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Short Communications

Liver L-Alanine–Glyoxylate and L-Serine–Pyruvate Aminotransferase Activities: an Apparent Association with Gluconeogenesis

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In an earlier comparative survey of liver L-alanine–glyoxylate aminotransferase activities, limited to non-carnivorous mammals, outstandingly high activities were found for rabbit and pig. It was surmised that these might be connected with a requirement for extra glycine in the formation of bile salts (Rowsell, Carnie & Taktak, 1966); a characteristic of these two species is the conjugation of bile acids virtually exclusively with glycine (Haslewood, 1964).

However, our survey has been extended to include a range of carnivores and here also, in every case, we find high L-alanine–glyoxylate aminotransferase activity. Crude liver homogenates were prepared in 0·1M-potassium phosphate buffer, pH 7·4, in a Potter-type test-tube homogenizer with a Teflon pestle; assay incubations under N_2 at 37°, sampling into trichloroacetic acid and determinations of pyruvate were as indicated by Rowsell, Carnie & Snell (1969). L-Alanine–glyoxylate aminotransferase activities derived from linear progress curves were as follows, expressed (as throughout this paper) in μmoles of pyruvate/hr./g. of liver ± S.E.M. (no. of animals): cat, 1493 ± 153 (4); dog, 2024 ± 155 (5); common frog, 5484 ± 469 (7); common toad, 4900 (1); salamander, 5370 (1); roach, 3320 (1) [cf. rat, 214 ± 6 (17); mouse, 297 ± 7 (12); sheep, 230 ± 34 (4)].

For the carnivores listed above conjugation of bile acids is not with glycine (Haslewood, 1967), and one of the possibilities we have pursued is that the significant association for the high activities here may be with gluconeogenesis; in this respect, presumably, the carnivore must be especially well equipped, taking a diet low in carbohydrate but protein-rich.

We investigated whether enhanced gluconeogenesis in the rat was accompanied by increased liver L-alanine–glyoxylate aminotransferase activity, in the first place making a study of neonatal animals. The capacity for hepatic gluconeogenesis in the rat is acquired at birth; it rapidly reaches a maximum at about 5 days *post partum* and then at about 20 days begins to decline to the adult value (Ballard & Oliver, 1965; Yeung & Oliver, 1967*a*; Vernon, Eaton & Walker, 1968). This is paralleled by an initial increase and subsequent decline in the activities of several hepatic enzymes involved in gluconeogenesis (Yeung & Oliver, 1967*b*; Vernon & Walker, 1968; Walker, 1968).

We have assayed for liver L-alanine–glyoxylate aminotransferase activity in young developing rats, an inbred black-hooded strain. For assays before birth foetal ages were assessed from measurements of body length (Greengard & Dewey, 1967). With the small amounts of tissue available a more sensitive assay was desirable: incubations and sampling were as before, but pyruvate formed was now determined with excess of NADH and lactate dehydrogenase [Boehringer Corp. (London) Ltd., London W.5] by measuring the decrease in E_{340} in the presence of 0·3M-tris–HCl buffer, pH 8·3, with a tris/glyoxylate molar ratio greater than 100:1. Under these conditions glyoxylate does not reoxidize NADH, but a rapid complete consumption of pyruvate is apparent. Results are assembled in Fig. 1; they show a rapid increase in liver L-alanine–glyoxylate aminotransferase activity after birth, then, starting at the time weaning begins, a decrease to the adult value of 214 ± 6 (17), i.e. following fairly closely the rise and fall reported for gluconeogenic capacity.

In the same study liver L-alanine–2-oxoglutarate aminotransferase (EC 2.6.1.2) activities were also measured. Again unfractionated homogenates were assayed; with incubations at 37° under N_2, substrate concentrations and sampling into trichloroacetic acid as for L-alanine–glyoxylate aminotransferase assays, pyruvate was determined with lactate dehydrogenase. The course of development of this enzyme activity was in marked contrast with that observed for L-alanine–glyoxylate aminotransferase: before birth the activity was relatively low, 70–100 μmoles of pyruvate/hr./g. of liver for 5–2 days before birth [cf. adult rat, 1195 ± 33 (6)]. There was no dramatic increase in the neonatal period and the approach to the adult value did not get under way until the time of weaning (Fig. 1).

