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# The Effect of Dietary and Hormonal Conditions on the Activities of Glycolytic Enzymes in Rat Epididymal Adipose Tissue

By E. D. SAGGERSON and A. L. GREENBAUM
*Department of Biochemistry, University College London, Gower Street, London W.C.1*

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1. Measurements were made of the activities of nine glycolytic enzymes in epididymal adipose tissues obtained from rats that had undergone one of the following treatments: starvation; starvation followed by re-feeding with bread or high-fat diet; feeding with fat without preliminary starvation; alloxan-diabetes; alloxan-diabetes followed by insulin therapy. 2. In general, the activities of the glycolytic enzymes of adipose tissue, unlike those of liver, were not greatly affected by the above treatments. 3. The 'key' glycolytic enzymes, phosphofructokinase and pyruvate kinase, were generally no more adaptive in response to physiological factors than other glycolytic enzymes such as glucose phosphate isomerase, fructose diphosphate aldolase, triose phosphate isomerase, glycerol 3-phosphate dehydrogenase, phosphoglycerate kinase and lactate dehydrogenase. 4. Adipose-tissue pyruvate kinase did not respond to feeding with fat in a manner similar to the liver enzyme. 5. Glyceraldehyde phosphate dehydrogenase had a behaviour pattern unlike the other eight glycolytic enzymes studied in that its activity was depressed by feeding with fat and was not restored to normal by re-feeding with a high-fat diet after starvation. These results are discussed in relation to the requirements of adipose tissue for glycerol phosphate in the esterification of fatty acids. 6. A statistical analysis of the results permitted the writing of linear equations describing the relationships between the activities of eight of the enzymes studied. 7. Evidence is presented for the existence of two constant-proportion groups amongst the enzymes studied, namely (i) glucose phosphate isomerase, phosphoglycerate kinase and lactate dehydrogenase, and (ii) triose phosphate isomerase, fructose diphosphate aldolase and pyruvate kinase. 8. Mechanisms for maintaining the observed relationships between the activities of the enzymes in the tissue are discussed.

Adipose tissue readily metabolizes carbohydrate to yield both fatty acids and glycerol, both of which are stored as triglyceride. Under different physiological conditions both the absolute rates of synthesis and the relative proportions of these two products synthesized may vary. Such alterations in the metabolic pattern of the tissue may be reflected by changes in the activities of some, or all, of the enzymes of the lipogenic pathways. (The term 'activity' is used throughout to denote the maximal activity of enzymes measured *in vitro*.) Although several studies have been reported on adaptive changes in epididymal adipose-tissue ATP–citrate lyase, malate dehydrogenase (NADP) and hexose monophosphate-pathway dehydrogenases in response to various dietary and hormonal conditions (Young, Shrago & Lardy, 1964; Wise & Ball, 1964; Kornacker & Ball, 1965; Hollifield & Parson, 1965; Anderson & Hollifield, 1966; McLean, Brown & Greenbaum, 1968), with the exception of several studies confined to the hexokinases (Moore, Chandler & Tettenhorst, 1964; McLean, Brown, Greenslade & Brew, 1966; Katzen, 1966; Borrebaek, 1966) there have been surprisingly few reports concerning measurements of adipose-tissue glycolytic enzyme activities. Measurements of the tissue activities of all the glycolytic enzymes have been reported by Shonk & Boxer (1964) and Shonk, Koven, Majima & Boxer (1964) in rat and human adipose tissues, and by Durr & Dajani (1964) in the tail fat of the Syrian sheep. Measurements of a few glycolytic enzymes were reported for rat epididymal adipose tissue by Weber, Hird, Stamm & Wagle (1965). However, in these cases enzyme measurements were only made under one physiological state. Pogson & Denton (1967) have reported some studies of the effects of dietary changes and alloxan-diabetes on the activities of a small number of epididymal adipose-tissue glycolytic enzymes.

The work of Vogell *et al.* (1959) and Pette, Luh &

Bücher (1962) has led to the suggestion that the tissue activities of several glycolytic enzymes in several mammalian tissues may be in constant proportions to each other. Shonk *et al.* (1964) have proposed the existence of two glycolytic constant-proportion groups in human adipose tissue. In view of the constant-proportion hypothesis, and the proposal by Weber, Singhal, Stamm, Lea & Fisher (1966) that in the liver glycolysis may be, at least partially, regulated by large synchronous alterations in the activities of glucokinase, phosphofructokinase and pyruvate kinase (the 'key' glycolytic enzymes) under the control of a single 'functional genetic unit', it was thought that a study of the relationships between the tissue activities of adipose-tissue glycolytic enzymes over a range of physiological conditions would indicate, first, whether the 'key' glycolytic enzymes were as specially adaptive in adipose tissue as they are suggested to be in liver (Weber *et al.* 1966), and, secondly, whether there is a synchrony or constant proportion between the activities of the 'key' glycolytic enzymes and any other glycolytic enzymes in the adipose tissue.

## MATERIALS AND METHODS

*Chemicals.* All nucleotides, sugar phosphates, enzymes, triethanolamine hydrochloride and tris base were obtained from Boehringer Corp. (London) Ltd., London W.5. Monobarium DL-glyceraldehyde 3-phosphate diethyl acetal and dicyclohexylammonium dihydroxyacetone phosphate dimethyl ketal were converted into glyceraldehyde 3-phosphate and dihydroxyacetone phosphate respectively with Dowex 50 ($H^+$ form) as instructed in the leaflets supplied with these reagents by Boehringer. Sodium salts of fructose 6-phosphate and 3-phosphoglyceric acid were prepared by treatment of the barium salts with equivalent amounts of sodium sulphate. Dithiothreitol was obtained from Calbiochem Ltd., Los Angeles, Calif., U.S.A. Sodium pyruvate was a product of E. Merck A.-G., Darmstadt, West Germany. Insulin was obtained from Boots Pure Drug Co. Ltd., Nottingham, and was a six-times-recrystallized product containing 22·2 units/mg. All other chemicals were of A.R. grade.

*Animals.* Male albino rats of an inbred strain were used in all experiments. The animals were maintained on cube diet 41B (Bruce & Parks, 1949) and supplied with water *ad libitum*.

*Treatment of animals.* All animals weighed between 145 and 155g. at the start of an experiment. Tissues were obtained from animals that had been treated in seven different ways:

(*a*) Control animals. These were maintained throughout on the 41B diet *ad libitum*.

(*b*) Starved animals. All starvation was for a period of 72hr. with water supplied *ad libitum*.

(*c*) Animals starved and re-fed with a high-fat diet. These were starved as above and re-fed with a high-fat diet consisting of: 56% corn oil (Mazola; Brown & Polson Ltd., London E.C.4), 1·1% sucrose, 24% casein, 4% dried yeast, 0·3% wheat germ (Bemax; Vitamins Ltd., London W.6), 9·6% purified wood cellulose (Solka-Floc; Johnson, Jörgensen and Coettre Ltd., London E.C.4), 4% Hawk–Oser salt mixture (Hawk & Oser, 1965), 1% sodium alginate solution (10%, w/v, in water). In all cases re-feeding with this diet was for a period of 72hr.

