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Received July 4th, 1968

BBA 73052

Observations on the permeability of thymocytes to non-electrolytes

In the course of experiments with calf thymus nuclei isolated in sucrose solutions a [^{14}C]sucrose-impermeable fraction of the particles was found. This fraction corresponds to intact cells since Ficoll isolation of nuclei substantially reduces the sucrose-impermeable fraction and the cellular fraction of these preparations has a much higher sucrose-impermeable space[1]. The present experiments further support the contention that the sucrose-impermeable fraction corresponds to intact cells since the permeability of this fraction to malonamide is identical to that of intact thymocytes isolated by passing the tissue through a screen by the method of BLECHER AND WHITE[2].

The present communication reports on this validation and in addition furnishes the permeability constants for several non-electrolytes suspended in a sucrose medium containing Ca^{2+} and Mg^{2+}.

Techniques. The suspensions of nuclei and thymocytes were isolated by a method where the tissue is homogenized in a Waring blendor and centrifuged as previously described[3] and detailed in another communication[4]. These preparations are likely to contain 30 % intact cells. The substances used were labeled with ^{14}C and were purchased from New England Nuclear Corp. (Boston, Mass.), except for [^{14}C]erythritol which was obtained from Nuclear-Chicago Corp. (Chicago, Ill.).

Aliquots of 2 or 3 ml were incubated in 12-ml tubes of cellulose nitrate. All samples were incubated at 0° and shaken in a Dubnoff shaker (130 cycles/min). At zero time, 50 μl of the isotope solutions were added. The incubation medium consisted of 0.4 osmolal sucrose, 0.02 M Tris buffer, 5 mM $CaCl_2$ and 5 mM $MgCl_2$ at a pH of 7.4. In addition to the ^{14}C-labeled compounds, the medium contained 50- to 200-fold excesses of non-radioactive penetrant intended to saturate possible binding sites. The ^{14}C-labeled penetrant was added in the order of decreasing period of exposure. In this fashion all tubes were shaken for the same length of time, but the incubation time in the presence of the penetrant was varied. At the end of the incubation period all tubes were centrifuged at 12800 $\times$ g for 5 min in a Lourdes LRA refrigerated centrifuge. The supernatant was immediately poured off and saved for counting. The

cellulose nitrate tube portion not in contact with the pellet was cut off, and the remaining portion was blotted with absorbent paper. The pellets were extracted overnight with 1.00 ml 15 % trichloroacetic acid or 2 M HCl at 0–5°.

The radioactivity in the sample was measured with a gas-flow counter (Nuclear-Chicago, model D-47) from a wet sample on filter paper or with a liquid-scintillation counter (Packard, model 3003) (see ref. 1). The total solution volume was determined with [^{14}C]glycerol and the volume external to the particles with [^{14}C]sucrose (see ref. 1). These were previously found to be unchanged by the extensive incubation periods. In order to calculate permeability constants, it was necessary to estimate the radius of the spherical particles. Cell radius was measured by photographing the thymus suspension in a Petroff–Hausser bacteria counter under a light microscope. The particle diameters were measured from photographs at a final magnification of about 1500 ×. In three different experiments measuring 89, 148 and 202 particles in a final total of 19 photographs, the mean particle radii were 2.7 ± 0.2 μ, 2.8 ± 0.2 μ, 2.8 ± 0.2 μ. The mean particle water volume used in the calculations was corrected for the volume of internal solids (21 %, see ref. 1). The particle sizes may significantly differ from that of intact cells since there is no way of distinguishing nuclei from intact cells in these mixed preparations without complex manipulations (see ref. 1). However, the difference should not be great since the cytoplasmic ring is about 0.3 μ in thickness as viewed with the electron microscope (see ref. 4).

