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# CHARACTERISTICS OF THE MEMBRANE TRANSPORT OF SUGARS IN THE LENS OF THE EYE

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SUMMARY

1. Membrane transport of the nonmetabolized glucose analog 3-*O*-methyl-D-glucose was measured *in vitro* in the lens of the eye of the rat.

2. Transport leads to complete equilibration of the sugar and conforms to Michaelis–Menten kinetics, with a very high $K_m$, around 90 mM. These results, together with the chemical specificity established earlier, are consistent with the operation of a facilitated diffusion process. There was no significant contribution of a parallel diffusional pathway.

3. The specific blocker of sugar transport phloretin acts in the lens as a noncompetitive inhibitor with a $K_i$ of about 0.6 mM.

4. Various agents and treatment known to stimulate sugar transport in muscle and adipose tissue were ineffective in the lens. These include insulin (also when given *in vivo*), proteolytic enzymes, decreased extracellular $Na^+$, changes in intracellular $Na^+$ and $K^+$ (inhibition of the $Na^+$ pump), inhibition of energy metabolism by uncouplers of oxidative phosphorylation or inhibitors of glycolysis, and adrenaline. Thus, sugar transport in the lens resembles that in the mature mammalian erythrocyte.

5. These data support the generalization that metabolic and hormonal modulation of sugar transport is present only in tissues where transport is rate-limiting and where glucose utilization varies in response to functional activity.

## INTRODUCTION

The lens of the eye is well suited for the *in vitro* study of membrane transport; since it has no vascular supply, incubation of isolated lenses will approximate physiological conditions. In addition, the transparency, appearance, and weight of the lens provide easy means for checking the condition of the tissue in the course of an experiment. Earlier studies, reviewed by Patterson[1], suggested that sugars may enter the lens by facilitated diffusion. As free intracellular glucose was found in the lens, its

Abbreviations: EGTA, ethyleneglycol-bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid; CMPS, *p*-chloromercuriphenylsulfonic acid.

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transport does not appear to be rate limiting for utilization[2-4]. In view of the above, it was surprising that Ross[5] reported a stimulation of glucose uptake by insulin in rabbit lenses, an effect normally seen only in tissues where glucose transport is rate limiting for its utilization. This finding has been confirmed by some[6], but not by other[2,7,8] workers. A better understanding of the transport of glucose, the main substrate of lenticular metabolism, would also provide additional data for a comparison of the characteristics of sugar transport and metabolism in various cell types.

We have recently reported[9] measurements of sugar transport in the lens, using radioactively labelled non-metabolized glucose analogs which permit the study of transport independently of subsequent metabolic conversion. Several metabolized and nonmetabolized sugars appeared to enter by a single facilitated diffusion process; some other sugars did not utilize this transport process. The specificity pattern conformed to that in other mammalian tissues where sugar transport takes place by an equilibrating process. For example, the lens showed preference for D-xylose over L-xylose, and for L-arabinose over D-arabinose, excluded L-glucose and α-methyl-D-glucoside and was more sensitive to the specific inhibitor phloretin than to its glycoside, phlorhizin.

The aim of the present study was to investigate the kinetics of sugar transport in the lens and to determine whether this process is subject to hormonal and metabolic regulation, as is the case in muscle and other tissues[10,11].

## METHODS

The methods for incubation and analysis of the tissues have been described in detail previously[9]. Briefly, male Sprague–Dawley albino rats of about 150–200 g were killed by cervical dislocation and the globes were removed immediately and kept in ice-cold buffer. The lenses were dissected out from the posterior side and incubated individually with gentle shaking at 37 °C in 25-ml flasks. Usually, 45 min of preincubation were followed by an incubation period of 1 h. The incubation medium consisted of Krebs–Henseleit[12] bicarbonate buffer, pH 7.4, equilibrated with 95 % $O_2$–5 % $CO_2$ and containing one-half of the specified $Ca^{2+}$ concentration. The preincubation medium also contained 5.5 mM D-glucose; the incubation medium contained 1.0 mM D-glucose, a mixture of $^{14}$C-labelled (0.125 μCi/ml) and unlabelled 3-*O*-methyl-D-glucose and [$^3$H]inulin in tracer amounts (0.625 μCi/ml), serving as extracellular marker. Other additions were made as indicated. After incubation the lenses were weighed, digested in NCS solubilizer (Amersham/Searle) (a quaternary ammonium base) and the radioactivity of the tissue digest and of medium samples was determined by double-label liquid scintillation counting[13]. The determinations of $Na^+$ and $K^+$ were done by atomic absorption spectrophotometry. The tissue water content was determined in separate groups of lenses by drying to constant weight at 80 °C under vacuum. An average tissue water content of 62.5 % of wet weight was used for all calculations unless stated otherwise.

Trypsin was obtained from Nutritional Biochemicals Corp., collagenase (crude and chromatographically purified) from Worthington Biochemical Corp. and crystalline zinc insulin from Connaught Medical Research Laboratories, Toronto.

