In roots all lipids were increased. A number of lipid substances in the total extractable materials were measured.

3. Protein concentration was decreased in treated plants and carbohydrate fraction concentrations were increased reciprocally.

4. The esterified sterols of the shoots exhibited fast-reacting characteristics to the Liebermann-Burchard reagent.

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Ascorbic Acid and Aging in the Rat

UPTAKE OF ASCORBIC ACID BY SKIN AND BONE MARROW, AND ITS CONCENTRATION IN VARIOUS ORGANS

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Ascorbic acid participates in several metabolic reactions, and has been considered to be of particular significance in the aging process. Even though it is not an essential dietary requirement for mammals other than primates and guinea pigs, its metabolic functions are the same in all. In man its concentration in the blood cells declines with age in the adult (Kirk & Chieffi, 1953). The hepatic ascorbic acid concentration in rat increases rapidly during the first 3 weeks of extra-uterine life, declines sharply during the next 5 weeks and then remains relatively constant (Mourhouse & Guerrant, 1952). Several workers (Piez & Likins, 1957; Sinex, Van Slyke & Christman, 1959; Stetten,

1949; Stone & Meister, 1962) have shown that it is involved in the mechanism of hydroxylation of collagen. The administration of ascorbic acid to deficient guinea pigs enhances collagen synthesis (Gould, 1960; Robertson, Hiwett & Herman, 1959), but there is no evidence that it is required for the synthesis of other proteins. It has been suggested by Gross (1959) that ascorbic acid may be required for the maintenance of collagen. In view of the above observations, the uptake of ascorbic acid in the skin and bone marrow and its concentration in different organs of rats of various age groups have been studied to elucidate its role in the aging process.

MATERIALS AND METHODS

Animals. The albino rats used in the present studies were 2, 10 and 50 weeks old. These age groups were chosen because the 2- and 10-week-old rats are in a period of rapid growth whereas the 50-week-old rats have ceased to grow. These rats were fed on standard Anidiet 'A' obtained from Poona, India, and were kept at 24°.

Preparation of the tissues for the uptake of ascorbic acid. The animals were killed by dislocation of the neck. Several pieces of the skin were removed immediately, the hairs were plucked by hand and the skin was kept at 0° in beakers. The skin was then cut into thin sections with a razor blade. The weight of the skin used each time for incubation was approx. 1 g. A parallel control with exactly the same amount of skin was incubated in Ringer phosphate-bicarbonate (Krebs & Henseleit, 1932) adjusted to pH 7.4 with phosphate buffer without ascorbic acid. A few such control experiments showed that the skin did not give out any ascorbic acid, and hence this set of controls was discontinued.

The bone marrow was taken only from the limb bones by breaking them at the distal end and then injecting 2 ml. of the incubation medium with a syringe fitted with a 25-gauge needle. The fluid was then drawn out with the syringe. The wet weight of the marrow was determined by subtracting the weight of 2 ml. of incubation medium from that of the extracted incubation medium plus marrow. The weights of the bone marrow taken each time from the 2-, 10- and 50-week-old rats were 5–10, 30–40 and 50–60 mg. respectively. However, for comparative evaluation, the uptake of ascorbic acid by the bone marrow in each case was expressed as $\mu\text{g.}$ of ascorbic acid/10 mg. of bone marrow.

Incubation medium. Ringer phosphate-bicarbonate (Krebs & Henseleit, 1932) adjusted to pH 7.4 with phosphate buffer and containing 25 $\mu\text{g.}$ of ascorbic acid/ml. was used for the study of the uptake of ascorbic acid by both the skin and the bone marrow. In addition, the medium contained cysteine (1 μM) to prevent oxidation of ascorbic acid and EDTA (50 mM) to remove any heavy-metal ions that might cause oxidation of ascorbic acid. The total volume of the incubation medium for the skin was 50 ml. and for the bone marrow was 20 ml. The samples were incubated for 120 min. at 37°; the gas phase was air. Some preliminary experiments showed that cysteine did not interfere with the development of colour during the assay of ascorbic acid by the 2,4-dinitrophenylhydrazine method of Roe (1954a, b). Oxidation of ascorbic acid occurred if the concentration of cysteine was lower than 1 μM . To account for any oxidation that might occur despite the presence of cysteine, a parallel control containing ascorbic acid and cysteine but not the tissue was set up each time an experiment was performed.

Glass-distilled water was used throughout the experiments and the chemicals used were of analytical grade obtained from British Drug Houses Ltd., Bombay.

