Biochimica et Biophysica Acta, 426 (1976) 675–687
© Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

BBA 77251

EFFECT OF MALEIC ACID ON THE KINETICS OF α-METHYL-D-GLUCOSIDE UPTAKE BY ISOLATED RAT RENAL TUBULES

KARL S. ROTH, SHING MEI HWANG and STANTON SEGAL

Division of Biochemical Development and Molecular Diseases, Children's Hospital of Philadelphia and Department of Pediatrics and Medicine, University of Pennsylvania School of Medicine, Philadelphia, Pa. (U.S.A.)

(Received September 30th, 1975)

SUMMARY

α-Methyl-D-glucoside has been used to study the in vitro mechanism of the effect of maleic acid on sugar transport, using isolated rat renal tubule fragments. 6 mM maleate maximally inhibits the ability of the tubule to establish a concentration gradient for this model sugar with no evidence of ultrastructural changes. This inhibition is due to a 100 % increase in efflux, as well as to a 50 % decrease in influx with more prolonged incubation. The data presented here are consistent with those of other workers, but their work does not explain our results, which therefore deserve further investigation by other techniques.

INTRODUCTION

The renal cortical transport of α-methyl-D-glucoside has been previously characterized in rat [1] and in human [2]. α-Methyl-D-glucoside appears to share many of the transport characteristics, in both species, of the dietary hexoses D-galactose and D-glucose; it is competitively inhibited by both these sugars, its transport is active against a concentration gradient, and it is both Na^+ and energy dependent. Because of all of these features, in addition to which it is non-metabolizable, α-methyl-D-glucoside appeared to be an excellent choice of substrate, in an attempt to study the in vitro effect of the transport inhibitor, maleic acid, on sugar transport. It has been previously reported that upon injection of maleic acid into experimental animals there arises a glycosuria and aminoaciduria of large proportion, which is renal in origin [3, 4]. The latter workers examined the kinetic effects of maleic acid on transport of α-aminoisobutyric acid by rat kidney cortex slices. Their results suggested that, for this model amino acid, maleic acid impaired the ability to form a concentration gradient by affecting efflux out of proportion to influx and, further, that this effect was reversible. In this paper we report a study of the kinetic alterations induced by maleic acid in the sugar transport by the isolated kidney tubule.

EXPERIMENTAL

Sprague-Dawley rats were obtained from Charles River, Wilmington, Mass. Adult males weighing 150–200 g were fed ad libitum on Purina Chow. α-Methyl-D-[U-^{14}C]glucoside (52.2 Ci/mol) was obtained from Calbiochem, Los Angeles, Calif. Purity was assessed at 100 %. [*carboxy*-^{14}C]Inulin was obtained from New England Nuclear, Boston, Mass. Unlabeled α-methyl-D-glucoside was obtained from Pfanstiehl Co., Waukegan, Ill., with purity assessed at 100 % by gas-liquid chromatography. Maleic acid was obtained from Eastman, 98 % pure, made to 6 mM and neutralized before use. Collagenase, grade II, was obtained from Worthington Biochemical Corp., with a specific activity of 161 units/mg. Fetal calf serum, obtained from Flow Labs, was divided into 10-ml aliquots and stored frozen until used, to prevent bacterial growth.

Determination of sugar accumulation

The preparation of the isolated renal tubules was performed with modifications of the technique of Burg and Orloff [5]. Rats were stunned and decapitated. Injection of collagenase solution into the intact kidney via the renal artery as an initial preparative step was omitted, since we were repeatedly unable to demonstrate a difference in α-methyl-D-glucoside uptake between tubules prepared from a pre-injected and a non-preinjected kidney from the same animal. The kidneys were quickly removed and the capsule stripped and placed in cold Krebs-Ringer-bicarbonate buffer containing 10 mM sodium acetate*. Using a Stadie-Riggs microtome, two slices of cortex were taken from each of the lateral aspects of the kidney; the remainder of the cortex was then dissected free. The tissue was then finely minced, and placed in a 0.375 % collagenase solution (w/v) of Krebs-Ringer-bicarbonate buffer, 0.5 ml per kidney. The digestion mixture was gassed with 95 %O_2/5 %CO_2 for 60 s, capped and gently agitated for 45 min, using a small stirring bar, at room temperature. Iced buffer was then added to prevent further digestion which may lead to metabolic alterations [7], and the mixture was spun at 40 × *g* in an International model UV centrifuge for 1.5 min, following which the supernatant was discarded. The pellet was washed three times with iced buffer, spinning at 40 × *g* for 1.5 min. The final pellet was resuspended in Krebs-Ringer-bicarbonate buffer and filtered through three layers of surgical gauze. Final volumes of suspension were between 20 and 40 ml, with 1 ml of fetal calf serum added per 20 ml of suspension. Each ml of final suspension contained 15–20 mg of tissue obtained from a total of six kidneys.

