ments. RPF was consistently reduced by angiotensin infusion, and GFR and sodium excretion also tended to decrease although less consistently. However, such changes in renal hemodynamics and sodium excretion have previously been shown to be associated with stimulation rather than inhibition of renin secretion(3,4). This is particularly evident as regards the infusion of catecholamines (during "static aortic clamping") or stimulation of the renal nerves, since these procedures produce renal function changes quite similar to those of angiotensin, but stimulate renin secretion(4). It seems likely, therefore, that the inhibitory effect of angiotensin may be exerted directly upon the renin-secreting cells either by angiotensin itself or by some substance liberated extrarenally as a result of the elevated plasma angiotensin. It is interesting that this type of inhibition may be analogous to the enzyme repression which occurs in wholly intracellular metabolic systems.

*Summary.* Intravenous infusion of angiotensin II (6.3-200 ng/kg min) inhibited renin secretion in dogs under conditions of basal or elevated renin secretion. This inhibition was independent of changes in mean renal arterial blood pressure. We propose a negative feedback effect of circulating angiotensin on renin secretion.

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Received June 9, 1965. P.S.E.B.M., 1965, v120.

## Intestinal Transport of D-Xylose.*† (30548)

T. Z. CSÁKY AND P. M. HO

*Department of Pharmacology, University of Kentucky College of Medicine, Lexington*

The general concept of the intestinal absorption of D-Xylose has recently undergone considerable change. It was recognized early that the rate of xylose absorption is much lower than that of glucose or galactose, and that the transport of xylose was not influenced as much by metabolic inhibitors as that of the two hexoses. It was therefore assumed that, while the absorption of glucose and galactose proceeds "preferentially" involving the "vital" functions of the cell, xylose was taken up by simple passive diffusion(1).

It was shown that the intestinal transport of D-Xylose is carrier-mediated, and that this pentose shares a common carrier with glucose(2). Recently it was discovered that there is an active transport mechanism for intestinal transport of xylose although the capacity of the xylose pump is rather low (3,4).

The structure of the carrier (or better, carrier sites) on the plasma membrane is not known. However, one can obtain some information by studying the structural specificity of the substrates to be transported by the same or similar sites. This is best revealed when examining the mutual competition for a given carrier site between the various substrates of related structure. Since the transport of xylose is carrier-mediated, and since xylose itself does not readily undergo metabolic changes during the transport process, it was tempting to undertake such an investigation using xylose in competition with other simple sugars and sugar-alcohols.

* Aided by U.S.P.H.S. Research Grants.

† The preliminary experiments of this work were carried out at the University of North Carolina by one of us (T.Z.C.) in collaboration with Dr. Lawrence Zollicoffer.

