The Pentose Phosphate Pathway of Glucose Metabolism

HORMONAL AND DIETARY CONTROL OF THE OXIDATIVE AND NON-OXIDATIVE REACTIONS AND RELATED ENZYMES OF THE CYCLE IN ADIPOSE TISSUE

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1. Measurements were made of the activities of the enzymes of the pentose phosphate pathway concerned in both the oxidative (glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase) and the non-oxidative (ribose 5-phosphate isomerase, ribulose 5-phosphate epimerase, transketolase and transaldolase) reactions of this pathway, together with hexokinase and phosphoglucose isomerase, in adipose tissue in a variety of nutritional and hormonal conditions. 2. Starvation for 2 days caused a significant decrease in the activities of all the enzymes of the pentose phosphate pathway, with the exception of glucose 6-phosphate dehydrogenase, when expressed as activity/2 fat-pads; only the activities of ribose 5-phosphate isomerase and ribulose 5-phosphate epimerase were significantly decreased on the basis of activity/mg. of protein. Re-feeding with a high-carbohydrate or high-fat diet for 3 days restored the activity of all the enzymes of the pentose phosphate pathway to the range of the control values, with the exception of transketolase, which showed a marked 'overshoot' in rats re-fed with carbohydrate. Starvation for 3 days caused a marked decrease in the activities of glucose 6-phosphate dehydrogenase and transketolase. 3. On the basis of activity/two fat-pads, alloxan-diabetes caused a marked decrease, to about half the control value, in the activities of all the enzymes concerned in the pentose phosphate pathway, transketolase showing the smallest decrease; hexokinase and phosphoglucose isomerase activities were also decreased. Treatment with insulin for 3 and 7 days raised the activities to normal or supranormal values, transketolase showing the most marked 'overshoot' effect. On the basis of activity/mg. of protein the activity of none of the enzymes was significantly decreased in alloxan-diabetes; transketolase and transaldolase activities were raised above the control values. With insulin treatment for 3 or 7 days the activities of all the enzymes were significantly increased, except that of ribulose 5-phosphate epimerase at the shorter time-interval. Glucagon treatment did not alter any of the enzyme activities expressed on either basis. 4. Thyroidectomy caused a decrease of 30–40% in the activities of enzymes of the pentose phosphate pathway, except for transketolase activity, which fell to 50% of the control value. Little change occurred in adipose-tissue weight or protein content. 5. Adrenalectomy caused a decrease of 40% in the activity of glucose 6-phosphate dehydrogenase and of 20–30% in the activities of the remaining enzymes of the pentose phosphate pathway; hexokinase activity was also decreased. Treatment with cortisone for 3 days did not significantly raise the activity from that found in adrenalectomized rats. Treatment of normal rats with high doses of cortisone had no significant effect on the activities of the enzymes of the pentose phosphate pathway in adipose tissue. 6. The changes in enzyme activities are discussed in relation to: (a) the concept of constant-proportion groups of enzymes; (b) the known changes in the flux of glucose through alternative metabolic pathways; (c) the pattern of change found in liver with similar hormonal and dietary conditions.

The rapid response of adipose tissue to changes in hormonal patterns *in vivo* and *in vitro* or to the
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supply of glucose and lipid indicates that this tissue is of great significance in the overall homeostatic mechanism of the body. The importance of the pentose phosphate pathway in adipose-tissue

metabolism and the hormonal control of this pathway is shown by studies with ^{14}C -labelled glucose (see Ashmore, Cahill & Hastings, 1960; Jeanrenaud, 1961; Ball & Jungas, 1964; Flatt & Ball, 1965). Katz, Landau & Bartsch (1966) have used five different methods for calculating the contribution of the pentose phosphate cycle to glucose metabolism by using labelled glucose and have found that with stimulation by insulin the average value was 25%, in the presence of growth hormone the contribution was 14% and in the presence of adrenaline it was 7%.

Another approach to the problem of the hormonal control of pathways of glucose metabolism in adipose tissue is the measurement of metabolite and cofactor concentrations and the study of activities and properties of the enzymes involved in the two pathways of glucose metabolism (Denton, Yorke & Randle, 1966; McLean, Greenbaum, Brown & Greenslade, 1967; Weber, Banerjee & Ashmore, 1960; Weber, Hird, Stamm & Wagle, 1965; Schmidt & Schmidt, 1960; Durr & Dajani, 1964; Denton & Randle, 1966; Pogson, 1968; Novello & McLean, 1968). The findings that both glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are present in outstandingly high concentrations in adipose tissue, on a protein basis some ten times those in liver (Weber *et al.* 1960, 1965; Schmidt & Schmidt, 1960; Hernandez & Sols, 1963; Hollifield & Parson, 1965; Novello & McLean, 1968) and that these oxidative reactions are under hormonal and dietary control (Ball & Jungas, 1963; Young, Shrago & Lardy, 1964; Hollifield & Parson, 1965; Leveille & Hanson, 1966; Hanson & Ziporin, 1966) are further evidence for the importance of the pentose phosphate pathway in adipose-tissue metabolism.

At present relatively little is known about the activity and control of enzymes catalysing the non-oxidative reactions of the pentose phosphate cycle in adipose tissue, i.e. of ribose 5-phosphate isomerase, ribulose 5-phosphate epimerase, transketolase and transaldolase. These enzymes are of importance in the recycling of pentose phosphate to glucose 6-phosphate and in the formation of ribose 5-phosphate required in such processes as RNA synthesis and nucleotide formation. To determine whether the oxidative and non-oxidative parts of the pentose phosphate cycle changed in parallel, i.e. behaved as a constant-proportion group of enzymes, or varied independently, measurements were made of the activities of all the enzymes of the complete cycle, together with those of hexokinase and phosphoglucose isomerase, in a variety of hormonal and dietary conditions. These include the effects of pancreatic hormones, of pituitary hormones, of thyroxine and of adrenal hormones, together with studies of the whole enzyme pattern

in starved rats and starved rats re-fed with high-carbohydrate or high-fat diets.

