements and changes in methodology and instrumentation are being continually effected to provide greater accuracy, precision, or labor and cost savings.

The screening battery which we perform is shown in table I. This battery provides us with an estimate of the functional status of many of the biochemical systems of the body such as carbohydrate, protein, and nucleic acid metabolism and the status of the heart, liver, kidney, thyroid, parathyroid, and bone. All determinations are made on serum without taking precautions to insure aseptic techniques.

Our system differs from many other multiphasic analytical units in that it consists of 10 single channel Auto Analyzers* operating off of a single sampler (figure 1). Specimens are analyzed for 10 different clinical chemical determinations at a rate of 60 sera per hour. With this "Multi-10" Analyzer we analyze

*Auto Analyzer - Technicon Instruments Corporation, Chauncey, New York

Figure 1. MULTIPHASIC ANALYTICAL UNIT

TABLE I

AUTOMATED BATTERY OF CLINICAL CHEMICAL DETERMINATIONS

Glucose	Alkaline Phosphatase
Urea Nitrogen	Conjugated Bilirubin
Creatinine	Cholesterol
Uric Acid	Protein Bound Iodine
Calcium	Total Protein
Phosphorus	Albumin
SGOT	Protein Electrophoresis

simultaneously for glucose, urea nitrogen, creatinine, uric acid, calcium, phosphorus, serum glutamic-oxalacetic transaminase (SGOT), alkaline phosphatase, total or direct bilirubin, and a bilirubin blank. Less than two milliliters of serum is required for these analyses. The analytical results of the various tests are displayed on the 10 dual-pen recorders; for this unit alone 600 peaks per hour are displayed.

Cholesterol is conducted on a separate unit since we make an isopropyl alcohol extract of the serum before analysis. Protein bound iodine determinations are likewise made on separate units since the serum must be pretreated before analysis.

Originally, we used Auto Analyzers for the total protein and albumin determinations. Blanks were found to be necessary for both tests and, therefore, four Auto Analyzers were required for these two tests. Even using blanks, however, with the HABA dye method for albumin, we were unable to correlate the values with those obtained by other methods. We have now discarded the Auto Analyzer methods of analysis for total protein and albumin and use a refractometer (Bausch & Lomb) for total protein analysis and an electrophoretic separation of serum proteins on cellulose acetate for albumin and globulin fractions. Quantitation of the fractions is accomplished by use of Analytrols*.

The net result of all this instrumentation and all these tests is that we are spending as much time calculating results, typing reports, maintaining quality control records, etc. as we do in performing the various tests. Hence, we have found it necessary to purchase a computer which will be tied in with the recorders of all Auto Analyzers and the Analytrols. The computer will calculate the standard curve for each test, check all control sera to determine whether we are in control, calculate and correct for interaction or carryover in each test, correct for drifting baselines in each test, store the data until all tests on a particular serum are completed, and then type out a final report. This computer system should be functional in late 1967.

*Analytrols - Beckman Instrument Company, Fullerton, California

I would now like to direct our attention to some of the major problems that are critical in continuous flow automated battery systems. These problems are not necessarily unique to these systems but they have presented formidable problems which must be recognized and solved by anyone engaged in this type of chemical effort.

One of the first problems encountered was that of obtaining a proper standard. We have not been able to prepare successfully a single set of chemical standards for all the tests that we are performing, nor is there available commercially serum standards which fit our requirements. Hence, we had special lots of human serum prepared and lyophilized to meet our specifications. This posed a bigger job than we had anticipated, but we now have a set of eight serum standards which span the complete range of values for all tests. This required individual standardization of eight different standards for 17 different values. Needless to say, this is a task which we prefer to do only once every 14-16 months; this has been accomplished by purchasing 80 liters of lyophilized serum manufactured to our specifications.

Another problem which became apparent rather quickly upon conducting batteries of tests and one to which automated equipment provides no answers is the lack of uniformity and the mishandling of specimens. Our specimens originate not only in our laboratories, but also from a number of local and rural hospitals, from doctors' offices both local and remote, and from animal laboratories, the farthest of which is in Western Germany. We have learned to expect specimens that are hemolyzed, lipemic, nonfasting, clotted, or treated with any one of several anticoagulants and preservatives. Some may have been in the mail for days or in a delivery vehicle at 100-120 F for one to four hours.

Since the greatest variable in the sample is its condition when it is received at the laboratory, it is imperative that laboratory personnel know what constitutes an adequate sample and insist that a proper sample be received. No sample should be analyzed unless valid and meaningful results can be obtained. For this reason, it was essential to determine and evaluate any adverse effects that occur when specimens have not been handled properly.

Several studies were designed to determine the effects of room and elevated temperatures as well as conditions in mailing material for clinical chemical determinations. In these tests the serum was separated from the clot after 30 minutes at room temperature and divided into aliquots. These aliquots were allowed to stand at room temperature, incubated at elevated temperatures, allowed to stand in closed delivery vehicles at temperatures up to 120 F for six hours, and mailed from various rural areas to the laboratory.

To determine whether there was a significant change in a particular analysis, the difference between two results was compared with the standard deviation (S. D.) for the procedure. If a value varied from the value obtained on fresh serum by more than three S. D., the effect was termed significant. The standard deviation of the blind duplicate specimens for each procedure are shown in table II.

The most dramatic changes occurred, as one would expect, in the glucose fractions (table III). This was most notable in the room temperature experiments in which four percent of the samples showed changes in serum glucose after standing for 24 hours. As the time of standing at room temperature increased, the percentage of samples was affected. Interestingly enough, these decreases in serum

glucose were not apparent after maintaining the specimens at elevated temperatures (98-120 F) for six hours. Mailed specimens, however, showed a significant decrease in serum glucose values.

TABLE II

STANDARD DEVIATION OF BLIND DUPLICATES FOR VARIOUS ANALYSES

Analysis	Standard Deviation	Change Required For Significance
Glucose (mg/100 ml)	2.6	8
BUN (mg/100 ml)	0.6	2
Creatinine (mg/100 ml)	0.06	0.2
Uric Acid (mg/100 ml)	0.17	0.5
Calcium (mg/100 ml)	0.3	1.0
Phosphorus (mg/100 ml)	0.18	0.6
Total Protein (g/100 ml)	0.11	0.34
Albumin (g/100 ml)	0.11	0.34
Globulin (g/100 ml)	0.08	0.30
SGO Transaminase (Units)	2.1	7
Bilirubin (direct) (mg/100 ml)	0.02	0.07
Alkaline Phosphatase (Bessey-Lowery Units)	0.2	0.7
Cholesterol (mg/100 ml)	5.8	18
PBI (mcg/100 ml)	0.57	1.8

One of the interesting facts disclosed in these studies is the lack of uniformity of reduction of serum glucose in all specimens held at room temperature (table IV). Also, the magnitude of the change in glucose content varied markedly among the samples.

Urea nitrogen, uric acid, total protein, SGOT, alkaline phosphatase, and PBI did not appear to be affected by either 48 hours at room temperature or by mailing. In view of the bulk of information from various experiments, it is believed that the two alkaline phosphatase specimens which were altered in the two-day mailed samples were change variations rather than a true age-temperature effect.

TABLE III

EFFECT OF AGING OF SERUM ON VARIOUS ANALYSES

Treatment of Specimen (serum)	Percentage of Samples Affected by Treatment										
	Glucose	BUN	Creatinine	Uric Acid	Total Protein	Albumin Globulin	SGOT	Alkaline Phosphatase	Bilirubin (direct)	Cholesterol	PBI
1) Standing											
(A) Room Temperature											
24 hours	4					20			13	5	
48 hours	38					50			?	5	
4 days	92		8			67	4		?	8	
6 days	100		63		8	100	4		?	20	
(B) 98 F for six hours						7			33		
(C) 120 F for six hours						8		7	100	7	
2) Mailing											
(A) One day	8					56			23		
(B) Two days	21		7			64		14	29	14	

TABLE IV

STABILITY OF GLUCOSE IN SERA STORED AT ROOM TEMPERATURE

Age of Specimen	Serum Glucose Values (mg/100 ml) Sample Number											
	1	2	3	4	5	6	7	8	9	10	11	12
Fresh Serum	92	84	84	49	104	84	124	78	86	141	100	99
24 hours	95	86	79	50	100	82	119	73	78	132	94	96
48 hours	44	28	76	48	97	82	118	64	81	133	31	95
72 hours	0	0	35	0	35	82	0	0	36	52	0	0
168 hours	0	0	0	0	0	0	0	0	0	0	0	0

Underlined values indicate significant changes from previous analysis.

The marked effects observed on the albumin and globulin fractions of serum are not believed to be as significant as it would appear from inspection of the tables. These variations were due in part to the great variability and difficulty with the HABA dye methodology for albumin. These marked changes have not been found in mailed specimens which are subjected to quantitation by electrophoresis on cellulose acetate.

The decrease in conjugated (direct) bilirubin values was anticipated since no precautions were taken with any sample to avoid exposure to light. The decrease in serum cholesterol, on the other hand, was unexpected and unexplained. The change, although observed in only 5-10 percent of the samples and usually of small magnitude (-10%), is believed to be real.

Early in our studies it was found that freezing of serum did not alter the values of any of the components with which we were concerned (table V). This included the fractions of serum proteins as determined by electrophoresis. Therefore, this procedure of shipping frozen sera is our method of choice for mailed specimens. When such mailed specimens reach the laboratory in a frozen or "refrigerated condition" meaningful results can be obtained.

In table V are summarized the data from two of four experiments (total of 52 subjects) which show that serum specimens, when properly prepared and kept refrigerated for seven days, are stable for all analyses performed in our battery. In two of these studies aliquots of the refrigerated sera were analyzed after one, two, three, and seven days of refrigeration. In all cases there appeared to be no significant difference between the values obtained on fresh serum and on the refrigerated serum.

Particular emphasis was placed on the effects of allowing serum to stand on the clot (table VI). It can be seen that one-third of the samples are adversely affected after standing on the clot for only two hours - one and one-half hours longer than normal. Standing on the clot for four hours causes a decrease in glucose in more than 50 percent of the specimens; standing on the clot for 24 hours affected all specimens. No further decreases were observed, however, until after four days on the clot at either refrigerated or room temperatures (tables VII, VIII).

Bilirubin was adversely affected when serum was allowed to stand on the clot. Since these samples were not protected from fluorescent laboratory lights, it is not certain whether the reduction was due to light or standing on the clot, or both. In some specimens bilirubin values were decreased by 30-50 percent. Only one specimen showed a 10 percent decrease in cholesterol in these experiments.

Specimens that were refrigerated on the clot showed similar patterns for glucose as did those held at room temperature (table VI). However, the only other determination affected by such treatment was cholesterol (10 percent decrease in 15 percent of the specimens). It was found that the decrease of 20-30 mg/100 ml in glucose in refrigerated clotted specimens occurs in all samples within 48 hours; no further change was noted within the succeeding five days (table VIII).

Our experience with what happens to chemical values when serum is allowed to stand on the clot led us to investigate some of the anticoagulants and preservatives. We have studied the classical anticoagulants and preservatives and have found none of them to be completely satisfactory for mailed specimens for either

TABLE V

EFFECT OF FREEZING ON SERUM CHEMICAL VALUES

Age of Specimen	Mean Value of 24 Sera												
	Glucose	BUN	Creatinine	Uric Acid	Total Protein	Albumin	SGOT	Alkaline Phosphatase	Conjugated Bilirubin	Cholesterol	PBI	Calcium	Phosphorus
Fresh Serum	85	13.4	.95	4.1	8.3	4.9	17	2.6	0.14	230	6.2	9.8	2.9
Frozen 1 Week	85	13.1	.95	4.0	8.2	5.0	19	2.6	0.12	225	6.3	9.8	2.8
Refrigeration 1 Week	87	13.0	1.00	4.0	8.3	5.1	19	2.7	0.13	222	6.2	9.7	2.9

TABLE VI

ANALYSES AFFECTED BY ALLOWING SPECIMENS TO STAND ON CLOT

Treatment of Specimen	Percentage of Samples Adversely Affected										
	Glucose	BUN	Creatinine	Uric Acid	Total Protein	Albumin	SGOT	Alkaline Phosphatase	Bilirubin (direct)	Cholesterol	PBI
Standing on Clot											
Room Temperature for											
2 hours	33								7	7	
4 hours	54								28	7	
48 hours	100					40			?	8	
96 hours	100			50		75			?	8	
Refrigerated for											
24 hours	32									13	
48 hours	100									33	
96 hours	100			17						16	

TABLE VII

STABILITY OF GLUCOSE IN BLOOD SPECIMENS STORED ON THE CLOT AT ROOM TEMPERATURE

Age of Specimen	Serum Glucose Values (mg/100 ml) Sample Number											
	1	2	3	4	5	6	7	8	9	10	11	12
Fresh Serum	92	84	84	49	104	84	124	78	86	141	100	99
24 hours	73	67	64	42	88	73	102	55	64	100	66	76
48 hours	71	63	61	39	87	65	101	52	62	115	69	71
72 hours	65	63	59	37	85	65	99	51	61	114	69	69
96 hours	65	63	29	36	54	64	98	20	58	112	12	69
168 hours	0	0	0	40	0	53	100	0	0	0	0	0

Underlined values indicate significant changes from previous analysis.

TABLE VIII

STABILITY OF GLUCOSE IN BLOOD SPECIMENS STORED ON THE CLOT AT 8 C

Age of Specimen	Serum Glucose Values (mg/100 ml) Sample Number											
	1	2	3	4	5	6	7	8	9	10	11	12
Fresh Serum	92	84	84	49	104	84	124	78	86	141	100	99
48 hours	69	59	59	36	83	56	96	49	59	111	66	69
72 hours	66	61	58	34	84	54	101	47	59	111	69	67
96 hours	72	60	61	33	85	57	101	46	60	114	67	68
168 hours	70	62	63	37	83	60	100	48	61	117	69	72

Underlined values indicate significant changes from previous analysis.

hematology or chemical examinations. Ethylenediaminetetra-acetic acid (EDTA), heparin, fluoride, heparin-fluoride, citrate, oxalate, quaternary ammonium compounds, alkylating agents, enzyme inhibitors, and antibacterial agents have been used and no single material or combination has proved to be an effective preservative-anticoagulant that does not interfere with some test in the battery (figures 2-17, tables IX and X).

It is apparent from these studies that when a specimen is properly handled, valid results can be obtained on serum. Care must be taken, however, to separate serum from the clot within 30 minutes after drawing the blood and maintaining the serum at refrigerated temperatures until analysis.

TABLE IX

EVALUATION OF BLOOD SMEARS MADE FROM SPECIMENS TREATED WITH ANTICOAGULANTS AS COMPARED WITH SMEARS MADE FROM CAPILLARY BLOOD

Treatment	Time of Standing Before Preparation of Smear		
	10 Minutes	1 Day	2 Days
Heparin	Good	Good	Fair
Oxalate	Fair	Unsatisfactory	Unsatisfactory
Citrate	Excellent	Unsatisfactory	Unsatisfactory
Heparin-Fluoride	Poor (Platelet Degeneration)	Unsatisfactory	Unsatisfactory
EDTA	Excellent	Good	Unsatisfactory

TABLE X

EFFECT ON ANALYSIS IN SERUM OR PLASMA

Additive to Whole Blood	Glucose	BUN	Creatinine	Uric Acid	Calcium	Phosphorus	SGOT	Alkaline Phosphatase	Bilirubin, Conjugated	Cholesterol	PBI	T-3	Total Protein	Albumin	Globulins
Heparin	<	0	>	0	0	>	>	0	0	0	0	0	0	0	0
EDTA	<	0	0	<	<	>	>	<	0	0	0	0	0	0	0
Fluoride	<	0	>	<	<	>	>	<	UN	<->	0	>	<->	<	>
Heparin-Fluoride	<	0	>	<	<	>	>	<	UN	<->	0	>	<	<	>
Thymol	<	0	>	<	<	>	>	0	UN	0	0	>	>	<	>
Thymol-Fluoride	<	0	>	<	<	>	>	<	UN	<->	0	>	<->	<	>
Citrate	<	0	>	<	<	>	>	<	UN	<	0	0	0	<	0
Oxalate	<	0	>	0	<	>	>	<	UN	>	0	0	>	<	>
Azide	<	<	>	0	<	<	>	<	0	<	<	>	0	0	0
Quaternary NH_4^+	<	0	>	0	<	0	>	0	0	0	0	0	>	0	0

0 = no effect; > = increase in value; < = decrease in value; UN = unable to analyze; <-> = decrease followed by increase with time

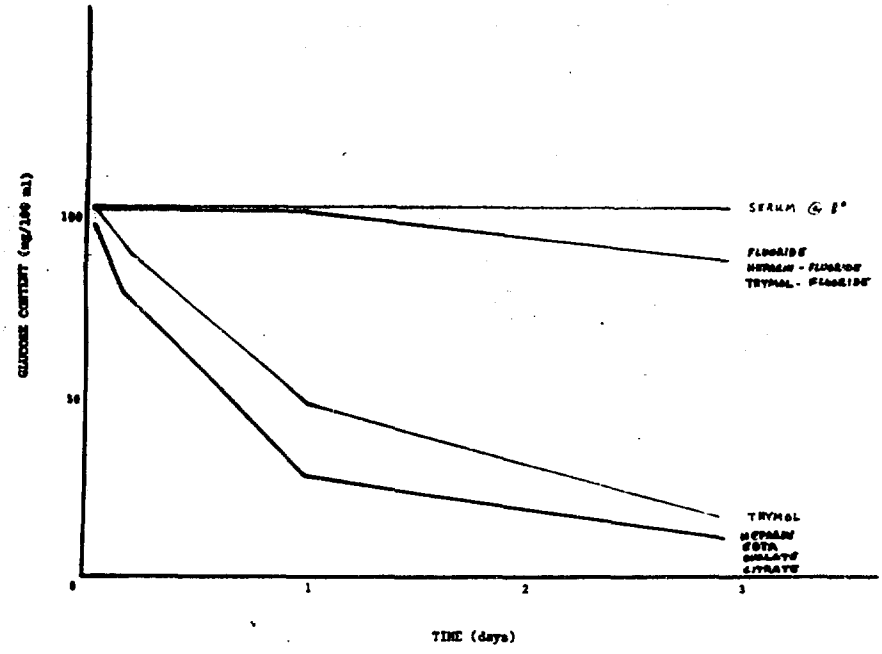

Figure 2. EFFECT OF ANTICOAGULANTS AND PRESERVATIVES ON BLOOD GLUCOSE

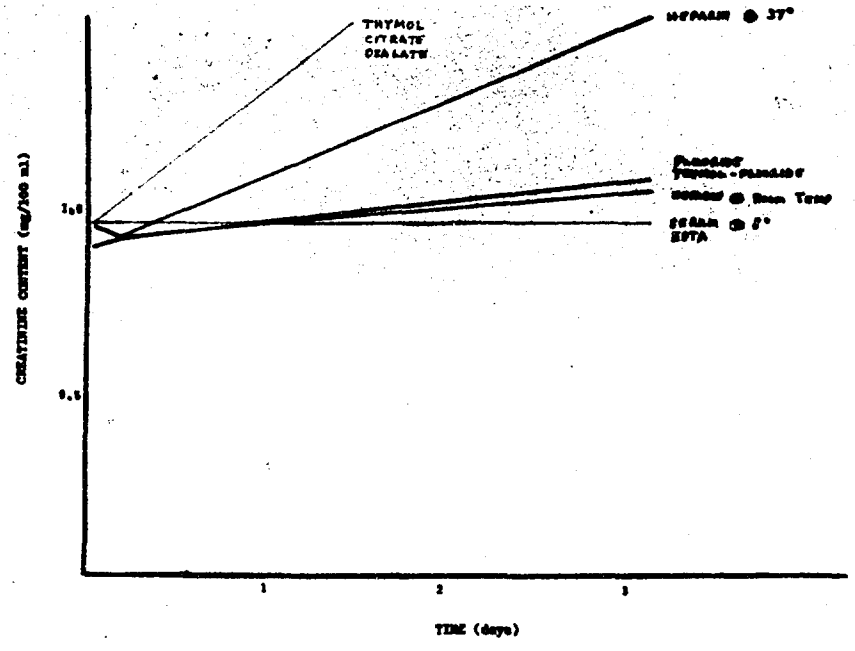

Figure 3. EFFECT OF ANTICOAGULANTS AND PRESERVATIVES ON BLOOD CREATININE

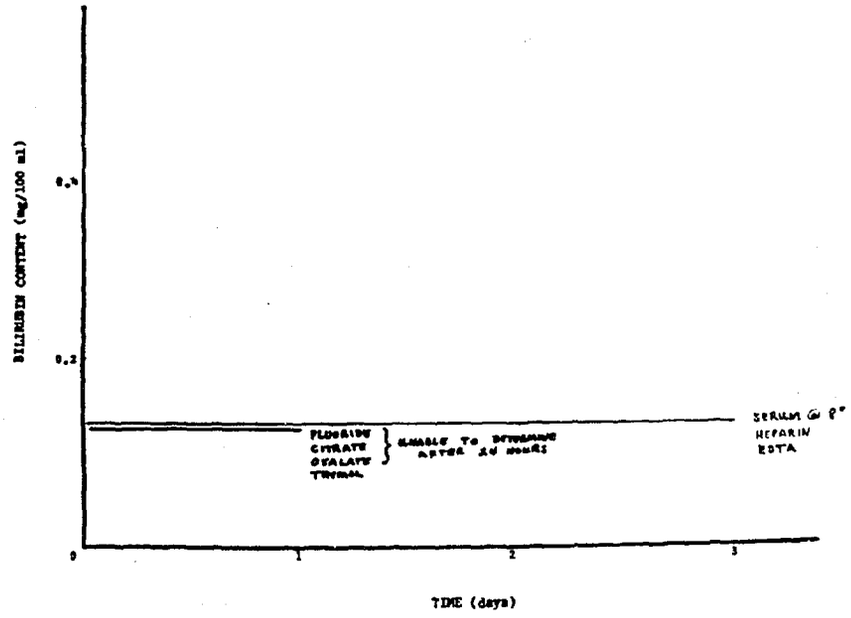

Figure 4. EFFECT OF ANTICOAGULANTS AND PRESERVATIVES ON BILIRUBIN CONTENT OF BLOOD

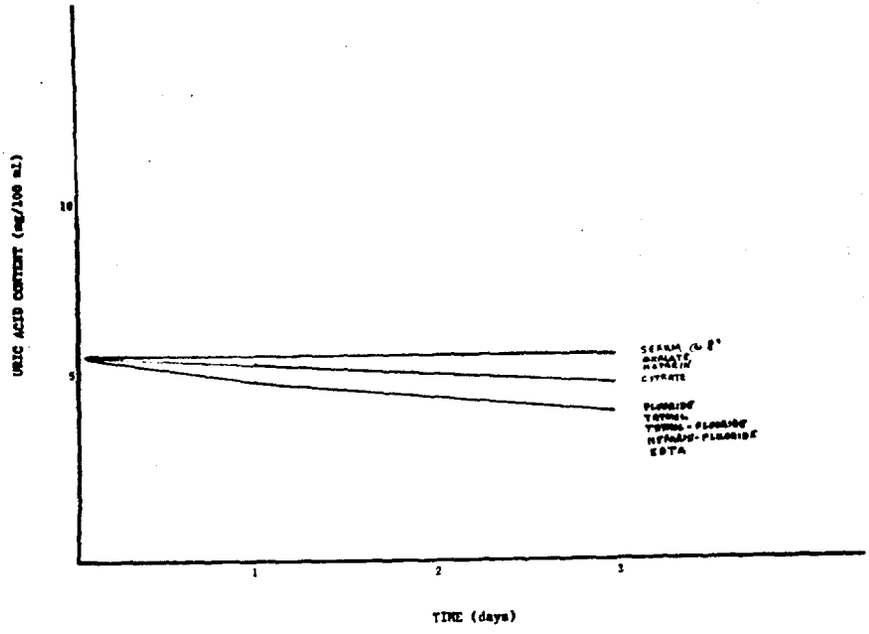

Figure 5. EFFECT OF ANTICOAGULANTS AND PRESERVATIVES ON BLOOD URIC ACID

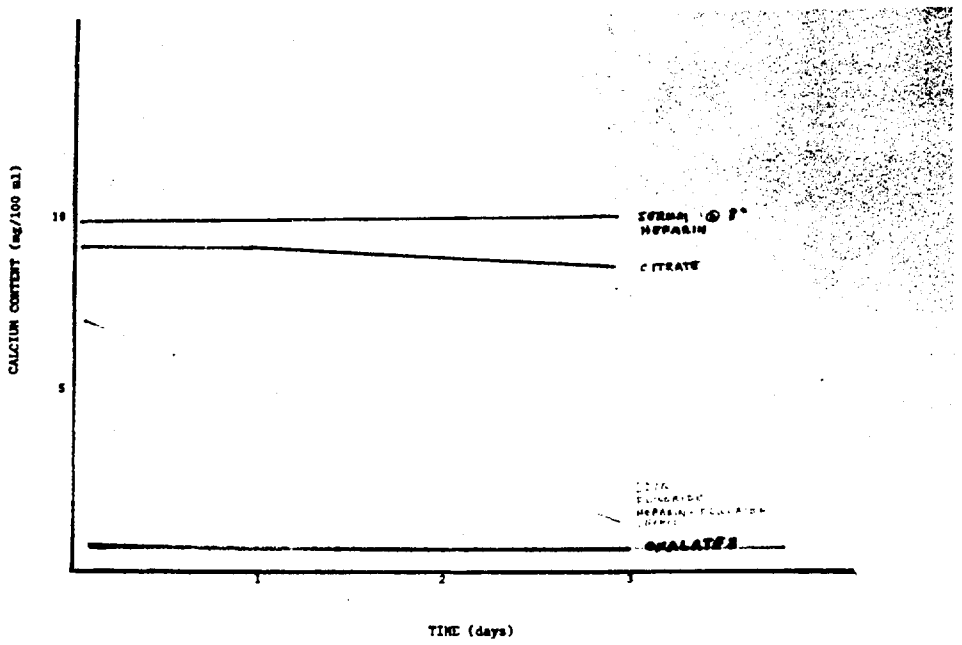

Figure 6. EFFECT OF ANTICOAGULANTS AND PRESERVATIVES ON BLOOD CALCIUM

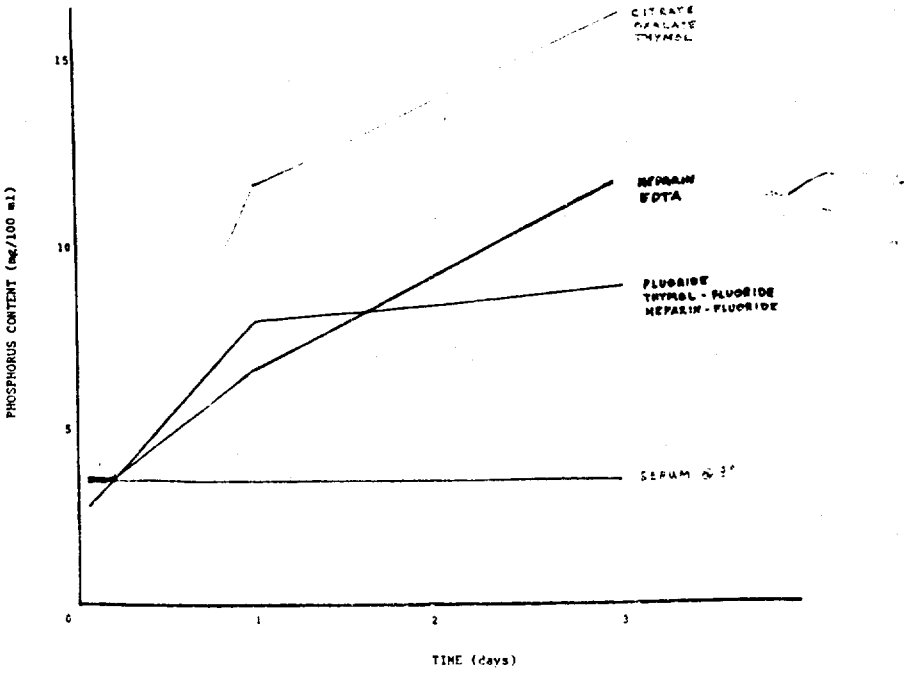

Figure 7. EFFECT OF ANTICOAGULANTS AND PRESERVATIVES ON BLOOD PHOSPHORUS

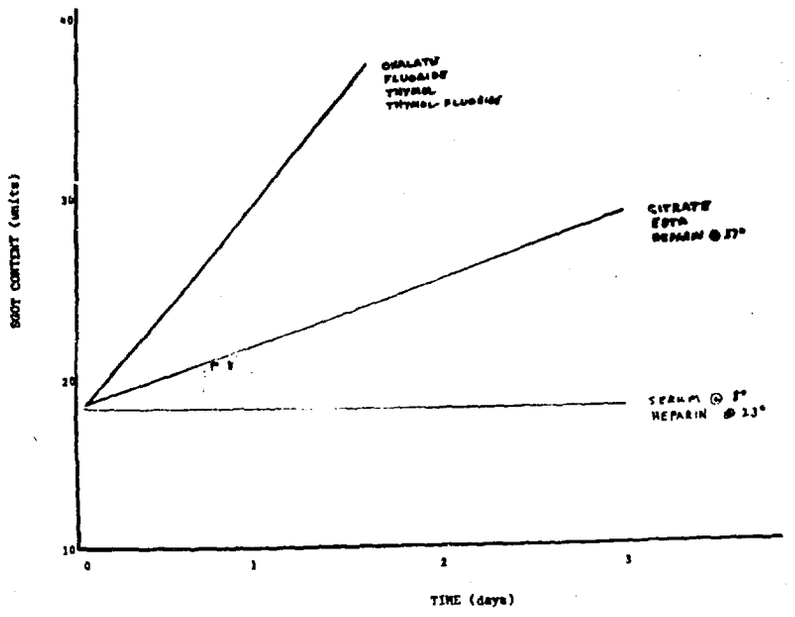

Figure 8. EFFECT OF ANTICOAGULANTS AND PRESERVATIVES ON SGOT CONTENT

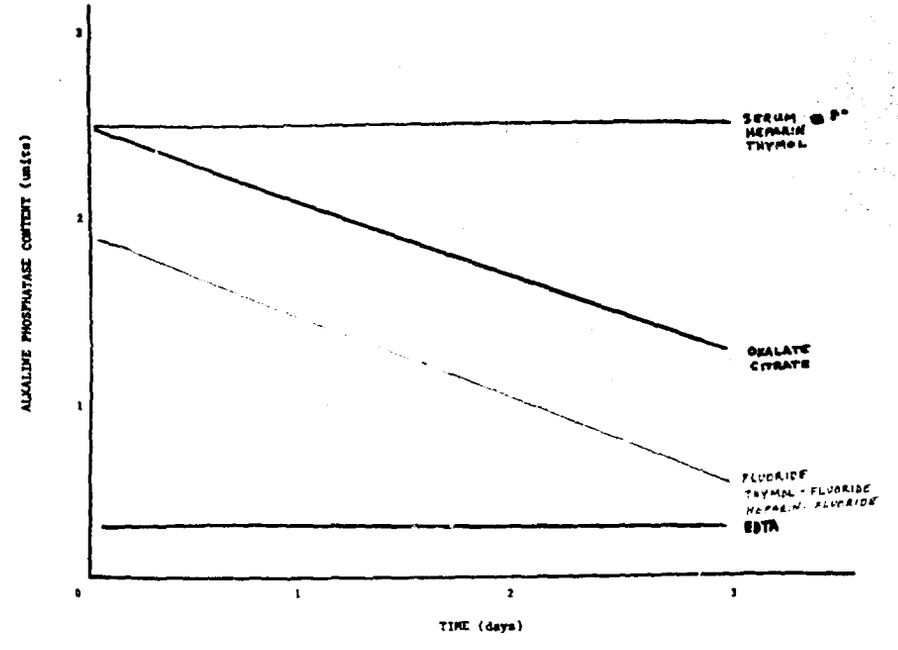

Figure 9. EFFECT OF ANTICOAGULANTS AND PRESERVATIVES ON BLOOD ALKALINE PHOSPHATASE

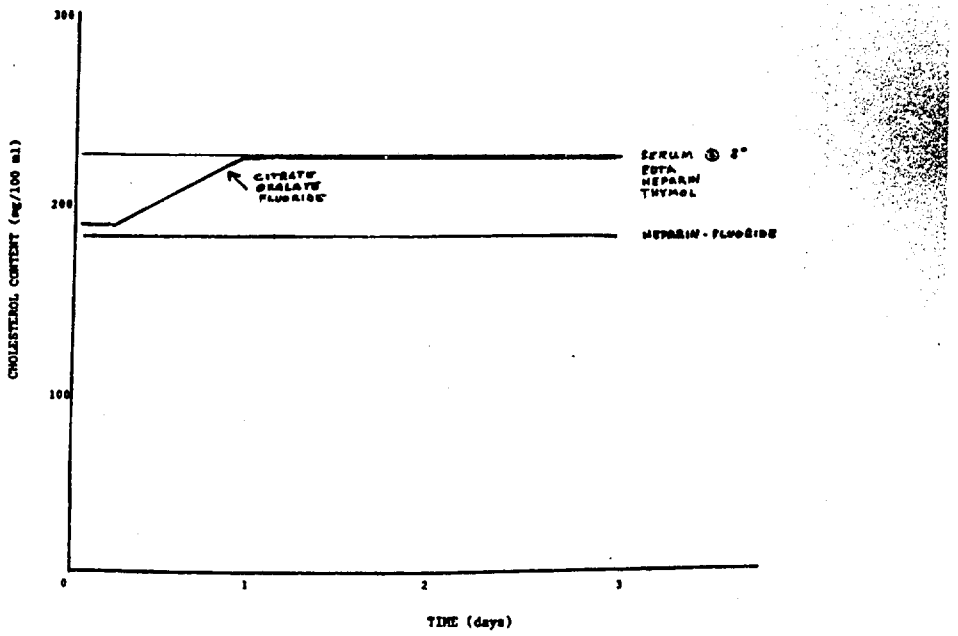

Figure 10. EFFECT OF ANTICOAGULANTS AND PRESERVATIVES ON BLOOD CHOLESTEROL

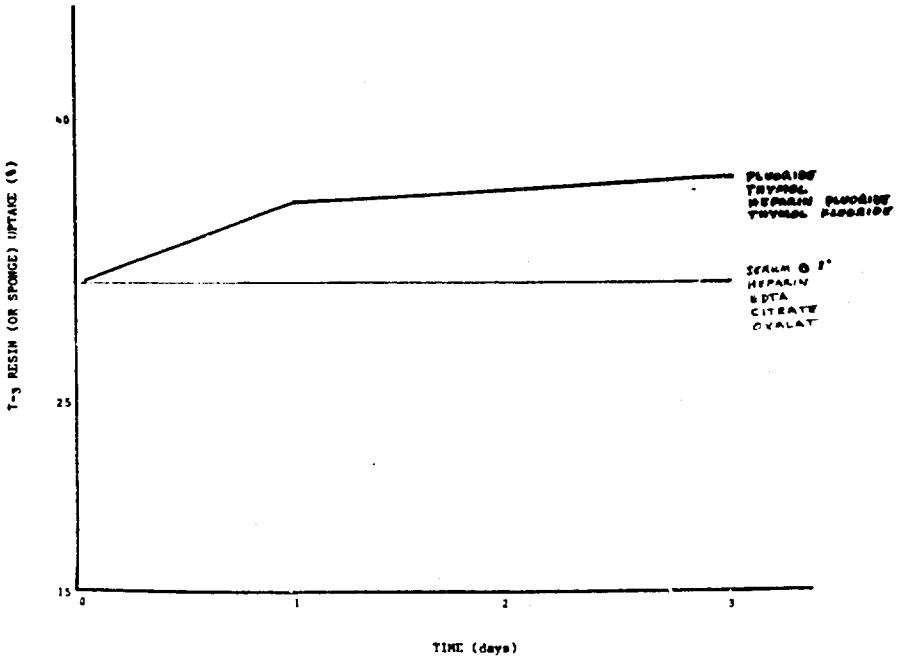

Figure 11. EFFECT OF ANTICOAGULANTS AND PRESERVATIVES ON $T-_3$ UPTAKE

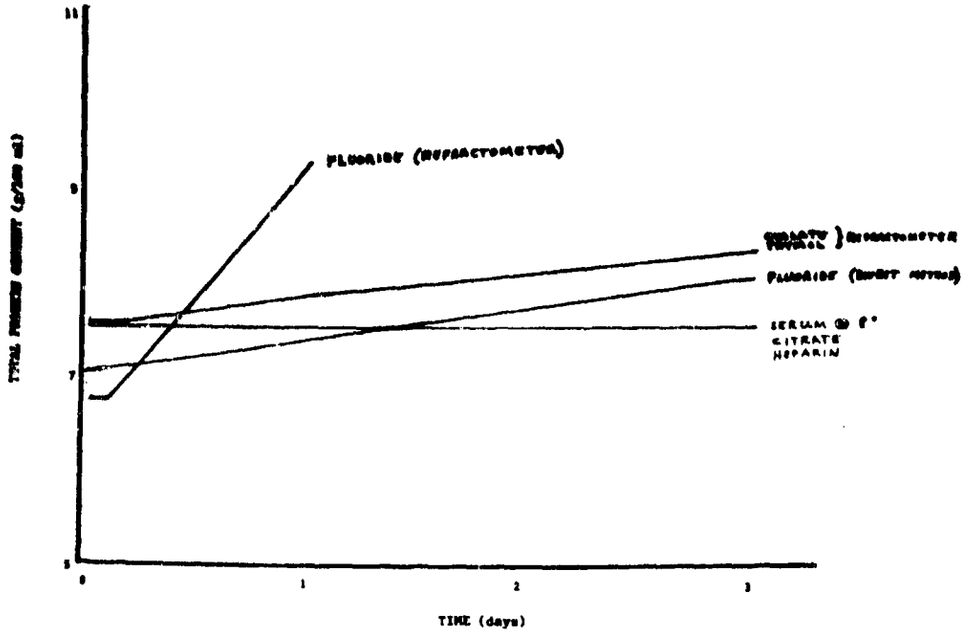

Figure 12. EFFECT OF ANTICOAGULANTS AND PRESERVATIVES ON TOTAL PROTEIN

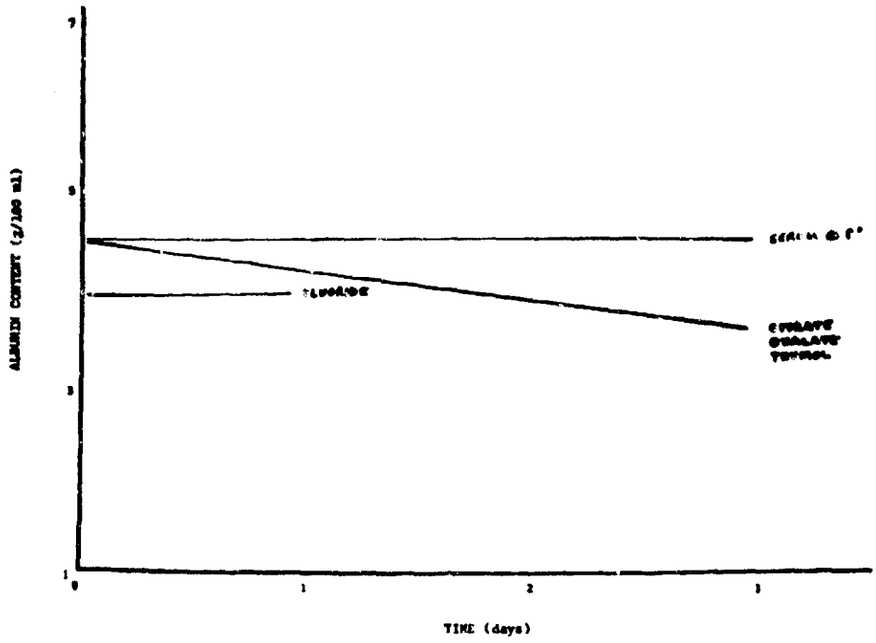

Figure 13. EFFECT OF ANTICOAGULANTS AND PRESERVATIVES ON ALBUMIN CONTENT OF BLOOD

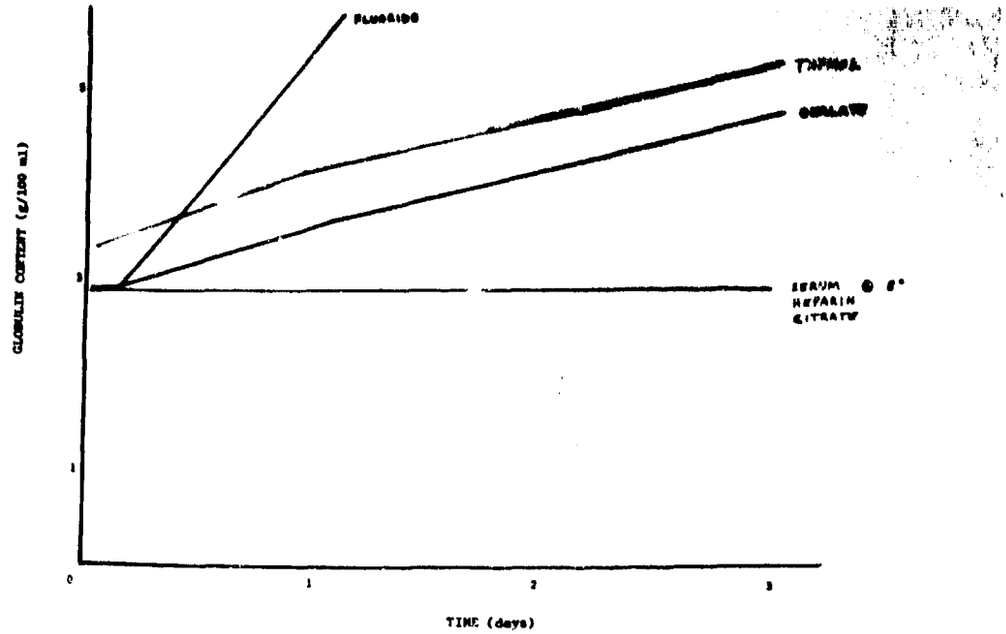

Figure 14. EFFECT OF ANTICOAGULANTS AND PRESERVATIVES ON GLOBULIN CONTENT OF BLOOD

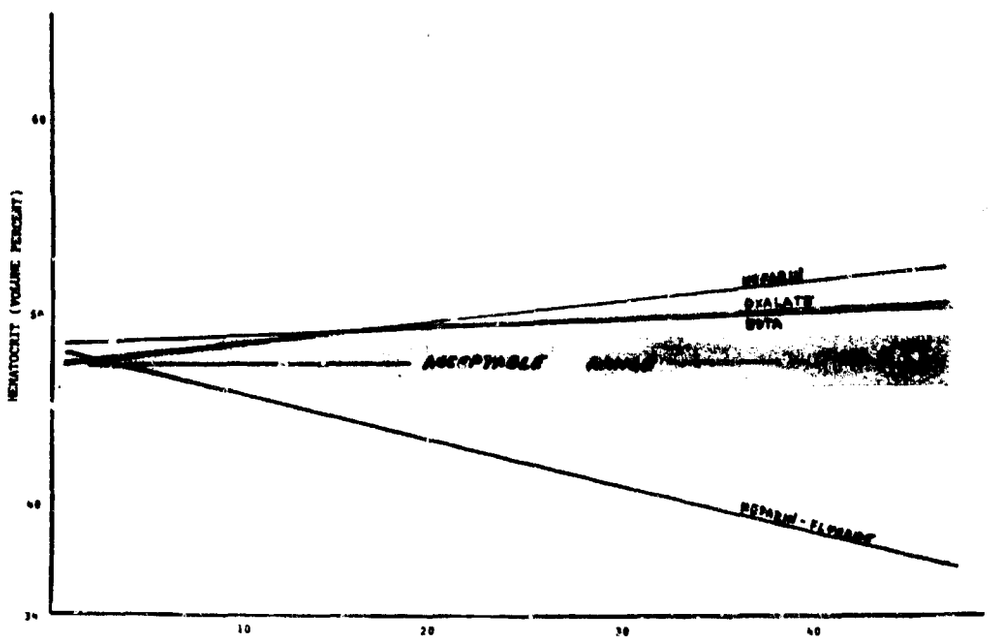

Figure 15. EFFECT OF ANTICOAGULANTS ON HEMATOCRIT

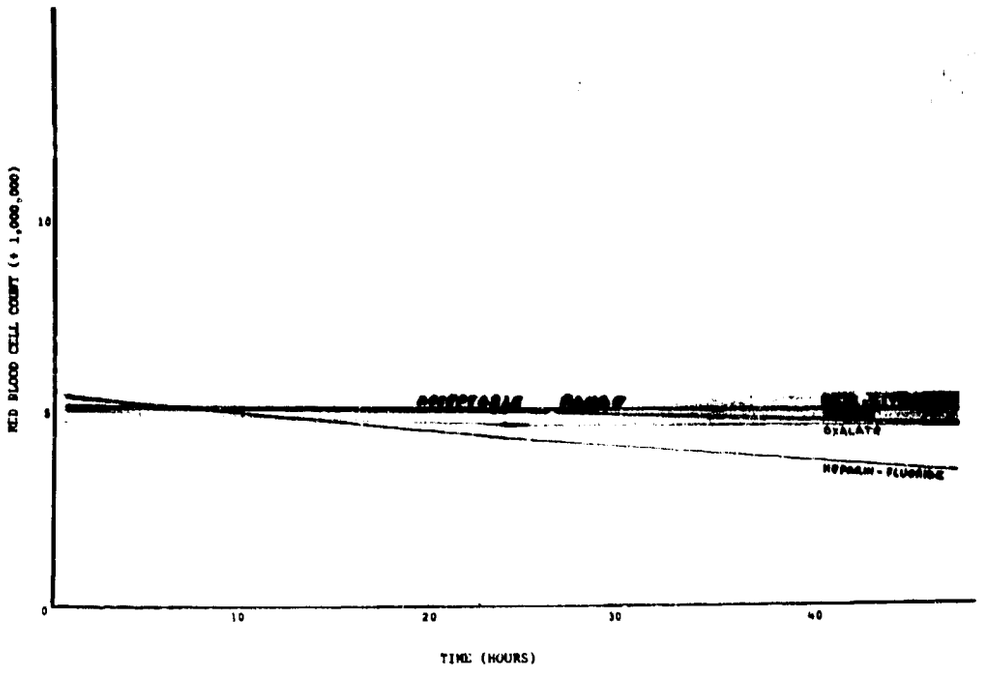

Figure 16. EFFECT OF ANTICOAGULANTS ON RED BLOOD CELL COUNT

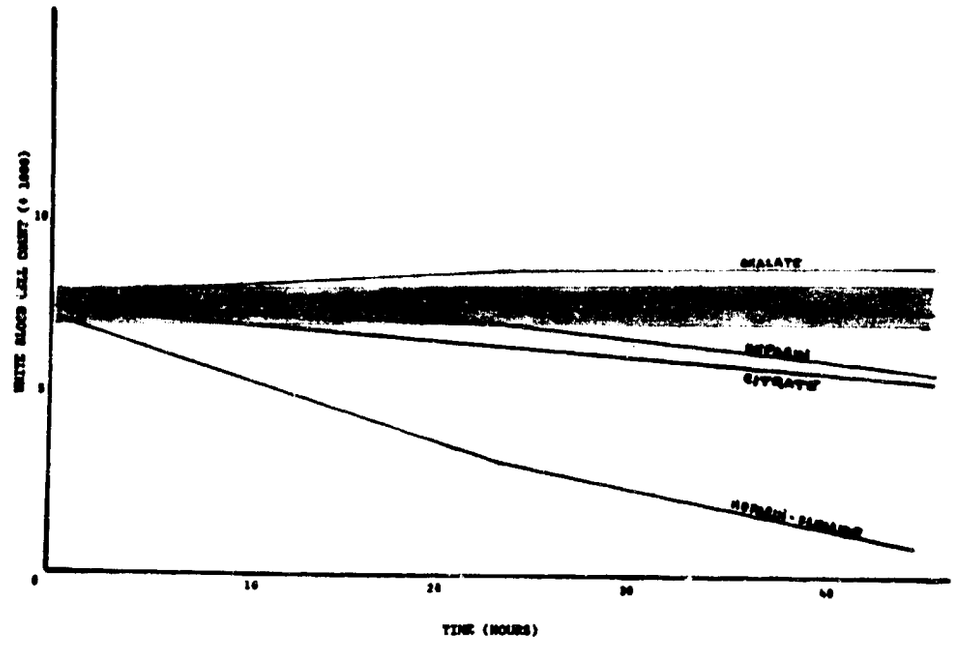

Figure 17. EFFECT OF ANTICOAGULANTS ON WHITE BLOOD CELL COUNTS

EXPOSURE TO SATURATED ETHYLENE GLYCOL VAPORS UNDER SPACE CABIN CONDITIONS

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INTRODUCTION

The toxicity of ethylene glycol upon oral ingestion has been extensively investigated. The LD_{50} for rats and guinea pigs by ingestion was found to be approximately seven g/kg (Lang, et al, 1939; Smyth, et al, 1941). Mice were much more tolerant with an LD_{50} of about 14 g/kg (Bornmann, 1954). A number of cases of fatal human poisoning resulting from drinking solutions of ethylene glycol have been recorded, and these indicate that the lethal dose in humans is about 1.5 g/kg (100 ml of ethylene glycol for a 160 pound individual). Death occurring shortly after ingestion appears to be due primarily to central nervous system depression, while individuals experiencing delayed deaths exhibit organ damage, primarily to kidney and brain. No chronic toxic effects were seen in three Rhesus monkeys fed low levels of ethylene glycol (0.2-0.5% blended with monkey chow) for three years (Blood, et al, 1962).

Previous work on the inhalation of ethylene glycol vapor has shown little toxicity, if any (Browning, 1953; Wiley, et al, 1936), although some indications of production of slight narcosis were obtained in one study (Browning). In these investigations, rats and mice were exposed to essentially saturated vapor concentrations for up to eight hours a day during five-day and sixteen-week periods. One investigation (Troisi, 1950) reported toxic effects of ethylene glycol inhaled by women workers in an electrolytic condenser factory. The work involved coating aluminum and paper with a mixture containing 40% ethylene glycol, 55% boric acid, and 5% ammonia at 105 C. Nine of 38 women exposed to the vapors suffered frequent attacks of loss of consciousness and nystagmus and five of these showed an absolute lymphocytosis. Further examination revealed five additional cases of nystagmus among the other 29 workers. Proper enclosure of the system to prevent inhalation of vapors resulted in complete recovery of all affected individuals. The women in this case were exposed to much higher concentrations of ethylene glycol than in the animal experiments as indicated by the fact that the vapor pressure of the glycol at 25 C (77 F) is 0.10 mm leading to a vapor concentration of 130 ppm or 330 mg/m^3; while at 105 C (221 F), the vapor pressure is 40 mm, equivalent to a vapor concentration of 53,000 ppm (5.3% by volume) or 134 g/m^3. Undoubtedly, because of vapor

pressure depression in the electrolyte mixture and condensation on cooling, the workers were not exposed to concentrations of this magnitude, but certainly higher than would normally be the case.

Because of the interest in ethylene glycol as a heat transfer medium in aerospace applications, it was decided to investigate the effects of continuous exposure of animals to saturated vapor concentrations in a 5 psia of 100% oxygen atmosphere at a nominal temperature of 73.2 F. Under these conditions, ethylene glycol has a saturation concentration of 254 mg/m^3 (equivalent to 100 ppm at 760 mm Hg).

MATERIALS AND METHODS

Table I lists the species and numbers of animals used in the study. The protocol demanded an initial exposure period of 14 days in the Thomas Domes (McNerney and MacEwen, 1965). If at the end of this period no overt symptoms of toxicity appeared, the study was to have continued for 60 days. However, recent experiences with fire in 100% oxygen atmospheres necessitated aborting this experiment prematurely so that a critical review of fire safety and extinguishing capability could be conducted. The maximum term of exposure for any species was, therefore, 21 days. Weight and clinical chemistry determinations were made at the conclusion of 14-day exposures and at termination for varying periods from 15 to 21 days for all species as shown in table I. This occurred because rabbits and guinea pigs were unavailable for inclusion at the beginning of the experiment and were placed in the chamber at later dates.

Additionally, a simple test for mouse behavior was discovered in the literature (McNamara, 1963), which, hopefully, would be able to detect performance decrements caused by borderline toxicity. The test involved observation of the ability of mice to descend a vertical bar and to right themselves on a horizontal bar. Ethylene glycol vapors at the same concentration were also admitted into a chamber containing monkeys negatively reinforced on a continuous avoidance task and discrete avoidance tasks involving visual or auditory cues (Wolfle, 1966).

TABLE I

ANIMAL EXPOSURES TO ETHYLENE GLYCOL

ANIMALS (all male)

Type	Control	Test	Total Exposure
Rhesus monkeys	2	4	21 days
Beagle dogs	2	4	21 days
Dutch rabbits	8	10	18 days
Hartley guinea pigs	20	30	15 days
Sprague Dawley rats	30	30	21 days
ICR mice	30	30	21 days

The ethylene glycol vapor was generated in a system diagrammed in figure 1. Air was drawn into the system under the pull of dome vacuum at a rate of five liters/minute to sweep out the vapors of glycol generated in the flask by the introduction of 24.5 ml/hour liquid glycol using the dual syringe feeder. This is equivalent to 266 mg/m^3 glycol concentration in the chamber. The 10-foot long coil down line from the flowmeter was wound with nichrome resistance wire to preheat the air entering this vaporizing flask. The major pressure drop in the system occurred at the needle valve between the flask and the dome. This valve was initially opened to give a flow of six liters/minute through the system with the flowmeter needle valve completely open, whereupon the flowmeter valve was restricted until a flow of five liters/minute was obtained. Using this technique, the generation system was essentially at atmospheric pressure and the problems associated with delivering a liquid continuously into a vacuum at closely controlled flow rates were avoided. The whole system was heated by means of heating mantles and resistance wire to 120 C which was sufficient to keep all the glycol in the vapor state until it joined the chamber atmosphere stream. Air was chosen to sweep the vapor rather than oxygen in the interest of safety, even though the concentration of the glycol in the feed stream was about one percent, well below the two percent flammability level usually quoted for compounds of this order of molecular weight. In any event, the air from the contaminant generation system diluted the chamber oxygen by less than one percent, acceptable under our conditions.

Two techniques were developed for the analysis of ethylene glycol chamber concentrations. Each of them depended on the atmosphere sample being taken by means of a gas sampling bulb of 320 ml volume. In one, a gas chromatographic technique, the glycol was absorbed into a one ml sample of methanol pipetted into the bulb. Fifty microliters of this sample were injected into a Perkin-Elmer Model 154 Gas Chromatograph under the following conditions:

Column - 4 feet 1/4 inch Porapak Q
Temperature - 130 C
Carrier gas (nitrogen) flow - 60 ml/minute
Detector - Hydrogen flame

Through the use of Porapak Q containing no liquid phase, ethylene glycol is eluted in a reasonable time and no problems of substrate bleeding are encountered.

A colorimetric procedure was also developed which was a modification of an analytical technique for ethylene glycol in blood and body tissue (Harger and Forney, 1959). In our method, the glycol was absorbed into water and periodic acid solution added to oxidize the glycol to formaldehyde. Schiff reagent (fuchsin decolorized by the addition of sodium metabisulfite and hydrochloric acid) is added and the absorbance measured at 555 mμ after 25 minutes. Although both these techniques gave good results with known concentrations, early chamber determinations were rather imprecise. It was theorized that droplets of ethylene glycol which had condensed in the sampling lines were being swept into the sampling flasks giving inordinately high results. A change in technique which included decreasing the length of sampling lines and washing the line with water between samplings effectively remedied the situation. Using these methods of introduction and analysis, we achieved an average concentration of 264 mg/m^3 over the whole period of exposure. This compares with the desired concentration of 254 mg/m^3.

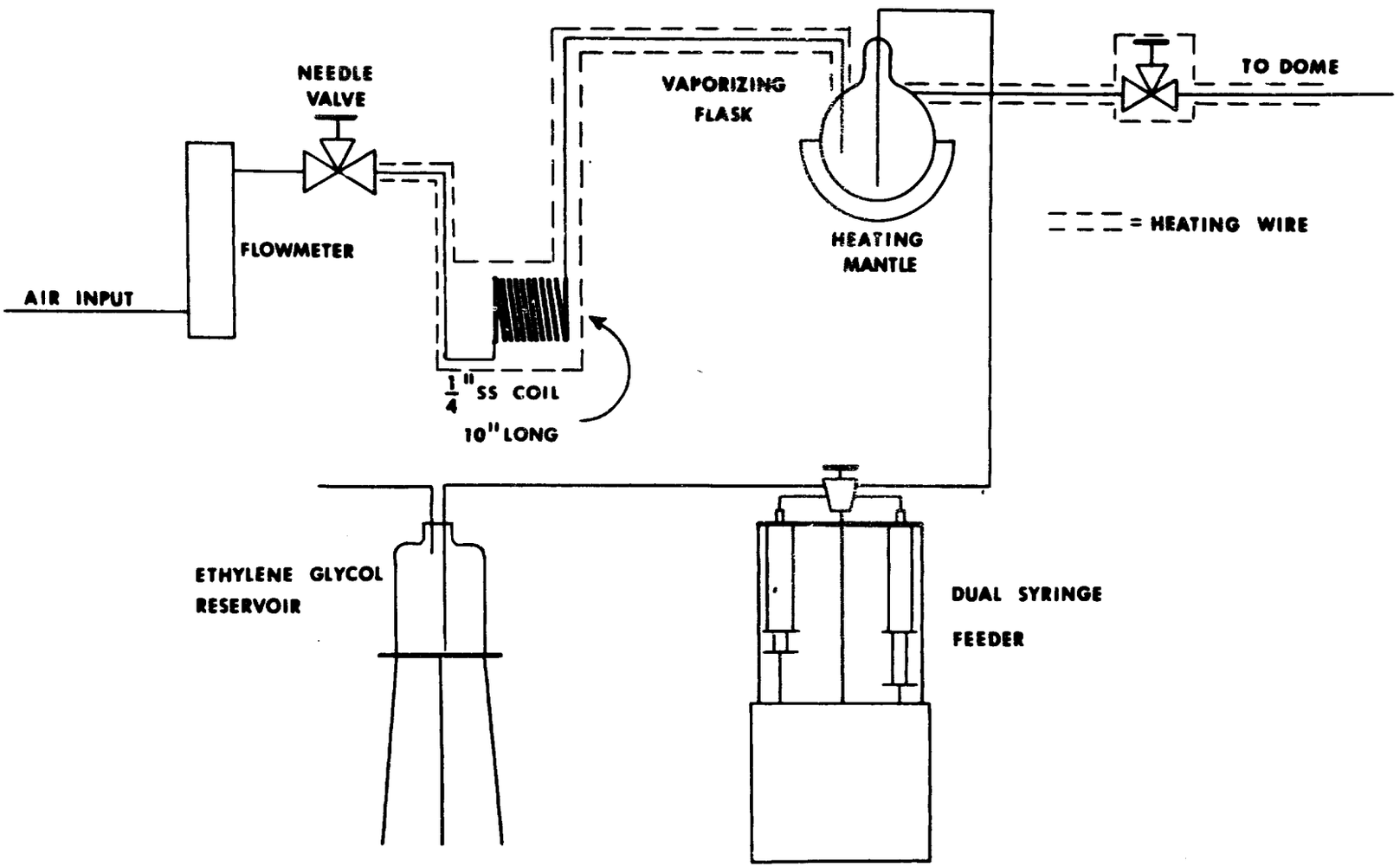

Figure 1. ETHYLENE GLYCOL GENERATOR

A major problem associated with the use of a saturated vapor of a contaminant is that if any chamber surfaces have temperatures lower than that for which saturation is calculated, the contaminant will condense out on these surfaces. In our situation, it is generally true that the temperature exterior to the domes is slightly lower than that inside. This resulted in extensive condensation on the windows, hindering observation of the animals during the test. Actually, condensation was not limited to the walls of the chamber, but a thin film of glycol appeared to be deposited on all surfaces of the chamber.

The ethylene glycol used in this study (B. P. 196-198 C) was unprotected by antioxidants. It was suggested that oxidation of the glycol to aldehydes might introduce a sufficient concentration of these materials to cause respiratory irritation. Although the study ended before chemical detection of aldehydes could be attempted, no odor of these materials was noted by chamber technicians when they removed their oxygen masks in the exposure chamber. Since the odor threshold of the aldehydes which might be produced from ethylene glycol (formaldehyde, ketene, glyoxal and glycolaldehyde) are well below toxic levels, it appears probable that any effects produced during the exposure were due to ethylene glycol rather than its oxidation products.

RESULTS AND DISCUSSION

Body and Organ to Body Weight Ratios

Animals were weighed at preexposure, after two weeks exposure, and at the termination of the experiment. Groups of rats, guinea pigs, and rabbits were also killed according to this schedule, dogs and monkeys at termination, and the organ and body weights measured and the ratios calculated. Organs weighed included heart, lung, liver, spleen, kidney, and brain.

Table II compares the average weight variations of control and exposed animals during the period of exposure. In dogs, monkeys, and rabbits, the exposed animals showed either no weight loss or showed a higher weight gain when compared with controls. Rats and guinea pigs, however, showed significant apparent exposure effects. Statistical comparison using the Student "t" test indicated that the rat body weights after two weeks exposure were significantly lower at the 97% level and at the 99% level after three weeks. The weight loss in exposed guinea pigs, although not studied for any length before exposure, appears to be affected to a much greater degree. (Guinea pigs lost weight during the two weeks they were exposed to the glycol while control animals exhibited a sharp weight gain.) The differences between control and exposed guinea pig body weights were highly significant (over 99.9%). These results are illustrated in figure 1 where average body weights of control and exposed rats and guinea pigs are plotted against dates. The first four points on the rat plot were taken preexposure and show that weight gain was identical in the two groups. After two weeks exposure, however, the control rats had gained much more weight; the weight difference still persisted after the exposed rats had been returned to normal conditions for four days.

Tests of significance of the differences between the organ to body weight ratios of control and exposed animals were made. As mentioned previously, dogs and monkeys showed no differences between control and exposed groups. Table III summarizes the organ to body weight parameters which did show significant

TABLE II

INFLUENCE OF ETHYLENE GLYCOL EXPOSURE ON BODY WEIGHT GAIN

Species	No.	Average Weights Pre-exposure	Post-exposure	Diff.	Comparison of Means Student "t"	Significance
Rats						
14-day Controls	20	147g	268g	+121g	2.507	97%
14-day Exposed	10	146	226	+80		
21-day Controls (a)	10	147	289	+152	5.198	> 99%
21-day Exposed	11	145	251	+106		
Dogs (b)						
Controls	4	4.93 kg	4.69 kg	-0.24 kg		Non-significant
Exposed	4	5.05	5.0	-0.05		
Monkeys (b)						
Controls	4	1.96 kg	1.78 kg	-0.18 kg		Non-significant
Exposed	4	1.65	1.59	-0.06		
Guinea Pigs (c)						
Controls	10	360g	479g	+119g	10.763	> 99.9%
Exposed	10	394	382	-12		
Rabbits (d)						
Controls	6	1337g	1418g	81g		Non-significant
Exposed	7	1230	1386	156		

(a) Postexposure weights measured four days after removal from chamber.
(b) Exposure time was 21 days.
(c) Exposure time was 15 days.
(d) Exposure time was 18 days.

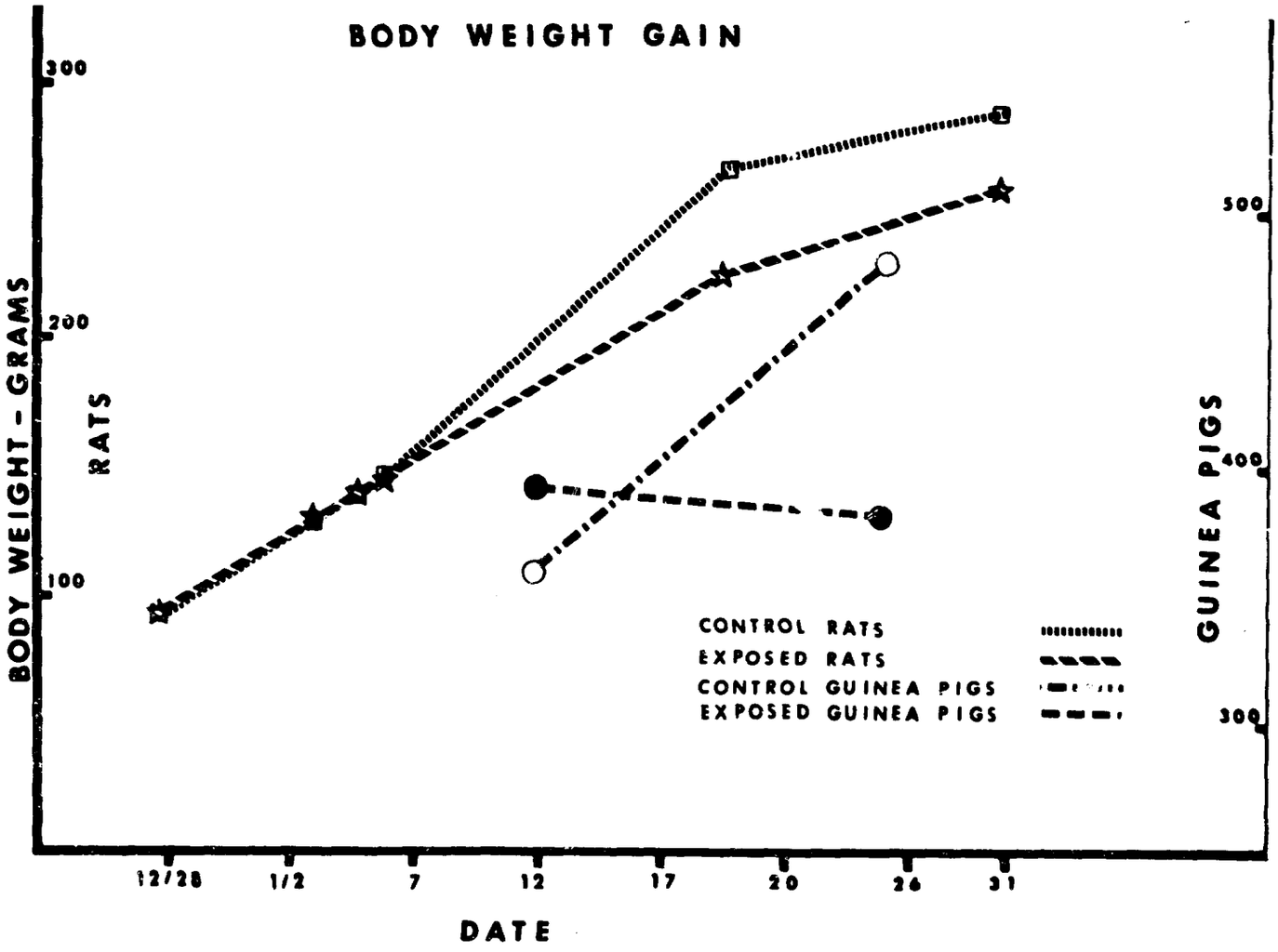

Figure 2. WEIGHT GAIN OF RATS AND GUINEA PIGS EXPOSED TO ETHYLENE GLYCOL.

TABLE III

SIGNIFICANCE OF ORGAN TO BODY WEIGHT RATIO DIFFERENCES EXPOSED VS. CONTROLS

Species	Organ	14-Day Exposure	Test Termination
Rat			21-Day Exposure
	Heart	-	99%
	Spleen	-	95%
	Kidney	-	98%
	Brain	-	99%
Guinea Pig			15-Day Exposure
	Lung	>99%	-
	Liver	-	>99%*
	Spleen	-	97%*
Rabbit			18-Day Exposure
	Lung	-	97%

*Ratio higher in Control

differences -- any difference with a level of significance below 95% was deemed trivial and is omitted. Higher levels are listed, as are notations as to whether the ratio is higher in exposed or control animals. The first column in the table lists values after two weeks exposure and the second column values at test termination, ranging from 15 to 21 days.

The significance of the values calculated for the guinea pigs must be discounted, since the apparent highly significant lung to body weight ratio difference calculated for the 14-day exposure group disappears for the group sampled the day after; and the liver and spleen ratios for the 15-day exposure indicate relative enlargement of the organs in the control animals, an unlikely occurrence. In view of the foregoing, one must look upon the statistical interpretation of the organ to body weight ratios with a jaundiced eye; undoubtedly differences or biases in organ isolation techniques and in animal preparation may have had a profound effect on the results. Nevertheless, it is true that the number and significance of the organ to body weight ratio differences in the rats exposed for 21 days appear to lend some confidence to the conclusion that they reflect the action of some toxic mechanism.

Clinical Chemistry

The blood of the larger animals in the experiment (dogs, monkeys, and rabbits) was subjected to clinical chemical analysis and hematological examination following essentially the same schedule as organ to body weight determinations.

None of the hematological parameters, i. e. HCT = hematocrit, HGB = hemoglobin, RBC = red blood cell count, and WBC = white blood cell count, showed any significant change from controls upon exposure (table IV). An apparent increase in average WBC in exposed rabbits was due to one individual whose count was almost tripled - the other two exposed animals had normal counts. Table V lists the clinical chemical parameters, total protein, albumin, globulin, SGPT = serum glutamic-pyruvic transaminase, SGOT = serum glutamic-oxalacetic transaminase, BUN = blood urea nitrogen, and creatinine. All animals showed values which were within normal limits except the exposed rabbits who showed a highly significant elevation in BUN values and changes in serum protein parameters.

TABLE IV

HEMATOLOGY OF ANIMALS EXPOSED TO ETHYLENE GLYCOL FOR TWO WEEKS

	Rabbits		Dogs		Monkeys	
	Control	Exposed	Control	Exposed	Control	Exposed
Number	1	3	2	4	2	4
HCT (vol %)	42	34	46.5	44.5	41.5	41.3
HGB (Gm %)	12.0	10.0	15.3	14.8	11.8	12.8
RBC (10^6 cells/mm^3)	6.48	5.61	6.61	5.99	5.26	5.23
WBC (10^3 cells/mm^3)	6.5	9.96[a]	12.8	12.1	17.2	13.2

(a) Individual values - 6.5, 6.7, 16.7

Table VI details BUN, creatinine and serum protein results in the rabbit. BUN has increased significantly in the exposed rabbits after both 14- and 18-day exposures. Creatinine, on the other hand, shows no increase at all. The indications from these data are that the rabbits were beginning to develop renal malfunction due to glycol exposure, but that the injury was minimal at the termination of the experiment since blood creatinines were not out of line. Creatinine is more readily excreted by the kidney than is urea, and levels will not usually rise in earliest renal damage. The kidney is damaged more easily in rabbits by agents acting at this site than in most other laboratory animals.

Consideration of rabbit serum protein values affords an indication of metabolic disturbance of nitrogenous materials. Absolute globulin values rose significantly after both 14- and 18-day exposure. This increase was reflected in higher total serum protein concentration. In contrast, the serum albumin level in exposed and control animals did not consistently differ, demonstrating no practical difference after 18 days exposure.

The method of Harger and Forney previously mentioned was applied to the analysis of ethylene glycol in the blood of control and exposed monkeys and dogs after 14 days of the experiment. In all cases the results calculated as ethylene

TABLE V

CLINICAL CHEMISTRY OF ETHYLENE GLYCOL-EXPOSED ANIMALS

	Rabbits				Dogs				Monkeys			
	14-Day		18-Day		14-Day		21-Day		14-Day		21-Day	
	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed	Control	Exposed
Number	3	4	6	7	2	4	2	4	2	4	2	4
Total Protein (Gm %)	5.8	7.2	6.4	7.1	7.0	6.6	6.7	7.0	7.5	7.7	8.5	8.1
Albumin (Gm %)	3.3	3.9	3.7	3.7	3.5	3.5	3.5	3.5	4.1	4.5	4.7	4.9
Globulin (Gm %)	2.5	3.3	2.7	3.4	3.5	3.1	3.2	3.5	3.4	3.2	3.8	3.2
SGPT RF Units	29.7	28.0	39.7	35.6	28.0	33.5	35.0	37.7	48.5	25.0	29.5	31.7
SGOT RF Units	34.7	40.5	57.5	58.0	22.5	27.5	37.5	40.0	45.0	34.0	35.5	54.0
BUN (Mg %)	20.0	27.5	14.2	28.3	26.3	16.9	27.5	23.7	23.7	17.5	21.0	19.7
Creatinine (Mg %)	1.17	1.23	1.40	1.36	0.85	0.85	0.95	0.89	1.0	1.1	1.1	1.2

TABLE VI

RABBIT CLINICAL CHEMISTRY PARAMETERS AFFECTED BY EXPOSURE

	14-Day Exposure		18-Day Exposure	
	Value	Significance	Value	Significance
		BUN		
Control	20.0 mg%		14.2 mg%	
Exposed	27.5	98%	28.3	>99%
		CREATININE		
Control	1.17		1.4	
Exposed	1.23	50%	1.36	24%
		TOTAL PROTEIN		
Control	5.8 gm%		6.4 gm%	
Exposed	7.2	>99%	7.1	94%
		ALBUMIN		
Control	3.3		3.6	
Exposed	3.9	96%	3.7	23%
		GLOBULIN		
Control	2.5		2.7	
Exposed	3.3	>99%	3.4	97%

glycol were higher in the exposed animals than in the controls. The absorbances measured and consequent glycol levels were so low that it would be difficult to ascribe any quantitative significance to the data. For instance, the highest absorbance difference between control and exposed animals amounted to only 0.030 absorbance units equivalent to 7 mg% ethylene glycol.

Mortality

Four of the 40 exposed mice and two of the exposed rats died during the course of the experiment. No control mice and one of 20 rats under 5 psia 100% oxygen control conditions died. There was a short period when one cage of exposed mice suffered a malfunction in the water delivery system and it is possible that this was at least partially responsible for the higher mortality in exposed mice. Gross and histopathologic findings (Esparza, 1967), however, testify to an adverse response of this agent for exposed mice.

Performance Tests

One of the 12 trained monkeys exposed to ethylene glycol vapors appeared to suffer an absolute decrement in performance, but this may not have been related to inhalation of glycol vapors. There was locally heavy condensation of ethylene glycol on various surfaces, and this may have had a deleterious effect on performance. In addition, equipment malfunctions, which may have been in part due to glycol condensation, presented themselves. No conclusions concerning the effect on performance of monkeys by inhalation of ethylene glycol can be drawn at this time.

The test for mouse behavior was essentially that of McNamara. The control performance test bars were set up just outside the window of the test chamber and the exposure test bars just inside so that the actions of the two sets of mice could be observed simultaneously. Figure 3 is a photograph of a typical side by side test. Intraperitoneal injections of ethanol, propylene glycol, and ethylene glycol (each 50% in distilled water) were made at the level of 0.1 ml/mouse (also 0.25 ml/mouse for the glycols); performance of these animals was observed to provide some measure of overt disturbance of the behavior of intoxicated animals. The major point of interest of these experiments was the variability of the reactions of individual animals; some showed minimal effects, others were completely unable to grasp either the horizontal or vertical bars.

When control and exposed mice were made to perform side by side, there did appear to be more assurance on the part of the control animals in accomplishing both phases of the test. This was not consistent because some exposed animals performed better than their control counterparts, but generally it seemed that the exposed animals exhibited some hesitancy in descending the vertical bar. Of course it is possible that this was due to slippery surfaces caused by glycol condensation or absorption. However, performance decrement was not as obvious in the ability to right themselves on the horizontal bar, an action conceivably more severely limited by a slippery surface.

SUMMARY

Examination of all parameters used to show toxic effects indicates that monkeys and dogs were unaffected by exposure to 264 mg/m^3 of ethylene glycol for 21 days, although there exists the chance that the performance of one monkey trained in avoidance tasks was negatively affected.

Rats demonstrated a significant decrease in absolute weight gain as well as increases in heart, spleen, kidney, and brain to body weight ratios.

The body weight gain of guinea pigs was very adversely affected. After two weeks, the exposed animals exhibited a weight decrease.

Elevated BUN values in exposed rabbits gave evidence of early kidney injury after only 14 days. In addition, total serum protein levels were higher in exposed animals and, since albumin values remained constant, this would imply that globulin was responsible for total increase.

Simple performance tests given to mice seemed to show a decrement on the part of exposed animals. However, it is possible that this was due to slippery test surfaces caused by condensed or absorbed ethylene glycol.

Figure 3. BAR TEST FOR MOUSE BEHAVIOR
Left side - Control; Right side - Exposed

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TERMINOLOGY AND ASSUMPTIONS OF STATISTICAL MANIPULATION

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INTRODUCTION

Although statistics is a dialect of English used by most of the scientific community, at gatherings of scientists there is a remarkable lack of communication. This arises partly from semantics and partly from a nonuniform application of principles. For a while it was thought that the introduction of the engineer-mathematician, with his wonderful machines and vocabulary, would create a more pristine approach to research. Instead the problem has been increased by at least one order of magnitude $(\frac{10^{n+1}}{10^n})$.

This lack of a standardized terminology creates difficulties in assessing the value of a study and in making comparisons with the results of other studies. It also leads to erroneous interpretations. The nonuniform application of principles results in the disregard of germaine evidence, the drawing of conclusions from inconclusive evidence, and in inferences contraindicated by the data. The problem is very real: two investigations of published research in the field of medicine found that approximately 95 percent of the studies employing statistical methods had conclusions not supported by the data and over 50 percent had conclusions contrary to those indicated by the data.

The purpose of this presentation is threefold: (1) to suggest standard definitions for some of the more commonly abused statistical terms, (2) to discuss the assumptions underlying parametric and nonparametric methods, with particular emphasis on the application of such methods to research in toxicology, and (3) to point out some of the pitfalls in machine data processing.

DEFINITIONS

Probability

It has been said that "probability is the most important concept in modern science, especially as nobody has the slightest notion what it means" (Stevens, 1951)

Although the word has been linked with a diversity of notions, for the purposes of this Conference it is suggested that it be restricted to the a priori concept and the frequency theory. The former is exemplified by the assignment of a probability of 1/52 to the turn of a given playing card and is induced from the "principle of indifference". The "principle of indifference" dictates that when any of several discrete events may occur, they are all regarded as being equally likely. The frequency theory of probability is the notion that the probability of an event is the relative frequency of its occurrence in a large sample. This is the notion that underlies most statistical methods.

The symbol for probability is P and should be in association with the probability level, e.g., $P \leq .05$. This may be interpreted as indicating that the differences noted in the study could have resulted from pure chance only five times (or less) in every one hundred replications.

Significance

"Significance" is given to the testing of hypotheses and determines whether the hypothesis is accepted or rejected (Chandler, 1957). The term should not be used without a qualifying adjective.

Statistical significance is based upon the probability level which the investigator believes is required to disprove the null hypothesis. This level is arbitrarily established prior to the collection of the data and the investigator must have a "whim of iron". The results are significant or they are not; there is no "almost significant" or "would be significant with more subjects". The probability levels generally employed are the .05 (5%) and the .01 (1%).

Clinical significance is based upon the experience of the investigator and health and safety considerations for the subjects. A treatment which in itself causes harm to even one patient in a hundred may be clinically significant in the eyes of the investigator. Clinical significance very often is in no way related to statistical significance.

Biological significance is based upon a knowledge of the mechanism(s) under investigation, and often is related to clinical significance. A statistically significant finding, on the other hand, frequently is biologically meaningless.

Confidence

The admixing of the concepts of confidence and significance has become so common that one typically hears expressions indicating that experimental results were significant at the "5% level of confidence". The concept of confidence should never be associated with the testing of hypotheses (Chandler, 1957). It is reserved for discussions of interval estimation and is the faith one has that an interval established by a sampling process actually contains the parameter of interest. It is seldom used in the reporting of research results.

PRINCIPLES

The evaluation of data collected in toxicological research may take any of several forms: the classic thesis-antithesis-synthesis approach; the systemized

observation, such as the phenotype-genotype-value judgment method; or the application of probability theory (Sommer, 1963). Since 1920, this last has been the most accepted method. There are, however, several assumptions which must be met before the results of statistical manipulation may be considered valid and these are:

1. The most powerful tests are those which have the strongest and most extensive assumptions. If the assumptions are not met, the test is weakened to the point of yielding invalid conclusions.

2. The measurement level (scale) must be known and the test appropriate to that scale. The parametric tests (t-ratios, F-ratios, correlation coefficient, etc.) cannot be applied to data measured in nominal or ordinal scales because the system known as arithmetic is not isomorphic to those scales.

3. In the case of correlation analyses, the shape of the regression line must be known; the correlation coefficient (r) applies only to linear data, the index of correlation (rho) is appropriate to curvilinear data, and the correlation ratio (eta) is used for "nonlinear" data. The indiscriminate use of the correlation coefficient leads to erroneous conclusions.

4. The parametric tests have the additional requirements that the observations are independent; that they are drawn from normally distributed populations; that these populations have the same variance, or in special cases, a known ratio of variances; and the sample size must be greater than six (Siegel, 1956).

Fortunately, there are comparable nonparametric tests for most of the parametric techniques.

MACHINE DATA PROCESSING

The ease with which any number of variables may be treated by machine has led to more concern over the functioning of the computer and the proper breadboarding of the machine than in meeting basic assumptions of statistical mathematics. The investigator planning to employ machine statistics must be especially wary of the following:

1. Statistical significance is not necessarily clinically or biologically meaningful.

2. The faith one has in the probability level of a statistic is the function of the number of statistics in the group. For instance, there is 70% probability of finding a "significant" correlation coefficient in a matrix of 25 intercorrelations just by chance alone. The probability of operant chance indicating two such correlations is 35%. Wilkinson (1951) has presented tables for determining the probability of obtaining n or more significant statistics by chance in a group of N for the .05 and .01 levels of probability. That this is not an uncommon occurrence is indicated by the fact that a survey of one particular journal

for one year indicated 17 articles which used the t test and 15 which used the correlation coefficient: the former were reported an average of 36 times per article and the latter 30 times. An average of eight statistics per article were reported as "significant" and of these, one would expect about two to be significant by chance alone. The principle of indifference does not permit any conclusion as to which two these are, so the faith one has in any generalization drawn from these results must be reduced accordingly. In another journal, one study reported 126 individual t tests with 17 being significant at the 10% level of probability. As 13 of the statistics could have occurred by chance at this level, these investigators have not appreciably advanced man's knowledge.

3. In setting up an intercorrelation matrix, there is a temptation to consider all variables as linearly related and, as a result, non-significant relationships have been reported when, in fact, there is a true curvilinear relationship.

4. The machine cannot discriminate among nominal, ordinal, interval, and ratio scale data and, as "numbers are numbers", an unwarranted importance is given to a meaningless value.

5. In machine data processing there is a tendency for the investigator to consider all variables as equally representative of the population.

There is a danger in the very ease of machine data processing. With the results of the "real problem" so readily available, one is not very eager to examine the raw data, to determine what assumptions underlie the particular test being employed and if these assumptions fully have been met, or to choose a test which is most appropriate to the data, particularly if it is not in the computer program. The serious investigator, however, must take the time to understand the characteristics of his data, for no amount of mathematical subtlety or statistical manipulation will make unreliable data trustworthy or establish a single truth in an empirical world.

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DISCUSSION

QUESTION: I just wanted to know if you made any correlation with the journal article. Did you find out how many papers were done by professional statisticians? There was a kind of inference about the Journal of the A. M. A.. Maybe someone who doesn't know anything about statistics put the papers together, which is going to happen occasionally.

DR. PIERSON: Unfortunately, these papers were put together by statisticians with data given to them by other investigators. Now a statistician knows the operation of the machine, he knows how to operate the cook books, but he doesn't know the assumptions underlying the physiological variations. As a consequence, you have computer work done by an expert.

DR. FAIRCHILD (Aerojet-General Corporation): There are reams of data presenting LD_{50}'s, LC_{50}'s, ED_{50}'s, MLD's, for a gamut of chemical substances, drugs, and gases, and, usually, they express a confidence limit (like 95%), that is, 95 out of 100 times this is going to occur. Do I understand you to say you don't agree with that concept?

DR. PIERSON: Yes, sir, because there are such reams of data. That's exactly why I presented the paper today. These are not statistically valid terms. The confidence level refers strictly to the sampling process. The significance of it statistically refers to the testing of hypotheses, and we have become quite sloppy in our terminology.

DR. FAIRCHILD: Then, if we have an LD_{50} of say 15 milligrams per kilogram, plus or minus 3.4, at the 95%?

DR. PIERSON: This may be clinically significant; statistically, it has no meaning. What I'm trying to get at is to purify the language. As I say, there is difficulty in communication. When somebody says something is significant with a 5% degree of confidence to a person who is interpreting confidence in the strict sense, this is gobbledy-gook. I am suggesting that there are many other terms, one tail vs. two tail test - - . The ones which have been most abused are "significance" and "confidence".

QUESTION: What do you call it if you don't want to call it confidence?

DR. PIERSON: It can be clinically significant if in the opinion of the evaluator it is meaningful. Confidence - to say I have confidence in these data says nothing of the testing of the hypothesis. When you test the hypothesis, you come up with something that is meaningful, i. e., significant or not significant.

DR. BACK (Aerospace Medical Research Laboratories): Usually the biologist talks about confidence limits, not as a single entity, not a single point on a curve. It is usually the data around a specific area. For instance, an LD_{50}, if it's 15 milligrams per kilo, it means one standard deviation on either side of that LD_{50} would be, usually, containing the confidence limits around that particular point. It is not a single point on a curve; it is a bunch of points on a curve, if you will.

DR. PIERSON: In that case, the confidence level is used exactly right because it indicates that the sample you have used has this much confidence of including the parameter in which you are interested. It is not, in that particular case, having anything to do with the testing of hypothesis.

DR. BACK: I have to agree that is the way most biologists use the term confidence limits. They don't usually say confidence level and pick out a single point. It's not a P value.

EFFECT OF AIR POLLUTION ON ALTERATION OF SUSCEPTIBILITY TO PULMONARY INFECTION

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Numerous secondary factors play a role in the causation of infectious disease. Exposure to cold, extreme fatigue, disturbance in the nutritional state, existence of previous diseases, and many other conditions are frequently significant in the establishment of the disease state. Such predisposing causes comprise the factors in the multiple causality principle (Top, 1964), and their importance is well established.

It is now known that certain inhaled gases may also alter susceptibility to infectious diseases of the lung. The role of an air pollutant as a predisposing agent was early suggested by Stokinger who noted an increased incidence of spontaneous pneumonias in guinea pigs undergoing exposure to ozone (Stokinger, 1957). Similarly, Hueter, et al, reported a preponderance of pneumonias in guinea pigs exposed to artificial auto smog (Hueter, et al, 1966). Ehrlich, et al, in a series of papers reported the experimental enhancement of bacterial pneumonias attributed to K. pneumoniae or Streptococcus by previous exposure to nitrogen dioxide or to ozone (Miller and Ehrlich, 1958; Purvis, et al, 1961; Ehrlich, 1963). Miller and Ehrlich (1958) reported that exposure to 1 to 9 ppm ozone for three hours enhanced the mortality rate from either Streptococcus or K. pneumoniae. The effect persisted six to 19 hours when the pollutant was administered before the bacterial aerosol, and up to 27 hours when the pollutant exposure followed the bacterial aerosol.

Nitrogen dioxide was also found to increase the susceptibility of mice and hamsters to K. pneumoniae resulting in higher mortality. Exposure of mice for two hours to 3.5 ppm effectively enhanced mortality, whereas a six-month exposure was necessary at the 0.5 ppm level (Ehrlich, 1966).

Experiments have been carried out in our laboratory with ozone, nitrogen dioxide, and photochemical auto smog. For these studies, streptococci group C isolated from a guinea pig were employed. Infection was achieved by application of an aerosolized culture to mice previously exposed to the gaseous agent.

By this system, electrostatically-generated ozone at 0.08 ppm or greater produced statistically significant enhancement of mortality after a three-hour exposure (Coffin and Blommer, Study of a Threshold Value for Ozone in an Infective System, In Press) (table I).

TABLE I

MORTALITY OF MICE EXPOSED TO STREPTOCOCCAL AEROSOL FOLLOWING OZONE

Ozone (ppm)	No. Mice per Group	Percent Mortality		
		Strep Only	Strep + Ozone	% Difference
.52	40	13	80	67*
.35	40	0	60	60*
.30	40	8	63	55*
.28	40	3	40	37*
.20	40	8	50	42*
.18	40	0	63	63*
.17	40	8	45	37*
.10	40	8	35	27*
.08	40	15	38	23*
.07	40	15	35	20
.07	40	8	5	-3

*$p < .05$

Similar experiments involving four-hour exposures of pathogen-free mice to predetermined concentrations of irradiated auto exhaust were carried out. The desired levels of carbon monoxide and total oxidant were achieved by altering the dilution with air and adjusting the intensity of illumination. The dilution of the auto exhaust is reflected by the carbon monoxide level, 100 ppm being equivalent

to a 500 to one dilution by filtered air, and 25 ppm carbon monoxide being equivalent to a 2,000 to one dilution. The minimal effective dose occurred when the experimental atmosphere contained 25 ppm carbon monoxide and 0.15 ppm total oxidant. Concentrations above this point invariably heightened mortality and below this point produced constantly negative results (Coffin and Blommer, Acute Toxicity of Irradiated Auto Exhaust, etc., In press) (table II).

TABLE II

MORTALITY OF MICE EXPOSED TO STREPTOCOCCAL AEROSOL FOLLOWING IRRADIATED AUTO EXHAUST

Pollutant (ppm)			Percent Mortality		
Carbon Monoxide	Oxidant	No. Mice per Group	Strep Only	Strep + Exhaust	% Difference
100	55	200	11	53	42*
75	41	30	7	53	46*
50	29	30	7	43	36*
50	28	30	13	60	47*
50	26	30	10	70	60*
25	16	30	7	40	33*
25	15	30	10	37	27*
25	14	30	0	7	7

*$p < .02$

Recent data also show that ozone has the propensity to alter the susceptibility of mice to D. pneumoniae. A fivefold increase in mortality was observed when ozone exposure was followed by aerosols of group III pneumococci.

Mechanism of Action

Exposure to a number of agents effectively increases the time required for the loss of cultivable bacterial cells from the lung; the so-called bacterial clearance rate. Stillman (1923) noted that this effect was achieved by administrations of alcohol and subsequent exposure to Diplococcus pneumoniae. Similar effects for a variety of agents were noted more recently by numerous investigators. Green and Kass (1964) showed that hypoxia, cold treatment, and exposure to alcohol will increase the persistence of viable bacterial cells in the lung. Ehrlich's

experiments showed that this so-called bacterial lung clearance is markedly prolonged by prior exposure to nitrogen dioxide (Ehrlich, 1966).

In our laboratory, pathogen-free white Swiss mice exposed to ambient air for six hours cleared greater than 99 percent of streptococci from their lungs, whereas those exposed to ozone cleared less than 50 percent in this time.

The ambient air-treated animals became negative after the six-hour period, whereas the cultivable bacteria were markedly increased in animals receiving a prior exposure to ozone. This striking increase was attributed to a growth phase, which began approximately four hours after exposure to the streptococci (Coffin, et al, Influence of Ozone on the Phenomenon of Lung Clearance, In Press) (figure 1).

When Serratia marcescens, a nominally nonpathogenic bacterium, was utilized, clearance was faster in the untreated animals. When Serratia marcescens followed ozone, the early phase of the clearance was markedly slowed; however, by the ninth hour, no significant difference was noted between animals receiving the ozone and those treated with ambient air (figure 1).

It may be postulated that bacterial clearance rates are governed by a summation of those forces that remove bacteria from the lungs or render them noncultivable in situ, i.e.: (1) exogenous clearance by means of ciliary activity and mucus flow; (2) endogenous clearance by means of lymphatic drainage; and (3) bacterial inactivation or death in situ - phagocytosis and destruction by intracellular enzymes, lysis by extracellular enzymes of mucus or other pulmonary fluids, or simply bacterial decay.

To postulate that there is a relationship between clearance rate and susceptibility to bacterial infection appears valid. A prerequisite to experimental verification of this postulate is the proper choice is an experimental modifier of clearance and the use of an organism capable of establishing itself as a pathogen by multiplying and invading the tissues. These criteria are met by ozone and the streptococcus treatment of mice. Bacterial species apparently plays a role in the alteration of the clearance phenomenon by ozone, as suggested by the marked difference between a fragile pathogenic Streptococcus and a tougher presumably nonpathogen S. marcescens (figure 1).

Experiments have been carried out to elucidate the effect of ozone in altering the presumed pulmonary in situ killing rate of bacteria. These will be reported in detail elsewhere (Coffin, et al, Effect of Ozone Exposure on the Pulmonary Cells, etc., In Preparation; Gardner, et al, Inhibition of Phagocytic Activity of the Pulmonary Alveolar Macrophage by Ozone Exposure, In Preparation). Studies of rabbits exposed to ozone show that when the pulmonary cells are washed out with balanced salt solution (pH 7.1) and numerically compared by means of differential counting the number of the rabbit heterophile leukocytes (polymorphonuclear leukocyte) in the washed out cells increases as a function of dosage. This phenomenon appears to be most marked at about six to nine hours after exposure and is still present in a diminishing amount at 24 hours (figure 2). Data also indicate a relative synchronous reduction in the percent of alveolar macrophage cells. Whether this latter change reflects an actual numerical reduction of these cells is unclear at this time.

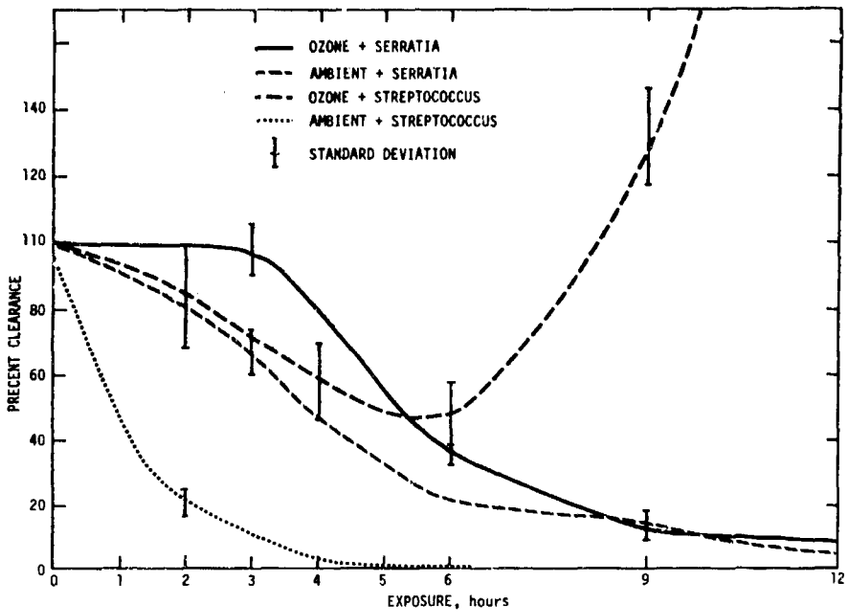

Figure 1. EFFECT OF OZONE ON BACTERIAL PERSISTENCE

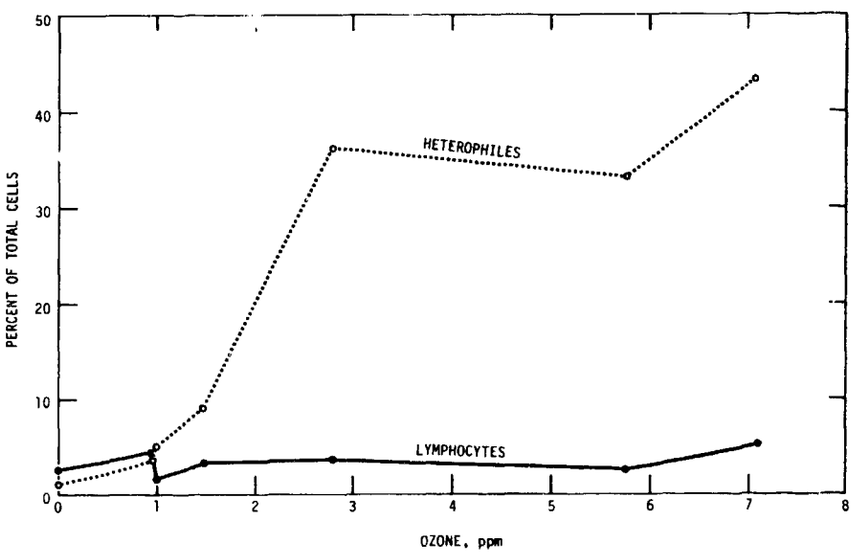

Figure 2. CELLULAR RESPONSE TO OZONE

The presence of the polymorphonuclear leukocyte in the washed out cells of the rabbit lung constitutes a sensitive method, however, for the detection of inflammatory ozone effect. The advantages of this method over perusal of histologic sections are manifestly improved sensitivity, reproducibility, and simplicity. Furthermore, this method is applicable to the computation of dose effect and can be carried on routinely by a technician.

In a series of in vivo experiments, observations indicated that exposure to ozone inhibits the ability of the rabbit pulmonary alveolar macrophage to phagocytize streptococcal cells. Briefly, the animals were exposed to ozone, removed from the chamber, placed under nembutal anesthesia, and given intratracheal injections of streptococci. They were held for 30 minutes with the head slightly elevated and then subjected to pulmonary lavage. Smears prepared from sedimented cells showed inhibition of phagocytic activity in animals previously treated with ozone (table III). The role of this phenomenon in the so-called bacterial clearance rate is still under investigation.

TABLE III

DEPRESSION OF PHAGOCYTOSIS BY OZONE

Ozone Dose (ppm)	Phagocytic Index x 100	Number Rabbits
None	498	20
0.67	442	6
1.25	341	6
2.50	303	6
3.75	281	6
5.0	286	6
9.5	291	6

Moderating Influences

A tolerance to ozone induced by repeated doses on succeeding days is now a well-known phenomenon (Stokinger, 1965). This interesting phenomenon appears operant also in pulmonary infection (table IV). Thus the mechanism by which ozone increases susceptibility to infectious agents apparently is capable of developing tolerance.

Temperature Variation

Probably many forms of secondary stress heighten the mortality from infectious disease produced by exposure to oxidant pollutants. Stress from prolonged

TABLE IV

DEVELOPMENT OF TOLERANCE TO THE MORTALITY ENHANCING EFFECT OF OZONE OF STREPTOCOCCAL INFECTION

No. Mice per Group	Percent Mortality From Streptococcal Aerosol Following Exposure to: Ambient Air	1 ppm O_3 x 3 hr	1 ppm O_3 x 3 hr Repeated 24 hr later
20	35	75	40
20	40	90	70
20	20	65	35
20	20	80	55
20	10	70	10
100	25%	76%	46%

($p < .001$) ($p < .001$)

exposure to cold following exposure to ozone and streptococci reportedly results in increased mortality (Coffin and Blommer, 1965).

Preliminary data, as yet unpublished, indicate that elevated environmental temperature following exposure to pollutant and bacterial aerosol accelerates the course of the pneumonic disease, so that deaths accumulate more rapidly and also suggest that mortality is heightened.

Discussion

Ozone, nitrogen dioxide, and artificially produced photochemical auto smog all induce a heightened susceptibility to infections of the lung by means of an aerosol of pathogenic organisms. From the viewpoint of environmental toxicology, this phenomenon may be looked upon as (1) an extremely sensitive parameter of effect of oxidant gases on the chemical and cytological milieu of the lung airway, and (2) a model that may mimic the actual predisposing influences of such pollutants in the causation of similar disease states in human beings. In the first instance, the increased mortality must reflect alterations in those mechanisms responsible for defending the lung against bacterial invaders. Furthermore, these same mechanisms may well be playing other roles, as for instance, removal or inactivation of irritating or carcinogenic substances. Data presented here indicate that ozone exposure reduces the capacity of the lung to rid itself of cultivable bacterial cells. It is possible that there is impairment of both (1) physical removal generally credited to ciliary activity and mucus flow, and (2) in

situ killing or loss of cultivability. There is ample evidence that irritating chemicals reduce the rate of mucus flow (Dalhamm and Strandbury, 1967; Tremer, et al, 1959); however, little is known concerning the action of these substances on the actual lung tissues likely to be responsible for inactivation of bacteria in situ. Green and Kass (1964) speculate that certain factors responsible for reduced pulmonary bacterial clearance may diminish the phagocytic activity of the pulmonary alveolar macrophage. Data from our laboratory show that this does in fact take place with ozone exposure in rabbits (Gardner, et al, Inhibition of Phagocytic Activity of the Pulmonary Alveolar Macrophage by Ozone Exposure, In Preparation). There is need for considerable work on a cytological and chemical level for elucidation of these mechanisms.

In regard to the production of a model mimicking the predisposing influences of oxidants in the cause of disease in human beings, no epidemiological or clinical evidence appears to exist linking bacterial pulmonary infections in man with oxidant air pollutants. On the contrary, epidemiological data do suggest that bacterial infections of the lower pulmonary tract of man may be more frequently encountered in atmospheres containing high concentrations of substances such as sulfur dioxide and sulfuric acid mist or a heavy load of solid particulates (Douglas and Waller, 1966; Lunn, et al, 1967). Therefore, there is urgent need for investigation of this atmosphere from the standpoint of the experimental infectious system described here. Conversely, there is need for complementary clinical and epidemiological studies in areas in which oxidant gases comprise the major source of pollution to determine whether these gases do in fact potentiate infection in man as they have been shown to do in animal experiments.

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DR. FAIRCHILD (Aerojet-General Corporation): Dr. Coffin, what was the ozone level that you showed on that clearance?

DR. COFFIN: We used, I think, about .7 on all the clearance studies. We don't know what the end point is for clearance; we'd like to determine this.

QUESTION: You are simply counting bacteria as being present or absent in these cells. Did you make any effort to determine the number of bacteria?

DR. COFFIN: We classified the cells containing bacteria in categories: no bacteria, from one to three bacteria, and so on, and then 10 or more, because when we got up to 10 we couldn't distinguish how many there were. I might say in this study we did also one other thing (because we felt that it was fairly liable to subjective slanting); each time we ran these studies, we used a control and two levels of ozone, and the examiner did not know which slide he was counting. Now, he could tell whether there had been an ozone exposure because of the presence of heterophils; but we used the two levels of ozone so he couldn't distinguish in a casual way between the two. The data still fit a fairly decent curve. We feel it's fairly valid in that regard.

KIDNEY TRANSLOCATION FOR TOXICOLOGIC EVALUATION

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INTRODUCTION

Standard toxicologic procedures involving gross observations of mortality, morbidity, and alteration of behavior are no longer adequate for the evaluation of new substances and the artificial atmospheres programmed for use in manned spacecraft. These test substances must now be validated as safe for prolonged, continuous human exposure, with contemplated durations of up to one year. To meet this requirement, any potential for low grade, subacute toxic effects on organ structure and function, and on the organism as a whole, must be ruled out. The most sensitive way to accomplish this is by a continuous evaluation of both morphologic and metabolic response to the test substance at a cellular level, correlated with evaluation of functional integrity of potential target organs. We have attempted to do this by utilizing the electron microscope for morphologic study, biochemical analyses of tissue samples for metabolic study, and appropriate in vivo function tests as indicated. In toxicologic studies, the liver and kidney are frequent target organs. By utilizing percutaneous biopsy techniques, tissue for morphologic and biochemical analyses can be obtained from these organs in unanesthetized animals before the test exposure, at selected intervals during exposure, and during a recovery period after exposure. Function tests can be done simultaneously. Consequently, test animals act as their own controls, a statistically desirable situation, and any response to the test substance can be evaluated in terms of severity, significance, progression, and reversibility. In addition, since these procedures can be accomplished without removing the test animal from the experimental environment, artifacts arising from interruptions of exposure for sampling purposes can be eliminated. Percutaneous biopsy of the liver of a laboratory animal is a simple procedure because of the size and accessibility of that organ. However, because of its relatively small size and retroperitoneal location, the kidney is not readily biopsied in this manner. For this reason, the use of renal biopsy as a clinical procedure for human patients is usually left in the hands of a few specially and extensively trained individuals in large medical centers. Even then the procedure requires the use of fluoroscopy or image intensification to guide the path of the biopsy needle, necessitating the availability of large nonportable equipment. It still

remains a relatively high risk procedure with the possibility of massive uncontrollable bleeding as a serious side effect and with a high yield of unsatisfactory specimens.

To alleviate this problem, others, interested in using percutaneous biopsy as a research technique for evaluating the kidney, had developed a two-stage, fairly complicated procedure for translocating a dog's kidney to a subcutaneous pocket without compromising that kidney's function (McCulley and Kraner, 1964). We have found that this procedure may be adapted for use with monkeys and can be reduced to a simple one-stage procedure that can be utilized by researchers without prior extensive surgical training.

METHOD

Kidney Translocation*

Macaca mulatta monkeys, weighing 2.7 to 8.0 kilograms, were each anesthetized with intravenous pentobarbital (approximately 30 mg/kg). An area approximately 15 centimeters square, in the left paravertebral region, immediately caudad to the costal margin, was then shaved and prepared for aseptic surgery. To prevent complications arising from aspiration of stomach contents during surgery, we have found it advantageous to withhold food for a 24-hour period prior to surgery and to utilize an endotracheal tube during surgery. With the monkey in a prone position, a three centimeter longitudinal skin incision was made parallel to and approximately three centimeters to the left of the spinal column, exposing the external oblique musculature. Because the musculature is relatively thin in this region, the left kidney was usually palpable through the skin. The incision was made slightly medial to the palpable kidney so that the skin closure suture line would not overlay the kidney and act as a potential irritant. By blunt dissection, the skin surrounding the incision was separated from the underlying musculature creating an extensive subcutaneous pocket. The incision was then carried through all abdominal muscle layers and the peritoneum, exposing the kidney. With gentle manipulation, the kidney was mobilized from the surrounding adherent connective tissue. Utilizing a purse-string suture which incorporated all three abdominal muscle layers and the peritoneum, an aperture about two centimeters square was created. When external pressure was applied to the abdomen on both sides of the incision site, the kidney extruded through this purse-string opening. Tightening the purse-string prevents the kidney from withdrawing through the opening to its natural intra-abdominal position. It is essential that the purse-string be drawn tightly enough to accomplish this, but loosely enough to prevent strangulation of the renal vasculature and the ureter. After pulling the loosened skin over the translocated kidney, the skin incision was closed with a continuous subcuticular stitch and the monkey returned to his cage. We have not found it necessary to dress or protect the surgical wound in any way.

Renal Function Tests

In each monkey, we determined the glomerular filtration rate by endogenous creatinine clearance, the effective renal plasma flow by sodium para-amino hippurate (PAH) clearance, the maximal proximal tubular secretory capability by Tmax PAH, and the maximal proximal tubular reabsorptive capability by Tmax glucose.

*"Kidney Translocation for Toxicologic Evaluation", designated as EC 6-67, can be obtained from Air Force Film Library Center (MAC), 8900 S. Broadway, St. Louis, Missouri 63125

The monkeys were anesthetized with iv pentobarbital (approximately 30 mg/kg). An indwelling urinary catheter was placed in the bladder, five French Premature Infant Feeding tubes in males and eight French Foley catheters in females, for the collection of urine. A 17-gauge intravenous catheter was inserted into the saphenous vein for infusions and a Cournand needle implanted in the femoral artery for obtaining blood samples. The needle was kept patent with a heparin-coated stylet between sampling periods. The monkeys were hydrated with physiological saline for 45 to 90 minutes prior to infusion of PAH to insure an adequate urine flow. To assure complete emptying of the bladder at each sampling period, bladder drainage was followed by two washes of 5 ml normal saline and one wash of 5 ml air. An equilibration period of 30-40 minutes was allowed after each loading dose of PAH and 15 minutes after the start of the glucose infusion before sampling periods began, thus providing adequate time for blood and urine levels of PAH or glucose to plateau. At the conclusion of these equilibration periods the bladder was emptied and washed. There were then three sampling periods of 15 minutes respectively for PAH clearance and Tmax PAH and three 10-minute periods for Tmax glucose.

Effective measurement of PAH clearance requires a level of 1-2 mg/100 ml plasma. To accomplish this, an initial loading dose of 12 mg/kg PAH was given intravenously and a sustaining infusion of 0.3 mg/kg/minute PAH was maintained throughout the sampling periods. To determine Tmax PAH, it was necessary to saturate the tubular cells. This was done with a loading dose of 300 mg/kg PAH followed by a sustaining infusion of 4.0 mg/kg/minute PAH. With these dosages PAH levels of about 20-50 mg/100 ml plasma were maintained. Creatinine clearance was measured simultaneously with PAH clearance and Tmax PAH. Tmax glucose determinations also required tubular saturation in excess of the glucose reabsorption threshold, i.e., glucose levels above 400 mg/100 ml plasma. This was accomplished with a constant iv infusion of 1.25 ml. of 50% glucose/minute.

Analytic Procedures

Creatinine determinations were performed by a modification of the Folin-Wu method (Technicon Autoanalyzer Methodology N-11b, New York); PAH determinations, according to the method of Bratton and Marshall, as modified by Smith et al (Smith, Finkelstein, Aliminosa, Crawford, and Graber, 1945). Glucose levels were measured enzymatically using the glucose oxidase method (Standard Methods Clinical Chemistry, 1965).

Percutaneous Biopsy

Percutaneous biopsies were performed on translocated kidneys with only topical anesthesia. Using 1% carbocaine, a small intradermal bleb was raised immediately caudad to the lower pole of the subcutaneous kidney. A sterile Vin-Silverman needle was then inserted through the skin bleb, into the kidney through the lower pole, and along the long axis. By rotating the cutting stylet, a core of renal tissue about 1 cm in length and 1 mm in diameter was obtained. After removal of the needle, manual pressure was maintained on the biopsy site for two to five minutes until bleeding ceased. No dressing was applied to the biopsy site.

Comparison of Normal Monkeys and Monkeys with Translocated Kidneys

Two groups of monkeys, one consisting of 20 animals (T-1), and the other of 16 (T-2), underwent the translocation procedure at the hands of two different teams of investigators working independently. Renal function tests were performed on each of these monkeys by members of the same teams that had performed their translocations. A minimum of 30 days was allowed for recovery from the surgical procedure before renal function testing was done. A third group of seven monkeys (control), surgically untouched, was evaluated by members of both teams to establish normal parameters of renal function. Because of technical problems arising with the analytical method for blood and urine glucose content, Tmax glucose was not evaluated in the control group.

RESULTS

With the exception of three monkeys that had to be subjected to subsequent nephrectomy and removed from the study because of infarction of the translocated kidney, there was no postoperative morbidity or mortality resulting from the translocation procedure. The infarctions noted were secondary to complete or partial strangulation of the renal vasculature, a consequence of excessive tightening of the musculo-peritoneal purse-string suture. We attribute this to operator error rather than to any deficiency in the procedure itself.

Table I summarizes the average renal function values obtained in each group. There is no statistically significant difference between the values obtained from monkeys with translocated kidneys by either team, or between those monkeys and the control group whose kidneys were left in their natural anatomic locations. We could find no indication that the translocation interfered in any way with the normal function of the affected kidney.

Examination of percutaneous biopsy material by light microscopy revealed no morphologic abnormalities in the translocated kidneys. Evaluation of samples obtained serially at weekly intervals for one month similarly revealed no morphologic abnormalities. Subsequent electron microscopic examination of biopsy tissue, obtained as late as six months after translocation, demonstrated only normal renal architecture even on the ultrastructural level. Biopsies obtained within 24 hours after performance of renal function tests, when examined electron microscopically, revealed vacuolization of the proximal tubular cells. This was interpreted by Dr. Willie Mautner of the Mount Sinai Hospital as an artifact induced by the fluid loading and osmotic diuresis which are part of the function test procedure. We did not determine how long after function tests this artifact persisted. The presence of these vacuoles does not hinder evaluation of cellular structure by an experienced renal pathologist.

SUMMARY AND CONCLUSIONS

A simple procedure for surgical translocation of a monkey kidney to an accessible subcutaneous pocket has been described. Morbidity and mortality from this procedure, properly performed, are nil. Overall renal function in monkeys followed for six months after surgery is unaffected. The renal function test

TABLE I

RENAL FUNCTION STUDY

GROUP	NUMBER OF ANIMALS	$C_{F/Kg.}$	$C_{PAH/Kg.}$	$Tm_{PAH/Kg.}$	$Tm_{GLUC/Kg.}$
T_1	20	$3.47 \pm .649$	21.92 ± 4.62	$2.63 \pm .490$	14.21 ± 4.62
T_2	16	$3.41 \pm .495$	20.59 ± 5.43	$2.75 \pm .415$	13.89 ± 4.03
CONTROLS	7	$4.03 \pm .488$	21.12 ± 3.06	$2.72 \pm .254$	- - -

NOTES:

1. Each monkey in groups T_1 and T_2 has one translocated kidney and one normal kidney.
2. $C_{F/Kg.}$ - Endogenous creatinine clearance in ml./min./kg., a measure of the glomerular filtration rate.
3. $C_{PAH/Kg.}$ - PAH clearance in ml./min./kg., a measure of the effective renal plasma flow.
4. $Tm_{PAH/Kg.}$ - Maximal PAH transport in mg./min./kg., a measure of the maximal proximal tubular secretory capability.
5. $Tm_{GLUC/Kg.}$ - Maximal glucose transport in mg./min./kg., a measure of the maximal proximal tubular absorptive capability.

procedure we have described does not permit us to determine whether function of the translocated kidney is completely unaffected, or slightly depreciated, with compensatory hypertrophy of the other kidney yielding overall normal renal function. Differential kidney studies, with independent evaluation of urine obtained from each kidney by individual ureteral catheterizations, are now underway to resolve this issue. However, light and electron microscopic studies of kidney tissue obtained by biopsy of the translocated kidneys of the monkeys described in this report, conclusively indicate that there is no morphologic change resulting from the translocation.

Preliminary experiments indicate that the procedure described herein represents a valid method for serial evaluation of morphologic, biochemical, and functional renal response to potential toxicants.

ACKNOWLEDGMENT

The authors wish to express their appreciation to Master Sergeant Joseph Young and to Technical Sergeant Edgar A. Hagan for their invaluable technical assistance.

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DISCUSSION

QUESTION: Dr. Kaplan, I assume the renal function studies were based upon a total urine sample, rather than from each of the two ureters from the normally located and translocated kidney?

DR. KAPLAN: That is correct.

SESSION II

HISTOPATHOLOGICAL EVIDENCES OF TOXICITY

Chairman

Dr. Frank M. Townsend
Laboratory Service
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ELECTRON MICROSCOPIC AND MORPHOMETRIC EVALUATION OF LUNGS FROM ANIMALS EXPOSED CONTINUOUSLY FOR EIGHT MONTHS TO 5 PSIA, 100% OXYGEN

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INTRODUCTION

In extension of previous studies on the effects of pure O_2 breathing on lung tissue ultrastructure and on critical dimensions of the pulmonary gas exchange apparatus, we have had the opportunity to investigate lungs of animals exposed at 6570th AMRL to 100% O_2 at 5 PSIA for about eight months. Following exposure some of these animals were allowed to recover in room air for 30 - 40 days. We should like to present in this report some findings obtained on these lungs by electron microscopy, using in part morphometric techniques.

As basis for the following report those findings from our previous studies (Kistler, et al, 1966; Kistler, et al, 1967; Kistler, et al, Third International Conference on Hyperbaric Medicine, 1966) which will be pertinent to today's presentation, are briefly summarized:

1. On adult rats which had been exposed to 98.5% O_2 at atmospheric pressure the first signs of damage were seen after 48 hours of exposure (Kistler, et al, 1966; Kistler, et al, 1967); these were

   - increase of the thickness of the air-blood barrier due to edematous enlargement of the interstitial space, and

- destructive changes of the capillary endothelial lining.

After 72 hours of exposure an aggravation of these findings was observed, i.e.

- the air-blood barrier was doubled in comparison with the control animals,
- there was a marked destruction of capillaries, and
- 65% of the alveoli were filled with a proteinaceous exudate containing in part blood cells and fibrin strands, as well as numerous macrophages.

2. Growing rats which had been exposed to 97% pure O_2 at a pressure of 5 PSIA showed no pathologic changes even after two weeks of exposure (Kistler, et al, 1966); except for an apparent increase of eosinophilic granulocytes within the lung capillaries.

Morphometric studies revealed

- a marked reduction in specific gas exchange surface.*

These findings were interpreted as adaptive rather than pathologic changes.

MATERIAL AND METHODS

Rats, monkeys, and dogs were exposed to 100% O_2 at 258 Torr in a controlled environmental chamber at 6570th AMRL for eight months. In test T the animals were sacrificed immediately after exit from the Thomas Dome. In test R the animals spent one more month in a chamber with room air.

There was a control group for each of the test groups which lived in ambient air for the same period of time (figure 1).

The methods of preparation and investigation have been extensively reported elsewhere (Kistler, et al, 1966). We deviated from our usual procedure only in one respect: the lungs were fixed in glutaraldehyde at 6570th AMRL and shipped to us in the fixative. Further processing took place in our laboratory in Switzerland. The unavoidable delay between fixation and embedding and the many hands involved in processing did not ensure optimal conditions; this must be realized retrospectively.

*The specific gas exchange surface is the fraction of the alveolar surface area which is available for gas exchange per unit body weight: S_a/W measured in m^2 / kg.

