Biochem. J. (1966) **100**, 458

Glucose Metabolism in the Mucosa of the Small Intestine

GLYCOLYSIS IN SUBCELLULAR PREPARATIONS FROM THE CAT AND RAT

BY L. M. SRIVASTAVA AND G. HÜBSCHER

Department of Medical Biochemistry and Pharmacology, University of Birmingham

(*Received* 22 *December* 1965)

1. Lactic acid formation in supernatant fractions of homogenates of cat or rat small-intestinal mucosa was measured under optimum conditions with glucose, fructose, glucose 6-phosphate, fructose 1,6-diphosphate or 3-phosphoglycerate as substrate. 2. Between 80 and 107% of the glycolytic activity of the homogenate was recovered in these particle-free preparations when glucose, fructose, glucose 6-phosphate or fructose 1,6-diphosphate was used as substrate. 3. Evidence was obtained that hexokinase and phosphofructokinase were the rate-limiting enzymes in the initial sequence of glycolytic reactions. The limitation of rate by hexokinase was much more pronounced in preparations from the cat than in those from the rat. 4. With subcellular preparations from cat or rat small intestine lactic acid was also formed from ribose 5-phosphate and at rates similar to those observed with glucose. 5. A higher rate of glycolysis was observed with glucose 6-phosphate as substrate with preparations from the proximal half of the small intestine of the rat as compared with the distal half. 6. Mucosal preparations from rats starved for 24–48hr. exhibited only about one-quarter of the glycolytic activity of those of fed control groups. The decreased rate of formation of lactic acid from either glucose or fructose was mainly due to a decrease in the activity of hexokinase(s). The activities of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase and a number of other enzymes were not significantly decreased by starvation. 7. The results are discussed in relation to metabolic control of glycolysis in other mammalian tissues.

Warburg, Posener & Negelein (1924) first reported a glycolytic activity in intestinal preparations. Dickens & Weil-Malherbe (1941) confirmed this observation and described a high rate of respiration and glycolysis in the mucous membrane of rat jejunum. They also showed that the Pasteur effect was absent from preparations of the upper half of the small intestine. There are numerous reports on the glycolytic activity of the small intestine (Rosenthal & Lasnitzki, 1928; Bumm, Appel & Fehrenbach, 1934; Weil-Malherbe, 1938; Lundsgard, 1940; Rosenthal, 1947; Wilson & Wiseman, 1954; Wilson, 1954, 1956; Hestrin-Lerner & Shapiro, 1954; Newey, Smyth & Whaler, 1955; Kiyasu, Katz & Chaikoff, 1956; Stern & Reilly, 1965; Clark & Porteous, 1965). However, in all of these investigations, everted sacs of the small intestine, the intact mucous membrane or cells of the mucosa were used and no attempts were made to study the sequence of glycolytic reactions in detail.

Some individual glycolytic enzymes have been described in the intestine. These are: hexokinase (EC 2.7.1.1) (Long, 1952, 1953; Csaky, 1953; Hele, 1953; Sols, 1956; Ginsburg & Hers, 1960; Nishikawara, 1961; De Torrontegui, 1961; Hänninen & Hartiala, 1964*a*), ketohexokinase (EC 2.7.1.3) (Cadenas & Sols, 1960), glucose phosphate isomerase (EC 5.3.1.9) (Alvarado, 1963), phosphoglucomutase (EC 2.7.5.1) (Villar-Palasi & Larner, 1960), fructose diphosphate aldolase (EC 4.1.2.13) (Schapira, 1961; Stern & Reilly, 1965), fructose 1-phosphate aldolase (Schapira, 1961) and lactate dehydrogenase (EC 1.1.1.27) (Stern & Reilly, 1965).

In the present investigation some properties of the glycolytic enzyme system of the intestinal mucosa of the rat and cat were studied at the subcellular level with particular reference to rate-limiting enzymes and to the effect of starvation. Some preliminary observations have been published (Hübscher & Sherratt, 1962).

MATERIALS AND METHODS

Animals. Adult rats weighing about 200g. were killed between 9 a.m. and 10 a.m. and were not deprived of food during the previous night. In some experiments litter mates were used. When the animals were starved they were kept in cages with a grid base to minimize coprophagy

but had free access to drinking water. In the initial experiments carried out to establish optimum conditions for glycolysis rats of either sex were used. In later experiments, particularly those involving starvation, female rats were used. Cats of either sex aged from 9 months to 2 years were used. They had no food for approx. 16hr. before the experiment.

Chemicals and enzymes. Glucose 6-phosphate (sodium salt), fructose 6-phosphate (sodium salt), ribose 5-phosphate (sodium salt), fructose 1,6-diphosphate (sodium salt), ATP, ADP, NAD, $NADH_2$ and yeast hexokinase (1500units/mg. of protein) were purchased from Sigma Chemical Co., London, S.W. 6, and glycerol 3-phosphate dehydrogenase (EC 1.1.1.8) (36units/mg. of protein), phosphofructokinase (EC 2.7.1.11) (20units/mg. of protein), glucose phosphate isomerase (390units/mg. of protein), lactate dehydrogenase (360units/mg. of protein) and 3-phosphoglycerate (tricyclohexylammonium salt) from Boehringer Corp., London, W. 5.

