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The Effect on some Enzymes of Rat Tissue of Diets Low in Fat Content

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1. Rats of two strains were kept on three different diets; one was a commercial diet of rat pellets, one contained about 80% of sucrose and 20% of casein and was supplemented with corn oil, and the third was a similar diet without the corn oil. 2. On the commercial diet, the specific activities of pyruvate kinase, glucose 6-phosphate dehydrogenase and fructose 1,6-diphosphatase in the livers of one strain of rats (strain A) were 1·5–3 times those in the other strain (strain B). When the diet high in sucrose and supplemented with corn oil was given, there were large increases in the specific activity of pyruvate kinase, glucose 6-phosphate dehydrogenase and fructose 1,6-diphosphatase in the livers of strain A rats. With strain B rats the increases were much smaller. Omission of corn oil from the diet caused a threefold increase in the specific activity of glucose 6-phosphate dehydrogenase in strain B rats, but had little effect on other enzymes. 3. The enzymes of the kidneys and hearts of strain A rats were also more active than those of strain B rats. In strain A rats, the specific activities of pyruvate kinase and fructose 1,6-diphosphatase in the kidney increased when the sucrose content of the diet was high, but in the kidneys of strain B rats there was little change. 4. In strain A rats, the specific activity of pyruvate kinase in the heart more than doubled with the high-sucrose–corn oil diet and increased threefold when corn oil was omitted. No changes were seen in strain B rats. 5. In strain A rats, omission of corn oil from the diet increased the ability of the kidneys to synthesize glucose from lactate. 6. In strain B rats, addition of corn oil to the diet resulted in a decrease in the liver in the specific activity of ATP citrate lyase and in the ability to incorporate acetate into lipid.

Many workers have shown that the composition of the diet influences the enzyme pattern found in the tissues. In general, enzymes utilizing a particular component of the diet increase when that component forms a larger fraction of the dietary intake. For example, enzymes of nitrogen metabolism increase in the rat when a high-protein diet is given (Knox & Greengard, 1965); again, glucose 6-phosphate dehydrogenase increases with increase in the carbohydrate content of the diet, though this response may be altered by substituting fat for part of the protein of the diet (Tepperman & Tepperman, 1963), and pyruvate kinase changes in response to a change in carbohydrate intake (Krebs & Eggleston, 1965). The type of carbohydrate (starch or sucrose) in the diet may also influence the enzyme pattern (Carroll, 1963, 1964). In the present work we have studied, in two different strains of rats, the effects on the activities of some enzymes of carbohydrate and fat metabolism of diets in which the carbohydrate was present either as starch or as sucrose. In view of the report by Allman & Gibson (1965) of changes in the activities of enzymes of fatty acid synthesis when mice deficient in essential fatty acids had their diet supplemented with corn oil or methyl linoleate, we have also investigated the effect of essential fatty acid deficiency on the same enzymes.

EXPERIMENTAL

Materials. All chemicals were of analytical grade or the purest available. $NADH_2$, NADP, ADP, phosphoenolpyruvate, glucose oxidase, peroxidase and lactate dehydrogenase were obtained from C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany. Glucose 6-phosphate, fructose 1,6-diphosphate and cysteine were from British Drug Houses Ltd., Poole, Dorset, and Diazyme was from Miles Chemical Co., Clifton, N.J., U.S.A.

Diets. Diet 1 was the commercial diet of rat pellets (Oxoid modified diet 86). The percentage distribution of carbohydrate, protein and fat was 70:25:5.

Diet 2 was a synthetic diet of the following composition: 20 parts of fat-free casein (British Drug Houses Ltd.),

