SUMMARY

The nonspecific hexokinases of rat intestinal epithelium were isolated by diethylaminoethyl column chromatography. The major isoenzymes of hexokinase which were found were types I and II, but, in addition, the presence of types III and IV in small amounts was suggested. The nonspecific hexokinases were clearly separated from specific fructokinase and from $N$-acetylglucosamine kinase, an enzyme previously believed to be absent from intestine. Guinea pig intestine differed from rat intestine in three significant ways. (a) There was a relative increase of type II hexokinase compared to type I, and there was an absence of types III and IV; (b) guinea pig type II hexokinase was more sensitive to $N$-acetylglucosamine inhibition than was rat type II; and (c) hexokinase specific activity of guinea pig intestine was one-third that of the rat.

Of the total hexokinase activity in both guinea pig and rat intestine, 50% was in a particulate form. Triton X-100 (0.5%) could solubilize 40% of this particulate hexokinase. The apparent solubilization of particulate hexokinase by 0.9 M KCl was counteracted by the marked inhibitory effect of KCl on both soluble and particulate hexokinases. KCl inhibition of hexokinase activity was proportional to KCl concentration and partly irreversible. The hexokinases of other rat organs were also inhibited by KCl. Purified intestinal epithelial microvilli had no demonstrable hexokinase activity.

There are conflicting reports in the literature on the changes of glycolytic enzymes of rat intestinal mucosa that result from dietary alterations and from metabolic disorders such as diabetes (1–4). Part of this conflict arises from the observations of Stifel et al. (1) that the intestine has a soluble, high $K_m$ hexokinase (i.e. glucokinase) that increases in activity with a high glucose diet. These workers also showed that intestinal fructokinase activity was increased by feeding fructose. These reported dietary effects on intestinal hexokinases, glucokinase, and fructokinase were based on changes of these enzyme activities in the crude 105,000 x g supernatant fraction. No studies were carried out on isolated enzymes or isoenzymes. Previous reports by Kataen and Schimke (5) indicated that the rat intestine has, predominantly, types I and II low $K_m$ hexokinases with a small, barely detectable amount of type III. These workers failed to detect any type IV hexokinase, which is the high $K_m$ hexokinase or glucokinase. In fact, type IV has not been detected in any tissue other than liver. Srivastava, Shakespeare, and Hubscher (6) also found types I and II in rat and guinea pig intestinal mucosa but did not detect types III or IV in either animal.

Prior to evaluating the mechanism of the presumed effects of diet and diabetes on intestinal glycolytic enzymes, we attempted to clarify some of the disparities in the literature by separating specific fructokinase and $N$-acetylglucosamine kinase from hexokinase isoenzymes and examining some of the properties of these enzymes.

Separation of the nonspecific hexokinases from specific fructokinase of the intestine has not been previously reported. Furthermore, $N$-acetylglucosamine, which inhibits hexokinase and is used in the usual spectrophotometric assay for hexokinase, has been considered not to be phosphorylated by the intestine (7). However, recently we reported that $N$-acetylglucosamine can, indeed, be phosphorylated in the intestine by two systems, one ATP-dependent, and the other a labile, phosphoenolpyruvate-dependent system (8). The present paper extends those observations.

This work shows that the two major intestinal hexokinase isoenzymes of rat and guinea pig, types I and II, can be separated from specific fructokinase and $N$-acetylglucosamine kinase. Rat is found to differ from guinea pig in the predominance of type II hexokinase rather than type I, and in the probable presence of small amounts of types III and IV. The major hexokinase isoenzymes of rat also differ from guinea pig isoenzymes in the degree of inhibition by $N$-acetylglucosamine. Contrary to expectations from the literature, high concentrations of KCl strikingly inhibit hexokinase activity. This raises questions concerning the use of KCl for solubilization of particulate hexokinase (4) and the use of KCl gradients in DEAE-cellulose chromatographic separation of hexokinase activities (5, 9). In addition, the hexokinases contribute at least 30% to the total fructose-phosphorylating activity. A simple method is presented for estimating specific fructokinase without interference by hexokinase.

