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Studies on Inorganic Phosphate, Inorganic Pyrophosphatase and Alkaline Nonspecific Phosphomonoesterase Levels in the Gastrointestinal Tract of the Rat

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A study was made of the concentrations of free inorganic phosphate in acid extracts of the mucosae of different segments of the small intestine of the male rat before and after feeding, and of the activities of inorganic pyrophosphatase and alkaline nonspecific phosphomonoesterase in total aqueous homogenates of the mucosae of various segments of the gastrointestinal tract and the liver.

The level of free inorganic mucosal phosphate was found to be roughly constant (about 10 μmole/g of dry weight) in the small intestine and feeding had no effect on it. The activity of inorganic pyrophosphatase was maximal at the oral end of the small intestine and decreased almost linearly along the intestine to about a fourth of the activity at the oral end. The other parts of the gastrointestinal canal resembled the aboral part of the intestine in their enzyme activity. The enzyme activity was lower also in the liver than at the oral end of the intestine. The activity of alkaline nonspecific phosphomonoesterase was maximal at the oral end of the intestine and declined rapidly to about one tenth in the middle of the intestine. The enzyme activity was of the same magnitude in the stomach as at the aboral end of the intestine and even lower in the liver.

A decrease occurs along the intestine in the capacity to absorb nutrients [1] and in certain synthetic processes.[2-3] On the other hand, a similar decline occurs in oxygen utilization,[4] in lactic acid dehydrogenase,[5] hexokinase [6] and adenosine triphosphatase activities, and in the adenosine triphosphate [7] and creatine phosphate contents.[8] It may therefore be concluded that the mucosa of the upper part of the small intestine needs a greater quantity of free inorganic phosphate than the other areas.

The aim of the present investigation was, firstly, to establish the inorganic phosphate contents in different segments of the mucosa of the small intestine of the rat and the effect of feeding on these contents [9] and, secondly, to study systematically the activity in the mucosa of different parts of the gastrointestinal canal of the inorganic pyrophosphatase which is responsible for the

splitting of the pyrophosphate[10] liberated in various biosyntheses and that of the alkaline nonspecific phosphomonoesterase which hydrolyses simple monoesters of phosphoric acid and is very high in the intestinal mucosa.[11,12]

METHODS

Male rats of the Wistar strain (weight range 195—225 g) were used as test animals. For the determination of the pre-prandial inorganic phosphate contents the small intestines of the animals were exposed one hour before feeding. For the post-prandial determinations of inorganic phosphate contents and also for the enzyme determinations the small intestines of the animals were prepared 3 h after feeding. For the inorganic phosphate determinations the animals and specimens were handled as described previously.[8] The tissue extracts were stored in melting ice. To 1 ml of the extract kept in melting ice was added first 1.5 ml of water and, after shaking, 0.5 ml of ammonium molybdate (1.5 %)-sulphuric acid (3 N) solution and a reducing reagent (7.5 mg/ml of water).[13] After storage in melting ice for 9 min the extracts were transferred to a 20° water bath for 1 min, and their absorptions were measured with a Beckman DU spectrophotometer at 660 mμ.

For the determination of the activity of alkaline nonspecific phosphomonoesterase and inorganic pyrophosphatase the specimens were prepared as described previously.[7] To study the enzyme activities, the scraped mucosa specimens were homogenised in 4 ml of water in an all-glass homogeniser of the Potter-Elvehjem type. For the inorganic pyrophosphatase measurements, homogenate diluted 1:40 with 0.05 M tris(hydroxymethyl)aminomethane-0.005 M $MgCl_2$ buffer was pre-incubated in a 38° water bath for 5 min. After this, 100 μl of the homogenate dilution was pipetted into 1 ml of the buffered substrate solution (0.0005 M $Na_4P_2O_7$ (E. Merck AG, *pro analysi*) in 0.005 M $MgCl_2$-0.05 M tris(hydroxymethyl)aminomethane(HCl)-buffer, pH 7.25) and incubated for 5 min in a 38° water bath. The reaction was stopped by adding 0.9 ml of 0.3 N trichloroacetic acid to the reaction mixture. The precipitate was centrifuged off, 1 ml of ammonium molybdate (0.75 %)-sulphuric acid (1.5 N) solution was added to the supernatant and the phosphate determination was continued as previously described.[7] The nonspecific alkaline phosphomonoesterase level of the samples was determined by adding 100 μl of diluted (1:80) homogenate to 1 ml of the buffer substrate solution (0.00225 M *p*-nitrophenylphosphate (B.D.H.), 0.01 M $MgCl_2$ in 0.05 M veronal buffer, pH 9.5) in a 38° water bath; after incubation for 5 min the reaction was stopped by adding 3.9 ml of 0.02 M NaOH to the reaction mixture. The absorption was measured at a wave length of 400 mμ. The dry weights of the tissue homogenates were also determined.