70 parts of household sugar (Tate and Lyle Ltd., Liverpool), and 5 parts of salt mixture. This consisted of salts in the following proportions by weight: NaCl, 100; $MgSO_4$, 315; $NaHPO_4,2H_2O$, 200; K_2HPO_4, 552; ferric citrate pentahydrate, 67; calcium lactate pentahydrate, 750; $CaH_4(PO_4)_2,H_2O$, 312; KI, 0·2; $CuSO_4,5H_2O$, 2·0; $MnCl_2,4H_2O$, 5·0. Vitamins were added to the diet to give the following amounts (mg./kg. of diet): thiamine hydrochloride, 2·0; riboflavine, 4·0; nicotinamide, 10·0; inositol, 50·0; choline chloride, 2000; biotin, 0·043; pyridoxine hydrochloride, 4·0; *p*-aminobenzoic acid, 25·0; calcium pantothenate, 24·0; vitamin A acetate, 7·5; DL-α-tocopherol, 50·0; calciferol, 0·04. The last three were added in tributyrin and the others in aqueous solution. In practice, large batches of diet minus vitamins were stored dry and the appropriate quantity of vitamins was mixed in, together with 10ml. of corn oil (Mazola, supplied by Brown and Polson Ltd., Esher, Surrey)/kg. dry wt. and 15% (w/w) of water, on the day of use. The percentage distribution of carbohydrate, protein and fat was 77:22:1 in the final mixture.

Diet 3 was diet 2 with the corn oil omitted. The percentage distribution of carbohydrate, protein and fat was 78:22:0 in the final mixture.

Animals. Strain A rats were males of a specific-pathogen-free Wistar stock supplied by Shell Research Ltd., Sittingbourne, Kent. Strain B rats were males of a Wistar strain supplied by A. Tuck and Son Ltd., Laboratory Animal Breeding Station, Rayleigh, Essex. Rats were caged separately in conditions of controlled temperature (23°) and humidity (50%) and given diet and water *ad libitum* up to killing, normally after 14 weeks, when clear-cut signs of essential fatty acid deficiency had appeared in the animals consuming diet 3. Random sampling of rats between 10 and 20 weeks of the dietary regime showed no significant changes of the enzyme activities measured within the dietary group. Rats were always killed at the start of the working day. Strain A rats went on to the diet when they were 4 weeks old and weighed 75–77g. They reached 380g. on diet 2 and 285g. on diet 3 after 14 weeks. Strain B rats went on to the diet when they were 4 weeks old and weighed 56–57g. They reached 260g. on diet 2 and 200g. on diet 3 after 14 weeks. The growth curves of rats on diet 1 were not plotted, but their weights at comparable ages were similar to those of rats on diet 2.

METHODS

Preparation of tissue homogenates for enzyme measurement. The animals were killed by stunning and decapitation and the organs were rapidly removed, dissected free from fatty and connective tissue, washed free from extraneous blood and cooled to 0°. For the measurement of pyruvate kinase, glucose 6-phosphate dehydrogenase, fructose 1,6-diphosphatase and glucose 6-phosphatase, approx. 1g. of liver or kidney or the whole heart (approx. 0·75g.) was minced in a Fischer mincer and the pulp transferred to a weighed glass Potter–Elvehjem homogenizer. The pulp was then homogenized in 3vol. of ice-cold 0·154M-KCl containing 0·66mM-EDTA. Of this homogenate, 0·5ml. was added to 4·5ml. of water for the measurement of pyruvate kinase activity; a further 0·5ml. sample was added to 4·5ml. of the original homogenizing fluid for the estimation of glucose 6-phosphate dehydrogenase and fructose 1,6-diphosphatase, and finally a 0·5ml. sample was added to 4·5ml. of 0·1M-sodium citrate buffer, pH6·5. The first two dilutions of the homogenate were centrifuged at 35000*g* for 10min. and the sediment was discarded. No loss of enzyme resulted from the centrifugation. For the measurement of ATP citrate lyase and malic enzyme a liver sample was homogenized in 9vol. of 0·4M-KCl prepared in aq. 20% (v/v) ethanol.

Preparation of kidney slices. These were cut with a Stadie–Riggs slicer from the kidney cortex at a thickness of 0·25mm.; the first slice was discarded.