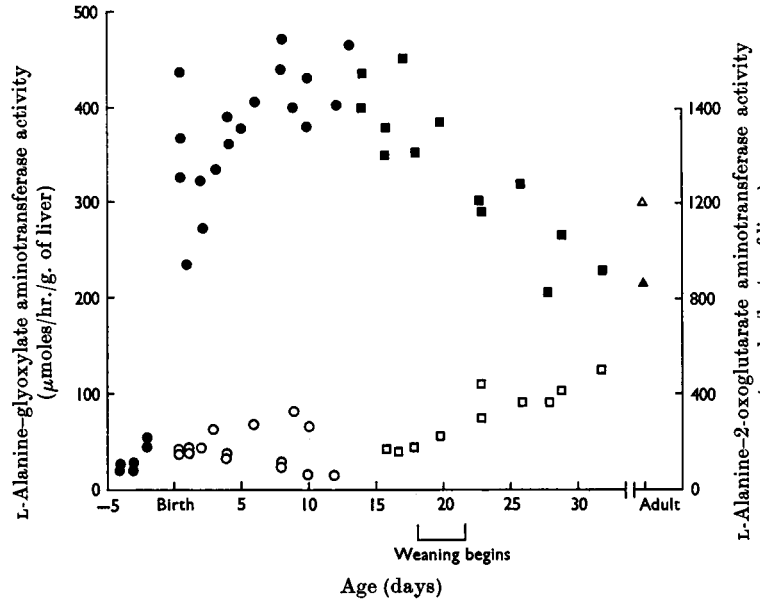

Fig. 1. Development of L-alanine–glyoxylate aminotransferase (●, ■ and ▲) and L-alanine–2-oxoglutarate aminotransferase (○, □ and △) activities in rat liver. ● and ○, Each assay with pooled liver from three to six rats; ■ and □, with a single liver or two pooled; ▲ and △, mean for assay with respectively 17 or six normal adult males (12–14 weeks old). For further details see the text.

In fact these particular results merely confirm previous reports on liver alanine–2-oxoglutarate aminotransferase activity in the neonatal rat (Yeung & Oliver, 1967*a*; Vernon *et al.* 1968).

Hepatic capacity for gluconeogenesis in the rat increases after glucagon treatment (Exton & Park, 1968; Struck, Ashmore & Wieland, 1966), and we have found a similar response in liver L-alanine–glyoxylate aminotransferase activity. Glucagon (Eli Lilly and Co. Ltd., Basingstoke, Hants.) was injected intraperitoneally (250 μg./100g. body wt.) into intact 80–100g. rats. After 24hr. liver L-alanine–glyoxylate aminotransferase activity had increased by 60 (±7 S.E.M.)% for ten fed rats in comparison with saline-injected controls where liver activity was 226±4 (10). By contrast no significant change was observed under these conditions in liver alanine–2-oxoglutarate aminotransferase activity after glucagon injection.

Our findings seem to suggest some connexion between the reaction catalysed by L-alanine–glyoxylate aminotransferase and gluconeogenesis. Any interpretation of the significance of this association must, however, depend on the direction in which this transamination reaction appears likely to proceed *in vivo*.

Thompson & Richardson (1967) record zero for the reverse reaction with glycine and pyruvate as substrates with the enzyme purified from human liver, employing [^{14}C]glycine and measuring any [^{14}C]glyoxylate formed. We have observed a very low rate of glyoxylate accumulation (0·11 μmole/hr./g. of liver) on incubating a frog liver homogenate at a final tissue concentration 2·5% (w/v) under N_2 at 37° with glycine and pyruvate each 20mM, taking samples into metaphosphoric acid and determining glyoxylate as described by Dekker & Maitra (1962). With alanine and glyoxylate as substrates in incubations with pig, dog, rat or rabbit liver we find that glycine and pyruvate are equivalent; for glycine determinations sampling and treatment of extracts with hydrogen peroxide to remove glyoxylate were as described by Rowsell *et al.* (1969), followed by separation by paper chromatography before quantitative ninhydrin analysis (Rowsell, 1962). Tentatively we conclude that the physiological function of L-alanine–glyoxylate aminotransferase is not in the transdeamination of glycine but in the conversion of glyoxylate into glycine.

Adopting this view two major problems confront us: what is the metabolic source of glyoxylate in animals, and what is the fate of the glycine formed? These are the themes of prime importance in our further development of this work, our speculation being that, under the conditions of gluconeogenesis,

L-alanine–glyoxylate aminotransferase has a role in the metabolic sequence:

? → glyoxylate → glycine → serine $\xrightarrow{a}$ pyruvate ----→ glucose

or

→ serine $\xrightarrow{b}$ hydroxypyruvate → glycerate → 2-phosphoglycerate ----→ glucose

As yet we have not sought for evidence that might link a glycine conversion into serine with gluconeogenesis, but this conversion is catalysed by normal rat liver preparations (Sato, Kochi, Sato & Kikuchi, 1969).

However, consistent with one of our suggested metabolic sequences (*b*, above) are results we are accumulating for L-serine–pyruvate aminotransferase activities in liver homogenates. With serine and pyruvate as substrates, concentrations in incubations under N_2 at 37° were as indicated for L-alanine–glyoxylate aminotransferase assays; 4·5ml. samples were taken into 0·5ml. of 72% (w/v) $HClO_4$, and after centrifugation supernatants were neutralized with 4M-KOH and recentrifuged before determination of hydroxypyruvate with excess of NADH and spinach glycerate dehydrogenase [Boehringer Corp. (London) Ltd.] at pH7·4 in 0·1M-potassium phosphate buffer. From calculations based on linear progress curves the following pattern of activities has emerged: considerably higher for cat or dog than for mouse or rat, in μmoles of hydroxypyruvate/hr./g. of liver ± S.E.M. (no. of animals) respectively 117 ± 23 (3), 144 ± 12 (4), 4·6 ± 0·2 (3) and 1·3 ± 0·1 (5). There was a 10–15-fold elevation, compared with the adult value, in the six neonatal rats tested (4–14 days old); and a 15–20-fold increase 24hr. after glucagon injection with six 80–100g. rats in comparison with six saline-injected controls, which gave values within the normal adult range.

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