The results are expressed as percentage penetration which indicates the sugar concentration in the intracellular water as a percentage of the concentration in the medium. The extracellular space was determined individually for each lens from the distribution of inulin. Within each experiment, lenses were assigned to the various treatments on a completely random basis, and Duncan's New Multiple Range Test[14] was used to determine the significance of differences between means of groups of lenses incubated under different conditions. With a few exceptions each treatment group consisted of a minimum of 4 lenses. Because of the known effect of age on lenticular metabolism, rats were matched for age in each experiment.

## RESULTS

In earlier experiments[9], using 5.0 mM 3-*O*-methylglucose, accumulation of the sugar against the concentration gradient was never observed; this suggested that

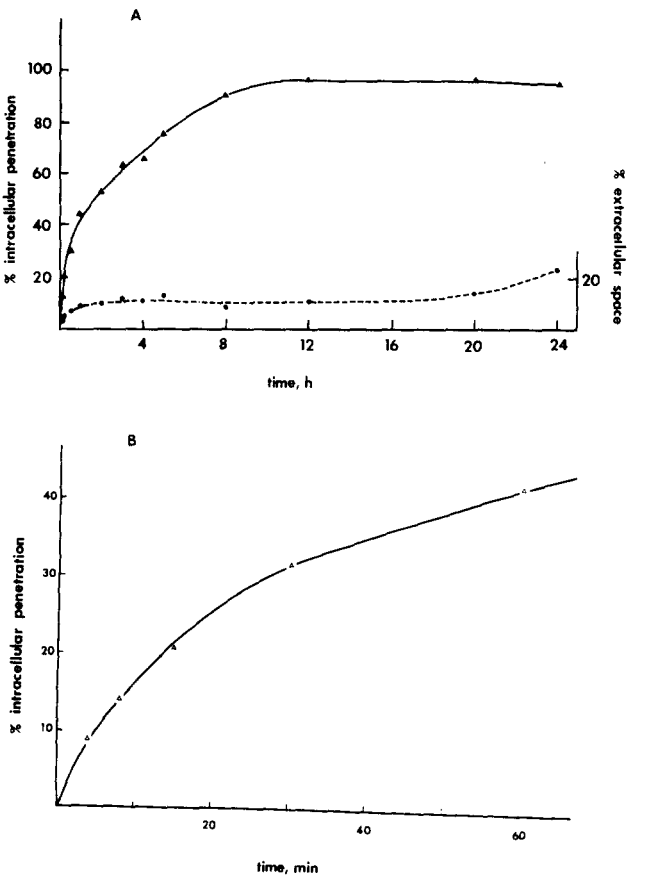


Fig. 1. (A) The time course of penetration of 5.0 mM 3-*O*-methyl-D-glucose (△—△) and of inulin distribution (●---●). Lenses which were incubated for more than 4 h received additional D-glucose and fresh $O_2$–$CO_2$ was blown into the flasks several times during the course of the experiment. For other details see Methods. The standard errors have been omitted as they were smaller than the symbols of the data points. (B) The time course of penetration of 3-*O*-methylglucose during the initial 60 min of incubation.

sugar entered the lens by facilitated diffusion. However, in hamster small intestine active transport of certain sugars could be demonstrated only at very low concentrations[15]. Therefore, we compared the penetration in the lens of 0.1 and 5.0 mM 3-*O*-methylglucose over a period of 24 h. The results, expressed in terms of percentage penetration, were identical at all incubation times. As seen in Fig. 1A, the sugar was fully equilibrated after approximately 12 h, and its intracellular concentration never exceeded equilibrium. The lenses remain in good condition for up to 20 h, as seen from the stable and low values for the extracellular space. Fig. 1B shows part of these results on an expanded time scale, indicating that the penetration rate shows only minimal deviation from linearity for the first 15 min, when the penetration reached is less than 20 %. This value will not be exceeded at higher sugar concentrations, as the slope of the rate against concentration (Figs 2A and 2B) is constant or decreases but never increases. Thus, the penetration in 15 min is a close reflection of initial entry at all concentrations and may be used for the determination of kinetic constants.

Fig. 2A shows the dependence of initial entry rate upon sugar concentration when lenses were incubated for 15 min with 3-*O*-methylglucose at concentrations ranging from 0.5 to 25 mM. In this experiment the NaCl concentration in the media was decreased from 145.2 to 132.7 mM, and constant osmolarity was maintained by adding appropriate amounts of sucrose. There was no clear evidence for saturation at

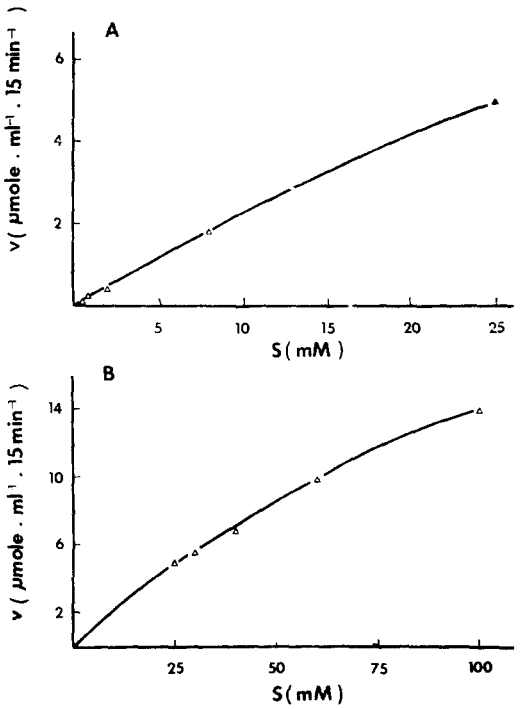


Fig. 2. The effect of the concentration of 3-*O*-methyl-D-glucose on its initial velocity of entry. Incubation time, 15 min. (A) Concentrations of substrate from 0.5 to 25 mM. (B) Concentrations of substrate from 25 to 100 mM.

