The Effects of Age and Sex on Glycolic Acid Metabolism in Rats[1] (35965)

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On a diet free of exogenous glycolic acid, male rats excrete more urinary oxalic acid than females do; a diet containing 2% glycolic acid produces hyperoxaluria in male rats, but not in females (1). In addition, glycolic acid is more toxic to male rats than to females (2). It has been suggested that these findings are related to the higher liver level of glycolic acid oxidase in adult male rats, apparently the result of enzyme induction by testosterone (3, 4).

Glycolic acid oxidase catalyzes the conversion of glycolic acid to glyoxylic acid (5). Several enzymes, among them glycolic acid oxidase, can catalyze the further oxidation of glyoxylic acid to oxalic acid (6, 7). An important alternate fate of the intermediate, glyoxylic acid, is transamination to glycine (8).

If the greater oxalic acid excretion of male rats, compared to females, is the result of higher liver levels of glycolic acid oxidase one might also expect a sex difference in the conversion of glycolic acid to glycine when increased amounts of glyoxylic acid become available for transamination as well as for further oxidation to oxalic acid. If glycolic acid oxidase does not catalyze the rate limiting reaction in the oxidation of glycolate to oxalate, the increased activity of this enzyme may not be a cause of the greater oxalate excretion in male rats compared to females.

This study was done to determine the effects of age and sex on the conversion of glycolic acid to glycine and to oxalic acid in rats *in vivo*.

Methods. Male and female rats of the Wistar strain were weaned at 3 weeks of age and fed the following diet *ad libitum*: (%) cornmeal, 42.1; skim milk, 18.4; linseed meal, 12.0; wheat germ, 7.5; yeast (unirradiated), 7.1; crude casein, 3.8; alfalfa meal, 1.5; iodized NaCl, 0.4; salt mixture of $CaCO_3$ plus trace elements, 0.4; irradiated yeast plus calcium pantothenate, 0.4; Wesson oil, 6.4. Each kilogram of irradiated yeast contained 6 g of calcium pantothenate. Radioactive compounds were obtained from commercial sources and used without further purification. The rats were housed in pairs in metabolism cages so that 24-hr urine collections could be made following intraperitoneal injections of radioactive solutions. Labeled compounds were used in trace amounts, dissolved in 0.5 *M* sodium benzoate. Each animal was injected with 0.5 ml of solution/100 g of body weight. Depending on the expected degree of conversion of the labeled compound to the product, 0.5 to 5.0 μCi/100 g of body weight were injected; the specific activities of the compounds were: glycolic acid, 52; glycine, 15; benzoic acid, 2.25 mCi/mmole.

Radioactive urinary oxalic acid was determined by a carrier technique as previously described (9), and radioactive urinary hippuric acid was determined by a carrier technique described elsewhere (10). All samples were counted in a Packard Tri-Carb liquid scintillation counter.

Results. As the rats matured, the conversion of labeled glycolic acid to urinary hippuric acid was more than doubled in both males and females (Table I). Only part of this increase can be explained on the basis of greater conversion of benzoic acid to hippuric acid, which increased approximately 50% between the ages of 4.5 weeks and 11.5 weeks. The most marked change was seen in the conversion of labeled glycine to hippuric acid

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TABLE I. Conversion of Labeled Compounds to ^{14}C-Hippuric Acid *in Vivo*.

^{14}C compound injected	Age of rats (weeks)	Male (%)	Female (%)	p
Glycolic-1-^{14}C acid sodium salt	4.5	9.1 ± 1.0[a]	9.5 ± 1.6[a]	NS[b]
	5.5	12.2 ± 2.0	15.7 ± 1.6	NS
	7.5	18.3 ± 0.9	19.8 ± 2.5	NS
	12	23.5 ± 1.0	21.1 ± 1.5	NS
Glycine-1-^{14}C	4.5	1.6 ± 0.7	4.0 ± 0.7	<.05
	7.5	11.3 ± 1.1	11.6 ± 1.1	NS
	11.5	16.4 ± 0.6	19.4 ± 1.2	NS
Benzoic (carboxyl-^{14}C) acid	4.5	67 ± 4	72 ± 3	NS
	7.5	87 ± 2	79 ± 4	NS
	11.5	96 ± 2	96 ± 1	NS

