Levels of Enzymes of the Direct Oxidative Pathway of Carbohydrate Metabolism in Mammalian Tissues and Tumours

By GERTRUDE E. GLOCK AND PATRICIA McLEAN

Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London, W. 1

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In a previous publication from this laboratory (Dickens & Glock, 1951), data of a semi-quantitative nature were given on the distribution of enzymes of the direct oxidative pathway of carbohydrate metabolism in several mammalian tissues. Apart from this work, however, and the quantitative data of Horecker & Smyrniotis (1951) on the 6-phosphogluconate dehydrogenase activities of some acetone-dried tissues, the distribution of the enzymes of this pathway has not been systematically investigated in animal tissues. Following the development of quantitative methods of assay of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (Glock & McLean, 1953) and of methods for quantitatively estimating the breakdown of ribose 5-phosphate, the enzyme activity levels in these consecutive stages of the direct oxidative pathway have been determined in a variety of normal mammalian tissues and tumours. The results are reported in the present paper.

MATERIALS AND METHODS

D-Glucose 6-phosphate (G 6-P), 6-phosphogluconate (6-PG) and D-ribose 5-phosphate (R 5-P). These were available as those preparations of the barium salts used previously (Glock & McLean, 1953).

Triphosphopyridine nucleotide (TPN). This was prepared and analysed as described previously (Glock & McLean, 1953). It contained 75% TPN and no diphosphopyridine nucleotide (DPN).

Preparation of tissue supernatants. All tissues were disintegrated in 9 parts of ice-cold isotonic KCl (0.15 M-KCl containing 8 ml. 0.02 M-KHCO₃/l.), either in a Potter glass homogenizer or in a Nelco homogenizer (Measuring and Scientific Equipment Ltd., London); centrifuging and dialysis of the supernatants were then carried out as before (Glock & McLean, 1953). In the case of erythrocytes, washed cells from 10 ml. heparinized blood were haemolysed and diluted with water up to 10 ml.; 1 part of haemolysate was treated with 9 parts of isotonic KCl and centrifuged and dialysed as above. Plasma was diluted with 9 vol. isotonic KCl and then treated similarly. Since tissue disintegration was carried out in isotonic solution, no corrections were necessary for the dehydrogenase activities of erythrocytes, the degree of haemolysis in all cases being negligible.

Estimation of the activities of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. Dehydro-

genase activities of dialysed tissue supernatants were determined spectrophotometrically according to the method of Glock & McLean (1953) using an amount of enzyme solution which reduced TPN at a rate not exceeding 0.015 μ mole/min. The reaction mixture consisted of 0.5 ml. 0.1 M-MgCl₂, 0.5 ml. 0.25 M glycylglycine buffer (pH 7.6 for G 6-P dehydrogenase and pH 7.6 and 9.0 for 6-PG dehydrogenase), usually 0.02–0.10 ml. tissue supernatant and 0.2 mg. TPN in a total volume of 2.4 ml. The reaction was started by the addition of 0.1 ml. 0.05 M substrate to both cells, the blank mixture not containing TPN.

Breakdown of ribose 5-phosphate. This was followed at 30° in a system containing 0.1 ml. 0.05 M R 5-P (Na salt), 0.5 ml. 0.25 M glycylglycine pH 7.6, 0.5 ml. 0.1 M-MgCl₂ and 0.2 ml. tissue supernatant in a total volume of 2.5 ml. After 10 and 20 min., 0.5 ml. digest was removed and introduced into 4.5 ml. 0.3 N-HClO₄. After spinning, pentose was determined by the orcinol method in 1 ml. portions of the supernatant as described previously (Glock, 1952). Incubated controls were included omitting the substrate. When necessary, pentose values were corrected for sedoheptulose interference, employing Dische's unpublished modification of the orcinol reaction. We are indebted to Dr Z. Dische for details of this method and also to Dr J. K. N. Jones for a sample of sedoheptulosan which was used as a standard.

Two pH activity curves for the breakdown of R 5-P by liver supernatants both in 0.05 M glycylglycine and in 0.01 M tris(hydroxymethyl)aminomethane ('Tris') are given in Fig. 1. The pH optimum in glycylglycine is at pH 7.6 and in 'Tris' at approx. pH 8.0. The effect of variation in enzyme concentration on the rate of breakdown of R 5-P by liver supernatants is shown in Fig. 2. The initial reaction rate is proportional to enzyme concentration, provided that not more than approx. 50% of the R 5-P is broken down.