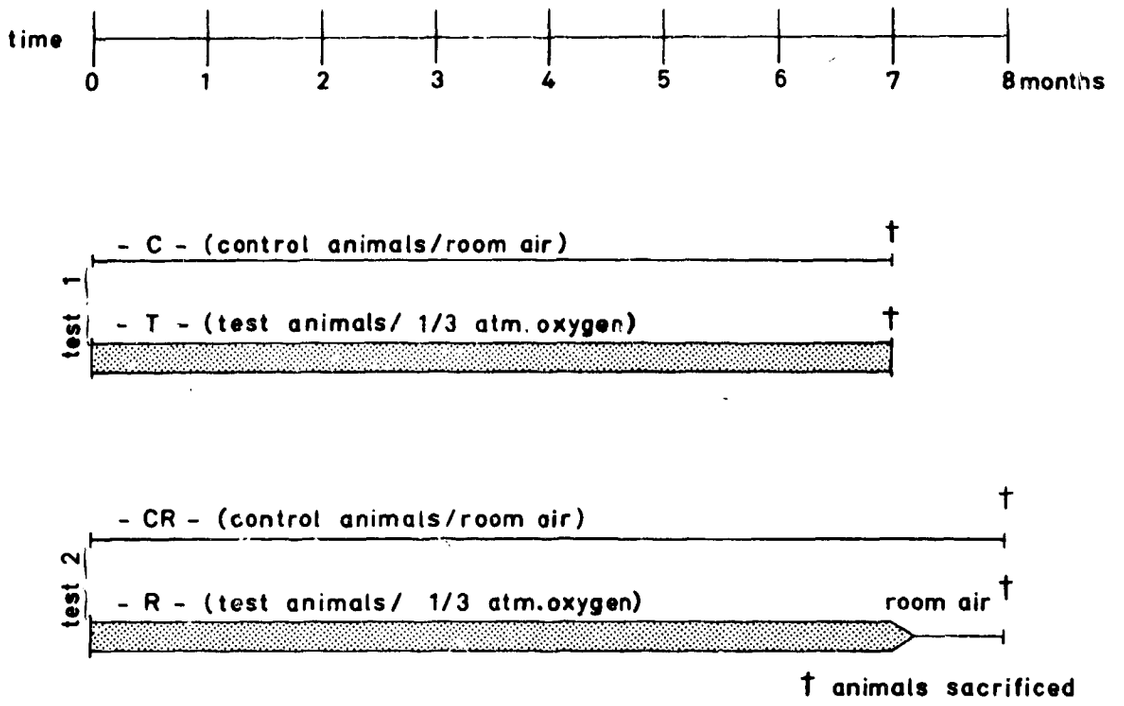

Figure 1. TIME SCALE OF THE EXPERIMENT
T: test animals exposed to 100% O_2 at 5 PSIA for eight months and sacrificed immediately after exit from chamber
C: control animals kept in room air under otherwise identical conditions
R: animals exposed to 100% O_2 at 5 PSIA for eight months and sacrificed after a recovery period of 30 - 40 days in room air
CR: control animals kept in room air for nine months

RESULTS

a. Rats

In their reports at the 2nd Annual Conference on Atmospheric Contamination in Confined Spaces in 1966 Drs. Back, Harper, and Hagebusch presented the general pathology of the animals from this series. With respect to rats, Dr. Hagebusch noted that all animals, controls included, suffered from chronic murine pneumonia.

In our electron microscopic study of these rats we found an obvious thickening of the air-blood barrier at the alveolo-capillary level which was due to accumulation of interstitial edema fluid.

This observation pertained as well to control as to test rats. In the morphometric determination of the mean thickness of the air-blood barrier this caused a wide scattering of the control values, which reached clearly pathological levels

(figure 2). Normal values of $\bar{\tau}$ *, determined in our laboratory during the past years (figure 2) ranged from 1.33 μ to 1.63 μ with an average of 1.5 μ (S.E. $\pm$ 0.026 μ). As shown in figure 2, the values of $\bar{\tau}$ determined for control rats from this series were widely scattered and reached up to 2.6 μ ; this is a clearly pathologic figure.

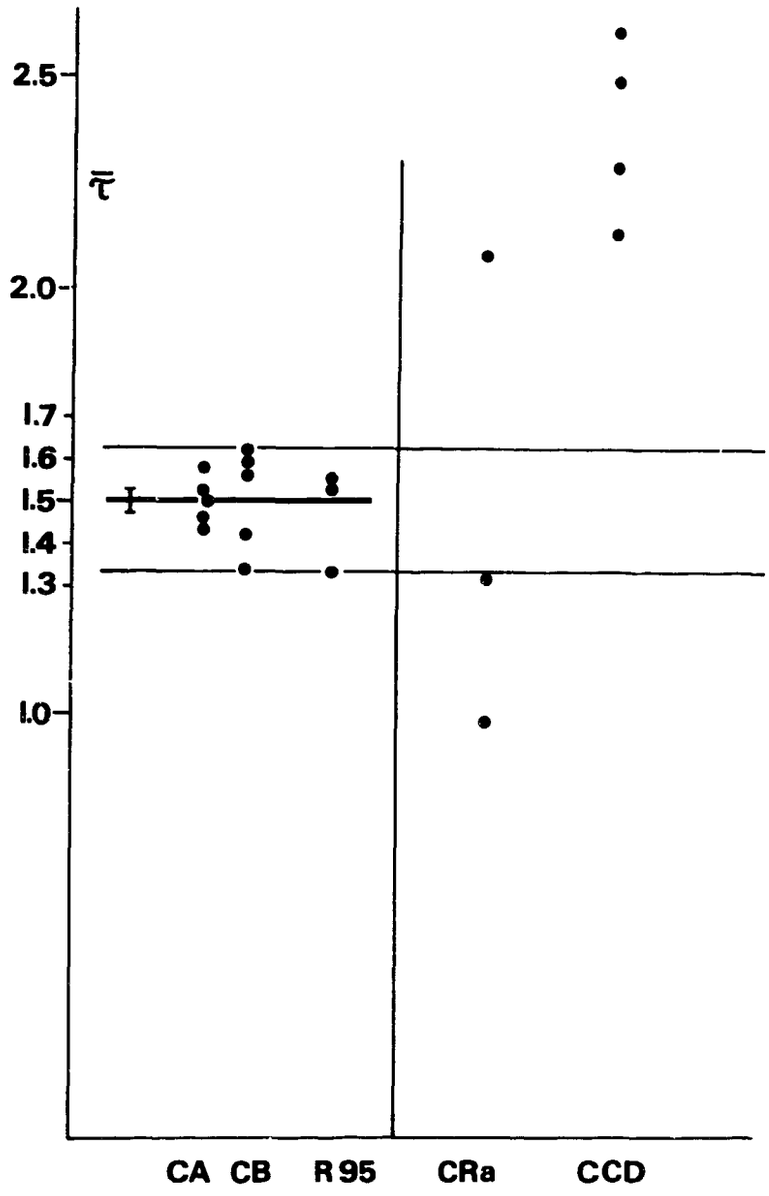

Figure 2. COMPARISON OF ARITHMETIC MEAN THICKNESS OF AIR-BLOOD BARRIER OF RATS IN THIS STUDY (CRa and CCD) WITH RANGE OF NORMAL VALUE (CA, CB, R 95).

* $\bar{\tau}$ is a measure of tissue mass per unit area of alveolar surface.

Under these circumstances it is evident that no conclusions can be derived from this material on the effect of O_2 breathing.

The finding of interstitial edema can be interpreted either as an effect of murine pneumonia or as a result of inadequate preparation. The latter cause cannot be excluded with certainty, since in this study we deviated from our usual procedure, as mentioned above. However, the adequate quality of preparation found in monkey and dog lungs would rather favor the first cause; since murine pneumonia is known to be an interstitial pneumonia, an interstitial reaction would not be surprising.

b. Monkeys

In the monkeys Dr. Hagebusch found infestation with lung mites in one control and one experimental animal.

We found mites and filaria (figure 3), mast cells in the interstitial space (figure 4) and an unusually high number of leucocytes, especially eosinophilic granulocytes in the blood vessels as well in controls as in test animals. With this amount of pathology in the control animals it appears impossible to draw any conclusions from this material on the effects of O_2 breathing on the monkey lung.

c. Dogs

The control dogs were rated to be normal by Dr. Hagebusch. Our own studies failed to demonstrate any pathology in the control dogs of our series. The morphometric determination of various parameters for these controls showed them to lie within the normal range. The series of dog lungs derived from this experiment appears, therefore, qualified to reveal effects of O_2 breathing on lung tissue. Hence, we have subjected these preparations to a complete electron microscopic and morphometric examination.

Changes in dog lungs related to O_2 breathing

a. Test T: Exposure to 100% O_2 at 258 Torr for eight months and sacrifice at exit

The examination by electron microscopy revealed many instances where the interstitial space was enlarged by accumulation of edema fluid as is seen in comparing figures 5 and 6. This edema separates the endothelium from the other tissue elements. The basement membrane remained closely apposed to the endothelial cells. Along the lines of junction between adjacent cells we find a zone of markedly augmented density when compared to the controls (figures 7 and 8).

Corresponding to the occurrence of edema, the morphometric evaluation revealed a thickening of the air-blood barrier (figure 10) with an increase of the arithmetic mean thickness from 1.45 μ in control animals to 2.24 μ as well as of the harmonic mean thickness* from 0,52 μ to 0.81 μ after eight months. As shown in figure 10, this barrier thickening is primarily due to enlargement of the

*The harmonic mean thickness τ_h is a measure of diffusion resistance.

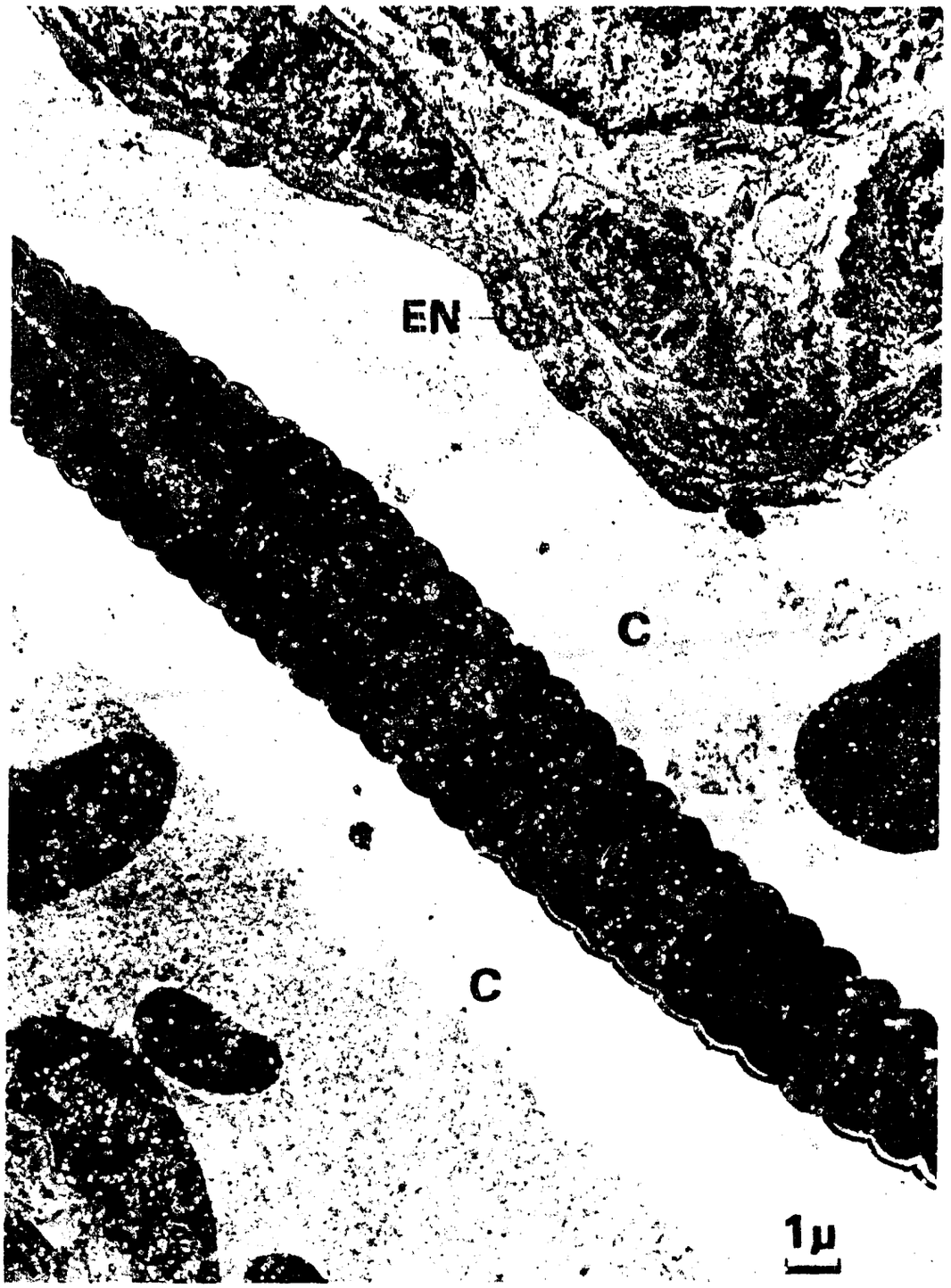

Figure 3. MONKEY CONTROL LUNG (electron micrograph). Filaria in small pulmonary arteriole.
Magnification: 8 400 x.

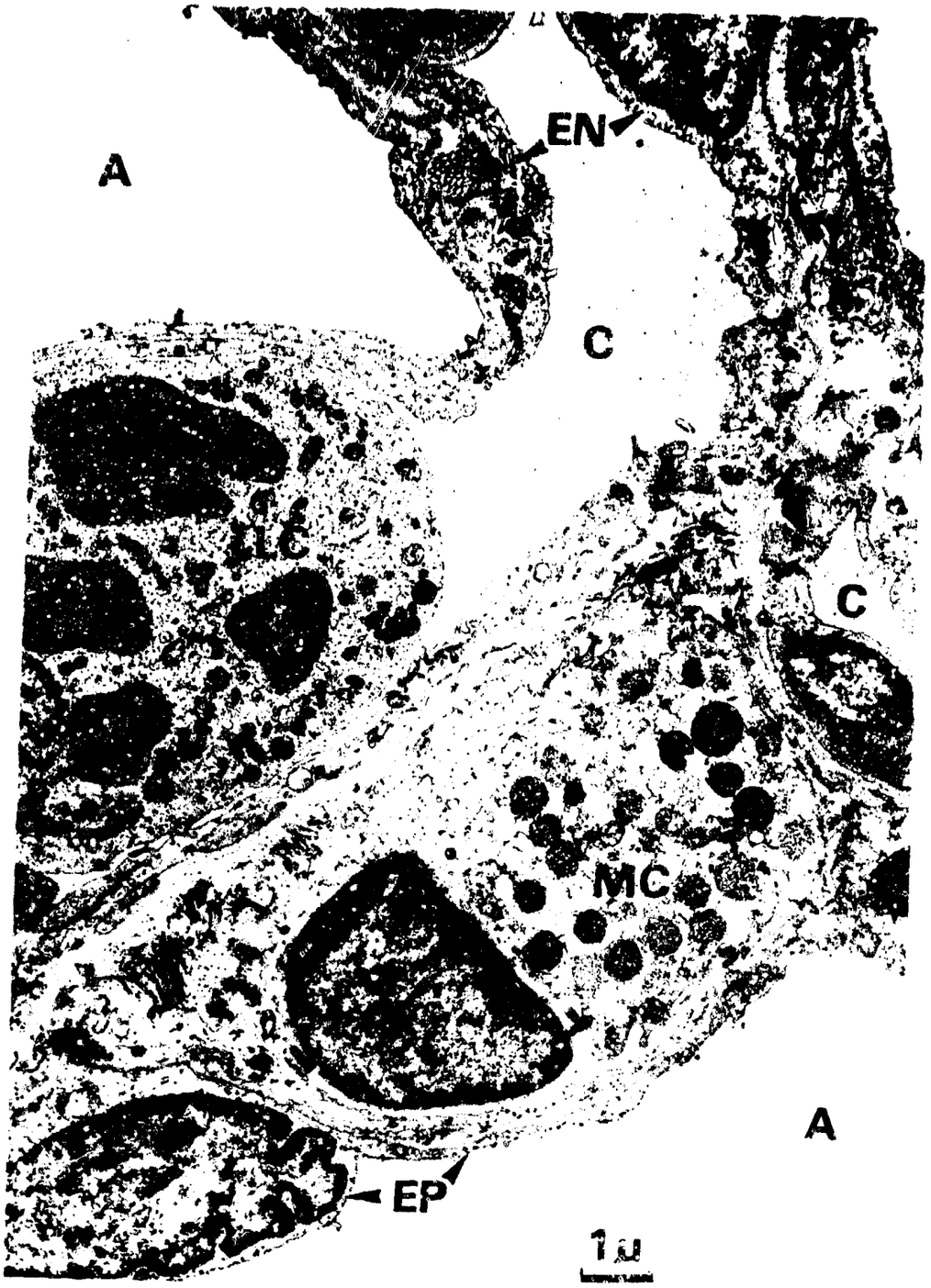

Figure 4. MONKEY CONTROL LUNG (electron micrograph). Neutrophil granulocyte in capillary and mast cell in interstitium. Magnification: 10 750 x.

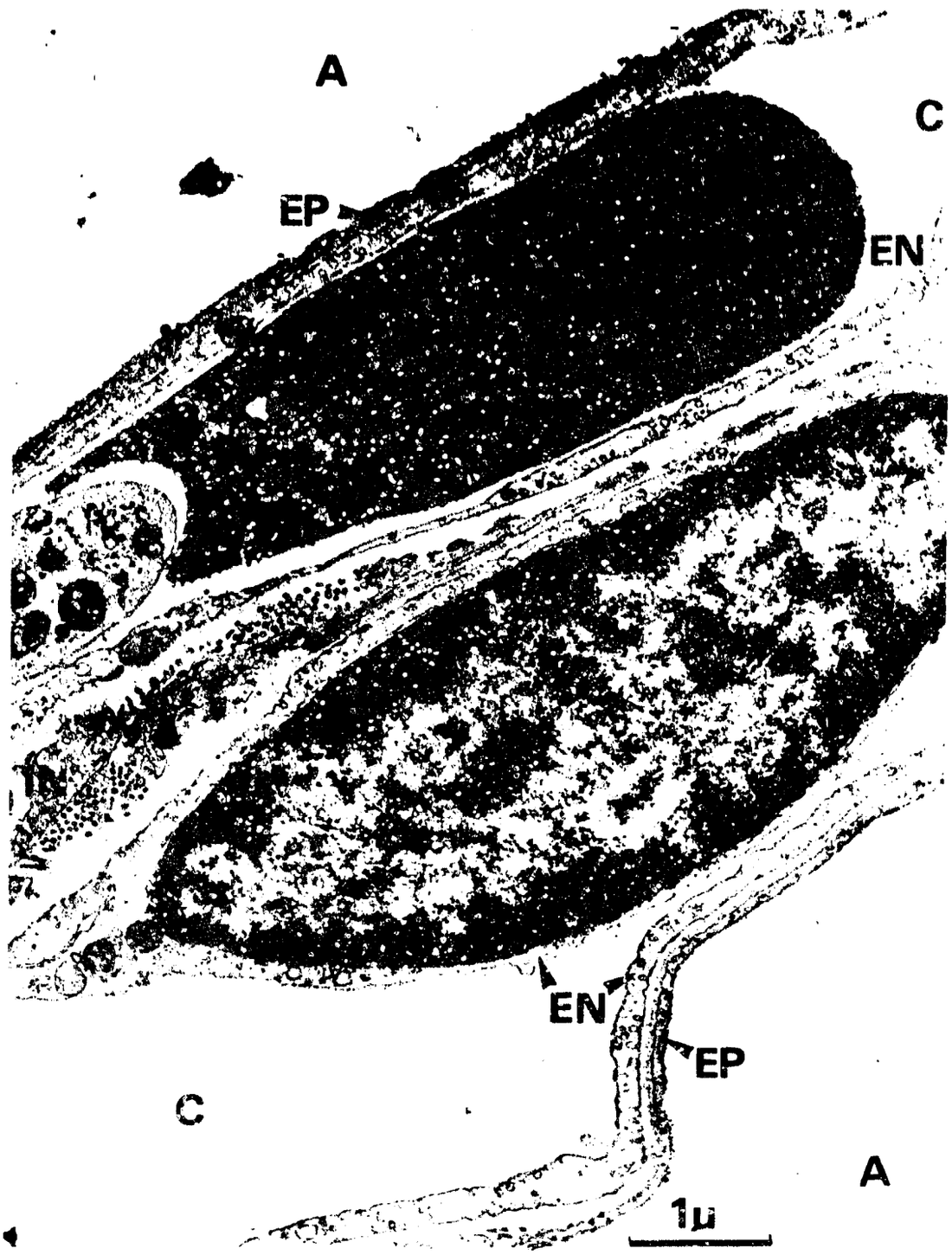

Figure 5. DOG CONTROL LUNG -C-. Interalveolar septum. Two capillaries are accidentally sectioned. Interstitium compact. Air-blood barrier narrow.
Magnification: 22 750 x.

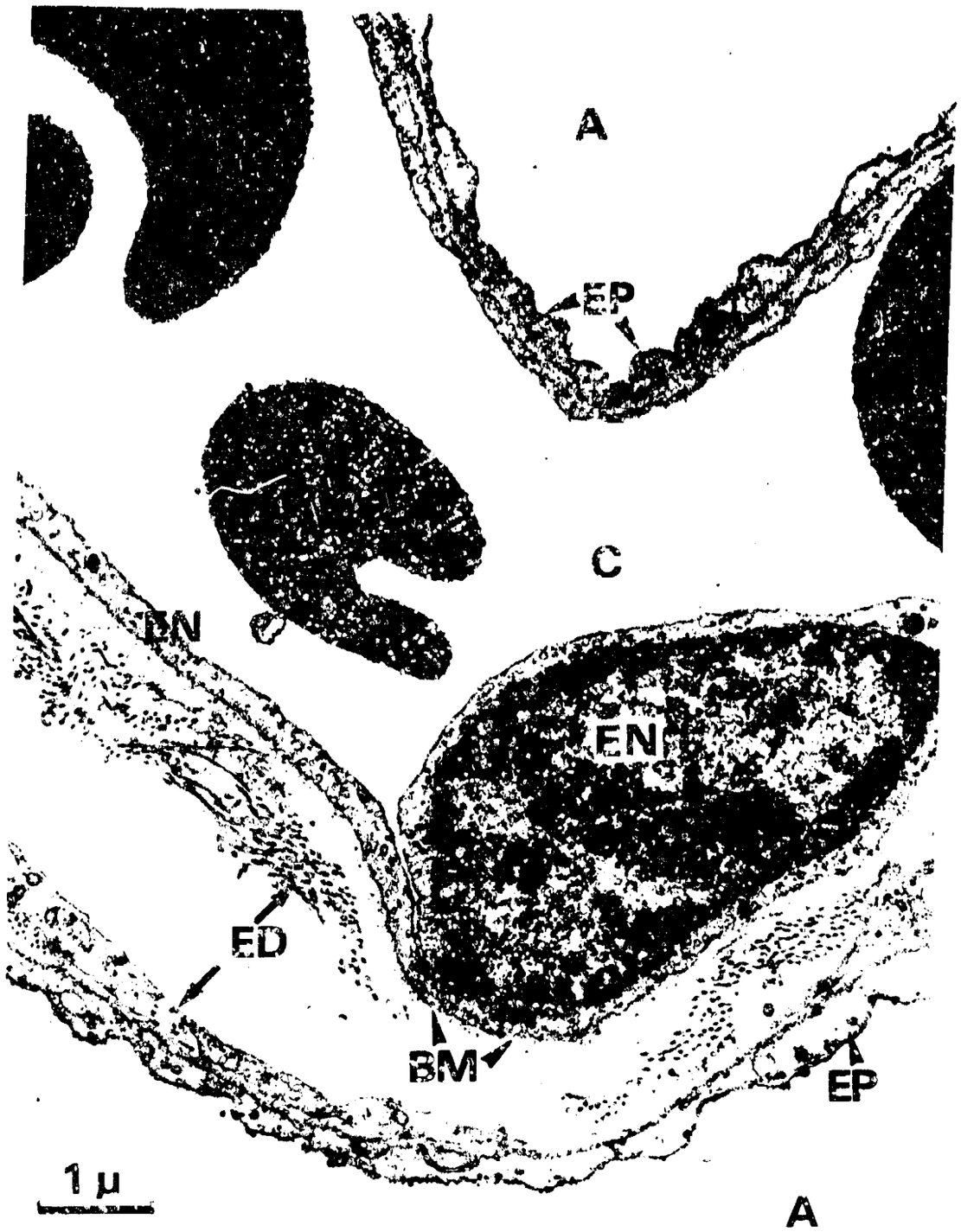

Figure 6. DOG TEST LUNG -T-. Alveolar capillary. Edematous enlargement of interstitial space. Magnification: 17 500 x.

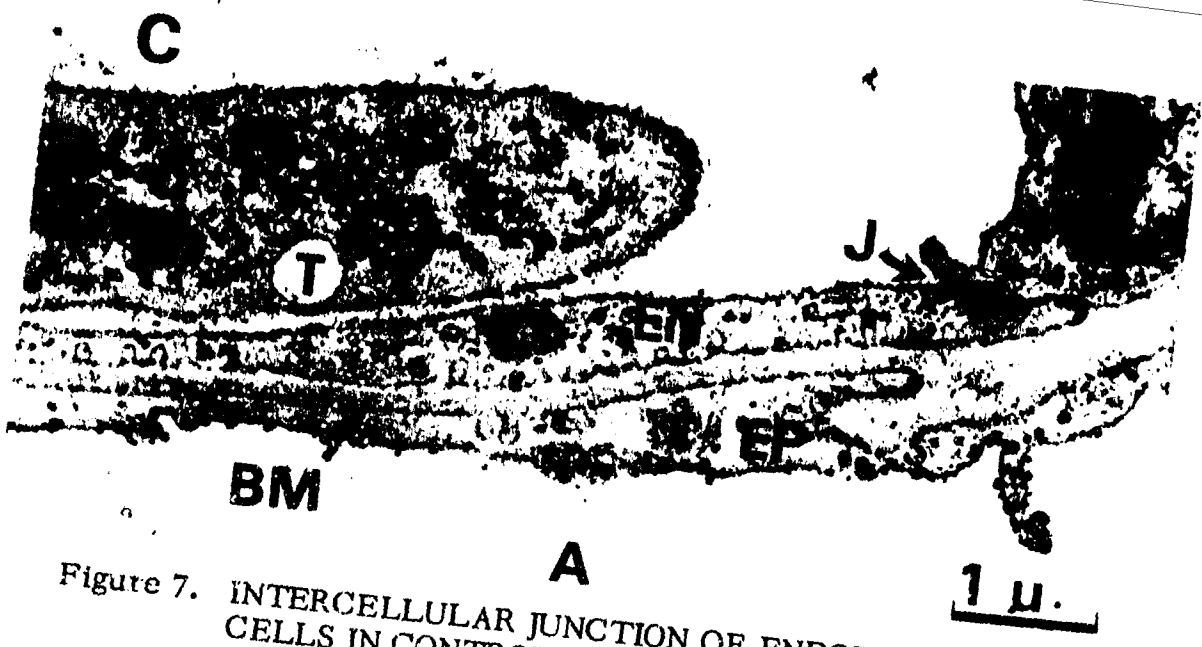

Figure 7. INTERCELLULAR JUNCTION OF ENDOTHELIAL CELLS IN CONTROL DOG -C-. Magnification: 17 500 x.

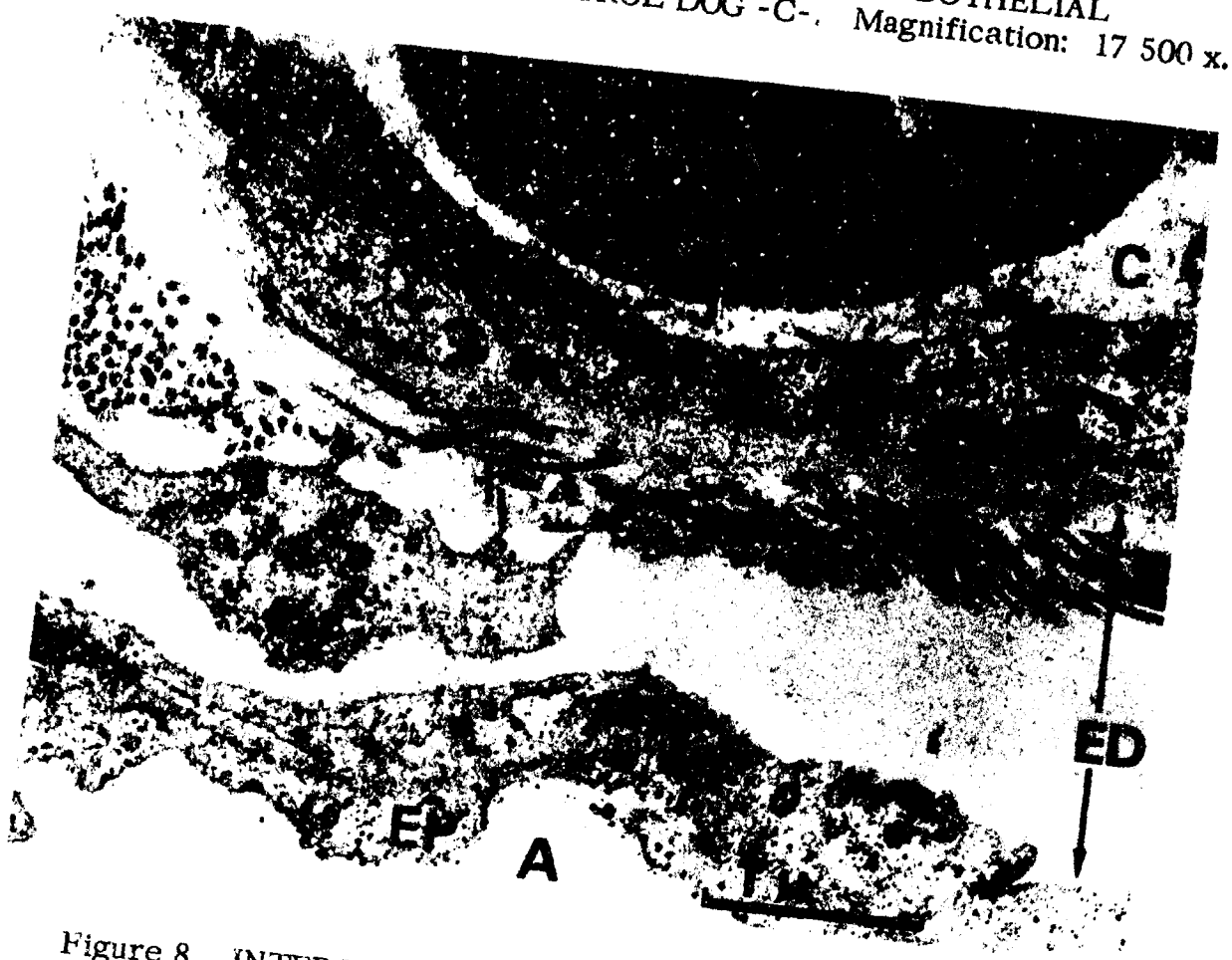

Figure 8. INTERCELLULAR JUNCTION OF ENDOTHELIAL CELLS IN TEST DOG -T-. Band of increased contrast along junction. Magnification: 27 300 x.

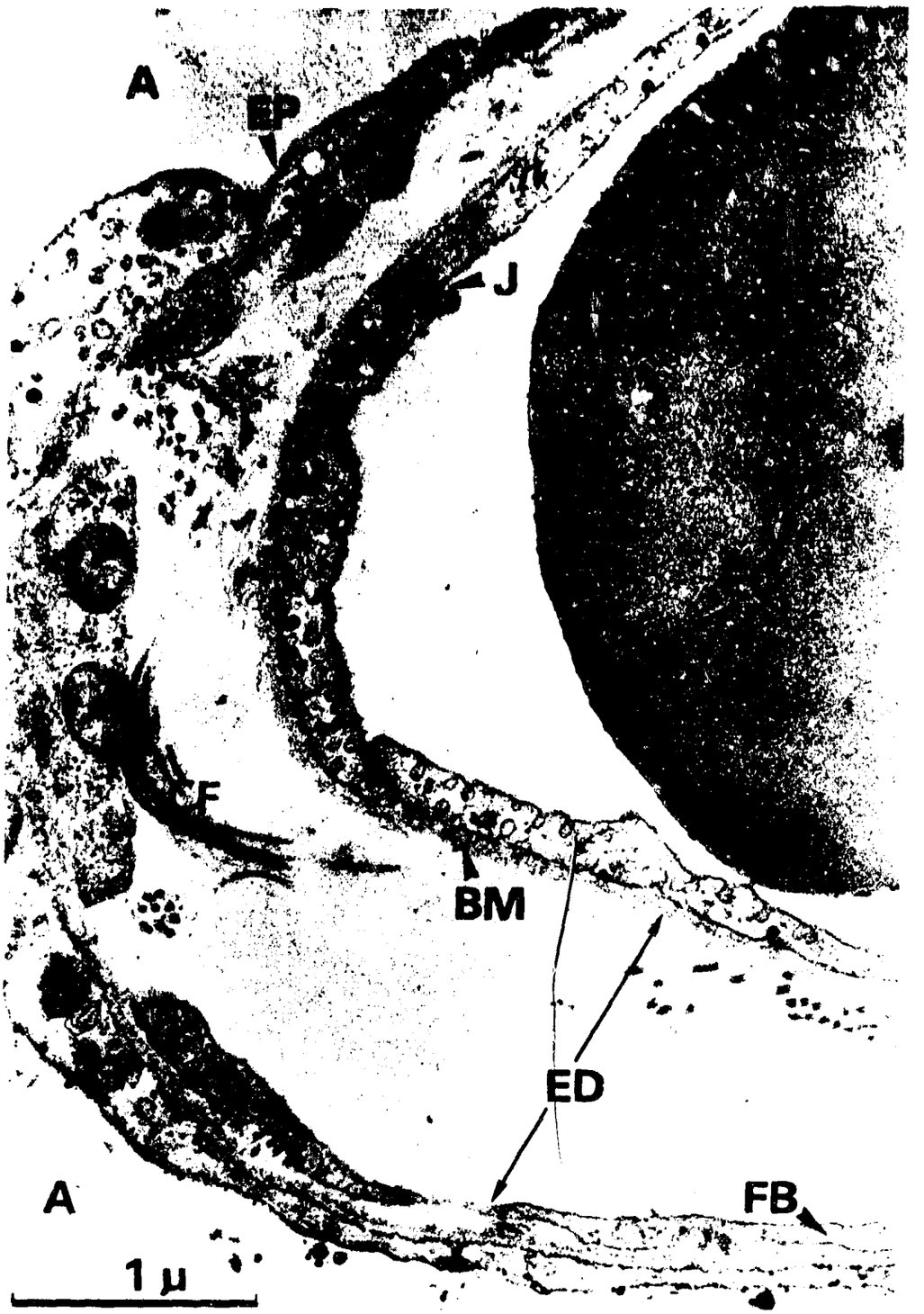

Figure 9. TEST DOG -R-. Alveolar capillary. Interstitial edema persists in some regions of barrier. Magnification: 39 000 x.

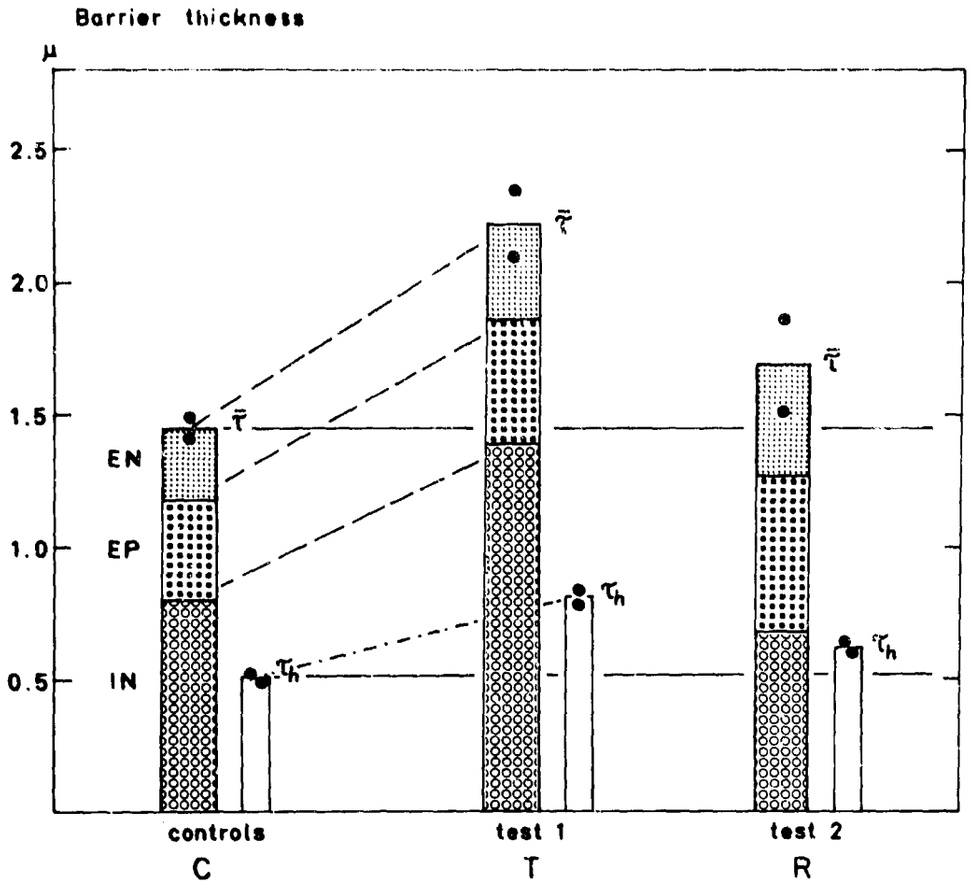

Figure 10. CHANGES OF BARRIER THICKNESS MEASURED BY ARITHMETIC MEAN ($\bar{\tau}$) AND HARMONIC MEAN (τ_h). Note enlargement of interstitial space in T and partial recovery in R.

interstitial space while alveolar epithelium and capillary endothelium remain nearly unchanged. The specific gas exchange surface (S_a/W) was found to drop from normally 3.8 m^2 / kg to 2.7 m^2 / kg after eight months of O_2 breathing at 258 Torr, that is to 70% of the control value (figure 11).

b. Test R: Exposure to 100% O_2 at 258 Torr for eight months and sacrifice after recovery in room air for 30 - 40 days

In electron micrographs the interstitial space of the alveoli was found to be mostly free of edema. However, in some places the interstitial edema appears to have persisted (figure 9). The morphometric evaluation revealed an evident recovery in comparison with the animals of Test T, and that with regard to the increased interstitial space as well as to the increased arithmetic and harmonic mean barrier thickness (figure 10). The relative volume of interstitium is at the control level and the average thickness of the alveolo-capillary barrier is reduced to near normal, though not completely.

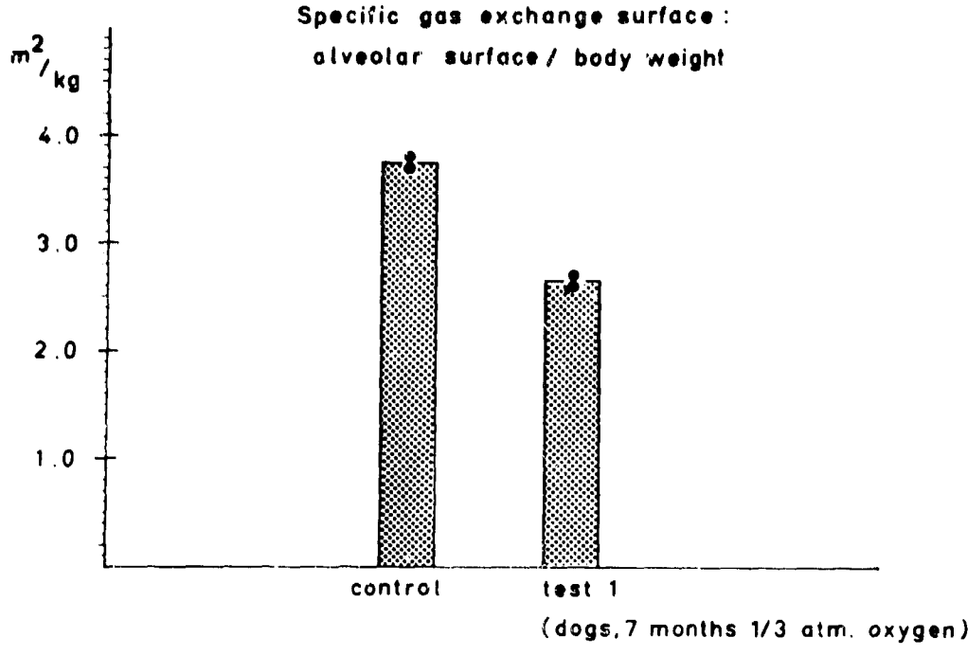

Figure 11. REDUCTION OF SPECIFIC GAS EXCHANGE SURFACE

For unknown reasons the lung volumes of this group are very different from those of the other animals, so that the lung volume-to-body weight ratio is more than double. A comparison of the specific gas exchange surface of these dogs with the group of Test T appears, therefore, not justified.

DISCUSSION

Our previous studies on oxygen toxicity to rat lungs (Kistler, et al, 1966; Kistler, et al, 1967) had revealed a thickening of the air-blood barrier from 1.5 μ to 3 μ after two to three days of pure O_2 breathing at 760 Torr (Kistler, et al, 1967), whereas no significant changes in barrier thickness were noted after two weeks of O_2-exposure at 258 Torr (Kistler, et al, 1966). It was, therefore, surprising to observe a barrier thickening by some 50% in dogs which were left in a pure O_2 atmosphere at 258 Torr for eight months.

The electron microscopic investigation of tissue fine structure detected focal areas of marked interstitial edema associated with an intensification of contrast at endothelial cell junctions. Both findings corresponded to the cytological damage found in rat lungs exposed to 760 Torr O_2 for two days (Kistler, et al, 1967).

On the basis of the available material and data it is certainly not possible to decide whether the interstitial edema is a chronic, possibly even progressive, damage developing during a certain period of low pressure O_2 exposure, or whether

it might be a precipitous reaction of lung tissue to change in O_2 partial pressure or to total pressure of the atmosphere upon removal from the Thomas Dome. However, the striking agreement of fine structural damage with the cytological changes observed after two days of 760 Torr O_2 breathing (Kistler, et al, 1967) is strong evidence for a gradual build-up of endothelial cell damage under the influence of elevated O_2 partial pressure in this experiment.

Growing rats raised for two weeks in 258 Torr O_2 reduced their specific gas exchange surface from 3.5 m^2 / kg to 2.5 m^2 / kg (Kistler, et al, 1966). An identical reduction of this parameter was observed in this study. The conclusion arrived at for the growing rat lungs (Kistler, et al, 1966), namely that the specific gas exchange surface is adapted to the O_2 partial pressure conditions in the atmosphere appears to pertain likewise to the present study on young dog lungs raised for eight months in 258 Torr O_2. It should be particularly noted that the degree of reduction of the specific gas exchange surface was practically identical in both experiments.

CONCLUSIONS

Prolonged exposure of young dogs to pure O_2 at 258 Torr resulted in double damage to the lung. Reduction of the specific gas exchange surface is interpreted as adaptation to a higher O_2 head pressure in ambient air, while the changes observed in the alveolo-capillary tissue barrier appear to be related to endothelial cell damage in the sense of true oxygen toxicity (Kistler, et al, 1967).

These damages were partially restored after one month of room air breathing following oxygen exposure. It is possible, though not proved, that complete remission could be attained after a prolonged recover period.

This study has clearly established that the dog lung is damaged by prolonged O_2 breathing at 5 PSIA. It is not known whether these findings can be projected into the human lung. Nevertheless, they lead to the conclusion that prolonged breathing of oxygen may be a serious hazard to man even at 5 PSIA.

SUMMARY

Rats, monkeys, and dogs were exposed to 100% oxygen at 258 Torr in a controlled environmental chamber for eight months. One group was sacrificed immediately after exit, another group was allowed to recover in room air for one month before sacrifice. The lungs were processed for electron microscopic and morphometric evaluation.

Lungs of rats were suspected of severe murine pneumonia, while lungs of monkeys were massively infected by filaria and mites. The lungs of dogs exposed to O_2 for eight months showed thickening of the alveolo-capillary tissue barrier due to interstitial edema and a reduction of the specific gas exchange surface. These changes are interpreted as oxygen toxicity to endothelium and as adaptation to higher O_2 supply in relation to the reduction of specific gas exchange surface.

In animals exposed for eight months and followed by one month of recovery in room air, the normal lung structure was largely, though not completely, restored.

Key to symbols on electron micrographs

A	Alveolar space	EP	Epithelium
BM	Basement membrane	FB	Fibroblast
C	Capillary lumen	IN	Interstitium
CF	Collagen fibrils	J	Intercellular junction
ED	Edema fluid	LC	Leucocyte
EN	Endothelium	M	Mast cell
		T	Thrombocyte

Note:

Fixation of lungs with glutaraldehyde followed by O_sO_4 causes a granular precipitate to form along cell membranes particularly of alveolar epithelium and erythrocytes. So far it has not been possible to eliminate this aesthetic defect. Glutaraldehyde fixation was used because of the necessity of shipping fixed lungs from the United States to Switzerland and because of good geometric fixation of lung structure.

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DISCUSSION

QUESTION: Can you explain why an edema lasting for eight months will not show any other morphologic changes? This is very hard to conceive. It's more a question whether this is an artifact.

DR. THOMAS (Aerospace Medical Research Laboratories): We left that door open because, unfortunately, at the present time we don't have the room in these domes to sacrifice the animals in the same atmospheric condition as they were exposed. In the new facility the four individual air locks will be interconnected with an operating room and then we can bring down animals from any of the domes into the operating room, do the sacrifice, or the punch biopsy, or whatever they do, without changing the pressure and the composition of the atmosphere. Then if we don't get that edema, we can write it off as an artifact. There's a good strong possibility of this. But Dr. Lewerenz also spoke of endothelial damage. Could you account for it with the sudden change in pressure?

DR. LEWERENZ: We cannot decide if it is on account of the removal from the Thomas dome or if it is progressive damage. That will be decided after the new procedure.

DR. KAPLAN (Aerospace Medical Research Laboratories): You have to remember that we're at a disadvantage because up until now the longest duration exposure that we had was two weeks and then we jumped all the way to eight months. I don't think we're assuming that there was edema for eight months. It would be very nice if we had specimens at six months and at four months so that we would know just what happened, but, frankly, we didn't expect there would be anything at eight months. This came as a surprise to us and we had not obtained intervals, so we have this big gap between two weeks and eight months where we don't know what happens. It's quite possible this was a consequence of the sudden removal back to room air. Certainly we know that animals who lived for eight months in this pure oxygen environment made certain adaptations to it, and having become accustomed to it, the sudden removal back to room air may have been somewhat of a traumatic thing. However, the monkeys were similarly removed and treated and they did not show this. Of course the monkey lungs were infested with parasites which makes them difficult to evaluate, but I don't see that parasitic infestation necessarily would protect them against the traumatic effect that could cause edema formation. In addition, I think the fact that these findings match so clearly those which have been seen at 15 PSI oxygen after 48 to 72 hours (in a situation where we know we are dealing with oxygen toxicity) and on animals that have been sacrificed in the exposure dome without removing them suggests this is indeed an oxygen effect.

DR. COULSTON (Albany Medical College): Why do you call this damage? Why do you call it damage? You keep stating that the cells are damaged. Is there any evidence that the cells are damaged?

DR. LEWERENZ: I am surprised that you ask that question because all the time I tried to point out that there was a damage. Did you ever see any normal lung tissue with such a great interstitial edema?

DR. COULSTON: Let me ask this then. Where is the damage in the cell? I'm trying to help; I'm not trying to criticize. The work is beautiful, but there are a lot of people sitting in the audience who are going away with the idea that you got a lot of damage. Were the mitochondria changed, microsomal bodies changed, were the lysosomes changed? Where was the damage? What happened?

DR. LEWERENZ: I must confess, I cannot really decide it now and here. There are certain hypotheses that the endothelial cell damage takes place at the membrane, referring to the work of Danon last year, but until now we do not really know the mechanism of the edema coming into the interstitial space.

PATHOLOGY OF ANIMALS EXPOSED FOR 240 DAYS TO A 5 PSIA 70% OXYGEN AND 30% NITROGEN ATMOSPHERE

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INTRODUCTION

This study is part of a comprehensive evaluation of a mixed gas environment. It deals with the histopathology of dogs, monkeys, rats, and mice exposed to an environment of 70% oxygen and 30% nitrogen at 258 mm Hg (5 psia) for 240 days.

METHOD

General experimental procedures and chamber operation for this experiment are presented elsewhere in the Conference Proceedings. Routine tissues were taken at Wright-Patterson AFB and submitted for histopathological evaluation. Pathologic alterations were graded and the distribution of the lesions was noted for each animal. In addition, control and experimental animals were compared as groups for a subjective evaluation.

RESULTS

Dogs

Eight dogs from the experimental group and four from the control group were studied. All animals survived. Three experimental animals showed focal chronic periportal hepatitis of the type frequently found in dogs. Focal chronic pyelonephritis was present in two experimental animals and one control animal. Lungs from both groups showed focal chronic bronchitis and bronchiolitis. One animal from the experimental group showed more marked chronic bronchiolitis. In general the pulmonary pathologic alterations were much less severe in both groups than are usually noted in dogs kept for relatively long periods in toxicological evaluation of environmental conditions and chemical compounds.

Monkeys

Four monkeys from the experimental group and two control monkeys were submitted for evaluation. Two exposed animals showed mild focal chronic pyelonephritis.

One animal in each group showed mild chronic periportal hepatitis. Both entities are frequently encountered in monkeys. No lesions were encountered which could be related to the mixed gas environment.

Rats

Fourteen rats were examined from the mixed gas environment. Eleven rats served as controls. Seven experimental animals which were submitted for histologic evaluation died prior to terminating the experiment. One died from the control group.

Chronic murine pneumonia was present in most of the rats from both groups and in most animals there was an interstitial as well as a peribronchial component. In this respect the two groups were approximately the same. Three experimental animals showed pulmonary pathology which was qualitatively and quantitatively worse than that seen in controls. These changes consisted of bronchiectasis, acute bronchopneumonia, hemorrhage, and edema. All three of these animals died prior to termination of the experiment. Other animals that died showed pulmonary changes like those noted in the control rats. Hepatic sinusoidal congestion was noticeable in three experimental animals. Inflammatory changes in the kidney were present in four experimental animals and three control animals.

Mice

There were nine mice in the experimental group; four died prior to the termination of the experiment. There were seven in the control group; two died prior to termination of the experiment. Endemic pulmonary disease was comparable in the two groups. This consisted of chronic murine pneumonia, focal chronic hepatitis and interstitial nephritis. Lymphoma was present in one exposed mouse. No pathologic alteration was noted which could be attributed to the mixed gas environment.

DISCUSSION

A review of tissues from four species in a mixed gas environment showed no definite pathologic alteration which could be attributed to the experimental conditions. Both experimental and control groups of monkeys and mice showed a similar incidence of endemic disease.

Rats from both experimental and control groups showed advanced chronic murine pneumonia. In most animals there was interstitial as well as peribronchial disease. Three of the seven exposed rats that died showed either bronchiectasis, acute bronchopneumonia, hemorrhage, or edema. These not infrequently occur as complications of chronic murine pneumonia. Therefore, it is difficult to interpret these findings. In evaluating the results of an experiment of several months duration there is always the possibility that the environment enhances the severity of naturally occurring endemic disease. This possibility cannot be excluded in rats.

One exposed dog showed a somewhat more marked chronic bronchiolitis than was noted in the control group. The lungs from the other seven exposed dogs appeared similar to the control dogs. Also three exposed dogs showed a mild

periportal chronic hepatitis. Some of the dogs in both groups had mild focal chronic pyelonephritis or cystitis. These are frequently encountered in dogs. No conclusions can be drawn from these findings. It is of interest that serum protein alterations were noted in some of the exposed dogs. This is more fully discussed elsewhere in the Conference Proceedings.

SUMMARY

Four species: dogs, monkeys, rats, and mice were exposed to a mixed gas environment for 240 days. Exposed rats showed an increased mortality as compared with the control group and pulmonary pathology was greater in three exposed rats. The type of disease present occurs endemically in this species. That the environment enhanced naturally occurring disease in rats cannot be excluded; however, on the basis of this experiment and previous experiments in which endemic disease has been prevalent, no conclusions should be drawn. No lesion was noted in the other three species which could be related to the experimental conditions.

DISCUSSION

COL. TOWNSEND: That brings out one of the old axioms of toxicology, that no clinical syndrome due to poisoning exists that cannot be mimicked by natural disease.

DR. THOMAS (Aerospace Medical Research Laboratories): Dr. Patrick, isn't it true, if I recall, that with the 5 PSI, 100% oxygen, we decided that the dog, which was the most sensitive species, had a definite destructive pulmonary lesion due to the oxygen? I think we said that 5 PSI oxygen was definitely toxic to the dog.

DR. PATRICK: That's right. Yes.

DR. THOMAS: These dog lungs you reported on were in pretty good shape?

DR. PATRICK: I intended to say this; I don't recall whether I did. Dogs carry a high load of chronic lung disease, usually, but this group of dogs was one of the best groups, both in the control and the exposed, that we have ever had, so we lucked out this time on the dogs. I see no change in dogs.

DR. THOMAS: Can we infer that since the dog is the most sensitive species as far as oxygen is concerned that we certainly don't see any damage due to oxygen in this study?

DR. PATRICK: I think that is right. That is a legitimate proposition.

DIFFERING EFFECTS OF IN VIVO HYPEROXIA ON ERYTHROCYTES

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The use of oxygen under high pressure (OHP) for medical and surgical purposes (Bean, 1945; Smith, 1964) and 100% oxygen for space cabin atmospheres (Roth, 1963) have stimulated renewed interest in, and provided a unique opportunity for study of, the biochemical and functional changes that occur during exposure of animals or humans to high oxygen environments. The possibility occurred to us that a hyperoxic environment might enhance oxidation of compounds in excess of that which would occur under normal physiologic conditions. A specific consideration was the possible in vivo peroxidation of unsaturated fatty acids.

Unsaturated fatty acids readily autoxidize in vitro to form lipid peroxides (Bieri and Anderson, 1960; Smith and Dunkley, 1962). The reaction takes place nonenzymatically in the presence of oxygen and ferrous ions. Studies in this laboratory and in others have linked in vitro peroxidation of erythrocyte lipid and hemolysis (Tsen and Collier, 1960; Kann, et al, 1964). Some observations had suggested that oxidation of unsaturated fatty acids might occur in vivo (Tsen and Collier, 1960; Taylor, 1958; Horgan and Philpot, 1954). However, the occurrence of lipid peroxidation in vivo had not been unequivocally demonstrated, and, therefore, its biologic significance was not established.

Previous studies carried out in this laboratory (Mengel, et al, 1964; Mengel, et al, Proc. Soc. Exp. Biol., 1964) suggested that hemolysis occurring in mice exposed to OHP resulted from peroxidation of erythrocyte lipid. Evidence suggesting that a similar hemolytic mechanism could occur in humans was obtained from our findings in a patient who developed hemolytic anemia after a brief period of exposure to OHP (Mengel, et al, 1965). The studies reported here demonstrate (1) in vivo peroxidation of RBC lipid, (2) its occurrence prior to RBC damage and lysis, and (3) other changes occurring as a result of O_2 per se rather than an effect of lipid peroxidation.

IN VIVO PEROXIDATION OF LIPID

Methods

Mice

Male and female strain DBA/2 mice (six to nine months old, average weight 25 g) were used in all experiments. For each experiment 20 mice of comparable age, sex, and weight were exposed to hyperoxia. Ten of these were taken from a group of mice that had been maintained on a tocopherol-deficient test diet for a minimal period of six weeks. The other 10, which had been fed a standard chow preparation, were each injected intraperitoneally with 0.5 mg of alpha-tocopherol acetate 0.5, three or 18 hours before OHP exposure. In each experiment an equal number of control mice of comparable age, sex, and dietary status but without exposure to hyperoxia were studied. The weight of mice in each study group did not differ appreciably.

Exposure to hyperoxia

Mice were placed in metal cages that had been coated with a saline-glycerine solution (fire safety precaution) and which contained no food, water, or combustible material. The test cages were placed in a hyperbaric chamber. This chamber had a volume of 12 cubic feet and provided constant circulation of the gaseous environment with continual flushing by 100% oxygen and absorption of expired CO_2. Chamber pressure was brought to 60 pounds per square inch absolute pressure with 100% oxygen over a period of five to 10 minutes and was maintained for 1.5 hours. No CO_2 could be demonstrated at several intervals tested with a micro-Scholander gas analyzer. Slow stepwise decompression was carried out over 20 minutes. Within one hour after removal from the chamber, each mouse was exsanguinated by cutting exposed axillary blood vessels. This method (one of several tested) enabled us to obtain the greatest volume of blood (average, 1 ml) per mouse. Blood was collected in heparinized pipettes and immediately cooled to 4 C.

Routine and special hematologic studies

Microhematocrits, reticulocyte counts, and Heinz body preparations were performed on individual and pooled blood samples (Cartwright, 1958). Plasma was examined for evidence of gross hemoglobinemia.

Lipid peroxide determinations

Lipid peroxides in erythrocytes were determined by measuring the pink chromogen (absorbance maximum 535 mμ) formed by the reaction of 2-thiobarbituric acid (TBA) with a breakdown product of lipid peroxides, malonylaldehyde (Hochstein and Ernster, 1964; Sinnhuber and Yu, 1958). Erythrocytes from mice in each study group were washed twice in physiologic saline. Then 0.16 or 0.2 ml portions of washed erythrocytes were mixed well with 1.5 ml 10% trichloracetic acid. The mixture was filtered through Whatman No. 1 paper. Thiobarbituric acid (0.67% in water) was added to portions of the filtrate (usually 0.6 or 0.8 ml) in a ratio of 1.2 to 1. The mixture was heated in a boiling water bath for 15 minutes, then cooled to room temperature. Absorption spectra were taken and the absorbance at 525 mμ recorded.

Lipid peroxide levels in plasma were determined by combining 1 ml of plasma with 1 ml of 10% trichloroacetic acid and filtering the mixture through Whatman No. 1 paper. One ml of the filtrate was mixed with 2 ml of the thiobarbituric acid solution and lipid peroxides were determined as described in the preceding paragraph.

Since it has not been possible to prepare a standard solution of unsaturated fatty acid peroxides, a standard absorption curve for malonylaldehyde was prepared using 1, 1, 3, 3-tetraethoxypropane (TEP), a compound that hydrolyzes to one mole of malonylaldehyde and four moles of ethanol (Sinnhuber and Yu, Food Technology, 1958). With this curve as a standard an absorbance of 0.1 in the TBA reaction was calculated to be equivalent to eight μ moles of malonylaldehyde. Although many other aldehydes and ketones give some color with the TBA reagent, they fade rapidly and have different absorption maximums or low extinction coefficients (Hunter, et al, 1963).

Saturated fatty acids do not peroxidize. Malonylaldehyde is derived primarily from those unsaturated fatty acids which contain three or four unsaturated bonds, such as arachidonate and linolenate (Dahle, et al, 1962). Since these represent only a fraction of the total unsaturated fatty acids in naturally occurring lipids, this method measures only a portion of the total peroxidized unsaturated fatty acids. For example, Hochstein and Ernster found that malonylaldehyde levels accounted for only approximately 5% of the total oxygen consumed during peroxidation of lipids in rat liver microsomes (Hochstein and Ernster, 1964). Most investigators agree, however, that this method may be used as a measure of lipid peroxidation (Wills, 1964; Wilbur, et al, 1949).

Results

The effect of in vivo OHP on mouse red cells is shown in table I. Before OHP exposure no significant differences of hematologic indexes were noted between tocopherol-deficient and tocopherol-supplemented mice. Mice that had been maintained on the tocopherol-deficient diet for four months had normal hematocrits and showed no evidence of hemolysis before OHP exposure. During OHP, hemolysis (fall of hematocrit and marked hemoglobinemia) occurred in tocopherol-deficient mice. Whereas hematocrit values varied among individual mice, each tocopherol-deficient mouse exposed to OHP showed clear-cut evidence of hemolytic anemia. No evidence of hemolysis during OHP was noted in mice supplemented with tocopherol 0.5, three or eight hours before exposure to OHP. Neither sex nor age (in the range studied, six to 10 months) affected lytic sensitivity to OHP. When blood of individual mice was studied, no correlation was noted between central nervous system manifestations and severity of the hemolysis in the tocopherol-deficient group.

Red cells in Wright's-stained blood films showed moderate size and shape variations with some fragmentation of cells only in tocopherol-deficient mice exposed to OHP. No significant numbers of spherocytes were seen in any of the blood films.

Lipid peroxides were present in erythrocytes obtained from tocopherol-deficient mice immediately after exposure to OHP. None were found in erythrocytes from tocopherol-deficient mice not exposed to OHP. No lipid peroxides were

TABLE I

HEMATOLOGIC VALUES AND LIPID PEROXIDE LEVELS IN ERYTHROCYTES FROM MICE*

Study Group	Hematocrit %	Percent Reticulocytes	Appearance of Plasma	Lipid Peroxides
Tocopherol-deficient (14)	45-50	0.2-1.5	Normal	0
Chow-fed, tocopherol-supplemented (14)	44-51	0.2-1.6	Normal	0
Tocopherol-deficient + OHP (14)	14-24	9-16	Bright red	36-50
Chow-fed, tocopherol-supplemented + OHP (14)	47-50	0.2-1.4	Normal	0
Tocopherol-deficient, tocopherol-supplemented + OHP (5)	46-49	0.1-1.2	Normal	0

*Values were obtained from pooled blood samples of 10 mice in each experimental group.

The number after each group indicates the number of experiments; + OHP designates those mice exposed to oxygen under high pressure.

Millimicromoles malonylaldehyde per milliliter erythrocytes

detected in erythrocytes of tocopherol-supplemented mice either before or after OHP. Plasma of tocopherol-deficient mice exposed to OHP contained only trace amounts of lipid peroxides.

Further studies were carried out to determine whether the lipid peroxides found in erythrocytes of tocopherol-deficient mice exposed to OHP had been formed in vivo during exposure of the mice to OHP, or in vitro as the erythrocytes were manipulated in the presence of atmospheric oxygen. Erythrocytes of tocopherol-deficient mice formed large quantities of lipid peroxides in vitro and were lysed when exposed to (1) bubbled oxygen at 37° for six to 12 hours, (2) 100% oxygen at 60 pounds per square inch absolute pressure at 37 C for one and 12 hours, (3) 0.1% hydrogen peroxide at 37 C for three hours, or (4) ultraviolet radiation (42 cm below two Westinghouse Sterilamps G1 T8 in round bottom quartz flasks for six hours at 25 C.

To determine the effect of prior in vivo tocopherol-deficient mice were each given 0.5 mg of alpha-tocopherol acetate intraperitoneally one hour before bleeding. Blood was collected in pipettes that had been rinsed with physiologic saline containing alpha-tocopherol emulsified in Tween-80 and physiologic saline (0.5 mg per ml),

and all subsequent steps of the TBA test were performed with solutions containing alpha-tocopherol (0.5 mg per ml). When these red cells were subjected to the oxidant stresses listed above, each of which is capable of peroxidizing lipid, no significant lysis or lipid peroxidation occurred.

Since tocopherol as we used it had prevented in vitro lipid peroxidation by these agents, we reasoned that it should also prevent any in vitro peroxidation of lipid by atmospheric oxygen in erythrocytes of mice exposed to OHP. Accordingly, tocopherol-deficient mice were exposed to OHP in the routine manner and given 0.5 mg ip of alpha-tocopherol immediately after decompression. One hour later blood was collected in tocopherol-rinsed pipettes, and lipid peroxides were determined by using solutions containing alpha-tocopherol and saline mixtures as described above. As shown in table II, there were no differences in lipid peroxide levels between the tocopherol-deficient mice that were bled immediately after OHP and those which were given the tocopherol after OHP but before bleeding. No additional lipid peroxide formation occurred when erythrocytes of these animals were subsequently exposed to H_2O_2, oxygen, and ultraviolet radiation. Thus, the lipid peroxides found in erythrocyte of tocopherol-deficient mice exposed to OHP must have been formed in vivo.

TABLE II

LIPID PEROXIDE LEVELS IN MOUSE ERYTHROCYTES*

Mouse Study Group	Lipid Peroxides
Tocopherol-deficient	0
Tocopherol-deficient + OHP	32-39
Tocopherol-deficient + OHP (given tocopherol before bleeding)	34-38
Tocopherol-deficient + OHP (given tocopherol before bleeding, blood collected and washed in tocopherol-saline mixture)	32-41
Tocopherol-supplemented (before and after OHP)	0

*Values were obtained on pooled blood of 10 mice in each experimental group, and the range of three separate experiments is given.

Millimicromoles malonylaldehyde per milliliter erythrocytes

SEQUENCE OF EVENTS AFTER PEROXIDATION OF RBC LIPID

Methods

Mice

Mice similar to the previous study were used.

Exposure to hyperoxia

Exposure to hyperbaric oxygen was carried out in a cylindrical hyperbaric chamber six inches in internal diameter, 16 inches long, with a total volume of 450 cubic inches. In each experiment 10 tocopherol-deficient mice were placed directly into the chamber, which was then flushed at normal atmospheric pressure with 100% oxygen for five minutes. Compression with 100% oxygen to 60 pounds per square inch, absolute (four atmospheres), was carried out over a period of 15 minutes, and pressure was maintained for 60 minutes. Continual circulation of the gaseous environment was maintained throughout exposure by a constant influx of pure 100% oxygen, and by constant efflux at a rate of 10 liters per minute. Fifteen minutes were allowed for slow, stepwise decompression. Total exposure time to 100% oxygen was 95 minutes.

Studies of red cells

Blood was obtained from ether-anesthetized mice, immediately after exposure and at regular timed intervals thereafter, by severing axillary vessels which had been surgically exposed. Hematocrits, reticulocyte counts, Wright's-stained blood films and Heinz body preparations were performed on blood collected in microhematocrit tubes from individual mice. Red cell counts were performed using a Model F-Coulter Counter.

Osmotic fragility of red cells was tested using the Osmotic Fragiligraph* as described by Danon (1963). Determinations were carried out within five minutes after the blood was obtained. Cumulative and derivative curves were inscribed in each study and values expressed as the salt concentrations at which 50% hemolysis occurred.

RBC lipid peroxides were determined on heparinized blood pooled from two mice as noted previously. Lipid peroxide determinations were performed on specimens of plasma and urine by the same method, using the same volumes.

Results

The course of hemolysis after exposure of the tocopherol-deficient mice to OHP is illustrated in figure 1. Immediately after exposure there was no evidence of gross hemolysis and the hematocrit was slightly higher ($P < .05$) than it had been prior to OHP. Ten minutes after exposure, while the mice were maintained at normal atmospheric conditions, hemolysis began, indicated by the appearance of visible hemoglobin in plasma and by a slight fall in hematocrit ($P < .025$). Thirty minutes after exposure,

*Kalmedic Instruments, New York, New York

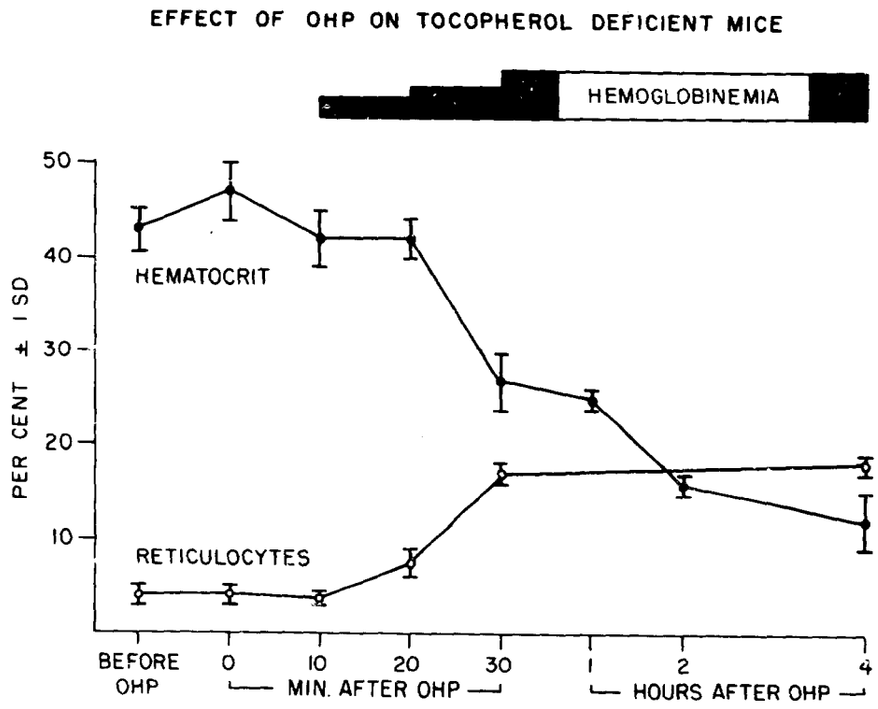


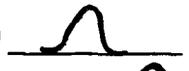
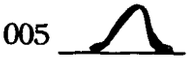
Figure 1. HEMATOLOGIC EFFECT OF HYPERBARIC OXYGEN ON TOCOPHEROL-DEFICIENT MICE. Each point represents the mean of 4 to 10 determinations (each in duplicate) carried out on individual mice. The bars above and below each point indicate ± one standard deviation.
Note that hemolysis began after exposure to hyperoxia, while the mice remained at normal atmospheric conditions.

the hematocrit had fallen to 30%, plasma was bright red, and hemoglobinuria was observed. Hemolysis was progressive, with the hematocrit falling to 12% four hours after OHP, and all animals died five to six hours after OHP. During the hemolytic phase reticulocytes increased to a high of 18%.

The concomitant changes of erythrocyte morphology and osmotic fragility are summarized in table III. Immediately after OHP, red cell morphology appeared normal, the MCV was not significantly altered, but there was an increase of osmotic fragility. Thereafter a significant increase of MCV and an increasing number of spherocytes in blood films was accompanied by an additional increase in cumulative osmotic fragility, apparently due to a distinct population of osmotically fragile red cells as identified by the derivative curve inscribed by the Fragiligraph. During the phase of most rapid hemolysis (from 20 minutes to two hours post-OHP) large numbers of ghosted and/or fragmented red cells were observed in blood films. Disappearance of significant numbers of spherocytes (i. e. at two hours post-OHP) was accompanied by a decrease of MCV and a return of cumulative osmotic fragility to normal with a single population of cells as determined by the derivative curve. No methemoglobin and no Heinz bodies were present in RBCs of these mice before OHP, or at any time after exposure to OHP.

TABLE III

CHANGES IN ERYTHROCYTES AFTER EXPOSURE TO OHP

Time	RBC Morphology	MCV	Osmotic Fragility
Before OHP	Normal	45 ± 3	.43 ± 0 
After OHP			
Immediately	Unchanged	46 ± 1	.47 ± 0 
10 min.	Moderate size and shape variation 20-30% spherocytes	55 ± 2	.495 ± .005 
20 min.	Same as 10 min.		.50 ± 0 
30 min.	60-70% spherocytes Moderate no. of ghosts and fragments, moderate polychromasia	52 ± 3	.50 ± .008
1 hour	Same as 30 minutes		.50 ± .005 
2 hours	Many ghosts and fragments Few spherocytes Moderate polychromasia	43 ± 4	.42 ± .005 
4 hours	Almost no ghosts Moderate size and shape variation Marked polychromasia	44 ± 2	.38 ± 0 

The relationship between lipid peroxides and lysis is shown in figure 2. Before exposure neither RBCs nor plasma contained measurable levels of lipid peroxides. Immediately after exposure and before hemolysis began, relatively large quantities of lipid peroxides were found in red cells but not in plasma. At 10 minutes after OHP, when hemolysis apparently began, there was a significant decrease in red cell lipid peroxide levels which occurred concomitantly with the initial appearance of lipid peroxides in plasma. Thereafter there was a progressive decrease of both red cell and plasma lipid peroxide levels. Four hours after exposure to OHP, neither RBCs nor plasma contained detectable amounts of lipid peroxides.

One hour after exposure, urine contained a substance which on acid hydrolysis and heating with 2-thiobarbituric acid formed a pink pigment which absorbed maximally at 535mμ, and was, presumably, malonylaldehyde.

EFFECT OF HYPEROXIA ON RED CELLS OF NORMAL MICE

Methods

Mice

Male and female strain DBA/2 mice (all between four and six months of age) were maintained on standard chow diets. Mice of the same age, sex, and average weight were used in each study. For special studies some mice received 0.5 mg alpha-tocopherol acetate each, given daily for five days prior to study.

Exposure to hyperoxia

Groups of 10 mice were exposed to 100% oxygen at pressures of 60 psia (lb./sq. in. absolute) for 90 minutes. The chamber used had a volume of 424 cubic inches. The chamber was first flushed for five minutes at 15 psia and the pressure was gradually increased to the desired level over a 15-minute period. Stepwise decompression was completed in 15 minutes. For studies involving larger numbers of mice, a larger chamber was used. This chamber had a volume of 12.56 cubic feet.

Studies of blood

All mice were exsanguinated by severing the right axillary vessels. For most studies blood was collected immediately using heparinized pipettes and the sample tubes were kept at 4 C. Subsequent studies were carried out on individual or pooled blood samples. Hematocrits, hemoglobins, reticulocyte counts, and osmotic fragilities were determined by standard methods.

Erythrocyte glycolytic intermediates

These were determined by modifications of the methods described by Shafer and Bartlett (1962) and Bartlett (1959). Fifty ml of samples of pooled heparinized blood from 100 mice were used for each study. Erythrocytes were washed three times with cold physiologic saline. Packed erythrocytes (12.5 ml) were added with constant vigorous mixing to two volumes of 10% trichloroacetic acid (TCA). After centrifugation and filtration of the supernate the precipitate was reextracted with two volumes of 5% TCA. After combining extracts, TCA was removed with four extractions of two volumes of ether. The ether was then removed by bubbling nitrogen

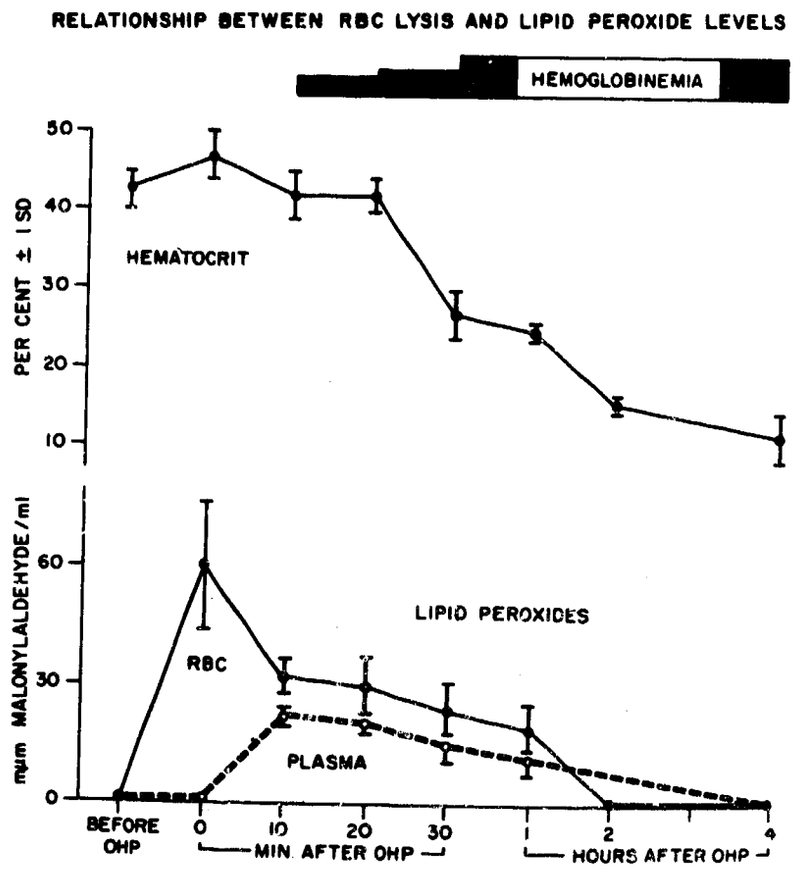


Figure 2. RELATIONSHIP BETWEEN LIPID PEROXIDE LEVELS AND LYSIS. The hematocrit values are those of figure 2. For lipid peroxide levels the points represent mean values of from 2 to 8 separate determinations (each in duplicate) using pooled blood of two mice and the bars indicate ± one standard deviation.

through the solutions. After neutralization, extracts were passed through a 1 x 20 column of Dowex-1 x 8-formate resin. The columns were then eluted with a linearly increasing concentration of 0 to 3.5 N ammonium formate buffer, pH 3.0 at a rate of 3-4 ml/min. Fractions were collected in 10 ml volumes and each fraction was analyzed for total phosphorus content. In addition, compounds were identified by elution position as determined by standard applications previously performed in this laboratory and by specific analytic methods (Bartlett, Colorimetric Assay Methods for Free and Phosphorylated Glyceric Acids, 1959; Bartlett, Phosphorus Assay in Column Chromatography, 1959). Quantities of compounds determined in this system were expressed as micromoles phosphorus/ml of erythrocytes.

Erythrocyte adenosine triphosphate (ATP)

ATP determinations were made by a modification of the method of Beutler and Baluda utilizing the firefly-luminescence method (1964). All chemicals and reagents were prepared exactly as described by them. Blood taken from individual animals (0.8 ml) was mixed immediately with 0.2 ml cold ACD and determinations were carried out within 10 minutes. One-tenth ml of blood (diluted 4:1 V/v with ACD) was added to 2.9 ml of iced tris-borate buffer in a graduated 15 ml test tube. The hemolysate was immediately placed in a boiling water bath for five minutes and then transferred to an ice bath after restoring the volume to 3 ml with distilled water. Two-tenths ml of hemolysate were added to 2 ml of ice-cold diluted firefly extract and the tube was inverted five times. After exactly one minute, the emitted fluorescence was read at 560mμ. The ATP content was determined by reference to a standard curve and was expressed as μ moles/Gm of hemoglobin.

Adenosine diphosphate (ADP) and adenosine monophosphate (AMP)

These determinations were carried out according to the methods of Lipmann (1941) and Bucher (1953), using DPN-DPNH linked changes in forward and backward reactions between phosphoenolpyruvate and lactate.

Splenectomies were carried out under ether anesthesia through a left upper quadrant abdominal incision.

The animals received tetracycline-HCl (.02 ml/Gm) daily, postoperatively for five days. All studies of these animals were carried out two weeks after splenectomy.

Results

In control animals exposed to OHP there were increases in hematocrit and osmotic fragility (table IV). These effects were not eliminated by splenectomy or prior tocopherol supplementation. In fact, splenectomized mice had an increase of MCV and a greater percent increase of hematocrit. The effect of OHP on RBC, ATP, and ADP is shown in table V. ATP content was consistently and significantly increased while ADP content was concomitantly decreased.

The effect of 100 percent oxygen at 60 psia for one hour on phosphorylated erythrocyte glycolytic intermediates of chow-fed mice is shown in table VI. No evidence of hemolysis was noted. As determined in this laboratory by the chromatographic method cited, levels of phosphate compounds in mouse erythrocytes were the

TABLE IV

EFFECT OF IN VIVO HYPEROXIA ON CHOW-FED MICE

Study Group	Hct. %	Retics. %	Osmotic Fragility	MCV μ^3
Control	44 ± 2	3.0	.46 ± .01	46 ± 4
Control + OHP	50 ± 3	3.3	.49 ± .01	47 ± 4
Splenectomy	41 ± 3	3.2	.45 ± .01	46 ± 4
Splenectomy + OHP	49 ± 4	3.4	.49 ± .03	51 ± 4
Tocopherol Supplemented + OHP	50 ± 2	3.1	.49 ± .02	---

TABLE V

EFFECT OF OHP ON RED CELL ATP AND ADP

Study Group	ATP μM/gm Hgb.	ADP μM/gm Hgb.
Control	5.3 ± .3	.37 ± .05
Control + OHP	7.3 ± .4	.19 ± .06

Values represent means ± 1 SD

same in two different groups of normal mice. The most significant variations from normal in the mice exposed to OHP were increases in erythrocyte ATP and hexose monophosphate compounds, with concomitant decreases in levels of fructose-1, 6-diphosphate, the triosephosphates, and triphosphopyridine nucleotide (TPN). The remainder of the measured compounds were not significantly altered.

These observations suggested a block between fructose-6-phosphate and fructose-1, 6-diphosphate, a step mediated by the enzyme phosphofructokinase. The effect of OHP on RBC phosphofructokinase activity is shown in table VII. Fifty percent decreases of enzyme activity were observed after OHP.

DISCUSSION

Although the clinical and histopathologic features of oxygen toxicity have been described in detail, the primary mechanism of cell damage by high oxygen tensions has not been elucidated. Aside from studies relating to erythropoiesis, the in vivo effect of increased oxygen tension on erythrocytes has received little attention. Its

TABLE VI

CHANGES OF PHOSPHATE COMPOUNDS OF ERYTHROCYTES IN CHOW-FED MICE EXPOSED TO 100 PERCENT OXYGEN AT 60 PSIA FOR ONE HOUR

Compound	Normal Mice #1	Normal Mice #2	OHP Mice	Percent*
Inorganic phosphorus (P)	.14	.11	.12	96
AMP	Trace	Trace	Trace	--
Adenosine diphosphate (ADP)	.34	.32	.32	99
Adenosine triphosphate (ATP)	.24	.21	.60	270
Diphosphopyridine nucleotide (DPN)	.24	.24	.22	92
Triphosphopyridine nucleotide (TPN)	.24	.26	.13	52
Fructose, 1-6, diphosphate	1.28	1.27	.72	56
Diphosphoglyceric acid	7.3	7.9	8.7	115
Triose phosphates	.21	.22	.10	47
Hexose mono-phosphates	.24	.26	.80	320

Quantities of metabolites are expressed as μ moles phosphorus/ml of erythrocytes. Data of normal mice represent levels in pooled blood of two separate groups of mice. OHP mice denotes blood from those mice exposed to hyperoxia.

*Concentration of mice exposed to OHP divided by concentration of normal control mice.

TABLE VII

EFFECT OF HYPEROXIA ON RBC PHOSPHOFRUCTOKINASE

Study Group	Enzyme Activity Eu/ul cells/minute
Control	22.9 ± 2.0
Control + OHP	12.2 ± 1.2

relevance to human clinical situations had not been considered until recently when several volunteers maintained in simulated space capsule environments (100% O_2 at low atmospheric pressures) developed evidences of red cell damage (increased osmotic fragility) and hemolysis (fall of hemoglobin and evaluation of reticulocytes and indirect-reacting bilirubin), and one patient developed hemolytic anemia after a brief exposure to OHP. The latter patient's red cells were similar to those of tocopherol-deficient mice with regard to increased lytic sensitivity to H_2O_2, increased lipid peroxide formation by H_2O_2, and their in vivo sensitivity to hyperoxia. Whether his susceptibility reflected a tocopherol-deficient state or some alteration of fatty acid content in his erythrocytes was not decided. Many other studies were helpful only in ruling out various possibilities.

Work in other laboratories and our own studies had linked high oxygen tensions, erythrocyte lysis, and lipid peroxidation in vitro, and previous studies in this laboratory had suggested their relationship in vivo. The present studies established the fact that peroxidation of erythrocyte lipid can occur in vivo during exposure to oxygen under high pressure. These effects were noted only in mice fed a tocopherol-deficient diet.

A frequent criticism of earlier studies of lipid peroxidation had been the failure to consider the ability of atmospheric oxygen to peroxidize unsaturated fatty acids in vitro. Since determinations of lipid peroxide content always involved manipulations during which tissue was exposed to atmospheric oxygen, it always seemed possible that any lipid peroxides found could have been formed in vitro. Our observation that levels of erythrocyte lipid peroxides were not decreased when alpha tocopherol was administered after OHP, but before exsanguination (a maneuver we proved effective in preventing in vitro lipid peroxidation), established their formation in vivo. They were not formed as a result of hemolysis, since lipid peroxides were demonstrated in remaining intact erythrocytes.

Subsequent results of this investigation have helped to clarify the relationship between peroxidation of RBC lipid and hemolysis in tocopherol-deficient mice exposed to OHP. Proof that peroxidation of RBC lipid preceded hemolysis came from the finding that RBCs contained large quantities of lipid peroxides immediately after exposure to OHP, before hemolysis began. That the onset of overt hemolysis (first discernible hemoglobinemia) and the attendant initial decrease in RBC lipid peroxide content coincided with the first appearance of lipid peroxides in plasma, suggested that lipid peroxides were released into plasma from damaged RBCs.

The sequence of events in changing red cell morphology and osmotic fragility suggested that a particular population of cells had been damaged, undergone sphering, and were ultimately destroyed. The marked hemoblobinemia and hemoglobinuria and the large numbers of ghosts and red cell fragments indicated that hemolysis was predominantly intravascular.

An unexpected finding in these studies was the speed and magnitude of the reticulocyte response after hemolysis. Morphologically these were classic reticulocytes, most of which appeared to be very primitive. Preliminary morphologic studies of the bone marrow have revealed an obvious increase in the number of erythropoietic elements, maximal at the time of peak reticulocytosis, 30 minutes after exposure to OHP. This finding, also unexpected, is not currently understood and will require additional study.

The finding that hemolysis can be delayed in onset, beginning after exposure to OHP, broadens current concepts of oxygen toxicity. We had previously observed a similar pattern of hemolysis in a human after exposure to OHP, and had postulated that this was a form of delayed oxygen toxicity. Other investigators have also alluded to the possibility that some manifestations of oxygen toxicity might be delayed in onset, beginning after exposure to OHP. However, the current investigation has provided the first direct demonstration of a form of delayed oxygen toxicity.

In contrast to these results, no hemolysis occurred in chow-fed mice (those resistant to peroxidation of lipid). There were changes of RBC membrane (increased MCV and osmotic fragility) and phosphorylated glycolytic intermediates. These changes reflected changes in cells themselves rather than a shift of cell populations. The metabolic effects were shown in part at least to result from inhibition of the RBC enzyme phosphofructokinase.

SUMMARY

1. Hemolytic anemia induced by exposure to OHP is associated with peroxidation of RBC lipid.

2. RBC lipid peroxidation occurs *in vivo* before the onset of hemolysis and is responsible for hemolysis.

3. Hemolysis mediated by peroxidation of RBC lipid can begin after exposure to OHP and progress under normal atmospheric conditions.

4. During OHP, O_2 *per se* increases RBC ATP, hematocrit, and osmotic fragility.

5. The increase of ATP is an effect of cell metabolism, probably via inhibition of phosphofructokinase.

6. Therefore, although O_2 *per se* produced transient metabolic and physical changes in red cells, irreversible damage and lysis only occurred when peroxidation of lipid has taken place.

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DISCUSSION

QUESTION: Did you notice any acanthocytes or membrane defects in the red cell other than spherocytic change?

DR. MENGEL: Every once in awhile we see a cell that appears to have features of an acanthocyte. This is not a dominant - what you have seen appears to be dominant. However, I think it is fair to point out that there is a 10-minute period there which we are currently exploring. That is to say there is that zero to 10-minute period, and in that 10 minutes spheres had already formed. We are concerned with the fact that it is now known that spiculated red cells or acanthocytes with tiny filamentous processes may, in point of fact, be broken in the circulation, leaving a membrane hole which simply fills up with water, and so we are about to embark on a study which will have, instead of zero to four-hour sequence, every 15-second sequence, for instance, during that zero to 10-minute period.

DR. BACK (Aerospace Medical Research Laboratories): Have you run through the gamut yet, going up in pO2 from normal pressure of oxygen up to say 25 millimeter mercury increments, or is this an all or none thing that you've done so far?

DR. MENGEL: The data that I presented to you is clearly all or none. We are in the process of coming back down the scale and are currently down to two atmospheres for 30 minutes in these animals, which will still produce red cell damage and lysis. We hope, of course, to ultimately construct that mythical and magical curve that will relate X species of animal with so much tocopherol catalase on board, so much exposure, where the likelihood of tissue damage will be such and such. This is the area that we are now exploring.

THE EFFECT OF PROTRACTED BREATHING OF A TWO-GAS ATMOSPHERE ON HEPATIC ULTRASTRUCTURE

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INTRODUCTION

The livers of rats, dogs, and monkeys exposed to 100% oxygen at 5 psia for 235 days showed ultrastructural evidence of increased organelle turnover, characterized by residual pericanalicular pigment and autophagic vacuoles containing mitochondria. The minimal degree of hepatocytic alterations suggested they were adaptive in nature and not detrimental to the organism (Klion, et al, 1967). Biochemical data were indicated with normal oxidative phosphorylation (Riesen, 1956). The present study concerns similar electron microscopic studies performed on the livers of rats, purebred beagles, and monkeys exposed to an atmosphere of 70% oxygen and 30% nitrogen at 5 psia for eight months.

MATERIAL AND METHODS

Specimens of liver from rats, dogs, and monkeys were obtained either by needle biopsy or laparotomy after mild Nembutal anesthesia. The tissue was fixed in 1% osmium tetroxide, buffered with veronal acetate or s-collidine, dehydrated, and embedded in Epon 812. Sections were cut with an LKB Ultrotome, stained with lead citrate or lead hydroxide, and examined with a Hitachi HS7 electron microscope. One micron sections, embedded in Epon and stained with PAS and toluidine blue were examined with the light microscope.

RESULTS

Thick sections examined with the light microscope and low power electron micrographs showed the hepatic plates to be intact, without evidence of necrosis. Cells varied in density, reflecting individual differences in hydration and glycogen.

Glycogen was decreased most prominently in the monkeys and rats and to a lesser degree in the dogs. Fat droplets were inconstant in both experimental and control animals and was probably related to dietary intake.

Mitochondria varied in size and shape. However, bizarre forms were unusual and infrequently the mitochondria were clumped and the outer membranes coapted. Mitochondrial cristae were shortened and increased in numbers and lay free within the matrix, at times aligned in parallel (figure 1). Occasionally they traversed the whole diameter. Although control dogs demonstrated variation in mitochondrial size and abnormalities of the cristae, in the animals exposed to the two-gas atmosphere the mitochondria were at times pencil-shaped or enlarged and clumped. In addition, increased amounts of pigment resembling lipofuscin and autophagic vacuoles containing mitochondria in various stages of disruption were found in the pericanalicular areas. Mitochondrial alterations were least in the monkeys. The rough endoplasmic reticulum and number of polyribosomes were normal in all three species. Partly due to glycogen depletion, the smooth endoplasmic reticulum was prominent. However, in the liver of monkeys in whom decreased glycogen was most pronounced there were clumps of hypertrophic, smooth endoplasmic reticulum (figure 2).

Microbodies were increased and were seen near other single membrane enclosed bodies having a dense osmiophilic core, probably lipid. Both were most numerous in the rat (figure 3), and were located predominantly in the pericanalicular area. Autophagic vacuoles, also pericanalicular in distribution, were common and contained mitochondria in various stages of degeneration as well as elements of smooth endoplasmic reticulum and glycogen (figure 4). Macrophages within the sinusoids were enlarged and contained phagocytic material (figure 5).

The endothelial lining was normal and collagen fibers within the Disse space were not increased. Bile canaliculi and ducts showed no abnormalities.

COMMENTS

The livers of animals after prolonged exposure to a two-gas system compared with those after similar exposure to pure oxygen demonstrated a greater degree of glycogen depletion and an increase in the number of microbodies, lipid bodies, and autophagic vacuoles (table I). Data concerning pure oxygen exposure were obtained from previously published material (Klion, et al, 1967; Schaffner, 1966; Schaffner and Felig, 1965; Schaffner, et al, 1966). Except for the greater depletion of glycogen, organelle abnormalities were least in the monkeys. Similar species variations were noted in previous studies. This emphasizes that species sensitivity which must be taken into account when extrapolating observations to man (Schaffner, 1966). Decreased amounts of glycogen within the hepatocytes may reflect stress to the organism under the altered atmosphere. Furthermore, the many autophagic vacuoles containing degenerated mitochondria and fragments of the endoplasmic reticulum suggest increased organelle turnover.

Microbodies were increased in number. In addition to urate oxidase, microbodies contain D amino acid and L hydroxy acid, oxidases, and catalase. The association of oxidases and catalase suggests they are sites of hydrogen peroxide metabolism and prompted the term "peroxisome" (DeDuve and Bauduin, 1966). Although their function is not established, they may participate in glucogenesis and

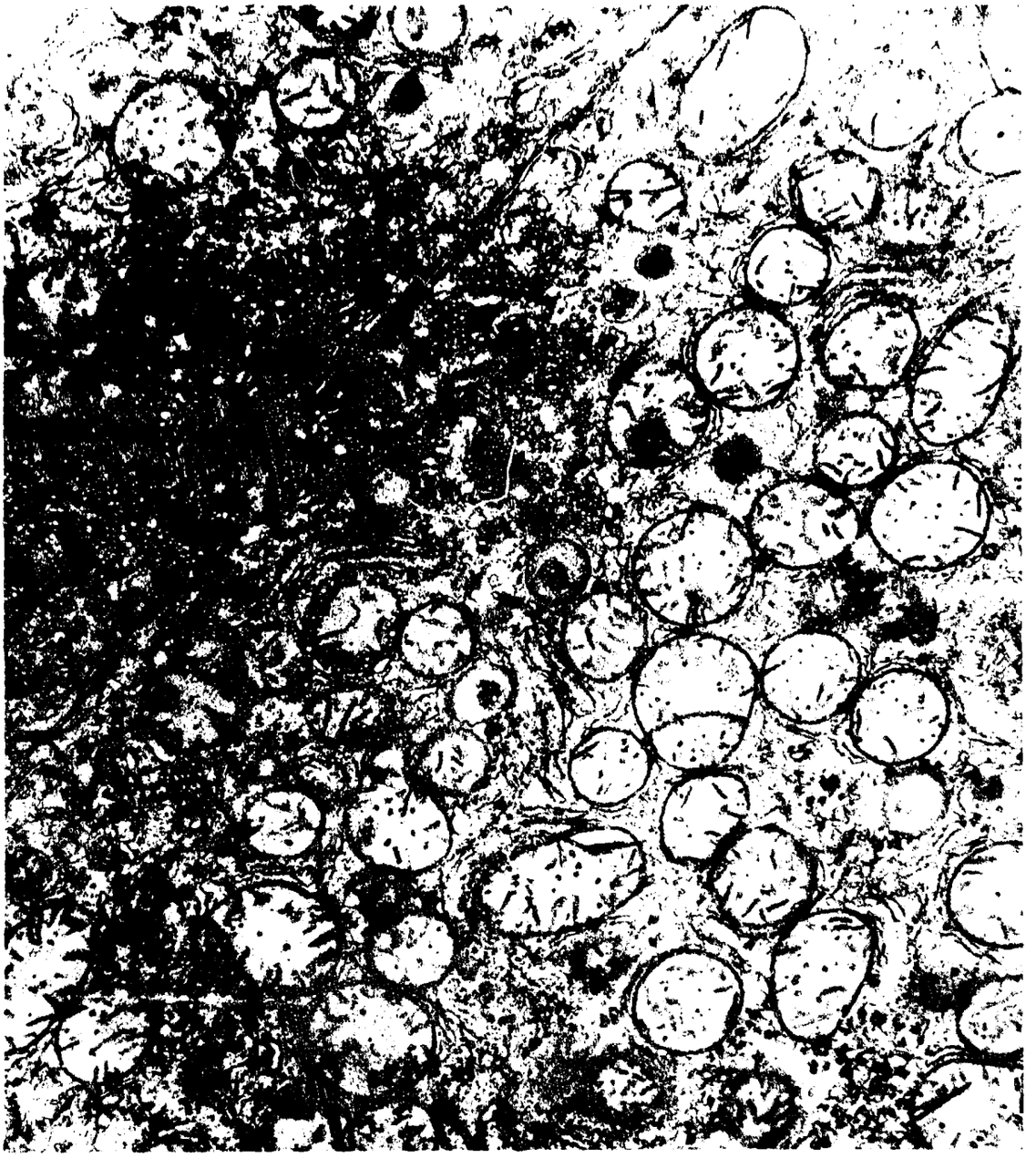

Figure 1. MANY MITOCHONDRIAL CRISTAE IN THE RAT ARE SEPARATED FROM THE INNER MEMBRANE AND LIE FREE WITHIN THE MATRIX. Several are aligned in parallel (X 18, 000).

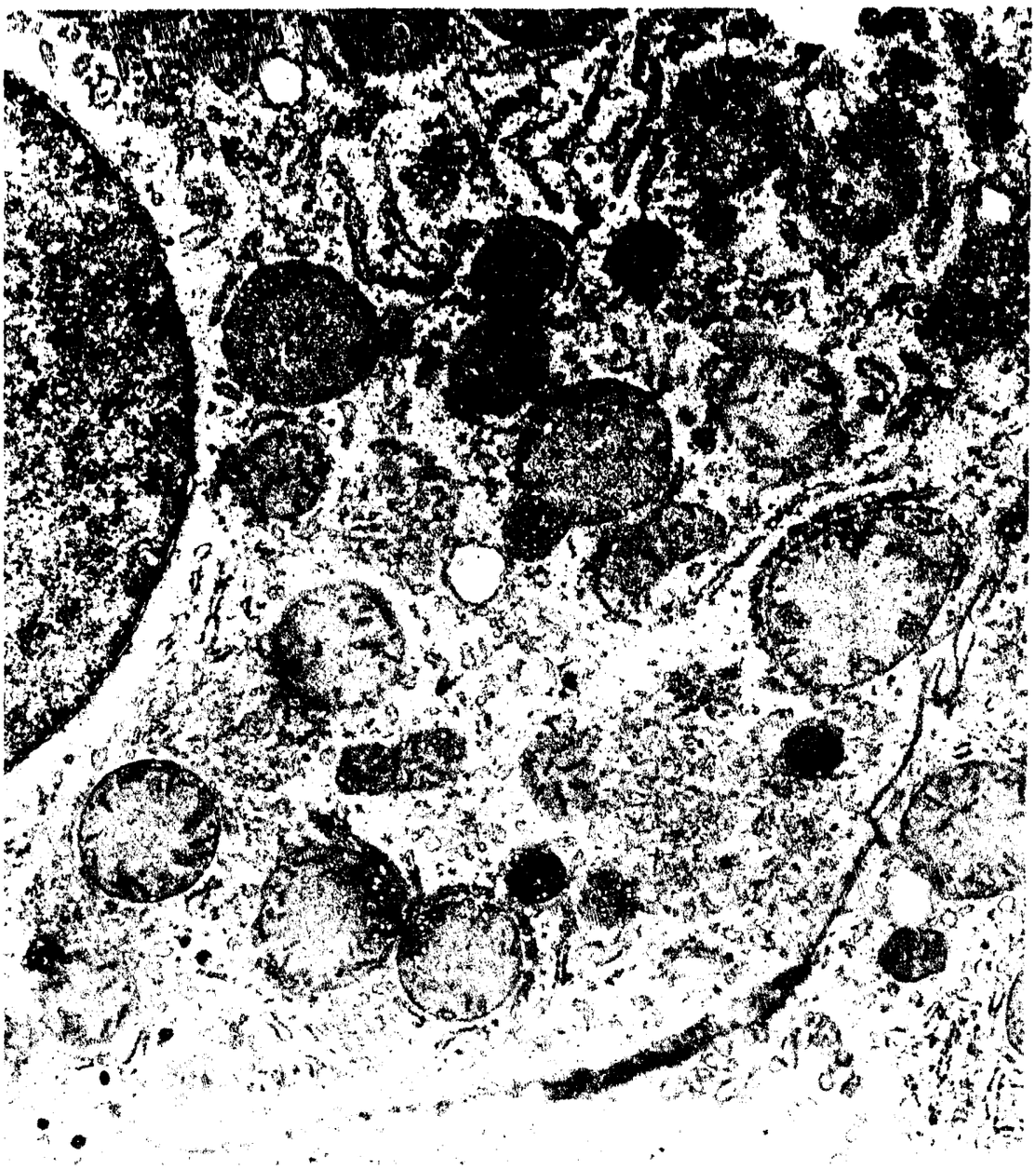

Figure 2. IN MONKEY LIVER, AREAS OF HYPERTROPHIC SMOOTH ENDOPLASMIC RETICULUM ARE PROMINENT. Glycogen is absent. Note also the mitochondrial cristae are shortened and separated from the inner membrane (X 18, 000).

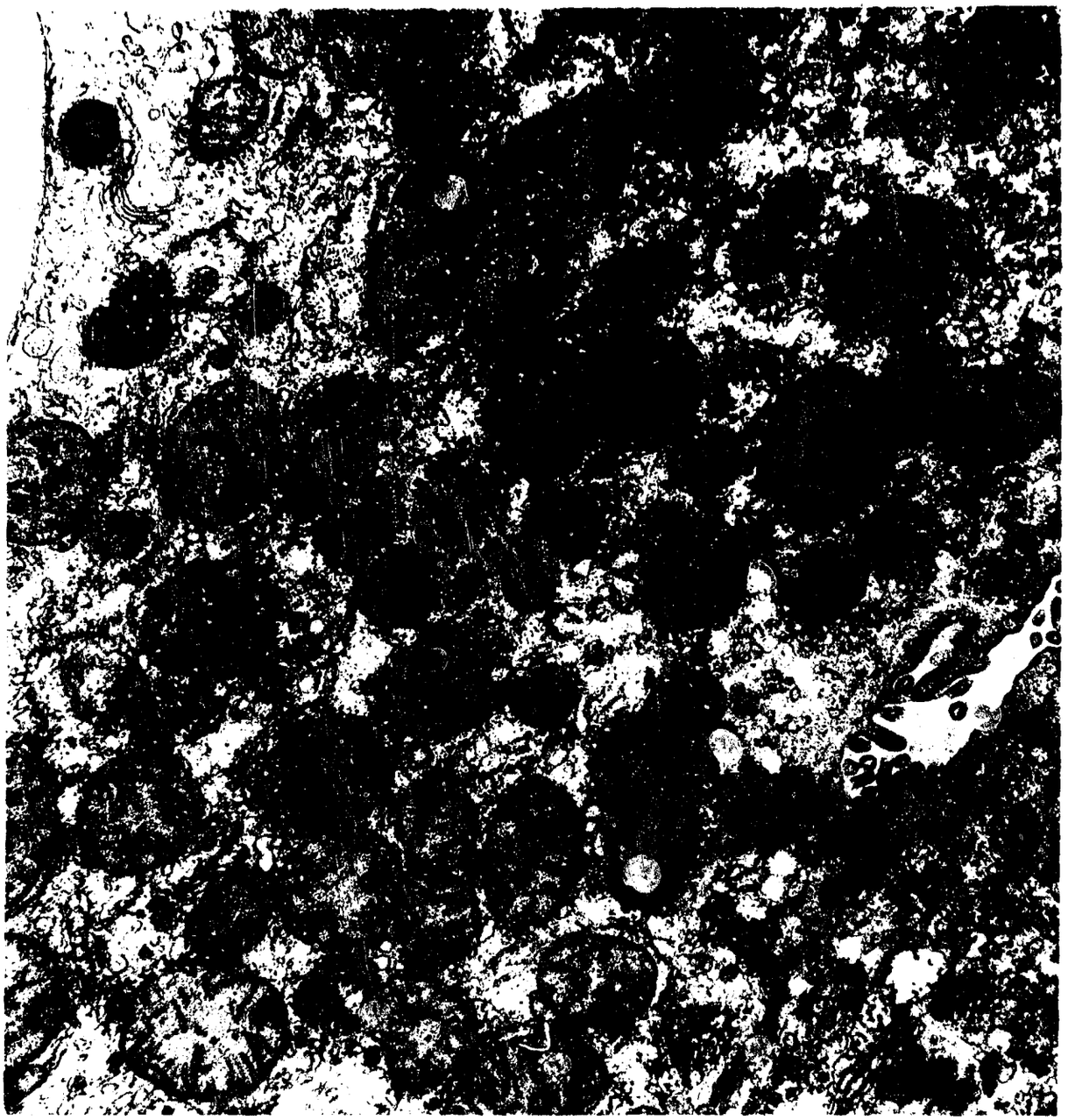

Figure 3. MICROBODIES (M) ARE INCREASED IN THE RAT LIVER AND ARE ADJACENT TO SEVERAL LIPID-CONTAINING BODIES WITH DENSE OSMIOPHILIC CORES (L) (X 25, 000).

Figure 4. AUTOPHAGIC VACUOLES ARE NUMEROUS IN THE PERICANALICULAR ZONE OF THE RAT HEPATOCYTES, AND CONTAIN MITOCHONDRIA IN STAGES OF DEGENERATION IN ADDITION TO SMOOTH ENDOPLASMIC RETICULUM AND GLYCOGEN (X 14, 000).

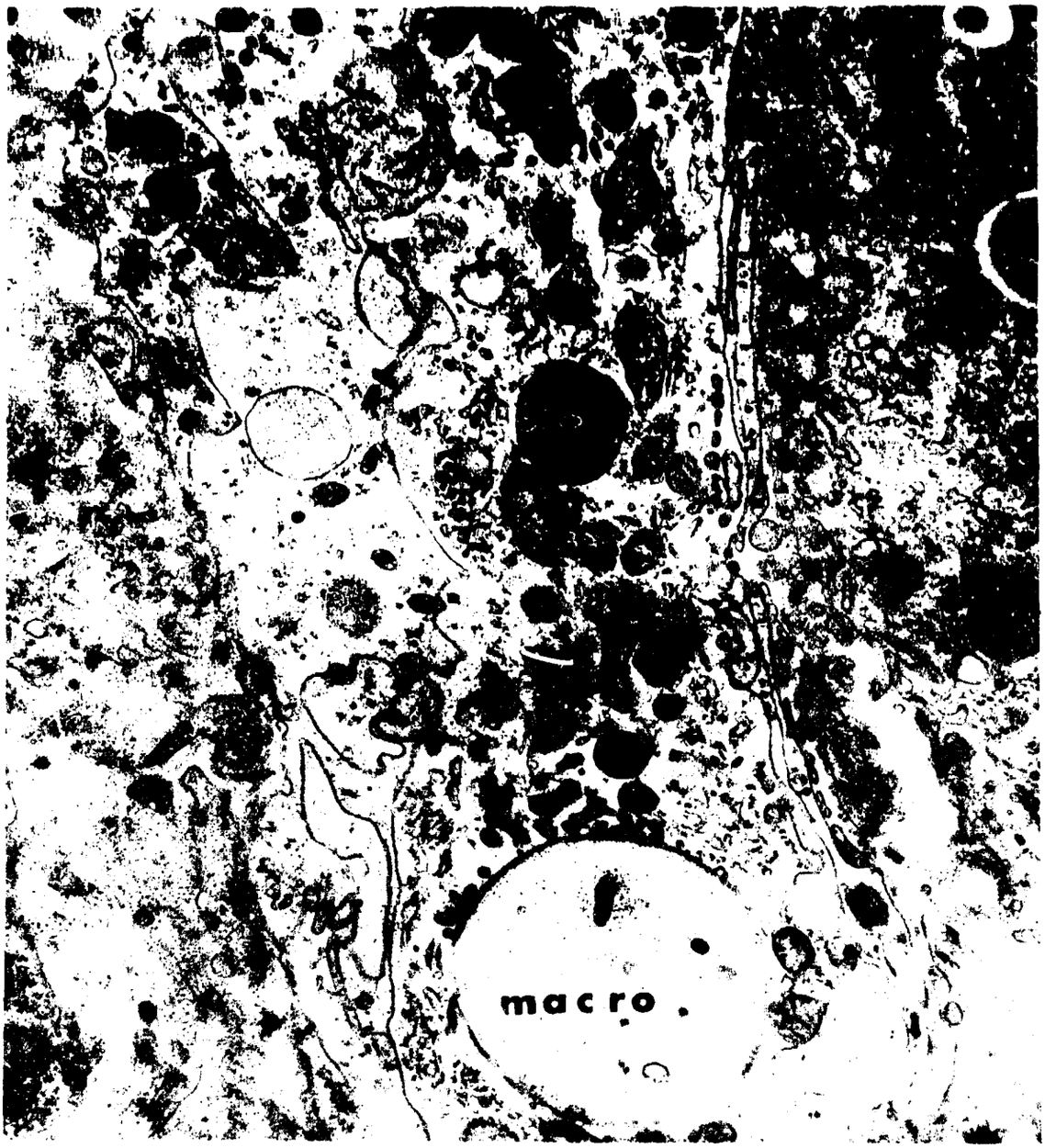

Figure 5. A LARGE MACROPHAGE (KUPFFER CELL) WITHIN A MONKEY LIVER SINUSOID WITH SEVERAL PHAGOSOMES (p) (X 18, 000).

TABLE I

HEPATIC ULTRASTRUCTURE AFTER PROLONGED EXPOSURE AT 258 mm TO:

	100% OXYGEN *	70% OXYGEN 30% NITROGEN
Glycogen	↓	↓↓
Mitochondria		
Variation in size	↑	↑
Variation in shape	↑	↑
Cristae	Normal	↑
Rough Endoplasmic Reticulum	Normal	Normal
Smooth Endoplasmic Reticulum	↑	↑↑
Microbodies	↑	↑↑
Fat-containing Bodies	↑	↑↑
Lysosomes		
Residual Pigment	↑	↑
Autophagic Vacuole	↑	↑↑

*Data was obtained from previous published material (Klion, et al, 1967; Schaffner, 1966; Schaffner and Felig, 1965; Schaffner, et al, 1966).

cellular oxidative processes. Experimentally they increase following administration of chlorophenoxyisobutyrate, a drug which lowers plasma lipids and cholesterol (Svoboda and Azarnoff, 1966). The increased number of microbodies and lipid-containing bodies may be a reflection of altered peroxidation of lipids, and would appear to be a fruitful path for biochemical investigation.

SUMMARY

The livers of rats, dogs, and monkeys exposed to 70% oxygen and 30% nitrogen at 5 psia for eight months showed ultrastructural alterations of the mitochondria, glycogen depletion, and increased numbers of autophagic vacuoles. This suggested increased stress and accelerated organelle turnover which was more prominent than in similar studies performed after 100% oxygen at 5 psia. The increased number of microbodies and lipid-containing vacuoles may represent altered peroxidation.

ACKNOWLEDGMENT

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DR. SCHAEFER (U. S. Naval Medical Research Laboratory): May I ask how sure can you be, in view of what we heard about this animal material, about glycogen depletion? For instance, just to compare exposed animals under the two-gas system, compared with the oxygen system? You haven't compared them with the controls; and you always find various stages in any animal exposure, phases where the glycogen goes up and the fat goes down, another phase where the glycogen is down and the fat content is up. How reliable is this statement in a two-gas system? You have greater glycogen depletion than in 100% oxygen, on the basis of animal material which is very limited, and you don't know whether you got this result just taking the animals out of the 100% oxygen, out of the dome.

DR. KLION: I would like to comment on a couple of things. One, I would agree that the only way one could be absolutely sure that there's glycogen depletion is by quantitative analysis. We showed last year that there was some glycogen depletion, at least from a qualitative point of view on the pure oxygen animals, and I only compared the two just to show differences between what we had last year. Both of them, from a qualitative point of view, had decreased amounts of glycogen present in the liver cells. I don't think it was due to the fact they were taken out of the chamber for biopsy, since within 20 minutes one does not see glycogen depletion within the liver.

DR. ROTH (Lovelace Foundation for Medical Education and Research): Were these the same strains or same stocks of animals as used in the 100% oxygen? This would be a very critical factor in view of the substrain differences seen in oxygen toxicity.

DR. KLION: As far as I know, they were.

QUESTION: Have you ever looked at the levels of catalase in the cell, for instance, and tried to correlate catalase level with the changes you see in the lipid?

DR. KLION: No. Our biochemist is involved in other things, but we would like to see someone do this.

PATHOLOGY OF ANIMALS EXPOSED TO CARBON TETRACHLORIDE IN AIR AND IN 100% OXYGEN AT 5 PSIA

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INTRODUCTION

Previous experiments in this laboratory have shown a possible potentiation of the toxic effects of carbon tetrachloride (CCl_4) when administered as a contaminant in a high oxygen content atmosphere (Harper and Robinson, 1966). In order to help determine the validity of that observation as well as to continue the evaluation of contaminant-space cabin atmosphere interactions on biological systems, the present experiment was performed.

Materials and Methods:

Details of the facilities and methods used in animal exposure and care have been reported elsewhere (Thomas, 1965). Briefly, to reiterate, groups of Rhesus monkeys (Macaca mulatta), purebred beagle dogs, rats, and mice, of both sexes, were exposed, by inhalation, continuously for 90 days to the industrial threshold limit value (TLV) of carbon tetrachloride (63 mg CCl_4/m^3 atmosphere).

One group of animals received its exposure in air of normal oxygen content. The other group was exposed in a space cabin-type atmosphere composed of 100% oxygen at 5 psia (258 torr). Similar nonexposed animals were kept in the Toxic Hazards Division's animal holding facility as controls. The animals were observed continuously for the entire length of the experiment. The monkeys and dogs were bled biweekly and extensive laboratory tests performed on the samples. The results of clinical pathologic data have been reported (MacEwen and Geckler, 1966). The very few animals dying during the course of the experiment were removed from the exposure chamber and necropsied promptly. At the end of the experiment, all exposed and control animals were killed painlessly by an overdose of anesthesia. Complete gross necropsies were performed on all animals and complete microscopic examination done on all of the large animals and representative rats and mice. Standard tissue processing techniques were followed and special stains used where required.

RESULTS

There was no significant mortality (table I) associated with the contaminant (CCl_4). All of the deaths that did occur appeared to be the result of natural disease or nonspecific stress.

TABLE I

ANIMAL MORTALITY

Species	Controls	Exposed	
	Air	100% O_2 5 psia	Air
Mice	0/80	1/39	0/40
Rats	3/100	0/50	1/50
Dogs	0/8	0/8	0/8
Monkeys	0/4	0/4	0/4

General Observations:

In all of the species used, significant pathologic changes which could be associated with the contaminant were limited to the liver. A considerable number of other lesions were found but they occurred sporadically and in the control group as well as the exposed group and represented naturally occurring disease.

The dogs were the most resistant of the animals used to the hepatotoxic effects of CCl_4 as judged by morphologic changes. The remaining species all had rather marked liver changes. There were noticeable morphologic differences in the pattern of liver damage as seen in the monkeys, rats, and mice compared as species. The mice showed the greatest amount of damage with occasional animals bordering on cirrhosis. The rats and monkeys showed more hepatic fat accumulation than the mice but much less condensation of the reticular stroma and considerably less regenerative activity.

Pathology:

Monkeys:

All animals showed pale livers with increased prominence of the hepatic lobular architecture at the time of necropsy. There was slightly more variability in the degree of hepatic injury in the group exposed at ambient (room air) conditions compared to those exposed at altitude (100% O_2 5 psia). Typically there was marked central lobular fatty change which, for the most part, presented itself in the form of swollen, foamy cytoplasmed hepatocytes. Large cytoplasmic fat vacuoles and rare fat "cysts" were also present. These larger accumulations of fat were most often seen in the peripheral lobules of the liver and were more randomly distributed in the deeper portions of the parenchyma. Occasional acidophilic, hyaline, round bodies were present in the liver cords or rarely in the sinusoids but overt necrosis was not present. There appeared to be little regenerative activity. Fibrosis or collapse of

the reticular stroma was essentially absent. No significant difference could be seen between the 100% O_2 5 psia group and the ambient group.

Lesions seen not specifically associated with the contaminant included lung mites (Pneumonyssus), focal individual cellular necrosis of hepatocytes, chronic interstitial pneumonia, and chronic portal triaditis of the liver.

Dogs:

Dogs were the least liable of any of the species used to the hepatotoxic effects of CCl_4 as judged by morphologic changes. The dogs of both 100% O_2 and ambient exposed groups showed similar lesions and no significant difference could be noted between the two groups. The liver lesion seen in the dog consisted of slight central lobular fatty change in the form of enlarged foamy cytoplasmed hepatocytes. There was a panlobular swelling of liver cells with clear foamy spaces in the cytoplasm which did not stain for fat or glycogen. Overt regenerative activity or necrosis was not seen.

Lesions not associated with the contaminant included focal chronic interstitial pneumonia, parasitic granulomata in the lungs caused by a nematode (Filaroides milksi), chronic interstitial nephritis, and chronic lymphocytic pyelitis.

Rats:

No discernible difference was present between the 100% O_2 5 psia and ambient groups. The hepatic lesion typically was severe central lobular fatty change involving 50-90% of the hepatic lobule. Much of the fat was present as large vacuoles rather than the foamy cytoplasm seen in previously mentioned species. In total fat content, as measured by special stain, the rats did, however, have about the same total stainable lipid as did the monkeys. Occasional eosinophilic, round, hyaline bodies were present, but widespread necrosis was not seen. A moderate amount of regenerative activity was present.

Lesions not associated with the contaminant included chronic murine pneumonia in all stages, often with lung abscesses, occasional minute foci of hepatocellular necrosis, and acute pyelonephritis.

