Effects of Vitamins A and D on the Biosynthesis of L-Ascorbic Acid by Rat-Liver Microsomes

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1. The synthesis of L-ascorbic acid from either D-glucuronolactone or L-gulonolactone by liver microsomes of rats is decreased under conditions of hypervitaminosis A; under hypervitaminosis D the synthesis from D-glucuronolactone is increased and that from L-gulonolactone is not affected. 2. The microsomal conversion of L-gulonolactone into L-ascorbic acid is impaired in liver tissues of rats made deficient with respect to either vitamin A or vitamin D when compared with the controls maintained on stock diet.

It is known (Burns, Peyser & Moltz, 1956; Chatterjee, Chatterjee, Ghosh, Ghosh & Guha, 1960*a,b*) that the enzyme system catalysing the conversion of D-glucuronolactone and L-gulonolactone into L-ascorbic acid is entirely located in the microsomal fractions of rat liver. Further, it has been reported that, when B vitamins are deficient, the urinary excretion as well as the tissue reserve of ascorbic acid are markedly diminished (Roy, Roy & Guha, 1946; Chatterjee, Roy & Guha, 1955; Ray, Gyorgy & Harris, 1935; Sure, Theis & Harrelson, 1939; Schwartz & Williams, 1952*a,b*). Chatterjee & Kar (1960) noted that the synthesis of L-ascorbic acid *in vitro* by the microsomal enzymes of liver of rats fed on diets deficient in thiamine, riboflavin, pyridoxine, pantothenic acid or folic acid is also diminished and that synthesis cannot be restored by the addition of the vitamins, or their coenzyme forms, to the enzyme system *in vitro*. In vitamin A deficiency there occurs a decrease in the ascorbic acid contents of the tissues of rats (Sure *et al.* 1939; Jonsson, Obel & Sjöberg, 1942). Also, under conditions of hypovitaminosis A symptoms of scurvy appear that cannot be cured by the administration of ascorbic acid (Meyer & Krehl, 1948). Mapson & Walker (1948) noted that the rise in urinary excretion of ascorbic acid, as a result of the administration of Chloretone to rats, was not much affected in vitamin A deficiency when compared with pair-fed controls. Ascorbic acid is interrelated with vitamin D (Lecoq, Chau-chard & Mazoue, 1944, 1945), and a very prominent role of vitamin E in the synthesis of ascorbic acid has been established (Caputto, McCay & Carpenter, 1958; Kitabchi *et al.* 1959; Caputto, Trucco & Kitabchi, 1961; Ghosh & Kar, 1963). The present

paper reports observations on the effects of hyper- and hypo-vitaminoses A and D on the synthesis of L-ascorbic acid by rat-liver microsomes.

MATERIALS

L-Gulonolactone was prepared as described by Chatterjee *et al.* (1960*a*). D-Glucuronolactone was purchased from California Corp. for Biochemical Research (Los Angeles, Calif., U.S.A.).

METHODS

Preparation of microsomes. Rats were killed by decapitation and microsomes were prepared from the livers by the method of Chatterjee *et al.* (1960*a*).

Incubation media. The test system contained sodium phosphate buffer, pH 7.4 (20 mM), D-glucuronolactone (10 mM), microsomal dispersion (0.25 ml., equivalent to 250 mg. of fresh liver) and KCN (50 mM). The total volume was 2.5 ml. and the mixture was incubated at 37° for 1 hr. When gulonolactone (5 mM) was used as substrate KCN (1 mM) was added to the test system to prevent oxidation of L-ascorbic acid formed in the medium. Though cyanide at a higher concentration inhibits the synthesis of L-ascorbic acid, a lower concentration of cyanide (1 mM) does not. The experiments were performed in the presence of pyrophosphate, pH 7.4 (20 mM), since in its absence there is a diminished formation of L-ascorbic acid from L-gulonolactone by rat-liver microsomes (Chatterjee, Kar, Ghosh & Guha, 1961).

Ascorbic acid and protein in the microsomal preparations were estimated as described by Chatterjee *et al.* (1960*a*).