[a] Values represent the mean ± SE of samples from six groups of two rats.
[b] NS = not significant.

as the rats matured (10-fold increase for males, 5-fold increase for females). This increase is much too large to be accounted for on the basis of increased glycine-benzoate conjugation alone. There was a significantly greater conversion of labeled glycine to radioactive urinary hippuric acid in female rats compared to male rats, at 4.5 weeks of age.

At 12 weeks of age, but not at 4.5 or 7.5 weeks, male rats converted significantly more labeled glycolic acid to oxalic acid than female rats did. The difference was almost twofold (Table II).

Discussion. Increased labeling of a product following the administration of a labeled precursor can result from several causes, as follows:

a. dilution of the labeled precursor in a smaller metabolic pool of unlabeled endogenous precursor;

b. decreased conversion of the precursor or an intermediate to an alternate product;

TABLE II. Conversion of Glycolic-1-^{14}C Acid to ^{14}C-Oxalic Acid *in Vivo*.

Age of rats (weeks)	Male (%)	Female (%)	p
4.5	0.49 ± 0.05[a]	0.50 ± 0.05[a]	NS[b]
5.5	0.80 ± 0.14	0.92 ± 0.10	NS
7.5	0.76 ± 0.06	0.73 ± 0.09	NS
12	0.44 ± 0.02	0.24 ± 0.02	<<.001

[a] Values represent the mean ± SE of samples from six groups of two rats.
[b] NS = not significant.

c. decreased degradation of the product;

d. increased activity of an enzyme which catalyzes the conversion of the precursor to the product.

The results shown in Table I do not support the first two possibilities as explanations for the increased conversion of glycolate to oxalate by mature male rats. Essentially the same percentage of injected glycolate was excreted as labeled hippuric acid by male rats as by females, suggesting similar glycolic acid pool sizes and similar glyoxylic acid transamination rates in the two sexes. The third possibility listed above would not apply in the case of oxalic acid, since this product is metabolically inert in rats (8).

The results shown in Table I therefore support the suggestion of Richardson (3) that sexual maturation, with the resulting increased androgen secretion, increases oxalate synthesis in male rats, compared to females, through increasing the activity of glycolic acid oxidase. Richardson, however, reported a significant sex difference in liver glycolic acid oxidase activity in rats 51 days old; in this study, no difference was found in the *in vivo* conversion of glycolate to oxalate at that age (7.5 weeks).

The marked increase in the conversion of injected radioactive glycine to labeled hippuric acid with age may be related to extensive metabolism of glycine for purposes other than hippuric acid synthesis (for example, protein and purine synthesis) in younger rats

of both sexes. The sex difference seen at 4.5 weeks may reflect a more rapid growth rate in male rats compared to females at this age. The glycine content of rat tissues varies with age (10) but the magnitude of this variation is much less than the increase in hippuric acid labeling from glycine, shown in Table I. Similarly, the greater labeling of hippuric acid after injection of labeled glycolate than after injection of labeled glycine may reflect the larger number of tissues that can metabolize glycine via alternate pathways. Glycolic acid oxidase is found only in the liver.

The increased conversion of benzoic acid to hippuric acid with age appears to be related to the development of the hippuric acid-synthesizing system demonstrated in homogenates of rat liver, although approximately adult values were attained at 1 month of age *in vitro* (11).

No explanation can be given at present for the increase, in both sexes, of the conversion of glycolate to oxalate between the ages of 4.5 and 5.5 weeks, and the subsequent decrease in values after 5.5 weeks. Possibly this represents another example of the development of an enzyme system, which subsequently declines as the rats mature further. Glycolic acid oxidase activity may be controlled by entirely different factors in young rats compared to sexually mature rats, in which testosterone appears to be an important regulator of this enzyme.

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