Effect of Castration on the Metabolism of L-Ascorbic Acid in Rat Prostate

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Summary

An appreciable decrease in the contents of prostatic ascorbic acid and dehydroascorbic acid along with an increase in diketogulonic acid was seen in rats ten days after castration. Castration caused a decrease in the activities of such biosynthetic enzymes as L-gulono-γ-lactone oxidase and D-glucuronolactone-δ-hydrolase with no significant alteration in the activity of L-gulono-γ-lactone hydrolase in the rat prostate. The activity of dehydroascorbatase, one of the degrading enzymes was, however, found to be elevated in castration. The implication of these results has been discussed.

Key Words castration, ascorbic acid, rat prostate

Various studies have implicated testicular secretions as being involved in ascorbic acid metabolism in animals. Tissue distribution and urinary excretion of ascorbic acid in rats have been demonstrated to be profoundly influenced by castration (1, 2). Hepatic biosynthesis of ascorbic acid has been shown to become disordered in rats under this condition (3–5). Also an earlier report from our laboratory showed elevation in the activities of the enzymes involved in ascorbic acid catabolism in both liver and kidney of castrated rats (6).

We recently demonstrated active ascorbic acid metabolism in animal prostate (7–9). In view of the reported dependence of sex accessory glands on androgens (10) and the implication of male sex hormone involvement in ascorbic acid metabolism in liver and kidney of animals, it was thought necessary to evaluate the effects of castration on the content of ascorbic acid and its metabolism in rat prostate.

MATERIALS AND METHODS

d-Glucurono-δ-lactone, d-gulono-γ-lactone, reduced GSH, ascorbic acid and bovine serum albumin were procured from Sigma Chemical Company, St. Louis, Missouri, U.S.A. L-Gulono-γ-lactone was obtained from INC Pharmaceuticals, Cleveland, Ohio, U.S.A. Dehydroascorbic acid and diketogulonic acid were prepared by the method of Kagawa et al. (11) and Kagawa (12), respectively. All
other chemicals used were of analytical grade. Forty-eight male albino rats (200–250 g) maintained on a stock laboratory diet (13), were divided into two groups. The animals from group II were bilaterally castrated, while those from group I acted as sham operated controls. The initial and final body weights of the two groups were recorded. Ten days after castration, the rats were killed by decapitation. Prostates were removed, weighed and blotted dry. The experimental design was such that the ascorbic acid metabolites, \( L \)-gulono-\( \gamma \)-lactone oxidase, hydrolases and catabolizing enzymes, were estimated in separate subgroups of six animals each in both sham operated control and castrated groups, since the amount of prostate was inadequate for all the estimations together.

**Enzyme assay.** A 10% homogenate of prostate was prepared in an isotonic sucrose solution and centrifuged at 10,000 g for 20 min, and the supernatant thus obtained was further centrifuged at 100,000 g for 1 h to yield soluble and microsomal fractions. The following enzymes were then estimated.

\( L \)-Gulono-\( \gamma \)-lactone oxidase [EC 1.1.3.8] was assayed by a modification of the method of Salomon and Stubbs (14). The incubation mixture consisting of microsomes suspended in 0.05 mol Tris-HCl buffer (pH 8.2) equivalent to 120 mg prostate, 0.00042 mol of \( L \)-gulono-\( \gamma \)-lactone, and 0.02 mol of GSH adjusted to pH 8.2 with NaHCO\(_3\) (all concentrations final, total volume 2 ml) was incubated at 25°C for 1 h. The reaction was stopped by addition of 1 ml of 10% TCA. The activity was measured in terms of ascorbate production (15) and the specific activity was expressed as \( \mu \)mol of ascorbic acid formed/mg protein/h under the assay conditions.

\( D \)-Glucurono-\( \delta \)-lactone hydrolase [EC 3.1.1.19] and \( L \)-gulono-\( \gamma \)-lactone hydrolase [EC 3.1.1.18] were assayed by the method of Isherwood et al. (16). The incubation mixture contained 9 mg of \( D \)-glucurono-\( \delta \)-lactone or \( L \)-gulono-\( \gamma \)-lactone, 0.1 mol of phosphate buffer (pH 7.4), 0.05 mol of manganese chloride, 0.05 mol of cysteine hydrochloride and an enzyme source equivalent to 100 mg of prostate (microsomal fraction for \( D \)-glucurono-\( \delta \)-lactone hydrolase and soluble fraction for \( L \)-gulono-\( \gamma \)-lactone hydrolase). Final volume was 5 ml at 37°C. The amount of lactone hydrolyzed per min was estimated by the method of Hestrin (17). One unit of the enzyme activity was defined as an amount of the enzyme hydrolyzing 1 \( \mu \)mol of \( D \)-glucurono-\( \delta \)-lactone or \( L \)-gulono-\( \gamma \)-lactone per min under the assay conditions.