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 from both cat and rat were done as described by Hübscher, West & Brindley (1965) except that in most experiments the mucosal scrapings were homogenized in 0·3M-mannitol, pH7·4, instead of 0·3M-sucrose. This change was necessitated in view of the presence of invertase in mucosal preparations, leading to a high endogenous glycolytic activity. In initial experiments where optimum concentrations of a number of cofactors and substrates were determined, 0·3M-sucrose, pH7·4, was used for the preparation of the subcellular fractions, which were dialysed against 200vol. of 66mM-sodium phosphate buffer, pH7·4, for 6hr. before use.

Estimation of glycolytic activity. In most experiments the glycolytic activity was measured by determining the amount of lactic acid produced. In a few experiments, the amounts of L-3-glycerophosphate and pyruvate produced were determined as well.

The basic assay system contained (final concentrations) in a total vol. of 1ml.: sodium phosphate buffer, pH7·8 (40mM), ATP (5mM), NAD (1mM), $MgCl_2$ (16mM), nicotinamide (6·6mM), suitable quantities of the enzyme preparations and one of the following substrates: glucose (25mM), fructose (25mM), glucose 6-phosphate (10mM), fructose 6-phosphate (10mM) or ribose 5-phosphate (10mM). The reaction was started by the addition of enzyme and, after incubation for 30min. at 30°, it was stopped by adding 1ml. of 7% (v/v) $HClO_4$. After centrifugation in a bench centrifuge, a sample of the supernatant was withdrawn for the determination of lactate, L-3-glycerophosphate or pyruvate.

When the production of lactic acid from fructose 1,6-diphosphate was measured, either ATP (5mM) or ADP (4mM) was present in the basic assay system and fructose 1,6-diphosphate (10mM) replaced the substrates named above. Similarly, when 3-phosphoglycerate was used as substrate, ATP and NAD were omitted from the basic assay system and replaced by ADP (6mM) and $NADH_2$ (2mM) and 3-phosphoglycerate was present at 10mM.

Lactate and L-3-glycerophosphate (Hohorst, Kreutz & Bücher, 1959) and pyruvate (Bücher, Czok, Lamprecht & Latzko, 1963) were determined enzymically.

Other determinations. Alkaline phosphatase (EC 3.1.3.1) was determined by the method of Hübscher & West (1965). Phosphoprotein phosphatase (EC 3.1.3.16), β-galactosidase (EC 3.2.1.23), succinate dehydrogenase (EC 1.3.99.1), protein and DNA were estimated as described by Hübscher *et al.* (1965). Glucose 6-phosphate dehydrogenase (EC 1.1.1.49) and 6-phosphogluconate dehydrogenase (EC 1.1.1.44) were assayed by the method of Glock & McLean (1953).

EXPERIMENTAL AND RESULTS

In initial experiments, optimum conditions were established for the formation of lactic acid in the particle-free supernatants from homogenates of the mucosa of cat and rat small intestine. Typical plots of reaction rate versus concentration were obtained for glucose, fructose, glucose 6-phosphate, fructose 1,6-diphosphate, 3-phosphoglycerate, ribose 5-phosphate, ATP, NAD and magnesium chloride as well as for ADP and $NADH_2$, the last two being studied when 3-phosphoglycerate was used as substrate. However, when lactate formation was measured from fructose 1,6-diphosphate, optimum reaction rates were observed over a narrow concentration range of ADP, higher concentrations being inhibitory (see Fig. 1). ATP was found to be less effective than ADP, this probably being due to the absence of adenosine triphosphatase (EC 3.6.1.3) from the particle-free supernatants. This assumption is supported by the observation that, in whole homogenates, the rate of formation

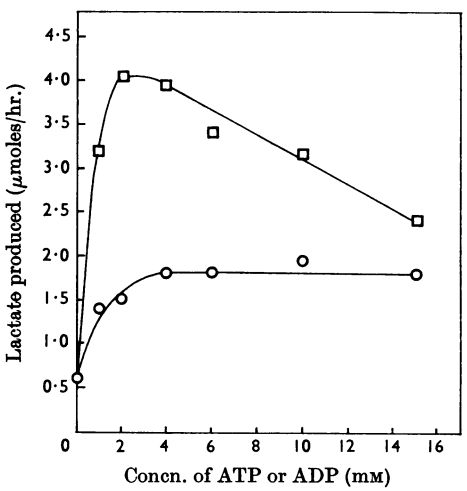

Fig. 1. Effect of varying the concentrations of ATP (○) and ADP (□) on lactate formation from fructose 1,6-diphosphate by the supernatant fraction of homogenates of cat intestinal mucosa. The assay system was that described in the Materials and Methods section.

of lactate from fructose 1,6-diphosphate was the same with 5mM-ATP or 4mM-ADP.

Nicotinamide had a slightly stimulating effect (15–20%) over the concentration range 6·6–66mM. A broad pH optimum between pH 7·4 and 8·5 was obtained for the formation of lactate from either glucose or glucose 6-phosphate.

