STUDIES OF TISSUE PERMEABILITY

I. THE PENETRATION OF SUGARS INTO THE EHRLICH ASCITES TUMOR CELLS∗

BY ROBERT K. CRANE, RICHARD A. FIELD,† AND CARL F. CORI

WITH THE TECHNICAL ASSISTANCE OF MARY LOUISE ROBERTS

(From the Department of Biological Chemistry, Washington University School of Medicine, St. Louis, Missouri)

(Received for publication, July 23, 1956)

Recent studies of carbohydrate metabolism have focused attention on the process of penetration of sugars into mammalian cells as a possible point of action of insulin (1). It would be of decided advantage in an investigation of this problem if one could work with a preparation consisting of single cells. A good deal is known about the penetration of sugars into erythrocytes (2), but so far an insulin effect on these cells has not been demonstrated.

The Ehrlich ascites tumor cells, which possess a remarkable capacity for glycolysis and for survival under conditions in vitro, seemed to be an appropriate choice of experimental object. In this paper, experiments are reported which characterize the penetration of sugars into the tumor cells, and it may be anticipated here that every effort to show an effect of insulin on the sugar uptake of these cells failed. One might therefore ask what characteristics of permeability a cell must possess if it is to be influenced by insulin. Therefore, it is of importance to investigate not only cells such as muscle, which are known to be acted upon by insulin, but also cells which, like the tumor cells and leucocytes, do not respond to insulin. It will be shown in this series of papers that there exist very striking differences in the sugar uptake of the tumor cells as compared to that of the muscle cells, which make it possible to give at least a partial answer to the question raised above.

EXPERIMENTAL

Cells—Ehrlich carcinoma ascites tumor cells were obtained from Dr. Paul C. Zamecnik and from Dr. Irving S. Johnson. The supply was main-

∗Supported by an Institutional Grant from the American Cancer Society to Washington University.

A preliminary report of this paper was made at the 3rd International Congress of Biochemistry, Brussels, 1955.

† Postdoctorate Research Fellow, United States Public Health Service; Fellow of the National Cancer Institute.

† S. M. Krane and R. K. Crane, unpublished experiments.
tained by periodic intraperitoneal transfer of 0.2 ml. of ascitic fluid containing tumor cells into Swiss mice of the Jackson Laboratory strain. Cells were harvested after 5 to 9 days of intraperitoneal development and washed free of erythrocytes and ascitic fluid with ice-cold Krebs-Henseleit (3) bicarbonate buffer. Cell agglutination was prevented by the addition of heparin to the initial washing fluid. The final cell suspension was filtered through glass wool to remove debris and clumps. Cell concentration was determined by the measurement of packed cell volume (1100 X g, 15 minutes) as described by McKee et al. (4). A conversion factor of 4.2 X 10^6 cells per c.mm. of packed cell volume was used.

Compounds—Phlorizin was generously contributed by Dr. T. P. Nash, Jr., 3-O-methyl-d-glucose by Ayerst, McKenna and Harrison, Ltd., and d-allose and 1,5-d-sorbitan by Dr. N. K. Richtmyer. d-Fructose, d-galactose, d-xylose, d-lyxose, d-ribose, d-arabinose, L-arabinose, and L-sorbose were obtained from the Pfannstiehl Chemical Company, 2-deoxy-d-glucose from the Aldrich Chemical Company, d-glucosamine and N-acetyl-d-glucosamine from the Nutritional Biochemicals Corporation, d-glucose from the Mallinckrodt Chemical Works, and d-gulose from General Biochemicals, Inc. The following substances were further purified before use. 3-Methylglucose was recrystallized from methanol after decolorization with Norit A and removal of glucose by fermentation with fresh yeast. Galactose was recrystallized from 80 per cent ethanol, phlorizin from water, fructose from methanol, 2-deoxyglucose from isopropanol, and ribose from ethanol.

Methods—Variations of technique were required in order to satisfy the specific design of a given experiment, but in general the method was as follows.