Measurement of enzyme activities. Pyruvate kinase (ATP–pyruvate phosphotransferase, EC 2.7.1.40) activity was measured by a modification of the method of Bücher & Pfleiderer (1955). Two cuvettes each contained 2·9ml. of a mixture of $MgSO_4$ (8mM), tris–HCl buffer, pH7·5 (6·6mM), KCl (50mM), $NADH_2$ (0·15mM), phosphoenolpyruvate (0·33mM) and 0·3unit of lactate dehydrogenase. The enzyme sample, usually 0·05ml., was added to both cuvettes and the reaction was started by the addition of ADP (1·15mM, final concn.) to one of the cuvettes. The enzyme sample size was arranged to give a change of extinction of 0·05–0·25/min. and the reaction was followed in the Beckman DB spectrophotometer at 340mμ. The temperature of the reaction was 25° and the change in extinction was plotted on a Sargent SR recorder with a chart speed of 1in./min.

Glucose 6-phosphate dehydrogenase (D-glucose 6-phosphate–NADP oxidoreductase, EC 1.1.1.49) activity was measured by the method of Lohr & Waller (1963) and glucose 6-phosphatase (D-glucose 6-phosphate phosphohydrolase, EC 3.1.3.9) and fructose 1,6-diphosphatase (D-fructose 1,6-diphosphate 1-phosphohydrolase, EC 3.1.3.11) activities by the methods of Harpur (1963) and McGilvery (1955) respectively.

ATP citrate lyase [ATP–citrate oxaloacetate-lyase (CoA-acetylating and ATP-dephosphorylating), EC 4.1.3.a] activity was measured essentially by the method of Srere (1959) except that malate dehydrogenase was omitted since this was already in excess in the preparation. The measurements were made at 25°.

Malic enzyme [L-malate–NAD oxidoreductase (decarboxylating), EC 1.1.1.38] was measured by the method of Ochoa (1955).

Measurement of incorporation of acetate into tissue lipids. The method of measuring the incorporation of acetate into liver lipids was based on that of Allman & Gibson (1965). A 1g. sample of liver was homogenized with 4vol. of 0·1M-potassium phosphate buffer, pH7·5, and centrifuged at 20000*g* for 30min. A sample (0·1ml.) of the supernatant was incubated for 60min. at 37° in a medium of total volume 1·0ml., containing potassium phosphate buffer, pH6·5 (50mM), $MnCl_2$ (0·8mM), ATP (4mM), cysteine (8mM), trisodium isocitrate (10mM), NADP (0·2mM), CoA (0·1mM), $KHCO_3$ (20mM) and 2μc of sodium [1-^{14}C]-acetate (2·2mM). The gas phase was CO_2. The reaction was stopped with 3ml. of 10% (w/v) KOH. The mixture was saponified by heating in a boiling-water bath for 2hr., acidified with H_2SO_4 and the lipid extracted with three 10ml. portions of light petroleum (b.p. 40–60°). The combined extract was washed with three 30ml. portions of 5% (w/v) acetic acid and dried with anhydrous Na_2SO_4. A 5ml. portion of the light-petroleum extract was evaporated to dryness in a counting vial and 10ml. of liquid

scintillator NE213 (Nuclear Enterprises Ltd., Sighthill, Edinburgh, 11) was added. Samples were counted in an automatic liquid-scintillation counter (Nuclear-Chicago Corp. model 725). The initial activity (counts/min./tube) was determined by making a 1:100 aqueous dilution of the stock [1-^{14}C]acetate solution and counting 0·1 ml. in 10 ml. of a dioxan-based scintillator, NE220 (Nuclear Enterprises Ltd.). Under the conditions stated, the counting efficiency was the same for extracted lipid and acetate.

Measurement of glucose synthesis by kidney slices. This was exactly as described by Krebs, Bennett, de Gasquet, Gascoyne & Yoshida (1963).

Protein. This was measured by the biuret method, as described by Layne (1957).

RESULTS

Changes in activity of liver enzymes with change in diet. Table 1 shows the activities of some liver enzymes when rats of strains A and B were kept under different dietary conditions.