These results were used to devise the assay systems described in the Materials and Methods section. The initial experiments included rate determinations with respect to time of incubation and concentration of protein to ensure that all rates of glycolysis determined in subsequent experiments were obtained under zero-order conditions.

Intracellular localization of glycolytic enzymes. Previous experiments with glucose as substrate had established that, when nuclei-free homogenates of cat intestinal mucosa were fractionated into two particulate fractions and a particle-free supernatant, about 90% of the glycolytic activity of the homogenate was recovered in the supernatant fraction and the remainder in the two particulate fractions (Hübscher, Clark, Webb & Sherratt, 1963). The results summarized in Table 1 show that between 80 and 107% of the activity of homogenates of cat or rat small-intestinal mucosa was recovered in the particle-free supernatant when not only glucose but also fructose, glucose 6-phosphate or fructose 1,6-diphosphate was used as substrate.

Rate-limiting reactions. In both cat and rat preparations lactate production from the following substrates increased in the order: glucose, fructose, glucose 6-phosphate, fructose 1,6-diphosphate (Table 2). However, the differences in glycolytic rate obtained with glucose, fructose and glucose 6-phosphate were statistically significant only with preparations from the cat. The supernatants from cat mucosa had consistently and significantly higher activities with all substrates except glucose.

Table 1. *Recovery of glycolytic activity in the particle-free supernatant*

Experimental details are given in the text.

	Glycolytic acitivity of rat intestinal mucosa			Glycolytic activity of cat intestinal mucosa		
	Whole homogenate	Particle-free supernatant		Whole homogenate	Particle-free supernatant	
Addition	(μmoles of lactate formed/hr.)	(μmoles of lactate formed/hr.)	(% of homogenate activity)	(μmoles of lactate formed/hr.)	(μmoles of lactate formed/hr.)	(% of homogenate activity)
None (endogenous)	24·1	19·3	80	145	110	76
Glucose (25mM)	48·2	46·3	96	410	375	92
Fructose (25mM)	53·0	54·0	102	579	530	92
Glucose 6-phosphate (10mM)	57·9	61·7	107	917	796	87
Fructose 1,6-diphosphate (10mM)+ADP (4mM)	135·1	108·1	80	2220	1989	90

Table 2. *Comparison of rates of glycolysis in the supernatants of the intestinal mucosa of rat and cat with various substrates*

Experimental details are given in the text. Results are expressed as means ±S.D., except where only two determinations were done. The numbers of different preparations are given in parentheses. The particle-free supernatants obtained from homogenates of the mucosa of the whole length of a small intestine were analysed.

	Glycolytic activity (μmoles of lactate formed/hr./mg. of protein)	
Addition	Rat intestinal mucosa (particle-free supernatant)	Cat intestinal mucosa (particle-free supernatant)
None (endogenous)	0·23±0·06 (10)	0·25±0·07 (7)
Glucose (25mM)	0·85±0·18 (10)	0·89±0·16 (7)
Fructose (25mM)	0·95±0·21 (4)	1·40±0·22 (4)
Glucose 6-phosphate (10mM)	1·08±0·31 (8)	2·27±0·44 (7)
Fructose 1,6-diphosphate (10mM)+ADP (4mM)	1·56, 1·50 (2)	3·45±1·37 (4)
Ribose 5-phosphate (10mM)	1·01, 0·69 (2)	1·60±0·63 (4)

The ratio of lactate formation from glucose 6-phosphate to lactate formation from glucose was 1·27 for rat and 2·25 for cat. This may indicate that hexokinase activity is rate-limiting in intestinal glycolysis, especially in the cat intestinal preparation. The ratios of lactate formation from fructose and glucose were 1·12 and 1·57 for rat and cat preparations respectively. These observations suggest either that cat intestinal mucosa may contain a larger amount of specific ketohexokinase or that the substrate affinities of the non-specific hexokinases in the two tissues are different. The formation of lactic acid from ribose 5-phosphate proceeded faster in preparations from cat intestine than in those from the rat.

Since the glycolytic rates in cat intestinal preparations increased with the substrates glucose, glucose 6-phosphate and fructose 1,6-diphosphate, in that order, it seemed possible that hexokinase and either phosphoglucose isomerase or phosphofructokinase might be rate-limiting enzymes in the overall reaction. Evidence that hexokinase was rate-limiting was obtained by adding a trace amount of highly purified yeast hexokinase to the supernatant fraction (see Table 3), whereupon the reaction rates were the same with glucose plus hexokinase and with glucose 6-phosphate. This result was reproducible, provided that a small amount of hexokinase was added, but larger amounts of hexokinase did result in lower reaction rates. This might be due to the excessive utilization and removal of ATP by the hexokinase reaction so that the ATP concentrations for phosphofructokinase are limited. As equal reaction rates were obtained with glucose 6-phosphate, with glucose 6-phosphate plus glucose phosphate isomerase and with fructose 6-phosphate, it seemed likely that phosphofructokinase rather than phosphoglucose isomerase was the rate-limiting enzyme between glucose 6-phosphate and fructose 1,6-diphosphate. Evidence for this view was obtained by adding a trace amount of highly purified phosphofructokinase to the particle-free supernatant from cat intestinal mucosa (see Table 4). Phosphofructokinase in many tissues is known to be inhibited by excess of ATP (Passoneau & Lowry, 1962, 1963; Mansour, 1963; Underwood & Newsholme, 1965), though there is as yet no direct conclusive evidence that excess of ATP inhibits phosphofructokinase of intestinal preparations. The relative rates of lactate formation from fructose 6-phosphate plus phosphofructokinase and from fructose 1,6-diphosphate were therefore measured in the presence of ADP, thus relying on adenylate kinase (EC 2.7.4.3) for the initial formation of ATP required in the kinase-catalysed reaction. For comparison, the reaction rates observed in the presence of added ATP are also included.

