THE KETOKINASE ACTIVITY OF THE INTESTINAL MUCOSA*

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SUMMARY

The ketokinase activity of homogenates of intestinal mucosa has been found to involve two enzymes, a heat-stable ketokinase which has a high affinity for fructose and sorbose and a heat-labile ketokinase which shows low affinity for both ketoses. The ketoses appear to be phosphorylated by both enzymes in the 1 position.

The heat-stable ketokinase has properties similar to the liver ketokinase and its presence in the intestinal mucosa is compatible with the hypothesis that the mechanism of the transformation of fructose into glucose that takes place in the intestinal mucosa might be essentially the same that operates in liver.

INTRODUCTION

Phosphorylating activities specific for ketoses have been found to occur in liver and muscle. CORI *et al.* obtained preparations of muscle and liver that phosphorylate fructose but are inactive with glucose[1]. The ketokinase activity of the muscle preparation was subsequently shown to be due to the action of phosphofructokinase[2]. This enzyme has low affinity for fructose and sorbose and phosphorylates both free sugars in the 1 position. HERS, however, has isolated a ketokinase from liver which has high affinity for fructose and sorbose[3,4]. He has elucidated the mechanism by which fructose is transformed into glucose in liver. The first step in the chain of events is the phosphorylation of fructose by ketokinase to produce fructose-1-phosphate[5].

The observation that sorbose is phosphorylated by homogenates of rat intestinal mucosa was interpreted in this laboratory as indicating the presence of a ketokinase[6]. Our efforts were directed consequently towards the identification of such an activity. The evidence reported in the present paper shows that the intestinal mucosa possesses two enzymes capable of phosphorylating fructose as well as sorbose. One of them, a heat-stable ketokinase has been purified and the study of its properties has revealed a close similarity with liver ketokinase.

SALOMON AND JOHNSON[7] have proposed recently that the transformation of

Abbreviations: ATP, adenosine triphosphate; ADP, adenosine diphosphate; Tris, tris-(hydroxymethyl)aminomethane; TCA, trichloroacetic acid; NAGA, N-acetylglucosamine.

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fructose into glucose which takes place when fructose is transported across the intestinal tissues may follow the same pathway as that of liver. Our findings provide evidence for the presence in intestinal mucosa of one of the enzymes that would be required for such a mechanism to operate in intestinal tissue. It can not be decided at present, however, whether or not this is the physiological role of the intestinal heat-stable ketokinase.

MATERIALS AND METHODS

Glucose-free fructose was prepared by recrystallization from 80% methanol of a commercial fructose (Pfanstiehl). L-Sorbose was purchased from Merck and D-arabinose from Pfanstiehl. D-Mannoheptulose was a gift from Dr. N. K. RITCHMYER. ATP and ADP were obtained from Sigma Chemical Co. as the disodium salts.

Rat-mucosa homogenates

Fed albino rats weighing 150 to 200 g were stunned, decapitated and exanguinated, the upper two thirds of the small intestine were flushed with ice cold saline, opened lengthwise and the mucosa was scrapped off with a spatula. After weighing the mucosa was homogenized for 2 min in a Waring microblendor with 2 volumes of 0.005 M Versene neutralized to pH 7. Further fractionation of the homogenate will be described in the experimental section. The upper part of the small intestine of a horse was used when larger amounts of starting material were needed (purification procedure).

Measurement of the ketokinase activity

Most of the experiments were carried out as follows: small tubes containing ketose, ATP-Mg ($1.5 \cdot 10^{-2}$ M), NaF ($2.5 \cdot 10^{-2}$ M), Tris–maleate buffer ($1 \cdot 10^{-1}$ M, pH 6.5), KCl ($4 \cdot 10^{-1}$ M) and an appropriate amount of enzyme were incubated at 30° for varying lengths of time. The reaction was stopped by the addition of either 5 ml of 5% trichloroacetic acid or equal volumes of 0.3 N $Ba(OH)_2$ and $ZnSO_4$. The methods used to follow the reaction were: (a) substrate disappearance, (b) esterified ketose formation, (c) non-nucleotidic acid-labile esterified phosphorus formation and (d) the photometric indicator method (see ref. 6, 8 and EXPERIMENTAL). Ketoses were determined by the method of ROE[9] or DISCHE[10]. The method of FISKE AND SUBBAROW[11] was used to analyze inorganic phosphorus. The total phosphorus was determined by analysis of the inorganic phosphorus after wet ashing with sulfuric and nitric acids. Protein was determined by the method of LOWRY *et al.*[12].

