Biochem. J. (1966) **101**, 48

Glucose Metabolism in the Mucosa of the Small Intestine

ENZYMES OF THE PENTOSE PHOSPHATE PATHWAY

BY L. M. SRIVASTAVA AND G. HÜBSCHER
Department of Medical Biochemistry and Pharmacology, Biology Building, University of Birmingham

(*Received* 25 *April* 1966)

1. The occurrence of five enzymes of the pentose phosphate pathway in cell-free preparations of the mucosa of rat small intestine is described. These enzymes were found to be localized mainly in the supernatant fraction (6240000 *g*-min.). 2. The properties of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were studied with respect to K_m values for substrates and $NADP^+$, pH optima and the effects of *p*-chloromercuribenzoate and palmitoyl-CoA. Higher total and specific activities of these two dehydrogenases were noted in the proximal half of the small intestine of the rat than in the distal half. 3. The specific activities of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in the mucosa of the small intestine of the rat, cat, rabbit and guinea pig were compared. 4. In the rat the specific activities of ribose 5-phosphate isomerase, transketolase and transaldolase were higher in the supernatant fractions from the intestinal mucosa than in those from the liver. 5. The role of the pentose phosphate pathway is discussed in relation to the metabolism of hexose phosphates in the intestinal mucosa.

A considerable amount of work has been done on the enzymes of the pentose phosphate pathway in the liver, kidney, mammary glands and erythrocytes of various animals (Glock & McLean, 1953, 1954; Horecker, Smyrniotis & Klenow, 1953; Newburgh & Cheldelin, 1956; de Lowker & Prankerd, 1961; Noltmann & Kuby, 1963; Horecker, 1964) but there are only very few and preliminary reports on the occurrence of these enzymes in the mucosa of the small intestine. Nigam, Sie & Fishman (1961) examined a number of mammalian tissues including the intestine and demonstrated the presence of an enzyme system synthesizing heptulose phosphate from hexose phosphates and ribose phosphate. By using glucose labelled in specific positions, Landau & Wilson (1959) and White & Landau (1965) provided evidence for the occurrence of the pentose phosphate pathway in the intestinal mucosa of the hamster and man respectively. Further indirect evidence for the occurrence of the enzymes of the pentose phosphate pathway includes the formation of hexose phosphate from ribose 5-phosphate in preparations from the intestinal mucosa of the rat and guinea pig (Bell & Sherratt, 1965) and the formation of lactic acid from ribose 5-phosphate in subcellular preparations of the intestinal mucosa of various animals (Hübscher, Clark, Webb & Sherratt, 1963; Srivastava & Hübscher, 1966).

Though there are preliminary observations on the occurrence of glucose 6-phosphate dehydrogenase (EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44) in the mucosa of the small intestine of the rabbit (Glock & McLean, 1954), cat (Hübscher & Sherratt, 1962) and guinea pig (Hübscher *et al.* 1963), other enzymes of the pentose phosphate pathway have not been demonstrated in this tissue.

The object of the present work was to study the properties of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of the intestinal mucosa and to investigate the occurrence of ribose 5-phosphate isomerase, transketolase and transaldolase in this tissue.

MATERIALS AND METHODS

Animals. Female or male rats weighing 200–250g., adult male rabbits, adult guinea pigs and female and male cats aged from 9 months to 2 years were used.

Chemicals and enzymes. ATP, CoA, $NADP^+$, NADH, glucose 6-phosphate (sodium salt), 6-phosphogluconate (sodium salt), ribose 5-phosphate (sodium salt), fructose 1,6-diphosphate (sodium salt), glucose 6-phosphate dehydrogenase (165 units/mg. of protein), glycerol 3-phosphate dehydrogenase (EC 1.1.1.8) and triose phosphate isomerase (EC 5.3.1.1) were purchased from Sigma Chemical Co., London, S.W. 6. The last two enzymes were supplied as a mixture. Glucose phosphate isomerase (EC 5.3.1.9;

390 units/mg. of protein) and aldolase (EC 4.1.2.7; 9 units/mg. of protein) were obtained from Boehringer Corp., London, W. 5. Xylulose 5-phosphate and sedoheptulose 7-phosphate were kindly given by Professor B. L. Horecker, New York, and erythrose 4-phosphate by Professor C. E. Ballou, California. Purified 6-phosphogluconate dehydrogenase was prepared from liver by the method of Glock & McLean (1953). All other chemicals were of A.R. grade.

Preparation of subcellular fractions from the intestinal mucosa. The collection of the intestinal mucosa and the preparation of homogenates and subcellular fractions were done as described by Hübscher, West & Brindley (1965), except that when it was intended to assay ribose 5-phosphate isomerase, transketolase and transaldolase the mucosal scrapings were homogenized in 0·15M-KCl in 2mM-tris–HCl buffer, pH7·4, instead of 0·3M-sucrose. This change was necessary because the fructose derived from sucrose by invertase activity of the intestinal mucosa interfered with the estimations.