The reaction rates obtained in the presence of fructose 6-phosphate plus phosphofructokinase approached or exceeded those obtained in the presence of fructose 1,6-diphosphate. The addition of phosphofructokinase consistently increased the lactate formation from fructose 6-phosphate although the degree of stimulation varied for different supernatant preparations.

Table 3. *Effect of added yeast hexokinase on the formation of lactate from glucose in the particle-free supernatant of cat small-intestinal mucosa*

Experimental details are given in the text. The 2·5 μg. of yeast hexokinase or glucose phosphate isomerase correspond to 3·75 and 1·0 enzyme units respectively.

Addition	Glycolytic activity (μmoles of lactate formed/hr./mg. of protein)
None (endogenous)	0·28
Glucose (25 mM)	1·09
Glucose (25 mM) + yeast hexokinase (2·5 μg.)	3·45
Glucose 6-phosphate (10 mM)	3·49
Glucose 6-phosphate (10 mM) + glucose phosphate isomerase (2·5 μg.)	3·42
Fructose 6-phosphate (10 mM)	3·49

Table 4. *Effect of added phosphofructokinase on the formation of lactate from fructose 6-phosphate in particle-free supernatant from cat small-intestinal mucosa*

The assay system is that described in the Materials and Methods section. The 12 μg. of phosphofructokinase corresponds to 0·25 enzyme unit.

Addition	Glycolytic activity (μmoles of lactate formed/hr./mg. of protein)
Glucose 6-phosphate (10 mM) + ATP (5 mM)	2·11
Fructose 6-phosphate (10 mM) + ATP (5 mM)	2·17
Fructose 6-phosphate (10 mM) + ATP (5 mM) + phosphofructokinase (12 μg.)	2·94
Fructose 6-phosphate (10 mM) + ADP (4 mM)	2·06
Fructose 6-phosphate (10 mM) + ADP (4 mM) + phosphofructokinase (12 μg.)	3·17
Fructose 1,6-diphosphate (10 mM) + ADP (4 mM)	3·04
Fructose 1,6-diphosphate (10 mM) + ATP (5 mM)	1·61

Since the $NADH_2$ produced in the step catalysed by glyceraldehyde phosphate dehydrogenase (EC 1.2.1.9) may be utilized in the lactate dehydrogenase- or L-3-glycerophosphate dehydrogenase-catalysed reactions, a more complete assessment of glycolytic activity would be obtained by determining the amounts of lactic acid and glycerophosphate produced. This was done in the experiment summarized in Table 5. Approximately equal amounts of lactic acid and glycerophosphate were formed, showing that the true glycolytic rate in terms of substrate used was twice that calculated from lactic acid formation only.

Variation of glycolytic activity along the length of rat small intestine. Rat small intestines 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 done but the first segment can be assumed to comprise duodenum and upper jejunum, the second segment mainly jejunum, the third segment mainly ileum and the last segment ileum. Lactate formation was measured with glucose 6-phosphate or ribose 5-phosphate as substrate. The results quoted in Table 6 show that the total glycolytic activity decreased sharply between the second and third segments, smaller differences being observed between the first and second or third and fourth segments. The decrease in specific activity was less pronounced since the protein content of the segments decreased in a similar manner to the total glycolytic activity.

Effect of starvation on the glycolytic activity of rat intestinal mucosa. Starvation of rats (litter mates) for periods of 12, 24, 36 and 48hr. led to a marked decrease in glycolytic activity with either glucose or fructose as substrate. After 12hr. of starvation

Table 5. *Formation of lactate, glycerophosphate and pyruvate in the particle-free supernatant of cat small-intestinal mucosa*

Details of the procedures and assays are given in the text. The results obtained with two preparations are quoted.

	Glycolytic activity		
Addition	Lactate formed (μmoles/hr./mg. of protein)	L-3-Glycerophosphate formed (μmoles/hr./mg. of protein)	Pyruvate formed (μmole/hr./mg. of protein)
None (endogenous)	0·23	0·21	0·03
	0·15	0·11	0·01
Glucose (25mM)	0·82	0·73	0·07
	0·62	0·61	0·05
Glucose 6-phosphate (10mM)	1·58	1·75	0·06
	1·04	1·03	0·04
Fructose 1,6-diphosphate (10mM)	2·28	1·54	0·07
+ADP (4mM)	1·54	1·08	0·05

Table 6. *Glycolytic activity in the intestinal mucosa from different regions of rat small intestine*

Experimental details are given in the text. The mucosal scrapings of intestinal segments of a group of four litter mates were pooled and the average of results obtained with two such groups is quoted.

