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The concentration of D-glucose in the enterocyte and in the fluid absorbed by rat jejunum "in vitro"

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Fisiologia. — *The concentration of D-glucose in the enterocyte and in the fluid absorbed by rat jejunum "in vitro"* (*). Nota di VITTORIO CAPRARO, GIOVANNI ESPOSITO, ALIDE FAELLI, NICOLETTA PACCES e MARISA TOSCO, presentata (**) dal Corrisp. V. CAPRARO.

RIASSUNTO. — Usando una preparazione di digiuno rovesciato di ratto in cui il sacchetto intestinale è inizialmente vuoto, è possibile determinare la quantità e la concentrazione di D–glucosio e di sodio nel liquido assorbito. Una correlazione positiva tra trasporto netto transepiteliale di sodio e di D–glucosio viene confermata anche in queste condizioni. La concentrazione media di D–glucosio nelle cellule non sembra correlata con il trasporto netto dello zucchero, e rimane pressochè costante qualunque sia il suo trasporto transepiteliale. Queste osservazioni vengono discusse e possono suggerire o l'esistenza di un meccanismo metabolico–dipendente di estrusione del glucosio dalla membrana basolaterale o alternativamente una mobilità del glucosio attraverso la stessa membrana correlata direttamente o indirettamente e positivamente con la estrusione attiva di sodio.

INTRODUCTION

It has been demonstrated that in rat jejunum " in vivo " some monosaccharides (D-glucose, 3-O-methyl-D-glucose) are apparently absorbed against a cell to blood concentration gradient [1, 2]. The apparent sugar concentration (obtained by dividing net sugar by net fluid transport) in the " absorbed fluid " (what Diamond and Bossert [3] name the solute concentration in the " emerging fluid " from the intercellular channels) is even higher than that found in the blood, but such values will be affected by diffusional and convective components occurring within the tissue. The magnitude of these effects, which can be predicted using suitable mathematical models [4], will reduce, but not eliminate, the concentration difference between intercellular fluid and blood. The aim of the present paper is to present experimental data on D-glucose concentration in the fluid from rat jejunum "in vitro" and to relate these data to the contemporaneous sugar concentration in the cell water of the absorbing enterocyte.

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(**) Nella seduta del 12 aprile 1980.

METHODS

Male albino rats (Wistar strain, Charles River Italiana) weighing 200–280 g were used. The jejunum was isolated, everted and incubated following the procedure commonly used in this laboratory and reported in detail elsewhere [5]. Briefly, under barbituric narcosis the jejunum was isolated, washed and wiped on the serosal side and everted in a Krebs-Ringer-bicarbonate solution at 28 °C. The intestine was then fixed at one end with a thin polyethylene tube and cannulated at the other end with a glass cannula and immersed in 50 ml Krebs-Ringer-bicarbonate solution, containing 5.56 mM glucose

