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Ascorbic Acid and Aging in the Rat

UPTAKE OF ASCORBIC ACID BY TEETH AND CONCENTRATION OF VARIOUS FORMS OF ASCORBIC ACID IN DIFFERENT ORGANS

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1. The uptake of ascorbic acid *in vitro* by the teeth of rats showed a gradual decrease with age, indicating that the uptake may be related to collagen synthesis as in bone. 2. The concentration of total free ascorbic acid in various organs declined with age, but the rate of decline was different in different organs. In the spleen, however, it increased until maturity and then declined. 3. This decrease may be due to one or both of the following reasons: (*a*) the permeability of different tissues may decrease at different rates for ascorbic acid, or (*b*) the requirement for ascorbic acid may decrease at different rates. 4. The bound ascorbic acid declined with age in the skin, kidney, liver and brain after the age of 10–12 weeks, and in the spleen after the age of 26 weeks. 5. The concentration of dehydroascorbic acid and dioxogulonic acid declined with age in the skin.

Ascorbic acid participates in several metabolic reactions and is considered to be of importance in the aging process. Kanungo & Patnaik (1964) and Patnaik & Kanungo (1965) reported that the uptake of ascorbic acid by the skin and the bone of the rats of various ages may be related to the rate of synthesis of collagen in these tissues. The involvement of ascorbic acid in the synthesis of mucopolysaccharides in the skin has also been suggested by Patnaik & Kanungo (1966). Boyle, Bessey & Percy (1940) demonstrated a quantitative relationship between the rate of formation of dentine in the incisors of the guinea pig and the amount of ascorbic acid available in the food. The teeth become loose in extreme cases of scurvy as dentine is not produced in the gingiva (Muhler, 1959).

Several reports show that the various forms of ascorbic acid may have different metabolic functions in different tissues of the rat. Peterkofsky & Udenfriend (1965) showed that ascorbic acid may function as a cofactor of proline hydroxylase in the hydroxylation of proline. Martin & Mecca (1961) reported that dehydroascorbic acid is the transport form of ascorbic acid and enters the tissues as such. It has been suggested that the bound form of ascorbic acid has some enzymic function (Summerwell & Sealock, 1952). We decided therefore to study the uptake of ascorbic acid by the teeth, and the concentrations of various forms of ascorbic acid in different organs of rats of various ages, to evaluate its function in the aging of various organs.

MATERIALS AND METHODS

Animals. The albino rats (Wistar strain) used were kept as described by Kanungo & Patnaik (1964). Two-, 25- and 59-week-old rats were used for the study of the uptake of ascorbic acid by teeth. The ages of the rats used for the determinations of total free ascorbic acid, bound ascorbic acid, dehydroascorbic acid and dioxogulonic acid are mentioned in the legends for the Figures and the Table.

Preparation of the homogenate of teeth for the uptake of ascorbic acid. The upper and lower incisors and the lower cheek teeth were uprooted from the base and immediately transferred to beakers containing ice-cold Krebs–Ringer phosphate–bicarbonate saline (Krebs & Henseleit, 1932). The adhering muscles were removed from the teeth, which were then weighed, broken into pieces by a bone cutter and homogenized in 2·0ml. of ice-cold Krebs–Ringer phosphate–bicarbonate saline. The homogenate was transferred to the incubation medium (total volume 25ml.) and incubated at 37° for 3hr. For 2-week-old rats all the four incisors and all the lower cheek teeth were used for one set of experiments, whereas for the others (25- and 59-week-old rats) one incisor each from the upper and lower jaws and all the lower cheek teeth of one side served as one set of experiments. The weight of the teeth used for the incubation was 100mg. for 2-week-old rats and 200mg. for 25- and 59-week-old rats.

Incubation medium. Krebs–Ringer phosphate-bicarbonate saline adjusted to pH7·4 with $NaOH$–KH_2PO_4 buffer and containing ascorbic acid (0·143μM) was used for the uptake of ascorbic acid by teeth. In addition, the medium contained cysteine hydrochloride (12·6μM) to prevent oxidation of ascorbic acid and EDTA (5·0μM) to remove any heavy-metal ions. Parallel controls were set up each time, as reported by Kanungo & Patnaik (1964).