	Glycolytic activity							
Total protein (mg.).....	Segment I 59·0		Segment II 64·0		Segment III 40·4		Segment IV 40·6	
Addition	Total activity (μmoles of lactate/hr.)	Specific activity (μmole of lactate/hr./mg. of protein)	Total activity (μmole of lactate/hr.)	Specific activity (μmole of lactate/hr./mg. of protein)	Total activity (μmole of lactate/hr.)	Specific activity (μmole of lactate/hr./mg. of protein)	Total activity (μmole of lactate/hr.)	Specific activity (μmole of lactate/hr./mg. of protein)
None (endogenous)	7·6	0·13	10·3	0·16	4·5	0·11	2·6	0·06
Glucose 6-phosphate (10mM)	61·0	1·03	50·0	0·79	25·1	0·62	19·4	0·48
Ribose 5-phosphate (10mM)	52·8	0·89	55·6	0·87	22·5	0·55	24·1	0·59

Table 7. *Effect of starving and re-feeding on glycolytic activity of the mucosa of rat small intestine*

Experimental details are given in the text. Results are given as means ± S.D. for a group of three rats. The particle-free supernatant derived from homogenates of the mucosa of the whole small intestine was assayed.

		Glycolytic activity					
	Addition.....	None (endogenous)		Glucose (25 mM)		Fructose (25 mM)	
Group	Nutritional state of animals	Total activity (μmoles of lactate/hr.)	Specific activity (μmole of lactate/hr./mg. of protein)	Total activity (μmoles of lactate/hr.)	Specific activity (μmoles of lactate/hr./mg. of protein)	Total activity (μmoles of lactate/hr.)	Specific activity (μmoles of lactate/hr./mg. of protein)
I	Control (fed)	22·0 ±8·9	0·37 ±0·08	65·6 ±22·3	1·09 ±0·23	72·5 ±25·5	1·20 ±0·27
II	Starved for 12 hr.	6·7 ±2·6	0·12 ±0·03	25·2 ±2·9	0·46 ±0·04	26·3 ±2·7	0·48 ±0·04
III	Starved for 24 hr.	4·5 ±0·9	0·10 ±0·02	8·7 ±3·3	0·20 ±0·08	9·2 ±3·3	0·21 ±0·08
IV	Starved for 36 hr.	2·8 ±2·3	0·06 ±0·04	8·4 ±6·2	0·18 ±0·11	8·9 ±5·6	0·20 ±0·09
V	Starved for 48 hr.	2·3 ±0·9	0·06 ±0·02	6·6 ±4·2	0·17 ±0·10	8·3 ±5·1	0·22 ±0·13
VI	Starved for 48 hr. then fed and 6 hr. later killed	30·5 ±3·4	0·60 ±0·20	50·2 ±2·9	0·98 ±0·28	52·6 ±2·6	1·03 ±0·30

the total glycolytic activity was only about 40% and after 24–48 hr. of starvation about 20% of that obtained with fed rats (Table 7). A group of rats that had been starved for 48 hr. was re-fed and killed 6 hr. later. The glycolytic rate was found to have risen within 6 hr. to about 75% of the level of the fed control group. In the experiment quoted in Table 7, only the particle-free supernatants were assayed, but in other experiments it was found that total homogenates of the intestinal mucosa of starved rats exhibited similarly sharp decreases in glycolytic activity.

The decrease of glycolytic activity might have been merely an indication of a general decrease of metabolic activity of the intestinal mucosa during starvation. The results in Table 8 indicate that this was not so, since of four particulate enzymes tested only one seemed to show a significant decrease in activity. With the exception of alkaline phosphatase, the enzymes showed a slight decrease during starvation as did the total protein and DNA content. The changes in total enzyme activity were therefore not significant, with the exception of phosphoprotein phosphatase. But even this enzyme activity decreased only by 40% of that in fed rats as compared with a decrease by 74–87% in overall intestinal glycolytic activity in starved rats.

The observed lowering of glycolytic activity was further investigated by measuring lactic acid formation from glucose 6-phosphate and 3-phosphoglycerate as well as glucose (Table 9), and by using the particle-free supernatant of the same homogenates as were assayed for the enzymic activities quoted in Table 8. With glucose as substrate, specific as well as overall glycolytic activity decreased as in the first experiment of this type (Table 7), though not quite to the same extent. However, when either glucose 6-phosphate or 3-phosphoglycerate was used as substrate the total glycolytic activity decreased only by 14–17%, whereas the specific activity remained virtually unchanged. Similarly, total glucose 6-phosphate-dehydrogenase and 6-phosphogluconate-dehydrogenase activities decreased by 15–17% during starvation but their specific activities did not change. These results suggest that the decreased glycolytic activity observed during starvation was mainly due to decreased hexokinase activity. The addition of a small amount of crystalline yeast hexokinase to a particle-free supernatant prepared from the intestinal mucosa of a starved rat increased the rate of lactate formation from either glucose or fructose to almost that obtained with glucose 6-phosphate, thus supporting this contention.

