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THE CALCULATED GLUCOSE CONCENTRATION PROFILE IN THE INTERCELLULAR SPACES OF EVERTED JEJUNUM OF RAT

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Summary

On the basis of experimental data from isolated and everted rat jejunum and on extension of Diamond and Bossert's mathematical model, the glucose concentration in the intercellular spaces has been calculated and has been found higher (less than 2 mM) than that present in the serosal space.

The isolated and everted jejunal sac transports some sugars from the mucosal to the serosal side against a concentration gradient. Our aim is to see whether what we have previously named the apparent glucose concentration in the intercellular spaces [1] and what we name here the emergent fluid concentration are much different from the actual glucose concentration along the same spaces.

The basic procedure of the experiment has been reported elsewhere [2], however, some additional details will be given here. The everted jejunum of male albino rats (Wistar strain Charles River Italiana, 200—230 g body weight) was cannulated at one end and ligated at the other. The intestine was immersed in 50 ml Krebs-Ringer-bicarbonate solution with 5.5 mM glucose added and gassed with 95% O_2 and 5% CO_2: 2 ml of the same solution, but with poly ([^{14}C]ethyleneglycol) added as an extracellular marker was introduced into the cannula. The temperature was kept constant at 28°C throughout the 30 min experiment. It has been demonstrated that this temperature is suited for this preparation [3]. Net glucose and sodium salt transport are referred to 1 cm^2 intercellular surface and per second. The salts of sodium are not specified because they consist of several salts such as chloride, bicarbonate and lactate. The values are reported with their S.E.M.

From the cell number of villi per unit length of rat jejunum [4] corrected for goblet cells (30%) [5] it is possible to calculate the total number of

absorptive cells with reference to 100 cm which in turn corresponds to 1 g dry weight of intestine [5,6].

By assuming for the columnar absorptive cell a diameter of $4 \cdot 10^{-4}$ cm and a full length of $100 \cdot 10^{-4}$ cm it is easy to obtain the total area of lateral walls of the intercellular space ($2.45 \cdot 10^4$ cm^2) with reference to 1 g dry weight

The full length of the cell ($100 \cdot 10^{-4}$ cm) is an approximate measure which includes all the tortuosities of the lateral membrane (Diamond and Bossert, [7]); otherwise the length is $25—30 \cdot 10^{-4}$ cm.

The average net sodium transport of 8 experiments is $(187 \pm 34) \cdot 10^{-7}$ μosmol$\cdot$cm$^{-2} \cdot$s^{-1}, whereas that of net glucose and water transport is $(19.8 \pm 3.7) \cdot 10^{-7}$ μmol$\cdot$cm$^{-2} \cdot$s^{-1} and $(6.8 \pm 1.5) \cdot 10^{-8}$ cm$^3 \cdot$s$^{-1} \cdot$cm^{-2} (cm$\cdot$s^{-1}), respectively. All the calculations are based on the assumption that the site of solute input is distributed along the whole length of the lateral membrane; this has been demonstrated in the bullfrog gallbladder epithelium (Mills and Di Bona [10]) and in rabbit intestinal epithelium (Stirling [11]) by the aid of radioautographs of the tissue exposed to [^{3}H]ouabain. The average final sodium salt and glucose concentration in the serosal side is 274 ± 2 and 9.1 ± 0.6 mosM, respectively.

Net sodium transport does not include the entry through the tight junctions. On the other hand net glucose transport does not seem to be affected by this component [1]. In order to calculate (see later) the glucose concentration profile in the lateral spaces, we have taken into account the results of some single experiments. Figs. 1 and 2 refer to the values of one experiment in which the net glucose transport (N_1, μmol$\cdot$cm$^{-2} \cdot$s^{-1}) and the net sodium salt transport (N_2, μosmol$\cdot$cm$^{-2} \cdot$s^{-1}) are the closest to the above reported means (see legends to the figures).