Determination of ascorbic acid in the incubation medium. The method used was the same as that described for skin by Kanungo & Patnaik (1964) with a few modifications. The uptake of ascorbic acid was determined by taking samples of the incubation medium at 1 hr. intervals for a period of 3 hr., as it was observed that significant uptake occurred even after 1 hr., unlike that by the skin. The extinction of the colour developed after the addition of 2,4-dinitrophenylhydrazine reagent was read at 540 mμ in a Beckman model DB spectrophotometer. The uptake of ascorbic acid was expressed as μg. of ascorbic acid/100 mg. wet wt./3 hr.

Determination of total free ascorbic acid in various organs. The method of extraction of free ascorbic acid was the same as that of Kanungo & Patnaik (1964). The whole organs were used for heart, spleen and thymus. For the skeletal muscle (gastrocnemius) and lungs about 0·5 g. of tissue was used. The extraction volume for each of the above tissues was 15·0 ml.

Determination of bound ascorbic acid in various organs. The method was based on that of Summerwell & Sealock (1952) with the following modifications. A 1·0 g. portion of skin or liver was extracted thrice in a precooled mortar with 15·0 ml. of ethanol saturated with CO_2. The volume of ethanol used for a whole organ like the kidney, brain or spleen was adjusted according to the weight of the organ so that the ratio between the tissue and ethanol was 1:15. The homogenate was centrifuged at 6500*g* at 0° in an International High Speed refrigerated centrifuge for 15 min. and the supernatant was discarded. The residue was mixed with 7·5 ml. of 5% metaphosphoric acid and was kept in a boiling-water bath for 15 min. for hydrolysis. It was then chilled and centrifuged at 12500*g* for 20 min. The volume of the supernatant was determined. Samples were taken from the filtrate and oxidized with bromine. The rest of the procedure was the same as that described by Kanungo & Patnaik (1964). The extinction of the colour developed was measured at 540 mμ.

Determination of dehydroascorbic acid plus dioxogulonic acid in the skin. The composite determination of dehydroascorbic acid and dioxogulonic acid was done according to the method of Roe, Mills, Osterlings & Damron (1948) with the modification that the incubation was done at 100° for 10 min. as suggested by Tewari & Pandey (1964). The extinction of the colour developed was measured at 540 mμ. A stock sample of dehydroascorbic acid was prepared by oxidizing ascorbic acid solution with bromine as described by Roe (1954). This was taken as the standard for the composite determination of dehydroascorbic acid and dioxogulonic acid. Since incubation at 100° for 10 min. measures both dehydroascorbic acid and dioxogulonic acid (Tewari & Pandey, 1964), results are expressed as a measure of both and not of one or the other.

The concentrations of total free ascorbic acid, bound ascorbic acid and dehydroascorbic acid plus dioxogulonic acid in different organs were expressed as mg./100 g. wet wt. The standard deviations, standard error of the mean and the levels of significance were calculated according to the method of Garret (1956).

RESULTS

Fig. 1 shows that the uptake of ascorbic acid by the teeth of 2-week-old rats was higher than that by those of 25- and 59-week-old rats. The differences in the uptake by various age groups were highly significant. The concentration of total free ascorbic acid, which includes ascorbic acid, dehydroascorbic acid and dioxogulonic acid, in different organs declined with age (Figs. 2 and 3). However, in the spleen the concentration increased until the age of 14 weeks. It was lower again at 59 weeks (Fig. 2). The concentration of ascorbic acid in heart muscle declined more sharply with age than that of skeletal muscle (Fig. 3). In the thymus a sharp decline was observed until the age of 14 weeks. Owing to the involution of the thymus after the attainment of maturity (14 weeks), it was not possible to determine its ascorbic acid concentration in the older rats.