The alterations in the activities of enzymes of the pentose phosphate pathway in adipose tissue were also compared with the known changes in the flux of glucose through alternative pathways of metabolism and with the relative pattern of change found in liver in similar hormonal and dietary conditions (Novello, Gumaa & McLean, 1969).

METHODS

Materials. NADP⁺, NAD⁺, NADH, ATP (sodium salt), glucose 6-phosphate (sodium salt), ribose 5-phosphate (barium salt) and fructose 6-phosphate (barium salt) were obtained from Boehringer Corp., London W.5. The barium salts of the sugar phosphates were converted into their potassium salts by treatment with K_2CO_3 ; the excess of carbonate was removed by treatment with HCl and the solutions were adjusted to pH 7.2. Glucose 6-phosphate dehydrogenase (140 units/mg.), glycerol 1-phosphate dehydrogenase-triose phosphate isomerase mixture (the relative activities of the two components being 1:6) (10 mg./ml.) and phosphoglucose isomerase (390 units/mg.) were obtained from Boehringer Corp. L-Thyroxine was purchased from Sigma Chemical Co., St Louis, Mo., U.S.A. All other reagents were standard commercial products with the exception of the following sugar phosphates and enzyme preparations required in the assay of the non-oxidative reactions of the pentose phosphate cycle: erythrose 4-phosphate, ribose 5-phosphate free from pentulose, an equilibrium mixture of ribulose 5-phosphate and xylulose 5-phosphate, partially purified transketolase free from ribose 5-phosphate isomerase and ribulose 5-phosphate 3-epimerase, a preparation of a mixture of ribose 5-phosphate isomerase and ribulose 5-phosphate epimerase, and a preparation of ribose 5-phosphate isomerase free from ribulose 5-phosphate 3-epimerase and transketolase. These were prepared as described by Novello & McLean (1968).

Animals. Hormonal and dietary treatment. (a) Starvation and re-feeding with high-carbohydrate and high-fat diets. Adult male albino rats (initial body wt. 170–190 g.) were used and 26 animals were divided into four groups. A control group of eight animals received *ad lib.* the standard laboratory rat cube diet 41B containing 65% carbohydrate, 16% protein and 3% fat. The remaining animals were starved for 48 hr. Six were then re-fed with a diet high in carbohydrate (white bread; 83% carbohydrate, 14% protein, 3% fat); six received a diet high in fat (60% fat, 1% carbohydrate, 30% protein, 9% cellulose filler) for periods of 72 hr.; and six animals were killed at the end of the starvation period. Further groups of six animals were starved for a longer period (3 days) and then re-fed with the high-carbohydrate diet for 3 days as described above.

(b) Alloxan-diabetes and treatment with insulin. Adult male albino rats (initial body wt. approx. 220–250 g.) were starved for 48 hr. and then alloxan monohydrate was injected subcutaneously in a single dose of 20 mg. of alloxan/100 g. body wt. The alloxan-diabetic rats were then given 2 units of protamine-zinc-insulin daily for the first 5 days after administration of alloxan; this greatly increased the overall survival rate. Thereafter the insulin was withdrawn and the rats were maintained on the stock diet *ad lib.* for

3 weeks. After this period the alloxan-diabetic rats were divided into three groups. One group was maintained without further treatment; the second group received daily injections of protamine-zinc-insulin (2 units) for 3 days; and the third group was treated with 2 units of protamine-zinc-insulin for 7 days.

(c) Glucagon treatment. Young male albino rats (initial body wt. approx. 160 g.) were used. Three doses of glucagon, each of 100 μ g., were administered daily by subcutaneous injection. Control animals received injections of 0.9% NaCl.

(d) Adrenalectomy and treatment with cortisone. Young male albino rats (initial body wt. approx. 160–170 g.) were adrenalectomized or submitted to a sham operation. The adrenalectomized rats were given 1% NaCl in the drinking water and the control rats were pair-fed with the adrenalectomized animals with the normal laboratory stock diet. These animals were used 1 week later. A second group of adrenalectomized rats was given three doses of cortisone (100 μ g. of cortisone hemisuccinate each dose) subcutaneously and killed 3 days later; again a paired-feeding regime was maintained with a separate group of control animals.

(e) Cortisone treatment of normal rats. Adult male albino rats (body wt. approx. 170–190 g.) were used. Cortisone hemisuccinate was given subcutaneously, 5 mg. daily for 3 days, and the animals were killed on the fourth day.

(f) Thyroidectomy. Adult male albino rats (body wt. approx. 150–160 g.) were thyroidectomized or submitted to a sham operation under ether anaesthesia. Since removal of the thyroid gland in rats also entails the removal of the parathyroids embedded in them, calcium gluconate (1%, w/v) was given in the drinking water to alleviate the effects of parathyroidectomy. Control rats were pair-fed with the thyroidectomized rats, since there was a considerable decrease in food intake in the thyroidectomized rats. The animals were killed on day 11 after the operation.

Preparation of tissue homogenates. Adipose-tissue homogenates were usually prepared in a medium containing 0.15M-KCl, 5mM-MgCl₂, 5mM-EDTA and 0.1mM-dithiothreitol, adjusted to pH 7.4 with KHCO₃, by using the proportions 7 ml. of medium/pair of epididymal fat-pads. The homogenate was centrifuged at 100000g for 40 min. at 0° and the supernatant was taken and dialysed, with stirring, for 1–2 hr. against the same extracting medium. This dialysed preparation was used for the determination of all the enzyme activities.