Preparation of palmitoylcarnitine. This was prepared according to the method of Bremer (1962).

Preparation of acyl-CoA. Acetyl-CoA was prepared as described by Ochoa (1955) and palmitoyl-CoA as outlined by Smith & Hübscher (1966).

Determinations of protein and phosphate. These were carried out as described by Hübscher *et al.* (1965).

Enzyme determinations. With the exception of ribose 5-phosphate isomerase, all enzymic determinations were carried out at 30° in a Unicam SP.700 spectrophotometer with a constant-temperature cell housing. The reaction rates were determined continuously over a period of 5min. by determining the change in E_{340}. The reaction rates were linear with respect to time and proportional to the amount of protein added over the range quoted for each enzyme. All assays were done in duplicate.

(*a*) Determination of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. These determinations were based on the method of Glock & McLean (1953). Optimum conditions for the assay of both enzymes were established with the supernatant fraction of rat intestinal mucosa.

The assay system used for the determination of glucose 6-phosphate dehydrogenase contained in a final volume of 2·4ml. the following (final concentrations): glycylglycine–NaOH buffer, pH7·6 (50mM), $MgCl_2$ (20mM), $NADP^+$ (0·11mM), glucose 6-phosphate (2·1mM), purified 6-phosphogluconate dehydrogenase (30·8 units) and the preparation to be assayed (0·12–0·35mg. of protein). The reaction was started by addition of substrate and the reaction rate was measured by using a control from which only $NADP^+$ was omitted.

The assay system used for the determination of 6-phosphogluconate dehydrogenase was the same, except that (i) the pH of the buffer was 9·0, (ii) glucose 6-phosphate was replaced by 6-phosphogluconate (2·1mM) and (iii) purified 6-phosphogluconate dehydrogenase was omitted.

(*b*) Determination of ribose 5-phosphate isomerase (EC 5.3.1.6). This was based on the method of Axelrod & Jang (1954). The reaction mixture contained in a final volume of 0·5ml. the following (final concentrations): tris–HCl buffer, pH7·0 (80mM), ribose 5-phosphate (20mM) and the preparation to be assayed (0·03–0·12mg. of protein). The reaction was started by the addition of the substrate and was stopped after incubation for 10min. at 25° by adding 0·5ml. of 10% (w/v) trichloroacetic acid. After centrifugation in a bench centrifuge, a sample of the supernatant was withdrawn for the estimation of ribulose by the method of Dische & Borenfreund (1951). The amount of ribulose 5-phosphate formed was corrected for that obtained when the substrate was added immediately after stopping the reaction with trichloroacetic acid.

In the method of Dische & Borenfreund (1951) a coloured product formed from ribulose in the presence of carbazole is measured. To determine the extinction coefficient of the coloured product at 540mμ, ribose 5-phosphate (98% pure) was incubated with the enzyme and the reaction was allowed to proceed to equilibrium. From the known equilibrium constant of this reaction at 25° (Axelrod, 1955) the molecular extinction coefficient ($\epsilon_{540}^{1\,cm.}$) was calculated to be $20{\cdot}3 \times 10^3$.

(*c*) Determination of transketolase (EC 2.2.1.1). The assay of this enzyme was based on the method of Horecker & Smyrniotis (1955*a*) with either ribose 5-phosphate and xylulose 5-phosphate (assay system I) or xylulose 5-phosphate and erythrose 4-phosphate (assay system II) as substrates.

Assay system I. In this determination, sedoheptulose 7-phosphate and glyceraldehyde 3-phosphate are formed as products and the latter is determined in a coupled enzymic reaction with triose phosphate isomerase and glycerol 3-phosphate dehydrogenase.

The incubation mixture contained in a final volume of 0·8ml. the following (final concentrations): glycylglycine–NaOH buffer, pH7·5 (37·5mM), cysteine (12·5mM), NADH (0·5mM), xylulose 5-phosphate (0·5mM), ribose 5-phosphate (1·0mM), mixed crystals of triose phosphate isomerase and glycerol 3-phosphate dehydrogenase (100μg. of protein) and either intestinal supernatant (0·07–0·45mg. of protein) or liver supernatant (0·3–1·1mg. of protein). The reaction was started by the addition of substrates. In some experiments, the two substrates were replaced by ribose 5-phosphate (2mM). An assay system from which the substrates had been omitted served as control.

