UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

AD A040384

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: ARCSL-TR-77026	2. GOVT ACCESSION NO.
3. RECIPIENT'S CATALOG NUMBER	
4. TITLE (and Subtitle): CENTRAL, PERIPHERAL, AND HORMONAL EFFECTS OF SCOPOLAMINE IN MALE VOLUNTEERS	5. TYPE OF REPORT & PERIOD COVERED: Technical Report, December 1974 - March 1975
6. PERFORMING ORG. REPORT NUMBER: EB-TR-76109	
7. AUTHOR(s): George M. Vaughan, Leo L. Laughlin, Frederick R. Sidell, Kenneth M. Wilson, Mary K. Vaughan, Paul D. Woolf, Shiu F. Pang	8. CONTRACT OR GRANT NUMBER(s)
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Director, Chemical Systems Laboratory, Attn: DRDAR-CLL-MC, Aberdeen Proving Ground, Maryland 21010	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: Task 1W762718AD2103
11. CONTROLLING OFFICE NAME AND ADDRESS: Director, Chemical Systems Laboratory, Attn: DRDAR-CLJ-I, Aberdeen Proving Ground, Maryland 21010	12. REPORT DATE: May 1977 13. NUMBER OF PAGES: 31
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	15. SECURITY CLASS. (of this report): UNCLASSIFIED 15a. DECLASSIFICATION/DOWNGRADING SCHEDULE: NA

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

Medical effects of chemical agents; clinical evaluation of chemical agents

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Scopolamine	Human subjects
Performance	Serotonin
Confusion	5-hydroxyindoleacetic acid
Pupils	Hormones
Heart rate	Melatonin

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

Scopolamine (12 to 20 µg/kg) was given to seven men intravenously. Reduction in motor performance and in ability to add numbers was evident for 4 to 8 hours after injection. Delusions, hallucinations, slurred speech, and disconnected sentence structure lasted 1 to 3 hours. An initial mydriasis correlated with tachycardia and a subsequent mydriasis correlated with maximal decrement in ability to add numbers. Plasma luteinizing hormone (LH) peaked at 30 minutes in three out of four subjects; follicle stimulating hormone (FSH) but not LH was released in the remaining subject. No effects on testosterone, growth hormone, or thyrotropin were observed. Stimulation of cortisol release was greater after scopolamine than after control

(over)

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

20. ABSTRACT.

injection in each of four subjects. Smaller decrements in ability to add numbers seemed correlated with higher 5-hydroxyindoleacetic acid (5-HIAA) excretion, whereas higher levels of incapacitation seemed related to reduced 5-HIAA excretion. These findings are discussed in relation to a possible serotoninergic basis for scopolamine intoxication.

PREFACE

The work described in this report was authorized under Project/Task 1W762718AD2103, Clinical Evaluation of Chemical Agents. This work was started in December 1974 and completed in March 1975.

The volunteers in these tests are enlisted US Army personnel. These tests are governed by the principles, policies, and rules for medical volunteers as established in AR 70-25 and the Declaration of Helsinki.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council.

The use of trade names in this report does not constitute an official endorsement or approval of the use of such commercial hardware or software. This report may not be cited for purposes of advertisement.

Acknowledgment

We wish to thank the nurses and medical corpsmen of the Clinical Research Branch, Edgewood Arsenal, for their invaluable assistance in ward care of the subjects and in data collection and tabulation; and Ms. Louyse Lee for careful performance of pituitary and adrenal hormone assays.

CONTENTS

CENTRAL, PERIPHERAL, AND HORMONAL EFFECTS OF SCOPOLAMINE IN MALE VOLUNTEERS

I. INTRODUCTION.

The effects of scopolamine on the central nervous system (CNS) have been described recently in detail and shown to be related to inhibition of cholinergic mechanisms.[1] Although the central to peripheral potency ratio for scopolamine was about tenfold greater than that for atropine, the effects of scopolamine on peripheral sites (heart and pupils) were noted. The impression of a delay in full mydriasis past the point for expected action at iris receptor sites, and the suggestion of bradycardia (following upon the more dramatic tachycardia) after scopolamine were considered as possible central manifestations of drug action. The present study was undertaken to confirm the effects of the drug on performance, to examine pupil size and heart rate in more detail, and to illustrate the pattern of response in individual human subjects.

Furthermore, in this study the pituitary axis was examined because of the role played by the anticholinergic agent, atropine, in elucidation of the critical period of neural activity on the afternoon of proestrus.[2] When given at the beginning of the critical period, this drug inhibited the hypothalamic stimulation of the release of ovulating hormone from the pituitary. Atropine also inhibited sexual behavior in male and female rats.[3]

The effect of scopolamine on compensatory ovarian hypertrophy in mice was also investigated.

II. MATERIALS AND METHODS.

The volunteers in this study were enlisted US Army personnel, 19 to 25 years of age. These tests are governed by the principles, policies, and rules for medical volunteers as established in AR 70-25 and the Declaration of Helsinki. The subjects refrained from ingesting bananas, cheese, coffee, colas, tea, or any drugs during the days of testing. Except for subject 1 each man was given a control saline injection on day 1 and scopolamine on day 2. Performance and physiological parameters were followed in a similar fashion on both days. Scopolamine hydrobromide or saline was given in a single bolus intravenously through an indwelling venous catheter inserted 30 to 60 minutes prior to injection for baseline blood samples and subsequent sampling. After a light breakfast 2 hours earlier, injections were given at 0800 (subject 1), 0900 (subjects 2, 3, 4, and 5), or 1000 (subjects 6 and 7) hours.

At various intervals after injection, pulse was measured for 30 seconds by palpation of the radial artery, and pupil size was measured by use of an infrared-sensitive television pupillometer with a tenfold image magnification and an accuracy of 0.1 mm. Number facility (NF) tests were administered to assess cognitive performance,[1] and peg board (PB) (subjects 2, 3, 4, and 5) tests were given to assess motor coordination. Blood samples (20 ml each) were drawn in heparinized syringes and tubes; plasma was deep-frozen until assay. Subjects 6 and 7 were connected to an electrocardiograph (ECG) oscilloscope from which heart rates were obtained by counting QRS complexes for 30 seconds and/or by measuring the QRS interval on ECG strips. Mean pupil diameters (of left and right pupils measured one immediately after the other) were recorded in subjects 2, 3, 4, and 5; and only left pupil diameter was

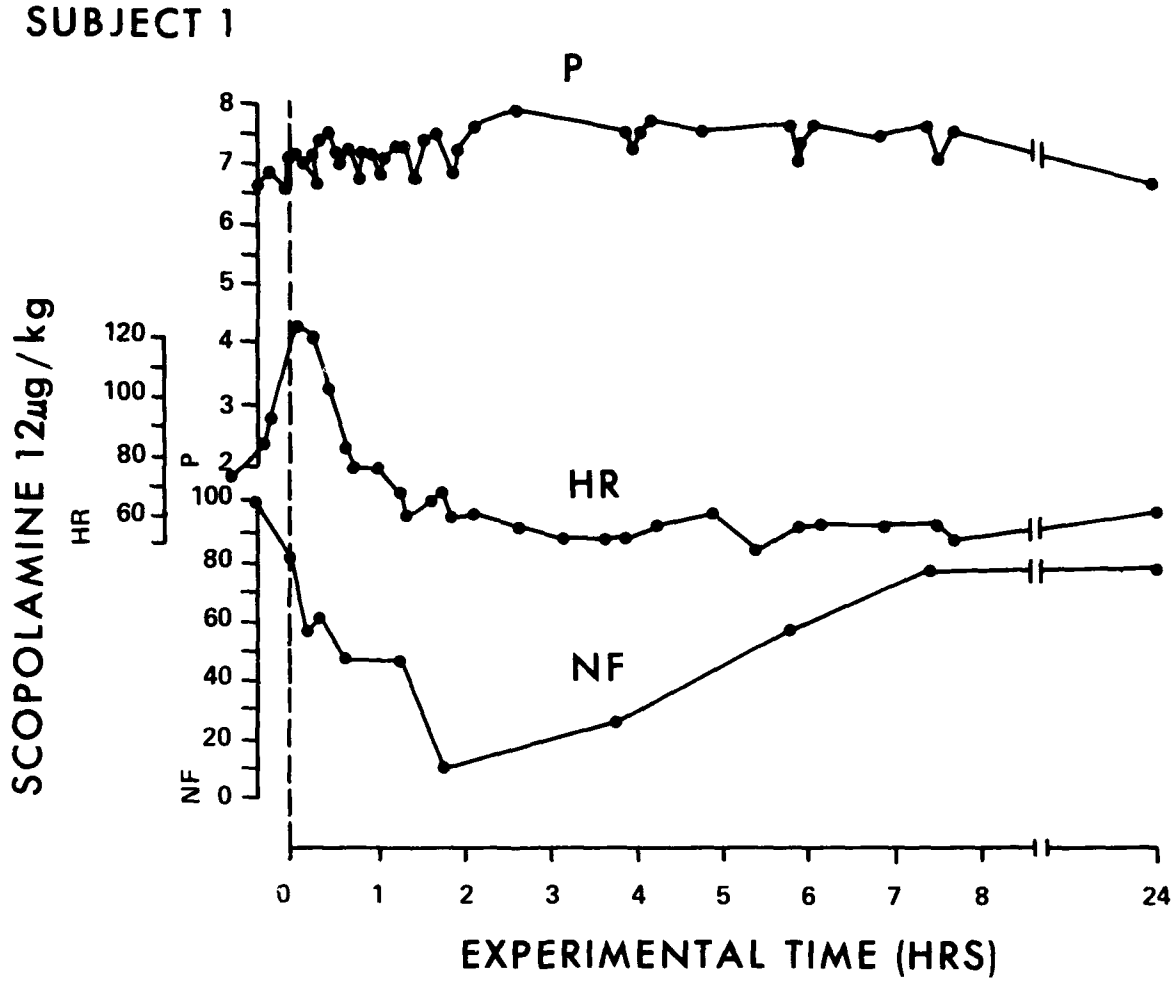
measured in subjects 1, 6, and 7. For subjects 6 and 7, a brightness discriminator attachment was added to the pupillometer in order to facilitate the readings. Urine was collected for various time intervals in subjects 2, 3, 4, 5, 6 and 7 to measure 5-hydroxyindoleacetic acid (5-HIAA), which was calculated in terms of milligrams excreted per hour.

