SOME ASPECTS OF VITAMIN C METABOLISM

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Within the past few years many investigators in the field of nutrition have emphasized the difference in effect on the body of a mild vitamin deficiency lasting over a considerable period of time, as compared with that of a more severe deficiency of but short duration. Since frequently the pathologic state thus induced is more severe in the former case, and requires a longer period for recovery, the importance of maintaining in the dietary adequate amounts of all essential nutrients is apparent.

Food sources. Recent reviews (1, 2, 3, 4, 5, 6) have shown a considerable percentage of industrial and rural populations with definite signs of deficiency of one or more vitamins or other nutrients which are directly traceable to dietary inadequacies. In many instances the omission of adequate sources of vitamin C or the improper handling of foods reduces the dietary intake of this vitamin to a dangerous degree.

While citrus fruits hold a place of pre-eminence in our diet as a source of ascorbic acid, the use of tomatoes probably can be considered the second most readily available source. A field grown vine ripened tomato may contain as much as 25 mgm. ascorbic acid (7). The use of tomatoes canned at the peak of the season frequently provides a food of higher ascorbic acid content than market available fruit in off seasons. Holmes, Jones and Richie (8) analyzed thirteen lots of late winter tomatoes and found an ascorbic acid content ranging from 2.5 to 22.0 mgm. per 100 grams of fruit. The average value of all lots was 8.8 mgm. per 100 grams. Tomatoes, canned at the height of the previous season gave an average ascorbic acid content of 14 to 15 mgm. per 100 grams. Ripe peaches (9), depending upon the variety, contain 3.84 to 12.86 mgm. ascorbic acid per 100 grams of fruit with the highest concentration directly under the skin. Varietal differences in ascorbic acid content are also found in red raspberries (10) and in new rather than in old potatoes (11). In some varieties of cabbage the ascorbic acid content decreases as the cabbage matures (12). Peppers on the other hand are an excellent source of vitamin C at all stages of ripeness, being highest when partially ripened, according to Lantz (13). He also found that the ascorbic acid content increases as the season advances. One variety, College no. 9, increased from 209 to 375 mgm. per 100 grams from August to November.

Methods of food preservation vary in their influence on the ascorbic acid content of the product. Canning has the advantage from the standpoint of the length of time the material will keep (14). Refrigeration is for short time preservation. Losses during drying and dehydration vary both with the method and material. Floyd and Fraps (15) have made a study of losses in ascorbic acid content in the commercial canning of Texas grapefruit juice. Tree ripened fruit, first grade, produces juice from 38 to 46 mgm. ascorbic acid per 100 grams. Canned fruit juices contained about 18.2 per cent less ascorbic acid than fresh juice. The inclusion of culls reduces the ascorbic acid content of the juice; with 10 per cent or less culls, the juice averaged 36.1 mgm. per 100 grams, while juice from 90 per cent culls contains but 29.5 mgm. ascorbic acid per 100 grams. Oxidative losses are low if pasteurization follows promptly after extraction (3½ minutes lapse, 6 per cent loss; 30 minutes lapse, 34.7 per cent loss). Of the methods of extraction of the juice (screws, burr press, rollers, graters) the screw extraction and burr (similar to domestic juice extractors) causes the greater destruction of ascorbic acid. Recent work in other laboratories indicates these processing losses to be rather high. Retention values of 98 to 99 per cent have been reported.* The loss of ascorbic acid in fruits and vegetables following maceration, as in the preparation of salad, may be appreciably decreased, according to McCay and Pijoan (16), by cutting or mincing with a plastic knife instead of one of metal.

The harvesting of leafy vegetables in the coolest part of the day (17), and packing of vegetables in crushed ice as soon as harvested and during transportation was found by Zepplin and Elvehjem (18) to decrease loss of vitamin C. Ascorbic acid was destroyed quite rapidly in spinach, chard, lettuce, and broccoli at room temperatures of 20 to 23°C; storage in a refrigerator favored its retention.