Assay system II. With xylulose 5-phosphate and erythrose 4-phosphate as substrates, glyceraldehyde 3-phosphate and fructose 6-phosphate are the products, of which the latter was determined in a coupled enzymic reaction with glucose phosphate isomerase and glucose 6-phosphate dehydrogenase. The reaction mixture contained in a final volume of 0·8ml. the following (final concentrations): glycylglycine–NaOH buffer, pH7·5 (50mM), $NADP^+$ (0·15mM), xylulose 5-phosphate (0·5mM), erythrose 4-phosphate (0·5mM), glucose phosphate isomerase (50μg. of protein), glucose 6-phosphate dehydrogenase (100μg. of protein) and the preparation to be assayed (0·17–0·54mg. of protein). The reaction was started by adding the substrates and again an assay system from which the substrates had been omitted served as control.

(*d*) Determination of transaldolase (EC 2.2.1.2). The assay system employed was based on those of Horecker & Smyrniotis (1955*b*) and of Venkataraman & Racker (1961). Either sedoheptulose 7-phosphate and glyceraldehyde 3-phosphate (forward reaction; assay system I) or fructose 6-phosphate and erythrose 4-phosphate (reverse reaction; assay system II) were used as substrates.

Assay system I. In this assay glyceraldehyde 3-phosphate was generated from fructose 1,6-diphosphate in the presence of aldolase, and the amount of fructose 6-phosphate formed

was determined in a linked enzymic reaction as outlined in the assay system II used in the determination of transketolase. The reaction mixture contained in a final volume of 0·8ml. the following (final concentrations): triethanolamine–HCl buffer, pH7·5 (50mM), $NADP^+$ (0·15mM), sedoheptulose 7-phosphate (0·5mM), fructose 1,6-diphosphate (1·0mM), aldolase (100μg. of protein), glucose phosphate isomerase (50μg. of protein), glucose 6-phosphate dehydrogenase (100μg. of protein) and the preparation to be tested (0·18–0·45mg. of protein). The reaction was started by the addition of sedoheptulose 7-phosphate and both substrates were omitted from the control. The same rates of reaction were obtained when only sedoheptulose 7-phosphate was omitted from the control.

Assay system II. The amount of glyceraldehyde 3-phosphate formed was determined in a linked enzymic reaction as outlined in assay system I of the transketolase reaction. The assay system contained in a final volume of 0·8ml. the following (final concentrations): glycylglycine–NaOH buffer, pH7·5 (50mM), NADH (0·5mM), erythrose 4-phosphate (0·5mM), fructose 6-phosphate (0·5mM), mixed crystals of triose phosphate isomerase and glycerol 3-phosphate dehydrogenase (100μg. of protein) and either intestinal supernatant (0·07–0·25mg. of protein) or liver supernatant (0·32–0·85mg. of protein). The reaction was started by addition of substrates, which were omitted from the control tube.

EXPERIMENTAL AND RESULTS

In previous work it was reported (Hübscher & Sherratt, 1962) that 93–98% of the glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase present in homogenates of the mucosa of the small intestine of the cat was recovered in the high-speed supernatant fraction. In the present work, a similarly high recovery of these two dehydrogenases was obtained in the supernatant fraction when intestinal preparations of the rat, rabbit and guinea pig were fractionated. It was therefore decided to use this subcellular fraction for the study of the properties of the two dehydrogenases.

Properties of the glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in rat intestinal mucosa. In initial experiments, optimum conditions were established for the assay of these two enzymes. The optimum conditions finally obtained were used in subsequent experiments and are given in the Materials and Methods section.

The effect of varying the pH value of the reaction mixture on the reaction rate is given in Fig. 1(*a*), showing pH optima at about 7·6 and 9·0 for glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase respectively. Glucose 6-phosphate dehydrogenase had a slightly higher activity in glycylglycine buffer than in phosphate buffer. The reactions were only partially dependent on added Mg^{2+} (Fig. 1*b*).

Typical plots of reaction rate against concentration were obtained for both substrates and $NADP^+$. From these plots the K_m values were calculated (Lineweaver & Burk, 1934) and found to be $6·6 \times 10^{-5}$M for glucose 6-phosphate, $2·4 \times 10^{-4}$M for 6-phosphogluconate and $1·2 \times 10^{-5}$M and $3·8 \times 10^{-4}$M for $NADP^+$ in the reactions catalysed by glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase respectively.

