Hexokinase Specificity of Some Tumor Tissue Extracts*

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SUMMARY

The substrate specificity of hexokinase preparations from Ehrlich ascites cells, a spontaneous adenocarcinoma, Jensen sarcoma, and Walker carcinoma was studied with a number of sugars which differed in structure from glucose at one or more carbon atoms used as substrates. A marked variation in specificity was found. The preparations from Jensen sarcoma and Ehrlich ascites cells were relatively specific for glucose and some C-2 analogs. The preparations from the adenocarcinoma and Walker carcinoma were nonspecific and brought about phosphorylation of a number of sugars differing from glucose in many positions.

Inhibition of the hexokinase preparation from Ehrlich ascites tumor cells by altrose and talose was observed.

In 1957 Krebs (7) referred to two “pacemakers” of anaerobic glycolysis, the hexokinase reaction and the triosephosphate dehydrogenase reaction, and discussed the fundamental importance of these reactions to the entire metabolic economy of the organism. Many hexose analogs substituted in the C-2 position have been shown to have a particular capacity to serve as glycolytic inhibitors (2, 3, 5, 9, 23, 24). The importance of the hexokinase reaction can thus be clearly seen. It appeared, therefore, that a study of the hexokinase activity in various tumor tissues should prove of interest, especially in light of the difference in glycolytic rates of these tissues as compared with that of normal tissues. The pronounced requirement of tumor cells for glucose, whatever the underlying metabolic demand, may offer a possible avenue of attack for cancer chemotherapy.

Because of the structural relation of known competitive inhibitors to normal substrates, it seemed reasonable to assume that a similar relationship might exist between D-glucose and other aldohexoses and that, therefore, hexoses other than glucose might act as inhibitors of glycolysis. More specifically, since glucose serves as a substrate for a hexokinase, the specificity of these enzymes was of particular interest.

The present investigation entailed a study of the specificity of hexokinases derived from a number of tumor tissues. For this purpose, hexokinase preparations were obtained from Ehrlich ascites cells and a spontaneous adenocarcinoma of mice and from Jensen sarcoma and Walker carcinoma of rats. Studies were made of the ability of the rare aldohexoses, D-allose, altrose, gulose, idose, and talose, and of glucosamine, N-acetylgulcosamine, and 2-deoxyglucose to serve as substrates for, or inhibitors of, these enzyme preparations. The findings indicate distinct differences in the specificities of the hexokinases in the enzyme preparations. In studies of a similar nature (8), differences in the specificities of the hexokinases in preparations derived from normal rat tissues were observed.

MATERIALS AND METHODS

The sugars used were obtained commercially or prepared synthetically as follows: glucosamine hydrochloride was obtained from Pfaffstiehl Laboratories, N-acetylgulcosamine from Chas. Pfizer and Company, Inc., 2-deoxyglucose from Aldrich Chemical Company, Inc. The sugars were recrystallized from suitable solvents before use. The rare aldohexoses allose, altrose, gulose, and talose

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were prepared as described previously (8). Idose was prepared from xylose by the procedure of Isbell (6). Adenosine triphosphate (ATP) was obtained from Pabst Laboratories as the disodium salt. Ethylenediaminetetraacetic acid (EDTA) was obtained from the Eastman Organic Chemicals Division of the Eastman Kodak Company.

Assay procedure.—The method used for assaying the enzyme preparations was the constant pH microtitrimetric procedure of Schwartz and Myers (17), carried out as previously described (8). Incubations were carried out in 4-ml volumes at 30 °C and pH 7.4 with 0.02 m MgCl₂, 0.002 M ATP, 0.002 M EDTA, enzyme, and varying concentrations of the substrate. A unit of enzyme activity was defined as the amount of enzyme required to produce 1 × 10⁻⁸ acid equivalents per minute at 30 °C. For the determination of each Km value, the enzyme concentration was adjusted to produce about 25 × 10⁻⁸ acid equivalents per minute. Phosphatase and ATPase activities were inhibited during assay by the use of fluoride (15, 21) and n-octanol (13). The activity of these interfering enzymes was decreased further as a result of the freezing and thawing (13) entailed in carrying out the assays.

Enzyme preparation.—All enzyme isolation work was performed at 0°-5° C. Tumors were grown in female Sprague-Dawley strain rats or Strong A mice. Protein determinations were carried out by the method of Lowry et al. (11), or by digestion and nesslerization as described in Hawk, Oser, and Summerson (3), after dialysis against running water for 15 hours.