Blanching of vegetables preliminary to drying according to von Loesecke (17) aids in the preservation of vitamins, expels part of the contained oxygen and decreases the bacterial population. Blanching by steam at atmospheric pressures reduces the loss of mineral salts and vitamins as compared with hot water blanching. von Loesecke

*Personal communication from Dr. C. A. Elvehjem.

quotes the following figures from Chance as to loss of vitamin C by the two respective methods:

VEGETABLE	LOSS STEAM	LOSS HOT WATER
	per cent	*per cent*
Kale	19.7	43.6
Beets	14.8	36.6
Potato (white)	22.5	37.5
Cabbage	14.1	31.5

In a study of vitamin losses during dehydration and storage, Tressler, Moyer and Wheeler (19) found potatoes to retain but a trace of ascorbic acid. Beets lost one-third of their original content. Water blanching and dehydration of rutabagas caused an 85 per cent loss. Cabbage however, showed only 20 per cent loss of ascorbic acid during dehydration with practically no additional loss during storage at −40°F for three months.

Vitamin loss in large scale food preparation in defense plants and civilian cafeterias have recently been shown to be excessive. Heller, McCay and Lyon (20) found a 27 to 90 per cent loss in the ascorbic content of vegetables served in a cafeteria feeding 2500 people. Losses of other vitamins, while less were still high (thiamin 16 to 64 per cent, niacin, 2 to 61 per cent, and riboflavin 22 to 45 per cent). Similar results were obtained by Daum, Aimone and Hollister (21), who furthermore observed a greater loss of ascorbic acid to occur during the holding of vegetables for one hour on the steam table than resulted from cooking. Suggested remedies included a decrease in cooking and holding time, staggered preparation of foods, a more limited choice of vegetables and a preview of the menu. The use of whole boiled (22) potatoes instead of mashed conserves their vitamin C content even when held at steam table temperatures. Small amounts of sodium bicarbonate may be added in cooking fresh or tunnel frozen peas without causing additional loss of vitamin C (23). The cooking time is shortened, and unless the membrane surrounding the pea is broken as in plate freezing, only moderate losses occur by leaching into the cooking water. The uncontrolled adoption of this practice in the home is not be be recommended.

An unusual high source of vitamin C is rosehips, 100 grams of which contain 1200 to 1500 mgm. ascorbic acid.

Methods of analysis. The older methods for estimating the ascorbic acid content of urine, plasma, whole blood and its cellular constituents have been reviewed by Bessey (25) and later briefly by Ralli and Sherry (26). A critical study of the photometric methods of Mindlin and Butler (27) have been made (28, 29).

Technics for the determination of ascorbic acid in the presence of highly colored solutions, such as fruit extracts, have been developed for the Evelyn Photoelectric colorimeter by Bessey (30) and a potentiometric method by Harris and Olliver (31).

Aside from the handicap of colored solutions in titration procedures, various indophenol reducing substances other than ascorbic acid are encountered in the urine, feces, plant extracts, cereal products, milk powders and carmelized or fermented products. In a recent issue of Nutrition Reviews (32) it is stated that aside from ascorbic acid, 2:6 dichlorophenolindophenol will be non-specifically reduced by such substances as stanous and ferrous salts, sulfites, sulfhydrol compounds, sulphides, thiosulfates, reductinic acid and "reductones" (formed by splitting of sugars by heat or fermentation). According to Enders (32), reductones are formed when sugars are heated at a suitable pH and especially in the presence of protein. These substances are suspected of having a structure somewhat similar to ascorbic acid with an aldol type of condensation between carbohydrate and protein derivatives.

Methods have been proposed by Lugg (33), Mapson (34) and Snow and Zilva (35) based upon the differential rates of combination of ascorbic acid and other indophenol reducing substances with formaldehyde at pH 1.5 to pH 2. At this acidity "reductones" condense but slowly with formaldehyde. Ascorbic acid is determined by difference in titration for total indophenol reducing substances and the value obtained for "reductones" by extrapolation from a series of determinations made at stated intervals. This method does not distinguish between l-ascorbic acid and close analogues (d-glucoascorbic acid, d-araboascrobic acid, etc.). It has also been suggested (36) that ascorbic acid may be titrated by 2:6 dichlorophenolindophenol in presence of "reductones" if the reductones are estimated at high acidity (20 per cent HCl); total reduction (reductones and ascorbic acid) being estimated after dilution with water to appropriate acidity.

It remains for future work to determine if these proposed methods are superior to existing photometric methods (30) properly controlled for non-ascorbic acid reducing substances.

The titration of ascorbic acid using a xylene solution of 2:6 dichlorophenolindophenol has also been proposed (37). Interference due to SO_2 in fruit products may be eliminated by the use of hydrogen peroxide according to Levy (36).

