ASCORBIC ACID METABOLISM IN HYPERTHYROID RATS

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Male albino rats were fed thyroid powder (1%) and ascorbic acid, dehydroascorbic acid, and diketogulonic acid were determined in liver, kidney, and urine, while only total ascorbic acid was determined in blood, after 14 days. Activities of ascorbic acid-synthesizing enzymes D-glucurono-δ-lactone hydrolase, L-gulono-γ-lactone hydrolase, L-gulono-γ-lactone oxidase were estimated in liver. The activities of degrading enzymes dehydroascorbatase and 2,3-diketoaldonate decarboxylase were studied in liver and kidney. In hyperthyroid rats there was a significant decrease in the ascorbic acid level and dehydroascorbic acid content of liver, and urine. On the other hand there was marked increase in the diketogulonic acid of liver and kidney. The content of ascorbic acid and dehydroascorbic acid in urine of hyperthyroid rats decreased significantly, while there was an increase in diketogulonic acid content. There was a slight decrease in the total ascorbic acid in blood. A significant decrease was found only in activities of L-gulono-γ-lactone oxidase, while no change in the activities of D-glucurono-δ-lactone hydrolase and L-gulono-γ-lactone hydrolase occurred. An appreciable increase in the activity of dehydroascorbate, with unchanged activity of 2,3-diketoaldonate decarboxylase, was observed in liver and kidney.

It has been shown by earlier workers that certain hormones play an important role in ascorbic acid metabolism. Synthesis of ascorbic acid in liver is known to diminish in hypophysectomized rats (1), and administration of growth hormones to increase the activity of L-gulono-γ-lactone hydrolase (2). Recently it has been shown in our laboratory that adrenalectomy causes a decrease in the activities of ascorbic acid-synthesizing enzymes such as D-glucurono-δ-lactone hydrolase and L-gulono-γ-lactone oxidase with concurrent increase in the activity of ascorbic acid-degrading enzymes (3-4).

Various studies have indicated that ascorbic acid is associated with the metabolism of thyroid. This vitamin has been shown to accelerate the metabolism
of tyrosine, the precursor of thyroid hormones (5). Several workers have reported the effect of hypothyroidism or hyperthyroidism on the ascorbic acid status in animals. Most of them have, however, noticed in hyperthyroid animals either a decreased (6–7) or an increased (8–9) ascorbic acid content in blood or tissues. The metabolic pattern has, however, remained uncertain. The action of thyroid hormones on the activities of synthesizing and degrading enzymes of ascorbic acid has not been studied.

It was felt necessary, therefore, to examine the effect of hyperthyroidism on ascorbic acid, dehydroascorbic acid and diketogulonic acid contents of liver, kidney, blood, and urine and also the metabolism of ascorbic acid in rat liver and kidney.

MATERIALS AND METHODS

D-Glucurono-δ-lactone, and L-gulono-γ-lactone were obtained from Sigma Chemicals Co. Thyroid tablets were obtained from BDH, 2:3 diketogulonate was prepared by the method of KAGAWA (10).

Male albino rats (100–125 g body wt.) were fed on stock laboratory diet (11) and divided into two groups. The experimental group received 1% thyroid powder in laboratory stock diet (12) for two weeks. A pair fed normal group was kept as control. Rats of both groups were sacrificed at the end of two weeks by decapitation, and blood was collected in oxalated tubes for total ascorbic acid estimation. Liver and kidney were removed, rinsed in ice-cold water and blotted dry. Part of the tissue was homogenized in 9 volumes of isotonic sucrose. The homogenate was centrifuged at 10,000×g for 20 min to obtain tissue extract free of heavy particles. The supernate thus obtained was centrifuged at 100,000×g for 1 hr to yield microsomes and soluble fraction. And the following estimations were carried out.

Ascorbic acid, dehydroascorbic acid, and diketogulonic acid. These were estimated in liver, kidney and urine (24-hr urine samples collected in 10% oxalic acid) by the method of Roe et al. (13).

D-Glucurono-δ-lactone hydrolase (EC 3.1.1.19). The activity was estimated by the modified method of Salomon and Stubbs (1). The incubation mixture consisted of the following: microsomes equivalent to 8.75 mg liver, 0.04 M NaHCO₃, 0.04 M D-glucurono-δ-lactone, (final volume 2 ml), pH 7.6 25°C, 95% N₂–5% CO₂. Activity was determined manometrically by CO₂ evolution for 1 hr. The specific activity had been defined as the evolution of µliters of CO₂ per mg protein at 30-min intervals under the assay conditions.