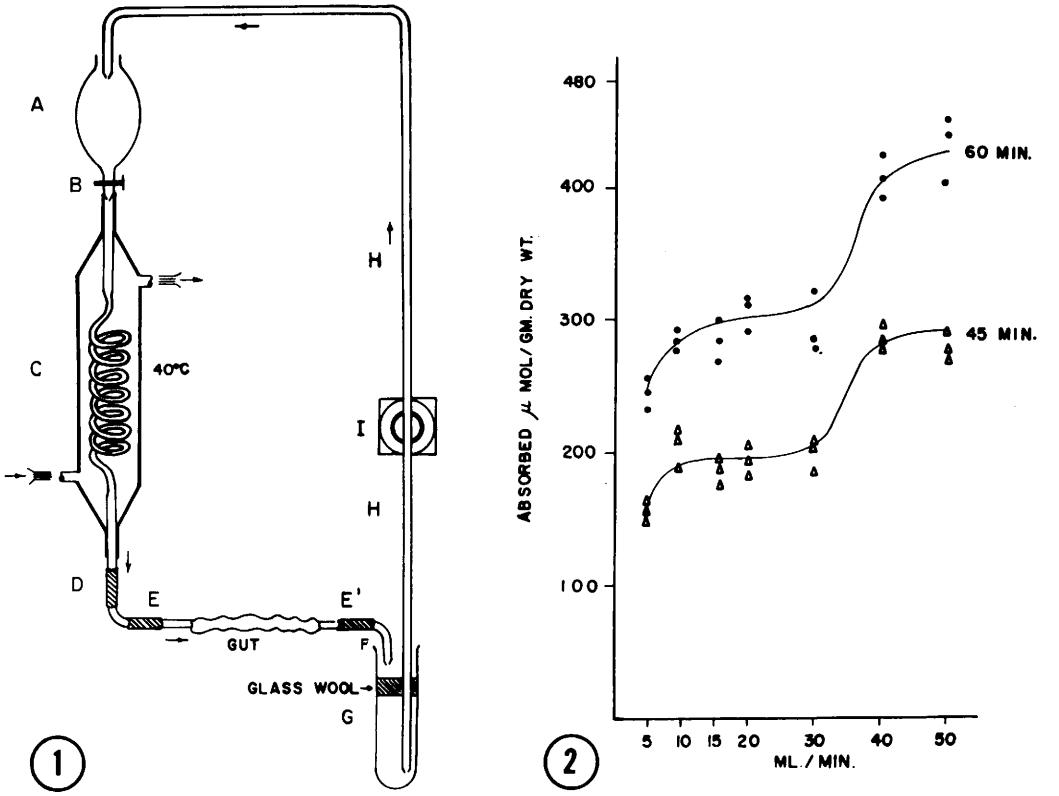


FIG. 1. Apparatus used in Method I. For explanation see text.
FIG. 2. Effect of rate of perfusion on absorption of glucose (2.8 mMol in isosmotic $Na_2SO_4$) from upper jejunum of the rat. Method I.

The experiments described here can be divided into two parts: (1) Intestinal absorption of glucose and xylose was compared by using 2 different *in vivo* techniques, and, (2) Rate of transport of xylose was quantitated in the absence and in the presence of a number of possible substrate competitors.

*Materials and methods.* Uniformly $C^{14}$-labeled D-Xylose was determined in a liquid scintillation spectrometer. The scintillation fluid consisted of 3 mg 2-5-Diphenyloxazole, 75 μg 1,4 bis-2(5-Phenyloxazolyl)-Benzene, 4.5 ml methanol + 7.5 ml toluene. 0.1-0.2 ml of xylose solution in water was introduced to this fluid.

Inert D-Xylose, D-glucose, D-fructose, D-arabinose, mannitol, xylitol, and phlorizin were commercial preparations of high purity. 3-0-Methylglucose (3-m glucose) and 3-0-methylfructose (3-m fructose) were gifts from Ayerst, McKenna and Harrison Ltd., Montreal, Canada. D-glucose was determined by the glucostat method(5); 3-m glucose by the Nelson copper reduction technique(6).

*Animal experiments.* The intestinal transport was studied *in vivo* in urethan-anesthetized (1.5 g/kg) adult white rats. The upper small intestine was perfused *in situ* with appropriate substrate solution using 2 different methods. Because the results obtained are strictly dependent upon the method used, the two methods will be described in detail.

*Method I:* The abdomen was opened, and a 10-12 cm segment of the appropriate anatomical region of the gut was exposed. Both ends of this segment were cannulated with glass cannulae and connected to the perfusion apparatus illustrated in Fig. 1. The apparatus consists of a reservoir (A) equipped with a valve (B) through which the rate of flow can be regulated (a separatory funnel can serve for such a purpose). The liquid flows by gravity through a condenser (C). Water from a 40°C bath is circulated through the

jacket of the condenser. Through a short polyethylene tube (D) the first glass cannula (E) is connected to (C). The gut segment is fastened between cannulae (E) and (E′). From (E′) the liquid flows through a polyethylene tube (F) into a large test tube (G). A plug of glass-wool serves as a filter to catch any debris passing from the gut. From (G) a polyethylene tube (H) carries the fluid through a finger tip motor (I) back into the reservoir (A). Depending upon the size of the reservoir, the reflux condenser, and the length of the tubing, the apparatus can accommodate any volume from 25 ml up.

The material from which the flexible tubing was made was not unimportant. According to our experience some tubing, after a period of use, may give off substances which interfere with various analytical assay processes such as the determination of glucose by glucose oxidase. For this reason a "blank run" should be made from time to time with the perfusion fluid recirculated through the system, but without the loop of gut.

Adequate mixing is of paramount importance in all flux experiments. It was expected therefore that the rate of perfusion, which influences the mixing, would modify the rate of absorption. To obtain quantitative data experiments were performed in anesthetized rats. A segment of the upper jejunum was perfused with 100 ml of isosmotic $Na_2SO_4$ solution in which 280 $\mu$M of glucose was dissolved. After perfusing periods of 45 and 60 minutes, respectively, the solution was analyzed for glucose. The disappearance of the sugar was calculated as absorption/gram of dry weight (d.w.) of the perfused intestinal loop. (Because of the great difficulty encountered in assessing the surface of the intestine, it is customary to relate the rate of absorption to the d.w. of the perfused gut whereby the necessary arbitrary assumption is made that in any given anatomical area the surface of the gut lumen is proportional with the d.w.) Fig. 2 shows the combined results of several experiments. There was an increase in rate of absorption when the perfusion rate was increased from 5 to 10 ml per minute. Between 10 and 30 ml/minute