All experiments were carried out at 37 °C with continuous gassing with either 95 %O_2/5 %CO_2 or 95 %N_2/5 %CO_2, in tubes identical to those described by Burg and Orloff [5]. Each ml of the incubation mixture contained 0.1 μCi of α-methyl-D-glucoside, with the unlabeled sugar added to give the desired concentrations.

At various times, 1-ml aliquots were removed from the incubation mixture in duplicate, added to tared tubes, cooled rapidly to 0° C, and centrifuged at 33 000 × *g* for 10 min in a Sorvall model RC2B centrifuge at 4 °C. The supernatants were

* 118.5 mM NaCl; 4.75 mM KCl; 2.53 mM $CaCl_2 \cdot 2H_2O$; 1.2 mM KH_2PO_4; 1.2 mM $MgSO_4 \cdot 7H_2O$; 130.8 ml glass distilled H_2O; 25 mM $NaHCO_3$; 10 mM sodium acetate; total volume 195 ml (pH 7.4).

removed for subsequent counting, and the pellet surfaces washed twice with iced buffer, removed with suction, and the tubes weighed to obtain the wet weight of the pellet. The cells were lysed by the addition of 1 ml distilled water and resuspended, followed by boiling for 3 min. The lysate was again centrifuged at $33000 \times g$ in the Sorvall. Aliquots (0.2 ml) of each cell lysate and its corresponding supernate were added to 10 ml phosphor/ethanol and counted in a Packard liquid scintillation spectrometer.

The volume of "trapped medium" in the pellet was established by the method of Burg and Orloff [5], adding [*carboxy*-^{14}C]inulin to the suspension immediately before sampling. The volume of intracellular fluid was taken as the difference between total tissue water, determined by overnight dessication, and the volume of "trapped medium". Tissue α-methyl-D-glucoside concentration and the distribution ratio (cpm/ml intracellular fluid to cpm/ml medium) were determined as previously described [7, 8].

Steady-state kinetic

In order to determine the amount of α-methyl-D-glucoside entering the cell per unit time, the suspension was preincubated with and without 6 mM maleate in the presence of 2 mM unlabeled sugar for sufficient time for the cells to achieve a steady-state distribution ratio. After 15 or 30 min of preincubation, tracer was added and samples were taken and treated in a manner identical to that described for uptake studies. Fractional rate constants were calculated by the method of Rosenberg et al. [9] for a two-compartmental system, as employed by McNamara et al. [8].

Direct measurement of efflux of sugar

The tubules were preincubated with and without 6 mM maleate for 30 min at 37 °C, as described earlier. Because the steady-state distribution ratio of maleate-treated tubules was shown in uptake experiments to be approximately 20 % of the control value at 30 min, it was necessary to preincubate the maleate-treated tubules at 5 times the α-methyl-D-glucoside concentration of the control (10 mM and 2 mM, respectively).

At the end of the 30 min period, aliquots were removed from each flask and the pellets counted to determine the maximum tissue concentration of the substrate. The remainder of the tissue from each flask was centrifuged at $10000 \times g$ at 4 °C for 2 min. Next, 2 ml of fresh buffer from two new incubation flasks containing 2 mM α-methyl-D-glucoside with and without maleate at 37 °C and continously gassed were added to the centrifuge tubes. Immediately on contact of buffer with the pellets, timing of efflux was begun. The pellets were rapidly resuspended in the 2 ml of their respective buffers and transferred immediately to the incubation flasks.

Aliquots were removed immediately for counting, and at regular intervals thereafter. Results were expressed as the percentage of the original isotope remaining in the pellet per unit time elapsed. Efflux rate constants were calculated from a semi-log plot of the data using a K curve.

Concentration dependence of uptake

The tissue was preincubated with or without 6 mM maleate for 30 min in Krebs-Ringer-bicarbonate buffer, at which time 0.1 μCi/ml of labeled α-methyl-D-

glucoside and sufficient unlabeled sugar to give the desired concentrations was added. Samples were taken from each incubation mixture at 5 min and treated as described above. A Lineweaver-Burk plot was made to determine the value of K_m with and without maleate, and the lines drawn using a Monroe 1775 model computer to obtain the best fit. All data was analyzed by Student's *t*-test, for statistical significance.

RESULTS

Concentrative uptake of α-methyl-D-glucoside

This sugar is accumulated by renal cortical tubule fragments in concentrations much greater than that in the incubation medium. Fig. 1 demonstrates the increase in distribution ratio plotted against time, and shows the effect of anoxia on the ability of the tubules to accumulate α-methyl-D-glucoside. The characteristics of the control curve in this system are significantly different from those reported previously for slices [1, 10]. While rat cortex slices achieve a steady-state distribution ratio over a time period of 30–45 min, it can be seen from Fig. 1 that this point is reached in 15–17 min by the tubules, and that the concentrative effect exerted by the tubules is greater than that shown by slices. Anoxia abolishes this concentrative ability of the tubule, as it does in the slice. Fig. 1 also shows the effect of 6 mM maleic acid on the accumulation of α-methyl-D-glucoside, clearly demonstrating a very different effect from that obtained in the anoxic preparation.