higher concentrations. Complete saturation could not be demonstrated even at concentrations up to 100 mM (Fig. 2B). In this second series the NaCl concentration was decreased to 95.2 mM and appropriate amounts of sucrose were added, providing for a uniform $Na^+$ concentration throughout each experiment. Besides, as shown below (Table V) partial replacement of $Na^+$ has no effect on sugar transport in the lens. The failure to reach saturation is traditionally explained by the existence of a diffusional pathway operating in parallel with the specific transport system. In an attempt to isolate the presumable contribution of diffusion to the overall uptake process, the transport system was inhibited with the specific inhibitor phloretin (Fig. 3). A relatively high concentration of phloretin, 0.5 mM, inhibited 3-*O*-methyl

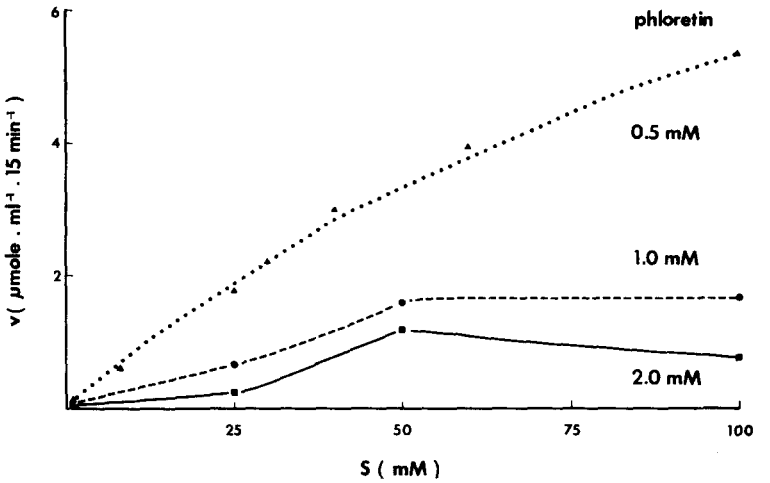


Fig. 3. The effect of various concentrations of phloretin on the initial velocity of entry of 3-*O*-methyl-D-glucose. Incubation time, 15 min.

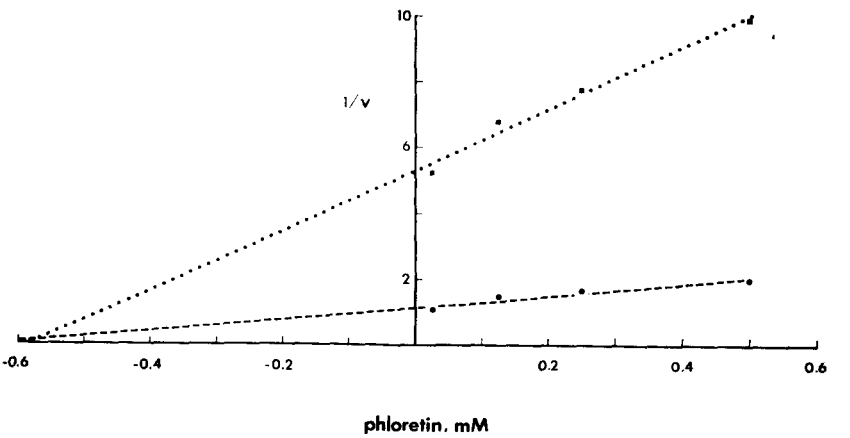


Fig. 4. Determination of the $K_i$ of phloretin by the Dixon[16] plot. Two concentrations of 3-*O*-methyl-D-glucose, 1.0 mM (■·····■) and 5.0 mM (●---●), were incubated with various concentrations of phloretin. Incubation time, 15 min. The straight lines were determined by regression analysis.

glucose penetration by approximately 50% (compare with Fig. 2B); higher concentrations of the inhibitor caused further progressive inhibition of sugar transport. The residual sugar penetration showed saturation which is incompatible with diffusion but agrees with the finding that phloretin acts in the lens of the eye as a noncompetitive inhibitor of sugar transport; as shown in Fig. 4, the two lines of the Dixon[16] plot intersect each other and the abscissa at the same point, indicating noncompetitive inhibition. The $K_i$ of phloretin thus obtained was approximately 0.6 mM. Furthermore, as shown in Table I, the penetration of 3-*O*-methylglucose was inhibited by D-glucose even in the presence of 0.5 mM phloretin. Unless a very high glucose concentration was used no significant inhibition was obtained, suggesting that the two sugars have similar high apparent $K_m$ values (see Tables II and III). It has been calculated that the usual addition of 1 mM glucose to the incubation media will inhibit the transport of 3-*O*-methylglucose (0.5 to 100.0 mM) by not more than 1.5%.

