SHORT COMMUNICATIONS

BBA 73026

Transport of *myo*-inositol in Ehrlich ascites cells

It has been demonstrated by several investigators that the Ehrlich ascites cells are capable of accumulating amino acids by an energy and sodium ion dependent mechanism[1–5]. Carbohydrates or their derivatives, however, are not known to be accumulated by these cells although it has been shown that several simple sugars enter the Ehrlich ascites cells by a carrier-linked mechanism[6,7]. Recently we have observed that *myo*-inositol is accumulated as such by Ehrlich ascites cell suspensions and that the mechanism for *myo*-inositol accumulation is highly specific. A report by HAUSER[8] has presented evidence that *myo*-inositol is actively transported by rat kidney cortex slices.

The procedures used in this study for preparing and incubating the Ehrlich ascites cells are identical with those reported in previous communications on studies of amino acid transport[5,9,10]. Cell suspensions were incubated for a specified time with [$^{14}C_6$]*myo*-inositol (New England Nuclear Corporation) as indicated in the text. After incubation the cell suspension was diluted threefold with cold Ringer medium and centrifuged for 2 min at 800 × *g*. The supernatant was poured off, the cell pellet recentrifuged and the remaining fluid wiped away with tissue paper. The cell pellet was extracted with 2 ml of 5% trichloroacetic acid. The trichloroacetic acid was removed by extraction 3–4 times with 5-ml aliquots of ether. A portion of the trichloroacetic acid extract was plated and counted with a Nuclear-Chicago thin end window flow counter. In initial experiments portions of the trichloroacetic acid extract were passed through columns of Dowex 50 (H^+) and Dowex 1(formate) and washed with water. In both cases, 85–100% of the initial radioactivity in the sample was recovered in the water wash. Both the tissue extract and the medium were subjected to paper chromatography before and after admixture with fresh [$^{14}C_6$]*myo*-inositol and non-radioactive *myo*-inositol. Chromatography was carried out in phenol–water (72:28, v/v)[11] or isopropyl alcohol–pyridine–glacial acetic acid (80:15:5, v/v/v)[12]. Following chromatography, the papers were scanned with a Packard radiochromatogram strip scanner model 7200. Then the papers were stained according to GORDON, THORNBURG AND WERUM[12] to detect *cis*-diols.

Only a single radioactive component was found in the tissue extract as well as in the incubation medium whose R_F coincided with that of [$^{14}C_6$]*myo*-inositol. The radioactive area also coincided with the spot given by the chemical stain for *myo*-inositol. These data indicate that the material accumulated by the cells is free *myo*-inositol and that there is little conversion to other water-soluble derivatives.

The typical data in Table I show that when suspensions of Ehrlich ascites cells are incubated with [$^{14}C_6$]*myo*-inositol there is an extensive accumulation of radioisotope in the tissue. It may be seen that under anaerobic conditions glucose is required to obtain accumulation whereas under aerobic conditions the accumulation proceeds nearly equally well in presence and absence of glucose. The accumulation is greatest in a bicarbonate medium containing glucose. In a phosphate medium, glucose causes a decreased uptake presumably because the pH is lowered[17]. The process may

TABLE I

TRANSPORT OF [$^{14}C_6$]*myo*-INOSITOL IN EHRLICH ASCITES CELLS

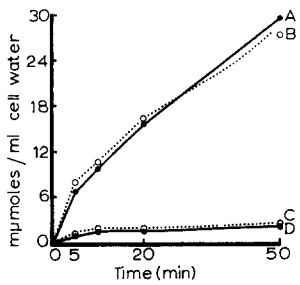
Ehrlich ascites cell suspensions were incubated for 60 min in Krebs–Ringer bicarbonate medium (Ca^{2+} free) or in Krebs–Ringer phosphate medium (Ca^{2+} free) at 37°. Approx. 10 mg dry weight of tissue were incubated in 3 ml of medium. [$^{14}C_6$]*Myo*-inositol was present at a concentration of 5 μM, specific activity $2.2 \cdot 10^6$ counts/min per μmole. In Expt. II, isoosmolar sucrose replaced the NaCl of the Ringer medium and all other components remained unchanged.

Expt. No.	*Conditions*	*Counts/min per 10 mg dry weight of tissue* $\times 10^{-3}$	*Ratio of the distribution of radioactivity between cell and medium*
I	95% N_2 and 5% CO_2 ($NaHCO_3$ buffer)	1.4	1.5
	95% N_2 and 5% CO_2 ($NaHCO_3$ buffer) + glucose (10 mM)	9.0	10.0
	95% O_2 and 5% CO_2 ($NaHCO_3$ buffer)	7.8	8.6
	95% O_2 and 5% CO_2 ($NaHCO_3$ buffer) + glucose (10 mM)	10.2	11.0
	Air (phosphate buffer)	6.1	6.7
	Air (phosphate buffer) + glucose (10 mM)	4.8	5.4
II	Control (air, phosphate buffer), normal Krebs–Ringer	5.6	6.2
	No K^+ (air, phosphate buffer)	1.8	2.0
	20 mM Na^+ (air, phosphate buffer)	0.5	0.6
	70 mM Na^+ (air, phosphate buffer)	1.6	1.8
	120 mM Na^+ (air, phosphate buffer)	4.2	4.6
III	Control (air, phosphate buffer)	6.6	7.2
	2,4-Dinitrophenol (air, phosphate buffer)	3.4	3.7

be inhibited by removal of Na^+, K^+ or the addition of 2,4-dinitrophenol. With an initial *myo*-inositol concentration between 2 μM and 50 μM, concentration ratios between cell and medium from 12 to 2 have been obtained in a Krebs–Ringer phosphate incubation medium. The following sugars and sugar alcohols (at a concentration of 10 mM) were found to be without effect on the uptake of *myo*-inositol: D-galactose, galactitol, 3-*O*-methyl-D-glucose, xylitol, L-xylose, L-ascorbic acid, 6-deoxy-L-galactose. Glycine too was found to be without effect.