Diamond and Bossert [7] have developed a mathematical model to take into account both diffusional and convective components of the transport of one solute along the epithelial intercellular spaces, in order to calculate its concentration profile. We extended their original model to the case of transport of two solutes. In our case, we do not consider the flow system as a right circular cylindrical channel, but as a space bounded by two parallel rectangular surfaces, with one side very long and the other length given by 'l' (intercellular space length which is assumed to be 100 μm). The distance between the two surfaces is 'h' (intercellular space width); 'h' is assumed to be 0.1 μm in order to have an intermediate value between 0.01 and 1 μm (see below). The space is open at the subepithelial side and assumed to be closed at the other end. This assumption is, of course, not valid with the exception of glucose, and will be discussed at the end of the paper.

Diamond and Bossert's Eqn. 1, written for two different solutes, then becomes

$$2N_j/h + D_j(d^2C_j/dx^2) - d/dx\,[C_j v] = 0 \qquad (1)$$

where $j = 1,2$ indicates respectively the first (glucose) and the second (sodium salt) solute, C_j, D_j and N_j their concentrations, free diffusion coefficients and net transepithelial transports (the last ones assumed to be constant on the whole intercellular space surface).

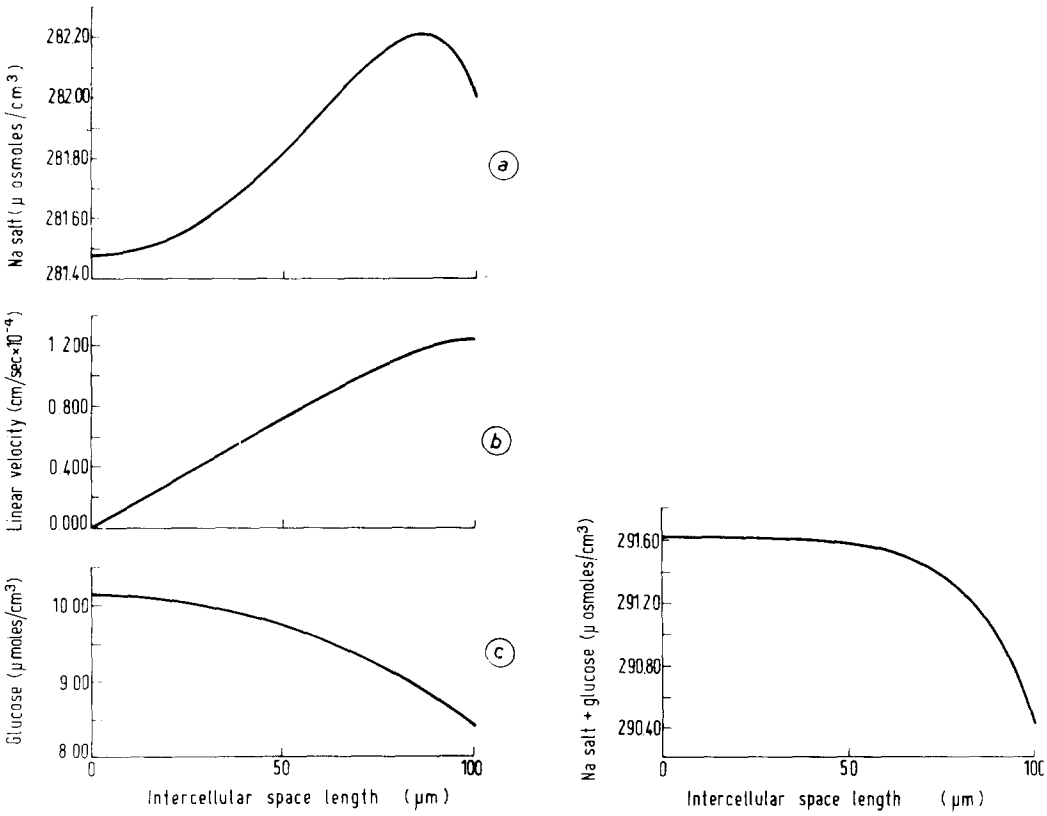

Fig. 1. (a) sodium salt osmolarity profile; (b) linear velocity of the fluid; (c) glucose molarity profile. Values of experimental parameters: $h = 0.1$ μm, $N_1 = 15.37 \cdot 10^{-7}$ μmol·cm^{-2}·s^{-1}, $N_2 = 195.30 \cdot 10^{-7}$ μosmol·cm^{-2}·s^{-1}, $D_1 = 0.5 \cdot 10^{-5}$ cm^2·s^{-1}, $D_2 = 10^{-5}$ cm^2·s^{-1}, $C_{1O} = 8.41$ μmol·cm^{-3}, $C_{2O} = 282$ μosmol·cm^{-3}, $C_{\text{cell}} = 290.41$ μosmol·cm^{-3}, $C_{\text{em}_1} = 24.43$ μmol·cm^{-3}, $C_{\text{em}_2} = 310.38$ μosmol·cm^{-3}.
The estimated value of P was $0.61 \cdot 10^{-4}$ cm·s^{-1}.

Fig. 2. Total osmolarity profile. Same value of parameters as Fig. 1.

Diamond and Bossert's Eqn. 2 becomes

$$dv/dx = (2P/h)[C_1 + C_2 - C_{\text{cell}}] \tag{2}$$

where 'v' is the linear velocity of the fluid, 'P' the osmotic permeability constant of the intercellular lateral walls and 'C_{cell}' the total cellular osmolarity (assumed to be the same as that of the free subepithelial space).