**Ehrlich ascites tumor hexokinase.**—Ascites fluid from tumor-bearing mice was harvested in Luster-old centrifuge tubes 10 days after inoculation, and the cells were separated from the fluid by centrifugation at 400 × g for 5 minutes. The layer of red blood cells was carefully removed by aspiration, and the precipitate was repeatedly washed with Krebs-Ringer bicarbonate buffer solution at pH 7.4 (20) until the supernatant fluid was clear and the tumor cells almost entirely free of red blood cells. One hundred ml of washed, packed cells was obtained from an original volume of 500 ml of ascites fluid.

The cells were homogenized for 5 minutes in a VirTis “45” blender with 2 volumes of a buffer consisting of 0.115 M KCl, 0.02 M K₂HPO₄, 0.01 M EDTA at pH 7.4. The homogenate was then centrifuged at 2000 × g for 30 minutes. The floating lipide layer and the precipitate were discarded, and the supernatant fluid was centrifuged at

### TABLE 1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Speed (×g)</th>
<th>Time (min.)</th>
<th>Homogenate (units/gm tissue)</th>
<th>Supernatant (units/gm tissue)</th>
<th>AMOUNT IN SUPERNATANT (PER CNT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ehrlich ascites cells</td>
<td>100,000</td>
<td>60</td>
<td>147†</td>
<td>105†</td>
<td>71</td>
</tr>
<tr>
<td>Spontaneous adenocarcinoma</td>
<td>100,000</td>
<td>60</td>
<td>208</td>
<td>105</td>
<td>51</td>
</tr>
<tr>
<td>Jensen sarcoma</td>
<td>20,000</td>
<td>45</td>
<td>304</td>
<td>357</td>
<td>54</td>
</tr>
<tr>
<td>Walker carcinoma</td>
<td>20,000</td>
<td>45</td>
<td>321</td>
<td>365</td>
<td>70</td>
</tr>
</tbody>
</table>

* Following an initial centrifugation at 600 × g for 2 minutes to remove unbroken cells and other debris.
† Expressed as units per 100 ml of washed, packed cells.

minute at 30° C. For the determination of each Km value, the enzyme concentration was adjusted to produce about 25 × 10⁻⁸ acid equivalents per minute. Phosphatase and ATPase activities were inhibited during assay by the use of fluoride (15, 21) and n-octanol (13). The activity of these interfering enzymes was decreased further as a result of the freezing and thawing (13) entailed in carrying out the assays.

**Hexokinase from a spontaneous adenocarcinoma.**—A mammary adenocarcinoma which had developed spontaneously in 21 mice was collected and found to weigh 50 gm. The tissue was homogenized and treated by the procedure described above. The supernatant fluid had a protein concentration of 14.5 mg/ml with a specific activity of 6.5 units/mg. The preparation was stable for several months at −15° C.

**Jensen tumor hexokinase.**—Whole tumors were excised from 26 rats, and most of the connective tissue was removed. The tumor tissue was washed in cold phosphate buffer at pH 7.4 to remove surface blood. After being washed, the tissue was

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1 Tissue kindly donated by Dr. Harry Monsen, Department of Anatomy, University of Illinois.

2 Tumor transplants were kindly donated by Dr. Kathryn Sydnor, Ben May Laboratories, University of Chicago.
 placed in petri dishes on ice, sliced longitudinally, and necrotic material was scraped away. The clean, soft, translucent tumor tissue was then carefully scraped away from any remaining connective tissue. The tissue, which weighed 115 gm., was homogenized in a Waring Blender for 3 minutes in 3 volumes of a buffer consisting of 0.115 m KCl, 0.02 m K2HPO4, 0.01 m EDTA at pH 7.4. An initial centrifugation at 800 × g for 30 minutes removed all the heavy material. The supernatant fluid was centrifuged at 20,000 × g for 45 minutes. The clear supernatant fluid so obtained was used for the assays (Table 1). Fluid having a protein concentration of 42 mg/ml with a specific activity of 4 units/mg was obtained. This preparation was stable for several months at −15° C.

Walker tumor hexokinase.2—From 95 gm. of tumor tissue, obtained from 25 rats, a soluble hexokinase (Table 1) was prepared by the procedure described above. A clear supernatant fluid having a protein concentration of 60 mg/ml with a specific activity of 3 units/mg was obtained. This preparation was stable for several months at −15° C.

