Effect of Sodium on Passive Permeability of Non-Electrolytes Through the Intestinal Wall[1]

We wanted to investigate whether, besides the well-known specific effect of sodium on the intestinal transport of sugars and aminoacids[2–7], there is another unspecific effect on the resistance of the intestinal barrier to the passage of hydrosolubles, non-metabolizables and passively diffusing substances such as thiourea and acetamide.

Sprague-Dawley albino male rats, semistarved over a period of 15 days, were used[8,9]. Everted jejunal sacs were incubated for 1 h ($^1/_2$ h of preincubation and $^1/_2$ h of experiment) at 28 °C in a Krebs-Henseleit solution added with glucose 13.9 mM and equilibrated with a gas mixture of 95% O_2 and 5% CO_2. This is regarded as a basic perfusion fluid. 40 ml of this basic perfusion solution added, according to the experiments, with acetamide (mol. wt. 59.07) or thiourea (mol. wt. 76.12) at a concentration of 10 or 20 mM, were used as a mucosal perfusion fluid.

Two millilitres of the same solution, added with trace amount of ^{14}C labelled compound and of tritiated inulin, were introduced into the everted sac; this is regarded as a serosal perfusion fluid.

The ^{14}C and ^3H radioactivities were measured by mean of a liquid scintillation spectrometer and the serosal glucose concentration by an enzymatic method[10]. From these measurements, the transported glucose (μmoles/g of dry weight/h), the transmural serosa-mucosa fluxes ($\dot{n}$) (mmoles/g dry weight/h) of the labelled substances and their contemporaneous mean concentration gradient (ΔC), were calculated.

Knowing the flux ($\dot{n}$) and supposing a constant volume of the system spaces, ω (that is the mobility of the solute) was calculated according to the equation:

$$\dot{n} = \omega \, RT \, \Delta C \, .$$

However, the spaces of the system are not constant because of the contemporary fluid flux from the mucosal to the serosal side, so that a drag effect must be taken into account. In order to evaluate approximately a maximal theoretical drag effect we have assumed a reflection coefficient (σ) equal to zero, so that the above equation becomes:

$$\dot{n} + \dot{v} \, (1 - \sigma) \, \bar{c} = \omega \, RT \, \Delta C$$

where $\bar{c}$ is a mean of the concentration of the solute in the 2 compartments[11,12].

Besides control experiments with the previously described incubating fluids, other experiments were performed on the same group of animals in which the sodium chloride of the incubating medium was substituted with choline-HCl or tris-HCl (the only sodium present in the incubating fluid is that due to the bicarbonate which is 25 mM). In order to evaluate the metabolic activity of the intestinal sac in the different perfusion fluids (i.e. sodium chloride replaced with choline or tris) we carried out another set of experiments by using a polarographic method to determine the oxygen consumption and an enzymatic method to determine the lactic acid production[13,14].

The following 2 tables show that a decrease in sodium concentration of the perfusion fluid not only reduces the net active transport of glucose from the mucosal to the serosal side, but also seems to reduce the passive mobility of molecules such as acetamide and thiourea.

It must be pointed out here that in the semistarved rat intestine the basic oxygen consumption does not vary throughout the experimental period[9]. Furthermore, in the absence of sodium chloride in the perfusion fluid, the basic oxygen consumption and the lactic acid production are not substantially modified. Therefore neither basic metabolism nor pH modification can explain the lowering of the passive permeability of the intestinal barrier. As far as the decrease of permeability of the passively diffusing substances is concerned, a possible expla-

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Table I

Perfusion fluid	No. of experiments	Net glucose transport (μmoles g^{-1} h^{-1})	ω of acetamide (mmoles g^{-1} h^{-1} Atm^{-1}) in the absence of drag flow	ω of acetamide (mmoles g^{-1} h^{-1} Atm^{-1}) in the presence of a maximal theoretical drag flow
Krebs and Henseleit added with glucose 13.9 mM and acetamide 10 or 20 mM	8	210.8 ± 40.8	0.80 ± 0.06	0.86 ± 0.06
Same fluid NaCl substituted with Tris-HCl	6	113.1 ± 31.4	0.58 ± 0.06	0.60 ± 0.06
Same fluid NaCl substituted with Choline-HCl	6	113.3 ± 34.3	0.60 ± 0.04	0.61 ± 0.03

Acetamide, mol. wt. 59.07; semistarved rats, average percent weight decrease 25.7 ± 1.1%.