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METHODS

General Methods and Materials—All sugars and sugar phosphates were purchased from Mann. Trisodium phosphoenol-pyruvate, disodium-ATP, disodium-ADP, and other nucleotides were obtained from Sigma; pyruvate kinase, glucose 6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase were from Boehringer Mannheim; ion exchange cellulose was from Reeve Angel Company, New York, New York; ion exchange resins were from Bio-Rad, Richmond, California; and Sephadex was from Pharmacia. All radioactive compounds were purchased from New England Nuclear. Purity was checked by high voltage electrophoresis in 0.05 M pyridine-acetate buffer, pH 6.5. N-Acetyl-1-14C-glucosamine contained an impurity and was routinely treated with a mixed ion exchange resin which effectively removed this impurity. All other chemicals were from common commercial sources.

Enzyme Assay—Hexokinase activity was determined spectrophotometrically according to the method either of Sharma, Manjeshwar, and Weinhouse (10) or of McLean and Brown (11). These are coupled assays in which NADPH, and the increase in optical density at 340 nm is observed. The reaction was run at 23–25°. In these assays three cuvettes are used. The first is the blank which contains N-acetylglucosamine, a hexokinase inhibitor, to correct for any endogenous ATP-dependent NADP reduction. The second cuvette contains 0.5 mM glucose, and the third contains 100 mM glucose. The rate difference between the second and third cuvettes has been considered a crude estimation of high K_m hexokinase, the so-called glucokinase (10). A radioactive assay for hexokinase was also used, especially in estimating column fractions. In this assay, the enzyme preparation (usually 0.1 ml) was added to 0.2 ml of total volume reaction mixture containing 10 mM ATP; 10 mM MgCl_2; 50 mM Tris- HCl buffer, pH 7.4; and 10 mM uniformly labeled glucose-14C (approximately 1500 dpm per millimole). Incubation was usually for 30 min at 37°. The reaction was stopped by placing the tubes in ice and adding 1 ml of 0.04 M barium acetate in 70% ethanol. The precipitate, containing the phosphorylated sugar, was washed with barium acetate- ethanol solution on a Millipore filter (type SM cellulose acetate, 5 μ pore). After drying the filter, retained radioactivity was counted in a Nuclear-Chicago planchet counter.

N-Acetylglucosamine kinase and fructokinase were measured with the use of the radioactive assay for hexokinase with substitution of the respective radioactive sugars. For estimation of fructokinase, without interference from nonspecific hexokinase, the crude supernatant was acid-treated according to Adelman, Ballard, and Weinhouse (12). The pH was lowered to 5.1 by addition of cold HCl with rapid stirring, and the resultant precipitate was removed by centrifugation at 20,000 × g for 20 min. The pH of the subsequent supernatant was then adjusted to 7.4 with KOH. This treatment effectively removed at least 75% of the hexokinase activity. Within the limitations of estimating fructokinase in the crude supernatant, it appeared that close to 70% of the original fructokinase activity was intact after mild acid treatment, and that the only product found was fructose-1-P (see “Results”). Protein was determined by the method of Lowry et al. (13).

Tissue Preparation—The usual enzyme preparations were made from male guinea pigs weighing approximately 250 g obtained from Elm Hill Breeding Laboratories, Chelmsford, Massachusetts, and from male and female rats weighing 175 to 200 g obtained from Charles River Laboratories, N. Wilmington, Massachusetts. The animals were sacrificed by cervical dislocation, and the small intestine was removed. After rinsing the lumen with 150 to 200 ml of ice-cold 0.154 M sodium chloride, the intestine was everted and blotted on paper towels, and the mucosa was removed by gentle scraping. The mucosal scrapings were suspended in 2.5 or 10 volumes of 0.01 M potassium phosphate buffer, pH 7.4, containing 5 mM EDTA and 0.5 mM dithiothreitol. On occasion, 10 mM glucose was added to the buffer. After homogenizing with a Potter-Elvehjem motor-driven homogenizer, the material was centrifuged at 20,000 × g for 30 min or at 105,000 × g for 60 min. Protein concentrations of the enzyme preparations (10:1, volume to weight) varied from 3 to 5 mg per ml. Intestinal microvilli were prepared according to the method of Forstner, Sabesin, and Isselbacher (14).

Chromatography—DEAE-cellulose column chromatography was performed in a manner similar to procedures described by Grossbard and Schimke (9). The columns (1.0 × 18 cm) were equilibrated with 0.01 M potassium phosphate buffer (pH 7.4) containing 5 mM EDTA, 0.5 mM dithiothreitol, and 1 M KC1 and then washed with buffer without KC1. The sample was then applied, and the column was developed with 200 ml of a linear gradient from 0 to 0.5 M KC1 in 0.01 M potassium phosphate buffer containing 5 mM EDTA and 0.5 mM dithiothreitol.