EXPERIMENTAL

Production of hypervitaminosis A and hypervitaminosis D. Male albino rats (40–50 g.), which had been maintained on the stock diet of Ghosh & Kar (1963), were divided into

four groups of which group I received oral supplementation of vitamin A palmitate (60000 i.u./100 g. body wt./day) for 3 weeks, group II served as the pair-fed controls for group I, group III received oral supplementation of calciferol (20000 i.u./100 g. body wt./day) for 4 weeks and group IV remained as the pair-fed controls for group III.

Production of hypovitaminosis A. Male albino rats (35–40 g.) obtained from mothers maintained on vitamin A-deficient diet were divided into two groups with one group serving as pair-fed controls. The vitamin A-deficient group was maintained on a diet consisting of vitamin-free casein, 18 parts, corn starch, 58 parts, groundnut oil, 10 parts, Steenbock salt mixture no. 40 (Steenbock & Nelson, 1923), 4 parts, and dried brewer's yeast powder, 10 parts, with a supplement of 10 i.u. of vitamin D and 1 mg. of tocopherol/rat/day. The control animals were given, in addition, 20 i.u. of vitamin A palmitate/rat/day. The animals were placed on these diets for 4–5 weeks.

Production of hypovitaminosis D. Male albino rats (35–40 g.) were obtained from the mothers maintained on a diet consisting of casein, 18 parts, corn starch, 68 parts, groundnut oil, 10 parts, and Steenbock salt mixture, 4 parts, with a supplement of vitamin mixture and 50 i.u. of vitamin A/rat/day. The vitamin D-deficient group was maintained on a diet consisting of yellow maize, 76 parts, wheat gluten, 20 parts, CaCO₃, 3 parts, NaCl, 1 part, and containing vitamin mixture and a supplementation of vitamin A (20 i.u./rat/day). The control group received, in addition, 6 i.u. of vitamin D/rat/day. The animals were placed on this diet and kept in the dark for 4–5 weeks.

RESULTS

Effects of hypervitaminosis A and hypervitaminosis D on the conversion of D-glucuronolactone and L-

gulonolactone into L-ascorbic acid by rat-liver microsomes. Table 1 shows that in hypervitaminosis A the microsomal synthesis of L-ascorbic acid from both D-glucuronolactone and L-gulonolactone is decreased when compared with the controls. In hypervitaminosis D, however, synthesis from D-glucuronolactone is increased and synthesis from L-gulonolactone is not affected.

Effects of hypovitaminosis A and hypovitaminosis D on the conversion of L-gulonolactone into L-ascorbic acid by rat-liver microsomes. From the results in Table 2 it can be calculated that there is a significant decrease in the microsomal synthesis of L-ascorbic acid by rats on diet deficient in vitamin A or vitamin D when compared with their respective pair-fed control rats. The *P* values for vitamins A and D are <0.05 and <0.01 respectively. But when the results are compared with rats fed *ad libitum* on stock diet the significance is more pronounced in both cases (*P* < 0.01). The recoveries of microsomal protein did not vary much between the experimental and the control groups of animals.

DISCUSSION

It has been previously reported (Chatterjee & Kar, 1960; Chatterjee, Kar, Ghosh & Guha, 1960) that the enzymic activity of liver microsomes for the synthesis of L-ascorbic acid from either D-glucuronolactone or L-gulonolactone is markedly decreased as a result of deficiencies of several B vitamins and vitamin E. In all experiments, however, it was observed that the activity of the microsomes could be restored by giving the respec-

Table 1. *Biosynthesis of L-ascorbic acid by the liver microsomes of rats under hypervitaminosis A and hypervitaminosis D*

Experimental details are given in the text. Each result is expressed as the mean $\pm$ s.d. of five experiments, each on a different animal. The mean recoveries of microsomal protein (in mg./g. of fresh liver) were: (a) rats under hypervitaminosis A, 13.5 ± 2.3 ; (b) control rats for group (a), 15.2 ± 3.5 ; (c) rats under hypervitaminosis D, 14.2 ± 2.0 ; (d) control rats for group (c), 15.6 ± 1.5 .