(*d*) Animals fed with a high-fat diet without previous starvation. Feeding was for 72hr. The high-fat diet was as described above.

(*e*) Animals starved and re-fed with white bread. These were starved as above and re-fed with white bread as a high-carbohydrate diet. The suitability of the bread as a diet was judged by the rate at which rats that were re-fed after starvation increased their body weight. In this respect, white bread was found to be as effective as a previously published low-fat high-carbohydrate diet (Baker, Chaikoff & Schusdek, 1952). Re-feeding with bread was for various lengths of time, depending on the experiment.

(*f*) Alloxan-diabetic animals. These were starved overnight. Alloxan monohydrate (57mg./ml. in 0·1M-sodium acetate buffer, pH4·5) was administered by subcutaneous injection in the morning. Each animal receiving 190mg. of alloxan/kg. body wt. The animals were allowed unlimited access to water and diet 41B. At death (after 48hr.) the concentration of urinary glucose in the bladder was assessed by the use of Clinistix reagent strips (Miles Laboratories Inc., Buckingham, Bucks.). All animals with urinary glucose concentrations of 0·5% or greater were taken as provisionally diabetic and used as such. Blood was removed immediately after death from the dorsal aorta of these animals for blood glucose analysis. These analyses confirmed in all cases that the animals were diabetic. The mean concentrations of blood glucose found for seven estimations was $1105 \pm 152$mg./100ml.

(*g*) Alloxan-diabetic animals injected with insulin after 48hr. These were rendered diabetic as above. The diabetic state was assessed with Clinistix. All those animals with urinary glucose concentrations greater than 0·5% were subcutaneously injected with 2 units of insulin in 0·5ml. of water (1 unit of protamine–zinc–insulin and 1 unit of normal insulin) in the morning. In the evening the animals received another 2 units of protamine–zinc–insulin. The animals were killed the next morning and blood was taken for blood sugar analysis as above. The mean blood sugar concentration found for this group of animals was $350 \pm 119$mg./100ml. (five estimations).

*Preparation of tissue extracts.* Animals were killed by cervical dislocation. The epididymal fat-pads were immediately excised, trimmed free of blood vessels as far as possible, rinsed and directly homogenized in 5ml. of ice-cold 0·15M-KCl in 4mM-NaF–2mM-EDTA–0·5mM-dithiothreitol–20mM-tris–chloride buffer (pH8·0)/g. of tissue. In every case the homogenate was made from the entire epididymal adipose tissue obtained from an animal, with the exception of a small piece of tissue (20–30mg.) that was set aside for determination of tissue nitrogen. Homogenization was in a glass Potter–Elvehjem homogenizer cooled in ice, fitted with a motor-driven Teflon pestle with a clearance of 0·5mm. The homogenate was centrifuged at 105000*g* for 30min. at 2–4° in the no. 50 rotor of a Spinco model L preparative ultracentrifuge. The aqueous fraction was decanted from the centrifuge tube and dialysed for 2hr. at 4° in 8/32in. Visking seamless dialysis tubing that had been previously boiled in 10mM-EDTA. Dialysis was against 100vol. of 4mM-NaF–2mM-EDTA–0·25mM-dithiothreitol–

50mM-tris–chloride buffer, pH 8·0. All dialysed high-speed supernatants were stood in ice until used, and all assays were performed within 6hr., during which time the enzymes were found to lose little activity.

Preliminary experiments showed that certain glycolytic enzymes were extracted in low yield from adipose tissue homogenized in 0·25M-sucrose-based media compared with yields from tissues from the same animals homogenized in 0·15M-KCl-based media. In particular, fructose diphosphate aldolase, triose phosphate isomerase and glyceraldehyde phosphate dehydrogenase were only extracted by 0·25M-sucrose in 22%, 54% and 61% yields respectively compared with controls extracted by 0·15M-KCl; glycerol 3-phosphate dehydrogenase and lactate dehydrogenase, on the other hand, were extracted in 89% and 95% yield respectively. Rehomogenization in a 0·15M-KCl-based medium of the fat-plug obtained by centrifugation of a homogenate made in 0·25M-sucrose yielded appreciable quantities of fructose diphosphate aldolase and, to a smaller extent, of glyceraldehyde phosphate dehydrogenase in the supernatant obtained by centrifugation of this second homogenate. Appreciable quantities of any enzyme could not, however, be extracted by 0·15M-KCl from the fat-plug resulting from the centrifugation of an initial homogenate in 0·15M-KCl. Presumably, with 0·25M-sucrose as homogenization medium certain enzymes are entrained in the fat-plug. The use of 0·15M-KCl appears to prevent this entrainment. In recent years there have been several reports on the association of various glycolytic enzymes with particulate cell fragments (Roodyn, 1956*a*,*b*, 1957; Green *et al.* 1965; Hultin & Westort, 1966). The degree of this association appears to depend on the enzyme concerned, and on factors such as the tissue concentration in the homogenization medium, the pH and the ionic strength of the medium. It is possible that similar effects are noted in this study, although poor yields of fructose diphosphate aldolase from adipose tissues homogenized in 0·25M-sucrose may also be due to an irreversible inactivation of the enzyme (Reshef & Heller, 1969).

Maximal yields of phosphofructokinase were only obtained when certain additions were made to the 0·15M-KCl homogenization medium. NaF was always present at a final concentration of 4mM in homogenization and dialysis media, as recommended by Denton & Randle (1966). The activities of none of the other glycolytic enzymes assayed in the KCl extracts were affected by the presence of NaF. It was also found necessary to buffer the 0·15M-KCl at pH 8·0. This is illustrated by an experiment, summarized in Fig. 1, in which one fat-pad was removed from each of a number of rats and homogenized in 0·15M-KCl medium buffered at pH 7·2. The other fat-pad from each animal was homogenized in one of a range of 0·15M-KCl media buffered at different pH values. The results indicated that recovery of phosphofructokinase from the tissue was pH-dependent and was greatest at pH 7·8–8·0. The recovery from tissues of all of the other glycolytic enzymes studied did not, however, appear to be pH-dependent between pH 6·5 and 8·5. Some evidence was obtained that the poor recovery of phosphofructokinase activity from the tissues below pH 7·8 is due to inactivation, rather than non-release of the enzyme from insoluble cell components. Phosphofructokinase activity in high-speed supernatants prepared at pH 6·6 was rapidly lost on standing in ice (more than 90% of the activity was lost in 8hr., whereas comparable extracts prepared at pH 8·0 only lost 20% of their activity). The rate of this inactivation at pH 6·6 could be lessened by dialysis for 90min. against a pH 6·6 medium containing 1mM-ATP, and almost completely arrested by dialysis for 90min. against a pH 8·0 buffer. Shonk & Boxer (1964) reported the need for a slightly alkaline medium for extraction of phosphofructokinase from rat tissues, and also that phosphofructokinase was stabilized by the presence of ATP in their assay procedure.