RESULTS

Rat Intestinal Enzymes—Separation of soluble hexokinase, fructokinase, and N-acetylglucosamine kinase of rat intestine was easily achieved by DEAE-cellulose chromatography with a KC1 gradient (Fig. 1). Fructose-phosphorylating activity was found in three peaks. The first peak had no activity with glucose as substrate. The product of this enzyme fraction was fructose-1-P as determined by descending chromatography in 1 M ammonium acetate, 95% ethanol, and 0.1 M EDTA (30:70:1) (15). The K_m for the formation of fructose-1-P with ATP in this peak was 1.25 × 10^-4 M as determined by a Woolf plot. These properties are compatible with those of a specific fructokinase. The two other peaks of fructose-phosphorylating activity were coincident with glucose-phosphorylating activity, and the products were fructose-6-P. This property is compatible with the nonspecific hexokinases of the low K_m type. High K_m hexokinase is reported not to phosphorylate fructose (16). N-Acetylglucosamine kinase activity was found in a separate peak which was clearly distinct from fructose- and glucose-phosphorylating activities.

Glucose-phosphorylating activity was found in two major peaks (Fig. 1). The first was a relatively sharp peak whose K_m (10^-4 M for glucose) and chromatographic position were consistent with type I hexokinase isolated similarly in other tissues by Grossbard and Schimke (9). The second peak appeared reproducibly as a sharp peak but with a prolonged tapering of activity. This suggested the possible presence of more than one hexokinase. Since type III is known to be inhibited by high concentrations of glucose, this fraction was evaluated with 0.5 mM glucose. No clear separate peak was seen with activity greater than that obtained with 10 mM glucose. A large scale preparation was then made, and activity was evaluated after elution from a correspondingly larger capacity DEAE-cellulose column. The pattern obtained is shown in Fig. 2. Peak 2 was subdivided as shown, and the respective labeled fractions were pooled. When these subdi-
The relative ratios of hexokinase subtypes and the eluting pattern seen on DEAE-cellulose chromatography for rat and transphosphorylating activity was assayed for three substrates ○ ○, fructose; ● ●, glucose; and ▲, N-acetylglucosamine. Two peaks have both glucose- and fructose-phosphorylating activity corresponding to type I and type II hexokinase iso-enzymes. A separate peak of fructose-phosphorylating activity without glucose-phosphorylating activity was eluted early and represents specific fructokinase. N-Acetylglucosamine-phosphorylating activity emerged in the later fractions separate from any other phosphorylating activities.

Guinea Pig Intestinal Enzymes—The ease of separation of rat soluble fructokinase, hexokinases, and N-acetylglucosamine kinase was in marked contrast to the poor separation of the corresponding enzyme activities from guinea pig intestine (Fig. 4). The early part of the fructose-phosphorylating activity (Fraction 13 of Fig. 4A) yielded fructose-1-P as product, whereas the later fraction (Fraction 24, Fig. 4A) gave fructose-6-P. In between these fractions, various ratios of these products were obtained. Thus, despite the poor separation, fructokinase activity could be detected apart from nonspecific hexokinase. However, guinea pig N-acetylglucosamine kinase activity could not be clearly separated from type II hexokinase by DEAE-cellulose chromatography (Fig. 4B), although, as in the rat, type I hexokinase of guinea pig was easily separated from type II. An attempt was then made to separate type II activity from N-acetylglucosamine kinase. The activities from the DEAE-cellulose column corresponding to type II were pooled, concentrated, dialyzed, and then eluted from a Sepha-
dex G-100 column with the use of the phosphate-EDTA-dithio-
threitol buffer. By this method, the elution of type II hexokinase was followed by a separate and distinct peak of N-acetylglucos-
amine kinase activity.

The relative ratios of hexokinase subtypes and the eluting pattern seen on DEAE-cellulose chromatography for rat and
plots. Subdivisions C and D have kinetics suggesting both types of constants were determined with both Lineweaver-Burk and Woolf plots. Subdivisions C and D have kinetics suggesting both types III and IV but in extremely small and probably insignificant amounts.