3.0 ml. of Krebs-Henseleit bicarbonate buffer, pH 7.4, 0.2 ml. of 0.25 M raffinose, the desired amount of a 0.25 M solution of the sugar to be studied, and 0.9 per cent NaCl to make a final volume of 5.2 ml. were placed in a 15 ml. centrifuge tube. The tube and contents were brought to the desired temperature by incubation for 10 minutes. The experiment was then started by the addition of 2.0 ml. of a 20 to 30 per cent suspension of cells, also preincubated to insure temperature equilibrium. The contents of the tubes were mixed by stirring throughout the incubation. In experiments of short duration which required a more rapid mixing of the components, the sugar solution, increased in concentration to 0.5 M, was layered in the bottom of the 15 ml. centrifuge tube, the cell suspension was added, and, at the appropriate time, the layers were mixed by rapid stirring. To terminate an incubation, the tubes were centrifuged at 1500 X g for 7 minutes. In order to account in part at least for the continued exposure of the cells to the incubation medium during the early part of
the centrifugation, the centrifugations were begun 10 seconds prior to the recorded interval of time.

After centrifugation, the supernatant liquids were decanted from the packed cell plugs and the tubes were thoroughly drained and their side walls wiped dry with absorbent tissue. A barium hydroxide-zinc sulfate filtrate (5) of the supernatant liquid was prepared. The packed cell mass was suspended in 4.0 ml of 0.19 M ZnSO₄ and homogenized by alternate withdrawal into and ejection from a hypodermic syringe through a No. 18 gauge needle. The suspensions were again homogenized after the addition of 4.0 ml of 0.3 N Ba(OH)₂, and the protein-free solution was obtained by centrifugation. Aliquots of both filtrates were analyzed for the sugar under study. Galactose and other reducing sugars were assayed by the Somogyi (6) or Nelson (5) methods, raffinose and fructose by the ketose method of Roe et al. (7), ribose by the Roe pentose method (8), and 1,5-sorbitan by a slight modification of the periodate method of Salo (9). Phlorizin was assayed by its absorption of light in the Beckman model DU spectrophotometer at a wavelength of 285 mm, corrected for the relatively small absorption contributed by the cells. The washed cells did not contain assayable amounts of reducing substances, nor did they contribute reducing substances to the medium in which they were incubated.

Some experiments were carried out with D-galactose-1-C¹⁴. The radioactivity of the filtrates from these experiments was assayed by means of an end window counter on aliquots of the fluids dried on a hot plate in standard planchets. In all cases, sample counting was in a range 50 times greater than background.

The volume of incubation medium entrapped in the interstices of the packed cell mass was calculated from the amount of raffinose found in the filtrate prepared from the packed cell mass. The amount of the sugar under study contained in this volume was then subtracted from the total found in the packed cell mass. The sugar present in excess was assumed to be intracellular, and its concentration per ml. of intracellular fluid was calculated² on the assumption that 80 per cent of the packed cell volume is water (4). The validity of this correction for entrapped medium can be assessed from the following information: The raffinose volume and the volume of distribution of thiosulfate are nearly identical. The raffinose correction accounts completely for the amount of sugar found in the packed

² For example, in a typical experiment it was assumed that 0.5 ml. of packed cells contained a total of 0.4 ml. of water, part extracellular and part intracellular. The concentration of raffinose in the medium was 0.007 M. The amount of raffinose found in the packed cell mass was 0.56 μmole, corresponding to a volume of medium of 0.08 ml. The volume of intracellular water was thus 0.4 — 0.08 = 0.32 ml.
cell mass after a 15 second incubation at 0° (see below). The raffinose volume does not change throughout extended incubation at temperatures up to 20°. At higher temperatures, there is some increase in the raffinose volume during incubation which may be attributable to swelling of the cells and imperfect packing during centrifugation. The raffinose volume is highly reproducible and is not appreciably influenced by centrifugation at higher forces (2000 × g) for a longer period of time (15 minutes).

Results

Reversible Penetration—Of the various compounds studied with respect to penetration into the ascites tumor cells, some compounds, namely raffinose, phlorizin, and N-acetylglucosamine, did not penetrate into the cells. Of the remaining ones tested, 3-methylglucose, galactose, allose, 1,5-sorbitan, xylose, lyxose, glucosamine, 2-deoxyglucose, gulose, ribose, D-arabinose, L-arabinose, and L-sorbose penetrated the cells rapidly and, as none of them was dissimilated to an appreciable extent, they accumulated in the intracellular water. Glucose and fructose also penetrated the cells rapidly, but their utilization by the cells made the study of their accumulation within the cells difficult. They are discussed separately later.

