Increased Liver L-Serine–Pyruvate Aminotransferase Activity under Gluconeogenic Conditions

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Rat liver L-serine–pyruvate aminotransferase activity exceeds markedly the normal adult value (*a*) in the neonatal period, (*b*) after glucagon injection and (*c*) after alloxan injection, observations that reinforce the suggestion from comparative findings that the aminotransferase has a role in gluconeogenesis. Some findings, however, argue in favour of L-serine dehydratase as the enzyme of gluconeogenesis from L-serine.

With a wide range of mammalian species liver L-serine dehydratase (EC 4.2.1.13) activity increases systematically for decreasing adult body size, i.e. for increasing basal metabolism (Rowsell *et al.*, 1965, 1972). The distribution pattern for liver L-serine–pyruvate aminotransferase is quite different: body size is not a significant factor; high activity is associated with carnivorous animal species, and a role in gluconeogenesis has been suggested (Rowsell *et al.*, 1972). On this interpretation, under conditions known to be accompanied by increased gluconeogenesis it would be reasonable to expect an elevated L-serine–pyruvate aminotransferase activity in rat liver (where normal adult activity is much lower than with, e.g., cat or dog). Aspects of the required investigation are now reported, and observations on the variation in L-serine dehydratase are included in view of the popular belief that this enzyme is responsible for gluconeogenesis from L-serine.

Rats were allowed free access to food (22% protein) and water at all times. L-Serine–pyruvate aminotransferase activity was determined with crude liver homogenates prepared in 0.1 M-potassium phosphate buffer, pH 7.4, by measuring the rate of hydroxypyruvate formation (Rowsell *et al.*, 1969). L-Serine dehydratase activity was determined with liver homogenates prepared in 0.01 M-potassium phosphate buffer, pH 7.8, containing 5 mM-EDTA; Greenberg's (1962) procedure was followed but with sampling to ensure measurement of the initial rate of pyruvate formation.

Liver L-serine–pyruvate aminotransferase activity was assayed in young developing rats, the course of development being followed until they were completely weaned. Foetal ages were assessed from measurements of body length (Greengard & Dewey, 1967). Results obtained for a blackhooded inbred strain are presented in Fig. 1. They show a rapid increase in activity at birth to a value severalfold that of the adult and the maintenance of relatively high

activity throughout the suckling period. The same pattern of development was observed with a strain of Sprague–Dawley rats; however, although activity in the adults is higher (see Table 1) the plateau in the neonatal animals was at about the same value as for blackhooded rats (see Fig. 1). The perinatal increase and subsequent decline in activity closely resemble what is observed for gluconeogenic capacity measured with rat liver slices (Ballard & Oliver, 1965; Yeung & Oliver, 1967*a*; Vernon *et al.*, 1968) and for the activities of hepatic enzymes specifically involved in gluconeogenesis from pyruvate (Yeung & Oliver, 1967*b*; Vernon & Walker, 1968; Walker, 1968), observations that are taken to imply an adaptation to an exclusively milk diet, high in fat and protein-rich but low in carbohydrate (see, e.g., Walker, 1971).

Fig. 1. Rat liver L-serine–pyruvate aminotransferase activity as a function of age

From before birth to 20 days *post partum* assays were with pooled liver from three to 13 rats (blackhooded strain) and from 20 days with a single liver or two pooled. Adult value: mean $\pm$ S.D. for ten males.

It may be significant that glycerate dehydrogenase (EC 1.1.1.29) activity shows a similar course of development in rat liver (Johnson *et al.*, 1964). In this study the assay substrates were D-glycerate and NAD⁺, but the results obtained appear to signify that rat liver must be especially well equipped in the neonatal period for the reduction of hydroxypyruvate to D-glycerate, the direction strongly favoured by the equilibrium position (Holzer & Holldorf, 1957).

There is considerable disagreement among the several published reports on the neonatal development of L-serine dehydratase activity in rat liver, but it is relevant here to note that no study has shown a sustained elevation for the suckling period. Yeung & Oliver (1971) find a peak of activity at 2 days *post partum*, a fall to below the adult value by 5 days and a subsequent slow increase only after weaning. Evered & Roffe (1971) and Goswami *et al.* (1972) report a peak of activity at 20–26 days, when weaning has normally started. According to Snell & Walker (1972), there are two peaks, at 2 and 18 days, and in their study also for most of the suckling period activity did not exceed the adult value (K. Snell, personal communication).

Liver L-serine-pyruvate aminotransferase and L-serine dehydratase activities were assayed in 80–120 g rats after a single intraperitoneal injection with glucagon (Eli Lilly and Co. Ltd., Basingstoke, Hants., U.K.). Results presented in Table 1 show an increase in L-serine-pyruvate aminotransferase activity evident at 6 h (the earliest time at which assays were made); with Sprague-Dawley rats by 24 h activity was 8-fold the control value and about 17-fold with black-hooded rats. We agree with Greengard & Dewey (1967) in finding no significant increase in L-serine dehydratase activity in response to glucagon with normally fed rats at this stage of development (Table 1).