Thus, the inhibition of guinea pig hexokinase by N-acetylglucosamine varied roughly one-third that found in rat intestine as measured in crude homogenates (Fig. 5, bottom). N-acetylglucosamine kinase by Sephadex column chromatography as well as that isolated originally on DEAE-cellulose columns. Guinea pig type II hexokinase was completely inhibited by glucosamine at concentrations that still left 40% of original activity intact. Guinea pig type II hexokinase, however, was much more sensitive to inhibition by N-acetylglucosamine. This was true for guinea pig hexokinase isolated from Sephadex as well as guinea pig and rat types I and II obtained from the DEAE-cellulose column were evaluated for inhibition by N-acetylglucosamine (Fig. 5). Both rat types I and II were inhibited similarly, with a rapid decline to 45% of original activity in the presence of 10 mM N-acetylglucosamine (Fig. 5, top). Thereafter, increasing concentrations of N-acetylglucosamine produced a more gradual inhibitory effect so that, even at 200 mM N-acetylglucosamine, 20% of the activity remained. Guinea pig type II hexokinase, however, was much more sensitive to inhibition by N-acetylglucosamine. This was true for guinea pig type II isolated free of N-acetylglucosamine kinase by Sephadex column chromatography as well as that isolated originally on DEAE-cellulose columns. Guinea pig type II hexokinase was completely inhibited by N-acetylglucosamine at concentrations that still left 40% of guinea pig type I hexokinase activity intact (Fig. 5, bottom). Thus, the inhibition of guinea pig hexokinase by N-acetylglucosamine clearly differed from the inhibition pattern seen with the rat hexokinases.

Guinea pig intestinal mucosal hexokinase activity was generally one-third that found in rat intestine as measured in crude homogenates (Table I). In both animals, approximately 50% of the activity in the homogenates was soluble without addition of any solubilizing agents. The soluble intestinal hexokinases of guinea pig differed from those of rat in the ratio of type I to type II, but this difference was not as great as reported by others (6). In the rat, 60% of the total soluble hexokinase was type II, whereas in the guinea pig it constituted 36% of the total soluble hexokinase activity eluted from DEAE-cellulose columns.

The fructose-phosphorylating activity of the nonspecific hexokinases accounted for 35 to 65% of the total fructose-phosphorylating activity eluted from the columns. The variation was principally due to an effect of diet on specific fructokinase (1). Evaluation of the products formed with uniformly labeled fructose-1-14C by the total phosphorylating activity present in the 105,000 × g crude supernatant fraction showed that at least 30% of the phosphorylated products were fructose-6-P and fructose 1,6-diphosphate. These products were identified separately by descending chromatography (13) and by high voltage paper electrophoresis (0.05 M pyridine acetate buffer, pH 6.5, 5,000 volts) (8). Thus, it appears that hexokinase contributes significantly to the total fructose-phosphorylating activity of the soluble cell fraction.

Effect of KC1—Particulate hexokinase was further studied by the use of agents reported by Katzen, Soderman, and Wiley (4) and others (17, 18) to solubilize particulate hexokinase. 1 M. M. Weiser, H. Quill, and K. J. Isselbacher, unpublished experiments.
Fig. 5. N-Acetylglucosamine inhibition of rat (top) and guinea pig (bottom) intestinal hexokinase isoenzymes. Both the spectrophotometric assay and the radioactive assay were used as described under “Methods,” usually with 0.1 ml of enzyme preparation (3 to 4 mg of protein per ml). O——O, type I; U——U, type II. The source of the enzyme activity was an (NH₄)₂SO₄ (40 g per 100 ml) precipitate of the corresponding peaks from the DEAE-cellulose column. In addition, guinea pig type II was separated from N-acetylglucosamine kinase activity by passage through a Sephadex G-100 column (see text). The N-acetylglucosamine inhibition pattern for guinea pig type II was essentially the same whether N-acetylglucosamine kinase was present or not. An explanation for the increased sensitivity of guinea pig type II hexokinase is not apparent.