Mice:

The ambient exposed group showed slightly more variation in the morphology and severity of the hepatic changes than did the 100% O_2 5 psia exposed group. However, when averaged, there was probably little difference between the groups. The lesion typically consisted of central lobular fatty change of both the foam cell and vacuolar type. Necrosis as evidenced by hyalinized cells and Mallory bodies was increased in the mice as compared to the other species but was still far from a striking feature. There was much more of a tendency toward collapse of the reticular stroma with secondary formation of fibrous septae between portal tracts. By far the most regenerative activity occurred in this species. Often there was partial disruption of the lobular architecture secondary to regenerating cell cords and occasionally nodules were formed with no orientation to the surrounding liver parenchyma. The hepatocytes varied greatly in size and intensity of staining both of the nucleus and cytoplasm. Many binucleated or multinucleated cells were seen.

Lesions not associated with the contaminant included focal, chronic interstitial pneumonia, and pulmonary edema.

DISCUSSION

Similar experiments reported at this Conference last year tended to point toward an additive, or possibly synergistic, action of CCl_4 and increased oxygen tension (Harper and Robinson, 1966). The experiment being reported at this time would appear to be at variance with that concept. That is, little if any difference was found between animals exposed in air as compared to those exposed at 5 psia (258 torr) of 100% O_2. The simplest explanation for this finding is that there is no additive effect of increased O_2 tension on CCl_4 toxicity. However, the marked difference in murine mortality, as well as significant differences in morphology between high O_2 and air-exposed animals reported by Harper and Robinson (1966), would make that explanation somewhat doubtful. Differences in the dose of CCl_4 used (594 vs. 63 mg/m^3) could partially explain the difference in results. The difference in length of exposure is also, perhaps, of some significance. The animals in this experiment were exposed for 90 rather than 14 days. Animals exposed to an atmosphere of increased O_2 tension have been shown to undergo certain changes, probably adaptive, both morphological and biochemical (Kistler and Caldwell, 1966; Schaffner, 1966; Mautner, 1966; Kaplan, 1966). If these animals became physiologically adapted to the increased O_2 tension, then the possible additive effect of O_2 could have been, at least partially, obviated. Work on the basic mode of CCl_4 toxicity has implicated oxidative phenomena as being responsible, at least in part, for the mechanism of its toxicity (DiLuzio and Costales, 1956). If the animals adapted to one oxidative agent, then perhaps they also partially adapted to another. This may also have played a part in the variance between the two experiments. This is, of course, pure speculation, and further work must be done to elucidate completely the mechanisms involved in this problem.

As an aside, it certainly should be stated that the eight-hour TLV for CCl_4 is much too high in a closed system with 24-hour per day exposure. All of the species used exhibited some liver changes which in three species (monkeys, rats, and mice) were severe.

SUMMARY

Monkeys, dogs, rats, and mice were exposed to the Threshold Limit Value of carbon tetrachloride (63 mg/m^3) continuously for 90 days in either room air or a space cabin atmosphere (5 psia, 100% oxygen). All animals developed fatty livers but little or no difference was noted between the altitude and ambient groups. No other significant pathologic change associated with the contaminant could be seen.

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DISCUSSION

DR. ROTH (Lovelace Foundation for Medical Education and Research): Was there any specific difference between the type of lipid stains that you used on the altitude ones and those on ambient air and CCl_4?

DR. SOPHER: Well, now, as a lipid stain, we used oil red-o in propylene glycol, and this was the only stain that we did use at the time. This was on formalin fixed tissue cut on the cryostat and stained for approximately one hour. We found no difference in lipid staining between the altitude and ambient groups. The only difference that we really could say occurred is that the liver damage seen in the animals exposed under ambient conditions appeared to have a little more variation to it than that in the animals exposed in the oxygen atmosphere. In other words, if one were to map it out and say four plus, three plus, two plus, we would end up with more three pluses, say, in the oxygen atmosphere and four plus and a one plus and a two plus in the ambient. Totaled together, the damage together was probably about the same.

QUESTION: Since you did a complete autopsy and histologic survey, did you find anything in the adrenals with the exposure to carbon tetrachloride of this magnitude? We have always found some changes in the adrenal medulla. Changes in the liver with oxygen (as previously described) and with carbon tetrachloride are very similar.

DR. SOPHER: I was unable to appreciate any changes that might have occurred in the adrenal. The adrenal was examined only in the larger animals. In the small animals it generally was taken with the kidneys and a random section may or may not include it. There may well have been a change, but as I say, I was unable to appreciate it in our material.

QUESTION: I think it is important to realize that we see animals adapt to a wide variety of materials and atmospheres, as we have shown with oxygen. When you're dealing with a substance like carbon tetrachloride, which is a poor inducer and causes damage which is the classical carbon tetrachloride lesion, one might always expect this type of lesion to occur, no matter what atmosphere you're dealing with. You have to separate what we call damage from what we call adaptation, which really doesn't reflect damage.

DR. SOPHER: Well, this was what the experiment was designed to try and do. As I say, last year it was reported that the animals exposed within a 5 PSI 100% oxygen atmosphere showed more damage, more stainable lipid, more necrosis than did the animals exposed in ambient air. One other variable existed, though; last year the animals were exposed to considerably more carbon tetrachloride than we used. This year it was 63 milligrams per cubic meter. At that time it was close to 600 mg/m^3.

DR. RIESEN (IIT Research Institute): I'd like to suggest that in the case of carbon tetrachloride, it is quite possible that very small traces might be growth factor in a sense. Dr. Smythe has shown that very small trace quantities of carbon tetrachloride can stimulate growth in rats. This is very much smaller quantities than you're using here. In the case of oxygen, on the other hand, which is essential to metabolize, when we give five times its dose, it is completely lethal. On the basis of these considerations I wonder if you can completely separate these two.

DR. SOPHER: It's a good question. I have no answer for it. I think we did show that the effects in this case were not additive, and this is what we set out to do, to show if they were or were not. I think we showed they are not in this particular case. The explanation for it is one of choice, I think.

DR. PATRICK (Laboratory for Experimental Biology): You didn't run a concurrent study at the 600 mg/m^3 level to see if you could reproduce last year's results?

DR. SOPHER: We did not, but this is in the mill.

DR. FAIRCHILD (Aerojet-General Corporation): Actually, Dr. Riesen asked what I was going to ask. I think, to reiterate, we should keep in mind that we are possibly working with different mechanisms, and changes in structure of the liver can occur while some other system can be affected by an additive effect of the irritant action of the oxygen. Brody's hypothesis on carbon tetrachloride mechanisms shows that you have a very severe stimulation of the adrenal medulla and sympathetic nervous system, greater release of catecholamines, and this can also occur with oxygen. Perhaps you're having an additive effect here.

PATHOLOGY OF ANIMALS EXPOSED CONTINUOUSLY FOR 90 DAYS TO O_3 OR NO_2 IN AMBIENT AIR OR 100% OXYGEN AT 5 PSIA

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Toxicological experiments were conducted to evaluate the biological response of animals exposed continuously for a 90-day period in either ambient air or 100% oxygen at 5 psia to threshold limit values (TLV) of ozone (O_3) and nitrogen dioxide (NO_2) (MacEwen and Geckler, 1966). Threshold limit values are based on intermittent daily exposures of industrial workers while these animals were exposed continuously, thereby increasing the exposure factor by at least three times. This does not include an unknown factor related to the accumulative effects of continuous versus intermittent exposure.

These toxicological experiments were conducted by Aerojet-General Corporation at the Toxic Hazards Research Unit (THRU) of the Aerospace Medical Research Laboratories, Wright-Patterson AFB, Ohio, under the terms of Contract AF 33(657)-11305. Gross pathologic examinations were done by pathologists of the Aerospace Medical Research Laboratories and histopathological examinations were done by the Laboratory for Experimental Biology, St. Louis, Missouri, under the terms of Contract AF 33(615)-2941. The purpose of this report is to briefly review the toxicological results and describe the pathologic lesions seen in these experimental animals.

MATERIALS AND METHODS

Animal exposure facilities have been reported (Thomas, 1963), as have the methodological details for this particular series of experiments (MacEwen and Geckler, 1966). In brief, monkeys (M. mulatta), beagle dogs, rats, mice, and

sometimes guinea pigs were exposed continuously in Thomas Domes to TLV concentrations of either O_3 or NO_2 at either ambient pressure or 5 psia for 90 days. The nominal TLV concentrations for O_3 and NO_2 are 0.2 and 9.4 mg/M^3, respectively, while actual measured concentrations were 0.19 and 9.3 mg/M^3, respectively. Biweekly hematological and clinical chemical examinations were done on blood and serum samples from monkeys and dogs; similar examinations were done on samples from rats at the termination of the experiment.

Dead animals were removed from the domes as soon after death as possible. Even so, several of the mice were cannibalized before they could be removed. Dogs and monkeys that survived the exposure and the controls were euthanatized with overdoses of intravenous barbiturate anesthesia; rats, mice, and guinea pigs were euthanatized with ether. Necropsy examinations were made on all animals and weights of the heart, lung, liver, kidneys, and spleen (also brains of monkeys) were recorded. Tissues from all organ systems from monkeys and dogs were preserved in buffered formalin while only selected tissues (heart, lung, liver, kidney, and spleen) were saved from rats, mice, and guinea pigs. Fixed tissues were embedded in paraffin, sectioned at five microns, and stained with hematoxylin and eosin. Each case was examined by a pathologist and the results reviewed by at least two others. Short descriptive diagnoses were recorded for each case and the degree and distribution of each pathologic change was graded on a one to five scale. These diagnoses were then tabulated for the heart, lungs, liver, kidney, and spleen; numbers of observations where the degree of change was moderately severe (three), severe (four), or extreme (five) were also noted.

RESULTS

Gross Pathology

Mortality rates have been reported (MacEwen and Geckler, 1966) and are repeated in tables I through V.

Gross lesions in most of the animals were due to endemic disease and not related to exposure to either O_3 or NO_2. Incidental gross findings in the monkeys included widespread lung mite infestations, occasional focal hemorrhages in the lungs, nodules of Eosophagastomum sp. in the intestines, and two instances of chronic liver disease. Of the two dogs that died, only the one exposed to ozone in air had red, wet, heavy lungs. The other dog exposed to ozone at altitude had to be sacrificed on day-70 because of extensive traumatic injuries. Incidental gross lesions seen in other dogs included parasitic nodules in the lungs, pneumonitis, partial collapse of a portion of the lung (usually the left cardiac lobe), and chronic nephritis.

Only control rats died and these deaths were related to severe pneumonia. Chronic murine pneumonia was seen in all groups, but the incidence and severity varied. Mild congestion and occasional focal hemorrhages were evident in the lungs of a few rats.

Mice dying following exposure to O_3 and NO_2 had dark red, wet, heavy lungs. It was not possible to examine all the mice that died following exposure to ozone in air since four of the seven that died were cannibalized.

TABLE I

MICROSCOPIC LESIONS FOUND IN MONKEYS EXPOSED FOR 90 DAYS TO O_3 OR NO_2 AND IN CONTROL MONKEYS

AGENT	O_3		NO_2		CONTROL		
CONCENTRATION (mg/M^3)	0.19	0.19	9.3	9.3	---	---	---
PRESSURE (mm Hg)	260	720	260	740	740	740	740
ATMOSPHERE COMPOSITION	100% O_2	Air	100% O_2	Air	Air	Air	Air
Mortality	0/4	0/4	0/4	0/4	0/2	0/2	0/2
Examined	4	4	4	4	2	2	2
HEART:							
Chronic Myocarditis	2	1	2	1			1
Myocardial Fibrosis						1	
LUNGS:							
Atelectasis	3(3)	1	3(3)	4(1)			2(2)
Emphysema	3(3)	4(1)	4(3)	4	2	2(1)	2(2)
Lung Mite Disease	3(1)	4(3)	1(1)	4	2(1)	1(1)	1(1)
Congestion	3		2(2)				
Alveolar Hemorrhage					1(1)		
Alveolar Edema			2(2)			1	
Pneumonia	1		1		1(1)		
Interstitial Pneumonitis	1(1)		2(1)				1
Focal Abscesses				2			
Bronchitis	1		2	1	2(1)		2(1)
LIVER:							
Congestion			1				
Cytoplasmic Vacuolization			1				
Fatty Change		1(1)	1(1)				
Focal Hepatitis		1		1			1
KIDNEY:							
Nephritis		1					
Pyelonephritis			1				1
Parasitic Granuloma					1		
SPLEEN:							
Acute Congestion	3				1		1(1)

(Numbers in parentheses indicate cases in which lesion was moderately severe, severe, or extreme.)

TABLE II

MICROSCOPIC LESIONS FOUND IN DOGS EXPOSED FOR 90 DAYS TO O_3 OR NO_2 AND IN CONTROL DOGS

AGENT	O_3			NO_2		CONTROL		
CONCENTRATION (mg/M^3)	0.19	0.19		9.3	9.3	---	---	---
PRESSURE (mm Hg)	260	720		260	740	740	740	740
ATMOSPHERE COMPOSITION	100% O_2	Air		100% O_2	Air	Air	Air	Air
Mortality	0/8*	1/8		0/8	0/8	0/4	0/4	0/4
Examined	7	1	7	8	8	4	4	4
HEART:								
Chronic Pericarditis				1				
LUNGS:								
Atelectasis	7(5)		7(7)	5(5)	7(7)		4(4)	4(3)
Emphysema	7(7)		7(3)	7(5)	7(7)	3(3)	3(3)	3(2)
Congestion			3	1(1)			4(4)	
Alveolar Edema			2(1)				2(1)	
Acute Hemorrhagic Pneumonia		1(1)						
Pneumonia	1(1)			1(1)		2(2)	1(1)	
Interstitial Pneumonitis	7(7)		7(5)	7(4)	8(8)	3(3)	3(3)	4(1)
Hemorrhage							1(1)	2(2)
Chron Bronchitis	6(6)	1(1)	3(2)	3(1)	5(4)		2(1)	1
Chron Peribronchitis			1(1)					
Chron Fibrosis	1(1)				3(3)	3(3)		4(2)
Parasitic Granulomas	2(2)			2				1
LIVER:								
Congestion		1(1)	1	3			1	
Parenchymatous Degeneration				1				
Focal Hepatitis					2			1(1)
KIDNEY:								
Congestion	1(1)						1	
Chronic Interstitial Nephritis				1				

TABLE II (CONT'D)

MICROSCOPIC LESIONS FOUND IN DOGS EXPOSED FOR 90 DAYS TO O_3 OR NO_2 AND IN CONTROL DOGS

AGENT	O_3			NO_2		CONTROL		
KIDNEY (Cont'd):								
Glomerulosclerosis				1(1)				
Chronic Pyelitis	3(3)			2	1	1(1)	1	
Chronic Pyelonephritis	1(1)			2(1)				
SPLEEN:								
Congestion	4(4)	1(1)	4(4)	2(2)	2(2)	1(1)	2(1)	4(3)

*One dog was sacrificed on day-70 because of extensive trauma, results of microscopic examination not included below.

(Numbers in parentheses indicate cases in which lesion was moderately severe, severe, or extreme.)

TABLE III

MICROSCOPIC LESIONS FOUND IN RATS EXPOSED FOR 90 DAYS TO O_3 OR NO_2 AND IN CONTROL RATS

AGENT	O_3		NO_2		CONTROL				
CONCENTRATION (mg/M^3)	0.19	0.19	9.3	9.3	---	---	---		
PRESSURE (mm Hg)	260	720	260	740	740	740	740		
ATMOSPHERE COMPOSITION	100% O_2	Air	100% O_2	Air	Air	Air	Air		
Mortality	0/50	0/50	0/50	0/50	2/50	0/50	1/50		
Examined	10	20	10	10	2 13	11	1 10		
HEART:									
Chronic Myocarditis	2	2			1 5				
LUNGS:									
Chronic Murine Pneumonia	10(4)	18(10)	10(7)	8(1)	1(1) 13(7)	11(10)		10(10)	
Purulent Pneumonia							1(1)	1(1)	
Purulent Bronchitis							1(1)		
Atelectasis	8(1)	17(9)	8(6)	9(2)	2(2) 5(1)	2(2)		5(4)	
Emphysema	7(1)	7(1)	6(4)	3	2(1) 6(4)	10(6)	1(1)	7(6)	
Congestion	7(2)	7(3)	7(6)	4(1)	1 9(4)	3(1)			
Alveolar Edema		3(1)	1	1	1(1)				
Hemorrhage	4	3	1	2	2	1	1(1)	1(1)	
Arteriolar Thickening		1						1(1)	
LIVER:									
Congestion	2(1)	4	3	3	2	5(3)	1	5(5)	
Cytoplasmic Vacuolization	1	1							
Pericholangitis						1		1	
KIDNEY:									
Congestion		5(1)	5		2	5(3) 4	1(1)	9(7)	
Tubular casts						1			
Mineralization					1				
Retention cysts						1			
SPLEEN:									
Congestion	1(1)	3(1)		9	1	4(4)		8(8)	
Fibrosis							1(1)		

(Numbers in parentheses indicate cases in which lesion was moderately severe, severe, or extreme.)

TABLE IV

MICROSCOPIC LESIONS FOUND IN MICE EXPOSED FOR 90 DAYS TO O_3 OR NO_2 AND IN CONTROL MICE

AGENT	O_3			NO_2				CONTROL		
CONCENTRATION (mg/M^3)	0.19		0.19	9.3		9.3		---	---	----
PRESSURE (mm Hg)	260		720	260		740		740	740	740
ATMOSPHERE COMPOSITION	100% O_2		Air	100% O_2		Air		Air	Air	Air
Mortality	2/40		*7/40	2/40		1/40		0/40	0/40	0/40
Examined	2	5	5	2	5	1	5	5	5	6
LUNGS:										
Chronic Murine Pneumonia			2			1	2	4		6(1)
Atelectasis		1	1		2	1	5	2	1	1
Emphysema			4				1	1		1
Congestion	2(2)	2(1)	4(3)	2(2)	4(4)	1(1)	1	4(3)	5(1)	
Alveolar Edema	2(2)	1(1)		2(2)						
Hemorrhage	1(1)	4(4)	3(2)	2(2)	3(3)			1		1
Interstitial Pneumonitis		2(1)	3(1)					3	1	2
Pneumonia			2	1						1(1)
Thickened Septae		1(1)								
LIVER:										
Congestion	2(1)	4(4)	5(1)	1	3(1)		2		2	1
Nuclear Changes	1(1)									
Hepatitis				1		1(1)				
Focal Granuloma								3		
KIDNEY:										
Congestion		4(3)	1		1(1)	1(1)	3	1		4
Chronic Pyelonephritis			1							
Chronic Pyelitis								1		
SPLEEN:										
Congestion								1		1

*All 7 were cannibalized or extensively autolyzed and not examined microscopically.

(Numbers in parentheses indicate cases in which lesion was moderately severe, severe, or extreme.)

TABLE V

MICROSCOPIC LESIONS FOUND IN GUINEA PIGS EXPOSED FOR 90 DAYS TO O_3 AND IN CONTROL GUINEA PIGS

AGENT	O_3				CONTROL		
CONCENTRATION (mg/M^3)	0.19		0.19		---	---	
PRESSURE (mm Hg)	260		720		740	740	
ATMOSPHERE COMPOSITION	100% O_2		Air		Air	Air	
Mortality	2/6		4/6		0/3	2/4	
Examined	2	2	4	2	3	2	2
HEART:							
Edema	1(1)		1				
Hemorrhage	1(1)				1		
Fatty Change					1		
Myocarditis		2(1)	4		2	1	
Endocarditis					2		
Pericarditis							1
LUNGS:							
Atelectasis	1(1)			1(1)	2		
Emphysema			3(1)	2(2)	3(2)	1(1)	2(1)
Congestion	1(1)		3(1)				
Alveolar Edema	2(2)	1(1)	1				
Hemorrhage	1(1)	1(1)	3(1)			1(1)	
Interstitial Pneumonitis				1	3(2)	1(2)	1
Pneumonia	1(1)	1(1)	4(4)	1(1)		2(2)	2(1)
Abscesses	1	1(1)					
Adenomatous							1
Fibrosis	1(1)	3(3)		2(1)		1	
Bronchitis	1	2(1)			2(1)		
Pleuritis	1(1)	1(1)					
Medial Hypertrophy Arteries		1(1)					
LIVER:							
Edema	1(1)						
Congestion	2(2)		3(1)	2		1(1)	

TABLE V (CONT'D)

MICROSCOPIC LESIONS FOUND IN GUINEA PIGS EXPOSED FOR 90 DAYS TO O_3 AND IN CONTROL GUINEA PIGS

AGENT	O_3		CONTROL	
LIVER (Cont'd):				
Parenchymatous Degeneration			1	
Fatty Change		2(2)	1	1
Focal Hepatitis		1(1)		1
Focal Necrosis				
KIDNEY:				
Edema	1(1)			
Congestion	1(1)			
Chronic Interstitial Nephritis	1	2		
SPLEEN:				
Congestion	1(1)			
Edema	1(1)	2(1)		
Fibrosis	1(1)	2(1)		

(Numbers in parentheses indicate cases in which lesion was moderately severe, severe, or extreme.)

Almost all of the guinea pigs had gross lesions of active chronic pneumonia. Those that died following exposure to O_3 had red, wet areas of hemorrhage and edema occupying the non-pneumonic areas. Of the two control guinea pigs that died, one had a severe peritonitis and the other had severe confluent pulmonary hemorrhages.

Histopathology

Histopathological results are given in tables I through V according to species. Where there were deaths during the experiment, the data are divided into two columns, one for those animals that died and the other for those that survived the exposure and were subsequently euthanatized.

None of the exposed monkeys died; most of the lesions were seen in the lungs and were similar, on a qualitative basis, to lesions seen in the control monkeys and other monkeys used in our laboratory as controls for other experiments.

One dog died as a result of O_3 exposure at 720 mm Hg and had an acute hemorrhagic pneumonia that was typical of exposure to O_3. Other lesions due to endemic disease were similar to those seen in the control dogs and other unexposed dogs in our laboratory.

There were no deaths in rats as a result of exposure to either O_3 or NO_2 although two control rats died of endemic disease. Chronic murine pneumonia was present in all groups of rats at the termination of the experiments and was moderately severe or worse in at least 40 percent of the rats in six of the seven groups.

Two mice died as a result of exposure to ozone in 100% oxygen at 5 psia; seven died in O_3 in air at 720 mm Hg, but could not be examined histologically. Two mice died that were exposed to NO_2 in 100% oxygen at 5 psia and one died in NO_2 in air at 740 mm Hg. All exposed mice that died and were examined had typical pulmonary lesions expected from O_3 or NO_2. Lesions of endemic disease were relatively minor in these mice. Ether euthanasia probably accounted for some of the congestion seen in the lungs of the mice as well as in the rats and guinea pigs.

Two guinea pigs died that were exposed to O_3 in 100% oxygen at 5 psia and four died in O_3 in air at 720 mm Hg. Two control guinea pigs also died. Endemic disease was widespread and further analysis of these data is not warranted.

DISCUSSION

Review of Toxicology Results

Mortality data were unremarkable except for the deaths at 720 mm Hg pressure in the O_3 experiments where mice seemed to be more sensitive than the other three species. There were also deaths recorded in a small number of guinea pigs exposed to O_3. Most of the deaths occurred during the first half of the 90-day exposure, suggesting some degree of adaptation in the survivors. There were no significant differences in organ to body weight ratios.

Analyses of clinical laboratory data showed slight rises in SGPT and SGOT from serum of dogs exposed to O_3 and NO_2 and a slight rise in SGPT in monkeys exposed to NO_2. These were qualitative changes and not statistically significant.

The data are consistent with the hypothesis that the animals first respond to the atmospheric contaminant and then adapt to the experimental environment. There were also no statistically significant differences between the data from animals exposed to contaminants at reduced pressure in 100% oxygen and those exposed at ambient atmospheric pressure (MacEwen and Geckler, 1966).

Pathology

Mortality was low in all experiments except for the high mortality in guinea pigs exposed to O_3. Even though guinea pigs are supposedly highly susceptible to O_3 toxicity, the mortality in these experiments is of questionable significance due to the high incidence of marked endemic disease. Gross lesions in the lungs of the dog and mice that died were typical of the pulmonary irritants, O_3 and NO_2, in that there was massive hemorrhage and edema of the lungs. These gross observations were confirmed on microscopic examination. Endemic disease in dogs, monkeys, rats, and guinea pigs was present in both exposed and control animals, making subtle changes impossible to identify. In the mice exposed to O_3 in ambient air, there was an apparent increase in pulmonary lesions due to experimental conditions.

There were indications in some experiments, for instance the dogs exposed to O_3 at 5 psia, that the extent and severity of endemic disease was greater in exposed dogs as compared to controls and was possibly accentuated as a result of the exposure. However, it is emphasized that valid conclusions such as this cannot be drawn because of the extensive changes seen in control dogs.

SUMMARY

Pathology results support the conclusions drawn by the toxicologists who stated that "it does appear clear that the TLV for space applications may not be radically different from industrial TLV if only the factors of continuous dosage, reduced pressure, and pure oxygen atmosphere are considered" (MacEwen and Geckler, 1966).

REFERENCES

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HISTOPATHOLOGICAL STUDY OF EXPERIMENTAL ANIMALS EXPOSED TO A SATURATED VAPOR CONCENTRATION OF ETHYLENE GLYCOL IN A SPACE CABIN ATMOSPHERE

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INTRODUCTION

Toxicological results have been reported on animals exposed by inhalation in a 100% oxygen atmosphere at 258 mm Hg (5 psia) to a saturated vapor concentration of ethylene glycol for periods up to 22 days elsewhere in these proceedings. The purpose of this report is to review briefly the pathologic lesions of ethylene glycol poisoning and describe the morphologic changes seen in the above animals.

PATHOLOGY REVIEW

The toxicity of ethylene glycol is well known, even though it rarely occurs in industry. Most of the reported cases resulted from accidental or suicidal ingestion of the ethylene glycol type of antifreeze (Pons and Custer, 1946; Grant, 1952; Morini, 1954).

The pathology in such cases is largely confined to the kidneys although, in some instances, lesions are seen in the central nervous system. The kidneys are markedly congested and microscopic examination reveals numerous calcium oxalate crystals in the tubules and a variable degree of epithelial damage (figure 1). The crystals are usually intraluminal, although in experimental cases they have been observed within the epithelium and even in the interstitium (Vaille, et al, 1963). Proximal and distal convoluted tubules are most commonly involved but occasionally crystals are also seen in the collecting system. The morphology of the crystals is quite varied as they may be rounded, polyhedral, needle-like, or more often resembling sheaves of wheat. They are colorless or yellowish-brown and are strongly birefringent when examined under polarized light (figure 2). Their chemical nature can be determined by X-ray diffraction or by histochemical techniques (Macaluso and Berg, 1959; Fanger and Esparza, 1964).

Figure 1. CALCIUM OXALATE CRYSTALS IN THE KIDNEY TUBULES, IN A HUMAN CASE OF ETHYLENE GLYCOL POISONING DUE TO INGESTION OF ANTIFREEZE. (H & E - 400 X)

Figure 2. HUMAN KIDNEY; CALCIUM OXALATE CRYSTALS UNDER POLARIZED LIGHT. (H & E - 400 X)

The mechanism responsible for the formation and deposition of the crystals in the kidneys is not clearly understood, but it is known that oxalic acid is a product of the metabolism of ethylene glycol.

The pathologic changes in the brain, in the most severe instances, have included a meningo-encephalitis with a considerable exudation of inflammatory cells along the meninges and the Virchow-Robin spaces, scattered ring hemorrhages, and nerve cell changes (figure 3) varying from moderate satellitosis to frank chromatolysis and ghosting of cells. Oxalate crystals have also been described around the blood vessels in brain substance and the pia-arachnoid. These changes are not always present and often a careful examination will be entirely negative or show only mild edema and congestion. The paucity of changes in the central nervous system frequently contrasts with the severity of the clinical picture, suggesting that some biochemical or subcellular abnormalities must occur before structural changes develop. In recent work with experimental ethylene glycol poisoning, changes of the cerebral lactic dehydrogenase have been reported (Tremblay and Dufour, 1966).

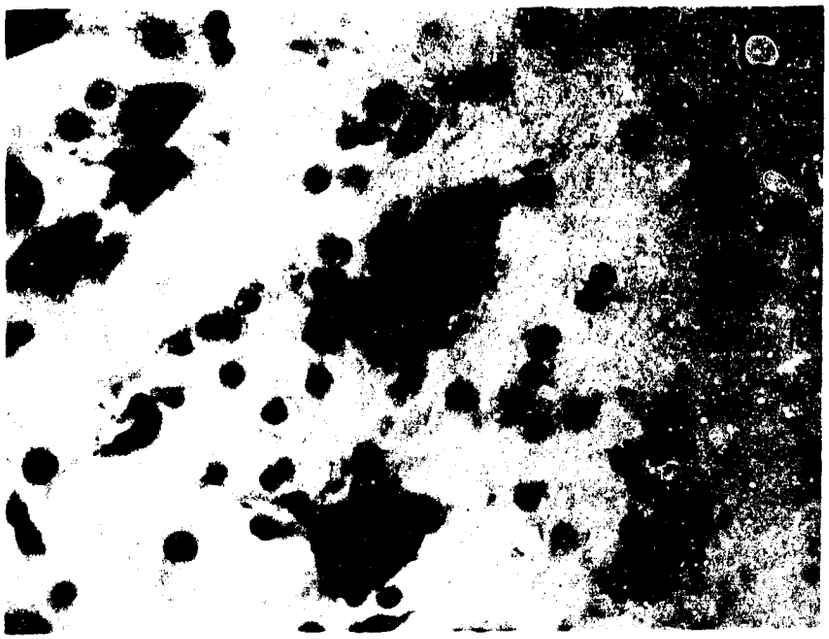

Figure 3. SECTION OF PARAVENTRICULAR NUCLEUS, SHOWING SATELLITOSIS OF UPPER NEURONE AND DEGENERATIVE CHANGES IN THE ONE BELOW. (Same case as Figures 1 and 2) (Nissl - 630 X)

Although rare cases of chronic intoxication due to the industrial exposure of inhaled ethylene glycol vapor have been reported (Triosi, 1950), nothing is known about the effects it may have upon tissue structures. Therefore, this project was undertaken with this purpose in mind.

Three of the four mice that died before the fourteenth day also showed a severe pneumonitis, whereas the fourth had considerable pulmonary edema. One of these early deaths was the only animal to have calcium oxalate crystals deposited in the convoluted tubules, and rarely in the collecting tubules, of the kidneys (figure 4).

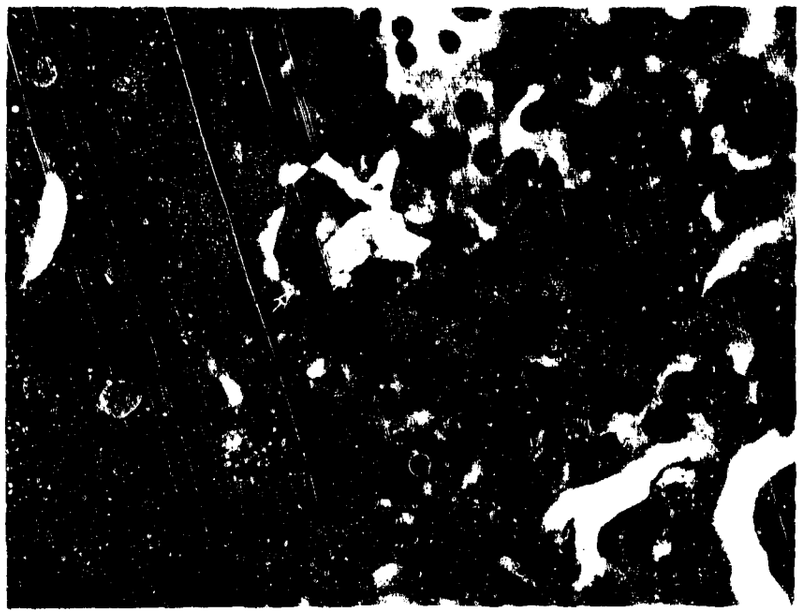

Figure 4. MOUSE KIDNEY - CALCIUM OXALATE CRYSTALS IN THE RENAL TUBULES. (H & E - 630 X)

Rats

A total of 20 exposed rats were examined. Two died before the fourteenth day, 10 were sacrificed on the fourteenth day, and 8 more on the twentieth day. Five and 10 control rats were sacrificed on the fourteenth and twentieth day, respectively (table II). In addition, seven control rats were maintained in a 100% oxygen atmosphere at 5 psia. One of these rats died before the fourteenth day, the remaining six were sacrificed on the twentieth day.

The two exposed rats that died before the fourteenth day showed marked pulmonary edema, congestion, and hemorrhage. In addition, one had an occasional oxalate crystal in the kidney tubules.

Eight of the 10 rats examined after the 14 days exposure to ethylene glycol vapor showed a mild to moderate degree of murine pneumonia. Similar findings, although of a lesser degree, were present in two of the five control rats.

In the 20-day group, seven of eight rats showed moderate to marked pneumonic infiltration, while five of the 10 control rats showed only a mild inflammatory infiltrate (figures 7 and 8).

RESULTS

The materials and methods, as well as the clinical observations and laboratory data, have been reported in the previous paper by Mr. Vernot. In brief, mice, rats, guinea pigs, rabbits, dogs, and monkeys were exposed to a saturated vapor concentration of ethylene glycol in a pure oxygen atmosphere at 5 psia. Control animals of the same species were maintained simultaneously in room air as were seven rats in 100% oxygen at 5 psia. Part of the rats, mice, and guinea pigs were sacrificed on the fourteenth day and the remainder were killed on the sixteenth day (guinea pigs) or the twentieth day (rats and mice). Rabbits were sacrificed on the seventeenth and twentieth day of exposure, and all dogs and monkeys on the twenty-second day. All monkeys and dogs and representative individuals of the other species were necropsied immediately after death and samples of the major organs were fixed in buffered formalin and processed as routinely for the preparation of H & E sections.

Mice

A total of 24 exposed mice were studied (table I). Four died before the fourteenth day, 10 were sacrificed on the fourteenth day, and 10 were sacrificed after an exposure of 20 days. Five control mice were included in each group. Gross and microscopic examinations revealed pulmonary hemorrhages, congestion, pneumonitis, and bronchiolitis with greater frequency and severity in the exposed than in the control animals. Six of 10 and eight of 10 mice, in the 14- and 20-day groups, respectively, had varying degrees of pneumonitis and bronchiolitis, as compared to only one of 10 control mice (figures 5 and 6).

TABLE I

EFFECT OF INHALED ETHYLENE GLYCOL ON THE LUNGS OF MICE

	No. of Mice	DEGREE OF PNEUMONITIS		
		Mild	Moderate	Severe
Spontaneous Death (T)	4	0	0	3
14-Day Group (T)	10	1	2	3
14-Day Group (C)	5	0	0	0
20-Day Group (T)	10	3	4	1
20-Day Group (C)	5	1	0	0

(T) - Test

(C) - Control

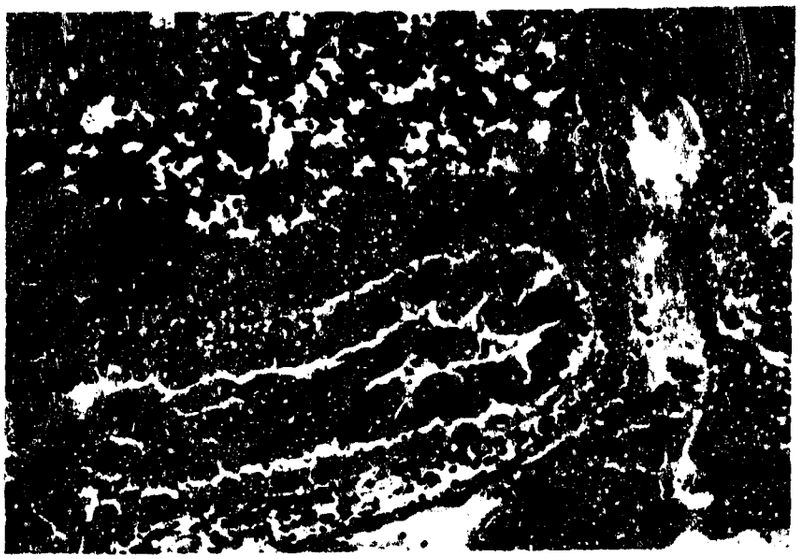

Figure 5. MOUSE LUNG - 14-DAY EXPOSURE TO ETHYLENE GLYCOL VAPOR, SHOWING SEVERE PERIBRONCHIAL AND ALVEOLAR INFLAMMATORY EXUDATE. (H & E - 125 X)

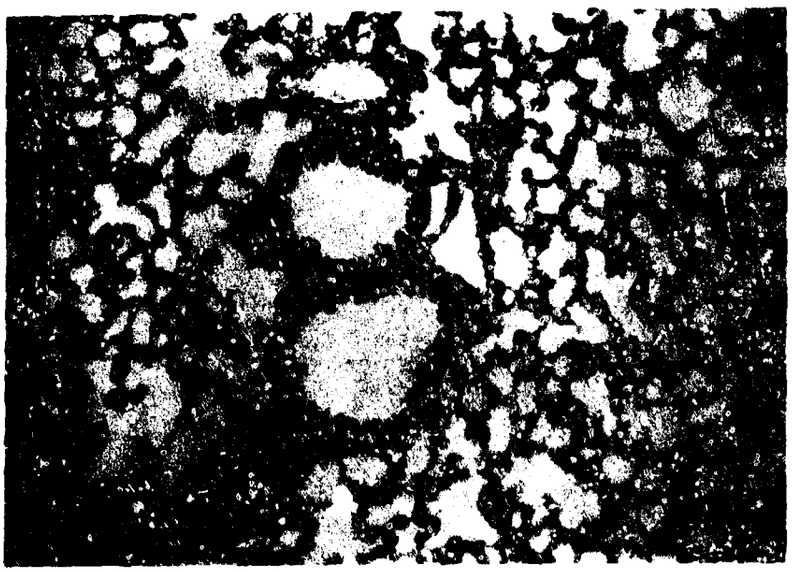

Figure 6. MOUSE LUNG - CONTROL MOUSE. (H & E - 125 X)

In the altitude control group, the rat that died early in the experiment showed severe pneumonia, hemorrhage, and edema. The other six had a mild interstitial and peribronchial infiltrate, which was nevertheless more pronounced than in the control animals kept at room air.

TABLE II

EFFECT OF INHALED ETHYLENE GLYCOL ON THE LUNGS OF RATS

	No. of Cases	DEGREE OF PNEUMONIA		
		Mild	Moderate	Severe
Spontaneous Death	3	0	2 (T)	1 (C)*
14-Day Group (T)	10	4	3	1
14-Day Group (C)	5	2	0	0
20-Day Group (T)	8	2	2	3
20-Day Group (C)	10	4	1	0
Altitude Group (C)	6	4	2	0

*Altitude Control rat

(T) Test

(C) Control

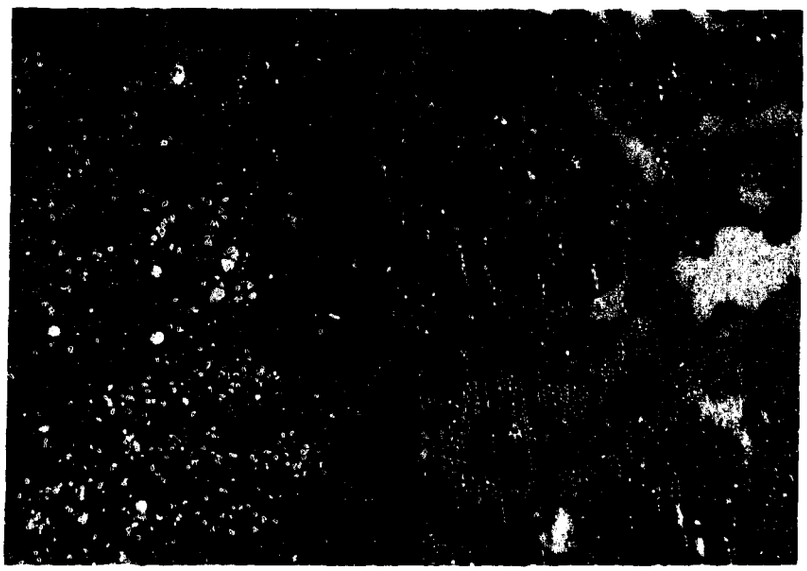

Figure 7. RAT LUNG - RAT EXPOSED FOR 20 DAYS. SEVERE INTERSTITIAL PNEUMONITIS AND BRONCHIOLITIS, WITH SQUAMOUS METAPLASIA (arrows). (H & E - 125 X)

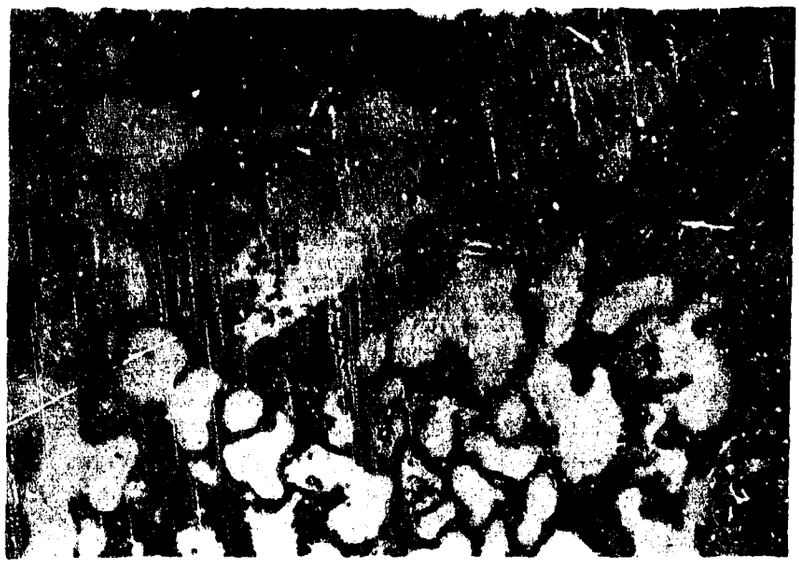

Figure 8. RAT LUNG - CONTROL. (H & E - 125 X)

Guinea Pigs

Of the 20 exposed guinea pigs, 10 were sacrificed on the fourteenth day, 10 more on the sixteenth day. There were five controls in each group. Variable degrees of pulmonary hemorrhage, congestion, pneumonitis, and often foreign body reaction due to aspiration were common findings. No significant difference was observed in either the incidence or severity of the lesions between the test and the control animals.

Rabbits

Ten exposed rabbits (three for 17 days and seven for 20 days), and eight control rabbits were studied. The pathologic changes seen in the lungs did not differ greatly between the exposed and control animals. An interesting incidental finding in these animals was the presence of a meningoencephalitis due to Nosema sp. (formerly Encephalitozoon cuniculu) in four instances, and the presence of numerous liver granulomata consistent with coccidiosis (Eimeria stiedae).

Dogs

Four exposed and two control dogs were studied. None of them had significant pathologic lesions.

Monkeys

All four monkeys exposed for 22 days and two control monkeys revealed no pathologic changes that could be related to the experiment. Incidental findings were the presence of focal nonspecific myocarditis in two exposed monkeys and lung mite

disease in the other two. In addition, two exposed and one control monkey had filaria within the pulmonary blood vessels.

SUMMARY

It appears that only mice and rats had changes related to the exposure. The pulmonary findings in the mice and rats suggest that the inhalation of ethylene glycol vapor acts as an irritant for the bronchiolar and alveolar epithelium, leading to a chemical pneumonitis and bronchiolitis.

In the altitude control group of rats, interstitial pneumonitis and murine pneumonia similar to that seen in exposed animals (although of a lesser severity) was present. This indicated that altitude, by itself, may play a contributory role in the pathogenesis of the pulmonary lesions as has been previously observed (Harper and Robinson, 1966). The very infrequent occurrence of calcium oxalate crystals in the kidneys suggests that very low levels of the contaminant were present in the blood. In conclusion, this work has demonstrated that, under the conditions described, the inhalation of ethylene glycol does not present a serious hazard to monkeys, dogs, guinea pigs, and rabbits, while there is probably some hazard to rats and mice.

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DR. COULSTON (Albany Medical College): Just so we can educate the audience, what was the parasite in the rabbit?

DR. ESPARZA: Coccidiosis.

COL. TOWNSEND: The chair wishes to express sincere appreciation to all of the speakers for the fine papers they have presented and recognize the tremendous amount of effort that has gone into the preparation and study of this material. I think we can state that at least as far as anatomical and biochemical changes are concerned we perhaps got on the first rung of the ladder of elucidating some of these. We have seen the problems that intercurrent infection can raise to cloud the issue, and we have seen the variability of different species, different response to potential toxic materials. So, in spite of all the work to be done, we can all go home feeling that there is still something else to do, a great deal to do. This has been a very inspiring afternoon for me. I have thoroughly enjoyed it and I want to say that the audience has been most attentive. It's been my pleasure to be your chairman and to be with you today.

SESSION III

EVALUATION OF CABIN MATERIALS

Chairman

Dr. R. Palmer Cox
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EXTRAPOLATION OF ACCELERATED GAS-OFF DATA TO AMBIENT CONDITIONS

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INTRODUCTION

For several years various spacecraft manufacturers have been studying the outgassing properties of organic materials for the qualification and the selection of space cabin materials. A common criterion used by the different manufacturers has been the use of an elevated temperature for procuring gas-off data. A major problem for the designer of a new space cabin is the degree to which this background accelerated gas-off data can be utilized under specific system environmental conditions and specific mission requirements.

Outgassing may result from a number of basic processes such as diffusion followed by the evaporation of monomer or low molecular weight polymers, plasticizers, catalyst residues, and solvents; desorption of material from carbon black or other fillers; oxidation and thermal degradation. Combinations of these processes will be operating in almost any material which is outgassing, and, in addition, there may be secondary reactions of the contaminants. At a given temperature, it is to be expected that various contaminants will have different outgassing rates because of the different mechanisms involved.

The purpose of the reported investigation was to assess the reliability of elevated temperature testing as a rapid means of screening organic materials intended for space cabin use. In this study, samples of each material were tested at 70 F, 155 F, and 200 F, with overall exposure times of 60, 10, and three days, respectively. Analyses of the test atmosphere for total volatile organic contaminants and for carbon monoxide were performed at several different times during each test. Curves were constructed relating contaminant generation to exposure time for each material and each temperature.

The resulting generation rate comparisons will be useful in establishing the space cabin contaminant control requirements relative to actual material usage temperatures and mission duration.

EXPERIMENTAL PROCEDURES

Twenty-two samples of proprietary space cabin organic materials representative of different generic classes were investigated. Typically the material was cut into strips approximately one-inch wide and placed in a two-liter, round-bottomed Florence flask. The flask was then necked down to 1/4 inch OD, and a valve was attached by means of a glass-to-metal seal. This container was then pumped down to a rough vacuum, partially filled with oxygen, pumped down again, and refilled with 100% oxygen to give a total pressure of 260 mm Hg at the test temperature. Approximately 10 grams of material was used whenever practical; in the case of materials such as coatings, a much smaller amount, applied to a substrate, was used, and the results were adjusted accordingly.

Each sample was analyzed for total organics and carbon monoxide with a gas chromatograph set up for the particular analysis. Samples of the flask atmosphere were taken directly from the flasks into the sampling loops of the chromatographs to minimize adsorptive loss of the contaminants. Since each aliquot was less than one ml, there was a negligible effect on the pressure within the flasks. Sampling times, together with the corresponding test temperatures, are shown in figure 1.

The gas samples were analyzed for total organics using an Aerograph gas chromatograph with a flame ionization detector, and a flow restrictor in place of the usual partioning column. Compressed air was used for the carrier gas, rather than nitrogen or helium, in order to minimize the perturbation of the baseline response caused by the oxygen in the sample. The comparison standards were made up by pressure dilution, and consisted of methane in argon at 260 mm Hg pressure. Results were calculated and expressed as micrograms of methane equivalent per gram of sample. (For actual materials testing it has been found more convenient in some cases to express outgassing in different units; for instance, contaminant generation rates for coatings are better expressed as μ g/unit area, and results on wire as μg/unit length.)

In the analysis for carbon monoxide, hydrogen was used as a carrier, and the sample was passed through a Linde Molecular Sieve 5A column, to separate other contaminants. The carrier gas was then passed through an external oven in which the carbon monoxide was reduced to methane by hydrogenation at 360 C in presence of a reduced nickel catalyst on alumina. The gas stream was then passed through the flame ionization detector and the methane content was determined. This method for carbon monoxide analysis was recommended by the Monsanto Research Corporation (Pustinger, 1965).

RESULTS

The variety of materials tested, which included epoxies, silicones, polyurethanes, butyls, and others, resulted in offgassing rates that were highly variable.

Total Organics

Table I shows the observed outgassed organics after three days at 155 F and after 30 days at 70 F for the 14 materials whose organic contaminant production was high enough to be reliable in this comparison. The ratios of these values are

TABLE I

TOTAL ORGANICS OUTGASSED VS. TIME AND TEMPERATURE

Total Organics Outgassed (μg/gram)

Material	155 F/3 Day	70 F/30 Day	Ratio 155/70 F
Dacron-reinforced mylar film	18.1	1.0	18.1
Polyurethane-cotton stretch fabric	43.3	3.0	14.4
Polyurethane potting compound	5.3	0.7	7.6
Butyl elastomer	30.3	18.7	1.62
Saran 18 Film	18.8	27.7	0.68
Polyvinylidene fluoride film	36.6	3.9	9.1
Polyurethane elastomer	13.0	3.3	3.94
Silicone potting compound	2.7	7.6	0.36
Nitrile-phenolic adhesive	105.0	4.7	22.4
Teflon-glass tie cord	5.0	2.3	2.17
Polycarbonate sheet	7.4	0.8	8.8
Polyester sheet	1.5	0.4	3.75
ABS sheet	4.5	3.3	1.36
Dry lubricant film	2.9	5.9	0.49

given in the third column. In three cases the ratio was less than one, that is, there was more contaminant production after 30 days at room temperature than after three days at 155 F. However, none of these materials approached the maximum acceptable value after three days at 155 F of 111 μg of total organics per gram of sample as methane. For the other 11 materials this ratio varies from 1.36 to 22.4. A high ratio may be indicative of poor thermal stability.

The data resulting from the 200 F exposure were treated in the same way, and as a rule the ratios of the 200 F outgassing values to those at room temperature values were grossly higher than for the 155 F comparison. Several materials which had fairly low outgassing values at room temperature evolved especially large quantities of organic materials at 200 F.

The average total organics outgassed at the different temperatures have been plotted against time in figure 1. The time scale for the 70 F curve has been shifted

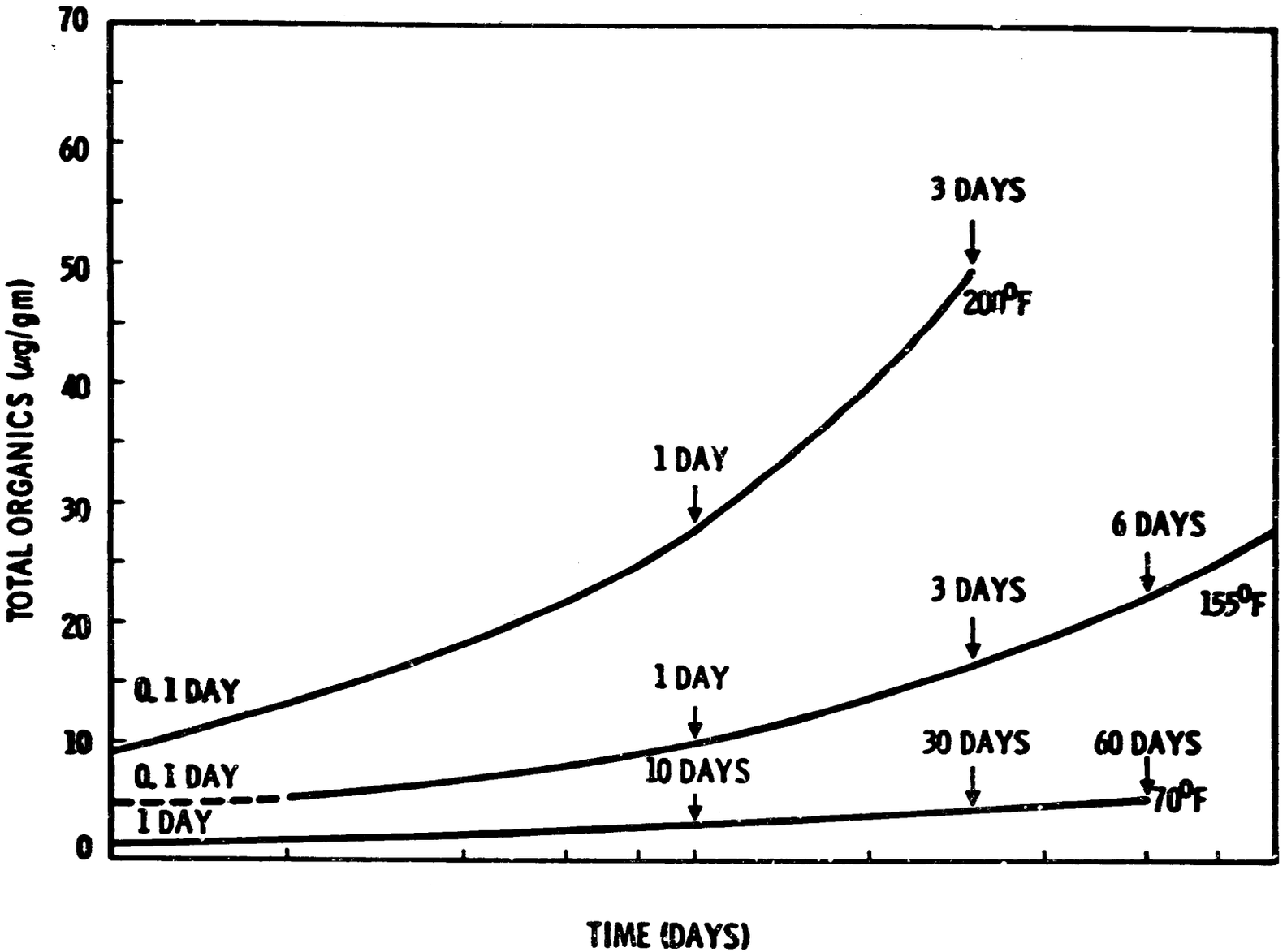

Figure 1. AVERAGE TOTAL ORGANICS OUTGASSED AT DIFFERENT TEMPERATURES VS. TIME

so that the 30-day point corresponds to the three-day points on the higher temperature curves. The 155 F curve is about three times as high as the 70 F curve when the time scales are shifted in this way, and the 200 F curve is perhaps 10 times higher than the 70 F curve. These differences constitute safety margins in the elevated temperature screening procedures.

The variation of the average total organics outgassing rates of the materials has been plotted against time for the three different testing temperatures in figure 2. There is generally a decrease in this rate with the passage of time -- an aging effect observed also by the Monsanto group (Pustinger, 1965). Thus measurements on freshly prepared samples may well give unduly high results.

Carbon Monoxide

Table II summarizes the results obtained for outgassed carbon monoxide after three days at 155 F and after 30 days at 70 F for the 10 materials out of the 22 materials tested which outgassed a detectable level of carbon monoxide after 60 days at 70 F. The ratios of these values are given in the third column. In three cases the ratio was less than one, which represents a greater production of carbon monoxide after 30 days at 70 F than after three days at 155 F. In two cases the ratio was one, representative of equivalent production of carbon monoxide under both experimental conditions. In five of the 10 cases, more carbon monoxide was outgassed in three days at 155 F than in 30 days at 70 F. The ratio between the respective quantities ranged from 1.7 to 4.0. Only one of the test materials failed the qualification outgassing criteria of a maximum of 5 μg per gram of carbon monoxide after three days at 155 F.

The carbon monoxide ratio data results from the 200 F tests relative to the 70 F tests were similar to those for total organics in that these ratios were much greater than the 155 F ratios.

DISCUSSION

There appears to be little possibility of evaluating the outgassing properties of materials simply on the basis of chemical type. As an example, of the two epoxies tested, one was very low and one was very high in the quantity of organic material produced. Of the five silicones tested, four were low and one produced a large quantity of organic material. A sample of Teflon-impregnated fiber glass tie cord had one of the highest carbon monoxide generation rates of all the materials tested. Vinyls tend to be considerably above the average in organic outgassing, but all materials of this type that were tested were satisfactory under the present qualification criteria. One generalization which might be made is that black materials should be regarded with suspicion; they often contain carbon black, which in turn may contain considerable quantities of adsorbed carbon monoxide and methane.

CONCLUSIONS

On the basis of the reported investigations, the total organics and carbon monoxide outgassing rates of nonmetallic materials at 155 F were generally an order of magnitude or more greater than at 70 F. A temperature of 155 F thus

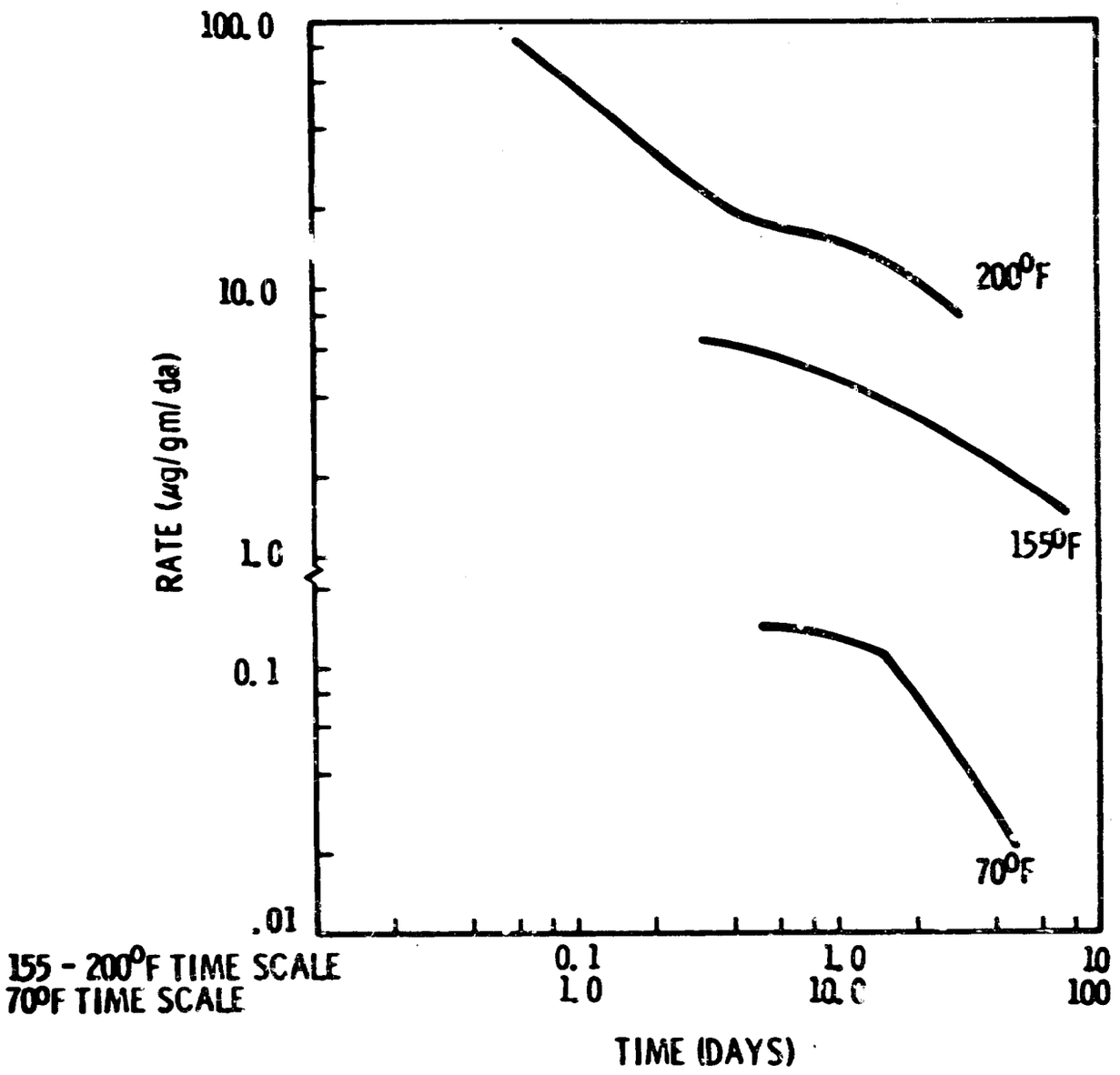

Figure 2. AVERAGE GENERATION RATES OF TOTAL ORGANICS VS. TIME AND TEMPERATURE

TABLE II

CARBON MONOXIDE OUTGASSED VS. TIME AND TEMPERATURE

Material	Carbon Monoxide Outgassed (μg/gram) 155 F/3 Day	70 F/60 Day	Ratio 155/70 F
Silicone adhesive	0.1	0.4	0.3
Polyhalo carbon packaging	6.3	12.7	0.5
Polyolefin/polyamide/carbon	1.2	0.3	4.0
Molybdenum disulfide lubricant	3.5	1.2	3.0
Teflon glass tying cord	2.7	0.7	4.0
Polyurethane electrical foam	0 1	0.1	1.0
Butyl elastomer	3.8	5.7	0.7
Polyurethane elastomer	1.9	1.1	1.7
Epoxy plastic	3.6	0.8	4.0
Silicone glass laminate	0.6	0.6	1.0

appears to be a realistic condition for accelerated testing. In terms of 30-day missions this approach often results in a "built-in" safety factor since higher values were observed in the great majority of cases after three days exposure at 155 F as compared with a 30-day exposure at 70 F. In contrast, the correlation between the outgassing behavior at 200 F and at 70 F is not nearly so meaningful. This means that the large volume of 200 F accelerated testing data available is of limited value for estimating outgassing rates under actual usage temperatures for specific missions.

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DISCUSSION

DR. THOMAS: I might not have understood you on this. Is this an average of all kinds of materials that you tested, or is this a specific organic material?

DR. COX: This is the average of multiples of a larger series of materials.

DR. THOMAS: When you say micrograms of gas-out product formed per material per day, is that figure in the first row corrected for 24 hours, based on a 0.1 day test? Is this a figure that you get during one tenth of a day?

DR. COX: One tenth of a day.

DR. THOMAS: But is the 90 actually showing this multiplied so it is corrected for 24 hours, since you expressed it in micrograms per day?

DR. COX: Yes, that is right. In this large a sample of materials we certainly have, in terms of individual materials, very big differences in individual outgas rates. This is a problem we must face when we are looking at five, six, or seven hundred specific materials that have undergone accelerated testing for a selection program.

We have another very definite problem of the same nature in terms of the differences in the outgassing rates from different batches of supposedly identical materials, and this certainly raises the magnitude of the required overall material testing program.

QUESTION (Honeywell): Bob, I have three questions: One, were there any coating materials in that group? Two, were these materials prescreened and judged acceptable for spacecraft use? Three, do you have similar data for carbon monoxide generation?

DR. COX: The series of materials here did include three coating materials. These materials are generally on the accepted spacecraft acceptable list. Due to the interruption of the testing program, we have limited carbon monoxide data. So I did not wish to discuss that at this time.

QUESTION: You mentioned the outgassing; do you also consider in your criteria the ease of absorbing the outgassed material?

DR. COX: That is a good point. The outgassing tests are done in a static-enclosed system and in terms of a manned space cabin, we are involved with a dynamic system in which we have built-in safety factors for taking care of the outgassing rates, in contrast to the static test system where you do have a chance to set up a true in and out gas exchange equilibrium.

QUESTION: In looking at these data, it occurs to me that with such things as plasticizers, accelerators, and plastics, and so on, you have maybe a total amount that will come out over a total mission time. Also with materials that absorb on the surface, you have a total. I'm wondering that in designing your removal equipment, couldn't you take into account the capacity of your absorbent

that must be able to take care of the total that will be desorbed in that time? This, plus the fact that your high rate of outgassing is at the beginning, might give you two ways of looking at your total protective equipment.

DR. COX: That is very true on a log plot of this data. You get a fairly linear relationship, and in terms of long-term missions, with a normal usage temperature gas-off rate certainly decreases quite rapidly with time.

A THERMOGRAVIMETRIC APPROACH FOR SCREENING CABIN MATERIALS

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Materials proposed for use in manned space cabins must be tested under simulated environmental conditions to identify and measure volatile contaminants. We have performed many such tests on candidate materials under Air Force programs during the last several years. The tests are time-consuming, however, and an initial screening of the materials is desirable to discover the materials that give an abundance of volatile contaminants, and those that give practically none. On this basis, materials could be recommended for either disqualification or conditional acceptance. Materials with moderate amounts of contamination could be tested further to identify and measure individual contaminants.

We recently began a screening program on space cabin materials, using thermogravimetric measurements. The screening is summarized in figure 1. Weight loss from approximately 10 g of a material is continuously recorded, and the temperature is programmed from ambient to 68 C over a four-hour period. The temperature is maintained at $68 \pm 0.5^{\circ}$ for 20 hours, or until the weight remains constant for two hours. All TGA measurements are made in prepurified nitrogen at 5 psia. Materials that yield volatiles from 0.001 to 1% of their weight, excluding water, are taken for further testing.

A TGA system was constructed which incorporates a Cahn RH recording electrobalance. The balance (figure 2) was selected because it will accept up to 100 g without breaking vacuum. Sensitivity is 10^{-6} g. The precision depends on the total load and the amount of weight change, but is approximately equal to 10^{-4} of the total change in weight.

The weighing mechanism of the balance is mounted in a vacuum-tight glass bottle provided with three "O" ring ball-joint ports for attachment of accessory tubes. The mechanism is constructed of materials compatible with high vacuum and corrosive atmosphere. Beam and magnet assemblies are goldplated; no organic adhesives are used. The other materials are principally goldplated aluminum and alnico, platinum, ceramic, and glass. Thus the balance can be used in almost any atmosphere desired, including dry acid and alkali vapors such as anhydrous ammonia and hydrochloric acid. Reactive hydrocarbons likewise do not affect the balance. A pressure of 10^{-7} torr can be maintained with little difficulty.

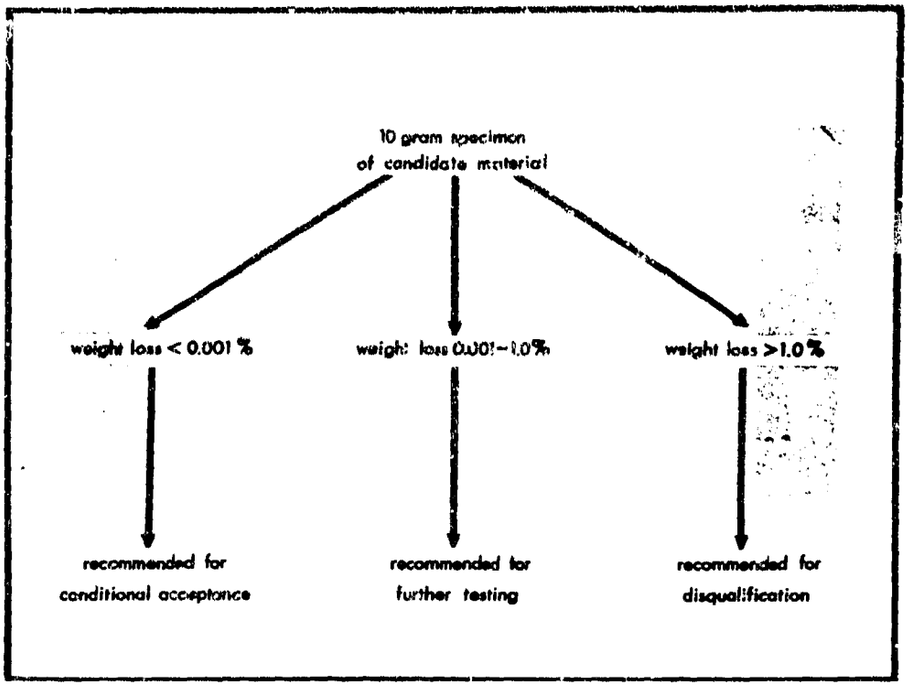

Figure 1. THERMOGRAVIMETRIC SCREENING

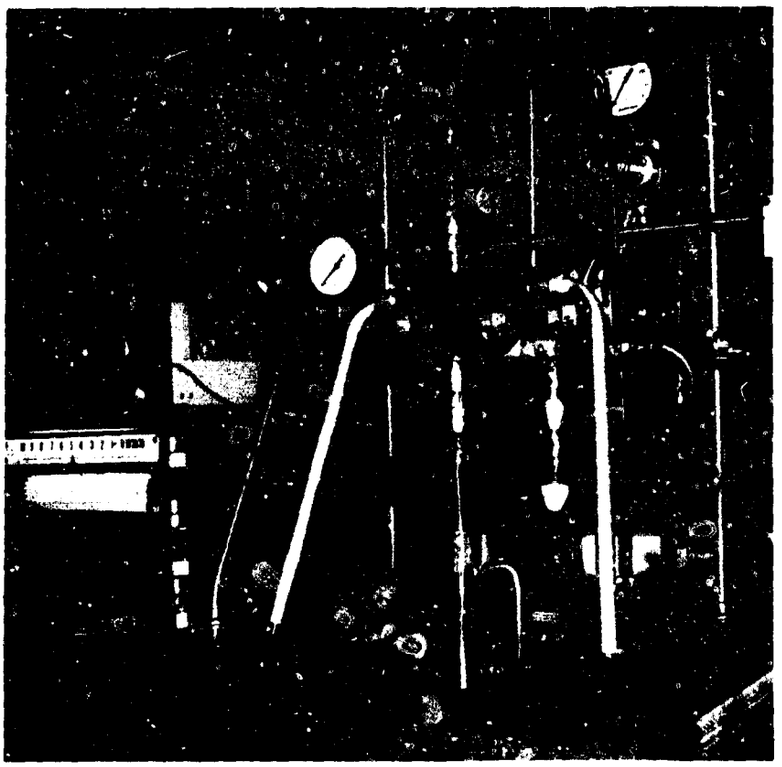

Figure 2. CAHN ELECTROBALANCE IN TGA SYSTEM

The principle upon which the balance operates is simple. A change in weight rotates the beam which is suspended on an elastic metal ribbon. The small flag on the right-hand end of the beam uncovers the cathode of a phototube, thus increasing the phototube current. This current is amplified and applied to the coil attached to the center of the beam. The coil is in a magnetic field, so current through it exerts a moment on the beam, restoring it to balance. The coil current is thus an exact measure of sample weight in accordance with Ampere's law.

Readout is obtained on a mass dial and strip chart recorder trace, both of which are calibrated in grams or milligrams. Any of 16 different recorder spans can be applied at will while recording. The entire balance system is exceptionally rugged, and readings are unaffected by vibration, temperature, or other environmental conditions.

The arrangement of the components of the TGA system is shown in figure 3. The sample is heated with an oil bath rather than a furnace. The slow program rate of one degree every six minutes is more easily achieved by programming the oil bath than by using a heating unit directly. The temperature is programmed by using an F & M Power Proportioning Temperature Programmer which has been specially modified to obtain the slow rate.

Since our interest is in sample weight loss exclusive of water, the amount of water released by the sample must be measured. This is accomplished by passing the vapors from the sample chamber through a tube packed with calcium carbide. There the water vapor generates acetylene gas which is trapped in a sampling loop cooled with liquid nitrogen. At the end of the run, the loop is removed and the amount of acetylene is measured by gas chromatography.

Though the system is at 5 psia, prepurified nitrogen is continuously flowing through it. The nitrogen is introduced into the balance chamber and flushed down over the sample and out through the calcium carbide reactor toward the pump. Not only is the flow not sufficiently brisk to interfere with the mass measurement, but rather the reverse is true. At times aerodynamic "noise" is produced when a sample is heated in a large diameter tube of a static system at a pressure of between 150 and 760 torr. The noise is usually much less in a flowing gas stream. A thermocouple directly above the sample container gives an accurate reading of temperature.

Before entering the system, the nitrogen is dried by passing through drierite and P_2O_5. The pressure in the system is maintained at 5 psia by a Matheson Absolute Pressure Regulator. The nitrogen flow can be measured by a flowmeter positioned in front of the drying tube (not shown) or at the exhaust end immediately before the pump. The flow is adjusted by partially throttling the pump with needle valve K.

The complete TGA unit is shown in figure 4. Note that the sample tube extends down through the table top into an oil bath directly below. The oil bath is on a support which can be readily lowered or raised. The movement of the bath support operates against a counter-balance, thereby facilitating its manipulation.

An attempt is made to remove surface water from the material before running a TGA analysis by storing it for 48 hours in a desiccator charged with drierite. This technique establishes a uniform starting condition for the samples, since previous storage conditions would vary considerably from sample to sample.

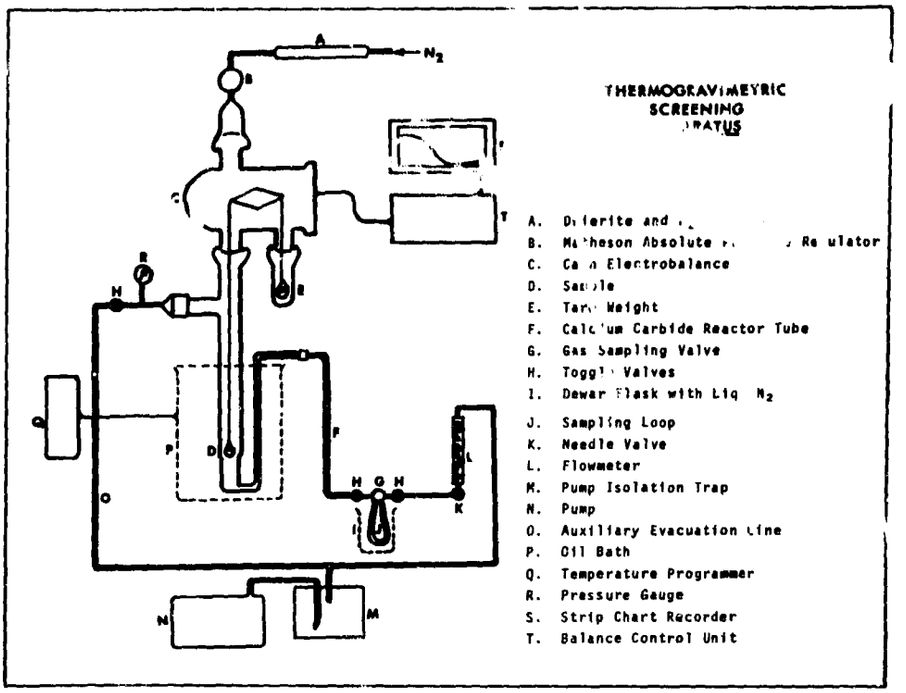

Figure 3. TGA SCHEMATIC

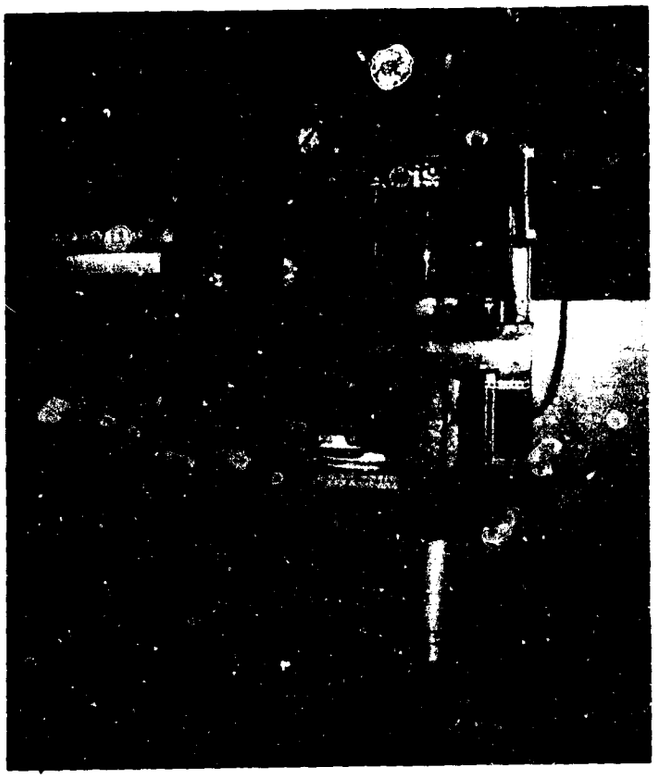

Figure 4. COMPLETE TGA SYSTEM

Figure 5 is a plot of the weight loss of a 2-g specimen of a polymeric foam. Usually 10-g specimens are tested but due to the large volume/weight ratio of the foam, only 2 g could be accommodated. Note the rapid drop in weight at the beginning of the run, even though the temperature is still quite low. This is perhaps associated with the rupturing of gas-filled bubbles at the surface due to the reduced pressure. Later tests on this material showed the blowing agent to be Freon-11 (fluorotrichloromethane), a rather heavy gas. Rupture of the gas-filled bubbles caused some of the solid material to be lost from the crucible in the form of fine dust. Collection of this dust for weighing was difficult because of the static charge associated with this type of material. It was estimated to amount to 2-3 mgs. The formation of the dust could perhaps, in itself, be considered a significant atmospheric contamination. Since only 0.2 mgs of the total 5 mgs weight loss is due to water, the screening indicated further testing of this material in a space cabin simulator.

Figure 6 is a TGA curve of butyl rubber. It will be noted that this material does not begin to lose weight until the temperature has risen considerably, thus indicating that in this case reduced pressure does not significantly affect the material. Once the material starts to lose weight, it continues to do so for the entire 24 hours. Screening of the material also indicates that further testing should be performed. Later gas-off studies indicated the major volatile contaminant to be isobutylene and some di- and triisobutylene.

Figure 7 shows the program of gas-off studies performed on a material if it has been indicated by the TGA screening. Approximately 10 g of the material is placed in a 9-liter boro-silicate glass bottle. In the ground glass mouth of the bottle a gas delivery tube is inserted through a greaseless teflon sleeve. The gas delivery tube has two teflon stopcocks. Where possible, the material is tested as received. If the material requires preparation, this is performed according to the manufacturer's directions. Paints and coatings are applied to aluminum foil substrates. Each chamber is then filled with breathing-grade oxygen at 5 psia and a relative humidity of 20-40%. Three tests are made: one at 68 C for 72 hours, one at 25 C for 30 days, and one at 25 C for 60 days.

As in the past trace contaminant studies, mass spectrometry and gas chromatography are the principal analytical tools used. Our laboratory has recently acquired an extremely versatile, fast scan mass spectrometer, the CEC 21-104 (figure 8), enabling us to couple a gas chromatograph directly to the mass spectrometer. This capability to obtain mass spectra directly and continuously on each component as it elutes from the GC column simplifies component identification and makes it possible to obtain mass spectra of GLC peaks that follow each other too closely to make collection of a clean fraction possible.

The mass range covered by the instrument is mass 1 to 2000 and, although it is a single focusing instrument, it can resolve some of the doublets commonly encountered, such as the doublet from nitrogen and ethylene at mass 28.

The pressure in the analyzer section of a mass spectrometer must not exceed the 10^{-6} torr range. Therefore, only a small portion of the GC carrier gas and sample can be introduced. The method used to accomplish this is shown in figure 9. After the material emerges from the GC column, it passes through a stream splitter. The flow to the mass spectrometer is further restricted by passing it through a

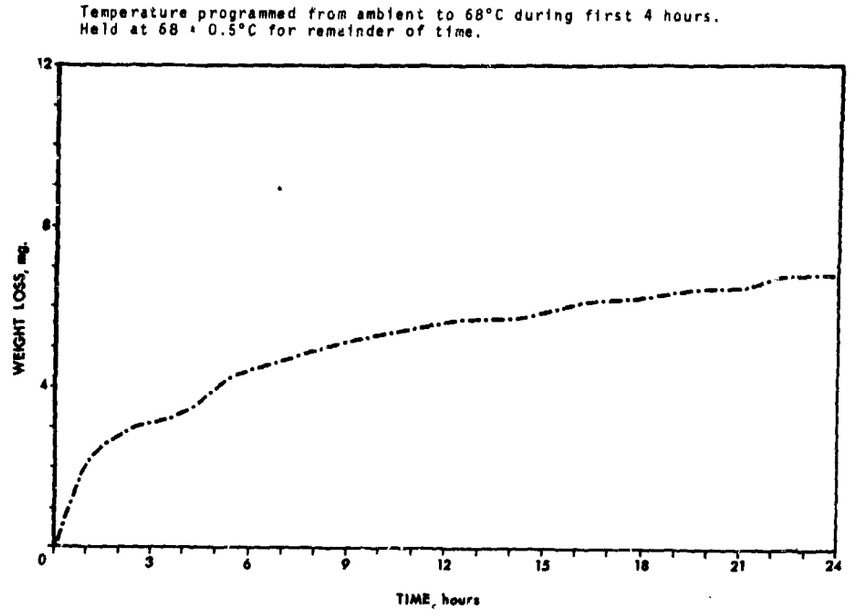

Figure 5. TGA CURVE OF POLYMERIC FOAM, 2 GRAM SPECIMEN

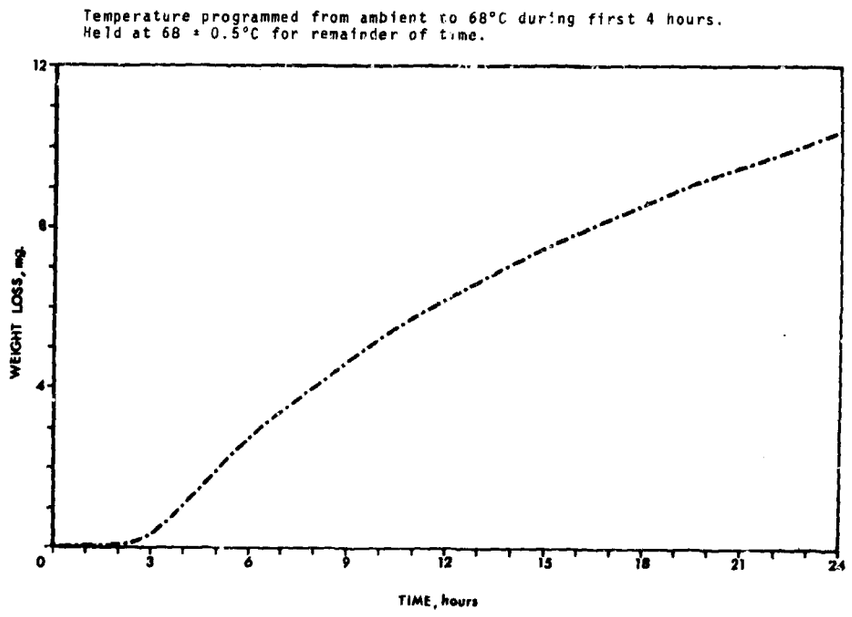

Figure 6. TGA CURVE OF BUTYL RUBBER, 10 GRAM SPECIMEN

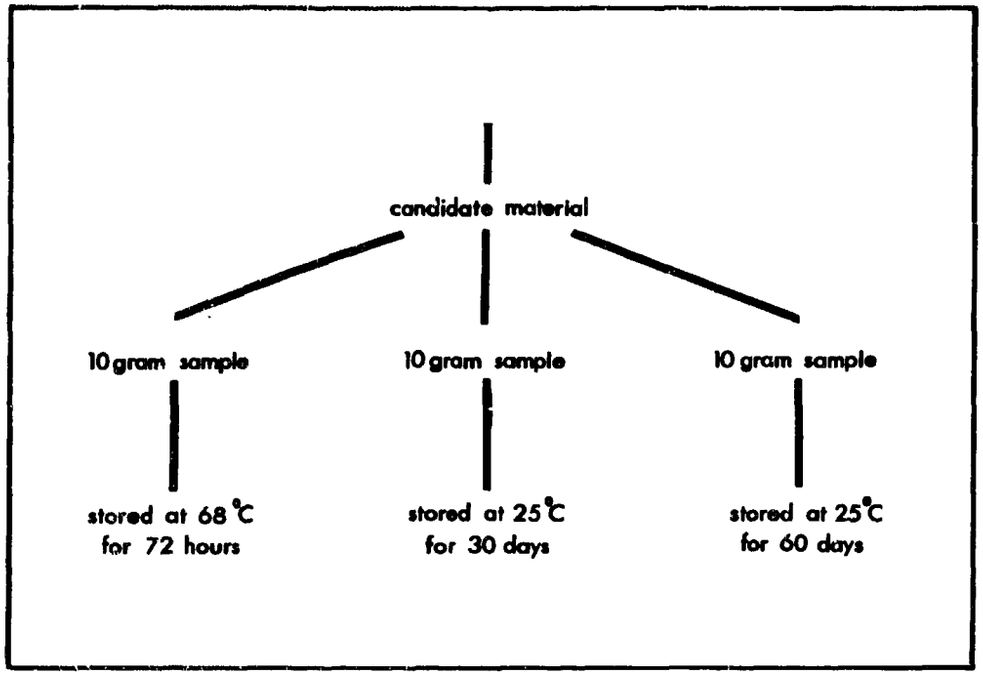

Figure 7. GAS-OFF STUDIES

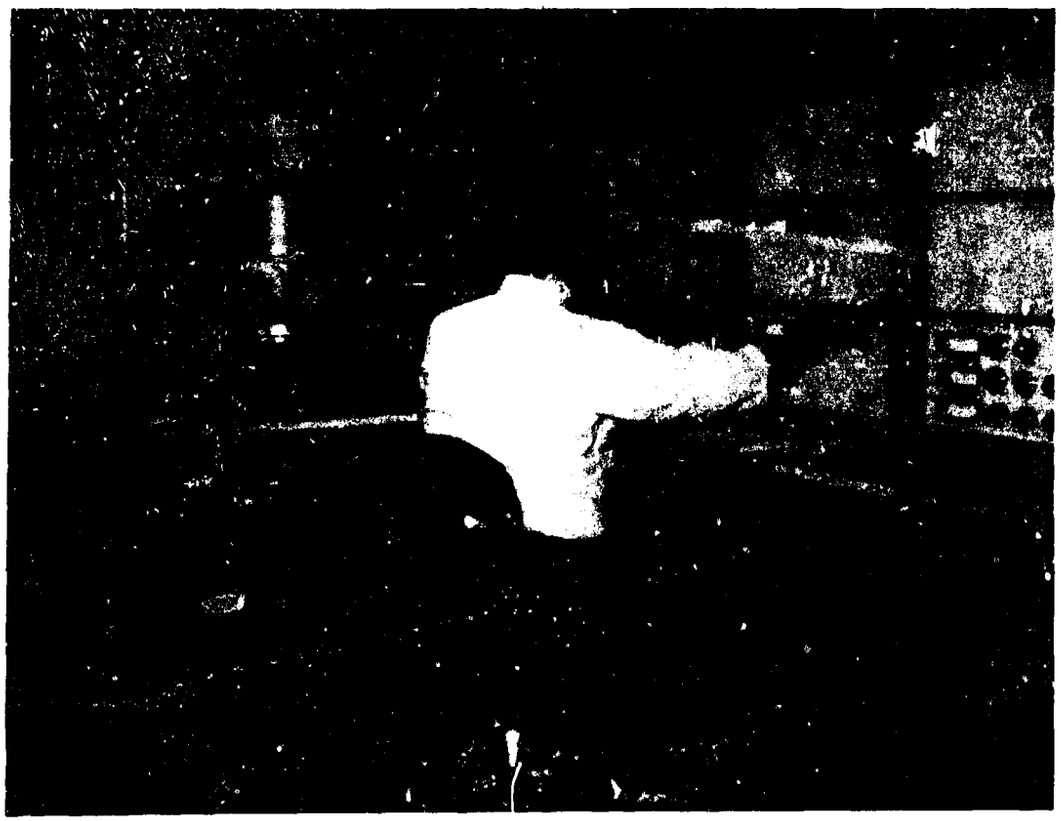

Figure 8. CEC 21-104 RAPID SCAN MASS SPECTROMETER

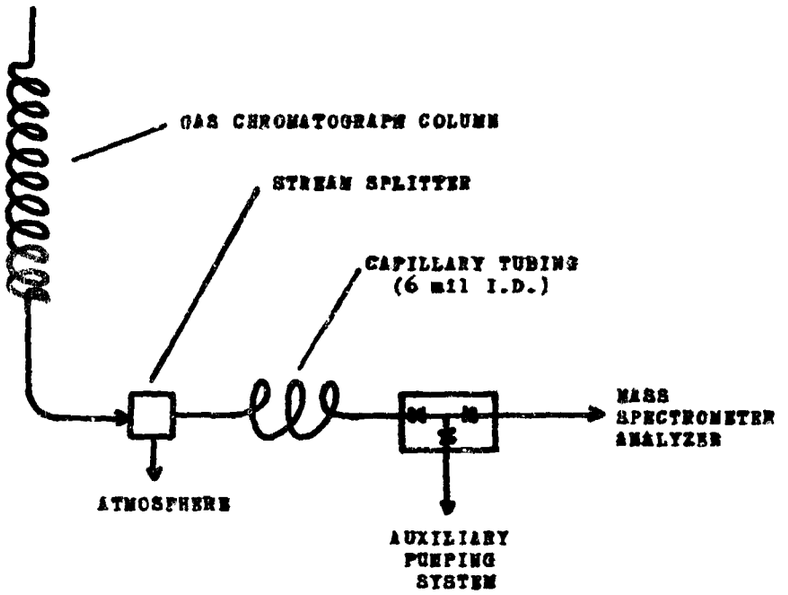

Figure 9. COUPLED GAS CHROMATOGRAPH AND MASS SPECTROMETER

10 grams Silicone Potting Material		10 grams Adhesive Material		10 grams Corrosion Preventive Resin	
Acetic Acid	(0.03 mg)	3-Methylhexane	(7.2 mg)	Ethanol & Isopropanol	(11 mg)
Ethanol	(0.02 mg)	2-Butanone	(23 mg)	n-Propanol	(7.8 mg)
n-Butanol	(0.004 mg)	Ethylacetate	(16 mg)	sec-Butanol	(19 mg)
Xylene	(0.005 mg)	Toluene	(40 mg)	2-Methyl, 2-Butanol	(3.4 mg)
		Xylene	(12 mg)	Xylene	(1.4 mg)
		C_3 Alkylbenzenes	(0.06 mg)		
		C_6-C_7 Monoolefins	(7.2 mg)		

Figure 10. TYPICAL GAS-OFF PRODUCTS AT 25 C IN 5 PSI OXYGEN - Storage Time 30 Days

five-foot length of SS 6 mil ID capillary tubing. Finally, a system of three valves allows the amount of effluent entering the analyzer to be adjusted so that a pressure of approximately 10^{-6} is obtained. These valves, the capillary, and the inlet tube are heated to 150-200 C. Ten scan speeds are available, from 0. 2 sec. to 300 sec. per octave. With the electron multiplier we are able to detect components at one part per million in the GC carrier gas.

Figure 10 shows some trace contaminant products and their amounts, detected from several candidate materials. These appeared after 30 days storage in 5 psia oxygen. The weight in milligrams of each component detected is given in parenthesis after the compound name.

The method of screening remains in the development stage. Considerably more data will have to be accumulated before specifications for acceptance or rejection of candidate material can be rigidly established. At this time, the weight loss data are only used as a means for preliminary and conditional screening of materials with further qualification being dependent on additional gas-off experiments.

DR. HARRIS (NASA, MSC): I would like to know how well your books balanced when you compared your weight loss with your water and total organics excluded.

MR. HODGSON: Generally it is pretty good. We are disturbed a little bit by the fact that we believe some materials are given off that do not necessarily always stay in the atmosphere. So we do get somewhat more weight loss than we can account for all the time in the atmosphere. These may be some recondensation. It really requires a little further study.

QUESTION: Have you looked at the surface area of these materials? There is quite a bit of difference when you use spheres of material or something with a larger surface area.

MR. HODGSON: Yes. We go to great pains to try to keep a uniformity in the surface area, and if we have a sheet of polymer we arrange that all of the surface area is exposed at the same time. In other words, we don't pile it up in a stack so that it doesn't have all the surface area gassing off at the same time. We try to spread it out. We haven't made any surface measurements though.

DR. COX (Douglas Aircraft): I would like to make just one comment on this study. It seems to me that it represents a very fundamental development. Up to this time the initial screening of materials has been based primarily on an odor test, and a development of this type certainly has a high potential with respect to an initial screen and maybe even batch screen of materials. The identification of the specific organics that are outgassed certainly gives much more useful information than a total organic outgassing fraction.

TOXICOLOGICAL SCREENING OF 100 SPACE CABIN MATERIALS

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INTRODUCTION

Since last year's report on the toxicological screening of the gas-off products of space cabin materials (Culver, 1966), a number of additional studies have been completed, including seven of one-week exposure duration and one of 60 days duration. Although the methodology and experimental protocol used for all of these tests will be described, the main purpose of this presentation is to report the results of the 60-day continuous exposure of rodents to the gas-off products of a group of 100 Apollo space cabin construction materials.

MATERIAL AND METHODS

Test System

Figure 1 shows an individual closed-loop system used for this experiment. There are three of these units inside a Thomas Dome, which is maintained at 5 psia and 100% oxygen. This flow diagram does include modifications and changes made in the system since last year's fire. However, a full description and the significance of these alterations is the subject of a later presentation.

The gas-off products in oxygen are circulated through the system by means of two motor-driven blowers installed in parallel for redundancy. To avoid overheating, each blower motor is automatically controlled and alternately operated every 30 minutes. From there, the atmosphere passes through one of two lithium hydroxide scrubbers. This portion of the system is set to alarm at 0.5 percent CO_2. However, when the level reaches 0.2 percent during daylight operation, the unused scrubber is switched into service by means of manually controlled valves. The cycling gas then passes through and is monitored by a flowmeter at a set rate of 4 CFM. With the appropriate sampling lines, oxygen and CO_2 concentrations are monitored continuously by means of a paramagnetic oxygen analyzer and an infrared carbon dioxide analyzer located outside of the Thomas Dome. After passing a static pressure switch which activates an alarm if the flow falls below 10 inches of water pressure, the gas then goes into the oven in which the gas-off products are evolved

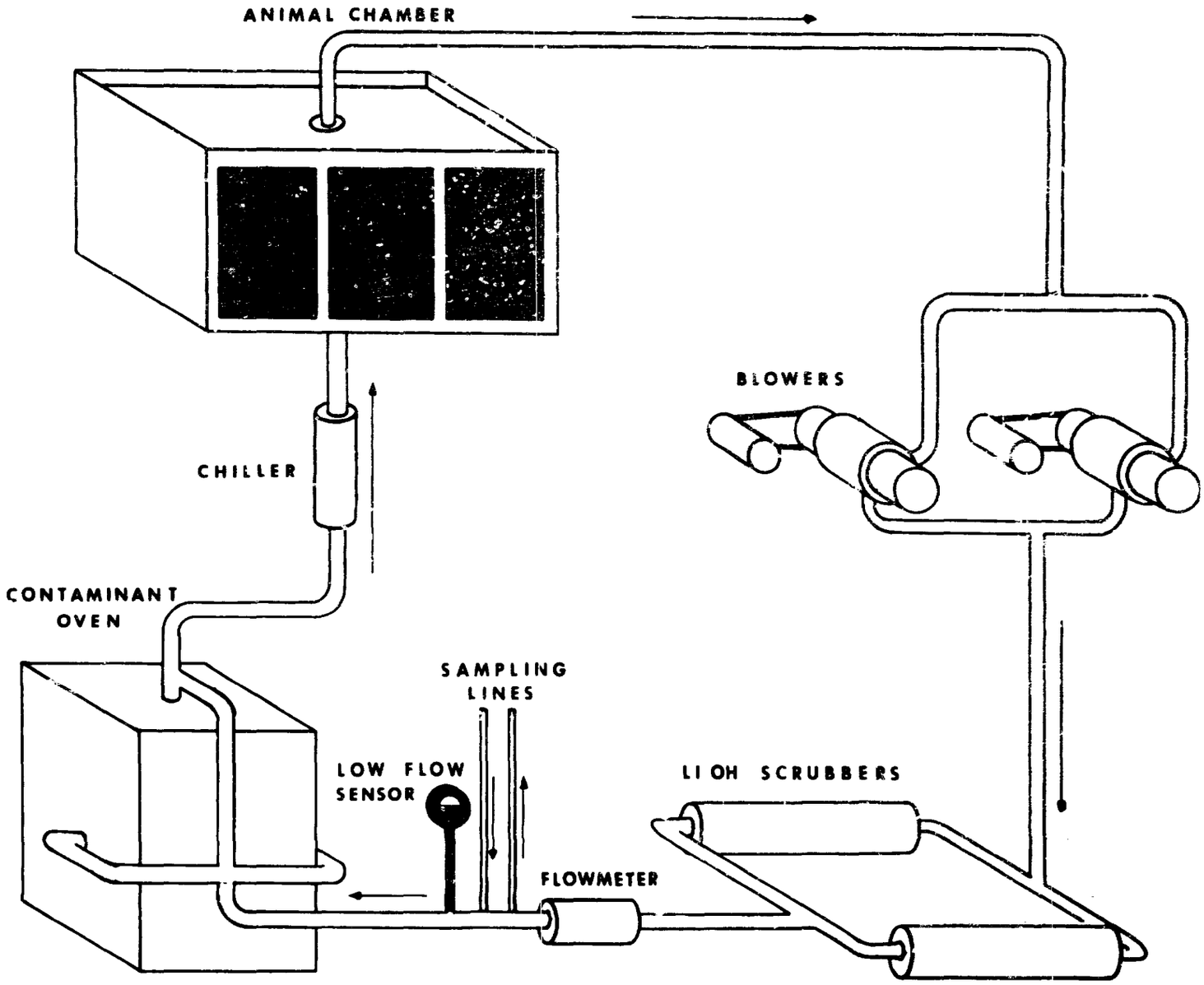

Figure 1. CLOSED-LOOP LIFE SUPPORT SYSTEM - TOXICITY TEST

from the test materials at a controlled temperature of 155 F + 5. To avoid possible overheating of the animal chamber, a bypass line is provided through which part of the flow is directed past the oven. Next, a water chiller which operates at 45 F effectively removes water vapor from the gas flow just prior to its entry into the animal chamber, thus facilitating 50% relative humidity control within the chamber. Two relief valves are located on the chamber. One is set to open to the dome atmosphere when the flow falls to minus 0.3 inch of water pressure because of the natural reduction in total gas volume in the loop system. The other valve is alarmed and opens if the gas flow exceeds 1.5 inches of water pressure.

Heat generated in the dome envelope by the ovens and blower motors in the console shown in figure 2 is dissipated by means of a water chiller positioned in the top near center of the dome. This arrangement provides a cooling effect on the loop system and aids in the 75 F $\pm$ 5 temperature control of the animal chamber.

ANIMALS

Groups of 20 rats (110-135 gm) and 25 mice (15-30 gm), all male, were used in this study. One set was exposed in the closed-loop life support chamber while a second set was housed in the dome and received only 100% oxygen at 5 psia. Another set of controls was maintained at ambient conditions. From receipt to test time, all of the rats were isolated in preconditioning chambers apart from the regular animal colony. All experimental subjects, once selected and grouped by random procedure, were observed and weighed at least twice during the pretest period.

In keeping with the experimental protocol, the exposed animals were frequently observed for symptomatology. Death occurring in any of the rodent groups was recorded. At the completion of the 60-day period, all surviving rats were weighed and again placed in the preconditioning chambers. All animals were weighed weekly for four weeks posttest. Groups of four or five of each species were sacrificed following each of the posttest weighing dates. Animals that died during, or were serially sacrificed after, the experiment were submitted for necropsy. Rat organ weights which included heart, lung, liver, spleen, and kidneys were determined at sacrifice for statistical comparison of organ to body weight ratios.

MATERIALS

The Apollo materials tested were designed as Group "J" and comprised various subgroupings, each of which had already been screened during the seven-day continuous exposures.

Table I lists the general use categories which cover most, if not all, of the materials used in this 60-day experiment. A comprehensive treatment of this subject was included during last year's Conference (Hodgson and Pustinger, 1966).

The raw materials were supplied by the Air Force and prepared according to manufacturers' instructions, and, as much as possible, in keeping with experimental specifications. For example, some epoxy compounds, as well as marking

Figure 2. APOLLO CONSOLE

TABLE I

GENERAL CATEGORIES - SPACE CABIN MATERIALS

60-DAY RODENT EXPOSURE

Adhesives	Greases and Lubricants
Electrical Insulation	Molding Materials
Finishes, Coatings, and Marking Materials	Plastic Laminates
Foams	Potting and Sealing Compounds

materials and other substances, were prepared in the laboratory from the parent reagents or solvents, then allowed to cure or dry at various times and temperatures as required or suggested. Relatively nontoxic liquids such as silicone oil were tested while metals per se were not. However, conductive epoxy resin containing finely divided silver in matrix was a test item. Insulation "spaghetti", various coatings and finishes intentionally painted on aluminum foil substrate were representative sample preparations. Particle size of solid samples was not rigidly controlled. Samples of powders and pellets were tested in their original state, while other solids of larger dimensions were reduced to quarter-inch aggregates.

The test materials in most cases were 10 gm quantities, weighed to the nearest mg. It was impossible, of course, to prepare the samples and make all 100 weighings on the day the study was started. As an alternative, the majority of determinations was performed within five days preexperiment. Stainless steel cups and trays, as well as aluminum "boats", contained the test items in the oven throughout the test period. At the conclusion of the 60-day gas-off period, weight determinations were a comparatively simple task. All 100 samples were weighed on the same day the experiment was terminated.

RESULTS AND DISCUSSION

Weight Loss of Test Materials

In table II, the individual materials are itemized by Air Force Code Number. The initial weights are shown, as well as the posttest weight changes, in grams. The last column expresses the losses as percentage change.

Material nonavailability precluded the use of 10 gram quantities in some cases, while in other situations physical condition of a few test items necessitated the use of more than the prescribed quantity.

As indicated, eight of the samples actually showed slight weight gains. Accelerated oxidation reaction, due to the oxygen rich environment, was a distinct possibility, which could account for these weight increases. Three samples showed

TABLE II

APOLLO SPACE CABIN MATERIALS
(60-Day Study)

AGC Group J

Air Force Code Number	AGC Subgroup Designation	Initial Weight of Material (Grams)	Weight Change (Grams)*	Percentage Change*
005	A (N=14)	10.005	1.991	19.90
018	"	10.083	0.036	0.36
019	"	9.993	0.119	1.20
020	"	9.371	0.637	6.80
022	"	10.168	1.656	16.29
024	"	9.946	+0.171	+1.72
025	"	7.918	0.599	7.55
026	"	0.917	0.107	11.70
030	"	10.144	0.029	0.29
032	"	9.418	0.087	0.93
033	"	10.083	0.157	1.56
035	"	10.771	0.073	0.73
042	"	10.004	0.113	1.13
054	"	10.026	0.005	0.05
006	B (N=14)	10.007	0.074	0.74
012	"	10.016	0.006	0.06
013	"	10.259	1.097	10.68
014	"	10.126	1.822	17.99
016	"	10.119	1.253	12.37
029	"	10.033	+0.002	+0.02
034	"	10.102	0.001	0.01
038	"	9.981	0.134	1.34
039	"	10.051	0.016	0.16
044	"	9.955	0.497	4.98
046	"	5.876	0.380	6.46
059	"	9.944	0.077	0.71
060	"	10.123	0.193	1.89
062	"	10.042	0.039	0.39
118	C (N=4)	10.075	0.234	2.32
119	"	9.976	0.017	0.17
127	"	10.065	0.209	2.08
131	"	9.996	0.008	0.08
045	D (N=6)	9.937	0.063	0.63
105	"	10.123	0.080	0.79
115	"	10.460	0.156	1.52
120	"	10.017	0.119	1.19
130	"	9.983	0.553	5.54
140	"	10.025	0.008	0.08

TABLE II (cont'd)

Air Force Code Number	AGC Subgroup Designation	Initial Weight of Material (Grams)	Weight Change (Grams)*	Percentage Change*
203	E (N=7)	10.055	0.933	9.28
206	"	10.014	0.028	0.28
209	"	9.955	+0.001	+0.01
228	"	10.212	+0.075	+0.74
235	"	9.964	0.143	1.43
247	"	9.991	0.084	0.84
325	"	9.943	1.323	13.33
053	F (N-12)	10.092	0.004	0.04
207	"	10.017	0.041	0.41
215	"	9.928	0.002	0.02
223	"	9.928	0.470	4.75
226	"	10.010	+0.009	+0.09
240	"	10.292	0.017	0.13
244	"	10.020	+0.003	+0.03
249	"	10.129	0.001	0.01
259	"	3.416	0.202	5.92
266	"	10.166	0.014	0.14
314	"	3.266	0.098	0.30
317	"	10.027	0.207	2.06
023	G (N=16)	10.149	0.004	0.04
037	"	10.055	0.114	1.13
063	"	10.100	0.043	0.04
066	"	10.035	0.118	1.18
069	"	8.152	0.096	1.17
123	"	10.045	0.000	0.00
135	"	10.060	0.259	2.57
141	"	10.666	1.099	10.31
221	"	9.968	0.038	0.38
245	"	10.020	0.252	2.50
258	"	9.989	0.941	9.42
308	"	9.985	0.135	1.35
312	"	10.132	0.101	0.99
321	"	9.954	0.265	2.62
353	"	10.031	+0.041	+0.41
359	"	9.942	0.069	0.69
064	H (N=15)	10.031	0.026	0.26
067	"	10.002	0.085	0.85
071	"	10.082	0.009	0.09
072	"	42.936	0.001	0.00
112	"	10.042	0.272	2.71
134	"	10.006	0.110	1.10
142	"	10.359	1.509	14.58
222	"	10.000	0.040	0.40
232	"	9.987	0.164	1.64

TABLE II (cont'd)

Air Force Code Number	AGC Subgroup Designation	Initial Weight of Material (Grams)	Weight Change (Grams)*	Percentage Change*
251	H (N=15)	10.029	0.014	0.14
305	"	11.439	11.398	99.50
309	"	9.967	0.719	7.21
335	"	6.361	0.187	2.94
337	"	10.027	0.014	0.14
361	"	9.990	0.278	2.78
065	I (N=12)	9.956	0.085	0.85
068	"	10.038	0.058	0.58
103	"	9.999	0.016	0.16
143	"	10.378	1.312	12.68
302	"	10.025	0.003	0.03
316	"	10.072	0.031	0.31
329	"	9.992	0.034	0.34
336	"	9.964	+0.066	+0.66
340	"	10.007	0.000	0.00
358	"	10.070	0.092	0.91
362	"	10.008	0.097	0.97
373	"	10.122	0.045	0.44

*Weight changes are losses unless otherwise noted.

no weight change. Forty-five lost one gram or less, 10 between one to two, and 15 between two to seven, with the remainder, except for one, showing various weight losses from approximately seven to 20 grams. The one exception, a fluorolube compound number 305 in Subgroup H, demonstrated a 99.5% weight loss during the test period. At the conclusion of one of the preliminary seven-day tests, another sample of this compound demonstrated a 68% weight loss. As a recheck, still another sample of material was placed in a laboratory oven in ambient air and normal pressure. A 20% weight reduction occurred within 24 hours. The vapor pressure of this substance is obviously less than the Freons but certainly as great or greater than that of water.

PERFORMANCE OF TEST SYSTEM

The closed-loop life support system previously described here and elsewhere (Culver, 1966; MacEwen and Geckler, 1966) operated almost flawlessly for the duration of the test period. The wet-bulb thermocouple probe located in the animal chamber malfunctioned sporadically during the course of the experiment, causing erroneously high relative humidity recordings.

The dome equipment and control system functioned well except for one minor incident. On the first day of animal exposure, a vacuum pump failure occurred. The problem (electrical in nature) was corrected, however, and normal operation

was restored in a few minutes without change in pressure or oxygen concentration within the dome and life support system.

SYMPTOMATOLOGY

The groups of exposed rodents were observed, although uneventfully, every hour throughout the duration of the experiment for any overt manifestations of toxic response. The negative results, of course, do not detract from the value of this type of continuous observation which will be utilized profitably in future uninterrupted inhalation studies.

MORTALITIES

Few deaths occurred in the groups of 20 rats and 25 mice during the course of the 60-day experiment. One rat in the exposed group died, but only after the study was completed, when the dome was repressurized to ambient conditions. Gross postmortem examination revealed severe chronic murine pneumonia of all lobes with multiple abscesses and adhesions in evidence. In light of the obviously moribund condition of this animal, it was not surprising that the animal could not withstand the stress "of the sudden removal . . . back to ambient air", resulting in "an effective relative hypoxia" (Kaplan, 1966). Indeed, it is reasonably speculated that this rat would have succumbed to the disease sooner had it not been for the benefit of the oxygen enriched atmosphere during the course of the experiment.

On the 14th exposure day, one rat died in the dome control group. Death was probably due to a broken extremity observed on removal. An ambient control rat was found dead on the 45th day of the experiment. Autolysis prevented the determination of the cause of death.

Two rats, one each in the exposed and ambient control groups, were eliminated at the third and fourth posttest sacrifice periods, respectively, inasmuch as incisor malocclusion and subsequent inanition contributed to severe weight losses.

No mortality occurred in the sets of 25 mice that constituted the exposed and dome control groups. One mouse was removed from the ambient controls and sacrificed because of moribund condition. This was a direct result of infected lacerations on the sacral area. Wounds of this nature, inflicted by cage mates, have been observed frequently among groups of male mice during other continuous inhalation studies of 90 days or more. This aberrant behavior among groups of male mice is apparently age-related but cannot be fully explained at this time.

PATHOLOGY

The necropsy findings showed no evidence of pulmonary injury that could be directly attributed to the effect of the gas-off products of the test materials. Chronic murine pneumonia (CMP), although observed in only one of the exposed rats, did occur to a much greater extent in both surviving control rat groups. The incidence of this disease was 26% and 18%, respectively, for the dome and ambient controls. The problems associated with a high incidence of CMP in experimental

rat populations was the subject of discussions in papers presented at the last two annual Conferences (Innes, 1965; Harper, 1966).

Other necropsy findings included several observations of mild congestion and slight reddening of the lungs; this was attributed to etherization of rodents for sacrifice purposes. This technique is no longer used, but the alternative, that of an overdose of barbiturate, is also open to question as the best technique of sacrifice.

No incidence of CMP was observed in any of the surviving mice groups; however, a significant number did show mild congestion and slight reddening of the lungs, as previously noted in rats.

BODY WEIGHT DATA

The growth curve for rats is presented in figure 3. It can be seen that the mean weight gains closely parallel each other. A second scrutiny would suggest, perhaps, significant differences. However, statistical comparison of the mean body weight gains evidenced no significance at the 95% level of confidence; the large standard deviations, indicated for all experimental periods, tended to negate any seemingly real difference.

The body weight data for mice are presented in figure 4 for the same periods shown for the rats. Again, as with the rats, the mean weight gains of the experimental groups of mice parallel each other closely; and, as before, at second glance some of the differences in weight gain would appear to be real, but statistical treatment of the data showed nonsignificance at the 95% level of confidence.

Examination of the body weight data of rats (table III) once again reveals a definite trend. The mean percent weight gains shown indicate that dome-housed rats (both the exposed and controls) gained weight at a slower rate than did those housed under ambient conditions. For example, Group J exposed and dome controls exhibited mean weight gains of 108 and 103%, respectively, during the 60-day period; by contrast, this value for ambient controls was 169%. These differences are also seen in comparing the last data lines where mean % gains over initial weights are 290, 263, and 320 in the same order as before. A similar trend, although less pronounced, was also observed in the mouse data; for example, 35.8 and 25%, as compared with 45.6%. At this point, it must be stressed that these comparisons do not imply differences between weight gains of rodents exposed to the Apollo materials and those of dome controls, inasmuch as the values are very comparable; rather, the implication from the data is that the rodents housed within the dome in an atmosphere of 100% oxygen at 5 psia did not attain growth comparable to that exhibited by the controls housed under ambient conditions. An additional, perhaps expected, weight trend was also noted. The data show that once outside the dome environment, there was a compensatory gain as early as the first week posttest; this is indicated by the tabulated mean percent gain over that recorded at the conclusion of the 60-day test period. The one-week gains for dome-housed rats were 8.5 and 10.3% as opposed to 2.7% for the same period with rats under ambient conditions. Similarly, values for mice were 5.2 and 14.1% compared to 1.7%.

The most obvious assumption that can be made from these observations is that both the exposed and dome controls had undergone a stress that perhaps can

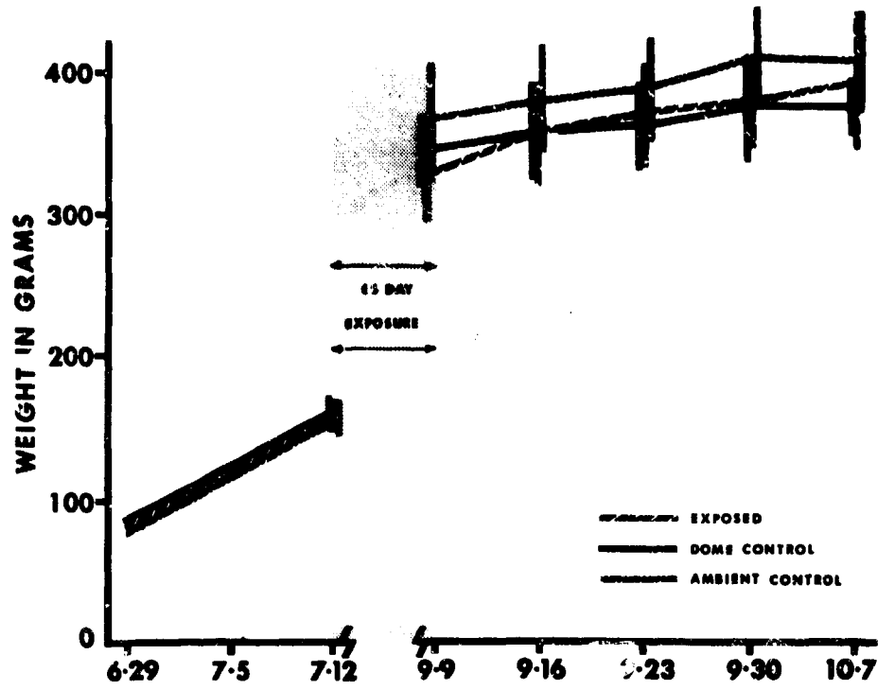

Figure 3. MEAN BODY WEIGHT CHANGE - RATS

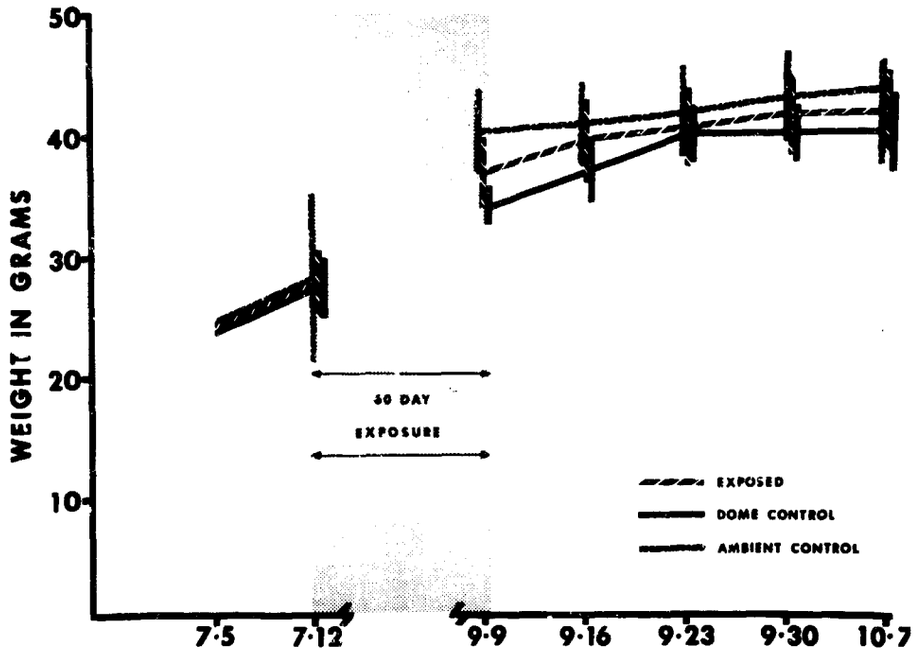

Figure 4. MEAN BODY WEIGHT CHANGE - MICE

TABLE III

BODY WEIGHT DATA - MALE RATS

60 DAY APOLLO MATERIALS SCREENING TEST

		PRE-TEST		TEST	POST TEST			
		7-5	7-12	9-9	9-16[1]	9-23[1]	9-30[1]	10-7[1]
MEAN % GAIN OVER PREVIOUS WEIGHT	EXPOSED	43.0	21.0	108.0	8.5	4.4	2.1	2.1
	DOME CONTROL	38.0	30.3	103.0	10.3	3.17	2.4	0
	AMBIENT CONTROL	46.0	29.1	169.0	2.7	2.5	6.3	-1.8
MEAN % GAIN OVER INITIAL WEIGHT	EXPOSED	43.0	75.0	290.0	311.0	339.0	351.0	360.0
	DOME CONTROL	38.0	78.7	263.0	301.0	314	325.0	325.0
	AMBIENT CONTROL	46.0	88.5	320.0	335.0	344.0	384.0	375.0

[1] - POST EXPOSURE SACRIFICE DATES

be attributed to the 100% oxygen - 5 psia environment. Close evaluation of weight measurement and other parameters in future tests will confirm or refute this speculation.

COMPARISON OF ORGAN TO BODY WEIGHT RATIOS

Statistical comparisons of organ to body weight ratios are the last criteria of toxic response which remain to be reported.