RESULTS AND DISCUSSION

Since the assay procedure was dependent on the liberation of H+4, control experiments were carried out to determine the extent of interference by ATPase and other acid-producing systems which may have been present in the enzyme preparations and the effectiveness of the inhibitors used to repress their activity. At pH 7.4 glucose-6-phosphate is a stronger acid than phosphoric acid. Therefore, the enzymatic hydrolysis of glucose 6-phosphate brings about an increase in pH, rather than a liberation of H+. In previous work (8) with preparations containing more active phosphatases than was present in these preparations, the increase was found to be less than 0.1 pH units. For this reason, and because octanol, fluoride, freezing and thawing, and short periods at room temperature destroy the activity of glucose-6-phosphatase (13, 15, 21), interference by this enzyme was neglected. The results of these experiments showed that the activity of ATPase and other acid-producing processes which may have been occurring was very low or absent from the preparations. Even in those instances where the test substrate proved to be inert, or a very poor substrate, necessitating the use of large amounts of the enzyme preparation, acid production due to interfering processes was less than 5 per cent of the total. This was considered to be within the limits of experimental error under these circumstances, and the blank values were neglected.

The results of the substrate specificity studies are shown in Tables 2 and 3. The behavior of the test sugars as substrates is compared to glucopyranose, on the assumption that all the aldohexoses exist primarily in the pyranose form in aqueous solution (16). Michaelis constants (Km) were calculated by the method of Lineweaver and Burk (10). Comparison of these values provides information pertaining to the identity of enzymes isolated from different sources. Relative maximal rates, obtained from the expression

$$\text{Relative maximal rate} = \frac{V_{\text{max}} (\text{substrate})}{V_{\text{max}} (\text{glucose})}$$

are of value in comparing the behavior of an enzyme toward different substrates. The phosphorylation coefficient (18), calculated from the expression

$$\text{Phosphorylation coefficient} = \frac{V_{\text{max}} (\text{substrate})}{V_{\text{max}} (\text{glucose})} \times \frac{K_m (\text{glucose})}{K_m (\text{substrate})}$$

indicates the suitability of a substance as a substrate for hexokinase and serves to indicate the sensitivity necessary to assay enzyme activity.

Values indicating the extent of phosphorylation of a substrate in a given time are shown in Table 3. To obtain these values, assays were carried out under conditions of optimal concentration of all constituents except the substrate, the concentration of which was limiting. Comparison of these values gives information about the activity of an enzyme toward a variety of substrates and can also be used to ascertain conditions necessary for the determination of Michaelis constants.

Ehrlich ascites tumor cells.—The results of the substrate specificity studies are shown in Tables 2 and 3. Only glucose and deoxyglucose were good substrates in the ascites cell system. Of the other substances tested, gulose and allose were found to be poor substrates. Considering that a large excess of enzyme was required to demonstrate phosphorylation of these two rare aldohexoses, it is apparent that this enzyme preparation had a pronounced specificity and was, in fact, more highly specific than the other systems studied. From these experiments with relatively crude preparations it is not possible to state whether the activity observed was due to a mixture of kinases or a single nonspecific kinase. On the basis of specificity, however, it appears that the preparation from Ehrlich ascites cells was more likely to have consisted of a single kinase than the other preparations studied. It should be noted, however, that in similar studies (14) galactose did not serve as a
### TABLE 2
**SUBSTRATE SPECIFICITY OF HEXOKINASE PREPARATIONS**

Incubation mixtures contained substrate, enzyme, 0.08 M MgCl₂, 0.002 M ATP, 0.002 M EDTA, and n-octanol and were carried out at 30°C and pH 7.4.