The results of varying concentrations of maleate on α-methyl-D-glucoside transport are shown in Fig. 2. While 3 mM maleate was found to exert only a minimal effect on the accumulation of α-methyl-D-glucoside by renal tubules, 3 mM maleate

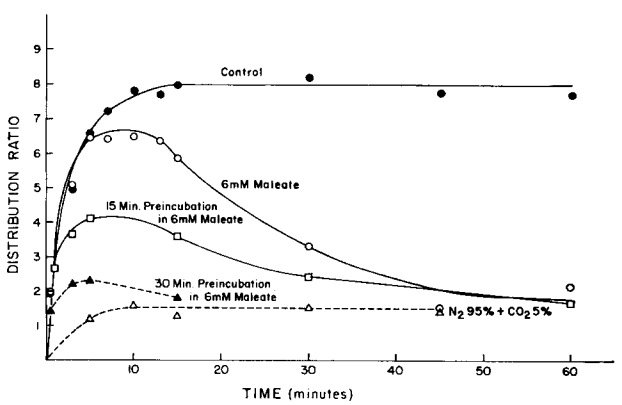


Fig. 1. Uptake of α-methyl-D-glucoside by isolated rat renal tubules. Tubules were incubated in 20 ml Krebs-Ringer-bicarbonate buffer with 10 mM sodium acetate (pH 7.4) containing 2 mM unlabeled sugar and 0.1 μCi/ml of label at 37 °C in special flasks (see text) which permitted continuous gassing. Uptake is designated by the distribution ratio, the ratio of cpm/ml intracellular fluid to cpm/ml medium. The 6 mM maleate curve (○) was obtained by addition of inhibitor (pH 7.0) to the above system, at 0 time. Preincubation curves were obtained by adding 6 mM maleate to the system 15 (□) and 30 (▲) min before adding labeled and unlabeled sugar. Anoxic conditions (△) were maintained by continuous gassing with 95% N_2/5% CO_2. Each point is an average of 3–5 incubations, the tissues representing 9–15 animals. "Trapped medium" and total water were 15.2% and 80%, respectively.

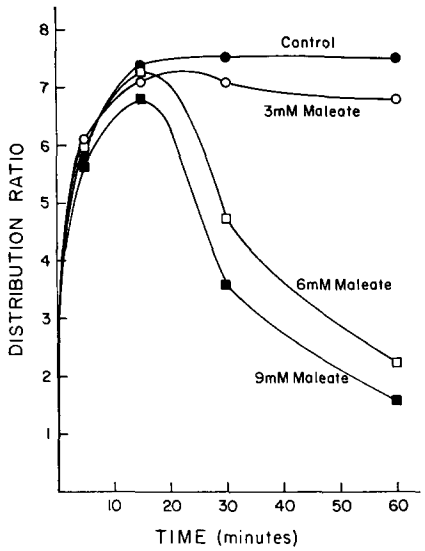


Fig. 2. Effect of varying concentrations of maleate on α-methyl-D-glucoside uptake.

has been shown to exert a marked effect on amino acid accumulation by the cortical slice [4].

The effect of addition of this concentration of maleic acid on the integrity of the cells was examined by three independent means. A determination of "trapped medium" space and total tissue water showed no differences attributable to maleate. Since Rosenberg and Segal [4] reported reversibility of the effect of maleate on cortical slice transport of amino acids, we tested our tubule preparation by a similar experiment. The results, shown in Fig. 3, demonstrate that the effect of 6 mM maleic