**Table I**

Comparison of rat and guinea pig soluble hexokinase activities

<table>
<thead>
<tr>
<th>Animal</th>
<th>Total hexokinase activity</th>
<th>Soluble hexokinase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nmoles/min/g tissue</td>
<td>% total</td>
</tr>
<tr>
<td>Rat</td>
<td>750</td>
<td>50</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>238</td>
<td>49</td>
</tr>
</tbody>
</table>

activity. However, our results differ from these observations, at least for intestinal hexokinase activity. As shown in Table II, Column A, 0.5% Triton X-100 by itself solubilized approximately 38% of the particulate hexokinase of rat intestine leaving the remaining particulate activity intact. Attempts to solubilize the particulate hexokinase in the presence of 0.9 m KCl as well as Triton X-100 always led to a considerable loss of activity (Table II, Column B). In view of this finding, the effect of KCl on hexokinase activity was examined.

As shown in Fig. 6, KCl is a very effective inhibitor of rat soluble hexokinase. A similar inhibition curve was obtained for guinea pig soluble hexokinase activity. The sources of the enzyme preparations were the 105,000 X g (60 min) supernatant and pellet. O——O, rat particulate hexokinase activity; U——U, rat soluble hexokinase activity. The spectrophotometric assay of Sharma et al. (10) was used (see “Methods”) with 0.5 to 0.7 mg of enzyme protein per assay.

**Fig. 6.** KCl inhibition of rat intestinal soluble and particulate hexokinase activity. The sources of the enzyme preparations were the 105,000 X g (60 min) supernatant and pellet. O——O, rat particulate hexokinase activity; U——U, rat soluble hexokinase activity. The spectrophotometric assay of Sharma et al. (10) was used (see “Methods”) with 0.5 to 0.7 mg of enzyme protein per assay.

**Table II**

Particulate and soluble hexokinases of rat intestines

Homogenizations and the spectrophotometric assay (with 0.2 ml of enzyme preparation) were as described under “Methods” and in Fig. 6. Centrifugations were done at 105,000 X g for 60 min. All precipitates were resuspended in original buffer plus 10 mM glucose; solubilizing agents were added with thorough mixing. In Column A the solubilizing agent is 0.5% Triton X-100; in Column B the solubilizing agents are 0.5% Triton X-100 plus 0.9 m KCl.

<table>
<thead>
<tr>
<th>Fraction and treatment</th>
<th>Total hexokinase activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>I. Homogenate</td>
<td>2250</td>
</tr>
<tr>
<td>Ia, supernatant</td>
<td>963</td>
</tr>
<tr>
<td>Ib, pellet</td>
<td>958</td>
</tr>
<tr>
<td>II. Pellet Ib treated with solubilizing agents</td>
<td></td>
</tr>
<tr>
<td>IIa, supernatant</td>
<td>365</td>
</tr>
<tr>
<td>IIb, pellet</td>
<td>500</td>
</tr>
</tbody>
</table>

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The demonstration of two major intestinal soluble hexokinase subtypes, types I and II, confirms the findings of Katzen and Schimke (5), and of Srivastava et al. (6). The latter, however, found only 5.8% of type I hexokinase in rat intestine in relationship to the total hexokinase activity eluted from a DEAE-cellulose column. On the other hand, Katzen et al. (4) recently stated that the normal rat small intestine has mainly type I activity with relatively little type II, as determined by staining intensity on starch-gel electrophoresis and by DEAE-cellulose chromatography. A lack of type II hexokinase in normal rat intestine is of importance since Katzen et al. (4) have found only 5.8% of type I hexokinase in rat intestine in relation to the total hexokinase activity.

No attempt was made to re-examine the distribution of the particulate hexokinase activity among the different cell particulate fractions. The hexokinase activity of microvillus preparations was estimated because there is some controversy as to whether the glucose-binding factors associated with intestinal microvilli are, in fact, hexokinases (19, 20). However, in our studies, not more than 0.1% of the total mucosal hexokinase activity could be detected in the crude microvillus preparation. The purified microvillus preparation, in fact, had no detectable hexokinase activity. The purification of microvilli involves exposure of the fraction to 0.1 M NaCl, a concentration which is ordinarily not sufficient to inhibit or solubilize hexokinase activity.

