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Strain Differences in the Activities of Rat Liver Enzymes

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1. The activities of four enzymes of glycolysis were assayed in the liver of five different strains of rats (four 'Wistar-derived', one Sprague–Dawley) kept on three different but very similar diets. 2. Major strain differences were found for the activities of pyruvate kinase (3-fold), α-glycerophosphate dehydrogenase (5-fold), glyceraldehyde phosphate dehydrogenase (3-fold) and triokinase (1·5-fold). 3. Although the initial activities of pyruvate kinase differed greatly the percentage responses to starvation or a diet high in soluble carbohydrate were of the same order in two strains. 4. The importance of considering strain differences is emphasized when making comparisons of measurements carried out in different laboratories.

When investigating the effects of a high ethanol intake (10% ethanol in drinking water) on the enzyme activities of Wistar rat liver it was noted that the activities of pyruvate kinase (EC 2.7.1.40) and α-glycerophosphate dehydrogenase (EC 1.1.1.8) were 3–5-fold higher and that of glyceraldehyde phosphate dehydrogenase (EC 1.2.1.12) about 2-fold higher than the corresponding activities found 2 years earlier in 'normal' rat liver under apparently identical conditions. There were also distinct though minor differences in the activities of triokinase (EC 2.7.1.28), alcohol dehydrogenase (EC 1.1.1.1) and aldehyde dehydrogenase (EC 1.2.1.3), but no significant differences were noted for fructokinase (EC 2.7.1.4), 6-phosphogluconate dehydrogenase (EC 1.1.1.43), glucose 6-phosphate dehydrogenase (EC 1.1.1.49), 'malic' enzyme (EC 1.1.1.40) and citrate-cleavage enzyme (EC 4.1.3.8).

A detailed study of the cause of the difference revealed that they were not connected with the ethanol intake but were due to strain differences. Until September 1966 the rats used had been bred by random pairing in the Department of Biochemistry, University of Oxford, from a strain of Wistar rats obtained in 1927 from Glaxo Laboratories. In September 1966 it became necessary to abandon breeding and as from that time Wistar rats were obtained from Scientific Products Farm, Ash Research Centre, Canterbury, Kent. In appearance the new and the old strain seemed identical.

In view of these experiences strain differences of enzyme activities were systematically investigated for the four enzymes (pyruvate kinase, α-glycerophosphate dehydrogenase, glyceraldehyde phosphate dehydrogenase and triokinase) that in the preliminary test showed the highest strain variations. Differences in two strains of Wistar rats have already been reported by Bartley, Dean, Taylor & Bailey (1967) for pyruvate kinase, glucose 6-phosphate dehydrogenase and fructose 1,6-diphosphatase.

EXPERIMENTAL

The following strains of rats were used: 1, Wistar; bred at the Departments of Biochemistry and Pathology, University of Oxford, originally obtained from Glaxo Laboratories Ltd., in 1927; average weight 280g., average age 9 weeks; 2, Wistar; obtained from Scientific Products Farm, Ash Research Centre, Canterbury, Kent; stated to be 'not carrying a pathogen burden'; average weight 240g., average age 8 weeks; 3, Wistar; bred in the Radcliffe Infirmary, Oxford, originally obtained from the Ministry of Defence, Allington Farm, Porton Down, Wilts.; average weight 305g., average age 9 weeks; 4, Wistar; referred to as Buffalo strain, obtained from Dr George Weber, University of Indiana Medical School; these rats were of different appearance from the other Wistar rats, the Buffalo Wistar rats having a shorter but wider head, short neck, broader squat body and coarser fur; average weight 470g., average age 36 weeks; 5, Sprague–Dawley; obtained from A. Tuck and Son Ltd., Rayleigh, Essex; average weight 250g., average age 8 weeks.

The stock diets were: 1, Spillers 'Small Laboratory Animal Diet'; 2, Oxoid 'Pasteurised breeding diet for rats and mice' (Oxoid Ltd., London S.E.1); 3, Oxoid 41B diet (based on the formula of Bruce & Parkes, 1949). The diets are very similar in composition and meant to provide in adequate amounts all required nutrient. The rats were kept for at least 1 week on the specified diet before the livers were examined. Tests showed that no further changes in the enzyme activities occurred on prolonged feeding for up to 1 month. The rats were killed before 10a.m. when their

stomachs still contained food. The animals were chosen at random from several litters.

Enzyme assays. Rats were stunned and bled from the neck, and their livers were removed and chilled on ice for a few minutes. A 1g. portion of liver was homogenized with 4ml. of 1mM-EDTA at 2°. Part of the homogenate was centrifuged at 30000*g* for 15min. The supernatants obtained and the remainder of the homogenates were stored at −18°. At this temperature the activity remained unchanged for several weeks, though frequent (about six times) thawing and freezing caused losses. Pyruvate kinase, α-glycerophosphate dehydrogenase and glyceraldehyde phosphate dehydrogenase were assayed in the homogenate and triokinase in the supernatants.

Pyruvate kinase was assayed by the method of Bücher & Pfleiderer (1955) as modified by Krebs & Eggleston (1965).

α-Glycerophosphate dehydrogenase and glyceraldehyde phosphate dehydrogenase were assayed by methods similar to those of Shonk & Boxer (1964) except that tris–HCl buffer, pH 7·4, was used.