We performed a first integration of Eqn. 1 between 0 and x taking into account the condition $dC_j/dx = 0$ and $v(0) = 0$ at the closed end (no solute and no water flux across the closed end). In this way Eqns. 1 and 2 reduce to the system of three first-order differential equations

$$\begin{aligned} dC_1/dx &= (1/D_1)[vC_1 - (2N_1/h)x] \\ dv/dx &= (2P/h)[C_1 + C_2 - C\,\text{cell}] \\ dC_2/dx &= (1/D_2)[vC_2 - (2N_2/h)x] \end{aligned} \tag{3}$$

with the initial conditions:
(i), $C_j = C_{jo}$ at the open end, where the values of 'C_{jo}' are the glucose and sodium salt concentrations of the subepithelial space; (ii), the value of 'v', '$v(l)$', at the open end of the space was determined by means of the relation

$$C_{emj} = \frac{2N_j l}{v(l)h}$$

where 'C_{emj}' is the experimental value of glucose or sodium salt emergent fluid osmolarity.

Conditions (i) and (ii) allow us to integrate the equations of system 3. The value of the parameter 'P' was determined by an iterative procedure in order to obtain $v(0) = 0$ at the closed end of the intercellular space.

Integration of equations of system 3 was numerically performed with the aid of a FORTRAN polyalgorithm for the solution of ordinary differential equations.

In Fig. 1(a, b, c) the computed results are represented as a function of the space length. The 0 value corresponds to the closed end of the intercellular space. Similar results were obtained by using the experimental values of other single experiments.

The value of 'P' was found to be $0.61 \cdot 10^{-4}$ cm $\cdot$ s^{-1} for the parameter values given in the legend to Fig. 1.

Smyth and Wright [12] found a value of P of $1.7 \cdot 10^{-4}$ cm $\cdot$ s^{-1} in rat small intestine. As this value refers to the whole epithelium, the real value of P should be certainly lower.

On this account we find that our model and the parameters used by us are consistent with acceptable values for the permeability constant of intercellular space wall. In fact a value of P of the order of magnitude of 10^{-4} cm $\cdot$ s^{-1} fit our experimental results, while in [13,14] a value of P of 10^{-1} cm $\cdot$ s^{-1} or less is necessary in order to explain the measured emergent osmolarity. This remarkable difference is due to the different experimental conditions employed and to a different value given to the parameters of the model.