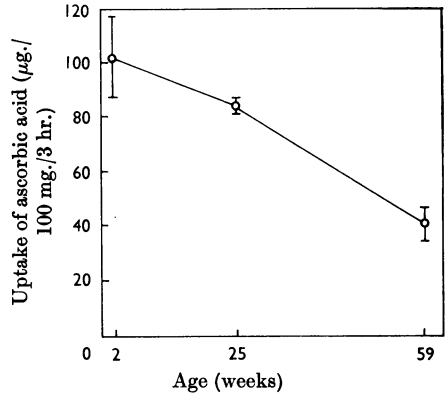

Fig. 1. Uptake of ascorbic acid by teeth of 2-, 25- and 59-week-old male rats. The experimental conditions are described in the text. Each point represents the mean of seven experiments and the vertical bars represent ±S.D.

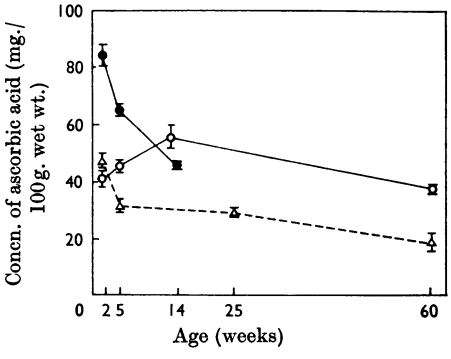

Fig. 2. Concentration of total free ascorbic acid in various organs of rats of different ages: ●, thymus (female rats); ○, spleen (both sexes); △, lungs (male rats). Each point represents the mean of seven to ten experiments and the vertical bars represent ±S.D.

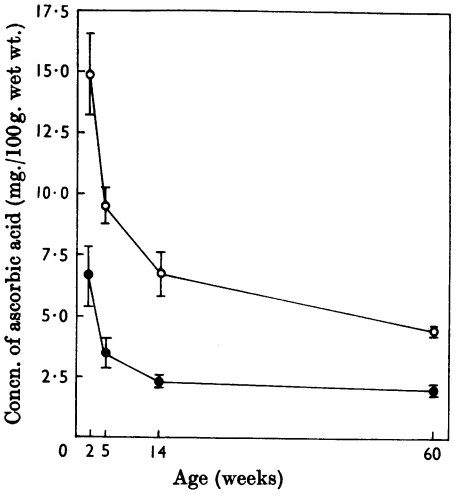

Fig. 3. Concentration of total free ascorbic acid in the heart (○) and skeletal muscle (●) of 2-, 5-, 14- and 60-week-old rats (both sexes). Each point represents the mean of five to seven experiments and the vertical bars represent ±S.D.

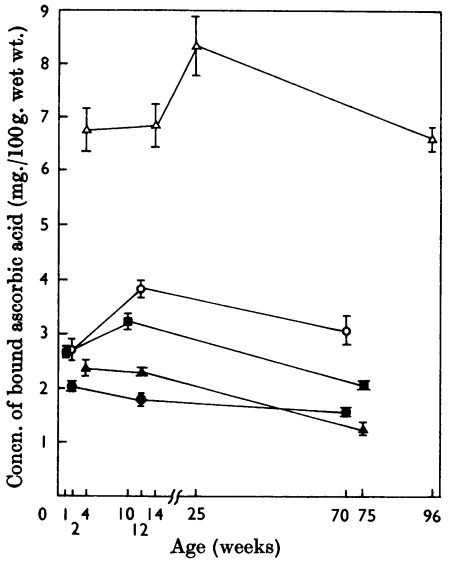

Fig. 4. Concentration of bound ascorbic acid in spleen (△), liver (○) and skin (●) of female rats and brain (■) and kidney (▲) of male rats of various ages. Each point represents the mean of four to nine experiments and the vertical bars represent ±S.E.M.

The concentration of bound ascorbic acid was significantly higher in the skin of 2-week-old rats than in that of 70-week-old rats (Fig. 4). Its concentration was lower in the liver of 2-week-old rats than in that of 12-week-old rats. However, the concentration was significantly lower in 70-week-old rats (Fig. 4). The concentration in the kidney declined with age. However, in the brain and the spleen there was an increase until 10 weeks and 26 weeks respectively. The concentrations were lower again at 75 and 96 weeks respectively.

The concentration of skin dehydroascorbic acid and dioxogulonic acid together was higher in the 5-week-old rats than in the 3-day-old rats. The concentration was significantly lower in the skin at 83 weeks than in that at 5 weeks (Table 1).