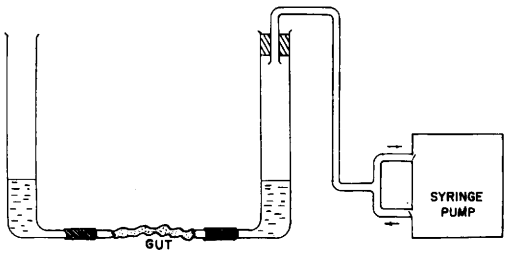


FIG. 3. Apparatus used in Method II. For explanation see text.

the increase in perfusion rate did not alter the rate of absorption, whereas upon further increase in perfusion rate there was a marked increment in rate of absorption. In the present experiments a rate of 20 ml/minute was chosen as a standard rate; this lies in the middle of the plateau (Fig. 2) and slight changes in either direction do not influence the rate of absorption.

*Method II:* A loop of intestine of the urethan-anesthetized animal was cannulated as in *Method I.* Each cannula was connected through a short polyethylene tubing to a small reservoir (about 15 ml) illustrated on Fig. 3. One of these reservoirs was connected to a pipetting machine and through this the liquid in the system was agitated by a 4 ml air displacement at a rate of 24 oscillations per minute (a Brewer Automatic Pipetting Machine, Model #120 by Baltimore Biological Laboratories was used but any other similar system can be utilized successfully). At this air displacement a 6 ml total volume is the minimum requirement, and 10 ml is the maximum. The rate of absorption (checked with the influx of 3-m glucose dissolved in isosmotic $Na_2SO_4$) was identical with both 6 ml and 10 ml total volumes. Higher volumes require higher air displacement for adequate mixing: *e.g.,* at a volume of 15 ml the optimum displacement was found to be 6 ml. When the volume is varied, air displacement and rate of absorption also vary (see Fig. 4).

General remarks applying to both methods: To minimize net water absorption, the basic perfusion fluid was an isosmotic $Na_2SO_4$. (In studies where the Na ion is to be replaced, other cations combined with sulfate can be used.) In numerous experiments in which

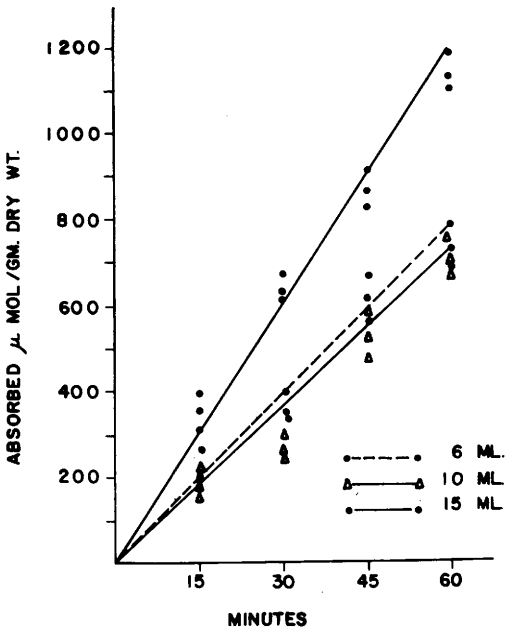


FIG. 4. Effect of volume on rate of absorption of 3-0-methylglucose (51.5 mMol in isosmotic $Na_2SO_4$) from upper jejunum of the rat. Method II.

the small intestine of rats was perfused with 100 ml isosmotic $Na_2SO_4$, after one hour the total volume recovered was 100 ± 2 ml. Evaporation should be avoided by covering all "open" places (A and G) with aluminum foil or thin layers of paraffin (Parafilm). Bleeding from the intestine can usually be avoided if the intestinal cannulae are coated with a thin layer of paraffin. Stubborn bleeding can be checked by adding thrombin (10 units per ml) to the perfusion fluid.

*Results.* 1. *Comparative study of intestinal transport of 3-0-methylglucose and of xylose using methods I and II.* The perfusing fluid was isosmotic $Na_2SO_4$ containing 50 mg% of either 3-m glucose or xylose. With *Method I,* the lumen to blood flux of 3-m glucose can be followed rather accurately. On the other hand practically no absorption of xylose can be demonstrated using this method. Fig. 5a demonstrates the comparative results obtained with the two sugars. The total volume of the perfusate in these experiments was 50 ml, subsequently 30 ml perfusates were used with D-Xylose, but the results were very similar to the one shown on Fig. 5a.