For all the penetrating, non-metabolized compounds except ribose and D-arabinose, the rate of penetration at 37° was too rapid to be measured by the techniques used. Consequently, the majority of experiments were carried out with incubation temperatures in the range 10–25°.

The ascites tumor cells do not possess a mechanism like that observed for amino acids (10) for the concentration of sugars in their intracellular water. Illustrative data for the ratio of distribution between medium and cells are shown in Table I. Equilibrium for penetration was attained when the water of the packed cell mass contained sugar, in this instance galactose, at the same concentration as the external medium. The speed of penetration is indicated by the fact that, at 37°, equilibrium was attained during 1 minute of incubation.

The kinetics of penetration of sugars into the cells are shown in Fig. 1. At 20°, 3-methylglucose and L-sorbose attained equilibrium within 20 minutes and ribose (point not shown) within 1 hour of incubation. Raffinose, on the other hand, did not penetrate the cells. The exit of sugars from the cells (Fig. 2) followed a pattern similar to that of entry. Xylose, the penetration of which is much more rapid than that of ribose (see below), likewise left the cells more rapidly than ribose. Although these results, in themselves, indicate that the processes of entry and exit have the characteristics of a single dynamic equilibrium, the point is most clearly established by the observation that galactose-1-C14 penetrated the cells rapidly when added after equilibrium had been attained with non-radioactive galactose.
TABLE I

Distribution of Galactose between Medium and Cells

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time of incubation</th>
<th>Galactose concentration</th>
<th>Ratio, cells to medium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>°C.</td>
<td>min.</td>
<td>Medium mole per l.</td>
<td>Packed cell mass mole per l.</td>
</tr>
<tr>
<td>10</td>
<td>120</td>
<td>$3.3 \times 10^{-3}$</td>
<td>$3.1 \times 10^{-3}$</td>
</tr>
<tr>
<td>14</td>
<td>66</td>
<td>$3.2 \times 10^{-3}$</td>
<td>$2.91 \times 10^{-3}$</td>
</tr>
<tr>
<td>37</td>
<td>1</td>
<td>$1.1 \times 10^{-4}$</td>
<td>$1.08 \times 10^{-4}$</td>
</tr>
<tr>
<td>37</td>
<td>15</td>
<td>$1.2 \times 10^{-4}$</td>
<td>$1.34 \times 10^{-4}$</td>
</tr>
<tr>
<td>37</td>
<td>15</td>
<td>$2.0 \times 10^{-5}$</td>
<td>$1.96 \times 10^{-5}$</td>
</tr>
</tbody>
</table>

Average ..................................................... 0.99

* These values are the concentration in the total water of the packed cell mass.

Fig. 1. The penetration of various sugars at 29°. The concentration used was 0.033 M for 3-methylglucose, L-sorbose, and ribose, and 0.007 M for raffinose. All the points shown have been corrected for entrapped medium by the raffinose volume found after 15 seconds incubation at 0°.

In attempts to influence the rate of penetration at a given temperature, it was found that the rate for 3-methylglucose remained unchanged in the presence or absence of oxygen, 5 per cent serum albumin, various aerobic substrates such as glutamate, or the hormone insulin.

Influence of Temperature—Owing to its slow rate of penetration at con-
venient concentrations, ribose was chosen for a study over the temperature range 0–36° (see Fig. 3). Of particular interest is the fact that at 0° there is almost no penetration. All the compounds tested exhibited a similar slow rate of entry at 0°, and, because of this, the sugar present in the packed cell mass from an incubation of 15 seconds duration at 0° could be used as a measure of trapped medium. The volume thus measured agreed precisely with the raffinose volume.

Fig. 2. The rate of exit of ribose and xylose from the ascites tumor cells at 20°. 5 ml. of a cell suspension (23 per cent) were incubated 20 minutes at 35° with 1 ml. of 0.5 M xylose or ribose, and cooled to 20°. 1 ml. of the incubated cell suspension was mixed with 9 ml. of Krebs-Henseleit buffer and centrifuged at the stated interval after mixing. Assays were made of the sugar in the supernatant fluid.

Temperature coefficients (Q10) were calculated from specific rate constants (see the next section). The results for ribose and for 3-methylglucose (Fig. 4) yielded a Q10 (20–30°) for the penetration process of 3.8 and 4, respectively. The value for the Q10 increased to disproportionately high values at lower temperatures, suggestive of an alteration in the physical properties of the cell membrane.