<table>
<thead>
<tr>
<th>Substrate</th>
<th><strong>EMBLECH ASCITES CELL</strong></th>
<th><strong>SPONTANEOUS ADENOCARCINOMA</strong></th>
<th><strong>JENSEN BAComa</strong></th>
<th><strong>WALKER CARCINOMA</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Km (mM)</td>
<td>Relative Maximal Rate</td>
<td>Phosphorylation Coefficient</td>
<td>Km (mM)</td>
</tr>
<tr>
<td>Glucose</td>
<td>4.0 × 10⁻⁵</td>
<td>1</td>
<td>1.5 × 10⁻⁴</td>
<td>3.7 × 10⁻⁴</td>
</tr>
<tr>
<td>2-Deoxyglucose</td>
<td>6.0 × 10⁻³</td>
<td>0.85</td>
<td>8.1 × 10⁻⁵</td>
<td>0.70</td>
</tr>
<tr>
<td>Gluconic acid</td>
<td>2</td>
<td>&gt;10⁻⁵</td>
<td>&gt;10⁻⁵</td>
<td>2.1 × 10⁻⁴</td>
</tr>
<tr>
<td>N-Acetylglucosamine</td>
<td>2</td>
<td>&gt;10⁻⁵</td>
<td>&gt;10⁻⁵</td>
<td>1.1 × 10⁻⁴</td>
</tr>
<tr>
<td>Allose</td>
<td>3</td>
<td>&gt;0.1</td>
<td>&gt;10⁻⁵</td>
<td>3.4 × 10⁻³</td>
</tr>
<tr>
<td>Taloase</td>
<td>2, 3</td>
<td>&gt;0.1</td>
<td>&gt;10⁻⁵</td>
<td>&gt;0.1</td>
</tr>
<tr>
<td>Dolose</td>
<td>3, 4</td>
<td>&gt;0.1</td>
<td>&gt;10⁻⁵</td>
<td>1.5 × 10⁻⁵</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.4 × 10⁻⁵</td>
</tr>
</tbody>
</table>

* Compared with d-glucose.
† An undetectable affinity (Km > 0.1 mM).
‡ An undetectable rate (<0.001 at 0.2 mM).

### TABLE 3
**PHOSPHORYLATION OF CARBOHYDRATES BY HEXOKINASE PREPARATIONS**

Incubation mixtures contained 0.002 M ATP, 0.002 M MgCl₂ and 0.002 M EDTA, and were carried out at 30°C and pH 7.4.

<table>
<thead>
<tr>
<th>Substrate Added (mM)</th>
<th><strong>EMBERCA ASCITES CELLS</strong></th>
<th><strong>SPONTANEOUS ADENOCARCINOMA</strong></th>
<th><strong>JENSEN BAComa</strong></th>
<th><strong>WALKER CARCINOMA</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Enzyme Added (units/ml)</td>
<td>Time (min.)</td>
<td>Amount Phosphorylated (per cent)</td>
<td>Enzyme Added (units/ml)</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.7</td>
<td>45</td>
<td>3</td>
<td>80</td>
</tr>
<tr>
<td>2-Deoxyglucose</td>
<td>0.76</td>
<td>45</td>
<td>3</td>
<td>56</td>
</tr>
<tr>
<td>Gluconic acid</td>
<td>0.58</td>
<td>90</td>
<td>10</td>
<td>0*</td>
</tr>
<tr>
<td>N-Acetylglucosamine</td>
<td>0.57</td>
<td>90</td>
<td>10</td>
<td>0*</td>
</tr>
<tr>
<td>Allose</td>
<td>0.95</td>
<td>90</td>
<td>240</td>
<td>3</td>
</tr>
<tr>
<td>Taloase</td>
<td>6.95</td>
<td>90</td>
<td>120</td>
<td>0†</td>
</tr>
<tr>
<td>Dolose</td>
<td>6.95</td>
<td>90</td>
<td>5</td>
<td>3†</td>
</tr>
<tr>
<td>Idose</td>
<td>7.8</td>
<td>135</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

* At substrate concentration of 1.16 μM/μl.
† At substrate concentration of 1.78 μM/μl.
substrate, but phosphorylation of fructose was demonstrated, suggesting that a specific fructokinase may also have been present. This is known to be the case in rat liver (4, 22).

Spontaneous adenocarcinoma.—Owing to the restricted amount of this enzyme preparation it was not possible to include studies with idose, glucosamine, N-acetylglucosamine, or deoxyglucose. Glucose, allose, altrose, gulose, and talose all served as substrates, indicating considerably less specificity than in the previous case. Of the four preparations described here, the hexokinase derived from this tumor showed the lowest affinity for glucose, having a $K_m$ value of $1.5 \times 10^{-4} M$, a fourfold increase over the other $K_m$ values reported. Whereas the high degree of specificity of the ascites cell preparation suggests the presence of a single hexokinase, the findings in this case suggest that more than one hexokinase with different specificities may be present rather than a single nonspecific enzyme. However, no evidence is available which would enable resolution of this point at this time.