Assay of enzymes. Hexokinase (ATP-D-hexose phosphotransferase, EC 2.7.1.1) was measured essentially by the method of Sharma, Manjeshwar & Weinhouse (1963) with modifications as described by McLean & Brown (1966). A unit of enzyme is defined as the amount catalysing the formation of 1 μ mole of glucose 6-phosphate/min. at 25°. Glucose 6-phosphate dehydrogenase (D-glucose 6-phosphate-NADP oxidoreductase, EC 1.1.1.49) and 6-phosphogluconate dehydrogenase [6-phospho-D-gluconate-NADP oxidoreductase (decarboxylating), EC 1.1.1.44] were measured by the method of Glock & McLean (1953). Ribulose 5-phosphate 3-epimerase (D-ribulose 5-phosphate 3-epimerase, EC 5.1.3.1), ribose 5-phosphate isomerase (D-ribose 5-phosphate ketol-isomerase, EC 5.3.1.6), transketolase (sedoheptulose 7-phosphate-D-glyceraldehyde 3-phosphate glycolaldehydetransferase, EC 2.2.1.1) and transaldolase (sedoheptulose 7-phosphate-D-glyceraldehyde 3-phosphate dihydroxyacetone transferase, EC 2.2.1.2) were

measured as described by Novello & McLean (1968). Phosphoglucose isomerase (D-glucose 6-phosphate ketol-isomerase, EC 5.3.1.9) was assayed by the method of Noltmann (1966). The rates of reduction of NADP⁺ or reoxidation of NADH were measured in a Unicam SP.800 recording spectrophotometer with a constant-temperature cell housing and scale-expansion accessory. In each case a unit of enzyme activity is defined as the amount catalysing the formation of 1 μ mole of product/min. at 25°. The results are given as milliunits/2 fat-pads or milliunits/mg. of protein.

Protein. This was determined in the dialysed high-speed supernatant fractions by the method of Lowry, Rosebrough, Farr & Randall (1951).

Statistical treatment. Results are expressed as units of enzyme activity $\pm$ s.e.m. The differences are regarded as being significant where Fisher's *P* is < 0.05; where *P* is greater than 0.1 this is shown as 'not significant' (N.S.). With adrenalectomized rats the food intake of the individual rats was quite variable, and here comparison was made between the adrenalectomized rat and the pair-fed control by using the method of corresponding pairs.

RESULTS

Effects of starvation and re-feeding with high-carbohydrate and high-fat diets. The results of experiments in which rats were starved for 48 hr. and re-fed with high-carbohydrate or high-fat diets for 3 days are given in Table 1. When considered on the basis of total activity in the two fat-pads it is clear that the activities of all the enzymes of the pentose phosphate pathway decrease significantly, except that of glucose 6-phosphate dehydrogenase, which although 30% lower than the control showed an unusually large variability in activity. Young *et al.* (1964) also report large variations in the activity of glucose 6-phosphate dehydrogenase in starved and re-fed rats. In the present experiments the smallest decrease is seen in transketolase activity, which is only about 20% lower than the control value. Examination of the results expressed as milliunits/mg. of protein shows that the activities of most enzymes of the pentose phosphate pathway are decreased in parallel with changes in adipose-tissue protein content and do not show differences expressed on this basis. The only enzymes whose activities decrease faster than the overall loss of tissue protein are ribose 5-phosphate isomerase and ribulose 5-phosphate 3-epimerase. These two enzymes are the most active of those comprising the pentose phosphate pathway, but even with the extra loss of these enzymes the relative pattern is not greatly changed. Transketolase remains the rate-limiting enzyme of the cycle when the activities are measured under optimum conditions.

Re-feeding with a high-carbohydrate or high-fat diet for 3 days results in restoration of the enzyme activities based on activity in the total fat-pad. Transketolase activity shows a more marked

Table 1. *Effect of starvation and re-feeding with high-carbohydrate and high-fat diets on the activities of enzymes of the pentose phosphate pathway in rat adipose tissue*

The results are expressed as milliunits/2 fat-pads or milliunits/mg. of protein in the supernatant fraction. Mean values $\pm$ s.e.m. are given together with values for Fisher's *P*. Where *P* is greater than 0.1 the value is quoted as N.S. (not significant). The experimental groups included rats starved for 48 hr. and then re-fed with either a high-carbohydrate diet or a high-fat diet for 3 days as described in the Methods section.

