Pyruvate Kinase of Hepatoma Morris 5123: Insensitivity to Dietary Changes

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At least two types of pyruvate kinase (ATP-pyruvate phosphotransferase, EC 2.7.1.40) have been identified in rat liver, which were named type L (liver) and type M (muscle) on the basis of their immunological properties (Tanaka, Harano, Morimura & Mori, 1965). The L-type enzyme is allosterically activated by fructose 1,6-diphosphate (Taylor & Bailey, 1967) and is subject to hormonal and dietary control (Tanaka *et al.* 1965; Suda, 1967; Tanaka, Harano, Sue & Morimura, 1967); in particular, it decreases during starvation and increases after feeding with fructose (Bailey, Stirpe & Taylor, 1968). The M-type enzyme is only slightly affected by these factors.

The nature of the pyruvate kinase of tumours has been investigated: the proportions of L- and M-type enzyme in several transplantable hepatomas have been studied by Lo, Farina, Morris & Weinhouse (1968). Taylor, Morris & Weber (1969)

reported that pyruvate kinase of the rapidly growing hepatoma 3924-A differs from rat muscle pyruvate kinase, as judged by the stability to inactivation at 25°C, by the electrophoretic mobility and by the sensitivity to various inhibitors (Cu²⁺, *p*-chloromercuribenzoate, ATP, alanine). Criss (1969) observed in well and in poorly differentiated rat hepatomas the occurrence of a new pyruvate kinase isoenzyme, whose electrofocusing mobility was different from that of muscle and M-type liver pyruvate kinase.

The purpose of the present investigation was to ascertain whether the pyruvate kinase of the hepatoma Morris 5123 underwent modification after dietary changes.

The hepatoma Morris 5123 (Morris, Sidransky, Wagner & Dyer, 1960) from a line maintained in this laboratory was transplanted subcutaneously in male rats of the Buffalo strain, fed on the stock

Table 1. *Pyruvate kinase activity of hepatoma Morris 5123 and host liver in normally fed, fructose-fed and starved rats*

Pyruvate kinase activity was determined as described by Krebs & Eggleston (1965) (KE method) or as described by Bailey *et al.* (1968) with 0.1 mM-phosphoenolpyruvate (PEP), with or without fructose diphosphate (FDP) (low-PEP method). The assay mixture contained, in a final volume of 3 ml: for the KE method, 67 mM-potassium phosphate buffer, pH 7.4, 0.15 mM-NADH, 1.3 mM-ADP, 8.0 mM-MgSO₄, 10 units of lactate dehydrogenase, 0.78 mM-phosphoenolpyruvate and 0.1 ml of supernatant; for the low-PEP method, 40 mM-triethanolamine-HCl buffer, pH 7.5, 3 mM-EDTA, 70 mM-KCl, 0.15 mM-NADH, 1.0 mM-ADP, 8.0 mM-MgSO₄, 10 units of lactate dehydrogenase, 0.1 mM-phosphoenolpyruvate and 0.1 ml of supernatant. Fructose diphosphate (0.5 mM) was added about 1 min after the reaction had been started with phosphoenolpyruvate. In preincubated samples the supernatant was added to the reaction mixture and kept at 25°C for 10 min before the reaction was started with phosphoenolpyruvate. Results are mean values, with the numbers of experiments given in parentheses.

Treatment	Pyruvate kinase activity (μmol/min per mg of protein)									
	Hepatoma					Host liver				
	Low-PEP method					Low-PEP method				
	KE method	No preincubation		10 min preincubation		KE method	No preincubation		10 min preincubation	
		-FDP	+FDP	-FDP	+FDP		-FDP	+FDP	-FDP	+FDP
Normally fed (4)	0.203	0.194	0.184	0.119	0.117	0.371	0.201	0.207	0.103	0.219
Fed on 20% fructose for 6 days (4)	0.213	0.193	0.188	0.122	0.131	0.814	0.467	0.482	0.197	0.511
Normally fed (4)	0.133	0.80	0.111	0.041	0.066	0.533	0.219	0.271	0.051	0.292
Starved for 3 days (4)	0.126	0.077	0.101	0.041	0.055	0.252	0.115	0.149	0.208	0.132

diet used in this laboratory. Fructose was offered to rats as a 20% (w/v) solution in drinking water. Livers and hepatomas were homogenized with water (1g/19ml) in a Potter-Elvehjem homogenizer, the homogenates were centrifuged at 105000g for 1h in an MSE 40 or a Beckman-Spinco L2/65K centrifuge, and samples of the supernatants were used for the assay of pyruvate kinase activity. This was performed at 25°C either by the method of Krebs & Eggleston (1965) at saturating concentrations of phosphoenolpyruvate to determine the total activity of the enzyme, or by the method of Bücher & Pfeleiderer (1955) with 0.1mM-phosphoenolpyruvate (Bailey *et al.* 1968) to study inactivation at 25°C and the effect of fructose diphosphate. The enzyme activity is expressed as μmol of NADH oxidized/min per mg of protein. Protein was determined by the method of Gornall, Bardawill & David (1949).

The results are summarized in Table 1. When rats were given 20% fructose the total pyruvate kinase activity did not show any change in the hepatoma, whereas it more than doubled in the host liver. Similarly, starvation did not affect the enzyme activity of the tumour, although the activity in the liver decreased by approx. 50%. Therefore it can be concluded that the enzyme present in hepatoma 5123 is not subject to dietary regulation.

This lack of response of hepatoma pyruvate kinase to dietary changes is presumably due to scarcity of the L-type enzyme, as it was observed in other transplantable tumours (Tanaka *et al.* 1965; Suda, 1967; Taylor *et al.* 1969; Criss, 1969). Preincubation at 25°C for 10min partially inactivated pyruvate kinase, more markedly that of liver than that from the hepatoma. Addition of fructose diphosphate after preincubation restored the enzyme activity of the liver supernatant, but was almost ineffective on the pyruvate kinase of the hepatoma. This supports the view that the pyruvate kinase present in hepatoma Morris 5123 is pre-

dominantly, if not entirely, of the M-type or of the new type observed by Criss (1969).

Suda (1967) reported that the pyruvate kinase of the liver of rats bearing the Walker tumour was predominantly of the M-type. This was not confirmed by our observations with rats bearing the hepatoma Morris 5123, since the effect of preincubation and of fructose diphosphate, as well as the response to dietary changes, demonstrated the presence of the L-type enzyme. It is possible that the Walker tumour, growing more rapidly than the hepatoma Morris 5123, induced a cachectic state in the animals, whose livers were probably in a condition similar to that of starved rats, i.e. largely deprived of type L pyruvate kinase.

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