Kinetics of Penetration—The shapes of the curves in Figs. 1 to 3 suggested that the kinetics of penetration might be those of a reversible first order process. Representative data are shown in Fig. 4, plotted against time in the form $-\ln(1/(1 - FE))$, in which $FE$ is the fraction of equilib-
Fig. 3. The penetration of 0.033 M ribose at various temperatures.

Fig. 4. The rate of penetration of 0.033 M ribose and 0.033 M 3-methylglucose at various temperatures. $FE$ = the fraction of equilibrium. Note the difference in the time scale for the two sugars.
rium. The straight lines connecting the experimental points for ribose did not intersect at the origin. This deviation could reflect as little as a 5 percent error in the measurement of the water content of the packed cell mass, and recalculation of the data on this assumption provided values which did extrapolate to the origin, without a significant change in the slopes of the lines.

The slope ($K$, $\text{min}^{-1}$), multiplied by the substrate concentration (in moles per liter), was used as a measure of the initial rate of penetration.

Owing to a large outside volume relative to cell volume, the substrate concentration did not change significantly during incubation.

First order rate constants and initial rates of penetration, in moles per liter per minute, were calculated for the other non-utilizable sugars from data similar to those in Fig. 1; these are listed in Table II. For the sake of comparison with other numerical values obtained with the Ehrlich ascites tumor cells, rates of penetration in Table II can be converted to the units, micromoles per $10^7$ cells per hour, through multiplication by the factor 115. For example, the initial rate of penetration of 3-methylglucose (0.0175 X 115) would be 2.01 μmoles per $10^7$ cells per hour.

3-Methylglucose has been used as the standard of comparison, and it may be seen that, at the particular concentrations and temperatures chosen, only 1,5-sorbitan and galactose penetrated more rapidly than 3-methyl-
ylglucose. On the other hand, some sugars, particularly glucosamine, ribose, and D-arabinose, penetrated at a much slower rate. The specificity of the cell membrane of the ascites tumor cell does not appear to be influenced in a predictable manner by configurational changes. The slower rates of penetration are, in general, exhibited by sugar molecules differing from glucose in configuration at carbon atom 2 or 3. A notable exception to this rule, however, is 3-methylglucose.

Effect of Concentration—In order to find out whether the process of penetration approaches a maximal velocity, such as is characteristic of enzyme-catalyzed reactions, experiments were carried out with different concentrations of 3-methylglucose, galactose, ribose, and L-sorbose, and the results were plotted according to Lineweaver and Burk. These plots show (Figs. 5 and 6) that temperature has a remarkable effect. At 10°, slopes and ordinate intercepts were obtained from which apparent $K_m$ values and maximal velocities could be calculated; these are listed in
Table III and would indicate that 3-methylglucose and galactose have relatively high "affinities" for the system, while ribose and L-sorbose exhibit low affinities. These differences disappear, however, at higher temperatures. In Fig. 5 at 20° the \(1/v \) versus \(1/S\) plot for 3-methylglucose passes through the origin, and this is also the case at 25° for ribose and L-sorbose (Fig. 6). Owing to the rapidity of penetration at the higher

### Table III

**Apparent \(K_m\) Values and Maximal Rates of Penetration at 10°**

The following values were calculated from the experiments in Figs. 5 and 6.

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Maximal initial rate</th>
<th>Apparent (K_m)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mole per l. per min.</td>
<td>mole per 10^6 cells per hr.</td>
</tr>
<tr>
<td>3-Methylglucose</td>
<td>0.0036</td>
<td>0.41</td>
</tr>
<tr>
<td>Galactose</td>
<td>0.0061</td>
<td>0.70</td>
</tr>
<tr>
<td>Ribose</td>
<td>0.003</td>
<td>0.34</td>
</tr>
<tr>
<td>L-Sorbose</td>
<td>0.011</td>
<td>1.27</td>
</tr>
</tbody>
</table>
temperatures, it is not possible to decide with certainty whether \( K_m \) becomes very large or infinite. The existence of competitive inhibition of penetration between pairs of sugars (see below) gives some reality to the apparent \( K_m \) values.