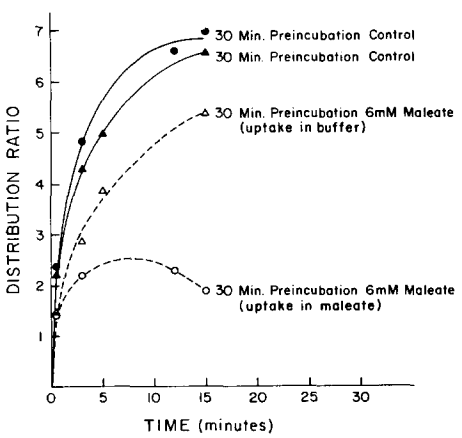
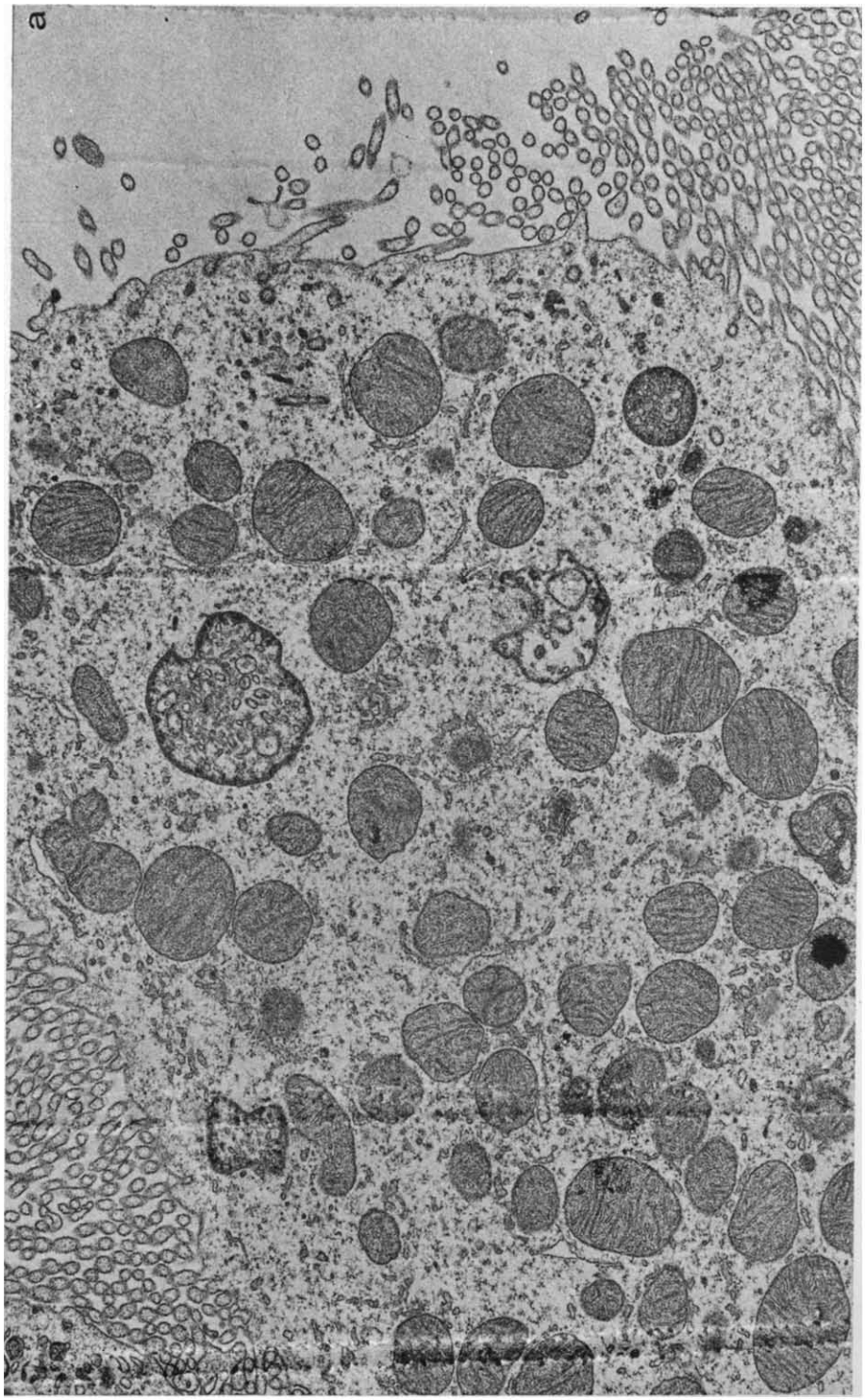


Fig. 3. Reversibility of the effect of 6 mM maleic acid on the uptake of 2 mM α-methyl-D-glucoside. Tubules were incubated as previously described with (△, ○) and without (▲, ●) 6 mM maleate. After 30 min preincubation, the tissue in one control (▲) and one experimental flask (△) was centrifuged and resuspended in fresh Krebs-Ringer-bicarbonate buffer, with subsequent addition of labeled and unlabeled sugar to all flasks, and the performance of a regular uptake study.