EXPERIMENTAL

Preliminary work

L-Sorbose was used as a specific substrate for the determination of the ketokinase activity in homogenates of rat intestinal mucosa that contain, in addition, a hexokinase. The hexokinase was assayed with glucose as substrate. The homogenate was centrifuged at 14,000 × g for 15 min and the phosphorylating activities of the supernatant were tested. Sorbose is phosphorylated 5 times faster than glucose by the supernatant while the phosphorylation of glucose by the whole homogenate is faster

than that of sorbose (Table I). The assumption that sorbose is not a substrate for hexokinase is confirmed by the lack of inhibition observed when the phosphorylation of sorbose was carried out in the presence of an equimolar concentration of NAGA.

TABLE I

THE KETOKINASE ACTIVITY OF THE INTESTINAL MUCOSA; REMOVAL OF HEXOKINASE

The homogenate prepared as described in the METHODS section was centrifuged at 14,000 × *g* for 15 min. The names Supernatant II and Supernatant I correspond to the supernatants obtained when the homogenate was adjusted to pH 4.5 with 0.1 *M* HCl prior to centrifugation and when the acidification was omitted, respectively. Supernatant II was adjusted to pH 6.5 immediately after centrifugation. Supernatant I was aged by incubation at 30° for 1 h (at pH 7.5). The phosphorylating activities (μmoles of sugar phosphorylated in 15 min at 30° by 1 ml of each preparation) were determined by the esterified sugar formation method[6]. The amount of sugar in the precipitate obtained with Ba-alcohol was analyzed by the ROE's method for ketoses[9] and glucose by the method of SOMOGYI[13]. The incubation mixture was analogous to the one described in the METHODS section except that the pH was 7.5, the Mg-ATP ratio was 0.5 and the KCl was omitted.

Sugar	*0.1 M sorbose*	*0.1 M glucose*	*0.1 M sorbose + 0.1 M NAGA*
Homogenate			
Phosphorylating activity	1.5	6.2	1.5

Sugar	*0.1 M sorbose*	*0.1 M glucose*	*0.1 M glucose + 0.1 M sorbose*
Supernatant I fresh (protein 14, mg/ml)			
Phosphorylating activity	1.0	0.2	1.2

Sugar	*0.1 M sorbose*	*0.1 M glucose*
Supernatant I aged		
Phosphorylating activity	0.5	0.0

Sugar	*0.1 M sorbose*	*0.1 M fructose*	*0.1 M fructose + 0.1 M NAGA*
Supernatant II (protein 5, mg/ml)			
Phosphorylating activity	0.8	2.0	2.0

On the other hand, the implication of a ketokinase activity different from the hexokinase, as responsible for the phosphorylation of sorbose is justified by the fact that the relative rates of phosphorylation of sorbose and glucose vary from one preparation to another. The aging of the supernatant I resulted in the loss of the ability to phosphorylate glucose while the preparation was still able to phosphorylate sorbose. The absence, however, of significative amounts of hexokinase in a preparation containing most of the original ketokinase activity was only achieved if the homogenate was acidified to pH 4.5 prior to centrifugation. The resulting supernatant (supernatant II) contains 80 % of the ketokinase activity recovered when the acidification is omitted (supernatant I) with a specific activity twice as high. Supernatant II phosphorylates fructose and no significative contribution of hexokinase to this

phosphorylation could be detected since an equimolar concentration of NAGA did not result in any inhibition.

At all concentrations tested fructose was phosphorylated about 2.5 times faster than sorbose (Table II). In the experiment described in Table II and all subsequent experiments the incubation mixture contained ATP-Mg in a ratio of 1 and 0.4 *M* KCl since they were found to result in maximum ketokinase activity. Under these optimal conditions phosphorylation of 0.1 *M* sorbose by the supernatant I was approximately of the order of 2.0 μmoles of sugar phosphorylated in 15 min at 30° by 1 ml of supernatant. The phosphorylation of 0.1 *M* mannoheptulose, which is 2.9 times slower than that of 0.1 *M* sorbose, could be established only under the optimal conditions.