Jensen sarcoma.—Only glucose and those derivatives of glucose modified at carbon 2 served as substrates in this enzyme preparation. The rare aldohexoses were completely inert. These results may be interpreted to indicate the presence of a rather specific kinase in this tissue. In an earlier investigation, mannose and fructose were found to be substrates for a Jensen sarcoma, but galactose was not (1). If it is assumed that the preparations were similar, these findings might also be interpreted to indicate the presence of a fructokinase, as well as some lack of specificity in the glucokinase. The results support the conclusion that modifications of glucose at carbon 2 are relatively unimportant in relation to the specificity of the enzyme, but modifications elsewhere in the molecule may markedly decrease or eliminate enzyme action (8).

Walker carcinoma.—The results show that all the sugars tested except idose served as substrates. The $K_m$ value for glucose was found to be comparable to that in the other systems studied. A considerable lack of specificity of the enzyme preparation is indicated and illustrates the marked variation of specificities of the hexokinases derived from different sources. The lack of specificity of this preparation can again be interpreted to indicate the presence of either one nonspecific enzyme or more than one enzyme with different specificities.

The data in Table 2 show that, in almost every instance, sugars which served as substrates in this preparation had a greater affinity (lower $K_m$ values) for the enzyme than they did for the enzymes of the other preparations in which they also were substrates. In spite of the lack of specificity and the high degree of enzyme-substrate affinity, idose was found to be inert in this enzyme preparation, as well as in the other systems studied.

In previous studies with hexokinase preparations from normal tissues (8), a fairly regular pattern of specificity was observed in all tissues, which permitted certain conclusions to be drawn associating carbohydrate structure with enzyme specificity. However, each of the preparations obtained from abnormal tissue, described in this report, had its own specificity characteristics, and generalizations relating structure to specificity are difficult to make because many inconsistencies were found in these studies. Studies with the normal tissues indicated that the structure of the carbohydrate at carbon 2 had little influence on hexokinase activity, but a change from the glucose configuration at carbon 3 (allose) or carbon 4 (galactose) resulted in decreased activity. Changes at carbons 2 and 4 (talose) decreased activity still further, and changes at 2 and 3 (altrose) or 3 and 4 (gulose) resulted in a loss of all activity. With the exception that galactose was phosphorylated in a system prepared from rat liver, the order of suitability of the rare hexoses as substrates for the normal tissues studied was allose, talose, altrose, and galactose.

In the present studies, the lack of influence on enzyme activity due to changes in structure at carbon 2 applied consistently only to 2-deoxyglucose. Glucosamine was not phosphorylated by the preparation from Ehrlich ascites cells, although it was found to be a good substrate in all other preparations studied except that from rat kidney (8). Conversely, N-acetylglucosamine was actively phosphorylated by the preparations from Jensen sarcoma and Walker carcinoma, although it was inert in the other systems in which it was tested. This was an unexpected finding in the case of the Jensen tumor, which otherwise showed a high degree of specificity, because it would appear that the bulk of the acetyl group would be likely to prevent activity. Phosphorylation of N-acetylglucosamine by hexokinases derived from a number of sources has, however, been reported by others (12, 18, 19).

Almost no further correlations can be drawn from the data, either by comparing the tumor tissues with the results obtained from normal rat tissues (8), or by comparing the tumor tissues only to each other. Considering only the preparations from ascites cells and Jensen sarcoma, both of which showed a high degree of specificity in their action toward the rare sugars, differences in
behavior nevertheless were found, the principal one being that gulose was not a substrate in the Jensen sarcoma preparation, but was a substrate, though a poor one, in the ascites-cell system. The preparations from Walker carcinoma and the adenocarcinoma, which both exhibited a low degree of specificity, also showed unique characteristics in their behavior toward the rare sugars other than idose. In the preparation from the adenocarcinoma the order of suitability of the rare sugars as substrates was altrose, gulose, allose, and talose, whereas in the preparation from Walker carcinoma the order of suitability was altrose, talose, allose, and gulose. In both these preparations, greater hexokinase activity was found toward sugars differing in configuration from glucose at two positions than toward allose, which differs from glucose only at carbon 3. In the studies with hexokinases from normal rat tissues (8), allose was found to be a better substrate than any of the other rare hexoses. Thus, the principal clear-cut conclusions that can be drawn from these observations are that loss of the hydroxyl group of glucose at carbon 2 affected these hexokinases only slightly, but changes from the glucose configuration at carbons 2, 3, and 4 (idose) caused a loss of all activity. Changes from the glucose configuration between these extremes, involving any one or any two of the carbon atoms, resulted in a pattern of behavior which was more or less unique for each of the enzyme systems studied. It is apparent that the hexokinases of these systems differ considerably in their capacity to act on sugars other than glucose. In view of these differences it is remarkable that they are all similar in their action on glucose and 2-deoxyglucose and that, furthermore, this activity, as judged by $K_m$ values, is similar to that of hexokinases derived from a wide variety of other biological sources.