Determination of ascorbic acid in the incubation medium. Samples (2 ml.) of the incubation medium containing the skin slices were removed at intervals of 30 min. starting from zero time (immediately after the tissue was placed in the medium) for a period of 120 min. With bone marrow, 3 ml. samples were removed and centrifuged at 1000 rev./min. (150 g) for 5 min. to sediment the bone marrow. Then 2 ml. of the supernatant was taken for the assay of ascorbic

acid and the remainder was immediately placed back in the medium. Each of the 2 ml. samples was diluted with 2 ml. of 8% (w/v) trichloroacetic acid. The ascorbic acid was oxidized to dehydroascorbic acid by adding 2 drops of bromine water to the samples. The excess of bromine was removed by aeration. The rest of the procedure for the determination of ascorbic acid was the same as that of Roe (1954a, b). The extinction of the colour developed after the addition of hydrazine-thiourea reagent and conc. H_2SO_4 was read in a Klett-Summerson colorimeter fitted with a green filter. The concentration of ascorbic acid ($\mu\text{g./ml.}$) in each sample was determined from a standard curve which was linear and was drawn each time. Calculations for the total concentration of ascorbic acid in the medium were made after taking into account the decrease in volume due to the removal of 2 ml. samples.

Determination of ascorbic acid in various organs. The organs used were skin, liver, brain and kidney. These were taken from the same rats that were used for the study of the uptake of ascorbic acid. The method of extraction of ascorbic acid from the organs was similar to that of Roe (1954a, b). Weighed quantities of the organs were ground with 10 ml. of 6% (w/v) trichloroacetic acid in a mortar containing acid-washed sand. The supernatant was decanted and the process repeated with the residue with 10 ml. and then 5 ml. of 6% trichloroacetic acid. Then 4 drops of bromine water were added to the supernatant for the oxidation of the ascorbic acid. The solution was then stirred and filtered. Excess of bromine was removed from the filtrate by bubbling air. Samples (2 ml.) of this filtrate were taken in duplicate for the determination of ascorbic acid by the 2,4-dinitrophenylhydrazine method of Roe (1954a, b). The concentration in each organ was expressed as $\mu\text{g.}$ of ascorbic acid/100 g. wet wt. of tissue.

RESULTS

Uptake of ascorbic acid by skin and bone marrow.

Fig. 1 shows that maximum uptake of ascorbic acid by the skin of 2-week-old rats occurred in the

Fig. 1. Uptake of ascorbic acid by the skin ($\mu\text{g./g.}$ wet wt.) of 2-, 10- and 50-week-old rats. The volume of the incubating medium was 50 ml. and the initial concentration of ascorbic acid 25 $\mu\text{g./ml.}$ Averages of five experiments for each of the 2-, 10- and 50-week-old rats were taken. ○, 2-week-old rats; ●, 10-week-old rats; ×, 50-week-old rats.

first 30 min. There was a sharp decline in the uptake after 30 min. and no further uptake occurred after 90 min. of incubation. In contrast, the skin of 10- and 50-week-old rats did not show any uptake at all throughout the incubation period.

With bone marrow (Fig. 2), maximum uptake was again obtained in 2-week-old rats. The uptake by bone marrow of 10- and 50-week-old rats was about one-sixth of that of the 2-week-old rats. There was no significant difference in the uptake between the bone marrow of 10- and 50-week-old rats at any period between 0 and 120 min.

Fig. 2. Uptake of ascorbic acid by the bone marrow ($\mu\text{g./10 mg. wet wt.}$) of 2-, 10- and 50-week-old rats. The volume of the medium was 20 ml. and the initial concentration of ascorbic acid 25 $\mu\text{g./ml.}$ Averages of five experiments for each of the 2-, 10- and 50-week-old rats were taken. ○, 2-week-old rats; ●, 10-week-old rats; ×, 50-week-old rats.

Fig. 3. Concentration of ascorbic acid ($\text{mg./100 g. wet wt.}$) in (a) skin, (b) liver, (c) brain and (d) kidney of 2-week-old (□), 10-week-old (▨) and 50-week-old (■) rats. The averages of five experiments for each of the 2-, 10- and 50-week-old rats were taken.

Concentration of ascorbic acid in the organs. Fig. 3 shows that the concentration in the skin of 2-week-old rats was more than twice that of 10- and 50-week-old rats. Thus it appears that the higher concentration of ascorbic acid reflects the higher uptake by the skin. The difference in the concentration in the skin of 10- and 50-week-old rats was not significant. In all the other organs examined, the concentration was significantly higher in the 2-week-old rats than in the 10- and 50-week-old rats. Also, the differences in the concentration were significant in all three groups of rats.