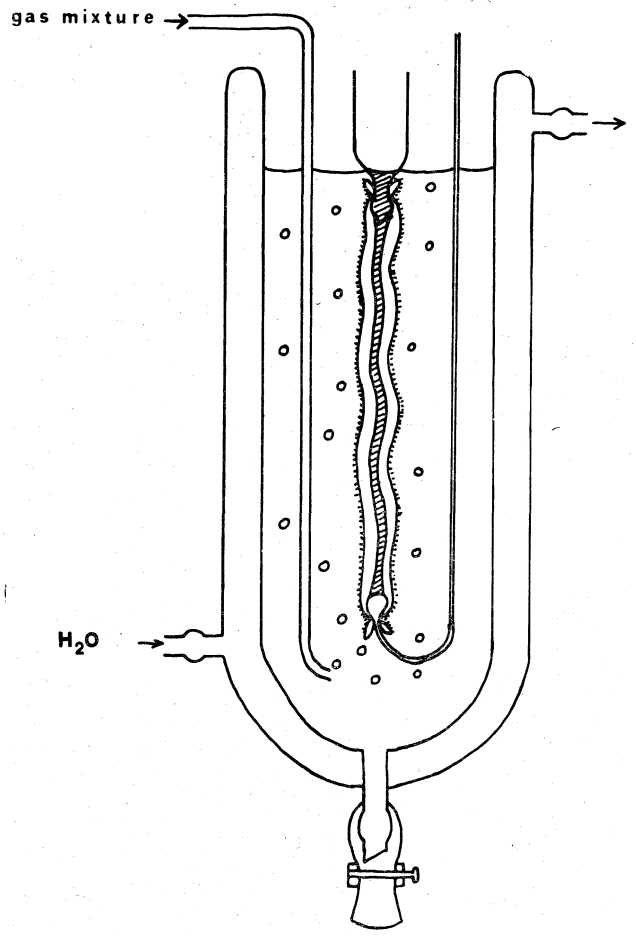


Fig. 1. – Scheme of the equipment used in the present experiments. The everted jejunum of rat is tied at the lower end to a thin polyethylene tube whereas the upper part is fixed to a glass cannula. The hatching represents the fluid collected in the serosal compartment at the end of the experiment. The drawing also shows the water inlet and outlet which keeps the temperature constant at 28 °C by circulating through a water jacket. A gas mixture is bubbled into the mucosal compartment via another polyethylene tube.

gassed with 95 % O_2 and 5 % CO_2 and kept at 28 °C throughout the experiment which lasted 60 min (Fig. 1). The serosal side was initially kept empty; from time to time the fluid emerging in the serosal compartment was mixed by gently raising and lowering the thin tubing. At the end of the experiment all the serosal fluid was collected in a preweighed weighing-bottle by gently squeezing the everted gut, after a rapid blotting of the mucosal side on filter paper. After weighing the weighing-bottle, transport of fluid was calculated per gram dry weight of total intestine and per hour incubation. The mucosa was immediately scraped off at 0 °C, added to 3 mM monoiodoacetic acid in order to prevent glucose breakdown [6] and the intestinal cells were broken by osmotic shock and by subsequent freezing at — 20 °C and thawing [7, 8]. After centrifuging and deproteinizing, samples of the supernatant of the mucosal and serosal fluids were taken for the determination of glucose and Na concentration.

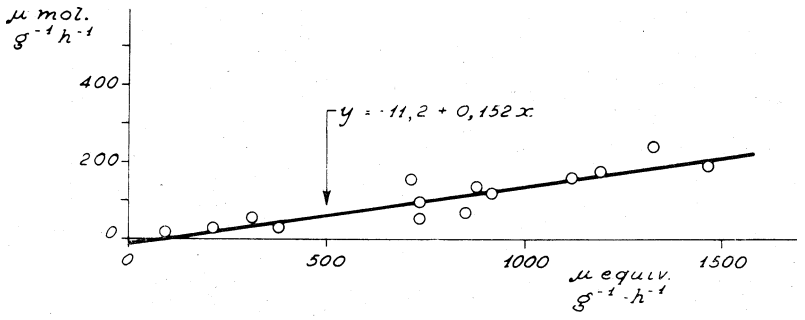
A very small loss of glucose due to glycolysis cannot be excluded, but in any case, when the intestinal sac is exposed to a constant mucosal glucose concentration, as in the present experiments, the glycolysis seems to be independent of sodium trasport [9]. Therefore, the possible absolute error is the same in every transport condition.

Glucose and sodium transport are measured in μmoles or μequiv/g dry weight of total intestine per hour. Cell glucose concetrantion is expressed in mmoles/l of cell water; the latter is obtained by subtracting from the total tissue water of scraped mucosa the extracellular water obtained in previous experiments by using ^{3}H-sucrose as an independent marker of serosal extracellular space and ^{3}H-polyethyleneglycol 900 as a mucosal extracellular marker. We could not use any serosal extracellular marker during the present experiments because the serosal compartment is initially empty. As reported elsewhere [10], the mucosal extracellular space is roughly one quarter of the serosal one determined with sucrose which seems in our hands to be one of the best extracellular serosal space markers.

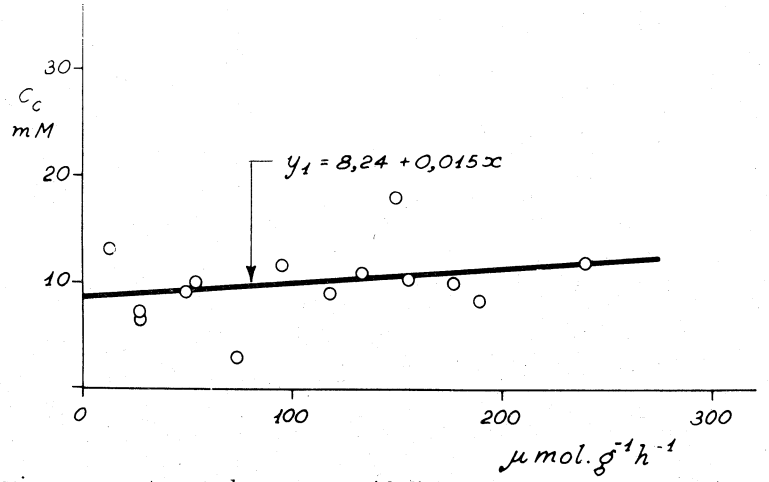
Histological specimens of the scraped mucosa and of the remaining tissue clearly indicate that the entire epithelium covering the villi is present in the scraped mucosa and that the crypts are present in the remaining tissue (Pl. I, *a*, *b*, *c*).