Luteinizing hormone (LH),[4] follicle stimulating hormone (FSH),[5] human growth hormone (HGH),[6] and thyroid stimulating hormone (TSH)[7] were measured in plasma by radioimmunoassay. Cortisol was measured by competitive protein-binding.[8] Testosterone was measured by radioimmunoassay using the commerical test set from Wien Laboratories, Succahanna, New Jersey. Five-HIAA was measured according to the method of Undenfriend, *et al.*[9] for urinary 5-HIAA with a modification of the Hycel kit procedure. Plasma melatonin was measured by bioassay according to the method of Ralph and Lynch.[10]

In order to test the effect of scopolamine on the compensatory ovarian hypertrophy (COH) response of young mature Swiss-Webster mice, a single injection of diluent (distilled water) or one of three doses of scopolamine in a volume of 0.1 ml was given intraperitoneally on the day of unilateral ovariectomy. Ten days later the remaining ovary was removed, and the weight increment attained in this ovary was expressed as a percent of the weight of the first ovary and was termed COH. Ovarian weights were corrected for body weight.

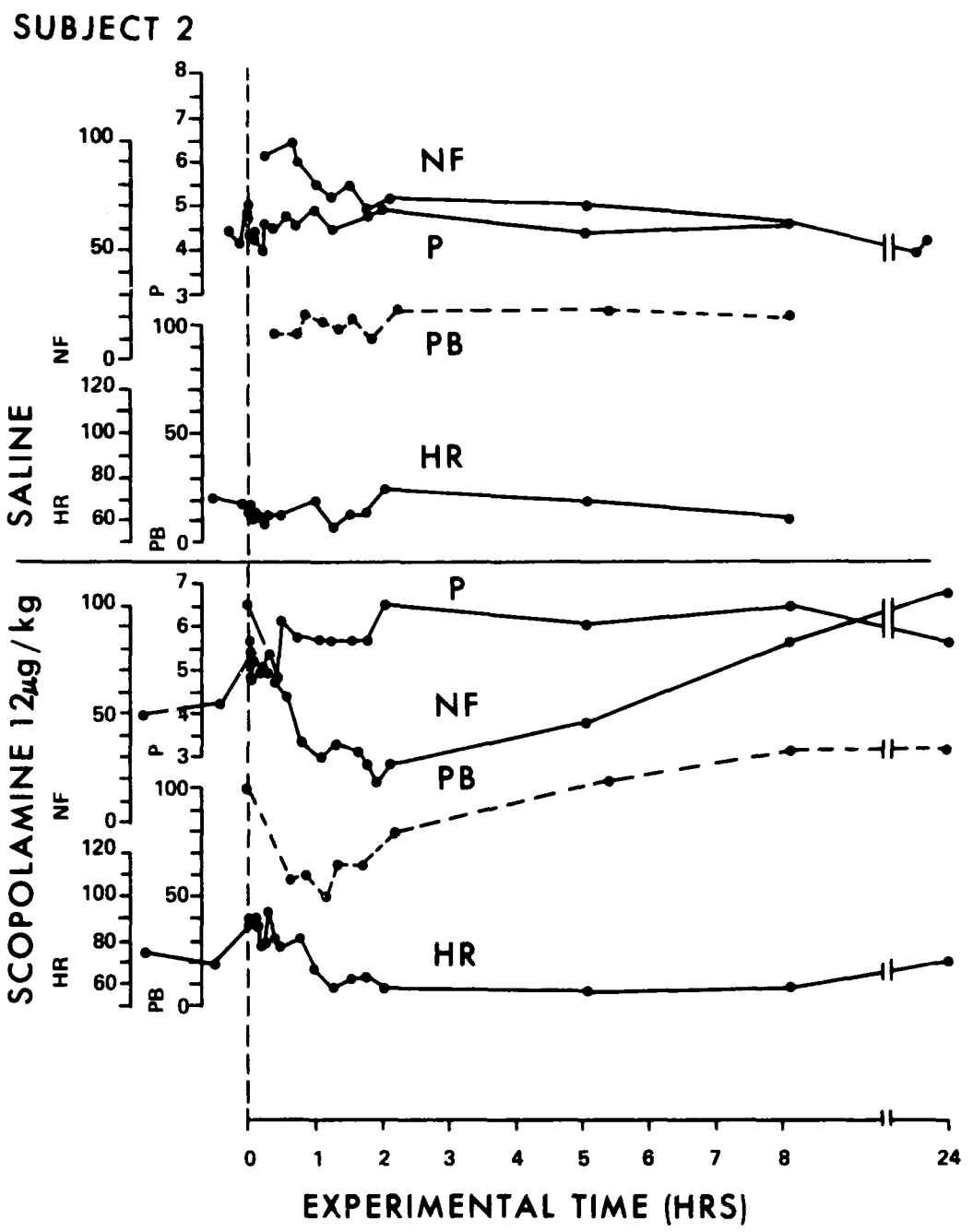
III. RESULTS.

Figures 1 through 7 depict the time course of the performance and physiological responses to saline and scopolamine. Pupil size began to rise within 2 to 3 minutes of injection of scopolamine (P_1). After this, it leveled off by 12 minutes (figure 8). There was a second rise in pupil size (P_2) culminating in the maximal pupil size change (P) at 1 to 2 hours. In subject 3, who had the smallest NF decrement, there was no definite second rise, although between 1 and 2 hours the pattern seemed slightly altered. There was a great deal of variability, and by 24 hours pupil size was still not quite to baseline, except in subject 1, who was measured in the dark. The others were measured in fluorescent light (12 footcandles). The total maximal change (P) in pupil size varied between 1.05 and 3.8 mm in diameter, and the maximal P_2 ranged from 0 to 2.1 mm. In subjects 1, 2, 3, 4, and 5 heart rate peaked in 1 to 5 minutes, and in subjects 6 and 7, who were monitored continuously by ECG, it peaked by 1 minute. Tachycardia (heart rate above baseline) continued for 40 to 60 minutes, and the maximal elevation in heart rate ranged from 24 to 54 beats per minute (bpm) (table 1). The pulse rate returned to a level 10 to 20 bpm below baseline for several hours except in subject 5, whose pulse rate returned to baseline. Examination of curves for the control day revealed no tendency for bradycardia below baseline. In subject 7, there was no difference between measuring heart rate by counting for 30 seconds and by measuring QRS intervals for ECG strips. In the latter method, there is great variability even when only seconds elapse between measurements (figure 7).



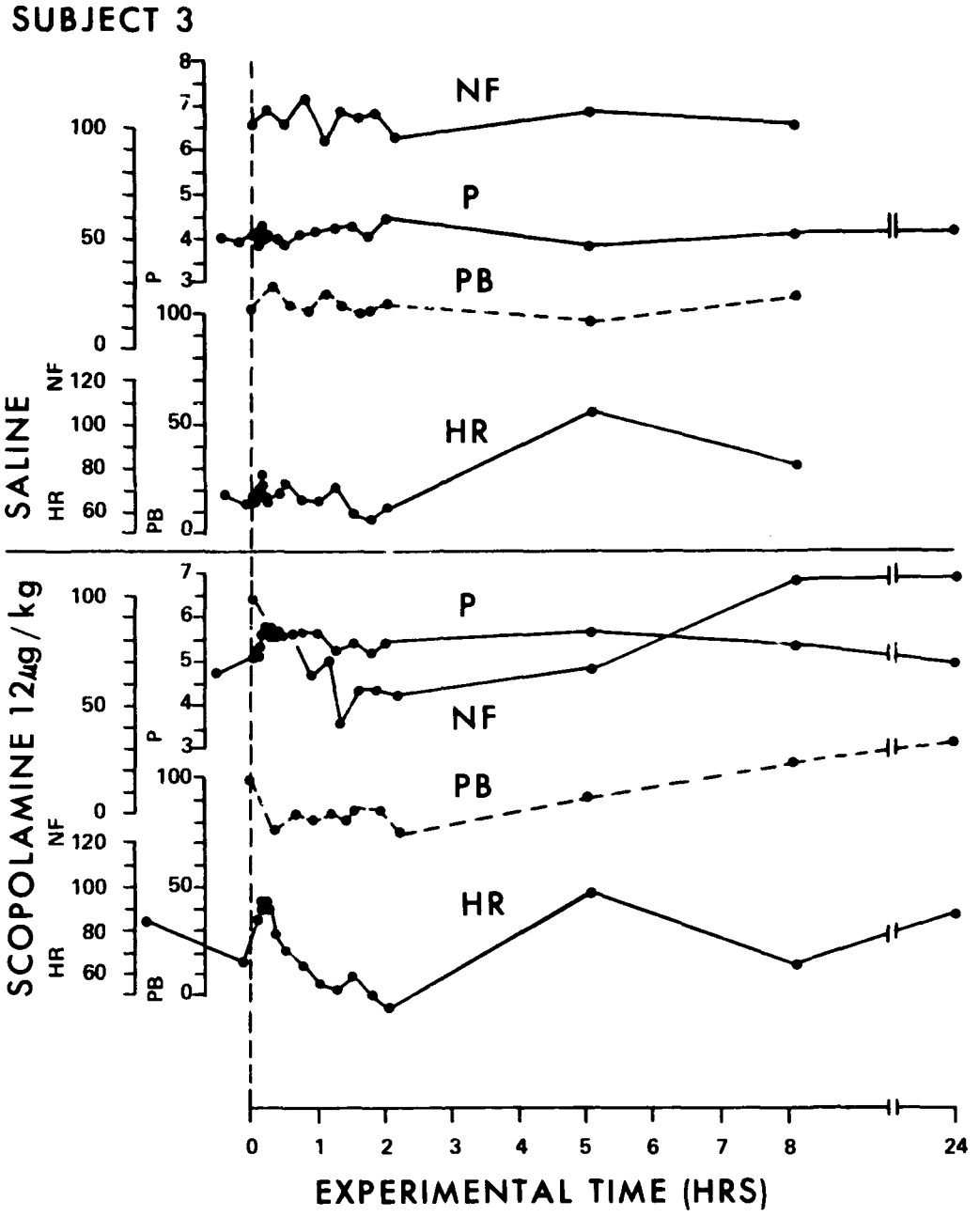
Legend: P, pupil diameter (mm)
NF, number facility
HR, heart rate (beats/minute)