DISCUSSION

The optimum concentrations of ATP, ADP, NAD and Mg^{2+} and of a number of substrates determined in this investigation to obtain maximum glycolytic rates with subcellular preparations of cat

Table 8. *Effect of starving and re-feeding on protein and DNA content and on some enzymes of the mucosa of rat small intestine*

Experimental details are given in the text. Homogenates prepared from the mucosa of the whole small intestine were assayed. All values quoted are means ± S.D. of results from six rats. The control group was given rat cubes *ad libitum* and was killed between 9 and 10 a.m. The starved group was deprived of food for 36 hr. The re-fed group was starved for 36 hr., then given rat cubes *ad libitum* and killed 22 hr. later.

	Control	Starved	Re-fed
Total protein (homogenate) (mg.)	224·0 ± 66·9	172·0 ± 21·9	194·5 ± 26·7
Total DNA (μg. of DNA P)	946·7 ± 200·0	837·5 ± 192·2	904·2 ± 124·2
Total phosphoprotein-phosphatase activity (μmoles of P_i/hr.)	7·7 ± 3·8	4·6 ± 1·4	8·2 ± 3·5
Total alkaline-phosphatase activity (μmoles of P_i/hr.)	830·2 ± 161·8	868·9 ± 268·0	965·4 ± 160·9
Total β-galactosidase activity (μmoles of galactose/hr.)	119·7 ± 19·3	100·7 ± 14·7	114·6 ± 12·1
Total succinate-dehydrogenase activity (μmoles of succinate/hr.)	154·1 ± 35·4	126·9 ± 21·9	158·7 ± 15·8

Table 9. *Effect of starving and re-feeding on glycolysis and on glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of rat intestinal mucosa*

Particle-free supernatants derived from the mucosa of the whole small intestine were assayed. They were prepared from the homogenates assayed in Table 8. The values quoted are means ± S.D. of results obtained from six rats.

	Control		Starved		Re-fed	
Addition (substrate)	Total activity (μmoles/hr.)	Specific activity (μmoles/hr./mg. of protein)	Total activity (μmoles/hr.)	Specific activity (μmoles/hr./mg. of protein)	Total activity (μmoles/hr.)	Specific activity (μmoles/hr./mg. of protein)
Glycolytic activity (formation of lactate)						
None (endogenous)	19·9 ± 7·2	0·23 ± 0·06	2·9 ± 2·2	0·04 ± 0·03	21·4 ± 7·8	0·22 ± 0·07
Glucose (25 mM)	74·6 ± 19·7	0·89 ± 0·18	19·8 ± 5·8	0·26 ± 0·09	69·9 ± 8·7	0·73 ± 0·07
Glucose 6-phosphate (10 mM)	89·5 ± 28·7	1·07 ± 0·34	76·3 ± 25·8	1·03 ± 0·39	83·6 ± 13·4	0·87 ± 0·15
3-Phosphoglycerate (10 mM)	150·7 ± 27·1	1·79 ± 0·11	125·4 ± 13·5	1·70 ± 0·31	168·8 ± 10·3	1·77 ± 0·19
Enzyme activities (reduction of NADP)						
Glucose 6-phosphate dehydrogenase	438·2 ± 99·8	4·64 ± 0·87	373·9 ± 66·8	5·09 ± 1·23	425·2 ± 46·9	4·45 ± 0·64
6-Phosphogluconate dehydrogenase	384·8 ± 67·8	4·13 ± 0·27	310·3 ± 41·5	4·17 ± 0·55	347·0 ± 54·6	3·62 ± 0·55

and rat intestinal mucosa are different from those employed to measure glycolytic rates in rat-heart homogenates (Horn, Haugaard & Haugaard, 1965), in preparations of rabbit brain or lactating mammary gland (Terner, 1952), in rat-brain mitochondria (Brunngraber & Abood, 1965) or in homogenates of rat and guinea-pig liver (Lea & Walker, 1965). The differences are mainly that intestinal preparations require a higher concentration of ATP (or ADP) and Mg^{2+} than the other tissues. Marked differences of optimum substrate concentrations were also noted. Thus 10 mM-glucose gave maximum reaction rates with intestinal preparations, whereas Lea & Walker (1965) reported that with homogenates of guinea-pig liver maximum reaction rates were approached only at 100 mM-glucose, and Terner (1952) added as little as 0·25 mM-glucose to his assay systems.

Lea & Walker (1965) reported that after fractionation of rat-liver homogenates the total glycolytic activity of the particle-free supernatant was about 2–3 times that of the original homogenate when glucose was used as substrate. They further showed that the lower activity of the homogenate was due to glucose 6-phosphatase activity of the endoplasmic reticulum. Mucosal homogenates of rat small intestine have barely detectable glucose 6-phosphatase activity and those of cat small intestine have specific activities of 0·5 μmole hydrolysed/mg. of protein (homogenate)/hr. (G. Hübscher, unpublished work), whereas homogenates of rat liver have specific activities of 4·5 (Sedgwick, 1965). The finding that hexokinase and phosphofructokinase are the rate-limiting steps of glycolysis in cat small-intestinal mucosa may be compared with similar observations reported for erythrocytes (Minakami, Saito, Suzuki & Yoshikawa, 1964), kidney slices (Wu, 1965) and rat and guinea-pig liver homogenates (Lea & Walker, 1965).