DISCUSSION

In the present studies, parallel experiments for the determination of uptake of ascorbic acid by the skin and bone marrow and for the determination of its concentration in the skin, liver, brain and kidney were undertaken in 2-, 10- and 50-week-old rats to evaluate the role of ascorbic acid at different ages. Fig. 1 shows that ascorbic acid is taken up by the skin of 2-week-old rats only. In rats, collagen synthesis reaches the maximum level towards the 35th day of post-uterine life (Wirtschaffter & Bentley, 1962). Also, ascorbic acid is required for the hydroxylation of proline and possibly lysine that are needed for the synthesis of collagen. Therefore the uptake of ascorbic acid by the 2-week-old rats may be related to the synthesis of collagen. The skins of 10- and 50-week-old rats do not show any uptake as the synthesis of collagen may be assumed to have ceased in the skins of these rats. These skins show two important biochemical features: (a) the absence of uptake of ascorbic acid; (b) the concentration of ascorbic acid is lower than in other organs examined, even at the 2-week-old stage when rapid collagen synthesis occurs in the skin. Since rats synthesize their own ascorbic acid, this cannot be due to a deficiency of ascorbic acid. It appears, therefore, that the requirement for ascorbic acid by the skin ceases after the synthesis of collagen has reached its maximum level, or the hydroxylation mechanism has been completed. Therefore ascorbic acid is not required for the maintenance of collagen, for, if it were required even after the completion of collagen synthesis, the skins from the 10- and 50-week-old rats would take up ascorbic acid, as does the bone marrow of these rats.

The uptake of ascorbic acid by the bone marrow of the three different age groups of rats shows that the uptake by that of the 2-week-old rats is about fivefold greater than those of the other two groups. The rate of uptake and the total amount of uptake (Fig. 2) are similar in the 10- and 50-week-old rats. This indicates that the metabolic requirement in these two groups of rats may be the same, whereas,

in the 2-week-old rats, ascorbic acid either performs additional functions or performs the same functions as in the other two groups but at accelerated rates. Since the bone marrow is predominantly engaged in the production of blood cells, it is possible that some relationship may exist between the rate of production of blood cells and the quantity of ascorbic acid available to the bone marrow.

The concentrations of ascorbic acid in the various organs show a general decrease with age. The liver, which is mainly responsible for its synthesis, also shows a lower concentration of ascorbic acid: this may be due to a decrease in the activities of the enzymes which synthesize it. This may result in lower concentrations in other organs also. The reason for its lower concentration in the liver of older guinea pigs and in the blood cells of older men (Mourhouse & Guerrant, 1952; Kirk & Chieffi, 1953) kept on a normal ascorbic acid diet is not known. Our studies on the rat show that this lower concentration in the organs may be due either to a decrease in its rate of synthesis in the liver or to a decreased rate of its uptake by the organs.

SUMMARY

1. Parallel experiments to determine the uptake of ascorbic acid by the skin and bone marrow and for the determination of its concentration in the skin, liver, brain and kidney were undertaken in 2-, 10- and 50-week-old rats to evaluate the role of ascorbic acid at different ages of the animal.

2. Uptake of ascorbic acid occurred in the skin of 2-week-old rats. There was no uptake by the skin of the other two groups. It was concluded that ascorbic acid is not required for the maintenance of collagen in the skin.

3. The bone marrow of all the three groups of rats showed uptake of ascorbic acid, but that in the 2-week-old rats was fivefold greater. It is suggested that there may be some relationship between the rate of production of blood cells by the bone marrow and the quantity of ascorbic acid available to it.

4. There was a general decrease with age of the concentration of ascorbic acid in various organs, including the liver. It is possible that there may be a decrease with age in the activities of the enzymes responsible for the synthesis of ascorbic acid in the liver.

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The Effects of Thiosulphate and Oxygen Concentration on Tetrathionate Oxidation by *Thiobacillus X* and *T. thioparus*

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Tetrathionate is readily oxidized to sulphate by a number of aerobic and anaerobic thiobacilli (Baalsrud & Baalsrud, 1954; Parker & Prisk, 1953; Vishniac & Santer, 1957; Santer, Boyer & Santer,

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1959; Jones & Happold, 1961). From time to time, however, there have been reports that some strains of the aerobic bacterium, *Thiobacillus thioparus*, are unable to metabolize this polythionate (e.g. Tamiya, Haga & Huzisige, 1941; Parker & Prisk, 1953).