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The Pentose Phosphate Pathway of Glucose Metabolism

INFLUENCE OF A GROWTH-HORMONE-SECRETING PITUITARY TUMOUR ON THE OXIDATIVE AND NON-OXIDATIVE REACTIONS OF THE CYCLE IN LIVER

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1. Measurements were made of the activities of enzymes of the pentose phosphate cycle, glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, ribose 5-phosphate isomerase, ribulose 5-phosphate epimerase, transketolase and transaldolase, as well as of the related or competing enzymes glucokinase, hexokinase, phosphoglucose isomerase and phosphoglucomutase, in control rats and in rats bearing the growth-hormone- and prolactin-secreting pituitary tumour MtTW5, to study the effect of high endogenous concentrations of growth hormone on this pathway in liver. 2. There was a twofold increase in liver weight. Glucokinase activity/g. of liver decreased to half the control value in the experimental group, although on a total liver basis it remained unchanged. Hexokinase activity increased in parallel with the liver weight, so that the total activity was doubled in rats with a high endogenous concentration of growth hormone. No differences in response were found between heat-stable and heat-labile forms of hexokinase. 3. The activity/g. of liver of the two oxidative enzymes of the pathway decreased slightly in the experimental group, but this was offset by the increase in liver weight, and the resultant effect was a 50% increase in the total activity. 4. Of the non-oxidative enzymes of the cycle the most marked increase on a total liver basis was in ribose 5-phosphate isomerase activity, to 2·5 times the control value. Ribulose 5-phosphate epimerase activity showed the smallest increase. Transketolase and transaldolase activities were also increased. The latter is the rate-limiting enzyme of the non-oxidative reactions of the cycle in these animals. 5. The results are discussed in relation to the glycolytic pathway and synthesis of glycogen, and more particularly to the increased requirement for ribose 5-phosphate for RNA synthesis.

Pituitary hormones have been implicated in the control of the pentose phosphate pathway of glucose metabolism (Huggins & Yao, 1959; Benevenga, Stielau & Freedland, 1964; MacLeod, Bass, Huang & Smith, 1968; Novello, Gumaa & McLean, 1969). It has been shown that hypophysectomy decreases the activities of both glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase and that there is a differential effect of thyroxine on the restoration of the activities of these enzymes, incomplete restoration being found for glucose 6-phosphate dehydrogenase (Huggins & Yao, 1959). These results pointed to a requirement for more than one pituitary hormone in the maintenance and function of the pentose phosphate pathway. The effects of pituitary hormones on transketolase have also been investigated, and it was found that hypophysectomy caused a 50% fall in its activity (Novello *et al.* 1969; Benevenga *et al.* 1964). Thyroxine caused only partial restoration of this enzyme and greater effects were found after treatment with growth hormone (Novello *et al.* 1969).

Despite this evidence for the influence of growth hormone on the activity of enzymes of the pentose phosphate pathway in liver it has nevertheless proved very difficult in the past to show effects of growth hormone on these enzymes in a normal animal, and treatment of adult female rats with large doses of growth hormone failed to produce any marked change in either glucose 6-phosphate dehydrogenase or 6-phosphogluconate dehydrogenase activity (Glock & McLean, 1955). Further, there has been no study of the complete cycle of enzymes of the pentose phosphate pathway including the two oxidative reactions and the non-

oxidative steps, ribulose 5-phosphate 3-epimerase, ribose 5-phosphate isomerase, transketolase and transaldolase, in growth-hormone-treated rats.

A different approach to this problem is provided by the use of rats bearing a transplantable pituitary tumour secreting growth hormone, thus providing an animal exposed to a continuously high concentration of the hormone (MacLeod, Allen & Hollander, 1964*b*; MacLeod, 1965). MacLeod *et al.* (1968) used such animals in a study of the changes in pathways of glucose metabolism and lipid synthesis, and showed that both the specific activity and the total activity of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were increased in rats bearing growth-hormone-secreting tumours. It was decided to extend this work to a study of the complete cycle to determine if the non-oxidative enzymes of the pathway were controlled by growth hormone and, in particular, how the enzymes concerned in the formation and removal of ribose 5-phosphate were influenced in relation to the known effects of this growth-hormone treatment on liver nucleic acid.