The intracellular distribution of the enzymes concerned with the breakdown of R 5-P by liver suspensions was determined using the same fractional centrifugation procedure as was employed previously (Glock & McLean, 1953). As in the case of G 6-P and 6-PG dehydrogenases, all of this activity was located in the soluble fraction of liver suspensions.

Units of enzymic activity. A unit is defined as that quantity of enzyme which, in the case of G 6-P and 6-PG dehydrogenases, reduces 0.01 μ mole TPN/min. at 20° (Glock & McLean, 1953), or in the case of R 5-P breakdown, splits 0.01 μ mole R 5-P/min. at 20°. Temperature coefficients, experimentally determined between 20 and 30°, of 1.7 for G 6-P and 6-PG dehydrogenase activities (Glock & McLean, 1953) and of 1.5 for R 5-P breakdown, were used to convert experimental values obtained at room temperature or 30° into the corresponding values at 20°.

Fig. 1. Effect of pH on the breakdown of pentose phosphate by rat liver. Enzymic activity was determined, as described under 'Methods', on 0.2 ml. liver supernatant in 0.05 M glycylglycine buffer, ○—○; and in 0.01 M-tris-(hydroxymethyl)aminomethane ('Tris') buffer, ●—●.

Fig. 2. Enzyme concentration/activity curves for pentose phosphate breakdown by rat liver. Enzyme activity was determined at 30° in 0.05 M glycylglycine buffer as described under 'Methods' with the indicated amounts of rat-liver supernatant.

Enzymic breakdown of TPN. At the end of the G 6-P dehydrogenase assay, Zwischenferment (G 6-P dehydrogenase; Kornberg, 1950) was added and the reduction of TPN followed to completion. In all cases, reduction was almost theoretical, indicating negligible breakdown of TPN by tissue supernatants at room temperature.

Phosphatase. Activity towards G 6-P, 6-PG and R 5-P was tested under the conditions of the assay procedures by following the liberation of inorganic phosphate. In no case was there more than 3% hydrolysis of substrate in 60 min. at 30°.

Resynthesis of hexose monophosphate. This was determined on the HClO₄ supernatants from the R 5-P breakdown experiments at the end of a 6 hr. incubation period, after neutralizing with KOH, cooling to 0°, and removal of KClO₄ by centrifuging (see Slater, 1953). Hexose monophosphate was estimated with Zwischenferment (Kornberg, 1950). As stated previously (Glock, 1952), our preparation of Zwischenferment (cf. Seegmiller & Horecker, 1951) contained isomerase, so that both G 6-P and F 6-P (fructose 6-phosphate) were estimated. The optimum concentration of Mg²⁺ ions, both for resynthesis of hexose monophosphate and for R 5-P breakdown was at a final concentration of 0.02 M, the concentration of Mg²⁺ used in the assay procedure.

RESULTS

Activities of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in tissues

Levels of dehydrogenase activities, expressed in units/g. tissue, in normal mammalian tissues are given in Tables 1 and 2. Since 6-PG dehydrogenase shows optimum activity at pH 9.0 (Glock & McLean, 1953), values for this dehydrogenase are given both at pH 7.6 and pH 9.0. High levels were found for both dehydrogenases in the adrenal glands, particularly in the adrenal cortex, in lymphatic tissue in general (spleen, thymus and lymph nodes), and in whole rat embryos in the early stages of development. In addition, the consistently high values found in the livers of adult female rats and in lactating mammary glands suggest that the levels of activity of both dehydrogenases are correlated with female sex hormones. Very low activities of both enzymes were found in skeletal muscle.

The results for mammary gland tissue in different physiological states of activity (Table 2) are of particular interest. The levels of activity of both dehydrogenases increase rapidly from the end of pregnancy to the end of lactation, this increase being approximately 60-fold for G 6-P dehydrogenase and 20-fold for 6-PG dehydrogenase, and then fall to very low levels in the involuting gland.

The results obtained with a variety of experimentally induced and spontaneous tumours are given in Table 3. All these values fall within the range of levels in normal tissues and are relatively high for the lymphomas and low for sarcomas.

Table 1. Levels of enzymes of the direct oxidative pathway of carbohydrate metabolism in mammalian tissues

(A unit of enzyme activity is defined as that quantity of enzyme which, in the case of G 6-P and 6-PG dehydrogenases, reduces 0.01 μ mole TPN/min. at 20°, or, in the case of R 5-P breakdown, splits 0.01 μ mole R 5-P/min. at 20°. Figures in parentheses are number of animals.)