TABLE II

THE KETOKINASE ACTIVITY OF THE INTESTINAL MUCOSA; EFFECT OF SUBSTRATE CONCENTRATION ON THE RATE OF PHOSPHORYLATION OF FRUCTOSE AND SORBOSE

The activity was assayed with the standard incubation mixture described in the METHODS section and by the esterified ketose formation method[7]. The enzymic preparation used was the supernatant obtained by centrifugation at 14,000 × *g* for 15 min of the homogenate adjusted to pH 4.5 (supernatant II).

Sugar concentration M	*Ketokinase activity*	
	Sorbose	*Fructose*
0.02	1.2	3.2
0.04	1.4	3.5
0.1	1.5	3.8
0.4	1.9	4.0

* μmoles of sugar phosphorylated in 15 min at 30° by 1 ml of enzymic preparation.

The ketokinase activity was determined indiscriminately by esterified ketose formation or by esterified phosphorus formation and in many instances by both and the complete agreement observed indicates that the product of the action of the ketokinase on sorbose and fructose is the ketose monophosphate. Moreover, the rate of acid hydrolysis of the sorbose monophosphate produced corresponds to that of sorbose-1-phosphate. The ester formed when 0.1 *M* sorbose was used, was hydrolyzed at the following rate: 40 % of the esterified phosphorus was hydrolyzed in 11 min at 100° in 0.1 *M* sulfuric acid and 85 % of it in 1 h.

Thermal fractionate inactivation

The heat inactivation of the ketokinase activity was studied using the supernatant obtained by centrifugation of the homogenate at 14,000 × *g*. The comparison of the rates of inactivation at different temperatures provided strong evidence for the presence of two enzymes with ketokinase activity in rat intestinal mucosa. The values obtained (Table III) indicate that the rate of inactivation of the ketokinase activity increases very sharply with an increase in temperature within the range of 50 to 60° and the range of 75 to 85°. The rate of thermal inactivation is independent from the temperature, between 60 and 75°. These results can be interpreted assuming that two ketokinases are present, one which is completely inactivated by 5 min of heating at 70°, and another which is not appreciably inactivated at temperatures

TABLE III

THERMAL FRACTIONATE INACTIVATION

Aliquots of supernatant I were used. They were heated at different temperatures for 5 min. Immediately after heating they were transferred to an ice bath. For the assay of the residual phosphatase and ketokinase activities the aliquots were adjusted to the same volume, the contents of the assay mixture were added (with 0.2 *M* sorbose, see the METHODS section) and except several unincubated controls, they were incubated at 30° for different lengths of time. The total volume was 1.1 ml in experiment I and 1.25 ml in experiment II. The reaction was stopped by the addition of 5 ml of 5% TCA. After treatment with Norit A charcoal and filtration, aliquots of the filtrates were used to determine inorganic phosphorus before and after 1 h of hydrolysis in 0.1 *N* sulfuric acid at 100°. The difference in inorganic phosphorus between controls and incubated tubes was used to calculate the phosphatase activity in each tube (in separate experiments it was found that the appearance of inorganic phosphorus corresponds essentially to enzymic ATP hydrolysis). The difference in esterified phosphorus (inorganic phosphorus after acid hydrolysis minus inorganic phosphorus before acid hydrolysis) between controls and incubated tubes served to calculate the ketokinase activity in the same tubes.

Thermal inactivation (5 min of heating)		*Assay of the residual activity*		*Ketokinase activity (μmoles of phosphorus esterified/ml of extract/15 min)*	*Phosphatase activity (μmoles of inorganic phosphorus formed/ml of extract/15 min)*
Expt.	*Temperature*	*Extract used (ml)*	*Time of incubation (min)*		
I	—	0.2	60	2.0	3.75
I	50	0.2	60	2.25	2.1
II	50	0.2	60	1.95	3.0
I	55	0.2	90	1.6	1.15
I	60	0.5	90	1.0	0.65
II	60	0.5	60	0.9	0.5
I	65	0.5	90	0.8	0.4
I	70	0.6	120	0.95	0.3
II	70	0.5	90	0.9	0.15
I	75	0.6	120	0.95	0.05
II	75	0.5	90	0.95	0.02
II	80	0.6	120	0.1	0.02
II	85	0.8	120	0.0	0.02

below 75° but is completely inactivated by 5 min of heating at 85°. The names heat-labile ketokinase and heat-stable ketokinase will be subsequently used in this paper to distinguish these two enzymes. We concluded also from these data that by means of a short heating at a suitable temperature, a preparation of heat-stable ketokinase could be obtained that was free from phosphatase and heat-labile ketokinase.

