Support of Water Absorption by Rat Jejunum *in vitro* by Glucose in Serosal Fluid. (23278)

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The relationship between glucose and absorption of water by the rat small intestine *in vitro* exhibits some interesting and puzzling features. Water transport is negligible unless glucose is added to the bathing fluid, and no other substance has thus far been found which can adequately substitute for glucose in this respect(1,2,3). Possibly in the absence of added glucose, the mucosa has no substrate to provide the energy to sustain water transport; yet rat small intestinal mucosa has been reported to have a high endogenous respiration(4) and can transport at least methionine against a concentration gradient without added glucose(5). It has also been reported that to support water absorption in surviving rat intestine, the glucose must be supplied from the mucosal fluid; glucose added to the serosal medium (up to 2% in concentration) did not appreciably augment the water absorption occurring with glucose in the lumen or promote water absorption when mucosal medium is glucose-free(2). Yet *in vivo*, water absorption proceeds from glucose-free solutions placed in the intestine, and the normal route of entry of blood glucose into mucosal cells is presumably through their serosal poles. Failure of glucose in the serosal fluid to stimulate water-absorption in the surviving intestine has been taken to indicate that the non-mucosal tissues present a barrier to diffusion of glucose which cannot be demonstrably overcome even when the glucose concentration is more than 20 times that normally present in the body fluids. On the other hand, since glucose is absorbed by the surviving intestine from the mucosal to the serosal fluid, the diffusion barrier is certainly not absolute. Hence one would expect that by increasing sufficiently the concentration in the fluid bathing the serosa, the mucosa could be supplied with enough glucose to bring about water absorption. Otherwise, leaving secondary effects aside, one would be forced to consider some rather implausible explanations for the observations. It therefore appeared important to examine the relationship between water absorption and glucose concentration in the fluid bathing the serosa of the rat surviving intestine.

Methods. The procedure of Fisher & Parsons(6) was followed with minor modifications. Unfasted male albino rats 200-400 g were employed, and ether was the anesthetic. Jejunal loops ranging in length from 30-55 cm were used. Krebs bicarbonate-Ringer fluid was made up according to Umbreit *et al.* (7). The rinsing fluid was 155 mM NaCl. The initial volume of the mucosal fluid was 25 ml; that of the serosal fluid, 40 ml. Whenever the composition of the serosal medium was altered, an isosmotic solution of the new solute was substituted for an equivalent volume of isotonic NaCl, so that initially the fluids bathing both sides of the intestine were always isosmotic (approximately 300 mM per K of water). All fluids were gassed with 5% CO_2 in O_2 at 38° before use. The experiments were carried out at 38°C; and 5% CO_2 in O_2, saturated with water vapor at the same temperature, was employed for aerating and mixing the fluids. The absorption time was 1 hour and the distension pressure was about 20 cm of water. Water absorption was calculated from the sum of the changes in weight in the serosal fluid and the intestinal wall. Based on control experiments, a small correction was made for the combined effects of evaporation or condensation, incomplete drainage of the chamber, and excess fluid adhering to the loop. The final water content of the tissue was estimated by slitting the loop open longitudinally, thoroughly blotting it on filter paper and drying it to constant weight at 100°C. Initial water content was measured on a series of loops taken down at what would otherwise have been the beginning of the absorption period.

TABLE I. Effect of Increasing Glucose Concentration in Isosmotic Serosal Fluids on Water Absorption when Mucosal Fluid Contains Approximately 1/10 Isosmotic Glucose.

Glucose in serosal fluid, mg %	Approx. fraction of osmotic pressure contributed by glucose	No. of exp.	Water absorption, μl/cm/hr
0	0	4	122 ± 13
530	1/10	11	124 ± 4
2660	1/ 2	8	124 ± 10
4100	4/ 5	4	92 ± 2

Results. The data in Table I confirm the observations of Fisher(2) that provided the mucosal fluid contains adequate amounts of glucose (customarily an initial concentration of some 500-600 mg%), additional glucose up to ½ isosmotic concentration in the outer fluid does not appreciably affect water absorption. When the serosal fluid contained 4/5 isosmotic glucose, the water absorption was reduced from 124 ± 10 μl/cm/hr (standard error of the mean) to 92 ± 2 μl/cm/hr.

The effects of adding glucose to the serosal fluid when the mucosal fluid was glucose-free are shown in Fig. 1. With increasing glucose in the serosal fluid, the rate of water absorption increased approximately linearly from a slightly negative value (which is probably real and possibly due to secretion)* until the outer fluid contained ½ isosmotic glucose. At this point however, the average rate (81 μl/cm/hr) was still only 65% of that measured when glucose was present only in the mucosal fluid (124 μl/cm/hr), and no further augmentation in absorption occurred when the serosal glucose concentration was raised until all the NaCl in the Ringer's was replaced by glucose.

Water absorption did not occur when galactose, xylose, or mannitol was substituted for glucose in the serosal fluid (Table II). To this extent, the stimulation of water absorption was specific for glucose. Galactose and xylose can penetrate the mucosal cells, at least from the lumenal pole, since they can be absorbed(8,9), and galactose can probably be utilized to some extent(8). Mannitol is not appreciably absorbed from the rat intestine *in vivo*(10), and presumably would be confined to the extracellular spaces.