Group no.	...	...	...	...	Control	Starved (48 hr.)	Starved (48 hr.), re-fed with high- carbohydrate diet	Starved (48 hr.), re-fed with high- fat diet	Fisher's <i>P</i> for comparison of groups					
									1 versus 2	1 versus 3	1 versus 4	2 versus 3	2 versus 4	3 versus 4
No. of animals	...	...	...	...	1	6	3	4						
Body wt. (g.)	...	...	...	...	183 $\pm$ 6	126 $\pm$ 5	129 $\pm$ 6	166 $\pm$ 3						
Wt. of adipose tissue (2 fat-pads) (g.)	...	...	...	...	1.33 $\pm$ 0.08	0.49 $\pm$ 0.10	0.77 $\pm$ 0.04	1.22 $\pm$ 0.18						
Protein content (mg./2 fat-pads)	...	...	...	...	13.6 $\pm$ 0.6	10.0 $\pm$ 0.4	11.5 $\pm$ 0.5	13.6 $\pm$ 1.4						
Milliunits/2 fat-pads														
Hexokinase	...	...	...	...	115 $\pm$ 8	69 $\pm$ 8	158 $\pm$ 26	165 $\pm$ 20	<0.01	N.S.	<0.05	<0.01	<0.01	<0.01
Glucose 6-phosphate dehydrogenase	...	...	...	...	423 $\pm$ 51	285 $\pm$ 57	469 $\pm$ 72	485 $\pm$ 49	<0.01	N.S.	N.S.	<0.05	<0.05	<0.05
6-Phosphogluconate dehydrogenase	...	...	...	...	640 $\pm$ 49	396 $\pm$ 58	573 $\pm$ 63	590 $\pm$ 52	<0.01	N.S.	N.S.	N.S.	<0.05	<0.05
Transketolase	...	...	...	...	157 $\pm$ 13	121 $\pm$ 12	264 $\pm$ 18	170 $\pm$ 15	0.06	<0.001	N.S.	<0.001	<0.001	0.01
Transaldolase	...	...	...	...	300 $\pm$ 22	204 $\pm$ 23	311 $\pm$ 36	278 $\pm$ 24	0.01	N.S.	N.S.	<0.05	<0.05	0.05
Ribose 5-phosphate isomerase	...	...	...	...	873 $\pm$ 78	439 $\pm$ 34	817 $\pm$ 71	1014 $\pm$ 87	<0.001	N.S.	N.S.	<0.001	<0.001	<0.001
Ribulose 5-phosphate 3-epimerase	...	...	...	...	1890 $\pm$ 208	1000 $\pm$ 86	1700 $\pm$ 108	2367 $\pm$ 213	<0.01	N.S.	N.S.	<0.001	<0.001	<0.001
Phosphoglucoisomerase	...	...	...	...	8820 $\pm$ 1255*	6720 $\pm$ 750	5980 $\pm$ 586	13260 $\pm$ 839	N.S.	0.06	0.01	N.S.	<0.001	<0.001
Milliunits/mg. of protein														
Hexokinase	...	...	...	...	8.32 $\pm$ 0.40	6.88 $\pm$ 0.84	13.4 $\pm$ 1.8	12.1 $\pm$ 0.6	N.S.	<0.05	<0.001	<0.01	<0.001	<0.001
Glucose 6-phosphate dehydrogenase	...	...	...	...	31.4 $\pm$ 2.5	28.0 $\pm$ 4.5	39.9 $\pm$ 5.1	36.6 $\pm$ 0.9	N.S.	N.S.	0.08	0.100	0.09	0.09
6-Phosphogluconate dehydrogenase	...	...	...	...	46.9 $\pm$ 1.7	39.8 $\pm$ 5.9	49.0 $\pm$ 4.0	43.8 $\pm$ 0.8	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.
Transketolase	...	...	...	...	11.5 $\pm$ 0.6	12.0 $\pm$ 1.1	23.0 $\pm$ 1.8	12.6 $\pm$ 0.7	N.S.	<0.001	N.S.	<0.001	N.S.	N.S.
Transaldolase	...	...	...	...	21.9 $\pm$ 0.8	20.4 $\pm$ 2.2	26.6 $\pm$ 2.4	20.6 $\pm$ 0.9	N.S.	0.09	N.S.	0.09	N.S.	N.S.
Ribose 5-phosphate isomerase	...	...	...	...	64.1 $\pm$ 4.1	43.8 $\pm$ 1.9	70.0 $\pm$ 3.3	75.3 $\pm$ 2.5	<0.001	N.S.	0.05	<0.001	<0.001	<0.001
Ribulose 5-phosphate 3-epimerase	...	...	...	...	138 $\pm$ 11	100 $\pm$ 7.8	147 $\pm$ 5	176 $\pm$ 5	<0.05	N.S.	0.010	<0.001	<0.001	<0.001
Phosphoglucoisomerase	...	...	...	...	653 $\pm$ 72*	996 $\pm$ 329	529 $\pm$ 26	1002 $\pm$ 67	N.S.	N.S.	<0.01	N.S.	N.S.	N.S.

* Six values only in these two groups.

increase to well above the control value when rats are re-fed with a high-carbohydrate diet. A tendency to show an 'overshoot' effect is also noted for hexokinase activity with both diets; this is perhaps more clearly seen when the results are expressed as milliunits/mg. of protein.

In the present experiment the enzymes of the pentose phosphate pathway tend to behave as a constant-proportion group of enzymes with the exception of transketolase. It is noteworthy that the high-fat diet is almost as effective as the high-carbohydrate diet in promoting the restoration of the enzymes of this pathway.

Other reports in the literature on changes in glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities in adipose tissue on starvation and re-feeding have shown rather more dramatic changes than those found here, and it was considered that the period of starvation might be critical; periods of 3 or 4 days' starvation were used by Ball & Jungas (1963) and by Young *et al.* (1964). The effects of a 3-day starvation period followed by a 3-day re-feeding period on four key enzymes of the pentose phosphate pathway are shown in Fig. 1. There is a very marked and significant decrease in both glucose 6-phosphate

dehydrogenase and transketolase activities between 2 and 3 days' starvation. The response of 6-phosphogluconate dehydrogenase and transaldolase activities is not so dependent on the time of starvation and little further decrease follows the extra 24 hr. starvation period. The 'overshoot' of transketolase activity when rats starved for 48 hr. are re-fed with a high-carbohydrate diet is abolished when more prolonged periods of starvation are used (see Fig. 1).

Effect of alloxan-diabetes with and without insulin treatment and of glucagon treatment. The results of these experiments are shown in Fig. 2 and Table 2. When the results are expressed as milliunits/2 fat-pads (see Fig. 2) then a very clear picture emerges of a marked decrease, in most cases to less than 50% of the control value, in activity of all the enzymes concerned in the pentose phosphate pathway, including hexokinase and phosphoglucose isomerase. Transketolase, the rate-limiting enzyme of the pathway when measured under optimum conditions, shows the smallest decrease. Insulin causes a marked increase in the activities of all the enzymes; after 3 days of treatment all are restored to normal or supranormal values, a further increase occurring after 7 days of treatment;

Fig. 1. Effect of different periods of starvation (2 or 3 days) and re-feeding with a high-carbohydrate diet for 3 days on the activities of enzymes of the pentose phosphate pathway in adipose tissue. All results are expressed as units/2 fat-pads and are given as percentages of the appropriate control values. Each group contained six rats. The symbols represent: ○, control value; ●, rats starved for 2 days; ▲, rats starved for 2 days and re-fed with a carbohydrate diet for 3 days; ●, rats starved for 3 days; ▲, rats starved for 3 days and re-fed with a carbohydrate diet for 3 days. The vertical lines at each point give the s.e.m. values, for clarity shown in one direction only. The horizontal line is at the control value of 100%. Values related to the period of starvation are shown joined by the solid line; values related to the period of re-feeding are shown joined by the broken line.