METHODS

Animals. The transplantation of the pituitary tumours (MtTW5) into female rats was as described by MacLeod, Allen & Hollander (1964*a*). This pituitary tumour secreted growth hormone and prolactin. The rats were used 5 weeks later when the implanted tumours were approx. 2 cm. × 4 cm. Food and water were allowed *ad lib.* During this period the body weight of the tumour-bearing rats increased considerably more than did that of the control rats and there was a marked increase, almost twofold, in the liver weight (see Table 1).

Materials. The substrates, cofactors and ancillary purified enzymes used were as described previously (Novello & McLean, 1968; Novello *et al.* 1969).

Preparation of tissue homogenates. Liver homogenates were prepared in a medium containing (final concentrations) 0·15 M-KCl, 5 mM-$MgCl_2$, 5 mM-EDTA and 0·1 mM-dithiothreitol adjusted to pH 7·4 with $KHCO_3$, by using the proportions 1 g. of liver to 9 ml. of medium. The homogenate was centrifuged at 100 000*g* for 40 min. at 0° and the supernatant was taken and dialysed, with stirring, for 1–2 hr. against the same extraction medium. This dialysed preparation was used for the determination of all the enzyme activities.

Assay of enzymes. Hexokinase (ATP–D-hexose 6-phosphotransferase, EC 2.7.1.1), the enzyme with a low K_m for glucose, and glucokinase (ATP–D-glucose 6-phosphotransferase, EC 2.7.1.2), the enzyme with a high K_m for glucose, were 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 μmole of glucose 6-phosphate/min. at 25°.

A portion of the dialysed extract was heated at 45° for 1 hr. The insoluble protein was removed by centrifugation and the supernatant fraction used for hexokinase assay. Grossbard & Schimke (1966) showed that type I hexokinase loses no more than 10% of its activity when heated in the absence of glucose, whereas type II loses more than 90% of its activity, and type III loses about 50% of its activity with this same treatment. This was used to distinguish between changes in the various isoenzymes of hexokinase that might occur in the treated animals, and is similar to the procedure used by McLean, Brown, Walters & Greenslade (1967).

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 methods 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 dihydroxyacetonetransferase, 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 measured by the method of Noltmann (1966).

The rate 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 μmole of product/min. at 25°.

Determination of protein. Protein in the dialysed high-speed supernatant fractions was determined by the method of Lowry, Rosebrough, Farr & Randall (1951), with bovine serum albumin as standard.

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 value is $< 0{\cdot}05$; where P is greater than 0·1 this is shown as not significant (N.S.).

RESULTS

The changes in the activities of enzymes of the pentose phosphate pathway and of hexokinase and glucokinase in rats bearing pituitary tumour MtTW5, which secretes predominantly growth hormone, are shown in Table 1 as units/g. of liver and as total activity in the whole liver. A different picture is presented by these two methods of expression because of the marked increase (twofold) in the liver weight in the group with high endogenous concentrations of growth hormone.

Certain of the enzyme activities increased in parallel with the liver weight and thus remained relatively unchanged when expressed as units/g. of liver but were highly significantly increased on the basis of total activity. These were hexokinase and the enzymes for the non-oxidative reactions of the pentose phosphate cycle, ribose 5-phosphate isomerase and transaldolase, which showed the most striking increases, and ribulose 5-phosphate epimerase and transketolase showed somewhat smaller increases. No evidence was found for any dif-

Table 1. *Enzymes of the pentose phosphate pathway in livers of rats bearing the hormone-secreting pituitary tumour MtTW5*

Tissue extracts were prepared in a medium containing (final concentrations) 0·15 M-KCl, 5 mM-$MgCl_2$, 5 mM-EDTA and 0·1 mM-dithiothreitol, adjusted to pH 7·4 with $KHCO_3$. The whole homogenate was centrifuged at 100000*g* for 40 min. and the enzyme activities and protein content were determined in the dialysed supernatant fraction. Enzyme activities are given in units defined as the amount catalysing the formation of 1 μmole of product/min. at 25°. The results are given as means ± S.E.M. Fisher's *P* values are given; where *P* is greater than 0·1 the value is quoted as N.S. (not significant). The experimental animals (growth-hormone-treated group) received transplants of the pituitary tumour MtTW5 5 weeks before being killed for enzyme determinations. The tumours secreted predominantly growth hormone together with some prolactin.