a

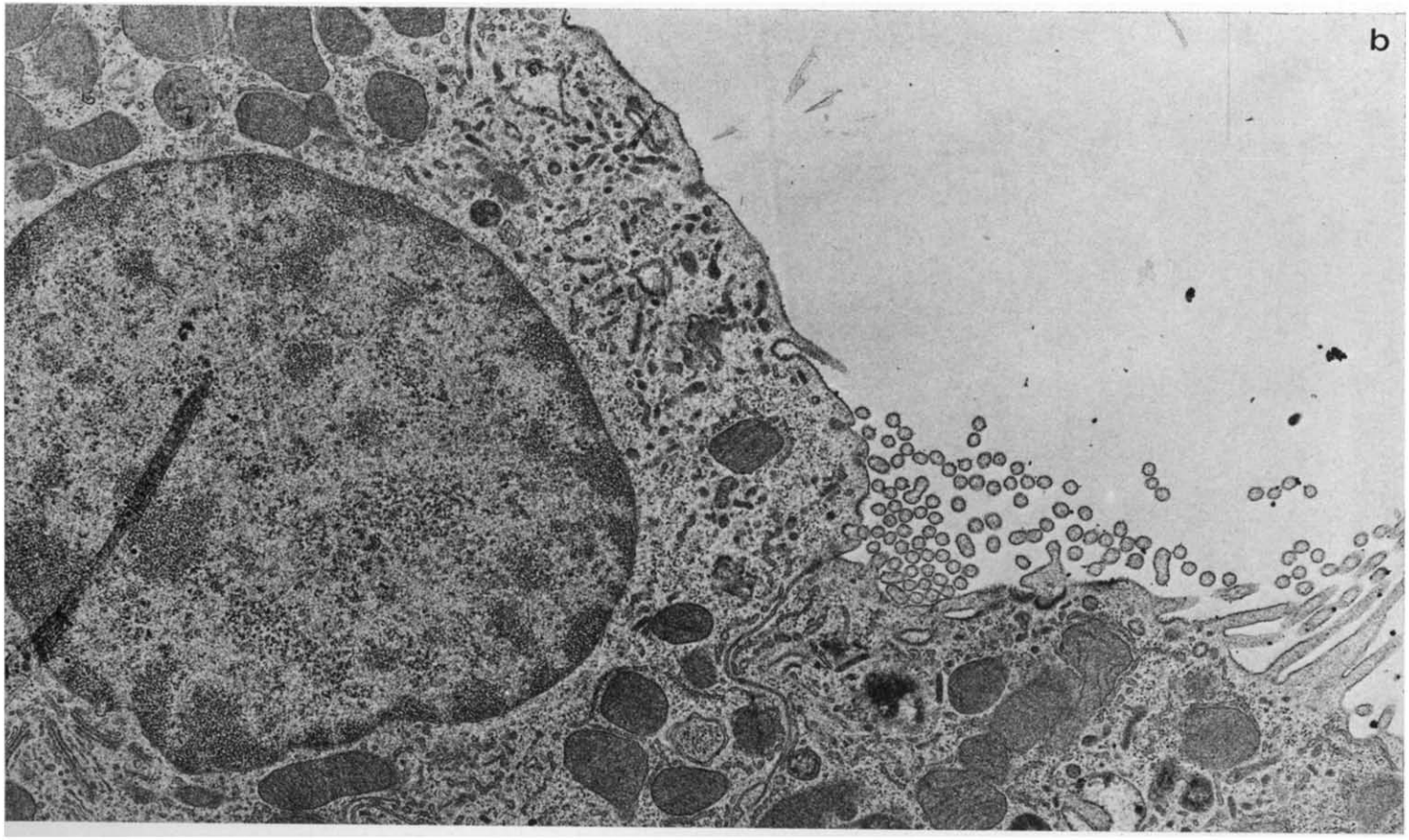


Fig. 4. Electron micrographs of tubules incubated for 30 min at 37 °C with continuous gassing with 95 % O_2/5 % CO_2 with (A) and without (B) 6 mM maleic acid. Specimens were fixed in 1 % Millonig buffered OsO_4 for 30 min and dehydrated in graded alcohol, then embedded in epoxy resin. Sections were doubly stained with uranil acetate and lead citrate and viewed in electron microscope type Phillips 300. No differences were visible, either in brush border or in mitochondria, which were attributable to maleate. Magnification × 14175.

acid on α-methyl-D-glucoside accumulation by the tubules is reversible. Finally, electron microscopy was used to assess ultrastructural changes attributable to maleate. For this study we used tubules which had been incubated for 30 min with and without maleic acid, since this time interval is required to demonstrate the maximal effect of maleate on α-methyl-D-glucoside transport in our system. Figs. 4A and 4B demonstrate the fact that no ultrastructural changes take place in the tubules during this period of time.

Kinetic effects of maleic acid

Since the uptake curve demonstrated a decrease in net uptake of α-methyl-D-glucoside with time in the presence of 6 mM maleic acid, we examined the influx and efflux components separately, in order to determine the effect of maleate on each. By using steady-state kinetic technique, we were able to measure influx directly, although we could not do so earlier than 15 min after beginning the incubation, since this is the time at which the steady state is achieved. These results are shown in Fig. 5. The fractional rate constants calculated from these data are presented in Table I. At 15 min, in the presence of 6 mM maleate, the steady-state distribution ratio is reduced from 7.07 to 2.7, with no measurable change in the influx rate constant. Hence, this decrease in net uptake is a result of an increase in the rate of loss from the cells, represented in Table I by a 100 % increase in the efflux rate constant within 15 min. Significantly, however, the distribution ratio in the maleate-treated cells is further reduced to 1.83 within 30 min, with no change in the efflux rate constant, but a 50 % fall in the influx rate constant. Hence, we must conclude that the initial effect of maleate is to increase efflux of accumulated substrate within the first 15 min, while any change occurring in the distribution ratio after this time is attributable to a decrease in the rate of influx to the cell from the medium.