The mean kidney to body weight ratio of rats exposed to Group J materials showed a significant difference (at the 0.05 level) when this value was compared with that of the dome controls following the second posttest sacrifice. A difference was also noted when the mean value for heart to body weight ratio of the exposed group was compared with that of the ambient control at the fourth sacrifice period. Equally unexplained differences were found in comparisons of the liver to body weight data between the dome and ambient controls following the second and fourth posttest sacrifice dates. No additional significant differences were found in comparisons of any other organ to body weight ratios among the three rat groups at any of the various sacrifice periods. No meaning of toxicological significance can be attributed to the preceding positive findings. Unrelated organ to body weight data comparisons were also found in several of the seven-day experiments. Causative factors, such as human and procedural error, will be discussed in another presentation.

SUMMARY

In summary, the results of this experiment have shown that, based upon the criteria used for assessment of effect, no measurable toxic response was observed in rodents exposed continuously for 60 days to the gas-off products of 100 Apollo space cabin materials in a 100% oxygen - 5 psia environment. The functional reliability of the closed-loop, life support system working in conjunction with the dome chamber and its associated controls has been adequately demonstrated by the successful physical completion of this study. The environmental parameters of this system can be modified for other studies which may require other experiment conditions of varying pressures and gas mixtures. Correctly conceived and properly executed, both biologically and mechanically, future toxicological screening of the remaining 400 Apollo materials should present no significant problem.

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QUESTION: Outside of the weight change of the material tested, you did no atmospheric sampling of materials within the animal chamber to identify the gas-off products?

MR. HAUN: No. Routine sampling wasn't within the scope of this program. It would have been very interesting but we did not do it.

QUESTION: Within the dome exposure chambers, were the control housing units similar to these, rather than the open mesh or screen type of units?

MR. HAUN: The housing units for the controls in the dome were different. They were hanging, wire-mesh cages as opposed to the test animals which were housed in the life support system.

QUESTION: Solid side?

MR. HAUN: Yes, leak proof.

QUESTION: Do you feel that this may have contributed to the weight gain, the difference in the type of caging?

MR. HAUN: I think, as I said before, we noticed a subnormal weight gain of both the exposed animals and the dome controls as compared with the ambient controls, and these animals were very similar in their weights, so I can't see really that the difference in caging mattered so much with these two groups of animals. There are some variables concerned that we have noticed; for example, we have noted that the animals exposed to the Apollo materials within the enclosed chambers don't seem to eat as much as the other animals.

QUESTION: Do you feel that you would have gotten the same results had you used equal aliquots of the materials based on their weight use in a cabin rather than using equal weight?

MR. HAUN: No. This was a bit of a problem. As I said, we only used 10 gram quantities in most cases here. Material availability was somewhat of a problem. As you say, it probably would have been better to use these weight proportions as they are used in the cabin, but for this initial 60-day screening, this is the best we could do at the time. Mr. Egan in the next presentation will mention more about weights of materials used.

7-DAY TOXICITY SCREENING PROGRAM

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INTRODUCTION

A 7-day toxicity screening program has been established at the Toxic Hazards Research Unit to evaluate candidate space cabin materials. Data are available in the literature detailing the relative toxicities of many of these compounds. However, space cabin atmospheres and excessive heat may effect changes in this information. Living organisms may be adversely influenced by an oxygen-rich, reduced pressure environment contaminated with gas-off products. To this end, 7-day continuous exposure trials were initiated in order to screen some 500 candidate compounds.

Materials and Methods

Utilizing a Thomas Dome containing three life support systems and related ovens for gas-off product generation, seven tests have been completed on approximately 25% of the candidate cabin materials. Details pertinent to the design of the Thomas Dome and the complement life support systems within the dome have already been reviewed at this meeting. With regard to the control of the environmental conditions within the dome and the closed-loop systems, the following parameters were monitored and automatically controlled:

1. Pressure: 260 mm Hg + 5 mm
2. Oxygen: 99.95% + .05%
3. Carbon dioxide: 00 - .05%
4. Temperature: 75 F + 5%
5. Relative humidity: 50% + 15%

The ambient control groups were maintained in an atmosphere whose temperature and relative humidity limits were the same as those in the dome. The dome environmental regulatory mechanisms were described at the previous symposia (Culver, 1966; Thomas, 1965).

A typical experiment in this series included the following animal population:

1. Exposed group - 20 rats, 25 mice
2. Dome control - 20 rats, 25 mice
3. Ambient control - 20 rats, 25 mice

All rats were male, Sprague-Dawley variety, whose initial weight range was 110-130 grams. Mice weights were 20-25 grams at the outset of the test.

As many as three different groups of compounds can be tested simultaneously in the three life support systems. There can be a total of three exposed sets of rodents in any one test. A control group of rats and mice was situated outside the life support loops, but within the Thomas Dome itself. An additional group of rodents was maintained at ambient conditions. In the last two experiments, a third control group was incorporated into the program. The latter group, termed the positive control, was placed within one of the life support systems and subjected to all conditions relative to the exposure complex with the exception of environmental gas-off products. All rodents were maintained in a similar temperature and relative humidity atmosphere.

Upon receipt, all rats were identified, weighed, and isolated. Groups of 25 mice were weighed and isolated in a similar fashion. This procedure was accomplished two weeks before the start of any experiment. Exactly one week preexposure, a second weight was recorded and the rats were randomly grouped into sets of 20; the mice into sets of 25. A third weight recording was added just prior to the actual placement of the rodents within the dome area. At the conclusion of the trial, all animals were weighed and isolated.

Exactly one week postexposure, all animals were weighed, and a sequential necropsy of the rodents was initiated according to the following plan:

One week postexposure:	Weigh all animals, sacrifice five rats and five mice from each group
Two weeks postexposure:	Weigh all remaining animals, sacrifice five rats and five mice from each group
Three weeks postexposure:	Weigh all remaining animals, sacrifice five rats and five mice from each group
Four weeks postexposure:	Weigh remaining animals and submit all for sacrifice

Gross pathology examinations were obtained on all rodents. Heart, lung, liver, spleen, and kidney weights were recorded for rats only. All animals were observed throughout the experiment, especially during exposure and postexposure periods, and any abnormal clinical symptoms were recorded.

A brief comment concerning the nature of the test compounds should be noted. Basically, any compound applicable to space cabin construction, incorporated as equipment, or utilized for interior modifications, may be considered for this screening program. Inks, dyes, resins, plastics, epoxies, and many more generic groupings have been included in this report.

A standard criterion was employed to determine the quantity of test material positioned in each oven. When the proposed use of the material in the actual Apollo capsule exceeded one pound, a 100 gram sample was employed. If the projected use was equal to or less than one pound in the capsule, the oven sample was 10 grams. Weights of all compounds placed in the ovens were recorded at the start and termination of any experiment. A more detailed description of the material preparation has been presented earlier in the symposium by Mr. Haun.

Results and Discussion

The discussion section of this paper concerns general observations applicable to all seven Apollo experiments. Dome operations were uneventful during the experiments. All parameters were maintained within prescribed limits.

With regard to the test materials, various weight modifications were observed at the completion of all tests. A green ink exhibited a 23.3% gain in weight. This was probably due to some water uptake. An epoxy-ester compound showed no change in weight. Any number of explanations may be offered for this finding; low physical and/or chemical activities or an equilibrium between oxidation reactions and water uptake rates. Finally, a flurolube compound was observed to have lost more than 68% of its initial weight after seven days. An oxidative-type reaction is the probable explanation for this phenomenon. Nearly 67% of all test compounds exhibited a weight change of less than 1%.

Approximately 680 rats and 850 mice were utilized for these screening tests. Of this number, nine rats and one mouse died or were sacrificed prior to the termination of the study. The mouse was removed from the study due to accidental injury. Four rats included in the groups exposed to the gas-off products were eliminated from the study before completion of the postexposure sacrifice periods. The remaining five rats were members of various control sets. Evidence of malocclusion and pneumonia rendered these rats unsuitable for inclusion in the study.

Graphical representation of rat growth is indicated in figure 1. One experiment is utilized to show typical growth patterns of rats as recorded for the exposed group and the positive, dome, and ambient control sets. The graph shows the mean values of the exposed group (K) and the positive, dome, and ambient control sets from a point one week prior to the start of the exposure to a point four weeks postexposure. It can be seen that the growth rates of all four experimental groups were normal. However, all three groups subjected to a 100% oxygen - 5 psia atmosphere showed growth depression during and at the termination of exposure, followed by a compensatory recovery phase. This phenomenon can be viewed in a different manner by observing the graphical treatment in figure 2. The mean and standard deviation (S.D.) of each group is included. The horizontal lines through the vertical S.D. bars for each group represent the 95% Confidence Limits computed about the ambient control mean. Using this as a reference point, it is possible to determine the length of time taken by each group before it returned to ambient control limits. The positive control appears to have overcompensated in the final observation. This could be due to an actual physiological process or to an experimental error. The exposed group showed the same growth depression noted in the other oxygen-reduced pressure groups. However, its mean growth rate appeared to return to the ambient limits.

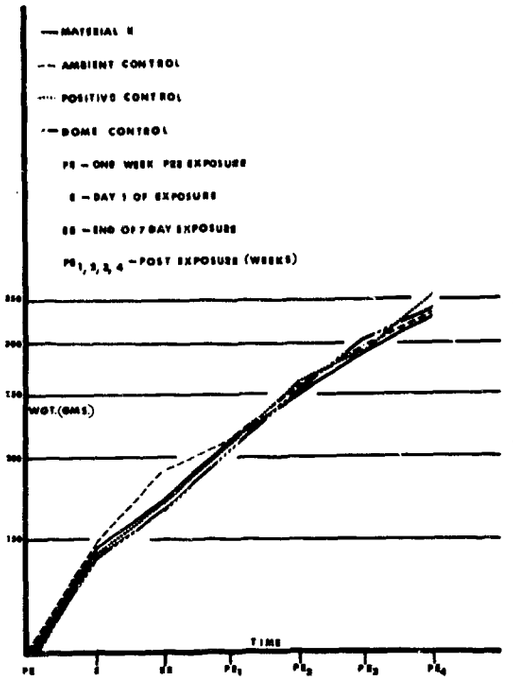

Figure 1. RAT GROWTH CURVES

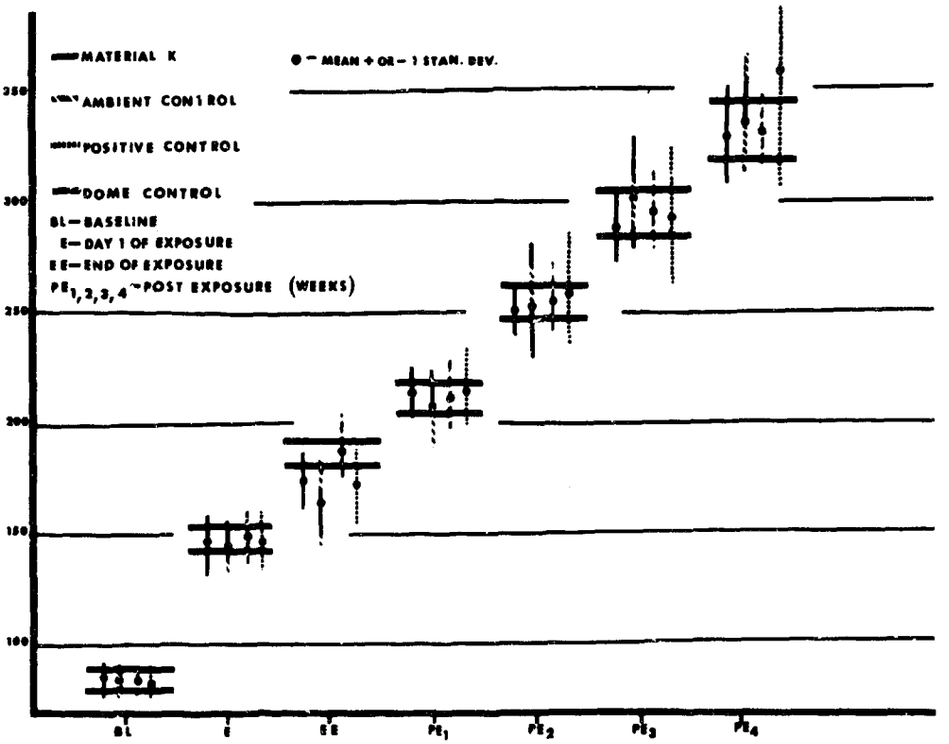

Figure 2. RAT GROWTH SHOWN FOR WEEKLY INTERVALS: Mean $\pm$ 1 S. D. of each group superimposed upon ambient control 95% Confidence Limit.

A typical graph of mice growth is indicated in figure 3. Here again, the 100% oxygen and reduced pressure environment showed some influence of apparent growth depression upon mice. All groups appeared to return to within the 95% Confidence Limits computed about the ambient control mean as depicted in figure 4.

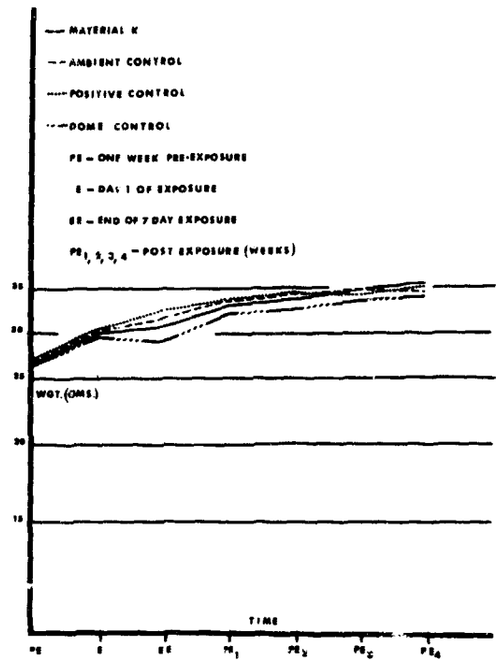

Figure 3. MICE GROWTH CURVES

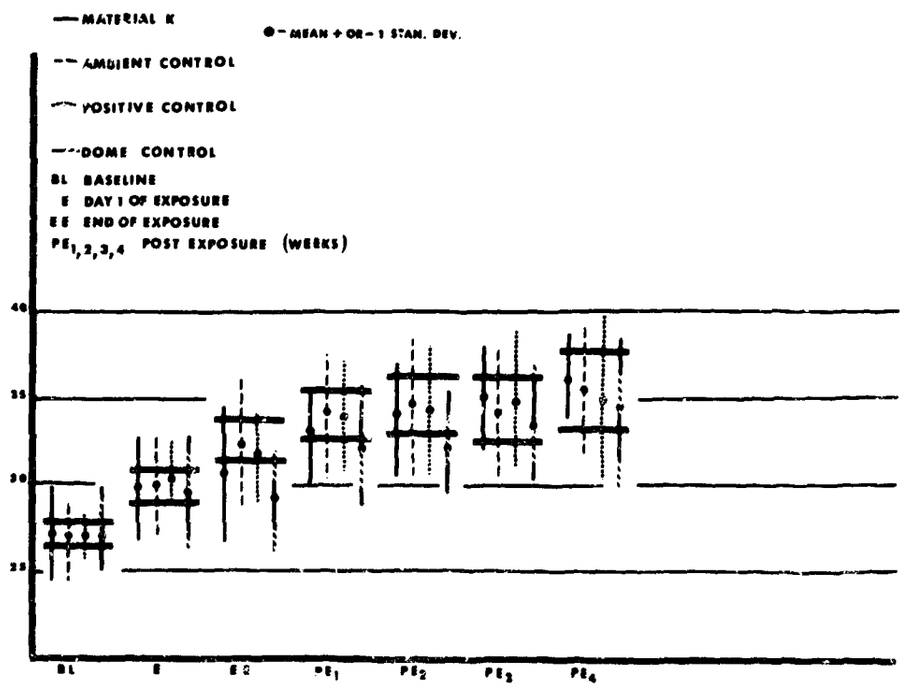

Figure 4. MICE GROWTH SHOWN FOR WEEKLY INTERVALS: mean $\pm$ 1 S.D. of each group superimposed upon ambient control 95% Confidence Limit.

Again, these graphic illustrations are representative of the typical rodent growth patterns seen in the Apollo experiments to date. The depression in growth following the termination of the exposure trial is characteristic of this test series. With the exception of this peculiarity in the growth curves, none of the test groups showed any manifestation of a toxic response. One group (F) showed a remarkable dropoff in weight during the last two postexposure observations. In light of this finding, the entire F group of materials was rescreened in two additional experiments, but no similar growth retardation phenomena was detected. A series of subsets were established and screened. These also showed no adverse effects on growth curves in either mice or rats. However, any conclusions here must be made in light of the fact that although the types of materials were identical in each experiment, they did not necessarily originate from the same batch source. Future plans call for the comparison of various batches to determine if indeed there is any significant difference.

A source of valuable information in these experiments was the organ-body weight ratio. As an integral part of each necropsy, heart, lung, liver, spleen, and kidney weights were recorded. Using the "t" test, statistical evaluations were made comparing the exposed group to each of the control sets. This information, coupled with the data on growth curves and pathology reports, furnished much of the basis for determining whether the test materials elicited any toxic response.

The mean organ-body weight values are listed in tables I-VII. Most of the positive "t" tests (95% Confidence level) occurred with the liver and kidney data. The affirmative tests involving the heart, spleen, and lung values were fairly small in number.

A close examination of the information recorded on tables I-VII indicated that the means included in some positive "t" tests vacillated, with respect to each other, during the four-week postexposure observation period. Positive 't' tests rarely repeated themselves during succeeding observation periods. Any toxic manifestation should have demonstrated some regular pattern of organ involvement, that is, duration and direction of response.

Several cases of positive "t" tests were resolved on the basis of the pathology reports. Lung infection, splenic engorgement, and enlarged heart were found to be regularly implicated in the positive tests.

CONCLUSION

In summary, it is possible to state that none of the materials screened at 100% oxygen - 5 psia atmosphere exhibited any toxic manifestations as demonstrated by negative symptomatology and pathology, normal growth curves, and inconclusive "t" tests on organ-body weight ratios.

TABLE I

Organ-Body Weight Means

Organ	Sacrifice Period	Exposed I	Exposed II	Exposed III	Dome Control IV	Ambient Control V
Heart	1	.438	.385	.408	.490	.414
	2	.373	.432	.492	.453	.424
	3	.378	.373	.298	.337	.306
	4	.351	.411[A]	.376	.311[A]	.392
Lung	1	.794	.738	.791	.747	.873
	2	.756	.744	.700	.718	.706
	3	.672	.684	.702	.597[A]	.751[A]
	4	.675	.665	.715	.567	.546
Liver	1	4.85[A]	4.82[B]	4.90[C]	4.38[D]	5.40[ABCD]
	2	4.09[A]	4.11[B]	4.44[C]	4.92[ABC]	4.67
	3	4.17	4.27	3.67	4.50	4.59
	4	4.49	4.51	3.26	4.49	4.11
Spleen	1	.611	.451	.493	.386	.461
	2	.474	.412	.597	.512	.552
	3	.477	.609	.461	.481	.479
	4	.476	.424	.309	.589	.321
Kidney	1	.943	.929	1.050	.965	1.030
	2	.897	1.010	1.040	1.040	1.040
	3	.871	.961	.906	.899	.837
	4	.993	.913	.905	.879	.840

A, B, C, D - Pairs of organ-body weight means involved in positive "t" tests (95% Confidence level)

TABLE II

Organ-Body Weight Means

Organ	Sacrifice Period	Exposed I	Exposed II	Exposed III	Dome Control IV	Ambient Control V
Heart	1	.370[A]	.424	.442	.461[A]	.438
	2	.380	.396	.412	.415	.403
	3	.415	.403	.411	.386	.385
	4	.431	.363	.367	.384	.379
Lung	1	.826	.801	.746	.846	1.07
	2	.856	.704	.706	.730	.883
	3	.687	.920	.813[A]	.612	.562[A]
	4	.691	.650	.743	.781	.607
Liver	1	4.45[B]	5.61[AC]	4.74	4.78[A]	4.98[BC]
	2	4.99	5.02[AB]	4.70	4.52[B]	4.51[A]
	3	4.77	5.15	4.50	4.64	4.46
	4	4.96	5.03	4.64	4.86	4.39
Spleen	1	.462	.630	.439	.490	.591
	2	.510	.587	.599	.489	.548
	3	.553	.487	.589	.666	.471
	4	.630	.471	.402	.410	.450
Kidney	1	.922	1.102[A]	1.020	.985[A]	1.062
	2	1.034	1.037	1.085	.952	.854
	3	.975	1.020	.947	.852	.954
	4	.944	1.003	.922	.873	.905

A, B, C - Pairs of organ-body weight means involved in positive "t" tests (95% Confidence level)

TABLE III

Organ-Body Weight Means

Organ	Sacrifice Period	Exposed	Exposed	Dome Control	Ambient Control
Heart	1	.473[A]	.331[BC]	.424[AB]	.451[C]
	2	.411	.402	.361	.362
	3	.449[A]	.405	.365[A]	.368
	4	.379	.407	.343	.341
Lung	1	.826[A]	.691[B]	.669[A]	.989[B]
	2	.918	.639	.722	.675
	3	.821[A]	.819	.589[A]	.739
	4	.613	.944	.741	.802
Liver	1	5.23	3.85	4.36	5.00
	2	5.37[AB]	2.90[C]	3.98[A]	4.81[BC]
	3	5.37[AB]	4.15	4.28[A]	4.73[B]
	4	5.12[AB]	3.69	3.98[A]	4.32[B]
Spleen	1	.767	.453	.664	.683
	2	.585	.665	.551	.475
	3	.729	.712	.554	.639
	4	.570	.701	.559	.722
Kidney	1	.998	.846[A]	.949	1.021[A]
	2	.967	.848	.879	.976
	3	1.080[A]	.908	.915[A]	.985
	4	1.020	.891	.943	.913

A, B, C - Pairs of organ-body weight means involved in positive "t" tests (95% Confidence level)

TABLE IV

Organ-Body Weight Means

Organ	Sacrifice Period	Exposed	Exposed	Exposed	Dome Control	Ambient Control
Heart	1	.359	.434	.370	.478	.367
	2	.396	.352	.395	.370	.390
	3	.365	.306[A]	.338	.403[A]	.346
	4	.277	.290	.309	.310	.309
Lung	1	.729	.722	.726	.776	.637
	2	.742	.769	.706	.827	.759
	3	.818	.655	.674	.749	.698
	4	.561	.622	.565	.549	.588
Liver	1	4.71	5.15[A]	5.14	4.60[A]	5.13
	2	5.12[B]	4.94	5.20[A]	4.71	4.63[AB]
	3	5.18	4.77	5.21[A]	4.76	4.45[A]
	4	4.59	5.06[AB]	3.96	3.85[A]	4.18[B]
Spleen	1	.369	.436	.471	.329	.517
	2	.542	.451	.460	.461	.499
	3	.552[A]	.319[B]	.417[C]	.470[B]	.226[AC]
	4	.312	.336	.319	.272	.357
Kidney	1	.954	.987	.941	.938	.890
	2	1.100[A]	.932	1.000	.939[A]	.971
	3	1.030	.916	1.042	1.021	.888
	4	.825	.860	.873	.776	.871

A, B, C - Pairs of organ-body weight means involved in positive "t" tests (95% Confidence level)

TABLE V

Organ-Body Weight Means

Organ	Sacrifice Period	Exposed	Exposed	Exposed	Dome Control	Ambient Control
Heart	1	.481[A]	.467	.466	.384[A]	.434
	2	.368	.341	.410	.369	.333
	3	.332	.377	.426[A]	.373	.317[A]
	4	.301	.327	.340	.325	.310
Lung	1	.720	.722	1.183	.748	.759
	2	.691	.686	.716	.720[A]	.594[A]
	3	.727	.702	.822	.668	.713
	4	.645	.625	.610	.697	.599
Liver	1	4.96	5.03	4.44	4.73	4.68
	2	3.88	4.65[A]	4.54[B]	3.64	3.92[AB]
	3	4.62	4.45	4.59	4.49	4.69
	4	5.11	4.77	4.78	4.83	4.67
Spleen	1	.460[A]	.413	.452	.364[A]	.410
	2	.465[A]	.423	.420	.295[A]	.310
	3	.365	.368	.416	.396	.307
	4	.324	.307	.350[A]	.319	.469[A]
Kidney	1	1.051	1.030	.967	1.001	1.041
	2	.928[B]	.946	.983[A]	.853	.823[AB]
	3	.930	.923	1.075	.991	1.005
	4	.939	.952	.941	.951	.997

A, B - Pairs of organ-body weight means involved in positive "t" tests (95% Confidence level)

TABLE VI

Organ-Body Weight Means

Organ	Sacrifice Period	Exposed	Positive Control	Dome Control	Ambient Control
Heart	1	.410	.392	.464	.418
	2	.357	.438	.344	.355
	3	.366	.365	.353	.331
	4	$.365^{A}$	$.325^{C}$	$.289^{ABC}$	$.379^{B}$
Lung	1	.873	.860	.854	.740
	2	.667	.630	.742	.623
	3	$.684^{AB}$	.587	$.540^{A}$	$.519^{B}$
	4	.695	.687	.701	.714
Liver	1	5.10	5.36	5.10	4.58
	2	4.84^{A}	4.79	4.41	4.23^{A}
	3	4.54	4.07	4.29	3.87
	4	5.31^{AB}	4.94^{B}	5.13^{C}	4.39^{AC}
Spleen	1	.501	.573	.474	.465
	2	$.483^{AB}$	.387	$.322^{A}$	$.355^{B}$
	3	.503	.362	.398	.375
	4	.454	.417	.366	.378
Kidney	1	1.101	1.081	1.120	1.081
	2	.953	.959	.923	.932
	3	.929	.924	.900	.902
	4	1.007	.998	.993	1.031

A, B, C - Pairs of organ-body weight means involved in positive "t" tests (95% Confidence level)

TABLE VII

Organ-Body Weight Means

Organ	Sacrifice Period	Exposed	Exposed	Positive Control	Dome Control	Ambient Control
Heart	1	.411[A]	.315	.369	.299[A]	.362
	2	.343	.334	.359	.329	.324
	3	.319	.334	.335	.374	.351
	4	.344	.361	.354	.319	.348
Lung	1	.812	.658	.739	.740	.716
	2	.645	.711	.837	.642	.639
	3	.749	.747	.648	.708	.640
	4	.609	.517	.568	.642	.626
Liver	1	4.94	4.88	5.31	4.63	4.85
	2	5.56	4.19	5.65[A]	4.61[A]	4.6'
	3	4.90	4.97	5.17	5.39	5.03
	4	4.71	4.95	4.94	5.17	5.29
Spleen	1	.413	.461	.538[A]	.441	.353[A]
	2	.477	.434	.604	.454	.469
	3	.418	.374	.379	.510	.404
	4	.336	.333	.429	.435	.428
Kidney	1	.978	1.040	1.071	.940	.941
	2	1.122[A]	.964	1.020	.987	.938[A]
	3	.901	1.041	.973[A]	1.020	.976[A]
	4	.950	.944	1.001	.871	1.001

A - Pairs of organ-body weight means involved in positive "t" tests (95% Confidence level)

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DR. SCHAEFER (U. S. Naval Medical Research Laboratory): I think experimental evidence with exposure of animals to various different environments shows that there are phases in the changes of organ to body weight ratios, ups and downs in every case, related to water changes in the organs and the lung. I wonder on what basis the postulation was made that you should expect a continuous atrophy in order to find an effect of any space cabin material? I would never expect this with any material.

MR. EGAN: We felt if there was any toxic involvement in either the liver or the kidney it would be indicated by either a change in the mean ratio between the organ and the body weight of the group of animals exposed to that material, or by the gross pathology or the histopathology. Now whether this is going to be indicated by an atrophy or hypertrophy is something else. In the light of this vacillating organ-body weight ratio, we are undertaking an experiment where we are investigating whether this is indeed so, particularly whether this is true of the animals that we are using and in the conditions that we are using these animals.

QUESTION: Were these all the same sex? All male?

MR. EGAN: That is right.

QUESTION: For the rat, a 100 gram rat is a young rat. It has a sharp growth curve at that point. Did I understand you to say that you were using a 25 gram mouse?

MR. EGAN: That is right.

QUESTION: He is pretty much of an adult at that point if you were looking for sensitive changes in the growth rate. Maybe you should have come down to a 15 or 20 gram mouse.

MR. EGAN: Well, with regard to the rats, they come into our laboratory with a weight of approximately 110 grams. When they eventually get into the experiment itself their weight is approximately 150 grams, and they can go all the way up to well over 200 grams and still be considered fairly immature. As Dr. Harris pointed out last year, these rats can be used during the log phase of their growth. For that reason we feel that using them at any weight during this level isn't going to really influence the response that we get.

QUESTION: I agree that this was a proper selection for the rat, but I was questioning the mouse.

MR. EGAN: In terms of mice, these weights that we are using will level off at approximately 38 to 43 grams, and this is toward the end of the actual four-week postexposure observation, so that when we actually start using them their weight is around 25 grams, but after seven days they haven't really increased that much; they are adults. They aren't as mature as the rats are.

QUESTION: That explains why you are seeing the great increase in weight.

MR. EGAN: That is possible.

COMMENT: If I can mention something about these mice: in these studies, it is very difficult to use mice that are too small. If you work with mice you will know what I mean.

DR. FAIRCHILD (Aerojet-General Corporation): I would like to just substantiate one of the points that Mr. Egan was attempting to make here. I think he had so much material that he had to rush through it. This very erratic showing of T test significance was the very basis for making the judgment that we really had no toxicity. Part of the time the controls are showing a heavier organ; the next time you check it, in the second sacrifice, maybe it's the experimental that is showing the change. Sometimes the difference in the two controls is significant, whereas the experimental isn't, so we have this tremendous variation with the indication that perhaps methodology and necropsy are going to have to have a great improvement with a complete exsanguination before these organs are handled and weighed.

SESSION IV

INSTRUMENTS AND DETECTION TECHNIQUES

Chairman

Mr. Raymond A. Saunders
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Washington, D. C.

SESSION IV

Opening Remarks

MR. SAUNDERS: Welcome to the fourth session of this Conference which concerns instruments and detection techniques for the determination of trace organic contaminants in closed atmospheres.

There are two entirely different frontiers of exploration today which take man out of his natural element and require a closed environmental atmosphere. These, of course, involve man's activities in space and under the sea. The instrumental needs of these two uses of closed atmospheres may not be exactly the same, but they are certainly similar. Information concerning the contaminant makeup of these atmospheres is presently derived in two ways: from on-board monitoring equipment and from remote laboratory analyses of samples taken from the atmosphere.

Both Navy and NASA have relied upon life support systems to remove or control contaminants and have only used on-board analytical equipment for monitoring a few known contaminants of special interest. Remote laboratories have provided all of the information as to the actual contaminant constitution of these atmospheres. This approach has been adequate so far, but as submarine and space voyages extend to longer and longer times, the crews must become less dependent upon some remote laboratory and rely to a much greater extent on more sophisticated on-board equipment for detailed analyses of their own atmospheres.

When a crew is confronted with a troublesome contaminant in a closed atmosphere, the biggest part of resolving the difficulty is often the identification of the offending compound. Once this has been accomplished, locating the source of the contaminant and controlling its generation or concentration may be relatively easy.

The MESA experiment which we discussed here last year is a case in point. That experiment was a simulated thirty-day flight. The crew became ill with unusual symptoms, the obvious victims of an atmospheric toxicant. Now you know that the control personnel wanted to solve that problem, to save the experiment, and to avoid an abort, but without any immediate knowledge of what contaminants were present, they were completely in the dark. The several modifications that were made in the environmental control system in an effort to eliminate the difficulty were futile. In fact some of them actually worsened the situation. They had to depend upon a remote laboratory for an atmospheric analysis, of course, and the results were not available quickly enough to serve as the basis for remedial action.

One might say, therefore, that the MESA experiment aborted for lack of an on-board analyzer which could have permitted identification of each cabin contaminant. That information would have allowed the crew to deduce the source of the only unusual contaminant present, which, as it turned out, was the toxicant. Knowing the source of the toxicant, the crew could have quickly made the proper modification in the environmental control system and prevented its continued formation.

This is the goal we must set our sights on next - adequate on-board analytical systems capable of resolving complex contaminant mixtures and permitting the identification of each component.

The equipment and techniques presently in use for the analyses of closed atmospheres and some of the instrumentation being contemplated or developed for future use are the subjects of the next few papers.

INSTRUMENTATION USED FOR THE SHIPBOARD ANALYSIS OF SUBMARINE ATMOSPHERES

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Walter D. Smith
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and
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INTRODUCTION

The earliest published work on atmosphere analysis in submarines appears to be that of Belli and Torcello in 1908 in Italy. To quote Miller and Piatt, 1960, "They made a careful study of the chemical alterations in the air, and reported Oxygen, Carbonic Anhydride, Hydrogen, Sulphurous Anhydride, and volatile hydrocarbons." Since that time, however, the submarine has developed into a sophisticated weapon requiring more men, complex equipment, and longer submergences. Whereas the normal dive time during World War II was measured in hours, today, with our modern nuclear powered fleet, the normal submergence time is often as long as 60 days and sometimes longer. The fact that longer dives are possible now is due to a large extent to the detection, analysis, and control of the contaminants that exist in the atmospheres of the submarines.

The U. S. Navy first gave air purification serious consideration in 1914. At that time they were concerned primarily with the buildup of CO_2 and H_2. In 1929, following the disasters of two boats in 1925 and 1927, the Naval Research Laboratory (NRL) became involved in research on submarine habitability.

Up to World War II, essentially all control of the atmospheres in submarines was designed for emergencies, with routine control by ventilation to the outside atmosphere. After World War II, with the development of nuclear power, thinking was toward longer dives, which created a need to develop better detection and control of the contaminants. This paper will be concerned primarily with the development of the analytical methods and instrumentation used to monitor the atmosphere within the submarines since World War II.

In 1956 a program was initiated to determine the identity and concentration of the contaminants in nuclear submarines. As a result of that program, it was ascertained that the major composition of the atmospheric contaminants was

hydrocarbon in character. These contaminants were produced from a wide variety of factors, including human effluents, cigarette smoke, refrigerant leakages, paints, cooking, degreasing solvents, etc.

Development of Instrumentation

From the study initiated in 1956, it was determined that the principal components that would require monitoring to insure the safe operation of the submarine were carbon dioxide (CO_2), oxygen (O_2), hydrogen (H_2), carbon monoxide (CO), and the hydrocarbons. The detection of CO was a problem because of the extremely low levels desired for its control in the atmosphere. Today that level is set at 25 ppm. Of the available physical detection methods, the use of the positive filter (nondispersive) method of infrared (IR) spectroscopy appeared to be the best method capable of detecting low concentrations of CO.

Thus NRL chemists designed and built the first Atmosphere Analyzer, the Mark I, and installed it aboard the NAUTILUS (Thomas, 1960). It utilized a combined IR portion for CO, CO_2, and a generalized hydrocarbon analysis; a paramagnetic oxygen unit of the Pauling type with electrostatic balancing; and a simple thermal conductivity unit for hydrogen. The signals from this unit were fed into panel meters and to a strip chart recorder.

Within a very short time, the Mark I was replaced by another NRL-built atmospheric analyzer, the Mark II. This unit differed from its predecessor in that the IR analysis channels were completely separate units. As a result of the performance of the Mark II, further changes were deemed necessary. Principally these were: greater sensitivity of the CO unit, removal of the recorder, and the elimination of the total hydrocarbon channel. The latter change was made because the results were influenced too greatly by the continuous relatively high concentration of methane often present in the submarine atmosphere which was included in the figure for total hydrocarbon concentration.

These changes were included in the Mark III. This unit and the subsequent units, Mark IV and V, were manufactured by Beckman Instrument Company for the U. S. Navy. In the Mark III the hydrocarbon channel was changed to a Freon-12 (F-12) analyzer for which there was a need since F-12 was used as a refrigerant and was often present in the atmosphere. The sensitivity of the CO as well as for F-12 was enhanced by increasing the gas sample pressure from ambient to 100 psig.

The Mark III was superseded by a Mark IV analyzer which is similar in principal to the Mark III. The Mark IV was supplied in three models for the different classes of submarines. These models incorporate the following channels as needed: CO, CO_2, F-12, F-11, O_2, H_2, and an ultraviolet channel that may be used to detect ozone, mercury vapor, and hydrocarbons (Thomas, 1963).

Back-up Instruments

Before continuing with the discussion of the primary atmospheric sensors, I wish to discuss briefly the presence of the back-up instruments available on board the nuclear submarines. These instruments may be described as being portable and, therefore, may be used to spot check various compartments of the boat for

suspected sources of contamination. The O_2 analyzer employs the same basic principle as that used in the Mark III unit, the paramagnetic property of O_2. For CO_2, a Dwyer unit is used. This unit operates by first trapping a fixed quantity of gas sample in a chamber. This trapped sample is then exposed to a potassium hydroxide solution which results in the complete removal of CO_2 by a chemical reaction. This in turn reduces the volume of the trapped gas sample and the amount of volume change is related to the concentration of CO_2 present.

The presence of CO is determined by a detector tube which uses a color reaction of CO in a known volume of air with a chemical, a complex silicomolybdate compound catalyzed by palladium sulfate on silica gel. The change of coloration is then compared to a color chart to determine the concentration of CO. Similar chemical detector tubes are used to detect other possible contaminants. For the detection of H_2, a portable thermal conductivity detector is used.

The Mark V Atmosphere Analyzer

Since the advent of gas chromatography in the early 1950's tremendous advances have been made in its design and application. Among the inherent advantages of such a system are its increased sensitivity and potentially greater reliability.

The Mark V atmospheric analyzer utilizes the principle of gas chromatography and is designed for automatic operation and analysis with a cycle time of 15 minutes. The analysis includes the components and range of concentration given in table I. It accomplishes this task by the use of seven columns, five are used to achieve the desired elution of the components of interest and the other two columns serve as stripper columns to prevent unwanted contaminants from entering into the analytical columns and detectors.

TABLE I

Components	Range of Concentration
H_2	0-5%
O_2	0-25%
N_2	0-100%
F-12	0-2000 ppm
CO_2	0-3.3%
CO	0-125 ppm
F-11	0-2000 ppm

The flow diagram for the Mark V analyzer is presented in figure 1. The sequence of events is as follows:

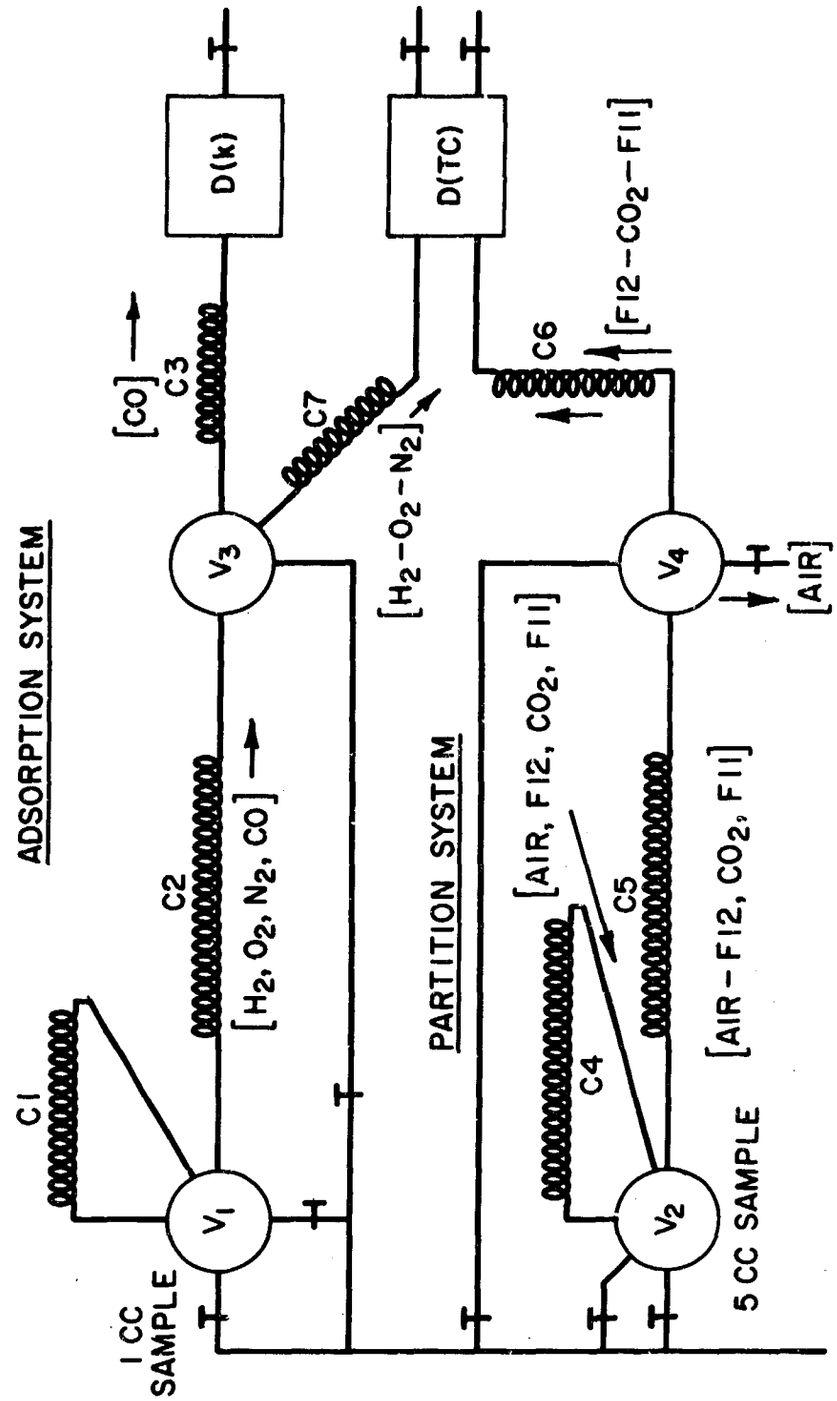

Figure 1. FLOW DIAGRAM FOR MARK V ATMOSPHERIC ANALYZER

At the start of the analysis, with helium as the carrier gas, V_1 injects a 1 cc sample into the adsorption column system. This system separates and elutes H_2, O_2, N_2, and CO. Column #1 functions as a stripper column. It allows the permanent gases to pass and retains all other components such as CO_2, F-12, F-11, and hydrocarbons. These contaminants are backflushed from the column after the passage or elution of the permanent gases from Column #1. Columns 2, 3, and 7 separate the components of interest before they enter the detector. There are two detectors used in the adsorption system. The components H_2, O_2, and N_2 are routed from Column #2 by valve V_3 to Column #7 and then into the thermal conductivity detector (TC). Valve V_3 then switches and routes the CO from Column #2 to Column #3, which increases the separation of CO and N_2, and then to a Karmen ionization detector.

The partition system separates and detects Freon-12, CO_2, and Freon-11. Valve V_2 injects a 5 cc sample into Column #4. The components of interest are allowed to elute and then that column is backflushed in the same manner as Column #1. The air peak is separated from the components of interest on Column #5 and is then vented by V_4 to the outside. This is done to protect the detector filaments. V_4 then directs the components onto Column #6 where they are separated and are eluted into the other side of the TC detector. The elutions of the components from Columns #6 and #7 are timed such that one side of the TC detector always serves as a reference.

The prototype of the Mark V Atmospheric Analyzer has been in operation at the Naval Research Laboratory for several months. Further units of the analyzer are being installed in the nuclear-powered submarines.

The Total Hydrocarbon Analyzer

As mentioned previously, in 1956 NRL began an extensive investigation to determine the identity and concentration of the organic contaminants on board the nuclear submarines. It was determined that the composition of organic contaminants was quite complex (Nestler and Smith, 1958; Saunders, 1959). In addition, it was ascertained that the aromatic hydrocarbons present in the atmosphere amounted to approximately 25 to 30% of the total hydrocarbons present (Johnson, et al, 1964).

Most of the early data for hydrocarbons was obtained at NRL by steam desorption of exposed activated carbon (Nestler and Smith, 1958). Though this method was adequate at that time in giving qualitative as well as quantitative information, it had several deficiencies. One of these is quite obvious, that is the long time delay for the shipment of the exposed carbon to the laboratory and the completed analysis. Another drawback was the possibility of "cracking" the higher molecular weight components due to the high temperatures used in the steam desorption process.

A more serious defect is that at low concentration or loading, desorption from the carbon is not quantitative. The lower the concentration of hydrocarbons in the atmosphere, the lower the loading on the carbon, and the less quantitative is the recovery. This is due to the retention of a certain amount of the hydrocarbons by the carbon, defying the desorptive effect of high temperature, steam, or vacuum.

The Bureau of Medicine and Surgery has lowered the maximum acceptable concentration of hydrocarbons to a point where the steam desorption technique could not produce adequate information. For instance, the allowable limit for total hydrocarbons has been lowered to 40 mg/m^3. At this low level, analysis by the desorption of charcoal is inadequate because a large percentage of the adsorbed hydrocarbon remained as a residue on the charcoal. Thus, a need was quite evident for the development of a total hydrocarbon analyzer to be used on board the submarines.

Because of this need, NRL chemists designed and built an atmospheric analyzer to monitor the total hydrocarbon concentration of the submarine atmosphere. Needless to say, this instrument has been named the Total Hydrocarbon Analyzer (THC Analyzer).

This instrument is basically a gas chromatograph utilizing as its detection sensor a hydrogen flame ionization detector. In contrast to the normal carrier gases used with such a gas chromatograph, viz., helium or nitrogen, compressed air is used as both the carrier gas as well as the support gas for the combustion process with hydrogen. In addition, the THC Analyzer employs a backflush technique which regroups the components on the separation column.

The advantages of using compressed air as a carrier gas are: (1) compressed air is readily available aboard the submarines. By using this air, which is "cleaned" by using a catalytic burner, the logistics of using a bottled carrier gas is eliminated; (2) methane is one of the desired components to be detected in the atmosphere. Normally, by employing helium as a carrier gas and using a liquid phase partition column, both the air peak and methane would be eluted at the same time. However, by using air, the effect of the air peak on the detector is nullified and, consequently, the methane can be sensed without interference.

The backflush technique is not at all new. In 1958, Schwenk and Weber described the use of this technique to analyze refinery gas samples containing C_5 and higher hydrocarbons by reversing the column flow to remove the components of interest and collecting them by adsorption and weighing (Schwenk and Weber, 1958). The backflush technique is used for the THC Analyzer for two reasons. First, it is desired to allow methane and F-12 to elute from the column to separate them from the "total hydrocarbon" peak, because it is often the case that either one of these compounds, always present in the atmospheres of nuclear submarines, may more than equal in concentration the total concentration of all other hydrocarbons. In the second place, the "total hydrocarbon" content comprises several hundred individual compounds, each of which may be present in minute amounts. By backflushing, these compounds are grouped together in a single measurable peak.

As shown in figure 2, the analyzer is contained in a 16-inch cubical case. In the top half of the front panel are the controls used to direct the flow of the carrier gas through the analyzer, and a sample port into which the atmosphere sample is introduced. The bottom half of the unit houses the controls for operating the amplifier, off-on power, and the temperature controller for the oven. A 1-mv strip chart recorder is used to record the events from the analyzer.

Figure 3 illustrates a typical flow diagram used in backflush chromatography. As shown, the carrier gas flows through the sample injection valve into a 4-way valve. The injected sample is routed by the 4-way valve to the separation column.

Figure 2. NRL TOTAL HYDROCARBON ANALYZER

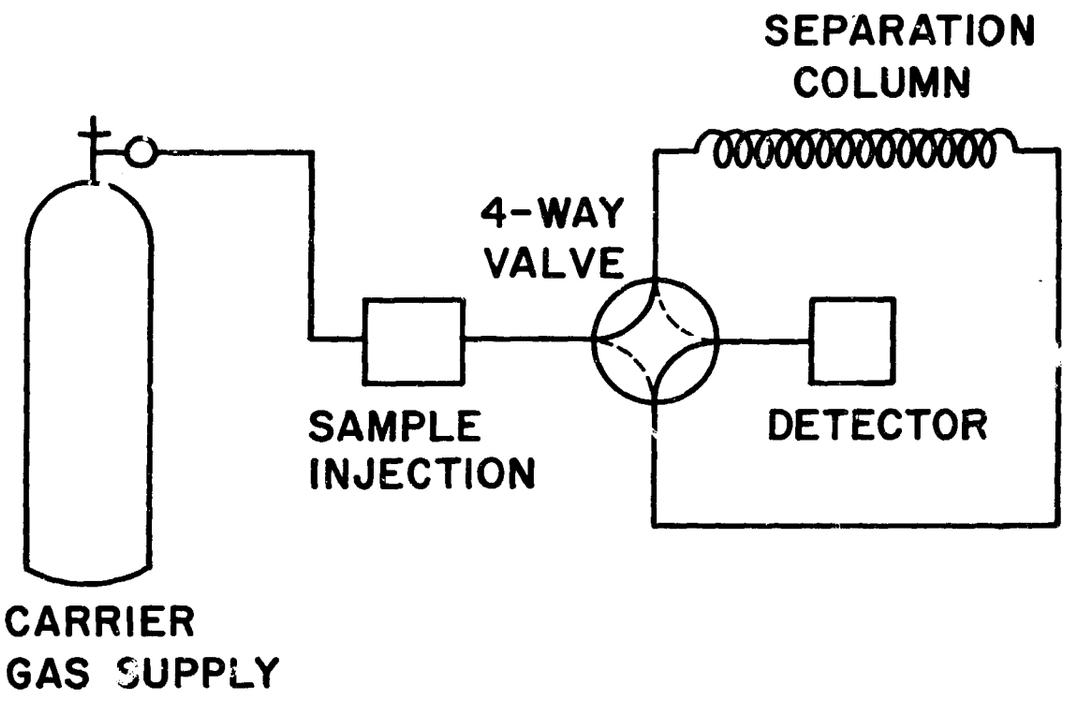

Figure 3. BACKFLUSH CHROMATOGRAPHY

Then the sample is either allowed to continue its normal path to the detector or is backflushed to the detector. The backflush flow is indicated by the dashed lines.

The THC Analyzer is designed to operate in the manner described. A sample of the submarine atmosphere is injected onto the column. The more volatile components, methane and Freon-12, are allowed to elute from the column to the detector and then the system is backflushed to obtain the total hydrocarbon peak. Figure 4 illustrates this operation with a sample of a submarine atmosphere. As was pointed out earlier, the methane and Freon-12 concentrations are often relatively high aboard the submarine and it is desirable that these components not be included in the total hydrocarbon picture. In addition, this serves as a backup analysis for Freon-12.

Since the analyzer is a gas chromatograph, the unit can be and is operated in the normal fashion in order to detect individual contaminants. Of particular interest, other than the presence of the hydrocarbons in the atmosphere, is the detection of certain compounds which have been found in the submarine atmosphere as listed in table II. Figure 5 shows a chromatogram of some of the individual components included in the total hydrocarbon peak illustrated in figure 4.

TABLE II

OTHER CONTAMINANTS OF INTEREST

R-11
R-12
R-113
R-114B2
Benzene
Methyl Chloroform
Tetrachloroethylene
Trichloroethene
Vinylidene Chloride

The performance of the laboratory THC Analyzer to date has proven its feasibility aboard the nuclear-powered submarines. In addition to detecting the total hydrocarbons present, it can detect individual components in the atmosphere with a sensitivity of less than 1 ppm for many of the volatile contaminants present in the atmosphere. It is anticipated that private industry will be requested by the Navy to build similar units to be installed in the nuclear submarines.

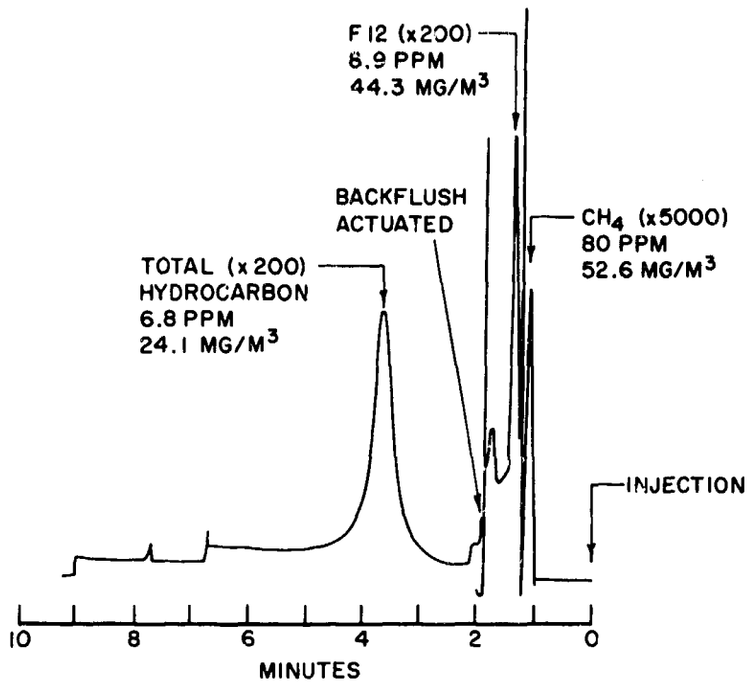

Figure 4. NORMAL BACKFLUSH MODE

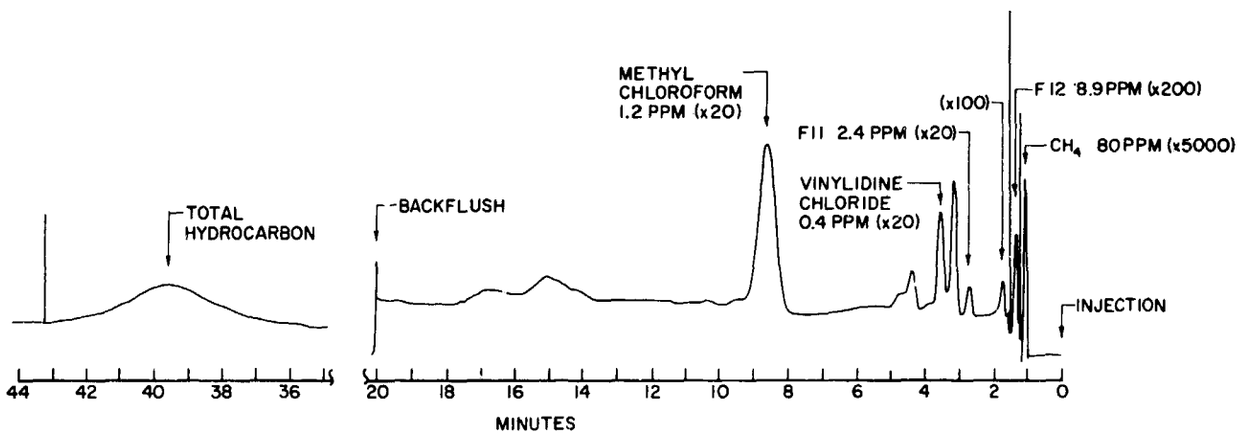

Figure 5. DELAYED BACKFLUSH MODE

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DISCUSSION

QUESTION: It might be of interest if you could give size, weight, and power requirements for either the Mark IV or V.

MR. EATON: The weight is about 500 pounds, and its size is about the size of this lectern. I didn't have a slide to show you the Mark V, but the signals are paired into panel meters and these meters are more or less peak pickers; it just follows the peak up as it normally does in the chromatograph, and when it gets up to the maximum, of course, these meters hold until the next 15-minute analysis. The power requirement, I think, is about 500 watts.

MR. MOBERG (Aerojet-General Corporation): I am interested in your comments on the charcoal; the lack of quantitative desorption; and what tests you used to indicate that certain amounts were still left on the charcoal after either vacuum heating or steam desorption techniques were used.

MR. EATON: This was done by more or less weighing the charcoal before and after removing the contaminants on the charcoal. Perhaps this wasn't clear, but at the higher concentrations or loading of the charcoal the error was somewhere within about five to six percent of what the actual quantitative measurement was. When you got down to your lower loading, the error jumped up to perhaps over 10 percent of what the actual concentration was. There always remains, and we were aware of this, a "hill" on the charcoal. We could not get this off. At the higher loadings this was fine. We took into account that there was this much left on the charcoal. When we started cleaning up the submarines we got, consequently, lower loadings and we couldn't see down to what the Bureau of Medicine and Surgery asked us to do - down to 40 milligrams per cubic meter. Charcoal just did not give up enough quantitative or qualitative information. Certainly some contamination did remain on the charcoal.

DOUBLE RESONANCE MICROWAVE SPECTROSCOPY

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Microwave spectra arise, as is well known, from the interaction of electromagnetic radiation with the rotational motion of gaseous, polar molecules. The rotational motion being quantized, absorption of radiation occurs at discrete frequencies at which the radiation energy is equal to the energy separation between rotational levels and, therefore, suitable to induce transitions (figure 1).

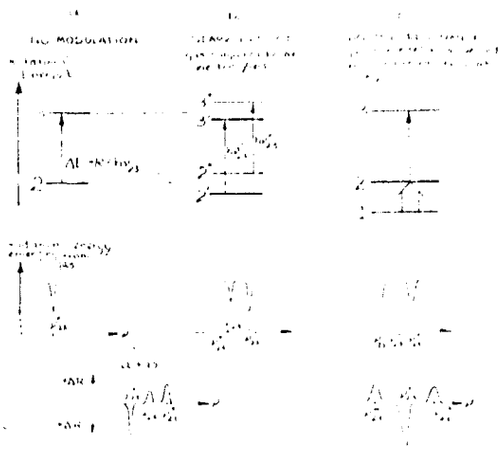

Figure 1.

Since the relative position of the energy levels depends critically on the structure of the molecule, one can identify a compound by observing one or several of its microwave absorptions. This provides the basis for the use of microwave spectroscopy as an analytical tool.

Unfortunately, the described principle cannot be applied without considerable experimental refinement because of the weakness of the absorption lines. It is necessary to employ modulation techniques for increasing the detectability of absorptions, i. e., the sensitivity of the spectrometer. The most commonly used

technique is based on the molecular Stark effect and was first introduced by Hughes and Wilson in 1948. In this technique the gas is subjected to an electric field under whose influence the rotational levels are replaced by sets of slightly shifted sublevels giving rise to different absorption frequencies. By applying the electric field periodically, the zero field line and the frequency shifted "Stark components" are switched on and off with the repetition rate of the field. Thus, an intensity modulation of the microwave radiation is obtained at the position of the lines, and this, in turn, enables frequency selective amplification and detection to be used. In this way, the high sensitivity of microwave spectrometers is achieved.

An alternate, and for analytical purposes, superior, modulation scheme is based on the phenomenon of double resonance. This effect, which finds wide application in nuclear magnetic resonance, has been introduced into microwave spectroscopy by Battaglia and his co-workers, and it might be described (not explained) as follows:

> Let there be three energy levels '1', '2', '3' such that allowed transitions occur from '1' to '2' and from '2' to '3'. If one now irradiates the gas with radiation suitable to observe the transition $2 \rightarrow 3$ and simultaneously applies a second intense microwave radiation with such a frequency as to induce many transitions from $1 \longrightarrow 2$, then the effect of this "pump radiation" at ν 1, 2 on the signal observed around ν 2, 3 is to change both the intensity and line shape of the $2 \longrightarrow 3$ transition. The change in intensity can be made plausible in a semiclassical picture as due to the change in the number of molecules in the rotational level '2'. There is, however, no such simple picture for the change in line shape which arises from the quantum mechanical behavior of the molecular system under the influence of the two radiations. Let it, therefore, suffice to say that the original absorption line is split into a doublet on account of double quantum transitions.
>
> Having thus achieved a change in the absorption signal at ν 2, 3 due to the radiation at ν 1, 2, all one needs to do to obtain molecular modulation is to apply the pump radiation periodically, in exact analogy to the Stark modulation technique. From this analogy it can also be deduced that double resonance spectroscopy preserves the high sensitivity typical for Stark spectrometers.

Equivalence to an existing technique, however, would hardly justify the development of a new technique. Let us, therefore, examine in what ways double resonance modulation differs from Stark modulation.

As one proceeds from small, and linear or highly symmetric molecules, to larger and asymmetric molecules, the microwave spectra become increasingly dense and complex; and this is even more the case when a mixture of different molecules is to be analyzed. Since Stark modulation affects all the energy levels of all molecules present, all absorption lines will be detected in a Stark system. The great number of absorptions will cause frequent overlapping and interference; therefore, Stark spectroscopy becomes unfeasible. Double resonance modulation, on the other hand, occurs only when the frequency of the pump radiation coincides very closely with the frequency of an absorption line and becomes effective only on

transitions with an energy level in common with the pump transition. Since there are usually not more than one or two "signal lines" connected with a given "pump line", the double resonance spectrum remains discrete and no interference occurs. In addition, the selective modulation makes double resonance spectroscopy so highly specific that even in a mixture one single double resonance suffices completely to identify the compound from which it arises.

It is thus seen that the double resonance method is superior to the Stark modulation technique because it does not suffer from the drawbacks that have so far prevented microwave spectroscopy from becoming an effective analytical tool.

According to the modulation scheme described, a modulated double resonance spectrometer consists of the following basic components: a high power microwave source providing the periodically changing pump radiation, a second microwave source providing the signal radiation, an absorption cell containing the gas under examination, and a detecting system for observing the double resonance absorption. A more detailed description of such a system is given in the block diagram shown on the slide (figure 2 - not reproducible). Another slide (figure 3 - not reproducible) shows a photograph of the actual spectrometer.

This system was built for NASA, Langley, with the goal of experimentally evaluating the capability of double resonance spectroscopy for detecting trace contaminants or analyzing mixtures of gases. In these experiments, a group of five molecules, which were selected by NASA, was used as test samples. They were dimethyl sulfide, methylene chloride, propionaldehyde, n-propyl chloride, and propionic acid (figures 4 - 8).

Clearly, before double resonance spectroscopy can be applied for identifying these compounds, the following questions have to be answered: first, what are the pump and signal frequencies at which a double resonance is obtained for the gases in question? Second, how sensitive is the double resonance signal amplitude against pressure variations, and in what pressure range can the double resonance be observed? Third, how does the double resonance signal change with temperature? To answer these three questions, the five contract molecules were first studied when present in the absorption cell as pure samples.

Let us first consider the selection of a double resonance in, say, propionic acid. This molecule is quite asymmetric ($\varkappa \sim -.72$) and shows at least 2000 absorption lines in the microwave range. But despite this great number of lines, there are only a few transitions in the 12GHz - 18 GHz band which are connected to another transition in the 26GHz - 40 GHz range. The strongest of these double resonance connections are the $6_{24} \longrightarrow 6_{33}$ transition at 30436.6MHz and the $5_{24} \longrightarrow 6_{15}$ line 28186.3MHz which are both connected to the $6_{15} \longrightarrow 6_{24}$ line at 17269MHz. These two double resonances are shown on figure 9.

What happens to the double resonance line when the sample pressure is varied can be seen from figure 10 which shows traces obtained on n-propyl chloride at pressures of 11μ, 30μ, and 140μ, respectively. The overall pressure dependence for this molecule is shown on figure 11. It is seen that the double resonance signal amplitude first increases with the number of molecules in the cell.

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Figure 4.

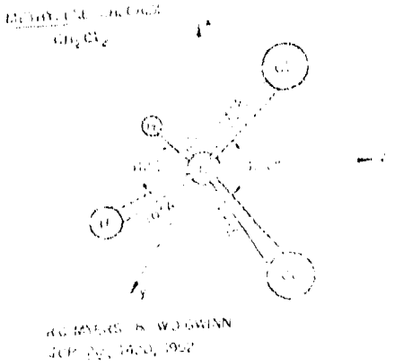

Figure 5.

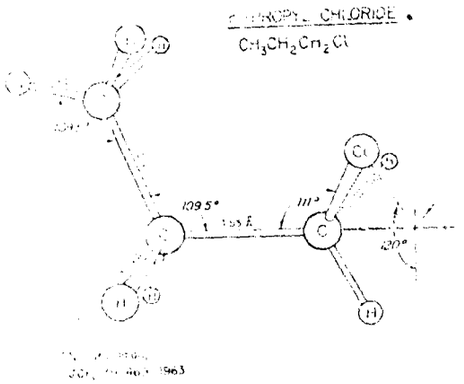

Figure 6.

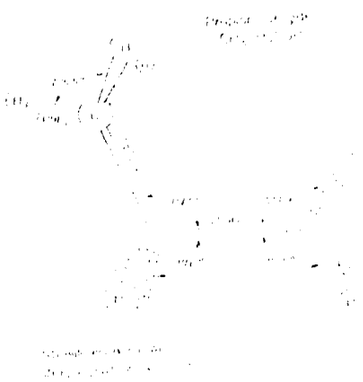

Figure 7.

Figure 8.

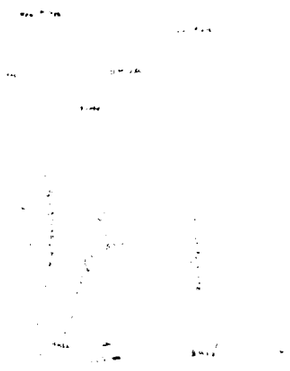

Figure 9.

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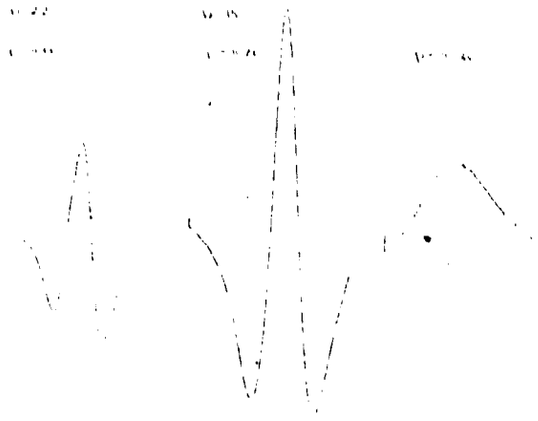

Figure 10.

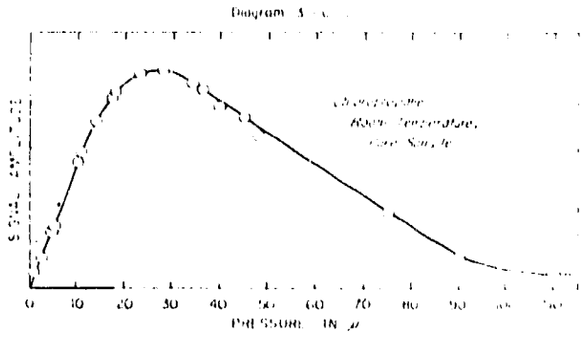

Figure 11.

At ~ 25μ Hg the molecules start influencing each other, and the signal no longer increases; instead, after going through a maximum at ~30μ, it decreases linearly. At 120μ-150μ the signal has lost the typical double resonance lineshape, and at still higher pressure it is so broad and weak that it can no longer be distinguished from the background noise.

Changing the temperature of the sample by 100 degrees enhances the signal because of the increase in population of the ground vibrational state.

The data concerning the best double resonances, pressure range, and temperature are collected for the five contract molecules in figure 12.

After establishing the basic information about the five test molecules, the smallest amount of gas traceable by the double resonance technique was determined in the following way: samples of propionaldehyde, methylene chloride, and dimethyl sulfide were progressively diluted in air and the signal versus pressure curve was reinvestigated for each degree of dilution. This process was continued until it became difficult to detect the double resonance signal. A typical result out of this series of experiments is presented in figure 13, which shows the pressure curves obtained for a pure sample, a 1:100 and a 1:300 diluted sample of propionaldehyde. (Notice the change in the position and width of the optimum pressure range.) Recorder traces of signals obtained on the most diluted samples studied are seen in figure 14 along with the corresponding traces from the pure samples.

On the basis of these experiments one has to answer the question after the smallest detectable amount as follows: with the instrument at our disposal, double resonance lines can be picked up from samples which are 1:300 diluted in air. In other words, a contaminant in an atmosphere of 760 mm Hg could be identified as soon as the partial pressure of the contaminating gas rises to ~2 mm Hg.

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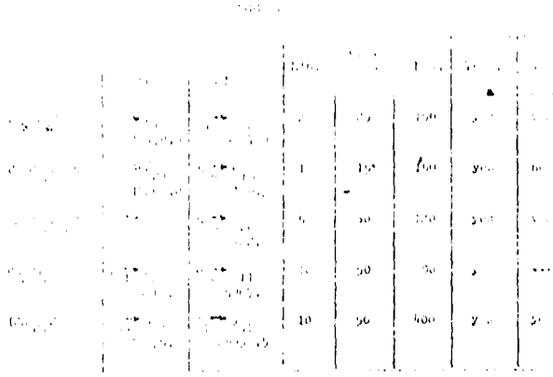

Figure 12.

Figure 13.

(We should like to add at this point that the quoted value for the threshold of detectability could be pushed down at least by a factor of 5 if some technical refinements were made to the spectrometer. We are, furthermore, aware of an additional technique which would allow us to eliminate large portions of the diluting air and to decrease the threshold of detectability down to about 10μHg.)

The effectiveness of double resonance in analyzing mixtures of gases was tested in two experiments of the following type: first, three, and then four, of the test gases were dosed simultaneously into the absorption cell and it was tried to detect the double resonance indicative of each compound. The results of these experiments are shown in figure 15. It is seen that all three (or four) signals could be obtained clearly. The total sample pressure in these experiments was ~ 50μHg.

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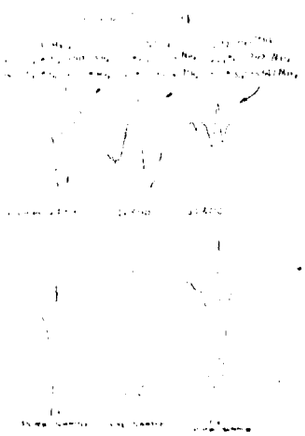

Figure 14.

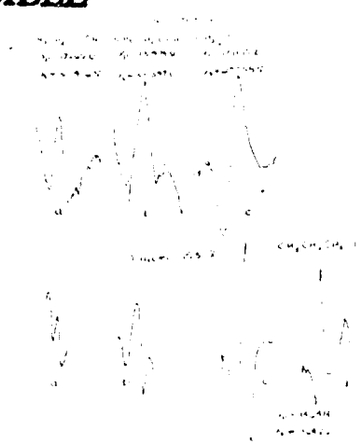

Figure 15.

The important feature in these figures is the complete absence of lines in the vicinity of the double resonance signals. Remembering that the four compounds together would show a total of ~5000 microwave absorption when investigated on a Stark spectrometer, it becomes clear that double resonance spectroscopy is not only superior to Stark spectroscopy but also ideally suited for a quick analysis of gas mixtures. The major results of this research may be summarized as follows:

1) The sensitivity of double resonance modulated spectrometers is equal to the sensitivity of Stark spectrometers, and could be increased easily by a factor of ~10.

2) Even in the presence of several compounds producing numerous overlapping and interfering microwave absorptions, double resonance signals will not be obscured or falsified. One single double resonance connection will suffice to identify unambiguously the compound from which it arises.

DISCUSSION

DR. KATCHMAN (Miami Valley Hospital): What happens with aromatic nuclei - benzene?

DR. STIEFVATER: Microwave spectroscopy is peculiar to polar molecules. They have to have a dipole moment. This is the essential part. That could be an electric dipole moment; it could be a magnetic dipole moment, but it has to have something to provide interaction with the radiation.

MR. ADAMS (Brooks Air Force Base): Is there a path length limit to the cell?

DR. STIEFVATER: Well, in general, sensitivity goes up as one goes to longer cells.

QUESTION: How long could you make a cell?

DR. STIEFVATER: Our cell is a hundred foot, is a coiled X Band waveguide. The length is a hundred foot, and this is something, again, which makes it superior to the Stark Spectroscopy, because in the Stark cell you need a tremendously big generator to create this field.

QUESTION: Is there a theoretical limit before you get overlap or get into a doppler effect?

DR. STIEFVATER: You don't get overlap anyway, even if you make your cell infinitely long, because there is only one line.

QUESTION (Aerojet-General Corporation): You suggested that there was only one line for each compound. Are you referring to a single line for an isomer or different isomers; or is this single line related to molecular weight? What is it related to beside dipole?

DR. STIEFVATER: As I tried to point out, the position of the microwave lines of a compound depends on the structure. If you do ordinary spectroscopy you must measure quite a number of lines before you can say (because of these frequencies) that this has to be such and such a compound. If you have a mixture, the lines start overlapping and identifying a compound by measuring lines becomes very difficult and takes a long time. Now the double resonance technique works only on connected transitions; therefore, if you pump one transition you usually get only one signal out, unless there are two or three connected to it, but three is the most I have ever run into. Now compared to three thousand or five thousand, well, that's a reduction in time and work by a thousand.

ANALYSIS OF SPACE VEHICLE ATMOSPHERE BY GAS CHROMATOGRAPHY

Sam S. Brody

Melpar, Inc.
Falls Church, Virginia

The gas chromatograph described here is a completely automatic system which has been flight qualified for use on missions aboard the Apollo spacecraft. The function of the Apollo Gas Chromatograph is to provide quantitative analysis for both the trace contaminants and the major components in the cabin (or suit) atmosphere. This instrument operates on a repetitive analytical cycle without attention for a minimum of 15 days. Operation includes automatic attenuation, drift-free baseline operation, and a feature which permits analysis of a minor component when it is on the trailing edge of a major component. Melpar has delivered six of these units to NASA.

A photograph of the unit is shown in figure 1. A titanium sphere for helium carrier gas storage and a two-stage pressure regulator are mounted within the supporting framework. A space exhaust port at the lower right of the front panel is connected to space vacuum so that spent helium carrier gas and sample may be evacuated. The "From Suit" and "From Cabin" ports are the sources of the two samples for this instrument. The "To Suit" port is used to circulate one sample while the other is being analyzed. The power switch in the upper right is used for turning on the instrument and for operation of the valves with the oven off. The sample selector valve is used to analyze either cabin or suit atmospheres. In the "Auto" position, the cabin and suit samples are sequentially programmed. The helium fill port is utilized to load the helium sphere with 6000 psig helium prior to flight. A calibration button is provided to check proper operation of the amplifiers. The electrical connector at the lower left is utilized for incoming power, two telemetry output channels, and an output to an oxygen readout meter.

The gas chromatographic instrument basically consists of a helium reservoir, a programmer, sample and column selector valves, sample injection valves, three partitioning columns, three cross-section ionization detectors in a single housing, an oven with proportional temperature control, and an amplifier assembly. The programmer controls the valve operations and sequentially selects the appropriate detector. The sample selector valve allows either cabin or suit sample to circulate through the injection valve. The injection valve injects a known volume of sample into the column system at periodic intervals. The column selector valves are used to prevent the flow of helium and sample through two of the three columns when they are not in use.

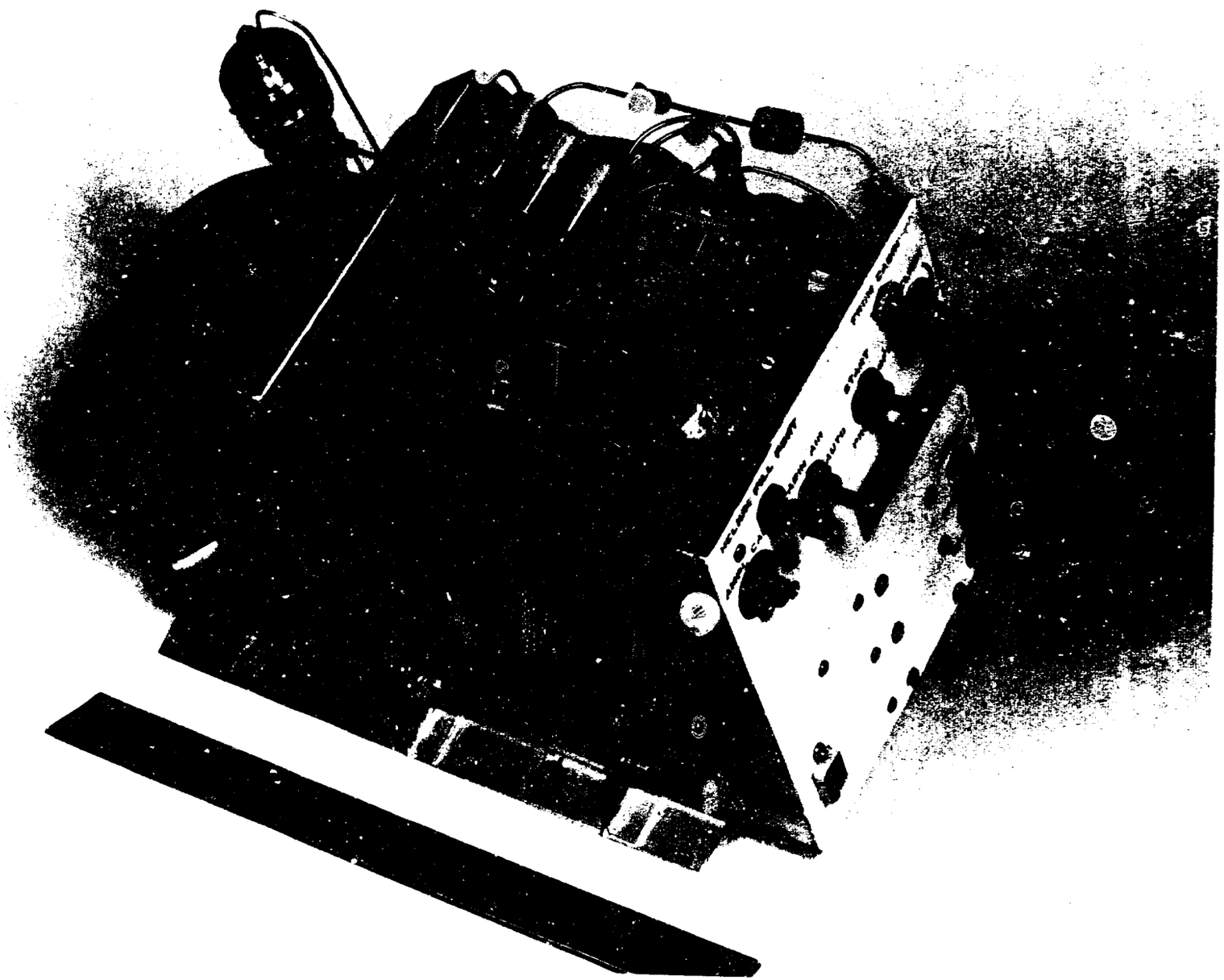

Figure 1. APOLLO GAS CHROMATOGRAPH

This instrument, like other gas chromatographic instruments, relies on the elution times of sample peaks for their qualitative identification. The certainty of correct identification is greatly enhanced through the use of two different liquid-phase partitioning columns which provide markedly different elution times. This instrument is capable of quantitatively analyzing samples containing as much as 100% oxygen and as little as a few ppm of organic contaminants, thus providing a very large dynamic range.

The columns consist of: (1) molecular sieve for permanent gas separation; (2) carbowax-amine coated Teflon for the separation of CO_2, H_2O, alcohols, and certain other organic compounds; (3) carbowax-coated chromosorb for separating the bulk of the probable organic compounds. The cross-section detector housing includes three chambers, one for each of the columns. The amplifier assembly automatically maintains the baseline at the zero level, except when a compound is eluted, and provides automatic attenuation of compound peaks present in concentrations greater than 50 to 100 ppm. The amplifier also provides for a rapid return to baseline of the trailing edge of major peaks so that minor peaks on the trailing edge may be measured at high sensitivity.

A schematic diagram of the gas system assembly is shown in figure 2. Each of the two sample source lines (upper right corner of figure 2) is supplied with a filter to remove particulate matter. The "From Cabin" line contains an emergency shutoff valve, V_2, which is closed by the pressure-sensing switch whenever the cabin pressure falls below 2.0 psia. This valve is necessary to prevent suit decompression in the event that cabin pressure is lost. The sample selector valve, V_1, allows either the suit or cabin sample to flow into the injection valve system. The other sample is circulated through a needle valve to the "To Suit" port. The sample to be analyzed flows through a filter and a check valve to the injection valve. Depending upon the valve positions of V_3, V_4, and V_5, this sample will flow through one of the 1 ml sample loops or the 0.2 ml sample loop to the metering device assembly. The sample is then allowed to flow out the space exhaust port.

The helium carrier gas flows through the injection valve assembly and through a check valve to the column selector valves, V_6, V_7, and V_8. In most cases, only one valve is open so that helium will flow through only one column at a time. Each column is preceded by a metering device assembly to control the carrier flow rate at 12 ml per minute. Each column is connected to a separate detector chamber. The detectors are followed by individual needle valves which maintain the detector pressure at 14.7 psia when the system is in operation. The needle valves are followed by helium shutoff valves V_9, V_{10}, and V_{11}. These valves always are in the same position as the corresponding column selector valves V_6, V_7, and V_8, so that any column-detector assembly is either open at both ends or closed at both ends. An emergency shutoff valve, V_{12}, is used between the rear of the system and the space exhaust port to prevent deterioration of the system by the space vacuum when the system is turned off. All of the switching valves in this system and the regulator were purchased from the Kidde Company.

The development of the columns and detector for this instrument has been previously reported by Huebner, Eaton, and Chaudet. *

*Huebner, V. R., H. G. Eaton, and J. H. Chaudet, Journal of Gas Chromatography, p. 125, April 1966.

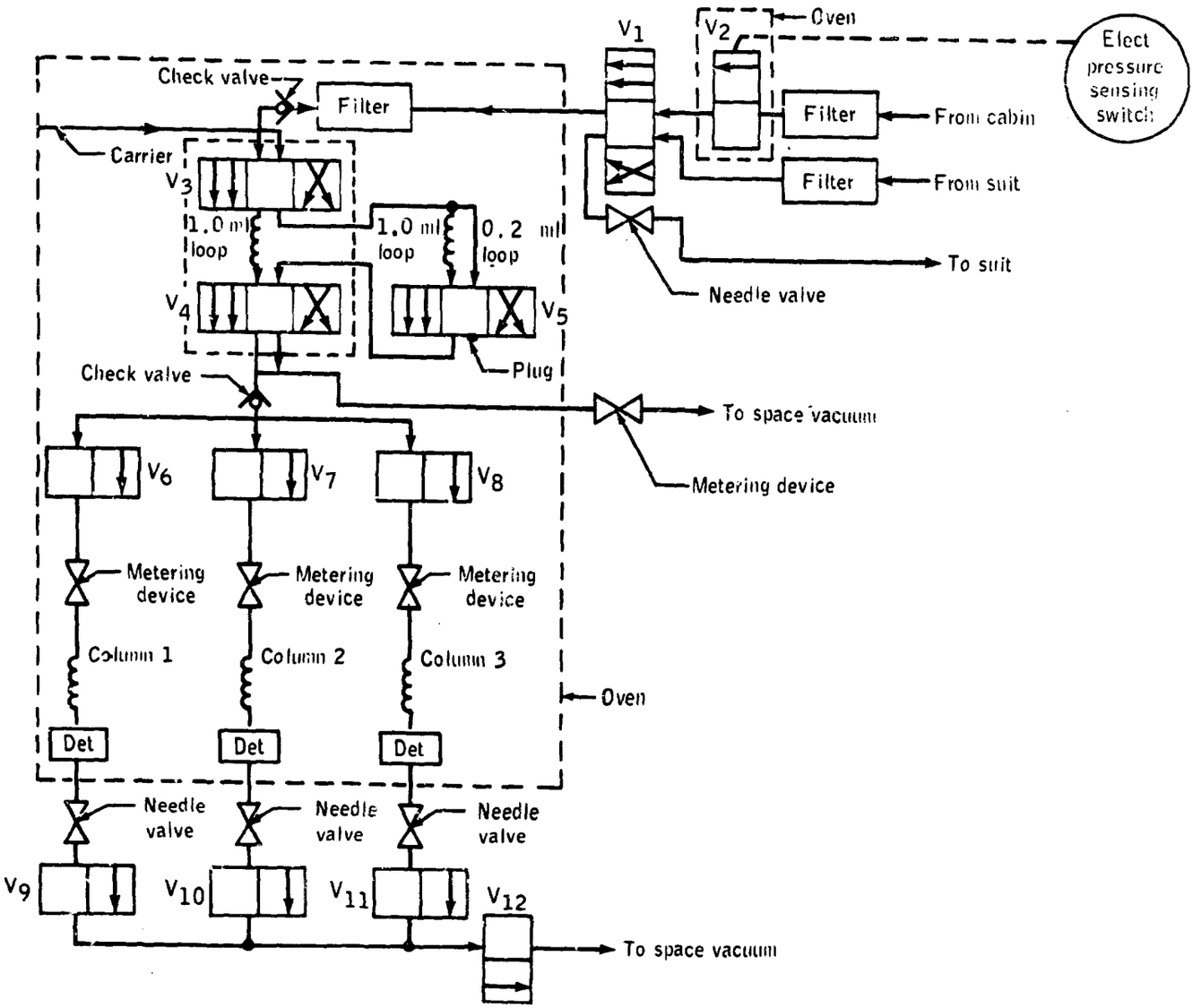

Figure 2. ANALYZER SCHEMATIC

All three columns are of 3/32" O.D., .073" I.D., stainless steel tubing; each has a flow rate of approximately 12 ml per minute and all are thermostated at 65 C an is the detector.

The permanent gases, hydrogen, oxygen, nitrogen, methane, and carbon monoxide are eluted from the eight-foot, 5 A molecular sieve column (column 1) in the order listed, within seven minutes. First a smaller sample (0.2 ml) is injected into column 1 for the purpose of analyzing the oxygen partial pressure. Next a large sample (1 ml) is injected into column 1 for the purpose of analyzing the minor components.

Column 2 is 12 feet long and contains 10% amine 220-carbowax 20M on Teflon 6. Column 3 is 12 feet long and contains 10% carbowax 20M on chromosorb G. After the sample has been analyzed twice on column 1, a new sample is introduced into column 2 for analyses and finally into column 3. A typical chromatogram is shown in figures 3, 4, and 5. These chromatograms are complicated by the automatic attenuation and by the coding indicators between four and five volts. With a little practice however, the chromatograms are not difficult to read. All chromatographic information is shown between zero and four volts. The signals between four and five volts are used as a code for indicating the attenuation (x1, x15, or x225).

Table I shows the relative retention times of several compounds on columns 2 and 3, and the absolute retention times on column 1. The absolute retention times for the reference cyclohexane on column 2 is 4.9 minutes and on column 3 it is 3.7 minutes. Typical calibration curves are shown in figures 6 and 7. Maximum noise level in these instances was 0.2V. Table II shows the result of a statistical evaluation of precision of response and retention time for a number of compounds at three different concentrations.

In conclusion, some of the design and performance details of an automatic, flight qualified gas chromatograph for use in the Apollo spacecraft have been described.

The instrument described in this paper was developed for the National Aeronautics and Space Administration, Houston, Texas, under Contract NAS 9-2518. The author would like to thank NASA and particularly John Lem, the technical monitor, for allowing the inclusion of this paper in the proceedings.

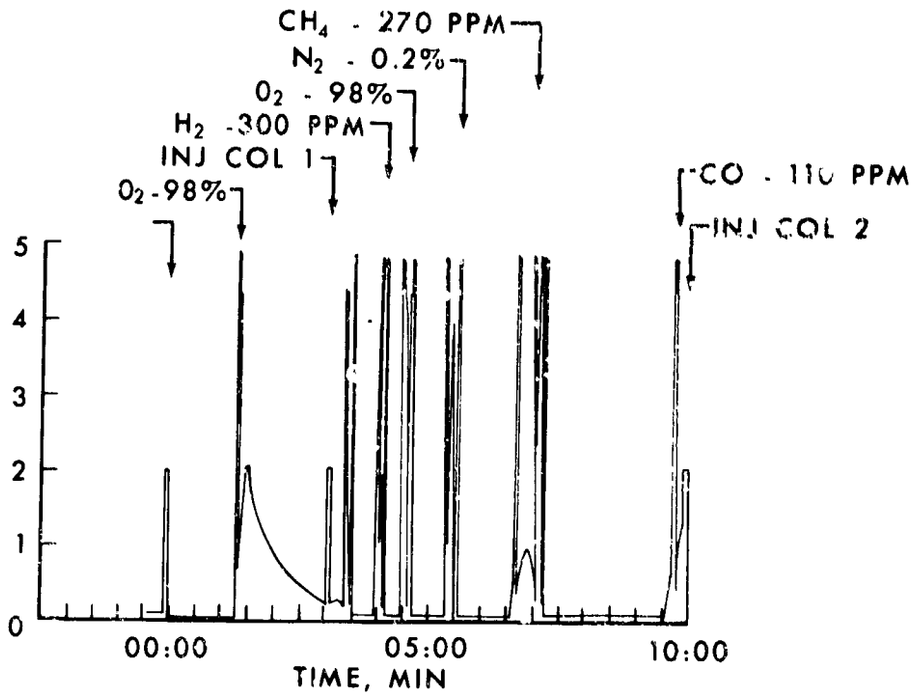

Figure 3. TYPICAL CHROMATOGRAM - COLUMN 1

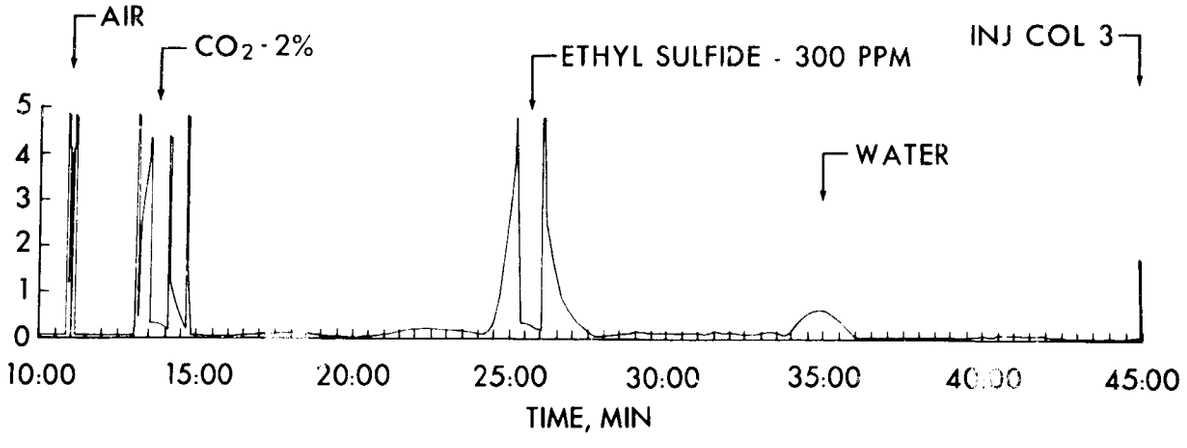

Figure 4. TYPICAL CHROMATOGRAM - COLUMN 2

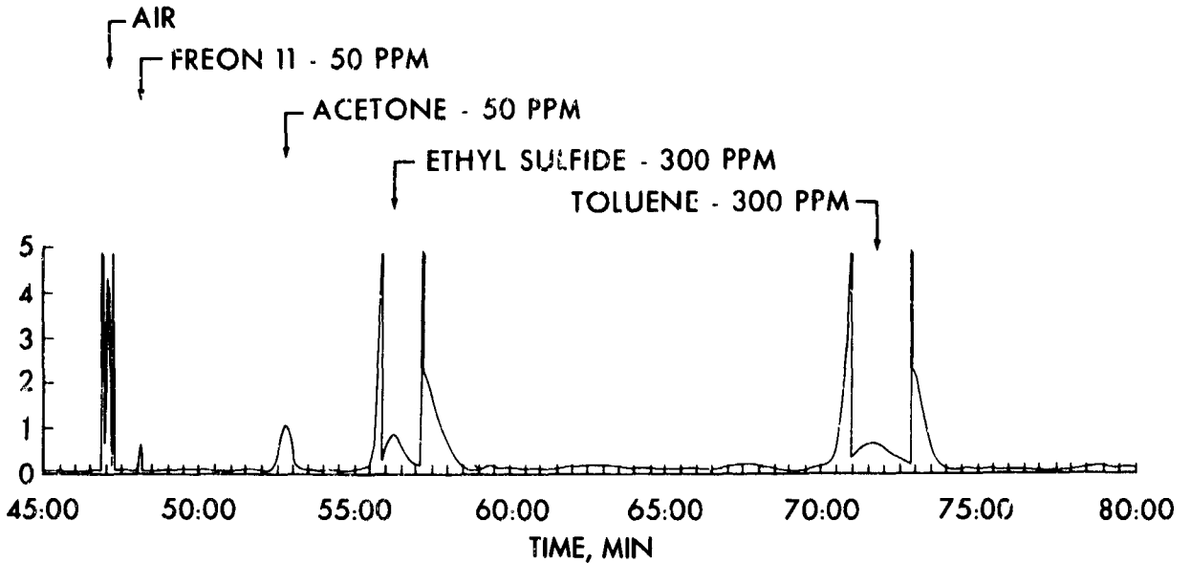

Figure 5. TYPICAL CHROMATOGRAM - COLUMN 3

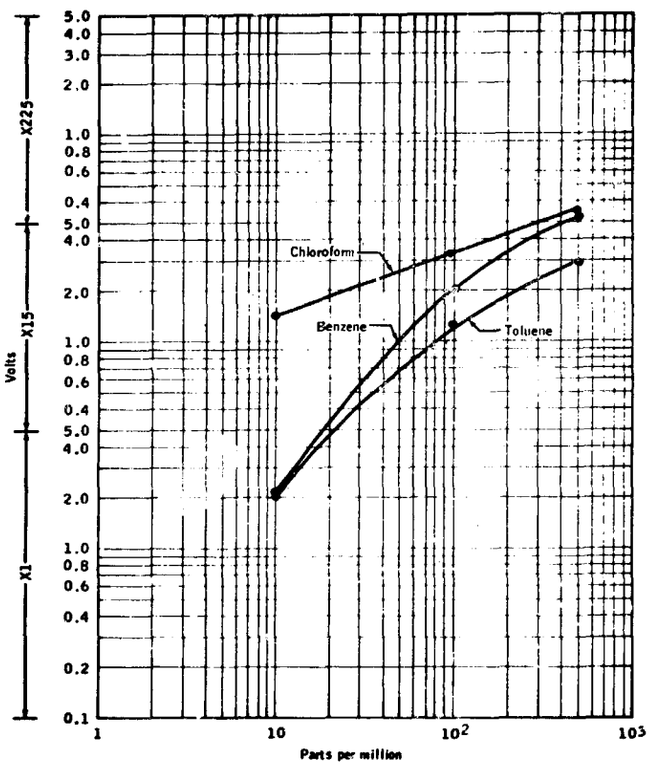

Figure 6. CALIBRATION CURVES, COLUMN 2

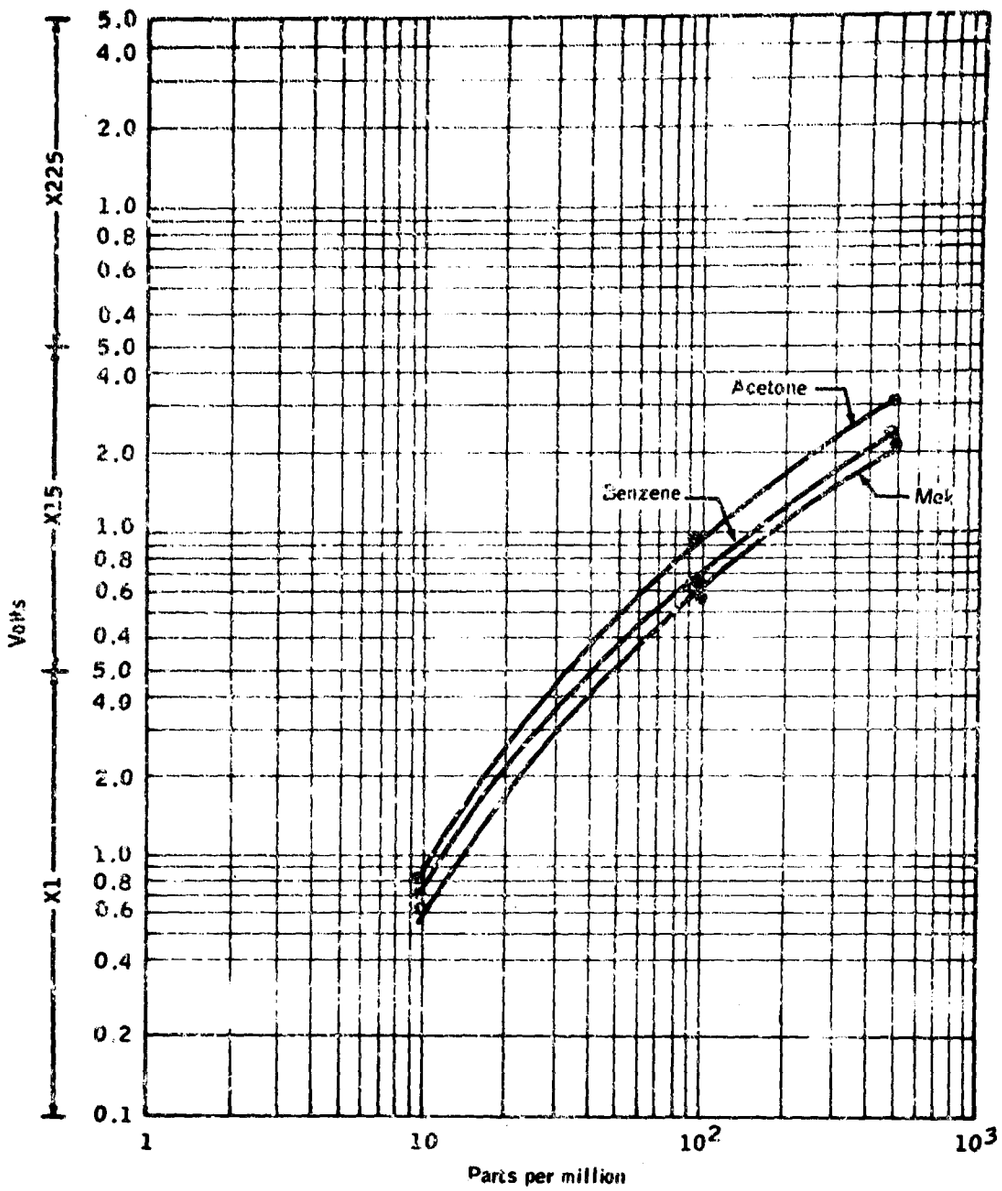

Figure 7. CALIBRATION CURVES, COLUMN 3

TABLE I

TYPICAL RELATIVE RETENTION TIMES

Compound	Column 1	Column 2	Column 3	Compound	Column 2	Column 3
Hydrogen	0.7			Isopropyl Alcohol	2.0	3.4
Oxygen	1.3			Ethanol	2.2	3.37
Nitrogen	2.1			Propyl Acetate	2.2	4.57
Methane	3.3			Water	2.36	--
Carbon Monoxide	5.7			Acetyldehyde	2.36	0.83
Acetylene		0.04	0.11	Chloroform	2.69	6.24
Freon 12		0.05	0.05	Trichloroethylene	2.71	5.4
Freon 14		0.08	0.05	Butyraldehyde	2.76	2.7
Freon 22		0.10	0.26	Isobutylacetate	3.2	6.05
Carbon Dioxide		0.10	0.03	Toluene	3.95	7.[illegible]
Pentane		0.19	0.19	Propanol	5.08	6.79
Freon 11		0.27	0.39			
Isoprene		0.33	0.51			
Methyl Mercaptan		0.44	0.65			
Hexane		0.47	0.35			
Dimethyl Sulfide		0.49	1.09			
Chloropropane		0.56	1.04			
Isopropyl Ether		0.57	0.56			
Acetone		0.61	1.67			
Cyclohexane		1.0	1.0			
Ethyl Acetate		1.08	2.22			
Methylene Chloride		1.13	3.39			
Dimethyl Hydrazine		1.16	2.7			
Heptane		1.19	0.76			
Butanone		1.31	2.91			
Methyl Chloroform		1.44	2.3			
Methanol		1.5	2.78			
Ethyl Sulfide		1.76	3.04			
Benzene		1.97	4.0			

Note: Retention times are relative to Cyclohexane for Columns 2 and 3. Times for Column 1 are from sample insertion.

TABLE II

ACCURACY DATA

Column 2

Compound	Concn PPM	Voltage Response Deviation (average ± 2σ)	Error %	Attenuation	Retention Time (min) (average ± 2σ)	Error %
Acetone	500	3.71 ± 0.16	±4.3	15	4.8 ± .09	±2.0
Methylene Chloride	500	2.66 ± 0.16	±6.0	15	7.9 ± .15	±1.9
MEK	500	2.36 ± 0.19	±8.1	15	8.8 ± .16	±1.8
Benzene	500	0.24 ± 0.014	±5.8	225	12.3 ± .25	±2.0
Chloroform	500	0.27 ± 0.020	±7.4	225	17.0 ± .44	±2.6
Toluene	500	2.93 ± 0.34	±12.	15	26.3 ± .95	±3.6
Acetone	100	2.25 ± 0.52	±23.	15	4.8 ± .17	±3.5
Methylene Chloride	100	1.54 ± 0.37	±24.	15	7.9 ± .11	±1.4
MEK	100	1.15 ± 0.20	±17.	15	8.9 ± .076	±0.85
Benzene	100	2.02 ± 0.37	±18.	15	12.3 ± .12	±0.98
Chloroform	100	3.20 ± 0.13	±4.1	15	17.0 ± .31	±1.8
Toluene	100	1.28 ± 0.05	±3.9	15	26.5 ± .29	±1.1
Acetone	10	1.12 ± 0.30	±27.	1	4.9 ± .14	±2.8
Methylene Chloride	10	1.22 ± 0.55	±45.	1	8.0 ± .13	±1.6
MEK	10	0.52 ± 0.38	±73.	1	8.9 ± .11	±1.2
Benzene	10	2.21 ± 0.64	±29.	1	12.4 ± .12	±0.97
Chloroform	10	1.43 ± 0.60	±42.	1	17.7 ± .31	±1.8
Toluene	10	1.97 ± 1.2	±61.	1	26.4 ± .55	±2.1

Column 3

Compound	Concn PPM	Voltage Response Deviation (average ± 2σ)	Error %	Attenuation	Retention Time (min) (average ± 2σ)	Error %
Freon 22	468	3.27 ± 0.12	±3.7	15	2.9 ± 0.08	±2.8
Hexane	500	3.24 ± 0.24	±7.4	15	3.3 ± 0.09	±2.7
Chloropropane	500	2.66 ± 0.31	±12.	15	6.3 ± 0.17	±2.7
Acetone	500	3.12 ± 0.36	±12.	15	9.6 ± 0.15	±1.6
Methyl Ethyl Ketone	500	2.17 ± 0.29	±13.	15	16.0 ± 0.43	±2.7
Methylene Chloride	500	1.87 ± 0.20	±11.	15	18.0 ± 0.50	±2.8
Benzene	500	2.15 ± 0.23	±11.	15	20.6 ± 0.39	±1.9
Freon 22	94	1.79 ± 0.17	±9.5	15	2.8 ± 0.20	±7.1
Hexane	100	1.92 ± 0.19	±9.9	15	3.2 ± 0.00	±0.0
Chloropropane	100	1.19 ± 0.11	±9.2	15	6.3 ± 0.13	±2.1
Acetone	100	0.79 ± 0.27	±34.	15	9.7 ± 0.075	±0.77
Methyl Ethyl Ketone	100	0.56 ± 0.19	±34.	15	15.9 ± 0.076	±0.48
Methylene Chloride	100	0.62 ± 0.080	±13.	15	17.9 ± 0.075	±0.42
Benzene	100	0.62 ± 0.14	±23.	15	20.5 ± 0.12	±0.59
Freon 22	9.4	0.76 ± 0.14	±18.	1	2.9 ± 0.11	±3.8
Hexane	10	1.03 ± 0.32	±31.	1	3.3 ± 0.00	±0.00
Chloropropane	10	0.90 ± 0.29	±32.	1	6.4 ± 0.066	±1.0
Acetone	10	0.79 ± 0.29	±37.	1	9.9 ± 0.18	±1.8
Methyl Ethyl Ketone	10	0.63 ± 0.15	±24.	1	16.1 ± 0.13	±0.81
Methylene Chloride	10	0.60 ± 0.16	±27.	1	18.0 ± 0.094	±0.52
Benzene	10	0.74 ± 0.078	±11.	1	20.6 ± 0.11	±0.53

Note: Retention times are from sample injection

GASEOUS MONITORING FOR THE INTEGRATED LIFE SUPPORT SYSTEM AT LANGLEY RESEARCH CENTER

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The Integrated Life Support System (ILSS) is an engineering concept and, in fact, a functioning test chamber. It was designed to provide for all the needs of four men, in a safe comfortable atmosphere, for intervals of time up to 90 days without resupply. It is, therefore, a prototype physical-chemical system to support life by reclaiming for reuse water and oxygen. It is the first to completely integrate within a test bed all the components for a closed environmental system, including man.

The Integrated Life Support System was conceived in late 1960 to emphasize the problems of integrating existing subsystems designed to operate at zero gravity. By the summer of 1963, a contract for the construction of an experimental hardware, breadboard-type system was let to General Dynamics, Convair Division. There were no stringent qualifications placed on the component parts because the total system was an experimental design. State-of-the-art hardware was procured and qualified in subsystem integration. Before delivery of the packaged systems, the builder conducted various performance tests, including a man-machine operation. There was limited gas monitoring equipment used in support of these tests. The unit was delivered to Langley Research Center (LRC) in August 1965 where it was installed and brought into operation.

The test bed is an 18-foot-diameter steel chamber arranged into two levels (figures 1 and 2). The top level is for crew quarters and activities. The lower level is for the life support subsystems. The atmospheric pressure can be controlled from a near-vacuum to a positive pressure. Entrance into the test bed can be accomplished without loss of internal environment integrity through an air lock. The following are the various subsystems found within and associated with the test bed: thermal control, atmosphere control, water management, waste management, personal hygiene, food management, and, finally, instrumentation and control measures (Armstrong, 1966).

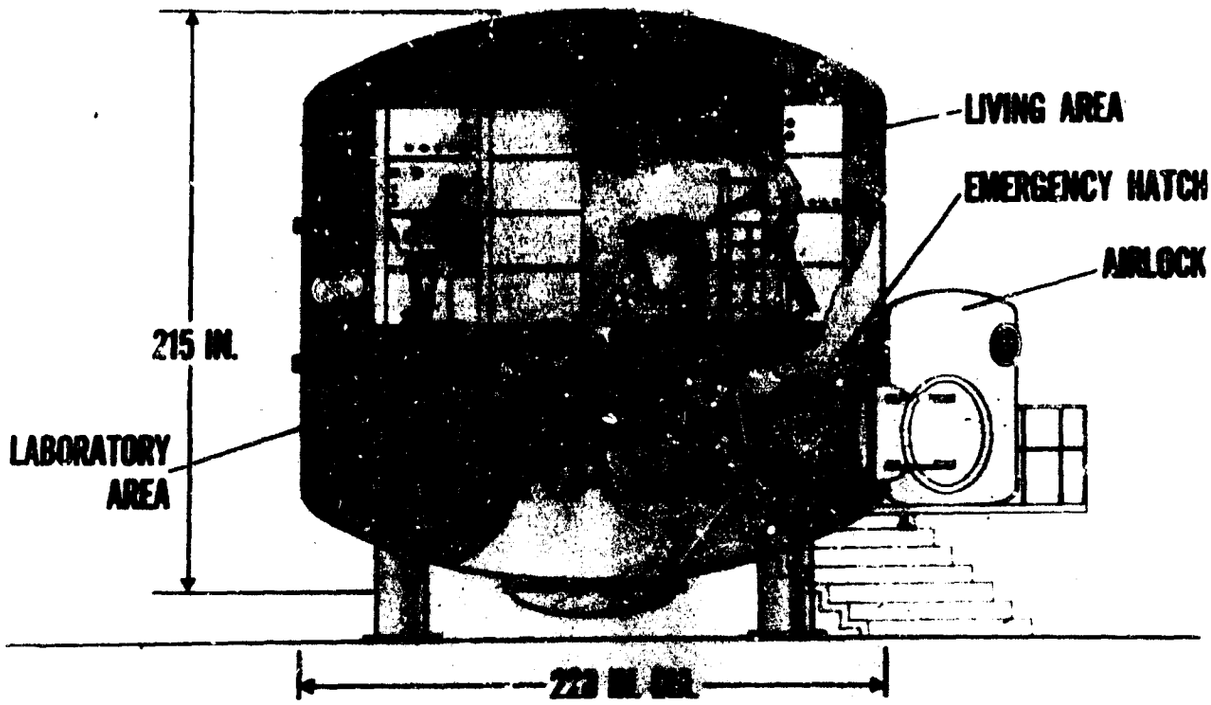

Figure 1. LIFE SUPPORT SYSTEM TEST BED - INTERIOR

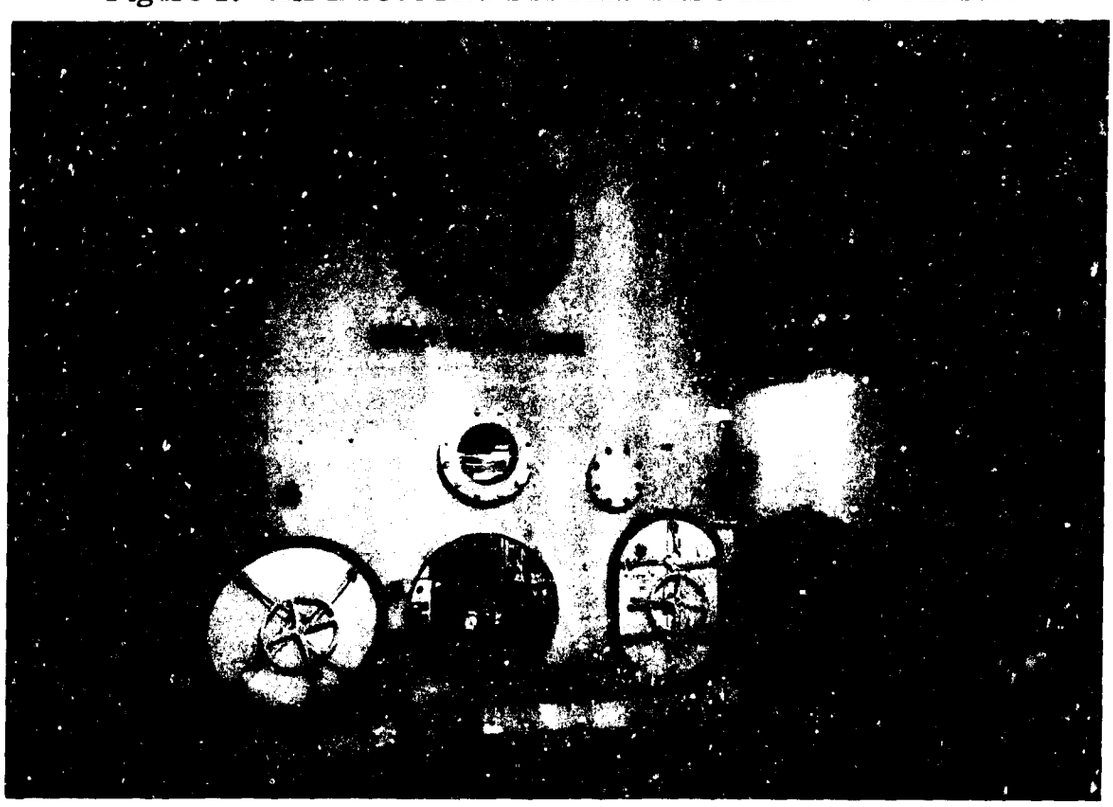

Figure 2. LIFE SUPPORT SYSTEM TEST BED - EXTERIOR

The thermal control subsystem has three interrelated control circuits to meet the requirements of integrated temperature control. The brayton nuclear reactor, which appears to be electrical power source of choice, has its waste heat simulated in a heat-transfer circuit. Space radiators are simulated by a low-temperature circuit for heat dissipation. The cabin air environment is maintained by the air circuit. It is worth noting that the heat load and the electric power loading are integral parts of the integration and simulation program.

The atmospheric control system regulates the composition of the cabin atmosphere by regulating water vapor, carbon dioxide concentration, regeneration of oxygen, removal of trace contaminants, and air circulation within the cabin.

The oxygen regeneration subsystem reclaims all the oxygen in the carbon dioxide generated by the crew and returns it into the cabin. The carbon byproducts are either collected or discharged to the outside. The regeneration subsystem has integrated into it the following components: (a) a carbon dioxide concentration unit for carbon dioxide removal from the cabin air, (b) a Bosch reduction unit which converts carbon dioxide and hydrogen to water and carbon, (c) a Sabatier reactor which converts carbon dioxide and hydrogen to water and methane (this unit was planned as a backup unit for the Bosch reactor), and (d) an electrolysis unit which converts water into oxygen and hydrogen; the hydrogen is passed to the reduction units.

Trace atmospheric contaminants are removed by continuously passing a fraction of the cabin air through catalytic burners and charcoal filters. The primary function of the catalytic burner is to oxidize carbon monoxide, hydrogen, and methane. The charcoal filters are in the system to remove higher molecular weight trace gases. There are several fiber glass filters to remove particulates from the air.

The cabin air circulation subsystem functions to maintain crew comfort requirements and heat-transfer requirements. In zero gravity operation, it is to function to prevent concentration gradients from being established.

The water management subsystem consists of two identical evaporation units for normal water recovery from urine, atmospheric water vapor condensate, and used wash water. The waste water is chemically treated at the time of collection to prevent bacterial action and chemical decomposition. Stored water, in conjunction with a standby multifiltration unit, is available for emergencies.

Evaporation is used for primary water purification. It is vaporized by recycling hot air through enclosed saturated wicks. A centrifugal water separator downstream of a condensing heat exchanger removes the water from the airstream and pumps it to holding tanks. The multifiltration unit used employs activated charcoal filters, an ion-exchange resin bed, and bacterial filters.

The waste management subsystem collects and vacuum-dries feces at an elevated temperature; collects and transports urine to the water reclamation subsystem. The dried feces and other wastes are stored in tightly sealed canisters.

The personal hygiene subsystem is relatively rudimentary. An interesting item in this subsystem is the "zero-g-sponge squeezer". It allows loading the sponge with water and also freeing it of water.

Food management also is rather rudimentary. The most important component is the food preparation console which provides hot and cold water by variable metering dispensers.

Finally, the instrument and control system permits safe, controlled, manned, and unmanned operation of the test bed. The functions of the system are to: (1) sense and read out physical quantities (i.e., pressure, flow, temperature, etc.), (2) control variables for stable subsystems operation, (3) failure warning, (4) provide manual and automatic overrides to protect equipment from destruction.

This system is a composite of equipment panels, onboard status panels, a ground control console, linked by a failure warning and alarm system to provide information on the status of the various subsystems.

Gaseous monitoring experience for LRC personnel began in 1963 with the prematurely terminated Manned Environmental System Assessment (MESA I) study at The Boeing Company in Seattle. The men within the chamber were made ill by gaseous contaminants and the test was terminated. This test was the dramatic example needed to make the aerospace community aware that gas monitoring capabilities were necessary and that materials selection was important. A complete stripping and material selection of the space cabin simulator furnishings permitted a successful 30-day test to be carried out in 1964. Gas monitoring equipment at that time did not generally have the sensitivity to detect the low levels of gaseous contaminants that have been reported to be in simulator environments. In an attempt to overcome this limitation, a Karman detector was built into a process gas chromatograph for the on-line monitoring of MESA II, again at Boeing in Seattle (Anon., NASA CR-134, 1964).

In an attempt to illustrate the evolution of the gas monitoring system associated with the ILSS, several related topics will be presented. They are (1) analytic equipment and procedures, (2) sampling, and (3) support activities.

Carbon monoxide and many organic compounds have been reported to be in the atmospheres of space cabins, space cabin simulators, and nuclear submarines. Their concentrations have been low from an analytical chemistry point of view. This does not mean the concentrations are without biological significance. Thus, instrumentation of high sensitivity was sought. In a number of instances the sensitivity requested exceeded that available at the time. Single gas detectors, multicolumn gas chromatographs, and wet chemistry procedures were the chosen pathways.

Single gas monitoring should be a relatively simple matter. There are sensitive detectors for such gases as oxygen, carbon dioxide, and carbon monoxide, but with an added requirement of being able to function equally with two different total gas pressures, there must be some redesigning. The paramagnetic detectors for oxygen monitoring had internal compensation built into them to meet this requirement. Single gas, infrared monitors were chosen to monitor for carbon dioxide in the range 0 to 2.5 percent, and for carbon monoxide in two ranges, 0 to 50 ppm and 0 to 100 ppm. The lower range necessitated redesign and increased cell length. The carbon monoxide maximum concentrations were chosen to coincide with the Threshold Limit Value of the American Conference of Governmental Industrial Hygienists and submarine experience.

A general purpose thermal conductivity detector, process gas chromatograph was secured to monitor various stages of gas processing in the oxygen reclamation subsystem and also the gaseous environment. The sampling streams are fed into the chromatograph from the transfer panel. Ten sampling streams are fed into the chromatograph from the transfer panel. By connecting a flexible tube to a sampling port, any additional location within the test bed may be sampled. This process chromatograph has four columns. Two identical columns are to strip out the higher molecular weight organic compounds and retard water vapor. Carbon dioxide is retained and eluted from one column. The last column is used for separating hydrogen, oxygen, nitrogen, methane, and carbon monoxide.

