Stimulation of the Water Transport in the Jejunum of the Rat by Ethyl Acetate (35236)

T. Z. Csáky,[1] G. Esposito, A. Faelli, and V. Capraro
Istituto di Fisiologia Generale, Università di Milano, Milano, Italy

The transport of water across epithelial layers is coupled with the transport of solutes (1–3). If an isosmotic solution is placed into the lumen of the intestine, net water transport or water absorption occurs as a consequence of a net solute transport. The solute primarily involved in the transport of water is the sodium ion which is actively transported into the intercellular spaces, causing these to become hyperosmotic; consequently, water is drawn osmotically into these spaces and then transferred into the serosal compartment or into the circulation. Such "local osmosis" has been demonstrated in various epithelial cells (4) and recently also in the rectal pads of insects (5).

The sodium transport, which is responsible for most of the net water transport, is an active energy-requiring process. *In vivo* measurements in human indicated that the ileum is capable of net water absorption against some 10 times higher salt concentration gradient than the jejunum (6). A less striking topographic difference was recorded in the rat intestine *in vitro* (7).

In the absence of a source of energy, the rat small intestine suspended in an isosmotic medium *in vitro* transports little water. On the other hand, if glucose is added to the medium the sodium and water transport is greatly stimulated (8). Surprisingly, in the rat jejunum, intermediates of glucose metabolism, such as pyruvate or acetate, cannot stimulate the sodium and water absorption (9).

These findings spurred speculation whether the metabolism of glucose in the intestinal epithelium is perhaps different from that in other tissues. Alternatively, it could be assumed that the pyruvate or acetate ions, because they are mostly ionized at the pH of the tissues, cannot enter the epithelial cells, while glucose passes readily across the cell membrane with the help of the specific carrier. We report below the results of experiments in which the latter possibility was explored for acetate.

The experiments were based on the following consideration: if acetate could be converted by esterification to a lipid soluble product, it would enter the epithelial cell in such form. The intracellular esterase will then split the acetate ester, releasing the free acetate. Ethyl acetate was chosen for testing this hypothesis because it is easily obtainable commercially in a high degree of purity, it is non-ionized and fat-soluble. Therefore, we examined whether ethyl acetate can stimulate the water transport in the rat jejunum *in vitro*.

Sprague-Dawley male rats, weighing approximately 180-200 g and fasted for 16 hr, were killed by a blow to the head. The jejunum was rapidly removed, immersed in an oxygenated, 30° temperature Krebs–Ringer bicarbonate solution, and everted. One end of the everted gut was mounted on a cannula while the other end was closed. Two ml of Krebs–Ringer bicarbonate was placed into the serosal sac and the entire gut was then immersed into a Krebs–Ringer bicarbonate solution, gassed with 95% O_2, 5% CO_2, and kept at 30°. From time to time, the intestinal loop was removed, gently blotted with filter paper, and weighed on an analytical balance. From the increase of the weight, the up-take of fluid was calculated. Experiments were

[1] Visiting Professor 1968/69. Permanent address: Department of Pharmacology, University of Kentucky, College of Medicine, Lexington, Kentucky 40506.

TABLE I. Fluid Uptake (mg/g of Dry Tissue) of Intestinal Loops Incubated with or without Ethyl Acetate or Glucose, Respectively.

	Incubation period (min)			
	15	30	45	60
Control[a] $n = 9$	981 ± 169	1483 ± 265	1825 ± 273	2016 ± 293
+ Ethyl acetate, 28 m*M* $n = 8$	1724 ± 270	2613 ± 343	3000 ± 505	3225 ± 593
+ Glucose, 14 m*M* $n = 8$	2291 ± 326	2749 ± 407	3484 ± 552	3957 ± 663

[a] Basic medium: Krebs–Ringer bicarbonate solution; n = number of experiments; values are means ± SEM.

carried out without any substrate and with 14 m*M* glucose or with 28 m*M* ethyl acetate, respectively. The substrates were placed in both the serosal and the mucosal medium.

The results reproduced in Table I show that glucose markedly enhanced the weight increase, thus the fluid uptake, of the intestinal loops. Ethyl acetate, in approximately equimolecular concentration (1 mole of glucose ≅ 2 moles of acetate), stimulates about the same extent as glucose.