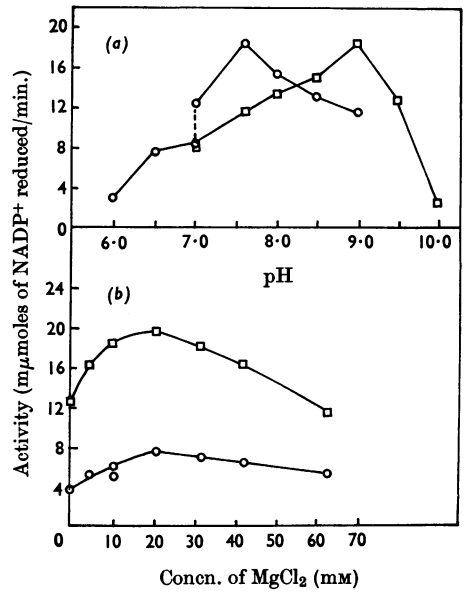

Fig. 1. Effect of (*a*) pH and (*b*) $MgCl_2$ concentration on glucose 6-phosphate dehydrogenase (○) and 6-phosphogluconate dehydrogenase (□). (*a*) The pH values of the reaction mixture were varied as indicated, 0·25M-phosphate buffer being used at pH6·0–7·0 and 0·25M-glycylglycine–NaOH buffer at pH7·0–10·0. The supernatant fraction from the intestinal mucosa of a female rat was used. (*b*) The concentration of $MgCl_2$ was varied as indicated. The supernatant fraction from the intestinal mucosa of a male rat was used.

After preincubation with *p*-chloromercuribenzoate for 5min. both enzymes were inhibited. Inhibitions of 50 and 90% were obtained at concentrations of 0·06mM and 0·25mM respectively with glucose 6-phosphate dehydrogenase. The corresponding values for 6-phosphogluconate dehydrogenase were 0·04mM and 0·10mM respectively. The addition of cysteine or β-mercaptoethanol only partially reversed the inhibition of 6-phosphogluconate dehydrogenase caused by *p*-chloromercuribenzoate (Table 1). The same concentrations of these thiol compounds did not reverse the inhibition of glucose 6-phosphate dehydrogenase.

In view of the observations of Eger-Neufeldt, Teinzer, Weiss & Wieland (1965) on the inhibitory

Table 1. *Effects of cysteine and β-mercaptoethanol on* p-*chloromercuribenzoate-induced inhibitions of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase*

The assay systems were those described in the Materials and Methods section. Other additions were made as indicated.

Enzyme tested	Additions	Relative rate (% of control)
Glucose 6-phosphate dehydrogenase	None	(100)
	p-Chloromercuribenzoate (0·25 mM)	12
	p-Chloromercuribenzoate (0·25 mM) + cysteine (10 mM)	12
	p-Chloromercuribenzoate (0·25 mM) + mercaptoethanol (60 mM)	13
6-Phosphogluconate dehydrogenase	None	(100)
	p-Chloromercuribenzoate (0·125 mM)	8
	p-Chloromercuribenzoate (0·125 mM) + cysteine (10 mM)	31
	p-Chloromercuribenzoate (0·125 mM) + mercaptoethanol (60 mM)	52

Table 2. *Effect of palmitoyl-CoA and related compounds on glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase*

Experimental details are given in the text.

Addition	Inhibition (%)	
	Glucose 6-phosphate dehydrogenase	6-Phospho-gluconate dehydrogenase
Palmitoyl-CoA (0·062 mM)	41	65
Palmitoyl-CoA (0·15 mM)	60	75
Palmitoyl-CoA (0·23 mM)	86	77
Palmitoylcarnitine (0·125 mM)	< 3	< 3
Acetyl-CoA (0·17 mM)	< 3	< 3
CoA (0·42 mM)	< 3	< 3
Palmitic acid (0·54 mM)	< 3	< 3
ATP (8·3 mM)	7	< 3
ATP (1·7 mM)	18	< 3

action of long-chain acyl-CoA on glucose 6-phosphate dehydrogenase, the effect of palmitoyl-CoA and some related compounds was studied. Both dehydrogenases were inhibited by low concentrations of palmitoyl-CoA (Table 2). Structurally related compounds such as palmitoylcarnitine, acetyl-CoA, CoA or palmitic acid were not inhibitory, even at concentrations above those giving more than 50% inhibition with palmitoyl-CoA. Higher concentrations of ATP slightly inhibited glucose 6-phosphate dehydrogenase but not 6-phosphogluconate dehydrogenase.

Comparative studies of the distribution of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. Two aspects were studied. First, the distribution of the two dehydrogenases along the length of the small intestine of the rat was investigated, and, secondly, the activity of the enzymes in the intestinal mucosa of various species was compared.

In the first investigation, small intestines of rats were divided into four equally long segments (numbered from proximal to distal end) and the mucosa of each segment was collected separately. Histological examination of the segments was not performed but, as mentioned by Srivastava & Hübscher (1966), the first segment can be assumed to comprise duodenum and upper jejunum, the second segment mainly jejunum, the third mainly ileum and the last segment ileum.

Table 3 shows that total as well as specific activity of both dehydrogenases decreased stepwise from the proximal to the distal segment, though the differences observed were statistically significant only between the first and third or the second and fourth segments.