Two samples are taken simultaneously from each sample stream: one 300 microliters and the other 3 milliliters in size. The smaller sample is used for gases in high concentration. The larger sample is used for the low concentration gases. The chromatograph is programmed to handle the samples in sequence at intervals of 10 minutes. A complete sampling cycle requires 200 minutes. The following table illustrates the sampling sequence, the programmed calibration ranges for each gas, and sample stream.

TABLE I

PROCESS GAS CHROMATOGRAPH, CONCENTRATION RANGES

Stream	Sample inject	H_2O	CO_2	H_2	O_2	N_2	CH_4	CO
1	Small		0 - 100	0 - 100	0 - 100	0 - 100	0 - 100	0 - 100
	Large	0 - 10	0 - 5	0 - 5	-	-	0 - 5	0 - 0.1
2	Small	-	0 - 100	0 - 100	0 - 100	0 - 100	0 - 100	0 - 100
	Large	0 - 10	0 - 5	0 - 5	0 - 5	0 - 5	0 - 5	0 - 5
3	Small	-	0 - 100	0 - 100	0 - 100	0 - 100	0 - 100	0 - 100
	Large	0 - 10	0 - 5	0 - 5	-	-	0 - 5	0 - 5
4	Small	-	0 - 100	0 - 100	0 - 100	0 - 100	0 - 100	0 - 100
	Large	0 - 10	0 - 5	0 - 5	-	-	0 - 5	0 - 5
5	Small	-	0 - 100	0 - 100	0 - 100	0 - 100	0 - 100	0 - 100
	Large	0 - 10	0 - 5	0 - 5	-	-	0 - 5	0 - 5
6	Small	-	0 - 100	0 - 100	0 - 100	0 - 100	0 - 100	0 - 100
	Large	0 - 10	0 - 5	0 - 5	-	-	0 - 5	0 - 0.1
7	Small	-	0 - 100	0 - 100	0 - 100	0 - 100	0 - 100	0 - 100
	Large	0 - 10	0 - 5	0 - 5	-	-	0 - 5	0 - 5
8	Small	-	0 - 100	0 - 100	0 - 100	0 - 100	0 - 100	0 - 100
	Large	0 - 10	0 - 5	0 - 5	-	-	0 - 5	0 - 5
9	Small	-	0 - 100	0 - 100	0 - 100	0 - 100	0 - 100	0 - 100
	Large	0 - 10	0 - 5	0 - 5	-	-	0 - 5	0 - 5
10	Small	-	0 - 100	0 - 100	0 - 100	0 - 100	0 - 100	0 - 100
	Large	0 - 10	0 - 5	0 - 5	-	-	0 - 5	0 - 0.1

NOTE: Concentrations in percent by volume

The Karman detector gas chromatograph that was mentioned earlier was further modified as the result of the experience acquired during the MESA II test. The concentrations of the "permanent" gases so overloaded the electrometer system that subsequent peaks were undetected. The chromatograph was modified and programmed to shunt these gases out of the detector-gas stream. This modification permits the Karman process chromatograph to function as a trace contaminant detector. The plumbing to the trace analysis chromatograph allows it to be used in two ways. It may be operated independent of the process chromatograph for atmospheric sampling. It may be operated in conjunction with the process gas chromatograph for trace analysis in the process streams of the various subsystems. The trace analysis chromatograph has been calibrated for certain organics that have been reported in the aerospace and submarine literature. Listed in the following table are the compounds and the concentration ranges for which the instrument was calibrated.

TABLE II

Compound	Range in ppm
Acetone	0 - 10
Methanol	0 - 20
Benzene	0 - 10
Trichloroethylene	0 - 10
Methane	0 - 120
Carbon monoxide	0 - 30
Hydrogen	0 - 300

Methane could be a major contaminant arising from man and machine. Thus, analytic information on methane and all other hydrocarbons would be highly useful. A total hydrocarbon analyzer is used to give us this additional information.

The total hydrocarbon analyzer is a flame ionization detector device. Col. Thadeus Domanski (USAF) in cooperation with LRC personnel decided that a first approximation to organic materials in the atmosphere could be had by determining the total hydrocarbons. Methane equivalents were chosen as the means of expressing this concentration. The difference between this value and the methane value from the Karman gas chromatograph would be the approximate concentration of the contaminants. This instrument operates on-line and gives real-time information. These results have more immediate meaning than those that have been generated by trapping procedures.

Construction of instrumentation can pose unexpected problems. One instrument that was delivered to Langley Research Center and one constructed at Langley were plumbed with copper tubing. There was sufficient surface to catalyze the conversion of organic compounds. This resulted in divergent readings from several monitoring locations. Replumbing with stainless steel tubing corrected this problem. Helium carrier gas contamination posed a problem in the early stages of putting the chromatographs into operation. Helium delivered in tank cars was bottled and used. It was discovered that the water vapor concentration was sufficient to poison the chromatograph columns. Assayed helium with a guaranteed analysis of 99.999 percent helium was substituted.

Reliable sampling of gases from the various locations in the test bed continues to be a problem. Many of the deficiencies are recognized. A problem not recognized by many who have not had to sample organic gas mixtures is the length of tubing (surface exposure) to which the gases are exposed. We have 37 lines about 40 feet long running from the test bed to a transfer or patchpanel. This surface exposure poses a sampling uncertainty and an analytical nightmare. Although care has been taken to prevent condensation within the tubes, there are thermal gradients and associated adsorption and desorption sites. Thus, the sample analyzed has a high probability of not being the one drawn originally. Efforts are being directed toward a solution of this sampling problem. The transfer or patchpanel is an interconnecting link between the test bed and the atmospheric monitoring areas. It was designed to simplify sample stream selection and delivery to the analytical equipment, cryogenic sampling system, and the wet chemistry area.

The cryogenic sampling system was designed for LRC by Atlantic Research Corporation. It permits trapping samples at various low temperatures with regulated flow rates. The sample trap may be easily replaced through the use of quick disconnect couplings. Samples are drawn through the stainless steel traps at a reduced pressure of 400 torr in an attempt to reduce oxygen trapping. With the removal of the traps from the cryogenic traps, they begin to warm, the pressure increases. For safety purposes the pressure is kept within the safe pressure range of the bottle. Temperature changes, pressure changes, and release may alter the sample before analysis.

The wet chemistry area was established to analyze for the more odoriferous byproducts of man and some from the processes. From the results of this work, it was felt that the requirements for automatic equipment could be established. So far, no equipment has been requested to handle these analyses. The table below lists the compounds monitored and the procedure used to analyze for it.

TABLE III

Compound	Method
Ammonia	a. Acid neutralization (Williams and Miller, 1962) b. Ninhydrin reaction
Sulphur dioxide	Colorimetric (Stephens and Lindstrom, 1964)
Hydrogen sulfide	Lead sulfide precipitation (Smith, et al, 1961)
NO NO_2	Colorimetric (Anon., ASTM D1607-60, 1964)
Mercaptans	Colorimetric (Pennfoy, et al, 1964)

Additional support equipment is immediately available to assist in identifying contaminants. This exists in two F and M 810 gas chromatographs: an infrared spectrophotometer with a 10-meter cell, and a research gas analysis laboratory. In other parts of LRC, additional support equipment is found in the form of microwave spectrometers and a high-resolution mass spectrograph. These have not been used to any great extent in ILSS support.

The F and M 810 gas chromatographs are two-column, flame ionization detector, general purpose instruments. They are calibrated for a large number of organic compounds reported in the aerospace literature.

The research laboratory has gas chromatography and infrared spectrophotometry capabilities. This laboratory was established by Dr. Robert M. Bethea for comprehensive analytical work. In addition to the analytical procedures established, a computer program was developed to reduce the time for complete analysis of contaminants.

The complexities of putting a fully integrated life support system into operation are manifested in the time it has taken from delivery and our first closed-door test. A number of open-door tests were performed to check the functioning capabilities of the individual subsystems and the supporting equipment. As a result of these tests, a major subsystem improvement program was started. As the improvements were completed, the subsystems were again evaluated. Completion of improvements and satisfaction with the evaluation results permitted proceeding on to the next step in the testing program, closed-door integrated system operation.

The first closed-door test for the ILSS at Langley Research Center was accomplished during the 7-day period from January 31, 1967 to February 7, 1967. Several major objectives were obtained in this test, one of which was atmospheric monitoring experience with all life support equipment in operation and the hatches closed.

The most significant observation in atmospheric monitoring was the low level of contamination. The baseline for total hydrocarbons before the hatches were closed was 3 ppm (parts per million). After hatch closure, it slowly rose to stabilize at 6 ppm. There was one excursion above this level to a value of 15 ppm. The contaminant control system appeared to remove this unidentified contaminant.

The nonadapted nose is still the most sensitive detector for odors. This was illustrated again in this test. A small mechanical pump used for atmospheric bacteriologic sampling discharged some oil vapors into the chamber air. The odor was most disagreeable to men who had to enter the chamber. Discontinued use, removal of the pump, and the contaminant control system returned the atmosphere to an acceptable point. None of the analytical equipment detected the material - not even the total hydrocarbon analyzer.

An unplanned contaminant removal system has been in nearly constant use for over a year. Filtered air driven by a powerful blower has kept the chamber flushed out, also preventing accumulation by adsorption and absorption. Many contaminants have been eliminated by preventing admission into the chamber and by materials selection.

We do not have a complete analysis of our contaminants, nor do we know how man will disturb the system. We are preparing to define and attempt solutions of these and other problems of atmospheric contamination in the ILSS.

SUMMARY

The Integrated Life Support System was conceived to study the problems of integrating regenerative equipment designed to operate in a negligible gravitational field. It is the first to fully integrate the three major contributors to atmospheric contamination: man, machine, and materials.

Aerospace literature has been replete with many lists of organic compounds that may have been in space cabins and space cabin simulators. Some of these compounds are real; others are perhaps artifacts of sampling and sample handling. Nonetheless, the choice of instrumentation for ILSS contaminant monitoring was predicated on the assumption that there might be a problem. Several types of sensitive instrumentation have been employed to monitor this potential problem, the most notable being the total hydrocarbon analyzer. This instrument gives a first approximation of the contaminant problem. The results appear to be more reliable than the results, till now, from more sophisticated instrumentation and sampling procedures. Gas chromatography, infrared and mass spectroscopy, and microwave analysis will give the final definition of the contaminants. Before these instruments can truly be effective in contaminant definition, the problem of sampling and sample handling will have to be solved.

The limited contaminant problem experienced during the 7-day closed-door test of the ILSS illustrates the effectiveness of the contaminant removal system. A fact which should not be overlooked in contaminant removal in the ILSS was the unplanned but virtually continuous forced air flushing of the chamber for one year. The nonadapted nose is still the most sensitive detector for odoriferous compounds in small quantities. During the test procedure, a disagreeable odor was observed. A microbiological sampling pump had been in operation. The air contaminant had not been detected by the analytical instrumentation. Discontinued use and removal of the pump, coupled with the trace contaminant removal system, discharged the odor within eight hours.

Much data are yet to be derived from this test chamber on man, machine, and material interactions.

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MR. WILLARD (Honeywell Aeronautical Division): Was the atmosphere normal ambient or was it pure oxygen?

DR. WILSON: Normal, ambient.

QUESTION: The base line that you reported of three parts per million - was this for the unoccupied chamber?

DR. WILSON: Unoccupied chamber with the doors open out to the atmosphere. That reminds me, I forgot to mention that we did have a very effective contaminant removal system operating for one whole year. We have had a filtered air blower, blowing through this chamber at a pressure so that there was no ingress of outside air except what might trail along under lab coats or clothes. We have had the blower operating for one whole year on this chamber, blowing out everything that might have been coming off all the plastics and all the processes, so there was no adsorption or desorption propositions being laid down someplace else and then being washed away.

QUESTION: You mean this system was essentially degassed for a year?

DR. WILSON: Yes.

QUESTION: Was there any control exercised over the materials going into this system?

DR. WILSON: That was one of the points I forgot to mention. We have been fairly careful in the selection of the materials going into the chamber, and we have watched this quite closely.

QUESTION: By wet test?

DR. WILSON: No, we have followed the guidelines of the Navy, the testing procedures for Apollo and Mercury for the selection of devices. When they said something was inadmissible to these systems, we kept it out.

COL. WESTLAKE (Space Systems Division): I was wondering if that was a single or double wall chamber?

DR. WILSON: Single wall.

QUESTION: And you mentioned 10 psi?

DR. WILSON: Yes.

QUESTION: As well as ambient?

DR. WILSON: Ambient, yes.

QUESTION: At 10 psi you would have an inboard leak then, is that right?

DR. WILSON: That is right. The test for this leak was found to be a little over a pound a day. We have not operated at 10 psi yet.

APPROACHES TO CONTINUOUS ANALYSIS OF EXPOSURE CHAMBER ATMOSPHERES

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INTRODUCTION

One of the most important aspects to be considered when exposing laboratory animals to various concentrations of toxic contaminants is that of sampling and analyzing chamber atmospheres for achievement of necessary contaminant control. A convenient method is that of "batch" analysis, where a chamber sample is drawn into a midget bubbler containing some absorbing solvent, and the resulting solution is subjected to chemical analysis. During the early work done on monomethylhydrazine (MMH) in this laboratory, a "batch" technique was developed which entailed condensing MMH with p-dimethylaminobenzaldehyde (DMBA) and measuring the absorbance of the product. This was a variation of a procedure used for MMH in blood described by Reynolds and Thomas (1965). A number of difficulties were experienced while attempting to analyze exposure chamber atmospheres for MMH using this technique. Sometimes there appeared to be degradation of the MMH on the fritted glass bubbler. Occasionally reagents were prepared which did not give expected results with known MMH solutions. In addition, analytical techniques differed among technicians with the result that significant variations existed among replicate analyses of samples from the same chamber. Because of these errors, it was difficult for the toxicologist to correlate mortality data with chamber concentration. When acute exposures are being tested, it is important that the contaminant concentration be known exactly over the whole period of exposure, which may be as short as five minutes. The toxicologist must know the mean concentration, the time to achieve final concentration, and fluctuations in concentrations. Since it normally takes more than five minutes to absorb a "batch" sample in a midget bubbler, it is impossible to gain any knowledge of these parameters. In an attempt to overcome these limitations, the possibilities of continuous analysis were investigated. The obvious advantage of continuous analysis is that the measurement represents the concentration in the chamber at any instant rather than an average over a period of time. Thus, it is possible to measure the time to achieve equilibrium concentration as well as fluctuations during an exposure.

MATERIALS AND METHODS

Since our laboratory was in possession of two types of continuous analyzers, the Technicon Auto-Analyzer and the Mine Safety Appliances Billion-Aire, it was decided to apply both instruments to the problem. The operation of the Billion-Aire depends upon the formation of a solid aerosol by combining, in this case, trifluoroacetic acid (TFA) vapor with MMH pumped from the exposure chamber. This aerosol captures electrons in a radium source, and the decrease in electron current is a measure of MMH concentration. The Auto-Analyzer, as used in this application, absorbs MMH from a gaseous stream pumped from the exposure chamber into a liquid containing a material which will either develop color, or decrease color, after reaction with the MMH. The transmittance of the solution is measured continuously in a flow colorimeter and can be related to the concentrations of the MMH.

The Billion-Aire has certain advantages over the Auto-Analyzer. No standard solutions need to be prepared, and the instrument has fewer separate components which might need frequent maintenance and replacement. There were some possible disadvantages to the Billion-Aire. The sensitivity of the instrument was so great that in the MMH concentration range of interest (50-300 ppm) it was found necessary to dilute the sample ten times with air. Since previous work had shown that MMH is unstable in air, there was uncertainty as to whether good accuracy could be achieved with the technique. Standard bag samples would also be needed to calibrate the instrument, and it was thought that the standards would have to be prepared in nitrogen. The effect of nitrogen on the Billion-Aire was unknown due to the difference in electron capture cross-section of nitrogen vs. oxygen. The Billion-Aire requires calibration and the analytical data supplied by the instrument would be no more accurate than the prepared standard bag samples. If air oxidation or destruction of MMH was reproducible, it would never be detected, and erroneous results would be obtained for exposure chamber concentrations. Therefore, a stoichiometric method of analysis, i.e., one which gave predictable results for MMH concentrations, was needed to analyze the standard bag samples accurately and to supply us with a backup method in case we were unable to use the Billion-Aire. The Auto-Analyzer was found to provide the required stoichiometry.

Previous work had shown that MMH could be titrated in the same manner as hydrazine (Audrieth and Ogg, 1951) with a standard solution of iodine if the pH is maintained between 7.0 and 7.4 according to the following equation:

$$CH_3NHNH_2 + 2\,I_2 \rightarrow N_2 + 3\,HI + CH_3I \;^{*}$$

On this basis, a continuous titration could be established employing the Auto-Analyzer using a standard iodine solution as the absorbing or scrubbing medium according to the flow diagram shown in figure 1.

Air from the exposure chamber is pumped through the scrubber coil, the flow rate being adjusted by means of the needle valve immediately following the flow meter. Concurrently, iodine solution is pumped through the scrubber using the peristaltic Auto-Analyzer pump. A portion of the solution, after it has contacted the chamber air, is pumped into a flow colorimeter which directs a signal to a

*Products were not identified

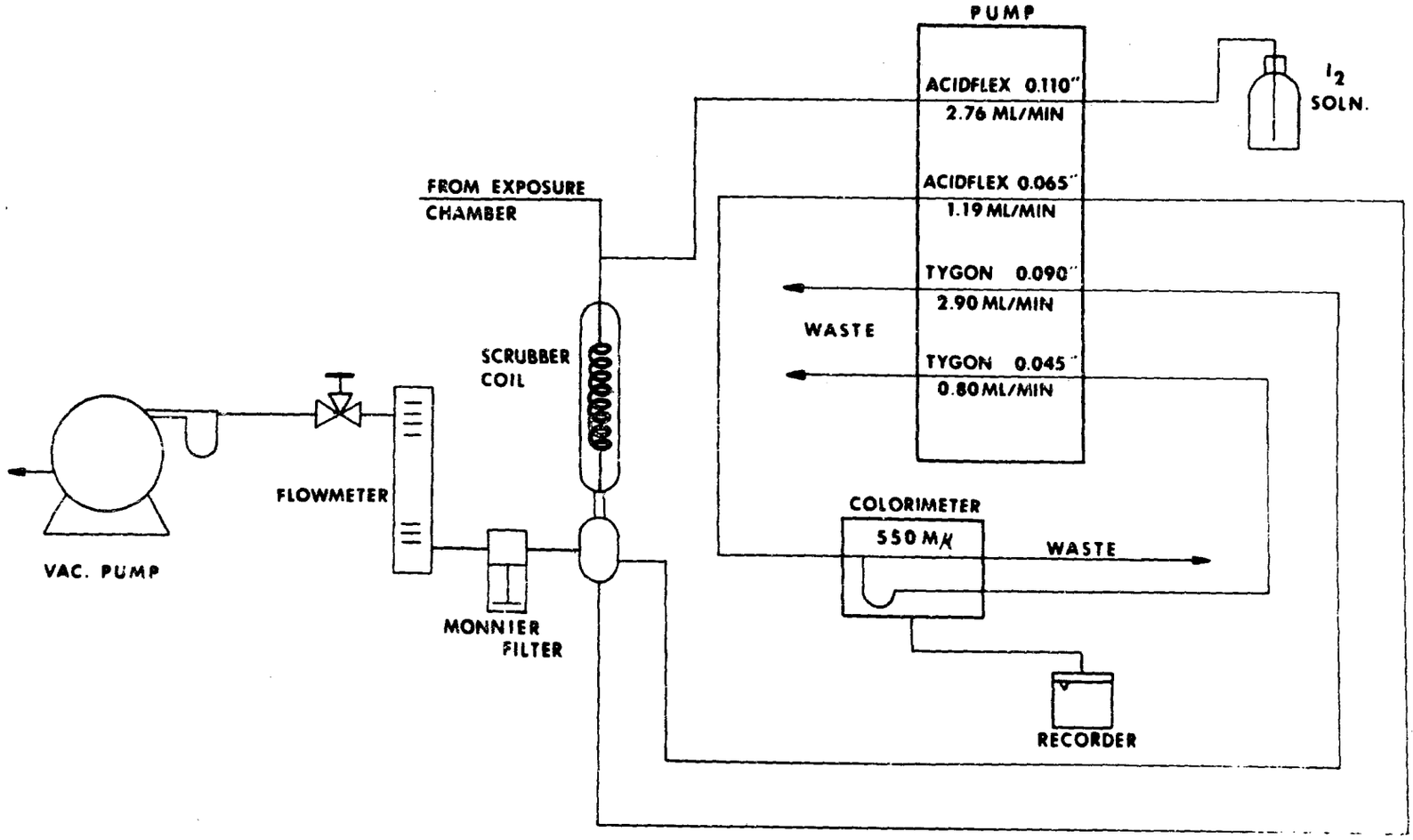

Figure 1. FLOW DIAGRAM FOR TECHNICON AUTO-ANALYZER

ratio recorder where the transmittance is continuously recorded. All Auto-Analyzer tubing in contact with iodine solution prior to entering the colorimeter is "acidflex" (a fluorocarbon elastomer). Tygon forms a complex with iodine and would remove iodine from solution or release it depending upon its concentration, thus invalidating any quantitative interpretation.

RESULTS AND DISCUSSION

Auto-Analyzer Method

A solution of 0.1N iodine in 0.25N potassium iodide was prepared and diluted 1-100 and 1-200. Absorption curves of the two solutions were run from 420 mμ to 600 mμ. Figure 2 demonstrates that Beer's law is followed over the whole wavelength range although no peak is obtained. A series of iodine solutions ranging in concentration up to 0.002N were introduced into the system. Ten minutes were required to reach equilibrium when concentrations were changed. Beer's law was followed very well as illustrated in table I. A 10% change in concentration gave a 10% change in absorbance. Distilled water was passed through the system before and after the iodine solutions and showed essentially zero absorbance in both cases.

TABLE I

IODINE CONCENTRATION VS. "A-A" ABSORBANCE

CONC. IODINE SOLN. (N)	EQUIVALENT MMH CONC. (PPM)	% T	ABS.
0.0020	0.000	25.1	0.602
0.0011	76.0	47.3	0.325
0.0010	84.5	50.8	0.294
0.0004	135	77.1	0.113
0.00036	139	79.2	0.101
0.000 Initial	169	100.0	0.000
0.000 Final	169	99.5	0.001

In order to further examine the variation of absorbance of iodine with changes in concentration, solutions of 0.0002N, 0.0004N, and 0.0006N I_2 equivalent to MMH concentrations of 152, 135, 118 ppm, respectively (± 17 ppm or ± 12.5% relative), were sampled in random order at three-minute intervals by the Auto-Analyzer. In this experiment we attempted to determine the maximum variability in absorbance for a particular concentration. The absorbances as measured after a ten-minute lag time are presented in figure 3. The individual absorbance measurements for each concentration are summarized in table II. The extreme and rapid concentration

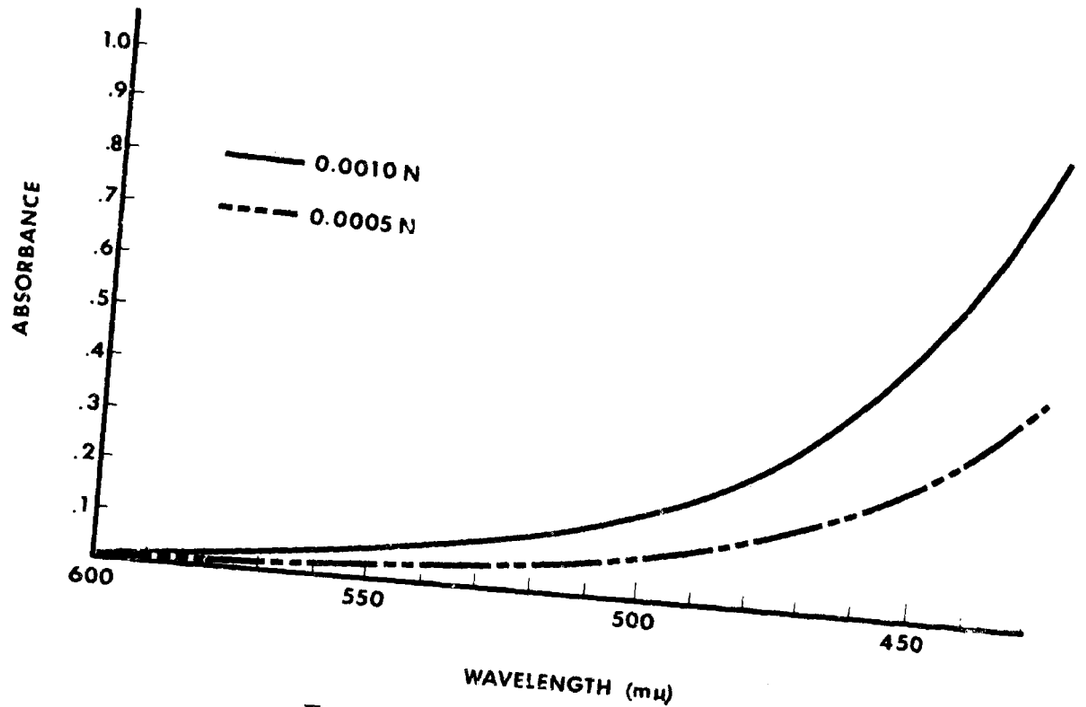

Figure 2. % T VS. TIME

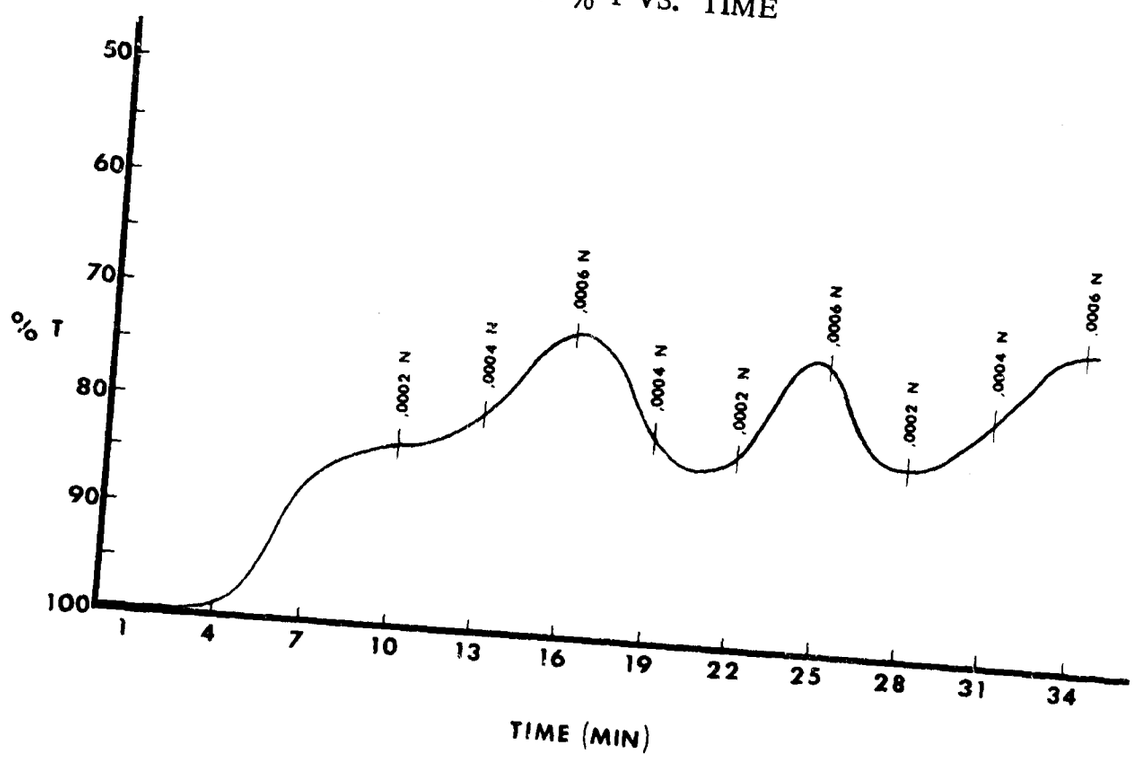

Figure 3. ABSORBANCE VS. WAVELENGTH

variations of this experiment introduced a maximum standard deviation of only ± 10%.

TABLE II

ABS. OF I_2 SOLNS. VS. THREE-MINUTE CONC. CHANGE

Normality (N)	0.0002	0.0004	0.0006
Number of Measurements	6	6	6
Average Absorbance	.075	.102	.135
Range of Absorbance	.066 .082	.088 .118	.130 .142
Standard Deviation	.0055	.0101	.0045

Drawing air containing no MMH through the system decreased the absorbance of the iodine solution. Table III shows that the reduction of absorbance was proportional to the flow rate of air pulled through the absorber. Either the iodine was being reduced by airborne particulates or was being lost by volatilization as free iodine. Installing a high efficiency filter in the air line removing particulate matter had no effect on the loss of iodine and it was, therefore, assumed that the loss was due to vaporization of the iodine into the air stream. This vaporization was ascribed to a significant concentration of free iodine in the Auto-Analyzer solution due to an insufficient amount of potassium iodide. In order to decrease the free iodine, a high concentration of potassium iodide was maintained in each dilution by adding 40 grams of solid potassium iodide to give a 0.5N potassium iodide solution.

TABLE III

ABS. IODINE VS. AIR FLOW

CONC. I_2 (N)	AIR FLOW (CC/MIN.)	%T	ABS.	EQUIL. TIME (MIN.)
0.002	0.0	19.4	0.712	14
0.002	100	22.3	0.652	16
0.002	200	24.9	0.602	10
0.002	400	30.6	0.514	9
0.002	400*	30.6	0.514	9

*High efficiency air filter

The effect of this increase of iodide concentration was tested on air streams containing no MMH and on standard MMH samples prepared in mylar bags. These mylar bags were constructed with approximately 200 liters volume capacity. Known concentrations of MMH were prepared by calculating the amount of liquid MMH to add to 200 liters. The bags were filled using a wet test meter and the calculated volume of MMH was injected. For concentrations of 100, 200, and 300 ppm MMH, volumes of 45, 90, and 135 μl, respectively, of liquid MMH were required. Table IV compares the absorbance of an iodine solution (0.5N in potassium iodide) when no air was being pulled through the absorber with the absorbance obtained with an air flow rate of 200 ml/min. Also listed in table IV are the absorbances of standard bag samples with nominal concentrations of 300 ppm. It appeared that the increase in potassium iodide concentration eliminated the loss of iodine into the air stream.

TABLE IV

ABS. IODINE VS. AIR FLOW

CONC. I_2 (N)	AIR FLOW (CC/MIN.)	%T	ABS.	MMH (CONC.) THEOR.	MMH (CONC.) MEAS.
.004	None	4.3	1.376	0	0
.004	200	4.2	1.386	0	0
.002	200	18.4	0.734	0	0
.004	200	25.5	0.593	300	195
.004	300	50.0	0.300	300	177

However, the measured concentration of MMH from the standard bag samples, calculated as shown in table V, was still considerably lower than nominal. Either or both of two effects might have occurred. The pH of the unbuffered iodine solution might have been too acid for stoichiometric reaction and/or the MMH might have decomposed before it reached the absorber. Buffering the iodine solution with 0.1N phosphate buffer, pH 7.0, increased the measured concentration of a nominal 300 ppm MMH bag sample from 195 ppm to 268 ppm. This showed that more iodine was being reduced by the MMH under these conditions. Furthermore, when the tygon tubing connecting the mylar sample bag to the Auto-Analyzer was shortened, the measured concentration of MMH again increased from 268 to 298 ppm. Since this final absorbance was equivalent to theoretical MMH within the precision of the technique, conditions for the precise and accurate analysis of MMH had been established. Although the time to reach equilibrium was still too lengthy for our purposes, stoichiometry was being achieved. If the sample air flow was increased, perhaps the time to reach equilibrium would be shortened. In order to use these higher flow rates, however, the iodine concentration would have to be increased since there would be a greater quantity of MMH being pulled into the absorber column in a given time. Furthermore, after increasing the iodine concentration from 0.004N to 0.012N, the wavelength of measurement had to be changed from 480 $m\mu$ to 550 $m\mu$; the absorbance of the 0.012N iodine solution was too high to be read

TABLE V

CALCULATION OF MMH CONC. FROM I_2 SOLN. ABS.

$$\text{ppm MMH} = \frac{A \cdot K \ (ABS_1 - ABS_2) \cdot 24.5 \cdot 10^6}{4\,B}$$

where

A = Flow rate of I_2 soln. (ml/min.)

K = N I_2/abs. of N I_2

ABS_1 = Initial abs. of I_2

ABS_2 = Abs. of I_2 soln. after reaction with MMH

B = Flow rate of chamber air through absorber (cc/min.)

on the Auto-Analyzer chart at 480mμ but could be read quite precisely at 550mμ where the absorbance was 25% of that at 480mμ. All other conditions of solution preparation were unchanged. Table VI shows that under these new conditions the equilibration time was decreased from 25 minutes to 10 minutes from time of concentration change, and from 20 minutes to six minutes from the time of first pen movement. Thus it was demonstrated that the effect of air flow on equilibration time had been substantially eliminated. When the sample line was preconditioned with MMH and timing initiated as iodine entered the absorber, the equilibration time from the first movement of the pen was further decreased from six to three minutes. However, the equilibration time from concentration change remained the same, at 10 minutes, which was the required time for a given volume of iodine solution to move through the system.

TABLE VI

SAMPLE FLOW VS. EQUIL. TIME

Conc. MMH (ppm)	300	300	300*
I_2 (N)	0.004	0.012	0.012
Sample (cc/min.)	200	500	500
Equil. Time (min.)			
From Conc. Chg.	25	10	10
From Pen Move.	20	6	3

*Sample line preconditioned with MMH

Billion-Aire Method

The Billion-Aire could be calibrated unambiguously because the Auto-Analyzer was capable of analyzing standard bag samples accurately. Previous conversations with Mine Safety personnel indicated that the upper limit of unsymetrical dimethylhydrazine (UDMH) concentrations capable of analysis by the Billion-Aire under normal conditions was 35 ppm. Since our concentration of MMH was approximately 10 times this, dilution of our sample with air was necessary. This 10 to one dilution was accomplished as shown in figure 4. All lines and valves were stainless steel unless otherwise noted. A tee was attached to the sample inlet line; one leg leading from the sample volume and the other open to air for dilution. A flow meter was installed in the air line and a fine metering valve was installed between the tee and the sample volume to control the flow of undiluted sample into the tee. In addition, a flow meter was also added to the sample line to measure the sample air flow. The total air flow through the instrument as shown by the total flow meter remained unchanged whether the sample valve was opened or closed. Diluting the sample was accomplished by adjusting the total flow to 10 liters/minute and the sample flow to one liter/minute. Since it was desirable to make up MMH standards in nitrogen to avoid oxidation, pure nitrogen was delivered into the sample line and diluted in the same manner as the sample. Nitrogen had no effect on the Billion-Aire readings and could be used as the atmosphere in the standard mylar bag. Standard samples of 100, 200, and 300 ppm MMH were prepared and connected to the instrument. After adjusting the instrument so that the 300 ppm standard gave a meter reading of 80, the 200 and 100 ppm standards gave meter readings of 68 and 51, respectively. The calibration curve as shown in figure 5 was obtained with these data. At these concentrations, a statistical comparison of the standard bag samples on a day to day basis gave 95% confidence limits of $\pm$ 5% relative.

Figure 6 compares the equilibration time of a standard bag sample and a "Rochester" type exposure chamber of the same concentration. Since contaminant introduction into the "Rochester" chamber began at zero time, the difference in the two curves represents the time necessary for the chamber to reach equilibrium. Figure 6 also demonstrates that the instrumental equilibration time for the Billion-Aire is only two minutes. This compares with 10 minutes obtained using the Auto-Analyzer and demonstrates the faster response of the Billion-Aire.

SUMMARY

The reaction of iodine with MMH was adapted to the analysis of MMH vapor in air using the Technicon Auto-Analyzer. By adjustment of pH and potassium iodide concentration, stoichiometric reduction of iodine in solution was accomplished. This method permitted the absolute measurement of MMH concentrations in standard bag samples which were then utilized for calibration of the Mine Safety Appliances Billion-Aire analyzer.

The sample inlet system of the Billion-Aire was modified to give a 1-10 dilution of the chamber air stream. Under these conditions, the Billion-Aire gave contaminant concentration readout within two minutes of introduction into the exposure chamber. Constant use of the Billion-Aire over the last five months has shown the precision of the technique to be $\pm$ 5% relative at the 95% confidence level.

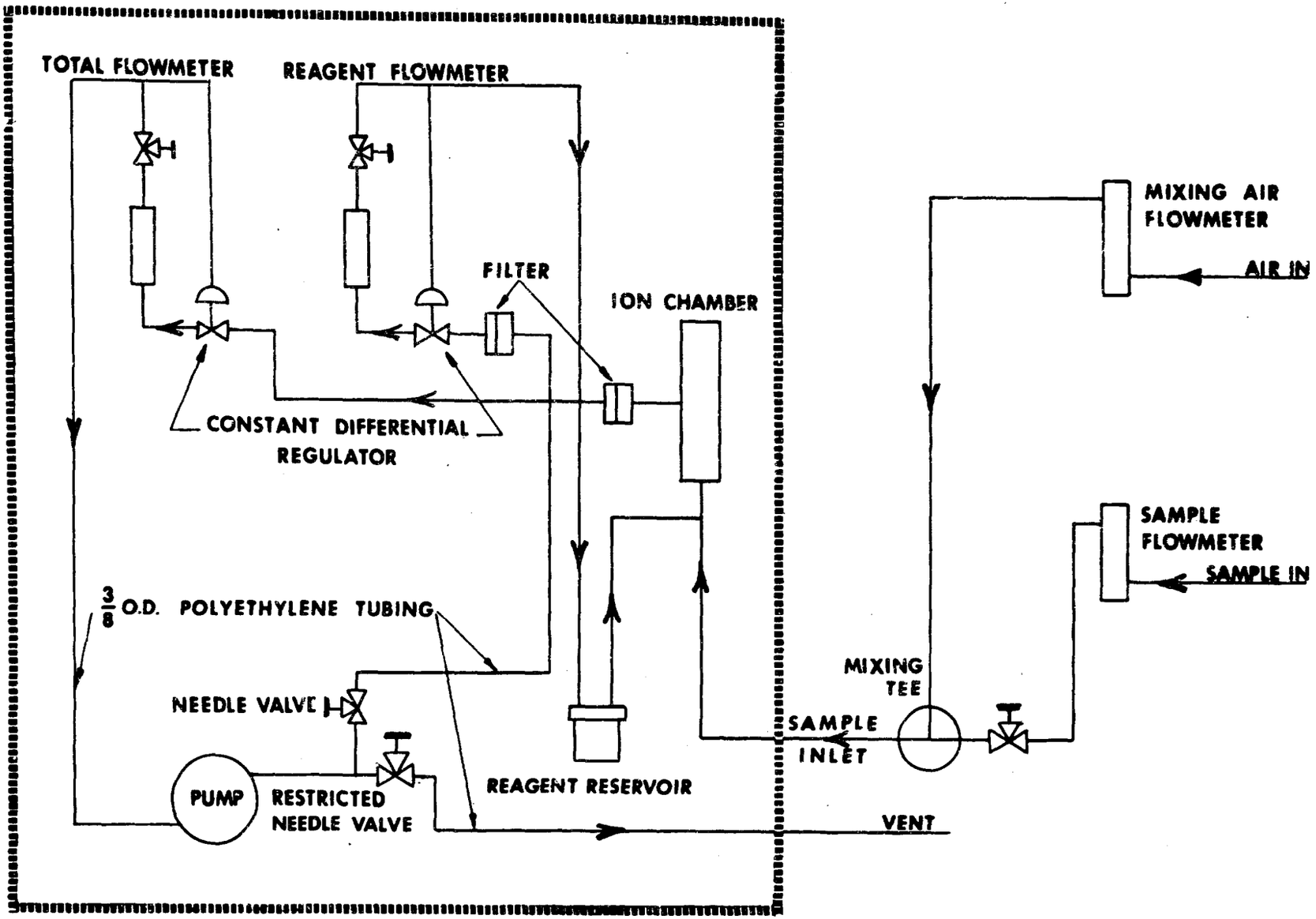

Figure 4. FLOW DIAGRAM FOR MSA BILLIONAIRE

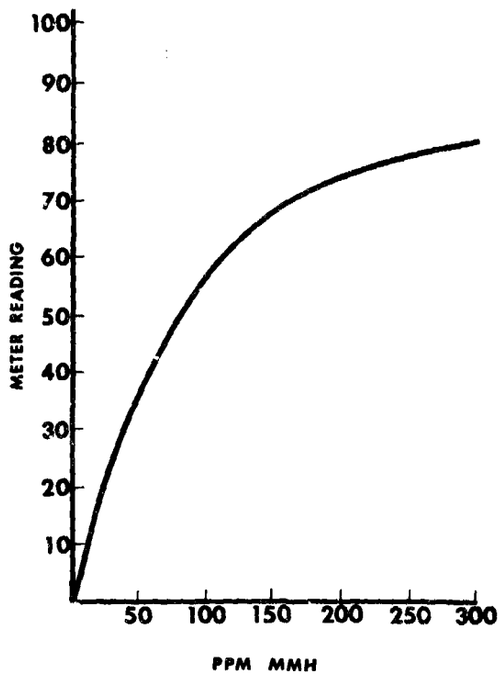

Figure 5. CALIBRATION CURVE FOR MMH

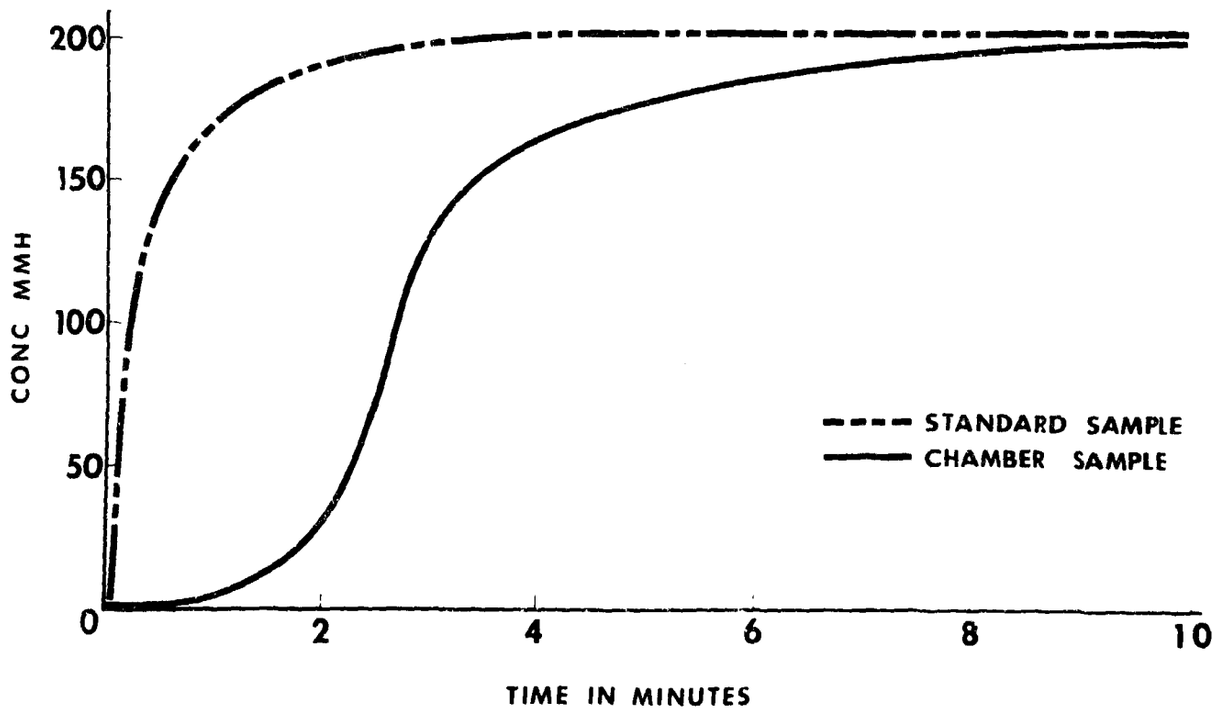

Figure 6. CONCENTRATION VS. TIME

REFERENCES

1. Audrieth, L. F. and B. A. Ogg; The Chemistry of Hydrazine; 160, Wiley, New York (1951).

2. Reynolds, B. A. and A. A. Thomas; A Colorimetric Method for the Determination of Hydrazine and Monomethylhydrazine in Blood; Amer. Ind. Hyg. Assoc. Jour., 26:527, 1965.

QUESTION: Have you checked the effect of moisture content on the zeroing of the Billion-Aire?

MR. GEIGER: We have checked it with room air; we've checked it with our bag samples containing no contaminants; and we've also checked it hooked up to the chambers and we notice that the zero holds constant in all three cases.

QUESTION: We have had somewhat different experience with it.

MR. STRANGE (Mine Safety Appliances Company): If the Billion-Aire becomes contaminated, there can be a moisture effect, but we have run humidity studies from 10% R. H. to saturated and find practically no difference. If you do run into something like that, there is an indication that the chamber should be cleaned.

A REVIEW AND FORECAST OF GAS CHROMATOGRAPHIC - MASS SPECTROMETRIC ANALYSIS OF ATMOSPHERIC IMPURITIES

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When the concept of gas-liquid chromatography was first advanced by Martin and James in 1952, the tremendous number of potential applications of the technique were immediately recognized. Indeed, there was a period of time when many thought that this type of chromatography was the Utopia of analytical techniques. It did not take long, however, to discover that gas-liquid chromatography, like all analytical methods, had several severe shortcomings. For example, it is essentially impossible to identify each of the peaks in figure 1 from the chromatographic data alone.

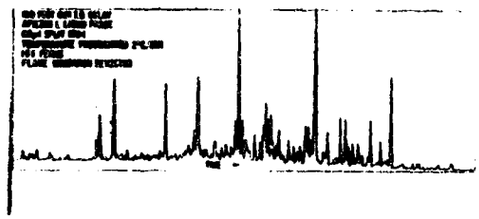

Figure 1. CHROMATOGRAM OF DESORBATE GEORGE WASHINGTON CHARCOAL FILTER BED

Researchers faced with the unenviable task of analyzing such mixtures experience a feeling of frustration when trying to identify the multitude of peaks in such a complex mixture. The ideal solution to this problem is to have a gas chromatographic detector which will uniquely identify the eluted components as well as sense their presence. Fortunately, such a detector has been available for a number of years; it is a mass spectrometer.

The mass spectrometer had been used for the qualitative and quantitative analyses of gaseous mixtures for many years prior to the introduction of gas-liquid

chromatography, and while the analyses were satisfactory for single compounds or simple mixtures, analyses of only moderately complex mixtures either failed completely or were extremely tedious and time-consuming. Many workers recognized the complimentary natures of the gas chromatograph and mass spectrometer; the former was an ideal separator and quantitative instrument, while the latter is unexcelled for qualitative identification. The obvious marriage of the two instruments was retarded by the fact that the chromatograph normally operates at a pressure of one atmosphere or above, while the working pressure of a mass spectrometer is about 10^{-9} atmospheres.

The first solution to combining a gas chromatograph with a mass spectrometer was to trap the chromatographic component as it emerged from the column and then to manually transfer the substance to a mass spectrometer, where it was introduced into this instrument by conventional methods. The major advantages of trapping chromatographic peaks prior to mass spectrometric identification are: (a) rapid recording of the mass spectrum is not required so that better accuracy can be obtained and a less elaborate mass spectrometer can be employed; (b) the sensitivity of the mass spectrometer is not reduced by dilution of the sample with carrier gas; (c) compounds may be separated on several chromatographs using different columns and then identified with one mass spectrometer; and (d) more than one type of spectral identification can be employed (e.g., IR, UV, or NMR spectra). On the other hand, the trapping approach has the disadvantages: (a) collecting every member of a mixture containing a very large number of components such as is shown in figure 1 is a horrendous task; (b) some of the mixture's components may be present in concentrations which are too minute for successful collections; and (c) the sample may be hydrolyzed, oxidized, or decomposed by some other mechanism during the collection procedures.

The disadvantages of trapping chromatographic peaks for mass spectral analysis are eliminated when the two instruments are directly combined; however, some of the advantages are also lost. Nonetheless, for the analysis of complex mixtures, direct effluent monitoring by a mass spectrometer is the most powerful means yet developed. The first gas chromatographic peak monitoring with a mass spectrometer was reported by Gohlke in 1959. Gohlke used a packed column chromatograph with a time-of-flight mass spectrometer to demonstrate the tremendous analytical capabilities of the combination. This pioneering work was followed by a series of papers on the analysis of flavor extracts by the research team headed by McFadden and Teranishi at the Western Regional Research Laboratory. These workers, reporting analyses of the complex mixtures with highly varying concentrations employed high resolution open-tubular columns in their gas chromatograph and beautifully demonstrated the simplicity with which such a gas chromatograph can be coupled to a mass spectrometer.

The technique used by both Gohlke and McFadden, et al, to reduce the gas chromatograph's pressure to a value that was not deleterious to the mass spectrometer was to split the gas chromatograph effluent into two portions - one part either being sent to a gas chromatograph detector or discarded, and the second part entering the mass spectrometer. For the next few years, publications appearing in the literature dealing with gas chromatographic - mass spectrometric analyses continued to employ this method of stream splitting to reduce the gas chromatograph's pressure prior to introducing the effluent into the mass spectrometer. Then, in 1963, Ryhage and his collaborators reported the first successful usage of a molecular separator (a device which preferentially removes the carrier gas) between a

gas chromatograph and a mass spectrometer. Ryhage's separator, an adaptation of the uranium isotope separator of Becker, employed two jet stages whose entrance and exit orifices and intervening distances are extremely critical for successful operation of the jet.

Ryhage's publication of his use of a molecular separator was followed by a series of papers dealing with molecular separators based on various other physical methods. The first of these subsequent separators, developed by Watson and Biemann in 1964, was based on the preferential effusion of the lightweight carrier gas through a porous tube emersed in a vacuum jacket. This separator was followed by the separators developed by Lipsky, Horvath, and McMurray, and by Llewellyn and Littlejohn in 1966. The Lipsky separator is based on the diffusion of the carrier gas through minute holes in a long piece of thin-walled Teflon tubing, while the Llewellyn separator uses a silicone rubber diaphragm to block the helium but not the eluted compound. The physical principle employed for this last separator is based on the extremely low solubility of helium in the rubber diaphragm. Thus, this carrier gas does not permeate readily through the membrane (permeability = diffusivity x solubility). Finally, Cree has developed a molecular separator based on the effusion of gas through a sintered metal tube.

The main objection to all of the separators, particularly for use in trace analysis, is that none of the separators, with the possible exception of the Llewellyn type, removes only the carrier gas. Each removes some sample, and when the sample is a low molecular weight molecule such as methane, the loss is indeed quite serious. At present, work is underway at several laboratories to improve the separators discussed above and perhaps develop new ones which will be more suited for use in the trace analysis of atmosphere contaminants. Some of the parameters which could improve the separators are: (a) the variation of the pressure restrictors in the entrance and exit of each of the separators; (b) variation of the length and type of polymer tubing used in the Lipsky separator or the porosity of the glass tube in the Watson-Biemann separator or the pore size in the Cree separator; and, finally, (c) the pressure, temperature, and flow conditions of all of the separators should be thoroughly examined for the optimum operation parameters.

I believe that the Llewellyn-type separator suggests an excellent means of concentrating the impurities in an air sample because nitrogen and oxygen also have limited solubility in a silicone rubber diaphragm compared to impurities usually found in confined spaces. Table I illustrates the wide variation of permeability between organic materials and normal atmospheric constituents. Such a concentration method would have many advantages over the presently used adsorption-desorption methods. It would certainly be lighter in weight and could be incorporated readily into the batch-inlet of any analytical instrument used to monitor the enclosed atmosphere.

Of the currently available commercial mass spectrometers, none seems to be completely adequate for the analysis of complex mixtures of trace impurities. All manufacturers advertise combined gas chromatographic operation capabilities; however, only two manufacturers are routinely making available the complete combination, and one of these instruments has only recently become available (1967). A gas chromatograph is easy to combine with a mass spectrometer for analysis of routine samples, but such a combination for use in trace analysis is altogether another story. Even the manufacturers who have commercial chromatograph - mass

TABLE I

MEASURED PERMEABILITY - DIMETHYL SILICONE RUBBER

GAS	P_r*	GAS	P_r*
Nitrogen	28×10^{-9}	n-Hexane	940×10^{-9}
Helium	35×10^{-9}	n-Octane	860×10^{-9}
Oxygen	60×10^{-9}	n-Decane	430×10^{-9}
Hydrogen	66×10^{-9}	Ethylene	135×10^{-9}
Carbon Dioxide	320×10^{-9}	Benzene	1910×10^{-9}
Freon 11	1500×10^{-9}	Phenol	1080×10^{-9}
Freon 12	138×10^{-9}	Toluene	913×10^{-9}
Methane	94×10^{-9}	Pyridene	2100×10^{-9}
Ethane	250×10^{-9}	Acetone	1980×10^{-9}
Propane	410×10^{-9}	Ammonia	586×10^{-9}
n-Butane	900×10^{-9}	Water	3800×10^{-9}
n-Pentane	2000×10^{-9}	Hydrogen Sulfide	650×10^{-9}

*P_r (at 25 C, 760 mm Hg) = $\frac{\text{cm} \times \text{cm}}{\text{sec} \times \text{cm} \times (\text{cm Hg } \Delta P)}$

spectrometer combinations have had very trying experiences attempting to analyze complicated atmospheric contaminant mixtures such as the one shown in figure 1. When contemplating use of a gas chromatograph - mass spectrometer system for trace analysis, I feel the use of a dual ion source mass spectrometer (where one source is to be used as a chromatograph detector, while the second is employed in the conventional manner to produce the ions which constitute the mass spectrum) has advantages, such as increased sensitivity and concurrent observation of the chromatograph peak and mass spectrum, that far outweigh its disadvantage of increased instrument complexity. Thus, in my opinion, the dual ion source type of instrument is the best mass spectrometer available for direct gas chromatographic peak monitoring.

While many state-of-the-art improvements such as better ion detectors, improved vacuum systems, solid state electronics, etc., have been made in the last few years, mass spectrometers still remain large, delicate, and complex instruments. The field of mass spectrometry is extremely capricious, however, as there are cyclotron, quadrupole, and monopole mass spectrometers now available

and the quality and capabilities of these instruments are improving rapidly. The advantages of these mass spectrometers are their rugged simplicity and the fact that their mass resolution is essentially independent of the physical size of the mass spectrometer. Thus, these types of mass spectrometers lend themselves readily to miniaturization.

In my opinion, the time is approaching when a high resolution ($M/\Delta M \geq 3000$) mass spectrometer might be reduced in size to the dimensions of the transistor radio available today. By the time the size and cost of high resolution mass spectrometers are appreciably reduced, most of the mass spectral data will be sent directly to a computer for interpretation. Today, many laboratories are recording their mass spectral data on tape and then sending the tape to a computer center for analyses. Only a few laboratories have an on-line connection between their mass spectrometer and the computer. However, it will not be long before this arrangement will be the rule and not the exception. With a high resolution mass spectrometer, it will be easy to identify the components of a mixture by exact mass measurement. As an example, nitrogen, ethylene, and carbon monoxide all have nominal mass 28 for the molecular ion and in most of today's mass spectrometers appear as one peak. In a high resolution mass spectrometer, these gases are separated into three distinct peaks. Thus, if the mass spectral resolution is sufficient, any compound can be identified by the elemental composition calculated from the exact mass measurements.

I believe the routine atmospheric control system for confined spaces in the not-too-distant future will contain some compact means to concentrate the contaminants of the system's atmosphere prior to the actual analysis of the sample. From the concentrator, the impurities will pass into a general purpose gas chromatographic column. This column will probably be an improvement of the support-coated open-tubular column already available. The stationary phase of the column will be sufficiently nonvolatile that any column bleed will be negligible, thus greatly reducing the background in the detector, and as the separated components emerge from the gas chromatograph they will be directly introduced into a high resolution mass spectrometer. There will be no need for a molecular separator in this system because the vacuum system of the mass spectrometer will be sufficient to maintain an adequate pressure. The data from the mass spectrometer will be fed directly by some method into a computer which will be programmed to subtract any instrumental background from the data, identify the exact mass of every ion observed in the spectrum, fit the fragmentation ions together to form the original molecule, and record the concentration of the impurity constituent. This system will be packaged in less than a one cubic-foot volume and still have the ability to identify and characterize every member of at least a 100-component mixture in less than five minutes! Such a system does not really lie too far in the future because most of the basic concepts are sufficiently developed now to permit such an analysis; only hardware and a good deal of practical engineering are really required to make this system a reality.

The uses of such a system are almost endless. For example, it could be incorporated into the atmospheric control system of spacecrafts, nuclear submarines, and underwater living quarters. However, by the time such a system is operational, I am sure that many new uses will be anxiously awaiting its arrival.

QUESTION: Dr. Saalfeld, which spectrometer would you envision as fitting into this conjectural forecasting here?

DR. SAALFELD: I would think probably the megatron type. I don't want to name any brands, but this to me seems to appear to have the greatest possibility for both being compacted and remaining relatively high resolution.

DR. STIEFVATER (Research Systems, Inc.): What is the ratio of transmission of the Llewylln separator?

DR. SAALFELD: From what we have done on this type of separator, it is quite limited. We have had some small diaphragms and we've got the transmission at about 50 percent. I feel that the Llewylln, when it finally can be adequately tested, may be higher. I'm not certain. The thing that worries me most about the Llewylln type separator is sample holdup in passing through the diaphragm.

OPEN FORUM

Frederick Coulston, Ph. D.
Albany Medical College
Moderator

DR. COULSTON: It is a great privilege for me to be here and to chair this kind of a discussion. It is not exactly new to me, but it is always a great challenge. This is indeed the moment of truth. This is when the people who have sat in the audience can now ask questions, make themselves heard, and expect a reasonable answer, if it is possible at all, from the speakers.

I am reminded that a few weeks ago I was on the witness stand as part of a team trying to help resolve a problem of drug toxicity. As I sat on the witness chair, the lawyer asked me questions, and he was a very interesting lawyer, like the Hollywood-type lawyer, very dapper, very smart, and he had very piercing eyes, and he glared at you, and he had the habit of moving back and forth across the audience. And finally I began to feel hypnotized, literally, so I took my eyes away from him. He didn't like that, and he said to me, "Dr. Coulston, you look at me", and I turned to the Judge, it was a Supreme Court Judge, and I said, "Judge, do I have to look at him?" He said, "No, Dr. Coulston, you do not. You can look at the jury, you can look out the window, and occasionally I would appreciate it if you would turn around and look at me." So, by the same token then, with this beautiful oil painting behind me, I would appreciate it if you feel good, look at the grass; if you feel despondent, the mist in the valley; if you feel elated, the clouds; if you want to be isolated and all alone (like I feel up here) look at the peak of the mountain. But occasionally, please, look at me.

The way we will conduct this discussion forum is rather simple. With the help of the program Chairmen, we will just go through the presentations of the program by sections. We'll ask each of the Chairmen of the various Sessions to participate by reading questions that were directed at their Sessions. Since Dr. Townsend is not here, I will act on his behalf with the questions for his Session. I will ask for discussion related to the question. By that I mean please don't raise questions that don't fit what we are talking about. Then I will ask for unrelated questions from time to time whereby we will try to keep an orderly process in going through the various sessions of the day; otherwise we will get hopelessly mixed up and not make any sense out of our discussions.

At the end of the formal questions, I will throw the floor open to miscellaneous questions and there we can just talk about anything, whether it be space capsule or whatever we wish. So without further ado, then, we will turn to the first part of the program which was chaired by Dr. Roth, and Dr. Roth can start by asking questions.

DR. ROTH: The first question is directed to Dr. Fairchild. Relative to the interpretation of significance of hematologic and other supposed changes in animals exposed for eight months to the two-gas system, were the diets identical?

DR. FAIRCHILD (Aerojet-General Corporation): As I interpret the question, were the control animals on the same diet as the experimental animals in the two-gas system? Yes, they were.

QUESTION: Yesterday we heard the stocks were essentially the same, so that is not a factor. Now there is a third question.

DR. STOKINGER (U. S. Public Health Service): I don't think that question was quite answered. What I meant to ask in that question was this: you had that broad spectrum of deviations in those animals that comprised a group of 200 dogs, and that was the question. What was their diet in comparison to yours?

DR. FAIRCHILD: That clears it up a little bit. Dr. Stokinger has reference to the fact that we were showing a three sigma band with a median limit based upon analysis of the control animals, and then we plotted by random selection groups of four for each experiment, our experimental or exposed. Then we had a third band which was superimposed to indicate how these would relate to a total large population, which was usually shown as overlapping.

Now your question then would be: were the diets of these animals which gave the experimental plots and their controls for three sigma limits the same as these animals on this 202 dog study conducted by Maj. Robinson of the Aero Med Lab? Having just joined the unit in September, I can speak for the experimentals and controls in saying these were on the same diet, and I would be rather positive in saying those 202 were also, but I would have to refer that to either Mr. Haun or Maj. Robinson, or perhaps Dr. MacEwen.

DR. MAC EWEN: The answer to that is yes, they were on the same diet.

DR. ROTH: This is again to Dr. Fairchild. Why were the animals exposed for eight months to oxygen or to the two-gas system not sacrificed in the atmosphere in which they were exposed? The domes have enough space to sacrifice animals.

DR. FAIRCHILD: Well, I beg to differ in that really the domes don't have that much space when you are going through this many animals. But possibly a better answer to this would be that, similar to the eight-month study of one hundred percent oxygen at 5 psia, using the same animal complement, we wanted to have exact duplication of protocol with our mixed gas for interpretation of comparative results. Now it was pointed out yesterday that in the new installation future experimentation will certainly take this into account. There will be studies made with animals which can be sacrificed in the interconnecting air lock surgical suite system; they will not have to be removed from within their exposure environment. I can fairly well predict that when this time comes there will be a lot of comparison of this type of a sacrifice versus the older one which is being done now to see if, indeed, these rapid pressure changes are producing an artifact effect to eliminate what is not a true toxic change from the prolonged exposure.

DR. ROTH: Another question that covers technique, and this could be to Drs. Fairchild, Kaplan, Patrick, or Robinson: is the contamination of animal feeds within the dome cages by the test materials such as ethylene glycol, ozone, NO_2, et cetera, considered a factor relative to exposed versus controlled food consumption, body weight gain ratios and/or toxicity?

DR. FAIRCHILD: I'll take a crack at that first because I have already anticipated this type of question. In fact, we were discussing this with the engineers

two days ago in the design of the new systems for housing animals in the Manned Orbital Lab screening program. The idea came up that we could possibly use trained animals and put food in small compartments. It is no problem at all to train rats to just hit a bar when they want to get a pellet of food; in this way you would have ruled out contamination. As far as contamination in the mixed gas and pure oxygen studies is concerned, there was no problem at all. In reference to the glycol study, the chamber technicians were definitely instructed to give the animals (rabbits, guinea pigs, rats, and dogs) as much food as they would consume for a feeding period and then to remove the leftovers. Especially with the glycol where you are getting it intragastrically, you could have an additive effect; in fact, most of the cases in the literature are from people who have chosen to use this instead of alcohol as an intoxicant; not really very much by inhalation. So this was definitely an attempt to rule out this effect. Again, it was a very short-term exposure. Had it been a long exposure, it would have been a very serious thing, in that we would have had to have absolute control in ruling out food contamination.

DR. COULSTON: Dr. Roth, I think you know one of the problems that is going on here that I see. What do we mean by toxicity? Are we talking about irreversible changes observed in a biochemical system? Are we talking about changes in tissues that can't come back to normal, or are we talking about physiologic changes?

In modern toxicology, we tend to feel that physiologic changes - and I mean it just in that sense - like a muscle swells from exercise, or doesn't; these kinds of things we are not apt to call a toxic phenomenon, but rather an adaptation on the part of the animal or man to the particular environment he finds himself in. I think one of the important points that we should try to clear up this afternoon, if all the speakers will agree, is when they use this word toxicity or toxic manifestation or damage, that they define what they mean, and in that way there will be no misunderstanding. Now a lot of these findings that I have been listening to for the last several days we would not necessarily call toxic phenomena. Certainly if you have a pneumonitis, and there are tremendous changes going on in the lung, you have to call this pathology, and you have to say that it's due to something. But the mere fact that a transaminase goes up a little bit and if it's not backed up by some good pathology or reason for it this doesn't necessarily mean that it's a toxic manifestation. That is the way the animal adapts itself to that particular substance in a particular environment.

I think if we bear this in mind as we discuss things here we'll all understand each other a little better.

DR. STEMMER (University of Cincinnati): I would like to make a remark to your statement about adaptation. Adaptation in the classical meaning means how the body takes care of the toxicant, and in time it might detoxify the material in greater amounts and shorter time. I think what you are talking about in adaptation in the term as you used it was a stimulation to a hyperactivity which not necessarily means that it is because of the toxicant that is given, but because of other organs that are involved in this process. And one has to define the target organ and the responding organ as two different systems, and most of what was said to be a toxic effect yesterday in the discussion was probably a stimulation of activity in a responding organ but not the target organ itself, especially where we had the hundred percent pure oxygen exposure.

DR. COULSTON: I think that is a very valid point, and it bears out the type of thing I have been trying to say in the last few minutes. And I would go further as we develop our discussion; there is a lot of new knowledge about enzyme systems and stimulation or induction of enzyme systems, and these enzyme systems can be induced or they can be depressed. They can go either way. So it is not always stimulation, I'm sure you will agree, but it can also be a depression. Everyone sitting in this room is exposed to toxicants right now; right now my cigarette is helping, and I am sure just sitting in the room with people helps too. The point I am trying to make is that we handle these compounds very successfully. You may have to go outside once in a while and get some air or a drink of water to help handle these compounds in the air, but the fact is we do, and we live, we survive in it; and man, indeed, can live in an environment of his own choosing, provided he knows the rules of the game. So the point that Dr. Stemmer and I are trying to make is that we should be very careful in using the words toxic, toxicity, damage. I think we have always to bear in mind one cardinal rule: is this the normal way the body would handle it? And after a period of a day or an hour, it's gone, done with. The body is adapted to it - no more worries about that chemical. I will develop this as we go along.

DR. KAPLAN (Aerospace Medical Research Laboratories): I think you have to carry that a little further for our purposes. I don't think it is adequate just to divide things into adaptive mechanisms, in other words an effect and a toxic change. I think you also have to consider the degree of toxic change and I think you can have true toxicity in an organ, not an adaptive change, but an inability of that organ to adapt and, therefore, damage. But yet because of the nature of the organ, the extent of the damage could be such that it is insignificant in terms of the overall function of that organ and the well-being of the organism. To stick with the same example, this question of oxygen and the lung, I think the finding of interstitial edema that Dr. Lewerenz presented is not adaptive. I think it is toxic. But I think it is not clinically significant, because we have evidence that that degree of interstitial edema is not enough to interfere with the animals' pulmonary function; so, therefore, I think even though this would be toxicity and not adaptation, it still has to be differentiated further into meaningful or significant or deleterious toxicity and the degree of toxicity that is such that the organism can tolerate it.

DR. ROTH: I think there is one other thing to add. One can have an organ that has adapted to a given stress and yet that organ would be more susceptible to a secondary factor. Since this seems to be the major theme of the work going on here, multiple toxicities, I think that we also have to consider this as a major factor in our evaluation of the animal. How well is he able to handle secondary stresses of a toxic chemical or physiological nature?

DR. COULSTON: I think what you and Dr. Kaplan say is right - no one would argue. A man makes his own decision as to what he sees. This is an objective, we hope - summation of data that is presented to him by his own research, by the knowledge of the scientific community. But I would ask you, suppose I give you a good pinch, a very hard pinch, say on your arm, and you get edema. We are not going to call this toxicity, are we? You see this is the dilemma. You have every right to call what you see - changes occurring in the lung of an edema nature, if you believe it is truly edema - a toxic effect of the oxygen; that is all right, nobody is going to argue. But then one has to say: we know as pathologists, histologists, biochemists, that edema fluid is an adaptive process of the host, animal, or man,

and that sooner or later if the noxious stimulus, if I may use the term, the excess oxygen under pressure is taken away, the edema fluid would go back. Now it is very hard to consider edema fluids. That was a big point yesterday, and I think once again the doctor from Cincinnati raised the question: why don't you see inflammatory cells there? Why don't you see breakdown of ground substance with electron microscopy? And he even raised the horrible specter that it might have been artifact. I say "horrible specter" because I don't know whether it is or not. The point is: in order to say that something is indeed toxic, you have to be certain. You have to be certain. You have to explore all the normal physiological events that can occur. Yet you have every right to call it what you wish, and this is why we publish papers and people disagree with us - this is what the word "research" means, to do it over and over again - re-search - that's the very meaning of the word. So I'm with you. I agree with what you say. Do we have a related question?

DR. RIESEN (IIT Research Institute): This is related to what we have been discussing. It seems to me we should emphasize in this connection, as I discussed yesterday, that all substances are toxic or not by virtue of their dose, and generally a substance may be a metabolite or an innocuous substance depending upon the dose administered, be it oxygen or anything else. This is now something I hold to very strongly. I think, secondly, given multiple stresses, generally there will be a maximum which individuals of the species homo sapiens or the monkey, or any other species, will generally be able to tolerate in a given environment. However, as you would have with an individual astronaut, there will be individuals who will have very wide latitude in the maximum that we have, and so I think that we best consider these wide variations. Perhaps if you did pinch me, I might get a welt, I don't know.

DR. COULSTON: Yes, some people would and some people wouldn't, that's quite true.

DR. ROTH: There is one other generally recurrent question that I received: why are experimental and control animals not treated prophylactically with antibiotics and other drugs to rid them of parasites prior to experimentation? The number of disease conditions is a major factor in inefficiency of experiments presented.

DR. COULSTON: This is a very pertinent point and is worthy of a few minutes of our total discussion of this group. Who of the speakers would like to try, or of the Chairmen, would like to try taking this on?

DR. THOMAS: Dr. MacEwen would be a good one.

DR. MAC EWEN: I think that after listening to the presentations yesterday we were left with a slightly erroneous impression that all of the animals used were extremely unhealthy, sick animals, and this is not true. These animals were examined periodically by a group of veterinarians. The incidence of endemic disease was no greater than one would normally expect to see. I think that when you are looking for very minimal effects, you tend to notice the endemic processes more intensely; only when you are looking for no effects or extremely slight effects do you notice that your ability to define health is obscured.

DR. ROTH: The basic question was why not prophylactically treat these animals because of the diseases they have and get rid of them and don't worry about these subtle changes that do interfere with these subtle interpretations?

DR. MAC EWEN: The incidence of lung mites I know of no treatment for; the other specific coccidiosis occurred only in a very few animals, is that not so?

DR. COULSTON: Malaria came up. Let me help out a little bit here. When you set up an experiment you have to ask yourself a question: do you want a population that is relatively a normal population? A normal population is a sick population. You know people get sick and people die; monkeys get sick and monkeys die; rats live and they die. They get all kinds of diseases. So you can say to yourself at the start, if you are smart you'll say to yourself, what do I want? Do I want an animal, if I'm studying oxygen in lungs, do I want normal lungs or lungs that have infection, like the astronauts might have? They are certainly going to catch colds if they stay up there long enough or get an allergy or get some kind of a reaction to something. They are just human beings, just like everybody else. The point that I'm trying to make is that you can predetermine what you are going to do with the animals that you want. Do you want pathogen-free animals? They are available - they are perfectly available. I don't mean germ-free animals now, I mean pathogen-free rats and mice. They are available. You can get strains of mice and rats that are free of any kind of pneumonias that will live for two years without any respiratory disease, almost 99.9 percent of them. We have such a strain at Albany. We use it for certain purposes. But there are many good toxicologists who say, well this isn't a good test, we should have animals that have pneumonia; you should have animals that have a few worms in them; and so, you have a choice. Now the choice is here. I don't think that you have to apologize that your animals have developed pneumonia. I think it is rather unfortunate that your monkeys have filariasis. I think it is unfortunate that your monkeys have such an overbearing burden of mites, which does not occur in my colony at Albany. At Albany we rarely see mites. I'll bet you, as I sit here, and I am going to stick my neck out, that you got monkeys from Pakistan.

DR. BACK (Aerospace Medical Research Laboratories): You stuck your neck out.

DR. COULSTON: Where do they come from?

DR. BACK: All over. From a number of different places, the East Coast, the West Coast. Many of them were born in this country. Dr. Innes came to us and wanted to give us animals without any lung mites and I have a whole group of them. He guaranteed that the Green Monkey would not have lung mites; and yet they have as large an incidence of lung mites as our colony does here; and we bought them because they are not supposed to have any. Now once we get monkeys or dogs or rats or mice, we keep them around for observation. Some of our dogs have been around for six to eight months; some of our monkeys have been around from a year to a year and a half, and during that time we put maybe $200.00 worth of medication in these animals. We test them for liver and kidney function and so forth. The dogs get shots over and over again. We look at them for TB. We unfortunately can't do lung biopsies, but we treat these animals as if they were humans, and they have so much "gunk" in them that many times by the intense medication we elevate SGPT's and SGOT's just to get all these diseases out of them and then we have to wait around until they calm down a little bit while the laboratory results go back to normal. Finally, then, we think as our veterinarians do that we have a fairly good animal to start with. We have gone out and bought pathogen-free animals and we kept them around the laboratory for quite some time trying to figure out whether

they would have less incidence of pulmonary disease; unfortunately this is not so. These, after being around awhile, did, indeed, come up with horrible looking lungs, so it makes no difference how we keep our rodents, where we get them from - whether they are Harlans or whose they are - we keep them under the best conditions and, despite this, we get lung changes. Now this isn't only in our laboratory, because I have about a half million dollars worth of contracts out around the country and every one of the people who are doing work for me have animals that have poor looking lungs and that has always been our problem, and I don't know where you are going to find such large numbers of animals with perfect lungs.

DR. COULSTON: Monkeys that come from the area of New Delhi do not have filariasis and there is no way for the animal to transmit the disease in the laboratory - I hope there's no way - unless you've got the right vector around here. We buy monkeys only from the New Delhi area. I'm giving you a good tip now. The only thing that they can have is an occasional amebiasis. They usually have a high incidence of balantidium; they will have some ascarids occasionally, and occasionally a tapeworm. They very often have esophagastomum; but if you buy monkeys from good dealers, and I insist on using that phrase, you will practically not see esophagastomum. You know this is a little hookworm in the peritoneal cavity. This can be cleaned out of your colony pretty well with good cleaning practices anyway. But the point is, a monkey is like we are. There is nobody in this room that doesn't have some amoeba of some kind or some parasite of some kind and we shouldn't expect them to be otherwise. The thing that worries me is not the lung mites. I don't think they do that much damage. They don't change your interpretation of the lung tissue, unless you have terrible infestation. The only thing that would worry me is filariasis, which can change a great deal of the physiology of the animal. But if I saw a microfilaria floating around in the blood of one monkey out of 50, I wouldn't be concerned about that either. So I think we are in agreement.

DR. SCHAEFER (U. S. Naval Medical Research Laboratory): In regard to the prophylactic treatment of these animals, there was the statement made that these animals are treated so much that they even have abnormal liver function tests. How do you evaluate, later on, your data?

DR. COULSTON: I was afraid someone would ask that.

DR. SCHAEFER: We actually got to the point that when animals are in the experiment, we don't treat any. If they get sick, they get sick; if they die, they die; but we don't introduce an additional factor (or even several factors) in the evaluation of data by treating animals.

DR. COULSTON: I think that is a dangerous game in its own right. When you have a valuable animal like a chimpanzee, you must treat it like a human being would be treated in a hospital. If it has a disease, and there is a known drug for the disease, I see nothing wrong in treating the disease and in clearing the condition up, at least putting it in a subclinical state prior to your experiment, if that is your wish. You may not wish to do that. As I pointed out, you may even wish to leave the animal like you do. That's perfectly all right. On the other hand, you may wish to clean up certain diseases which may interfere with your interpretation. I think you have every right to do that. We routinely treat our monkeys and try to clean them up. We certainly don't want them to get pneumococcal infection. We don't want them to get a lot of diseases that monkeys get, meningitis and whatnot, because

for one thing it is a hazard to the handler as well as a hazard to the experimental procedure which will follow. So, again, it is a question of judgment as to what you are trying to do. Are there any related questions?

DR. MAC EWEN: When Dr. Back talked about treating the animals with drugs, this was during quarantine period. Now after we bring in new animals we routinely deworm them. And in the process of deworming them, you do cause an increased or elevated SGOT or SGPT level and anybody that followed these enzymes during the deworming process has seen it. It wouldn't deworm them if it didn't, probably. The animals are not given therapeutic drugs during experimentation for several reasons, one of them being that we are trying to follow them clinically with biochemistry and hematological evaluations, and we don't want to mask any effect that we might get from the exposure with the drug effect.

DR. COULSTON: I would even argue with this. I see nothing wrong, if your animals for some reason or other develop a vitamin deficiency, in giving them some vitamins, provided you give the controls vitamins also. I see nothing wrong in beefing up the diet, which you find may be deficient, say in vitamin C for monkeys. They are supposed to have the normal content of vitamin C. I see nothing wrong with giving the monkeys prophylactic vitamins in this case.

If one particular animal on a very valuable test develops pneumonia, like a monkey or a chimp, I see nothing wrong in treating the disease which has nothing to do, really, with your toxicology, so long as you state it, so long as you tell in your report exactly what you did. I see nothing wrong with that either.

DR. MAC EWEN: Perhaps one of the pathologists could answer this a little bit better. I believe the incidence of filaria is rather rare in our monkeys. Dr. Sopher maybe can answer that.

DR. COULSTON: We didn't get that impression from what we saw yesterday.

DR. SOPHER (Aerospace Medical Research Laboratories): We've looked at a rather large number of monkeys even in the short time I have been with the Lab, and filaria has been seen twice, one more than was seen in Dr. Weibel's Lab. This is perhaps out of a couple hundred monkeys.

DR. COULSTON: You see how an erroneous impression can be given to an audience? This is the value of a discussion period like this.

DR. SOPHER: And I might just add on the lung mite problem that virtually all of our Rhesus monkeys have lung mites, but this might vary from one or two to a half a dozen or more. I've never seen one that was so badly infested that there weren't normal areas of lung in the animal, so it depends upon what section is selected. Certainly what you are going to see if one selects an area filled with mites is going to be abnormal. If one selects an area in a lobe that is not infected with mites, then I don't think one is going to tend to see mite damage.

DR. COULSTON: I think this is right. It is normal in a way that you, the pathologist, understand. You would not interpret it in any wrong way.

DR. THOMAS: Quickly, to clarify a point on this treatment of animals. Sometimes you've got a monkey developing diarrhea, and there is no reason why you can't put something in his drinking water, not when you are down past the five-month mark in an eight-month experiment. You are not going to scrub it, this costs too much money and time. When you talk about clean animal colonies, I start to challenge people. We do 7000 necropsies each year, and if you do 7000 necropsies each year your chances of getting a bad animal are much greater than if you use 40 rats a month. Sure, you can be very selective. You buy 200, sacrifice 50 percent, and you've got your incidence of lung disease, but not when you are buying three or four thousand. The animal breeding business is in a sad shape. The availability of healthy animals is one of our greatest problems.

DR. BACK: The point here was that should we or should we not use prophylactic treatment of animals, and it is a matter of judgment, and we have to do it pure and simply by good judgment. If it is something that looks like it's chronic and it is not going to get any better, the thing we do usually is pull them out of the experiment. Now I have monkeys from Holloman that have been working for quite some time, and we keep seeing hematocrits going down, hemoglobin going down, and sed rates going up, and the animal is clinically sick. Can you treat them at altitude? No, you just can't do anything but take them out of the experiment. When we get them out of the test sometimes even then we can't find out what's wrong with them; and the fact of the matter is I've got two such monkeys right now, very valuable monkeys because they work perfectly well with the hematocrit down and hemoglobin at three and a half, but they can sure work. But the problem here is that you have to use your best judgment about treating them. I didn't mean to imply that our veterinarians don't know how to treat dogs and monkeys. They surely do. The point I was making is that on baselines we must make sure that at least on the three latest baselines the animals fall in the normal range. We've got a lot of data on that. About 200 dogs and 200 monkeys all out of the same area, and we are getting more all the time. The point is that when you do treat them, you do increase SGOT's and PT's and LDH's many many times, because that is what treatment does for them while you are getting them in shape. This doesn't mean that you've got overt pathology. I'm sure this happens in clinical medicine. You treat a man and his SGOT may well go up for a short time, but that doesn't mean that you've knocked his liver out or his kidney or anything else.

DR. COULSTON: I think this is quite right. We are becoming more and more aware of the fact that we must treat each animal as if it were a patient in a hospital, a hospitalized human being. When we begin to do this, we will learn a great deal more about the animal. I would far rather see, in the long run, fewer numbers of animals used, but used wisely, than tremendous numbers of animals to get statistics and not look at the animals often enough.

DR. SOPHER: On the matter of prophylaxis, we ran one experiment on this. It was an acute high concentration oxygen toxicity experiment. As you well know, the animals develop tremendous pulmonary edema in the first few days and often die. At one time we thought perhaps they were getting a bacterial secondary infection. So we set up two groups of closely matched rats, put one on the outside and one in the dome, and split each group in half again. This was a total of 200 rats. There were 50 rats in each group. Fifty rats on the outside and 50 rats on the inside of the dome exposed to oxygen were put on prophylactic tetracycline in the drinking water, and we thought this was a fairly innocuous drug, and they were

getting no more than a therapeutic dose in their water. Well, surprising enough to us, virtually every rat inside the dome on tetracycline died. The others survived. Now here we introduced a variable where no one knew what was going to happen. Had this been an oxygen experiment of any importance, other than just to see what the effect of prophylaxis would have been, we would have been in severe trouble. We had introduced a variable that we didn't know what effect it might exert on oxygen toxicity.

DR. COULSTON: These are very important questions. Now the reason I am allowing so much time on this is because in various discussions I have had with people, this concerned them a great deal; because every man gets up and says, well, too bad the lungs were infected with murine pneumonia, or something like that. The point is that one is no better off in an experiment of this kind, whether it is humans or animals, than his understanding of the man or the animal that he is working with, and all this means is that you recognize the condition that you have set in your experiment; and the condition to start with is the baseline of your animal. A lot of people have gone to tremendous extremes. A very eminent toxicologist-pathologist insists upon biopsying all his animals before putting some on test. He looks at the kidney, he looks at the lung, he looks at the spleen, and he looks at the liver, and if they all look normal then he goes ahead and proceeds with his experiment. Well, this is an extreme. I think he probably does more damage in the long run than good. The fact remains he does this and he is really a very eminent man.

DR. KAPLAN: I don't want to belabor this. I think we could talk about it forever, but since the question has arisen from the various oxygen exposure studies, all of which I have been involved in at one point or another, as a point of information I just want to set the record straight as to what was done with the various animals for lung work and all the other work. The monkeys were derived from various places as Dr. Back has noted. As the monkeys arrived at the animal facility (which I think is as good an animal facility as there is anywhere), they went through a physical examination which exceeds what I had to pass to enter the Air Force. The monkey distributor must remain there as the monkeys are initially examined and any monkeys that are found to be deficient are sent back on the same truck. They are not even taken. Beyond this, they go through a minimal three-month quarantine period during which time they have had PPD's, various blood tests, and other things are checked. They are only used after this minimum of three months, and because of the usual delays it's usually much longer than that. Mite problems I think Dr. Sopher has put in its proper perspective. It is not that great. In the case of Dr. Lewerenz' Lab, we have an unusual situation. These monkeys with their mites and their filaria are quite adequate for electron microscopic examination just to look at the picture; however, we have to remember that they are doing a very complex morphometric analysis that involves trying to evaluate what is happening to the total alveolar surface, to the total capillary bed of the lung, which they are expressing then in specific gas exchange surfaces, and, therefore, endemic effects which may have inflammatory effects, such as the presence of the mites, can affect things like that, because the methodology for that in itself involves trying to evaluate the entire lung. So for that you cannot select a little piece and look at it under the microscope.

DR. COULSTON: Dr. Kaplan, the thing that worries me is why didn't you know that the animal had filaria. It is easy enough to diagnose.

DR. KAPLAN: The presence of filaria, because it is not something that we come across very frequently, was something that I don't believe anybody was aware of at the time this was done.

DR. COULSTON: Well, I appreciate that.

DR. KAPLAN: I'm not trying to excuse that, I'm just trying to explain why the filaria, for the morphometric analysis, are more significant than in other situations. Now to try to resolve this, also the question raised earlier by sacrificing the animals inside the exposure chamber, we have obtained 10 supposedly parasite-free monkeys that were bred in Texas. These were exposed to 100 percent oxygen last fall and were sacrificed inside the exposure chamber. Dr. Weibel and Dr. Lewerenz' Lab have this tissue right now. They are working on this, and we hope with this to be able to evaluate first of all what effect, if any, or what artifact, if any, is created by removing the animals from the dome before sacrifice, by comparing our previous tissue with the tissue that we get in these animals sacrificed in the dome - of course, not for an eight-month study but for our earlier one atmosphere work where the damage is much more extensive - and also to try to determine whether there is any difference in the overall picture between the mite-free animals and the mite-infested animals.

Now as far as the rodents go, we try to keep these for awhile and exclude the hackers, the coughers, the sniffers, and the wheezers. Beyond this, other than that one study that we attempted with prophylaxis, we have not done anything, and our experience, as related, has been very bad in terms of endemic murine pneumonia.

DR. COULSTON: This is tough. I think, just in generalities, the problems we are talking about are of the utmost importance for any animal researcher. I do think, though, that certain things can be done. I think we have discussed these rather fully. I would like to call on this wonderful group of people from St. Louis. Would you like to discuss this a moment? You've had a lot of experience in this area.

DR. PATRICK (Laboratory for Experimental Biology): We've looked at thousands of animals through the years for this Facility and for a number of the pharmaceutical outfits. I have yet to see, consecutively, in a given species, good animals from anywhere. When we are using drugs in the pharmaceutical industry, we are not so much concerned with murine pneumonia in rodents as we are with the studies for the Air Force here. There is one point that I want to bring up that we have gone through this afternoon concerning animals - the difference between a disease-free animal and a disease-resistant animal. Which one are we talking about? I think that we can get animals, perhaps with a great deal of effort, that are free of disease at the time the experiment starts. We have had much less difficulty with chronic murine pneumonia in acute experiments when weanling rats were brought in and kept a day or two in a chamber and sacrificed. The problem that I wonder if we are going to solve so readily is the one in which we keep animals for eight months. This we might be able to solve, I'm not sure; perhaps we can find disease-resistant rodents. There are tremendous variations in dogs also. In some instances we've had dogs on long-term studies such as the one that I reported on in mixed gas studies where the lungs in these dogs were very good. I would say, as far as dogs go, they were the best that we have probably ever seen from any source. On other occasions, dog lungs have given real problems concerning

interstitial pneumonia. I don't know the etiology of this. I don't know of any treatment. I have been through this with Dr. Thomas and the other people over there too. We've talked about it at length. I finally have come to the conclusion, and I think the rest of my group has too, that on long-term studies we are going to have to adapt ourselves. I don't know of any other solution. As a pathologist, my job would be a lot easier if every animal that I looked at had no chronic lung disease, chronic hepatitis, pylonephritis, interstitial nephritis, or what have you. We have to try to do the best we can; but, as I mentioned, I wonder if in these long-term studies, we are realistic if we think we are going to reach our goal. We just have to do the best we can and Dr. Coulston, I think, has some animals, some information that might be of use to us. I would like to see some of your animals put in the chamber for eight months - just a few of them. I'm sure you don't have a colony of tens of thousands to farm out. This is a real problem, keeping a resistant-free colony such as this, because if you get in a few animals from another supplier, you are in sad troubles again. But we just have to try to do the best we can and I want to make one point: if there is information available, I think we want to get to it.

DR. COULSTON: I think we had better leave this. We've got a long way to go, so would you proceed, please?

DR. ROTH: There are several other general questions that cover the first two Sessions, and I think we'll take those up next.

Did you monitor the trace contaminants to which the animals were exposed so that you could give a daily average mean concentration? And also, record daily the fluctuations of trace contaminants? If this has not been done, the peak concentrations could have been produced during the first day of exposure and the subsequent recovery period of 59 days with little or no exposure. Under these conditions no interpretation of data is possible. There are several other questions to the same point, about monitoring trace contaminants continuously during these exposures.

DR. COULSTON: Who wants to try this? It's an important point.

DR. THOMAS: Whoever asked this question, I assume he is talking about the 60-day Apollo study. He said 59 days plus one. We have 120 materials in that drying oven at constant temperature. Of these 120 materials, most of them have been looked at by Monsanto, just bottled up-looking at the gas-off products, and the gentleman who made the last presentation on matching the gas chromatograph with the mass spectrometer made his point very clearly. If you ask me whether I tried to monitor about 600 volatile contaminants in there and keep them at a steady level, I think I'd throw up my hands. There was no such attempt made. The only time when we were going to check what's in that recirculating system is if we had an overt sign of toxicity. This was not the case. Would we have had toxicity, we would have collected freezeout samples and would have tried to pin down everything in there. If symptomology coincided with the effects of a certain compound we would have had an answer. But what you are asking for to be done routinely is a half million dollar analytical program for every 60-day run.

DR. COULSTON: I think that's his point. The point is that maybe you need a lot more support. I will be very frank with you. If these compounds were new drugs or food additives, you couldn't get to first base with the Food and Drug

Administration with this kind of data. You would indeed have to monitor it, and have to explain it; and I see nothing different with this type of research than what one would do for a commonly used pesticide, food additive, or anything else. If you don't have this support, then somebody is wrong; somebody ought to give you the support, because I think what is at risk here is another horrible, horrible tragedy that could occur. I think we have honestly to do everything that is possible for us to do. And if you say it costs another half million dollars, I could care less. The point is, it is important or it isn't important. It is necessary or it isn't necessary. And this was what the question that was being raised is pointing out.

DR. FAIRCHILD: May I interject? I think the question is a very valid one. So is Dr. Thomas' answer. But I will agree, it would be nice. It would be nice if we could say that in a 60-day study the animals received 89 percent of the gas-off in the first three days, and during the rest of the study they were not receiving any gas-off. I think this was the real point of the question, not to attempt to identify every component, but just to see how long the real exposure was.

DR. THOMAS: I think he said he would like to know what's in that mixture.

DR. FAIRCHILD: All right, break it down by components, but it would be impossible to do that absolutely.

DR. COULSTON: Many people have gone to the Food and Drug Administration with pesticides and said it would be impossible. And they'd say, "If you want the thing on the market, you do it." And so two years go by and you do it. Somehow or other, if you want that product to go on the market, that's all there is to it. There is no question about it.

Now there are many people in this audience who know exactly what I am talking about. I'm just raising a horrible specter here. Are you doing enough? I think this is what the man was driving at. I would say that in a program involving some 23 billion dollars, which I read in the paper this morning, I don't think it's unduly too much to ask for; that we do the best that the art, the science allows us to do to protect all the people in the future that will go up into space or down in a submarine or sit in an airplane and fly to Europe, or sit in this room. I think the whole question now becomes very, very pertinent - are we doing enough?

MR. WILLARD (Honeywell Aeronautical Division): I very timorously raise my voice, bearing in mind the phrase "fools step in where wise men fear to tread". I'm not a toxicologist, I'm an engineer. I believe from the discussions I have had that I'm one of the few here who is directly concerned with hardware selection; and so perhaps my concern comes more from ignorance of toxicology. However, I can't help but have a very overwhelming feeling that the kinds of screening tests that are being reported here, and I hesitate to use the phrase, I feel are entirely superfluous. I feel rather strongly that the chemical tests, the gassing analyses, the simulator flights, and the manned flights that we have had to date have more than adequately established the qualification of the materials' gassing characteristics as they now stand. However, there are two very substantial areas that raise a specter in my mind. One of these is what happens under malfunction conditions? How much of these higher concentrations can the personnel stand before the mission must be aborted? The other question is, what are the more subtle effects, rather than gross toxicological effects, of some of these contaminants? For example, can

they cause euphoria, depression, or some other psychologically debilitating effect? I would feel, from the standpoint of the hardware engineer who has already shipped Apollo hardware, that a more pressing need would be for information concerning emergency conditions.

DR. COULSTON: Now I am going to say something off the record here. I think, sir, that the first part of your question is a very naive, very dangerous thought. It says, in essence, that you can take any chemical, you don't have to do animal experimentation, or do very little of it, and put it right into man. This is true, hypothetically, in 999 times out of 1,000. You could start with an infinitesimally low dose of a chemical in man, directly, right from the chemist's test tube, and gradually double the dose until you get side effects or trouble. But, sir, in that one case in 1,000 you'll kill or hurt somebody; and the same thing applies here, where such great efforts are being made to protect the astronaut, protect the workers in the laboratory, protect the chemists and engineers. I think a reasonable amount of basic understanding of the toxicology, including the safety evaluation, has to be done. If you don't, you are going to have an analogous situation to the thalidomide tragedy in your spacecraft or your submarine. This can happen, don't kid yourself. It is going to happen. The only thing you can hope to do is work like heck and spend time, time; not money, time. The heck with the money. As much time in evaluation of safety as you possibly can, with the best brains you can find, to protect the people. I grant you, you've put people up into space; but, would you have put them up into space without sending a few monkeys up first? Would you have done it? Ask yourself this soul-searching question. Now the second part of the question I am going to leave open for anyone else that wants to answer it. Would you like to rebut what I said? You are entitled to a rebuttal.

MR. WILLARD: Very briefly, the flight of the monkeys was much shorter than the two-day man flight and, of course, it was a simulated flight that preceded that.

DR. COULSTON: Well, I have got an answer to that. You had no business putting man up for as long as you did, without having the backup animal capability, but I hope you did. I don't know whether you did. I just hope you did. And I listen to this 60-day program and it frightens me to death when you are talking about putting guys up for a year. What good then is a 60-day experiment? And that was what this man was trying to drive at actually.

DR. SCHAEFER: Dr. Thomas, I raised this question since I feel I am in the same boat with you. I am concerned with submarine problems and I have the same situation. That is the reason I raised this question of monitoring. I think we have to look at the problem from an overall point of view of air pollution. If we do, at all, any such experiments (and the same holds true for me), we have to have this information on the magnitude of daily exposure, because then we can use it in overall plans for threshold limit values. If you don't have them, we can't use them, and I think we have to find a way, when we can't monitor all the trace contaminants at once (which I agree with you would be very difficult to do with the present conditions in which all of our laboratories are; I need as much support as you do), we at least should find a way to monitor groups of substances continuously. This is my problem.

DR. THOMAS: Here is a rebuttal to a rebuttal. No, honestly, I would be delighted to do this as every researcher would be delighted to do continuous monitoring and a better job, but keep in mind that the Monsanto results have indicated unequivocally that there is a significant difference in gas-off products from one batch of the very same material to the other; such as the amount of polymer and monomer in a plastic, how long it has been polymerized, and so on. Now if we would have the actual hardware, and we have said this years before - the most valid thing to do would be to measure the gas-off products in the actual cabin they are going to fly and to hook it up to an animal exposure chamber and recycle the gas-off products from the whole system, with all subsystems going. Unfortunately, gentlemen, these capsules are too expensive and hard to come by. You just can't get one. But that would be the best way to do it. You could do it once and have it over with.

DR. COULSTON: Go get one then. But that's easy for me to say.

DR. THOMAS: All right, I'll write my mother. (Laughter)

DR. COULSTON: If it's an important thing, that's what you should have. I'm going to bring this up at this point, at this moment, and this is vitally important. There is experience in World War II, with the antimalaria program, which was the number one world problem medically and biologically in World War II, and unfortunately it is back perhaps the same again for this Vietnam War. The point at issue here is that we started out very naively with these new drugs and chemicals to be used as antimalarials. Do you know what we used to do? We used to run a seven-day rat study, maybe a 30-day rat study, do a few dogs, and then go to a man. Because after all, clinical investigators are good enough. They can tell when somebody is getting hurt. The trouble is that we hurt too many volunteer prisoners, and so very quickly we made a change. I'll tell you one story, and I hope this bears the point out. The substance known as Plasmacid? It was used in antimalarial programs in animals and it had great hope as an antimalarial. At the University of Chicago where I was at the time, I happened to be playing squash. (Believe it or not, I used to play when I was younger.) And in talking to the man who was Associate Professor of Pharmacology, I said "Hi. I hear you are running a couple of monkeys on plasmacid?" He said, "Yes." He said, "Gee, they are all going blind." I said, "The next day we are supposed to go out to the prison and give prison volunteers this very compound, and you are telling me the monkeys are going blind." With that I went and got the clinician and called Washington. Believe it or not, a lot of the clinicians in Washington and in other cities decided that maybe we should go ahead. But the overall opinion was that we shouldn't go ahead. Now, about six months later, in searching the early Russian literature, we found that plasmacid had indeed been put in human beings, and that it had indeed made humans blind. You know what the dose of this stuff was? One to two milligrams per kilogram orally. That's all that was necessary, and relatively few number of doses, maybe four or five doses. And so, until we have the experience and knowledge, it is very difficult to say what some of these trace amounts of these things that you people are concerned with are really going to do. All I am trying to emphasize is that we should do the best we know how. It isn't a question of just money, it is a question of time and knowledge.

DR. ROTH: There have been several questions addressed to Dr. Fairchild. I will include them as one big question. Are the four-week cycles seen in total

protein constant enough to be considered a biorhythm? Second, may the total protein increases with time in your controls not be a normal aging phenomenon? Lastly, since the globulin fraction is responsible for significant increase in total protein, how do you account for the failure of electrophoresis to demonstrate any change in globulin?

DR. FAIRCHILD: In answer to the first question, we submitted our data to NASA for computer analysis. We soon became aware of the pattern of cycling and had wondered about it. The cycling was rhythmic as you saw on the plots shown on the slides, and the constancy was sufficient to call it a biorhythm; there was a very puzzling aspect about it, however, in that two distantly related species of animals showed cyclic rhythms with the same phase. Computer analysis didn't really tell us much, but the fact that both controls and experimentals cycled to the same extent indicated a uniform trend. We didn't look any further into it until it was seen that dogs exposed to the mixed gas atmosphere began to depart from normal by giving inverted A/G ratios. This, of course, concerned us very much.

Relative to the second question concerning the association of total protein increase with aging, it will be recalled that control animals did not show an increase such as that exhibited by the exposed dogs. The slide depicting serum total protein had a stippled-crosshatch band which represented value limits (UCL and LCL) for the control animals, and another band with crosshatching which gave limits obtained from protein determinations on another very larger group of "normal" control dogs. The limits, or width of the bands, represent three standard deviations. It will be recalled that protein determinations for the dogs exposed to the mixed gas atmosphere were point plotted against the three sigma limit bands, and after several months, the great majority of values fell outside and above three standard deviations for control values. So we can see that, definitely, the shift in total protein was a finding peculiar only to the dogs confined to the mixed gas atmosphere. Had age been a factor, this would also have been reflected in determinations for control animals; it would merely broaden the three sigma limits so that plots for exposed dogs would have fallen within the boundaries of control. Furthermore, the other eight-month study referred to, that is, 100% oxygen at 5 PSIA, did not reveal a significant change in serum total protein for either experimental or control dogs, and the same time period was involved.

In regard to the last question, which is more difficult to answer intelligently, I must again refer to some of the graphic representations. First, it is once again pointed out that total serum protein fractionation by electrophoresis gave higher A/G ratios than the wet chemistry method. This was readily seen on the slide giving data on serum protein fractionation. Similar statistical treatment was used to compare A/G ratios derived by either of two methods, that is, by salt precipitation or electrophoresis. The data to the left of center were derived by sodium sulfate precipitation, whereas data on the right were calculated from electrophoresis determinations. It will be recalled that each sample was subjected to both methods and that the results were then treated identically insofar as statistical evaluation was concerned. The apparent difference between the two methods was demonstrated by values derived from samples of each of 21 "normal" dogs. The mean plot for salt precipitation, as noted by a circle-dot and one standard deviation was considerably lower than that for electrophoresis (diamond-dot).

I did not mean to give the impression that the electrophoresis method of fractionation showed no inverted A/G ratios. To the contrary, there were some, but

in many cases certain high values negated the lows. It was seen in the plots in the last figure that values by electrophoresis were considerably more scattered than were those by salt precipitation.

Although protein fractionation by electrophoresis is thought to be the most reliable method, there is an inherent arbitrariness of any method. Serum protein has a number of physical and chemical properties, one or more of which serve as bases for a fractionation procedure. Thus, it isn't surprising that a separation taking advantage of one set of properties yields somewhat different results than a procedure utilizing some other set. The salt precipitation method used in these studies was not the best, but the important thing to consider is that the procedure did show a difference between exposed and control dogs; a difference that was not seen in the 100% oxygen - 5 PSIA study using the same methods for protein and albumin determinations.

It should be pointed out, again, that the inherent penalty of small samples may have, indeed, given misleading clues; the control dogs (N = 4) had rather high A/G ratios by the salt precipitation determination. This was readily seen in the last figure; mean A/G ratio was above 1.5, whereas determinations for the group of 21 normal dogs gave a mean A/G ratio well below 1.2. Even so, the value for the 21 dogs was a single point in time while that for the four control dogs represents the mean and deviation for four samples collected biweekly for three months. Hopefully, the latter is more representative than the former.

DR. COULSTON: I'm not so sure that the salting out technique is any better than the electrophoretic technique.

DR. FAIRCHILD: I didn't mean to imply that at all. Had we used the sodium sulfite precipitation, however, we would have gotten more complete fractionation of the globulin from the samples, hence more accurate albumin measurements. The advantage, of course, with electrophoresis is the quantification of each constituent. I am not a clinician, but I am told that in most diseases which cause shifts in A/G ratios that it doesn't necessarily reflect a rise in total protein. Rather the globulins will increase and the albumin will decrease. The fact that the total protein of exposed dogs was rising, however, must mean something.

DR. COULSTON: It does, very much.

Many of the questions in Dr. Townsend's Session relate to this business of why didn't you get a better animal and what can you do about it, and I think that we have an obligation to read the questions of the people who asked them.

Now here is a question that I think is rather good, and it is addressed to Dr. Patrick and Dr. Kaplan. With regard to pulmonary edema in dogs exposed to 100% oxygen, 5 PSIA, would sacrificing a group of dogs in the dome, and a second group removed from the dome, not answer the question as to whether or not pressure change was responsible for the edema observed? We have discussed this a little bit. Would you care to discuss it any further?

DR. KAPLAN. I think the answer is no. I don't believe the edema resulted from the pressure change, but more likely because the animal had adjusted over an eight-month period to an environment that had an oxygen tension approximately 50%

greater than the air to which he was being suddenly removed. Now beyond that, you could only speculate. We don't know whether spending eight months breathing pure oxygen at 258 mm does anything to the lung which is not visible under the ultrastructural examination. The lung may have adapted, if I may use that term, to this new environment, and suddenly removing it to air, where oxygen pressure drops down to 150 mm, could create a situation that might be, for lack of a better term, a relative hypoxia, even though we wouldn't normally call air hypoxic.

Now, applying ourselves at the moment to the question in terms of the liver mitochondria, we know that at 5 psi there is no breakdown - well I should say - we have not been able to find at 5 psi of oxygen any breakdown in liver mitochondrial functions. However, perhaps after the mitochondria have adapted for a long time to that high oxygen tension, again if they were suddenly removed to a situation where they were allowed relatively less oxygen for cellular respiration, there again might be a relative hypoxia.

We have done shorter term exposures with lower oxygen tensions where the pressure change in bringing the animal back to air was the same and did not see this edema formed due to the pressure change itself.

DR. COULSTON: I think this approach at the molecular level is what you have to do to get at it. I'm very pleased to hear this is being done. This is such a vital question to the whole program I would entertain a little further discussion on this point.

MR. BROOKSBY (NASA, Ames Research Center): Was there any edema in the perivascular spaces of these lungs or was it only in the interstitium?

DR. COULSTON: This is a very leading question, and he obviously knows what he is asking. Who wants to answer that? That's a very important point.

DR. KAPLAN: Could the question be repeated?

QUESTION: Was there any edema in the perivascular spaces or was the edema that you saw only in the interstitial area?

DR. COULSTON: That's a loaded question. Who wants to answer? It's a good question.

MR. BROOKSBY: The reason I asked the question was that Norman Staub at the University of San Francisco Medical Center has been doing a lot of work recently on the mechanism of edema clearance in the lungs and he has shown that edema that arises in the interstitium will move to the perivascular space and then will go out of the lung. If this is a chronic condition there should be a good deal of edema in the perivascular spaces of these lungs.

DR. COULSTON: A very pertinent question. Who will answer that question? Dr. Lewerenz?

DR. LEWERENZ (University of Bern): I will try to answer this question. I'm not quite sure there is edema. I got this impression in looking at the photos, at the micrographs, in the morphometric evaluation, but we have to find out this point to define it in the computer program.

QUESTION: But you should be able to see it?

DR. LEWERENZ: I saw it. I saw it on the electron microscope, but I can't say to what degree.

DR. COULSTON: If I might talk about electron microscopy. It is difficult to determine edema with electron microscopy. You can do very well with a light microscope, probably better. Membranes, in the little knowledge we have of ultrastructure, normally change with very little effort; even our own technique can change them around a little bit. So it is extremely difficult to determine edema. I think this would almost have to be answered on the basis of biochemical studies and light microscopy.

DR. SCHAEFER: I would like to ask the question of why do you call it edema? It looked to me more like a submicroscopic interstitial emphysema. I asked that question yesterday.

DR. COULSTON: Yes, of course, this is what we are saying. I think you are right.

DR. SCHAEFER: I raise the question again. Can't you determine extracellular fluid space in these animals? That would be quite easy to do.

DR. COULSTON: It is not easy to do it with the electron microscope, when you get to that fine a point. Maybe Dr. Lewerenz can answer.

DR. LEWERENZ: First I should like to return to the question on the perivascular edema. Which vessels are you speaking of, the greater ones in the lung or which ones? The edema I demonstrated to you yesterday is, in fact, perivascular. It is around the smallest blood vessels in the lung tissue.

MR. BROOKSBY: This would be pericapillary edema. Now Staub has introduced edema into the lungs and he finds that the normal process of getting rid of this fluid - if that is what it is - is that the fluid migrates to the corner spaces of the lung and then it migrates to the larger vessels and there collects around the perivascular spaces and then moves via some mechanism outside of the lung; and that perhaps the oxygen toxicity syndrome we see is that when this system gets overloaded, fluid leaks out into the alveolus, and the alveolus fills up with fluid and the animal becomes anoxic and dies. Now if this is a chronic condition of fluid exudate into the alveolar wall, then this fluid should appear just by looking at it with the light microscope around the larger vessels in the lung. You should be able to see it very easily on a slide.

DR. LEWERENZ: I can't say on light microscopy but I saw it on electron microscopy. Now on this second part of the question, if it is edema or emphysema, I must confess I cannot answer. I can show you the picture, and I can tell you what looks like fluid on an electron micrograph and what looks like gas; and this accumulation looks like fluid.

MAJ. ROBINSON (Aerospace Medical Research Laboratories): I can speak from the light microscopic standpoint. We followed the progression of the oxygen lesion by light microscopy for two weeks. Now the acute response as we have

described it occurs in about three or four days. In two days' time, we can find small amounts of edema in scattered alveolae and there are many times you can find this in any lung that you look at. But by the fourth day, there is a massive exudation of this edema fluid into the alveolae and at the same time there is a so-called perivascular accumulation, as well as distention of the peripheral lymphatics with this edema fluid. If the animal lives through this and goes on to the proliferative phase, then the edema, as you say, Mr. Brooksby, is resolved in some manner and whether this is through the perivascular lymphatics or how, I really can't say.

DR. COULSTON: Part of this problem - the word edema is like the word stress - means about a dozen different things to different people. We don't allow the word "stress" to be used in Albany. We throw it out. Because if somebody says an animal was stressed, and then you ask the man, what did you mean, he might say, "Oh, you know, it lost some weight, didn't feel good, didn't eat right, was stressed." We don't allow this kind of a word. Now edema unfortunately is in the same category, like a cloudy swelling. You can get 10 different definitions as to what it means and what we are talking about. So can you define this edema fluid in some way? Is it an edema like that you would see in pneumonia or is it watery edema or is it viscous, what is the pH of it, et cetera, et cetera?

MAJ. ROBINSON: We have defined edema as what is apparently an extravascular accumulation of blood serum or plasma. Now to finish up on Mr. Brooksby's question on the eight-month animals, this so-called edema that we saw was quite focal in the septa and it certainly wasn't to the point where we had seen it in the acute responses where it had gotten to the perivascular spaces or to the peripheral lymphatics, to finish up the story.

DR. COULSTON: Dr. Kaplan, I have a question for you and Dr. Lewerenz. Electron microscopy studies on rats exposed to a hundred percent oxygen at one atmosphere pressure reported by Dr. Weibel and Dr. Kistler previously, indicated more severe changes than described yesterday. The mild interstitial edema demonstrated in dogs exposed to a hundred percent oxygen for eight months at 5 psia was similar to initial mild reversible changes in rats exposed to the hundred percent oxygen atmosphere environment. Please elaborate further on this matter; for example, were the changes described in the dogs yesterday capable of causing functional impairment and death, or were they adaptive changes, reversible, and of no serious consequence?

DR. KAPLAN: I think that's very pertinent because I think what we are doing here is getting involved in a semantic discussion at the expense of missing the significance of these findings. We are defining edema here as an accumulation of fluid in tissue space. We don't know whether it is a serum or whether it is plasma, or what else it is. To answer the emphysema question; it is fluid, not air, or oxygen. It is fluid. We know this by correlation with light microscopy; we know this, as Dr. Robinson has pointed out, by following the progression of what happens to these lungs over a period of time, to the point where they are really grossly, obviously, wet edematous lungs.

Now carrying this one step further to the question that you are raising. The effect that Dr. Lewerenz described yesterday is as she pointed out, identical, at least morphologically, to the very earliest incipient oxygen effect seen when you expose either a monkey, or a dog, or a rat to a hundred percent oxygen at the toxic

and lethal one atmosphere total pressure. We have not carried any animals beyond eight months at 5 PSIA 100% oxygen, so we cannot say factually that it does not progress any further than that. However, we believe that it probably would not progress further than that. I think the question was raised yesterday, and I believe you raised it, about it being difficult to believe that this edema was present for such a long period of time. I think probably that it couldn't have been present very long. Certainly you would have seen other changes. The fact that there was just this edema, this accumulation of fluid in the interstitial space, with no other changes except this density at the junction of the capillary endethelial cells (the significance of which we do not understand) led us to think that perhaps this was indeed an effect of taking the animals out of the oxygen and returning them to air before sacrificing them. I call this an effect rather than an artifact because I don't think it is. If you are going to expose astronauts to 5 PSI oxygen for eight months, they at some time too will have to return to room air, and whether they get lung damage during exposure or while they are returning, is interesting, but it is also a little bit beside the point. The point is that they get lung damage; so it is not then necessarily an artifact caused by the removal but perhaps an effect of removal. Maybe we should concentrate more not on the question of whether prolonged exposure to pure oxygen at 5 PSI is dangerous, but whether after prolonged exposure to pure oxygen at 5 PSI a sudden return to ambient air would be dangerous, or both of these things. We did not, unfortunately, do any kind of function tests at the conclusion of that eight-month study. It only seems that we don't know the best way to carry out a study until we have already finished it and it is too late. However, we have constantly added things to our studies, like the fact that we have to sacrifice the animals inside the dome rather than outside, and lately we have been doing other things like arterial pO_2's, pCO_2's, and arterial pH's. We can infer, I think, that the degree of edema seen after eight months was not detrimental in terms of lung function for the following reasons: (1) because the monkeys themselves did not have any kind of overt respiratory distress, they appeared perfectly normal in terms of their breathing; (2) because arterial blood gas studies that we have done on monkeys exposed to one atmosphere of oxygen at the point where their lung changes are the same as with 5 PSIA oxygen at eight months, namely, around 48 to 72 hours, reveal that their arterial oxygen, their arterial CO_2, and pH's are normal. We don't begin to see the development of hypoxia resulting from profusion and ventilation problems in the lungs of monkeys exposed to one atmosphere oxygen until sometime around 90 or 96 hours, depending upon the individual resistance of the animal. So we can say that the kind of picture that is shown early in our one atmosphere study is not associated with any kind of lung deficit in terms of the ability of the lung to carry oxygen into the blood stream and remove carbon dioxide, or in overt clinical appearance of the animals. By parallel we might say (and we can't be sure about this) that the changes that were seen in these 5 PSIA 100% oxygen, eight-month animals - whether you want to call them toxicity, adaptation, or effect - probably are not significant clinically. Again raising the point that was brought out in the paper on statistics given yesterday that there is a difference between statistical and clinical significance, I think these may be of statistical significance because they appeared only in the exposed animals and not in the controls. They are probably not clinically significant. As far as reversibility goes, as Dr. Lewerenz reported, after the 30- to 40-day recovery period, although the changes were not entirely gone, they were almost entirely gone. Later work which we have not yet published, done on one atmosphere oxygen animals, again if we may correlate, seems to indicate that if animals are removed from the exposure before they enter the proliferative phase Dr. Robinson was talking about, even though they have severely damaged lungs, plural effusion,

alveolar exudate, hemorrhage, breakdown in capillaries, epithelial cellular infiltration, they undergo almost complete recovery over a period of a couple of months. However, if they are maintained until they enter this later phase of proliferation of the alveolar lining cells, cellular infiltration and deposition of collagen in the interstitium occurs. Then, although they recover completely in terms of clinical appearance and their ability to diffuse gases, they do have residual interstitial scarring. On that basis, we would presume that if we had continued to follow these animals beyond 40 days, we would have seen complete recovery.

DR. ROTH: Is it known how much of the contamination was removed by the dehumidification and heat control system, that is, the chiller, in the seven- and 60-day animal exposure, or in any of the others?

DR. MAC EWEN: No attempt was made to determine how much of it was removed. The system used for removal is the same as that used in the environmental control system of the spacecraft so that we are trying to scale-model a spacecraft atmosphere. That is why the units used were 10 grams and a hundred, based on total consumption or use of that material in a space cabin, as near as it could be determined. The removal in the chiller was kept minimal by chilling only a small fraction of the atmosphere and chilling it minimally to about 45 - 50 degrees. Less than 10 percent of the atmosphere actually went through the chiller, the rest was blended back with it.

DR. COULSTON: There is one question addressed to Dr. Robinson. As much as we would like to agree with you that the industrial TLV's for ozone and NO_2 are satisfactory for space use, your statement should be qualified to "on the basis of histologic (morphologic) findings for 90 days". Other criteria must be considered; that is, we have found that animals acquire a tolerance to the acute effects of NO_2 at 5 ppm, and, further, that this tolerance triggers chronic pulmonary effects eventually. Dr. Robinson?

MAJ. ROBINSON: Yes. I would like to point out that, first of all, the reason that the TLV's were used for these compounds was to test the changes in the animals, or to see if there was a difference in the animal exposures at ambient versus altitude, and at 5 PSI oxygen. As I pointed out this morning in review of the toxicological results, there were none, and in my conclusion I think I verified the toxicological results in stating that there was no difference when reduced pressure and the hundred percent oxygen atmosphere was involved. I fully realize there are more sensitive methods of testing the reaction of biological systems to these compounds, as very aptly pointed out by Dr. Coffin, but please keep in mind that the purpose of our experiment was to use the TLV levels for comparison of tolerance during continuous versus intermittent exposures. So I don't think you can apply these things directly to your TLV problems nationwide.

DR. COX (Douglas Aircraft Company): I have one question here which was asked by two different people who have not identified themselves. I would like to address the question to Dr. Thomas. Would the continuous threshold limit values for widely studied contaminants, such as ozone, nitrogen dioxide, and carbon tetrachloride be much different if based purely on data from continuous exposure at ambient pressure in air, rather than based on data from 5 PSIA hundred percent oxygen or 5 PSI oxygen-nitrogen mixture?

DR. COULSTON: Whoever asked the question, would he like to discuss it? Whoever asked the question, do you want to make yourself known? (Pause) I guess not.

DR. FAIRCHILD: I didn't ask it. I just wanted to bring out a point. I think the point Capt. Sopher brought up that tetracyline produced a completely different response at 15 PSIA oxygen than it did at atmospheric air is an answer right there - that you can't assume that anything happening at atmospheric air is going to happen in oxygen environment.

DR. THOMAS: That's why we did the comparative series on NO_2, ozone, and carbon tetrachloride.

DR. COULSTON: Let's be very careful. When you give an antibiotic to animals, many animal species, depending upon where they are in the evolutionary tree, react quite differently. You can kill, for example, a rabbit or a guinea pig with relatively small amounts of penicillin or a tetracycline. A hamster also is in this class. This is one of the enigmas we don't quite understand - how this happens. Yet with a Rhesus monkey or with a dog we can give tremendous doses, relatively speaking. You have to have an awareness of which animal species, which class of animals you are giving your antibiotic to. You can get into all kinds of trouble making generalizations. That is all I'm trying to say.

DR. FAIRCHILD: I didn't mean to generalize. I just wanted to say it is an excellent example, and we are using isolated incidents to show that here is a drug given to the same species. Half of this group was kept in atmospheric air and the other half at near atmospheric hundred percent oxygen. Those in the high oxygen concentration receiving tetracycline all died; those not receiving the drug survived; those at atmospheric air receiving the same dose didn't respond at all.