An alternative explanation for the failure of transport to reach saturation would be that the concentration of substrate is low in relation to the apparent $K_m$ of transport. This is supported by the data in Tables II and III. The precise values of kinetic constants are subject to some uncertainty because the extracellular marker, and presumably the test sugar as well, are not fully equilibrated in the extracellular space during a 15-min incubation period (see, *e.g.* Fig. 1). With such a short incubation

TABLE I

THE EFFECTS OF PHLORETIN AND D-GLUCOSE ON THE PENETRATION OF 3-*O*-METHYL-D-GLUCOSE

Incubation time, 1 h. An amount of NaCl, osmotically equivalent to 150 mM sugar was omitted to maintain constant osmolarity. When only test sugar was present, sucrose was added instead of the competing sugar.

| | *% Sugar penetration ± S.E.* | |
|---|---|---|
| | *Control* | *0.5 mM phloretin* |
| 50 mM 3-*O*-methyl-D-glucose | 36.84 ± 0.41 | 13.40 ± 0.22 |
| 50 mM 3-*O*-methyl-D-glucose + 100 mM D-glucose | 24.62 ± 1.23* | 7.30 ± 0.08* |

* $P < 0.01$.

TABLE II

MAXIMUM ESTIMATES FOR $V$ AND $K_m$ BY THE THREE MOST COMMON GRAPHICAL PROCEDURES

$K_m$ values in mM. $V$ in $\mu$moles·ml$^{-1}$·15 min$^{-1}$.

| *Range of substrate concentrations:* | | *0.5–25 mM* | *25–100 mM* | *0.5–100 mM* |
|---|---|---|---|---|
| Lineweaver–Burk[17] | $K_m$ | 55.42 | 119.84 | 59.14 |
| | $V$ | 13.99 | 33.17 | 15.39 |
| Hofstee[18] | $K_m$ | 155.45 | 128.55 | 111.61 |
| | $V$ | 28.70 | 35.07 | 28.22 |
| Woolf[19] | $K_m$ | 135.87 | 137.21 | 113.19 |
| | $V$ | 33.50 | 36.86 | 28.54 |

time the apparent extracellular space was around 4 % as opposed to between 6 and 7 % at complete equilibrium. Thus, the true concentration of test sugar at the cell membrane is probably lower than in the bathing medium and, moreover, is unlikely to be uniform throughout the extracellular space of the lens. The $K_m$ calculated on the "maximum assumption" that the 3-*O*-methylglucose is completely equilibrated within the extracellular space, is therefore probably an overestimate. A more realistic value is probably obtained from the "minimum assumption", that the sugar concentration in the extracellular space is decreased to the same extent as the distribution of the extracellular marker, *i.e.* to 4/6.

TABLE III

ESTIMATES FOR $V$ AND $K_m$ BY THE METHOD OF BLISS AND JAMES[20]

CI, 99% confidence interval. $K_m$ values in mM; $V$ values in $\mu$moles·ml$^{-1}$·15 min$^{-1}$.

| *Range of substrate concentrations:* | | *0.5–25 mM* | *25–100 mM* | *0.5–100 mM* |
|---|---|---|---|---|
| Maximum estimates | $K_m$ | 142.63 | 142.62 | 110.20 |
| | CI | 101.56–224.93 | 84.11–284.71 | 88.09–139.99 |
| | $V$ | 34.99 | 37.90 | 28.05 |
| | CI | 23.29–46.69 | 22.01–53.78 | 23.85–32.25 |
| Minimum estimates | $K_m$ | 97.51 | 69.46 | 67.16 |
| | CI | 69.63–152.97 | 40.97–138.61 | 53.69–85.33 |
| | $V$ | 34.96 | 37.90 | 28.05 |
| | CI | 23.38–46.54 | 22.02–53.78 | 23.85–32.26 |

Table II summarizes the values of apparent $K_m$ and $V$ obtained graphically according to the three accepted methods of Lineweaver–Burk[17], Hofstee[18] and Woolf[19]. Using the method of least squares, the parameters were calculated separately for three separate experiments encompassing the ranges of substrate concentration from 0.5 to 25 mM, 25 to 100 mM and 0.5 to 100 mM. The results all fitted straight lines but showed wide variations, particularly with the Lineweaver–Burk plot. These plots also have the disadvantage that they involve transformation of the data which makes statistical evaluation very difficult.

The method of Bliss and James[20], does not involve transformation of the data, which are instead directly fitted to a hyperbola by the method of maximum likelihood. A PDP-8 computer was used to calculate the parameters by this method for the three concentration ranges and with the two assumptions for the external substrate concentration. The results are shown in Table III together with their 99 % confidence limits. The variability of the data is much less than with the graphic procedures and the values of maximum estimates for the complete substrate range compare very well with the values obtained from the Hofstee and Woolf plots for the complete substrate range and from the Lineweaver–Burk plot for the higher substrate range. Thus, depending on the initial assumptions, the $K_m$ for 3-*O*-methylglucose lies between 67 and 110 mM. This is in the range of the highest substrate concentration tested and consequently explains satisfactorily the failure to demonstrate complete saturation in Figs 2A and 2B.