A comparison of the specific activities of the two dehydrogenases in the supernatant fraction (6 240 000 *g*-min.) obtained from the mucosa of the small intestine of four species indicated that the preparation from the rat had the highest specific activities (Table 4). Only the specific activities are quoted because the total activities of the two dehydrogenases were merely an expression of the size of the small intestine, which was of course related to the size of the animal. In all male animals, 6-phosphogluconate dehydrogenase had a higher specific activity than glucose 6-phosphate dehydrogenase. No sex differences were obtained with the cat and the sex differences seen with preparations from rats were statistically insignificant. However, in some preparations from male rats the specific activity of 6-phosphogluconate dehydrogenase was twice that of glucose 6-phosphate dehydrogenase (compare Fig. 1*b*).

Ribose 5-phosphate isomerase in rat intestinal mucosa. In initial experiments, optimum conditions

Table 3. *Glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in the mucosa from different regions of rat small intestine*

Results are expressed as means ± S.D. of four preparations from female animals.

Segment no.	Total protein (mg.)	Glucose 6-phosphate dehydrogenase: Total activity (μmoles/min./ total supernatant fraction)	Glucose 6-phosphate dehydrogenase: Specific activity (mμmoles/min./ mg. of protein)	6-Phosphogluconate dehydrogenase: Total activity (μmoles/min./ total supernatant fraction)	6-Phosphogluconate dehydrogenase: Specific activity (mμmoles/min./ mg. of protein)
1	31·8 ± 2·4	3·87 ± 0·47	121·6 ± 16·5	3·37 ± 0·36	105·9 ± 5·0
2	33·2 ± 5·4	3·29 ± 0·64	99·0 ± 4·7	2·91 ± 0·53	87·6 ± 5·3
3	28·0 ± 5·1	2·38 ± 0·57	84·9 ± 10·9	2·01 ± 0·51	71·9 ± 11·0
4	24·4 ± 2·9	1·90 ± 0·41	77·7 ± 17·8	1·71 ± 0·33	70·2 ± 18·7

Table 4. *Comparison of glucose 6-phosphate-dehydrogenase and 6-phosphogluconate-dehydrogenase activities in the intestinal mucosa of the rat, cat, rabbit and guinea pig*

Results are expressed as means ± S.D., with the numbers of preparations in parentheses. The supernatant fractions obtained from homogenates of the mucosa of the whole length of a small intestine were analysed.

Animal	Sex	Specific activity (mμmoles/min./mg. of protein): Glucose 6-phosphate dehydrogenase	Specific activity (mμmoles/min./mg. of protein): 6-Phosphogluconate dehydrogenase
Rat	Male	51·4 ± 16·0 (6)	71·9 ± 12·6 (6)
Rat	Female	74·7 ± 13·6 (6)	69·0 ± 10·5 (6)
Cat	Male	28·1 ± 3·2 (3)	60·4 ± 1·5 (3)
Cat	Female	17·4 ± 5·6 (5)	62·7 ± 10·6 (5)
Rabbit	Male	20·6 ± 2·9 (3)	40·3 ± 6·0 (3)
Guinea pig	Male	34·1 ± 7·3 (3)	44·5 ± 7·3 (3)

were established for the assay of this enzyme in the supernatant fraction from rat small intestine. A K_m value of $1·7 \times 10^{-3}$ M was obtained for ribose 5-phosphate. Rate determinations with respect to time of incubation and concentration of protein were also made with the supernatant fraction of rat liver to ensure that this fraction too was assayed under zero-order conditions. A cell fractionation showed that 87% of the ribose 5-phosphate-isomerase activity of homogenates of rat intestinal mucosa and liver was recovered in the supernatant fraction.

The results given in Table 5 show that the specific activity of ribose 5-phosphate isomerase in the supernatant fraction from the intestine was about six times that of the corresponding fraction from liver. The specific activity of the liver preparation was similar to that reported by Lang & Hartmann (1958). The total activity of the liver was higher than that of the intestinal mucosa.

Transketolase and transaldolase in rat intestinal mucosa. Because some of the substrates were available in only limited amounts, optimum conditions for the assay of these enzymes could not be established. However, in each assay the reaction rate was proportional to the amount of protein added and linear with respect to the time of incubation, thus indicating zero-order conditions. An analysis of the intracellular distribution of transketolase and transaldolase showed that in each case 93–108% of the activity found in nuclei-free homogenates of the intestinal mucosa or liver was recovered in the supernatant fraction. This sub-

Table 5. *Ribose 5-phosphate isomerase in the supernatant fraction of rat intestinal mucosa and liver*

Results are presented as means ± S.D. of six preparations from male animals. The supernatant fractions obtained from homogenates of the mucosa of the whole length of a small intestine and of the whole liver were analysed.