A method for estimating ascorbic acid using whole blood or urine has recently been proposed by Roe and Kuether (38). Blood is deproteinized with trichloracetic acid, the ascorbic acid in the

filtrate is oxidized by norit to dehydroascorbic acid and a stable colored derivative formed with 2:4 dinitrophenylhydrazine in the presence of sulphuric acid. The color is read in a photoelectric colorimeter. The method is apparently specific and not affected by keto-acids. Glucose, fructose, pentoses and glucuronic acid do not interfere at levels ordinarily encountered in blood or urine.

The use of hydrogen sulphide in methods requiring the estimation of dehydroascorbic acid is not without its disadvantages. Recently electrolytic reduction (39) has been advocated, while another method proposes the use of a suspension of B. coli in glucose media (40) for the same purpose. The reduced ascorbic acid is then estimated by reduction of 2:6 dichlorophenolindophenol.

Plasma and blood methods. Following the earlier studies on hypovitaminosis C in which urinary excretion (41) of ascorbic acid was used as a criterion of depletion, methods measuring fasting blood plasma levels were developed (42, 43, 27).

Plasma was used to avoid difficulties arising from oxyhemoglobin of whole blood, which was later shown to enter into a coupled oxidation with ascorbic acid during deproteinization (44). By saturating blood with carbon monoxide before precipitation with metaphosphoric acid, Butler and Cushman (45) were able to estimate ascorbic acid in whole blood by oxidation-reduction dye methods. The use of lead acetate (46) with metaphosphoric acid deproteinization has recently been recommended for removal of sulphydral derivatives in whole blood analysis.

Because of the variation of plasma ascorbic acid levels with food intake, it has been recommended (45) that either whole blood or white-cell platelet ascorbic acid content be employed as a more reliable single index of body tissue saturation.

Kruse (48) has recently recommended the examination of the gingiva by means of the biomicroscope as a means of detecting early signs of vitamin C deficiency.

Requirements and utilization. Jolliffe has recently pointed out that "the diagnosis of nutritional failure cannot be limited to clinically manifest anatomical lesions. The preclinical states, as represented by tissue depletion, biochemical 'lesions' and altered physiology, hold greater import because they precede and are more common than the anatomical lesions (47)." Estimations of tissue saturation have been based upon measurements of the ascorbic acid level of the plasma, the whole blood, the white-cell platelet layer as well as tolerance tests based upon changes in plasma level and urinary excretion in response to oral or parenteral doses of ascorbic acid. Thysell (49) in a study of 233 subjects, showed blood levels of ascorbic acid of 1.0 to 2.0 mgm. per 100 ml. to follow the daily intake of more than 100 mgm. ascorbic acid, with other respective blood levels to intakes as follows: 0.4–0.8 mgm. per cent with 50–100 mgm. intake; 0.2–0.6 mgm. per cent with 30–50 mgm. intake; 0.0–0.4 mgm. per cent with 15–30 mgm. intake; and 0.0–0.2 mgm. per cent with less than 15 mgm intake. While it is generally agreed that the maintenance of fasting plasma ascorbic acid levels of 0.7 mgm. per cent or above is indicative of normal tissue saturation, low plasma values (0.0–0.4 mgm. per cent) unless found upon repeated examination, need not indicate a state of marked tissue depletion, and should not be taken as evidence of an impending scorbutic state. When such values are found, a determination of the ascorbic acid content of whole blood (45) or of the white-cell platelet layer may furnish evidence of the severity of tissue depletion. From studies on experimental human scurvy (50) low ascorbic acid values in cellular blood constituents would indicate a prolonged period of inadequate vitamin C intake.

As a result of placental transfer, the plasma ascorbic acid of the infant at birth is frequently higher than that of the maternal blood (51, 6, 52). Plasma levels at birth average above 0.60 mgm. per cent but decrease by nearly 50 per cent during the first 24 hours. On artificial diets without adequate vitamin C supplements, these values remain low, but with adequate intake either as a supplement or breast milk, they may attain values of 1 mgm. per cent at two weeks of age (53). A postpartum drop has also been observed in the mother (54).