The remainder of this paper deals with the question whether sugar transport in the lens is subject to hormonal and metabolic regulation, as is the case in muscle, or is independent of any such modulation, as is the case in the erythrocyte. Some of the experiments described below were done on decapsulated lenses to ensure that the various agents had unimpeded access to the fibre membranes. Decapsulation was performed by treatment with either crude or pure collagenase or with trypsin; completeness of decapsulation was confirmed by microscopic examination. As seen in Table IV, Expts B and C, decapsulation by itself had no effect on the penetration of 3-*O*-methylglucose. This agrees with earlier work suggesting that the barrier to sugar diffusion is at the level of the fibre membranes[2].

TABLE IV

THE EFFECT OF INSULIN, COLLAGENASE AND TRYPSIN ON THE PENETRATION OF 5.0 mM 3-*O*-METHYL-D-GLUCOSE

Incubation time, Expts A and E, 30 min, Expts B, C and D, 60 min, Expt F, 15 min. Collagenase was present during 2 h of preincubation only. Trypsin was present during preincubation and incubation in Expt C and during preincubation only in Expt E. The lenses were washed in a large volume of buffer after decapsulation when incubated in the presence of insulin. In the *in vivo* experiment (F) insulin, 10 units/100 g wt, was injected intraperitoneally while the control rats received an equivalent volume of saline. The animals were killed either 45 or 90 min after injection.

| *Expt* | | % *Penetration* ± *S.E.* |
|---|---|---|
| A | Control | 31.77 ± 0.27 |
| | Insulin, 25 munits/ml | 32.05 ± 0.57 |
| B | Control | 38.23 ± 0.83 |
| | Collagenase pure, 1 mg/ml | 40.20 ± 0.50 |
| | Collagenase crude, 1 mg/ml | 38.58 ± 1.39 |
| C | Control | 38.23 ± 0.83 |
| | Trypsin, 0.2 mg/ml | 39.25 ± 1.02 |
| D | Collagenase pure, 1 mg/ml | 39.80 ± 1.34 |
| | Collagenase pure, 1 mg/ml + insulin, 100 munits/ml | 40.37 ± 1.00 |
| E | Trypsin, 0.4 mg/ml | 30.23 ± 0.48 |
| | Trypsin, 0.4 mg/ml + insulin, 100 munits/ml | 29.40 ± 0.62 |
| | Trypsin, 0.05 mg/ml | 30.00 ± 0.83 |
| | Trypsin, 0.05 mg/ml + insulin, 100 munits/ml | 30.23 ± 0.19 |
| F | *In vivo* | |
| | Control | 19.04 ± 0.28 |
| | Insulin, 45 min | 17.93 ± 0.71 |
| | Insulin, 90 min | 19.19 ± 0.33 |

As shown in Table IV, Expt A, 25 munits/ml, a concentration of insulin giving maximal stimulation of sugar transport in skeletal muscle[21], did not alter sugar penetration in the lens. In this experiment insulin was present during a 150-min preincubation period as well as during incubation with the sugar. The failure of insulin to affect sugar transport is not due to its inability to reach at least the superficial fibre membranes, since a very high concentration of insulin, 100 munits/ml, was without effect

after decapsulation with collagenase or trypsin (Table IV, Expts D and E). It is unlikely that this lack of insulin effect is due to degradation of the hormone since the lenses were thoroughly washed after decapsulation. Furthermore, insulin had no effect after decapsulation using a chromatographically purified preparation of collagenase, an enzyme which is known not to attack insulin.

In view of reports that the *in vivo* administration of insulin increased glucose utilization subsequently measured *in vitro*[7], rats were injected with 10 unit of zinc insulin per 100 g body wt and killed 45 or 90 min after injection. Sugar transport was then measured in isolated intact lenses. These animals showed evidence of frank hypoglycemia but the *in vitro* penetration of 3-*O*-methylglucose in the intact lenses was not altered (Table IV, Expt F).

In view of a report that the hypoglycemic biguanide drug, phenformin, stimulated glucose uptake in isolated rabbit lenses[8], we have investigated the effect of this drug on the transport of 3-*O*-methylglucose. 0.1 and 0.2 mg/ml of phenformin and 0.1 mg/ml of another biguanide, methformin, did not affect the penetration of 3-*O*-methylglucose into rat lenses during a 1-h incubation period. Such high concentrations of phenformin stimulate glucose uptake in muscle through their effect to inhibit oxidative metabolism and to stimulate glycolysis[22].

Treatment of muscle and adipose tissue with trypsin and some other enzymes affects sugar transport and the response to insulin of these tissues[23,24]. This does not appear to be the case in the lens since the presence of trypsin in the incubation medium after decapsulation had no effect on the transport of 3-*O*-methylglucose (Table IV, Expt C).