Figure 1. Time Course of Responses After Injection of Saline Or Scopolamine in a Volunteer at Zero Experimental Time



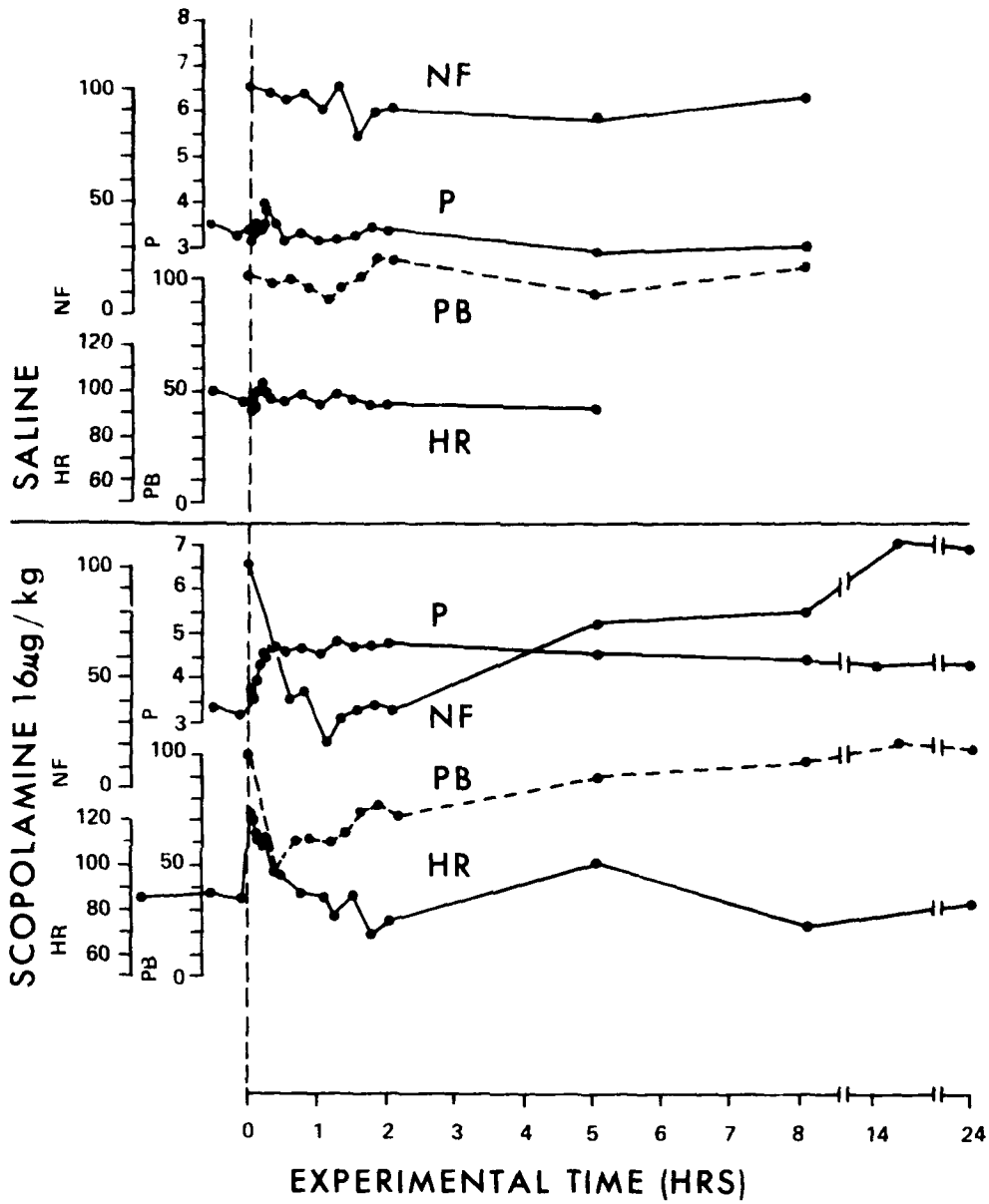
Legend: P, pupil diameter (mm)
NF, number facility
PB, peg board performance (percent baseline)
HR, heart rate (beats/minute)

Figure 2. Time Course of Responses After Injection of Saline Or Scopolamine in a Volunteer at Zero Experimental Time



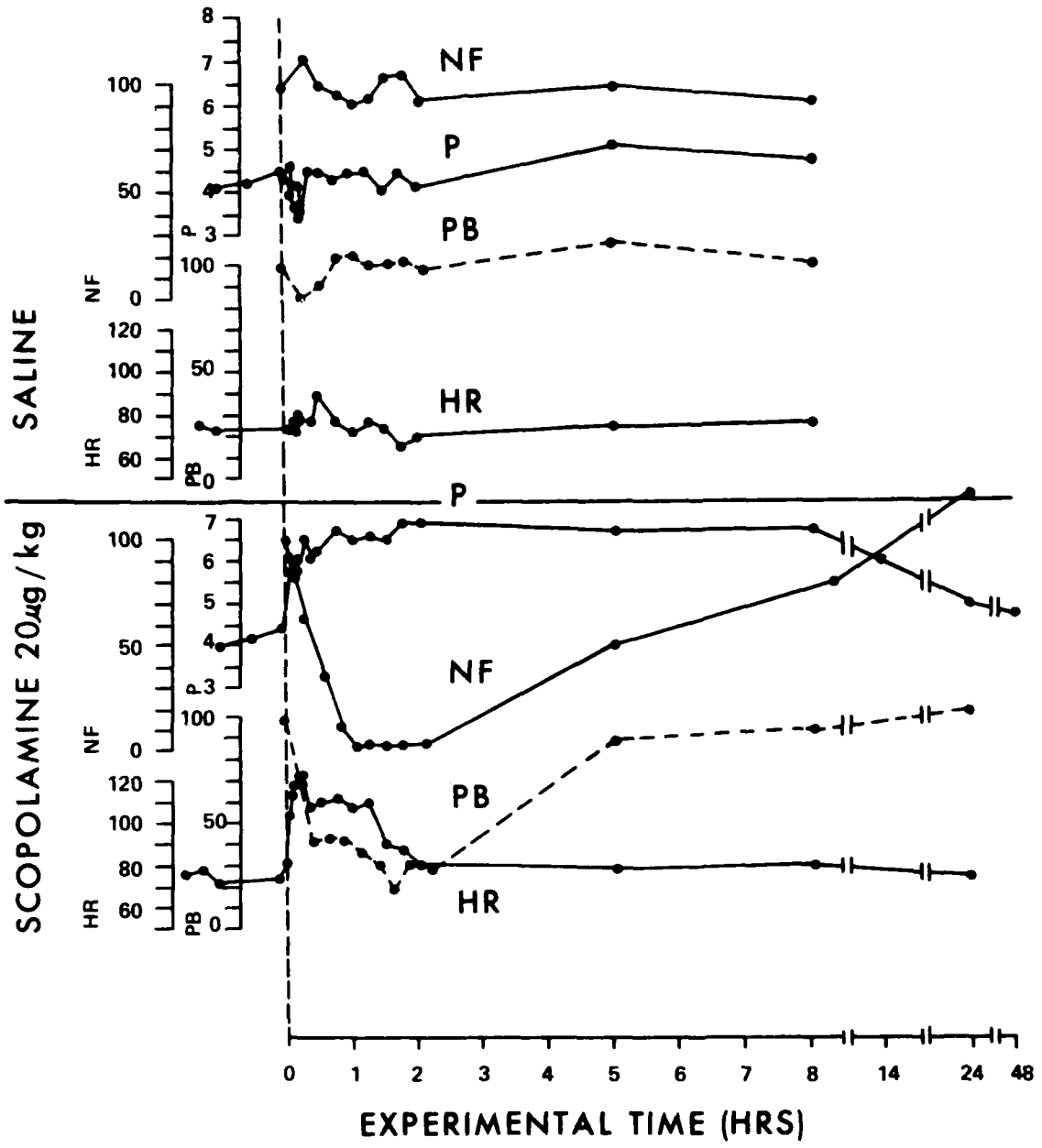
Legend: P, pupil diameter (mm)
NF, number facility
PB, peg board performance (percent baseline)
HR, heart rate (beats/minute)