	Enzyme activity (units/g. of liver)			Total enzyme activity (units/whole liver)		
	Control	Growth-hormone-treated group	Fisher's *P* value	Control	Growth-hormone-treated group	Fisher's *P* value
No. of animals	6	6				
Initial body wt. (g.)	155 ± 3	115 ± 3				
Final body wt. (g.)	167 ± 5	212 ± 6	< 0·001			
Liver wt. (g.)	6·55 ± 0·23	13·33 ± 0·47	< 0·001			
Protein content (mg./g. of liver)	95 ± 1	88 ± 9	N.S.			
Glucokinase	2·02 ± 0·12	1·12 ± 0·09	< 0·001	13·2 ± 0·9	14·9 ± 1·0	N.S.
Hexokinase: total (types I + II + III)	0·31 ± 0·05	0·35 ± 0·02	N.S.	2·04 ± 0·36	4·71 ± 0·41	< 0·001
Hexokinase: heat-stable (types I + 0·5 III)	0·23 ± 0·03	0·25 ± 0·02	N.S.	1·48 ± 0·24	3·29 ± 0·29	< 0·001
Hexokinase: heat-labile (types II + 0·5 III)	0·11 ± 0·02	0·11 ± 0·01	N.S.	0·73 ± 0·17	1·42 ± 0·21	< 0·05
Glucose 6-phosphate dehydrogenase	2·07 ± 0·19	1·50 ± 0·22	0·07	13·5 ± 1·3	20·2 ± 3·2	0·08
6-Phosphogluconate dehydrogenase	6·12 ± 0·53	4·35 ± 0·52	< 0·05	40·1 ± 3·9	58·0 ± 7·6	0·05
Ribose 5-phosphate isomerase	3·27 ± 0·18	3·79 ± 0·32	N.S.	21·3 ± 1·1	50·3 ± 4·0	< 0·001
Ribulose 5-phosphate epimerase	6·43 ± 0·69	4·79 ± 0·46	0·07	42·6 ± 5·6	63·9 ± 6·8	< 0·05
Transketolase	2·32 ± 0·03	1·95 ± 0·10	< 0·01	15·2 ± 0·6	25·8 ± 0·7	< 0·001
Transaldolase	1·13 ± 0·01	1·14 ± 0·03	N.S.	7·4 ± 0·3	15·1 ± 0·5	< 0·001
Phosphoglucose isomerase	59 ± 2·5	37 ± 1·8	< 0·001	386 ± 26	495 ± 24	0·01
Phosphoglucomutase	75 ± 3·7	58 ± 6·1	< 0·05	492 ± 35	773 ± 94	< 0·05

ferential effect of the hormone on the various isoenzymes of hexokinase. These were partially resolved by their different stabilities to heating at 45° for 1 hr. in the absence of glucose (Grossbard & Schimke, 1966) and it was clear that the heat-stable forms and heat-labile forms behaved in an exactly similar way. The relative activities of the heat-stable forms (type I + 0·5 type III) to the heat-labile forms (type II + 0·5 type III) remained unchanged. In marked contrast with this, the glucokinase content/g. of liver decreased sharply to half the control value in the livers of rats with high endogenous concentrations of growth hormone, and thus the total activity in the livers of these animals was completely unchanged.

The two oxidative enzymes of the pentose phosphate cycle, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, were intermediate in behaviour, showing a small decrease in activity/g. of liver, which was on the borderline of significance (Table 1). This was compensated for by the increase in liver weight: the activities of these two enzymes were a little less than 50% higher than those of the controls on the basis of total activity in the liver. Phosphoglucose isomerase and phosphoglucomutase could also be included in this category of enzymes, showing only about a 50% increase in activity on the basis of total activity in the liver.

DISCUSSION

The present results, on the activities of enzymes of the pentose phosphate pathway of metabolism and of hexokinase and glucokinase, may be considered in relation to the formation of ribose 5-phosphate required for RNA and DNA synthesis, the formation of NADPH required in reductive synthetic processes such as lipid synthesis, and the glycolytic pathway, gluconeogenesis and glycogen synthesis. These processes have been shown to be influenced by growth hormone and certain aspects of these have been studied in rats bearing the growth - hormone - secreting pituitary tumour MtTW5 (Weber, Singhal, Hird & Furth, 1966; MacLeod *et al.* 1968).