	Total activity (μmoles/min./ total fraction)	Specific activity (mμmoles/min./ mg. of protein)
Intestinal mucosa	38·4 ± 8·7	322 ± 59·0
Liver	56·0 ± 18·6	51·9 ± 6·2

Table 6. *Transketolase and transaldolase in the supernatant fraction of the intestinal mucosa and liver of the rat*

Results are expressed as means ± S.D., with the numbers of preparations from male animals in parentheses. The supernatant fractions obtained from the mucosa of the whole length of the small intestine and the whole liver were assayed.

	Transketolase			Transaldolase	
Substrate used (or generated).....	Ribose 5-phosphate + xylulose 5-phosphate	Ribose 5-phosphate	Erythrose 4-phosphate + xylulose 5-phosphate	Sedoheptulose 7-phosphate + glyceraldehyde 3-phosphate	Erythrose 4-phosphate + fructose 6-phosphate
Intestinal mucosa					
Total activity (μmoles/min./fraction)	2·09 ± 0·43 (5)	2·06 ± 0·38 (7)	1·83 ± 0·27 (6)	1·83 ± 0·42 (6)	5·59 ± 1·33 (6)
Specific activity (mμmoles/min./mg. of protein)	20·2 ± 4·8 (5)	20·3 ± 4·0 (7)	18·0 ± 3·5 (6)	17·6 ± 2·9 (6)	54·7 ± 12·7 (6)
Liver					
Total activity (μmoles/min./fraction)	18·1 ± 3·0 (5)	10·2 ± 2·4 (7)	12·5 ± 2·8 (5)	—	11·6 ± 1·9 (6)
Specific activity (mμmoles/min./mg. of protein)	19·8 ± 3·6 (5)	10·6 ± 2·3 (7)	13·6 ± 3·0 (5)	—	12·8 ± 2·8 (6)

cellular fraction was used for subsequent experiments.

The assay of transketolase was carried out with either ribose 5-phosphate and xylulose 5-phosphate (assay system I) or erythrose 4-phosphate and xylulose 5-phosphate (assay system II) as substrates. Similar activities were obtained with both assay systems with intestinal preparations. These were as active or slightly more active than the preparations from liver. Further, only the preparations from the intestine had the same activities when ribose 5-phosphate and xylulose 5-phosphate were replaced by ribose 5-phosphate. This result denotes that ribose 5-phosphate isomerase and xylulose 5-phosphate epimerase are likely to be very active in the intestinal mucosa. As shown above the first of these two enzymes is more active in the intestinal mucosa than in the liver (Table 5). Its specific activity in the intestinal mucosa is about 15 times that of the transketolase (assay system I) (Table 6).

With erythrose 4-phosphate and fructose 6-phosphate as substrates (assay system II), the transaldolase had a higher specific activity in the supernatant fraction from the intestinal mucosa than in that from the liver. Comparison of the two specific activities obtained for the intestinal transaldolase in assay systems I and II showed that the forward reaction was slower than the reverse reaction. Similar findings were reported for yeast preparations by Venkataraman & Racker (1961), who stated that the lower rates in the forward reaction could be due to an inhibition of phosphohexose isomerase by erythrose 4-phosphate and that this inhibition could be overcome by cysteine. However, the addition of cysteine (12·5 mM) to the assay system had no effect on the transaldolase with either intestinal or liver preparations.

DISCUSSION

The results of this study give evidence that five of the enzymes of the pentose phosphate pathway occur in the mucosa of the small intestine of the rat. The presence of xylulose 5-phosphate epimerase may be deduced from indirect evidence. Other enzymes participating in the conversion of pentose phosphate into glucose 6-phosphate, such as fructose diphosphate aldolase (EC 4.1.2.13) (Schapira, 1961; Stern & Reilly, 1965) and glucose phosphate isomerase (EC 5.3.1.9) (Alvarado, 1963), are also known to occur in the intestinal mucosa of the rat. The observation that almost all of the activity of glucose 6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, ribose 5-phosphate isomerase, transketolase and transaldolase present in mucosal homogenates was recovered in the supernatant fraction is in agreement with reports on the intracellular distribution of those enzymes in other mammalian tissues (Glock & McLean, 1953, 1954; Newburgh & Cheldelin, 1956).

In the intestinal mucosa, the subcellular compartment made up by the soluble proteins of the cell contains not only the enzymes of the pentose phosphate pathway but also those of the glycolytic pathway (Srivastava & Hübscher, 1966). Since the two pathways have some sugar phosphates in

common, channelling of some intermediates from one pathway to the other may occur, and the formation of lactic acid from ribose 5-phosphate in the supernatant fraction of rat intestinal mucosa (Srivastava & Hübscher, 1966) indicates this.