RESULTS

About 83 % of the total inorganic phosphate was removed in the first extraction by the method employed. The recovery of inorganic phosphate added to the tissue extracts was 78 % (range 75—83 %). The use of melting ice in the phosphate determination slowed down the hydrolysis of creatine phosphate, which is otherwise very labile in acid ammonium molybdate solution,[14] to about a fourth of rate at room temperature (see also Ref. 15). On the other hand, the use of the ice bath also reduced considerably the range in which the colour intensity varies linearly as the phosphate concentration.

The recovery of phosphate added to the reaction mixtures in the inorganic pyrophosphatase activity determinations was 85 % (range 80—88 %). The enzyme activity was preserved well when the samples were stored in melting ice: approximately half of the activity remained after 3 days. The pH for optimal enzyme activity in the test conditions was 7.25 and the activities of the enzyme preparations were maximal at a magnesium chloride con-

Table 1. Contents of inorganic phosphate, (in μmole per g of dry weight) (± S.E.M.), in the mucosae of different segments of the small intestine before and 3 h after feeding. The distance from the pylorus to the anus is taken to be unity.

	Before feeding		3 h after feeding	
Site of sample	μmole/g	± S.E.M.	μmole/g	± S.E.M.
0.04	10.5	0.53	10.9	0.53
0.10	10.7	0.41	9.54	0.27
0.19	9.69	0.57	9.10	0.40
0.28	10.2	0.34	9.18	0.46
0.37	10.1	0.47	9.80	0.50
0.55	9.05	0.50	9.75	0.63
0.73	9.83	0.52	12.7	0.70
0.91	12.1	0.62	14.5	0.42

centration of 5 mM. Further addition of pyrophosphate did not accelerate the reaction. The reaction proceeded linearly during the incubation period. Crystals slowly separated from the buffered substrate solution during storage,

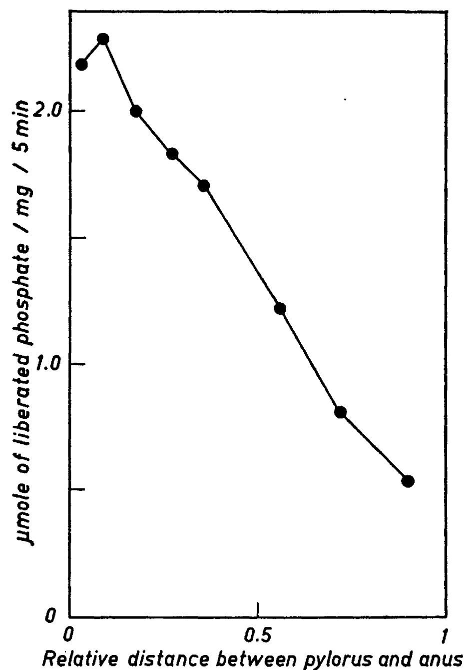

Fig. 1. Activity of inorganic pyrophosphatase in the mucosae of different segments of the small intestine of the rat. The distance from the anus to the pylorus is taken to be 1.

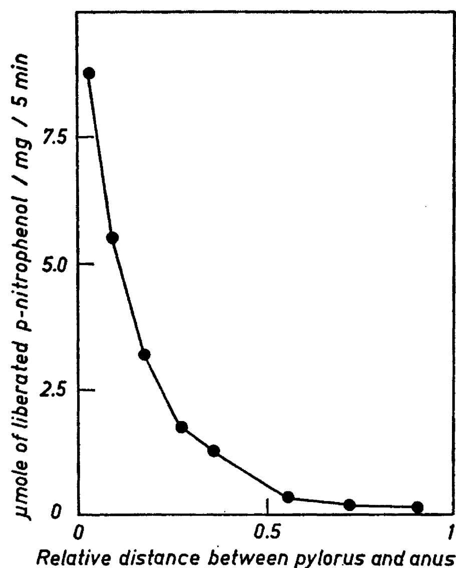

Fig. 2. Activity of alkaline nonspecific phosphomonoesterase in the mucosae of different segments of the small intestine of the rat. The distance from the anus to the pylorus is taken to be 1.

Table 2. The activities of inorganic pyrophosphatase and alkaline nonspecific phosphomonoesterase per unit of dry weight (± S.E.M.) in the mucosae of the gastrointestinal canal and in the liver.

	Inorganic pyrophosphatase		Alkaline nonspecific phosphomonoesterase	
	Liberated phosphate		Liberated p-nitrophenol	
	(mole/mg/5 min) $\times 10^8$		(mole/mg/5 min) $\times 10^8$	
		± S.E.M.		± S.E.M.
Stomach:				
lesser curvature	36.5	4.0	15.1	2.1
greater curvature	52.2	6.3	17.4	2.1
pylorus	36.5	4.1	20.6	2.7
membranous stomach *	15.2	2.0	9.4	0.99
Appendix	53.6	6.1	37.0	3.0
Large intestine	57.8	5.7	29.4	2.0
Liver	155	21	0.82	0.08

* whole wall

and consequently the latter was always prepared just before the determinations were begun. When tissue specimens were preincubated as described, their content of free inorganic phosphate increased during about 5 min.