L-Gulono-γ-lactone hydrolase (EC 3.1.1.18). The activity was estimated by the modified method of Salomon and Stubbs (1). The incubation mixture consisted of the following: soluble fraction equivalent to 3.75 mg liver, 0.04 M NaHCO₃, 0.07 M L-gulono-γ-lactone, (final volume 1 ml), pH 7.6, 25°C, 95%
N₂–5% CO₂. Activity was determined manometrically by CO₂ evolution for 1 hr. The specific activity had been defined as the evolution of μliters of CO₂ per mg protein at 30-min intervals under the assay conditions.

L-Gulono-γ-lactone oxidase (L-gulono-γ-lactone oxidoreductase EC 1.1.3.8). The activity was estimated by the modified method of SALOMON and STUBBS (14). The incubation mixture consisted of the following: microsomes suspended in 0.05 M Tris (hydroxymethyl) aminomethane-HCl buffer, pH 7.6, equivalent to 120 mg liver; 0.00042 M L-gulono-γ-lactone, 0.02 M glutathione adjusted to pH 7.6 with NaHCO₃ (all concentrations final), total volume 2.0 ml, were incubated for 1 hr under O₂ at 25°C. After 1 hr, 1 ml of 10% trichloroacetic acid (TCA) was added to stop the reaction, and the activity was measured by ascorbate production (13). The specific activity had been defined as μmoles of ascorbic acid formed per mg protein per hr under the assay conditions.

Dehydroascorbatase. The activity was estimated by the method of KAGAWA et al. (15). The incubation mixture consisted of the following: soluble fraction equivalent to 100 mg of tissue, 10 μmoles of dehydroascorbate (freshly prepared by bromine oxidation of ascorbate). 0.3 μmoles of glutathione in Tris-malate buffer (200 μmoles) pH 6.8 (final volume 3.0 ml), 37°C. The reaction was stopped after 5 min of incubation by 1.0 ml of 20% metaphosphoric acid +2% SnCl₂, and the remaining dehydroascorbic acid was rapidly reduced with H₂S. The specific activity had been defined as μmoles of diketogulonate formed per mg of protein per 5 min under the assay conditions.

2, 3-Diketoaldonate decarboxylase. The activity was estimated by the method of KAGAWA (10). The incubation mixture consisted of the following: soluble fraction equivalent to 100 mg of the tissue, 0.00025 M diketogulonate, 0.025 M phosphate buffer pH 6.8 (final volume 2.5 ml), 37°C, N₂. The enzyme activity was determined manometrically by CO₂ evolution for 1 hr. The specific activity had been defined as μliters of CO₂ evolved/mg protein at 30-min intervals under the assay conditions.

Protein. Protein was determined by the method of LOWRY et al. (16) using bovine serum albumin as the standard.

RESULTS

It is evident from Table 1 that the thyroid-fed rats significantly lost body wt. (P < 0.001). There were slight decreases in the contents of ascorbic acid (reduced) and dehydroascorbic acid of liver and kidney of rats following thyroid feeding for two weeks. However, thyroid caused a very significant increase (P < 0.001) in diketogulonic acid in both tissues. The total ascorbic acid content of the blood also showed a slight decrease. The metabolic pattern of ascorbic acid in urine showed a marked increase (P < 0.001) in the diketogulonic acid with appreciable changes in the contents of both ascorbic acid (reduced) and dehydro-ascorbic acid (P < 0.01).
| Group          | Body weight | Liver | Kidney | Urine | Blood |
|---------------|-------------|-------|--------|-------|-------|
|               | Initial     | Sacrifice | AA (mg/100 g fresh) | DHA (mg/100 g fresh) | DKA (mg/100 g fresh) |
| Normal (control) | 105         | 130     | 28.1   | 5.6   | 2.6   |
|               | ± 8         | ± 7     | ± 0.92 | ± 0.12| ± 0.08| ± 1.81|
| Hyperthyroid   | 104         | 80a     | 19.2b  | 3.3c  | 9.8a  | 14.7 |
|               | ± 7         | ± 5     | ± 0.71 | ± 0.25| ± 0.90| ± 1.01|

Values are expressed as mean ± SEM of six rats.