Direct measurement of the efflux rate constants with and without maleate after 30 min of preincubation gave results (Fig. 6) that are compatible with those

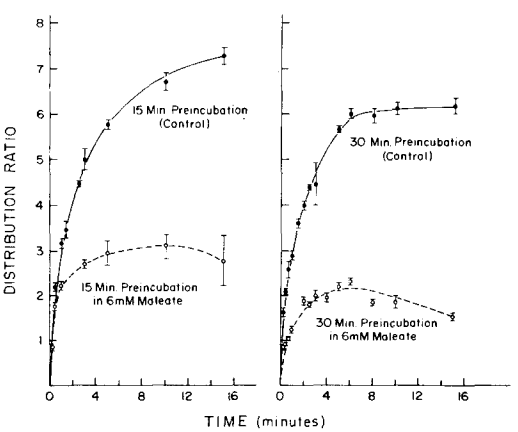


Fig. 5. Steady-state kinetic analysis of α-methyl-D-glucoside transport. Tubules were incubated as previously described for 15 or 30 min with (○) and without (●) 6 mM maleate in the presence of 2 mM unlabeled sugar. Labeled sugar was added (0.1 μCi/ml) after preincubation, and an uptake study performed. Data are plotted as the mean ± standard error of the mean for each point. All points represent triplicate experiments.

derived from the steady-state method, although the absolute values from each method cannot be compared. Control studies showed an efflux rate constant of 0.93(min^{-1}), and maleate-treated tubules gave a value of 2.73(min^{-1}).

TABLE I

STEADY-STATE KINETIC PARAMETERS OF α-METHYL-D-GLUCOSIDE TRANSPORT

Triplicate experiments were carried out at 37 °C as described in text. All calculations are based on 100 mg of tissue. Statistical analysis was performed using Student's *t*-test. Fractional rate constants are expressed as the mean values ± standard error.

Medium $\underset{\lambda_{MI}}{\overset{\lambda_{IM}}{\rightleftharpoons}}$ Intracellular pool

Conditions	Time preincubation (min)	Steady-state distribution ratio	Steady-state intracellular pool (μmol)	Fractional turnover rate (min^{-1})	
				λ_{IM}	λ_{MI}
Control	15	7.07	0.9615	0.014 ±0.000	0.616 ±0.0109
	30	6.55	0.8840	0.013 ±0.0003	0.663 ±0.0243
Maleate	15	2.70	0.3672	0.011 ±0.0009	1.125** ±0.0675
	30	1.83	0.3060	0.006* ±0.0000	1.027** ±0.0843

* Differs from control value and from the 15 min maleate value, $P < 0.02$.
** Differs from control values, $P < 0.02$.

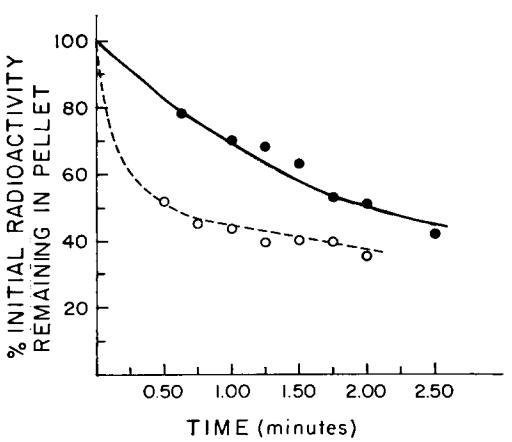


Fig. 6. Effect of 6 mM maleic acid on efflux of α-methyl-D-glucoside. Tubules were preincubated for 30 min with (○) and without (●) 6 mM maleate and sufficient labeled and unlabeled sugar to achieve approximately equal intracellular concentrations in both flasks, as described in text. The tubules from each flask were then separated from the media by 2-min total centrifugation time and the tissue transferred to fresh buffer containing 2 mM unlabeled sugar with (○) and without (●) 6 mM maleate. Appearance of isotope in the medium was measured with respect to time, a semilogarithmic plot made of the data, and efflux rate constants calculated by *K*-curve analysis.