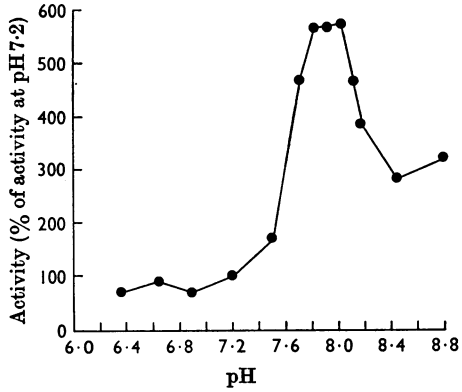


Fig. 1. Effect of varying the pH of the homogenization medium on the recovery of phosphofructokinase from adipose tissues. Tissues were homogenized in 0·15M-KCl in 4mM-NaF–2mM-EDTA–0·5mM-dithiothreitol–10mM-tris–10mM-imidazole buffer, adjusted to the required pH. High-speed supernatants were prepared, and assays for phosphofructokinase activity were performed as described in the text.

Re-extraction with a 0·15M-KCl medium at pH 8·0 of the fat-plug centrifuged out of a pH 6·6 homogenate yielded little phosphofructokinase activity. It is therefore unlikely that phosphofructokinase has a pH-dependent association with particulate components of the homogenate under these conditions.

Freezing of dialysed high-speed supernatants was avoided, since this led to considerable inactivation (more than 90%) of phosphofructokinase.

*Assays of glycolytic enzymes.* All the enzyme assays were coupled to systems involving the oxidation, or reduction, of one of the nicotinamide–adenine dinucleotides. The assay methods used were essentially similar to those previously reported (Wu & Racker, 1959; Shonk & Boxer, 1964; Pogson & Denton, 1967), with minor modifications in some cases. The enzyme measurements were made by recording rates of change in extinction at 340nm. with a Unicam SP.800 recording spectrophotometer at 25°. All reactions were carried out in 3ml. in cuvettes of 1cm. light-path, and in all cases the rates were constant over the measured period (3–5min.). Preliminary experiments indicated that in all cases the rates of reaction were proportional to the amounts of dialysed high-speed supernatant employed, and that the concentrations of substrates and pH of buffers yielded maximal activities. Reactions were begun by addition of the necessary amount of dialysed supernatant. Simultaneous blanks were carried out by addition of tissue extract to an identical cuvette from which a substrate was omitted.

The contents of the 3ml. reaction mixtures used for assay of each enzyme are listed below, together with the amount of dialysed high-speed supernatant generally used in each assay. The pH values quoted refer to the final pH of the cuvette contents after the addition of all substrates and supplementary enzymes.

(*a*) Medium for assay of glucose phosphate isomerase (EC5.3.1.9): 200$\mu$moles of tris–chloride buffer, 10$\mu$moles of $MgCl_2$, 2$\mu$moles of $NADP^+$, 3$\mu$moles of fructose 6-phosphate, 40$\mu$g. of glucose 6-phosphate dehydrogenase; pH7·9; 0·02ml. of dialysed high-speed supernatant.

(*b*) Medium for assay of phosphofructokinase (EC 2.7.1.11): 100$\mu$moles of tris–chloride buffer, 600$\mu$moles of KCl, 15$\mu$moles of $MgCl_2$, 7·5$\mu$moles of glucose 6-phosphate, 6$\mu$moles of AMP, 2$\mu$moles of ATP, 0·5$\mu$mole of NADH, 10$\mu$g. of triose phosphate isomerase, 25$\mu$g. of glycerol 3-phosphate dehydrogenase, 20$\mu$g. of glucose phosphate isomerase, 130$\mu$g. of fructose diphosphate aldolase; pH7·4; 0·2–0·4ml. of dialysed high-speed supernatant.

(*c*) Medium for assay of fructose diphosphate aldolase (EC4.1.2.13): 200$\mu$moles of tris–chloride buffer, 2$\mu$moles of fructose 1,6-diphosphate, 0·5$\mu$mole of NADH, 10$\mu$g. of triose phosphate isomerase, 25$\mu$g. of glycerol 3-phosphate dehydrogenase; pH7·4; 0·1–0·2ml. of dialysed high-speed supernatant.

(*d*) Medium for assay of triose phosphate isomerase (EC5.3.1.1): 200$\mu$moles of tris–chloride buffer, 6·6$\mu$moles of glyceraldehyde 3-phosphate, 1$\mu$mole of NADH, 100$\mu$g. of glycerol 3-phosphate dehydrogenase; pH7·4; 0·005ml. of dialysed high-speed supernatant.

(*e*) Medium for assay of glycerol 3-phosphate dehydrogenase (EC1.1.1.8): 800$\mu$moles of triethanolamine–HCl buffer, 15$\mu$moles of EDTA, 2$\mu$moles of dihydroxyacetone phosphate, 1$\mu$mole of NADH; pH7·4; 0·02ml. of dialysed high-speed supernatant.

(*f*) Medium for assay of glyceraldehyde phosphate dehydrogenase (EC1.2.1.12): 200$\mu$moles of triethanolamine–HCl buffer, 1m-mole of semicarbazide, 10$\mu$moles of EDTA, 10$\mu$moles of $MgCl_2$, 40$\mu$moles of 3-phosphoglycerate 10$\mu$moles of ATP, 1$\mu$mole of NADH, 100$\mu$g. of phosphoglycerate kinase; pH8·0; 0·05–0·10ml. of dialysed high-speed supernatant.

(*g*) Medium for assay of phosphoglycerate kinase (EC 2.7.2.3): 200$\mu$moles of triethanolamine–HCl buffer, 1m-mole of semicarbazide, 10$\mu$moles of $MgCl_2$, 40$\mu$moles of 3-phosphoglycerate, 10$\mu$moles of ATP, 1$\mu$mole of NADH, 100$\mu$g. of glyceraldehyde phosphate dehydrogenase; pH7·4; 0·02ml. of dialysed high-speed supernatant.

(*h*) Medium for assay of pyruvate kinase (EC2.7.1.40): 100$\mu$moles of tris–chloride buffer, 240$\mu$moles of KCl, 50$\mu$moles of $MgCl_2$, 10$\mu$moles of phosphoenolpyruvate (potassium salt), 2$\mu$moles of ADP, 0·5$\mu$mole of NADH, 100$\mu$g. of lactate dehydrogenase; pH7·4; 0·02–0·04ml. of dialysed high-speed supernatant.

(*i*) Medium for assay of lactate dehydrogenase (EC 1.1.1.27): 200$\mu$moles of tris–chloride buffer, 4$\mu$moles of sodium pyruvate, 1$\mu$mole of NADH; pH7·4; 0·02ml. of dialysed high-speed supernatant.

All supplementary enzymes used in the assays were diluted on the day of use from stock solutions in 2·5M-$(NH_4)_2SO_4$ into 4mM-NaF–2mM-EDTA–0·25mM-dithiothreitol–50mM-tris–chloride buffer, pH8·0, and dialysed against 100vol. of the same medium for 5hr. at 4°. This treatment removed virtually all $(NH_4)_2SO_4$ from the enzyme preparations. This precaution was taken since $(NH_4)_2SO_4$ added with the required amounts of supplementary enzymes produced slight inhibitions in many of the assays. Severe inhibition of triose phosphate isomerase was found in the presence of $(NH_4)_2SO_4$. The time-course of the triose phosphate isomerase assay was also found to be non-linear in the presence of $(NH_4)_2SO_4$. Sulphate has also been shown to be an activator of adipose-tissue phosphofructokinase by Denton & Randle (1966).