Figure 3. Time Course of Responses After Injection of Saline Or Scopolamine in a Volunteer at Zero Experimental Time



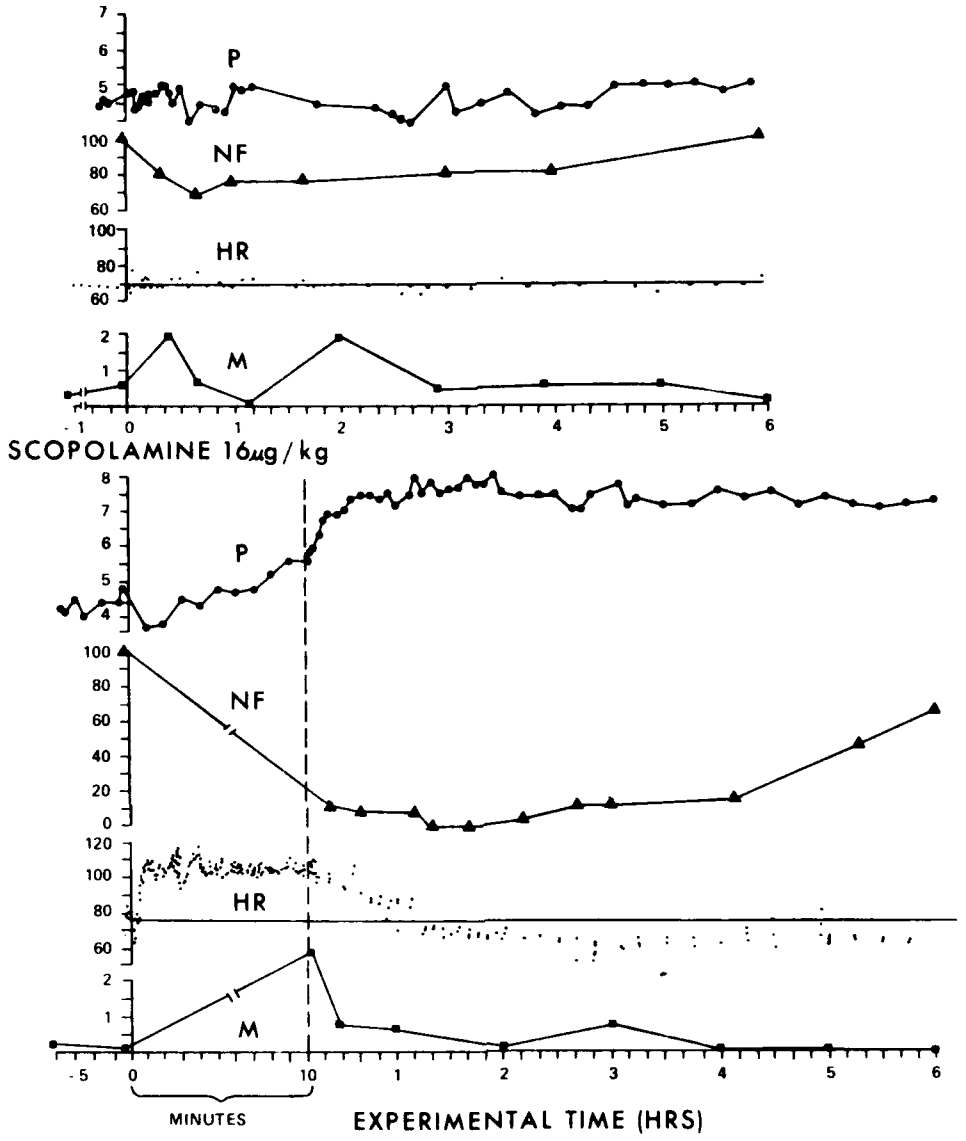
Legend: P, pupil diameter (mm)
NF, number facility
PB, peg board performance (percent baseline)
HR, heart rate (beats/minute)

Figure 4. Time Course of Responses After Injection of Saline Or Scopolamine in a Volunteer at Zero Experimental Time



Legend: P. pupil diamter (mm)
NF. number facility
PB. peg board performance (percent baseline)
HR. heart rate (beats/minute)

Figure 5. Time Course of Responses After Injection of Saline or Scopolamine in a Volunteer at Zero Experimental Time



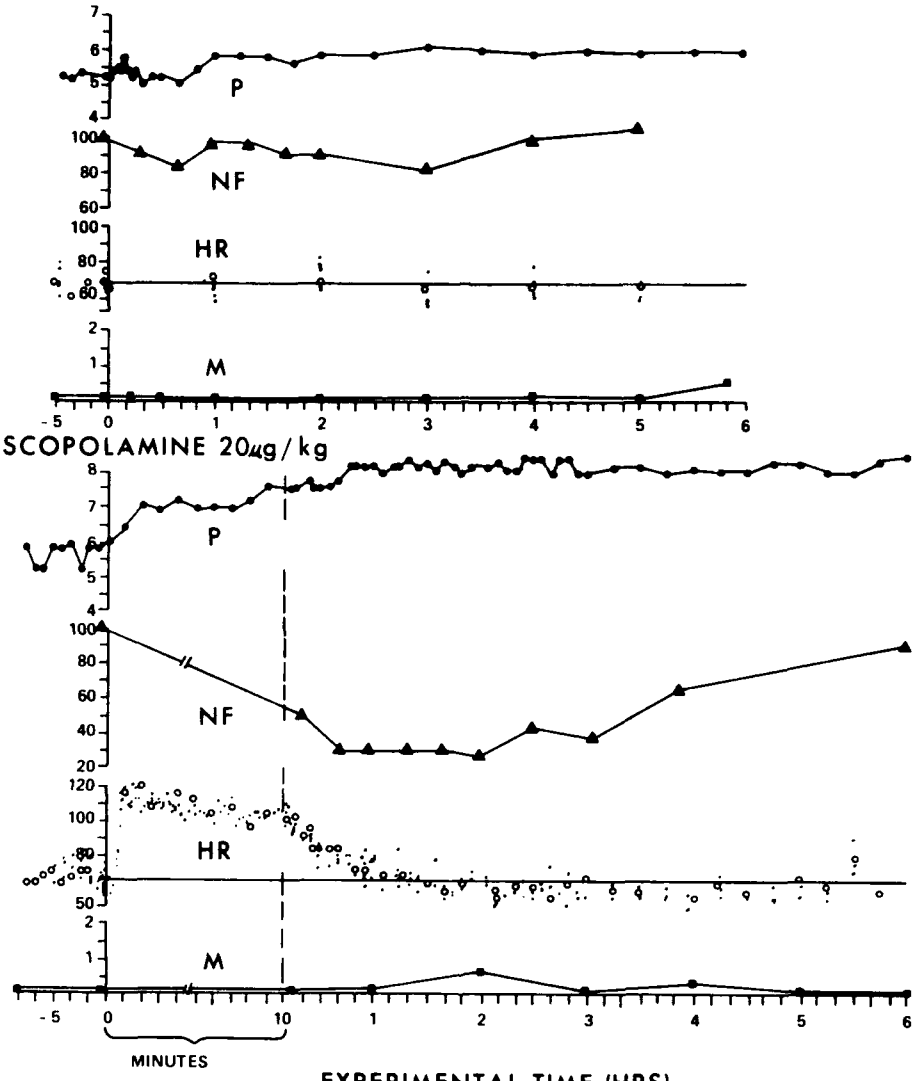
Legend: M, plasma melatonin (ng/10 ml)
P, pupil diameter (mm)
NF, number facility
HR, heart rate (beats/minute)

Figure 6. Responses of Subject 6 Who Was Given 16 μg/kg of Scopolamine

Note the expanded time scale (0-10 minutes) shown only for the scopolamine day. Heart rate was taken from ECG strips in this case. The horizontal line in the HR graph indicates the mean heart rate prior to injection.

SUBJECT 7

SALINE



Legend: M, plasma melatonin (ng/10 ml)
P, pupil diameter (mm)
NF, number facility
HR, heart rate (beats/minute)

Figure 7. Responses of Subject 7 Who Was Given 20 μg/kg of Scopolamine

Note the expanded time scale (0-10 minutes) shown only for the scopolamine day. In this case, the open circles represent heart rate taken by counting QRS on the monitor for 30 seconds, and closed circles indicate heart rate taken from ECG strips.