It is possible to look at these results in another way. The abnormally high temperature coefficients for the rate of penetration in the range 0-20° have been commented upon. The extrapolated maximal velocity for 3-methylglucose (Fig. 5) is 10 times greater at 15° than at 10°. One would hesitate to attribute such temperature coefficients to enzyme-catalyzed reactions or to a "carrier" type of mechanism of sugar transport, but it would not be too difficult to imagine temperature effects of this magnitude if they involved the lipide-protein layers of the cell membrane. For example, such a membrane, with "pores" of the dimensions of a sugar molecule, would be markedly influenced in its permeability by the degree of hydration it undergoes at different temperatures. It is not intended to offer further speculation about this problem at the present stage of our information.

**Table IV**

_Inhibition of Penetration_

<table>
<thead>
<tr>
<th>Inhibitor sugar</th>
<th>Test sugar</th>
<th>Duration of incubation</th>
<th>Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>3-Methylglucose</td>
<td>mole per l.</td>
<td>0.007</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0.007</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0.033</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0.0033</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0.0167</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0.0033</td>
</tr>
<tr>
<td>3-Methylglucose</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0.0167</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0.0167</td>
</tr>
<tr>
<td>Galactose</td>
<td>Ribose</td>
<td>mole per l.</td>
<td>0.033</td>
</tr>
<tr>
<td>&quot;</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0.033</td>
</tr>
<tr>
<td>L-Sorbose</td>
<td>Ribose</td>
<td>&quot;</td>
<td>0.033</td>
</tr>
<tr>
<td>Ribose</td>
<td>L-Sorbose</td>
<td>&quot;</td>
<td>0.033</td>
</tr>
<tr>
<td>Xylose</td>
<td>Xylose</td>
<td>&quot;</td>
<td>0.0167</td>
</tr>
<tr>
<td>L-Sorbose</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0.0167</td>
</tr>
</tbody>
</table>

*Competitive Inhibition*—Although it was possible to obtain apparent \( K_m \) values at 10° (Table III), it was doubtful whether \( K_m \) values existed at temperatures of 20-25°. Most of the experiments on competitive inhibition (Table IV) were carried out at the latter temperatures, but there was some resemblance to the \( K_m \) values at 10° in so far as the order of inhibitors was 3-methylglucose > galactose > ribose > L-sorbose. Glu-
sugar (0.007 M), which inhibited the penetration of 3-methylglucose (0.0167 M) 40 per cent, was also a stronger inhibitor of ribose and L-sorbose than 3-methylglucose. When several molar ratios of inhibitor and test sugar were tried, the results were consistent with a competitive type of inhibition. The pair, xylose and L-sorbose, showed mutual inhibition of penetration at 20°, but there was no mutual inhibition when the pair, ribose and L-sorbose, was tested at 25°. It seems noteworthy that this system exhibits the properties associated with competitive inhibition. If this criterion were strictly applied, one would have to say that the pathway of penetration for all the hexoses and pentoses is the same.

**Inhibition by Phlorizin**—Phlorizin did not penetrate into the cells. Nevertheless, the presence of phlorizin in the external medium produced a significant reduction in the rate of penetration into the cells of all the sugars tested. The final equilibrium concentration of the penetrating sugar was not changed. In contrast to the mutual inhibition phenomenon described above for two penetrating sugars, the extent of inhibition by phlorizin appeared to be independent of the relative concentrations of the penetrating substance and inhibitor (Table V).

**Penetration of Rapidly Metabolized Sugars**—The relationship between penetration of the three fermentable hexoses, glucose, fructose, and mannose, and their glycolytic breakdown will be described in a separate paper. Direct measurement of the penetration of these sugars is made difficult by their rapid utilization, which continues while the cells are being separated from the medium by centrifugation. The effects of the post-incubation metabolism were minimized by dilution at the end of the experiments with 8 volumes of medium (containing all the components as used for the incubation) cooled to 0°, then by centrifugation at the same temperature and fixation with reagents cooled to 0°. When the penetration of fructose was measured, ribose in place of raffinose was used as an indicator of entrapped medium and was added with the cold diluting medium to minimize its penetration into the intracellular water.

