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# SEX VARIATION IN ASCORBIC ACID CATABOLISM

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**Abstract:** Male and female albino rats of same age and body weight were pair fed with laboratory stock diet and ascorbic acid, dehydroascorbic acid and diketogulonic acid were determined in the liver and urine, while in blood only ascorbic acid was estimated . Male rats had concentration higher of ascorbic acid in liver and urine as compared with females, while there were no significant variations in the contents of dehydroascorbic acid and diketogulonic acid. Hepatic and renal 2, 3-diketoaldonate decarboxylase, and hepatic dehydroascorbatase were also found to be significantly higher in male rats. Similar sex variations were also observed in ascorbic acid cataboism in guinea pigs without any differences in urinary ascorbic acid contents.

**Key words:** ascorbic acid metabolites, ascorbic acid degrading enzymes, male female rats, sex variation

## INTRODUCTION

Marked sex difference in the ascorbic acid contents of certain tissues has been reported earlier (1, 2). Higher enzyme activities of ascorbic acid biosynthesis in male as compared to female rats have also been shown (3). However, investigations to study any sex variations on the mortality of guinea pigs under ascorbic acid deficiency (4), as well as other stress conditions (5, 6), have yielded contradictory results. It was therefore felt necessary to study the effects of sex variation on the ascorbic acid catabolism in rats and guinea pigs, so as to assess the ascorbic acid status, and further to resolve the contradictory results observed on guinea pig mortality.

## METHODS

*Materials :* Reduced glutathione, bovine serum albumin, maleate and tris were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A. Dehydroascorbic acid and diketogulonic acid were prepared by the methods of Kagawa et al (7) and Kagawa (8) respectively. All other reagents and chemicals used were of analytical grade.

Albino rats and guinea pigs of either sex, and of similar ages and weights were pair fed on laboratory stock diet (9) and diet prescribed by Nandi et al (10), respectively. At the end of two weeks, all animals were sacrificed by decapitation and blood collected in heparinized tubes. Total ascorbic acid in blood and ascorbic acid and its metabolites in urine (collected in 10% oxalic acid solution, one day prior to sacrifice) were estimated by the method of Roe (11).

Liver and kidney were removed, rinsed in ice-cold water and blotted dry. A portion of these tissues were homogenized in 9 vol isotonic sucrose and centrifuged differentially (12) to obtain the soluble supernatant fraction. The activities of dehydroascorbatase and 2, 3–diketoaldonate decarboxylase were assayed as described elsewhere (13).

The other portions of the tissues were taken in 0.5% $SnCl_2$ – 5% m-phosphoric acid solution, homogenized and processed for the assay of ascorbic acid, dehydroascorbic acid, and diketogulonic acid by the method of Roe (11).

Protein was estimated by the method of Lowry et al (14), using bovine serum albumin as standard.

## RESULTS

The effects of sex variations on the contents of ascorbic acid and its metabolites in liver and urine of rats are presented in Table I. It is evident that the hepatic and urinary contents of ascorbic acid are significantly

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TABLE I: Ascorbic acid (AA), Dehydroascorbic Acid (DHA) and Diketogulonic Acid (DKA) Contents of Liver, Urine and Blood in male and female rats.

| Sex | Liver | | | Urine | | | Blood |
|---|---|---|---|---|---|---|---|
| | AA | DHA | DKA | AA | DHA | DKA | Total AA |
| | (mg/100 g fresh tissue) | | | (mg/dl) | | | (mg/dl) |
| Male Rats | 29.83 ±1.80 | 4.7 ±0.21 | 1.90 ±0.19 | 24.675 ±1.12 | 11.40 ±1.21 | 5.16 ±0.08 | 1.368 ±0.038 |
| Female Rats | 19.34** ±0.92 | 4.17 ±0.11 | 1.60 ±0.09 | 12.60 ±2.25 | 8.77 ±1.50 | 5.10 ±0.03 | 1.140* ±0.035 |

Values expressed are mean ± SEM of six rats; * P < 0.01 as compared to male rats; **P < 0.001 as compared to male rats

higher in males as compared with females (P < 0.001). However, contents of dehydroascorbic acid and diketogulonic acid in liver and urine are unaffected by sex variation. Total ascorbic acid in blood is also found to be higher in males than in females (P < 0.01). A similar pattern in catabolic profile of ascorbic acid is evident in the liver and kidney of guinea pigs, as recorded in Table II. However, urinary ascorbic acid contents in guinea pigs was fond to be free from sex variation.

The results in Table III & IV indicate that the ascorbic acid catabolic profile is almost similar in both rats and guinea pigs. In either case, males are found to possess higher activities of hepatic dehydroascorbatase and 2, 3-diketoaldonate decarboxylase, as compared with females (P< 0.02). Although the activity of renal 2, 3–diketoaldonate decarboxylase is found to be higher in males, there is no significant sex variation in the activity of renal dehydroascorbatase.