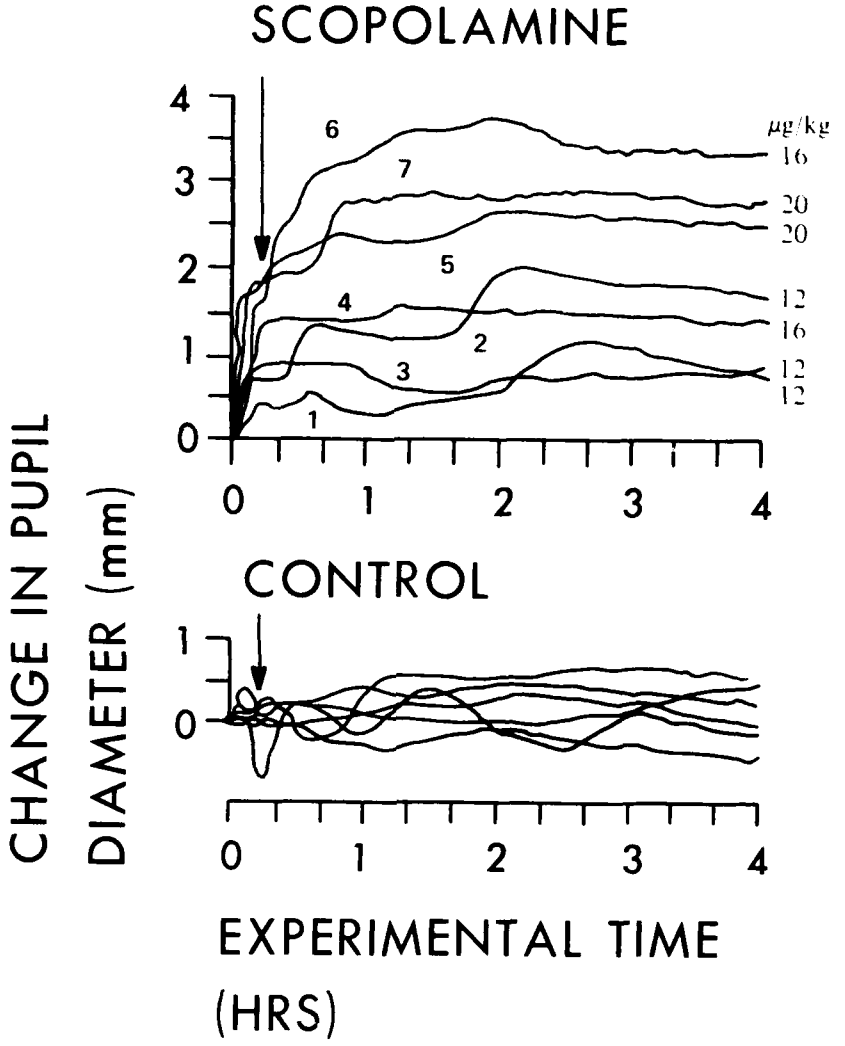


Figure 8. The Time Course of Pupillary Dilation Following 12, 16 or 20 μg/kg B. Wt. Scopolamine

The numbers by the curves indicate individual subjects. The arrow indicates 12 minutes after injection, when the initial rise in pupil diameter (P_1) after scopolamine is completed in all subjects. The second rise (P_2) is small and begins late (1 to 2 hours) in subjects 1 and 4, but is large and begins early in subject 6. Subject 7 has the nearest approximation to a possible idealized curve with both components well separated and easily visible.

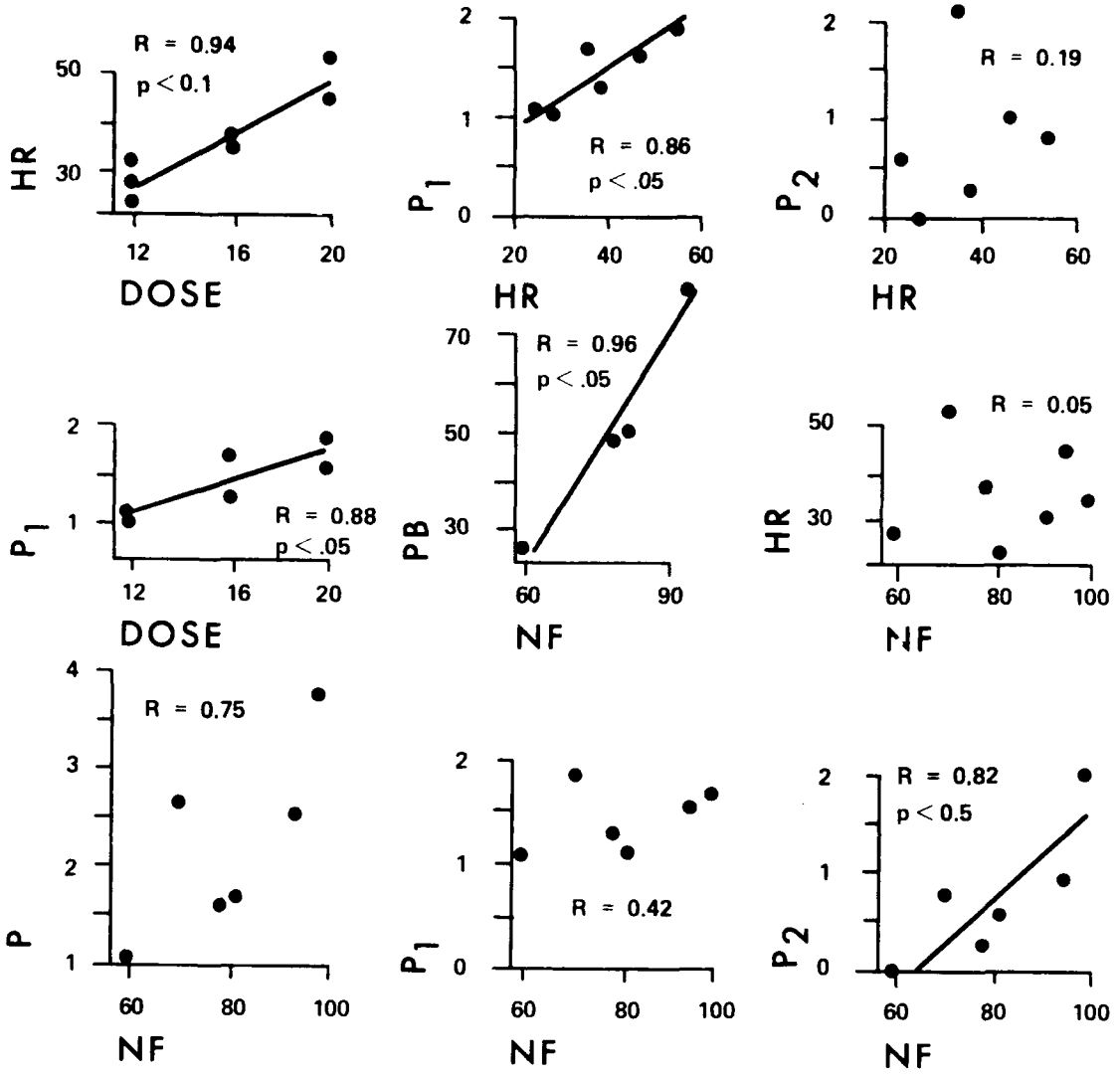
Table 1. Dose of Scopolamine Hydrobromide and the Resulting Maximal Changes from Baseline in Various Parameters

Subject No.	Dose	HR	P_1	P_2	P	NF	PB
	μg/kg	bpm	mm			% reduction	
1	12	32	0.3	0.9	1.2	90	–
2	12	24	1.1	0.6	1.7	81	51
3	12	28	1.05	0	1.05	59	26
4	16	38	1.3	0.3	1.6	78	49
5	20	46	1.6	1.0	2.6	94	80
6	16	35	1.7	2.1	3.8	98	–
7	20	54	1.9	0.8	2.7	70	–

Measurements for NF and PB showed the expected variability and decline after scopolamine. Although the time course corresponded to that described earlier,[1] there was no significant dose-response relationship noted because of the small number of subjects given each dose and the relative proximity in magnitude of the doses to one another. However, the maximal decrement in these two parameters of CNS effect (NF and PB) correlated with each other (figure 9). In general, the NF approached minimal level at about the time of P_2 and began slowly to return toward baseline at about the time the heart rate dropped below baseline. A series of linear regressions on the parameters listed in table I showed that whereas P_1 correlated with heart rate but not NF, P_2 correlated with NF and not heart rate (figure 9).

Figure 10 shows the results of the hormone assays. Although no effect of scopolamine was observed on TSH, HGH, or testosterone, cortisol showed a rise at 30 to 60 minutes that was greater than that seen with control injections. Luteinizing hormone peaked 30 minutes after scopolamine injection in subjects 2, 3, and 4. The timing of the peaks and their absence on control days indicate that they did not represent spontaneous bursts of LH. In subject 5 there was a large FSH elevation after scopolamine injection without a rise in LH. (See table 2 for comparison of area under the curves for cortisol and LH.) Because of inadequate numbers of control samples for subject 3, his values were not included. Since there were only three subjects to compare in this way, no statistical analysis was done. Though there seemed to be no dose-related response to cortisol, it appears that LH release was inversely related to the dose given. Melatonin was measured in subjects 6 and 7 who were part of another test group in which the effects of drugs on this putative pineal hormone were studied.[11] Scopolamine had apparent effect on melatonin levels.

The excretion of 5-HIAA for 2 hours after the injection of scopolamine appeared greater at lesser degrees of incapacitation when expressed as percent of that excreted during respective control periods (figure 11). In the four subjects for whom there are longer periods followed with urine collection, the relationship persisted for 24 hours.



Legend: HR, heart rate increment
P_1, initial rise in pupil size
P_2, second rise in pupil diameter
P, total increase in pupil size ($P_1 + P_2$)
NF, number facility
PB, peg board (as percent reduction from baseline)

Figure 9. Correlation Coefficients for Linear Regressions on Various Parameters

Only the comparisons that were statistically significant ($p < 0.05$ except HR versus dose which was $p < 0.01$) have regression lines drawn. For each parameter is plotted the maximal change seen after scopolamine injection. Since subject 1 was measured in the dark and conditions were therefore different from those for the others, his values were not used for pupil size comparisons.