DR. COULSTON: This is one of the most important points to come out of the last two days. If this is indeed true, then everything that we have been saying about doing more work in this area is justified. Because sooner or later you are going to have men up in space in an oxygen-rich environment who are going to have to take medicines, and one of the most widely used of these medicines would be an antibiotic. This should be explored at great length.

DR. SCHAEFER: They have done some studies, studying drug effects at altitude and at atmosphere. They found that central acting drugs like amphetamine had a sevenfold increase in effect at altitude compared with atmospheric conditions, so this is a very important aspect.

DR. THOMAS: As far as the Air Force is concerned, there is a project on at the School of Aerospace Medicine for studying the effects of drugs under altitude conditions.

DR. ROTH: We have just completed a study for NASA for reviewing the treatment of medical and surgical emergencies for present and future space vehicles, and, frankly, we've been upset by the lack of consideration of the drugs being given and the amount of thought that has gone into what drug and side effects has been very minimal, and I think the point that was raised today should be enough to stimulate a lot more thinking along this line.

DR. COULSTON: Thank you very much. I think it is very crucial.

DR. RIESEN: I think you people would be interested in the result we got on hyperbaric oxygen, while it is a little higher than one atmosphere. This was at two atmospheres above ambient. Two substances which have been proposed as protective against oxygen toxicity, which in this case would be clearly defined toxicity, central nervous symptoms in terms of convulsions, and cellular effects, probably in terms of oxidative phosphorylation are succinate, for one, by the Duke Group, and gamma-aminobutgrate as another by a group in Canada. These, administered individually to rats, tend to protect, respectively, against the CNS effects or against the cellular effects. But when put together they are a highly lethal combination in male rats that are very heavy; in male rats that are light they are not. In female rats, no effect, heavy or light. So this points out to me at least, in the case of hyperbaric oxygen, and in the case of rats, all the same species and same supplier, that we have to clearly define that these are essentially metabolites, not drugs as such, except for the dose. And so I think we have to keep clearly in mind the very precise conditions under which all these things are administered in order to even talk about these things.

DR. COULSTON: That's a good point, a recognized point in toxicology. We know a great deal about this, usually by accidents such as eating a piece of cheese after you've taken a monamine oxidase inhibitor. You'll practically die, and some people have. Look out when you eat that wonderful cheddar cheese. The point that is being raised is that this is an area about which I'm delighted to hear that the Air Force and NASA, and the submarine people are looking into. I don't think they are looking into it enough, because I know a little bit about what is going on. Dr. Cox, can we have one more important question?

DR. COX: I have one very pertinent question here directed to Mr. Hodgson of Monsanto. In the TGA System for screening cabin material, what were the criteria for (1) rejection, (2) pass, (3) go for further gas-off studies? What was the basis for selecting these criteria? Were any validation studies attempted?

MR. HODGSON (Monsanto Research Corporation): Well actually, our contracting organization made this decision for us, so I cannot state what the basis for criteria was. I think the system would work at any break point and that is the important thing as far as you are concerned.

DR. COULSTON: Now I would like to throw this forum open to one or two miscellaneous questions, to things that bother you the most.

DR. EKBERG (General Electric Company): Rhythms have been alluded to a few times in the Conference; and, in particular, reference to 24-hour rhythms or Circadian rhythms, if you will. I wonder if the experimenters have made their measurements at the same time of day?

DR. COULSTON: This is a good point, which has occurred to a lot of people in the room. There is a lot known about these rhythms. You can change parasites from dividing, just by changing the light in the room, and a question could be asked whether they had the light on 12 hours and off 12 hours in these domes. You can ask a lot of questions like this. I think this is a very important point and certainly I am sure the people have considered it.

DR. BACK: Well not always, but almost always. It depends somewhat on circumstances. However, the lights are on in these domes all the time. Now, as far as feeding is concerned, the monkeys that we use on psychopharmacology experiments go to work at eight o'clock in the morning and they are finished at three-fifteen in the afternoon. They eat at three-twenty in the afternoon. On Saturdays and Sundays they still eat at the same time. They only eat for a half hour and so the food is never in there longer than that and it doesn't get contaminated. So we have thought about these things. In all domes, food is usually taken every morning, because our people in the laboratories have to get the day's work done at the end of the day, so we take bloods the first thing in the morning so the blood work is done at that particular time. I say that for the most part we have a very tight routine through which all animals go.

DR. COULSTON: Be sure in your report you describe this in detail. You know this is terrible, what they do to the poor monkeys. They are going to have either a Union on their neck or the United States Government for unfair labor practices, one or the other. Seven days a week - you've got to give them a day of rest don't you?

DR. BACK: They don't work two days, but they eat those days.

MR. MOBERG (Aerojet-General Corporation): I'm not speaking as a medical man but I feel a little miffed at the fact that this is a medical conference. There was a lot of time given to discussions of instrumentation and measurements and the complexity of sampling and the complexity of analysis data, and yet there is not an equivalent radio time given to this problem.

DR. COULSTON: I see your point. I should say two things: one, very few questions came in on this point, less than, I am sure, four percent; but, secondly, I did say that this would be carried over until tomorrow. We don't intend to neglect Mr. Saunder's discussion, or other people's discussions, so why don't you give this point right now.

MR. MOBERG: I think there is one very important point that I would like to bring out, at least now, in spite of receiving probably much criticism from the medical profession. Each of us looks at the other profession as not quite as important as ours; and, of course, the medical profession deals with human life directly and the analytical chemist only indirectly, and yet we get the blame if anything goes wrong. There has been quite a bit of discussion raised at least outside of the doors of the Conference about data that does not seem to agree, particularly on the contaminant data, and I think that this is not necessarily the instrument itself that is furnishing the data. It is the processing of the data - the sample handling for one - which is of vital importance. Secondly, the diagnosis of the data is also vitally important, almost as much as a medical man would diagnose a case, and each one would look at it differently; but even more so, they would not like to look or diagnose a case without knowing some case history, and too often too little information is passed on to the professional analytical man to give him background as to what might be expected, what interferences might be present in the system, what other instrumental difficulties might have arisen in the test before he received the samples. Had this information been passed on to him, there might have been much more intelligence in handling this data. And this is a recommendation, a request, that the analytical people might make to at least the medical people here and to some of the

people not dealing in the handling of numbers or to those getting numbers from the analytical laboratories. That is a vital point.

DR. COULSTON: What you say, of course, is very true, and I don't think there is anyone in the room that would disagree with you. I think the liaison between the various groups, be it biologists, pharmacologists, pathologists, toxicologists, biochemists, and between the bioengineering groups, is essential and we've got to cross over. These are multidisciplinary approaches to a problem, and there can be no sitting inside your discipline any longer. We are talking about a total, intact, unanesthetized animal, and you must explore him in that very sense.

MR. MOBERG: Thank you. After Ray Saunders gives his paper tomorrow, I would like to cover some of this data that has been presented on the trace contaminants and compare the types of data, the numbers. I'm speaking a little from experience in working with Brooks Air Force Base, and I think maybe, as you people have defended your positions in interpreting data, I'd like to do that too.

DR. COULSTON: Again I repeat, the questions did not come in, and so we have no way of doing it. You people should have asked a lot of questions. But I am sure Dr. Thomas will take this into consideration tomorrow, and I am certain it will be very successful.

DR. ROTH: Here is a general question directed to the Wright-Patterson people. Review of contaminants found in closed systems shows the majority of hydrocarbons, ketones, esters, alcohols, et cetera, to fall into the broad category of narcotics or anesthetic agents. Although many are present at fractional to a few milligrams per cubic meter, additive effects could be postulated. How come more effort has not been put into the continuous exposure, ground and altitude toxicology of these combined narcotics?

DR. BACK: As a matter of fact, we just haven't had time. We have been doing some of the basic work on pulmonary irritants in one and two gas systems and we've only got so many domes. But that's one of the things that we have on the agenda. We noticed that most of them are depressant type compounds, alcohols, ketones, and so forth; that's where we are going, but it will take time. It will come but just wait a while.

DR. COULSTON: Gentlemen, I want to tell you that this has been one of my greatest pleasures in my scientific career. This has been a very stimulating audience. You have asked pertinent and soul-searching questions, and I think it augurs well for the future of our program in aerospace and submarine medicine. Thank you very much.

SESSION V

MEASUREMENT OF BEHAVIORAL RESPONSES

Chairman

Dr. M. D. Fairchild
Department of Pharmacology
University of California
Los Angeles, California

QUANTITATIVE ANALYSIS OF SPONTANEOUS ACTIVITY: EFFECTS OF LONG-TERM CONTINUOUS EXPOSURE TO SPACE CABIN ATMOSPHERES

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Wright-Patterson Air Force Base, Ohio

Ever since the altitude facility started to operate, we have been documenting the spontaneous activity of dogs in the Thomas Domes. We felt that a normal and happy bunch of beagle dogs moving around unrestricted in their pen should result in a lot of activity, indicating a state of well-being. As part of the clinical observation of these animals, we have instituted time-lapse photography to give us a permanent record on the status and position of the animals within the pen every 30 seconds during the experiment. We also hoped that decreased spontaneous activity could be an early indicator of toxicity with the exception of CNS stimulants, which would create increased activity and agitation in the early phase.

Documentation by time-lapse photography using existing light proved no problem. Quantitation of the information and data handling is another story. A picture every 30 seconds amounts to 2,880 frames per 24-hour period, and in the case of an eight-month exposure run this becomes somewhat of a problem.

Looking for the easy way out, I was searching for mechanization of the evaluation process and the elimination of any eyeball strain to the operator of the equipment. The final process which has evolved is illustrated as a flow diagram in figure 1. The time-lapse camera records the scene of the dog pen in the dome every 30 seconds. There are usually four dogs moving around freely in a fenced area of approximately one-quarter of the dome floor or roughly 30 square feet. Since the floor is white and the grill work forming the bottom of the pen is stainless steel, there is quite a good contrast between the dogs and their environment. The 16 mm film is removed at the end of each 24-hour period at eight o'clock in the morning and is sent for processing and a positive print. When the print is received it is projected at 16 frames/second (fps), and approximately one-fifth of the pen area is scanned for activity information by a photocell. This is a very important point, because if the entire pen area were scanned, the photocell would always receive the same dark to light ratio since the four dogs are always there, and, therefore, the total amount of reflected light would not change. As the dogs move around, the scanned area can be empty, partially or fully occupied by a dog, thus changing the dark to light ratios which, in effect, reflect the degree of activity.

If one frame is identical to the next one, there is no activity; if it is slightly different, there is slight activity; and if it is entirely different, it represents maximum activity. For example, if the scanned area is occupied by a black dog for six consecutive frames, the photocell receives no light for that length of time and we would say that there was no activity in that period. If, in the next six frames, the dog should gradually move out of the scanning area, there would be a gradual change from black to grey (stainless steel grill work) and, therefore, the photocell would receive a gradually increasing amount of light indicating moderate activity. By the same token, if a dark frame should alternate with a grey frame, there would be maximum change of light intensity from frame to frame, such as could be caused by the black dog moving in and out of the scanned area every 30 seconds, which would indicate maximum activity. In the latter instance, the photocell would have no output on the dark frame and maximum output on the grey frame.

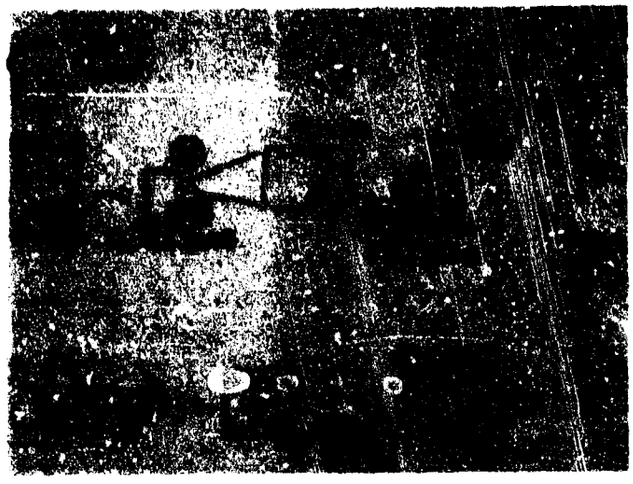

Figure 1. FLOW PROCESS AND EQUIPMENT USED IN THE EVALUATION OF SPONTANEOUS ACTIVITY

Continuing on the flow chart, you can see that the output of the photocell is used to drive a light-powered transistorized audio-frequency oscillator (figure 2) whose output varies from 40 to 4000 cps (cycles per second), depending on the amount of light falling on the photocell. Thus, if there is no change in activity, the pitch of the sound is also unchanged. Maximum activity causes maximum variation in pitch. The modulated audio is then recorded on a simple audio tape recorder for later quantitation by a pen recorder or it can be directly transcribed without the aid of any tape recorder if one is willing to sacrifice resolution. The use of the tape recorder is not only convenient from the point of phasing workloads, but also increases resolution of the activity record if the tape is played back at a slower speed than recorded. This expands the time scale threefold if the recording was done at 7.5 inches per second (ips) and the play back is done at 1-7/8 ips. This time expansion is important for two reasons: first, it allows the recorder pen to follow the potential changes more accurately; and second, the recorder response does not have to be the oscillographic type. By the simple trick of using the audio output for modulating the intensity of a small flashlight bulb and picking

up this modulated light by a second photocell, a rather inexpensive D-C millivolt chart recorder can be used for the graphic write out of the activity record. This tape to pen converter must be enclosed in a light-tight black enclosure and is illustrated in figure 3.

Figure 2. LIGHT-POWERED TRANSISTORIZED AUDIO FREQUENCY OSCILLATOR

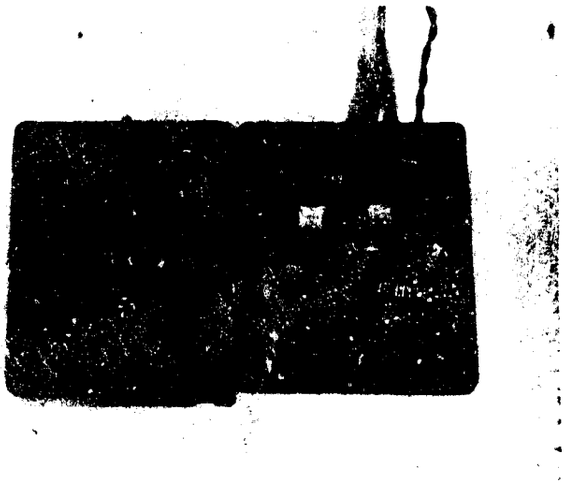

Figure 3. LIGHT BULB AND PHOTOCELL COMBINATION TO CONVERT SIGNAL FROM A-C TO D-C

In essence then, during the recording period, we have been transforming optical information picked up by a photocell (D-C output) driving a variable pitch audio frequency oscillator (A-C output) into audio information recorded on magnetic tape. In the play back phase, we are starting with the audio information on the tape (A-C output), which we are using to drive a small flashlight bulb whose light output is proportional to the variation in audio information, which, in turn, is picked up again by a photocell (D-C output) and recorded by a simple strip chart recorder.

To illustrate the difference in the picture information from frame to frame during a maximum activity period, figure 4 shows the situation in the dog pen on six consecutive frames. This example was selected because there was only one dog in the pen, which makes for easier understanding, and because the chamber technician also got into the scanned area, supplying a great amount of light reflected from his white coveralls. While figure 4 shows the entire frame in each case, figure 5 illustrates only the scan area in the same frames. As mentioned earlier, if the entire frame were scanned, activity would be indicated by the scanning process only in the top and bottom frames at the right where the dome entrant got into the picture. As can be seen from figure 5, the scanned area contains different picture information on each frame, indicating substantial activity.

Figure 4. A SIX-FRAME SEQUENCE OF THE DOG PEN AREA AS SEEN BY THE TIME-LAPSE CAMERA

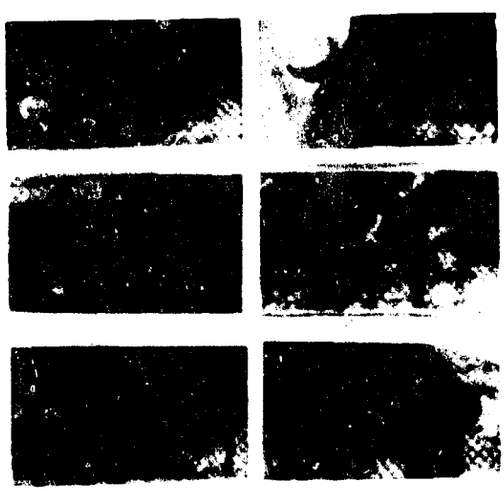

Figure 5. THE AREA SCANNED FOR ACTIVITY BY THE PHOTOCELL FROM THE SAME SIX-FRAME SEQUENCE ILLUSTRATED IN FIGURE 4

There is one problem in using photographic processes in general. This is the day-to-day variation in processing of the negative and in printing the positive copy. This results in sufficient variations in the contrast of the positive print from reel to reel to introduce a great deal of artifact during the scanning process. A print with a great deal of contrast and one with normal contrast from the same negative would yield a high vs. normal activity record. The reason for this is that even minor changes in picture information cause greater variation in the light intensity received by the photocell if the contrast is great. To circumvent this difficulty, two areas are scanned from each frame; one which is 1/4 of the pen area where the dogs move about, and one outside of the pen area where there is never any movement. Figure 6 shows the pen record from the chart recorder comparing the two

scan areas from a print with extremely high contrast. Record A is the area outside the pen and record B is the activity record. The excursions in record A are due to the artifact induced by the high contrast print and represent variation of density of a neutral grey area in the dome due to extraneous light reflections through the dome windows. Still, this noise level is substantially below the activity level produced by the movement of animals on record B. Subtracting the noise level from the activity level compensates for the variations in development techniques. Figure 6 also represents the total activity for a 24-hour period and, therefore, contains all the information from 2,880 frames.

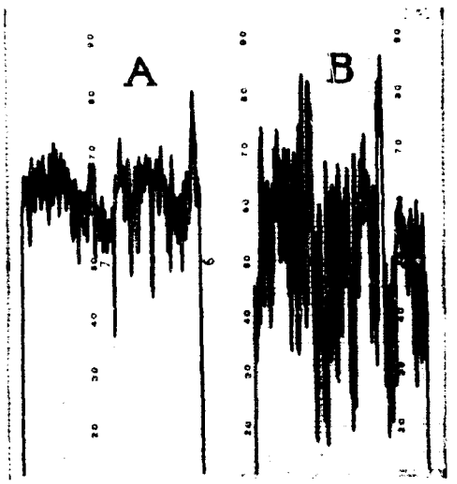

Figure 6. PEN RECORD FROM STRIP CHART REPRESENTING 24 HOURS OF SPONTANEOUS ACTIVITY (B) AND NOISE LEVEL (A)

Table I summarizes the recording characteristics of the system. The remaining noise component after correcting for the variations in development is approximately $\pm$ 10% and is furnished by a fixed component consisting of the light chopper of the projector (interrupting the light beam three times during one frame) plus amplifier noise. The variable component is furnished by slight variations in light intensity, decibel response of the tape, and nonuniformity of the magnetic coating. Reproducibility by scanning the same film again and again is within 10%. The reproducibility of transferring the tape record to chart record is within 2%.

The quantitative evaluation of the chart record is done by elution of the colored ink (which is water soluble) and spectrophotometric measurement of the optical density of the eluates. We refer to this process as a "length-of-trace" analysis and it deserves some explanation.

The pen record of a number of frames with no activity would be a straight line since the light input to the photocell would not change, and, therefore, there would be no change in the entire process chain. To further elaborate, there would be no change in the output of the audio oscillator, the pitch of the recorded tone would be steady, the audio output driving the small flashlight bulb would be constant, resulting in a steady glow of the filament, and the photocell picking up the light from this bulb would have a steady D-C output at a fixed level to the recorder pen.

TABLE I

CHARACTERISTICS OF THE RECORDING PROCESS

TIME LAPSE PHOTOGRAPHY:	One frame every 30 seconds Ambient light	
PROJECTION:	16 FPS	
TAPE:	7.5 IPS	
CHART:	2.5 cm/min	
NOISE:	± 10%	
(Noise components,	Fixed:	chopper, 3/frame tape DB response amplifiers
	Variable:	development light intensity)
REPRODUCIBILITY:	± 2% (from tape to chart)	

Therefore, no activity always equals no change in pen movement or the shortest possible trace. Whether the pen is at the bottom of the chart or at the top is a function of the amount of grey to black ratio in the scanned area. If, for example, a black dog would occupy half of the scan area, leaving the other half grey, while he is in this position the pen record would indicate a straight line at the middle of the chart. If he moves to cover the whole scan area and stays there for awhile, the straight line would drop to the bottom portion of the chart and remain there. On the other hand, if he decided to get out of the scan area and not to return there for awhile, the straight line would move to the top portion of the chart and remain there. It is easy to perceive that activity, therefore, is indicated by upward and downward deflections of the pen on the chart record. A black frame followed by a grey frame would cause the pen to oscillate between the bottom and the middle of the chart. A white frame (such as the coverall of the dome entrant), followed by a black frame, would make the pen travel from the top of the chart to the bottom, the maximum possible change in activity, and, therefore, the maximum possible distance to be traveled by the pen. It is quite obvious then that the length of the trace is directly proportional to the amount of activity.

In the actual process of performing this "length-of-trace" analysis, the activity records are separated from the noise records and the ink tracings are eluted in 500 ml of water, separately. The optical density of the eluates is measured at a suitable wavelength, depending upon the color of the ink used, and the Activity Index (I_a) is calculated as follows:

$$I_a = 10 (T_a - T_n) \text{ where:}$$

T_a = O. D. of activity tracing

T_n = O. D. of noise tracing

To date we have analyzed data from the eight-month 5 psia 100% oxygen exposure study. The pertinent parameters for this study have been reported in the previous Conference proceedings. Although time-lapse photography does provide continuous coverage on a 24-hour, round-the-clock basis, the data presented here are samplings of spontaneous activity for full 24-hour periods two weeks apart during the study. Figure 7, therefore, represents 17 separate 24-hour periods of activity evenly spaced from the beginning to the end of the exposure run. The envelope which fits over these data seems to indicate a peak of activity in the middle of the exposure period and a fairly even level of activity most of the time except for a decrease in activity around the end of the exposure. This trend may or may not be significant from the standpoint of oxygen effect. Since we do not yet have true control data, the variation of activity level might be explained by other factors such as seasonal variation or the aging process. These dogs are young pups (about six months old) when they are placed in the chamber and they mature somewhat in the eight-month period of the exposure.

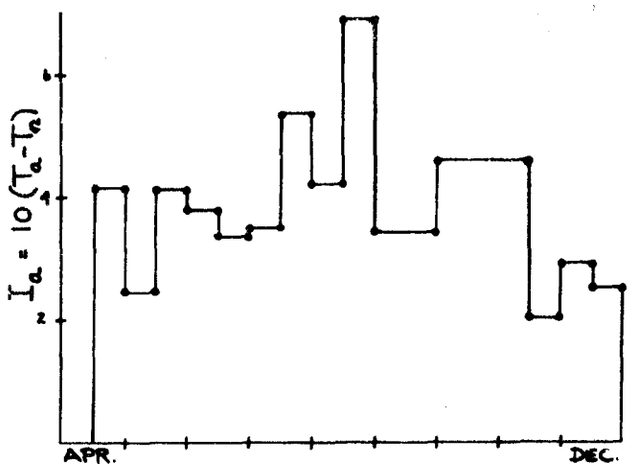

Figure 7. ENVELOPE OF SPONTANEOUS ACTIVITY LEVELS DURING EIGHT MONTHS EXPOSURE TO 5 PSIA 100% OXYGEN. SEVENTEEN 24-HOUR OBSERVATION PERIODS PLACED TWO WEEKS APART

The actual Activity Index averaged 3.87 during this study with a low of 2.00 and a high of 6.90. Looking at the seasonal trends, the data points in the spring period show an average I_a of 3.80 with $\pm$ 10% reproducibility range of 3.42 to 4.18. Similar calculations for the summer period are 4.50 (4.05 to 4.95) and for the fall 3.60 (3.24 to 3.96). There is considerable overlap in these ranges with only the summer period showing an elevated trend. We do plan to evaluate these data further by more frequent sampling of the material on hand and by instituting a suitable control study in a few weeks.

The data reduction aspects of this study are illustrated in table II. If one would have to use a visual and manual checking method by comparing one frame to another, scoring results on a zero to four plus basis, about five frames could be scored per minute. Therefore, to evaluate a total of almost 49,000 frames taken

during the seventeen 24-hour periods, a technician would be busy for a whole month. With the present system, only six hours are required for the entire job. There is considerable economy in the volume of records. Tally sheets for the 9,000 bits of data are eliminated, and the total records consist of one 1600-foot reel of time-lapse movies, a standard seven-inch reel of tape, and one 7-foot section of a strip chart record. Considering that this was biweekly sampling only, if the existing data were to be evaluated on a daily basis, a technician could easily evaluate each 14-day portion of the study in one day. Since the tape is reusable, the only storage problem is that of the film which can be periodically discarded as it is transcribed into chart record.

TABLE II

DATA REDUCTION ASPECTS OF THE STUDY ILLUSTRATED IN FIGURE 7

FRAMES/DAY = 2,880 TOTAL FOR 17 DAYS = 48,960

FILM LENGTH = 1,600 ft. TAPE LENGTH = 1,800 ft.

CHART LENGTH = 7 ft. PRESENTED AS I_a = 1 page

TIME FOR PREPARING I_a PRESENTATION:

SPLICING OF FILM	1 hr.
PROJECTION AND TAPE RECORD.	1 hr.
REPLAY FOR CHART RECORD.	1 hr.
ELUTION + MEASUREMENT	3 hr.
	6 hr.

TIME FOR MANUAL CHECK (5 frames/min): 163 hr.

Our future plans are to continue documenting each study with time-lapse photography, with the exception of the cabin materials tests. As we gain more experience with the method and its virtues and limitations, we will optimize certain parameters. It appears that sampling may be revised from 30-second intervals to 60-second intervals on the basis of comparing workday periods with weekend periods, where we are now using 60-second intervals to reduce overtime for photographic personnel loading the cameras. On the other hand, in the study of the toxic effects of CNS stimulants, we may find that short bursts of five-second interval samplings each minute or so apart may yield more meaningful information to detect agitation, tremors, and overt convulsions. We firmly believe that these parameters can be of major importance in increasing the sensitivity of this behavioral method and can support clinical observation of the animals at the same time. Spontaneous activity measured by other methods (activity cages, light beam interruptions, etc.) does not furnish complete information in a visual field and, therefore, is inherently limited in application. Time-lapse photography overcomes these disadvantages and will become a very powerful tool if used properly.

The equipment involved in evaluation of the film footage is rather primitive and is all "home-brewed" gadgetry. Consequently, we plan to keep all our footage until highly sophisticated equipment specifically for this purpose is developed. We are working on this. The increasing availability of integrated circuits holds great promise in this area. With increased response times we hope to eliminate the tape recording step and work directly through logic circuits. Ultimately, we hope to eliminate the photographic process and use the screen of a closed-circuit TV system for picking up the information from the scan area and process it digitally from frame to frame. This would negate the importance of sampling intervals, eliminate the chopper effect, and would further increase the accuracy and sensitivity of our observations.

QUESTION: Is the activity index proportional or linear to the amount of activity? I don't know how one would express the amount of activity, but do you have a thought on that?

DR. THOMAS: Yes, sir. You're measuring ratios of light against dark areas. Let's take a theoretical case. If you would have a dog with a white belly and dark back, and you would put him in front of the camera, immobilized, showing black color on his back, nothing would change in the picture information. If you would turn him around every 30 seconds, you get a completely white frame against a completely black frame, right? So, it's linear.

QUESTION: Would you consider this equal to or preferable to the light beam interruption method?

DR. THOMAS: I don't think that it's equal to and I don't think it's preferable to. The reason why we went this way is that the light beam interruption method does not give you the amount of information we get from this process, because from the movies you can make clinical observations also. Moreover, the light beam interruption method is very difficult in the dome situation. As a matter of fact, technically it is not feasible.

RESPONSES OF TRAINED MONKEYS TO DIFFERENT GASEOUS ENVIRONMENTS

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I shall discuss briefly some of the work we have accomplished during the past year using trained monkeys under varying conditions of exposure. These conditions are shown in table I.

TABLE I

EXPERIMENTAL EXPOSURE CONDITIONS USING TRAINED MONKEYS

Atmosphere	Altitude	Ambient	Duration	Dates
67% O_2/32% N_2	x		90 days	7 Mar. - 5 June
68% O_2/32% N_2 + Ozone (0.2 mg/m^3)	x		90 days	6 June - 3 Sep.
Air		x	14 days	4 Sep. - 18 Sep.
Vivarium		x	35 days	18 Sep. - 21 Oct.
Air		x	28 days	24 Oct. - 18 Nov.
68% O_2/32% N_2 + N_2O_4 (9.4 mg/m^3)	x		37 days	21 Nov. - 27 Dec.
100% O_2	x		13 days	27 Dec. - 10 Jan.
100% O_2 + Eth. Glycol (275 mg/m^3)	x		18 days	10 Jan. - 27 Jan.

Since most of the participants at this Conference have seen our facilities, you know that we are working with specially designed and made instruments, which were constructed at Holloman Air Force Base. They include 12 cages placed in one Thomas Dome so that each cage is situated in front of a single dome window. The programming equipment is solid state and was also built at Holloman and shipped to us (Wolfle, 1966). The animals are programmed to work eight hours a day. They work for 15 minutes out of every hour, for five days a week, as long as the experiment is in progress. They do not work during the weekends. They start at eight o'clock in the morning and they finish at three fifteen in the afternoon, at which time they are fed. We feed them only during the time it takes to clean cages and perform other daily maintenance tasks in the dome. We treat these animals as individual cases rather than as a group, and perform clinical laboratory tests on them routinely (table II).

TABLE II

CLINICAL LABORATORY TESTS PERFORMED ROUTINELY

Hematology	Chemistry
WBC	Sodium
Differential Count	Potassium
RBC	Calcium
Hemoglobin	Total Protein
Hematocrit	Albumin
Sedimentation Rate	Total Phosphorus
	SGOT
	SGPT
	Alkaline Phosphatase
	LDH
	A/G Ratio

While the chamber technicians are in the dome, we try to do our routine clinical tests very early in the morning. This means that three men have to be in the dome by six o'clock in the morning to bleed the animals so that by eight o'clock the monkeys can begin to work. In addition to the blood studies, our pathology branch personnel also take anal and oral swabs so that we can get a good picture of the microflora population in these animals.

Our first exposure atmosphere was 68% O_2/32% N_2, total pressure 260 mm Hg. The oxygen partial pressure varied somewhat but averaged 175 mm Hg. Flow rate was 22 cfm and temperature, depending on whether it was summer or winter, ranged from 70 to 78 degrees F. The humidity was approximately 50%, sometimes a little higher, sometimes a little lower. We had 91 operating days, from 7 March to 5 June. These animals had to be prebreathed at 100% oxygen for two days on the way to altitude in order not to get the bends and in order to become acclimatized before we switched over to the 68% oxygen-32% nitrogen. During the 90-day exposure to mixed gas atmosphere, there were no detectable changes in performance of these animals.

After 91 days we decided to initiate the ozone exposure without bringing them back to ground level. We just started by adding ozone, on 6 June, at a concentration of 0.2 mg/m^3. We ran the ozone exposure for 90 days, until 3 September 1966. Again we saw absolutely no changes in the animals' performance.

The next two weeks were run under ambient conditions; i.e., we brought them back to ground level, worked them from 4 through 18 September. Once again, there was no discernible change from their prebaseline or altitude levels, or altitude levels after ozone was added.

Since we were beginning to see a number of individual changes in hematocrit and hemoglobin, we transferred the animals to the vivarium for about five weeks. Occasionally an animal would also stop eating. At this time we received four newly trained animals from Holloman, so we sent four of the old animals back to Holloman. This changed our monkey complement to eight old and four new monkeys.

They were returned to the chambers after five weeks rest in the vivarium and were worked in the dome for four weeks at ambient conditions. Then we took them to altitude at 100% oxygen, 5 psia. After they had become acclimatized to altitude for one day, we switched over to the mixed oxygen-nitrogen atmosphere on the next day. The following day we added N_2O_4 at approximately 9 mg/m^3. This experiment was stopped after 37 days of exposure, on the 27th of December, when we learned that there was an urgent need to study ethylene glycol. We realized that we had to return to a one-gas system because the requirement for the ethylene glycol experiment was in 5 psia oxygen. Therefore, we stopped the N_2O_4 and switched to 5 psia oxygen for 13 days, until 10 January, and then started exposure to saturated vapors of ethylene glycol.

As you heard from Mr. Vernot yesterday, we ran the ethylene glycol experiment for 18 days. We started exposures to ethylene glycol in Dome 1 earlier, with the idea that exposures of monkeys, dogs, guinea pigs, rabbits, rats, and mice in Dome 1 would precede the trained animal experiment by a few days. If we found any overt toxicity in Dome 1, we could avoid serious damage to the more valuable Holloman monkeys.

Since there were no obvious differences between performance of these monkeys, I have arbitrarily chosen two individuals to illustrate the results. The animals push a left (audio) and a right (visual) lever in their discrete avoidance test. At the top of figure 1 are the right and left lever press plots, showing about 0.5 for the audio and 0.9 seconds for the visual as the amount of time it takes for this animal to respond. At the bottom half of figure 1 are the continuous avoidance tasks, and they are recorded as the average number of lever presses per minute for a days work session. This particular animal (Duane) normally pushes the left lever approximately 25 times per minute and the right lever approximately 65 times per minute. On 21 December, the exposure to N_2O_4 in a two-gas system was already 31 days completed. This animal was one of the new shipment from Holloman and, therefore, had never been previously exposed to anything. This was his first experience. After the premature termination of the N_2O_4 experiment, we changed to 5 psia oxygen; note that there is practically no change in his performance. All of the animals continued to work as though nothing had changed.

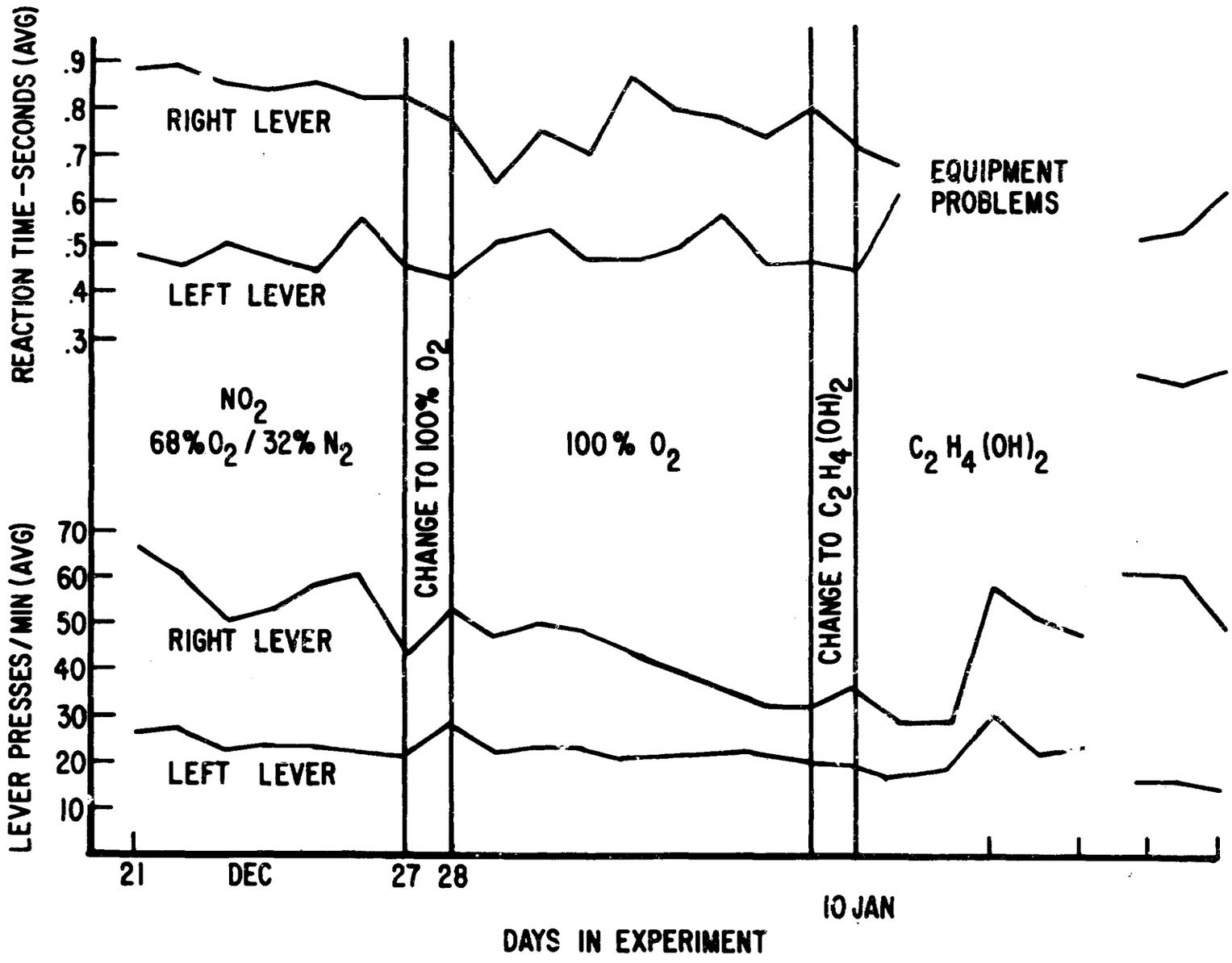

Figure 1. DUANE - NO. 433

With the change to ethylene glycol, we had problems. This material really gummed up much of the equipment in the dome, resulting in erratic lever action, and you should note that Duane started taking shocks in the audio and visual discrete avoidance tasks. Notice that when he started slowing down in one area, he started to compensate by working faster on the two continuous avoidance tasks. We called for assistance from Holloman, and they got some of the problems straightened out so that we ended up with the animals not showing very much real decrement, although they were extremely excited by that time, not knowing whether or not they would get shocked. We had lots of problems with the equipment.

In contrast, look at figure 2. This is another animal, Bugs. This animal has been used for approximately three years in various exposures to monomethylhydrazine, unsymmetrical dimethylhydrazine, and to decaborane, two or three times. I don't know what his liver looks like, but I can show you what his clinical chemistry picture looks like. But note how well he works. His right and left lever presses, discrete and auditory tasks, are fantastically overlapping. In fact, right and left lever presses at the bottom of figure 2 show that he is just as good, frequently, on the left side as on the right, and that he's almost ambidextrous.

Now, let me show you something about the clinical laboratory results. Note the results of the blood samples taken from all the animals on 15 December 1966 (table III). The values under J9 are from Bugs. He has a hematocrit of 26 at this time and has been going down constantly for six months. He has a hemoglobin of 7.8, a RBC of 4.54 million, and a SGPT of 330. His LDH is not remarkably high; about average for the type of monkey that we use.

Note animal R25, which is the one Mr. Vernot talked about yesterday as the one that deteriorated somewhat. This animal refused to eat and had to be removed from the ethylene glycol tests. She looked very ill although she continued to work, and her laboratory tests are relatively normal.

At the end of the ethylene glycol experiment (table IV), animal J9 (Bugs) has a hematocrit of 20, hemoglobin of 5.6, RBC of 3.3 million, and SGPT of 340, and he still works like a demon. In summary, there is no good correlation between the clinical state of the animal and its ability to perform well.

If, indeed, we can work monkeys under almost any conditions with no change of performance, why do we do it? We have many reasons for continuing this kind of work. It is certainly not enough to know what the hematology and clinical chemistry picture of an animal is, either at the cellular level or the peripheral circulation level. We must also know whether the animal can indeed perform in his environment. We look at the animals daily in these domes, and some of them look perfectly fine, but we do not know whether they can react or not. This is one simple way of evaluating their capabilities. Another consideration is that we want to establish sufficient baseline data in this area. Very few investigators today are using this type of measurement, and even this may be too gross; it may not be sensitive enough to measure small changes. We must continue to evaluate the merits and limitations of such techniques. After we have established historic baselines, we would like to implant brain electrodes in these animals, require them to work at the same time, so that we can record changes in electroencephalograms and try to change the response by direct injections of compounds into specific portions of the central nervous system as the animals are being exposed and as they are working in the altitude environment.

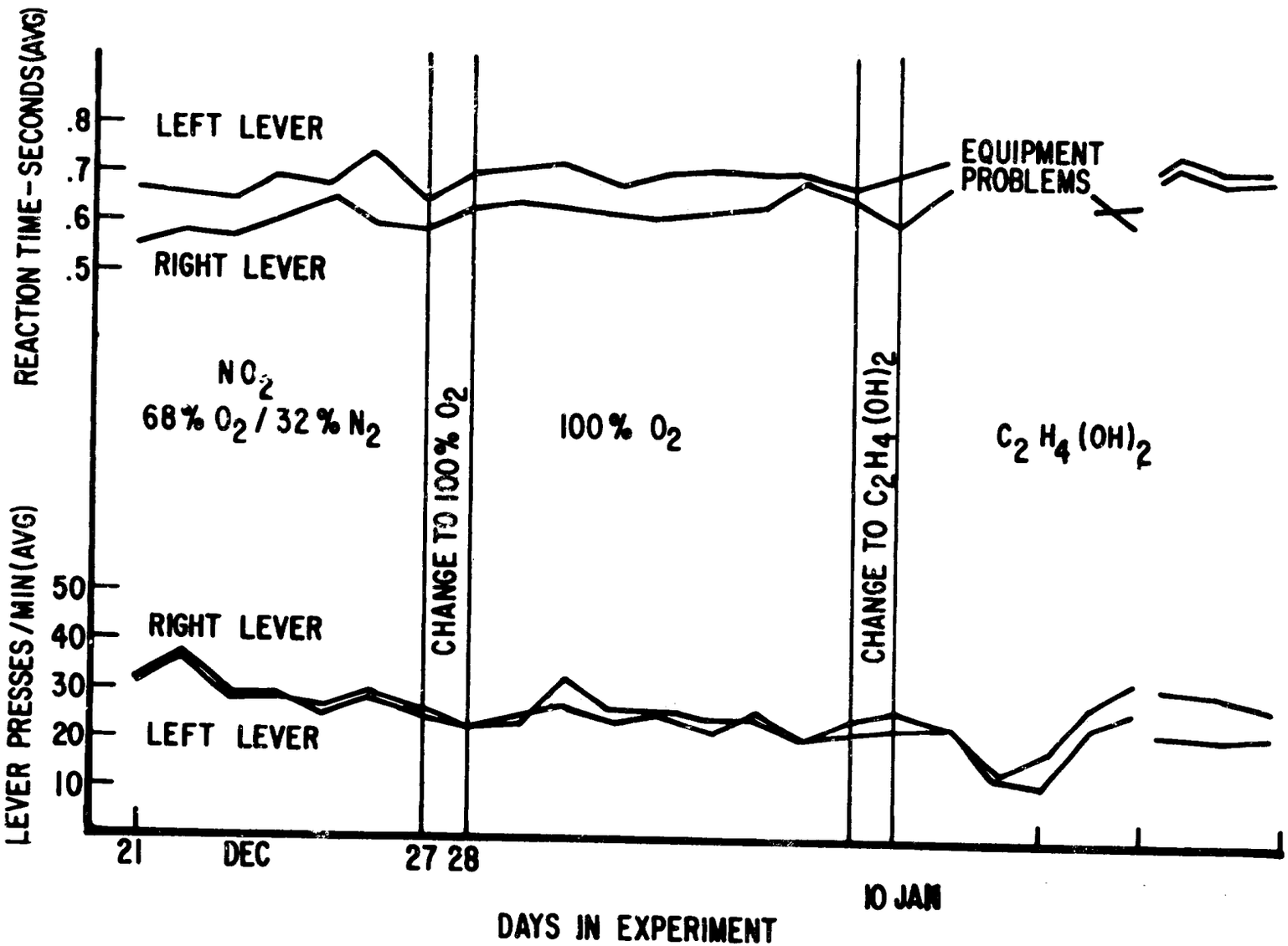

Figure 2. BUGS - NO. J9

TABLE III

DATE 15 December 1966

SPECIES Holloman

TEST	ANIMAL NUMBER											
	433	441	M62	J9	408	427	J8	M55	M56	M52	M58	R25
HCT	43	41	39	26	44	39	35	36	42	38	42	35
HGB	13.2	12.8	12.4	7.8	13.6	12.4	9.9	11.4	13.2	12.0	13.6	10.8
RBC	5.47	5.21	4.76	4.54	5.66	4.66	5.03	4.41	5.50	4.82	5.07	4.55
WBC	13.2	12.8	12.4	7.8	13.6	12.4	9.9	11.4	13.2	12.0	13.6	10.8
DIFFERENTIAL SED RATE	0	0	0	4	0	0	1	0	0	0	1	1
DIFFERENTIAL Blasts												
DIFFERENTIAL Myelocytes												
DIFFERENTIAL Bands												
DIFFERENTIAL Lymphocytes												
DIFFERENTIAL Monocytes												
DIFFERENTIAL Eosinophiles												
DIFFERENTIAL Basophiles												
Sodium	145	147	146	154	147	143	150	143	150	146	147	142
Potassium	4.5	4.7	4.1	5.0	4.5	4.5	5.0	4.4	4.8	4.7	4.5	3.4
Calcium	5.8	5.8	5.4	6.4	6.0	5.7	6.6	5.6	5.8	5.9	6.2	5.5
Total Protein	7.4	7.2	6.8	7.8	8.0	7.2	7.6	7.2	7.8	7.0	7.8	7.4
Albumin/Globulin	4.1/3.3	4.1/3.1	4.8/2.0	4.8/3.0	5.0/3.0	5.1/2.1	4.8/2.8	4.8/2.4	5.1/2.7	4.6/2.4	4.8/3.0	4.8/2.6
SGPT	93	250	50	330	46	93	115	39	42	93	58	93
SGOT	50	120	55	74	50	45	50	45	55	64	45	55
Alkaline Phosphatase	14.0	15.0	12.0	2.6	8.2	1.0	1.8	5.8	27.0	9.0	16.0	0.4
Total Phosphorus	6.5	6.5	4.8	3.1	5.7	4.8	4.6	5.0	5.2	4.6	5.0	2.3
LDH	1440	1240	720	860	750	780	780	520	600	780	440	820

TABLE IV

DATE 25 January 1967 SPECIES Holloman

TEST	ANIMAL NUMBER											
	433	441	M62	J9	408	427	J8	M55	M56	M52	M58	R25
HCT	42	36	46	20	40	39	31	34	41	33	39	34
HGB	12.8	10.8	13.6	5.6	11.4	11.4	9.1	10.8	12.0	9.9	11.7	10.5
RBC	5.47	4.76	5.80	3.34	4.84	4.83	5.18	4.27	4.66	4.12	4.86	4.90
WBC	12.6	13.0	14.4	13.1	7.9	8.5	12.4	6.7	11.3	14.5	14.4	11.2
SED RATE	0	1	3	1	0	0	6	1	1	1	0	-
DIFFERENTIAL Blasts												
Myelocytes												
Bands												
Lymphocytes												
Monocytes												
Eosinophiles												
Basophiles												
Sodium	145	150	149	147	148	145	153	146	148	146	145	150
Potassium	4.0	4.7	5.3	5.0	4.7	4.5	4.5	4.3	5.1	5.4	4.7	2.5
Calcium	5.4	5.9	6.1	5.7	5.5	6.1	6.3	5.5	5.9	6.2	6.1	6.6
Total Protein	8.0	7.4	8.5	8.2	7.8	7.8	8.7	7.4	7.6	7.4	7.6	8.0
Albumin/Globulin	4.4/3.6	4.2/3.2	4.6/3.9	4.6/3.6	5.0/2.8	4.3/3.0	4.6/4.1	4.4/3.0	4.8/2.8	4.4/3.0	4.6/3.0	4.8/3.2
SGPT	58	270	66	340	50	104	138	50	50	104	50	42
SGOT	40	74	55	84	55	84	74	50	50	84	40	55
Alkaline Phosphatase	19.0	17.5	19.5	2.4	12.5	7.0	3.8	11.0	28.0	14.0	19.0	1.4
Total Phosphorus	5.4	5.6	6.8	5.0	5.8	5.4	4.1	4.6	6.1	5.8	5.6	2.2
LDH	1960	1760	1300	1800	1620	1460	1400	1330	1360	1060	1160	820

This technique is not a panacea; it will not tell us everything. No one approach, per se, will give us the complete answer to the toxicity of a compound. This is just one additional method to assess pharmacological or toxicological changes.

REFERENCES

1. Wolfle, Thomas L.; Psychopharmacological Evaluation of Primates Exposed to 5 PSIA 100% Oxygen Atmosphere; Proceedings of the 2nd Annual Conference on Atmospheric Contamination in Confined Spaces; AMRL-TR-66-120, pp 223-235, December 1966.

DR. GELLER (Southwest Foundation for Research and Education): All I got from your procedural methodology was your avoidance behavior. Could you tell me what your other procedure was? What other procedures were there, some sort of a positive reinforcement?

DR. BACK: These are all avoidance tests. It's very simple. Left and right lever continuous avoidance and two discrete avoidance tasks - one auditory and one visual. In other words, a little white light or a buzzer comes on behind a button and he must push it in two seconds or he's shocked. I didn't go through all this; it had been described.

DR. GELLER: I was just curious, because I think you might get greater sensitivity if you used discriminations that involved just positive reward situations. This is not the most sensitive technique to use.

DR. BACK: We certainly recognize this. This may get a little philosophical here, but what I was going to say is the reason we use negative reinforcement is that we didn't want to introduce appetite as a problem. We already have animals that won't eat under these circumstances and we wanted to make the worst possible situation so that if we did get decrement it would really show up, because if you press an animal, if you make him work, he'll work until he just can't work anymore. In other words, we're loading the dice against us in this instance and, again, it's another way of getting a quick screen. We wanted to know if these animals could indeed work in 275 mg per cubic meter ethylene glycol. Indeed, they can work, if they're prodded. Some of these animals went off their feed immediately when we put in ethylene glycol, and I am sure that you don't need a positive reward situation to show decrement, because I'm sure they wouldn't work for food when they weren't hungry. They work when you push them a little bit.

METHODS FOR ASSESSING EFFECTS OF DRUGS ON BEHAVIOR

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The methods employed by behavioral pharmacology have been derived primarily from B. F. Skinner (1938) and his followers. These researchers make use of operant conditioning methods which have certain advantages over gross observational techniques (Scheckel, 1966).

1. The use of automated programming and recording equipment precludes the possibility of observer bias and permits analysis of behavior in an objective fashion.

2. With these techniques, one may condition behavior in laboratory animals which remains stable over long periods of time. The effects of experimental manipulation of an animal's environment on a stable behavior pattern may be studied in a purely objective manner.

3. Different behavior may be conditioned in the same animal to allow for the possibility that a drug may selectively effect one behavior and not the other.

4. Since data for each animal is obtainable before and after drug administration, an animal can serve as his own control and large samples of animals are, therefore, not required.

A description of the following two experimental models which have been used for the purpose of chemically manipulating behavior in laboratory animals will illustrate their potential applicability for assessing the behavioral effects of a space environment. A method first reported in 1960 (Geller and Seifter, 1960) has proved to be useful in a number of laboratories for the preclinical evaluation of "minor tranquilizers". The behavior which has been referred to as "conflict" is known to be sensitive to many behavioral variables as well as to pharmacologic manipulations. Briefly it is as follows: hungry rats are given lever-pressing training in small sound-resistant chambers, containing a lever, an automatic feeding device for the delivery of a liquid food reward, a speaker for the delivery of

auditory stimuli, and a grid floor for the delivery of electric shocks. The rats first learn to press the lever for a liquid food reward, which is obtainable after every lever press (crf). They are then switched to a two-minute variable interval (VI) schedule where rewards are obtainable on the average of once every two minutes (Ferster and Skinner, 1957). When lever-pressing rates become relatively stable, clearly audible but nonaversive 1850 cy/sec tones of three minutes duration are introduced at 15-minute intervals as a signal that every lever response will be reinforced with food (crf). The tone thus signals a change over from a relatively undesirable schedule (two-minute VI) to one with a higher payoff (crf). After these contingencies are well established, conflict is induced by punishing with 60-cycle AC grid shock any lever response made during a tone presentation. The hungry animals are simultaneously rewarded with food and punished with shock for any response made during the tone periods. Since the animals must balance the positive features of a high reward "payoff" against the negative aspects of accepting aversive electric shocks, the procedure is regarded as conflict producing. Low response rates during the tone periods may be obtained by appropriate manipulation of the shock intensity. Baseline data obtained under control conditions are shown in the top cumulative record of figure 1. This record represents the responses of a rat as they cumulate throughout an experimental session. Each time the pen reaches the top of the record, 550 responses have cumulated and the pen automatically resets. The conflict trials, which are indicated to the rat by the tones, are shown on the record by the offsets of the pen. The numbers, as well as the upward pips of the pen during the tone periods, indicate responses that are simultaneously rewarded with food and punished with shock. The variable-interval response rates between the tone periods are utilized as controls for nonspecific or undesirable effects of the drugs. Changes in intertrial VI rate might reveal motor side effects produced by overstimulation, incoordination, or ataxia. In this way, one is able to evaluate the "primary" effects of a drug relative to its side effects.

During the past nine years, we have used the "conflict" technique for evaluating those compounds which have been classified as "minor tranquilizers". The compounds have been shown to have a specific effect on punished behavior as in the "conflict" test. In the lower cumulative record of figure 1 are data which illustrate the effects of a 135 mg/kg IP dose of meprobamate on "conflict" behavior in the rat.

During the tone periods the hungry rat was willing to accept aversive electric shocks in order to obtain food rewards. During the predrug control session (upper record) the rat accepted only one shock during three trial periods.

Similar data for a 5 mg/kg dose of pentobarbital are shown in figure 2. Extensive attenuation of conflict was obtained only during the first two trials. The pentobarbital effect was of much shorter duration than was the meprobamate effect.

The effects of several doses of chlordiazepoxide (CDAP) on "conflict" behavior in the rat are shown in figure 3. Attenuation of conflict was obtained with the 7.5 and 15 mg/kg doses of chlordiazepoxide. Under the drug, the hungry rat was willing to accept shocks during the tone periods in order to obtain food. At 30 mg/kg of chlordiazepoxide, lever-pressing behavior was reduced to almost zero. Direct observation of the rat revealed an ataxic animal that had difficulty in standing on the grid floor of the experimental chamber.

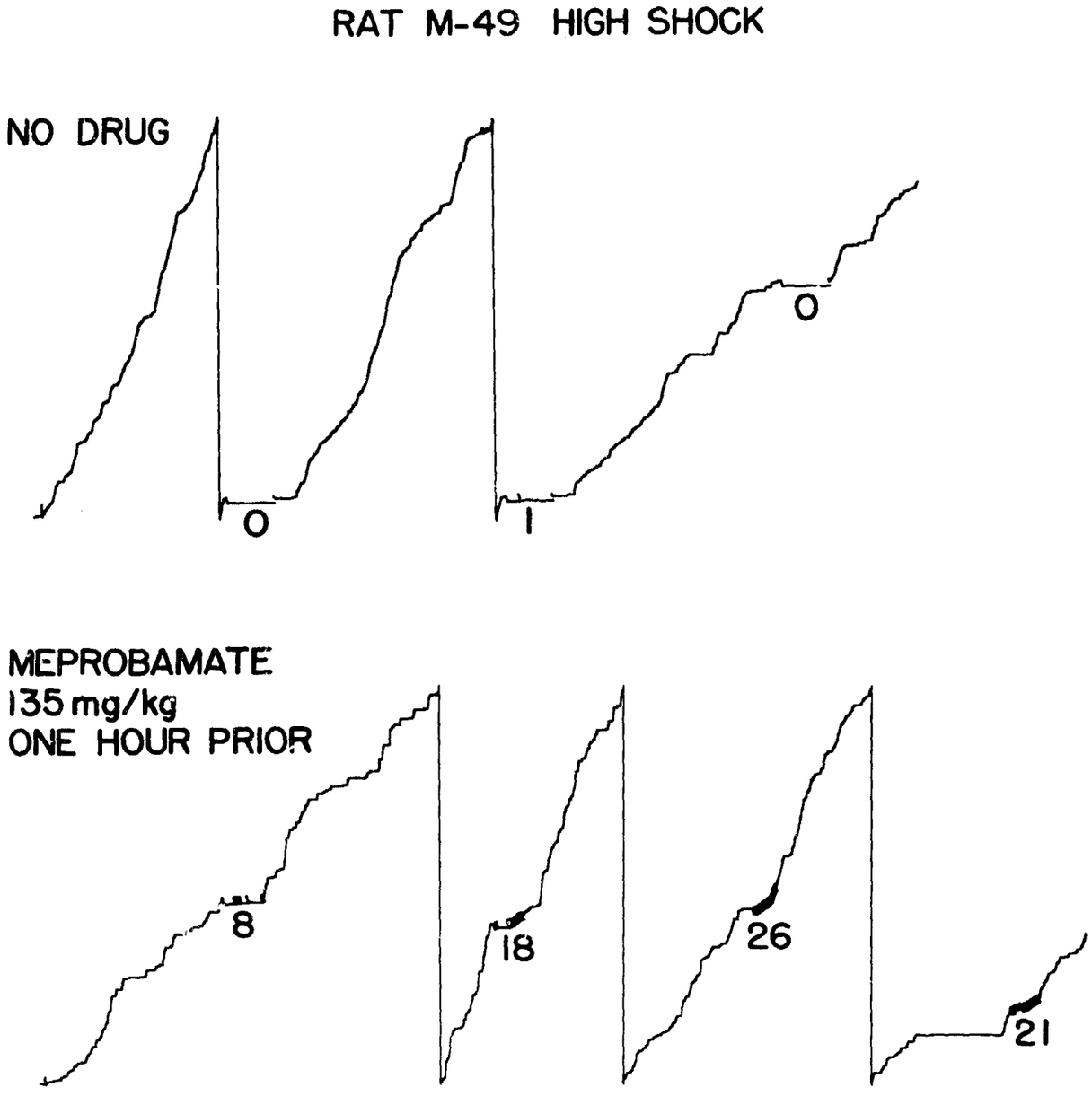

Figure 1. THE EFFECT OF 135 mg/kg MEPROBAMATE ON EXPERIMENTALLY INDUCED CONFLICT IN THE RAT. Pen offsets indicate conflict trials; numbers and upward pips of the pen represent responses that were simultaneously rewarded with food and punished with shock.

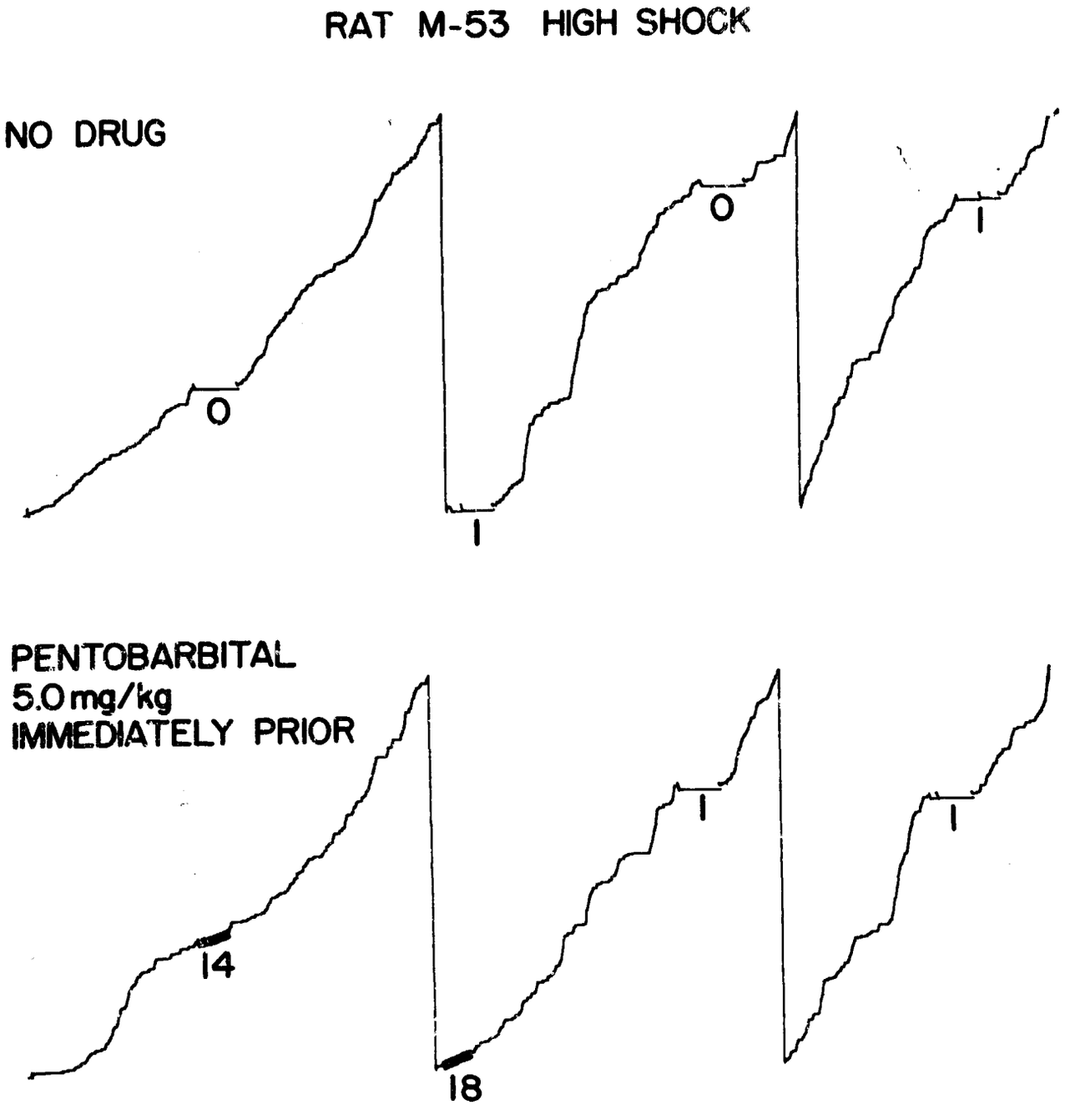

Figure 2. THE EFFECT OF 3 mg/kg PENTOBARBITAL ON EXPERIMENTALLY INDUCED CONFLICT IN THE RAT.

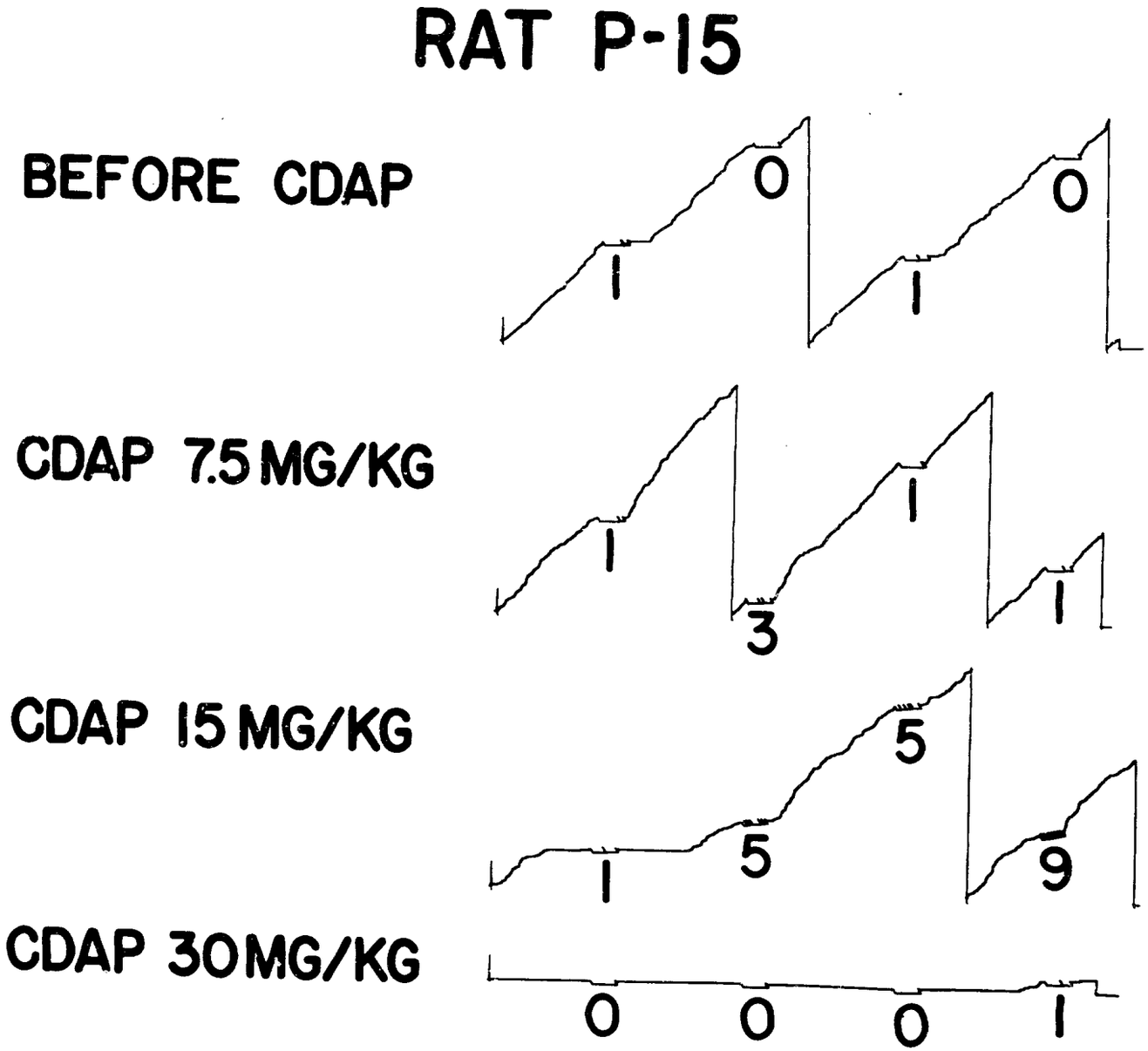

Figure 3. THE EFFECT OF CHLORDIAZEPOXIDE (CDAP) ON EXPERIMENTALLY INDUCED CONFLICT IN THE RAT.

Reproducibility of conflict behavior, as well as chlordiazepoxide effects in another species, are illustrated in the cumulative records of figure 4. Each record represents approximately half of a six-hour experimental session. The experimental animal, a cynomolgous monkey, was seated in a restraining-chair device and pressed a lever to obtain solid food pellets. The effects produced by a 30 mg/kg dose (route of injection I. M. via gastrocnemius muscle) were qualitatively similar to effects produced in the rat. The monkey, when under the influence of the drug, pressed the lever in the presence of the tone and accepted shocks in order to obtain the food pellets. As may be seen in the records, the drug effects persisted as long as seven days postinjection whereas in our rat studies the behavioral effects were dissipated 24 to 48 hours postinjection. These findings correlated well with the reported metabolic fate of CDAP (Koechlin and Schwartz, 1961). These authors found a rapid excretion of CDAP by the rat, but a much longer retention of the drug by primates.

In table I are listed the drugs which will attenuate to some degree "conflict" behavior of laboratory animals. The compounds, with the exception of reserpine, have anticonvulsant potential. Acute administration of all these compounds, with the exception of reserpine, will produce attenuation of "conflict" behavior.

TABLE I

DRUGS THAT INCREASE SHOCKS TAKEN IN CONFLICT

1. meprobamate	6. emylcamate
2. isopropyl meprobamate	7. chlordiazepoxide
3. sodium phenobarbital	8. diazepam
4. sodium pentobarbital	9. oxazepam
5. hedonal	10. trimethadione

11. reserpine

A second procedure which has been used for investigating drug behavior interactions is called DRL or differential reinforcement of low-rate responding. The technique involves a precise timing discrimination in laboratory animals. We have used DRL to study effects of acceleration forces. The method used for subjecting animals to acceleration forces of two G has been described previously (DeMarco and Geller, 1964). The behavioral test chamber was the same as described above.

Three male albino rats of Sprague-Dawley strain, approximately four months old at the start of the experiment, served as subjects. They were gradually reduced to 80% of their original body weights and were maintained at the reduced weights by limited feedings after each experimental session. The rats were given daily two-hour training sessions in the behavioral test chambers. They were first trained to space their lever responses at least 20 seconds but no more than 40 seconds apart in order to obtain a drop of sweetened, condensed milk. The

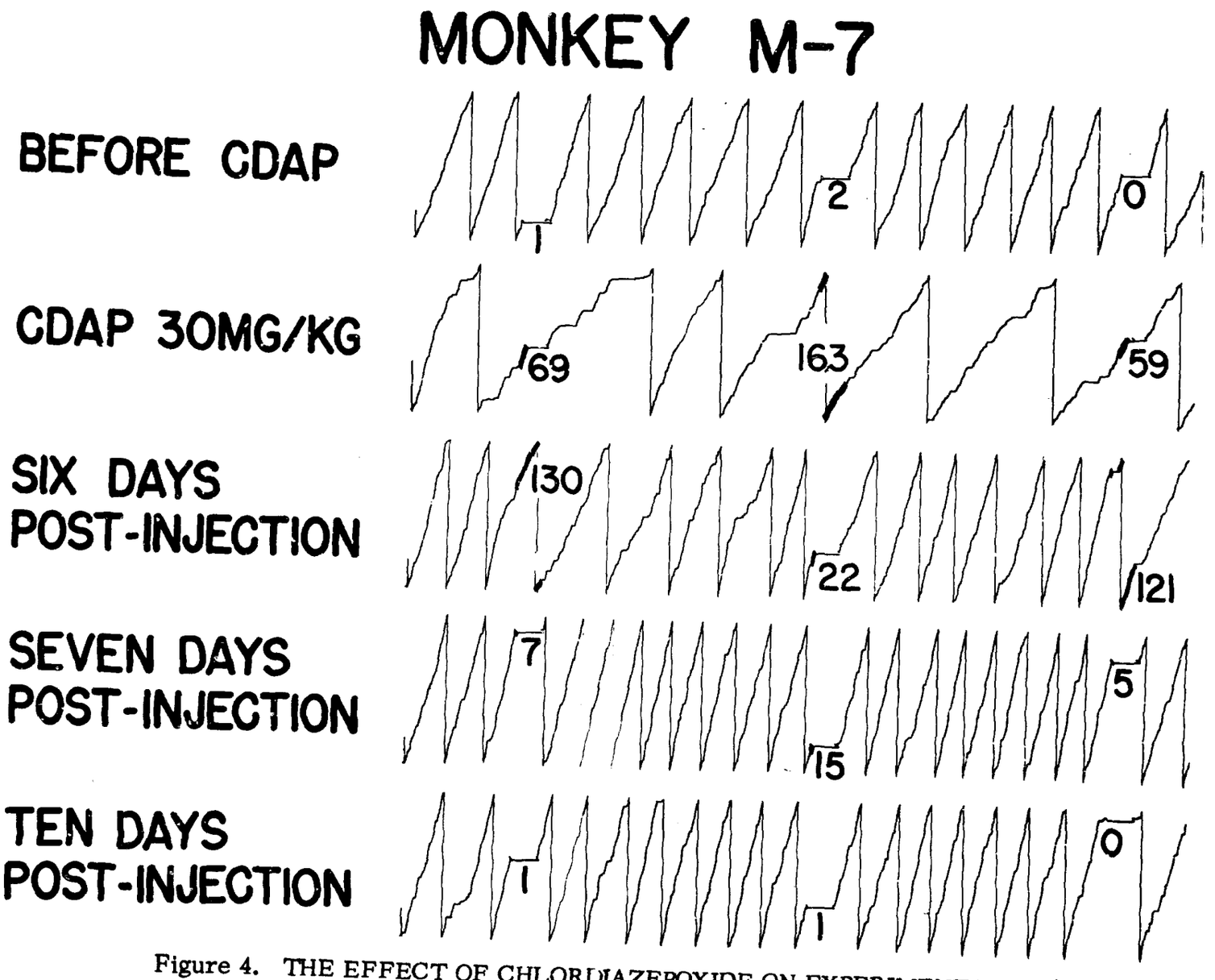

Figure 4. THE EFFECT OF CHLORDIAZEPOXIDE ON EXPERIMENTALLY INDUCED CONFLICT IN THE MONKEY.

20-second period of eligibility (20-second limited hold) after the 20-second timing period had elapsed, was progressively reduced over many weeks of training until a four-second limited hold was in force.

The final experimental conditions for assessing the effects of G forces on timing behavior required that responses be spaced at intervals of 20-24 seconds in order to produce reinforcements. Responses which occurred before or after the four-second "payoff" period were not reinforced and merely initiated a new timing cycle. The timing behavior which results under these conditions is conventionally described by a distribution that gives the relative frequency of the time between successive responses (interresponse times or IRT's). For this study, the IRT's were grouped into two-second categories and cumulated over the two-hour experimental sessions. Records were also kept of the average daily response rates and the number of reinforcements obtained. After sufficient training, the timing behavior of the rats sharpened so that a peak in the IRT distribution occurred at the reinforced intervals (20 to 22 and 22 to 24 seconds), or in some instances at the interval just preceding the reinforced ones.

Centrifugation was initiated for the desired time duration when the IRT's for the rats had stabilized and the total number of reinforcements obtained in each session was roughly constant. Two of the rats were spun in a centrifuge once every two weeks just prior to a session in the behavioral test chamber. The time durations of the spinnings for these rats were 4, 2, 1, 3, 5 and 2, 4, 3, 1, 5 minutes respectively. Following each centrifugation, a rat was immediately placed into the behavioral test chamber for a two-hour experimental session. The timing behavior after centrifugation was compared with the timing behavior of the rat for the pre and postcentrifugation days. Thus, each animal served as its own control. After the first series of acceleration exposures, a second series was initiated at weekly intervals. A third rat, AK-2, was centrifuged at weekly intervals at time durations 1, 2, 3, 4, 5, 3 and 2 minutes respectively. In figure 5 are shown IRT distributions for Rat AL-65 after centrifugation at 100 rpm for three and four minutes. The top six figures show data obtained during the first centrifugation series. Examples of the baseline performance may be seen in the precentrifugation and postcentrifugation histograms. The large proportion of IRT's in the 0 to 2 second category represents, for the most part, bursts of responses, but the remainder of the distribution gives a good picture of the timing behavior. The distributions may be seen to peak at or just prior to the rewarded interval (white columns) and to decline sharply at either side.

The crosshatched distributions were obtained on the days the animals were centrifuged. During the first series of spinnings, a very pronounced disruption of the timing behavior occurred. When centrifuged at 100 rpm for four minutes, the rat was unable to perform satisfactorily in the behavioral test chamber. The IRT distribution was disrupted, the response rate fell from 774 on control to 94, and reinforcements were reduced from 57 on control to 0 after centrifugation. The three-minute centrifugation also disrupted the timing behavior. Reinforcements were reduced from 96 on control to 50 after centrifugation. It should be noted that for the four-minute control data, the animal was not functioning as well as during the three-minute spinning control periods. The four-minute data was obtained at an earlier stage of the experiment, approximately two and one-half months prior to the three-minute data. The performance of these animals tends to become better with continued training, so that the rats earn more reinforcements during

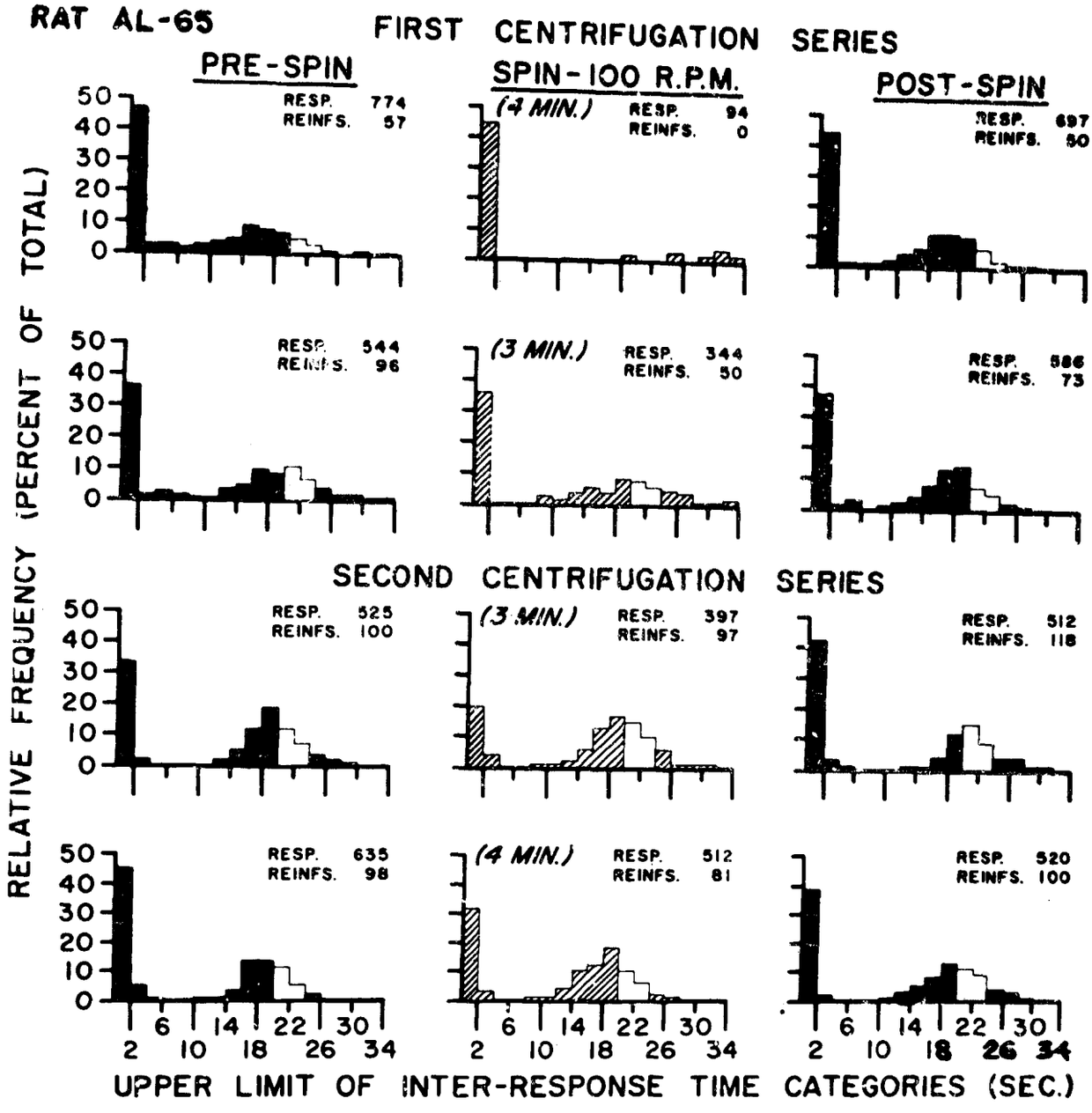

Figure 5. EFFECTS OF CENTRIFUGATION ON TIMING BEHAVIOR. Frequency distributions of interresponse times for a typical rat. The timing behavior is represented by solid histograms for control days and crosshatched histograms on centrifugation days. The white columns indicate the reinforcement intervals.

the later stages of the experiment. The data obtained during the second series of centrifugations show that the spinnings were virtually ineffective in disrupting the timing behavior. Following centrifugation at 100 rpm for three minutes, Rat AL-65 earned 97 reinforcements as compared with 100 and 118 obtained in the precentrifugation and postcentrifugation sessions. The four-minute centrifugation data is quite similar. Reinforcements were reduced slightly, from 98 on control to 81 after centrifugation, while the IRT distribution remained fairly intact.

In figure 6 are plotted the average number of reinforcements obtained by each rat during the first and second series of centrifugations. The data obtained for AL-65 and AL-66 during the second series represent the last four spinnings of a series of eight. The dotted lines across the graphs represent mean number of reinforcements obtained during all the precentrifugation and postcentrifugation control sessions. The data for AL-66 shows that the amount of disruption in timing behavior as reflected in number of reinforcements obtained is directly related to the duration of spinning. However, for the second series of spinnings, very little, if any, disruption of the timing behavior occurred. Reinforcements obtained were only slightly below control values. Apparently the rat had developed a tolerance to the effects of the centrifugation.

The same is true for Rat AL-65. With the exception of the five-minute spinning, the disruption of timing behavior appears to be directly related to the duration of centrifugation. The five-minute spinning occurred last in this series, and the rat adjusted to the effects of the acceleration forces at this point. These adaptation effects are evident during the second centrifugation series, as reflected in the number of reinforcements obtained under each condition. Reinforcements obtained under each spinning condition closely approximate the mean control value.

The bottom record shows, in essence, the same effects for AK-2, who was initially spun at weekly intervals. For this rat, adaptation effects occurred after the third centrifugation. After being centrifuged for four and five minutes, this rat obtained more reinforcements than after spinnings of two or three minutes. Even though the disrupting effects of the centrifugation are less after spinnings of four and five minutes, the number of reinforcements obtained was still considerably below control values.

In summary, we have described two methods which have had wide applications for the study of drug effects upon behavior. We have shown the disruption of a temporal discrimination in rats subjected to the stress of acceleration forces. Repeated exposures to the accelerations resulted in the development of tolerance to the G forces and a concomitant lessening of the disruption of the temporal discrimination. We suggest the possible use of techniques such as these for assessing the behavioral effects of a space environment.

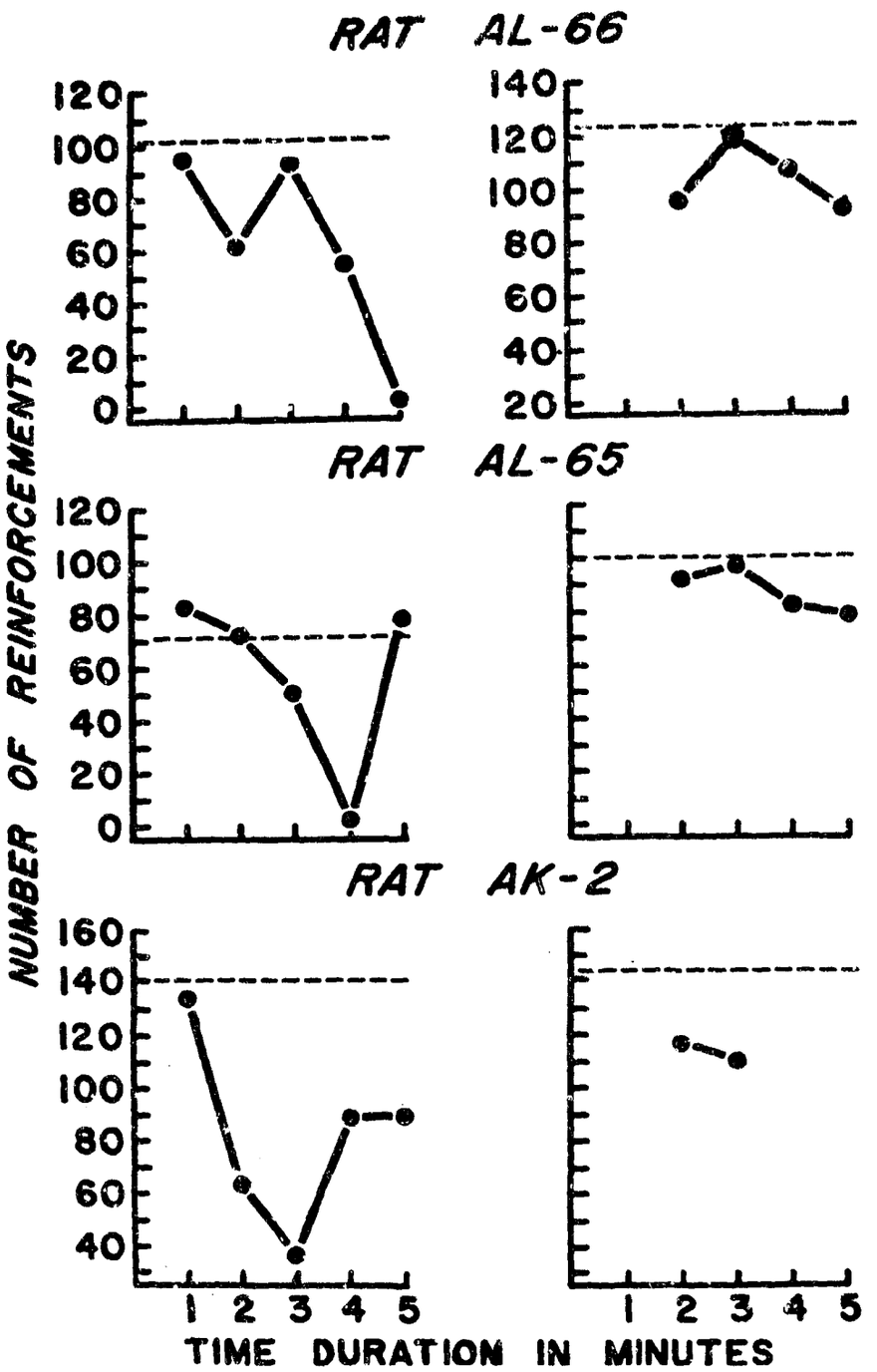

Figure 6. AVERAGE NUMBER OF REINFORCEMENTS OBTAINED BY INDIVIDUAL RATS UNDER EACH CENTRIFUGATION CONDITION. The dotted lines represent mean control values of reinforcements earned during precentrifugation and postcentrifugation sessions.

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DISCUSSION

DR. KAPLAN (Aerospace Medical Research Laboratories): I noticed on your slide that morphine was listed as one of the drugs that gave a negative response, both antianxiety effects and also reduction in pain perception. How do you account for that?

DR. GELLER: I didn't want to get involved in this discussion. All the drugs that I have tested over a ten-year period now intensify this conflict in the animals on the low shock baseline, are drugs that have convulsant potential, or are, in fact, convulsants. I think there are some other activities here going on, whether it's a change in synaptic transmission - what it is I don't know, but phenothiazines do this, morphine does this, and amphetamine does this. Of the drugs which are known to produce convulsions or potentiate the action of other drugs in producing convulsions, all intensify conflict. They may have antianxiety reactions, as morphine supposedly has, but I think the critical dichotomy here is convulsant versus anticonvulsant. The only one that doesn't seem to fit the ill is reserpine. Reserpine is a good antianxiety agent but isn't used as such because of other undesirable effects. Reserpine causes convulsions and does fall into this only if you have administered this over a long period of time.

DR. FAIRCHILD (University of California): Is there evidence of a general nature that you can say, say in a group of tranquilizers - librium, equanil, and some of these that you have listed - that the monkey is always closer to man in the responses. I know this is a function of metabolism, but I mean on your responses that you get here?

DR. GELLER: The work that I have done, behaviorally, on responses to the acute effects, to acute administration of the drug, I would think we could do the same thing. It does have predictive value. We are able to predict clinical dose relative to standard compounds, and we have been able to predict side effects with this technique. This is reproducible over species. I don't see where one has any advantage over the other, with regard to the behavior, with the exception of the fact that the length of time in the system may be different across different species.

SOME NEUROPHYSIOLOGICAL AND BEHAVIORAL EFFECTS OF UDMH IN THE CAT

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Dr. Fairchild presented data from two separate papers. The papers are printed here in their entirety. The first, titled "Subconvulsive Effects of 1, 1-Dimethylhydrazine on Locomotor Performance in the Cat: Relationship of Dose to Time of Onset", was authored by M. B. Sterman, Ph. D. and M. D. Fairchild, Ph. D., and published as AMRL-TR-67-66; the second, titled "Effects of 1, 1-Dimethylhydrazine (UDMH) on Evoked Cerebral Neuroelectric Responses", was authored by W. R. Goff, T. Allison, Y. Matsumiya, M. B. Sterman, and M. D. Fairchild, and published as AMRL-TR-67-67.

SUBCONVULSIVE EFFECTS OF 1, 1-DIMETHYLHYDRAZINE ON LOCOMOTOR PERFORMANCE IN THE CAT: RELATIONSHIP OF DOSE TO TIME OF ONSET

SECTION I

INTRODUCTION

In the cat, a characteristic sequence of behavioral and electrophysiological signs has been found to precede the onset of general seizures following the parenteral administration of 1, 1-dimethylhydrazine (UDMH) in a time course directly related to dose (Fairchild and Sterman, 1964). In other neurophysiological studies of this compound, detectable effects were consistently noted at doses well below these convulsive levels (Fairchild and Sterman, 1965). Low dose effects, however, were not accompanied by any overt symptoms of illness. The major difficulty encountered in assessing the behavioral consequences of these low doses was the tendency for performance to become more variable once UDMH was introduced into the situation. To quote a previous communication (Fairchild and Sterman, 1965), "as drug test sessions continued --- all aspects of performance tended to become more labile (although) reasonable periods of time were allowed between drug tests and there was no obvious

change in the general physiological condition, weight or feeding patterns of the cats" (page 29). Thus, it would appear that some subtle variable, inherent in the most judicious of behavioral studies on UDMH, has masked any clear determination of preconvulsive effects.

Recent neurophysiological investigations of sensory evoked potentials and monosynaptic reflex activity in response to UDMH exposure have indicated a graded increase in excitability leading eventually to convulsions, but interrupted periodically by episodes of recovery or suppression of activity (Goff et al, in press). It is possible that such an alternation of central nervous system excitability would confound any biological measurements obtained without prior knowledge of its characteristics. A determination of the subconvulsive effects of UDMH on the central nervous system has been one of the primary objectives of this laboratory, particularly as related to integrated behavior. We, therefore, examined the implications of cyclic alterations in brain excitability in response to UDMH within this context.

SECTION II

METHODS

Five adult cats were trained to stable performance in a runway apparatus designed to detect subtle changes in central nervous system functions. Integrated locomotor behavior may be easily quantified in this runway by reference to the time required to run, alternately, between two enclosed chambers (figure 1). The apparatus and training procedures employed have been described in detail elsewhere (Fairchild and Sterman, 1965; Sterman and Fairchild, 1966).

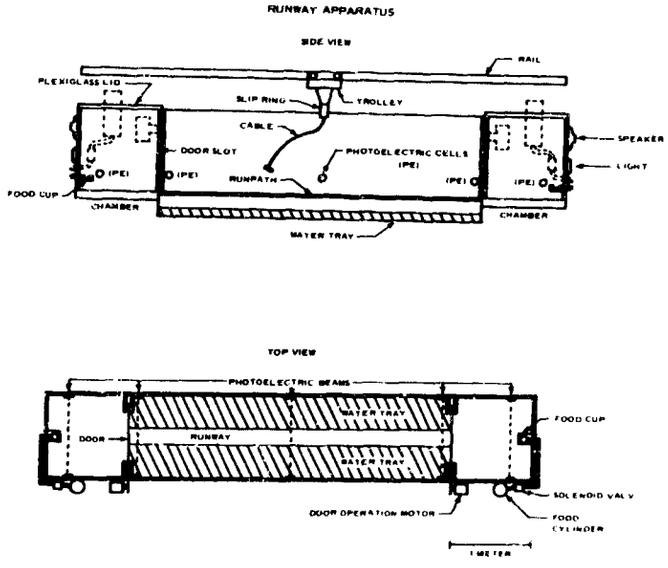

Figure 1. DIAGRAM OF SIDE AND TOP VIEW OF RUNWAY APPARATUS. The two chambers are identical and serve alternately as start and goal boxes as the animal runs between them. Note photoelectric cells along run-path which were used to time performance. Mirrors suspended overhead were used to observe behavior during the experiments.

To achieve the objectives of the present experiment, the five animals were run in a predetermined sequence which spanned a period of approximately six hours following the injection of either one cc of normal saline, or various doses of UDMH. Animals were started at periods of 30, 90, 150, 210, and 270 minutes following injection. Each of the five cats was run 60 trials and their starting time sequence was rotated in a counter-balanced design so that each animal appeared in three of the five time blocks near the beginning, middle, and end of the postinjection period.

Test sessions were initially conducted each 24 hours following saline injections until five days of control performance data were collected. Following the predrug control phase of the experiment, UDMH, diluted 10 to one with distilled water, was administered intraperitoneally to each animal in doses of four, eight, and 16 mg/kg body weight. Drug tests were conducted at 48-hour intervals with saline controls on alternate days.

SECTION III

RESULTS

Under normal conditions an animal negotiates the runway at stable velocities, demonstrating only a gradual decrease in speed as he becomes more satiated. Behavior does not change in relation to time of day. Previous studies have established the fact of long-term performance stability under normal conditions (Sterman and Fairchild, 1966). The mere introduction of UDMH into this test situation caused a general shift in performance during subsequent saline control measurements (figure 2). This residual effect of UDMH on control performance was not related to dose and the stability of controls during the course of the experiments indicated that UDMH had no cumulative effects. However, this alteration in control performance was a very real phenomenon since in four of the five animals there was a significant difference in predrug and postdrug control run times. These results are summarized in table I.

A possible explanation for this difference is apparent from a close inspection of figure 2. Each curve in this figure is generated by the mean deviation from predrug saline control data of each of six sequential 10-trial blocks obtained from the three animals tested in a given time period. It can be seen that in every time period the deviation is progressively greater for the last three blocks of trials. This trend reflected an interaction between the normal reduction in velocity associated with increasing satiety and some residual influence of UDMH. Thus, in control measurements obtained 24 hours after the administration of low doses of this compound, the slope of the normal satiety function is increased.

This effect is even more evident immediately after the administration of UDMH (figure 3). However, it is greatest in the first two time segments of measurement which cover a period up to 150 minutes postinjection. After 150 minutes, for doses of four and eight mg/kg UDMH, there is a progressive reversal of effects, such that by 270 minutes animals receiving the two lowest doses often demonstrated maximum velocities during the last 30 trials of the test measurement. This change accompanied a more general shift in performance velocity associated with those doses at that time. Since our primary concern was with these overall dose-time changes in velocity, the drug effects were subsequently expressed as deviations from the postdrug saline control values. The confounding caused by this interaction with satiety

TABLE I

ANALYSIS OF VARIANCE OF CONTROL RUNWAY VELOCITY DATA OBTAINED BEFORE AND AFTER THE INITIATION OF UDMH TESTING

Source	d. f.	Sum of Squares	Mean Square	F ratio
1) Cats	3	12.75	4.25	
2) Drug: Pre- vs. Post-	1	1.41	1.41	470.0**
3) Trial Blocks	5	9.24	1.85	
4) 1 x 2	3	0.01	0.003	
5) 1 x 3	15	0.62	0.04	
6) 2 x 3	5	0.35	0.07	
7) Residual	15	0.13	0.01	
Total	47	24.51		

**P = < 0.01

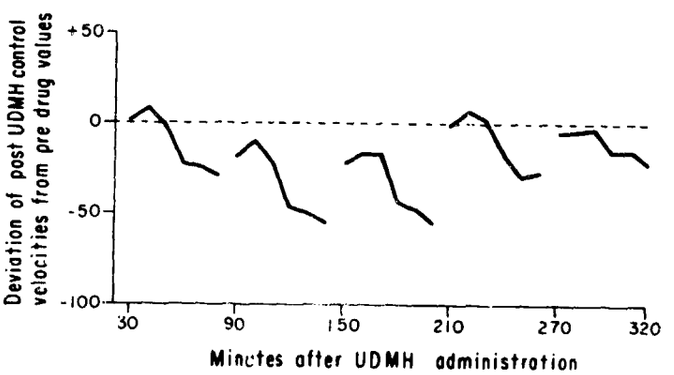

Figure 2. COMPARISON OF SALINE CONTROL VELOCITIES OBTAINED BEFORE AND AFTER THE INITIATION OF UDMH TESTING. The postdrug values are plotted here as deviations from their corresponding predrug levels. The data points represent the six trial block means of three different animals run in each of the five time periods after drug administration.

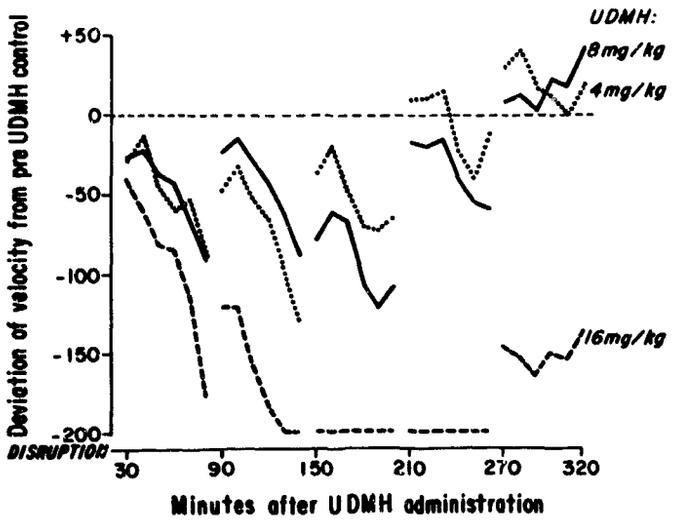

Figure 3. COMPARISON OF PERFORMANCE AFTER 4, 8, AND 16 mg/kg UDMH WITH PRETESTING SALINE CONTROL DATA

is thereby reduced because of a similar trend in the postdrug control data (figure 4). The interaction between satiety and UDMH was greatest during the earlier portion of the test period and, thus, in spite of this transformation of the data, it is still apparent in the 30- and 90-minute time blocks. Thereafter, the curves are flattened and indicate differential shifts in velocity in relation to the dose of UDMH administered.

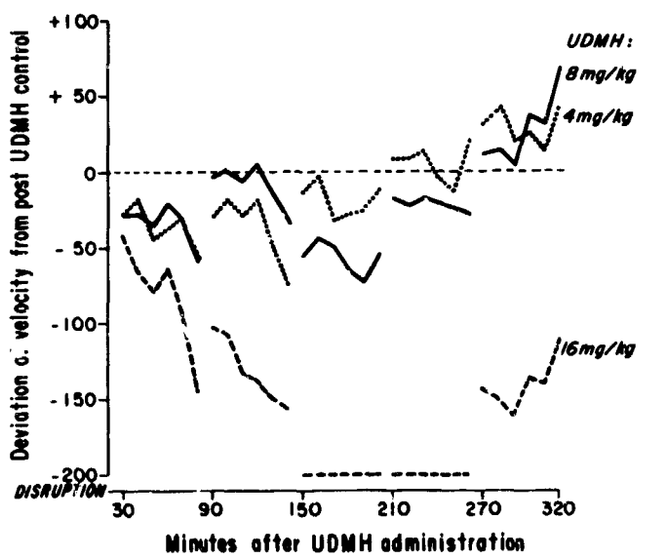

Figure 4. COMPARISON OF PERFORMANCE AFTER 4, 8, AND 16 mg/kg UDMH WITH POSTDRUG CONTROL DATA.

A statistically significa it reduction in performance velocity was noted 30 minutes after the injection of 16 mg/kg UDMH, and became progressively increased until, by 150 minutes, behavior was greatly depressed or totally disrupted. This effect persisted for the next two hours. Performance was either relatively normal but extremely slow, or it was terminated by a consistent pattern of behavior. The cat stopped on the runway short of the goal box and refused to enter. At this point he would either sit and vocalize or groom, or he would turn around and approach the opposite chamber, only to stop again short of its entrance. The animal appeared apprehensive, was often disinterested in food, and showed a slight visual-motor incoordination. When removed from the apparatus he would consume food eagerly and showed normal exploratory behavior. Visual motor coordination was still somewhat disrupted. This effect persisted for the next two hours. Variable results were obtained again in the last hour of the sequence, in that one animal remained disrupted, one animal was partially recovered, and the third was totally recovered. With the exception of an apparent disinterest in food and a slight visual-motor incoordination on the part of some of the animals, no emesis or other signs of distress were observed during the measurement period. Behaviorally the animals were occasionally irritable and somewhat hyperactive. In several instances a diarrhetic stool and intestinal residue were found in the home cage on the day following exposure at this dose level.

The administration of 8 mg/kg UDMH produced a complex effect upon runway performance. Initially, there was a moderate but significant reduction in velocity, followed by a recovery within the second hour. Performance was again depressed significantly during the third hour of measurement, but recovered once more in the succeeding hour. During the last test period, starting 270 minutes after injection, a reversal of effects was noted and indicated a reliable facilitation of performance. In two of the animals run during the third and fourth time periods after administration of 8 mg/kg, a disruption of performance identical to that described above for 16 mg/kg was noted during the last 10 or 20 runs of the session. These blocks were eliminated from the statistical treatment of data at this dose level.

The administration of 4 mg/kg UDMH exerted yet another characteristic effect upon subsequent runway performance. In this instance, velocity was mildly depressed 30 minutes after drug injection, but recovered gradually over the following two measurement periods. By 210 minutes postinjection, the animals were again running at normal velocities. However, in the last hour of measurement, a significant increase in velocity was registered.

In order to more clearly visualize the various dose-time relationships reported above, all data from the three animals run in each postinjection time period were combined and plotted together with the appropriate standard deviations in figure 5. It can quickly be seen from this figure that the doses of 4, 8, and 16 mg/kg UDMH each had distinctive effects upon performance during the time span of experimental measurement. A dose of 16 mg/kg had a monophasic effect, with a gradual depression of velocity eventually reaching disruptive levels three to four hours after administration and diminishing in the last hour of measurement. The 8 mg/kg dose had a polyphasic influence on subsequent performance with a two-hour cycle of depression and recovery followed by an enhancement of performance in the fifth hour after administration. Finally, 4 mg/kg caused a mild but protracted reduction in velocity which diminished gradually and gave way to an enhanced

performance at the end of the measurement period. In general, variability was greatest where the curves for the various doses were divergent and least where they converged. The statistical analysis of these data is presented in table II.

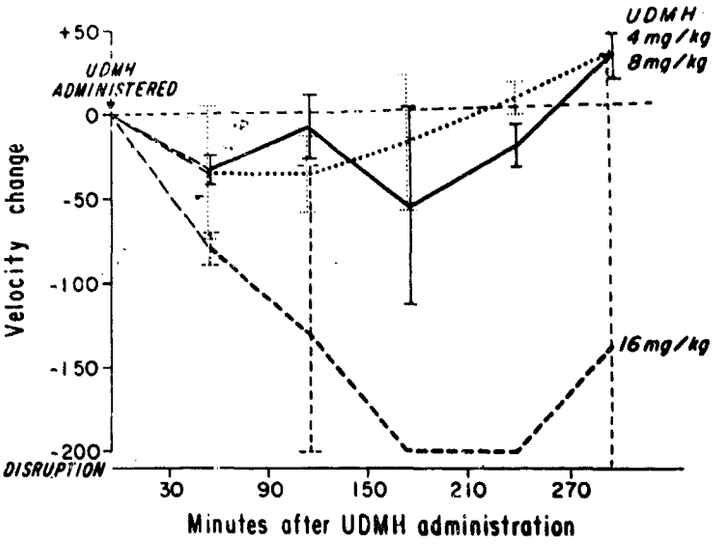

Figure 5. SUMMARY OF DOSE-TIME VELOCITY ALTERATIONS IN RESPONSE TO THREE SUBCONVULSIVE DOSES OF UDMH. Each point indicates the mean of six blocks of ten trials each combined for the three animals run in a given time period after UDMH injection.

TABLE II

STATISTICAL COMPARISON OF VELOCITY DATA OBTAINED AT THE FIVE TIME PERIODS AFTER SALINE OR UDMH INJECTION (N = 18)

Dose	Time Period (min.)	Mean	σ	t	Probability
4 mg/kg	30	-. 35	. 37	4. 07	< . 01
	90	-. 37	. 35	4. 44	< . 01
	150	-. 19	. 38	2. 13	< . 05
	210	+. 05	. 27	0. 85	< . 41
	270	+. 30	. 26	4. 99	< . 01
8 mg/kg	30	-. 34	. 24	6. 01	< . 01
	90	-. 09	. 27	1. 41	< . 18
	150	-. 57	. 53	4. 51	< . 01
	210	-. 22	. 21	4. 52	< . 01
	270	+. 28	. 30	3. 99	< . 01
16 mg/kg	30	-. 81	. 53	6. 51	< . 01
	90	-1. 31	. 92	6. 06	< . 01
	150	-2. 12	1. 14	7. 92	< . 01
	210	-2. 48	. 86	12. 19	< . 01
	270	-1. 40	1. 46	4. 08	< . 01

SECTION IV

DISCUSSION

Our previous experience with UDMH in the cat indicated that convulsions could occasionally be observed after a very long delay with doses as low as 20 mg/kg, but not with doses below that level (Fairchild and Sterman, 1965). For this reason, we chose in the present experiment to deal with subconvulsive doses of 4, 8, and 16 mg/kg. We can now state clearly that these low doses can significantly alter the locomotor performance of this animal in a predictable manner, and without being accompanied by any other signs of UDMH toxicity. The performance alterations noted were found to be consistent for a given dose and different for each of the three doses tested. Furthermore, these effects were registered as soon as 30 minutes after the injection of this compound.

Within the postinjection time period covered in this experiment (six hours), the dose of 16 mg/kg UDMH produced a gradual disruption of performance followed by a tendency toward recovery in the last hour of measurement. There is no way of knowing what subsequent changes in performance might have occurred, but based upon the characteristics of response at lower doses and with other measures of CNS function, one could predict a continuing oscillation of decrement and recovery with a possible increment in performance after a protracted period of time. Indeed, such was the case for 8 mg/kg within the scope of the present experiment. A similar, but less complex pattern, was registered in response to 4 mg/kg, which consisted of a single mild decrement in performance followed after some delay by an increment. Although the animals tended to show a consistent pattern of response following a given dose of UDMH, it is apparent, from an inspection of figure 5, that a large amount of individual variation exists. There is a tendency for variability to increase with increasing drug effect, and, conversely, to decrease as drug effects abate. Increased variability with pronounced drug activity is particularly evident at 16 mg/kg, while decreasing variability with time is evident for both the 4 and 8 mg/kg dose levels. The relatively small standard deviations encountered for the period of enhanced runway performance following the lower doses of UDMH is of interest. In dealing with the relatively small number of animals tested in each time block (N = 3) normally distributed data would not be expected and large variations at peak drug effects would express interaction of UDMH with a number of individual differences in both physiological and behavioral parameters.

Exposure to UDMH was found to exert a general influence upon the course of satiety in our experimental situation, without causing any specific anorexic effects. The slope of the satiety function was markedly increased after all doses during the first two postinjection hours. This effect was still apparent, to a lesser degree, 24 hours after these injections. Visceral or central disturbances associated with the metabolism of this compound may be responsible for this apparent interaction between UDMH toxicity and the mechanisms underlying the process of satiation to food.

These findings represent the first stable performance data ever collected with UDMH experimentation in this laboratory. As such, they support our original contention regarding the characteristic variability encountered in previous behavioral studies of this compound. A systematic alternation of central depression and

excitation, which these findings suggest is caused by UDMH, would confound any performance data obtained without a knowledge of its characteristics. We would propose that future attempts to evaluate the influence of UDMH on the performance of any specific task must consider this fact and incorporate its implications into the experimental design employed.

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EFFECTS OF 1, 1-DIMETHYLHYDRAZINE (UDMH) ON EVOKED CEREBRAL NEUROELECTRIC RESPONSES

INTRODUCTION

The purpose of these studies was to gain a clearer understanding of the neural mechanisms affected by UDMH intoxication. The experiments were carried out in two phases: in freely-moving cats, the behavioral and neurologic effects of 16 and 32 mg/kg UDMH i. p.; in paralyzed preparations, the neurologic effects of 64 mg/kg UDMH i. p. The latter experiments were indicated because the violent movements of nonparalyzed animals produce electrical artifacts which obscure evoked response records during seizure and, more important, these experiments provide data relevant to the sensorimotor feedback hypothesis of Fairchild and Sterman (1964).

PROCEDURE AND RESULTS

Twelve experiments were carried out on 10 animals prepared with chronic recording electrodes in various areas of the brain, and chronically implanted ulnar nerve stimulating electrodes. Our major findings from experiments in nonparalyzed animals are typified by figures 1a and 1b. Similar results have been obtained in a number of other experiments. This freely-moving, unanesthetized cat was given

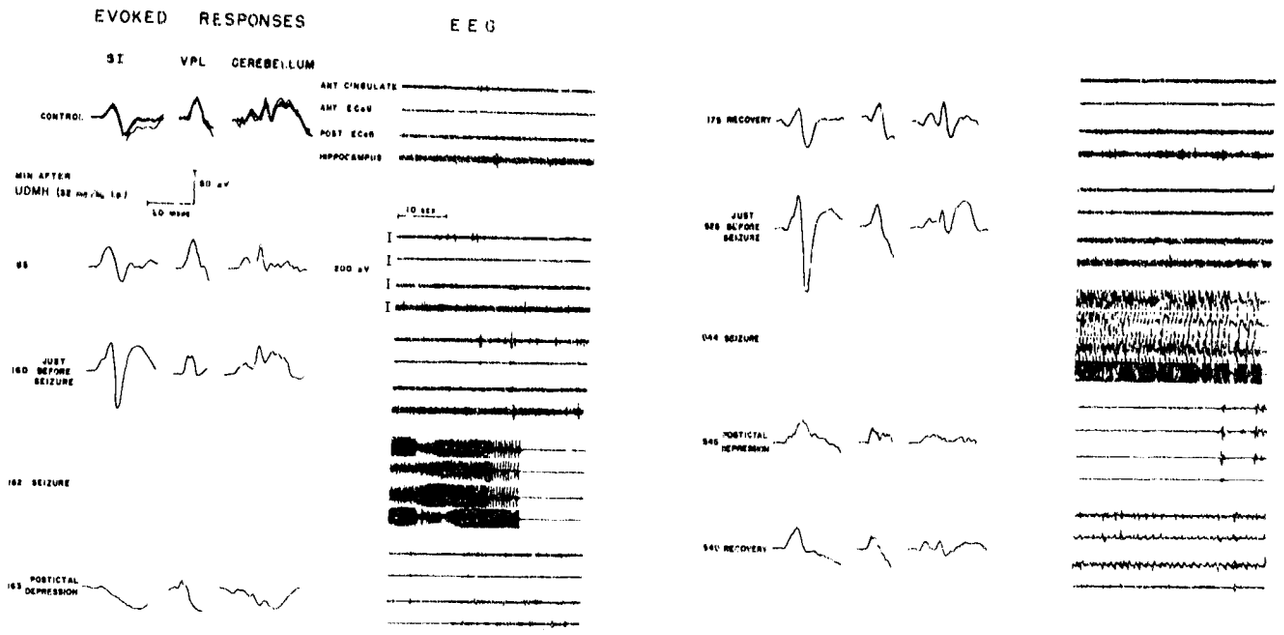

Figure 1. ELECTROENCEPHALOGRAPHIC (right column) AND AVERAGE EVOKED RESPONSE (left columns) CORRELATES OF UDMH IN AN AWAKE, FREELY-MOVING CAT WITH IMPLANTED ELECTRODES. Evoked responses are recorded from primary somatosensory cortex (SI), somatosensory thalamic relay nucleus (VPL) and forearm area of the anterior cerebellum. The responses were recorded with reference to a frontal sinus lead, and positive at the active electrode is recorded upward. Each average response consists of 15 individual responses. Blank spaces in evoked response columns indicate times when the response could not be accurately recorded due to paroxysmal EEG activity and/or movement artifact. EEG records during seizure and posticta phases are continuous.

32 mg/kg UDMH i. p. For a considerable period of time after UDMH administration, evoked potential amplitudes remained at control levels; the EEG was normal. By 160 minutes after UDMH administration, the negative phase of the primary response of somatosensory cortex (SI) had become extremely large; there is also some enhancement of the primary positive wave, but the thalamic relay nucleus response (VPL) and the response in the forearm representation in the anterior lobe of the cerebellum remained essentially at control levels. All EEG traces at this time were normal. Two minutes later (162 minutes) the cat went into a full-blown EEG and behavioral convulsion consisting of the usual tonic, clonic, and postictal depression phases. In this experiment it was impossible to record evoked responses during the seizure itself because of the extremely large paroxysmal EEG activity and movement artifacts.

With the exception of the primary positive SI response, the evoked potentials are virtually abolished during the postictal depression phase, but recover in a short time to control levels. With essentially the same sequence of evoked potential changes a second and third seizure ensued. Data for the second seizure are not presented, but as shown in figure 1b, the third seizure was presaged by a potentiated SI negativity at 525 minutes with convulsion occurring at 544 minutes. During the postictal depression all evoked activity is again severely depressed with the exception of the primary positive wave, which appears to be much more resistant to the paroxysmal neural events of the seizure and postictal phases. Immediately after the third convulsion the animal was sedated with Nembutal®.

Eight days later this animal was tracheotomized and venous cannulated under halothane, immobilized with Flaxedil®, and maintained on forced respiration. Wound margins were locally anesthetized with Xylocaine®, and the halothane was removed and its effects allowed to wear off. Evoked responses and EEG recordings were made as before. The animal was given 64 mg/kg UDMH i. p. The results are shown in figures 2a and b. Again, the only precursor of the first convulsion was an extreme augmentation of the SI primary negativity. The EEG at this time was not perceptibly abnormal. At the beginning of the tonic-clonic phase of the seizure the SI response remained large but was abolished during the second half of the seizure. Note that the VPL and cerebellar responses did not potentiate before the seizure. After the seizure the SI response quickly became abnormally large again, and the sequence of events before, during, and following the second seizure (figure 2b) was very much like that of the first seizure. Two additional seizures, not shown in figure 2, gave very similar results.

The limbic system in both man and animals is particularly prone to seizure activity. Fairchild and Sterman (1964) have previously suggested that UDMH-induced convulsions originate in the limbic system and then spread to the cerebral cortex. Two examples of such a progression, observed in the present investigation, are presented in figures 3a and b. These records are perhaps the clearest we have obtained, but similar results have been seen in several other animals. Seizure activity begins in the anterior cingulate gyrus and hippocampus, with minimal or no involvement of cerebral cortex until several seconds later. The examples shown here were "subseizures" with few or no behavioral concomitants. In both cases the EEG returned briefly to normal following this seizure activity, but within two minutes a full EEG and behavioral convulsion ensued.

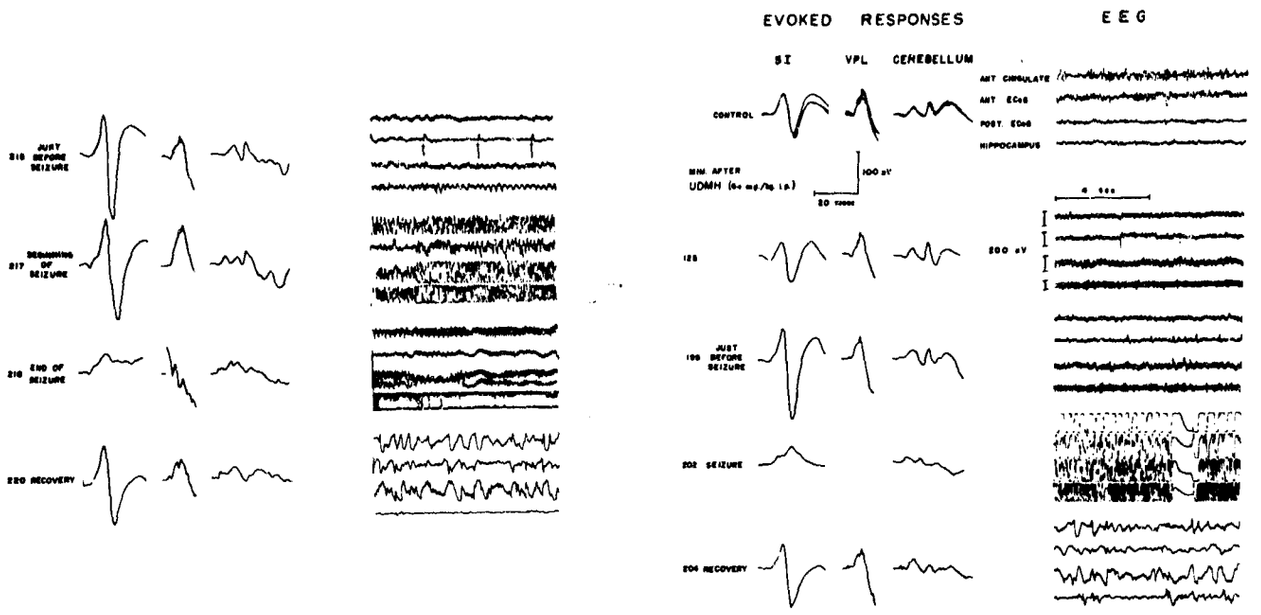

Figure 2. SAME ANIMAL AS IN FIGURE 1, BUT EIGHT DAYS LATER. Cat is immobilized with Flaxedil® and artificially respirated. EEG and evoked response recordings as in figure 1.