Purification of the heat-stable ketokinase

A survey of the ketokinase activity content in the intestinal mucosa of several species of mammals was carried out. Dog, sheep, cow and horse intestinal mucosa was found to contain approximately the same activity. Horse small intestine was chosen as starting material because the mucosa can be removed easily and also a single long piece of small intestine provided enough material to carry out the purification. The procedure for the purification of the heat-stable ketokinase consists essentially of a short heating at 70° followed by ammonium sulfate fractionation in order to concentrate the enzyme (Table IV).

In each step of the purification, the ketokinase activity was assayed by the esterified phosphorus formation with sorbose as substrate. In step 1 the ratio between the rates of phosphorylation of sorbose at $2 \cdot 10^{-1}$ *M* and $5 \cdot 10^{-3}$ *M* initial concen-

TABLE IV

PURIFICATION OF THE HEAT-STABLE KETOKINASE FROM HORSE INTESTINAL MUCOSA

Starting material: 460 g of scrappings of intestinal mucosa from the small intestine of a horse. The ketokinase activity is given in μmoles of 0.005 M sorbose phosphorylated by 1 ml of extract in 15 min. Step 1, preparation and centrifugation of the homogenate. A piece of small intestine (about 6 meters long) was taken from a slaughtered horse and chilled in ice. The contents were washed off with cold water and the intestine was opened lengthwise. The mucosa was removed carefully with a spatula in the cold room. The scrappings (460 g) were homogenized with 2 volumes of 0.005 M Versene in a Waring type blendor for 2.5 min. The homogenate was kept overnight at −10°. After thawing it was centrifuged at 14,000 × g for 15 min. The supernatant was removed and the precipitate discarded. Step 2, heating. Fractions of 100 ml of the supernatant (900 ml) were heated in a 250-ml Erlenmeyer placed in a water bath with continuous stirring. The temperature of the fluid rose up to 70° in 2 min and was maintained at 70° for 3 additional minutes. The Erlenmeyer was then cooled with ice water and the fluid was kept frozen overnight at −18°. After thawing the resulting clotty suspension was centrifuged at 14,000 × g for 15 min. The supernatant was removed and the precipitate discarded. Step 3, fractionation with ammonium sulfate. Solid ammonium sulfate (170 g) was added to the supernatant (870 ml) to give a 33% saturated solution. After allowing 12 h at 2° for precipitation it was centrifuged at 10,000 × g for 10 min. The precipitate was discarded and to the supernatant (920 ml) solid ammonium sulfate was added (98 g) to give a 50% saturated solution. This was left overnight at 2° and centrifuged as before. The precipitate was suspended in 0.005 M Versene and dialyzed against renewed water for 4 h.

Step	*Volume ml*	*Ketokinase activity µmoles/ml/15 min*	*Protein mg/ml*	*Purification*	*Recovery %*
1	900	1	13		
2	870	1	4	× 3.3	96
3	11	28	34	× 11.7	33

tration varied from one preparation to another from 2.0 to 3.25. In step 2 the ratio was consistently 2.0. These values indicate the presence in some step 1 preparations of a rather unstable ketokinase which was destroyed completely by heating at 70° for 5 min. This heat-labile ketokinase has apparently a low affinity for sorbose since the rate of phosphorylation at $5 \cdot 10^{-3}$ M sorbose was the same before and after heating.

The values obtained for the ketokinase activity with low sorbose concentration were used to ascertain the purification of the heat-stable ketokinase attained in each step of the procedure. It was assumed that with low substrate concentration the activity measured was exclusively due to the action of the heat-stable ketokinase even when both ketokinases are present, *i.e.* in the preparation obtained in step 1. The procedure presents a certain degree of variability in step 3 but always yielded in this step a preparation very suitable for the study of the properties of the heat-stable ketokinase. This preparation contains a relatively high ketokinase activity which is stable for months at −20°, and it is free from phosphatase, hexokinase and heat-labile ketokinase.

Properties of the heat-stable ketokinase

Affinity: The phosphorylation of fructose and sorbose by the heat-stable ketokinase was followed with the photometric indicator method[8].

The relative rates of phosphorylation of fructose and sorbose at different concentrations were determined by taking the reciprocal of the time required for a given

decrease in O.D. The results (Fig. 1) showed the K_m for fructose to be approx. $7 \cdot 10^{-4}$ M, the K_m for sorbose to be approx. $5 \cdot 10^{-3}$ M and the ratio V_{max} sorbose/V_{max} fructose to be approx. 2.0.