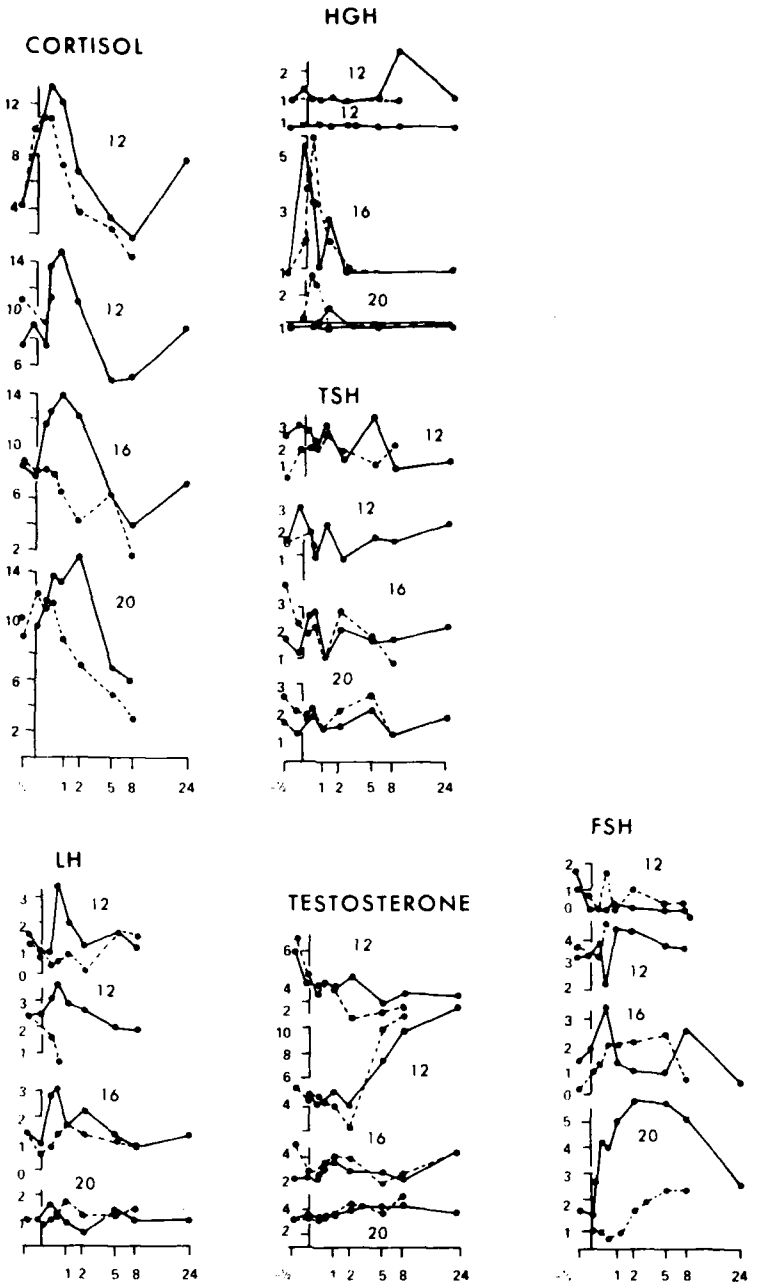


Figure 10. Plasma Hormones on Subjects 2, 3, 4, and 5

Usually, two samples were drawn before injection at time zero, the position of the ordinate axis. The numbers near the curves indicate the dose of scopolamine in μg/kg for each volunteer. Broken lines, control (saline) injection; solid lines, scopolamine injection. Cortisol, μg/100 ml; HGH (human growth hormone), TSH (thyroid stimulating hormone), LH (luteinizing hormone), FSH (follicle stimulating hormone), and testosterone, ng/ml. The abscissa is marked in hours.

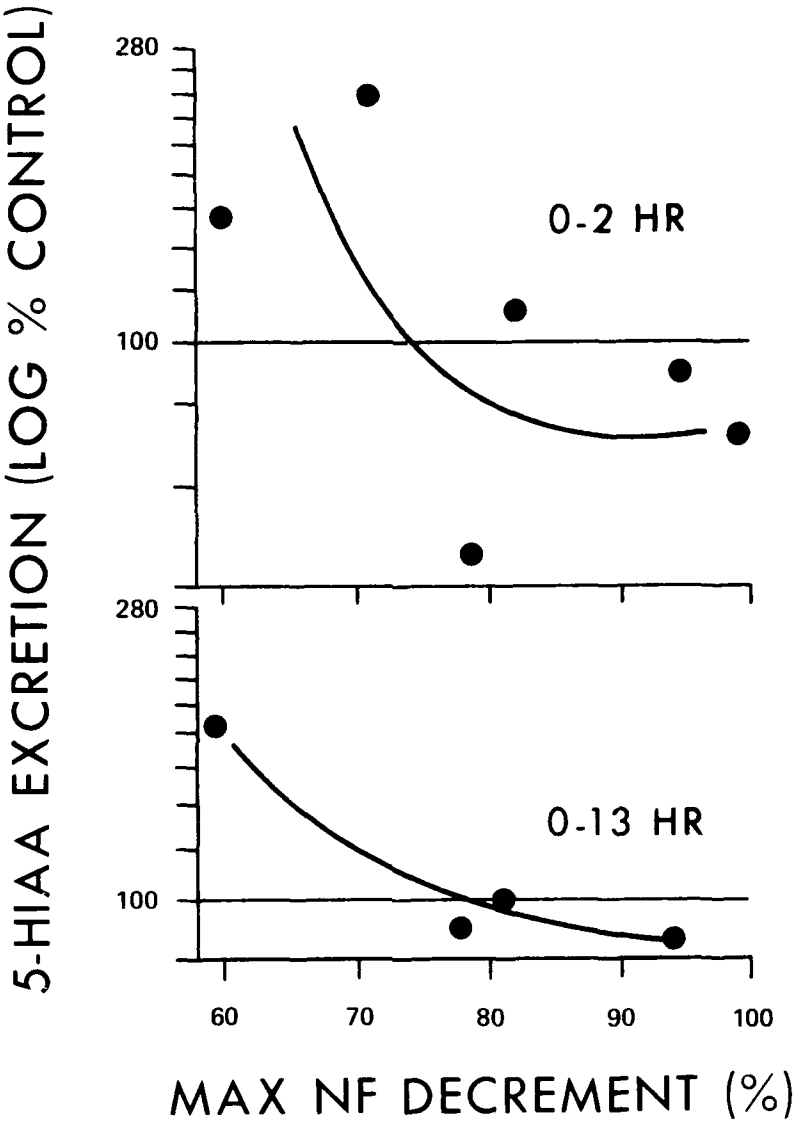


Figure 11. Five-HIAA Expressed as Log % Control, Using the Equivalent Time Periods for Control and Scopolamine Days, Related to Maximal NF Decrement

The relationship is depicted for two lengths of time after injection, 0 to 2 hours and 0 to 13 hours.

Table 2. Dose of Scopolamine and Hormone Response

Subject No.	Dose	Cortisol	Human Growth Hormone
	μg/kg	% control*	% control**
2	12	146	347
4	16	172	218
5	20	165	116

* Area under curve for 1 hour after injection.
** Area under curve for 8 hours after injection.

IV. DISCUSSION.

The CNS effects of scopolamine described by Ketchum, *et al.*[1] were evident in these studies. They included deterioration of the ability to place pegs into new holes on a board (PB), add numbers (NF), and to speak and communicate in a coordinated manner. Concrete interpretations of questions and inability to remember what was asked gave the experimenter the impression of confusion in the subjects. Subject 7 had a "very good trip" and afterwards wrote that the experience more closely resembled that due to a psychedelic agent than that due to marijuana. He and subject 5 described visual hallucinations, including insects crawling on a pencil and a nurse's arm floating across the room. Subject 2 described auditory hallucinations. In contrast to the very enjoyable experience of subject 7 who had previously had experience with psychotropic drugs, subject 6 (who denied any previous drug use) felt "weird" and intermittently frightened. He verbalized relatively infrequently during the period of drug effect, and aside from responses to direct questioning, he essentially was content to sit quietly in the pupillometer chair. On the other hand, subject 7 verbalized almost constantly beginning at about 5 to 15 minutes up until about 2 hours after injection. A subjective feeling of sleepiness in most of the volunteers seemed prominent only in the first 5 to 10 minutes. Most subjects described distortions of time and space.

It is conceivable that the elevations of cortisol could have been due to the stress and excitement of the drug effect. However, it is necessary to consider a specific action of scopolamine on the control of cortisol secretion in view of the cholinergic neural component in the control of adrenocortical function in animals.[12] Alternatively, since brain serotonin can be inhibitory to corticotropin release,[13] an antiserotonin effect of scopolamine could explain the cortisol results.

The correlation of P_2 with incapacitation, as well as its timing, suggests that this may be an effect of scopolamine on CNS pupil control mechanisms. This interpretation is consistent with the hypothesis of scopolamine-induced inhibition of serotoninergic mechanisms, since the action or serotonin in cat brain produces miosis,[14] the opposite of our observation (mydriasis). The interpretation of P_2 as a CNS effect, however, cannot be certain until experimental evidence shows that increased scopolamine concentration in aqueous humor or iris does not produce P_2, which could theoretically explain the results. Attributing only P_1 to a direct effect of

scopolamine on the iris is consistent with the occurrence of maximal rise in heart rate well within the period for P_1. Subsequent bradycardia could have represented a central effect, though there is no direct evidence for this.