The relative contribution of the pentose phosphate pathway in the utilization of glucose in human intestinal mucosa was reported by White & Landau (1965) to be about 5–10%, and the experiments of Landau & Wilson (1959) with the small intestine of the hamster gave values of 18–26%. However, the glycolytic activity of the intestinal mucosa of the rat has been shown to be markedly affected by the nutritional state of the animal though the activities of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase remained unchanged (Srivastava & Hübscher, 1966). Thus the relative importance of the pentose phosphate pathway may depend on the nutritional state of the small intestine. Further, the pentose phosphate pathway may function not only as an oxidative pathway but also as a non-oxidative pathway by reversal of the transaldolase and transketolase reactions. In the latter case, pentose phosphates are formed from fructose phosphate and triose phosphate without reduction of either $NADP^+$ or NAD^+ (Horecker, 1964). Evidence for a non-oxidative formation of pentose phosphates has been given for several animal tissues (Bernstein, 1953; Marks & Feigelson, 1957; Hiatt, 1957; Shuster & Goldin, 1958; Pontremoli, Bonsignore, Grazi & Horecker, 1960; Bonsignore, Pontremoli, De Flora & Horecker, 1961) and may also play a role in the intestinal mucosa.

The properties of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase in the mucosa of rat small intestine are in many respects similar to those in the mammary gland and liver of the rat (Glock & McLean, 1953; McLean, 1958). The observation that cysteine and β-mercaptoethanol were either ineffective or only partially effective in reversing the inhibition of the dehydrogenases caused by *p*-chloromercuribenzoate warrants further investigation. The reversal of the *p*-chloromercuribenzoate-induced inhibition of glyceraldehyde 3-phosphate dehydrogenase by thiol compounds is known to depend on the concentration of the inhibitor and the time of exposure (Velick & Furfine, 1963). Further, *p*-chloromercuribenzoate is reported to induce denaturation of glyceraldehyde 3-phosphate dehydrogenase as determined by changes in optical rotation and intrinsic viscosity of the protein molecule (Elödi, 1960). It seems probable that the binding of *p*-chloromercuribenzoate to the thiol groups of glucose 6-phosphate dehydrogenase led to conformational changes in the protein molecule that were not reversed by cysteine or mercaptoethanol.

It has been suggested that the concentration of long-chain acyl-CoA in a tissue may control the activity of the pentose phosphate pathway (Eger-Neufeldt *et al.* 1965). In the present investigation it was observed that both dehydrogenases of the pentose phosphate pathway were inhibited by palmitoyl-CoA. From comparison with the effect of palmitoylcarnitine it could be deduced that the inhibitory action of palmitoyl-CoA was not merely due to the surface-active properties of the molecule though a long alkyl residue was required. However, palmitoyl-CoA is unlikely to be of any great importance with respect to the regulation of the pentose phosphate pathway. There are two reasons for this. First, in addition to the two dehydrogenases of the pentose phosphate pathway, acetyl-CoA carboxylase (EC 6.4.1.2) (Bortz & Lynen, 1963), citrate synthase (EC 4.1.3.7) (Wieland, Weiss & Eger-Neufeldt, 1964) and six dehydrogenases (Taketa & Pogell, 1966) are also inhibited by long-chain acyl-CoA. Such a wide spectrum of inhibitions would not be expected from a compound exerting a specific control. Secondly, the formation of acyl-CoA in the intestinal mucosa is known to take place in the membranes of the endoplasmic reticulum (Senior & Isselbacher, 1960; Ailhaud, Samuel & Desnuelle, 1963; Hübscher *et al.* 1963) and the biosynthesis of complex lipids also proceeds predominantly in this membrane fraction (Gurr, Brindley & Hübscher, 1965; Brindley & Hübscher 1965). It seems therefore probable that long-chain acyl-CoA as an intermediate in the biosynthesis of lipids is retained within this membrane system and thus does not exert an inhibitory effect on glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase, which are localized in the subcellular compartment made up of the soluble proteins of the cell.

A research grant from the Medical Research Council in support of this work is gratefully acknowledged. We are also indebted to Professor B. L. Horecker, New York, and Professor C. E. Ballou, California, for the generous supply of sugar phosphates used in this work.

REFERENCES

Ailhaud, G., Samuel, D. & Desnuelle, P. (1963). *Biochim. biophys. Acta*, **67**, 150.

Alvarado, F. (1963). *Enzymologia*, **26**, 12.

Axelrod, B. (1955). In *Methods in Enzymology*, vol. 3, p. 363. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.

Axelrod, B. & Jang, R. (1954). *J. biol. Chem.* **209**, 847.

Bell, J. W. & Sherratt, H. S. A. (1965). *Biochem. J.* **96**, 61P.

Bernstein, I. A. (1953). *J. biol. Chem.* **205**, 317.

Bonsignore, A., Pontremoli, S., De Flora, A. & Horecker, B. L. (1961). *Ital. J. Biochem.* **10**, 106.