Fig. 2. Activities of enzymes of the pentose phosphate pathway and of hexokinase in alloxan-diabetic rats and alloxan-diabetic rats treated with insulin. All results are expressed as units/2 fat-pads and are given as percentages of the appropriate control value. Each group contained six rats. The control value is shown by the horizontal broken line; the S.E.M. is given by the shaded area. The vertical columns are: □, alloxan-diabetic rats; ▨, alloxan-diabetic rats treated with 2 units of protamine-zinc-insulin for 3 days; ▩, alloxan-diabetic rats treated with 2 units of protamine-zinc-insulin for 7 days. The vertical lines give the S.E.M. values, for clarity shown in one direction only. Fisher's *P* values for comparison among different groups are given at the foot of each column.

transketolase showed the most marked 'overshoot' effect.

The results shown in Table 2 indicate whether the enzymes change more or less rapidly than the overall change in tissue protein content. On this basis none of the enzyme activities are significantly decreased in alloxan-diabetes; in fact, transketolase and transaldolase activities appear to decrease more slowly than the amount of tissue protein as a whole, and are significantly raised. With insulin treatment for 3 days all the enzymes are significantly increased on the basis of activity/mg. of protein with the exception of the very active ribulose 5-phosphate epimerase; after treatment with insulin for 7 days the activities of the enzymes of the pentose phosphate pathway, now including ribulose 5-phosphate epimerase, are elevated above both control and alloxan-diabetic values. The marked increase in tissue weight and protein content after insulin treatment are also shown in Table 2.

Glucagon treatment, given by three daily injections each of 100 μ g. for 3 days, fails to alter the activity of any of the enzymes of the pentose phosphate pathway or of hexokinase or phosphoglucose isomerase when calculated as units/2 fat-pads or as milliunits/mg. of protein.

Effect of thyroidectomy on enzymes of the pentose phosphate pathway. The results of experiments in which rats were thyroidectomized and the activity of enzymes of the pentose phosphate pathway were measured in adipose tissue 11 days later are given in Table 3, both as milliunits/2 fat-pads and as milliunits/mg. of protein. The general pattern of change is the same when expressed on either basis, i.e. there is an overall decrease of about 30–40% in the activities of the enzymes measured, for those concerned with both the oxidative and the non-oxidative segments of the pathway. The most striking decrease is found in transketolase activity, which falls to 50% of the control value.

Effect of adrenalectomy and of treatment of adrenalectomized and normal rats with cortisone on enzymes of the pentose phosphate pathway. As shown in Table 4 adrenalectomy causes a decrease of 20–30% in the activities of all the enzymes of the pentose phosphate pathway in adipose tissue. Although the change in ribose 5-phosphate isomerase activity is not statistically significant it shows the same trend as the other enzymes of this pathway. The most marked decrease is in glucose 6-phosphate dehydrogenase activity, which falls by approx. 40%; hexokinase activity is also significantly decreased. Treatment with cortisone hemisuccinate (100 μ g. daily for 3 days) does not significantly alter the pattern from that found in the untreated adrenalectomized group with the exception of ribose 5-phosphate isomerase and ribulose 5-phosphate epimerase activities, which are further depressed.

Partly because of the smaller number of animals in these groups the differences between the adrenalectomized rats treated with cortisone and the appropriate pair-fed control animals are not statistically significant, with the exception of glucose 6-phosphate dehydrogenase activity. However, the enzymes all show approximately the same percentage decrease in activity as is found in the untreated adrenalectomized rats. It seems possible that longer periods of treatment are required to restore the activity of the enzymes in adipose tissue.

The converse of this treatment, administration of high doses of cortisone, was also used. It is found that there is relatively little effect on the enzymes of the pentose phosphate pathway, although there is a tendency for their activities to be increased in constant proportion; transketolase activity increases by 24% and hexokinase activity is also somewhat above normal, being increased by 43%. Although these differences are not statistically significant they do show the opposite trend to that found in the adrenalectomized group and therefore lend some support to the possible involvement of adrenal steroids in control of the pentose phosphate pathway in adipose tissue.

DISCUSSION

Effect of starvation and re-feeding with high-carbohydrate and high-fat diets. Katz *et al.* (1966), using five different methods of calculating the percentage contribution of the pentose phosphate cycle to glucose metabolism, have found that with a normal rat fed *ad lib.* the average value was 16%, whereas in starved-re-fed rats the value rose to 25%. It is interesting to compare the maximum potential rate of an enzyme measured *in vitro* in tissue extracts with the rate achieved *in vivo*, and such a comparison is possible between the present results and those of Katz *et al.* (1966), since these authors have calculated both the rate of glucose phosphorylation and of glucose 6-phosphate dehydrogenase from the results of their radioisotope experiments. The rate of glucose phosphorylation in the intact tissue was calculated to be approx. 3 μ moles/hr./g. and 14 μ moles/hr./g. in the normal and re-fed rats respectively (Katz *et al.* 1966), and the present measurements recalculated on the same basis gave values in adipose-tissue extracts of 10 μ moles/hr./g. and 25 μ moles/hr./g. respectively for the two groups. An even greater excess of potential enzyme activity was revealed for the rate of glucose 6-phosphate dehydrogenase: the results of Katz *et al.* (1966) for the intact tissue showed that the rate was 1.5 μ moles/hr./g. and 11 μ moles/hr./g. for the normal and starved-re-fed rats respectively whereas the extracted enzyme had a

Table 2. *Effect of alloxan-diabetes and insulin treatment on the activities of the pentose phosphate cycle in rat adipose tissue*

Values are given as means $\pm$ S.E.M. together with Fisher's *P* values. Where *P* is greater than 0.1 the value is quoted as N.S. (not significant). The alloxan-diabetic rats were used 3 weeks after administration of alloxan; the insulin-treated rats received daily subcutaneous injections each of 2 units of protamine-zinc-insulin for 3 or 7 days.