The activities of pyruvate kinase are less than those published by Krebs & Eggleston (1965), ranging from about one-quarter for strain B rats to about two-thirds for strain A animals. The rats fed on the synthetic diet of sucrose and casein supplemented with 1% of corn oil (diet 2) had a much higher hepatic pyruvate kinase activity than those on the commercial diet (diet 1), although the carbohydrate content of the diet was only 7% greater. Krebs & Eggleston (1965) found a similar rise in activity with an increase in the carbohydrate content of the diet of only 4%. Complete removal of fat from the diet (diet 3) resulted, in both strains, in a slight lowering of the pyruvate kinase activity, but the activity was still much higher than in rats fed on the normal stock diet (diet 1). Because the liver weights of the rats consuming the fat-deficient diet 3 were appreciably less than those of rats fed on diet 2, while the percentage of fat in the livers was considerably higher, there was much less total pyruvate kinase activity in the livers of the deficient rats. In both strains of rat the total activity of pyruvate kinase in the liver was only sufficient to deal with some 10–20% of the daily dietary intake of carbohydrate.

As with pyruvate kinase, there was a greater activity of glucose 6-phosphate dehydrogenase in the livers of the rats on diet 2 than in those on diet 1, and in strain B rats the specific activities were lower than in strain A rats. With diet 3 there was a slight further increase in specific activity in strain A and a very marked increase in strain B, but the specific activity of strain B was still only one-quarter of that of strain A. The total activity in strain B rats on diet 3 was almost double that of those on diet 2.

Fructose 1,6-diphosphatase is a key enzyme of gluconeogenesis and its activity might be expected

Table 1. *Enzyme activities in rat livers from male animals consuming different diets*

Animals of strain A were of a specific-pathogen-free Wistar stock (Shell Research Ltd.). Animals of strain B were of Wistar stock and were obtained from Tuck and Son Ltd. Diet 1 was the Oxoid modified diet 86, diet 2 was a synthetic low-fat diet supplemented with corn oil, and diet 3 was an unsupplemented low-fat diet. The protein content of the liver for rats on diet 1 was calculated from the data of Biran (1962). The values are the means ± S.D. from at least six different animals.

Enzyme	Diet 1: Specific activity (μmoles of substrate transformed/g. of protein/min.)		Diet 1: Total activity (μmoles of substrate transformed/total liver/min.)		Diet 2: Specific activity (μmoles of substrate transformed/g. of protein/min.)		Diet 2: Total activity (μmoles of substrate transformed/total liver/min.)		Diet 3: Specific activity (μmoles of substrate transformed/g. of protein/min.)		Diet 3: Total activity (μmoles of substrate transformed/total liver/min.)	
Strain......	A	B	A	B	A	B	A	B	A	B	A	B
Pyruvate kinase	121±21·7	40±9·8	340	90	780±92·9	97±20·1	2180	219	720±129	80±10·6	1560	113
Glucose 6-phosphate dehydrogenase	14±12·6	9±0·27	39	20	150±18·2	15±0·95	420	34	160±11·9	43±5·7	340	61
Fructose 1,6-diphosphatase	101±7·8	66±5·3	285	148	241±28·0	72±6·6	680	160	261±29·9	76±8·2	570	107
Glucose 6-phosphatase	—	49±3·3	—	110	—	65±3·1	—	149	—	81±2·8	—	127
Malic enzyme					96±15·6				93±6·3			

to change with the carbohydrate content of the diet. In fact, as first shown by Carroll (1964), it increases with the sucrose content of the diet. Thus with strain A rats the activity on diet 2 is more than twice that on diet 1, and is even higher in rats on diet 3. With strain B rats, diet 3 gave the highest specific activity, but this was only slightly higher than that with diet 2, which in turn was little above that with diet 1. The specific activity was much lower in strain B than in strain A and, as found with pyruvate kinase, the total activity was lower in rats on diet 3 than in those on diet 2 because of the smaller protein content of the livers. In the B strain of rats, the total activity on diet 3 was less than on diet 1.

Glucose 6-phosphatase was measured only in the B strain of rats. The activity was higher with diet 2 than with diet 1 and highest with diet 3.

Changes in the enzymes of the kidney with changes in diet. In the kidney the end product of gluconeogenesis is glucose rather than glycogen, and Krebs *et al.* (1963) have shown that in this organ the ability to synthesize glucose is influenced by the dietary regime. In the present study, kidneys from strain A animals on diet 2 synthesized carbohydrate from lactate at the rate of 79 μmoles/g. dry wt./hr. This is slightly lower than the value published by Krebs *et al.* (1963) for rats on a normal mixed diet, possibly because the strains of rats were different or, more likely, because of differences in the carbohydrate in the diet.