Lund and Kimble (52) gave intravenous injections of ascorbic acid in doses of 100 to 500 mgm. to women before delivery and noted a rapid rise in maternal and cord blood; the foetal plasma level lagging somewhat, but reaching an equal concentration in 1½ to 2 hours. If the women were injected earlier than 2 hours before delivery, the maternal blood level decreased but the cord blood level remained high for at least 12 hours. On the basis of their observation, they suggest that ascorbic acid passes from maternal to foetal circulation by diffusion, at periods of high concentration in the former, and is blocked by the placental barrier from re-entering the maternal circulation. This theory seems plausable and if corroborated, would dispose of the question of foetal synthesis. On the other hand, living membranes have repeatedly been shown to exhibit selective permeability (55), and recent work shows ascorbic acid capable of functioning at cell surfaces (56).

As shown by Levine, Marples and Gordon (57) premature infants exhibit a spontaneous defect in their metabolism of tyrosine and phenylalanine which is manifest by the excretion of p-hydroxy-

phenyllactic and p-hydroxyphenylpyruvic acids into their urines if fed artificial diets in which the protein intake is 5 grams or more per kilogram of body weight per day. The relationship of vitamin C to the metabolism of aromatic amino acid has recently been reviewed by Sealock (58) and therefore need no further discussion here. It has also been shown that premature infants receiving human milk retain a larger part of a saturation dose of ascorbic acid in their tissues than do premature infants given cow's milk (59).

Breast fed infants of mothers on adequate vitamin C intake will receive 10 mgm. ascorbic acid daily until lactation is fully established when 20 mgm. or more is provided, increasing to 50 mgm. when weaned at 9 to 10 months of age (60). Artificially fed infants require a minimum daily intake of 10 mgm. ascorbic acid to prevent symptoms of scurvy (61). Plasma ascorbic acid levels are higher in breast fed infants than in those fed an artificial diet with a supplement of two ounces orange juice daily (62).

Ascorbic acid requirements for various age groups as summarized by Smith (65) are: Infants 8 to 50 mgm. daily; children 22 to 100 mgm.; adults 28 to 100 mgm. or more. Recent studies employing various criteria of saturation with subjects on controlled ascorbic acid intakes, indicate the following daily amounts to be required: Boys 10 to 14 years—45 to 75 mgm (64, 65); girls 6 to 12 years—62 to 72 mgm. (66); college students under 25 years—over 100 mgm.; older college women (25 to 50 years)—below 100 mgm (67). Observations on 800 youths at an N.Y.A. center indicated a diet containing 75 mgm. ascorbic acid to be inadequate (68).

Studies by Storvich and Hauck (69) indicated that daily supplements of 65 to 150 mgm. ascorbic acid in addition to 10 mgm. in the basal diet were required by six normal adults to maintain tissue saturation. In later studies (70), 10 of 12 subjects showed renal thresholds at plasma levels of 1.10 to 1.30 mgm. per cent. Of six subjects receiving 74 mgm. ascorbic acid daily for 12 to 14 days, three showed saturation and three slight depletion of tissue reserves. The authors also point out that subjects with high renal threshold levels maintain higher plasma ascorbic acid levels on lower intakes of ascorbic acid than do individuals with low renal thresholds. It is implied that this has a corresponding influence upon tissue saturation. The effect of a possible renal retention of ascorbic acid should not be overlooked when investigating tissue vitamin C saturation in clinical material (71, 72).

In reviewing the literature, one is impressed with the relative agreement as to daily ascorbic acid requirements considering the various criteria employed. In establishing the degree of tissue saturation, the test dose may be given orally as in most cases cited above, or by intravenous injection (73). In the oral tests, some gauge the dose according to the weight of the subject (65, 74) while others administer an arbitrary amount (usually 300 or 400 mgm. ascorbic acid) and base the criterion of tissue saturation upon the excretion of 50 per cent of the test dose into the urine in the following 24 hours. The adoption of a standardized technic for saturation tests under suitable sponsorship would help future attempts at establishing the proper levels of daily requirements. It is also apparent that the present recommendations of the Committee on Foods and Nutrition of the National Research Council for daily allowances of vitamin C are conservative, especially if the body is to maintain suitable reserves to successfully resist drains placed upon it by the onset of an infectious disease or other emergency.

Recent observations on the ascorbic acid requirements of certain lower animals are of interest. Hens under demand of heavy egg production when fed a vitamin C deficient diet may develop avitaminosis C resulting in leg weakness (75). Ascorbic acid when added to purified diets and liver supplements exerts a growth promoting effect in chicks (76). Ascorbic acid has been shown to be of therapeutic value in treating certain types of sterility in the cow (77). Later work indicates that vitamin C has a direct stimulating effect in maintaining fertility in the bull, stallion, jack and boar (78). Holstein calves may be raised from birth on skim milk supplemented with vitamins A, C, and D when given access to hay and grain (79).