Group no.	...	...	...	...	Control group	Diabetic group	Diabetic group treated with insulin for 3 days	Diabetic group treated with insulin for 7 days	Fisher's <i>P</i> values for comparison of groups				
					1	2	3	4	1 versus 2	2 versus 3	2 versus 4	1 versus 4	
No. of animals					6	6	6	6					
Body wt. (g.)					263 $\pm$ 14	171 $\pm$ 9	205 $\pm$ 5	200 $\pm$ 8					
Wt. of adipose tissue (2 fat-pads) (g.)					2.76 $\pm$ 1.38	0.33 $\pm$ 0.10	0.77 $\pm$ 0.11	1.35 $\pm$ 0.15					
Protein content (mg./2 fat-pads)					21.1 $\pm$ 2.7	7.6 $\pm$ 1.2	16.3 $\pm$ 1.0	23.9 $\pm$ 1.7					
Blood sugar (mg./100 ml.)					105 $\pm$ 5	473 $\pm$ 44	299 $\pm$ 58	289 $\pm$ 45					
Millimoles/mg. of protein													
Hexokinase					10.4 $\pm$ 1.3	13.3 $\pm$ 2.2	31.4 $\pm$ 2.9	27.1 $\pm$ 2.1	N.S.	<0.001	<0.001	<0.001	
Glucose 6-phosphate dehydrogenase					30.9 $\pm$ 3.1	29.8 $\pm$ 1.7	67.2 $\pm$ 2.6	55.6 $\pm$ 10.1	N.S.	<0.001	<0.05	<0.05	
6-Phosphogluconate dehydrogenase					34.8 $\pm$ 4.6	38.0 $\pm$ 2.8	65.5 $\pm$ 2.0	52.4 $\pm$ 3.3	N.S.	<0.001	<0.01	<0.001	
Transketolase					7.1 $\pm$ 1.2	11.4 $\pm$ 1.0	18.0 $\pm$ 2.3	21.8 $\pm$ 1.3	N.S.	0.01	<0.001	<0.001	
Transaldolase					16.0 $\pm$ 2.0	22.3 $\pm$ 1.4	32.8 $\pm$ 2.0	34.3 $\pm$ 2.1	<0.05	0.001	<0.001	<0.001	
Ribose 5-phosphate isomerase					29.4 $\pm$ 5.7	17.9 $\pm$ 1.4	55.2 $\pm$ 4.1	47.5 $\pm$ 2.5	<0.001	<0.001	<0.001	<0.001	
Ribulose 5-phosphate 3-epimerase					64.4 $\pm$ 9.1	69.5 $\pm$ 6.4	80.7 $\pm$ 3.0	103 $\pm$ 5.0	N.S.	0.08	<0.01	<0.05	
Phosphoglucose isomerase					988 $\pm$ 202	770 $\pm$ 32	1980 $\pm$ 25	920 $\pm$ 20	N.S.	<0.001	<0.001	<0.01	N.S.

Table 3. *Effects of thyroidectomy on the activities of enzymes of the pentose phosphate pathway in adipose tissue*

Adipose-tissue extracts from normal and thyroidectomized rats were prepared in 7 ml. of medium containing 0.15M-KCl and 0.16M-KHCO₃. The whole homogenate was centrifuged at 100000g for 40 min. and the enzyme activities and protein contents were determined on the dialysed supernatant fraction. The control rats were pair-fed with the thyroidectomized group. The results are given as means $\pm$ S.E.M. together with Fisher's *P* values.

No. of animals	Control group 6	Thyroidectomized group 6	Fisher's <i>P</i>
Body wt.	156 $\pm$ 5	152 $\pm$ 5	
Wt. of adipose tissue (2 fat pads) (g.)	0.70 $\pm$ 0.01	0.59 $\pm$ 0.07	
Protein content (mg./2 pads)	11.6 $\pm$ 0.5	12.0 $\pm$ 0.7	
Milliunits/2 pads			
Glucose 6-phosphate dehydrogenase	826 $\pm$ 35	533 $\pm$ 26	< 0.001
6-Phosphogluconate dehydrogenase	722 $\pm$ 49	463 $\pm$ 55	< 0.01
Ribose 5-phosphate isomerase	868 $\pm$ 59	618 $\pm$ 70	< 0.05
Transketolase	284 $\pm$ 18	143 $\pm$ 24	< 0.001
Transaldolase	320 $\pm$ 18	204 $\pm$ 26	< 0.05
Milliunits/mg. of protein			
Glucose 6-phosphate dehydrogenase	72.5 $\pm$ 4.6	46.9 $\pm$ 2.2	< 0.001
6-Phosphogluconate dehydrogenase	60.8 $\pm$ 4.1	38.3 $\pm$ 4.5	< 0.01
Ribose 5-phosphate isomerase	74.8 $\pm$ 5.3	52.1 $\pm$ 6.0	0.01
Transketolase	24.0 $\pm$ 1.2	12.0 $\pm$ 1.0	< 0.001
Transaldolase	26.3 $\pm$ 2.6	16.3 $\pm$ 2.1	0.01

maximum velocity of 38 μ moles/hr./g. and 73 μ moles/hr./g. for these two groups.

Starvation and feeding with a high-fat diet decrease lipogenesis in adipose tissue and decrease glucose oxidation markedly. Lipogenesis is increased to the highest level on a carbohydrate fat-free diet (Hausberger & Milstein, 1958; Jeanrenaud & Renold, 1960; Jeanrenaud, 1961; Masoro, 1962). The present observations that the enzymes of the pentose phosphate pathway are almost equally restored by re-feeding with a high-carbohydrate or high-fat diet point away from adaptive changes in the enzymes of this pathway as a major factor in the control of adaptive hyperlipogenesis (Tepperman & Tepperman, 1965; Vagelos, 1964; Numa, Bortz & Lynen, 1965).