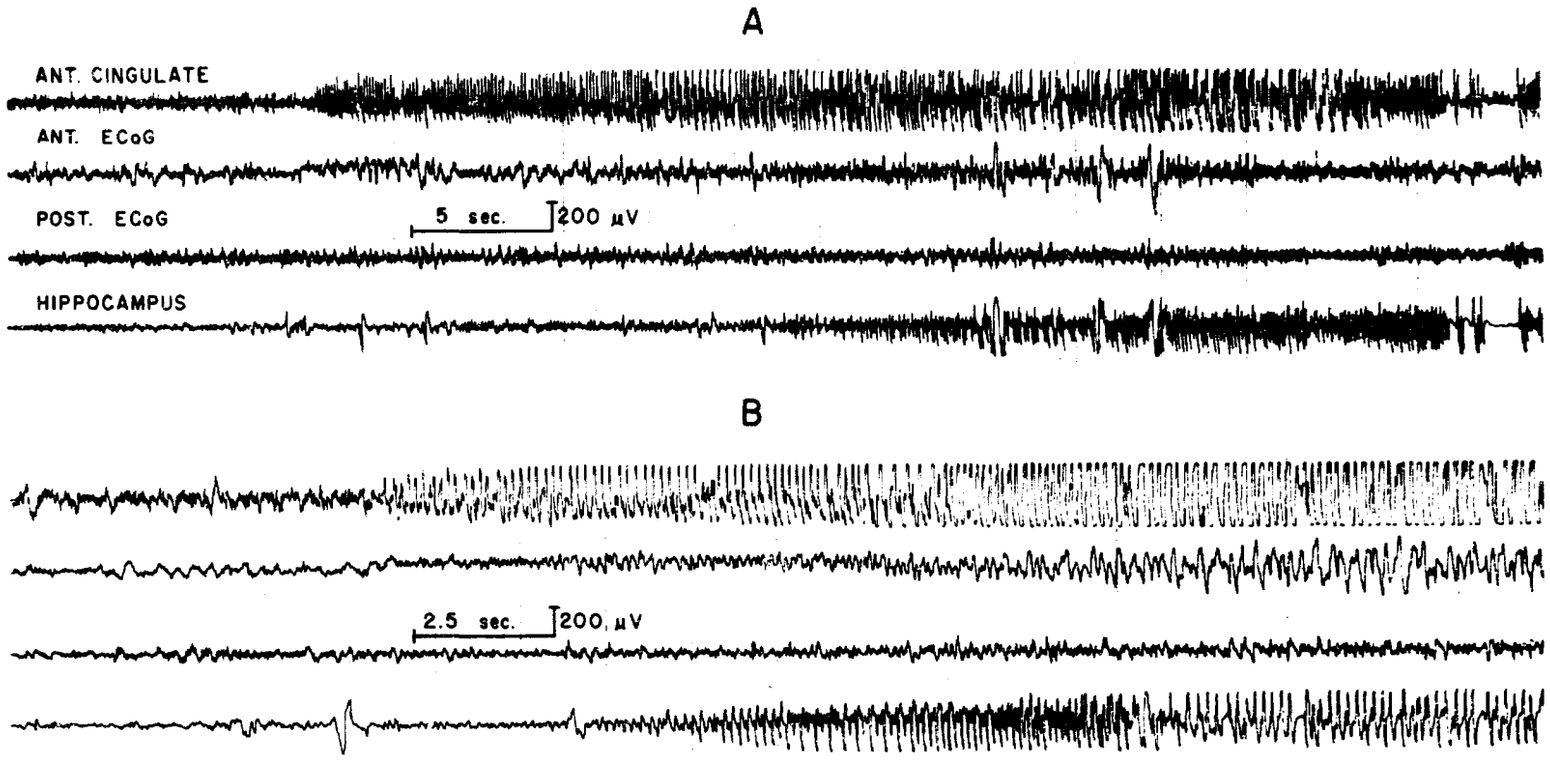

Figure 3. EEG TRACINGS FROM PORTIONS OF THE LIMBIC SYSTEM (ANTERIOR CINGULATE GYRUS AND HIPPOCAMPUS) AND FROM ANTERIOR AND POSTERIOR CEREBRAL CORTEX, SHOWING DEVELOPMENT OF SEIZURE ACTIVITY FIRST IN LIMBIC STRUCTURES WITH LATER SPREAD TO THE CEREBRAL CORTEX.

CONCLUSIONS

The following tentative conclusions and interpretations are drawn from these results.

Perhaps the most dramatic finding of this study is that cortical evoked response recordings predict when seizure will occur. When the primary SI negativity becomes exceedingly large it is probable that a seizure will follow within a few minutes. We emphasize that the EEG record and behavior preceding a seizure are usually within normal limits. Thus, the amplitude of the primary negativity is a sensitive, reliable, and, so far as we know, sole predictor of a seizure. Indeed this has proven very useful in practice because it allows the experimenters to mobilize themselves and the experimental apparatus to cope with the fast-moving events of the seizure, postictal, and recovery phases of the experiment.

In terms of underlying neural mechanisms, the extremely large response of the somatosensory cortex before a seizure is not due to an increase in sensory inflow to the cortex, because the VPL response, which represents the output of the somatosensory relay nucleus in the thalamus, is in our experience within normal (control) limits before a seizure. Thus, UDMH appears to increase intracortical neural excitability. We may further conclude that the drug acts primarily at axodendritic synapses, as it is known that the SI negativity is due to activation of synapses on the dendrites of somatosensory cortex pyramidal cells. The SI positivity which is much less affected by UDMH is, on the other hand, due to axosomatic activation. We also reason that UDMH acts in some manner by blocking inhibitory postsynaptic potentials because it has little effect on cerebellar evoked responses before a seizure. Purpura et al (1959) have shown that there are relatively few inhibitory synapses in the cerebellum. If UDMH exerted its effect by enhancing excitatory postsynaptic potentials, cerebral and cerebellar cortex responses should be affected. Thus, UDMH (or its active metabolic by-product) must be an inhibitory synapse blocking agent.

As suggested by Fairchild and Sterman, sensory-motor feedback is an important element in the production of seizures. In the freely-moving animal of figure 1, for example, the first seizure was seen at 162 minutes, whereas in the paralyzed condition this cat did not show a seizure until 202 minutes (figure 2), even though the dosage was twice as great. In practical terms this means that a person suffering exposure to UDMH should be kept as quiet as possible, as would be the case with other types of convulsants.

The paroxysmal neural activity due to UDMH poisoning appears to begin in the limbic system and then spreads to include the cerebral cortex. In this respect UDMH intoxication mimics the effects of temporal lobe epilepsy.

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2. Purpura, D. P., M. Girado, and H. Grundfest; Synaptic Components of Cerebellar Electrocortical Activity Evoked by Various Afferent Pathways; J. Gen. Physiol., 42: pp 1037-1066, 1959.

MEASUREMENT AND INTERPRETATION OF SPONTANEOUS AND EVOKED BRAIN POTENTIALS IN RATS

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The problems of assessing the electroencephalographic (EEG) record as it relates to varying states of consciousness will be discussed.

Evoked responses will be considered in regard to 1) origin, 2) influence of state variables, and 3) the significance of changes in the sequential components of the response from an informational standpoint.

The random component of well-developed (sinusoidal) REM sleep activity was investigated by converting the brain wave patterns into amplitude probability densities. This function has specific graphical distributions for different patterns, varying from a U shape for pure sine waves to Gaussian and Rayleigh distributions when randomness is a component of the pattern.

Neuroelectric phenomena must be presented in a reasonably quantitative manner but should not be fitted too rigidly to any one mathematical model. There should be flexibility in the choice of experimental design and of methods of data processing and analysis of EEG data.

Several papers from our laboratory have discussed recording and amplifying methods used in electro-biology with particular emphasis on the electrical activity of the brain (Xintaras, et al, Arch. Envir. Health, 1966; Xintaras, et al, Toxicol. Appl. Pharmacol., 1966; Xintaras, et al, 1967). The encephalogram (EEG), popularly known as "brain waves", is the time-varying voltage observed between two electrodes contacting separate points of the head, typically two points of the scalp. The predominant frequency range is from zero to fifty cycles per second. Peak amplitude ranges from 50-350 μV.

Considerable variation in the character of the signal may be observed under differing experimental conditions for a given subject. The irregularities and complexities of the EEG may have a real and important association with both normal

mental processes and with diseased conditions of the brain. Electroencephalography is used widely in clinical neurophysiological work as an aid in the diagnosis and treatment of certain brain disorders. However, the main tool of EEG classification is the eye of the experienced clinician observing the data directly as recorded by an ink writer. Even though the clinical electroencephalographer has made remarkable progress, more parametric aids of a quantitative objective nature would be welcome. It was with these considerations in mind that studies were undertaken in this laboratory to explore the utility of computers as aids in developing techniques of data processing for the electroencephalographer.

The reasons for applying computer techniques to quantitative study of the EEG fall into three main areas. The first involves storing the information in the EEG for later recall or transmission. The second is concerned with the need to assess changes in the EEG, when it is being used empirically in test procedures or experiments, as an indicator of changes in cerebral state. Finally, quantitative assessment and computer analysis of the EEG may be used in attempts to indicate the types of mechanisms which could or could not produce the observed phenomena.

Since the nature and organization of the brain mechanisms responsible for producing the waveforms of the electroencephalogram are not so well understood, computer techniques have been designed primarily to answer specific questions about the EEG - for example: can changes in the EEG be correlated with the time of occurrence of physical or physiological events? Which characteristics of the EEG may be so defined that subjects can be grouped according to their physiological, pathological, or psychological condition? The aim of such studies is to supplement the observations of experienced workers with objective information.

The purpose of the studies reported in this paper is to determine whether spontaneous brain wave activity and the sensory evoked response are sensitive and reliable measures of the effect of exposure to air pollutants. Altered functions of the central nervous system may be evident before clinical signs and symptoms have been induced by such exposure.

Moreover, if a normal physiologic function is time varying, the study of its rhythms will be essential for a definition of normalcy, and, hopefully, it may prove to be a sensitive gauge of early pathology as well.

METHODS AND MATERIALS

Sprague Dawley, young adult, male albino rats, weighing from 200 to 300 gm, were used in these studies.

Subcortical (superior colliculus) and cortical (visual area) electrodes were implanted stereotaxically in anesthetized rats. Figures 1 and 2 show the areas selected for implantation of electrodes and the method used to locate brain coordinates. The bipolar electrodes used as implants were enameled stainless steel wires attached to a 26-gauge hypodermic needle to provide rigidity when directed by the stereotaxic instrument. Recording points 0.5 mm in the vertical direction were used when signals from the superior colliculus were desired. For cortical records, No. 00 stainless steel screws were placed in the visual cortex (penetration of the dura was avoided). An indifferent electrode (#00) was placed 1-2 mm

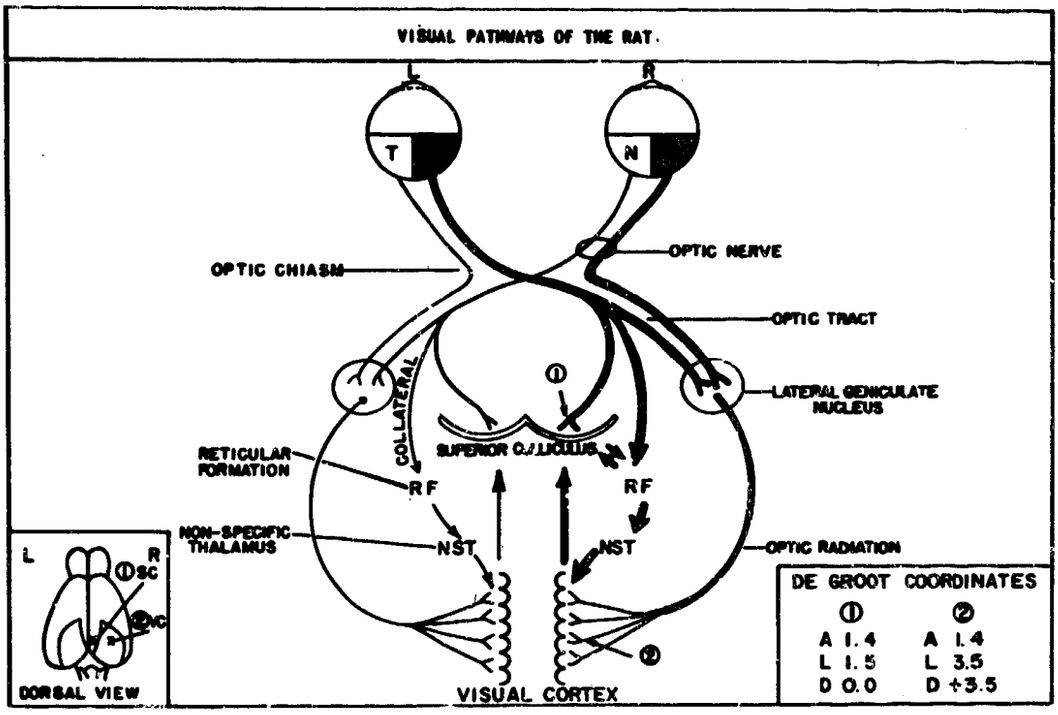

Figure 1. SIMPLIFIED DIAGRAMMATIC REPRESENTATION OF THE VISUAL PATHWAYS OF THE RAT

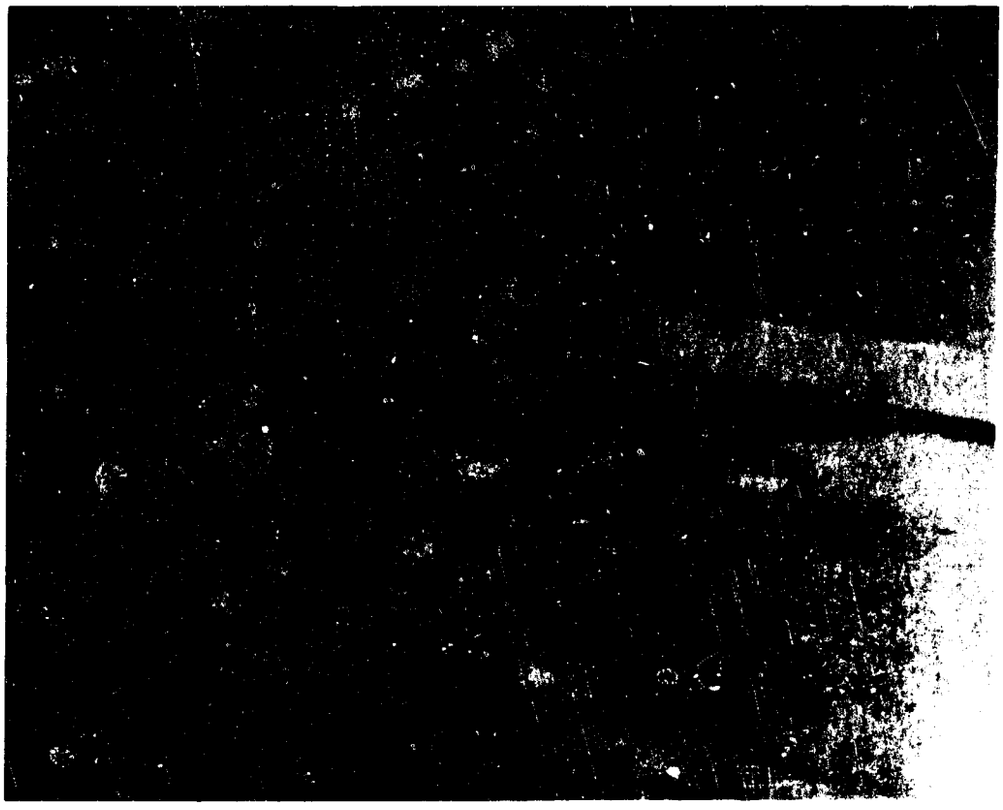

Figure 2. CLOSE-UP OF STEREOTAXIC INSTRUMENT SHOWING CHRONIC IMPLANT TECHNIQUE

anterior to the occipital crest. The superior colliculus was selected in addition to the visual cortex, because of its participation in complex sensorimotor integration, particularly related to visually guided behavior.

For confirming electrode sites in the superior colliculus the rat brains were removed from the skulls and placed in 10% formalin solution for several weeks and then sliced into 70μ -sections by the frozen method. The sections were stained with Sudan Black B and then used as photographic negatives and copied at an enlargement of about 12 times (figure 3). Cortical sites were confirmed by visual inspection.

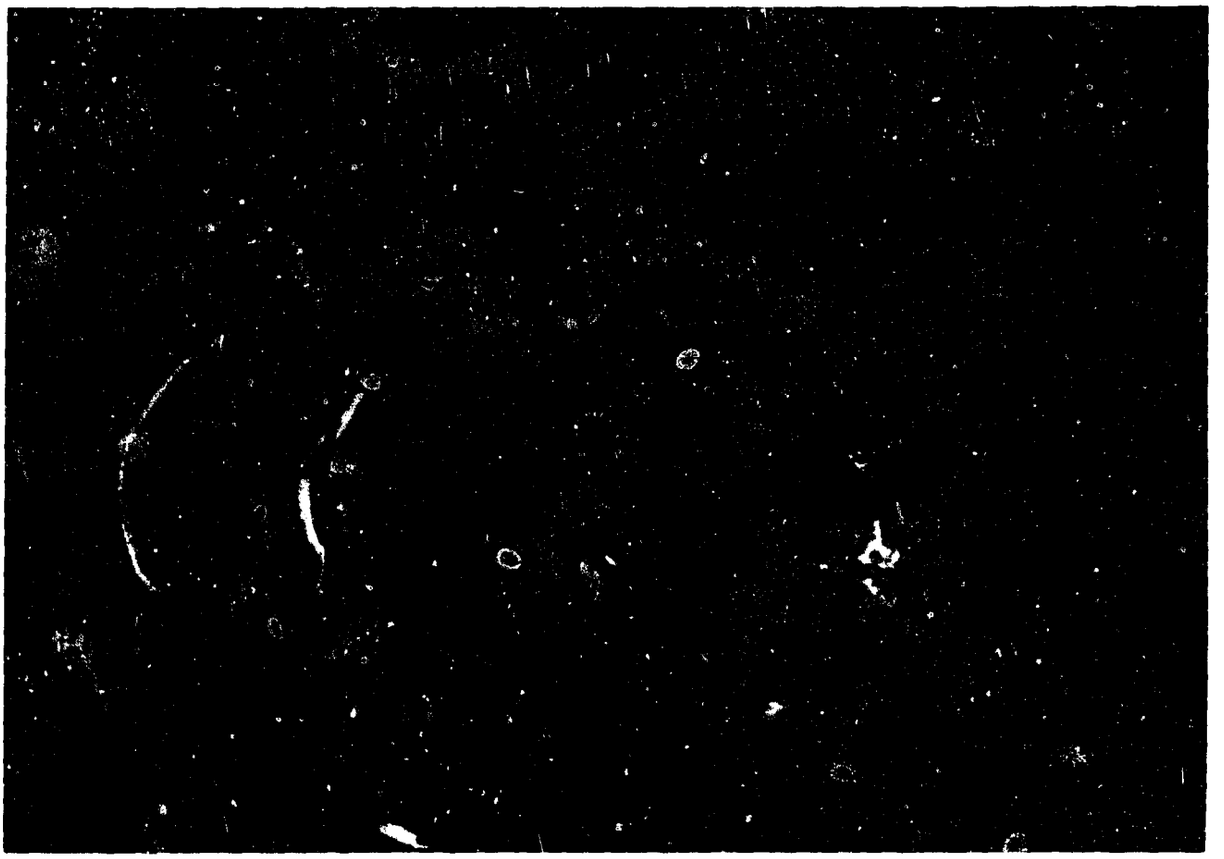

Figure 3. A CORONAL SECTION OF RAT BRAIN STAINED WITH SUDAN BLACK B AND SHOWING THE ELECTRODE TRACT PIERCING THE CEREBRAL CORTEX (RIGHT HEMISPHERE) AND THE TIP OF THE ELECTRODE TERMINATING IN THE STRATUM ALBUM MEDIALE OF THE SUPERIOR COLLICULUS.

The details of the exposure chamber and the electronic recording system are shown in figures 4 and 5. The primary exposure and testing chamber was a modified

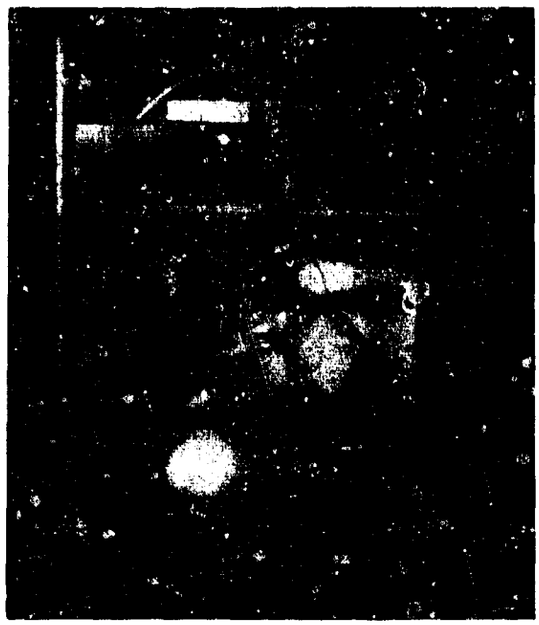

Figure 4. NEUROBEHAVIORAL TESTING AND EXPOSURE CHAMBER. The rat has just depressed the lever and will receive a food reward. Brain waves are simultaneously recorded. The stroboscope (source of light flashes) is in the foreground.

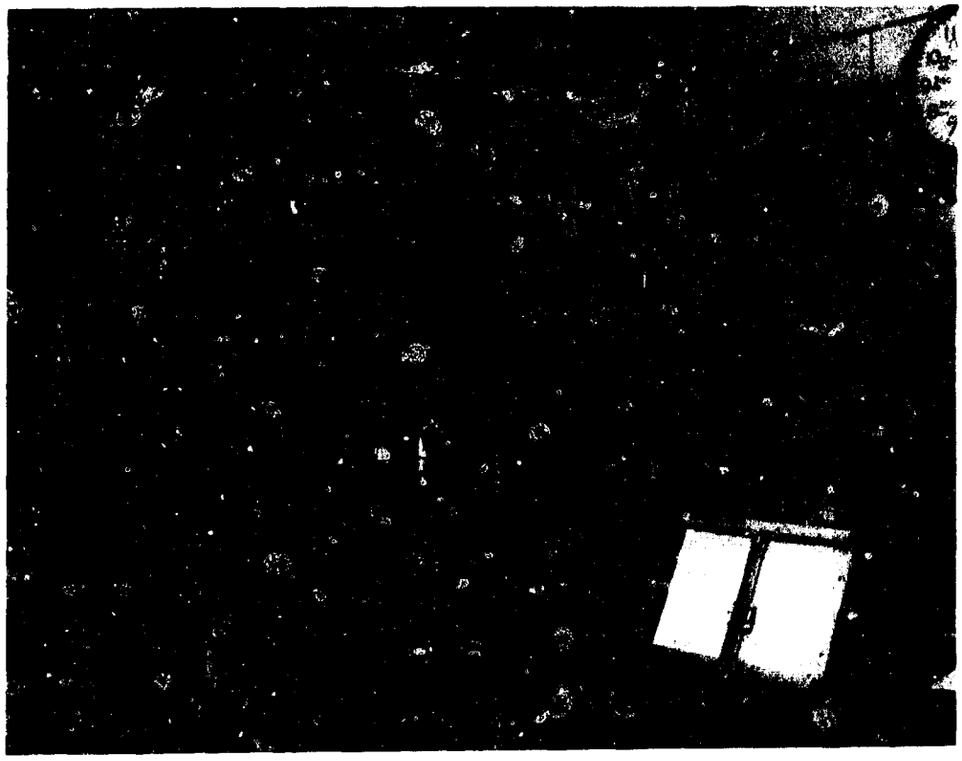

Figure 5. DATA RECORDING AND ANALYSIS SYSTEM USED IN THIS STUDY.

Skinner box designed to permit the simultaneous measurement of behavioral and electrophysiological responses and, in addition, to serve as a dynamic inhalation chamber. Five electrically shielded secondary chambers were also available. Each chamber housed one rat. In the secondary chambers water containing lead acetate (1.5 mg/cc H_2O) was available ad libitum. Observations were made regarding the rat's behavior (grooming, exploring, eating, etc.) via a closed-circuit TV system in order to relate changes in the electrophysiological records with changes in the rat's behavior. The computer was programmed to trigger a photostimulator, and a train of light flashes was presented to the rat housed in the primary test chamber. Brain potentials evoked in response to each light flash were either averaged on-line by computer and graphed on an x-y plotter or recorded on a tape recorder for later averaging. Conventional electroencephalograms were also obtained simultaneously from five rats housed in the secondary chambers (not subjected to flashes of light).

Pentobarbital was used as a standard for comparing the depressant effects of carbon monoxide. Killam (1962) noted that the depression of reticular activity by pentobarbital has been so reproducible from species to species that it has become an acceptable standard in neuropharmacologic research.

When a flash of light is delivered to the eyes, the potentials of the brain which can be correlated with the flash do not return immediately to zero, but go through a sequence of positive and negative phases before they die out.

The computational technique for summing these flash-evoked responses is shown in figure 6. The occurrence of the stimulus (light flash) and the starting point of the recording of the response must be synchronized. This was achieved by initiating both, simultaneously, by means of the computer. In Trace 1 the computer did not trigger the photostimulator, and, hence, the flash occurred along the entire baseline. The computer "saw" the neuroelectric potentials as random fluctuations and "averaged out" the components (the summing is algebraic).

Trace 2, however, illustrates the use of a time reference. The computer was programmed to trigger the photostimulator on the 20th ordinate (400 ordinates in each trace and each ordinate a memory location in the computer). The electrical potentials (disturbance), occurring after each stimulus, were added and stored in the computer. A record of these summed responses was provided by an x-y Plotter.

The complex electrical system used may originate, in itself, signals that may be synchronized with the stimulus and recorded, therefore, as artifacts in the record of the response. To assure that this did not occur, a simple test was performed. In the synchronized system, as depicted in Trace 2, an opaque material was placed over the flash unit. The curve, as shown in Trace 3, shows that no inherent artifacts were present.

In order to calibrate the amplitudes of the evoked response, a 10 μV pulse, as a positive square wave, was fed into the system. The output is shown in Trace 4.

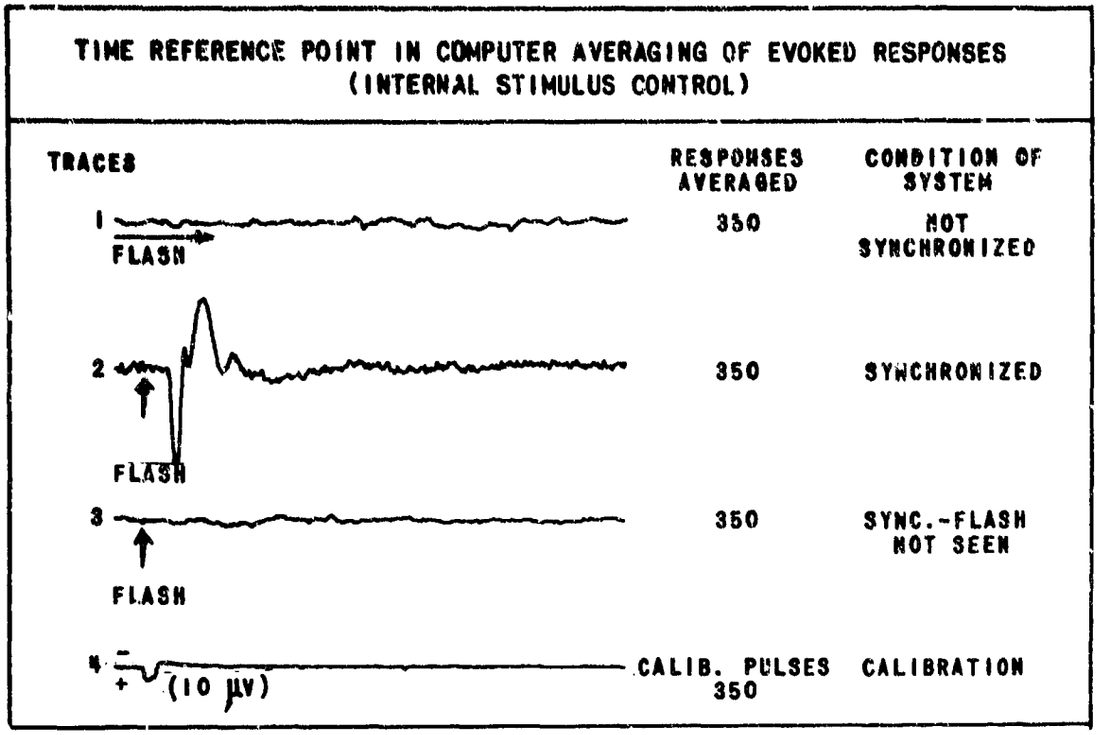

Figure 6. COMPUTER TECHNIQUE FOR SUMMING SENSORY EVOKED RESPONSES. The length (analysis time) of each trace is 500 milliseconds. The arrow indicates the onset of the flash (regulated by the computer).

RESULTS

Evoked Response Characteristics

Figure 7 is a typical averaged response to light flash stimulation evoked in the visual cortex of the rat during sleep behavior characterized by irregular slow waves of high amplitude in the visual cortex. The tracing is the average of 6,000 responses; analysis time is 250 milliseconds; stimulus (one light flash per second) occurs 12.5 milliseconds after the start of the trace; positivity at the active electrode gives a downward deflection in this graph. The evoked response to flash in the primary visual cortex shows a short-latency initial positive-negative complex which we have designated waves I and II. Wave I is not always seen. Immediately thereafter there are two positive deflections, waves III and IV. In the slow phase of sleep the amplitude of wave III is augmented and the amplitude of wave IV is reduced. The subsequent broad negative deflection, wave V, is increased in amplitude during the sleep phase and appears to be composed of several components.

The effect of pentobarbital on the cortical evoked response (visual cortex) is shown in figure 8. The upper tracing is a control and is the average of 30 responses; analysis time is 250 milliseconds; positivity is downward; stimulus (one flash per second) was continuous and all electrical potentials were recorded on tape for later

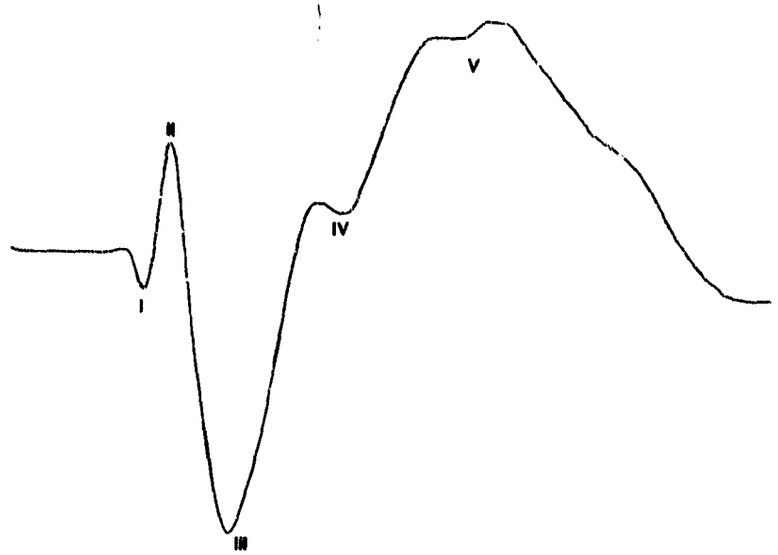

Figure 7. TYPICAL AVERAGED EVOKED RESPONSE TO FLASH STIMULATION FROM THE VISUAL CORTEX OF AN IMPLANTED RAT OBSERVED WHEN THE RAT SHOWS BEHAVIORAL AND ELECTROPHYSIOLOGICAL SLEEP.

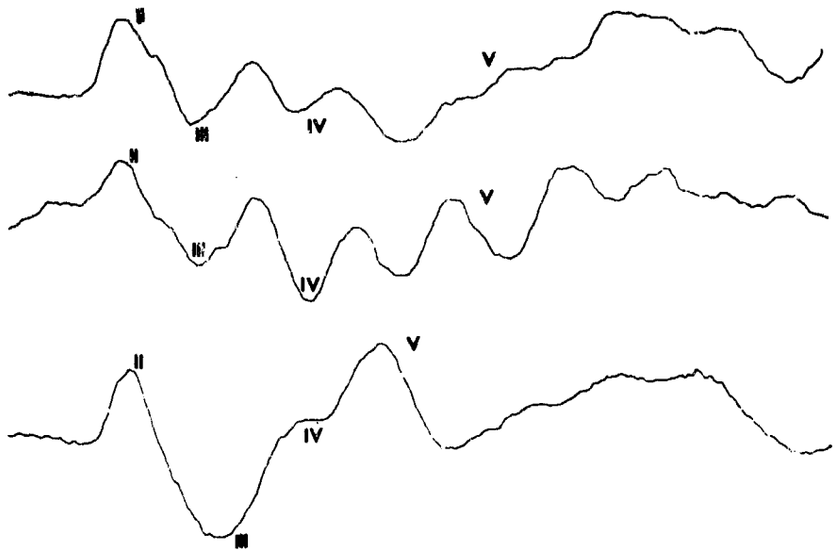

Figure 8. FLASH-EVOKED RESPONSES AVERAGED BY COMPUTER FROM AN UNBROKEN SERIES OF CORTICAL POTENTIALS (90 second sample). Each tracing is the average of 30 responses. The analysis time is 250 milliseconds and the flash rate is one per second. The first tracing is typical of an alert rat. The second tracing shows the immediate excitatory effect of an intraperitoneal injection of pentobarbital (5 mg/kg). The last tracing shows the depressant effect of the drug on certain components of the evoked response (elimination of wave IV and increase in the peak amplitude of waves II, III, and V).

averaging and to insure minimal interference with the subject. Waves II, III, IV, and V are prominent in the control tracing. The rat was given an injection of pentobarbital (5 mg/kg, intraperitoneally) immediately after the control tracing was recorded. The next two tracings show both the initial excitatory effect of pentobarbital and also its depressant effect. In the middle tracing, the increase in amplitude of wave IV and the two early components of wave V is apparent. This change occurs within 30 seconds after the injection and reflects an excitatory phase. The bottom tracing shows the depressant effect, i.e., increase in peak amplitude of waves II, III, and the early component of V, and the disappearance of wave IV immediately after the middle trace was recorded.

The effects of carbon monoxide on the evoked cortical potentials of a rat are shown in figure 9. The bottom trace was recorded when the rat was active in the test chamber (pressing a lever for food reinforcement). The upper tracing was recorded following an exposure of the rat to 100 ppm of carbon monoxide for two hours. The changes are obvious. The peak amplitudes of waves III and V are increased whereas the amplitude of wave IV is reduced to where there is no visible evidence of its presence in the waveshape.

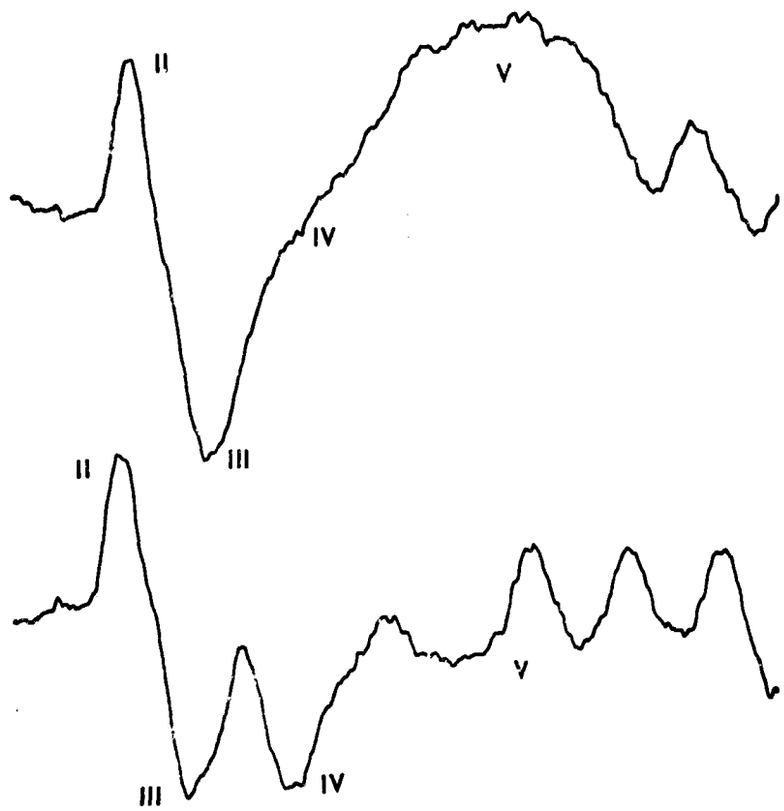

Figure 9. THE EFFECT OF CARBON MONOXIDE ON THE RESPONSE TO FLASH IN THE VISUAL CORTEX OF A RAT. The rat was exposed to carbon monoxide (100 ppm/two hours). Each trace is the average of 50 responses. The bottom trace is control and was recorded when the rat was actively depressing a level for a food reward. The upper tracing was recorded immediately following the exposure.

Figure 10 shows the changes in the evoked response in the transition from slow wave sleep to rapid eye movement (REM) sleep. The upper tracing was recorded when the rat was behaviorally and electrophysiologically asleep (simultaneous monitoring of the visual electrocorticogram indicated irregular high amplitude electrical potentials). The lower trace was recorded during paradoxical sleep (REM) characterized by regular slow waves of 6-10 cycles per second. The fundamental period of the upper trace is 160-170 milliseconds or about six cycles per second. Waves with a period of 25 milliseconds are evident in the lower trace (40 cycles per second). In addition, the fundamental 160-170 millisecond period is also noted but less striking than in the upper trace. Positivity is downward.

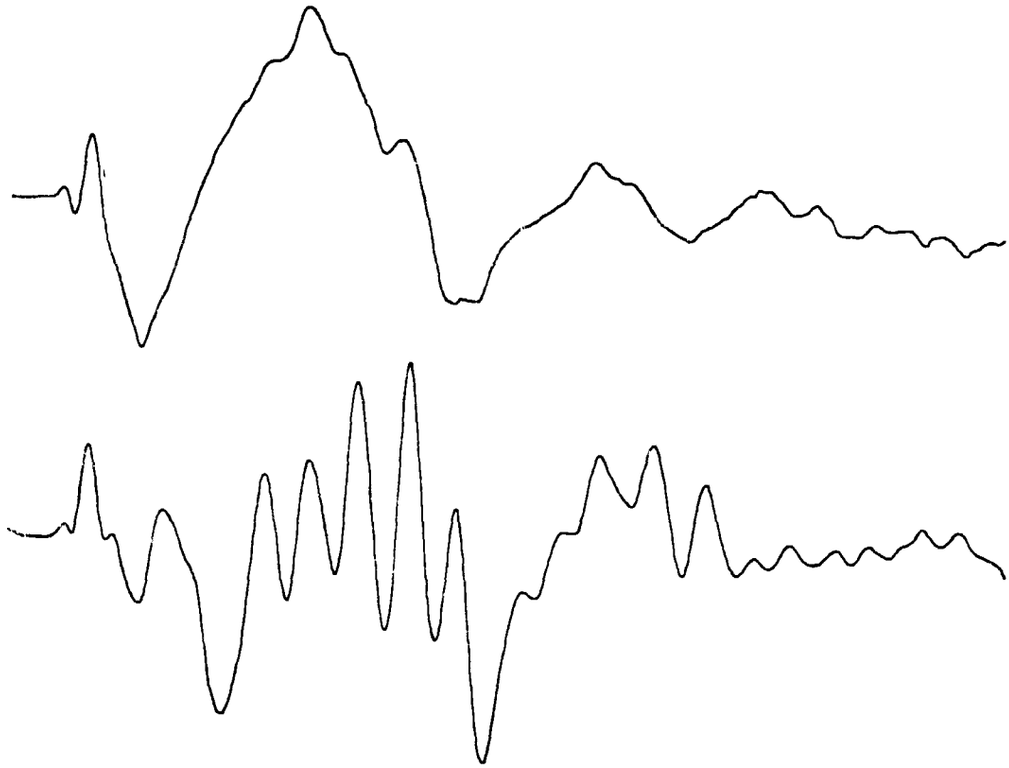

Figure 10. CHANGES IN EVOKED CORTICAL WAVEFORMS RECORDED DURING THE TRANSITION FROM SLOW-WAVE DEEP SLEEP TO RAPID EYE MOVEMENT (REM) SLEEP. The upper tracing is averaged from background EEG activity characterized by slow-waves and spindles. The lower tracing is averaged from REM sleep. Each tracing is the average of 50 responses; analysis time is 500 milliseconds; flash rate is one per second.

Amplitude Density Distribution

Electrophysiologically the rat enters the REM phase of sleep by way of slow-wave and sleep spindles. The duration of this pre-REM period may last several minutes (early morning sleep) or a few seconds (late afternoon sleep). A sample of pre-REM sleep is shown in figure 11. The slow-waves are characterized by a

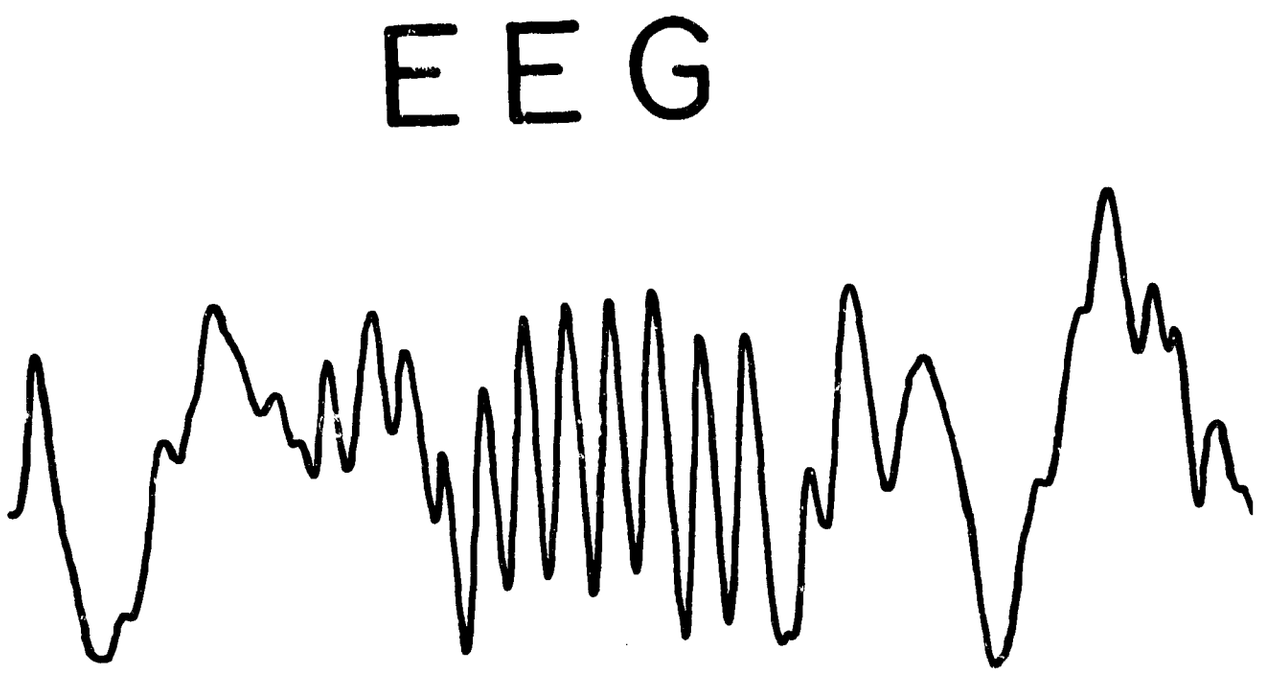

Figure 11. SPONTANEOUS EEG RECORDED FROM THE VISUAL AREA OF THE RAT DURING SLOW-WAVE AND SPINDLE SLEEP. The length of the trace is 1000 milliseconds. Note the high amplitude spindles (~25 cps) in the center of the tracing and the slow-waves (~6 cps) on either side of the spindle burst. Peak amplitude (trough to peak) is about 350 μV.

period of about 160-250 milliseconds and peak amplitude of 300-350 μV. A spindle burst is shown in the center of the trace and in this epoch the spindles have a period of 30-40 milliseconds (25-33 cycles per second). In this EEG sample the rat was not stimulated peripherally with photic stimuli. However, the evoked response to light flash when averaged from this kind of background activity would be similar to the waveshape shown in figure 10 (upper trace).

The EEG pattern of rapid eye movement sleep in an unrestrained normal adult rat is shown in figure 12. Both traces are records of REM sleep. The time base in the lower trace was expanded to show the quasi-sinusoidal characteristics of the waves. Although eye movements are predominant during this phase of sleep, other movements of the rat are usually observed, i.e., sudden movements of the ears, the vibrissae, the fingers, the tail, and the tongue. Irregular respiration is also noted. The REM phase is entered by way of 15 cycle per second "sleep spindles" and is characterized by regular waves of 6-10 cycles per second and by waves which seem to be summed with noise giving the appearance of deformed sine waves. REM epochs are more abundant during the later part of the sleep record,

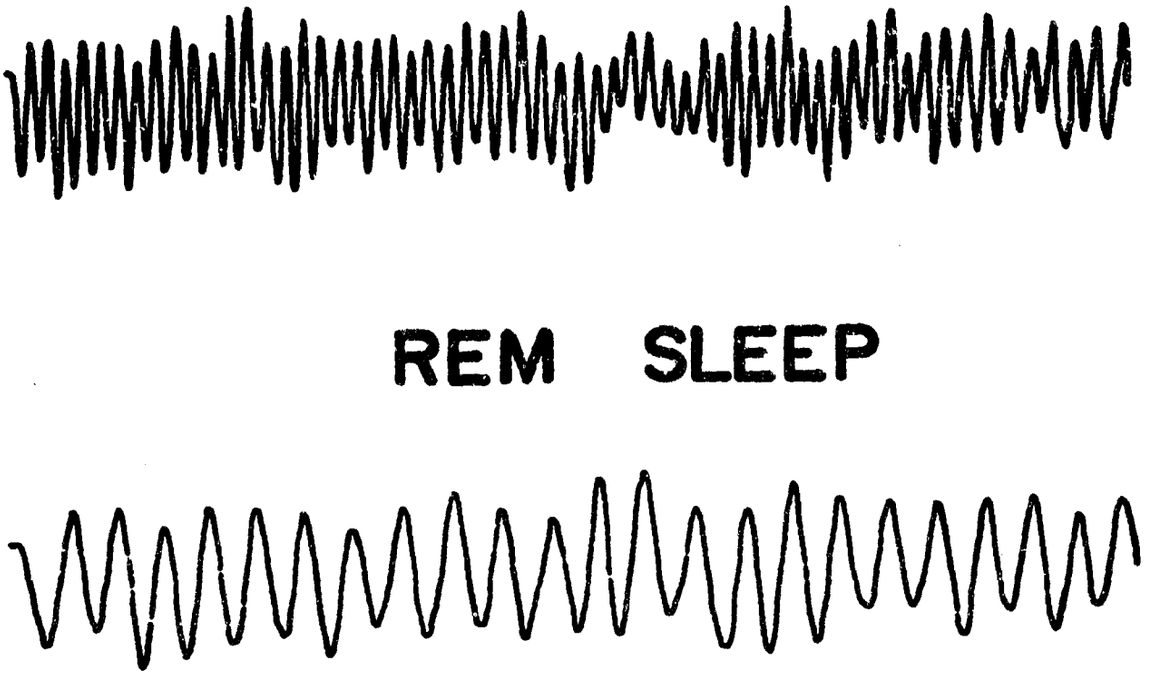

Figure 12. QUASI-SINUSOIDAL EEG PATTERN DURING RAPID EYE MOVEMENT (REM) SLEEP. The signals in the bottom tracing are from the top trace and were recorded with an expanded time base in order to show the regularity of the REM waves. Analysis time for upper tracing is eight seconds (frequency of signals about seven cycles per second). Amplitudes of these waves range from 80 - 110 μV.

i. e., late afternoon. The early portion of a REM epoch appears to be more stable (less noise) than the period preceding arousal of the rat.

The "noise" component (which may carry important information) of well developed REM sleep can be investigated by converting the EEG-REM pattern into an amplitude density distribution. These relate the different amplitudes of sequential points in the waveform to the frequency of occurrence of these amplitudes. Amplitude densities have specific graphical distributions for different patterns, varying from a U shape for pure sine waves to Gaussian and Rayleigh distributions when randomness is a component of the pattern.

Figure 13 demonstrates the computer technique for recording the amplitude density distribution of square waves. The analog signal to be sampled is a square wave with a voltage range of zero to +10 volts (upper trace). The dotted line indicates that gating signals are provided by an external pulse generator such that the instant a gating pulse is supplied the amplitude of the analog signal is sampled for

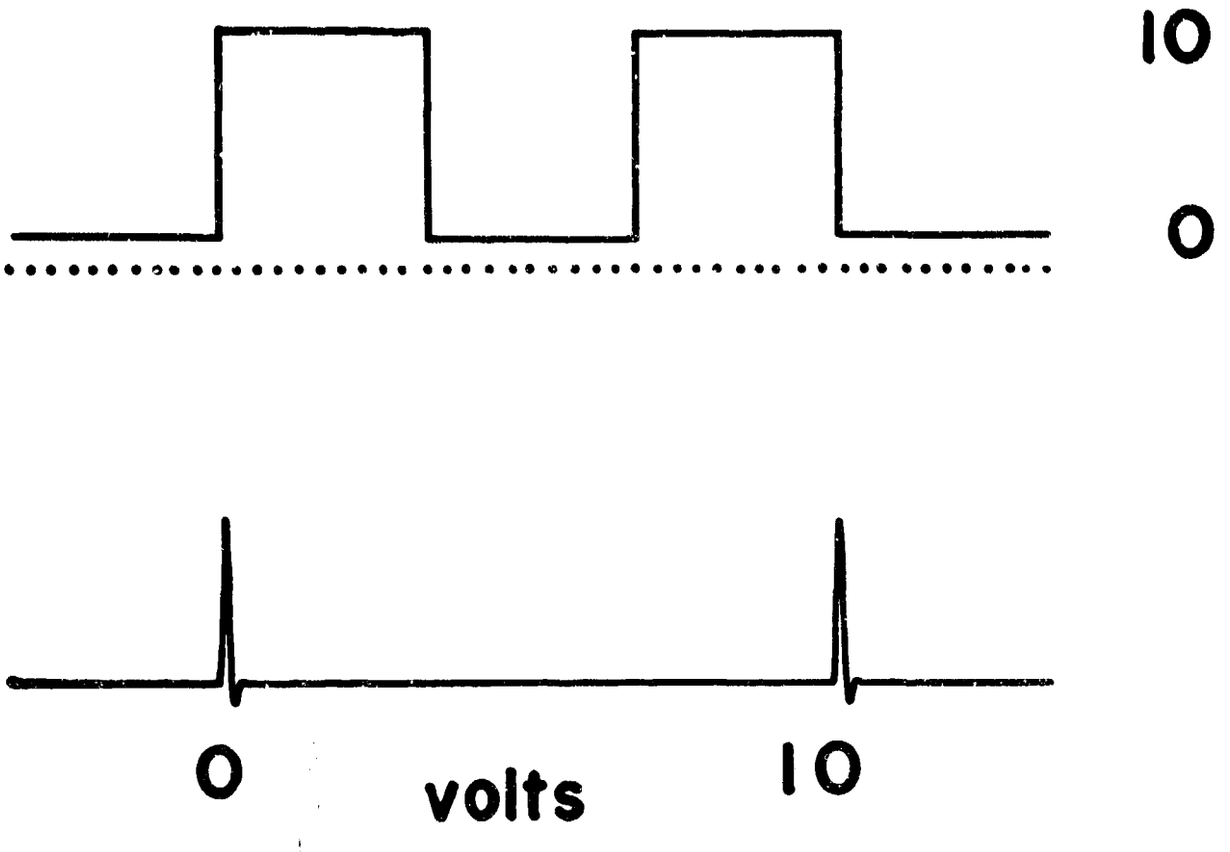

Figure 13. COMPUTER TECHNIQUE FOR OBTAINING THE DISTRIBUTION OF SAMPLED ANALOG AMPLITUDES. The height of the spikes in the lower trace reflects the frequency of occurrence (number of events) of the sampled signal voltages. The square wave in this example is sampled each time an external gating pulse (dot) is provided and the voltage information is transmitted to the computer for storage.

ultimate transmission to the memory of the computer. The lower trace shows the amplitude density distribution of the square waves. The horizontal axis is signal (square wave) amplitude in volts. The vertical axis is frequency of occurrence of sampled analog amplitudes. When the first few gating pulses were supplied the analog signal (the square wave) was at a voltage level of zero volts and consequently the computer added counts at the zero level (first spike of lower trace). At the 11th gating pulse the sampled analog signal reads +10 volts and counts will now be added at the 10 volt level in the lower trace (second spike).

If the density distribution in the lower trace had been normalized and the vertical axis labelled probability density the height of the spikes would be equivalent to 0.5 indicating that the amplitude of zero volts of the square wave occurred with probability of 0.5. The same probability level would apply to the amplitude of +10 volts. In a real on-line situation 1500 gating pulses per second are provided for sampling the analog signal.

Specific graphical distributions for several well known wave patterns are shown in figure 14. The amplitude density distribution for a square wave is easily recognized. The distribution for a sine wave is shown in the second trace and appears U shaped (this is expected theoretically). Triangular waves and noise are pictured in the third and last traces, respectively. In all distributions the signal amplitude was zero to +10 volts. The interpretation of these amplitude density distributions is as follows. The square wave, as also shown in figure 12, is sampled and the computer records how frequently the zero and the ten volts occurred. The distribution shows that no voltages between zero and ten volts were sampled and this accounts for the flatness of the baseline. The second distribution (sine) indicates that the sampled signal contains amplitudes not only at zero and ten volts but also voltages between these points and this accounts for the rise in the baseline. The distribution for the triangular wave indicates that the sampled voltages occur with equal frequency or probability (if the data had been normalized). Noise gives a density distribution as shown in the last trace.

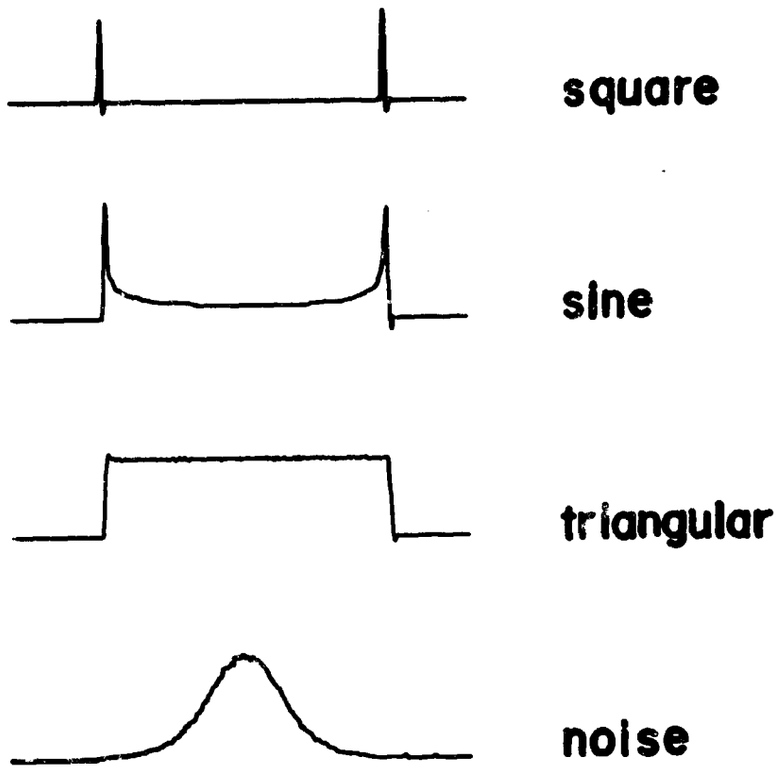

Figure 14. AMPLITUDE DENSITY DISTRIBUTION CURVES FOR SEVERAL FAMILIAR WAVES. The horizontal axis in each tracing is signal amplitude (0 to +10 volts). Vertical axis is frequency of occurrence of the sampled amplitudes.

The amplitude density distribution of a periodic signal summed with increasing levels of noise is shown in figure 15. Trace (a) is the amplitude density distribution of a triangular wave (x-axis is signal amplitude in volts, 0-10; y-axis is frequency of occurrence of the sampled amplitude). With a summing circuit a small amount of noise was added to the triangular waves (signal to noise ratio about 10:1) and the

distribution in Trace (b) was recorded. Additional noise was added to the triangular wave and the amplitude density distributions shown in Traces (c) and (d) were recorded (S/N is 5:1 and 1:1, respectively).

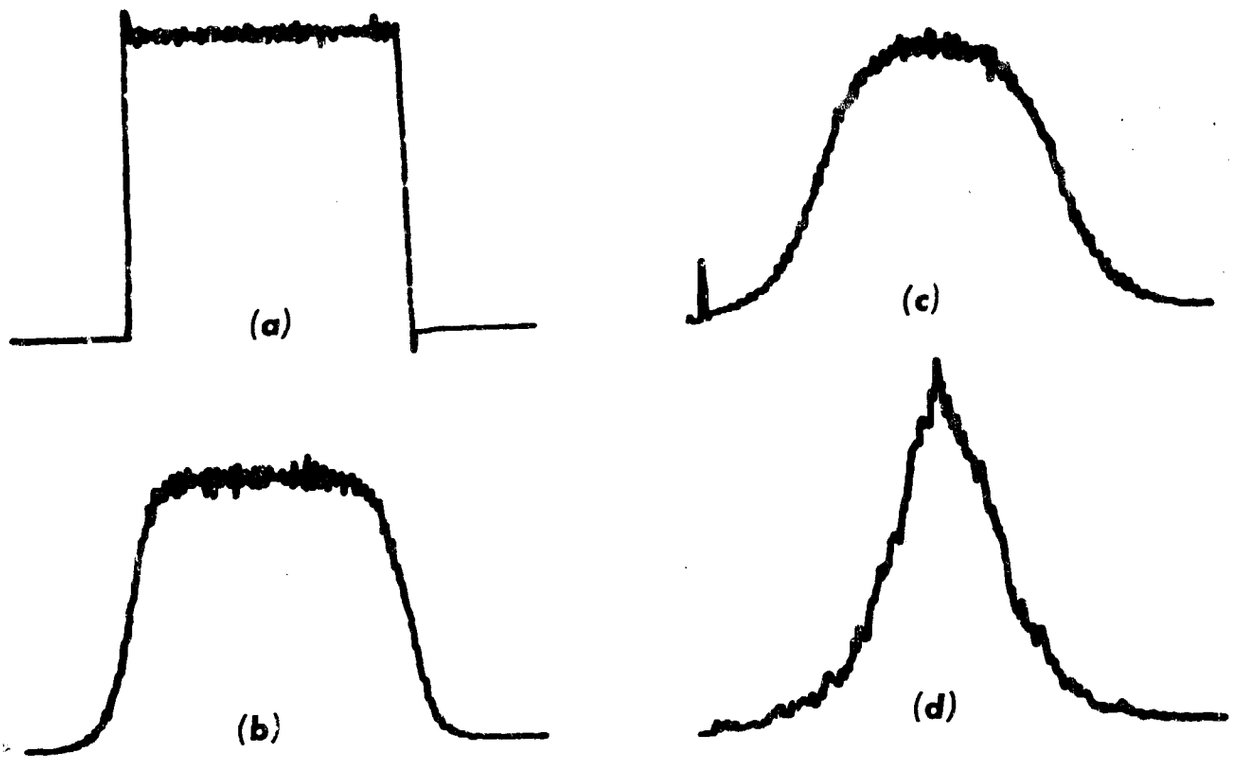

Figure 15. AMPLITUDE DENSITY DISTRIBUTION FOR AN ANALOG SIGNAL WHEN THE SIGNAL/NOISE RATIO IS ALTERED. Trace (a) is the density curve for a pure triangular wave. In Trace (b) the signal/noise ratio was made 10:1. The tracing in curve (c) is recorded when the ratio is changed to 5:1. In the last Trace (d) the signal to noise ratio is unity.

Figures 16 and 17 illustrate the application of computer techniques in assessing the amplitude density distribution of the rat's EEG's. Each trace represents a six-hour sample of continuously recorded brain waves. The distribution of amplitude densities in figure 16, the control rat, appears to be Gaussian distributed (with a slight skewness to the right). In the lead treated rat, figure 17, the density distribution appears to be more like a Rayleigh distribution. The skewness to the right is more apparent in this trace.

Since REM sleep appears quasi-sinusoidal (see figure 12) the amplitude density distribution would be expected to resemble a U shaped distribution. The control record shows, in figure 18, that the REM sleep patterns are indeed quasi-sinusoidal (the amplitude density distribution looks U shaped). In the lead treated animal, the amplitude density distribution of REM sleep patterns appears Gaussian.

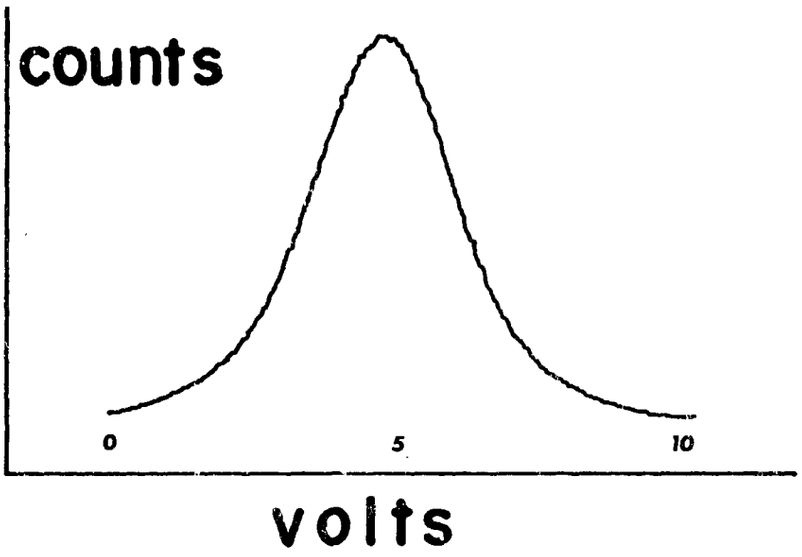

Figure 16. AMPLITUDE DENSITY DISTRIBUTION OF A SIX-HOUR EEG SLEEP PERIOD FROM A CONTROL RAT. Note that the distribution appears Gaussian (slight skewness to the right).

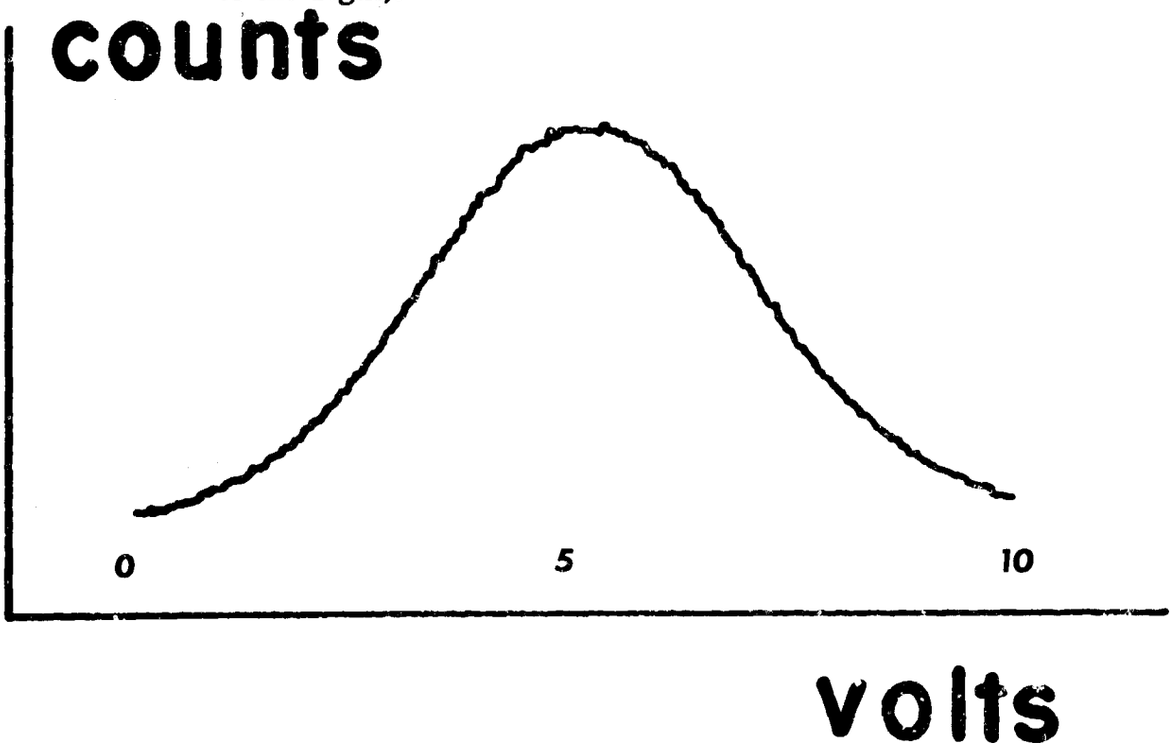

Figure 17. AMPLITUDE DENSITY DISTRIBUTION OF A SIX-HOUR EEG SLEEP PERIOD FROM A LEAD TREATED RAT (1.5 mgs/cc H_2O for four months). Note that the mean voltage in this curve has shifted to the right (higher amplitude) and the skewness on the right is more evident than in figure 16.

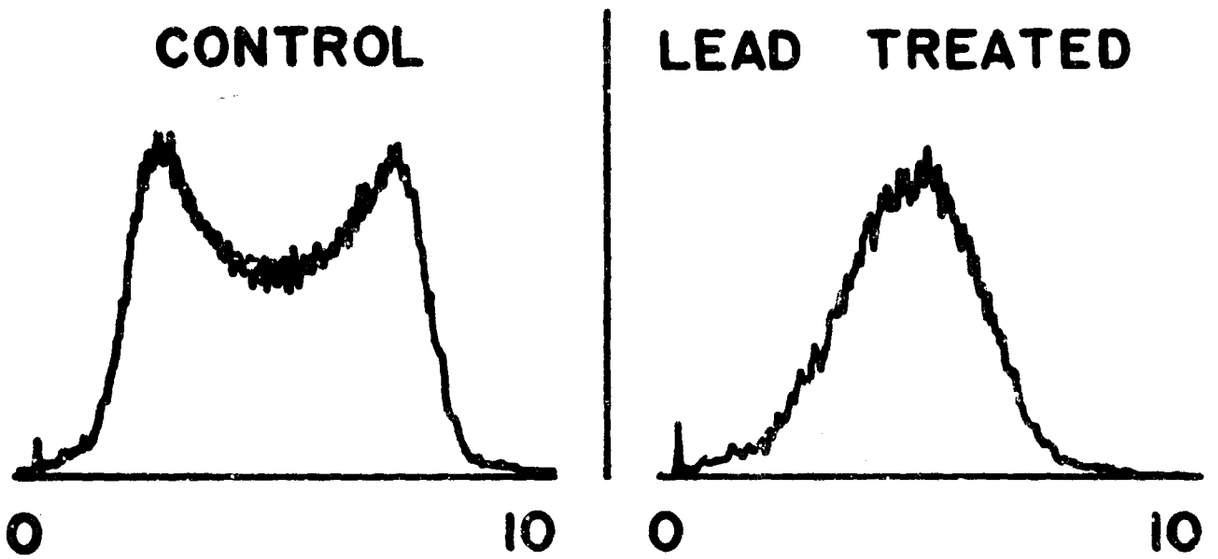

Figure 18. AMPLITUDE DENSITY DISTRIBUTION OF REM SLEEP IN A CONTROL RAT AND IN A RAT TREATED WITH LEAD ACETATE (1.5 mgs/cc H_2O/4 months).

DISCUSSION AND CONCLUSIONS

Averaged Evoked Response

Brain responses to flashes of light in the freely moving rat can be examined with on-line summation techniques (Xintaras, et al, Toxicol. Appl. Pharmacol., 1966). This development encouraged several studies to furnish additional information about response morphology, particularly, as it might vary as subjects move from alert wakefulness through drowsiness and into deeper stages of spontaneous or induced sleep.

Male rats were implanted with cortical and subcortical recording electrodes and placed in an electrically shielded chamber. Photic stimuli were presented to the rat, and conventional EEG recordings obtained. Cortical potentials evoked in response to each light flash were either averaged on-line by computer or recorded on a tape recorder for later averaging. Computer-summated responses were also graphically displayed by an x-y plotter.

In the transition from wakefulness to sleep, specific components of the evoked response were attenuated whereas others were markedly augmented. Carbon monoxide and pentobarbital induced changes in the response that appeared similar to the changes recorded during the subjects normal transition from wakefulness to

spontaneous sleep. Consistent relationships were noted between the spontaneous EEG and the magnitude of the visual evoked response. This was particularly evident with wave IV during REM sleep and with wave V during the onset of light sleep. The findings suggest that the alterations in the evoked response during light spontaneous or induced sleep may be associated with a general lowering of the level of vigilance and may be related to the integrative functions necessary to awareness and for processing sensory signals into something meaningful - perception.

Amplitude Density Distribution

Rapid eye movement (REM) sleep, in contrast to no-REM or slow-wave sleep, is a period of intense activity of the central nervous system. Since the most serious manifestations of lead intoxication are those resulting from cerebral involvement, a study was designed to investigate the effect of chronic lead absorption on the electroencephalographic (EEG) sleep record, especially the REM phase. REM sleep activity in the rat EEG appears as a quasi-sinusoidal pattern. Closer examination of REM sleep epochs reveals a degree of instant-to-instant variability of wave shape which appears to be random (also observed by Saunders, 1963, for the human alpha EEG pattern). In order to study the characteristics of this randomness in REM sleep the amplitude density distribution of the waveforms was determined. These densities have specific graphical distributions for different patterns varying from a U shape for pure sine waves to Gaussian and Rayleigh distributions when randomness is a component of the pattern.

For the REM amplitude density studies male rats were implanted with cortical recording electrodes and placed in electrically shielded chambers. Water containing lead acetate (1.5 mg/cc H_2O) was available ad libitum. Observations were made regarding the rat's behavior (alert wakefulness, quiet, sleep, etc.) via a closed-circuit TV system in order to relate changes in the EEG record with changes in the rat's behavior.

In the transition from wakefulness to sleep, the normal adult rat exhibited a typical succession of EEG changes similar to those found in man. The REM phase was entered by way of 15-20 cycles per second "sleep spindles" and was characterized by regular waves of 6-10 cycles per second and with amplitudes slightly higher than those found in the awake state. Lead acetate induced changes in the duration and stability of the REM periods. Excessive spindle-slow-wave complexes (figure 11) appeared during periods of REM sleep. Treated animals showed more REM sleep during the early sleep period as compared to control baseline data and, in addition, the regularity of REM sleep patterns (sinusoidal) were altered as shown in figure 18.

The findings suggest that the alterations in the REM phase in lead treated animals may be directly or indirectly associated with an impaired neural control system, and in particular with the mechanism responsible for controlling transitional changes in levels of consciousness.

Computer Techniques

Quantification of EEG data requires the detection of regularities that are commensurably related to the particular function of the nervous system being

investigated. We believe that the technique of computer averaging of sensory evoked responses can help us understand the statistical character of neural activity which is one of the essential features of the nervous system. The same can be said of the amplitude probability density function and of the amplitude probability distribution.

In defining "typical events" among those observed experimentally, the investigator must establish certain criteria of judgment. In order to decide whether an event is typical or whether two events differ, we must know something about the distribution of possible events. In our application of the technique of the evoked response in air pollution toxicology we have studied this distribution by observing evoked responses to a large number of identical flash stimuli and by repeatedly sampling the EEG.

Neural Mechanisms

The technique of the computer averaged sensory evoked response has been used by us in attempting to understand some of the mechanisms underlying the temporal factors in perception, i. e., fusion and critical flicker frequency.

In recent work, we have looked at the second derivative of the primary evoked response and have been impressed with the changes that occur in wave III of the response (figure 7). This wave may be related to a neural mechanism for habituation or blocking of incoming signals.

Lindsley (1961) has referred to a comparable system in humans, i. e., alpha excitability cycle, by means of which the cortex would protect itself from constant bombardment through all sensory channels.

Our most interesting data has come from comparing the second derivative of the evoked responses averaged from REM sleep and from an awake (alert) rat. Throughout the dream (REM) period wave III appears as a double wave with a peak to peak latency of about 40 msc (the fundamental period of REM sleep waves is about 160 msc). Analysis of our data shows that during certain epochs of REM sleep there are changes in the background EEG frequency of the order of 40 milliseconds. The phase in which the flash stimulus arrives at the cortex during REM sleep determines the characteristics of wave III in the evoked response. These signals, when differentiated, do not show high frequency components (~40 cps) for the 80 millisecond interval following the arrival of flash stimulation at the cortex. Our data suggests that in REM sleep the evoked response, although similar to the response recorded during wakefulness, lacks high frequency (40 cps) components in the 80 msc interval following the arrival of impulses over the primary visual pathway. Because of a phase shift in the arrival of the flash stimulation during REM sleep wave III appears as a double wave. During early REM epochs these waves would serve to block incoming sensory stimuli (via the brain stem collaterals). We suspect that the 40 cps activity reflects an excitation level of the brain and may be related to the integrative functions necessary to awareness and for processing sensory signals into something meaningful - perception.

Research Objectives

A major objective of our research is to establish a direct relationship between the responses of the experimental animal and those of man, in the appraisal of allowable concentrations of toxic substances in ambient air. We are currently designing studies involving time-limited perceptual phenomena in order to establish a correspondence between the psychophysical judgment in humans and the electrophysiological response in animals.

An additional objective is to develop and use the most appropriate methods available in order to understand the statistical aspects of neuroelectric activity. Dr. Barry L. Johnson, in our laboratory, has looked at the power spectral density of the evoked responses shown in figure 10 and his method of displaying the results seems to represent a most important advance in this area.

Neurophysiologists, with their mass of data, have been hampered to a large extent by a lack of adequate data processing facilities in their attempts to formulate general principles of behavior of the nervous system. The application of computers to biomedical problems often as part of an interdisciplinary effort, has alleviated this problem. Many other mathematical and statistical procedures have been developed as standard routines and are available for use in solving biomedical problems.

The nervous system is the body's primary data management system, and to investigate even a narrow area of this system requires high sampling rates and sophisticated computation methods. It is in this area that the most sensitive indexes of patient status may be found. It is an area which is extremely difficult to handle efficiently without utilizing applicable techniques of data processing.

ACKNOWLEDGMENT

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QUESTION: I would like to know what levels of carbon monoxide you were using.

DR. XINTARAS: We use 100 ppm for two hours. I've taken the system down to 30 ppm for two hours, and at that stage we are as confused as anyone else in trying to isolate the effect of exposure on the signals we were looking for, because it is so difficult depicting the effects of CO on evoked response. I try to look at it in terms of how it affects the total sleep pattern, because the excitation period in the evoked response is similar to the transitional period during the dream sleep. We are finding that at 100 ppm CO - again starting a little high for about a half hour exposure - the animal during this first night of sleep sleeps at a higher level than the normal animal, indicating perhaps at low levels of CO we are exciting the brain, contrary to what I would have wanted to believe.

QUESTION: Have you done any chronic studies, have you continued to watch the EEG?

DR. XINTARAS: On the lead treated animals we started six months ago. I have examined part of the data up to the four-month period. We've still got another two months. I intend keeping them for a full year. The major problem there is a full year's worth of data, five animals six hours a day with brain waves, I've already run out of space after about six months. We are interested in looking at brain waves to find if perhaps this level of lead caused the initial change and that the animal will come back to a baseline and that at necropsy the pathologists will tell us we see no morphological changes in the brain. Or I anticipate seeing some behavioral changes which I haven't seen to date, and our animals have been consuming one and a half milligrams of lead acetate per milliliter of water. I guess the rats have now been drinking maybe nine to 10 milligrams per kilogram. Incidentally, this concentration would have killed the dog a long time ago. The rats seem to be a very durable and resistant animal to lead acetate.

DR. FAIRCHILD: You know me well enough to know that I'm not trying to trick you. I'm just asking a real naive question. Because of this type of thing which you obtained with relatively slow acting barbiturate, in the 30 seconds you get this response. So then did you inject this animal with just a water vehicle or something and you found that you had a quantitative difference in the two potential curves?

DR. XINTARAS: We injected saline twice, because on a given day, when I told the technician to inject pentobarbital, somehow we got our signals crossed and the animal got a saline injection and I was a little disturbed that I didn't see the followup sleep pattern that I normally associated with the rat. We do see a little excitation in brain wave obviously when you pick up a rat and inject him IP, especially if your technique is horrible. But we've experimented with our rats to such a point now that you pick up the rat, you inject him, and everything is fine. One further point - we've given our animals pentobarbital and looked at the REM sleep component which is again that excitation component that we think varies during excitation states or sleep. We've eliminated in the sleeping animal the duration of

REM period which may go all the way from seven minutes to perhaps two-second episodes. Although durations are eliminated, in other words, no longer being sustained, but the frequency that one normally associates and the amplitude with REM sleep is still in the EEG. This is something that has always puzzled me. I think perhaps we should start another line of investigation to find out if the effect of pentobarbital doesn't involve elimination of the brain's ability to sustain a hyperactive state rather than eliminating it.

OPEN FORUM

Kenneth C. Back, Ph. D.
Aerospace Medical Research
Laboratories
Moderator

DR. BACK: I think you can all see that research on the use of psychopharmacological techniques is highly exciting. What I will try to do is to accept questions for the next 15 minutes on our portion, and then we will go back to the leftover questions from the other groups for the rest of the period. I'll start off by indicating that you have seen here this morning a number of approaches from a psychopharmacological point of view. You can use these techniques for two things; one of them is to get just plain performance data. Can an animal work in his environment and can you use this for studying dose response curves? The other thing that you can do with these techniques is to look at basic mechanisms. Now sometimes we can do both at the same time, but not always, obviously. The last three papers point toward mechanism. The kind of thing we have been doing in the domes has nothing to do with mechanisms. If the animal performs, fine; if he doesn't perform, you say he just can't react to his environment. You don't know why. In other words, the kind of behavioral work that we are doing with the monkeys in the dome is strictly an all-or-none function. Can the animal work or can't he? It has nothing to do with how this is taking place. On the other hand, you see that Drs. Geller, Xintaras, and Fairchild have been looking at basic mechanisms. Both are useful and we have to have both.

DR. FAIRCHILD (Aerojet-General Corporation): I would like to direct one question to Dr. M. Fairchild. Are the patterns obtained with hydrazine, which is acting high in the central nervous system, similar to an agent which acts much lower - such as strychnine sulphate - where you do get severe convulsions in somewhat of a different quantitative nature? Will you register similar types of patterns from some higher center?

DR. FAIRCHILD (University of California): I think this would depend a good deal on the agent involved. For instance, with UDMH itself, there is good evidence from the work of the Hine Laboratory that the reticular formation is essential for the development of these patterns of convulsions. If you section the brain serially going down, a decerebrate animal with a midcollicular cut will easily convulse with UDMH. If you cut further back so that you eliminate most of the reticular formation, the animal will not, so this fairly demonstrated that UDMH was involved at the cerebral cortex and we think, just by inference with no direct evidence, that this was a decreased inhibitory influence. Possibly these inhibitory influences are coming from reticular formation. The reticular formation not only excites, but depresses both downstream and upstream. I believe a little less is known about the upstream projections. I do know, for instance, that it will inhibit the masseteric reflex quite well, which is upstream, so I think that if we are going to try to pinpoint the locus of action of a drug at some level we would have to examine a lot of systems simultaneously. I didn't mean to infer that this action of UDMH was only at the cerebral cortex but it is certainly involved.

MR. WANDS (National Academy of Sciences): I would like to ask Dr. Fairchild if he has had any opportunity to also look at MMH?

DR. FAIRCHILD: No. In which system? For evoked potential?

MR. WANDS: Yes.

DR. FAIRCHILD: No, we just looked at UDMH. As a matter of fact, as I said, this was in the nature of an interim report and Dr. Goff has not managed to introduce any other drug and I would have hoped that he would have at least looked at metrazol for instance. From the literature, it is possible that metrazol has the same kind of an effect, at least in the thalamus and cortex; but to date, none of this has been done.

DR. BACK: As a side remark, next year's work will involve monomethylhydrazine. Unfortunately, it takes quite a bit of time. I think you can see, though, that we have to work in many different areas at the same time. We have to do the biochemistry and intermediary metabolism, we have to study performance, and it is just a large amount of work. I think that the work that Dr. Xintaras is doing will have direct application to our work in the future. This is a nice way to go for early warning. You know we are getting more and more questions about carbon monoxide in closed spaces, and this may be a good way to pick up early changes. I still don't know how sensitive it is going to be but it sounds exciting and is certainly something we must explore.

DR. GELLER (Southwest Foundation for Research and Education): I would like very much for you to further expound on the procedures you used. As we well know in drug work, the stronger the behavior that is built in the animal, and obviously you have strong behavior in the monkeys, the more difficult it is to break through with any sort of insult whether it be chemical or something else. We found this to be true many years ago when we worked with conditioned emotional response suppression techniques. We found we could blast right through our animals and attenuate this with electro convulsive shock, but when we tried it with drugs we couldn't get through. Now again, getting back to your animals, even using aversive kinds of stimulation, if the animals were not so well conditioned, or if they were conditioned to certain baselines where they weren't performing perfectly, I am sure there are many other ways in which we could make these techniques more sensitive and pick up these subtlties in effects of contaminants on behavior. Granted, you will run into problems using positive reinforcement techniques, but even in the aversive techniques there are simpler kinds of discriminations that could be used for picking up these subtlties. The DRL which I described, which is a very difficult procedure to work with, involves long hours of training, and is extremely sensitive to the slightest change in the environment of the animals; the slightest change will disrupt this. There are very simple kinds of discriminations, these go, no-go, discriminations that are also very sensitive, and I am thinking that maybe working with techniques such as these, you will be able to pick up disturbances in behavior that may well correlate with these physiological changes that you reported on.

DR. BACK: I think your point is well taken. We have a number of problems. Just to physically get the equipment located within the dome, and to get it bug-free, was quite a chore; in fact, we've just now gotten it bug-free by reprogramming the whole rack from beginning to end. Now we don't have any aberrant shocks coming

in. We did have problems with the instrumentation alone. Since we do have growing pains, further sophistication of testing will take some time. It takes people. In time we will get sophisticated enough to be able to pick up subtle changes. Again, it is a matter of philosophy. We had, for expediency sake, to get some data. We had to use what we had at the time, and it takes time to develop these things. It is not the total answer, there is no doubt about it. On the other hand, when you use positive reinforcement, certainly with drug studies - we have run our animals at Holloman by injecting things like decaborane, which as you know is a reserpine-like compound which depletes brain serotonin and catecholamines. The animals that were on positive reinforcement just went to pot in the first few hours. They just refused to eat. On the other hand, those which were negatively reinforced did indeed perform for a longer period of time and they came back again. A single dose of four milligram per kilo will knock an animal down for about 24 hours and keep him down, as far as performance is concerned, for about a week or so. We have been able also to demonstrate the difference between the continuous avoidance task and the discrete avoidance task with decaborane. This is plenty sensitive enough to show that continuous avoidance is the first thing blocked, and the next thing is discrete avoidance, and it goes in the order in which you might expect an anesthetic to go. The first thing that went was visual discrete avoidance, followed by auditory, and came back in just exactly the same way a week later. So it is sensitive to relatively high doses; but when you try to get real small subtle changes you are in trouble.

DR. COULSTON (Albany Medical College): Let me ask Dr. Geller a few questions. Dr. Geller, why do you call compounds like meprobamate a tranquilizer? The next question - drugs like pentobarbital are well known to be hypnotics, the action is well understood on what part of the brain it works. Meprobamate is understood as to where it works; librium we know where it works. They all work on all different parts of the brain; and yet this is not correlated into your program.

DR. GELLER: Let me answer the first one and try to see what we can do. I'm not quite certain I know where all these drugs do, in fact, act. I call them minor tranquilizers simply as a point of departure. They have been categorized as such by several people. The five-way classification of CNS acting drugs lists meprobamate, barbiturates. These are all nervous system depressants with different degrees of depression with relation to sedation. These drugs have been classified this way and are the drugs that are used clinically for the outpatient psychoneurotic groups as opposed to the major tranquilizers, such as phenothiazine or reserpine, which they have classed as major tranquilizers; plus several other categories, the antidepressants, the stimulants, and the hallucinogenics - this is the fivefold classification that I think was set up at one time by Wilbur Benson, et al, and also by Jonathan Cole, and this is for the purpose of expedience with some relationship to their clinical use. Now with regard to the specific activity of the mechanism where these drugs act, I'm not sure that anybody can really tell us completely the mechanism of action of a barbiturate. If you have reference to where these things act, I would be happy to hear them.

DR. COULSTON: Nobody knows exactly the mechanisms of action, but they know what part of the brain is involved. Be that as it may, I think that it is a little dangerous to call a drug like meprobamate any kind of a tranquilizer. I wouldn't want to stick my finger into the mouth of a monkey that received meprobamate; but I would be glad to do it in one that got chlorpromazine which is certainly a

tranquilizer. The point at issue is that to be a tranquilizer it has to be a compound in which the animal is relatively alert, but his behavioral pattern is changed.

DR. GELLER: That is absolutely correct. I can show you this with meprobamate. I'm not selling meprobamate, don't misunderstand me. You can give meprobamate at a dose range that does not debilitate the animal. This is why it is different from the barbiturates. In order to get the desired clinical effect, you must give the person a dose of the barbiturate where he cannot function in his everyday task.

DR. COULSTON: That's my very point, thank you. This is what I have been trying to get at.

DR. GELLER: I thought I had pointed this out in the discussion. Possibly I had not. This is the advantage of these drugs.

DR. BACK: This is highly exciting and I'd like to stay on it for quite some time, but we must go back and pick up where we left off yesterday with some general questions for all speakers.

"Because the problems of lung disease have caused problems of interpretation of some experiments, have you given consideration to using germ-free rodents or dogs derived by surgery from pregnant dogs? The large chambers used could be easily modified for nearly germ-free environment. Have you considered the use of the gerbil which has clean lungs?"

I'd like to start out myself with portions of this: we considered using germ-free rodents and dogs, but it is highly expensive for one thing; and number two, we are not quite set up to handle them either inside the domes or outside the domes because even if we had started out with germ-free animals we have to enter the domes daily to feed, clean, and manipulate, so I don't know how you are going to keep them germ-free for very long under these conditions. It is almost a physical impossibility. The gerbil, incidentally, we have not used because we have very little background data on the species. On most of the work we've done we've had to start out with animals that we knew something about, and we have very little data on the respiration of the gerbil, as far as I know. Maybe our vet officers can help me out on that? Do any of the vet officers have any idea about how we could go about using germ-free animals? Now pathogen-free, that's a different thing, and we are strongly entertaining the idea of going to pathogen-free, if we can ever get enough space to do it. Are there any comments?

DR. KAPLAN (Aerospace Medical Research Laboratories): This is almost beginning to sound like what we talked about yesterday for so long. I don't think, to be specific for a minute, that we want to use a germ-free or true notobiotic situation, at least in investigating the effects of oxygen on the lung. I think with the kind of pathology that you get with oxygen, there are certainly a number of other irritants in the lung; the interaction of the changes that occur in the pulmonary architecture, mucous secretion, cellular activity, and all of these things, the interaction between these changes and what happens with the normal flora probably play a large role in the outcome of what is happening in the animal's lungs. And I think if you start introducing animals that are truly germ-free, that don't have normal flora, you are going to end up with results that are useless, because I don't

think you are going to be able to apply them at all to any kind of a real situation. Now when you talk about trying to get pathogen-free animals so that you cut down on the incidence of murine pneumonia that's something else, but I think we talked about that yesterday.

DR. COULSTON: I agree with Dr. Kaplan in what he is saying, but if you want to do germ-free studies there are mechanisms that you could use. There are plastic tents available with glove sleeves that you put your hand in, or the use of the Cambridge Filter where you keep bacteria out. It's not expensive to do and it would be relatively simple. I think you could do it if you wanted to. I'm not so sure that you want to do it.

DR. BACK: No, I don't really want to do it.

DR. THOMAS: There would be no problem for studying the basic atmospheres that way, but if we add contaminants I don't know if we could get uniform concentrations under these tents. This might be a real problem for a specific contaminant. But for a basic synthetic atmosphere, I think it could be done.

We have here a philosophical question which I would like Dr. MacEwen to answer. It says that in toxicology the concentration of the contaminant is usually given in parts per million. This unit is dependent on earth equivalent atmospheric pressure and cannot be applied to a space cabin with subatmospheric pressure. A concentration of 3 ppm on earth would be 1 ppm at 5 PSIA; therefore the unit of ppm is misleading if the toxic effect is in most cases independent of the presence of other gases as long as it is a life-supporting environment. In order to be able to use the available information with minimal correction, the common concentration values must be converted into volume units.

DR. BACK: You will recognize that I gave all of our concentrations in milligrams per cubic meter, and it doesn't make any difference whether you are at altitude or on the ground, it is the same number of milligrams per cubic meter volume of space.

DR. THOMAS: Since the speaker identified himself, he can discuss it.

FROM THE FLOOR (not Dr. MacEwen): What we would like to do here is determine the volume equivalent for one part per million so that this is independent of the presence or absence of any other gas. If you use milligrams per cubic meter we have the same problem as the pharmacologists have now to convert into equivalents per liter. This corresponds, really, to the pharmacological unit of milliequivalents per liter; therefore, we would like to suggest to you, to use this unit or a similar one for conditions where we want to use varying atmospheric pressures.

DR. MAC EWEN (Aerojet-General Corporation): We went through this problem philosophically three years ago in the problem of applying parts per million directly to different atmospheric pressures. It is true that when you reduce your pressure to 5 PSIA, you are expanding your gas threefold, and you do change the part per million concept, but if you use the other standard term used by toxicologists and industrial hygienists, of milligrams per cubic meter, this applies in both cases and is fairly uniform. I see no particularly good reason at this point to introduce a new and confusing term into this area of toxicology and threshold limit values.

FROM THE FLOOR: I discussed this problem with Dr. Stokinger too, and he felt that it might be desirable to use the unit according to the toxic effects of the contaminants. If we have any additive actions of different compounds, we can use those units and we have a meaningful unit, the same as the pharmacologist has switched over to a more meaningful unit too.

DR. MAC EWEN: Milligram per cubic meter is strictly a matter of a weight per unit volume, and we do not consider this as volume of air but volume of the space which the milligrams are occupying. In essence, the lung sees the same number of molecules at either atmospheric pressure or any other pressure, if you use this milligram per cubic meter concept rather than parts per million.

DR. THOMAS: I would like to add to that. I think it is a real big help to use a familiar scale with which all of our industrial hygiene and physicians and everybody who works in occupational medicine and so forth are familiar. Milligrams per cubic meter gives us the backdrop, the reference. If somebody decides the level of 0.2 milligrams of ozone was toxic or not toxic at altitude, it immediately clicks in your mind, that this concentration is equivalent to what we use for an eight-hour TLV. I think we would have to reeducate everybody in the next 10 years and give everybody slide rules to recalculate all these standard reference values (the TLV's) or start publishing them in this new fashion.

FROM THE FLOOR: Dr. Thomas, I would just like to reemphasize some of the points that you were making just at the moment. We have a multidiscipline group here and our chemists have finally switched over and started reporting to you in units of milligrams per cubic meter, and if we have to start over again, we are going to be very confused among the various groups.

DR. BACK: The chemists have been using millimoles per cubic meter; that's the way I got most of my stuff back about five years ago and then I had to convert millimoles per cubic meter back to milligrams and back to parts per million. It becomes very confusing - I make at least five mistakes in every step.

FROM THE FLOOR: In our laboratory we have to go through this about once every three months. We sit down and go through all the conversions again to make sure that we are all talking about the same thing. However, I do think that one point that we should continue to emphasize is: where we are talking about this conversion in milligrams per cubic meter, due to the various types of instruments being used, the various pressures used, or changes in pressures during transfer of samples or before taking the measurements, we should state the measure, that we are measuring at 21.1C and 760 mm Hg, or whether we have obtained a cubic meter at a different place - how the sample was handled and then correct back for any pressure effects on the volume of the original sample.

DR. BACK: That's right. We continuously do this both on introducing the contaminant and in getting it back out again. We have this manipulation going both directions.

"There is a rumor that people have been exposed continuously to a near saturated atmosphere of ethylene glycol at the school in Aerospace Medicine at Brooks. Is this true and if so does anyone know the results? The second part of the question: in view of recent work on ethylene glycol, what is an acceptable level

for continuous two-week exposure?" I don't know where the rumor came from. I'm not aware of it. How about you, Dr. Bitter, are you aware of it?

DR. BITTER (USAF School of Aerospace Medicine): No. In answer to your question, I have no answer to it, and I suggest that if there are any answers Jim Conkle would possibly know about them. I hate to put this on your back. You don't know anything either?

MR. CONKLE (USAF School of Aerospace Medicine): I don't think it is true. It hasn't reached our building yet.

DR. BACK: If it were true, we would have heard about it. I don't believe it is true. Now, what about the acceptable levels for continuous two-week exposure? Dr. Fairchild, I can't remember what we recommended in our letter. Do you remember? Did we pick a level?

DR. FAIRCHILD: As far as I know we didn't pick a level. I think that because of aborting the whole run, we didn't come to realize many of our objectives and we felt that we were going to be sticking our neck way out trying to come through with a level.

DR. BACK: There is one other thing here. As you can see, we gave our monkeys a 275 milligrams per cubic meter dose, and we had to make a recommendation even before we did the work, so we had to make a "guesstimate". We predicted that we would see no toxicological change in rodents or monkeys or rats and, in fact, we didn't see very much of a toxicological change. The literature indicates that animals are just not as susceptible to ethylene glycol as humans are, and it sure looks that way. We predicted we would get relatively little effect and we did get relatively little effect. Since you can't induce too much toxicity in animals by inhalation, man must be ten to a hundred times more susceptible to the acute effects. I would put almost a zero tolerance on it. There should be none around, especially in view of the clinical data. The exposed women were intoxicated by relatively small doses. Ralph, do you have any comment?

MR. WANDS: I think the only thing I might say is that this question was referred to the Committee on Toxicology of the National Research Council and they came up with exactly the same comment you just made. In view of the existing literature and the real paucity of hard data, it was necessary, just in common sense of conservatism, not to recommend exposing people to any of this coolant if it could be avoided by an engineering procedure. This was one of the reasons, of course, why you people were asked to try to get some hard data; and again the problems that you ran into are quite obvious. I still think that this is a worthwhile thing. As far as the accidental exposure at SAM was concerned, this must be rumor, we've not heard anything about it. I would question whether it would be a very real situation again in view of the very low vapor pressure of ethylene glycol.

DR. BACK: We recommended going to another compound, propylene glycol rather than ethylene. If the system would tolerate a change from a physical-chemical point of view, we recommended substitution.

QUESTION: Would you clarify the positive application of standard statistical tests, that is t-Tests, and analysis of various correlation coefficients? Since you emphasized mainly the incorrect applications of statistical measures, I wonder

just how you define probability in general. Do you agree that statistics are to be used primarily to support conclusions, rather than form a basis for drawing conclusions?

DR. PIERSON (Lockheed Aircraft Corporation): Working backwards, first of all, the purpose of research as I understand it, is to uncover a truth. It is not to substantiate a preconceived conclusion or to draw conclusions from any particular set of data. Anything goes that aids you in understanding a given truth, whether it be experience, statistical manipulation, or witchcraft, as long as you get at a truth. This is the purpose of research. Consequently, you would not use statistics, as far as I can see, to substantiate any preconceived conclusions. I'm really not a statistician, I'm a physiologist, but unfortunately most of my work has been done with humans, so I can't draw conclusions for mice, beagles, rabbits, and chickens; but I would gather that the principles would be the same for animal investigation as for human investigation. Now we go one step back, and what was the question prior to that?

QUESTION: Clarify the positive application of standard statistical tests; your emphasis was mainly on the incorrect application of statistical measures. I wonder just how you define probability in general?

DR. PIERSON: Well, the standard statistical tests have been devised by mathematicians primarily, some by agronomists. But they have basic assumptions; and if these basic assumptions are met, then the test is valid. If the assumptions are not met, the test may be valid or it may not, but there is no way to determine. Now if you take a standard test such as the t-Test, and if you compare two related samples using a t-Test for independent samples, I would be very suspicious of the validity of the conclusions drawn from such an analysis. Right offhand, I think there are approximately 28 ways of using a t-Test and there are approximately 28 different formulas for a t-Test, including about three that were drawn by Student himself.

Now other tests are not as rigorous as the t-Test. The analysis of variance, for instance, has been shown to have a very wide range of sample distributions. Lindquist ran a hundred thousand IBM cards with a different distribution on each card. He compared the rigorous assumptions of the analysis of variance with skewed leptokurtic and platykurtic data. Unfortunately, he sets no limits. He just says it is not as rigorous a test as previously believed. So the test may be good, it may not be good; but you do not know unless you are sure you are meeting at least most of the basic assumptions. Again, I would like to repeat, you cannot add the numbers of a football team and say that the average football player is a tackle with a standard deviation of a quarterback; it just is not done - it can't be done. Is there any other part of that that has been left out?

DR. BACK: I think not.

QUESTION: Do you have any other comment as to the use of his statistics in general?

DR. BACK: I use them as a betting process. I'm betting that 90 percent or 95 percent of the time I'm going to get the same results the next time I do it. It is as simple as that, and if my data are homogeneous and they are not biased in some

certain way, I am going to be able to repeat my experiment within reason the next time I do it. We belabor statistics to death I think - many times you don't need statistics. There is no standard deviation in a monkey like Bugs. I don't need 50 thousand determinations to show that Bugs never moved from his left lever presses to his right lever presses as long as we used him under these conditions. I don't need statistics to prove that it is there; it is a straight line and there is no variation, very little variation. Now if all of a sudden he started taking lots of shocks, I don't need any statistics there, and if they are so subtle that you have to maneuver with any one of 16 million statistical tests, then there isn't very much there; and if you do do it you are going to repeat the experiment anyhow because it was too subtle to prove.

FROM THE FLOOR: As long as we are attempting to get at the truth, any tool is a good tool.

SESSION VI

LIFE SUPPORT SYSTEMS

Chairman

Dr. Toby Freedman
North American Aviation, Inc.
El Segundo, California

THE ATMOSPHERIC TRACE CONTAMINANT PATTERN IN SEALAB II

R. A. Saunders
M. E. Umstead
W. D. Smith
and
R. H. Gammon

Naval Research Laboratory
Washington, D. C.

The second of the SEALAB series of experiments has been completed and preparations are already underway for the third, which will take place late this fall off San Clemente Island, California. As you know, one of the multitude of tasks in these complex experiments is to determine the atmospheric contamination pattern in SEALAB. Two different sampling methods and follow-up analytical procedures are used to gain this information. Each has its limitations, but a combination of the two gives a pretty good overall picture.

One method is the direct chromatographic analyses of small samples of whole air (100 ml to 1 liter) taken from the SEALAB atmosphere without recourse to concentrating techniques. Analyses of this type are usually made at the scene to monitor the concentration of a few known contaminants of particular interest and importance. Whole air samples may also be taken for subsequent analyses in the laboratory and these provide, also by direct chromatographic examination, more detailed qualitative and quantitative information regarding the more concentrated trace contaminants.

Chromatograms of small whole air samples taken from closed environmental atmospheres typically display some 10 to 15 peaks representing perhaps 20 to 40 different contaminants. For any given set of column conditions there are a number of compounds which have identical retention times, hence in the gas-phase chromatographic separation of any mixture which comprises a wide range of compounds, there will be instances where some of the components of the mixture are not separated from others. Unfortunately, this fact diminishes the usefulness of the gas chromatograph as a positive means of identifying the constituents of such mixtures. True, the accuracy of identification can be increased by resolving the mixture on several different columns, but at the expense of a considerable increase in analytical complexity.

A more reliable technique is to recover the material eluted from the column during the time interval of interest and to obtain its infrared or mass spectrum, which is a positive method of identification for most compounds. Once the components

have been correctly identified, quantitative data can be obtained from the chromatogram. Even with this technique, however, the small size of the sample limits the lowest level of concentration at which contaminants can be detected. So, logically, a second method of analyzing the SEALAB atmosphere is to concentrate the contaminants by separating them from the large volume of air in which they are diluted. Cryogenic sampling is obviously not a feasible method for the SEALAB situation. But a practical alternative is to pass the air through a suitable adsorbent, such as activated charcoal, from which the contaminants can be recovered later. The big advantage of activated charcoal sampling, besides its simplicity and convenience, is that it permits qualitative identification of a large number of the less concentrated contaminants. Of course, the price one pays for all this valuable qualitative information is that, without calibration at least, the method is not suited to the production of quantitative data. Another disadvantage is that there may be contaminants in the atmosphere which are so tenaciously adsorbed on the charcoal that they cannot be recovered.

The SEALAB atmosphere was sampled using both of these methods. The whole air samples were analyzed by Merle Umstead and Walter Smith of the Naval Research Laboratory. These samples were taken in stainless steel gas bottles of 1.5 liter capacity at STP. The bottles were evacuated, sent down to SEALAB at a depth of 205 feet, and filled to ambient pressure by merely opening and then closing the valves. So after having been returned to the surface, the bottles contained about six atmospheres of positive pressure. Samples of the SEALAB atmosphere were taken daily at about the same hour each morning and analyzed shortly thereafter. Samples were analyzed on the surface support vessel which was moored almost directly above the submerged SEALAB. Selected samples spaced at intervals over the SEALAB operation were also retained for later more detailed analyses at the Naval Research Laboratory.

A Beckman GC-2A gas chromatograph equipped with a hydrogen flame detector was used aboard the support vessel. Two columns were employed, one packed with squalene and the other with polyethylene glycol 400. Both columns were operated at about 80 C. Each sample was chromatographed through both columns. The nonpolar squalene provided a general picture of the overall atmospheric contamination and separated the hydrocarbons present in higher quantities. The polar polyethylene glycol was useful for separating aromatic hydrocarbons and polar compounds from the other hydrocarbons present. Comparison of relative retention volumes through the two columns provided information as to the nature of the contaminants present, and made possible tentative identification of the major contaminants detected.

Analysis of the chromatograms of the whole air samples showed that the principal contaminants in the SEALAB II atmosphere consisted of hydrocarbons. These hydrocarbons ranged from methane up through those containing nine or 10 carbon atoms. They had an average molecular weight of about 100-120. Individual compounds tentatively identified aboard the support vessel included methane, cyclopentane, methylcyclopentane, n-hexane, cyclohexane, methylcyclohexane, n-heptane, n-octane, n-nonane, toluene, ethylbenzene, and o-, m-, and p-xylene. Ethyl alcohol was not unexpected, since it was used in SEALAB by the medical department.

The concentration of methane in these whole gas samples varied in an interesting way. This variation is shown in figure 1. There were three teams of divers of 10 men each which participated in the SEALAB II experiment. During the time the first team occupied SEALAB, the methane concentration built up fairly rapidly and then leveled off at an almost constant value for the last several days.

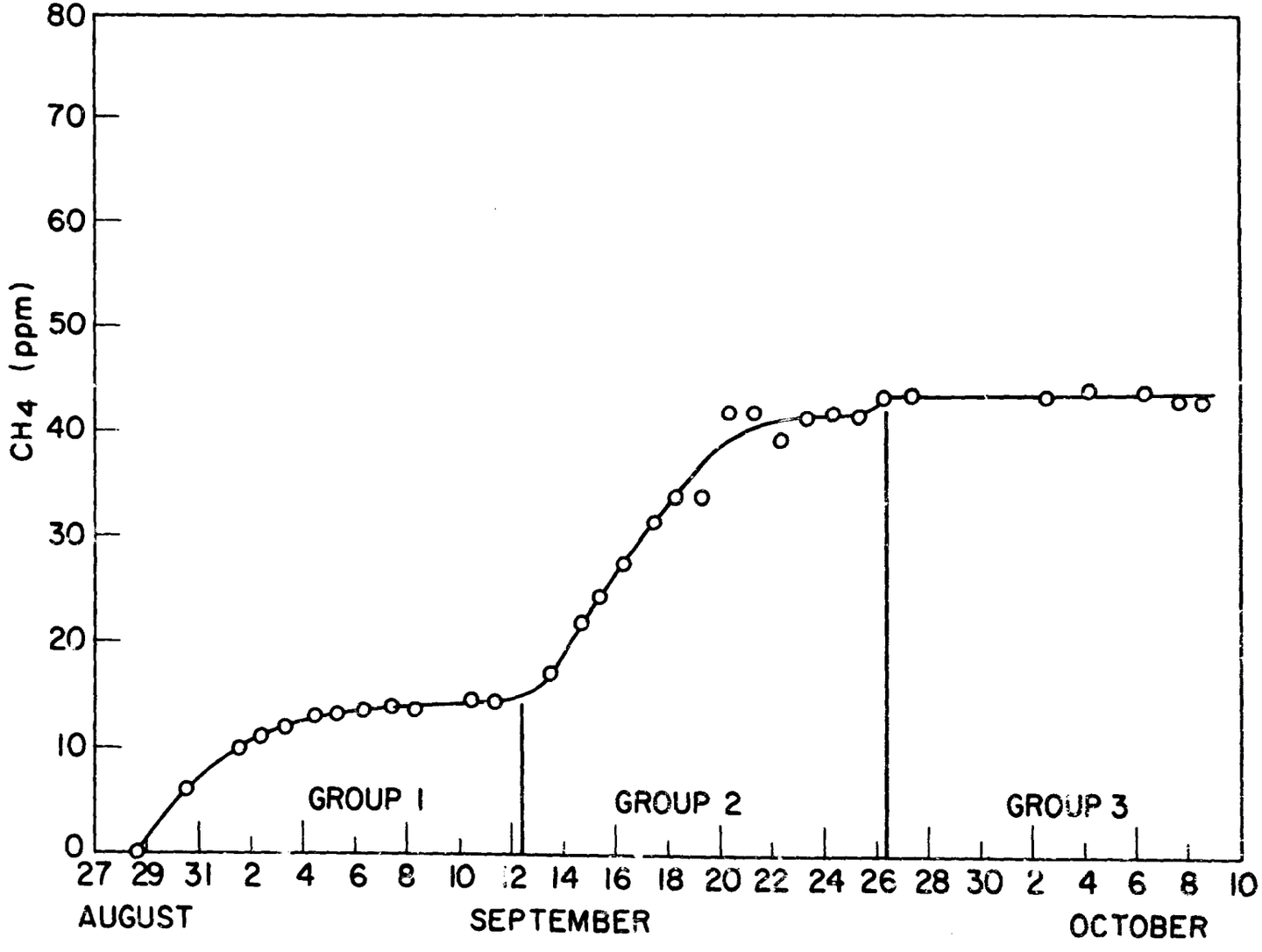

Figure 1. METHANE CONCENTRATION IN SEALAB II

At the end of the fifteenth day the first crew returned to the surface and the second crew took up residence in SEALAB. The methane concentration increased from the previous level and again stabilized at a higher value. The data are a little scattered at this point, but it does look as if there were another slight increase and leveling off when the third crew took over. Sorry to whet your curiosity but we prefer not to offer an explanation of these data at present.

A plot of the carbon monoxide concentration is shown in figure 2. These data happen to be based on laboratory measurements of whole air samples, but the CO concentration was also monitored at the scene. The concentration increased linearly for the first 15 days, which is not surprising. Extrapolation of the data for the first two weeks indicated the concentration would soon be above the desired upper limit, so the second team tried to slow the trend, mainly by involuntarily sacrificing their broiled steaks. Unfortunately, the air samples from the second two-week period were lost. The CO concentration was obviously brought under control, however, because during the occupancy of the third team, we see it is declining nicely. Hopcalite canisters at ambient temperature were placed on stream during this period, apparently without producing any significant effect.

As for the other contaminants - they were practically all hydrocarbons - the concentrations increased gradually but steadily for the first 10 days of the operation. The reason for this increase is interesting. Activated charcoal was used in the SEALAB air purification system, since it is an efficient means of controlling the concentration of trace organic contaminants. However, the charcoal in the main filter, which was scheduled for replacement at the beginning of the experiment and every 10 days thereafter, was actually only changed once. The change took place 10 days after SEALAB submerged. The original charge of charcoal in SEALAB had been in the system during construction and outfitting and was probably well loaded with volatile products from construction materials.

Although activated carbon can be an extremely efficient means of organic contaminant removal, care is required for its effective utilization. A carbon bed can even serve as a source of atmospheric contamination if improperly used. In previous work with nuclear submarines, it was found that if carbon had been installed during a construction or upkeep period, it rapidly became loaded with solvents from materials of construction, such as cleaning solvents, paints, and adhesives. If the ship was then sealed without changing the carbon, volatile substances were eluted from the carbon and contaminated the atmosphere.

This was undoubtedly the situation with SEALAB. The initial charcoal, installed during construction, may have been a primary source of atmospheric contaminants. After the charcoal was replaced on the tenth day, the concentration of the atmospheric trace contaminants quickly fell by more than a factor of 10. Although the contaminant concentration increased gradually during the remaining 35 days of the submergence, it never again approached the higher levels found prior to the installation of fresh carbon. The kind of contaminants present, however, remained the same. All this points out the importance of charging those environmental control systems, using it with fresh carbon immediately prior to the operation of any manned closed atmosphere system.

The analytical charcoal sample was taken on the thirtieth day of submergence - long after the main carbon filter had been changed. The contaminants detected in the desorbate from the analytical charcoal, therefore, were the volatile components

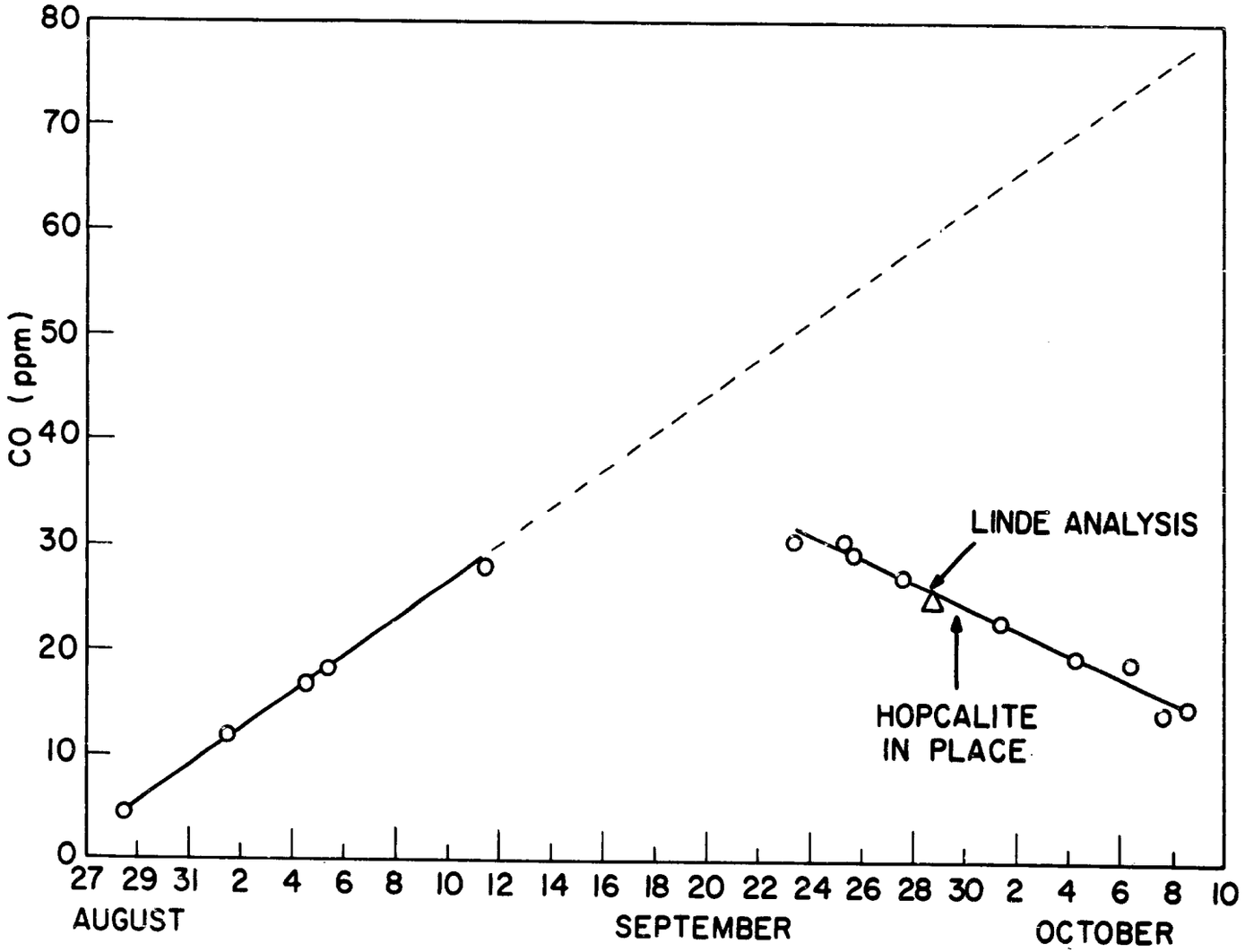

Figure 2. CARBON MONOXIDE CONCENTRATION IN SEALAB II

actually off-gassed during submergence from material sources aboard SEALAB and did not include any contaminant material which originated during the construction period.

The atmosphere sample was taken with the air contaminant sampler shown in figure 3. This device is five inches high and consists of a blower unit and a metal cartridge of adsorbent which screws into the top. The cartridge and blower unit are hermetically sealed from the motor. The flow rate through the cartridge is 1-1/2 CFM. This particular cartridge contained 40 grams of specially purified activated charcoal.

The desorbate from this cartridge was examined with the combination gas chromatograph - rapid-scanning mass spectrometer that Dr. Saalfeld described. The chromatogram of the whole desorbate is shown in figure 4. As is the case with most manned systems, the major contaminant recovered was carbon dioxide.

The amount of sample one can introduce into a gas chromatograph is limited and if one component, such as carbon dioxide, is a thousand times or better more concentrated than the others, the trace components may hardly be apparent. Such is the case here. But if we reduce the concentration of carbon dioxide by passing the sample through a small plug of lithium hydroxide, for example, we get a chromatogram like that in figure 5, whereon the peaks due to the other components are very much enhanced.

Only the more concentrated components on this chromatograph are labeled, but all of these peaks have been identified. Some of them, of course, represent more than one compound. All of the compounds previously identified in the whole air samples were also detected in the charcoal desorbate, except for a few which had unfavorable retention times. Of course, a good many additional compounds were also identified in the charcoal desorbate. A list of the contaminants in the SEALAB II atmosphere is given in table I.

The outstanding feature of this atmosphere is the fact that three or four of the more concentrated trace organic contaminants are C_5 through C_7 cycloalkanes. A few of these compounds were detected in SEALAB I and they are also present in some submarine atmospheres, but this is the first time they have been observed as dominant trace contaminants. Their probable source, therefore, is a matter of some interest.

Two possible sources which come to mind almost immediately are painted surfaces and the adhesive used for applying cork insulation to SEALAB's interior. The paint solvents used in SEALAB were regular mineral spirits which are rich (40% by volume) in cyclic hydrocarbons. However, the boiling range usually specified for such solvents, 150 C - 210 C typically, would presumably preclude all but traces of cyclic aliphatic hydrocarbons below C_8. A chromatogram of the lighter volatiles stripped at room temperature from a sample of mineral spirits (taken from the Navy Supply System but of unknown prime source) not surprisingly failed to show the presence of compounds in this molecular weight range. California crude oils are known to contain a higher proportion of cyclic hydrocarbons than other crudes, however, and it has been suggested that paint thinners derived from such oils would contain more aliphatic cyclic compounds than a similar product derived from an oil of different origin. While this is undoubtedly true, such a solvent would be subject to the same boiling range restrictions and, likewise, should contain no

Figure 3. ACTIVATED CHARCOAL AIR CONTAMINANT SAMPLER

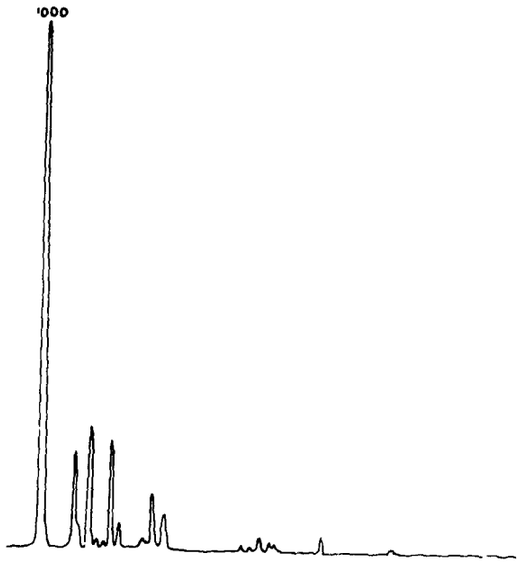

Figure 4. CHROMATOGRAM OF SEALAB II CHARCOAL DESORBATE

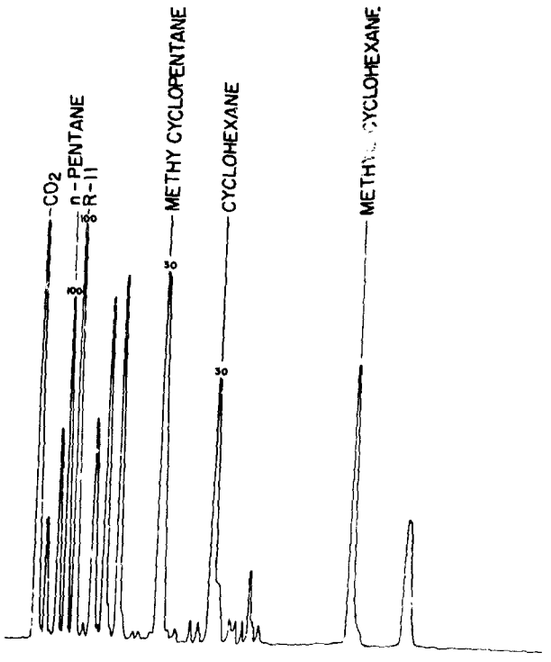

Figure 5. CHROMATOGRAM OF SEALAB II DESORBATE AFTER REDUCING THE CONCENTRATION OF CARBON DIOXIDE

TABLE I

CONTAMINANTS DETECTED IN THE ATMOSPHERE OF SEALAB II

	Contaminant	Detected in	
		Charcoal Desorbate	Whole Air
1.	Carbon monoxide	(a)	x
2.	Carbon dioxide	x	x
3.	Ethyl alcohol		x
4.	Acetone	x	
5.	Trichlorofluoromethane (refrigerant 11)	x	
6.	Dichlorodifluoromethane (refrigerant 12)	x	
7.	Difluorochloromethane (refrigerant 22)	x	
8.	Methylene chloride	x	
9.	Vinyl chloride	x	
10.	Vinylidene chloride	x	
11.	Methane	(a)	x
12.	n-Butane	x	
13.	n-Pentane	x	
14.	n-Hexane	x	x
15.	n-Heptane	x	x
16.	n-Octane	(b)	x
17.	n-Nonane	(b)	x
18.	Isobutane	x	
19.	Isopentane	x	
20.	Isohexane	x	
21.	Isoheptane	x	
22.	3-Methylpentane	x	
23.	2, 2-Dimethylpentane	x	
24.	2, 3-Dimethylpentane	x	
25.	2, 4-Dimethylpentane	x	
26.	Isoprene	x	
27.	3, 3-Dimethylpentene-1	x	
28.	Cyclopentane	x	x
29.	Cyclohexane	x	x
30.	Methylcyclopentane	x	x
31.	Methylcyclohexane	x	x
32.	cis-1, 2-Dimethylcyclopentane	x	
33.	trans-1, 2-Dimethylcyclopentane	x	
34.	1, 1, 3-Trimethylcyclopentane	x	
35.	Benzene	x	
36.	Toluene	x	x
37.	Ethyl benzene	(b)	x
38.	o-Xylene	(b)	x
39.	m-Xylene	(b)	x
40.	p-Xylene	(b)	x

(a) Activated charcoal has a low affinity for these compounds.
(b) These compounds had an unreasonably long retention time for the column and conditions used.

cyclanes below C_8. Painted surfaces do off-gas hydrocarbons into the SEALAB atmosphere, of course, but they are compounds above C_8 in molecular weight.