The fate of orally ingested ascorbic acid. Loss of ascorbic acid after oral ingestion has been shown to occur in infants during diarrhea and following saline catharsis (80). With subsidence of the diarrhea, the fecal losses decreased with corresponding increase in urinary excretion. It has also been shown that on daily ingestion of ascorbic acid in amounts varying from 73 to 1054 mgm., the fecal excretion averaged 5 to 13.8 mgm. (81). Since a large part of the difference, especially at higher intake levels is excreted through the kidney, the balance must either undergo bacterial destruction in the gastrointestinal tract, or be utilized in some method as yet unknown by body tissues. Secretion during lactation (60) is definitely established, but secretion in sweat probably does not occur (82, 83, 84). Excess utilization in hyperpyrexia of non-infective origin is doubtful (85), nor does a high intake increase work output or exert any favorable influence during exposure to high environmental temperatures (86).

Decomposition of ascorbic acid by bacteria in the rumen of cattle has been established. Kendall and Chinn (87) demonstrated its utilization by organisms of the mucosus capsulatus and entero-

coccus groups, isolated by special methods from the gastrointestinal tract and feces of human beings. They also found that certain non-ascorbic acid fermenting bacteria (B. alcoligenes, Flexner type B. dysenteriae) exert a "protective action" upon the breakdown of ascorbic acid. The simultaneous rapid fermentation of carbohydrate also prevents the breakdown of ascorbic acid. Young and Rettger (88) later include as ascorbic acid fermenting organisms members of the following genera,—Escherichia, Aerobacter, Salmonella, Eberthella, Streptococcus, Encapsulatus and Vibrio. These observations, while indicating the susceptibility of ascorbic acid to destruction by intestinal bacteria when studied in vitro, need not be viewed with alarm except possibly in certain individuals whose intestinal flora may be overrun with ascorbic acid fermenting organisms. We may find in this work an answer to the occasional failure of oral therapy (except in massive doses) and the prompt response upon parenteral administration. The importance of the sparing action of carbohydrates for ascorbic acid when subjected to simultaneous bacterial action should not be overlooked. Studies of absorption of ascorbic acid by the small intestine in the human being, by the intubation technic, shows that 188 to 374 mgm. of ascorbic acid can be absorbed in one hour by a segment of small intestine 45 cm. long of subjects in either the saturated or depleted states (89).

Some observations on human subjects during prolonged ascorbic acid depletion.[1] Recent studies in prolonged vitamin C depletion in the human subject have sought to gain information by available methods, of changes in body chemistry and physiology as depletion progresses to the scorbutic state. Surgical studies have been concerned with the relationship of ascorbic acid to wound healing. The excellent study on "Experimental human scurvy" by Crandon, Lund and Dill (50) served largely as a guide for the study to be briefly outlined here.

Twelve young men, ages 20 to 30 years, were placed on a basal diet inadequate in vitamin C, and containing minimal quantities of the vitamin B complex. Five subjects remained on this diet, five received daily supplements of the B complex in amounts recommended by the Committee on Foods and Nutrition of the National Research Council, and two, serving as controls, received daily supplements of the B complex and 75 mgm. ascorbic acid, which was later raised to 150 mgm. Calories, protein, minerals and other vitamins were supplied in adequate amounts. The experiment lasted for seven months. Observations were made of the plasma and white-cell platelet ascorbic acid content, urinary ascorbic acid excretion, complete blood chemistry, hemoglobin, cell count and metabolic urinary constituents.

The diet was selected from a cafeteria, choosing those articles which were known to be free from ascorbic acid or which by over cooking, with and without soda, and holding on the steam table were shown by chemical analysis to be low or lacking in vitamin C. In this respect, our investigation differed from that of Crandon, Lund and Dill. A civilian might conceivably select a diet similar to ours. The ascorbic acid content while calculated at zero, might occasionally rise to 5 or 10 mgm. per day as shown by analysis.

The average time required for the plasma ascorbic acid to fall to zero was 70 days. Crandon attained the same point in 41 days. The white-cell platelet ascorbic acid dropped more rapidly in subjects deficient in both vitamin C and B complex, than those deficient in vitamin C alone. Zero levels in both groups were obtained during the latter part of the fifth month on the depletion diet. Crandon required 122 days to reach zero white-cell platelet content.