		Enzyme activity (units/g. tissue at 20°)			
Tissue	Species*	G 6-P dehydrogenase	6-PG dehydrogenase		R 5-P breakdown
			pH 9.0	pH 7.6	
Adrenal gland	Rat (6)	163±25	350±28	185±9	425±29
Adrenal gland	Rabbit (1)	765	420	306	—
Adrenal cortex	Ox (2)	730	315	232	286
Adrenal medulla	Ox (2)	67	114	67	109
Spleen	Rat (4)	305±31	96±7	64±3	287±34
Spleen	Mouse (4)	106±16	84±8	47±3	393±53
Thymus	Rat (6)	98±1	87±9	49±5	359±14
Lymph nodes	Rat (1)	76	76	44	227
Liver	Adult female rat (12)	104±12	290±16	130±12	371±6
Liver	Adult male rat (18)	46±3	147±10	59±8	345±10
Liver	Young female rat (7)	55±9	122±8	62±4	232±17
Liver	Young male rat (7)	71±7	106±6	59±5	208±25
Lung	Rat (5)	88±6	106±7	59±3	282±44
Kidney	Rat (3)	69±7	62±5	56±3	259±9
Kidney cortex	Rabbit (1)	44	79	67	—
Kidney medulla	Rabbit (1)	32	39	27	—
Erythrocytes†	Rat (5)	40±2	34±1	18±0.4	212±11
Ovary	Cow (2)	30	52	35	108
Testis	Rat (4)	30±3	44±9	22±4	224±35
Prostate	Rat (6)	27±4	63±17	35±8	532±77
Seminal vesicles	Rat (4)	7±1	30±2	14±1	104±10
Placenta (20 days pregnancy)	Rat (1)	37	54	32	222
Brain	Rat (5)	32±3	22±1	11±1	75±3
Pituitary	Ox (3)	25	94	54	117
Duodenal mucosa	Rabbit (1)	20	54	58	—
Thyroid gland	Rabbit (1)	16	41	28	—
Cardiac muscle	Rat (6)	26±13	34±7	18±3	149±5
Skeletal muscle	Rat (6)	8±1	15±0.5	8±0.3	91±15
Skeletal muscle	Mouse (4)	19±5	25±8	15±4	209±49
Whole embryos					
10-day embryo	Rat (2)‡	186	140	71	430
20-day embryo	Rat (3)	50±6	45±9	30±1	198±20

* Adult male tissues were used, except where otherwise stated.
† Enzyme activities of erythrocytes are expressed as units/ml. whole blood. Plasma had no activity.
‡ Each value obtained from 7 pooled embryos.

Table 2. Levels of enzymes of the direct oxidative pathway of carbohydrate metabolism in the mammary gland of the rat in pregnancy, lactation and involution

Enzyme activity (units/g. tissue at 20°*)				
State of mammary gland	G 6-P dehydrogenase	6-PG dehydrogenase		R 5-P breakdown
		pH 9.0	pH 7.6	
20 days pregnancy	86	92	50	197
5 days lactation	191	156	131	353
10 days lactation	1728	673	409	940
16 days lactation	2105	800	449	1080
21 days lactation	5452	1734	883	1300
2 days involution	50	93	45	147

* Enzyme activities of the abdominal mammary glands are corrected for retained milk by the method of Folley & Greenbaum (1947).

Table 3. *Levels of enzymes of the direct oxidative pathway of carbohydrate metabolism in tumours*

(Figures in parentheses represent number of animals.)

Tumour	Enzymic activity (units/g. tissue at 20°)			
	G 6-P dehydrogenase	6-PG dehydrogenase		R 5-P breakdown
		pH 9.0	pH 7.6	
Mouse sarcoma 37 (5)	58 ± 11	60 ± 5	54 ± 2	—
Benzpyrene mouse sarcoma (3)	63 ± 14	98 ± 8	60 ± 7	128 ± 6
Benzpyrene mouse epithelioma (1)	126	137	84	289
Mouse squamous-celled carcinoma (3)	72 ± 4	71 ± 3	37 ± 4	121 ± 4
Spontaneous mammary adenocarcinoma C3H mice (1)	103	109	55	480
Mouse-lung carcinoma (1)	19	69	42	202
Spontaneous lymphoma C57 mice (1)				
Lymph nodes	290	140	97	378
Liver	222	153	129	344
Spleen	266	172	110	378
Walker rat carcinoma (1)	65	140	87	254
Spontaneous rat carcinoma (1)	127	193	148	306
A fowl tumour (3)	33 ± 9	63 ± 5	50 ± 5	281 ± 16