Concentration dependence of uptake

As reported by Segal [1] for rat cortical slices, saturability of the uptake of α-methyl-D-glucoside by rat renal tubules occurred at concentrations above 1.5 mM. The effect of maleic acid on this parameter of uptake is shown in Fig. 7. A significant decrease in V was observed (Table II). The apparent transport K_m was unchanged by maleic acid during the time period studied. No evidence was obtained for more than one K_m over a wide range of substrate concentrations.

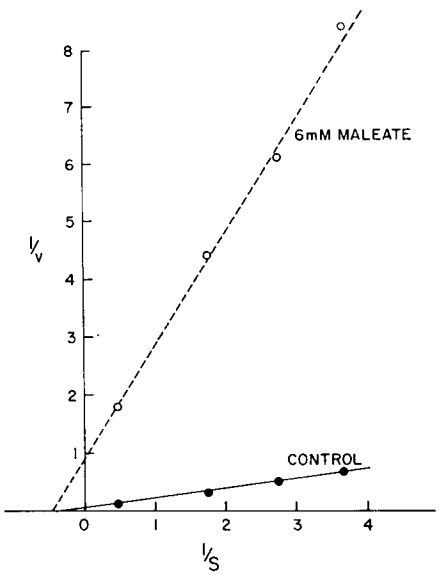


Fig. 7. Effect of 6 mM maleate on the concentration dependence of uptake of α-methyl-D-glucoside. Tubules were preincubated with (○) or without (●) 6 mM maleate for 30 min, as described in text. After preincubation, 0.1 μCi/ml of labeled glucoside and sufficient unlabeled sugar was added to give the desired concentrations. Incubation was carried out for 5 min with substrate, at which time samples were removed and treated as previously described. Points represent the means of triplicate experiments, and the line was fitted by computer.

TABLE II

EFFECT OF MALEATE ON PARAMETERS OF CONCENTRATION-DEPENDENT UPTAKE OF α-METHYL-D-GLUCOSIDE

Triplicate experiments were carried out at 37 °C as described in text, over a range of substrate concentrations including 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 2.0, 4.0 and 6.0 mM. Preincubation with maleate was performed for 30 min, and aliquots were removed for counting 5 min after addition of the glucoside. Data were analyzed using Student's t-test.

Conditions	K_m (±S. E.) (mM)	V (±S. E.) (mmol/l per 5 min)
Control	5.227±1.064	24.983±6.9876
Maleate	7.667±2.564	4.198±2.1131
	$P > 0.3$	$P < 0.05$
	$n = 3$	$n = 3$

DISCUSSION

The problem of the biochemical origin of the aminoaciduria and glucosuria of Fanconi's syndrome remains unresolved. The pathological evidence of involvement of the proximal tubule in patients with this syndrome [11], as well as the fact that glucose and amino acid reabsorption are usually considered to occur primarily in the proximal segment of the nephron have tended to focus the attention of investigators on this region of the renal tubule. Harrison [3] and others have indicated the renal origin of the glucosuria and aminoaciduria, while classical concepts of renal physiology have dictated acceptance of the theory that this renal origin lay in failure of the brush border surface of the proximal tubule to reabsorb filtered substances contained in the tubular lumen.

Early experimental in vitro evidence with kidney slices [4, 9], however, suggested the hypothesis that the aminoaciduria produced in vivo by maleic acid resulted primarily from an increase in outward movement of amino acid from the cell, rather than from a failure of uptake of the substance presented to the cell surface. Subsequent experimental data [12, 13] obtained by the micropuncture technique as well as by in vivo injection of labeled isotope and maleate have tended to support the hypothesis that the aminoaciduria is a result of increased rate of loss of accumulated substances from the cell induced by maleic acid.

Our results indicate that maleic acid induces a 100 % increase in the rate of loss of α-methyl-D-glucoside from the cell water, suggesting that the origin of the glucosuria in the maleate-treated rat follows the same general mechanism as that which has been demonstrated for the amino acids. Preliminary work by Silverman and Huang [14], using the multiple indicator dilution technique in dogs, tends to support our findings, since their results suggest that maleate produces glucosuria by increasing efflux of D-glucose across the brush border surface. The major difficulty in conceptualizing this mechanism for glucosuria lies in the fact that it is not easily possible to explain the existence of a free glucose pool intracellularly from which the glucose found in the urine derives. Although the 100 % increase in the rate of efflux which we have demonstrated is consistent with the large amount of glucose in the urine, it may be that the later and smaller (50 %) change in influx which we also demonstrated with maleate is the crucial event occurring in vivo in the rat to account for the glucosuria. If this were the case, it would not necessitate a hypothesis for the mechanism of flux through an intracellular pool of free glucose.