Table II

Perfusion fluid	No. of experiments	Net glucose transport (μmoles g^{-1} h^{-1})	ω of thiourea (mmoles g^{-1} h^{-1} Atm^{-1}) in the absence of drag flow	ω of thiourea (mmoles g^{-1} h^{-1} Atm^{-1}) in the presence of a maximal theoretical drag flow
Krebs and Henseleit added with glucose 13.9 mM and thiourea 10 or 20 mM	9	276.9 $\pm$ 37.4	0.56 $\pm$ 0.03	0.61 $\pm$ 0.04
Same fluid NaCl substituted with Tris-HCl	6	164.8 $\pm$ 45.1	0.42 $\pm$ 0.05	0.43 $\pm$ 0.05
Same fluid NaCl substituted with Choline-HCl	8	149.2 $\pm$ 34.6	0.42 $\pm$ 0.02	0.44 $\pm$ 0.02

Thiourea, mol. wt. 76.12; semistarved rats, average percent weight decrease 24.2 $\pm$ 2.0%.

nation is that sodium choline and tris affect, directly unspecifically and in a different way the physico-chemical properties of the cell membrane. Another possible explanation of our data is that of an indirect effect of the decreased Na^+ concentration on the membrane permeability. We have previously demonstrated that glucose transport depends on the intracellular concentration of glucose, i.e. the higher the intracellular concentration of glucose, with a consequent swelling of the cell, the more the glucose transport[15]. In the absence of sodium chloride, the intracellular accumulation of glucose is lower as well as the swelling of the cell. Now the hypothesis may be put forward that the degree of swelling is parallel with the permeability of the cellular membrane.

Zusammenfassung. Der Einfluss des Natriums auf die passive Permeabilität der Jejunum-Schleimhaut der Laboratoriumsratte gegenüber wasserlöslichen, nichtmetabolisierbaren und elektroneutralen Substanzen (Thioharnstoff, Azetamid) wurde untersucht. Wird das Natrium des NaCl in der Perfusionsflüssigkeit durch Tris- oder Cholin-Kationen ersetzt, so nehmen sowohl der transepitheliale Glukosetransport wie auch die Mobilität der geprüften Substanzen ab.

G. Esposito, A. Faelli and V. Capraro

Istituto di Fisiologia Generale dell'Università, Milano (Italy), 17 January 1969.

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Muscle Spindle Innervation in the Intertransverse Caudal Muscles of the Rat

The intertransverse caudal muscles in rat are interesting physiologically because β-axon excitation of the muscle spindles they contain results in an unusually intense and prolonged response from primary endings[1]. β-axons are motor to both intra- and extra-fusal muscle fibres, and their conduction velocities are intermediate to α-(purely skeletomotor), and γ-(purely fusimotor)axons.

These muscles are also useful experimentally since their conformation permits intramuscular structures to be studied with relative ease[2,3]. The histological work described here was done with the aim of explaining previous findings and of extending the usefulness of the preparation.

This paper has a precedent in the study of rat lumbrical muscles recently published in this journal by Porayko and Smith[4], who justifiably state that there has been relatively little work on rat muscle spindles as compared with those of cat. This is particularly true of rat fusimotor innervation.

Method. The intertransverse muscles of adult albino rats were stained with methylene blue (Boyd[5]), and gold chloride (Boyd[6]), but most of the work was done on preparations stained with silver (Ip and Barker[7]). Some muscles were stained for anticholinesterase activity, using acetyl thiocholine and butyryl thiocholine as substrates[8], and were subsequently stained with silver (Ip[9]). Nerve branches to the muscle were stained with buffered osmium tetroxide, sectioned and the component axon

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