Semicarbazide was included in the assays for glyceraldehyde phosphate dehydrogenase and phosphoglycerate kinase to trap the glyceraldehyde 3-phosphate formed by the reaction. If this is not done, overestimation of the enzyme activity may result due to the presence in the extracts of an active triose phosphate isomerase and glycerol 3-phosphate dehydrogenase.

*Determination of tissue nitrogen.* Samples (20–30mg.) of fresh adipose tissue were digested in 2·0ml. of nitrogen-free 9M-$H_2SO_4$, and the $(NH_4)_2SO_4$ so produced was estimated as $NH_3$ by the method of Fawcett & Scott (1960), modified by the use of potassium tartrate (Lubochinsky & Zalta, 1954).

*Calculation and expression of results.* Tissue enzyme activities were expressed as $\mu$moles of substrate utilized/hr./pair of fat-pads, or as $\mu$moles/hr./mg. of tissue N. These two methods of expression were thought to be better for comparison of enzyme activities between groups of animals in different dietary states than rates expressed per hr./g. of tissue, since the fat content (and therefore the weight) of the fat-pads varied widely between different dietary conditions. No correction was made for the contribution of the very small amount of adipose-tissue water (Crofford & Renold, 1965) to the total volumes of the homogenates.

## RESULTS

The effects of starvation, starvation and refeeding with a high-carbohydrate or a high-fat diet, feeding with fat, alloxan-diabetes and insulin therapy after alloxan-diabetes on the tissue activities of nine epididymal fat-pad glycolytic enzymes are shown in Tables 1 and 2. With the exception of glyceraldehyde phosphate dehydrogenase, all the enzyme activities appeared to respond similarly to the various treatments. Table 1 shows that enzyme activities per pair of fat-pads were generally decreased by approx. 50% by 72hr. of starvation (with the exception of glyceraldehyde phosphate dehydrogenase). Re-feeding with the bread diet caused gradual restoration of the activities. The feeding with the high-fat diet produced a slightly faster recovery of activity than did the bread diet. Feeding with the high-fat diet without preliminary starvation caused little change in the activities of any of the enzymes, except glyceraldehyde phosphate dehydrogenase. Glycolytic enzyme activities in tissues from alloxan-diabetic animals were decreased by approx. 50% per pair of fat-pads. Treatment of these animals with insulin over 24hr. resulted in a partial restoration of activities to 70–80% of the control value.

Table 1. *Variation of activities of glycolytic enzymes per pair of fat-pads with changes in dietary and insulin status*

Treatment of animals and assay of enzyme activities were as described in the Materials and Methods section. The values given are the means of the determinations ± S.E.M. All results are expressed as μmoles of substrate utilized/hr./pair of fat-pads. The following abbreviations are used in this and subsequent tables: HK, hexokinase; GPI, glucose phosphate isomerase; PFK, phosphofructokinase; ALD, fructose diphosphate aldolase; TPI, triose phosphate isomerase; GPDH, glycerol 3-phosphate dehydrogenase; G3PDH, glyceraldehyde phosphate dehydrogenase; PGK, phosphoglycerate kinase; PK, pyruvate kinase; LDH, lactate dehydrogenase.

| Status of animal | GPI | PFK | ALD | TPI | GPDH | PGK | PK | LDH | G3PDH | No. of animals | Body wt. of animals (g.) | Total wt. of fat-pads (mg.) | N content of pair of fat-pads (mg.) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Control | 694±38 | 20·5±1·3 | 37·3±2·6 | 4820±429 | 1357±104 | 579±31 | 279±20 | 997±70 | 218±26 | 28 | 178±3 | 1262±55 | 4·55±0·25 |
| Starved for 72 hr. | 403±47* | 6·8±1·3* | 16·4±2·7* | 1847±201* | 597±57* | 300±38* | 107±23* | 454±49* | 61±13* | 7 | 121±3 | 594±91 | 2·08±0·54 |
| Starved for 72 hr., re-fed with bread for 24 hr. | 414±31* | 15·4±2·2 | 20·1±2·0* | 2185±194* | 731±73* | 397±30* | 137±19* | 598±50* | 115±28* | 7 | 138±2 | 568±81 | 3·36±0·20 |
| Starved for 72 hr., re-fed with bread for 48 hr. | 498±30* | 15·5±0·9* | 22·7±2·4* | 3511±412* | 811±73* | 380±20* | 198±9* | 674±32* | 76±21* | 7 | 134±2 | 601±36 | 3·66±0·24 |
| Starved for 72 hr., re-fed with bread for 72 hr. | 446±53* | 14·7±1·1* | 28·6±2·2* | 3052±332* | 825±71* | 392±29* | 250±20 | 664±46* | 107±16* | 7 | 137±2 | 612±62 | 4·26±0·34 |
| Starved for 72 hr., re-fed with bread for 96 hr. | 550±47* | 15·9±1·3* | 38·0±9·9 | 3854±294 | 984±89* | 493±47 | 232±19 | 756±68* | 218±43 | 6 | 139±3 | 791±78 | 3·98±0·30 |
| Starved for 72 hr., re-fed with bread for 144 hr. | 758±74 | 24·4±2·7 | 53·1±6·9† | 5498±825 | 1684±206 | 747±76† | 429±55† | 996±95 | 288±61 | 7 | 154±2 | 1178±94 | 4·45±0·25 |
| Starved for 72 hr., re-fed with high-fat diet for 72 hr. | 592±41 | 18·6±1·5 | 32·2±2·3 | 4627±602 | 1100±83 | 580±45 | 236±27 | 974±73 | 67±16* | 7 | 159±2 | 1210±118 | 4·02±0·35 |
| Fed with high-fat diet for 72 hr. | 754±51 | 24·6±1·1† | 42·2±4·3 | 4865±446 | 1398±94 | 572±25 | 328±36 | 1074±43 | 111±14* | 7 | 156±4 | 1464±114 | 4·86±0·25 |
| Alloxan-diabetic (48 hr.) | 371±43* | 12·2±3·1* | 17·5±3·1* | 1470±170* | 646±88* | 382±46* | 151±24* | 560±64* | 94±16* | 7 | 135±4 | 926±84 | 3·11±0·20 |
| Alloxan-diabetic (48 hr.) + insulin (24 hr.) | 536±37* | 18·8±2·2 | 24·0±3·4* | 2290±185* | 1050±126 | 448±30* | 204±24 | 731±69* | 63±27* | 5 | 155±3 | 879±71 | 3·36±0·18 |

* Fisher's $P < 0.05$ for results less than the control value.

† Fisher's $P < 0.05$ for results greater than the control value.