The rise in gonadotrophins (LH in subjects 2, 3, and 4 and FSH in subject 5) was somewhat unexpected. In contrast, atropine blocked the release of LH and FSH in male and female rats, particularly if it was administered into the third ventricle where it had access to the hypophyseotropic areas of the hypothalamus.[15] Compensatory ovarian hypertrophy (COH) relies on FSH and LH,[16] and has been used to demonstrate the antigonadotrophic effects of certain agents in mice.[17] COH was inhibited by the lowest but not the higher coses of scopolamine (table 3), suggesting a response different from that in the volunteers, though with a similar peculiar relationship to dose. Release of FSH in the subject with the highest dose, with no simultaneous LH release, indicates it is possible to release FSH independent of LH. Although acetylcholine would appear to be stimulatory,[2,15] serotonin is considered to be an inhibitory coding element for gonadotropin release in the rat.[18,19] The peculiar responses observed in this study for gonadotrophin-related function might be explained by scopolamine interaction with both acetylcholine and serotonin. Further experimentation in mice and men, with greater attention to differential effects of dosage level and use of more specific amine blockers, should help resolve this question.

Table 3. Compensatory Ovarian Hypertrophy (COH) After a Single Injection of Scopolamine at the Time of Unilateral Ovariectomy in Mice

Dose	N	Mean COH	Standard Error
μg/mouse		%	
Vehicle	10	39.5	4.0
1	10	28.3*	3.5
10	10	51.9	8.2
100	9	33.6	7.3

* $p < 0.05$ versus either vehicle or 10 μg group (Student t test).

The suggestion of lower 5-HIAA excretion with greater degrees of incapacitation is interesting in view of the improvement in psychosis index in schizophrenic patients following presumed elevation of brain serotonin metabolism with the precurser 5-hydroxytryptophan and a peripheral decarboxylase inhibitor.[20] Although there are differences between endogenous and drug-induced psychosis, particularly in the predominance of the delirium component in scopolamine intoxication, nevertheless there are clinical similarities. Furthermore, there is evidence that increments of cerebral acetylcholine released during CNS stimulation can elevate brain serotonin in rats.[21] If this is true in humans, it is possible to speculate that, with smaller degrees of scopolamine incapacitation, sertoninergic function increased in an attempt to induce stability and resulted in an excretion of 5-HIAA greater than 100% of control values (figure 11).

With greater degrees of cholinergic inhibition in the CNS, synthesis or turnover of serotonin have been inhibited, depressing 5-HIAA excretion below 100% of control values. Alternatively, the stress of incapacitation could have elevated brain serotonin and 5-HIAA[22] and, with greater anticholinergic CNS effect, a possible antiserotoninergic effect could have become predominant.

The idea of cross-relationships between coding elements (represented by putative neurotransmitters) has already had precedent in relation to scopolamine intoxication: this effect (depressed performance) was potentiated with inhibition of catecholamine receptors by chlorpromazine.[1] We now consider the possibility that sertoninergic activity may participate as well. Perhaps agents such as tryptophan[23] or melatonin,[24] which elevate brain serotonin, might be used as therapeutic or prophylactic agents to counteract CNS toxicity caused by scopolamine. This speculation is based in part on the presumption that urinary 5-HIAA was related to brain serotonin. Although this is not known, there is evidence that it is the case: the finding by Vaughan, *et al.*[11] of a diurnal rhythm in human urinary 5-HIAA similar to brain serotonin cycles in rats[23] and mice;[25] obliteration of the 5-HIAA cycles in volunteers exposed to constant light,[11] and the observation that melatonin, which elevated brain serotonin in rats,[24] caused a greater than twofold rise in urinary 5-HIAA in three out of four Parkinsonian patients.[26] Furthermore, there is evidence that in animals the brain contributes a large (over one third of the total) proportion of excreted serotonin metabolic products, and this contribution results from relatively high turnover.[27] The melatonin-treated patients of Papavasiliou *et al.*[26] and those of Cramer *et al.*[28] were reported to show evidence of greater psychic stability while on melatonin. Perhaps melatonin could be used to counteract the instability of scopolamine intoxication, which seemed related to less 5-HIAA excretion in the more severe cases.

Since the number of volunteers was small in this study, the results are only suggestive. The timing and CNS-correlation of P_2, the effects of hormones, and the relationship of scopolamine to the serotoninergic system must be investigated further.

LITERATURE CITED

1. Ketchum, J. S., Sidell, F. R., Crowell, E. B., Aghajanian, G. K., and Hayes, A. H. Atropine, Scopolamine, and Ditran: Comparative Pharmacology and Antagonists in Man. Psychopharmacol. (Berl.) *28*, 121-145 (1973).

2. Evertt, J. W. Central Neural Control of Reproductive Functions of the Adenohypophysis. Physiol. Rev. *44*, 373-431 (1964).

3. Sourilac, A., and Sourilac, M. L. Les Effets de l'atropine sur le Comportment Sexuel du rat Femelle. J. Physiologie *61*, 407-408 (1969).

4. Schalch, D. S., Parlow, A. F., Boon, R. C., and Reichlin, S. Measurement of Human Luteinizing Hormone in Plasma by Radioimmunoassay. J. Clin. Invest. *47*, 665-678 (1968).

5. Boon, R. C., Schalch, D. S., Lee, L. A., and Reichlin, S. Plasma Gonadotropin Secretory Patterns in Patients with Functional Menstrual Disorders and Stein-Levinthal Syndrome: Response to Clomiphene Treatment. Am. J. Obstet. Gyn. *112*, 736-748 (1972).

6. Schalch, D. S., and Parker, M. L. A Sensitive Double Antibody Immunoassay for Human Growth Hormone in Plasma. Nature (London) *203*, 1141-1142 (1964).

7. Utiger, R. D. Radioimmunoassay of Human Plasma Thyrotropin. J. Clin. Invest. *44*, 1277-1286 (1965).

8. Murphy, B. E. P. Some Studies of the Protein-Binding of Steriods and Their Application to the Routine Micro and Ultramicro Measurement of Various Steroids in Body Fluids by Competitive Protein-Binding Radioassay. J. Clin. Endocrinol. Metab. *27*, 973-990 (1964).

9. Undenfriend, S., Titus, E., and Weissbach, H. The Identification of 5-Hydroxyindoleacetic Acid in Normal Urine and a Method for Its Assay. J. Biol. Chem. *216*, 499-505 (1955).

10. Ralph, C. L., and Lynch, H. J. A quantitative Melatonin Bioassay. Gen. Comp. Endocrinol. *15*, 334-338 (1970).

11. Vaughan, G. M., Pelham, R. W., Pang, S. F., Loughlin, L. L., and Wilson, K. M. Plasma Melatonin and Urinary 5-HIAA Cycles in Young Men: Modification by Environmental Lighting, Sleep, and Autonomic Drugs. Anat. Rec. *181*, 499 (1975).

12. Krieger, H. P., and Drieger, D. T. Pituitary-Adrenal Activation by Implanted Neurotransmitters and Ineffectiveness of Dexamethasone in Blocking This Activation. *In:* Influence of Hormones on the Nervous System, Proc. Int. Soc. Psychoneuroendocrinol., Brooklyn, pp. 98-106. Basel:Karger 1971.

13. Vermes, I., Telegdy, G., and Lissak, K. Inhibitory Action of Serotonin on Hypothalamus-Induced ACTH Release. Acta Phys. Acad. Sci. Hung. *41*, 95-98 (1972).

14. Koella, W. P. Serotonin and Sleep. Exp. Med. Surg. *27*, 157-168 (1969).

15. Libertun, C., and McCann, S. M. Blockade of the Release of Gonadotropins and Prolactin by Subcutaneous or Intraventricular Injection of Atropine in Male and Female Rats. Endocrinol. *92*, 1717-1724 (1973).

16. Howland, B. E., and Skinner, K. R. Effect of Hemiovariectomy on Serum FSH and LH Levels During the Oestrous Cycle in the Rat. J. Reprod. Fert. *32*, 501-503 (1973).

17. Vaughan, M. K., Reiter, R. J., Vaughan, G. M., Bigelow, L., and Altschule, M. D. Inhibition of Compensatory Ovarian Hyperthrophy in the Mouse and Vole: A Comparison of Altschule's Pineal Extract, Pineal Indoles, Vasopressin, and Oxytocin. Gen. Comp. Endocrinol. *18*, 372-377 (1972).

18. O'Steen, W. K. Suppression of Ovarian Activity in Immature Rats by Serotonin. Endocrinol. *77*, 937-939 (1965).

19. Kordon, C., Javoy, F., Vassent, G., and Glowinski, J. Blockade of Superovulation in the Immature Rat by Increased Brain Serotonin. Europ. J. Pharmacol. *4*, 169-174 (1968).

20. Wyatt, R. J., Vaughan, T., Galanter, M., Kaplan, J., and Green, R. Behavioral Changes of Chronic Schizophrenic Patients Given 5-Hydroxytryptophan. Science *177*, 1124-1126 (1972).

21. Campos, H. A., and Jurupe, H. A Histamine-Dependent Increase of 5-hydroxytryptamine in the Rat Brain *In Vivo*. Experientia *26*, 613-614 (1970).

