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A Sexual Influence on the Biosynthesis and Storage of L-Ascorbic Acid in Rats.* (32348)

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Previous investigations have demonstrated a marked hypophyseal influence on the biosynthesis of ascorbic acid in rats(5); an influence shown to be mediated by somatotrophic hormone(6). Perusal of some of the older literature reporting concentrations of ascorbate in tissues of male and female rats has given indication of a possible sexual influence as well. Several authors have reported higher concentrations of ascorbate in a few tissues of male rats in comparison with those from females of similar age(2,4,7). Although there has been no uniform agreement as to which tissues show the higher concentrations in males, such data do indicate a possible sexual influence on the biosynthesis and storage of ascorbic acid. To the best of our knowledge no comparison of specific cellular enzymes in the biosynthetic pathway has been made for the two sexes. This paper reports the first analyses of 4 hepatic enzymic activities involved in ascorbic acid synthesis in both males and females, and confirms the sexual difference in tissue ascorbate concentrations for several tissues.

Methods. Male and female rats of the Sprague-Dawley strain between 45 and 65 days of age were used. Animals were decapitated, exsanguinated, and tissue samples removed for analysis. Blood was collected during the exsanguination. Liver was divided into two parts; one part was used for determination of ascorbate concentration, and the other was homogenized and separated into microsomal and cytoplasmic fractions for enzymic assay. Tissues were weighed to the nearest milligram and homogenized in 10 ml of 10% trichloroacetic acid. One milliliter of serum was added to 2 ml of 10% trichloroacetic acid. Following removal of precipitated residue, all supernates were analyzed for total ascorbic acid content by a modification of the method of Lowry *et al*(3).

Enzymic assay conditions, substantially as described previously(6), were slightly modified to give linear initial reaction rates. The

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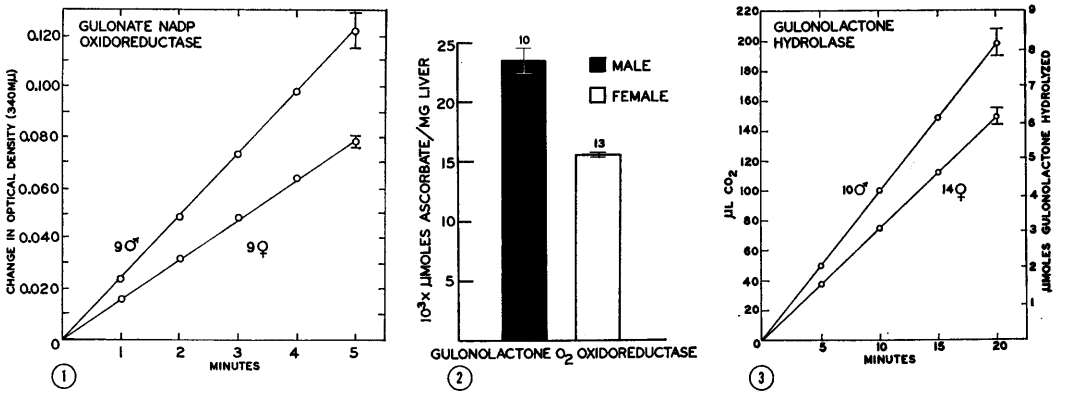

FIG. 1. Enzymatic activity of gulonate NADP oxidoreductase. Reaction rate is indicated by change in optical density (340 mμ) from oxidation of NADPH in reduction of glucuronate to gulonate. Brackets around final points are standard error of mean.

FIG. 2. Enzymatic activity of gulonolactone O_2 oxidoreductase. Reaction rate is indicated by product formation in oxidation of gulonolactone to ascorbic acid. Brackets are standard error of mean.

FIG. 3. Enzymatic activity of gulonolactone hydrolase. Reaction rate is indicated by evolution of CO_2 (left ordinate) following enzymic hydrolysis of gulonolactone to gulonic acid in bicarbonate buffer. At 25°C 24.3 microliters is equivalent to hydrolysis of one micromole of lactone (right ordinate). Brackets around final points are standard error of mean.

two hydrolases, gulonolactone hydrolase (formerly aldonolactonase), and glucuronolactone hydrolase (formerly uronolactonase), were assayed manometrically in Gilmont differential syringe manometers. Evolution of CO_2 following hydrolysis of the appropriate lactone to its free acid in the presence of bicarbonate buffer was taken as a measure of enzymic activity. Gulonate NADP oxidoreductase (formerly TPN-L-hexonate dehydrogenase) was assayed by measurement of $NADPH_2$ oxidation recorded as change in optical density (340 mμ) in a ratio-recording spectrophotometer with the cuvette chamber maintained at 37°C. The activity of gulonolactone O_2 oxidoreductase (formerly gulonolactone oxidase) was determined by analysis for ascorbic acid synthesis from gulonolactone at 37°C under a 100% O_2 atmosphere for 30 minutes. No attempts were made to determine initial reaction rates for this enzyme.