It was again found that capillary fragility measurements were an unreliable index of vitamin C deficiency (90). Crandon reports negative values by the same technic in his case. Serum phosphatase was unaffected by vitamin C depletion, remaining within a range of 1.89 to 3.91 Bodansky units for the depleted subjects, and 2.08 to 3.41 units for the normal controls. The observed decrease of phosphatase in the scorbutic infant (91) and scorbutic guinea pig (92) need not be considered contradictory to our results when it is remembered that phosphatase values in man are normally low after skeletal development has been attained. We were dealing with young adults 20 to 30 years of age.

Studies on work output were made by Dr. Eliot E. Foltz through the courtesy of the Department of Physiology, using an electrodynamic brake bicycle ergometer. Subjects in both depletion groups showed a measurable decrease in work output, that of the controls remaining constant. Evidence that vitamin C plays a minor part, if any, in the performance of work has been presented by Keys (93) in a recent review. Our subjects complained of severe fatigue during the last two months of depletion.

Neurological and psychological tests were made by Dr. G. K. Yacorzynski of the Department of

[1] The work described in this section was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Northwestern University. Unpublished data by C. J. Farmer, A. F. Abt, D. Y. Burrill, W. W. Carroll, M. Dentler, E. E. Foltz, D. O. Manshardt, J. A. Wolfer and G. K. Yacorzynski.

Nervous and Mental Diseases. His observations may be summarized as follows: The choice reaction time increased after the third to fifth months on the deficiency diet. Individuals making the greatest number of errors in choice reactions or whose reaction time shows great variation, appear to show the greatest debilitating effects of vitamin C depletion. The latter effect is associated with loss of interest or motivation. Characteristics such as aggressiveness, submissiveness, etc., become exaggerated during depletion. Under the conditions of our investigation, no measurable effect attributable to vitamin C depletion could be observed in certain tests including threshold of perception, coordination of motion on a pursuit meter and critical fusion frequency of visual flicker.

A study of oral changes attributable to acute ascorbic acid depletion was made by Dr. D. Y. Burrill of the Department of Oral Pathology, of the Dental School. The teeth of all subjects were scaled, the necessary fillings made and a program of oral hygiene instituted at the beginning of the study. Periodic checks and a final examination revealed no sponginess of gums or bleeding tendency. Examination of gums with the biomicroscope and slit lamp revealed no insacculation or capillary abnormalities. No changes in bone structure were revealed upon X-ray examination. The lack of oral pathology in our subjects, and the observation of but a single small gingival hemorrhage by Crandon, Lund and Dill (50) suggest that oral conditions frequently ascribed to an acute inadequate intake of vitamin C may in reality be due to a pre-existing caries or to improper oral hygiene. It cannot be questioned that severe oral lesions follow protracted depletion of vitamin C as evidenced by lesions around erupted teeth in the scorbutic infant (119), the interruption of the lamina dura, indicating a beginning atrophy of the alveolar bone as observed in x-rays by Crandon, and the familiar loosening of the teeth in the scorbutic guinea pig.

Studies of wound healing were made by Drs. J. A. Wolfer and W. W. Carroll of the Department of Surgery, and histological examinations by Dr. D. O. Manshardt, Pathologist of Passavant Memorial Hospital, on all depleted and control subjects during the seventh month of depletion. An incision 6 centimeters long was made through the skin and fascia of the left thigh. After properly suturing, biopsy sections were taken of the skin and fascia, from the 5th to 14th day of healing. The biopsy sections were removed in such a way that both cruciate and lineal closures of the skin were affected. The biopsy specimens were used for histological examination and also for measurement of the strength of tissue at the suture line [rupture factor = $\frac{\textit{grams to rupture at suture line}}{\text{area at suture line, (sq. mm.)}}$]. The rupture factor paralleled depletion as indicated by ascorbic acid saturation tests. Histological examination of the skin and fascia in the depleted subjects showed a marked deficiency of reticulum and collagen. (See Photomicrographs.) Similar results were obtained by Crandon.