For tests with alloxan, after intraperitoneal injection, each rat (including controls) was housed in a metabolic cage (Metabowl; Jencons Scientific Ltd., Hemel Hempstead, Herts., U.K.) to facilitate the collection of urine. After alloxan treatment there was clear evidence of glucosuria, semi-quantitative tests being made on 24 h urine samples with Clinistix (Ames Co., Stoke Poges, Bucks., U.K.). Results included in Table 1 confirm the finding, originally made by Ishikawa *et al.* (1965), that L-serine dehydratase activity increases in alloxan-diabetes (5-fold in the present experiments). L-Serine-pyruvate aminotransferase activity also increased, although to a smaller extent (3-fold).

The present observations add weight to the suggestion emerging from comparative studies that L-serine-pyruvate aminotransferase has a role in gluconeogenesis. In rat liver both hydroxypyruvate and D-glycerate are readily convertible into glucose (Dickens & Williamson, 1959, 1960), and a plausible

Table 1. *Effects of glucagon or alloxan on rat liver L-serine-pyruvate aminotransferase and L-serine dehydratase activities*

Strain	Time after injection (h)	L-Serine-pyruvate aminotransferase (μmol of hydroxypyruvate formed/min per g of liver)		L-Serine dehydratase (μmol of pyruvate formed/min per g of liver)	
		Glucagon-treated	Control	Glucagon-treated	Control
		(μmol of hydroxypyruvate formed/min per g of liver)	(μmol of hydroxypyruvate formed/min per g of liver)	(μmol of pyruvate formed/min per g of liver)	(μmol of pyruvate formed/min per g of liver)
(a) Sprague-Dawley	6	0.330 $\pm$ 0.025 (5)*	0.115 $\pm$ 0.007 (4)	1.78 $\pm$ 0.51 (5)	1.57 $\pm$ 0.45 (5)
	24	0.730 $\pm$ 0.040 (12)*	0.088 $\pm$ 0.007 (6)	1.66 $\pm$ 0.17 (12)	1.47 $\pm$ 0.08 (10)
	48	0.532 (1) 0.405 (1)†	0.085 (1) 0.108 (1)†		
Blackhooded	24	0.447 $\pm$ 0.044 (5)*	0.027 $\pm$ 0.001 (5)	Alloxan-treated 30.4 $\pm$ 1.6 (6)*	Control 6.0 $\pm$ 0.6 (6)
(b) Blackhooded	24	0.098 $\pm$ 0.004 (10)*	Control 0.032 $\pm$ 0.001 (8)		

† Female.

Rats were (a) 80–120 g body wt. or (b) 190–230 g body wt. and all males except as indicated. Test animals received a single intraperitoneal injection of (a) glucagon (250 μg /100 g body wt.) in the solvent supplied by the manufacturer or (b) alloxan (40 mg/100 g body wt.) in sterile 0.9% NaCl; controls were injected with an appropriate volume of sterile saline. Animals were killed for assays at the times indicated. Activities are given as means $\pm$ S.E.M. (numbers of observations in parentheses). Significance of results (Student's *t* test): *, *P* < 0.001.

pathway from L-serine can be visualized in terms of known metabolic reactions in liver:

It is difficult, moreover, to see what function a conversion into hydroxypyruvate could have other than to initiate the flow of serine carbon towards glucose.

It has been shown, even so, that liver L-serine-pyruvate aminotransferase activity does not invariably increase under conditions conducive to gluconeogenesis. Sallach *et al.* (1972), in a study with 60–80 g Sprague–Dawley rats, found no increase on starvation, nor with severe alloxan-induced diabetes. An increase was observed with mild diabetes, but this was not diminished in response to insulin. Under these conditions a considerable increase in liver L-serine dehydratase activity was observed and in diabetic rats activity was decreased to the normal value by insulin treatment. Moreover activity increases were observed for both L-serine-pyruvate aminotransferase and L-serine dehydratase in rats that had received multiple injections of glucagon. There are other reports of a glucagon-induced increase in L-serine dehydratase activity in rats, but the significance is problematic since the animals had been previously subjected to several days of protein starvation (see, e.g., Jost *et al.*, 1968).

The dehydratase conversion of L-serine into pyruvate (and ammonia) need not serve exclusively as a starting point for glucose formation. The acute dependence of L-serine dehydratase activity on body size in adult mammals (Rowell *et al.*, 1965, 1972) and the striking increase that occurs in rats well supplied with carbohydrate but exposed to cold (Rowell *et al.*, 1965; Nakagawa & Nagai, 1971) suggest a role for this enzyme other than in gluconeogenesis. Even under conditions of carbohydrate shortage a function, for example, in the provision of oxaloacetate for the tricarboxylic acid cycle could be of importance. The fact remains that a pathway from pyruvate to glucose is thoroughly established; to some degree, it would appear, L-serine dehydratase must contribute to glucose formation, and an elevation of its activity under circumstances associated with gluconeogenesis is at least consistent with such a role.

If there is a single enzyme that initiates glucone-

genesis from L-serine, observations presently available do not allow a clear unqualified identification; both L-serine-pyruvate aminotransferase and L-serine dehydratase remain candidates.

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