Table 2. *Variation of activity of glycolytic enzymes per mg. of tissue nitrogen with changes in dietary and insulin status*

The values given are the means of the determinations ± S.E.M. All results are expressed as μmoles of substrate utilized/hr./mg. of tissue N.

| Status of animal | GPI | PFK | ALD | TPI | GPDH | PGK | PK | LDH | G3PDH |
|---|---|---|---|---|---|---|---|---|---|
| Control | 156±6 | 4·56±0·21 | 8·23±0·37 | 1078±69 | 298±13 | 131±6 | 62·0±3·3 | 221±9 | 46·6±4·1 |
| Starved for 72 hr. | 266±64 | 4·41±1·17 | 12·30±4·40 | 1254±316 | 413±108 | 199±47 | 47·3±10·1 | 304±73 | 28·3±18·0 |
| Starved for 72 hr., re-fed with bread for 24 hr. | 124±8* | 4·57±0·57 | 6·17±0·28* | 657±58* | 247±19* | 119±8 | 40·7±4·6* | 179±11* | 32·6±6·7 |
| Starved for 72 hr., re-fed with bread for 48 hr. | 138±6* | 4·29±0·24 | 6·34±0·64* | 972±108 | 223±16* | 105±6* | 54·8±2·5 | 178±10* | 20·0±5·0* |
| Starved for 72 hr., re-fed with bread for 72 hr. | 106±7* | 3·48±0·17* | 6·86±0·56* | 714±60* | 197±16* | 93±5* | 60·0±3·9 | 158±9* | 24·5±2·6* |
| Starved for 72 hr., re-fed with bread for 96 hr. | 139±6 | 4·03±0·17 | 9·17±1·70 | 982±59 | 249±14* | 124±6 | 58·6±2·1 | 190±7* | 54·5±10·2 |
| Starved for 72 hr., re-fed with bread for 144 hr. | 172±15 | 5·56±0·58 | 12·08±1·51† | 1249±175 | 383±44 | 170±16† | 97·3±11·2† | 227±21 | 65·1±13·3 |
| Starved for 72 hr., re-fed with fat diet for 72 hr. | 150±8 | 4·86±0·31 | 8·13±0·38 | 1151±90 | 276±10 | 147±9 | 58·9±4·2 | 246±14 | 17·0±3·7* |
| Fed with fat diet for 72 hr. | 156±10 | 5·13±0·34 | 8·71±0·78 | 1023±116 | 291±23 | 119±6 | 67·9±7·0 | 224±15 | 23·7±4·3* |
| Alloxan-diabetic (48 hr.) | 121±9* | 3·83±0·74 | 5·67±0·76* | 479±38* | 211±22* | 126±12 | 49·0±6·0 | 183±13* | 31·2±4·9* |
| Alloxan-diabetic (48 hr.) + insulin (24 hr.) | 160±11 | 5·69±0·81 | 7·04±0·67 | 688±65* | 311±27 | 134±10 | 61·0±7·1 | 218±19 | 17·9±6·6* |

* Fisher's $P < 0·05$ for results less than the control value.

† Fisher's $P < 0·05$ for results greater than the control value.

Table 3. *Sample correlation coefficients for pairs of glycolytic enzymes*

Sample correlation coefficients were calculated from the data of Table 1.

| Enzyme | GPI | PFK | ALD | TPI | GPDH | PGK | PK | LDH | G3PDH |
|---|---|---|---|---|---|---|---|---|---|
| GPI | | 0·89 | 0·91 | 0·87 | 0·94 | 0·91 | 0·93 | 0·92 | 0·63 |
| PFK | | | 0·83 | 0·80 | 0·88 | 0·87 | 0·89 | 0·91 | 0·57 |
| ALD | | | | 0·81 | 0·94 | 0·88 | 0·93 | 0·85 | 0·75 |
| TPI | | | | | 0·85 | 0·88 | 0·84 | 0·90 | 0·58 |
| GPDH | | | | | | 0·91 | 0·94 | 0·90 | 0·77 |
| PGK | | | | | | | 0·91 | 0·90 | 0·68 |
| PK | | | | | | | | 0·84 | 0·74 |
| LDH | | | | | | | | | 0·55 |
| G3PDH | | | | | | | | | |

Expressed per mg. of N (Table 2), the changes in activities were not as great. With few exceptions, all activities lay within 70 and 130% of their respective control values, whatever the status of the animal. The treatments described thus appear to have caused little alteration in the activities of eight of the nine enzymes studied per mg. of tissue N.

The behaviour of glyceraldehyde phosphate dehydrogenase did not, however, fit the response pattern of the other eight enzymes. Starvation produced a greater decrease in activity and restoration of activity on re-feeding with bread was initially somewhat delayed compared with that of the other eight enzymes. Re-feeding with the high-fat diet after starvation did not restore the activity at all, whereas feeding with the high-fat diet without preliminary starvation significantly decreased the activity of this enzyme. The activity of glyceraldehyde phosphate dehydrogenase also did not respond to 24hr. of insulin therapy after alloxan-diabetes. This further differentiated the behaviour of this enzyme from that of the other eight studied.

To investigate the possibility that there may be constant proportions or some other form of linear relationship between the tissue activities of the nine glycolytic enzymes studied, sets of two-variable linear equations were derived governing the regression of the activity of one enzyme on the activity of each of the others in turn for a population of 46 animals selected from the data of Table 1. This population consisted of: seven animals starved for 72hr., seven animals starved and re-fed with bread for 144hr., seven animals starved and re-fed with the high-fat diet for 72hr., seven animals fed with the high-fat diet for 72hr. without preliminary starvation, seven alloxan-diabetic animals, five diabetic animals treated with insulin for 24hr. and six animals randomly selected from the control group. This provided a large population with a wide range of dietary and insulin status.

A sample correlation coefficient ($r$) was calculated for each regression analysis on each pair of enzymes. These correlation coefficients (Table 3) are high and positive (greater than +0·8, except where the activity of glyceraldehyde phosphate dehydrogenase is compared with that of another enzyme); therefore the null hypothesis that there is no linear correlation between the activities of the pairs of enzymes in this population can be rejected with a high degree of certainty in most cases. This treatment, however, only established the likelihood of the existence of positive linear correlations between the tissue activities of the possible 36 different pairs of the nine enzymes. The same experimental findings as were used to produce the results of Table 3 were therefore used in the derivation of the equations of the eight regression planes that simultaneously relate the tissue activity of each enzyme in turn to that of the other seven (the results for glyceraldehyde phosphate dehydrogenase were not used in these calculations). Eight sample multiple correlation coefficients ($r_{y/1,2...k}$) and regression equations are presented in Table 4. All the multiple correlation coefficients are close to unity (a value of 1·00 would be produced if all sample values lay precisely on the regression planes), thus indicating that these eight-variable linear equations should reliably describe the relationships between the tissue activities of the eight adipose-tissue glycolytic enzymes in question. The reliability of the linear equations of Table 4 can also be assessed by using them to predict the tissue activity of each enzyme in turn from the experimentally determined activities of the other seven enzymes. The results of such predictions are shown in Table 5. Estimates of the activities were made by using results from animals of a different experimental group for each enzyme. The experimental groups used were chosen arbitrarily, simply by commencing at the bottom left-hand corner of Table 1 and working diagonally up the table, taking a different experimental group and different enzyme each time. In most cases Table 5 shows that the linear