Table 1. *Concentration of dehydroascorbic acid plus dioxogulonic acid in the skin of rats of various ages*

Determinations were made as described in the text. Results are given as means +S.E.M., with the numbers of animals in parentheses.

Age (days)	Concn. of dehydroascorbic acid+dioxogulonic acid (mg./100g. wet wt.)	Significance of change (*P*)
3	2·711±0·315 (3)	<0·05
35 (5 weeks)	3·833±0·179 (3)	
586 (83 weeks)	2·958±0·222 (3)	<0·05

DISCUSSION

Our experiments show that the uptake of ascorbic acid by the teeth of young rats is significantly higher than that by those of 25- and 59-week-old rats. This may be due to a higher requirement of ascorbic acid at the early age for the synthesis of dentine, which contains a high proportion of collagen. This is in agreement with Boyle *et al.* (1940), who demonstrated a quantitative relationship between the rate of formation of dentine in the incisors of guinea pigs and the amount of ascorbic acid available in the food. Further, in the young teeth, enamel and dentine undergo constant turnover, in contrast with that of the adult, in which the turnover is very slow (Harper, 1961). So the decreased uptake of ascorbic acid by the teeth of 25- and 59-week-old rats may be due to their lower requirement for the synthesis of dentine, which slows down with age. The decreasing turnover of dentine may be due to the decline in the activities of odontoblasts with age. Thus the rate of synthesis of collagen in the teeth may be related to the uptake of ascorbic acid, as in skin and bone (Kanungo & Patnaik, 1964; Patnaik & Kanungo, 1965).

Kirk (1962) suggested that the age-related changes in the concentration of vitamins in the tissues may be due to their differential transport

into the tissues at various stages of development. He further stated that the decreased ascorbic acid concentrations in elderly human beings and old animals may be of some importance in connexion with the aging changes of tissues. However, the reason for such a change in the rat and other mammals for which ascorbic acid is not a vitamin, and which synthesize their own ascorbic acid, is not known. Chatterjee & Mcknee (1965) showed that the synthesis and concentration of ascorbic acid in the liver of rats is highest 14 days after birth. Then the concentration declines and stabilizes at a lower level. Since the rate of decrease in the concentration of ascorbic acid in the various tissues is different, it is unlikely that the concentration of ascorbic acid in the different tissues is entirely dependent on its synthesis in the liver. The decline in the concentration in different tissues may be influenced by two factors: (*a*) the permeability of different tissues may decrease at different rates, or (*b*) the requirement for ascorbic acid that participates in several metabolic functions besides collagen synthesis may decrease at different rates.

A more significant decline in the concentration of ascorbic acid is seen in the tissues in which the cells do not divide (skeletal muscle and heart) than in the liver, which has mitotic potential cells and in which there is a constant replacement of old cells by new ones (Kanungo & Patnaik, 1964). The rise in the concentration of ascorbic acid in the spleen up to the age of 14 weeks may be due to an increase in its activity for the production of antibodies. Since a simultaneous decrease in the concentration of ascorbic acid in the thymus was observed until this age, it is possible that the increase in the concentration in the spleen until this age may be due to its taking over the function of the thymus.

The protein-bound ascorbic acid in the skin and liver does not show similar age-related changes to those of free ascorbic acid. Summerwell & Sealock (1952) suggested that the bound ascorbic acid has some enzymic function. It is possible therefore that the increase in the bound ascorbic acid in the liver, brain and spleen may be due to increased binding with a specific enzyme protein. A decreasing capacity of the proteins to bind water with advancing age owing to cross-linking has been suggested by Bjorksten (1962). The decreased binding of ascorbic acid may be due to a similar effect.

The higher concentration of dehydroascorbic acid plus dioxogulonic acid in the skin of 5-week-old rats (Table 1) indicates their involvement in some metabolic function. Martin & Mecca (1961) found that dehydroascorbic acid is the transport form of ascorbic acid. Whether the higher concentration of dehydroascorbic acid plus dioxogulonic acid in the skin of 5-week-old rats is due to the higher rate of transport of dehydroascorbic acid or dioxogulonic acid or both to the site of collagen synthesis is not known.

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