The formation of lactate from ribose 5-phosphate may be best interpreted by assuming that ribose 5-phosphate isomerase, xylulose 5-phosphate epimerase and at least transketolase occur in the intestinal mucosa of the cat and rat. These enzymes are required to form triose phosphate from ribose 5-phosphate and thus would facilitate the entry of some of the ribose 5-phosphate into the glycolytic pathway. Such an interpretation is in agreement with the observations of Bell & Sherratt (1965). They described the specific reduction of NADP by ribose 5-phosphate with subcellular preparations of rat and guinea-pig intestinal mucosa, indicating the formation of glucose 6-phosphate from ribose 5-phosphate and thus the presence of the above-mentioned enzymes and of transaldolase.

A higher rate of glycolysis was noted in the proximal half of the small intestine (mainly duodenum and jejunum) of the rat as compared with the distal half (mainly ileum). Since glucose 6-phosphate was used as substrate the observed differences in glycolytic activity could not merely be a reflection of the distribution of the rate-limiting hexokinase. However, Hänninen & Hartiala (1964*a*) noted higher hexokinase activities in the proximal parts of small intestine of the rat as compared with the distal parts. These results are in agreement with previously reported regional differences of glycolytic activity observed with segments of the small intestine of the rat (Dickens & Weil-Malherbe, 1941) and everted sacs of the small intestine of the rat and hamster (Wilson & Wiseman, 1954). A higher metabolic activity of the duodenum and jejunum as compared with the ileum is not only seen in glycolysis. Glyceride biosynthesis is known to proceed at a higher rate in the upper small intestine (Dawson & Isselbacher, 1960). Similar regional differences have been reported for monoamine oxidase (EC 1.4.3.4) and dihydroxyphenylalanine decarboxylase (EC 4.1.1.26) (Klingman, Kardaman & Haber, 1964), α-glucosidase (EC 3.2.1.20) and β-fructofuranosidase (EC 3.2.1.26) (Wilson & Vincent, 1955), alkaline phosphatase (Malhorta & Philip, 1965) and for the concentration of creatine and creatine phosphate (Hänninen & Hartiala, 1964*b*). However, a higher metabolic activity of the proximal part of the small intestine does not seem to be a general phenomenon as shown by the distribution of acid phosphatase (EC 3.1.3.2) (Malhorta & Philip, 1965) and of β-galactosidase (Koldovský & Chytil, 1965).

Starvation was found to decrease the glycolytic activity of the mucosa of the small intestine of the rat. The glycolytic rate was decreased to 13–25% of that obtained with preparations from fed animals when glucose was used as substrate whereas it remained unchanged when glucose 6-phosphate or 3-phosphoglycerate was used as substrate. From this, and from the observation that the apparent block in glucose utilization could be overcome by adding a small amount of purified hexokinase, it was concluded that the decreased glycolytic activity was mainly due to decreased hexokinase activity. With fructose as substrate similar decreases in glycolytic activity to those found with glucose were recorded. Since fructose may be phosphorylated by hexokinase and a specific ketohexokinase it is possible that the latter enzyme is also decreased during starvation.

The fact that lactate formation from 3-phosphoglycerate did not change during starvation may indicate that pyruvate kinase (EC 2.7.1.40) of intestinal mucosa does not respond to starvation as do the enzymes from liver and kidney (Krebs, 1965; Wu, 1965).

The hexokinase activity of the intestinal mucosa during various dietary regimens was studied by Long (1953) and De Torrontegui (1961). Both workers reported a significantly lower hexokinase activity during a high-fat carbohydrate-free diet as compared with a high-carbohydrate fat-free diet. De Torrontegui (1961) also noticed that starvation for 24hr. did not bring about a change in hexokinase activity. In view of the recently described occurrence in rat liver of a hexokinase (not responding to starvation and with a low $K_{m\,(glucose)}$) and a glucokinase (EC 2.7.1.2) (responding to starvation and with a high $K_{m\,(glucose)}$) (Viñuela, Salas & Sols, 1963; Walker, 1964) the glucose concentration becomes a specially important factor in the assay of the kinases. Since De Torrontegui (1961) employed low glucose concentrations in his assay of hexokinase it is difficult to interpret his results.

We thank Professor A. C. Frazer for his interest in and support of this work and the Medical Research Council of Great Britain for a Research Grant. We are also indebted to Dr D. G. Walker for helpful criticisms of the manuscript.

REFERENCES

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

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

Brunngraber, E. G. & Abood, L. G. (1965). *J. biol. Chem.* **235**, 1847.

Bücher, Th., Czok, R., Lamprecht, W. & Latzko, E. (1963). In *Methods of Enzymatic Analysis*, p. 253. Ed. by Bergmeyer, H. U. New York and London: Academic Press Inc.

Bumm, E., Appel, H. & Fehrenbach, K. (1934). *Hoppe-Seyl. Z.* **223**, 207.

Cadenas, E. & Sols, A. (1960). *Biochim. biophys. Acta*, **42**, 460.

Clark, B. & Porteous, J. W. (1965). *Biochem. J.* **96**, 539.