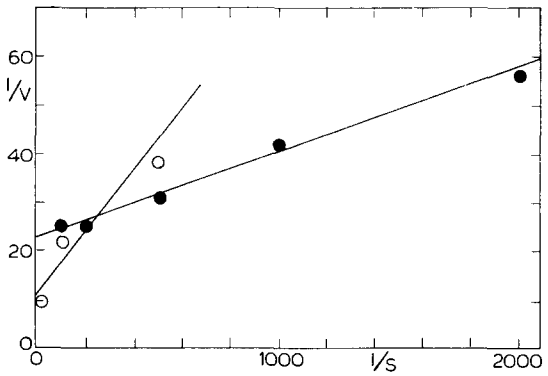

Fig. 1. Phosphorylation of fructose and sorbose at different concentrations by the heat-stable ketokinase. LINEWEAVER AND BURK representation; v is expressed in arbitrary relative values obtained by the photometric indicator method and S are molar concentrations. ○ ○ ○, sorbose; ● ● ●, fructose.

The phosphorylation of fructose was not appreciably affected by changing the concentration of ATP from 3 to $15 \cdot 10^{-3}$ M (Table V). This behaviour indicates that the affinity for the donor ATP may be as high as has been observed with other kinases[3,8]. The addition of ADP inhibits markedly the heat-stable ketokinase. The inhibition appears to be relieved at least partially by increasing the ATP concentration. The results obtained do not constitute enough evidence, however, to conclude that the inhibition by ADP is strictly competitive as it has been proved to be with brain hexokinase[8]. Liver ketokinase is also inhibited by ADP although the available reports are not in agreement on whether the inhibition is competitive[14] or non-competitive[15].

Reaction product: The product accumulated during the phosphorylation of sorbose by the heat-stable ketokinase was isolated as described in Table VI. The conclusion that the esterified sugar is sorbose monophosphate is supported by the results of the analysis of phosphate and ketose. That the product is sorbose-1-phosphate is indicated by the rate of acid hydrolysis of the phosphate group.

TABLE V

INHIBITION BY ADP OF THE PHOSPHORYLATION OF FRUCTOSE BY THE HEAT-STABLE KETOKINASE

Two series of tubes were prepared containing in 1.9 ml, 2 μmoles of fructose, 100 μmoles of Tris-maleate buffer, pH 6.5, 800 μmoles of KCl, 30 μmoles of $MgSO_4$ and the appropriate amounts of ATP to give the initial concentrations of 15, 7.5 and $3.7 \cdot 10^{-3}$ M. The tubes in the one series contained, in addition, 15 μmoles of ADP. To each tube 0.1 ml of the preparation obtained in step 3 of the purification procedure (diluted 8 times) was added. Except the unincubated controls, all the tubes were incubated at 30° for 30 min. They were deproteinized with $Ba(OH)_2$ and $ZnSO_4$ and ketose was determined by the method of DISCHE[10]. The phosphorylation of fructose was estimated by substrate disappearance.

ATP, average molar concentration $\times 10^3$	11.5	11.9	5.75	5.9	2.8	3.0
ADP, average molar concentration $\times 10^3$	0.21	6.1	0.25	6.1	0.2	6.0
Fructose phosphorylated (referred to 1 ml)	0.42	0.18	0.50	0.15	0.41	0.11

TABLE VI

ANALYSIS OF THE PRODUCT OF THE REACTION OF PHOSPHORYLATION OF SORBOSE BY THE HEAT-STABLE KETOKINASE

To a tube containing in a volume of 1.8 ml, 100 μmoles of sorbose; 600 μmoles of Tris-maleate buffer, pH 6.5; 800 μmoles of KCl; 75 μmoles of ATP and 75 μmoles of $MgSO_4$, 0.2 ml of the preparation obtained in the step 3 of the purification procedure were added. It was incubated at 30° for 2 h. 8 ml of 5% TCA were added, it was shaken with 1.5 g of norit A charcoal for 15 min and filtered. To 5 ml of the filtrate, phenolphtalein and 0.2 ml of 0.1 *M* barium acetate were added. After 5 min in the cold it was centrifuged and the supernatant was decanted. To 5 ml of the supernatant 20 ml of 96% alcohol were added and after 45 min in an ice bath, it was centrifuged. The precipitate was dissolved in 0.35 ml of 0.1 *N* HCl. 0.4 ml of water and 0.1 ml of 0.5 *M* K_2SO_4 were added. It was centrifuged, checked for excess barium and then neutralized and adjusted to 3 ml with water. Suitable aliquots were taken for the different determinations.