Triokinase was assayed by measuring the ADP formation in the reaction:

D-glyceraldehyde + ATP → D-glyceraldehyde 3-phosphate + ADP

Cuvettes contained (final concentrations), in a total of 3·0ml.: 67mM-tris–HCl buffer, pH 7·4, 5mM-$MgCl_2$, 5mM-ATP, 2mM-phosphoenolpyruvate, 0·13mM-NADH, 50mM-NaF, 5μl. each of purified pyruvate kinase and lactate dehydrogenase (C. F. Boehringer, und Soehne G.m.b.H., Mannheim, Germany) and 3·33mM-D-glyceraldehyde [Sigma (London) Chemical Co., London, S.W.6]. When all ADP contaminating the ATP had reacted (about 10min.) and any slight extinction changes in both test and blank cuvettes were equal, 0·1ml. of diluted liver supernatant (equivalent to 1mg. of original liver) was added to both cuvettes and readings were taken for about 15min. The blank contained all reagents except D-glyceraldehyde.

All enzyme activities are expressed as μmoles of substrate reacted/min./g. fresh wt. of liver at 25° and pH 7·4, and are given as means ± S.E.M. with numbers of observations in parentheses.

RESULTS

The results of the assays of seven groups of rats, involving five strains and three different stock diets, are given in Table 1. The activity of pyruvate kinase varied between 27 and 84. Although this enzyme can be much affected by starvation and a diet rich in soluble carbohydrate (Krebs & Eggleston, 1965) the three stock diets did not modify the activity of this enzyme. The activity of α-glycerophosphate dehydrogenase varied between 12 and 59, with diets 1 and 2 in strain 1, and diets 2 and 3 in strain 3 caused marked differences of the enzyme activity. Glyceraldehyde phosphate dehydrogenase activity varied between 78 and 225, with the diet making a major difference in strain 3. Triokinase showed relatively minor strain dif-

Table 1. *Enzyme activity in the livers of rats of different strains and on different diets*

See the text for strains, diets and methods of assay.

Date of assay	Strain no.	Diet no.	Enzyme activities (μmoles/min./g.) at 25° and pH 7·4			
			Pyruvate kinase	α-Glycerophosphate dehydrogenase	Glyceraldehyde phosphate dehydrogenase	Triokinase
1963	1	1	27·2 ± 2·6 (7♂)	12·0 ± 0·9 (4♂)	90 ± 1·7 (6♂)	—
1968	1	2	33·4 ± 5·3 (4♂)	27·1 ± 1·6 (4♂)	78 ± 18·1 (4♂)	1·83 ± 0·11 (4♂)
1968	2	2	60·8 ± 7·5 (1♂, 5♀)	57·7 ± 6·6 (1♂, 6♀)	91 ± 11·8 (7♀)	1·73 ± 0·09 (6♀)
1968	3	2	37·6 ± 4·7 (4♀)	25·3 ± 5·6 (4♀)	88 ± 14·8 (4♀)	1·91 ± 0·12 (4♀)
1968	3	3	38·4 ± 3·9 (4♀)	55·3 ± 3·9 (4♀)	139 ± 5·9 (4♀)	2·45 ± 0·13 (4♀)
1969	4	2	83·7 ± 11·7 (6♀)	59·1 ± 2·3 (6♀)	183 ± 33·2 (6♀)	2·56 ± 0·12 (6♀)
1969	5	2	66·1 ± 9·2 (6♀)	57·5 ± 1·2 (6♀)	225 ± 39·2 (6♀)	2·43 ± 0·12 (6♀)

Table 2. *Response of liver pyruvate kinase activity to dietary changes in two strains of Wistar rats*

The assay method and the high-carbohydrate diet were as described by Krebs & Eggleston (1965).

Strain no.	Diet no.	Pyruvate kinase activity (μmoles/min./g.)		
		Normal	Starved for 48hr.	High-carbohydrate diet for 3 days
1	1	27·2 ± 2·6 (7♂)	9·9 ± 0·7 (7♂)	96 ± 5·9 (6♂)
2	2	60·8 ± 7·5 (1♂, 5♀)	28·8 ± 1·9 (4♂, 4♀)	172 ± 22·1 (2♂, 4♀)

ferences, between 1·73 and 2·56. Generally the Buffalo strain and the Sprague–Dawley strain showed the highest activities of these four glycolytic enzymes.

The percentage responses of the pyruvate kinase activity of dietary changes (starvation or a diet rich in soluble carbohydrate) in two Wistar strains were very similar, though the activities on the standard diet differed by about 100% (Table 2).

DISCUSSION

The liver enzyme activities given by Shonk & Boxer (1964) for Holtzman rats are of the same order of magnitude as for the strains listed in Table 1, but the spectrum of activity differs from that of the present strains. In the Holtzman rats pyruvate kinase activity was the same as in strain 1 on diet 1. α-Glycerophosphate dehydrogenase activity was about the same as in strains 2, 4 and 5. Glyceraldehyde phosphate dehydrogenase activity was similar to that in strain 1 on diet 2.

Another strain of Wistar rats, studied by Bartley *et al.* (1967), obtained from Tuck and Son Ltd. showed lower pyruvate kinase activity (9 μmoles/min./g.) than any of the strains listed in Table 1.

The general lack of homogeneity of rat strains named Wistar has been emphasized by Porter (1966). The differences in the enzyme activities of strains derived from the original Wistar strain, and the effects of minor undefinable variations in the stock diets, are of importance when comparisons are made between measurements carried out at different laboratories or at different times. It is obviously essential to carry out controls simultaneously with the test experiments and not to rely on published data. It is noteworthy that, where tested, sex appeared to make no significant difference to the enzyme activities in the cases examined, though the variations seemed to be somewhat greater in female rats, presumably in connexion with the oestrous cycle.

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