On a fat-deficient diet (diet 3) the rate of gluconeogenesis from lactate by the kidney increased to 100 μmoles/g. dry wt./hr. It might be expected from this that the activities of enzymes consuming carbohydrate would decrease while those of the gluconeogenic enzymes would rise. As shown in Table 2, however, although pyruvate kinase activity decreases slightly in strain A rats on diet 3 compared with those on diet 2, there is a larger decrease in fructose 1,6-diphosphatase activity.

The amounts of enzymes in strain B were lower than in strain A, but the differences were not so marked as with the liver enzymes. In strain A the increases in pyruvate kinase and fructose 1,6-diphosphatase activities on changing from diet 1 to diet 2 were very similar to those that occurred in the liver of this strain, but strain B showed no difference in pyruvate kinase activity between diet 1 and diet 2, and fructose 1,6-diphosphatase activity was lower with diets 2 and 3 than with diet 1. Glucose 6-phosphatase activity was slightly higher in strain B rats on diets 2 and 3 than in those on diet 1, a similar but less marked change to that which occurred in the livers of this strain.

Changes in activity of heart enzymes with changes

Table 2. *Enzyme activities in rat kidneys from male animals consuming different diets*

The rats, the diets and the expression of the results are as described in Table 1.

Enzyme	Diet 1 Specific activity (μmoles/g. of protein/min.)		Diet 2 Specific activity (μmoles/g. of protein/min.)		Diet 3 Specific activity (μmoles/g. of protein/min.)	
Strain......	A	B	A	B	A	B
Pyruvate kinase	110 ± 11·3	93 ± 12·0	309 ± 44·2	93 ± 3·5	290 ± 31·2	118 ± 6·4
Glucose 6-phosphate dehydrogenase	24 ± 2·2	9·7 ± 0·54	21 ± 2·6	10 ± 0·56	19 ± 2·6	10 ± 0·84
Fructose 1,6-diphosphatase	168 ± 19·8	68 ± 5·6	314 ± 39·3	43 ± 5·3	256 ± 36·2	39 ± 7·3
Glucose 6-phosphatase	—	57 ± 1·9	—	71 ± 0·8	—	72 ± 2·5
Malic enzyme			11 ± 0·45		13 ± 0·99	

Table 3. *Enzyme activities in rat hearts from male animals consuming different diets*

The rats, the diets and the expression of the results are as described in Table 1.

Enzyme	Diet 1 Specific activity (μmoles/g. of protein/min.)		Diet 2 Specific activity (μmoles/g. of protein/min.)		Diet 3 Specific activity (μmoles/g. of protein/min.)	
Strain......	A	B	A	B	A	B
Pyruvate kinase	297 ± 68·9	164 ± 6	798 ± 97	160 ± 3·2	900 ± 122	163 ± 12·9
Glucose 6-phosphate dehydrogenase	3·3 ± 0·47	1·1 ± 0·05	2·5 ± 0·38	1 ± 0·14	2·6 ± 0·4	1·1 ± 0·08
Fructose 1,6-diphosphatase	141 ± 20·6	39 ± 4·4	245 ± 27·3	40 ± 11	261 ± 36·4	38 ± 14·6
Glucose 6-phosphatase	—	4·9 ± 0·54	—	3 ± 0·52	—	4·1 ± 0·15
Malic enzyme			36 ± 4·74		27 ± 2·8	

Table 4. *Metabolic activities in rat liver after different dietary regimes*

The diets were as described in Table 1 and the experiments were carried out with strain B rats. For Expt. 1 two animals were used for each measurement, and in Expt. 2 five animals were used for each measurement. Standard deviations are given for Expt. 2.