The cork adhesive used in SEALAB was a one part waterproof cement based on a combination of resin, drying oil, and a suitable non-toxic solvent. The more volatile components of the solvent were stripped from a sample of the adhesive at room temperature and examined with the gas chromatograph - rapid-scanning mass spectrometer combination. The volatile portion of the solvent was found to be a complex mixture of hydrocarbons which included cyclopentane, methylcyclopentane, cyclohexane, and methylcyclohexane. The latter was one of the major components. Benzene, toluene, and the isomeric xylenes were also detected along with normal- and isohexane, normal- and isooctane, and normal nonane. Since all of these compounds were also detected in the SEALAB atmosphere, it is apparent that the cork-insulation adhesive could have been a major contributor to the SEALAB II trace contaminant pattern. The large number of hydrocarbons off-gassed from the insulation adhesive and painted surfaces effectively obscured other contaminants developed at lower concentration by less profuse sources. The SEALAB II facility will be slightly modified and used again for the SEALAB III experiment and, since the cork adhesive and paint work will have had ample time for drying, hopefully more of the contaminants from other more interesting sources will be apparent.

The contaminants in the SEALAB atmosphere were determined in two ways; by on-site and laboratory chromatographic analyses of whole air samples, and by G. C. -mass spectral identification of constituents recovered from a charcoal air-contaminant sampler. Whole air sampling permits quantitative determinations of the more concentrated contaminants. Charcoal sampling yields qualitative results, but in considerably more detail. The two methods supplement each other nicely.

In all, 40 contaminants were identified. Many of them were hydrocarbons. A few of the more concentrated were cycloalkanes. The cork-insulation adhesive has been identified as a likely source and as a probable major contributor to the SEALAB II atmosphere.

ALTERATIONS IN A CLOSED-LOOP LIFE SUPPORT SYSTEM AFTER A FIRE

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INTRODUCTION

A system for the toxicological screening of space cabin materials, specifically Apollo materials, has been in use for the past two years at the Toxic Hazards Research Unit of the Aerospace Medical Research Laboratories at Wright-Patterson Air Force Base. At 0817 hours on 24 February 1966 a fire occurred in the portion of the system enclosed by a Thomas Dome. This paper presents a detailed description of the fire, its causes, the resultant damage to the system, and the consequent modifications.

I. Description of System

At the time of the fire, the total system consisted of the following major components:

1. Animal Exposure Chambers (three) (figure 1)
2. Environmental Support Console (figure 2)
3. System Control Panel (figure 3)
4. CO_2 and O_2 Monitoring and Alarm Panel (figure 4)
5. Thomas Dome Envelope (figure 5)

II. Experimental Conditions Before Fire

The experiment in progress when the fire occurred had been initiated at 1430 hours on the preceding day, 23 February 1966. That experiment consisted of the exposure of rats and mice to the gas-off products of Apollo space cabin construction materials subjected to 155 F temperature in the presence of 100% oxygen at 5 psia pressure. Three groups of experimental rats and mice were housed in closed-loop life support systems within the dome and each group was exposed to a separate mixture of Apollo construction materials. A control group of rats and mice located on the periphery of the dome, approximately six feet above the floor of the dome, was subjected to essentially the same environmental parameters of

oxygen pressure and temperature. Shortly before the fire, it was noted that some of the rats in chamber No. 3 were wet with a solution which was causing some skin burns and eye irritation. The cause of this problem was being investigated when the fire started.

Figure 1. ANIMAL EXPOSURE CHAMBERS (3)

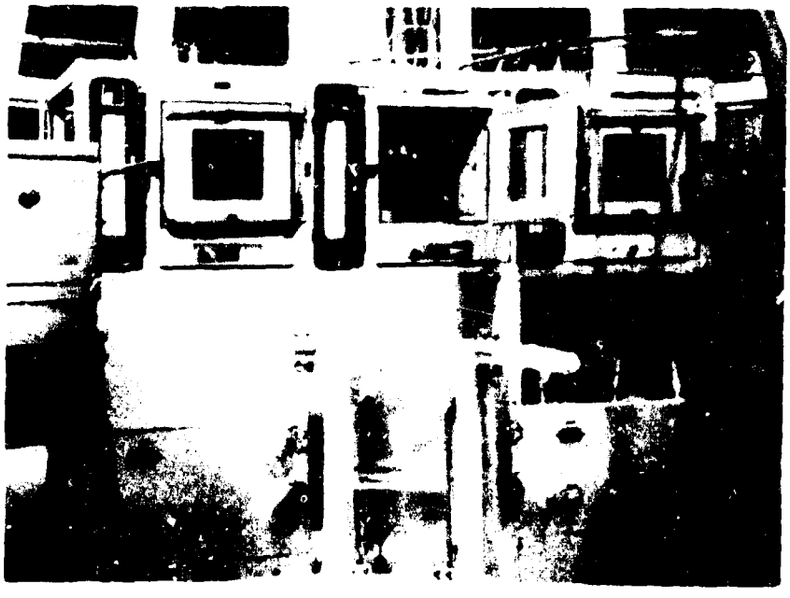

Figure 2. ENVIRONMENTAL SUPPORT CONSOLE

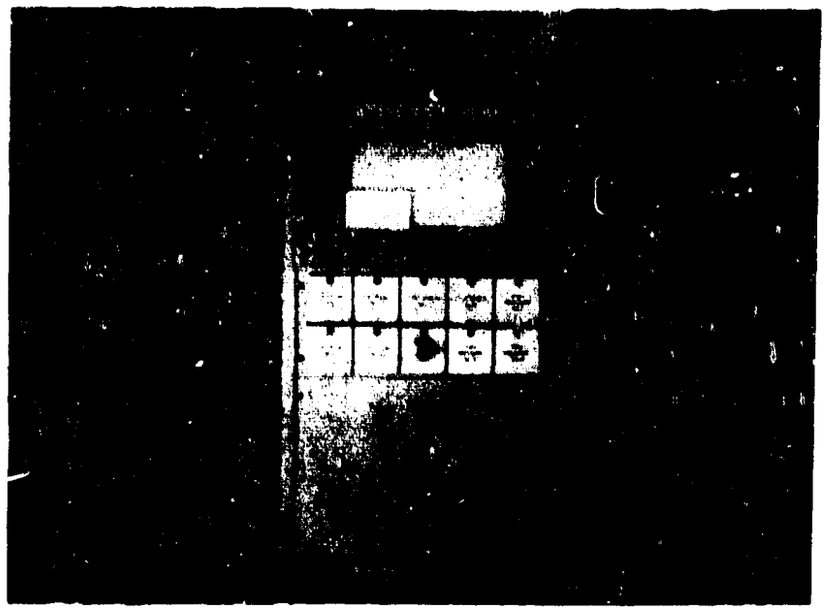

Figure 3. SYSTEM CONTROL PANEL

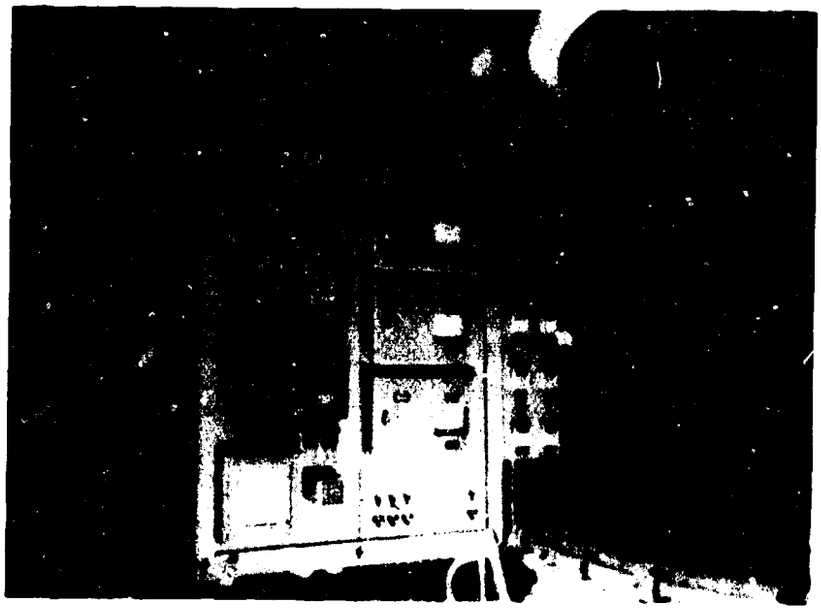

Figure 4. CO_2 AND O_2 MONITORING AND ALARM PANEL

Figure 5. THOMAS DOME ENVELOPE

III. Description of Fire

The immediate cause of the fire was due to the overheating of a rubber drive belt connecting a motor and atmospheric circulation pump at one life support system (figure 6). The pump had stalled due to internal mechanical failure. The motor continued to run with the drive pulley slipping on the belt. When the belt temperature reached its ignition point in the presence of pure oxygen it started to burn.

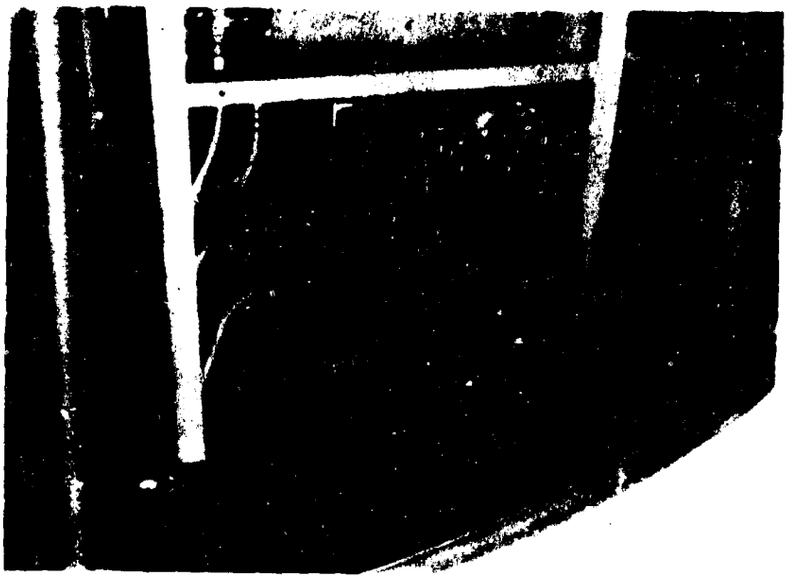

Figure 6. MODULE 3 PUMP SYSTEM AFTER FIRE

The force of the rotating pulley threw flaming pieces of laminated rubber and fiber into other portions of the Apollo environmental support console. These flaming pieces of drive belt ignited the two adjacent pump drive belts and the immediately adjacent polyvinylchloride-covered electrical cable (figure 7). The fire progressed at a slow rate from this point upward in the No. 3 module of the Apollo environmental support console and, ultimately, laterally across the top portion of the console (figure 8). The fire was extinguished approximately 20 minutes after ignition.

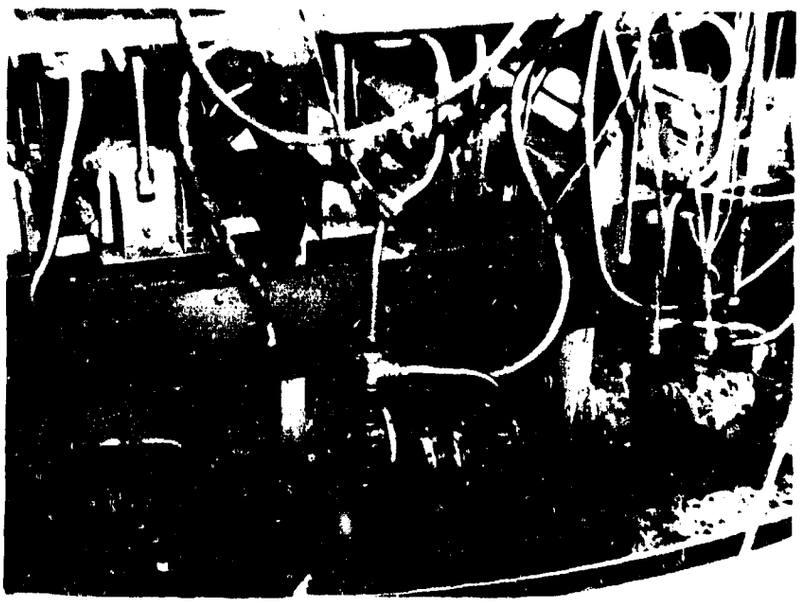

Figure 7. REAR OF ENVIRONMENTAL SUPPORT CONSOLE

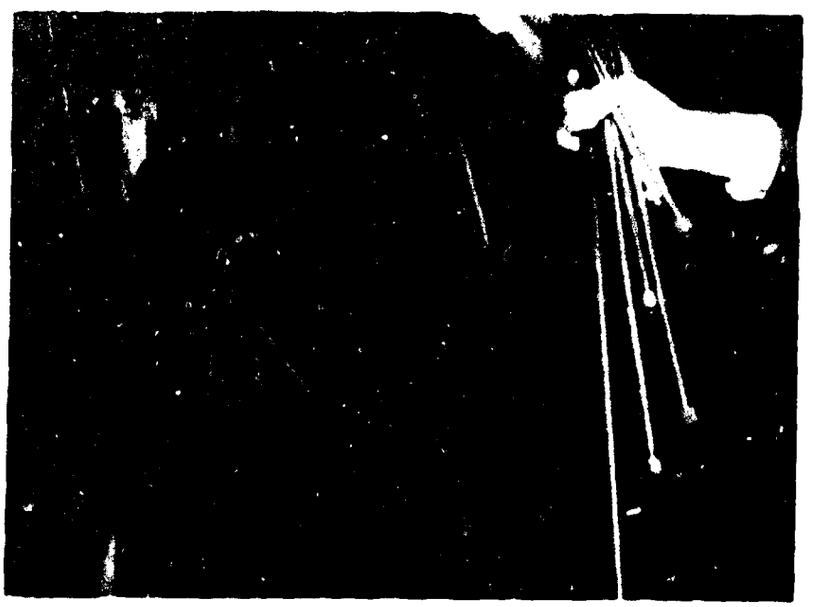

Figure 8. TOP SECTION OF ENVIRONMENTAL SUPPORT CONSOLE

Approximately 12 minutes after the fire began, the fusible linkage sprinkler head, set to operate at 135 F, opened, and the water deluge system began to wash down the sides of the dome. The water deluge did not appear to help extinguish the fire, since it was located and contained within the interior and immediately to the rear of the environmental support console. At this time, two windows were removed from the dome in order that the Wright-Patterson AFB Fire Department could enter the dome to extinguish the last portion of the fire with CO_2 hand extinguishers.

As previously stated, the immediate cause of the fire was the result of mechanical failure in one atmosphere circulation pump of a pair in module No. 3 of the Apollo environmental support console (figure 6). The reasons for the fire may be traced back to other primary causes which in turn produced the pump mechanical failure. All condensate traps on the support console were equipped with drains which required manual opening of a valve external to the Thomas Dome. This was not performed in the hours prior to the first indications of abnormal experimental conditions, as discussed in Section II. These conditions were a direct result of excessive accumulation of water in atmosphere circulation lines. This water ultimately flowed into the circulation pumps and on through the system, including the lithium hydroxide CO_2 scrubbers which are described in detail in Section VII. In the No. 3 module more water was accumulated in the condensate trap than in either of the other two modules. This may possibly have been a result of the drain system being common to all three, with the No. 3 condensate trap connected to the line last. Module No. 3 had sufficient water internally to enter the vacuum oven used for heating of the materials and resulted in a flow of lithium hydroxide solution through the air supply to the animal chamber. The accumulation of water in the circulation pump overloaded the carbon vanes, causing them to break (figure 9). The broken vanes jammed the rotor and stopped the pump. This action did not sufficiently overload the pump motor to trip its circuit breaker, consequently allowing the drive belt to continue rotation on the stationary pump pulley.

Figure 9. INTERIOR OF MODULE 3 AIR PUMPS

Immediately upon indication of the fire, all dome oxygen supply and vacuum control valves were isolated at the master control panel. Within 30 seconds after the domes were isolated, the main circuit breaker in the electrical supply to the altitude facility was opened to prevent facility power outage due to short circuiting of the burning cables. Significantly, at 0840, the other three Thomas Domes were back in normal operation, having suffered no change in pressure or oxygen concentration.

IV. Resultant Damage

The damage sustained during the 20-minute period of the fire was primarily due to the progressive combustion of approximately 71 feet of polyvinylchloride insulation on the electrical cable flexible conduit. Total damage to the Apollo testing systems material due to combustion was limited to the environmental support console in Thomas Dome No. 2. All six air pumps of the system were damaged to some degree and were returned to the manufacturer for repair (figure 10). Three of the six explosion-proof motors were damaged beyond repair. The electrical cable servicing the air pump motors and the vacuum heating ovens was damaged and had to be replaced. All auxiliary monitoring and alarm wiring was rubber and plastic insulated and was completely destroyed. In addition, all input lines such as chilled water, sampling lines, etc., were installed in a semirigid manner, as explained in detail in Section VI, and were damaged when the dome windows were removed to permit the fire-fighting squad to enter the dome.

Figure 10. DETAIL OF ENVIRONMENTAL SUPPORT CONSOLE PUMPS

V. Effect on Test Animals

The experiment in progress when the fire occurred included a total of 100 mice and 80 rats, as shown in table I. These were divided as follows: 20 rats and 25 mice as controls, and 60 rats and 75 mice as test subjects. Mortality resulting

from the fire was four rats and all 25 mice in the control cages (figure 11). All animals in the test chambers survived the fire.

TABLE I

ANIMAL MORTALITY DUE TO FIRE

	Rats	Mice
	No. Dead/Alive	No. Dead/Alive
Controls	4/16	25/0
Test	0/60	0/75

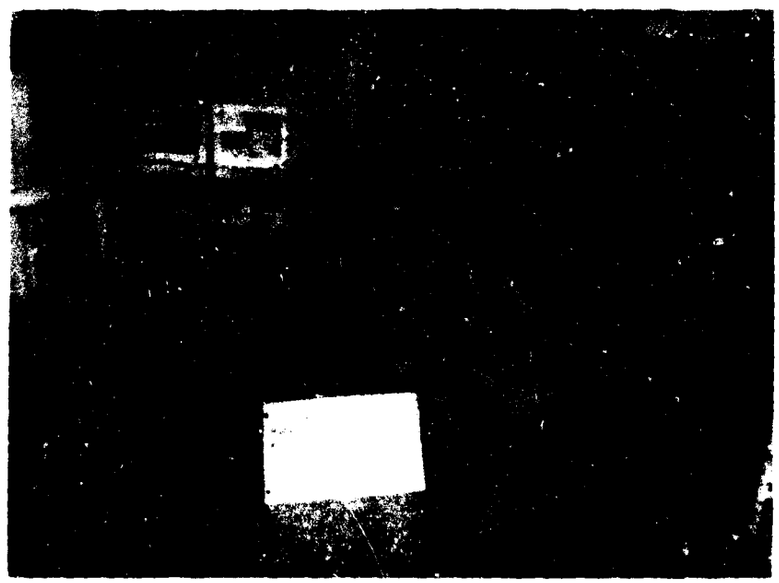

Figure 11. ANIMAL CAGES AFTER FIRE

Animal deaths were obviously due to proximity to the fire since cages were located on the periphery of the dome above the hottest portion of the fire (figure 12). There was no spreading of fire between the control cages.

VI. Areas Requiring Modification Related Specifically to Causing and Aiding the Fire

Several factors were involved in the occurrence of the fire in the Apollo materials screening system. These items are listed in table II. Each item was analyzed both individually and collectively as to its effect on the complete system.

Figure 12. LOCATION OF CONTROL CAGES IN DOME

TABLE II

AREAS REQUIRING MODIFICATION
RELATED SPECIFICALLY TO CAUSING THE FIRE

Item	Action Taken
A. Condensate Trap	Modified
B. Atmospheric Pump V-belt Drive	Replaced
C. Pump Motor Protective Circuit Breaker	Added
D. Flexible Electrical Conduit	Replaced
E. Alarm and Monitoring Wiring	Replaced
F. Penetration Plate Connections	Replaced

II A. Condensate Trap

The condensate traps in the original system were installed directly below the water chillers near the front panel of the environmental support console (figure 13). The condensate trap was connected to the chiller by 1/4-inch stainless steel tubing leading to the top of the trap. Each condensate trap was connected to a common drain line, which was

then routed to the exterior of the dome through the upper penetration plate (figure 14). A manual drain valve was located at the penetration plate with a return line installed to the exterior of the dome. On inspection of the condensate level in the trap, draining was accomplished by opening the exterior drain valve.

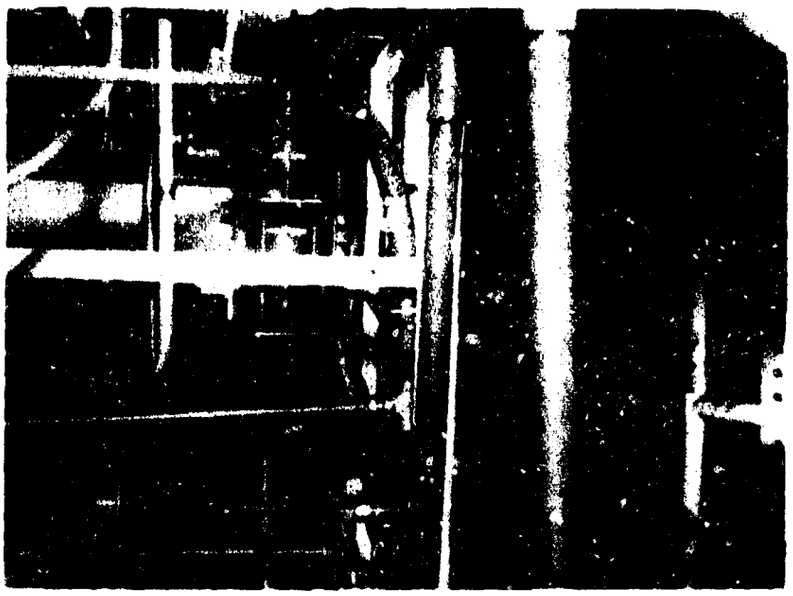

Figure 13. ORIGINAL LOCATION OF CONDENSATE TRAP

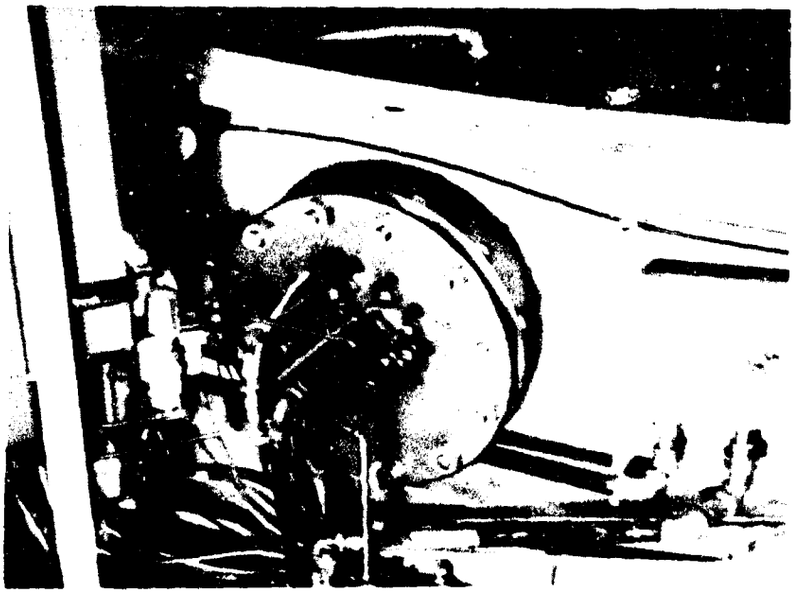

Figure 14. DOME PENETRATION PLATE

The location of the condensate trap in the interior of the environmental support console prevented adequate observation of the water level. The traps were removed from these locations and installed at the immediate rear of each module in the console (figure 15). Observation of the condensate level is now accomplished quite readily.

Figure 15. LOCATION OF MODIFIED CONDENSATE TRAP

To eliminate excessive accumulation of water due to failure to drain the condensate trap, each trap was equipped with an antisiphon drip leg (figure 15). A length of 3/4-inch stainless steel tubing approximately 12 inches long was sealed at the bottom. A 1/4-inch stainless steel line led from the bottom of the condensate trap through a stainless steel fitting at the bottom of the tube. A 1/4-inch hole was then bored into the side of the tube. This arrangement allows water to drain from the condensate trap into the drip leg and, after it attains a level of approximately 10 inches, to drain directly to the dome floor. Continuous draining of the condensate trap is thereby accomplished with no danger of excessive accumulation of water or backflow of dome atmosphere to the condensate trap.

II B. Atmospheric Pump V-belt Drive

When the air pump jammed, the electric drive motor continued to turn and drive the v-belt which continuously slipped on the stationary pump. Because the motor did not stop completely, there was insufficient overload current to

trip the protective circuit breakers and, as a result, power to the motor was not cut off. To eliminate this factor, the v-belt pulleys and belts originally installed on the pumps and motors were replaced with toothed belts and pulleys (figure 16). By using this type of drive system, any increase in mechanical load of the pump will be immediately transferred to the pump motor, resulting in a considerable increase in overload current.

Figure 16. MODIFIED PUMP DRIVE ASSEMBLY

II C. Pump Motor Protective Circuit Breaker

Electrical power to the pump motors was supplied to each set of pumps through a standard 20-ampere, single-pole, single-throw circuit breaker. An actual load test of the pump and pump motor showed that an excess of 25 amperes was required to trip the breaker. Since the normal operating current of the motors in the system is nine amperes, it was decided to add a circuit breaker to the system with a trip rating of 10 amperes (figure 17). Load testing with this circuit breaker resulted in a trip current of 11-12 amperes. Protection is achieved considerably faster and with less total current load present in the electrical wiring to the pump motors. A 1-inch red lamp assembly was installed parallel with the overload circuit breaker to provide visual indication of overload conditions.

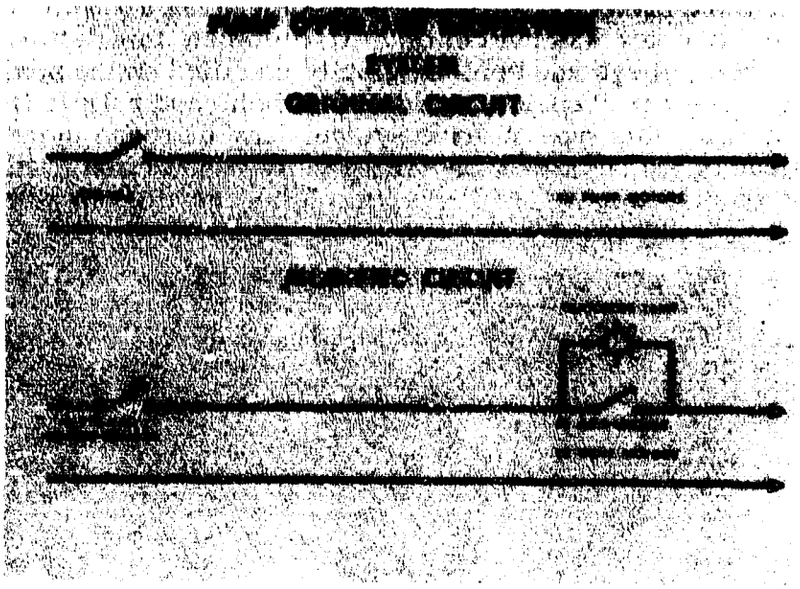

Figure 17. ORIGINAL AND MODIFIED OVERLOAD PROTECTION CIRCUITS

II D. Polyvinylchloride Flexible Electrical Conduit

All electrical power wiring in the dome was enclosed in polyvinylchloride flexible electrical conduit. This type of cable is rated fireproof under ambient conditions. In the 100% O_2 5 psia environment, it burned quite readily. A test was conducted subsequent to the fire with ignition under conditions similar to those present before the fire. Two-inch pieces of 1/2-inch diameter conduit were ignited in a small vacuum chamber and total combustion times were recorded. Figure 18 shows the unburned conduit, and after ignition in air, in 5 psia 100% O_2, and in 14.7 psia 100% O_2. When ignited in air, the material burned but would not support combustion and the fire ended rather quickly. Complete combustion resulted when the pieces were ignited in both 100% O_2 5 psia, and in 100% O_2 14.7 psia environments, with total combustion times of 222 seconds and 147 seconds, respectively. It was quite evident from these tests that use of this material provided inadequate fire protection. All electrical power lines were subsequently enclosed in rigid metal conduit, with explosion-proof junction boxes and motor fittings (figure 19). This eliminates the possibility of igniting the internal wiring if a fire starts in the dome, and will confine any fire that might possibly occur to the electrical lines themselves.

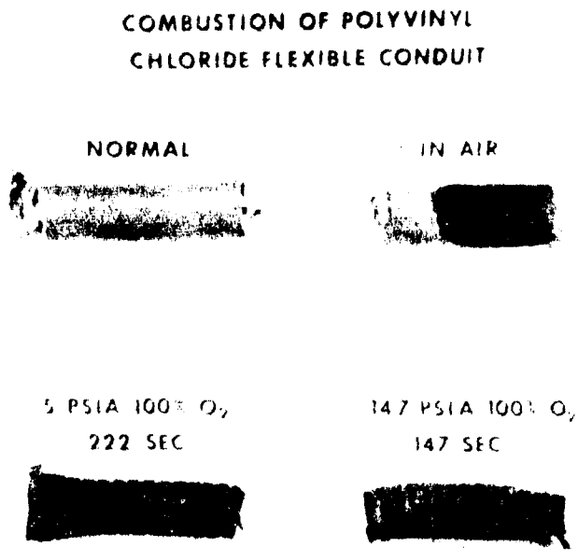

Figure 18. FLEXIBLE CONDUIT COMBUSTION TEST

Figure 19. MODIFIED ELECTRICAL POWER SYSTEM

II E. Alarm and Monitoring Wiring

There were nine two-conductor thermocouple leads, and three two-conductor alarm leads used originally in the system for temperature monitoring and pressure alarm purposes. All wiring was either rubber or plastic insulated. Combustion of the flexible conduit ignited this wiring, resulting in 95% loss. Although there was little or no possibility of ignition resulting from electrical shorts in this wiring because of the low voltages used, it was enclosed in separate rigid metal conduit to reduce the amount of combustible material which could be ignited from other sources (figure 20).

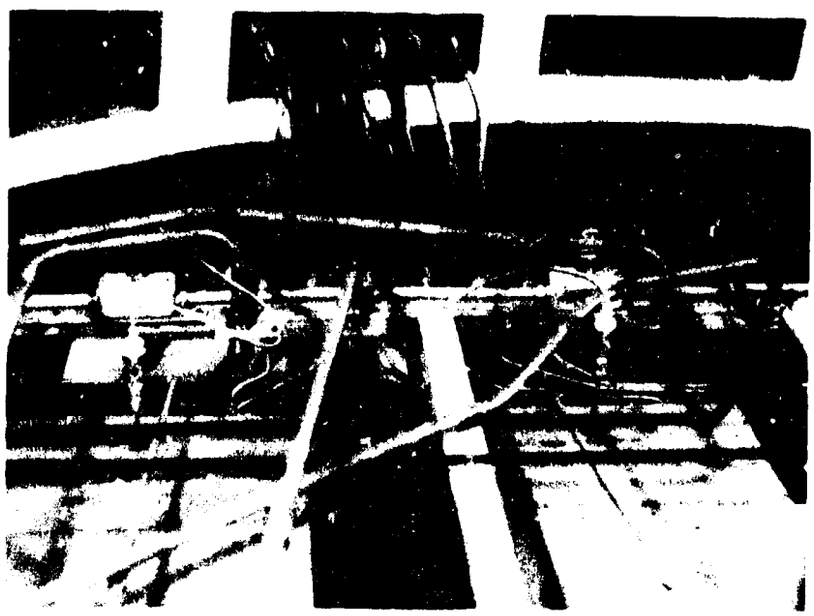

Figure 20. MODIFIED ALARM AND MONITORING SYSTEM

II F. Penetration Plate Connections

The Thomas Domes consist of a stationary bottom section with a top which may be raised using a crane (figure 5). Emergency procedures are predicated on the lifting of the dome top. One problem encountered during the fire was the difficulty in raising the top. This was attributed to the input plumbing connections from the penetration plate to the environmental support console. These lines consisted of six stainless steel tubes and two copper tubes. In case of emergency, it was assumed that the tubing would be sufficiently flexible to allow the dome top to be raised. When an attempt was made to raise the dome top, lateral pressure from these lines resulted in binding of the dome top in its guides, preventing further elevation of the top. These semirigid lines

were replaced with flexible metal braid tubing for both the electrical and plumbing inputs to the dome (figure 21). These have been in operation for several months and work very well.

Figure 21. FLEXIBLE PENETRATION PLATE CONNECTIONS

VII. Additional Modifications for Increased Operating Safety and Reliability

Although the fire damage incurred to the system was not major, it did result in destruction of approximately 80 percent of all interconnecting electrical and atmospheric supply lines, requiring a complete rewiring and plumbing of the system. Therefore, it was decided in the process of refitting the system that various other problems previously encountered but not considered critical could easily be eliminated at this time and the system redesigned for optimum performance. Table III lists the items selected for modification and improvement.

TABLE III

ADDITIONAL MODIFICATIONS FOR
INCREASED OPERATING SAFETY AND RELIABILITY

Item	Action Taken
A. Animal Chamber Waste Drain	Modified
B. Relative Humidity Monitoring System	Modified
C. LiOH Canisters	Replaced
D. Apollo Systems Control Console	Modified
E. Alarm Systems	Added

III A. Animal Chamber Waste Drain System

Due to the nature of operating conditions present when using altitude chambers, it is preferable to eliminate as much as possible the necessity for personnel to enter the chamber. As stated before, the installation of a continuous drain on the condensate trap eliminated the need for a dome entrance to drain it. During normal operations, the animal chambers in the Apollo system were equipped with two-inch drain lines with a two-inch manual drain valve. Applying similar methods as used on the condensate trap drain system (figure 22), the manual drain valve was removed and a two-inch pipe was installed in the bottom of the chamber. Cylindrical containers three inches in diameter and approximately 18 inches high were constructed. These containers were replaced under each animal chamber and the drain pipe extended to within three inches of the bottom of the container. Since the top of the container is open to the dome, continuous draining is effected to the dome floor.

Figure 22. ANIMAL CHAMBER WASTE DRAIN

III B. Relative Humidity Monitoring System

Relative humidity in the animal chambers is monitored by measuring wet-bulb, dry-bulb temperature depression. The probes for these parameters were originally installed below the floors of the animal chambers (figure 23). Erroneous readings were obtained from these locations because of the buildup of animal waste materials on the probes. The two probes were moved to a position above the test animals, eliminating any buildup of waste materials from the test

Figure 23. RELATIVE HUMIDITY PROBES

animals. In addition, the wet-bulb moisture socks were maintained in a moisturized condition by manual setting of a water valve so as to drip water onto the wet-bulb thermometer wick. With this arrangement, it was extremely difficult to achieve the optimum moisture condition for the wet-bulb thermometer. The manual water drain valve for wetting the wet-bulb wick was replaced by a continuous feed water reservoir which supplies water to the bottom of the wick, when then becomes saturated with moisture by capillary action. This arrangement worked quite well.

III C. LiOH Canisters

The LiOH canisters used in the original system, as shown in figure 24, utilized a glass envelope with O-ring seals at each end. The LiOH material was placed in a perforated stainless steel tube which was placed in the glass envelope. The procedure of filling the canisters was not only difficult but also resulted in the spreading of fine LiOH particles to the surrounding air. Another potential problem with this arrangement was the possibility of channeling of the gas flow between the internal cylinder and the glass envelope. The glass outer envelopes were replaced with stainless steel cylinders approximately four inches in diameter and 18 inches in length (figure 25). A fiber glass filter is placed at the exhaust of the canister to prevent migration of

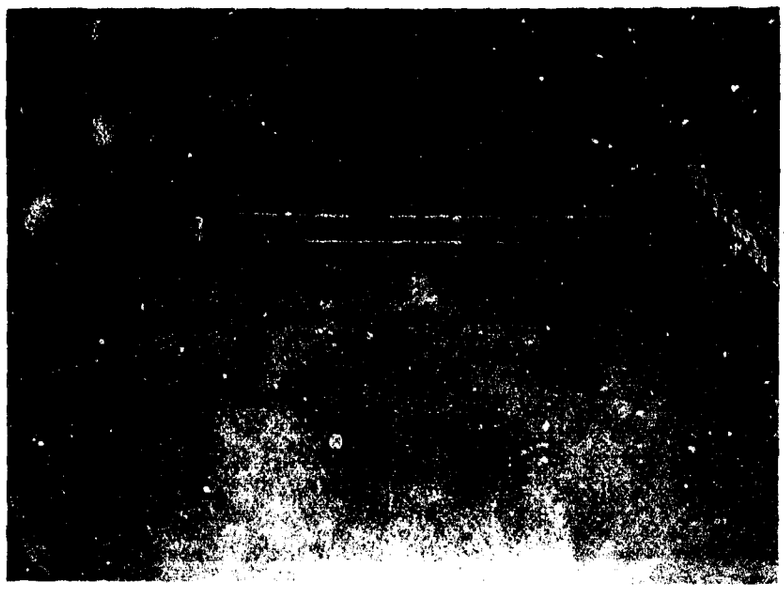

Figure 24. ORIGINAL LiOH CANISTERS

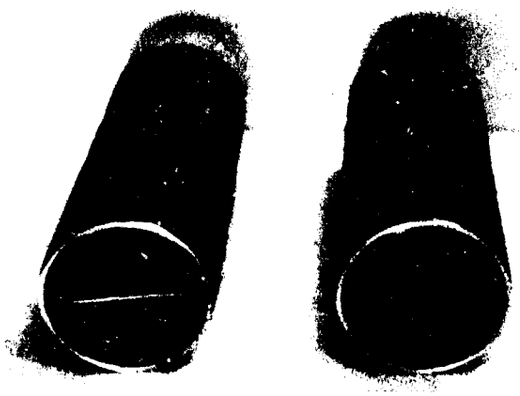

Figure 25. MODIFIED LiOH CANISTERS

fine particles of LiOH through the system. The input of the canister is fitted with a removable fine mesh screen. Replacement canisters are filled in an exhaust hood constructed for this purpose to prevent scattering of LiOH particles. The canister is now of one-piece construction and problems of air channeling around the LiOH have been eliminated.

III D. Apollo System Control Console

Several months experience in operating the Apollo system prior to the fire had revealed various aspects of the control system which showed need of improvement. It was deemed appropriate at this time to completely redesign the control console from both an operational and human engineering basis. Among the deficiencies considered were: inadequate indication of operating mode; insufficient control flexibility; limited alarm capability; and increased component overload protection.

The control console panel was completely redesigned (figure 3), with all switching modes labeled and visual indications provided. Switching for the dual pumps for each environmental support console now includes: automatic or manual modes of operation; manual operation of either pump continuously; an overload circuit breaker which trips at current loads greater than 12 amperes; and visual and audible alarms if this circuit breaker is opened (figure 26). Operating personnel, therefore, are alerted immediately if a malfunction occurs in any of the pumping systems. The three vacuum heating ovens are also provided with power switches, operating lamps, and protective circuit breakers on this panel.

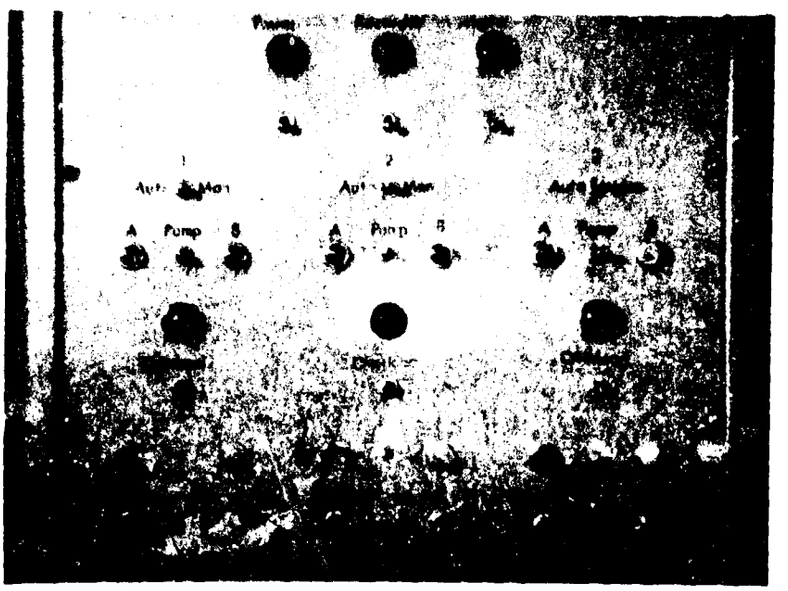

Figure 26. CONTROL CONSOLE SWITCHING PANEL

III E. Alarm Systems

Alarms provided on the original system were for animal chamber pressure. To achieve increased diagnostic alarming of the life support system, alarm relays were added to each module to cover loss of pump motor power and system low flow. A nine-point alarm system with three-inch square visual lamp panels and an audible horn was installed to provide coverage of malfunctions in the life support system (figure 27). Alarm indications of atmospheric conditions outside of present limits of more than 0.5% CO_2 and less than 98% O_2 are provided for by the CO_2 and O_2 monitoring and alarm panel (figure 4). In addition, a relay is connected to the alarm system to activate the main facility alarm system if any point is activated due to system malfunction.

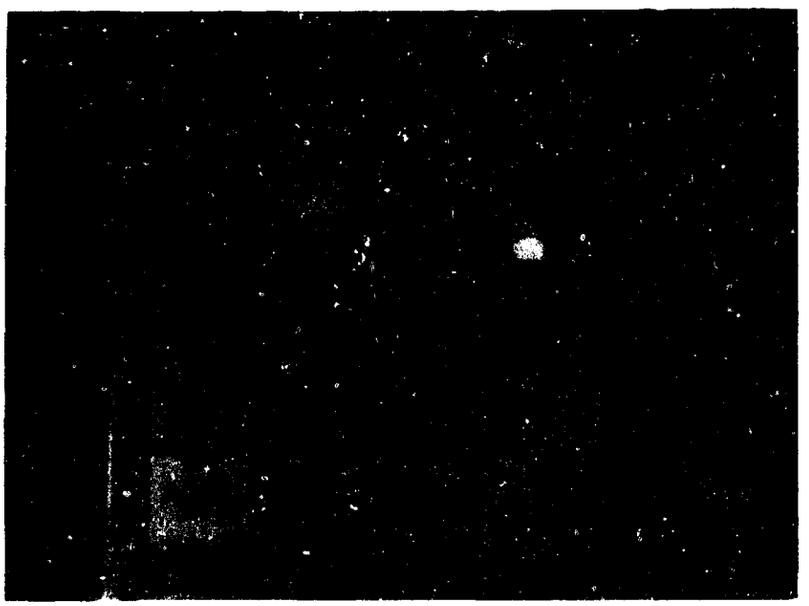

Figure 27. CONTROL CONSOLE ALARM SECTION

VIII. Discussion

Equipment has been designed and constructed for toxicity screening of the gas-off products emanating from space cabin construction materials. This equipment is incorporated into Thomas Dome No. 2 at the Toxic Hazards Research Unit of the Aerospace Medical Research Laboratories at Wright-Patterson Air Force Base. In February of 1966, on the second day of a scheduled seven-day experiment under 100% O_2 5 psia atmospheric conditions, a fire occurred. Damage to major components of the system was minimal, but approximately 90-95% of all electrical interconnections were destroyed. The causes of the fire were definitely identified, namely, an overfilled condensate trap, a jammed atmosphere pump and its associated drive belt, and inadequate overload protection of the pump drive motor. Subsequent modifications eliminated the potentiality of a fire reoccurring due to these factors. Simultaneously, modifications were accomplished to provide increased flexibility of operating controls, more definitive alarm protection, and increased operating reliability.

OPEN FORUM

James D. MacEwen, Ph. D.
Aerojet-General Corporation
Moderator

DR. MAC EWEN: Before we start, I might ask Dr. Thomas if we have received any questions regarding the other session this morning, the chemistry sessions, or the analytical chemistry sessions? Before we open the floor to general discussion of other sessions, we should give a few minutes to questions concerning the last two papers, which were both very fine presentations.

I might take the opportunity to ask one question that I am curious about. I was particularly interested in the curve presented showing the methane concentration in the SEALAB increasing with continuing days of exposure in the SEALAB and apparently with each group reaching an equilibrium point. I know that Mr. Saunders didn't want to discuss or speculate on the causes of this, but I wondered if he could indicate whether this has ever been shown before in a closed atmospheric environment with a complete recycling system.

MR. SAUNDERS: I don't know whether it has ever been shown before.

DR. MAC EWEN: I know that you have analyzed a number of other chamber operations like this, but I wondered if you had ever observed this?

MR. SAUNDERS: Methane is not one of the compounds that I usually follow. I don't know whether this effect has ever been observed before. I had a good reason for not speculating about what caused it: we don't know. That's the main reason.

DR. MAC EWEN: The floor is now open to question.

DR. ROTH (Lovelace Foundation for Medical Education and Research): I wonder if there are any chemical traps on the Apollo material test chambers, and if any of the toxic products of the burning were obtained? I extend the question to ask whether toxic products of burning were ever determined from ECS system of the Apollo that burned?

MR. JOHNSON (Aerojet-General Corporation): No, I don't believe there were any. There were no chemical traps, other than lithium hydroxide, involved in the Apollo material test system at the time of the fire. Some tests were conducted on the pumps to determine the presence of lithium hydroxide in the rest of the system. I think these were the only tests accomplished.

DR. MAC EWEN: The second part of the question was directed to the Apollo I fire. Would somebody like to answer that question?

ANSWER: There were no determinations made during the fire itself; after the fire, during simulation, there were detailed analyses made. This will be available in the final report.

MR. GROSSMANN (Atlantic Research Corporation): I would like to know how soon after the fire started was the dome vented to ambient air?

MR. JOHNSON: Repressurized, you mean?

QUESTION: Well it was at 5 psi.

MR. JOHNSON: I would say approximately less than two minutes.

QUESTION: The majority of the time the fire was in progress it was burning at 14.7 psi mixed gas?

ANSWER: Enriched oxygen, more than normal, but not a hundred percent.

QUESTION: If there were men in there at the time, how soon could they have been removed?

MR. VERNOT (Aerojet-General Corporation): As I said, a man could have let himself out through the hatch into the lock and that would have taken maybe 30 seconds, somewhere between 30 seconds and a minute.

DR. MAC EWEN: I might comment that the fire was extremely localized, that there was no fire on the other side of the dome where a man would normally be, and that the attendant inside the dome who is servicing any of the animals normally has an oxygen supply mask respirator, a breathing mask, and is connected to an oxygen supply of his own, so he would not normally be affected by smoke inhalation.

DR. BACK (Aerospace Medical Research Laboratories): The other thing is that the water deluge system didn't come along until quite late in the game, and that's why the fire kept going so well. The other thing is that the fire didn't generate a great deal of heat. None of the windows was broken in the fire. Had a man been in the dome, if he had stayed away from the fire he could have pulled the water deluge system himself. Even in our old system the bottom of the dome could have been covered with water two to three inches thick in a matter of a few seconds. We have more now, but even at that time.

DR. MAC EWEN: I might comment further that when this fire occurred there were people in attendance, including the laboratory regular shift operators. The other domes that were in operation were immediately isolated, the power supply was cut off because it was an electrical fire, and the dome repressurized. All this was done within about two minutes. The rest of the building was evacuated in case the fire broke out and spread. The fire was completely out within 20 minutes and the other domes were back in normal operation.

Maybe Mr. Johnson would like to discuss some of the additional fire prevention equipment or fire fighting equipment that was added in the way of fire quenching systems.

MR. JOHNSON: Well, this is one thing I didn't bring out too well in the paper. Subsequent to the fire we did add an external control for the water deluge system. Originally we had a manually controlled deluge head inside the chamber,

controlled by the dome entrant. We also had a temperature activated head. Subsequent to the fire, we added an outside manual controlled deluge head which the observer could immediately operate from outside the chamber in case of a fire. We also added a CO_2 system which would repressurize the dome, also manually operated at the control station.

LT. COL. WESTLAKE (SSD): I wanted to question Mr. Saunders about the presence of hydrogen in his SEALAB. Did you by chance measure hydrogen, Mr. Saunders?

ANSWER: That would have been Dr. Umstead that would have done that. I don't know whether he did it or not. Our analytical facilities are divided among two different groups.

QUESTION: Is he here?

ANSWER: No, he is not here.

QUESTION: In the techniques that you used for scrubbing and analysis, might you have overlooked hydrogen?

MR. EATON (Naval Research Laboratory): I am unable to answer that, I just joined the Lab in September. All this work was done before I got there.

DR. THOMAS: Were the parts per million figures on these concentrations compensated for back to normal one atmosphere pressure?

MR. SAUNDERS: They are for one atmosphere pressure. So the effective pressure in SEALAB would have been seven times higher than shown on the slide. This would make the carbon monoxide concentration above the level at which a crew would have experienced physiological effects like headaches, and they did have headaches, which is what tipped them off to the fact that the concentration was getting too high.

DR. BACK: What was the highest?

MR. SAUNDERS: I believe, according to the slide, it was 40 parts per million at one atmosphere.

DR. BACK: Maybe it would be a good idea for Mr. Johnson to indicate what has been done recently in the way of fire protection in terms of what we have now installed.

DR. MAC EWEN: Mr. Johnson, would you like to comment further on that?

MR. JOHNSON: We are still in the process of modifying our extinguishing systems to get increased reliability and increased efficiency on extinguishing fires. We increased our line into the chamber to a larger line. We have now installed five sprinkler heads inside the dome envelope in a circular pattern, which at this time gives considerably more protection to a dome entrant. It completely covers the dome area. The flow rate that we have in the system now is approximately one hundred gallons per minute activated by a pneumatic dump valve. It is approximately one gallon per square foot, or a little less.

MR. SAUNDERS: Suppose this system actuated and you had a man in the trap, would all this water flood him out and maybe even drown him?

MR. JOHNSON: This is a possibility if the air lock is open and the man is down in the air lock. You can get water down in the air lock; but normally in operation if the entrant is in the dome the air lock door is closed. We also have installed a water deluge head in the air lock, because the air lock is a potential hazard. If he was in the air lock, he's got a manual dump down there, a dive valve which is about two inches, so he is not going to drown. Water may fill up to about three feet, which won't hurt him.

QUESTION: Have tests been performed to show that the deluge system will put out a fire at 5 psi pure oxygen?

ANSWER: No they have not.

DR. THOMAS: We are planning such a test, as you might be aware, especially the people who were involved in looking into fire problems. You know there was a simulator fire at Brooks Air Force Base. As a result of that, our Headquarters is looking very seriously at other means of fire extinguishing. Apparently both water and some of the freons work very well if they are used with automatic fire detection devices. You can detect fire in about a hundred milliseconds and you can put it out within an additional 50 milliseconds. The amount of coverall burn was something in the order of one-half percent. So as soon as things get standardized and the best fire fighting agents defined, I'm sure we will be installing some automatic water system.

MR. LUSK (General Motors Corporation): I would like to ask Mr. Johnson if he has any idea what the ignition temperature of the belt is, at a hundred percent oxygen?

MR. JOHNSON: I'm not sure exactly what the ignition point of the belt was. I would imagine it could easily be determined from the manufacturer of the belt.

DR. ROTH: The auto-ignition temperature of solids doesn't change too much. The ignition point of solids at a hundred percent psi is not too much different than in air.

DR. MAC EWEN: There is a question concerning the analytical chemistry session. I'm not quite sure what is referred to here, but the question is this: "Please discuss the large differences reported by several laboratories in trace analysis." I presume this refers to the manned simulator runs at Brooks Air Force Base. Perhaps Mr. Conkle will discuss that, first with reference to the number of compounds, and secondly to the order of magnitude of differences in concentrations.

MR. CONKLE (Brooks Air Force Base): I think that this is one question that is rather loaded and I'll go to some of the other individuals in analytical chemistry to aid and assist in this. We have quite a few compounds in the samples that we have taken, and they were sent to several different laboratories and each laboratory has had its own analytical procedures that have been involved. Again, the levels of material are quite low, and we are working at the limits of detection

in the analytical sense. So at the present time we are measuring very low concentrations, and this difference in analytical results that we have is due to the various analytical procedures that are being used, the fact that there can be changes involved in the materials by the association of lithium hydroxide in system within a vehicle which is going to remove some of the compounds, water absorption of the compounds before they are trapped, the walls of the chamber will affect the amount of material that is being processed and trapped, and this continues all the way to the analytical procedures. I would like to ask Mr. Saunders to discuss this also in the variance that we do see between laboratories.

MR. SAUNDERS: Naturally the way the atmosphere is sampled has something to do with it, as well as the type of vessel that one takes a sample in, and what it is constructed of, because you get absorption on walls and that sort of thing. After an analyst takes his sample, gas chromatography is one of the techniques used, partly at least in any analysis, and everybody has his own favorite type of column. One analyst may use his own column, see contaminants which don't go through another analyst's column in a reasonable period of time, so he fails to detect them. Neither analyst would be wrong. They are both seeing things that are there, but they may not necessarily be in full agreement about what they do see. When you are working on the analysis of anything at extremely low levels, I think that in addition to the equipment you have, it is very much a matter of personal technique. One man might be more successful with a particular type of compound than another because of his technique. Then there is the matter of interpretation of data. You might sometimes be very sure of your data and report it; at some other time, in the case of a particular component, you may be less sure of it and not mention it. All our identifications are based on either infrared or mass spectra or both. We never use retention time data and so if we have a good infrared spectrum or mass spectrum and we successfully interpret it, we are sure of what we see. If we don't have a mass spectrum, we don't report it. If we haven't interpreted it to our satisfaction we don't report it. We may have seen many things which we don't report. Maybe Mr. Moberg can add something further to this if he is here?

MR. MOBERG (Aerojet-General Corporation): I think you've summarized it very well, Ray. The only addition that might be made is that with the many differences in techniques and many differences in sampling there are quite gross differences in transferring the samples to the chromatograph. If you consider the lines that you are using, the intermediate lines, and if you have desorbed these lines prior to use, and then introduce the sample through these lines, you will, in fact, get absorption again, and you could have losses of sample in this manner; and so you must take additional steps either through solvent washing, rinsing. When one recognizes this difficulty then you usually do something about it. I think the greatest difference is to try to look honestly at all the areas that may cause the difference, conscientiously, just as the medical people do in looking at the complex data that they see. In trying to interpret this data in light of the scheme of analysis, the source of the samples, and the type of components that may be expected, this should not affect the reporting of samples because you say I don't believe that compound can be present, so, therefore, it is not. But it should be added as part of the diagnosis of the whole problem just as a doctor likes to get some prior history on the patient while he is diagnosing the difficulty the patient is experiencing, because this always helps to lend credence to his final diagnosis.

MR. VERNOT: We analytical chemists like to pretend that we are very objective and that our results can be relied upon to a much greater degree than

toxicological or biochemical data, but the real fact is that this problem with analysis is not limited to microanalysis or to analysis of a very small concentration. I once worked in the baking industry, and they would send referee samples of wheat around for protein analysis and you did a protein analysis in a very simple way. You did a Kjeldahl analysis, people have been doing Kjeldahl analysis for close to a hundred years. After they had sent the referee samples around to all the laboratories they then had all the laboratories total up and send in what their results were. They didn't say what laboratories they were, but the variations were just fantastic. If you had a nominal 12 percent wheat protein, you'd get things going from six to 18 percent, and this was on a very good, very well-tested technique where you really wouldn't expect any problems. But you got it there, so if you've got problems there, you can see what the problems involved in analyzing trace quantities might be.

DR. MAC EWEN: If there are no more questions on this matter, we can open the floor now to any of the sessions that anybody would like to discuss.

DR. ROTH: One of the gaps I detected in the meeting was an attempt to relate the recent findings of the Gemini Program, the blood findings, to what we see in animals. I think during the last meeting Dr. Fischer presented the data on the decrease in the red cell mass amounting to 15 or 20 percent and associated plasma changes. Since then there has been evidence that associated with this decrease in red cell mass is a decrease in serum tocopherol, half to a third, with large spherocytes and spiked cells being present in the blood, suggesting that there is a hemolytic process of some sort going on. There is a clear absence of an associated bilirubin change. Dr. Mengel has presented a very nice system whereby one can simulate, by making animals deficient in tocopherol and adding oxygen, a red cell change. I would like to suggest that thought be given to titrating the several different species of animals with tocopherol deficiency and lower partial pressures of oxygen to give equivalent red cell changes, and then going through some of the organ analyses under these conditions which more closely simulate the humans. Of course, there might be tremendous organ differences, as there probably are, but at least the animals will be normalized with respect to red cell changes, and one might have a little more profitable comparison with humans - has this been considered?

DR. MAC EWEN: Dr. Kaplan, would you like to answer that?

DR. KAPLAN: We have a program in that direction with Dr. Mengel and we had hoped that we would have something to report at this meeting. Unfortunately, since we have been unable to do any experimental work since the first of February, we are at least three and one-half months behind our schedule. We have done some preliminary work exposing monkeys at one atmosphere. One atmosphere seems to be a good place to start filling in the gap, because the problem with 5 psi, the Gemini atmosphere, is that there is a good possibility that nothing definitive happens there. It is true that there is some decrease in tocopherol levels, but having discussed this with Dr. Mengel, it is clear that the decrease is not nearly extensive enough to cause in vivo hemolysis. This is what he tells me, compared to the kind of decrease that he has to get in his animals before he starts to see hemolysis. It is difficult to get in vivo hemolysis just where there is a tocopherol deficiency. The human patient that he got this in is an abnormal patient. All the rats that he talked about, remember, displayed in vitro hemolysis after a preconditioning by exposure to oxygen at high pressure. The same thing is true for humans, except for one individual, an elderly man he worked with who had abnormal findings before the exposure.

I don't remember exactly what the findings were, but I believe he already had some level of lipid peroxide in his blood before he was even exposed, indicating that there was something abnormal in this man's resistance to hemolysis. Anyway, in normal patients Dr. Mengel has not seen any in vivo hemolysis. In a large number of dogs that he has exposed to oxygen at high pressure, he also has not seen any in vivo hemolysis. Now the trouble with the hyperbaric situation is that it is so fast that he can't expose his animals or people very long because he starts running into trouble with CNS effects. So whatever is occurring is occurring so fast that all he is seeing is the end result. We felt that at one atmosphere the oxygen tension is high enough so that it is toxic and yet the events might proceed somewhat slower than they would at two or three or four atmospheres, and we might indeed be able to see just what the pattern is, what leads to what, and what precedes what. We are working in this direction.

Work done at Yale a few years ago on chronic malabsorbers by the gastroenterology people found that these individuals, because of their malabsorption syndromes, did have low body levels of tocopherol and their blood responded the same way as Dr. Mengel's experimental animals did, i.e. if you take their blood and challenge it in vitro with chemical oxidants like hydrogen peroxide, it hemolyzes and exactly the same sequence occurs, but even these individuals who are naturally deficient in tocopherol do not hemolyze in vivo. The red cell picture is extremely complicated because of the unique metabolic capabilities of the red cells as a non-nucleated cell; and also because of the many interactions that go on in the metabolic pathways in the red cells and the many available antioxidant defenses. Tocopherol is just one of these. There are many others, glutathione, the pyridine nucleotides themselves in the reduced state, all components that are preferentially oxidized to protect the other parts of the cell that can't tolerate oxidative damage. So we are trying to look into this and I hope we get something in a short time, but I suspect that it will take some doing before we really understand what is going on with the red cells.

DR. ROTH: There is an interesting human congenital defect where the serum protein that normally binds the tocopherol decreases. This results in acanthosis (spiked red cells), very similar to the cells that Dr. Fischer has seen with a decrease in red cell mass, and this decrease in red cell mass responds to added Vitamin E, and this might be a model regarding the sensitivity of human red cells to decreased tocopherol. There is a mild hemolytic condition in the absence of any elevated partial pressure of oxygen. So I mean we have a complete spectrum, and I think one might be able to juggle, in animals, both the tocopherol levels in the serum and the partial pressure of oxygen, to give a low-grade hemolysis, transient to a permanent-type, that might simulate a human. What happens in the rest of the cells of the body under these conditions isn't clear, but I think we would be starting at a closer baseline.

DR. MAC EWEN: Dr. Kaplan?

DR. KAPLAN: Just one last quick statement to bring this around into its full context. There are other factors in the Gemini flights that might have been responsible. There are factors such as restriction and inactivity, such as the weightlessness that occurred over the four to 14 days, depending upon which flight you are talking about. Factors such as accelerative forces during reentry, all of which affect cardiovascular dynamics and could have led to things like visceral

pooling of blood and stagnant hypoxia leading to hemolysis of the cells which stagnated - this kind of thing. We've talked about this with Craig Fischer and the others doen at NASA and there are a lot of things that are confusing, like the fact that so many land-based human exposures with the same oxygen conditions do not yield any hemolysis; also the fact that although we can't seem to get any clear information, there is some indication that the Russians who use air rather than oxygen may also have seen some changes in hematocrits or red cell count. I have some translations to this effect; others have information. I think you said you spoke with some of them and they knew nothing about it. We can't get a straight answer on that; but nevertheless, I think just because the Gemini astronauts lost red cell mass it is a little presumptuous to assume that this was entirely because of the pure oxygen environment and not related to other things that were also not normal, like weightlessness, acceleration force, and prolonged confinement. I think most likely it is a combination of all of these and that will only add to the complexity of trying to clear up what is doing on, but it wouldn't surprise me if when we eventually switch to mixed gas we might end up seeing the same sort of thing.

DR. MAC EWEN: Thank you, Dr. Kaplan. Any other questions?

MR. SAUNDERS: Somebody submitted a question in regard to medical and engineering operating design criteria for levels of spacecraft contaminants. Have any been set? If so, by whom, and on what basis? According to Dr. Harris, there have been no contaminant limits set for spacecraft. The Toxicology Advisory Committee of the National Academy of Science has this matter under consideration. The Navy does have a working committee which is setting limits for submarine contaminants and there have been limits set tentatively for perhaps 40 or 50 different components. If anyone is interested, they can perhaps get a copy of this list from the Chemistry Department of the Naval Research Laboratory.

QUESTION: What toxicological research has been done, or is being contemplated, to evaluate the mishmash of hydrocarbon contaminants in a submarine atmosphere?

MR. SAUNDERS: None, really. There hasn't been any experience so far that indicates that any of the things in a submarine atmosphere bothers any of the crew and they have experienced these contaminants for 90-day exposures at normal atmospheres; however, it would be foolish to assume that there may not be some long range effects. This is just something that has never been discussed here. This hasn't been done yet by the Navy. The Navy has from time to time discussed the possibility of following the various crew members and giving periodic physical examinations throughout their professional lifetime to see if anything develops.

One might think that being a crew member of a nuclear submarine would be a professional hazard, at least as bad as smoking. They are exposed to a great number of contaminants and they are breathing them continuously over a long period of time; so maybe after 20 years of exposure they might develop something, but they certainly don't develop anything immediately.

DR. BACK: I would like to throw open the discussion on the electron microscopic findings in the two-gas system and our data on increased protein in dogs. There may be a good correlation here. Would anybody care to speculate on a reason for it, considering that it is not artifact? We don't want to go back to this artifact business; but let's say for the sake of discussion it is true and it is real.

DR. ROTH: I think there is one thing that isn't clear in the literature, and this is the effect of inert gases on lipid membranes. The old Meyer-Overton theory regarding the inert gas effects has given way to clathrate hypothesis, the iceberg hypothesis, etc., but they all focus on the alteration of the membrane-water interface being a key factor. The presence of the nitrogen may add to the effect of slightly increased percent of oxygen, to give at the membrane surface an alteration which you see as a change in the electron microscopic pattern. Repeating the same thing with helium and other gases, at other tensions, may very well give you interesting pictures all the way across the board. At a membrane level you may be seeing the interaction between peroxidation and membrane changes due to inert gases in a much more fine way.

DR. BACK: For those of you who are interested, we are tailoring a rerun of the mixed gas study. Our dogs are getting older and this may be a factor; but we have selected animals with pronounced high A/G ratios. That is, we are selecting them for the fact that they have near 3 : 1 A/G ratios, and we feel that if we should try to induce reversal again, we ought to load the stack against us. So we are starting out with deliberately high A/G ratios and trying to reverse them. Our dogs are quite a bit older and if age happens to be a factor, why we are going to hurt, I'm afraid.

DR. MAC EWEN: The aging is due to the delay in starting the experiment?

DR. BACK: Yes.

APPENDIX

The following discussion is a summary of the state-of-the-art in oxygen toxicity research which has been going on at the 6570 Aerospace Medical Research Laboratories for the past five years. It represents a compilation of data and interpretation thereof of both contracted and inhouse efforts in this area. It also presents some unsolved problems and possible approaches to solution. The summary was prepared by Dr. Harold P. Kaplan prior to his departure from military service in the United States Air Force. The report was originally prepared as an internal information document only. However, since the information contained and discussed is so germane to the preceding papers of this Conference, the report has been edited and is included here for the benefit of our Conference participants.

STATUS REPORT: OXYGEN TOXICITY RESEARCH PROGRAM

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INTRODUCTION

This report summarizes research in mechanisms of oxygen toxicity and the safety of 5 psi pure oxygen atmospheres, carried out in the Toxic Hazards Division, Aerospace Medical Research Laboratories, between August 1965 and May 1967. Prior research, accomplished between 1962 and 1965, is referred to when indicated. Additional experimental programs are outlined where needed for completion of incomplete research projects. Finally, a continuing research program is proposed based on concepts established from the present program.

The research described herein utilizes morphologic and biochemical studies to investigate the effects of increased oxygen tensions on subcellular structure and metabolism. With this approach, an understanding of the basic mechanisms of oxygen toxicity and adaptation may be reached. Application of these findings to study of the 5 psi pure oxygen atmosphere (spacecraft atmosphere) will determine whether this is or is not a toxic atmosphere for man.

Note that throughout this report the following terms are used interchangeably:

1 atmosphere: 760 mm Hg total pressure: 15 psi
1/3 atmosphere: 258 mm Hg total pressure: 5 psi

Note: Much of the data reported herein derives from experiments which are as yet incomplete. These data, and many of the tentative conclusions derived from them, must be interpreted in this light and should not be construed as definitive.

OXYGEN TOXICITY PROGRAM

1. Cellular Biochemistry

   A. Oxygen toxicity at the cellular level (Contract AF 33(615)-5096, with IIT Research Institute, NASA supported)

Prior research:

Research performed under Contract AF 33(657)-9843 by the Department of Research of the Miami Valley Hospital, Dayton, under direction of Dr. Bernard J. Katchman, between 1 February 1963 and 30 June 1964, has been reported in AMRL-TR-65-173, "The Metabolic Activity of Intact Cell Dispersates from Normal and Oxygen Intoxicated Rats". This work was supported by the Air Force. Dr. Katchman examined liver cell dispersates obtained from rats exposed to pure

oxygen atmospheres at both 258 mm Hg and 760 mm Hg total pressure. Rats exposed to the 760 mm Hg atmosphere until death demonstrated 40% higher respiratory activity than controls. This increase in QO_2 was thought to represent uncoupling of oxidative phosphorylation in these cells, with about a 50% decrease in energy generative mechanisms. This was accompanied by a decrease in liver mass and cell size, but an increase in total tissue nitrogen. The only finding in cells from rats exposed to the 258 mm Hg atmosphere was a decrease in liver mass. There was no increase in QO_2 in these preparations, and, therefore, no suggestion of uncoupling.

Felig (1965) discovered that intraperitoneal injections of sodium lactate prior to exposure offered rats partial protection from oxygen toxicity, whereas other alkalinizing salts did not. He theorized that excess lactate might be acting by going to pyruvate and thereby replacing depleted NADH.

Present research:

Evaluation by IITRI of mitochondrial preparations obtained from liver cells of rats exposed to 760 mm Hg of pure oxygen for six to 96 hours confirmed that uncoupling of oxidative phosphorylation occurred in this atmosphere after 72 hours of exposure. In two initial experiments, P:O ratios dropped from control values of 3.12 (range 2.86 to 3.28) to 2.62 (range 2.43 to 2.76) in one, and to 2.17 (range 1.99 to 2.44) in the other, both after 72 hours of exposure. Later experiments utilizing matched control-experimental pairs, with computer analysis of data for variance, again revealed a significant decrease in P:O ratios at 72 and 96 hours of exposure. Mitochondrial preparations were also obtained from the livers of three rats that had been exposed to this toxic atmosphere for 96 hours and then allowed to recover in ambient air for three days, and from three rats exposed for 72 hours and allowed to recover in air for 10 days. Data obtained from these preparations suggested that there was complete recovery of oxidative phosphorylation. It is also of interest that no increase in QO_2 was observed at any time, and that uncoupling was more pronounced in preparations obtained from rats exposed in a recirculating chamber than in those obtained from rats exposed in the flow-through Thomas Domes. A detailed report of these findings is presented in the final report of the first year's work (1965-66) as AMRL-TR-66-206, and in the Proceedings of the 2nd Annual Conference on Atmospheric Contamination in Confined Spaces, AMRL-TR-66-120 on pages 178-199. Observations on rats exposed to 760 mm Hg of pure oxygen for longer than 96 hours have not been made.

P:O ratios and QO_2 determinations were made using Warburg techniques to monitor in vitro respiration of experimental and control tissues. Evaluation of liver pyridine nucleotide ratios, initiated to further explore the concepts presented by Chance (1965) that hyperoxia blocks reduction of the oxidized forms, leading to depletion of the reduced forms, and by Felig (1965) on the protective properties of lactate, yielded no abnormal findings.

Evaluation of rats exposed to 5 psi of pure oxygen for up to two weeks using P:O ratios has not revealed any evidence of uncoupling in mitochondrial preparations from liver cells. In fact, it appeared that between the third and seventh days of exposure there was an actual tightening of coupling, with P:O ratio higher than control values (see Proceedings of 2nd Annual Conference on Atmospheric Contamination in Confined Spaces, page 194). In mitochondrial preparations obtained from

the livers of rats, dogs, and monkeys exposed continuously to 5 psi of pure oxygen for almost eight months, there was no deviation in P:O ratios from simultaneous control analyses. If uncoupling of oxidative phosphorylation is the metabolic manifestation of oxygen toxicity at the cellular level, i. e., the functional correlate of the mitochondrial morphologic changes induced by exposure to high oxygen tensions, then we must conclude that the 5 psi oxygen atmosphere is not toxic to cellular respiration for up to eight months of exposure. Note, however, that this observation is necessarily restricted to cells exposed to hyperoxemia (pO_2 of about 150 mm Hg), and not to pulmonary parenchymal or pulmonary capillary endothelial cells, which are exposed to higher alveolar oxygen tensions (250 mm Hg). The reaction of these cells has not yet been studied.

In more recent work (September - November 1966) monkeys were exposed to 15 psi of pure oxygen (750 mm Hg) in a Thomas Dome for intervals from six hours to 12 days. Liver and kidney tissue was obtained from two monkeys at intervals of six, 48, 96, 168, and 288 hours, and from four controls exposed to ambient air. A slight decrease in liver P:O ratio was again seen at 96 hours of exposure, but the most dramatic results were with the kidney tissue, where P:O ratios fell to 60% of normal at 96 hours of exposure. Both liver and kidney mitochondrial P:O ratios were back to normal at 168 hours of exposure, with a secondary uncoupling seen at 288 hours of exposure. Liver and kidney ATP levels, determined for the first time during this experiment, fell to about 40% of normal at 48-96 hours of exposure, a finding consistent with uncoupling of oxidative phosphorylation. Kidney ATP levels were back to normal by 168 hours of exposure, but liver ATP remained depressed for the remainder of the exposure period. This difference in response is presently unexplained. Liver and kidney pyridine nucleotide analyses gave divergent results. However, all pyridine nucleotide levels, both reduced and oxidized forms, were consistently elevated above normal levels at six hours of exposure. It is theorized that this may be a factor in an early adaptive response on the part of the cell. Examination of pyridine nucleotide levels in the blood of these monkeys revealed no significant change after the first 96 hours of exposure, but then a dramatic increase in all forms to about 200% of normal at 168 hours and thereafter.

It appears reasonable that there are early adaptive changes in all cells in response to increased oxygen tensions. In animals exposed to the clearly toxic 15 psi atmosphere, adaptive response is inadequate or overcome. Exposure to the 5 psi oxygen atmosphere, however, is probably handled adequately by these adaptive mechanisms.

Preliminary work has been initiated to test the thesis that adaptation to a 5 psi atmosphere for prolonged periods of time would necessitate readaptation to the lesser O_2 partial pressure in ambient air, for safe return to normal air atmosphere. Similar adaptation might be required for optimal functioning in the 3.7 psi pressure suit atmosphere for EVA. Possibly, mitochondrial respiratory systems, having adapted to increased tissue oxygen tensions associated with habitation of a 5 psi pure oxygen atmosphere, would become relatively hypoxic if suddenly denied that extra oxygen by abrupt return to air, or to 3.7 psi of pure oxygen. To investigate this we have:

(1) Applied cellular respiration and oxidative phosphorylation parameters to mitochondrial preparations derived from rats exposed to pure oxygen at 15 psi for from 15 minutes up to six hours, looking for evidence of early adaptive

changes. After studying approximately six rats at each interval of exposure (15, 30, 60, 180, and 360 minutes), we have found no deviation from normal.

(2) Studied rats and monkeys exposed to air at about 18,000 feet altitude. Such exposure lowers the animals' arterial pO_2 from normal values of 80-100 mm Hg to about 35 mm Hg. This decrement is quantitatively similar to the reduction in arterial blood pO_2 experienced with removal from a 5 psi pure oxygen environment back to ambient air, viz. from 150 mm Hg to 80-100 mm Hg. We are utilizing this procedure to identify sensitive parameters of hypoxia that can be applied to studying the effect of return from hyperoxia to normoxia. This experiment is currently underway, with no definitive results as yet. However, preliminary findings indicate that there is a significant drop in ATP levels in liver tissue obtained from exposed rats. Examination of blood lactate/pyruvate levels in monkeys has so far resulted in paradoxical findings. Blood from eight monkeys obtained on three different occasions has suggested that there is a drop in lactate with concommitant rise in pyruvate in this hypoxic situation. The data are partially outlined in tables I and II.

TABLE I

BLOOD LACTIC AND PYRUVIC ACID FROM MONKEYS SUBJECTED TO AIR AT 380 mm Hg IN THOMAS DOME FOR APPROXIMATELY ONE HOUR

Animal No.	Control Lactic Acid uM/ml Values	Control Lactic Acid uM/ml Average	Exp. Lactic Acid uM/ml Values	Exp. Lactic Acid uM/ml Average	Control Pyruvic Acid uM/ml Values	Control Pyruvic Acid uM/ml Average	Exp. Pyruvic Acid uM/ml Values	Exp. Pyruvic Acid uM/ml Average
	11.12		7.96		.374		.56	
B-48 ♂		10.69		7.95		.379		.555
	10.25		7.94		.384		.55	
	10.04		9.92		.459		.71	
B-59 ♀		10.04		9.92		.459		.71
	10.04		9.92		.459		.71	
	13.20		6.12		.307		.370	
B-56 ♂		12.74		5.87		.309		.380
	12.28		5.61		.311		.390	
	13.77		4.24		.372		.320	
B-27 ♀		13.95		4.34		.377		.310
	14.07		4.44		.382		.300	

In addition, to improve the sensitivity of evaluations of oxidative phosphorylation, mitochondrial activity is now being evaluated with the Respiratory Control Index (RCI) in addition to the P:O ratio. The RCI is determined by polaragraphic technique, using a Gilson Oxygraph, rather than by Warburg technique. It measures the maximal ability of mitochondria to perform oxidative phosphorylation in the presence of abundant amounts of ADP, and, therefore, will demonstrate even small decrements in function. It is conceivable that the 5 psi oxygen atmosphere may affect oxidative phosphorylation to a minimal degree which is beyond the sensitivity of the P:O ratio, but which will be perceptible by interpretation of the RCI. The

Gilson Oxygraph will be used to reevaluate liver and kidney mitochondria from rats exposed to 15 psi for up to five days, and to 5 psi for up to three weeks. Additional observations will be made to support the contention that uncoupling is a reversible phenomenon in this situation.

TABLE II

RAT LIVER ATP VALUES FROM ANIMALS EXPOSED TO THE ABOVE CONDITIONS

	Control ATP Values	Averages		Exp. ATP Values	Averages
Group 1					
Animal 1	3.41		Animal 4	1.77	
	3.41	3.46		1.70	1.75
	3.56			1.77	
Animal 2	3.71		Animal 5	2.70	
	3.71	3.67		2.86	2.75
	3.63			2.70	
Animal 3	3.87		Animal 6	Lost	
	3.87	3.87			
	3.87				
Group 2					
Animal 1	3.55		Animal 4	2.81	
	3.55	3.55		2.74	2.77
	3.55			2.78	
Animal 2	3.76		Animal 5	3.18	
	3.69	3.70		3.18	3.18
	3.76			3.18	
Animal 3	3.08		Animal 6	2.49	
	3.19	3.12		2.49	2.45
	3.08			2.83	

Additional study of kidney mitochondrial preparations, utilizing RCI's and tissue ATP/ADP/AMP levels, will be necessary to confirm that uncoupling occurs in the kidneys of monkeys exposed to 15 psi pure oxygen atmospheres for four days, and that such uncoupling is a reflection of the mitochondrial morphologic changes seen by the electron microscope in the proximal tubular cells. These changes will then be correlated with proximal tubular function as measured by the renal function tests described later in this report. Similarly, nine monkeys have been prepared for confirmation of the erythrocyte pyridine nucleotide response described above. Each monkey has been bled on two occasions to provide two sets of preexposure control values, and has been subjected to a bone marrow aspiration (from the posterior iliac spine).

Proposed research:

Additional exposure of rats and monkeys to 5 psi of pure oxygen for a minimum duration of two weeks, and for longer durations, as feasible, is needed to validate (1) the impression that there is actual tightening of coupling with increased ATP production as an initial response to the increased tissue oxygen tension, and (2) the observation that there is no toxic breakdown of oxidative phosphorylation at this pressure using the more sensitive RCI. If the hypoxia studies described above yield evidence of measurable change, as they appear to be doing, the parameters utilized should be applied to the evaluation of animals exposed to 5 psi oxygen for a minimum of two weeks, and then rapidly decompressed to 3.7 psi, or returned to ambient air.

Gross observations of animals exposed to 5 psi of pure oxygen have failed to indicate any evidence of systemic toxicity. An apparent exception to this is the finding of morphologic changes in the lungs of dogs and rats exposed to this atmosphere continuously for eight months (see "Research in the Pulmonary Pathology of Oxygen Toxicity"). It would appear that the only site of potential toxicity from the 5 psi oxygen atmosphere is the lung, where tissue is exposed to higher oxygen tensions than elsewhere in the body, and where there might be oxidative alteration of surfactant, or damage to alveolar epithelium, capillary endothelium, or intracapillary erythrocytes. Since another eight-month exposure to this atmosphere is probably financially and logistically prohibitive, it is proposed that we study the effects of shorter exposures (two weeks - one month) to 5 psi of oxygen, by evaluating the integrity of pulmonary mitochondrial preparations obtained from exposed animals. An operational method for isolation of viable lung mitochondria has now been perfected by IITRI and would provide the most sensitive parameter yet available for evaluating the reaction of pulmonary parenchyma to a 5 psi oxygen environment.

In our investigations of the mechanisms of oxygen toxicity, we have determined that morphologic alteration in mitochondrial membranes leads to uncoupling of oxidative phosphorylation and subsequent decrease in cellular ATP production, essential for maintaining cellular integrity. Correlation of this finding with work done by Mengel and Danon on red cell membranes (see "Research in the Hematologic Effects of Increased Oxygen Tensions") and reports of changes in pulmonary surfactant in response to increased oxygen tensions (Fed. Proc. 26, 497, 1967, and AMRL-TR-65-136) and of changes in brain tissue lipid as preceding hyperbaric convulsions (Aerospace Med., 36, 1027, 1965) suggests that the initial insult may involve alteration of phospholipids in mitochondrial membranes. Indeed, peroxidation of phospholipid by the formation of an intermediate hydrogen peroxide, as suggested by Mengel, may be the common denominator of oxygen effect throughout the organism. Present biochemical concepts of David Green, et al, hold that all cellular enzymes are anchored to some membrane structure. Thus glycolytic enzymes are attached to the inner cell membrane, citric acid cycle dehydrogenases to the outer mitochondrial membrane, and electron transport respiratory chain enzymes to the inner mitochondrial membrane (cristae). Peroxidation of membrane phospholipid could alter structure to the extent of disrupting enzyme sites and altering electron flow.

It is therefore desirable to investigate the effect of increased oxygen tensions on mitochondrial membranes in liver, kidney, and other systemic organs. Phospholipids are easily extracted from mitochondria by aqueous acetone, for direct analysis. Mitochondrial preparations could be analyzed for the formation of lipid peroxides, and the effect of alpha tocopherol as a prophylactic agent (anti-oxidant) could be easily evaluated in this system.

This should be followed by an evaluation of the activity of key enzymes found associated with the mitochondrial membranes. Particularly worthy of emphasis is the possibility of inactivation of enzymes bearing sulfhydryl groups by the oxidation of these groups with subsequent formation of disulfide bonds. In addition, the effect of substituting succinate for alpha keto-glutarates as the in vitro substrate for evaluation of oxidative phosphorylation should be studied. This would help clarify the site of uncoupling, the role of the pyridine nucleotides, and significance of reversal of electron flow in the respiratory chain.

Additionally, the extreme sensitivity of central nervous system tissue to hypoxia and hyperoxia makes it desirable to evaluate the effect of small changes in oxygen tensions on CNS integrity. Determination of ATP/ADP/AMP levels in brain tissue should ultimately be included in the evaluation of possible adaptive problems associated with rapid changes in atmospheric oxygen tensions.

Further procedural modifications, which would increase sensitivity, specificity, and significance of analytic procedures described above, include the following:

(1) A method for separating medullary from cortical mitochondria in the kidney to enhance specificity and sensitivity of the study of proximal tubular cell mitochondria.

(2) Fluorometric methods for determination of tissue ATP/ADP/AMP, requiring only micro amounts of tissue. This would enable analysis of tissue obtained from experimental animals by percutaneous needle biopsy, without interruption of experimental exposure, anesthesia, or sacrifice. Serial determinations obtained from test organs of a given animal in this fashion would clarify pathogenesis, chronology, progression, and reversibility of lesions.

B. Research in the hematologic effects of increased oxygen tensions (Contract AF 33615-67-C-1482 with the Ohio State University Research Foundation; NASA supported)

Observations of data derived from examination of the blood of astronauts on the completion of Gemini 4, 5, and 7 Missions, revealed reversible decrease in circulating red blood cell mass. It was, and still is, unclear whether these changes were a consequence of exposure to the 5 psi pure oxygen spacecraft atmosphere, of confinement, of cardiovascular dynamic changes resulting from accelerative forces, or from prolonged weightlessness, or a combination of these (see Proceedings of the 2nd Annual Conference on Atmospheric Contamination in Confined Spaces, pp 200-222).

Even prior to these observations, research had been initiated to investigate the effect of increased oxygen tensions on the red cell and on erythrogenesis. In 1964, Dr. B. Katchman, under Contract AF 33(615)-1169 with the Miami Valley Hospital (Air Force supported), attempted to assay erythropoietin levels in rats exposed to 100% oxygen at 15 psi. He was unable to develop a bioassay that would reflect alterations in erythropoietin level of less than about 50% magnitude. Therefore, the results were inconclusive. However, work done by others (see Proceedings cited above) and observations of the Gemini astronauts, has suggested that there probably is some suppression of marrow activity in hyperoxic environments. This

may be a converse expression of the physiologic mechanisms which lead to secondary polycythemia in hypoxic environments.

In 1965, Dr. David Danon of the Weizmann Institute of Science in Rehovoth, Israel, working under Contract AF 61(052)-879 (Air Force supported), exposed rabbits to 100% oxygen at 15 psi total pressure.

He determined the alteration in age distribution of circulating red cells by density distribution, osmotic fragility, electron microscopy, and other parameters. He found that within 24 hours of exposure to the hyperoxic environment there was a marked increase in the number of "old" red cells in the circulation. This "accelerated" aging appeared to be maximal at 72 hours, and then reversed, with subsequent finding of increased proportions of younger cells in the circulation. There was no evidence of increased erythropoiesis during this interval. It was, therefore, postulated that the increased oxygen tension accelerated the red cell aging process, that senescent cells were then physiologically removed from the circulation, and that this consequently left a population consisting of the remaining young cells. His work is reported in detail in AMRL-TR-66-168, "Influence of Oxygen Toxicity on the Rate of Red Cell Aging".

Under the present contract with the Ohio State University Research Foundation, monkeys have been exposed to 15 psi of pure oxygen during September - November 1966. Blood was obtained from these monkeys after six hours, two, four, seven, and 12 days of exposure. This blood, and control blood samples obtained pre-exposure from the same monkeys, were transported to Dr. Charles Mengel's labs at Ohio State and analyzed for the following:

1. Hematocrits, hemoglobin, reticulocyte counts and Heinz body formation
2. Methemoglobin and plasma hemoglobin, osmotic and mechanical fragility
3. Autohemolysis, hemolytic sensitivity, serum bilirubin
4. Density distribution of red cells
5. Glycolytic intermediates, catalase, reduced glutathione, glutathione peroxidase, red cell glycolytic enzymes and intermediates, and red cell cholinesterase
6. Serum lipid peroxide levels

Evaluation of data from this small initial group is made with reservations. Arterial blood-gas analysis revealed that the red cells were not subject to continuous hyperoxia, but that because of severe pulmonary changes, an interval of hypoxemia prevailed somewhere between the fourth and eighth day of exposures. This hypoxic interval was unanticipated and, therefore, not taken into account in the original protocol for red cell analyses. However, the analyses suggest that with continued exposure there is a gradual decrease in serum tocopherol levels, or increase in lipid peroxide levels, an increase in red cell ATP levels, and possibly an increase in phosphofructokinase activity and in levels of reduced glutathione.

The latter two observations are converse to Mengel's findings in mice exposed to hyperbaric oxygen (three atmospheres) and, if valid, may represent a compensatory defense mechanism. In addition, in the six-hour to five-day interval there was evidence of change in density distribution, suggesting an increased proportion of older cells with subsequent reversal. These findings parallel those of Danon described above. The finding of increased ATP levels is interesting in light of the simultaneous finding of increased pyridine nucleotide levels in the same blood samples, when analyzed by IITRI personnel.

Proposed additional studies:

To enable significant conclusions to be drawn from the evaluation of blood exposed in vivo to one atmosphere of oxygen, it is necessary to repeat exposure of monkeys to that atmosphere for intervals from six hours to 12 days. With each blood sampling, simultaneous arterial blood pO_2 and pCO_2 levels must be established. To this end, we have already prepared nine female Rhesus monkeys for such an exposure. From each one we have already obtained preexposure blood on two separate occasions for complete analysis, and a bone marrow aspirate (from the posterior iliac spines) for evaluation. The advantage of using the 15 psi pure oxygen environment for these studies of red cell response to hyperoxia is clear. This creates a high enough oxygen tension to evoke a measurable response, as opposed to the 5 psi pure oxygen environment which may be completely benign, but, at the same time, a low enough oxygen tension to allow for mobilization of measurable antioxidant defenses as opposed to the 30-60 psi pure oxygen environment where changes occur too rapidly to permit the investigation of mechanisms. By using 15 psi exposures, we hope to be able to correlate the red cell damage occurring in animals exposed to hyperbaric oxygen, as documented by Mengel, with the confused and obscure hematologic reaction observed in the Gemini astronauts.

The present contract also called for exposure of monkeys to 5 psi of pure oxygen, with similar analysis of their blood. However, because of the delay resulting from the prohibition of experiments necessitating human exposures to pure oxygen environments, effective since 1 February 1967, we have been unable to complete the 15 psi portion of the contract, or even to begin with the 5 psi portion.

Because of the importance of the hematologic questions raised by the Gemini program, it is suggested that extensive evaluation of subhuman primates exposed to 5 psi of pure oxygen be initiated even if future programming disallows completion of the 15 psi experiments.

In addition, future plans include further study of the effects of hyperoxia on red cell aging, the response of macrophages and the reticulo-endothelial system in general to prematurely senescent cells, and the possible role of post-capillary sequestration of these cells in the phenomenon of decreased red cell mass seen in the Gemini astronauts.

2. Ultrastructural Changes

A. Research on the pulmonary pathology of oxygen toxicity (Contract AF 61(052)-941, with the University of Bern, through EOAR, NASA funded)

In 1964, rats were exposed to a 15 psi pure oxygen atmosphere for 24, 48, and 72 hours, and their lungs, along with those of control rats maintained in ambient air, were evaluated by Drs. Ewald Weibel and Gonzague Kistler at the University of Zürich in Switzerland, under Contract AF 61(052)-784, which was funded by the Air Force. The results of their findings are reported in AMRL-TR-65-66. In work done under the same contract, rats were exposed to 5 psi of pure oxygen for three, seven, and 14 days, and their lungs similarly examined. The results of this experiment are reported in AMRL-TR-66-103. In summary, at 15 psi interstitial edema appears after 48 hours of exposure, and this is followed at 72 hours by capillary endothelial damage, leukocytic infiltration and fibrin deposition in interstitial tissue, destruction of intracapillary erythrocytes, and there is simultaneous thickening of the air-blood barrier and reduction of specific gas exchange surface as a result of the morphologic damage described. However, the lungs of rats exposed to only 5 psi of oxygen, for as long as two weeks, show no morphologic changes. Morphometric evaluation of these lungs reflects a significant decrease in gas exchange surface, which in this instance is thought to represent an adaptive "oxygen effect" rather than toxicity. The above work utilized both light and electron microscopy as analytic tools, and a concise discussion of all the findings and implications appears in J. Cell Biology, 32, 605-628, 1967.

In April of 1966, Dr. Weibel moved to the University of Bern, and under the present contract has evaluated the lungs of dogs, monkeys, and rats exposed to 5 psi of oxygen for eight months and then sacrificed, and of a similar number, identically exposed, but then maintained in ambient air for 40 days after the completion of the oxygen exposure, prior to sacrifice. The results of this study were presented at the 3rd Annual Conference on Atmospheric Contamination in Confined Spaces in May 1967 by Dr. Weibel's associate, Dr. Margret Lewerenz. In summary, interstitial edema was found in the lungs of exposed dogs and rats, but not monkeys. Evaluation of monkey lungs was somewhat confounded by the presence of lung mites and filaria. In addition, morphometric evaluation of the dog lungs revealed a decrease in gas exchange surface quantitatively similar to that seen in the rats exposed to the same atmosphere for two weeks (see above). Unexpectedly, however, this change was not found in evaluation of the lungs from the rats exposed for eight months. These lesions were also present in the dogs and rats from the group allowed to remain in ambient air for 40 days after exposure, but to a much lesser extent, suggesting reversibility and significant healing. Since all the animals in this study were sacrificed after return to ambient air, it is unclear whether the lesions seen resulted from the oxygen exposure itself, or from the sudden removal to air after prolonged habitation of the pure oxygen environment. The similarity of these lesions to those seen in rats exposed to 15 psi of oxygen, after 48 hours, suggests that the effect was due to the oxygen.

Additional studies being performed under this contract include the evaluation of lungs from rats exposed to 5 psi of pure oxygen for up to 30 days, and of monkeys exposed to 15 psi of oxygen for up to 12 days, and of monkeys identically exposed and then allowed to recover in ambient air for several months. The results of the latter two studies are not yet reported. In addition, Dr. Weibel's group is examining the lungs of dogs, rats, and monkeys exposed to an atmosphere of 70% oxygen/30% nitrogen for eight months.

Proposed additional lung pathology studies:

Work accomplished in the past three years has done much to elucidate the pathogenesis of acute pulmonary oxygen toxicity. Work presently being done is

helping to elucidate the pathogenesis of subacute and chronic oxygen toxicity, a pathologic entity hitherto unappreciated. The latter has considerable civilian application in furthering the appreciation of risks inherent in excessive oxygen therapy. In addition, the basic findings appear to be broadly applicable to the understanding of mechanisms of pulmonary response to many topical irritants.

In the 5 psi work, the absence of significant pathology after two weeks of exposure is clear. The effects of exposure for up to 30 days is presently being evaluated. However, no data have been gathered in this program for the one-month to eight-month period. Since we are faced with evidence of morphologic changes at eight months, although they may not be functionally significant in terms of total lung impairment of function, it behooves us to investigate the intervening period to determine at what point these changes appear, and whether they are truly secondary to the oxygen tension.

In addition, results collected by Dr. Weibel have indicated that paradoxically, in the acute toxicity arising from 15 psi exposure, the capillary endothelium appears to be damaged before the alveolar epithelium, although the latter is exposed to a higher oxygen tension. The mechanisms initiating these reactions bear investigation.

B. Research on subcellular hepatic and renal effects of altered atmospheres (Contract AF 33(615)-3464 with Mount Sinai Hospital, New York, AF funded)

Work originally done by this contractor under Contract AF 33(615)-1849, between June 1964 and September 1965, was reported in the following publications: AMRL-TR-65-2, "Changes in Hepatic Structure in Rats Produced by Breathing Pure Oxygen", published in J. Cell Biology 27: 505-517, 1965; and AMRL-TR-66-90, "Electron Microscopy of Monkey Liver After Exposure of Animals to Pure Oxygen Atmospheres", published in Proc. Soc. Exper. Biol. and Med. 121: 1200-1203, 1966. In summary, this work describes mitochondrial changes resulting from exposure of rats and monkeys to pure oxygen at 760 mm Hg, 380 mm Hg, and 258 mm Hg total pressures. Evidence of resultant glycogen depletion, polyribosome formation, and increased numbers of autophagic vacuoles and lysosomes was found. In addition, a paper describing electron-microscopic evaluation of the lungs of rats exposed to 700 mm Hg of pure oxygen for 10 days, "Structure of Rat Lung After Protracted Oxygen Breathing", published in the Arch. of Pathology 83: 99-107, 1967, derived from this contract. This paper describes an increase in alveolar cells, increase in thickness of alveolar capillary endothelium, and decrease in alveolar capillary surface area. These findings are probably related to the "proliferative phase" of pulmonary oxygen toxicity which we have seen occur in animals surviving the first four to six days of exposure to 760 mm Hg of pure oxygen, and the associated acute pulmonary changes described by Weibel, et al.

Additional work done under the present contract is included in the reports by Schaffner and Mautner in the Proceedings of the 2nd Annual Conference on Atmospheric Contamination in Confined Spaces, pages 162-169 and 170-177. A report of the evaluation of livers of dogs, monkeys, and rats exposed to 5 psi of pure oxygen for eight months, AMRL-TR-66-61, "Hepatic Effects of Breathing Pure Oxygen for Eight Months Upon Rats, Dogs, and Monkeys", has been published in Aerospace Medicine 38: 273-274, 1967. In summary, evaluation of hepatic ultrastructure in animals exposed to 5 psi of oxygen for intervals varying from seven to 235 days,

reveals initial mitochondrial membrane change, reverting to almost normal by the 90th day of continuous exposure, with only evidence of increased organelle turnover thereafter. At 15 psi there is mitochondrial damage after only 24 hours, and it is more severe but qualitatively the same. A report of the evaluation of hepatic structure in animals exposed to a 70% oxygen/30% nitrogen atmosphere for eight months was presented by Dr. Klion at the 3rd Annual Conference on Atmospheric Contamination in Confined Spaces in Dayton on 9-11 May 1967. Nonspecific abnormalities were found, the significance of which is unclear. Kidney tissue from animals similarly exposed is still being examined. Dr. Mautner is also presently examining kidney tissue from monkeys exposed to 15 psi of oxygen for six hours to 12 days, in conjunction with renal function tests, and Drs. Schaffner and Klion are examining livers from rats exposed to 15 psi of pure oxygen for intervals from 15 to 360 minutes.

Proposed additional work:

Baseline evaluation of hepatic response to oxygen is adequate. Additional electron-microscopic support would be essential to further evaluations of hepatic cellular biochemistry. In addition, similar support would be useful in evaluating synergistic or additive effects of increased oxygen tensions with other potential toxicants, or of experimental mixed gas atmospheres. Additional renal electron microscopy is needed for correlation with renal function tests described under the "Inhouse" portion of this report, and also for correlation with renal mitochondrial respiration studies. In addition, the same comments applicable to study of potentially hepatotoxic agents above would apply to the use of electron microscopy in studying nephrotoxic agents.

3. Arterial Blood Gas Studies (Inhouse)

In evaluating morphologic, biochemical, and functional response of various organs and organ systems in animals exposed to 100% oxygen at one atmosphere total pressure, it has proved necessary to monitor arterial pO_2 at the time of organ sampling or testing. This is because of the extensive pulmonary damage which inevitably occurs after about three days of exposure. The associated alveolar exudate, interstitial edema, and capillary endothelial damage leads to ventilation, perfusion, and diffusion abnormalities. Consequently, although atmospheric oxygen tension remains extremely high, the amount of oxygen successfully diffusing from the lungs into the arterial blood is drastically reduced, and hyperoxemia rapidly changes to normoxemia and then hypoxemia. It is, therefore, invalid to assume that liver, kidney, endocrine or CNS tissue of an animal in the fourth through eighth day of exposure to one atmosphere of pure oxygen, is still hyperoxic. In conjunction with studies of the above organ systems, and of the red cell, we have attempted to establish an arterial blood gas curve for monkeys exposed to 15 psi of oxygen for up to 12 days. Simultaneously, this has also given us information about the functional significance of the pulmonary pathology which we have reported. We have been unable to complete this curve because of the prohibition of experiments involving exposure to pure oxygen atmospheres (since 1 February 1967), but data accumulated prior to that is summarized in table III.

TABLE III

Monkey	Preexposure (ambient air)		Exposure (15 psi O_2)			Recovery (ambient)		
	pO_2	pCO_2	Days	pO_2	pCO_2	Days	pO_2	pCO_2
OZ-4	91	45	2	610	--	None		
OZ-8	94	38	2	550	34	None		
B-51	94	42	2	575	38	None		
B-80	--	--	3	360	24	None		
B-54	102	43	4	80	84	None		
B-53	97	37	4	600	67	None		
IZ-0	84	44	7	62	61	None		
B-58	100	44	12	655	--	84	96	45

Analysis of the above data indicates that there is not sufficient pulmonary damage to interfere with gas exchange after two days of exposure. This is consistent with known observations of lung morphology. However, at three to four days there seems to be individual variability, probably due to natural variations in degree of lung damage between different monkeys. The low pCO_2 seen in B-80 at a time when there is definite decrease in pO_2, but still a hyperoxemic state, is probably secondary to hyperventilation induced by the pulmonary damage, and a reflection of the fact that CO_2 diffuses 30 times more easily than O_2, and, therefore, pulmonary damage sufficient to interfere with O_2 diffusion may not be sufficient to interfere with CO_2 diffusion. With this in mind, it is difficult to explain the high pCO_2 found in B-53 at a time when there was no evidence of interference with O_2 diffusion. The values listed for this animal are, however, the average of two independent sets of blood gas determinations obtained about an hour apart, which were in excellent agreement (pO_2: 650, 550; pCO_2: 55, 79). Therefore, they are considered to be valid measurements. Between the fourth and eighth days of exposure, all monkeys are visibly ill, with marked respiratory distress. About 60% of them die during this interval. Pathologic examinations have revealed that the degree of pulmonary architectural disruption, edema, and exudate is maximal at this time. It is, therefore, not surprising to find subnormal pO_2 levels and also evidence of CO_2 retention. In fact, since the measurements obtained on days four and seven were from living monkeys, it would be reasonable to assume that, had we measured blood gas levels on those that died during that period, shortly before their demises we would have found even lower values. I would postulate at this point that the specific mechanism of death in acute oxygen toxicity is severe hypoxia secondary to pulmonary damage. The big gap in our measurements is between the eighth and twelfth day of exposure. We know that during this period the classical acute pulmonary pathology of oxygen toxicity disappears, the exudate is resorbed, the edema is organized, and the lung enters a proliferative phase of being dry, but heavy, with thickened alveolar epithelium and scarring in the interstitium. With this the monkeys show marked clinical improvement. They are no longer in respiratory distress, eat and drink normally again, and demonstrate normal kinetic activity. The finding of a pO_2 of 655 at 12 days is, therefore, not unbelievable. However, if these monkeys are removed from the 750 mm Hg oxygen tension of the dome to ambient air, the proliferative changes become significant and cyanosis and coma rapidly intervene.

B-58, however, examined after having been weaned back to ambient air over a one-week period with gradually decreasing oxygen tensions, successfully made the transition. Two and one-half months later, she appeared completely normal, and had normal pO_2 and pCO_2 levels in ambient air. Postmortem examination of B-58's lung tissue revealed an almost normal lung, with disappearance of the proliferative changes, and only residual interstitial scarring and some slight interstitial cellular infiltrate.

Proposed additional studies:

It is necessary to fill in the gaps in our 0-12 day 15 psi oxygen exposure curve. Also, it is necessary to obtain arterial blood samples from unanesthetized monkeys, to avoid artifact secondary to anesthesia-induced respiratory depression, especially in sick monkeys. The above data was accumulated from monkeys anesthetized with intravenous Nembutal. As evidenced by the preexposure values, which are well within acceptable normal range, the anesthesia, which was kept light for this purpose, did not apparently induce artifact. However, it is an additional variable, best dispensed with. The significance of this work is twofold:

1) It is necessary to put studies of other organs obtained during possible hypoxic periods in necessary perspective.

2) The information gained is invaluable in interpretation of pulmonary response to high oxygen tensions, not only for our experimental purposes, but also for the increased clinical interest in pulmonary damage in human patients exposed to prolonged oxygen therapy or to hyperbaric oxygen therapy.

4. Renal Function Tests (Inhouse)

A colony of monkeys was prepared for evaluation by the surgical translocation of the left kidney of each monkey to a subcutaneous pocket. Each monkey was then subjected to a series of renal function tests consisting of glomerular filtration rate, renal plasma flow, maximal proximal tubular excretory capacity, and maximal proximal tubular reabsorptive capacity. The techniques have been described elsewhere in these proceedings (see page 81).

These procedures were adapted in the fall of 1966 to the study of monkeys exposed to pure oxygen continuously for as long as 12 days. The experiment called for preexposure evaluation of 12 monkeys, which were then divided into four groups of three, with each group to be reevaluated after one of the following intervals of exposure: two days, four days, seven days, and 12 days. However, because of the toxicity of the experimental atmosphere, a number of monkeys expired before the conclusion of the test interval, or during the test procedure. Consequently, we were able to evaluate only seven of the original 12 monkeys, with results as summarized in table IV (C: control, E: experimental).

Two monkeys were exposed, and then removed from the experimental atmosphere, and allowed a recovery period in ambient air. The results of their tests are set forth in table V (C: control, R: recovery).

TABLE IV

Monkey	Days Exposed	Cf/kg		C PAH/kg		Tm PAH/kg		Tm glucose/kg	
		C	E	C	E	C	E	C	E
OZ-4	2	3.2	3.3	16.4	30.3	2.78	3.64	14.7	10.1
OZ-8	2	3.4	5.1	23.6	27.7	2.39	3.38	15.0	22.2
B-51	2	3.7	2.8	18.5	10.4	2.79	1.80	18.4	11.6
B-53	4	4.1	0.9	22.6	10.5	2.11	0.65	23.2	1.9
IZ-0	7	3.2	3.0	14.7	35.2	2.62	2.45	13.2	9.1
B-58	12	2.5	3.6	17.6	30.0	2.12	2.37	9.2	15.1

TABLE V

Monkey	Days Exposed	Days of Recovery	Cf/kg		C PAH/kg		Tm PAH/kg		Tm gluc/kg	
			C	R	C	R	C	R	C	R
B-66	8	56	3.5	3.2	17.5	12.6	---	3.14	14.4	10.4
B-58	12	84	2.5	2.6	17.6	17.7	2.12	2.78	9.2	5.5

In all the above tables, kilograms of body weight was used as a common denominator to facilitate the comparison of monkeys of different weights and, therefore, different renal mass. Cf refers to the filtration clearance, or glomerular filtration rate in ml/minute. C_{PAH} is the PAH clearance, a measure of renal plasma flow, also in ml/minute. The Tm or T_{max} values are expressed in mg/minute. Tm glucose determinations were discontinued at the time of the "control" evaluations because of technical difficulties in the urine glucose analysis. Attempts are being made to clarify the problem and reinstate this portion of the function tests.

The evaluation of renal function in monkeys exposed to 15 psi of oxygen is also obviously incomplete. Although at first glance it would appear that there are no abnormal changes after two days of exposure in monkeys OZ-4, OZ-8, and B-51, closer scrutiny suggests that the moderate decrease in all parameters seen in B-51 might represent an early deficit, secondary to that monkey's higher susceptibility. This would be consistent with clinical observations that clearly indicate that tolerance to this atmosphere is subject to wide individual variation between animals. In B-53, exposed for four days, there is complete and marked disruption of function. Renal plasma flow is still about 50% of normal, this reduction probably attributable to the general debility of the animal, but the other parameters are disproportionately lowered to a much greater extent. This correlates chronologically with the finding of uncoupling of oxidative phosphorylation in the mitochondrial preparations obtained from the kidneys of monkeys exposed to the same atmosphere for the same four days. Proximal tubular function, measured by the T_{max} tests, is dependent on energy from oxidative phosphorylation. It has been shown in the past that known uncoupling agents, like dinitrophenol, will decrease T_{max} values, after administration to many species of animals.

Further evaluation of the data suggests that there is complete recovery of function in animals surviving the first week of exposure. The unusually high renal plasma flow (C_{PAH}) rates in these longer duration exposures may be a reflection of sensitive cardio-vascular dynamic changes associated with the stress of exposure, or with uninvestigated endocrinologic (adrenergic) changes.

Additional work needed for completion of these studies:

Urine for these analyses was collected by bladder catheterization, i.e., the analysate was a common pool of the output of both kidneys. Since it is quite possible that there is some restriction in activity of the left kidney arising from the translocation, but that this restriction is masked by compensatory hyperactivity of the right kidney, yielding overall normal renal function, further studies are planned to evaluate and validate the reliability and sensitivity of the procedures used. These include the following:

1. Renal function tests of individual kidneys by cannulation of each ureter on five monkeys with translocated kidneys and on five monkeys with kidneys in the normal anatomical position

2. Renal function tests on monkeys with unilateral nephrectomy to determine the compensatory change in the remaining kidney

3. Intravenous pyelograms of translocated kidneys

5. Histopathology (Inhouse)

Earlier inhouse work designed to evaluate pulmonary response to increased oxygen tensions was reported by Caldwell and Lee in AMRL-TR-65-136, "Effect of Oxygen Breathing on One Atmosphere on the Surface Activity of Lung Extracts in Dogs". They found that there was loss of surface activity in lung extracts obtained from dogs which had been exposed to 98% oxygen at one atmosphere total pressure for 54 hours. A more recent report, AMRL-TR-66-234, "Pathology of Oxygen Toxicity in 40 Macaca Mulatta", by Robinson, Harper, Kaplan, and Thomas, describes the pulmonary responses of monkeys exposed to pure oxygen atmospheres at pressures from 600 to 760 mm Hg, for as long as 14 days. Additional data on this subject was presented by Maj. Farrel Robinson at the 37th Annual Scientific Meeting of the Aerospace Medical Association in April 1966, and has since appeared in Aerospace Medicine 38: 481-486, 1967. In summary, these papers describe the progression of pulmonary responses from the acute to the proliferative stage (see discussion of arterial blood gas studies).

Recent clinical interest has turned to evaluation of pulmonary response of humans to prolonged oxygen therapy. In a letter to the editor titled, "Pulmonary Disease and Respirator Therapy", published in the New England Journal of Medicine 276: 1264, 1967, our experimental findings in monkeys are correlated with these clinical reports.

Preliminary evaluation of lung tissue specimens obtained from monkeys exposed to 15 psi of pure oxygen for up to 12 days, in the fall of 1966, demonstrates well the changes described in the above reports. In addition, two monkeys that had

been exposed for eight and 13 days respectively, and then allowed to recover in ambient air for 56 and 84 days, demonstrated remarkable recovery. The monkey exposed for 13 days did have some residual interstitial scarring, but apparently not enough to interfere with function. Tissue from these lungs is presently being evaluated by Dr. Ewald Weibel's group for electron-microscopic confirmation and elaboration of these findings. The data and conclusions derived will probably be applicable to understanding pulmonary response to pulmonary irritants in general, not merely in relation to oxygen toxicity.

SUMMARY

An integrated program utilizing morphologic, metabolic, functional, and clinical evaluation of animals exposed to artificial atmospheres has been undertaken to evaluate mammalian response to these atmospheres. Efforts have been concentrated on lungs, kidneys, liver, and erythrocytes, as potential target organs or sites of concentration, detoxification, or metabolic sensitivity. The program has included both Air Force and contractor personnel and facilities, and has been supported by both Air Force and National Aeronautics and Space Administration funds.

The 15 psi pure oxygen atmosphere has been lethal to 40 to 100% of animals exposed for as long as five days, depending on species, strain, age, weight, and other variables. The primary site of toxic reaction is the lungs, where oxygen tensions are highest. Secondarily involved at the cellular level are other organs. In the liver and kidney, the increased oxygen tensions have initiated morphologic changes on the ultrastructural level, primarily in mitochondria, which are metabolically reflected by partial uncoupling of oxidative phosphorylation in those organs, and may be functionally reflected by interference with tubular function, an energy dependent phenomenon, in the kidneys. These effects, and the as yet incompletely explained effects on the erythrocytes, are possibly commonly mediated by the peroxidation of unsaturated lipids in cellular and intracellular organelle membranes throughout the body.

The acute pulmonary lesions are often severe enough to interfere with ventilation, perfusion, and diffusion, thereby causing hypoxemia, the probable cause of death. Exudate in these pulmonary lesions resolves in those animals that survive the acute insult. The acute changes are replaced by chronic proliferative changes which interfere with diffusion to the extent of requiring abnormally high alveolar oxygen tensions to maintain adequate blood oxygen tensions. These lesions all appear to have the potential for reversibility upon removal of the animals from the experimental atmosphere. However, animals removed after the onset of the pulmonary proliferative changes will have residual interstitial scarring.

The changes seen in the animals exposed to the 5 psi pure oxygen spacecraft environment appear to be adaptive rather than toxic. The morphologic studies reveal ultrastructural changes in liver and kidney which we choose to call oxygen effect, rather than toxicity, because we are unable to demonstrate any metabolic or functional deficit arising from them. In the lung, no detrimental changes are seen except by electron microscopy after eight months of exposure. Ever these latter changes are reversible with return to air, and are apparently unassociated with any functional defect.

Much of the above, especially on the 5 psi level, is speculative; based on interpretation of preliminary or incomplete data. Much work still needs to be done on understanding cellular respiratory and energy producing mechanisms, erythrocyte reactions and adaptive mechanisms, and alterations in renal function and compensatory capabilities. It also is essential that the concept of adaptation to the 5 psi oxygen atmosphere as a potential hazard for later return to ambient air be further explored.

Tentatively, however, we conclude that the organism recognizes the 5 psi pure oxygen atmosphere as a foreign one, undergoes certain morphologic and metabolic alterations at the subcellular level to adapt to the change, and may have to undergo similar readaptive changes on return to ambient air. Within the scope of these changes we find no alterations that may be defined as toxic, if we take that to mean detrimental to or creating a significant hazard of morbidity or mortality for the exposed organism.

CLOSING REMARKS

DR. THOMAS: There are two things which I would like to underscore and put in the proper perspective.

All the speculations about the possible explanation of pathological, ultrastructural, and biochemical changes in the mixed gas 5 psia eight-month exposure study, intriguing as they may be, are only speculations. We will have to repeat this study to see whether these changes are true and reproducible. Certainly there is no obvious reason to explain these changes as far as partial pressure of oxygen and oxygen toxicity are concerned. Another thing which we will have to do is to compare the results of this new study with a true set of controls, i. e. with results from animals kept in the dome environment at ambient pressure air atmosphere. This will also be done. This is the only way to take care of all environmental variables.

The only other thing I should like to add to this, in retrospect, is that by listening to the papers and the discussions I would like to dispel a possible misunderstanding about the quality of our experimental animals and the validity of our conclusions which are based on the pathological findings. I am sure that what was presented in connection with endemic diseases in the experimental animals was nothing new or unusual to those of us who are familiar with animal research in general, and veterinary pathology in particular. Perhaps this surprised some people in the audience who are not biologically oriented. Perhaps we got a little bit involved in semantics with reference to what is a healthy animal or what is a suitable experimental animal. I wish to state that all the animals used in any of our experiments were apparently healthy. They were apparently healthy as far as we could tell, and this judgment was based on good veterinary medical examinations and on a complete clinical laboratory evaluation of these animals, performed several times before undergoing exposure. The rigidity of these examinations is in excess of that done on humans before they get a clean bill of health. I venture to say that a careful pathological study on victims of fatal accidents who received a clean bill of health a few days before their deaths would reveal just as many pathological changes in their tissues as we have found in our animals, except that they would be qualitatively different. The main thing is, and I am sure that my colleagues will agree, that we can always find areas in organs where the endemic disease does not obliterate the fine changes caused by the toxic exposure. I believe Dr. Patrick has said, in effect, that his work could have been much easier if endemic disease were absent, but endemic disease did not make it impossible to evaluate pathology that can be attributed to the exposure. As a matter of fact, there are only a few isolated instances, and these relate to the morphometric analyses of changes in the lungs, where some animal material could not be studied because of the extent of structural aberations due to lung mites and filaria.

One last point. The discussions were extremely stimulating and many people have commented that they wish that we could have had more time for these. I assure you that we will have more time programmed for such open sessions at our next conference.

I want to thank everybody for coming and participating in our Conference. I am more than ever convinced that audience participation and the informal atmosphere

we managed to keep are a real tribute to the good and free exchange of scientific ideas and technical information. I wish to thank both Col. Yerg and Col. Schafer for their stimulating Welcoming and Introductory Remarks. I want particularly to thank the Chairmen for their skilled mediation of the scientific sessions, and Dr. Coulston for his intriguing Banquet address, which related so specifically to the new trends we shall have to follow in pathology, to all our speakers for their fine presentations, and to all of our administrative personnel both from Aerojet-General and our Division who made the necessary arrangements before and during this Conference.

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13. ABSTRACT

This report is a complete compilation of the papers presented and the Proceedings of the 3rd Annual Conference on Atmospheric Contamination in Confined Spaces, sponsored by the Aerospace Medical Research Laboratories and held in Dayton, Ohio on 9, 10, and 11 May 1967. Major technical areas discussed by the invited speakers, members of the Open Forum and Conference attendees included toxicological evaluation of atmospheres and contaminants, histopathological evidences of toxicity, evaluation of cabin materials, instruments and detection techniques, measurement of behavioral responses, and life support systems. Included as an Appendix, but not presented at the Conference, is a status report on oxygen toxicity prepared by Dr. Harold Kaplan, which is considered sufficiently pertinent to the Conference proceedings that it should be of interest to all participants.

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