The recovery of p-nitrophenol added to the reaction mixtures when measuring the activity of alkaline nonspecific phosphomonoesterase was about 99 % (range 96—102 %). The enzyme activity diminished to about a tenth during 24 h in melting ice; maximal activation of the preparations occurred at a magnesium chloride concentration of less than 10 mM. The reaction proceeded linearly during the incubation period.

The activities of both inorganic pyrophosphatase and alkaline nonspecific phosphomonoesterase varied linearly as the dry weight content of the homogenate within the range of dry weight variation.

Ten animals were used in each study. Table 1 shows the contents of inorganic phosphate in the mucosae of different segments of the small intestine both before the animals were fed and 3 h after feeding. Fig. 1 and Table 2 show the activities of inorganic pyrophosphatase in the mucosae of sections of the gastrointestinal canal and Fig. 2 and Table 2 show the distribution of alkaline phosphomonoesterase activity.

DISCUSSION

Lundsgaard [9] reported a value of 1.31 mg of inorganic phosphorus per g of dry weight in the wall of the rat intestine. The same value was obtained by Kroeger and Edwards.[16] According to Shishova,[17] 0.66 mg of inorganic phosphorus per g is extracted from intestinal specimens. All these values are

higher than those obtained in the present study. It is possible that in the earlier studies labile derivatives of phosphoric acid decomposed during the determinations. In the studies of Kroeger and Edwards the ratio of acid-soluble phosphate to inorganic phosphate was roughly 4, whereas in the present study the ratio was found to be about 5.

The present authors were unable to confirm the decrease in the content of inorganic phosphate along the small intestine which was observed by Kroeger and Edwards.[16] The observed distribution of enzyme activities (see also Ref. 7) suggests that the derivatives of phosphoric acid decompose more readily in the oral than in the aboral part of the small intestine unless particular care is taken in the preparation of the animal. Feeding was not found to have any appreciable effect on the inorganic phosphate content of the mucosa of the small intestine in the present work. This is contrary to the findings of Lundsgaard,[9] and Kroeger and Edwards.

It was reported by Kay [18] that the mucosa of the small intestine contains inorganic pyrophosphatase. We have not been able to find in the literature a systematic study of pyrophosphatase activity in the gastrointestinal canal. In the present work, the enzyme activity declined nearly linearly along the small intestine to about a fourth of its level at the oral end. It was distinctly lower elsewhere in the region of the gastrointestinal canal than in the oral end of the small intestine. It was found to be slightly lower also in the liver.

According to Kay,[19] the activity of alkaline nonspecific phosphomonoesterase varies only slightly in the cat intestine. The enzyme activity is sligthly greater in the oral than in the aboral part of the small intestine of the rabbit.[20] Feher and his co-workers [21] showed that the activity is three times as high in the oral part of the small intestine of the rat as in the aboral part. The decrease in enzyme activity along the intestine has been confirmed histochemically.[22] Triantaphyllopoulos and Tuba [12] reported that the activity drops in the rat exponentially as a function of bowel length when β-glycerophosphate is the substrate. An even more rapid decrease in activity was established in the present study when the substrate used was foreign to the organism. The activity in the middle of the intestine was one tenth of the activity at the oral end.

The activities of both enzymes studied were thus maximal at the oral end of the small intestine and decreased along the intestine. If the levels of substrates of the enzymes or their rates of synthesis vary similarly on moving down the small intestine, the rate of the pyrophospholytic reactions must also be similar. These reactions are associated with biosyntheses (*e.g.* Ref. 10). The hydrolysis of inorganic pyrophosphate is considerably more rapid in the mucosae of the oral part of the small intestine than in the aboral part and probably speeds up the biosyntheses in the former more than at the aboral end of the intestine where the inorganic pyrophosphatase activity is lower.

A great deal of utilisable energy is lost in the reaction catalysed by pyrophosphatase. The distribution of enzyme activity in the mucosae of different sections of the small intestine also proves that consumption of energy is greater at the oral end than in other parts of the small intestine.

The roughly constant levels of inorganic phosphate in different segments of the intestine and the marked decrease in the activities of both inorganic

pyrophosphatase and alkaline nonspecific phosphomonoesterase along the intestine — the same is true of the enzyme which hydrolyses adenosine triphosphate [7] — probably signify a more rapid turnover of phosphate at the oral end of the intestine than in other parts of the gastrointestinal canal.

The inorganic phosphate may in some situations regulate the rate of oxidative phosphorylation for, according to the present study, its content in the mucosae of the small intestine is only slightly greater than the creatine phosphate content [8] and well over double the adenosine triphosphate content [23] in similarly prepared animals.

A preliminary report on the above work was published earlier.[24]

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