It may seem surprising that the sodium salt concentration profile decreases as the distance from the open end increases. Recently some authors [8] have experimentally determined the sodium concentration profile in the intercellular space; they found a biphasic pattern of this profile.

The peculiar sodium salt concentration profile in our case is presumably due to its diffusion coefficient which is higher than that of glucose. As a matter of fact, the concentration profiles shown by Diamond and Bossert (see also Ref. 9) all present a monotonic decrease.

However, as we are now dealing with two solutes, the result is not absurd, as long as the total osmolarity is still a decreasing function in the direction of the open end (Fig. 2).

As to the behaviour of glucose concentration profile (Fig. 1c), the glucose concentration in the intercellular space reaches a maximal difference which increases by the decreasing of 'h' value; at 'h' = 0.1 μm this value is 1.7 μmol $\cdot$ cm^{-3} (Fig. 3).

If tight junctions are open to sodium and water entry, we must generalize the above mathematical model [9,15].

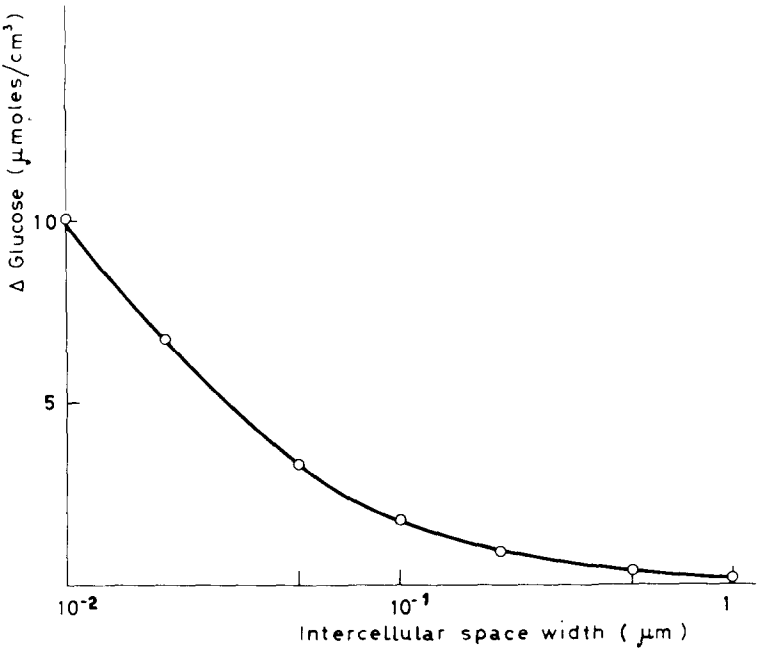

Fig. 3. Glucose maximal concentration difference in the intercellular space as a function of the intercellular space width (h).

Volume flow through the tight junctions can be calculated by using the Sackin and Boulpaep's equation 1c [9] rewritten for the case of two solutes. L_p^{α} and Δ_p^{α} were set equal to $2.6 \cdot 10^{-4}$ cm·s^{-1}·cm^{-1} H_2O and 1 cm H_2O, respectively. σ_s^a for sodium chloride is set equal to 0.7.

The ratio between the width of the tight junction and that of the intercellular spaces is supposed to be 0.05.

Under these conditions, with a sodium salt entry of 13% of the emerging flux at the serosal side we obtain a diffusion coefficient of sodium chloride through the same junction very close to that chosen in Ref. 9. The estimated value of P becomes $0.47 \cdot 10^{-4}$ cm·s^{-1} instead of $0.61 \cdot 10^{-4}$ cm·s^{-1}. Furthermore the glucose concentration in the intercellular space becomes only 1.5 μmol·cm^{-3} higher than that in the serosal space instead of 1.7 μmol·cm^{-3} in the ungeneralized model.

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