Results

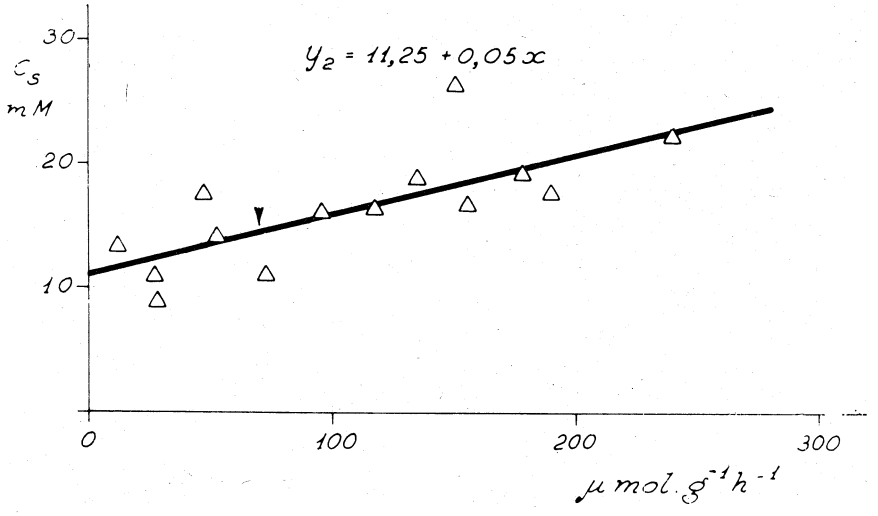
Table I gives the mean values of experimental concentration and transport. Mean concentration of D-glucose into cells refers to cell water of scraped mucosa after correction for the extracellular space. This concentration varies around a mean of 10 mM; moreover there is a very small correlation between cell concentration and net D-glucose transport (Fig. 2 *b*, $r = 0.32$). As Plate I shows, the scraped mucosa consists mainly of enterocytes and subepithelial cells of the villi; the enterocytes of the crypts are presumably absent. D-glucose



a) Relationship between net Na (abscissa) and glucose (ordinate) transepithelial transport.



b) The abscissa represents net glucose transepithelial transport whereas on the ordinate mean cell glucose concentration (C_c) is reported.



c) Relationship between net glucose transepithelial transport (abscissa) and emerging fluid glucose concentration (C_s) reported on the ordinate.

Fig. 2.

concentration of total emerging fluid is significantly higher than that of the cell and the concentration difference between these two values for each experiment is significantly higher than zero (Table I). This concentration is positively correlated with net D-glucose transport (Fig. 2 *c*, $P < 0.01$, $r = 0.75$).

TABLE I.

C_c mM	C_s mM	$\Delta C = (C_c - C_s)$ mM	Gluc. transp. μmoles $g^{-1} h^{-1}$	Na^+ transp. μequiv. $g^{-1} h^{-1}$
9.97±0.90	16.63±1.24	−6.63±0.77	107±19	780±112
(14)	(14)	(14)	(14)	(14)

The table shows the cell glucose concentration (C_c) and the concentration of the sugar in the final serosal fluid (C_s) which represents the fluid emerged from the cells into the intercellular spaces and then into the serosal compartment which was initially empty. These concentrations are expressed in μmoles per liter of cell water. ΔC represents the glucose concentration difference between cell and serosal compartment. Net transintestinal transports are given in μmoles/g dry tissue weight of total intestine per hour (glucose) and in μ equivalents/g dry tissue weight of total intestine per hour (sodium). Values ± M.S.E., with number of experiments in parentheses, are reported.