Table 4. *Multiple correlation coefficients and equations of regression planes relating the activities per pair of fat-pads of eight glycolytic enzymes*

Multiple correlation coefficients and regression equations were calculated from the data of Table 1.

| Enzyme | Multiple correlation coefficient | Compared with other seven enzymes: equation of regression plane |
|---|---|---|
| GPI | 0·968 | GPI = −1·48 PFK + 1·42 ALD + 0·31 LDH − 0·08 PGK − 0·001 TPI + 0·66 PK + 0·045 GPDH + 140 |
| PFK | 0·957 | PFK = −0·0034 GPI − 0·119 ALD + 0·025 LDH + 0·0025 PGK − 0·0015 TPI + 0·0537 PK − 0·0019 GPDH − 1·56 |
| ALD | 0·954 | ALD = 0·0149 GPI − 0·401 PFK + 0·0064 LDH + 0·0056 PGK − 0·001 TPI + 0·0537 PK + 0·0161 GPDH − 5·24 |
| TPI | 0·936 | TPI = −0·2186 GPI − 130·45 PFK − 23·83 ALD + 6·537 LDH + 1·852 PGK + 10·16 PK − 0·712 GPDH − 1273 |
| GPDH | 0·972 | GPDH = 0·247 GPI − 4·579 PFK + 8·551 ALD + 0·573 LDH + 0·0175 PGK − 0·0194 TPI + 1·530 PK − 9 |
| PGK | 0·952 | PGK = −0·110 GPI − 1·52 PFK + 0·717 ALD + 0·274 LDH + 0·0127 TPI + 0·752 PK + 0·0044 GPDH + 123 |
| PK | 0·980 | PK = 0·192 GPI + 6·72 PFK + 1·732 ALD − 0·282 LDH + 0·158 PGK + 0·0147 TPI + 0·0814 GPDH − 32·4 |
| LDH | 0·978 | LDH = 0·495 GPI + 17·75 PFK + 1·530 ALD + 0·319 PGK + 0·0522 TPI − 1·56 PK + 0·168 GPDH + 16 |

Table 5. *Comparison of direct measurements of enzyme activities per pair of fat-pads with estimates calculated from the equations of Table 4*

The results are expressed as the means of estimation ± S.E.M. The 'actual measurements' are the same as those recorded in Table 1, except for the 'control' data.

| Enzyme | Actual measurements | No. of estimations | Calculated values | Status of animals |
|---|---|---|---|---|
| GPI | 536 ± 37 | 5 | 518 ± 41 | Alloxan-diabetic + insulin |
| PFK | 12·2 ± 3·1 | 7 | 13·1 ± 2·1 | Alloxan-diabetic |
| ALD | 42·2 ± 4·3 | 7 | 41·5 ± 4·0 | Fed with high-fat diet for 72 hr. |
| TPI | 4627 ± 602 | 7 | 4456 ± 533 | Starved for 72 hr., re-fed with high-fat diet for 72 hr. |
| GPDH | 1684 ± 206 | 7 | 1677 ± 181 | Starved for 72 hr., re-fed with bread for 144 hr. |
| PGK | 380 ± 20 | 7 | 428 ± 20 | Starved for 72 hr., re-fed with bread for 48 hr. |
| PK | 107 ± 23 | 7 | 114 ± 20 | Starved for 72 hr. |
| LDH | 775 ± 102 | 6 | 848 ± 109 | Control |

equations of Table 4 do, in fact, effectively describe the relationships between the tissue activities of these eight enzymes in animals of various status.

## DISCUSSION

*Effects of dietary and hormonal treatments.* The enzyme activities for control tissues presented in Table 1 are generally somewhat higher than those presented for rat adipose tissue by Shonk & Boxer (1964) and by Pogson & Denton (1967), although the relative proportions of the enzymes appear to be similar. However, allowing for likely variations in animals and assay conditions between this and earlier studies, the measurements are essentially in agreement. Table 1 shows that triose phosphate isomerase [which was not assayed in adipose tissue by Shonk & Boxer (1964) or by Pogson & Denton (1967)] has by far the highest activity in the extracts. As far as we know, the only other reported measurement of triose phosphate isomerase in adipose tissue is that of Durr & Dajani (1964), who, working with the tail fat of the Syrian sheep, found this enzyme to be the most active of 12 glycolytic enzymes assayed. This high activity of triose phosphate isomerase compared with the other glycolytic enzymes has also been found in other rat and human tissues by Shonk & Boxer (1964) and by Shonk *et al.* (1964).

It is of note that the high glycerol 3-phosphate dehydrogenase/lactate dehydrogenase activity ratio found in every physiological state in this study

(1·1–1·4) was similar to the values obtained by Shonk & Boxer (1964) and by Shonk *et al.* (1964) for rat adipose tissue (1·0) and human adipose tissue (1·1) respectively. In other rat and human tissues this value is much smaller (Shonk & Boxer, 1964; Shonk *et al.* 1964; Bücher & Rüssman, 1964). Since the activity of lactate dehydrogenase in adipose tissue is not disproportionately low compared with that of other glycolytic enzymes, the high value of this ratio presumably reflects the special role of glycerol 3-phosphate dehydrogenase in adipose tissue as a supplier of one of the substrates of the esterification process.

It is possible that the tissue activities of the enzymes presented in Tables 1 and 2 may be slightly overestimated, since no allowance was made for any contribution to the enzyme activities by blood trapped in the tissue at the time of homogenization. Schmidt & Schmidt (1960) estimated that the blood content of human adipose tissue was 3%. Shonk *et al.* (1964) suggested that a blood content of this size in adipose tissues from fed rats would generally cause the tissue activities of glycolytic enzymes to be overestimated by 5–10% and, although all reasonable precautions were taken to remove blood from the tissues, it must be concluded that some overestimation of enzyme activities has occurred, particularly in the starved animals.

As far as we know, the only published report dealing with alterations in adipose-tissue glycolytic enzyme activities in response to changes in physiological conditions is that of Pogson & Denton (1967). However, the magnitude and direction of changes produced by starvation, starvation and re-feeding and alloxan-diabetes in that study were uncertain, since the measurements presented were made on animals fed with a normal diet and on animals fed with the same diet but which had been previously powdered. In some cases the differences between these two sets of control groups were larger than those between the control and the experimental groups. Nevertheless it is clear from the data of Pogson & Denton (1967) and the present study that the adipose-tissue 'key' glycolytic enzymes are not as responsive to starvation, re-feeding and changes in insulin status as those of liver. In this respect adipose tissue resembles rat heart muscle, in that the phosphofructokinase activity is virtually unchanged by alloxan-diabetes, starvation and hypophysectomy (Pogson & Randle, 1966). The results of Table 2, expressed per mg. of N, demonstrate more clearly how small the changes are, compared with the results expressed per pair of fat-pads in Table 1. The results of this study and that of Pogson & Denton (1967) also show that the changes that are produced in the activities of the adipose-tissue enzymes are not peculiar to the 'key' glycolytic enzymes, but that other glycolytic enzymes respond similarly. Weber *et al.* (1966) showed that starvation and alloxan-diabetes resulted in severe depletion of liver glucokinase, phosphofructokinase and pyruvate kinase. Re-feeding, or insulin therapy, resulted in very rapid restorations of activity: far more rapid than these effects become manifest in adipose tissues. These results led Weber *et al.* (1966) to propose that insulin was a specific inducer of the synthesis of these three hepatic enzymes. However, since Weber *et al.* (1966) did not report any parallel measurements of the activities of other glycolytic enzymes in their studies, a non-specific induction of all the liver glycolytic enzymes cannot be ruled out. Also, work by Takeda, Inoue, Honjo, Tanioka & Daikuhara (1967) has suggested that insulin is not an inducer of hepatic pyruvate kinase, and that the synchrony between the activities of the liver 'key' glycolytic enzymes reported by Weber *et al.* (1966) need not always hold.