One of the most striking alterations in the present

experiments was the very marked increase in total liver hexokinase activity in the rats with high endogenous concentrations of growth hormone; glucokinase activity remained completely unchanged. This differential effect on the two enzymes that phosphorylate glucose might have a marked influence on the pattern of glucose metabolism by liver under conditions of high and low blood sugar concentrations such as would occur after the ingestion of carbohydrate. The increased activity of the low-K_m hexokinase might lead to more efficient utilization of glucose at starvation concentrations of blood sugar, yielding a greater concentration of glucose 6-phosphate for metabolism by the main oxidative routes. Conversely, the fall in glucokinase activity, which is decreased by 50% when expressed as units/g. of liver, might cause a slower utilization of glucose by liver after the intake of carbohydrate and elevation of blood sugar concentration. This may perhaps be correlated with the fall in glycogen content/g. of liver which is also decreased by 50% in livers of rats bearing the growth-hormone-secreting pituitary tumour MtTW5 (MacLeod *et al.* 1968). In this context the fall in phosphoglucomutase activity/g. of liver found in the present experiments may also be correlated with the decreased glycogen concentration. A relative decrease in glucokinase is also in keeping with the postulated diabetogenic action of growth hormone after prolonged periods of administration (de Bodo & Altszuler, 1958; Marshall & Engel, 1960). Diabetes can be induced in rats bearing the tumour MtT, which secretes growth hormone, adrenocorticotrophic hormone and prolactin, after partial removal of the pancreas (Bates, Scow & Lacy, 1966). The present results suggest the possibility that under conditions where concentrations of growth hormone are raised, such as in starvation, or where the growth hormone/insulin ratio may be increased, as in diabetes (see Hales, 1968; McCann, Dhariwal & Porter, 1968), this hormone may play a role in the control of liver glucokinase and hexokinase and thus of the blood sugar concentration (DiPietro & Weinhouse, 1960; Niemeyer, Clark-Turri, Garces & Vergara, 1962; Walker & Rao, 1964; Blumenthal, Abraham & Chaikoff, 1964; Sols, Sillero & Salas, 1965).

Although the present results form a coherent pattern there are marked discrepancies in the literature about some of these changes. Weber *et al.* (1966) found an increase in glycogen content/g. of liver in animals bearing the growth-hormone-secreting pituitary tumour MtTW5, and MacLeod *et al.* (1968) did not find any decrease in glucokinase activity/g. of liver. It is possible that differences in the homogenization medium or assay system might be responsible for the latter finding, since the present results show a much higher glucokinase activity in the control liver. Further, no distinction was made between the high-K_m and low-K_m enzymes glucokinase and hexokinase in the earlier paper (MacLeod *et al.* 1968). In the present work, hexokinase activity behaved differently from that of glucokinase in the experimental group of animals, being unchanged perg. of liver and significantly raised on a total liver basis (Table 1), perhaps concealing in part any alterations in glucokinase activity.

Another point of action of growth hormone, which has an important role in the control of liver glucose 6-phosphate concentration and blood sugar, is on glucose 6-phosphatase, which has been shown to increase markedly in rats bearing the growth-hormone - secreting pituitary tumour MtTW5 (Weber *et al.* 1966). These authors also showed that fructose diphosphatase activity was increased, which points to a faster rate of gluconeogenesis in these treated animals. Possibly the most marked increase of all was in the activity of malate dehydrogenase (decarboxylating), which increased about tenfold on a total liver basis (Weber *et al.* 1966).

The two oxidative enzymes of the pentose phosphate pathway, glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, show only marginal changes in activity in the rats with high endogenous concentrations of growth hormone. There was a tendency for these enzymes to be decreased in terms of units/g. of liver, an effect offset by the increase in liver weight. The overall effect was an approximately 50% increase in the total liver activity of each of these enzymes. Since in earlier work on these enzymes more marked increases were found (MacLeod *et al.* 1968), it is possible that the whole metabolic response of the animals in the present experiments may be rather less than in the previous experiments; there was also no increase in the total lipid synthesis in livers from the present experimental animals (D. Gompertz, A. L. Greenbaum & R. M. MacLeod, unpublished work). On the basis of the total activities of the oxidative enzymes in liver it seems that the potential rate of ribulose 5-phosphate formation might be increased. However, this would depend on many controlling factors, among them the concentration of long-chain acyl-CoA, which is known to inhibit glucose 6-phosphate dehydrogenase at low concentrations (Eger-Neufeldt, Teinzer, Weiss & Wieland, 1965; Taketa & Pogell, 1966), and, more particularly, on the rate of reoxidation of NADPH, since there is evidence that the supply of $NADP^+$ is a rate-limiting factor for these oxidative reactions in liver as well as in many other tissues (Cahill, Hastings, Ashmore & Zottu, 1958; Brin & Yonemoto, 1958; Wenner, 1959; McLean, 1960).