Unlike the results obtained with *Method I,* excellent lumen to blood flux of D-Xylose could be demonstrated with *Method II* using a total of 10 milliliter xylose solution (Fig. 5b).

2. *Inhibition of intestinal transport of D-Xylose by various sugars and sugar alcohols.* In these experiments 50 mg% of $C^{14}$-labeled xylose were dissolved either in isosmotic sodium sulfate alone or in the presence

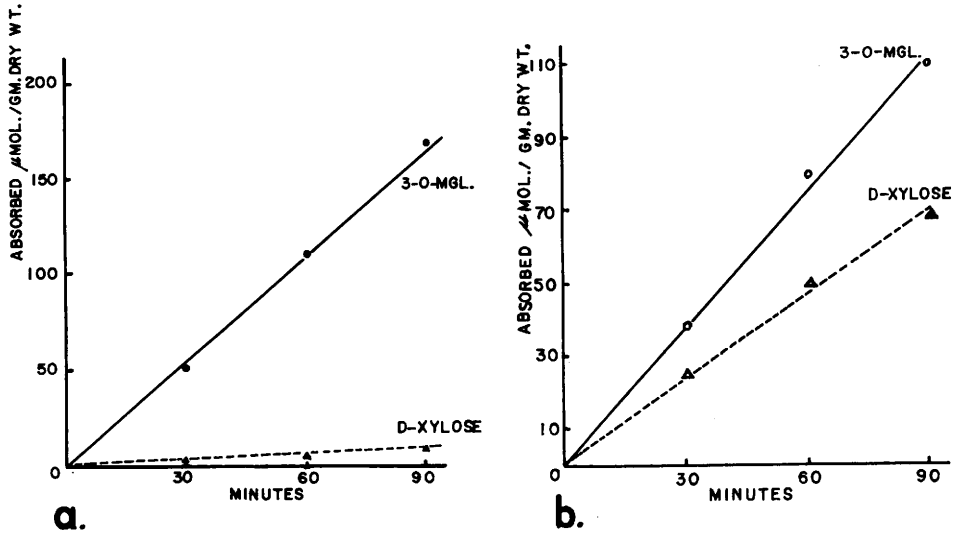


FIG. 5 a. Comparison between absorption of 3-m glucose (2.58 mM) and of D-Xylose (3.33 mM). Method I, perfusion with 50 ml isosmotic $Na_2SO_4$. b. Same, using Method II, perfusion with 10 ml isosmotic $Na_2SO_4$.

TABLE I. Inhibition of Intestinal Absorption of D-Xylose (3.33 mM Initial Concentration) by the Presence of Various Substances in the Perfusion Fluid. Values represent: μMol xylose absorbed/g dry gut/hr. Method II. 10 ml perfusion fluid was made isosmotic with $Na_2SO_4$. Control contained $Na_2SO_4$ alone.

| | Control | Mannitol 148 mM | Arabinose 148 mM | Xylitol 148 mM | Fructose 148 mM | 3-m Fructose 148 mM | Glucose 148 mM | 3-m Glucose 148 mM | Phlorizin $10^{-1}$ mM |
|---|---|---|---|---|---|---|---|---|---|
| | 52 | 70 | 39 | 23 | 25 | 27 | 25 | 21 | 7.2 |
| | 48 | 49 | 38 | 25 | 25 | 28 | 13 | 16 | 14.2 |
| | 46 | 55 | 26 | 25 | 21 | 27 | 14 | 17 | 23.6 |
| | 43 | 54 | | 38 | | 17 | 16 | | |
| | 57 | | | | | | | | |
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| | 69 | | | | | | | | |
| | 58 | | | | | | | | |
| | 57 | | | | | | | | |
| Avg | 57 | 57 | 34 | 28 | 24 | 25 | 17 | 16 | 15 |

of $10^{-4}$M phlorizin or alternatively in a 1:1 mixture of isosmotic sodium sulfate and isosmotic solutions of either mannitol, D-glucose, 3-m glucose, fructose, 3-m fructose, xylitol, or arabinose. The transport of D-xylose was followed by a decrease of radioactivity in the lumen. Table I summarizes the results of the experiments. It should be kept in mind that in these experiments, the inhibitor is applied in great excess to the xylose. Mannitol apparently does not inhibit intestinal absorption of xylose. The other agents inhibit significantly, ranging from 75% with phlorizin to about 40% with arabinose. The following is the scale of the inhibition in decreasing order: phlorizin > glucose > 3-m glucose > fructose > 3-m fructose > xylitol > arabinose.

*Discussion.* The description of the methods employed in the first part of this study needs no discussion. It should be pointed out, however, that when small perfusion volumes are used, as in *Method II,* particular attention should be paid to rigorous maintenance of a constant volume of the perfusing fluid as well as to the rate of agitation. This is clearly illustrated in Fig. 4.