Studies of the influx effect of 6 mM maleate show a dramatic decrease in V to about 16 % of control values, with no change in the apparent transport K_m. This finding implies a decrease in the number of available sites for transport or in the efficiency of the transport system for entry. The decrease in entry rate, accompanied by efflux being affected earlier and to a greater degree is consistent with earlier work [4, 9, 15] with metabolic inhibitors such as 2,4-dinitrophenol, which suggested that efflux phenomena are energy linked. A metabolic effect of maleic acid is also consistent with the work of several authors [16–18] who have demonstrated effects of maleic acid on the energy-related processes by which active transport is thought to occur. These studies indicated effects of maleic acid on renal membrane $(Na^+ + K^+)$-ATPase, on renal gluconeogenesis from precursors requiring coenzyme A in this synthetic process, and on substrate-level phosphorylation and/or other mitochondrial reac-

tions involving succinyl-CoA. The concentrations of maleate required to produce these effects have caused marked mitochondrial damage, thus making predictable the demonstrated effects on energy-related processes. However, we have demonstrated a significant perturbation of sugar transport without any evidence of damage to either brush border or mitochondria. In addition, we have shown that this is a reversible phenomenon which suggests that any theory which postulates the establishment of a covalent bond between maleate and sulfhydryl groups may not be applicable here.

Tate and Meister [19] have shown that maleate increases the rate of hydrolysis of γ-glutamyl donors in the cell, thus interfering with the cycle, which Meister has proposed to be involved with amino acid transport [20, 21]. However, any unifying hypothesis for the nature of the maleic acid effect on tubular transport should account for the aminoaciduria and glucosuria in similar ways, since the evidence now points to similar transport effects in vitro for both types of compound. If, in Tate and Meister's view, amino acid transport is slowed due to lack of donors in the γ-glutamyl cycle, then it would follow that influx should be slowed as the primary phenomenon. The bulk of evidence points to the contrary, that efflux is affected to a much greater degree. Perhaps the most reasonable way to reconcile these difficulties with the established fact is to speculate that there is a combined effect of maleate on the cell membrane-glutathione stability relationship [22] and its known metabolic inhibitor effects, the former accounting for decreased entry of sugars, the latter for the tremendous increase in efflux of sugars and amino acids.

ACKNOWLEDGEMENTS

This work was supported, in part, by Grant AM 10894 and by Training Grant HD 215 from the National Institutes of Health.

REFERENCES

1 Segal, S., Rosenhagen, M. and Rea, C. (1973) Biochim. Biophys. Acta 291, 519–530
2 Segal, S., Genel, M., Holtzapple, P. and Rea, C. (1973) Metabolism 22, 67–76
3 Harrison, H. E. and Harrison, H. C. (1954) Science 120, 606–607
4 Rosenberg, L. E. and Segal, S. (1964) Biochem. J. 92, 345–352
5 Burg, M. B. and Orloff, J. (1962) Am. J. Physiol. 203, 327–330
6 Guder, W., Wiesner, W., Stukowski, B. and Wieland, O. (1971) Hoppe-Seyler's Z. Physiol. Chem. 352, 1319–1328
7 Rosenberg, L. E., Blair, A. and Segal, S. (1961) Biochim. Biophys. Acta 54, 479–488
8 McNamara, P. D., Rea, C. and Segal, S. (1971) Science 172, 1033–1034
9 Rosenberg, L. E., Berman, M. and Segal, S. (1963) Biochim. Biophys. Acta 71, 664–675
10 Segal, S. and Rosenhagen, M. (1974) Biochim. Biophys. Acta 332, 278–285
11 Darmady, E. M. and Stranack, F. (1957) Brit. Med. Bull. 13, 21–26
12 Bergeron, M. and Vadeboncoeur, M. (1971) Nephron 8, 367–374
13 Bergeron, M. (1971) Rev. Can. Biol. 30, 267–272
14 Silverman, M. and Huang, L. (1974) Am. Soc. Nephrol., 7th Annual Meeting, Washington, D.C. Abstr., p. 84
15 Rosenberg, L. E., Downing, S. J. and Segal, S. (1962) J. Biol. Chem. 237, 2265–2270
16 Kramer, H. J. and Gonick, H. C. (1970) J. Lab. Clin. Med. 76, 799–808
17 Schaerer, K., Yoshida, T., Voyer, L., Berlow, S., Pietra, G. and Metcoff, J. (1972) Res. Exp. Med. 157, 136–152

18 Rogulski, J., Pacanis, A., Adamowicz, W. and Angielski, S. (1974) Acta Biochim. Polon. 21, 403–413
19 Tate, S. S. and Meister, A. (1974) Proc. Natl. Acad. Sci. U.S. 71, 3329–3333
20 Meister, A. (1973) Science 180, 33–39
21 Orlowski, M. and Meister, A. (1970) Proc. Natl. Acad. Sci. U.S. 67, 1248–1255
22 Kosower, N. S., Kosower, E. M. and Wertheim, B. (1969) Biochem. Biophys. Res. Commun. 37, 593–596