Development of Guanase and Xanthine Oxidase in Rat Liver[1] (34155)

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A number of enzymes have been noted to be altered in various tissues of multicellular organisms during cellular differentiation and maturation (1–3). These changes are an expression of the basic differentiation process which is thought essentially to involve differential control of gene expression at the level of transcription of DNA information into messenger RNA molecules which are then translated into enzymes and other proteins (4). It is not clear, however, whether control may alternatively be exerted at the level of translation or by other mechanisms (5). We here report marked alteration during maturation of rat liver of two enzymes of purine metabolism, guanase and xanthine oxidase, which are virtually absent at birth.

Methods and Materials. Rats of various ages maintained on a diet of Purina lab pellets and water *ad libitum* following weaning at 21 days were decapitated and exsanguinated. The livers were immediately removed, placed in ice, and then homogenized[2] in 10 vol of 0.25 *M* sucrose in a tissue grinder and a supernatant solution obtained after centrifugation at 100,000*g* for 30 min following preliminary centrifugations at 1200 and 18,000*g* for 30 min. The supernatant fraction contained virtually all the enzyme activity and was therefore used for enzyme assay.

Guanase and xanthine oxidase were assayed at 25° in a Gilford spectrophotometer with chart recorder by obtaining the initial rate of spectral change associated with conversion of guanine to xanthine (245 mμ) and xanthine to uric acid (290 mμ) (6, 7) (Fig. 1). The guanase reaction mixture (3 ml) contained 0.65 m*M* guanine in 90 m*M* Tris–Cl, pH 8.1, while the xanthine oxidase reaction mixture (3 ml) contained 13 μ*M* xanthine and 0.033% albumin in 78 m*M* K-pyrophosphate, pH 8.3. Protein was determined by the method of Lowry *et al.* (8). Enzyme activities are expressed as specific activities with respect to protein. Results were entirely similar when activities were expressed relative to liver weight.

Results. The adequacy of enzyme assays is indicated in Fig. 1 where direct proportionality of the quantity of spectral change with the quantity of enzyme present is evident. Guanase activity increased steadily and markedly from 1 to 40 days of age to a maximum of about 30-fold, declining slightly thereafter. On the other hand, xanthine oxidase scarcely increased until the 20–30 day period when a sharp, approximately tenfold rise in activity occurred, with little further change thereafter to 60 days of age (Fig. 2). No significant sex difference in the pattern of maturation was found.

Discussion. These findings are compatible with differential rates of transcription of the guanase and xanthine oxidase genomes with development, resulting in differing rates of synthesis of these enzymes (4, 9). This explanation remains to be proven, as does what signals the enzyme synthesis, presumably by binding specific genome repressors. Hormones have been shown to affect other enzyme systems pre- and postnatally, but whether they function in enzyme development as specific effectors for genome repressors is not clear (5). In this regard growth hormone has been

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[2] This and subsequent steps were done at 0°.

shown to increase guanase but not xanthine oxidase activity in rat liver (10), while various amino acids may stimulate or inhibit liver xanthine oxidase activity (11, 12). The marked rise in xanthine oxidase activity after weaning suggests that a dietary component may be causally related to the rise.

These results suggest that catabolic metabolism of guanine and xanthine is scarcely required by fetal liver, perhaps due to supporting maternal metabolism, but is turned on after birth in response to altered metabolic requirements by signals yet to be elucidated, which result in increased guanase and xanthine oxidase synthesis. It is of interest that these two enzymes which are closely related in purine metabolism have strikingly different patterns of development, suggesting that their synthesis occurs in response to different signals.

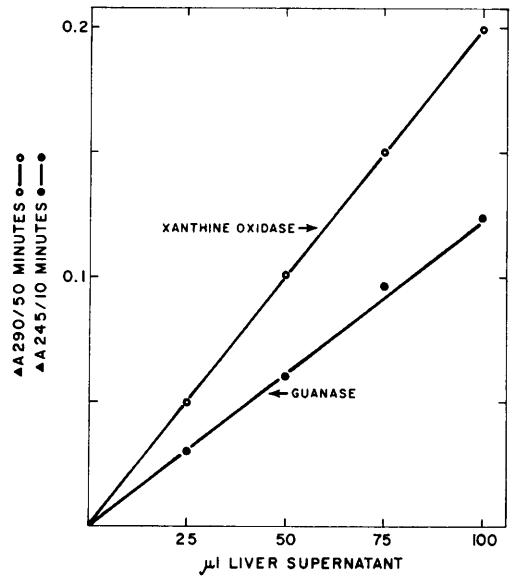

FIG. 1. Assay of xanthine oxidase and guanase in rat liver supernatant.

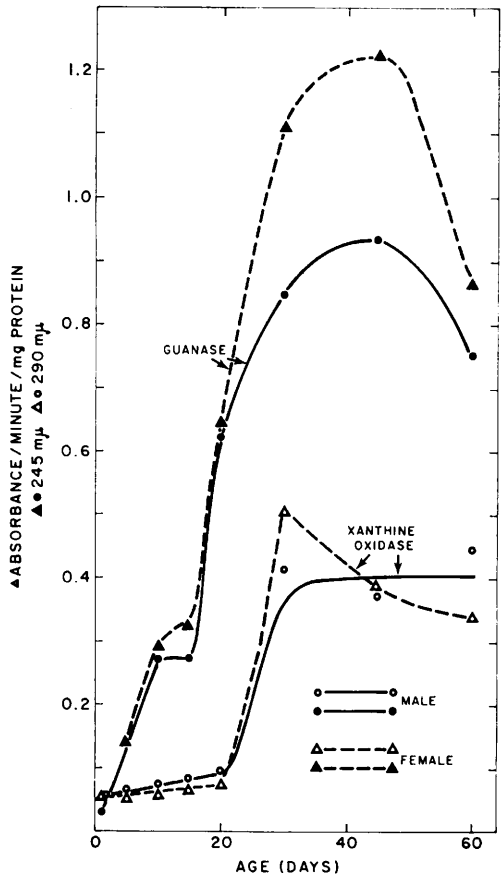

FIG. 2. Development of rat liver guanase and xanthine oxidase. Each point represents the mean determination of two animals, each done in duplicate.

Summary. Activities of the metabolically related rat liver enzymes, guanase and xanthine oxidase, increase markedly from birth to maturity. Temporal difference in the increases suggests that they are signaled differently.

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