Nature of the reaction product.—In each assay in which hexokinase activity was found, the incubation mixture was examined to determine the nature of the reaction product. According to the procedures previously described (8), it was found that hexose phosphate esters were formed. Because these esters also had reducing properties, it is reasonable to assume that they were the 6-phosphate esters. The appearance of these esters in the incubation mixtures indicated that hexokinase activity was indeed present.

Inhibition of hexokinases by the rare sugars.—Since a number of reports (2, 5, 9, 23, 24) have indicated that hexokinase can be inhibited by analogs of glucose, experiments were conducted to determine whether the rare aldohexoses would act as competitive inhibitors of glucose. These studies were carried out by determining the reaction velocity in assays in which the concentration of inhibitor was kept constant and the concentration of glucose was varied. In those experiments in which no inhibition occurred, calculations as described by Lineweaver and Burk (10) led to a result identical to the $K_m$. When inhibition occurred, an inhibitor constant ($K_i$) was calculated (10).

The only instances in which inhibition was observed was the inhibition of the hexokinase of Ehrlich ascites cells by altrose and talose (Table 4). In similar experiments with the preparations from Walker and Jensen tumors and the adenocarcinoma, no inhibitory effect of any of the rare hexoses was detected at concentrations of the rare sugars ranging from 7.8 to 77.8 μmoles/ml.

<table>
<thead>
<tr>
<th>Inhibitor hexose</th>
<th>Modified at carbon</th>
<th>Inhibitor concentration (μmoles/ml)</th>
<th>$K_i$ (μmoles/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allose</td>
<td>3</td>
<td>13.9</td>
<td>≈</td>
</tr>
<tr>
<td>Altrose</td>
<td>2, 3</td>
<td>13.9</td>
<td>6X10$^{-3}$</td>
</tr>
<tr>
<td>Talose</td>
<td>2, 4</td>
<td>13.9</td>
<td>3X10$^{-3}$</td>
</tr>
<tr>
<td>Gulose</td>
<td>3, 4</td>
<td>23.4</td>
<td>3.5X10$^{-3}$</td>
</tr>
<tr>
<td>Idose</td>
<td>2, 3, 4</td>
<td>21.8</td>
<td>≈</td>
</tr>
</tbody>
</table>

* In similar studies with preparations from the spontaneous adenocarcinoma and Walker and Jensen tumors, no instances of inhibition were found.
† Compared with D-glucose.
‡ An undetectable inhibition.

The rare aldohexoses were identified by gel electrophoresis. In no case was any sugar phosphate other than glucose 6-phosphate detected, regardless of whether or not the rare hexose had previously been found to be a substrate or inert, or whether it had an inhibitory action in these studies.
If it is assumed that two substances which serve as substrates for the same enzyme occupy the same active site on the enzyme surface, it is difficult to understand why inhibition did not occur in those instances in which the rare sugars were found to be substrates. A possible explanation for this apparent contradiction in behavior may lie in the conditions under which the experiments were carried out. In those assays in which the rare sugars were found to be substrates, the concentration of the sugar and of the enzyme was much higher than in the inhibition studies, and, as can be seen from the phosphorylation coefficients and Kₘ values in Table 2, the rare sugars were greatly inferior to glucose as substrates for these enzymes. Because glucose has such a high affinity for the enzyme and because low enzyme concentrations were used in the inhibition studies, the inhibitory effect of the rare sugars may have been decreased beyond the point of detection by the methods used.

The fact that altrose and talose, which were inert as substrates, could nevertheless exert an inhibitory effect, suggests that these substances could be bound to the active site of the enzyme, but activation and phosphorylation could not occur.

The results obtained in both the substrate specificity and inhibition studies clearly indicate that there are significant differences in the enzyme systems investigated, but these differences would not be readily apparent if only glucose were studied as a substrate.

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REFERENCES

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Charles F. Lange, Jr. and Paul Kohn