Liver also appears to differ from other tissues in its response to feeding of diets low in carbohydrate or high in fat (Vaughan, Hannon & Vaughan, 1960). Krebs & Eggleston (1965) found that feeding with a low-carbohydrate high-fat diet produced considerable decreases in the liver pyruvate kinase activity. Table 1 shows that feeding with a high-fat diet had no such effect in adipose tissue. Re-feeding with the high-fat diet after starvation was as effective as the bread diet in restoring pyruvate kinase activity. This difference in response of liver and adipose-tissue pyruvate kinases to feeding with fat can probably be explained by the finding by Tanaka, Harano, Sue & Morimura (1967) and Pogson (1968) that epididymal adipose-tissue pyruvate kinase is of the 'M' type, rather than the 'L' type, which is more responsive to physiological changes. The special liver form of pyruvate kinase may well reflect the gluconeogenic role of that tissue. Adipose tissue can carry out a partial reversal of glycolysis, as is shown by the incorporation of $^{14}C$ from [$^{14}C$]pyruvate into glyceride glycerol *in vitro* (Reshef, Niv & Shapiro, 1967; Kneer & Ball, 1968). However, Pogson (1968) has suggested that any 'gluconeogenic' demands by adipose tissue could be met by an allosteric interconversion of adipose-tissue pyruvate kinase, rather than by a change in the concentration of enzyme as appears to happen in liver.

Glyceraldehyde phosphate dehydrogenase showed a different behaviour pattern from that of the other glycolytic enzymes in that its activity was lowered by feeding with fat and was not restored by re-feeding with fat after starvation (Table 1). Under such dietary conditions the tissue may be called upon to supply considerable quantities of glycerol phosphate for esterification of incoming fatty acids. A decrease in the activity of glyceraldehyde phosphate dehydrogenase, which is the first enzyme after

a metabolic 'branch point', may thus be associated with a diversion of carbohydrate metabolism away from fatty acid synthesis into glycerol phosphate formation. Whether the fall in enzyme content, leading to a decreased oxidation of triose phosphate, is the primary event or whether it occurs secondarily as a result of some other control decreasing triose phosphate oxidation is not clear. It is noteworthy that the results of Shonk & Boxer (1964) indicate that the activity of glyceraldehyde phosphate dehydrogenase is lower compared with enzymes at the 'hexose end' of glycolysis (phosphoglucose isomerase, aldolase) in rat adipose tissue than in other rat tissues, such as heart or skeletal muscle, which are little concerned with the esterification of fatty acids, but which use carbohydrate for energetic reasons. These findings would seem to offer some support for the view that glycerol phosphate provision in adipose tissue is aided by a low activity of glyceraldehyde phosphate dehydrogenase.

*Possible existence of constant-proportion groups.* The results of Tables 3 and 4 suggest that, when considered in pairs, or all together, the activities of adipose-tissue glucose phosphate isomerase, phosphofructokinase, fructose diphosphate aldolase, triose phosphate isomerase, glycerol 3-phosphate dehydrogenase, phosphoglycerate kinase, pyruvate kinase and lactate dehydrogenase are linearly related to each other. Tables 4 and 5 also demonstrate the reliability of linear equations in describing the relationships between these eight enzymes. Such linear relationships, however, do not necessarily indicate the existence of constant proportions between the activities of these enzymes over the range of physiological conditions studied. In fact, inspection of the results shows that the relative proportions of some enzymes in the tissue will vary as their absolute concentrations are varied as a result of physiological factors. This is illustrated by Fig. 2, which shows the nature of the linear relationship between the activities of two pairs of enzymes, namely pyruvate kinase and fructose diphosphate aldolase, and glucose phosphate isomerase and fructose diphosphate aldolase. Fructose diphosphate aldolase and pyruvate kinase form a constant-proportion group, whereas fructose diphosphate aldolase and glucose phosphate isomerase do not. This is perhaps giving a slightly different interpretation to the term 'constant-proportion group' as originated by Pette *et al.* (1962). Plots of the type shown in Fig. 2 for all of the possible 28 different pairs of these eight enzymes are linear, as indicated by the results of Table 3; the best-fit straight lines in some cases pass through, or very near to, the origin, but in other cases intersect one of the axes at an appreciable distance from the origin, suggesting that a certain proportion of the activity of one of the pair of enzymes is invariable with respect to the other enzyme. In plots of this nature, using the data of Table 1, it appeared that triose phosphate isomerase possessed the least proportion of its activity that was invariable, i.e. plots of triose phosphate isomerase activity on the abscissa and another enzyme activity on the ordinate always gave positive intercepts on the ordinate. The activities per pair of fat-pads that corresponded to these ordinate intercepts, and their values expressed as percentages of the mean control activity for each enzyme, are shown in Table 6. Two groupings emerge from the data of this table. Glucose phosphate isomerase, phosphoglycerate kinase and lactate dehydrogenase have similarly high invariant proportions of their activity with respect to triose phosphate isomerase, and appear to form a constant-proportion group. Triose phosphate isomerase, pyruvate kinase and fructose diphosphate aldolase appear to be similarly related, but with much smaller invariable activities. Glycerol 3-phosphate dehydrogenase and phosphofructokinase do not appear to fit either of these groups. The absolute values of any invariable components cannot be established from the data at hand, since the triose phosphate isomerase itself may possess an invariable component that is masked by the nature of the graphs. Subtraction of the ordinate intercepts of Table 6 from the mean values of Table 1 for each enzyme in each physio-

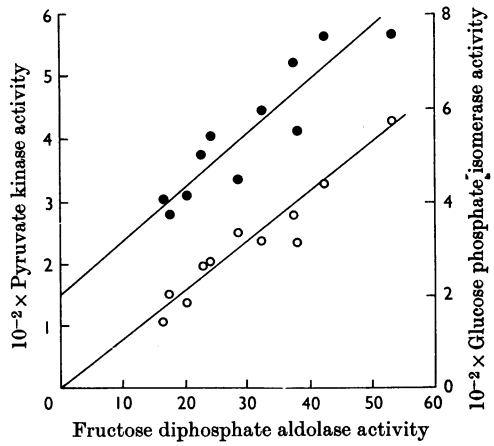


Fig. 2. Linear relationships between the tissue activities of fructose diphosphate aldolase and pyruvate kinase, and fructose diphosphate aldolase and glucose phosphate isomerase, in rat adipose tissues. The data used are those of Table 1. Each point represents the mean of several determinations of each enzyme activity, expressed as $\mu$moles of substrate utilized/hr./pair of fat-pads. ○, Pyruvate kinase against fructose diphosphate aldolase; ●, glucose phosphate isomerase against fructose diphosphate aldolase.