The activity of dehydroascorbatase was estimated by the method of Kagawa et al. (11). The incubation mixture consisted of a soluble fraction equivalent to 100 mg of prostate, 10 \( \mu \)mol of dehydroascorbate and 0.3 \( \mu \)mol of GSH in Tris-maleate buffer (200 \( \mu \)mol, pH 6.8, final volume 3 ml), at 37°C. The reaction was stopped after 5 min of incubation by the addition of 1 ml of 20% metaphosphoric acid in 2% SnCl\(_2\) and the remaining dehydroascorbic acid was reduced with H\(_2\)S. Specific activity was defined as \( \mu \)mol of diketogulonate formed/mg protein under the assay conditions.

The activity of diketogulonate decarboxylase was estimated by the method of Kagawa (12). The incubation medium contained a soluble fraction equivalent to

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100 mg prostate, 0.00025 mol of diketogulonate, and 0.025 mol of phosphate buffer pH 6.8 (final vol 2.5 ml) at 37°C. The reaction was stopped after 30 min by TLC and the remaining diketogulonate was measured. The activity was defined as µmol of diketogulonate utilized/mg of protein under the assay conditions.

Other assay. Ascorbic acid, dehydroascorbic acid and diketogulonic acid were estimated by the method of Roe et al. (18). One percent tissue extract was prepared in a solution of 10% SnCl₂: 5% HPO₃ mixed at the ratio of 1:19.

i) Determination of diketogulonic acid. H₂S was passed into the original SnCl₂-HPO₃ filtrate for 15 min. Powdered thiourea was added to it and CO₂ was bubbled into the filtrate for 5 min. Four ml of the aliquot and 1 ml of 2,4-dinitrophenylhydrazine were kept at 37°C for 3 h. The reagent was added to the blank after the incubation but not prior to it. When the incubation period was over, the tubes were placed in an ice bath and 5 ml of 85% H₂SO₄ was added dropwise. The tubes were well shaken and the color developed was measured at 540 nm.

ii) Determination of dehydroascorbic and diketogulonic acid. To 4 ml of the original SnCl₂-HPO₃ filtrate, 1 ml of 2% 2,4-dinitrophenylhydrazine was added and the resultant was kept at 37°C for 3 h. Five ml of 85% H₂SO₄ was added dropwise and readings were taken at 540 nm.

iii) Determination of dehydroascorbic, diketogulonic and ascorbic acid. A H₂S-saturated SnCl₂: HPO₃ solution was filtered. H₂S was removed by aeration through the filtrate. Sufficient bromine was added, the excess being removed by aeration. Powdered thiourea was added to the filtrate. Four ml of the aliquot was pipetted into the test tube, and treated with dinitrophenylhydrazine and H₂SO₄ as described above.

The value of the individual substance was calculated by deducting (a) iii–ii and (b) ii–i.

Protein was measured by the method of Lowry et al. (19), using bovine serum albumin as standard.

RESULTS

It could be demonstrated from Table 1 that castration caused a reduction in the body and prostatic weights (p<0.05). There were significant reductions in the contents of prostatic ascorbic acid (p<0.01) and dehydroascorbic acid (p<0.05), but prostatic diketogulonic acid was found to be significantly elevated (p<0.05), following castration.

The activities of such biosynthesizing enzymes as L-gulono-δ-lactone oxidase (p<0.001) and D-glucurono-δ-lactone hydrolase (p<0.05) were found to be significantly diminished in the prostate of the castrated rats (Table 2). However, the activity of prostatic L-gulono-δ-lactone hydrolase was found to be unaffected by castration.

It is demonstrated in Table 3 that the activity of diketogulonate decarboxylase remained practically unaltered while castration caused an elevation in the activity of
Table 1. Effect of castration on body wt., prostate wt., and contents of prostatic ascorbic acid and its metabolites in male albino rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Gain in body wt. (g)</th>
<th>Wt. of prostate (g)</th>
<th>Ascorbic acid (mg/100 g of tissue)</th>
<th>Dehydroascorbic acid (mg/100 g of tissue)</th>
<th>Diketogulonic acid (mg/100 g of tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Sham operated controls</td>
<td>58 ± 4.8</td>
<td>0.374 ± 0.01</td>
<td>6.84 ± 0.41</td>
<td>2.44 ± 0.32</td>
<td>1.51 ± 0.18</td>
</tr>
<tr>
<td>II Castrated</td>
<td>-43.35 ± 2.17*</td>
<td>0.218 ± 0.03*</td>
<td>4.55 ± 0.34**</td>
<td>1.48 ± 0.22*</td>
<td>2.1 ± 0.17</td>
</tr>
</tbody>
</table>

Values expressed as ± SEM of six animals. * p < 0.05. ** p < 0.01.