		Enzyme activities (μmoles of substrate transformed/min./g. wet wt. of liver)		Acetate incorporation into lipids (μmoles incorporated/hr./g. wet wt. of liver)
Expt. no.	Diet	Glucose 6-phosphate dehydrogenase	ATP citrate lyase	
1	2	4·8	1·4	
	3	22	3·0	
2	2	—	0·8±0·19	0·5±0·05
	3	—	1·6±0·12	1·5±0·11

in diet. Little change was expected in the activities of the heart enzymes and with strain B rats little change was found (Table 3). However, strain A rats showed some changes in enzyme activities that could be correlated with the dietary changes and the enzyme activities were again higher in this strain. The much higher activities of pyruvate kinase and fructose 1,6-diphosphatase that were found in rats on diets 2 and 3 than in those on diet 1 in liver and kidney were found also in the heart.

Changes in the enzymes of fat synthesis. Allman & Gibson (1965) showed that the addition of corn oil to the diet of mice suffering from essential fatty acid deficiency caused a substantial fall in the activities of enzymes concerned with fat synthesis. One of the enzymes that showed this change was glucose 6-phosphate dehydrogenase. The values in Table 4 show that in strain B rats both ATP citrate lyase and glucose 6-phosphate dehydrogenase activities in the liver were increased when essential fatty acids were absent from the diet. Further experiments (Table 4) showed that under the same conditions there was an increase in the incorporation of [1-^{14}C]acetate into lipid.

DISCUSSION

Effect of strain of rat on enzyme response to dietary change. These experiments show that the absolute magnitude of enzyme activity in an organ, and the response of the organ in changing its enzyme pattern with a change in diet, is dependent on the strain of animal used. Possibly because they were initially specific-pathogen-free, the tissue enzyme activities in strain A rats were much larger than in strain B rats, and the response of the tissue enzyme activities to dietary change was also larger. The large changes in the enzyme activities in strain A rats were clearly related to the amount or quality of the carbohydrate or fat in the diet.

Changes of enzymes with diet. In general the changes found in this study are in agreement with those described by Krebs & Eggleston (1965) in short-term experiments and by Carroll (1963, 1964) in experiments with animals kept on different diets for up to 4 weeks. Our animals were kept on their diets for 14 weeks and presumably were in a 'steady state' as far as their dietary intake and enzyme activities were concerned. Strain A rats on diets 1 and 2 ate similar amounts of about 20g. daily and, since the carbohydrate content of the diets was 70 and 77% of the dry wt. respectively, the difference in daily carbohydrate intake was only 1·4g. It is difficult to believe that the liver could respond to the small differences in portal blood glucose concentration that presumably existed on diets 1 and 2. Hearts and kidneys would be even less likely to be affected, since they receive only systemic blood. It seems more likely that the changes in enzyme activity are the result of the change in the type of dietary carbohydrate, e.g. starch to sucrose. Preliminary experiments support this suggestion. Three groups of rats were given diets of 66% of rice starch, 70% of sucrose and 70% of starch respectively. After 3 days the hepatic pyruvate kinase activities of the rats on the 70%-carbohydrate diets relative to the activities of those on the 66%-starch diet were 0·93 (70%-starch diet) and 1·36 (70%-sucrose diet).

Although the type and quantity of dietary carbohydrate is likely to be the main controller of pyruvate kinase activities, it is also possible that other components of the diet exert some control. It must be remembered that the diets producing high pyruvate kinase activities are fat-free and that in the experiments of Krebs & Eggleston (1965) the low-carbohydrate diet, which produces a low pyruvate kinase activity, is also a high-fat (25%) diet.

The increases in fructose 1,6-diphosphatase and glucose 6-phosphatase activities are similar to those found by Carroll (1963, 1964) and are pre-

sumably induced by an increase in blood fructose concentration, although it is likely that much of the fructose is converted into glucose in its passage through the intestine. The magnitude of the increase in fructose 1,6-diphosphatase activity of strain A animals is as great in the kidneys and heart as it is in the liver, although the concentration of fructose in the systemic blood is likely to be much lower than in the portal blood. The enzyme changes might be explained if it is assumed that the intestinal cells, in response to the diet, liberate a chemical messenger that can pass into the blood and affect all cells with which it comes into contact. The smaller response of strain B animals would then reflect a smaller production of the messenger substance by the intestine.

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