In addition there is a positive linear relationship between net glucose and sodium transepithelial transport with a reasonably good correlation coefficient (Fig. 2 *a*, $P < 0.001$, $r = 0.91$). The slope of the straight line is close to that previously found in rat and hamster jejunum "in vitro" [11, 12]. Such linear correlation indicates that the more the sodium is transported, the more the glucose is transported as well. The straight line passes through the origin of the coordinates, thus indicating that in the absence of sodium transport also the transported glucose is zero. The meaning of this correlation is presumably that there is a constant ratio between net glucose and sodium transepithelial transport and that the ability to transport Na and glucose of enterocytes is varying from preparation to preparation. As a matter of fact, the suprabasal oxygen consumption also increases with increasing transport activity [13]. In the light of the sodium-glucose cotransport hypothesis [14], this fact should mean that when the enterocytes are functioning well, mean cell glucose concentration should be high and viceversa. This is only partially verified by the experimental data obtained in the present experiments (Fig. 2, *b*), i.e. the mean cell glucose concentration increases slightly at high rates of transport, but this difference is not statistically significant. These data seem to suggest that the glucose entry across the brush border is just balanced by D-glucose extrusion through the basolateral membrane, whatever the glucose transport is.

DISCUSSION

Concerning the nature of the extrusion mechanism, several hypotheses could be put forward. First, the mechanism is an active, metabolically-dependent one [1, 2], just as it is for the sodium extrusion mechanism. Second, the mechanism is a chemically facilitated passive transport, but in this case the assumption is necessary that only the columnar cells of the tip of the villus can absorb and concentrate sugars [15] in such a way that the concentration difference between absorbing cells and serosal space is increasing together with the transport activity. Third, the metabolic activity favours D-glucose permeability through the basolateral membrane, and this is responsable for the linear relation between D-glucose and sodium extrusion. In this case however it is also necessary to assume a sugar concentration in the absorbing cells higher than in the serosal one. This concentration difference may remain nearly constant if the sugar permeability through the basolateral membrane is increasing together with the transport activity.

Considering the second possibility, each mean cell glucose concentration (C_c) is linked to the actual glucose concentration in the functioning enterocytes (tip of the villi) (C_t) by a factor " α ", higher than one, in order to account for a sugar accumulation in the absorbing cells of the tip: $C_t = \alpha C_c$. The value of " α " may vary with net transepithelial transport activity, but it presumably decreases with the glucose transport. As a matter of fact, mean cell glucose concentration remains nearly constant and the glucose concentration in the non-absorbing cells should increase since the glucose concentration in the serosal fluid (C_s) increases in the meantime, so that more and more sugar should enter into this type of cell. Let us assume, for a moment, that " α " remains constant during any transport condition. In this case the glucose concentration difference between the functioning enterocytes and the serosal fluid comes from the difference between the two linear equations (one of these corrected by " α ") shown in Fig. 2 b and c ($\Delta y'$):

$$\Delta y' = \alpha\, 8.24 - 11.25 + (\alpha\, 0.015\text{–}0.05)\, x$$

where " x " represents net glucose transepithelial transport expressed in $\mu\text{moles} \cdot g^{-1} \cdot h^{-1}$.

In order to give a physiological meaning to this equation, the slope of the straight line must be positive and the difference between the two intercept values must be nearly zero. By assuming an " α " value, for instace, of 4 we obtain a transport kinetics completely different from that of a passive, chemically facilitated transport. As a matter of fact, when glucose transport (x) is zero, there is still a concentration difference between cell and serosal compartment ($\Delta y'$) of 21 mM (see equation), a condition which is impossible in a passive process. Otherwise, if we reduce the value of " α " to 1.36 so that $\Delta y'$ becomes zero, at zero glucose transport the slope of the above linear function

is zero. In order to avoid this difficulty (i.e. in order to have a $\Delta y' = 0$ at zero glucose transport with a positive slope) we should suppose a statistical error in the determination of the intercept and slope values of y_1 and y_2 (the ordinate values of Fig. 2, *b* and *c*). By assuming an error of the slopes equal to the standard error, i.e. + 0.013 and — 0.012 for slopes of y_1 and y_2 respetively, and an error of the intercept value equal to the standard error, i.e. — 1.67 and + 1.59 for the intercept values of y_1 and y_2 respectively and assuming an " α " value of 1.7 we obtain:

$$\Delta y' = 1.7 \times 6.57 - 12.84 + (1.7 \times 0.028 - 0.038)\, x$$

This equation can reasonably fit the kinetics of a passive facilitated transport of glucose through the basolateral membrane of the functioning enterocytes. However, these errors correspond to a statistical probability much lower than 10 %.

A last possibility can now be examined, i.e. the behaviour of glucose concentration along the length of the villus core and the crypt. Glucose concentration in the serosal fluid (C_s, Fig. 2 *c*) may be different from the actual concentration at the basal pole of the enterocytes of the villus tip, and may be higher if a glucose concentrating process takes place at the level of the crypt due to the secretion of a glucose-free solution (external fluid circuit, [16]).