Bortz, W. M. & Lynen, F. (1963). *Biochem. Z.* **337**, 505.
Bremer, J. (1962). *J. biol. Chem.* **237**, 3628.
Brindley, D. N. & Hübscher, G. (1965). *Biochim. biophys. Acta*, **106**, 495.
de Lowker, W. C. J. & Prankerd, T. A. J. (1961). *Clin. chim. Acta*, **6**, 641.
Dische, Z. & Borenfreund, E. (1951). *J. biol. Chem.* **192**, 583.
Eger-Neufeldt, I., Teinzer, A., Weiss, L. & Wieland, O. (1965). *Biochem. biophys. Res. Commun.* **19**, 43.
Elödi, P. (1960). *Biochim. biophys. Acta*, **40**, 272.
Glock, G. E. & McLean, P. (1953). *Biochem. J.* **55**, 400.
Glock, G. E. & McLean, P. (1954). *Biochem. J.* **56**, 171.
Gurr, M. I., Brindley, D. N. & Hübscher, G. (1965). *Biochim. biophys. Acta*, **98**, 486.
Hiatt, H. H. (1957). *J. biol. Chem.* **229**, 725.
Horecker, B. L. (1964). In *Comprehensive Biochemistry*, vol. 15, p. 48. Ed. by Florkin, M. & Stotz, E. H. Amsterdam: Elsevier Publishing Co.
Horecker, B. L. & Smyrniotis, P. Z. (1955*a*). In *Methods in Enzymology*, vol. 1, p. 371. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
Horecker, B. L. & Smyrniotis, P. Z. (1955*b*). In *Methods in Enzymology*, vol. 1, p. 381. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
Horecker, B. L., Smyrniotis, P. Z. & Klenow, H. (1953). *J. biol. Chem.* **205**, 661.
Hübscher, G., Clark, B., Webb, M. E. & Sherratt, H. S. A. (1963). In *Biochemical Problems of Lipids*, p. 209. Ed. by Frazer, A. C. Amsterdam: Elsevier Publishing Co.
Hübscher, G. & Sherratt, H. S. A. (1962). *Biochem. J.* **84**, 24P.
Hübscher, G., West, G. R. & Brindley, D. N. (1965). *Biochem. J.* **97**, 629.
Landau, B. R. & Wilson, T. H. (1959). *J. biol. Chem.* **234**, 749.
Lang, K. & Hartmann, K. U. (1958). *Experentia*, **14**, 130.
Lineweaver, H. & Burk, D. (1934). *J. Amer. chem. Soc.* **56**, 658.
McLean, P. (1958). *Biochim. biophys. Acta*, **30**, 303.
Marks, P. A. & Feigelson, P. (1957). *J. biol. Chem.* **226**, 1001.
Newburgh, R. W. & Cheldelin, V. H. (1956). *J. biol. Chem.* **218**, 89.
Nigam, V. N., Sie, H. G. & Fishman, W. H. (1961). *Canad. J. Biochem. Physiol.* **39**, 1367.
Noltmann, E. A. & Kuby, S. A. (1963). In *The Enzymes*, vol. 7, p. 223. Ed. by Boyer, P. D., Lardy, H. & Myrbäck, K. New York: Academic Press Inc.
Ochoa, S. (1955). In *Methods in Enzymology*, vol. 1, p. 688. Ed. by Colowick, S. P. & Kaplan, N. O. New York: Academic Press Inc.
Pontremoli, S., Bonsignore, A., Grazi, E. & Horecker, B. L. (1960). *J. biol. Chem.* **235**, 1881.
Schapira, F. (1961). *Bull. Soc. Chim. biol., Paris*, **43**, 1357.
Senior, J. R. & Isselbacher, K. J. (1960). *Biochim. biophys. Acta*, **44**, 399.
Shuster, L. & Goldin, A. (1958). *J. biol. Chem.* **230**, 883.
Smith, M. E. & Hübscher, G. (1966). *Biochem. J.* (in the Press).
Srivastava, L. M. & Hübscher, G. (1966). *Biochem. J.* **100**, 458.
Stern, B. K. & Reilly, R. W. (1965). *Nature, Lond.*, **205**, 563.
Taketa, K. & Pogell, B. M. (1966). *J. biol. Chem.* **241**, 720.
Velick, S. F. & Furfine, C. (1963). In *The Enzymes*, vol. 7, p. 243. Ed. by Boyer, P. D., Lardy, H. & Myrbäck, K. New York: Academic Press Inc.
Venkataraman, R. & Racker, E. (1961). *J. biol. Chem.* **236**, 1876.
White, L. W. & Landau, B. R. (1965). *J. clin. Invest.* **44**, 1200.
Wieland, O., Weiss, L. & Eger-Neufeldt, I. (1964). *Biochem. Z.* **339**, 501.