TABLE V

THE EFFECT OF OMISSION OF $Na^+$ ON THE PENETRATION OF 5.0 mM 3-*O*-METHYL-D-GLUCOSE

Incubation time, 1 h. Isosmotic conditions were maintained by the addition of appropriate amounts of the substances listed. The $Na^+$-free media were buffered with 25.0 mM Tris, bubbled with 95% $O_2$–5% $CO_2$, and adjusted to pH 7.4.

| *Expt* | % *Sugar penetration* ± *S.E.* | | | |
|---|---|---|---|---|
| | *Normal* $Na^+$ | *Replacements for* $Na^+$: | | |
| | (*control*) | *Sucrose* | D-*Mannitol* | *Choline chloride* |
| | *50% of* $Na^+$ *omitted* | | | |
| A | 40.76 ± 0.79 | | | 39.85 ± 0.19 |
| B | 41.10 ± 0.46 | 39.66 ± 1.99 | 41.86 ± 0.89 | |
| | $Na^+$*-free* | | | |
| C | 40.76 ± 0.79 | | 35.04 ± 0.75* | 34.78 ± 1.11* |
| D | 42.03 ± 0.96 | 36.73 ± 1.16* | | |

* $P < 0.01$.

The effect of external $Na^+$ was studied by replacing half or all of the $Na^+$ salts in the medium with osmotically equivalent amounts of mannitol, sucrose or choline chloride. As shown in Table V, when 50 % of the $Na^+$ was thus omitted sugar transport remained unchanged. However, when all the $Na^+$ was omitted sugar penetration was significantly decreased. Under these conditions an increase in the extracellular

space with no change in lens weight was noted, suggesting some cell shrinkage. These observations are in contrast to those reported for skeletal muscle where osmotic shrinkage[25] and a moderate decrease in external $Na^+$ (ref. 26) caused a stimulation of sugar transport.

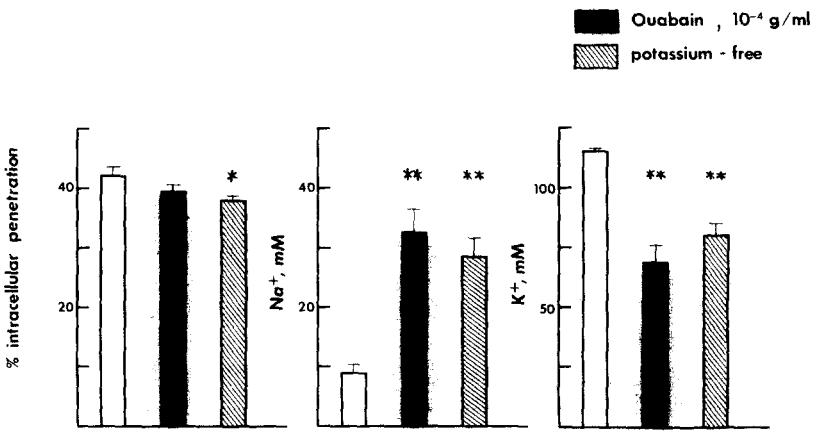


Fig. 5. The effect of ouabain and incubation in a $K^+$-free medium on the penetration of 3-*O*-methyl-D-glucose (5.0 mM) and on the concentrations of intracellular $Na^+$ and $K^+$. Both preincubation (3 h) and incubation (1 h) took place in the presence of ouabain ($10^{-4}$ g/ml) or in a $K^+$-free medium. Controls are indicated by the open bars. The vertical lines represent one standard error.
$^*P < 0.05$, $^{**}P < 0.01$.

In skeletal muscle sugar transport is stimulated when intracellular $Na^+$ is increased and intracellular $K^+$ decreased as a consequence of the inhibition of the sodium pump[21]. Preliminary experiments showed that very high concentrations of ouabain are required to inhibit ion transport in the lens; this is not unexpected in view of the known resistance of the rat to cardiac glycosides[27]. The lenses were therefore incubated with $10^{-4}$ g/ml ($1.17 \cdot 10^{-4}$ M) of ouabain, or in a $K^+$-free medium. As shown in Fig. 5, these treatments caused a strong inhibition of the $Na^+$-pump, as illustrated by the great increase in intracellular $Na^+$ and corresponding decrease in intracellular $K^+$. In these same lenses, ouabain had no significant effect on sugar transport. Incubation in $K^+$-free medium, while causing somewhat smaller changes in ion distribution, had a small but significant effect on sugar transport. However, lenses incubated in this medium showed a decrease in weight when compared to controls, indicating water loss. When results were corrected for this change in water content, the decrease in sugar penetration was no longer statistically significant. It would seem, therefore, that sugar transport in the lens is not dependent on the intracellular concentrations of $Na^+$ or $K^+$.

In muscle, sugar transport and its response to insulin are decreased by omission of $Ca^{2+}$ from the incubation medium[28,29]. In the lens omission of $Ca^{2+}$ from the incubation medium, as well as the addition of the calcium chelator ethyleneglycol-bis($\beta$-aminoethyl ether)-*N*,*N*'-tetraacetic acid (EGTA) had no effect on sugar entry during 1 h of incubation. The mean of differences ($\pm$ S.E.) between control and $Ca^{2+}$-free incubation was $-1.68 \pm 2.16$ %, and for $Ca^{2+}$-free with EGTA, $+0.67 \pm 1.51$ %. Neither was there any effect when the $Ca^{2+}$ concentration in the medium was doubled

(difference from control, $-0.90 \pm 0.94$ %). The extracellular space was not altered by these treatments.