22. Bliss, E. L., Thatcher, W., and Ailion, J. Relationship of Stress to Brain Serotonin and 5-Hydroxyindoleacetic Acid. J. Psychiat. Res. *9*, 71-80 (1972).

23. Wurtman, R. J. Effects of Physiologic Variations in Brain Amino Acid Concentrations on the Synthesis of Brain Monoamines. *In:* Frontiers in Neurology and Neuroscience Research. P. Seeman and G. M. Brown, eds. pp. 16-25. Tronto: The University of Toronto Press 1974.

24. Anton-Tay, F. Pineal-Brain Relationships. IN: The Pineal Gland, G.E.W. Wolstenholme and J. Knight, eds, pp. 213-227. London: Churchill Livingstone 1971.

25. Morgan, W. W., and Yndo, C. A., and McFadin, L. S. Daily Rhythmic Changes in the Content of Serotonin and 5-hydroxyindoleacetic Acid in the Cerebral Cortex of Mice. Life Sci. *14*, 329-338 (1974).

26. Papavasiliou, P. S., Cotzias, G. C., Duby, S. E., Steck, A. J., Bell, M., and Lawrence, W. H. Melatonin and Parkinsonism. J. Amer. Med. Assoc. *221*, 88-89 (1972).

27. Garattini, S., and Valzelli, L. Serotonin, pp. 62-63. Amsterdam: Elsevier 1965.

28. Cramer, H., Rudolph, J., Consbruch, U., and Kendel, K. On the Effects of Melatonin on Sleep and Behavior in Man. IN: Advances in Biochemical Pharmacology, Vol 11, pp. 187-191. New York: Raven Press 1974.

DISTRIBUTION LIST 3

Names	Copies
CHEMICAL SYSTEMS LABORATORY	
SAFETY OFFICE	
Attn: DRDAR-CLF	1
ERDA SUPPORT OFFICE	
Attn: DRDAR-CLE	1
PLANS & PROGRAMS OFFICE	
Attn: DRDAR-CLR-L	6
AUTHOR'S COPIES: Biomedical Laboratory	7
BIOMEDICAL LABORATORY	
Attn: DRDAR-CLL	1
Attn: DRDAR-CLL-B	1
Attn: DRDAR-CLL-M	1
Attn: DRDAR-CLL-V	1
DEVELOPMENTAL SUPPORT DIVISION	
Attn: DRDAR-CLJ-I	4
Attn: DRDAR-CLJ-M	1
MUNITIONS DIVISION	
Attn: DRDAR-CLN	1
Attn: DRDAR-CLN-E	1
PHYSICAL PROTECTION DIVISION	
Attn: DRDAR-CLW	1
Attn: DRDAR-CLW-P	1
RESEARCH DIVISION	
Attn: DRDAR-CLB	1
Attn: DRDAR-CLB-B	1
Attn: DRDAR-CLB-C	1
Attn: DRDAR-CLB-P	1
Attn: DRDAR-CLB-R	1
Attn: DRDAR-CLB-T	1
SYSTEMS ASSESSMENTS OFFICE	
Attn: DRDAR-CLY-A	1
Attn: DRDAR-CLY-R	1
DEPARTMENT OF DEFENSE	
Administrator Defense Documentation Center Attn: Accessions Division Cameron Station Alexandria, VA 22314	12
Director Defense Intelligence Agency Attn: DB-4G1 Washington, DC 20301	1
DEPARTMENT OF THE ARMY	
HQDA (DAMO-SSC) WASH DC 20310	1
Director Defense Civil Preparedness Agency Attn: RI Washington, DC 20301	1
CINCUSAREUR Attn: AEAGC-DS APO New York 09403	1
Chief, Office of Research, Development & Acquisition	
Attn: DAMA-CSM-CM	1
Attn: DAMA-ARZ-D Washington, DC 20310	1
Headquarters, Sixth US Army NBC Office Attn: AFKC-OP-PN Presidio of San Francisco, CA 94129	1
HQDA (DAMI-FIT) WASH, DC 20310	1
CINCUSAREUR Attn: AEAMD-PM APO New York 09403	1
OFFICE OF THE SURGEON GENERAL	
Commander US Army Medical Bioengineering Research & Development Laboratory Attn: SGRD-UBD-A Fort Detrick, Bldg 568 Frederick, MD 21701	1
HQDA (SGRD-HR) *Forrestal Building* WASH DC 20314	1
US ARMY HEALTH SERVICE COMMAND	
Commander US Army Environmental Hygiene Agency Attn: HSE-AA-L, Librarian, Bldg 2100 APG-Edgewood Area	1
Superintendent Academy of Health Sciences US Army	
Attn: HSA-CDC	1
Attn: HSA-IHE Fort Sam Houston, TX 78234	1
US ARMY MATERIEL DEVELOPMENT AND READINESS COMMAND	
Commander US Army Materiel Development and Readiness Command	
Attn: DRCDE-DM	1
Attn: DRCLDC 5001 Eisenhower Ave Alexandria, VA 22333	1
Project Manager for Chemical Demilitarization and Installation Restoration	
Attn: DRCPM-DR APG-Edgewood Area	1

DISTRIBUTION LIST 3 (Contd)

Names	Copies
Commander US Army Foreign Science & Technology Center Attn: DRXST-IS1 220 Seventh St., NE Charlottesville, VA 22901	2
Director DARCOM Field Safety Activity Attn: DRXOS-C Charlestown, IN 47111	1
Commander DARCOM, STITEUR Attn: DRXST-ST1 Box 48, APO New York 09710	1
Commander US Army Science & Technology Center-Far East Office APO San Francisco 96328	1
US ARMY ARMAMENT RESEARCH AND DEVELOPMENT COMMAND	
Commander US Army Armament Research and Development Command Attn: DRSAR-ASN Attn: DRSAR-IMB-C Dover, NJ 07801	1 1
CDR, APG USA ARRADCOM Attn: DRDAR-SEI-C Attn: DRDAR-QAC-R Aberdeen Proving Ground, MD 21010	1 1
US ARMY ARMAMENT MATERIEL READINESS COMMAND	
CDR, APG USA ARRCOM Attn: SARTE Aberdeen Proving Ground, MD 21010	1
Commander US Army Dugway Proving Ground Attn: Technical Library, Docu Sect Dugway, UT 84022	1
Commander Rocky Mountain Arsenal Attn: SARRM-EA Attn: SARRM-MD Denver, CO 80240	1 1
Commander Pine Bluff Arsenal Attn: SARPB-ETA Pine Bluff, AR 71611	1
US ARMY TRAINING & DOCTRINE COMMAND	
Commandant US Army Missile & Munitions Center & School Attn: ATSK-CD-MD Attn: ATSK-DT-MU-FOD Redstone Arsenal, AL 35809	1 1
Commandant US Army Military Police School Attn: ATZN-CD Fort McClellan, AL 36205	1
Commander US Army Infantry Center Attn: ATZB-CD-MS-C Fort Benning, GA 31905	1
Commandant US Army Ordnance & Chemical Center & School Attn: ATSL-CD-MS Attn: ATSL-DTG Aberdeen Proving Ground, MD 21005	1 1
US ARMY TEST & EVALUATION COMMAND	
Record Copy CDR, APG Attn: STEAP-AD-R/RHA APG-Edgewood Area, Bldg E5179	1
CDR, APG Attn: STEAP-TL APG-Aberdeen Area	1
Commander US Army Test & Evaluation Command Attn: DRSTE-EA APG-Aberdeen Area	1
Commander US Army Tropic Test Center Attn: STETC-MO-A (Tech Library) APO New York 09827	1
Commander Dugway Proving Ground Attn: STEDP-PO Dugway, UT 84022	1
DEPARTMENT OF THE NAVY	
Chief of Naval Research Attn: Code 443 800 N. Quincy Street Arlington, VA 22217	1

Names	Copies
Commander Naval Explosive Ordnance Disposal Facility Attn: Army Chemical Office Indian Head, MD 20640	1
Commander Naval Surface Weapons Center Attn: Tech Lib & Info Svcs Br White Oak Laboratory Silver Spring, MD 20910	1
Commander Naval Surface Weapons Center Dahlgren Laboratory Attn: DX-21 Dahlgren, VA 22448	1
Commander Nuclear Weapons Training Group, Atlantic Naval Air Station Attn: Code 21 Norfolk, VA 23511	1
Chief, Bureau of Medicine & Surgery Department of the Navy Attn: CODE 5 Washington, DC 20372	1
Commander Naval Weapons Center Attn: Technical Library/Code 233 China Lake, CA 93555	1
DEPARTMENT OF THE AIR FORCE	
HQ Foreign Technology Division (AFSC) Attn: PDRR Wright-Patterson AFB, OH 45433	1
Commander Aeronautical Systems Division Attn: ASD/AELD Wright-Patterson AFB, OH 45433	1
HQ, USAF/SGPR Forrestal Bldg WASH DC 20314	1
Commander Armament Development & Test Center Attn: DLOSL (Technical Library) Eglin AFB, FL 32542	1
NORAD Combat Operations Center/DBN Cheyenne Mtn Complex, CO 80914	1

Names	Copies
OUTSIDE AGENCIES	
Director of Toxicology National Research Council 2101 Constitution Ave, NW Washington, DC 20418	1
US Public Health Service Room 17A46 (CPT Osheroff) 5600 Fishers Lane Rockville, MD 20857	1