	μmoles	
Ketose	36	
Carbohydrate	33	
Total esterified phosphorus	36	
Acid hydrolysis in 0.1 N sulfuric acid at 100°		*% of total*
Phosphorus hydrolyzed in 5 min	6.6	15
10	12	33
60	33	90

Specificity: The phosphorylation of sorbose is inhibited by fructose. The rate of phosphorylation of an equimolar mixture of 0.05 *M* fructose and sorbose determined by the photometric indicator method[8] was about 40% of the rate of sorbose phosphorylation and approximately equal to the rate of fructose phosphorylation at the same concentration. The phosphorylation of 0.01 *M* sorbose was not inhibited by the presence of a concentration of D-arabinose 10 times higher. This result is in contrast with the pattern of specificity of brain hexokinase. Hexokinase is inhibited by pentoses which differ from the corresponding hexose substrate by the absence of the carbon on which the phosphate should be attached. In the case of the heat-stable ketokinase, it appears that the sugar must be a ketose in order to combine with the enzyme. It was not possible to detect with the photometric indicator method[8] any phosphorylation of 0.016 *M* mannoheptulose by the heat-stable ketokinase. The supernatant obtained by centrifugation of the homogenate phosphorylates 0.1 *M* mannoheptulose about 1/3 as fast as 0.1 *M* sorbose (see preliminary work). This indicates that mannoheptulose is a good substrate for the heat-labile ketokinase but not for the heat-stable ketokinase. This difference in specificity with respect to mannoheptulose between the two intestinal ketokinases has its parallelism in liver and muscle ketokinases. KUYPER[14] found that mannoheptulose is not phosphorylated by liver ketokinase and VILLAR-PALASI AND SOLS[2] have reported that mannoheptulose is substrate for muscle phosphofructokinase.

DISCUSSION

Because of the possible implication in the mechanism of transport of sugars from the intestine, attention has been directed towards the transformation of fructose into glucose that takes place in the intestinal mucosa[16–18]. SOLS AND ALVARADO* have

* A. SOLS AND F. ALVARADO, unpublished results.

searched insistently for the possibility of a direct transformation of fructose into glucose by homogenates of rat intestinal mucosa and have failed to detect any isomerization. Recently SALOMON AND JOHNSON[7] have proposed that the transformation of fructose into glucose in the intestine may follow the same path as that in liver and they have reported evidence in favor of this hypothesis. Our experiments show the presence in the intestinal mucosa of a heat-stable ketokinase which is very similar in its properties to the liver ketokinase. That the role of the heat-stable ketokinase in the intestinal mucosa is analogous to the function of liver ketokinase in the transformation of fructose into glucose, although suggestive, remains to be proved. Evidence has been obtained indicating that in the intestinal mucosa in addition to a heat-stable ketokinase there is a heat-labile enzyme which also phosphorylates ketoses. In homogenates of rat intestinal mucosa this latter enzyme is responsible for approx. 50 % of the total phosphorylation of 0.2 *M* sorbose. Both heat-stable and heat-labile ketokinases phosphorylate fructose and sorbose in the 1 position. Aside from their different heat-stability they differ in two main characteristics: (a) The heat-labile ketokinase has a very low affinity for fructose and sorbose in contrast with the high affinity of the heat-stable ketokinase for both sugars and (b) the ratio of the relative maximum rates of phosphorylation of sorbose and fructose by the heat-stable ketokinase is 2.0 and approximately 0.5 for the heat-labile ketokinase. It should be emphasized that the properties of the ketokinase activity present in homogenates and crude extracts of intestinal mucosa differ markedly from the properties of liver ketokinase. In fact it resembles the muscle ketokinase activity. Only after purification the presence of a ketokinase similar to the liver enzyme becomes evident. By analogy with the observations of VILLAR-PALASI AND SOLS[2] on the ketokinase activity of muscle it is suggested that the heat-labile ketokinase of the intestinal mucosa may be a marginal activity of the intestinal phosphofructokinase. Like muscle phosphofructokinase the heat-labile ketokinase of the intestinal mucosa has low affinity for fructose and sorbose and phosphorylates mannoheptulose which is not a substrate for the intestinal heat-stable ketokinase nor the liver ketokinase[15].

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