Results and discussion. Fig. 1-4 illustrate the activities of the 4 hepatic enzymes assayed. In each case the activity of enzymes from males was significantly higher than those from females ($p<0.001$). Brackets above and below points for the final values for each assay are the standard error of the mean.

Tissue ascorbate concentrations were also shown to be significantly higher in all of the tissues from males with the exception of adrenals and bone. Absence of a statistically significant difference in the adrenals may have been the results of unnoticed stress which could easily affect the concentration of ascorbic acid in the adrenals. A significant sexual difference in adrenal ascorbate has been recently reported by de Nicola *et al*(1). It is interesting to note that all tissues analyzed have higher concentrations of ascorbic acid than does blood. The sexual differences in tissue ascorbate may either reflect some sexually influenced active transport system in the tissues, or may depend upon a rate of uptake which is directly proportional to the concentration of the vitamin in the blood. If the

TABLE I. Ascorbic Acid in Tissues of Male and Female Rats.*

Tissue	Male	Female	p†
Plasma	1.6 ± .10	1.0 ± .04	.001
Liver	37.8 ± 1.45	24.8 ± 1.09	.001
Lungs	39.8 ± 1.52	31.9 ± 1.26	.005
Kidneys	19.8 ± .47	13.4 ± .25	.001
Spleen	63.0 ± 2.82	53.2 ± 2.18	.020
Muscle	7.3 ± .36	5.3 ± .15	.001
Bone	12.2 ± 1.12	10.9 ± .53	.40 ‡
Adrenals	449.5 ±20.93	385.6 ±28.10	.10 ‡

* Values represent averages of 6-8 samples (mg/100 g tissue ± S.E.M.).

† p values determined from Student's t test.

‡ Differences not statistically significant.

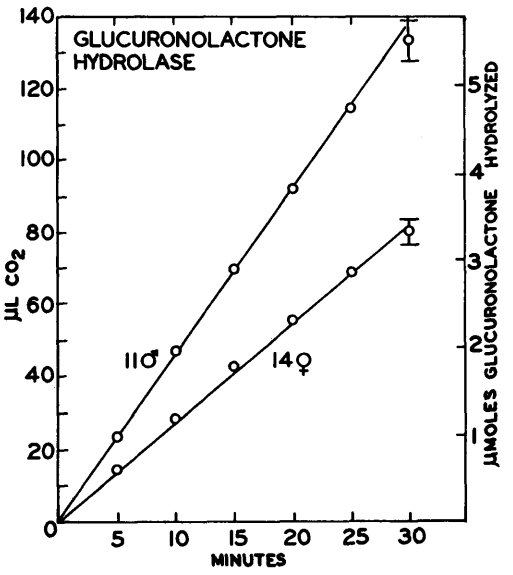

FIG. 4. Enzymatic activity of glucuronolactone hydrolase. Reaction rate is indicated by evolution of CO_2 (left ordinate) following enzymic hydrolysis of glucuronolactone to glucuronic acid in bicarbonate buffer. At 25°C 24.3 microliters is equivalent to hydrolysis of one micromole of lactone (right ordinate). Brackets around final points are standard error of mean.

latter is true, the differences in male and female tissue ascorbate would be direct consequences of the difference in blood ascorbate, which in turn could be the result of a sexual difference in hepatic biosynthesis. The hepatic enzyme differences reported here support this latter hypothesis. That is, the sexual differences in enzymic activities at the subcellular level could be the molecular basis for the differences in ascorbate concentrations found at the tissue level.

Summary. A sexual influence on the enzymes in the biosynthesis of ascorbic acid, and on the tissue concentrations of total ascorbate has been shown by comparisons between male and female rats. Males have significantly higher activities for the 4 hepatic enzymes assayed, and significantly higher ascorbate concentrations in 6 of the 8 tissues analyzed. The higher male enzymic activities at the subcellular level are thought to be the molecular basis for the higher concentrations of ascorbic acid at the tissue level.

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Radiosulfate Space in Humans at High Altitude.* (32349)

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It has long been known for many years that subjects acutely exposed to a high altitude environment lose weight(1,2), and it has been suggested(2) that this phenomenon may bear some relationship to the decrease in plasma volume which occurs under the same circumstances(2-6). However, we are not aware of any investigation concerning the extracellular space during acute exposure to high altitude. Picón-Reátegui(7) has reported a slight increase in the sucrose space in permanent residents of the high Andean region, in spite of the fact that their plasma volume is normal or moderately decreased(8). Data concerning the measurement of the intracellular space using radiosulfate are reported here. The observations have been carried out in sea level residents, before and

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