The changes in glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase activities are largely in accord with the findings of Ball & Jungas (1963), Young *et al.* (1964) and Hanson & Ziporin (1966); however, the magnitude of the changes in these enzyme activities on starvation and re-feeding is clearly related to the age of the rat (Ball & Jungas, 1963; Gellhorn & Benjamin, 1965; Rudman & DiGirolamo, 1967), and differences in the degree of response between the present results and those reported in the literature may perhaps be ascribed to this factor. The decrease in hexokinase activity is in accord with the findings of Moore, Chandler & Tettenhorst (1964), Katzen & Schimke (1965) and Gromova & Ilyin, (1965).

It seems that the overall pattern of response of

the pentose phosphate pathway is for the enzymes to behave as a constant-proportion group in these different nutritional conditions (Pette, Klingenberg & Bücher, 1962; Pette, Luh & Bücher, 1962), although transketolase, the rate-limiting enzyme, does tend to show some differences from the group as a whole when the animals are re-fed with a high-carbohydrate diet, a point of contrast with the liver enzymes examined under identical conditions (Novello *et al.* 1969). A further point of contrast between the behaviour of liver and adipose-tissue enzymes is that the well-known 'overshoot' effect was observed for liver glucose 6-phosphate dehydrogenase but not for the adipose-tissue enzyme in rats fed with the high-carbohydrate diet. An adaptive change, which is difficult to explain at the moment, is the more marked increase in phosphoglucose isomerase in rats fed with a high-fat diet that is seen in both liver and adipose tissue (Novello *et al.* 1969).

Starvation for 2-3 days significantly depressed the synthesis of RNA in rat adipose tissue; re-feeding the starved rat with glucose repaired the defect in RNA biosynthesis before the biosynthesis of monoenoic fatty acids was completely restored (Benjamin & Gellhorn, 1966). The changes may be correlated with changes in the activity of enzymes of the pentose phosphate pathway since an important function of this route of glucose metabolism is the supply of ribose 5-phosphate for RNA synthesis. It is not yet clear how far the oxidative and non-oxidative reactions contribute to ribose 5-phosphate synthesis in adipose tissue; in liver and

Table 4. *Effect of adrenalectomy and treatment with cortisone on the activities of enzymes of the pentose phosphate pathway in rat adipose tissue*

The results, as means $\pm$ s.e.m., are expressed as milliunits/2 fat-pads or milliunits/mg. of protein in the supernatant fraction. The control rats were pair-fed with the adrenalectomized or adrenalectomized+cortisone-treated group and here the Fisher's *P* values are given for the corresponding pairs of animals. For the control group versus the cortisone-treated group the normal Fisher's *P* value is given. The rats were adrenalectomized and 1 week later were given 100 μ g. of cortisone hemisuccinate daily for 3 days. Cortisone treatment of intact rats was by subcutaneous administration of 5 mg. of cortisone hemisuccinate daily for 3 days (see the Methods section).

No. of animals	Adrenalectomized				Adrenalectomized + cortisone-treated				Fisher's <i>P</i>	
	Control	Adrenalectomized	Fisher's <i>P</i>	Control	+ cortisone-treated	Fisher's <i>P</i>	Control	Cortisone-treated	Fisher's <i>P</i>	
Body wt. (g.)	165 $\pm$ 4	164 $\pm$ 5		170 $\pm$ 4	168 $\pm$ 4		182 $\pm$ 7	174 $\pm$ 7		
Wt. of adipose tissue (2 fat-pads) (g.)	1.15 $\pm$ 0.11	0.88 $\pm$ 0.12		1.18 $\pm$ 0.14	0.87 $\pm$ 0.04		1.23 $\pm$ 0.12	1.28 $\pm$ 0.15		
Protein content (mg./2 fat-pads)	15.4 $\pm$ 1.1	13.5 $\pm$ 1.0		13.9 $\pm$ 1.1	11.7 $\pm$ 0.4		15.8 $\pm$ 0.6	16.6 $\pm$ 0.8		
Milliunits/2 fat-pads										
Hexokinase	256 $\pm$ 60	188 $\pm$ 40	< 0.05	263 $\pm$ 92	144 $\pm$ 17	N.S.	211 $\pm$ 35	302 $\pm$ 45	N.S.	
Glucose 6-phosphate dehydrogenase	790 $\pm$ 112	477 $\pm$ 83	0.05	656 $\pm$ 190	331 $\pm$ 32	0.05	580 $\pm$ 63	634 $\pm$ 85	N.S.	
6-Phosphogluconate dehydrogenase	885 $\pm$ 79	594 $\pm$ 67	< 0.05	780 $\pm$ 155	532 $\pm$ 30	N.S.	1520 $\pm$ 155	1720 $\pm$ 210	N.S.	
Transketolase	234 $\pm$ 20	160 $\pm$ 24	< 0.001	205 $\pm$ 36	156 $\pm$ 13	N.S.	170 $\pm$ 20	211 $\pm$ 30	N.S.	
Transaldolase	420 $\pm$ 42	287 $\pm$ 36	< 0.001	325 $\pm$ 55	247 $\pm$ 14	N.S.	290 $\pm$ 25	327 $\pm$ 40	N.S.	
Ribose 5-phosphate isomerase	1230 $\pm$ 100	964 $\pm$ 103	N.S.	886 $\pm$ 172	680 $\pm$ 16	N.S.*	890 $\pm$ 45	1046 $\pm$ 140	N.S.	
Ribulose 5-phosphate epimerase	3430 $\pm$ 250	2760 $\pm$ 143	< 0.05	1950 $\pm$ 320	1720 $\pm$ 57	N.S.**	2370 $\pm$ 205	2540 $\pm$ 225	N.S.	
Phosphoglucose isomerase	14040 $\pm$ 1160	11800 $\pm$ 1180	N.S.	13900 $\pm$ 1770	11030 $\pm$ 1080	N.S.	9990 $\pm$ 995	9940 $\pm$ 795	N.S.	