In tissues where sugar transport is subject to regulation it is also stimulated by inhibition of energy supplies to the sodium pump[21]. This appears to be the mechanism whereby anoxia increases sugar transport in skeletal muscle. As shown in Table VI, Expt A, a high concentration of 2,4-dinitrophenol, 0.5 mM, had no effect on sugar penetration in the lens. However, in lenses decapsulated with collagenase, a lower concentration, 0.1 mM, of dinitrophenol and of another inhibitor of oxidative phosphorylation, 2,4-dinitro-*o*-cresol, had a small but significant inhibitory effect (Table VI, Expt B). The inhibitor of glycolysis, iodoacetic acid, 2.5 mM, inhibited sugar transport significantly in intact lenses and had an even stronger effect in lenses decapsulated with collagenase (Table VI, Expt C). Therefore, inhibition of energy metabolism in the lens does not stimulate sugar transport, a conclusion which is in agreement with the data in Fig. 5.

TABLE VI

THE EFFECT OF METABOLIC INHIBITORS ON THE PENETRATION OF 5.0 mM 3-*O*-METHYL-D-GLUCOSE IN INTACT AND DECAPSULATED LENSES

Incubation time, 1 h. Lenses were decapsulated by preincubation for 2 h with 1 mg/ml collagenase. 2,4-Dinitrophenol was present during preincubation and incubation in Expt A and during incubation only in Expt B; 2,4-dinitro-*o*-cresol and iodoacetate were present during incubation only.

| *Expt* | | % *Penetration* ± *S.E.* | |
|---|---|---|---|
| | | *Intact* | *Decapsulated* |
| A | Control | 32.33 ± 2.76 | |
| | 2,4-Dinitrophenol, 0.5 mM | 32.48 ± 1.18 | |
| B | Control | | 41.65 ± 0.75 |
| | 2,4-Dinitrophenol, 0.1 mM | | 35.80 ± 1.27 * |
| | 2,4-Dinitro-*o*-cresol, 0.1 mM | | 36.60 ± 0.37 * |
| C | Control | 41.65 ± 0.75 | 41.65 ± 0.75 |
| | Iodoacetate, 2.5 mM | 35.23 ± 0.55 * | 32.53 ± 0.55 * |

* $P < 0.01$.

It has been reported that in frog skeletal muscle[30] and in isolated adipocytes[31] adrenaline causes an increased entry of non-metabolized sugars which has been interpreted as a direct effect on the transport process. Experiments with intact and collagenase-decapsulated lenses, however, did not reveal any effect on sugar transport of adrenaline, the $\alpha$-adrenergic agent phenylephrine, the $\alpha$-antagonist phentolamine or the $\beta$-adrenergic agent, isoproterenol.

The effect of sulfhydryl group reagents is shown in Table VII. An inhibitory effect could only be demonstrated if the lenses were exposed to these agents for long periods or if they were decapsulated. The sulfonic acid derivative of *p*-chloromercuribenzoate, *p*-chloromercuriphenylsulfonic acid (CMPS), was used because this charged compound is believed not to penetrate across the cell membrane[32]. *N*-Ethylmaleimide has a number of specific effects on various cellular processes, and very brief exposure

to 1.0 mM of this agent has previously been found to specifically inhibit insulin-stimulated sugar transport in skeletal muscle[21]. As shown in Table VII, Expt A, under these conditions *N*-ethylmaleimide was ineffective in the lens. The need for long exposure to these agents to inhibit sugar transport (Table VII, Expt C) makes one suspect that the observed inhibition may be due to a non-specific effect.

TABLE VII

THE EFFECT OF SULFHYDRYL REAGENTS ON THE PENETRATION OF 5.0 mM 3-*O*-METHYL-D-GLUCOSE IN INTACT AND DECAPSULATED LENSES

Incubation time, 1 h. Lenses were decapsulated by preincubation for 2 h with 1 mg/ml collagenase. In Expt A CMPS was present during incubation and *N*-ethylmaleimide during the last 100 s of preincubation only. In Expt B CMPS and *N*-ethylmaleimide were present during incubation. In Expt C, CMPS was present during preincubation and incubation and *N*-ethylmaleimide during incubation.

| *Expt* | | *% Penetration ± S.E.* | |
|---|---|---|---|
| | | *Intact* | *Decapsulated* |
| A | Control | 43.90 ± 0.45 | |
| | CMPS, 2.5 mM | 41.45 ± 2.12 | |
| | *N*-Ethylmaleimide, 1.0 mM | 41.50 ± 1.61 | |
| B | Control | | 41.65 ± 0.75 |
| | CMPS, 2.5 mM | | 38.00 ± 0.51 * |
| | *N*-Ethylmaleimide, 1.0 mM | | 35.03 ± 0.73 * |
| C | Control | 39.20 ± 1.05 | |
| | CMPS, 0.125 mM | 35.03 ± 1.15 * | |
| | *N*-Ethylmaleimide, 1.0 mM | 24.78 ± 0.84 * | |

* $P < 0.01$.