- P < 0.001 as compared to control using student’s t test.
- P < 0.01 as compared to control using student’s t test.
- P < 0.05 as compared to control using student’s t test.
Table 2. Effect of hyperthyroidism on the activities of some ascorbic acid-synthesizing enzymes in rat liver.

Values are expressed as mean ± SEM of six rats. Respective enzyme activities are denoted in text.

| Group            | D-Glucurono-δ-lactone hydrolase | L-Gulono-γ-lactone hydrolase | L-Gulono-γ-lactone oxidase |
|------------------|---------------------------------|------------------------------|---------------------------|
|                  | Specific activity               |                              |                           |
| Normal (control) | 965 ± 30                        | 1376 ± 34                    | 1.26 ± 0.018              |
| Hyperthyroid     | 960 ± 45                        | 1360 ± 21                    | 1.21 ± 0.010             |

* P < 0.02 as compared to normal using student’s t test.

Table 3. Effect of hyperthyroidism on the activities of ascorbic acid-degrading enzymes in liver and kidney of rats.

Values are expressed as mean ± SEM of six rats. Respective enzyme activities are denoted in text.

| Group            | Liver                              | Kidney                             |
|------------------|------------------------------------|------------------------------------|
|                  | Dehydroascorbate                    | 2,3-Diketoaldonate decarboxylase    | Dehydroascorbate            | 2,3-Diketoaldonate decarboxylase |
|                  | Specific activity                   | Specific activity                   |                            |                                    |
| Normal (control) | 0.108 ± 0.013                      | 12.50 ± 0.64                      | 0.053 ± 0.008              | 5.92 ± 0.15                       |
| Hyperthyroid     | 0.264 ± 0.019*                     | 12.63 ± 0.63                      | 0.094 ± 0.010b             | 5.5 ± 0.080                       |

* P < 0.001 as compared to control using student’s t test.

There were no significant alterations in the activities of ascorbic acid synthesizing enzymes such as D-glucurono-δ-lactone hydrolase and L-gulono-γ-lactone hydrolase, while a slight decrease in the activity of L-gulono-γ-lactone oxidase (P < 0.02) was noted in hyperthyroid rats (Table 2). It is, however, interesting to note that there was a marked increase (P < 0.001) in the activity of the degrading enzyme dehydroascorbate with no change in the activity of diketoaldonate decarboxylase in liver and kidney of hyperthyroid rats (Table 3).

DISCUSSION

The alterations in the content of tissue ascorbic acid in hyperthyroid rats have been observed by many workers. Although a few earlier workers (8, 9) reported an increase in the tissue ascorbic acid content, the majority (6, 7, 17, 18) noted the opposite result. Our results indicate that hyperthyroidism in rats caused significant decrease in the urinary and tissue ascorbic acid content. In addition, the present communication also shows a tremendous increase in diketoalulonic acid with significant decrease in dehydroascorbic acid content of liver, kidney and urine in hyperthyroid rats. These observations of the ascorbic acid meta-
bolites indicate a decreased synthesis with an increased degradation of ascorbic acid in hyperthyroid rats.

Although growth hormone and corticotropin (2, 3) have been shown to affect both ascorbic acid synthesis and degradation, the role of other hormones on ascorbic acid synthesis and degradation is still obscure. It is evident from our studies that feeding rats with thyroid caused a significant alteration in the ascorbic acid degradation of liver and kidney. There is marked increase in the activity of dehydroascrobatase both in liver and kidney. The activity of the other degrading enzyme, 2,3-diketoaldonate decarboxylase, was, however, found to be unchanged. This explains the unusual increase in the tissue and urinary diketogulonic acid with consequent decrease in the urinary dehydroascorbic acid and ascorbic acid. This result thus also confirms the earlier suggestion of Scoz and Cataneo (17) that thyroxine in large doses causes stimulation of catabolic process of ascorbic acid.

It is also evident from our studies that with the exception of L-gulono-γ-lactone oxidase, the activities of ascorbic acid synthesizing enzymes (hepatic) do not change in hyperthyroid rats. There is thus very little change in the ascorbic acid synthesis in hyperthyroidism.

Thyroid-fed rats show an increased level of ketone bodies (acetoacetate) in liver (19). It has been shown that accumulation of ketone bodies in liver decreases the synthesis and increases the degradation of ascorbic acid in liver (20). Hence the thyroid action on the ascorbic acid metabolism can be thought to be mediated through the formation of ketone bodies. However, other possible explanations for the action of thyroid on ascorbic acid metabolism still remain to be investigated.

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