A marked susceptibility to wound infection, especially in the severer type of wound resulting from cruciate closure accompanied vitamin C depletion. (See Fig. 1, severely depleted subjects.) The dependence on adequate reserves of vitamin C in tissues for wound healing, and for other functions, has been shown by several investigators for both the human subject and experimental animals (50, 94, 95, 96, 97, 98, 99, 100). A distinction is made by Chambers and Cameron (101) between

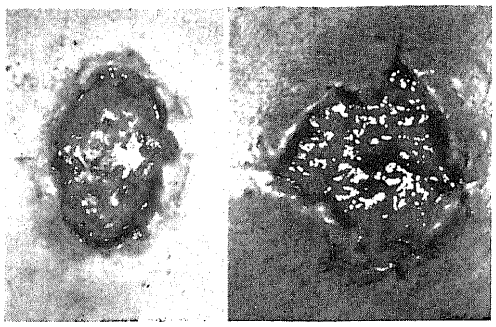

Fig. 1. Photographs of wounds in severe vitamin *C* depletion. Diet vitamin *C* deficient—*B* complex minimum. Mahoney (left) on eighth day and Mallette (right) on eleventh day after biopsy with cruciate closure.

the requirement of ascorbic acid for formation and maintenance of interstitial matrices (dentine, bone, collagen in connective tissue) and the non-essentialness of ascorbic acid for production and effectiveness of intercellular cement.

Periodic physical examinations were conducted by Commander A. F. Abt, U.S.N.R. No evidences of clinical scurvy were observed except hyperkeratotic papules surrounding the hair follicles on the lower extremities. The latter were observed by Crandon at an earlier date on his more rigourous diet. Although spontaneous capillary hemorrhages never occurred in our subjects, several areas around the wounds showed petechiae resulting from the slight trauma attending surgical manipulation. No such areas were observed on the normal control subjects.

Following the surgical studies, all subjects, except the two who were hospitalized because of severe wound infection, received by mouth a test

dose of 15 mgm. of ascorbic acid per kilogram of body weight. Their degree of tissue saturation was judged from the behavior of the plasma ascorbic acid curve, its magnitude of rise, and the hourly excretion of ascorbic acid into the urine, during the subsequent five hour period. Typical curves are given in figures 2 and 3. These, together with the results of the surgical studies, indicate the saturation test as described here to be a convenient and reliable index of tissue depletion. These studies further indicated that daily intakes of 100 mgm. ascorbic acid for one month provide adequate tissue saturation for a maximum rate of tissue repair in healthy young adults. Even lesser amounts are indicated by the behavior of one subject (Robley), emphasizing the rôle of individual variations, or possibly a less rigid adherence to a prescribed diet. These studies will be reported elsewhere in full at a later date.

acid may be related to the animal's detoxification processes.

Sulzberger and Oser (105) in 1935 showed that guinea pigs on diets inadequate in vitamin C, are more easily sensitized to neoarsphenamine by intracutaneous injection than controls on adequate diets.

In 1937, Dainow (106) reported a favorable influence of ascorbic acid upon arsphenamine tolerance in the human being. Many of his subjects were judged to be on inadequate vitamin C intake as evidenced by the lack of urinary excretion of this substance. Plasma ascorbic acid levels (107) are depressed in some cases and not in others (108) by administration of neoarsphenamine. It has been shown by Bundesen, Aron, Greenebaum, Farmer and Abt (109) that the dermatitis developed in human subjects when patch tested with various arsenicals may be prevented in a large proportion

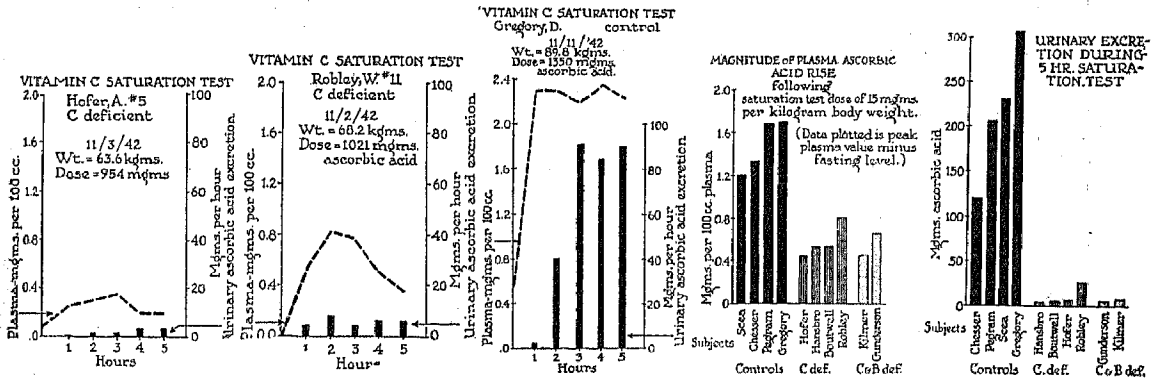

Fig. 2a Fig. 2b Fig. 2c Fig. 3a Fig. 3b

Figs. 2 and 3. Vitamin saturation tests

Miscellaneous conditions apparently influenced by ascorbic acid. In conclusion, a word should be said regarding the rôle of ascorbic acid in certain drug intoxications, the anemias and one or two other conditions.