## DISCUSSION

The present data, together with the results reported earlier[9], serve to characterize more completely the mechanism for sugar transport in the lens. Sugar transport does not lead to accumulation of substrate against the gradient and is therefore of the facilitated diffusion type, similar to that in various types of muscle, adipocytes and erythrocytes. The chemical specificity pattern also conforms to that established in the above tissues; it is fairly wide but still discriminates between optical isomers of various sugars, preferring the D-form (with the exception of L-arabinose). These conclusions were arrived at on the basis of measurements of penetration of the various sugars and of mutual competition between the different sugars belonging to this group[9]. Also, the specific inhibitor phloretin was more potent than its aglycone, phlorhizin[9], a situation identical with that in the tissues enumerated above but opposite to that in intestinal epithelial cells where the potencies of these two inhibitors are reversed. The data are consistent with the existence of a single transport system shared by most of these sugars; this, of course, does not exclude the possibility that some other sugars may enter by a separate system.

The concentration dependence of sugar entry is consistent with the operation

of a saturable mechanism conforming to Michaelis–Menten kinetics. The failure to reach complete saturation even at concentrations approaching 100 mM does not contradict this conclusion because the values for $K_m$ (Tables II and III) show that complete saturation cannot be expected with the substrate concentrations employed. The unexpectedly low affinity for sugars exhibited by this transport system is not unique; the $K_m$ of sugar transport in pancreatic islets was about 50 mM (Hellman *et al.*[33]). The alternate explanation, often invoked in similar cases, is that a parallel diffusion pathway significantly contributes to sugar entry. However, it seems unlikely that diffusion plays a major role in the penetration of sugars in the lens: When the transport of 3-*O*-methylglucose was progressively depressed by increasing concentrations of phloretin, saturation of the transport system was not abolished but became more evident, in agreement with the non-competitive nature of phloretin inhibition (Fig. 4). Furthermore, the chemical specificity of transport was maintained during substantial inhibition with phloretin (Table I). A third possibility to account for the failure to reach saturation at high substrate concentrations would be the operation of a second, low-affinity transport system such as that for amino acid transport in Ehrlich ascites tumour cells (Christensen and Liang[34]). No evidence supporting such an additional transport system was found, and the observation of a decreased maximal velocity in the presence of high concentrations of phloretin would speak against it.

The kinetic parameters as determined by graphic extrapolation (Table II) showed wide divergence between ranges of substrate concentrations, particularly with the Lineweaver–Burk plot. The main shortcoming of the Lineweaver–Burk plot, when unweighted data are used, is that the data at the lowest concentrations, which are the least reliable, have a disproportionate importance (Dowd and Riggs[35]). This was also found in the present study. However, the values obtained at the high concentration range with the Lineweaver–Burk plot are very close to those obtained at the complete concentration range, from 0.5 to 100 mM, with the Hofstee and Woolf plots. Far more consistent data are obtained by the method of Bliss and James[20], in which nontransformed data are used. (Table III). This method also permits calculation of confidence intervals for the means. The data for the complete range of concentrations show the least variability and are in good agreement with those obtained by the Hofstee and Woolf plots. Thus, a $K_m$ of about 110 mM and a $V$ of about 28 $\mu$moles$\cdot$ ml$^{-1}\cdot$ 15 min$^{-1}$ appears the most likely assuming that the sugar concentration in the intracellular space equals that in the bulk of the incubation medium (maximum assumption). On the other hand, as the apparent extracellular space after 15 min of incubation was about 2/3 of its equilibrium value, the sugar concentration in this space may have been lowered to the same extent. When thus recalculated (minimum assumption), the $K_m$ at the complete range of substrate concentrations was about 67 mM, ($V$ unchanged). The true $K_m$ of 3-*O*-methylglucose should be between these two extremes.

A number of various hormonal and metabolic factors are known to stimulate the specific sugar transport system in tissues, such as muscle and adipocytes, where sugar entry is rate-limiting for its metabolic utilization. These factors are ineffective in tissues such as the erythrocyte, where appreciable concentrations of free intracellular sugar are present. None of the agents and conditions examined in this study, insulin, proteolytic enzymes, changes in the extracellular or intracellular ion concen-

trations (and inhibition of the sodium pump), inhibition of energy metabolism by uncouplers of oxidative phosphorylation or inhibitors of glycolysis, or sympathomimetics, had a specific effect on sugar transport in the lens. Thus, the sugar transport mechanism in this tissue appears to be very similar to that in the mature mammalian erythrocyte.

The above data on sugar transport in the lens provide additional material for a correlation of transport and metabolism of sugars in various tissues. From this point of view, tissues may be divided into two main groups. In the first, exemplified by muscle, sugar transport is rate-limiting for its utilization and is subject to hormonal and metabolic control; in the second group, sugar transport also occurs by a specific mediated process but is neither rate-limiting nor influenced by insulin or other regulatory factors. The transport process appears to be integrated with metabolism in the sense that it has variable activity in those tissues where the rate of glucose utilization changes in response to functional activity or to increased deposition of energy reserves. This integration between transport and metabolism will be discussed in a separate paper.

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