### Table V

**Inhibition of 3-Methylglucose Penetration by Phlorizin at 10°**

<table>
<thead>
<tr>
<th>3-Methylglucose concentration (mole per l.)</th>
<th>Time of incubation (min.)</th>
<th>Rate, mole per l. per min.</th>
<th>Inhibition (per cent)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.068</td>
<td>10</td>
<td>0.0015</td>
<td>33</td>
</tr>
<tr>
<td>0.034</td>
<td>5</td>
<td>0.0024</td>
<td>42</td>
</tr>
<tr>
<td>0.017</td>
<td>3.5</td>
<td>0.0028</td>
<td>38</td>
</tr>
<tr>
<td>0.0085</td>
<td>2.5</td>
<td>0.0028</td>
<td>57</td>
</tr>
</tbody>
</table>
At 37° glucose and fructose accumulated in the cells because penetration was more rapid than utilization, and consequently penetration was not a rate-limiting step at that temperature. In view of the abnormally large temperature coefficient for penetration found with these cells, it was possible that penetration might become rate-limiting at lower temperatures.

After incubation with glucose for 10 minutes at 30°, the internal concentration was 85 per cent of the external medium concentration of 0.045 M. At 10°, after 2 minutes of incubation, no intracellular glucose was found, but 31 per cent of the external concentration was found intracellularly when the incubation was continued for 10 minutes. The accumulation of glucose in the cells indicates that the initial penetration rate exceeds the phosphorylation rate at both temperatures, but apparently by a greater margin at 30° than at 10°. The apparent $K_m$ of glucose at 10°, calculated from its inhibition of 3-methylglucose penetration (Table IV), is 0.0007 mole per liter.

By incubation of the Ehrlich cells at various temperatures with fructose the following information was obtained. No free intracellular fructose could be found after incubations at temperatures below 25°. At 30°, the intracellular fructose concentration rose in 5 minutes to 55 per cent of the external concentration of 0.088 M and remained at that level for 10 minutes, although at lower external concentrations the relative internal fructose concentration decreased. In the presence of a concentration of phlorizin known to produce 40 per cent inhibition of 3-methylglucose penetration, no fructose could be found inside the cells at any temperature or duration of incubation.

From these observations with glucose and fructose, it is reasonable to conclude that the internal concentration of a metabolizable sugar reflects a steady state between penetration and utilization. The effect of insulin on such a system should be detectable, if it were to accelerate a rate-limiting step; i.e., penetration in the case of fructose, utilization in the case of glucose. No such effect could be detected. In particular, addition of insulin (1 unit per ml.) failed to influence the net penetration rate of fructose at temperatures below 30° and the amount of free intracellular fructose at temperatures above 30°. Insulin did not cause a change in the extent of inhibition of fructose penetration by phlorizin. The properties which a cell must possess in order to be influenced by insulin have not so far been defined and appear to be absent from the ascites tumor cell.

### SUMMARY

1. The penetration of hexoses and pentoses into the Ehrlich ascites tumor cells is described by a reversible first order process, with an equilibrium ratio of inside and outside concentrations of unity. The rate of
penetration of twelve different sugars varied over a 20-fold range, which indicates that the cell membrane exhibits configurational specificity.

2. The rate of penetration is strongly influenced by temperature. Between 20–30°, the temperature coefficient is about 4 and becomes progressively larger as the temperature is dropped to 0°, at which temperature there is practically no penetration. Apparent Michaelis constants and extrapolated maximal velocities are demonstrable in measurements at 10°, whereas at 20–25° the ordinate intercept in a Lineweaver-Burk plot is close to the origin.

3. Competitive inhibition occurs between pairs of sugars, the order of inhibitors being glucose > 3-methylglucose > xylose > galactose. Phlorizin, without being able to enter the cell, acts as a non-competitive inhibitor of penetration.

4. Under steady state conditions at 10–37°, the penetration of glucose is not rate-limiting for utilization, whereas that of fructose is rate-limiting at temperatures below 30°. Insulin fails to increase the penetration of fructose at any temperature and does not appear to have an effect on permeability in these cells.

BIBLIOGRAPHY

STUDIES OF TISSUE PERMEABILITY: I. THE PENETRATION OF SUGARS INTO THE EHRlich ASCITES TUMOR CELLS

Robert K. Crane, Richard A. Field, Carl F. Cori and With the technical assistance of Mary Louise Roberts


Access the most updated version of this article at http://www.jbc.org/content/224/2/649.citation

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/224/2/649.citation.full.html#ref-list-1