Source of microsome preparation	Ascorbic acid synthesized (μ mole/mg. of protein)	
	From D-glucurono- lactone	From L-gulono- lactone
(a) Rats under hypervita- minosis A	0.04 ± 0.001	0.10 ± 0.01
(b) Control rats for group (a)	0.07 ± 0.002	0.20 ± 0.02
(c) Rats under hypervita- minosis D	0.07 ± 0.001	0.19 ± 0.02
(d) Control rats for group (c)	0.06 ± 0.001	0.21 ± 0.01

Table 2. *Biosynthesis of L-ascorbic acid the by liver microsomes of rats under hypovitaminosis A and hypovitaminosis D*

Experimental details are given in the text. Each result is expressed as the mean $\pm$ s.d. of five experiments, each on a different animal. The mean recoveries of the microsomal protein (in mg./g. of fresh liver) were: (a) rats under hypovitaminosis A, 14.6 ± 1.6 ; (b) pair-fed controls for group (a), 15.6 ± 2.0 ; (c) rats under hypovitaminosis D, 14.0 ± 1.5 ; (d) pair-fed controls for group (c), 16 ± 2.1 ; (e) normal controls fed on stock diet, 18 ± 3.2 .

Source of microsome preparation	Ascorbic acid synthesized from L-gulonolactone (μ mole/mg. of protein)
(a) Rats under hypovitaminosis A	0.11 ± 0.03
(b) Pair-fed controls for group (a)	0.17 ± 0.03
(c) Rats under hypovitaminosis D	0.065 ± 0.003
(d) Pair-fed controls for group (c)	0.156 ± 0.01
(e) Normal controls on stock diet	0.21 ± 0.03

tive vitamins to the deficient rats. The impairment in vitamin E deficiency was found to be due to the inhibition of the enzyme L-gulonolactone oxidase (Kitabchi *et al.* 1959). Table 1 indicates that hypervitaminosis A also leads to impairment in the microsomal synthesis of L-ascorbic acid from either D-glucuronolactone or L-gulonolactone in rats. When rats are fed on a vitamin D-rich diet microsomal synthesis of L-ascorbic acid from L-gulonolactone is not affected but synthesis from D-glucuronolactone is significantly increased. The cause of this increased synthesis is not clear. Rodahl (1949) noted that the administration of high doses of vitamin A to rats leads to the development of scurvy-like symptoms, and that the subsequent administration of large doses of vitamin C alleviated some of the scurvy-like symptoms but none of the symptoms of hypervitaminosis A. Significant decrease in the liver ascorbic acid content was also noted under hypervitaminosis A (Morehouse, Guerrant & Dutcher, 1952). Malathi & Ganguli (1964) have observed a marked decrease in the synthesis of ascorbic acid in hypovitaminosis A. The slight decrease observed in our experiments may be associated with the less severe vitamin deficiency. Previous studies on hypovitaminosis A in rats indicated lowering of the tissue concentrations of ascorbic acid (Meyer & Krehl, 1948), and Mapson & Walker (1948) attributed this decrease to the lowered food intake. However, in the experiments presented in Table 2 the effect of inanition has been eliminated by using pair-fed control animals. The mechanism of inhibition in the synthesis of L-ascorbic acid by microsomal enzymes as a result of hypervitaminosis A, hypovitaminosis A and hypovitaminosis D is not clear, but Chatterjee & Kar (1960) have suggested that structural derangement of the microsomes occurs at times of vitamin deficiency and that this brings about impairment of the enzymes rather than depletion of cofactors involved in the synthesis. This appears reasonable since it was also observed (Chatterjee & Kar, 1960) that neither the addition of the coenzyme forms of several B vitamins to microsomes isolated from the rats deficient in the respective vitamins nor the addition of normal supernatant fractions to the microsomes from deficient rats (and vice versa) could augment the decreased synthesis. It appears from the work of Caputto *et al.* (1961) that vitamin E may be specifically involved in ascorbic acid synthesis. On the other hand, it has been observed by Ghosh & Kar (1963) that the microsomal conversion of L-gulonolactone into L-ascorbic acid by liver tissues

of rats deficient in vitamin E is not affected. The synthesis is, however, pyrophosphate-dependent. These findings indicate that vitamins A and D are also not specifically involved in the biosynthesis of ascorbic acid.

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