Breakdown of ribose 5-phosphate

Values for R 5-P breakdown, as measured by disappearance of pentose, are also included in Tables 1, 2 and 3. With a few exceptions, all the values are very much higher than the corresponding G 6-P and 6-PG dehydrogenase activities, indicating that the enzymes involved in pentose phosphate metabolism do not control rate-limiting steps in this direct oxidative pathway. At least two enzymes were involved in the disappearance of pentose: first, a pentose phosphate isomerase concerned in the interconversion of R 5-P and ribulose 5-phosphate (Horecker & Smyrniotis, 1950; Horecker, Smyrniotis & Seegmiller, 1951); and secondly, a pentose phosphate splitting enzyme, distinct from aldolase, which degrades ribulose 5-phosphate (Horecker & Smyrniotis, 1952; Horecker, Smyrniotis & Klenow, 1953; Racker, De La Haba & Leder, 1953). Pentose phosphate isomerase is clearly not rate-limiting in the present experiments, since ribulose was never detected (by absorption at 540 m μ . in the orcinol reaction) in any of the incubation mixtures at 30°, although it could sometimes be detected at 20°.

Resynthesis of hexose monophosphate from R 5-P (see Horecker, 1953) was found to occur in all the tissues and tumours examined, including skeletal and cardiac muscle. This is in contrast to Sable (1952) who found no hexosemonophosphate formation from R 5-P by aqueous muscle extracts, but only an accumulation of ketopentose 5-phosphate, and suggested that the enzymes catalysing this synthesis were absent from muscle.

DISCUSSION

The most interesting findings arising from this investigation appear to be the very high levels of activity of both glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in the adrenal cortex and in lactating mammary glands. The very low levels of activity in muscle, on the other hand, indicate that this pathway is quantitatively of relatively little importance in this tissue. The metabolic significance of these findings is, however, obscure, and warrants further experimentation, particularly in the cases of the adrenal cortex and of the mammary gland.

The very rapid increase in the levels of activity of G 6-P and 6-PG dehydrogenases in the mammary gland from the end of pregnancy to the end of lactation, followed by a fall in the level of activity in the involuting gland, is not peculiar to these particular dehydrogenases. The same pattern of activity has been demonstrated by Folley & Greenbaum (1947) in the case of arginase activity and milk production, and by Folley & French (1949) for the Q_{O_2} (μ l. O₂/mg. dry wt./hr.) of mammary-gland slices. The pattern here described is different from that of alkaline phosphatase (Folley & Greenbaum, 1947), which increases during pregnancy and then remains constant throughout lactation. Since the weight of the mammary gland, after correcting for the milk content, is virtually constant in the rat throughout lactation (Folley & Greenbaum, 1947), these high values of G 6-P and 6-PG dehydrogenase activities appear to be connected with milk secretion rather than with growth of the gland. These results

also suggest that there might be active competition for glucose between the enzymes of this direct oxidative pathway and the pathway concerned with the synthesis of lactose, or with some other secretory activity of the gland.

Since one of the main functions of this direct oxidative pathway is probably to supply ribose 5-phosphate for incorporation into ribonucleic acid and coenzymes, it would be expected to play an important part in the metabolism of tumours and other rapidly dividing cells. The results of the present investigation, however, indicate that the G 6-P and 6-PG dehydrogenase activities of a variety of experimentally induced and spontaneous tumours all fall within the limits of the same activities in normal tissues, although no adequate controls are available. The present method of approach is limited, however, in that only the maximum enzymic capacities are determined, whereas in the intact cell other regulatory factors probably limit or control the activity of this pathway.

SUMMARY

1. Measurements of the activities of enzymes of the direct oxidative pathway of carbohydrate metabolism have been made in a variety of normal mammalian tissues and tumours.

2. Both glucose 6-phosphate (G 6-P) and 6-phosphogluconate (6-PG) dehydrogenase activities are very high in adrenal cortex, lactating mammary gland, lymphatic tissues in general (spleen, thymus, and lymph nodes), and in whole rat embryos in the early stages of development, and are strikingly low in muscle.

3. In mammary glands, the levels of activity of these two enzymes increase rapidly from the end of pregnancy to the end of lactation and then fall to very low levels in the involuting gland. The increases during lactation are approximately 60-fold

for G 6-P dehydrogenase and 20-fold for 6-PG dehydrogenase.

4. The G 6-P and 6-PG dehydrogenase activities of a variety of experimentally induced and spontaneous tumours fall within the range of normal tissues and are relatively high in lymphomas and low in sarcomas.

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