Additional observations. With 5% CO_2 in N_2 instead of 5% CO_2 in O_2 used as the aerating gas, and with ½ isosmotic glucose in the serosal fluid, water absorption was reduced by 80% (2 experiments). When bicarbonate was omitted from the mucosal fluid, significant water absorption did not occur (5 experiments; pH in lumen 4.85). Reducing the Ca in the Ringer's fluid by one-half (*i.e.* to 1.25 mM) had no effect on water absorption (3 experiments). Substitution of acetate or glycerol for glucose in both fluids did not stimulate water absorption (2 experiments each).

Discussion. Glucose supplied from the serosal fluid can apparently support water absorption from the surviving rat small intestine. The large concentration of glucose in the serosal bathing medium which is required to yield maximum water absorption is no doubt indicative of a high barrier either to diffusion of glucose from the serosa to the mucosal cells or to permeation into them, or both. To account for the finding that this maximum rate of water absorption is only about 65% of that observed when glucose is added to mucosal fluid, at least two explanations suggest themselves. The first is that, due to the diffusion barrier, it takes time for the establishment of an adequate glucose supply to the mucosa from the serosal fluid, so that even with large serosal fluid concentrations, the mucosa is operating with optimal glucose over only a fraction of the experimental period. Moreover, as soon as water absorption is initiated "solvent drag" would tend to oppose the glucose diffusion stream. On the other hand, with glucose in the lumen,

TABLE II. Effect of Isosmotic Outer Fluids Containing 1/2 Isosmotic Non-Electrolyte. Mucosal fluid glucose-free Krebs bicarbonate—Ringer.

Non-electrolyte in serosal fluid	No. of exp.	Water absorption, μl/cm/hr
Glucose	11	81 ± 6
Galactose	4	−1 ± 2
Xylose	4	−8 ± 1
Mannitol	4	−2 ± 2

* It is interesting in this connection that Wilson has recently reported water absorption by sacs of everted hamster jejunum incubated in sugar-free solutions(11).

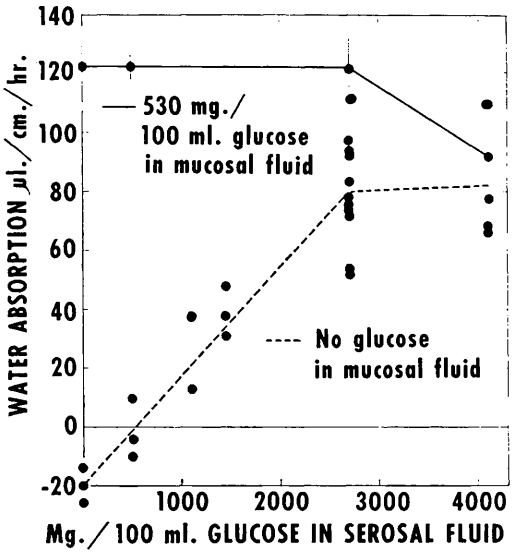

FIG. 1. Effect of increasing glucose conc. in serosal fluid on water absorption.

the substrate becomes immediately available to the mucosal cells.† Although increasing the serosal glucose level from ½ isosmotic to nearly 4/5 isosmotic fails to increase water transport further, this failure may be due to secondary deleterious effects associated with the extreme change in the composition of Ringer medium. Thus, with 4/5 isosmotic glucose in the serosal fluid, the rate of water absorption from glucose containing mucosal fluid is decreased, and there is no significant difference in water transport whether or not glucose is present in the mucosal fluid (Fig. 1).

While diffusion time must play a role, other factors might also be of importance. A second possible explanation for the observation under discussion is that absorption of glucose in some way carries water with it. It may be, in the limiting case, that the rate of isosmotic absorption of water and solutes other than glucose is the same whether glucose is provided from the mucosal or serosal fluids, but that with glucose present in the lumen, the absorbed glucose takes with it additional water to maintain osmotic equilibrium. An attempt was made to obtain evidence on whether such a factor was operating. With ½ isosmotic glucose in the serosal fluid 2 other absorbable sugars, galactose or 3-methyl glucose, were used in the mucosal fluid instead of glucose. With galactose the observed rate of H_2O absorption was 94 ± 8 µl/cm/hr, which is 13 µl/cm/hr greater than the value of 81 ± 6 µl/cm/hr found when the mucosal fluid was Ringer's; but the difference is not statistically significant. An average of 1.6 mg of galactose/cm/hr was absorbed which could have accounted for $\frac{1.6}{54} \times 1000 = 30$ µl of isosmotic H_2O/cm/hr. Essentially similar findings were obtained with 3-methyl glucose. The results were thus no more than suggestive.

Summary. Glucose in the Ringer's fluid bathing the serosal surface supports water absorption by isolated jejunal loops of rat intestine. The rate of water absorption increased with increasing glucose concentration up to ½ isosmotic. This maximum rate was about 65% of that found when 1/10 isosmotic glucose was present in the Ringer's fluid bathing the mucosa.

† The endogenous carbohydrate stores of mucosal cells appear to be very small. Total glycogen content of 4 typical jejunal loops excised and taken immediately for analysis(12) ranged from 0.6 to 2.2 mg.

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