**Discussion**

The inhibition of hexokinase activity by high KCl concentrations raises the possibility that the intestine actively phosphorylates N-acetylglucosamine. The inhibition of hexokinase activity by high KCl concentrations was shown by Harpur and Quastel (21) for brain hexokinase and also noted for intestinal hexokinase activity by Sols (22) and by Lange and Kohn (7). However, in contrast to the results presented here, these workers stated that N-acetylglucosamine was not phosphorylated by their crude preparations. Lange and Kohn (7), in fact, checked for phosphorylated products by descending paper chromatography and could not detect the formation of any N-acetylglucosamine-6-P. This seems unusual since the intestine actively synthesizes mucopolysaccharides containing N-acetylglucosamine and sialic acid. Recent work in our laboratory (8) did establish that the intestine actively phosphorylates N-acetylglucosamine and that the product is the 6-phosphate derivative. We have now isolated N-acetylglucosamine kinase as distinct from any nonspecific hexokinase activity in both rat and guinea pig.

Differences between guinea pig and rat intestinal hexokinase activities are evident not only in the differing proportions of the subtypes but also in the effect of N-acetylglucosamine inhibition. As shown in Fig. 5, neither type I nor type II of the rat was completely inhibited at 100 μM, whereas guinea pig type II was completely inhibited at 30 mM N-acetylglucosamine. An important finding was the inhibition of hexokinase activity by KCl. This KCl effect was not mentioned in the reports by Katzen and Schimke (5) and Grossbard and Schimke (9) in which they described the purification of the hexokinase subtypes on DEAE-cellulose column chromatography with a KCl linear eluting gradient. The apparent permanent loss of soluble intestinal hexokinase by high KCl concentrations raises the possi-
bility that other subtypes may be inactivated in the high KCl concentration area of the linear gradient. It may also be the explanation for the gradual tailing of type II hexokinase. Thus, there may be even more total type II hexokinase than indicated in these studies.

It is of interest that Grossbard and Schimke (9) had no difficulty demonstrating type IV hexokinase in liver by DEAE-cellulose column chromatography with a KCl eluting gradient. Type IV, in fact, is eluted best between 0.27 and 0.35 M KCl (9). As shown in Fig. 7, liver type IV hexokinase was not inhibited by KCl at these concentrations. Presumably, intestinal type IV hexokinase is also not inhibited by KCl and, therefore, would have been easily demonstrable if present in significant quantities. Intestinal particulate hexokinase does not appear to be irreversibly inhibited by KCl, and inhibition requires much higher KCl concentrations than are needed to inhibit the soluble hexokinases. Recently Katzen et al. (4) used KCl with Triton X-100 to solubilize maximally skeletal muscle (diaphragm), heart, and brain particulate hexokinase. However, these preparations could not be subsequently used for DEAE-cellulose column chromatography because of the interference of the salt; when the salt was removed prior to chromatography, the enzyme activity was significantly reduced. The data in Table II, which show that not all of the total intestinal particulate activity was recovered after solubilization with KCl, are consistent with the reduced enzyme activity noted by Katzen et al. (4) with the other tissue organs. This loss may be due to the inhibition by KCl once the activity is solubilized. Probably, the activity not inhibited by 0.9 M KCl is protected by some elements of the particulate state, thus suggesting that the solubilization partially involves a liquid crystalline phase of membrane components with Triton X-100 promoted by a high ionic media. However, with regards to the intestine, our data with Triton X-100 with or without KCl do not suggest an exposure of latent hexokinase activity as Katzen et al. (4) found for diaphragm, heart, and brain particulate hexokinase. That is, the activity found after extraction with Triton X-100 (Table II) is not significantly increased over that present before extraction.

Hexokinase activity associated with the microvillus fraction of the intestinal epithelial cell is detectable only in the crude preparation of this fraction. Presumably, this could be nuclear hexokinase activity since the activity is lost after the purification step with NaCl, and this step is known to remove contaminating nuclei (14). Another possibility, in view of our findings, is the solubilization or irreversible inhibition of hexokinase activity by exposure to NaCl. Although the concentration of NaCl used is relatively low (0.1 M), the microvillus-associated hexokinase may have become more susceptible to its effects by the repeated hypotonic treatment used in the preparation of microvilli. In any event, it would appear that glucose-binding activity associated with purified microvillus preparations is not due to an active hexokinase as has been suggested by Parsons (20). However, the binding of glucose by inactivated enzyme is not excluded.

In summary, techniques have been established for the isolation of specific glycolytic enzyme activities of the small intestine. These techniques permitted us to show in other studies that the observations of Stifel et al. (1) on dietary effects may not be correct. Some of the discrepancies may be due to the limitations that must be considered when determining a specific enzyme activity in crude preparations.

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M. M. Weiser, Helen Quill and Kurt J. Isselbacher


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