Csaky, T. Z. (1953). *Science*, **118**, 253.

Dawson, A. M. & Isselbacher, K. (1960). *J. clin. Invest.* **39**, 150.

De Torrontegui, G. (1961). *Biochim. biophys. Acta*, **50**, 164.

Dickens, F. & Weil-Malherbe, H. (1941). *Biochem. J.* **35**, 7.

Ginsburg, V. & Hers, H. G. (1960). *Biochim. biophys Acta.*, **38**, 427.

Glock, G. E. & McLean, P. (1953). *Biochem. J.* **55**, 400.

Hänninen, O. & Hartiala, K. (1964*a*). *Acta chem. scand.* **18**, 947.

Hänninen, O. & Hartiala, K. (1964*b*). *Acta chem. scand.* **18**, 1397.

Hele, M. P. (1953). *Biochem. J.* **55**, 857.

Hestrin-Lerner, S. & Shapiro, B. (1954). *Biochim. biophys. Acta*, **13**, 54.

Hohorst, H. J., Kreutz, F. H. & Bücher, Th. (1959). *Biochem. Z.* **332**, 18.

Horn, R. S., Haugaard, E. S. & Haugaard, N. (1965). *Biochim. biophys. Acta*, **99**, 549.

Hübscher, G., Clark, B., Webb, M. E. & Sherratt, H. S. A. (1963). In *Biochemical Problems of Lipids*, p. 201. Ed. by Frazer, A. C. Amsterdam: Elsevier Publishing Co.

Hübscher, G. & Sherratt, H. S. A. (1962). *Biochem. J.* **84**, 24 P.

Hübscher, G. & West, G. R. (1965). *Nature, Lond.*, **205**, 799.

Hübscher, G., West, G. R. & Brindley, D. N. (1965). *Biochem. J.* **97**, 629.

Kiyasu, J. Y., Katz, J. & Chaikoff, I. L. (1956). *Biochim. biophys. Acta*, **21**, 286.

Klingman, G. I., Kardaman, S. & Haber, J. (1964). *Life Sci.* **3**, 1355.

Koldovský, O. & Chytil, F. (1965). *Biochem. J.* **94**, 266.

Krebs, H. A. (1965). *Abstr. 2nd Meet. Fed. Eur. Biochem. Soc., Vienna*, p. 351. Vienna: Verlag der Wiener Medizinischen Akademie.

Lea, M. A. & Walker, D. G. (1965). *Biochem. J.* **94**, 655.

Long, C. (1952). *Biochem. J.* **50**, 407.

Long, C. (1953). *Biochem. J.* **53**, 7.

Lundsgard, E. (1940). *Fiziol. Zh. S.S.S.R.* **29**, 311.

Malhorta, O. P. & Philip, G. (1965). *Indian J. med. Res.* **53**, 410.

Mansour, T. E. (1963). *J. biol. Chem.* **238**, 2285.

Minakami, S., Saito, T., Suzuki, C. & Yoshikawa, H. (1964). *Biochem. biophys. Res. Commun.* **17**, 748.

Newey, H., Smyth, D. H. & Whaler, B. C. (1955). *J. Physiol.* **129**, 1.

Nishikawara, M. T. (1961). *Endocrinology*, **68**, 850.

Passoneau, J. V. & Lowry, O. H. (1962). *Biochem. biophys. Res. Commun.* **7**, 10.

Passoneau, J. V. & Lowry, O. H. (1963). *Biochem. biophys. Res. Commun.* **13**, 372.

Rosenthal, O. (1947). *Fed. Proc.* **6**, 286.

Rosenthal, O. & Lasnitzki, A. (1928). *Biochem. Z.* **196**, 340.

Schapira, F. (1961). *Bull. Soc. Chim. biol., Paris*, **43**, 1357.

Sedgwick, B. (1965). Ph.D. Thesis: University of Birmingham.

Sols, A. (1956). *Biochim. biophys. Acta*, **19**, 144.

Stern, B. K. & Reilly, R. W. (1965). *Nature, Lond.*, **205**, 563.

Terner, C. (1952). *Biochem. J.* **52**, 229.

Underwood, A. H. & Newsholme, E. A. (1965). *Biochem. J.* **95**, 868.

Villar-Palasi, C. & Larner, J. (1960). *Arch. Biochem. Biophys.* **86**, 270.

Viñuela, E., Salas, M. & Sols, A. (1963). *J. biol. Chem.* **238**, PC1175.

Walker, D. G. (1964). *Abstr. 6th int. Congr. Biochem., New York*, Abstract VI, p. 475.

Warburg, O., Posener, K. & Negelein, E. (1924). *Biochem. Z.* **152**, 309.

Weil-Malherbe, H. (1938). *Biochem. J.* **32**, 2257.

Wilson, T. H. (1954). *Biochem. J.* **56**, 521.

Wilson, T. H. (1956). *J. biol. Chem.* **227**, 751.

Wilson, T. H. & Vincent, T. N. (1955). *J. biol. Chem.* **216**, 1955.

Wilson, T. H. & Wiseman, G. (1954). *J. Physiol.* **123**, 126.

Wu, R. (1965). *J. biol. Chem.* **240**, 2373.