Ascorbic Acid Biosyntheses After Phenobarbital in Male and Female Rats[1] (37316)

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Previous studies have shown that drugs capable of inducing the microsomal biotransformational enzymes also increase the urinary excretion of ascorbic acid in those species that are capable of synthesizing the vitamin (1–8). The biochemical basis of these two seemingly unrelated induction phenomena is not known.

In 1960, Conney *et al.* (9) noted an increase in ascorbic acid excretion in male rats following phenobarbital treatment and attributed it to an increase in enzymic activity of hepatic UDP-glucose dehydrogenase, an enzyme in the glucuronate pathway. However, they observed decreased activity of gulonate-NADP-oxidoreductase and gulonolactone-O_2-oxidoreductase, two of the four enzymes in the ascorbic acid pathway (Fig. 1). Aarts (10) reported that male rats respond with a greater urinary excretion of ascorbic acid following the drug treatment than females. Stubbs and McKernan (11) reported a sexual difference in the four enzymes in the ascorbic acid pathway, with male rats having greater activity than females. This paper reports the first analyses of the four hepatic enzymic activities in the ascorbate pathway following phenobarbital treatment in rats of both sexes.

Materials and Methods. Male and female rats of the Sprague–Dawley strain between 45 and 52 days of age were used. There were six animals in each experimental and control group. Sodium phenobarbital was injected intraperitoneally for 5 consecutive days in a dosage of 100 mg/kg body weight. Control animals were injected with saline. Animals were decapitated between 9 and 10 AM, exsanguinated, and liver tissue samples of about 4 g were removed for analysis. The liver samples after being weighed to the

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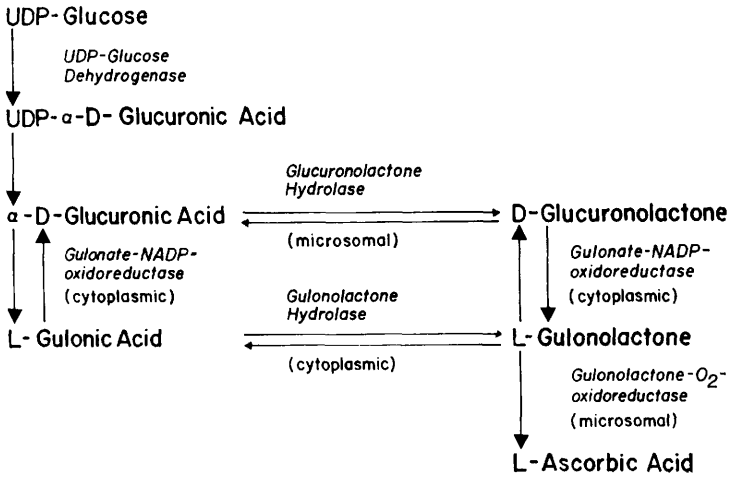

FIG. 1. Ascorbic acid biosynthetic pathway.