By assuming that 40 % of the absorbed fluid is excreted by the crypts, the actual extracellular glucose concentration at the basal pole of the enterocytes of the tip of the villus tends to remain 40 % lower than that in the serosal fluid i.e. when the serosal sugar concentration determined at a mean " x " value of 150 μmoles/g·h is 18.75, we have:

$$18.75 - 4 \times 1.87 = 11.0 \text{ mM}.$$

On the basis of this extracellular concentration, also the intracellular concentration should be changed, thus becoming higher than the previously reported one, i.e. at a mean " x " value of 150 μmoles/g·h, we obtain:

$$10.50 + 1.03 = 11.53 \text{ mM}.$$

Therefore the equations (Figs. 2 b and 2 c) become:

$$y_1 = 8.24 + 0.022\, x$$

$$y_2 = 11.25 - 0.0017\, x\,.$$

Assuming an " α " value of 1.36, the corrected difference ($\Delta y'$) becomes:

$$\Delta y' = (0.0220 + 0.0017)\, x.$$

This equation is also adequate to fit the kinetics of a passive facilitated transport of glucose through the basolateral membrane of the functioning enterocytes. This is true if we assume that 40 % of the absorbed fluid is excreted by the crypts; however, at least in dog [17] and man [18], the amount of fluid excreted seems to be approximately 20 % of the absorbed fluid. This value does not account for a kinetics of a passive facilitated transport.

Coming back to the nature of the D-glucose extrusion mechanism, two hypotheses seem possible. First, the mechanism is an active metabolically-dependent one, just as it is for the sodium extrusion mechanism.

Second, the metabolic activity directly or indirectly favours D-glucose chemically facilitated permeability through the basolateral membrane in such a way as to maintain the linear relation between D-glucose and sodium extrusion, notwithstanding the fact that the D-glucose concentration gradient remains nearly constant.

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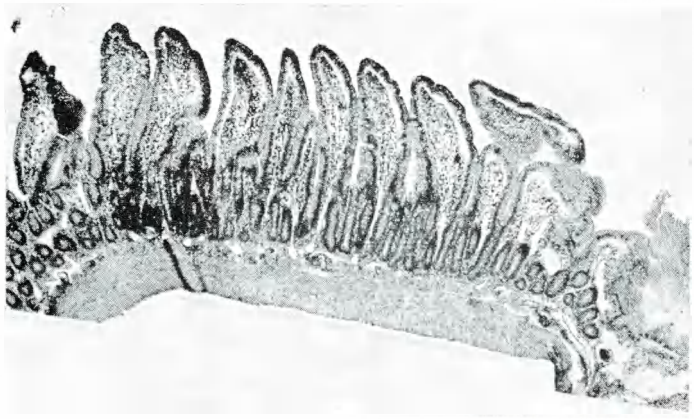
EXPLANATION OF PLATE I

a) Light microscope photograph of a transverse section of everted rat jejunum (magnification: ×80).

b) Microphotograph of mucosal scrapes of rat jejunum, clearly indicating the presence of the entire epithelium lining the villi and the absence of crypts (magnification: ×140).

c) Microphotograph of the remaining layer of rat jejunum after scraping, clearly indicating the presence of crypts alone (magnification: ×170). The tissue was fixed in Bouin solution and stained with haematoxylin–eosin.



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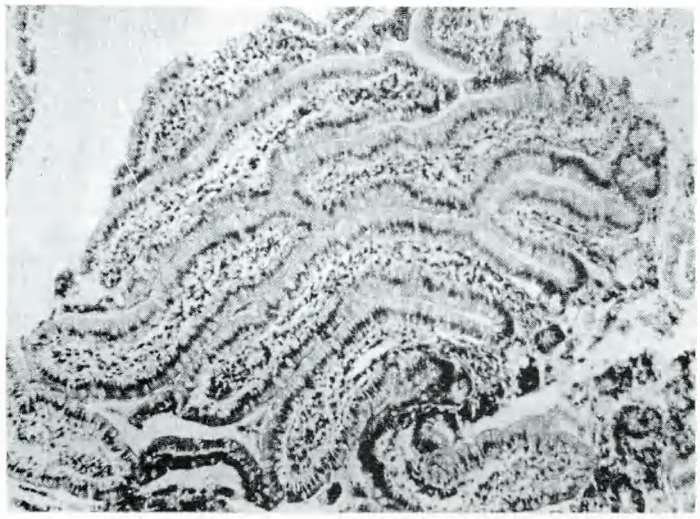

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