In the concentration reported in Fig. 5a and b (50 mg%) 3-m glucose is absorbed primarily by active transport whereas xylose passes primarily by facilitated diffusion aided by some active transport. Because of this difference in the case of xylose the local concentration gradient between the outside surface of the brush border and the intracellular space may be more critical than in the case of 3-m glucose. Consequently, with the latter sugar maximum absorption could be achieved regardless of the volume of the perfusate whereas with the pentose smaller perfusion volumes, well agitated, may cause a more efficient local mixing resulting in an increase of the rate of absorption. This may be the explanation why *Method II* is more suitable for the study of xylose absorption as indicated in Fig. 5a and b. Also this is likely the reason why the concept of the intestinal transport of xylose was until recently incorrect.

Furthermore the present experiments point to the importance of the selection of the proper method when studying the intestinal transport of various substrates. It could be expected that such an approach may be fruitful in gaining a more accurate insight into the processes involved.

In the second part of the present work, intestinal transport of D-Xylose was studied in the presence of $10^{-4}$M phlorizin and of large amounts of various sugars and sugar alcohols. The results indicate that with the exception of mannitol, the diverse group of aldohexoses, aldopentose, ketohexoses, and a pentose-alcohol, all inhibited markedly the transport of xylose. Based on numerous previous studies which used a similar approach, it is reasonable to assume that this inhibition is due to a partial competition between xylose and the sugar of alcohols for the transport receptor sites on the membrane.

The present results emphasize the difficulty in assessing a definite structure for a molecule

to qualify as a substrate for the xylose carrier. Apparently the hexose and pentose structures can share the carrier. The aldehyde structure is not an absolute necessity as xylitol inhibits the xylose transport the same way as arabinose. It is also interesting that ketose sugars may partially share the transport mechanism with xylose. The present results can be more easily explained by assuming a multiple attachment between the substrate and the carrier sites rather than assuming a rigid structural requirement for the carrier transport. Such a flexible concept will allow the assumption of a partial attachment of various structural groups within a given molecule. This would result in a wide variety of "affinities" and would allow a broad spectrum in the rate of mutual inhibition.

*Summary.* The intestinal transport of D-Xylose was studied in the rat using 2 different methods of *in situ* perfusion. The absorption of xylose is inhibited by the presence in the perfusate of any of the following substances (listed in decreasing order of their inhibitory ability): phlorizin > glucose > 3-0-methylglucose > fructose > 3-0-methylfructose > xylitol > arabinose. The results can be explained by assuming a xylose carrier site of a rather complicated nature on the epithelial membrane.

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Received June 21, 1965. P.S.E.B.M., 1965, v120.

## Effect of Dietary Orotic Acid on Liver Glycogen.* (30549)

HERSCHEL SIDRANSKY, ETHEL VERNEY AND D. S. WAGLE
*Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, Pa.*

Rats fed a purified diet containing 1% orotic acid develop a fatty liver with a periportal distribution of lipid(1,2). Addition of adenine to the diet prevents the fatty liver (2). The hepatic lipid accumulation is more severe in female than in male rats(3).

We have recently observed that in the orotic acid-fed rat, there is also an increase in liver glycogen as well as in liver lipid. A similar parallelism between increased lipid and glycogen in the liver has been described in human cases of kwashiorkor(4) and of glycogen storage disease(5,6) and also in experimental studies such as with amino acid or protein deficiency(7,8) and following administration of cortisone(9,10). This paper describes our data related to the increased liver glycogen in rats force-fed for 3 days a purified diet containing 1% orotic acid. The increased hepatic glycogen, unlike the increased hepatic lipid, is not prevented by adenine.

*Materials and methods.* Female rats of the Wistar (Carworth Farms) strain weighing 135 to 185 g were used. The animals were maintained with a commercial chow (Wayne Lab-Blox) before beginning the special diets. All animals had free access to water. In all experiments, several groups of 3 to 5 rats each of the same age and weight were used. The animals were housed in individual wire cages in an air-conditioned room maintained at 25.5°C.

The basal diet was the same as that used in earlier studies(3,11) and was composed of 16% vitamin free casein, 5% corn oil, 4% salts, 5% vitamin-sucrose mixture and 70% sucrose. In the force-feeding experiments rats were tube-fed according to the method of Shay and Gruenstein(12) using plastic tubes.

* This investigation was supported by U.S.P.H.S. Research Grant AM-05908 from Nat. Inst. of Arthritis and Metab. Dis. and CA-07465 from Nat. Cancer Inst.