*, ** *P* values for comparison of adrenalectomized + cortisone-treated rats with adrenalectomized rats are * < 0.05 and ** < 0.001 respectively; all other differences are not significant.

HeLa cells it would seem that the non-oxidative route predominates (Hiatt, 1957, 1958; Marks & Fiegelson, 1957).

Effect of alloxan-diabetes with and without insulin treatment and of glucagon treatment. Milstein (1956) studied the oxidation of [$1-^{14}\text{C}$]glucose and [$6-^{14}\text{C}$]glucose by adipose tissue *in vitro*, and from observations on tissues removed from alloxan-diabetic rats and insulin-treated alloxan-diabetic rats he concluded that in the diabetic state the oxidation of glucose by the non-glycolytic pathway was selectively depressed and that insulin treatment restored and enhanced both total oxidative capacity of the tissue and the relative importance of the non-glycolytic pathway. Although there are many difficulties inherent in this method (Landau & Katz, 1965; Flatt & Ball, 1965) studies by these authors, using a variety of different methods of calculation, have shown that the flux of glucose through the pentose phosphate pathway is increased by insulin. Katz *et al.* (1966) have shown that the contribution of the pentose phosphate pathway to total glucose metabolism rose from a control value of 16% to 25% in the presence of insulin. The present results on the changes in the activities of the enzymes of the pentose phosphate pathway in alloxan-diabetic rats and the 'overshoot' in the enzyme activities in the insulin-treated alloxan-diabetic rats are in keeping with the above results from radioisotope experiments.

The present findings that both the oxidative and non-oxidative reactions of the pentose phosphate pathway increase in insulin-treated alloxan-diabetic rats suggest that there is an increased capacity for recycling the ribulose 5-phosphate, formed oxidatively, back to glucose 6-phosphate. Another interpretation might be that there is an increase in the potential rate of ribose 5-phosphate synthesis by both routes, which might be a key factor in RNA synthesis, which may increase in this condition where there is a rapid increase in protein synthesis.

The present results on the changes found in hexokinase activity are in accord with the previous demonstrations of the effect of diabetes and insulin on this enzyme (Gromova & Ilyin, 1965; McLean, Brown, Greenslade & Brew, 1966; Katzen, 1967; McLean, Brown, Walters & Greenslade, 1967).

It is of interest to compare the pattern of response of a group of related enzymes in different tissues exposed to the same hormonal stimulus. With liver and adipose tissue, alloxan-diabetes causes a much more striking decrease in the activities of the enzymes of the pentose phosphate pathway in the latter tissue; this is particularly noticeable for the oxidative enzymes and for transaldolase (see Novello *et al.* 1969). In contrast with this the effect of insulin treatment of alloxan-diabetic rats is very

similar in both tissues; this is particularly true of the 3-day insulin-treatment period where marked 'overshoot' effects are seen and where the profile of response to insulin in the two tissues is very similar (see Fig. 2 of Novello *et al.* 1969 and Fig. 2 of the present paper). Longer periods of treatment with insulin (7 days) result in a return of the liver values towards the control values (see Osborn, Felts & Chaikoff, 1953) but a further increase in adipose-tissue values.

The response to glucagon was different in the two tissues: a significant decrease was found in ribose 5-phosphate isomerase, ribulose 5-phosphate epimerase and transaldolase activities in liver (Novello *et al.* 1969), but no changes were found in adipose tissue.

Effect of thyroidectomy on enzymes of the pentose phosphate pathway. The pattern of change of the activities of the enzymes of the pentose phosphate pathway in adipose tissue in thyroidectomized rats is somewhat similar to that found in liver (Novello *et al.* 1969), with the exception that transaldolase activity was not significantly changed in liver; again the enzymes measured tended to behave as a constant-proportion group of enzymes. There is considerable evidence that lipid synthesis is depressed in adipose tissue from thyroidectomized rats (Masoro, 1962; Jeanrenaud, 1961), and this may be associated with the fall in the rate of the oxidative reactions of the pentose phosphate pathway.

Effect of adrenalectomy and treatment with cortisone. The overall decrease in the activities of hexokinase and of enzymes of the pentose phosphate pathway in adipose tissue from adrenalectomized rats suggests the possibility that the flux of glucose through this pathway may fall and that there might be a concomitant decrease in lipogenesis. This view is strengthened by studies with lactating rat mammary gland, which has many similarities to adipose tissue, in which it has been shown that there is a fall in the oxidation of C-1 of glucose, in the incorporation of labelled glucose into lipid and in the decarboxylation of pyruvate in adrenalectomized rats (Greenbaum & Darby, 1964). However, Jeanrenaud & Renold (1960) have found that the metabolism of specifically labelled glucose to carbon dioxide and fatty acids was similar in tissues from adrenalectomized and normal animals whenever comparable nutritional conditions had been achieved. In the present experiments the rats were used 10 days after adrenalectomy whereas in those of Jeanrenaud & Renold (1960) only 2-5 days elapsed, and it is possible that the discrepancies could be accounted for on this basis.

Comparison of the effects of adrenalectomy on the pattern of change of enzymes related to the pentose

phosphate pathway in adipose tissue and liver reveals a marked difference in response of hexokinase and glucose 6-phosphate dehydrogenase: both these enzyme activities decrease significantly in adipose tissue, whereas in liver hexokinase activity is increased and glucose 6-phosphate dehydrogenase activity remains unchanged (see Table 4 of Novello *et al.* 1969). Thus two key enzymes concerned with the entry of glucose into metabolic pathways show different control characteristics depending on the tissue being studied, even though both tissues are presumably exposed to the same hormonal environment.

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