The experiments reported in Table I were also performed in a sodium-free Krebs–Ringer bicarbonate medium. The latter was prepared by substituting Tris-chloride for NaCl, and $KHCO_3$ for $NaHCO_3$. The results, presented in Table II, indicate that the unstimulated water transport was very much reduced in the sodium-free medium; moreover, neither glucose nor ethyl acetate produced any significant change in the low rate of water transport. These findings provide strong circumstantial evidence that the net water transport, and particularly its stimulation, is closely related to the transport of sodium.

In order to ascertain whether ethyl acetate exerted its stimulatory effect through the release of acetate by the intestinal esterase, the experiments were repeated in the presence of a known esterase-inhibitor, tetraethyl pyrophosphate (TEPP). Table III shows that TEPP itself did not influence the water transport in the controls; neither did it inhibit the stimulation of the water transport by glucose. However, the inhibitor completely eliminated the stimulatory effect of ethyl acetate on the intestinal water transport. Ethyl alcohol, when examined in the same molecular concentration, did not affect the water transport; thus it has to be concluded that it was the acetate ion which was released intracellularly from the ethyl acetate and which stimu-

TABLE II. Similar Experiments as in Table I but in the Absence of Sodium.

	Incubation period (min)			
	15	30	45	60
Control[a] $n = 4$	397 ± 68	334 ± 103	265 ± 99	292 ± 101
+ Ethyl acetate, 28 m*M* $n = 4$	513 ± 119	512 ± 73	518 ± 118	434 ± 112
+ Glucose, 14 m*M* $n = 4$	376 ± 98	332 ± 69	224 ± 91	147 ± 87

[a] Basic medium: Modified Krebs–Ringer bicarbonate (NaCl replaced by Tris Cl; $NaHCO_3$ replaced by $KHCO_3$); n = number of experiments; values are means ± SEM.

TABLE III. Similar Experiments as in Table I but in the Presence of an Esterase-Inhibitor (TEPP).

	Incubation period (min)			
	15	30	45	60
Control[a] $n = 4$	1210 ± 234	1940 ± 242	2167 ± 256	2319 ± 314
+ Ethyl acetate, 28 mM $n = 8$	1006 ± 118	1455 ± 205	1516 ± 205	1615 ± 200
+ Glucose, 14 mM $n = 8$	2046 ± 208	3146 ± 255	3726 ± 334	4155 ± 293

[a] Basic medium: Krebs–Ringer bicarbonate solution + tetraethyl pyrophosphate (TEPP), 10 mM; n = number of experiments; values are means ± SEM.

lated the water transport quantitatively to about the same degree as glucose.

It is therefore reasonable to assume that glucose stimulates the sodium and water transport in the jejunum of the rat through the energy released in the course of aerobic metabolism. Intermediary products of this metabolism can equally stimulate when produced intracellularly or when administered from the outside in such a form that they can readily reach the intracellular space.

This work was supported by grants from the U.S. Public Health Service and the Consiglio Nazionale delle Ricerche. One of us (T. Z. C.) thanks the U.S. Public Health Service for a special senior research fellowship.

1. Curran, P. F., and Solomon, A. K., J. Gen. Physiol. **41**, 143 (1957).

2. Diamond, J. M., J. Gen. Physiol. **48**, 1 (1964).

3. Parsons, D. S., Proc. Nutr. Soc. **26**, 46 (1967).

4. Diamond, J. M., J. Gen. Physiol. **48**, 15 (1964).

5. Wall, B. J., Oschman, J. L., and Schmidt-Nielsen, B., Science **167**, 1497 (1970).

6. Fordtran, J. S., Rector, F. C., and Carter, N. W., J. Clin. Invest. **47**, 884 (1968).

7. Parsons, D. S., and Wingate, D. L., Biochim. Biophys. Acta **46**, 170 (1961)

8. Lifson, N., and Parsons, D. S., Proc. Soc. Exp. Biol. Med. **95**, 532 (1957).

9. Parsons, D. S., Brit. Med. Bull. **23**, 252 (1967).

Received Sept. 11, 1970. P.S.E.B.M., 1971, Vol. 136.