Calculations of the permeability constants. The equations that can be used to calculate permeability constants for a spherical particle are well known (*e.g.* see ref. 6). The equations used in our calculations are integrations of Eqn. 1, a modified Fick diffusion equation, and they take the form of Eqn. 2 or 3

$$\frac{dS_i}{dt} = PA\left[\frac{S_0 - S_i}{V_0} - \frac{S_i}{V_i}\right] \quad (1)$$

$$-\log_e(1 - C_i/C_0) = P\frac{3}{r}t \quad (2)$$

$$-\log_e\left[1 - \beta\frac{S_i}{\alpha}\right] = \beta PAt \quad (3)$$

where P is the permeability constant; S_i the internal amount of penetrant; S_o, the initial external amount of penetrant; C_i, C_o, V_i and V_o are the corresponding concentrations and volumes; t is time; r, the radius of the particle; and A, the total cell-surface area. The constants of Eqn. 3, α and β are S_o/V_o and $(V_i+V_o)/V_iV_o$, respectively. Eqn. 2 makes the assumption that the external concentration does not change with penetration and was used for slow penetrants where the depletion of extracellular penetrant after 5 h of incubation was insignificantly small. Eqn. 3 was used for faster penetrants and takes into account the changes in external concentration of penetrant.

The kinetics of penetration for a typical experiment is shown in Fig. 1. The calculated permeability constants for several non-electrolytes are shown in Table I. The line of Fig. 1 intercepts at the ordinate above 0, contrary to the prediction of the equations. This is probably the result of some non-homogeneity of the preparation, perhaps the presence of damaged cells. Deviations from linearity occasionally occur

after 5 h of incubation. These are within the experimental error; the function plotted in the ordinate becomes easily affected by small deviations in the measured S_1, V_1 or V_0, when the system approaches equilibrium.

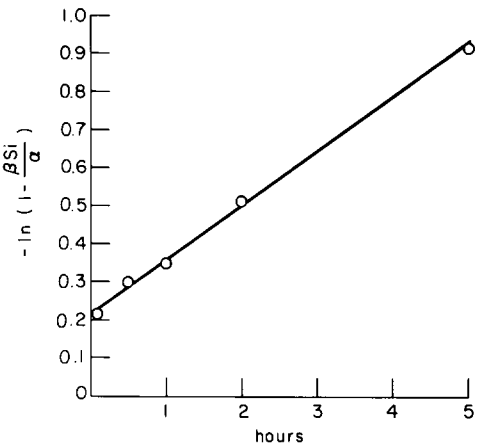

Fig. 1. Penetration kinetics of erythritol.

TABLE I

PERMEABILITY CONSTANTS OF INTACT THYMOCYTES AT 0°

The concentrations in mM were: malonamide, 0.7–4; erythritol, 0.2; arabinose, 0.2–0.4; mannitol, 0.2–1.

Expt.	*Permeability constants (cm/h × 10⁶)*					
	Malonamide	*Erythritol*	*D-Arabinose*	*D-Mannitol*	*Glycerol*	*Sucrose*
1	16	9.9	2.2	3.4	equilibrates	does not
2	19	10.2	2.1	1.5	within 5 min	penetrate
3	18		0.5	0.7		significantly
4	19		2.3	1.5		in 5 h
5	26		3.1			
Mean	20 ± 4	10.0	2.0 ± 0.9	1.8 ± 1.4	high	low

A calf thymus suspension reported to be rich in intact cells was prepared by first mincing the tissue and then pressing it through a 24-mesh stainless-steel screen (see ref. 2). The homogenate was then strained through a double layer of nylon hose. The filtrate was centrifuged at 600 × *g* for 10 min. The pellet was washed twice with the isolation medium. The permeability constant for malonamide of this preparation was found to be $23 \cdot 10^{-6}$ cm/h. This was about the same as the mean permeability constant for malonamide of the sucrose-impermeable particles of the preparation obtained by the regular method of Waring-blendor homogenization (Table I). The results presented confirm the conclusions of a previous study (see ref. 1) that the fraction is made up of intact cells.

The permeability constants shown in Table I, are not very different from other cell preparations (*e.g.* see refs. 5, 6 for the constants of *Chara ceratophylla*). However,

it should be noted that the medium used in the present experiments is low in electrolytes. The permeability of the cells in a more physiological environment may be significantly different.

This work has been supported in part by the U.S. Public Health Service (RG-9156).

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Received April 1st, 1968
Revised manuscript received June 26th, 1968

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