It has recently been reported that scorbutic guinea pigs show extensive hepatic damage when injected with hydrazine (102) while control animals receiving 30 mgm. ascorbic acid daily were completely protected. Evidence that pathologic changes may occur in the parenchymal cells of the liver, and in the proximal convoluted tubules, in the guinea pig as a result of scurvy has recently been reported (103). Longenecker, Fricke and King (104) have shown an increased excretion of ascorbic acid into the urine of albino rats after administration of certain hypnotics and antipyretics. While there was no evidence of conjugation of ascorbic acid with these drugs, it is suggested that the endogenous production of ascorbic

of cases, by admixture with ascorbic acid. Neoarsphenamine and mapharsen solutions exposed to air are quickly oxidized to a brownish-black color. The addition of ascorbic acid markedly retards this reaction. Recently McChesney, Barlow and Klinck (110) have shown that the toxicity of neoarsphenamine for albino rats is materially reduced by ascorbic, isoascorbic, d-glucoascorbic and p-aminobenzoic acids. The most favorable effect was obtained when the arsenical and protective agent were injected simultaneously in the same solution. The function of ascorbic acid appears to be primarily that of preventing oxidation, chiefly after injection. Much clinical data has accumulated in evidence of the amelioration of symptoms of toxicity when arsphenamines are administered with ascorbic acid (106, 111, 112, 113).

Studies on the relationship of vitamin C to anemia are numerous. Anemia usually ac-

companies the production of scurvy in the growing guinea pig (114). Administration of some source of vitamin C (115) improves the blood picture unless the animal has lost more than 25 per cent of its body weight or one-third of its hemoglobin (116). A specific erythropoietic action has been attributed to vitamin C (117), but other investigators have failed to associate anemia, at least in the adult, solely with a vitamin C deficiency (50) but concomitantly with infection, general malnutrition or iron deficiency (118, 119, 120). It has been shown that patients with pernicious anemia on adequate vitamin C intakes have a significantly lowered plasma ascorbic acid level while patients on similar diets with an iron deficiency anemia show normal levels (121). The administration of ascorbic acid concomitantly with liver therapy has been successful in causing remission of symptoms in a series of pernicious anemia patients following unsatisfactory response to liver therapy alone (122).

Many other important observations could be added, for example the development of oral lesions accompanied by an increase in the fusospirochetal flora by Macacus mulatta monkeys when maintained on diets deficient in certain members of the B complex but adequate in vitamins A, C, D, nicotinic acid and riboflavin (123). Glucoascorbic acid when fed as 10 per cent of the dietary has produced a condition in mice which the authors consider as the counterpart of scurvy in other animals (124). A final observation, which may be of importance in the field of aviation reports that exposure of human subjects at a simulated altitude of 18,000 feet for one hour every second or third day, disturbs the metabolism of vitamin C (125). The immediate effect was a decreased urinary excretion of ascorbic acid followed later by a compensatory excretion of large amounts of this substance. Guinea pigs injected with 100 mgm. ascorbic acid when exposed to a simulated altitude of 18,000 feet for 12 hours showed a higher plasma and muscle ascorbic acid content than control injected pigs remaining at normal atmospheric pressures.

Conclusion. An attempt has been made to bring together information as to food sources of vitamin C and the influence of certain methods of handling and cooking upon the ascorbic acid content. A brief review of methods for analysis of food, and body fluids is given. Requirements ascertained for various age groups are indicated. Studies on depletion of human subjects have furnished evidence indicating that tissue depletion requires a considerable period to affect adversely processes of surgical repair. It is also shown that an individual may inadvertently subsist on a diet which through processes of handling or poor selection of foods may without apparent physical symptoms, reduce his body stores of ascorbic acid to a dangerous degree, leaving no margin of safety in the event of any unusual demand. Brief discussions of the rôle of ascorbic acid in detoxification and the anemias is also given.

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