TABLE II: Ascorbic Acid (AA), Dehydroascorbic Acid (DHA) & Diketogulonic Acid (DKA) Contents of liver, kidney and urine of male and female guinea pigs.

| Sex | Liver | | | Kidney | | | Urine |
|---|---|---|---|---|---|---|---|
| | AA | DHA | DKA | AA | DHA | DKA | Total AA |
| | (mg/100 g fresh tissue) | | | (mg/100 g fresh tissue) | | | (mg/dl) |
| Male guinea pigs | 6.0 ±0.31 | 5.3 ±0.26 | 4.0 ±0.22 | 3.6 ±0.20 | 2.0 ±0.18 | 2.3 ±.0.20 | 21.0 ±1.75 |
| Female guinea pigs | 4.0* ±0.20 | 4.5 ±0.21 | 3.3 ±0.19 | 2.3** ±0.19 | 1.54 ±0.09 | 2.3 ±0.19 | 19.5 ±1.12 |

Values expressed are mean ± SEM of five animals; *P <0.01 as compared to male animals; **P<0.02 as compared to male animals

TABLE III: Activities of ascorbic acid degrading enzymes in liver and kidney of male and female rats.

| | Liver | | Kidney | |
|---|---|---|---|---|
| | Dehydroascorbatase | 2,3-diketoaldonate decarboxylase | Dehydroascorbatase | 2,3–diketoaldonate decarboxylase |
| | ← (specific activity) @ → | | | |
| Male rats | 0.109 ± 0.015 | 12.74 ± 0.69 | 0.058 ± 0.008 | 5.5 ± 0.090 |
| Female rats | 0.045 ± 0.012* | 8.56 ± 0.99 * | 0.041 ± 0.004 | 5.0 ± 0.074** |

Values expressed are mean ± SEM of six rats; *P<0.01 as compared to male rats ; **P< 0.02 as compared to male rats;

@ Units of specific activity were the same as described earlier (13).

TABLE IV: Activities of ascorbic acid degrading enzymes in liver and kidney of male and female Guinea pigs.

| | *Liver* | | *Kidney* | |
|---|---|---|---|---|
| | *Dehydroascorbatase* | *2,3-diketoaldonate decarboxylase* | *Dehydroascorbatase* | *2,3–diketoaldonate decarboxylase* |
| | ←——*(specific activity)* @ ——→ | | | |
| Male guinea pigs | 0.198 ± 0.013 | 11.400 ± 0.56 | 0.148 ±0.007 | 5.540 ± 0.09 |
| Female guinea pigs | 0.118 ± 0.010 ** | 8.740 ± 0.75 ** | 0.156 ± 0.011 | 2.720 ± 0.081* |

Values expressed are mean ± SEM of five animals; *P< 0.001 as compared to male animals; **P <0.01 as compared to male animals; @ Units of specific activity were the same as described earlier (13).

## DISCUSSION

The present findings confirm the earlier observations that male rats have higher concentrations of ascorbic acid in the blood, tissues and urine as compared with females (1,2,15,16). It seems probable that such higher ascorbic acid pool in male rats may be due to anabolic action of androgens. Stubbs and McKernan (3) had earlier demonstrated higher activities of ascorbic acid biosynthesizing enzymes in male rats and later on Stubbs et al (17) attributed this increase to androgens. Recently our studies showed an inhibitary action of estrogen on the hepatic microsoma enzyme L-gulono-r-lactone oxidase (18).

Effect of sex variation on the contents of ascorbic acid metabolites and the enzymes involved in its catabolism have been observed in the present study. Males possessed comparatively stimulated activities of the enzymes involved in ascorbic acid degradation, with liver showing maximum catabolic ability. However, the metabolites of ascorbic acid were not affected by sex variation. Though this latter finding did not correlate well with the stimulated activities of ascorbic acid catabolizing enzymes, it could be the result of the ascorbic acid biogenic capability of males exceeding its catabolic ability. Thus increased ascorbic acid pool in males probably caused no apparent change in the contents of its metabolites inspite of stimulated catabolism.

The relatively higher catabolism of ascorbic acid in male animals is not clearly understood. It may be that some specific factor (s) regulating ascorbic acid catabolism vary in the two sexes. Nathani and Nath (19), had demonstrated the role of mineralocorticoids in controlling the activities of ascorbic acid degrading enzymes, and its insufficiency was related to elevated catabolism (19-21). The earlier observation of Bankova et al (22) indicated relative decrease in corticoids of blood and urine in male rats. Also, progesterone, the female sex hormone was found to be an effective producer of aldosterone (23). Thus the relatively lower status of corticoids (especially mineralocorticoids), in male animals may have contributed towards elevated ascorbic acid catabolism.

Three different groups of workers have earlier recorded contradictory results on the effect of sex variations on guinea pig mortality under ascorbic acid deficiency (4-6). As against the observation of Barnes et al (5) and Jones et al (6), Odumosu and Wilson (4) demonstrated lesser mortality of female guinea pigs. However, these studies on sex variations were performed on guinea pigs under scorbutic condition or such nutritional stress conditions as low or high cholesterol imposition in diet. In order to correctly assess the relationship of sex dependent mortality with ascorbic acid status, it is necessary to evaluate the ascorbic acid catabolism in normal guinea pigs of either sex. The present study demonstrates that in guinea pigs, the effect of sex variation on the catabolic profile of ascorbic acid is almost similar to that observed in rats. This finding bears important implication. It may be that in guinea pigs (where hepatic biosynthesis of ascorbic acid is supposed to be negligible) males possessing higher catabolic activity could have higher requirement of ascorbic acid and thus be more prone to scrobutic condition. This is supported by the observations of

Odumosu and Wilson (4). In view of subsequent contradiction (5,6), to the findings of Odumosu and Wilson (4), and the present demonstration of higher tissue ascorbic acid pool of male guinea pigs, with no sex variation in the urinary ascorbic acid, despite higher degration, indicates the possibility of an alternative biogenic site for ascorbic acid in the male species.

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