The non-oxidative reactions of the pentose phosphate cycle show two noteworthy changes; these are the very marked increase (150%) in ribose 5-phosphate isomerase activity and the relatively small increase (50%) in ribulose 5-phosphate 3-epimerase activity. The probable effect of this change of pattern would be to promote the formation of ribose 5-phosphate at the expense of xylulose 5-phosphate, since these two enzymes compete with each other for the common substrate, ribulose 5-phosphate (see Scheme 1). A branch point, and therefore a control point, of metabolism may be considered to be present here, since ribose 5-phosphate may be converted into phosphoribosyl pyrophosphate and thus directed towards nucleotide and nucleic acid synthesis or may be utilized in the recycling reactions of the pentose phosphate pathway with xylulose 5-phosphate and transketolase. The pattern of enzyme change seems to favour the former route. There seems to be general agreement that RNA and DNA synthesis increases in livers of rats bearing the growth - hormone - secreting pituitary tumour, both from measurements of the concentration of nucleic acid and total nucleic acid of the liver and from measurement of the incorporation of [^{14}C]orotate into nucleic acids (Weber *et al.* 1966; MacLeod *et al.* 1968). Here again interpretation of the results requires more knowledge of the pathways of biosynthesis *in vivo*. There is considerable evidence that the non-oxidative reactions of the pentose phosphate cycle can function in the formation of ribose 5-phosphate from fructose 6-phosphate without intervention of the two oxidative NADP-linked steps (Hiatt, 1957, 1958; Fiegelson & Marks, 1957; Bonsignore, Pontremoli, Mangiarotti, De Flora & Mangiarotti, 1962). Thus the increase in transketolase and transaldolase activities, the latter being the rate-limiting reaction among the non-oxidative reactions in these animals, might also be interpreted as yielding increased amounts of ribose 5-phosphate by way of the reverse reactions of the cycle.

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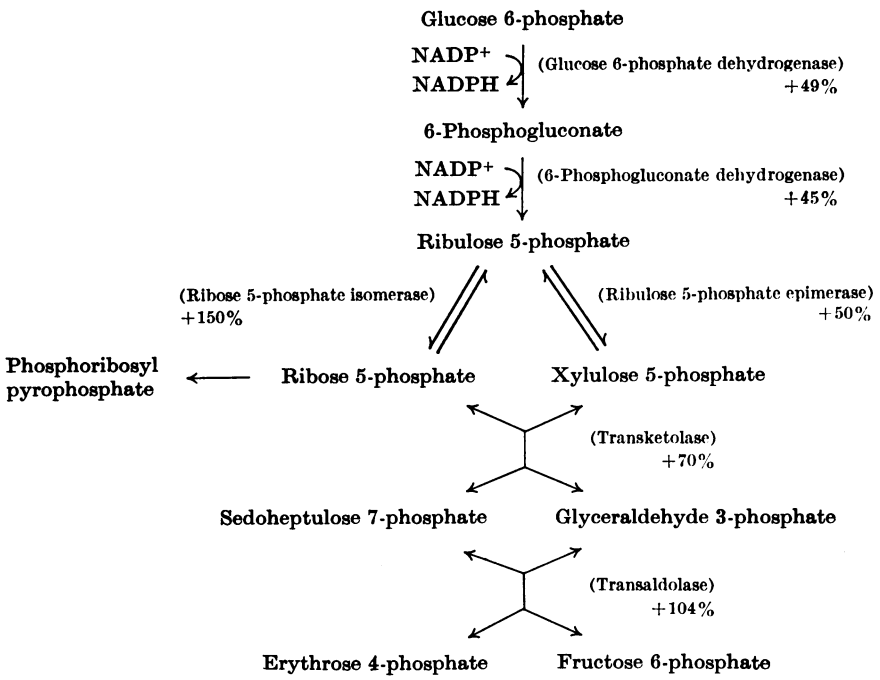

Scheme 1. Diagram of the oxidative and non-oxidative reactions of the pentose phosphate pathway showing branch points of the pentose phosphates. The numbers given below each enzyme name show the percentage change in activity of that enzyme in the experimental group, i.e. the rats bearing the growth-hormone-secreting pituitary tumour MtTW5, compared with the corresponding control value of 100%. These are calculated from the total units of enzyme activity in the whole liver.

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