Table 6. *Estimates of invariable portions of enzyme activities compared with triose phosphate isomerase activity*

| Enzyme | Ordinate intercept as μmoles/hr./g./pair of fat-pads | Ordinate intercept expressed as % of total activity of control |
|---|---|---|
| GPI | 200 | 29 |
| PGK | 156 | 27 |
| LDH | 251 | 25 |
| PFK | 3·94 | 19 |
| GPDH | 151 | 11 |
| ALD | 1·25 | 3 |
| PK | 3·4 | 1 |
| TPI | — | 0 |

Table 7. *Sample correlation coefficients for pairs of glycolytic enzymes*

The data used were those of Pogson & Denton (1967). The sample correlation coefficients refer to regression lines calculated for six pairs of experimental values in each case. The experimental values were each the mean of several determinations.

| Enzyme | HK | PFK | ALD | PK |
|---|---|---|---|---|
| HK | | 0·72 | 0·77 | 0·69 |
| PFK | | | 0·99 | 0·97 |
| ALD | | | | 0·98 |

logical state yielded a set of modified activity measurements for each enzyme. These modified activity measurements were found to be linearly related and to be in constant proportion to each other.

Examination of the results of Pogson & Denton (1967) also shows good linear relationships between the reported activities of phosphofructokinase, fructose diphosphate aldolase and pyruvate kinase, although the linearity of plots involving hexokinase was not so pronounced. This is illustrated in Table 7. Plots of these data with pyruvate kinase activity on the abscissa and the activity of another enzyme on the ordinate, similar to those described above, showed that hexokinase has a greater invariable component than phosphofructokinase, which in turn has a greater invariable component than fructose diphosphate aldolase. Pyruvate kinase appeared to have the least invariable component. Thus the degrees of invariability that can be assigned to the activities of phosphofructokinase, fructose diphosphate aldolase and pyruvate kinase from the data of Pogson & Denton (1967) are qualitatively in agreement with the results presented in Table 6, although the small number of experimental points obtained from their results must necessitate a cautious interpretation of the findings.

Various hypotheses could be advanced to explain the finding that some enzymes have portions of their total activity that are invariable over the range of physiological conditions employed in this study. One that appears possible is that some of the enzymes considered may exist in the form of two or more isoenzymes, one or some of which do not respond to the physiological changes produced in this study. The total activities of these enzymes in the tissue would then be composed of a variable and an invariable component, as shown in Table 6. Synchronous alterations in the concentration of the variable component (or isoenzyme) would, owing to each enzyme having a different invariable activity component, produce changes in the proportions of the total activities of the enzymes. These total activities could, however, be related to each other by linear equations of the type shown in Table 4. The variable components of the enzymes are proposed to maintain a constant proportion with each other, perhaps by a co-ordinated synthesis directed by a single genetic unit, whereas the synthesis of the invariable components may be directed by separate genetic units. It should be pointed out that constant proportions between enzyme activities could be achieved without the need to synthesize equimolar amounts of enzymes according to the scheme of Mier & Cotton (1966). It is not known what effects differing rates and modes of enzyme degradation would have upon this isoenzyme model.

Another hypothesis that should be considered is that the whole, or part, of the glycolytic pathway in adipose tissue may operate in more than one compartment of the cell. If the enzymes in only one compartment responded to a physiological stimulus by changing in concentration, the appearance of an overall variable and invariable component could again be produced.

Evidence for either of the above hypotheses is scant. At present hexokinase is the only glycolytic enzyme of rat adipose tissue for which investigation of multiple forms has been undertaken. There have been several reports that the type II (higher-$K_m$) hexokinase of adipose tissue is more variable in response to physiological changes than the type I enzyme (McLean *et al.* 1966, 1967; Borrebaek, 1966; Hansen, Pilkis & Krahl, 1967). A similar behaviour of type II hexokinase has also been demonstrated in rat mammary gland by Walters & McLean (1967).

Some evidence for 'variable' and 'invariable' glycolytic isoenzymes can be found in tissues other than adipose tissues and in organisms other than the rat. Tsoi & Douglas (1964) have found that yeast phosphoglucomutase exists in two forms, which are probably products of different genes since mutation at a particular locus leads to loss of only

one isoenzyme. Ray & Koshland (1962) also found two forms of the muscle phosphoglucomutase. Joshi *et al.* (1967) have demonstrated that the presence of two forms of phosphoglucomutase is apparently a general phenomenon in a wide range of muscle tissues, and that the 50% fall in liver and 25% fall in skeletal-muscle phosphoglucomutase due to starvation in rats can be accounted for by a decrease in the quantity of only one of the two isoenzymes. Yeast phosphoglucose isomerase (Nakagawa & Noltmann, 1967) and rabbit muscle phosphoglycerate kinase (Avramov & Repin, 1965) have been found to exist in multiple forms, and the literature on multiple forms of lactate dehydrogenase is extensive. The data of Table 6 suggest that these three enzymes possess an invariable component, or isoenzyme, in adipose tissue. Also pertinent to this argument is the fact that adipose-tissue pyruvate kinase appears to be only the 'M' type (Tanaka *et al.* 1967; Pogson, 1968), and that the adipose-tissue aldolase shows a very high fructose diphosphate/fructose 1-phosphate activity ratio (E. D. Saggerson & A. L. Greenbaum, unpublished work), similar to that of skeletal muscle, suggesting that adipose tissue possesses only the muscle type of aldolase (Rutter, 1961). The existence of only single forms of these two enzymes would support the data of Table 6. It is noteworthy that Hommes (1966) found the activities of yeast aldolase, triose phosphate isomerase and pyruvate kinase to be the most responsive to changes in glucose concentration in the medium among the yeast glycolytic enzymes.

Evidence for compartmentation of the glycolytic enzymes in adipose tissue, or in any other tissues, is at present even more scant. Landau & Sims (1967) have presented some evidence for the existence of two pools of glucose 6-phosphate in incubated diaphragms. Moses & Lauberg-Holm (1966) have also suggested the existence of separate pools of glucose 6-phosphate in ascites-tumour cells, and also interpret their findings as indicating a complex compartmentation of glycolysis. It is conceivable that the present results may be explicable both in terms of multiple forms of enzymes and compartmentation. Certainly this may be true for the NADP-linked malate dehydrogenases in mouse tissues (Henderson, 1966).

Verification of these suggestions would require studies of multiple forms of adipose-tissue glycolytic enzymes and of glycolytic compartmentation. It is probable that the present suggestions are, at best, simplifications of a complex picture, and that adipose tissue, with its less complicated metabolism, may well possess a simpler system of regulation of enzyme activities than other, more complex, tissues.

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