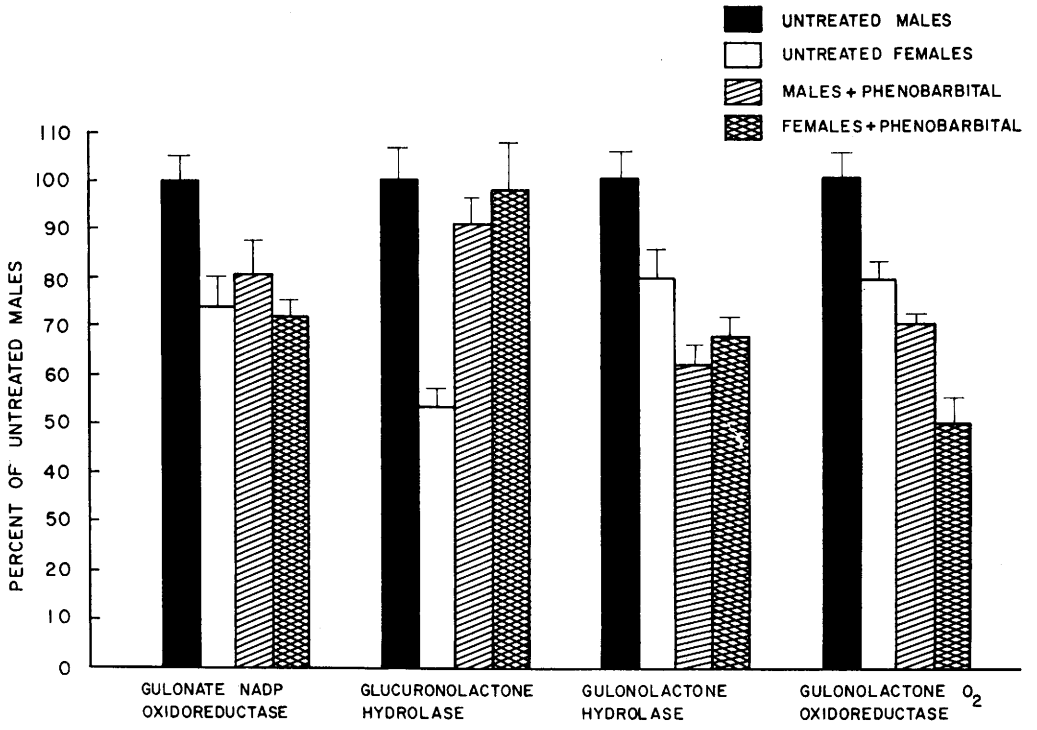

FIG. 2. Relative enzymic activities in males and females as percentage of the values in untreated males. Brackets indicate one standard error of the mean. There were six animals in each group. Enzymatic activity of gulonate-NADP-oxidoreductase is indicated by change in optical density (340 nm) from oxidation of NADPH in reduction of glucuronate to gulonate. (Control male 61.3 ± 3.1 change in optical density × 1000). Enzymatic activity of glucuronolactone hydrolase is indicated by evolution of CO_2 following enzymic hydrolysis of glucuronolactone to glucuronic acid in bicarbonate buffer. (Control males 179 ± 11.6 μl CO_2 in 20 min). Enzymatic activity of gulonolactone hydrolase is indicated by evolution of CO_2 following enzymic hydrolysis of gulonolactone to gulonic acid in bicarbonate buffer. (Control males 203 ± 10.7 μl CO_2 in 20 min.) Enzymatic activity of gulonolactone-O_2-oxidoreductase is indicated by product formation in oxidation of gulonolactone to ascorbic acid. (Control males 3.05 ± 0.16 μmoles of ascorbic acid.)

nearest milligram were homogenized in 0.25 *M* sucrose and separated into microsomal and cytoplasmic fractions by ultracentrifugation (12).

Enzyme assays were performed as described previously (11). Gulonate-NADP-oxidoreductase, a cytoplasmic enzyme, was assayed by measurement of NADP-oxidation as recorded by change in optical density (340 nm) in a ratio-recording spectrophotometer with the cuvette chamber maintained at 37°. The two hydrolases, glucuronolactone hydrolase, a microsomal enzyme, and gulonolactone hydrolase, a cytoplasmic enzyme, 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 enzyme activity. The activity of gulonolactone-O_2-oxidoreductase, a microsomal enzyme, was determined by analysis for ascorbic acid synthesis from gulonolactone at 37° under a 100% O_2 atmosphere for 30 min.

Results and Discussion. Figure 2 presents the activities of the four hepatic enzymes assayed. As previously noted (11), the activity of each of the four enzymes from untreated male rats was significantly higher ($p < .05$) than those from female rats.

In comparison between the activity in males treated with phenobarbital and untreated males, both gulonolactone-O_2-oxidoreductase (microsomal) and gulonolactone hydrolase (cytoplasmic) had significantly lower activity ($p < .001$) after phenobarbital treatment. Glucuronolactone hydrolase (microsomal) and gulonate-NADP-oxidoreductase (cytoplasmic) were found to have slightly lower activity after phenobarbital but the differences were not significant. Conney *et al.* (9) observed lower activity in male rats after phenobarbital in the case of gulonolactone-O_2-oxidoreductase and of gulonate-NADP-oxidoreductase. Our findings thus confirm those of Conney *et al.* (9) in the former case and it is possible that our findings might have also confirmed Conney *et al.* in the case of gulonate-NADP-oxidoreductase if a larger number of animals had been studied.

In females, as in the males, phenobarbital treatment was followed by a significant fall in gulonolactone-O_2-oxidoreductase activity. The corresponding activities of gulonate-NADP-oxidoreductase and gulonolactone hydrolase were essentially unchanged. An interesting finding was an *increase* in activity of glucuronolactone hydrolase, which reached the level of the normally higher, untreated males.

The sexual difference in urinary ascorbate levels following drug treatment (9) most probably is a result of hepatic ascorbate biosynthesis. From our data it is doubtful that the activities of the four enzymes assayed could singly or in combination explain the molecular basis for the increased ascorbate excretion. Furthermore, the decreased or unchanged activities of gulonolactone-O_2-oxidoreductase, gulonolactone hydrolase and gulonate-NADP-oxidoreductase suggest that these enzymes are not rate-limiting in ascorbate biosynthesis. That glucuronolactone hydrolase increased only in females despite the normally lower urinary ascorbate excretion in females compared with males, tends to reinforce the hypothesis that glucuronolactone hydrolase is involved in the minor pathway of ascorbate biosynthesis (13).

Summary. Male and female rats were treated for 5 days with phenobarbital and the four hepatic enzymes in the ascorbic acid pathway were assayed. In males, gulonate NADP-oxidoreductase were found to have decreased enzyme activity while glucuronolactone hydrolase activity remained unchanged. In females, gulonolactone hydrolase and gulonolactone-O_2-oxidoreductase had decreased enzyme activity, gulonate-NADP-oxidoreductase remained unchanged while glucuronolactone hydrolase was found to have increased twofold in activity.

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