Table 2. Effect of castration on prostatic ascorbic acid biosynthesizing enzymes in male albino rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>1-Gulono-γ-lactone oxidase (µmol ascorbic acid/mg of protein)</th>
<th>D-Glucurono-δ-lactone hydrolase (% lactone hydrolyzed/min)</th>
<th>L-Gulono-γ-lactone hydrolase</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Sham operated controls</td>
<td>0.332 ± 0.02</td>
<td>15.57 ± 0.30</td>
<td>16.08 ± 0.28</td>
</tr>
<tr>
<td>II Castrated</td>
<td>0.078 ± 0.02**</td>
<td>13.10 ± 0.45*</td>
<td>15.97 ± 0.34</td>
</tr>
</tbody>
</table>

Values are expressed as ± SEM of six animals. * p < 0.05. ** p < 0.001.

Table 3. Effect of castration on prostatic ascorbic acid catabolizing enzymes in male albino rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Dehydroascorbatase (µmol diketogulonate formed/mg protein)</th>
<th>Diketogulonate decarboxylase (µmol diketogulonate utilized/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I Sham operated controls</td>
<td>0.46 ± 0.03</td>
<td>5.90 ± 0.25</td>
</tr>
<tr>
<td>II Castrated</td>
<td>0.61 ± 0.03*</td>
<td>6.21 ± 0.13</td>
</tr>
</tbody>
</table>

Values expressed as ± SEM of six animals. * p < 0.01.

prostatic dehydroascorbatase (p < 0.01).

DISCUSSION

Salomon and Stubbs (14), and Jacobson and Havens (20) demonstrated a...
considerable decrease in the content of hepatic ascorbic acid following castration in rats and capons, respectively. Khandwekar et al. (6) later showed a decrease not only in the content of ascorbic acid but also in dehydroascorbic acid along with a concomitant increase in the content of diketogulonic acid in the liver and kidney of the castrated rat. Chinoy et al. (21) demonstrated for the first time a decrease in the ascorbic acid content of steroidogenic and ascorbic acid-storage tissues by histochemical methods in gonadectomized rats. Our present demonstration of derangement in the status of ascorbic acid and its metabolites in rat prostate following castration completely mimicked the situation earlier found in the liver and kidney of castrated rats.

While making an extensive probe into hepatic biosynthesis of ascorbic acid as well as ascorbic acid catabolism in the liver and kidney of castrated rats, Khandwekar et al. (4, 6) found a significant decrease in the activity of such microsomal enzymes of ascorbic acid biosynthesis as D-glucurono-δ-lactone hydrolase and L-gulono-γ-lactone oxidase along with an elevation in the activity of dehydroascorbatase, a catabolizing enzyme. Thus, they satisfactorily accounted for the alteration in the status of tissue ascorbic acid and its metabolites by enzymatic studies. Coincident with this finding, the prostate was also found to exhibit similar alterations in the enzymatic profile as described above. It may thus be inferred that testicular insufficiency had a similar influence on ascorbic acid metabolism irrespective of the sites involved.

Salomon and Stubbs (14) as well as Jacobson and Havens (20) showed androgen dependency of the entire biosynthesizing enzyme system of ascorbic acid. Khandwekar et al. (4) failed to confirm this finding. According to them, such microsomal enzymes as L-gulono-γ-lactone oxidase and D-glucurono-δ-lactone hydrolase only were found to be androgen dependent. Our present study of identical enzymatic profile in the prostate of castrated rats as described in liver by Khandwekar et al. (4) conclusively proves that only those enzymes involved in ascorbic acid biosynthesis which are microsomal in nature, are androgen dependent. Although it is difficult to account for the elevated activity of dehydroascorbatase in the prostate of castrated rats, it might be suggested that the effect was discrete in nature. It might be recalled that Khandwekar et al. (6) also showed a similar elevation in hepatic and renal dehydroascorbatase without any alteration in the activity of diketogulonate decarboxylase in castrated rats.

Evidence of gross alteration in prostatic metabolism of ascorbic acid in castrated rats is not only a new finding but also bears important implications regarding the relationship of ascorbic acid to the morphological integrity of the accessory sex glands. It might be remembered that Chinoy et al. (21) demonstrated damage in the morphological and structural integrity of the accessory sex glands following gonadectomy in rats. They also presented evidence that the above lesions paralleled a reduction in the contents of ascorbic acid and that ascorbic acid therapy of the castrated rats caused a significant restoration of ascorbic acid levels to normal. It might thus be possible that the loss in the prostate weight observed here
and the morphological lesions described by Chinoy et al. (21) under testicular insufficiency, were somehow related to the androgen-dependent ascorbic acid biosynthesis in the rat prostate.

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REFERENCES

8) Shah, S., and Nath, N. Biological Synthesis of ascorbic acid in goat prostate (communicated).