Inositol, in contrast to many amino acids, is taken up slowly by the Ehrlich ascites cells, nearly 90 min of incubation being required to attain a steady state. Moreover, once inside the cell the rate of leakage or loss into an inositol-free medium is also slow. If the initial intracellular [$^{14}C_6$]*myo*-inositol concentration is between 10 and 35 μM, and the cells are placed into an inositol-free medium, no more than 10% of the inositol initially present is lost from the cells within 10 min of incubation. The data in Figs. 1 and 2 show the influx and efflux respectively of [$^{14}C_6$]*myo*-inositol in the Ehrlich ascites cell suspensions.

It has been demonstrated with a number of transport systems that the absence of sodium ions from the incubation medium increases the apparent K_m value for uptake[13–15]. In accordance with these findings we observed a 10-fold increase in the apparent K_m value for *myo*-inositol uptake when the sodium ions of the medium (except for the sodium ions in the 10 mM phosphate buffer) were replaced by isoosmolar sucrose. For example, the apparent K_m for *myo*-inositol uptake in a normal Krebs–Ringer phosphate medium was found to be between 15 and 20 μM. In a medium in which all the NaCl of the Ringer solution was replaced by sucrose, the apparent K_m rose to 200. There was no alteration in the maximum uptake (v_{max}).



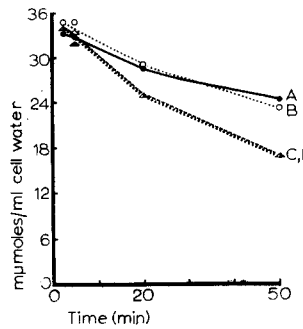


Fig. 1. Effect of prepacking with *myo*-inositol on the uptake of [$^{14}C_6$]*myo*-inositol. The cells were preincubated for 1 h in air at 37° in a Krebs–Ringer–phosphate medium in presence and absence of 5 μM inositol. Then the cells were centrifuged and each set of cells divided into 2 equal portions to obtain four cell pellets. Each pellet was resuspended in either 1 ml of isotonic Krebs–Ringer or in 1 ml of a solution in which the NaCl was completely replaced by sucrose. The cells were then added to a Krebs–Ringer–phosphate medium or a sucrose–phosphate medium (the NaCl of the Krebs–Ringer–phosphate having been replaced by sucrose) containing 5 μM [$^{14}C_6$]*myo*-inositol (specific activity 10^6 counts/min per μmole). Incubation was in air at 37° and 2-ml samples were taken at the times specified. A, preincubation without *myo*-inositol followed by incubation in Krebs–Ringer medium; B, preincubation with 5 μM *myo*-inositol followed by incubation in Krebs–Ringer medium; C, preincubation without *myo*-inositol followed by incubation in sucrose medium; D, preincubation with 5 μM *myo*-inositol followed by incubation in sucrose medium.

Fig. 2. Efflux of [$^{14}C_6$]*myo*-inositol from Ehrlich ascites cells. The cells were preincubated for 1 h at 37° in air in a Krebs–Ringer–phosphate medium (pH 7.4) containing 5 μM [$^{14}C_6$]*myo*-inositol (specific activity $1.28 \cdot 10^6$ counts/min per μmole). Just prior to the termination of the incubation period a 2-ml sample was taken, and the remainder of the flask contents was distributed equally among 4 tubes and centrifuged. Two of the cell pellets were resuspended in Krebs–Ringer and two in a sucrose–phosphate medium (see Fig. 1). The cells suspended in Krebs–Ringer were added to a Krebs–Ringer–phosphate medium and the cells suspended in a sucrose medium were added to a medium in which the NaCl of the Krebs–Ringer had been replaced by sucrose. A, cells incubated in Krebs–Ringer–phosphate; B, cells incubated in Krebs–Ringer–phosphate containing 50 μM *myo*-inositol; C, cells incubated in sucrose medium; D, cells incubated in sucrose medium containing 50 μM *myo*-inositol. All incubations were in air. 2-ml samples were taken at the times indicated. The initial content of [$^{14}C_6$]*myo*-inositol was 36 mμmoles/ml cell water.

Reduction of the sodium concentration to 95 mM and replacement by sucrose, increased the apparent K_m value to 45–50 μM.

Although *myo*-inositol is accumulated by Ehrlich ascites cells and the K_m for uptake is altered by the Na^+ concentration in the medium, *myo*-inositol, unlike amino acids, does not appear to undergo exchange diffusion. HELMREICH AND KIPNIS[16] have proposed that exchange diffusion is most likely to occur in a system where the K_m (K_2) on the inner surface of the membrane is similar in magnitude to the K_m (K_1) on the external side of membrane. Where K_2/K_1 is large, the system becomes essentially irreversible and exhibits no exchange. KIPNIS AND PARRISH[14] showed in rat diaphragm that by removing sodium ions from the incubation medium (and presumably increasing K_1 relative to K_2) they obtained exchange diffusion of α-aminoisobutyric acid whereas no exchange was observed in a normal Ringer medium. The data with inositol (Figs. 1 and 2) show that no exchange is evident in either sucrose or normal Ringer medium although as mentioned above, there is at least a 10-fold increase in the apparent K_m (K_1), value in a sodium-deficient medium. It is evident, however, that in a sucrose medium an increased loss of radioactivity from the tissue is apparent after 20 min of incubation. The presence of *myo*-inositol in the incubation

medium does not alter the extent or rate of this loss. In measuring uptake of *myo*-inositol over an extended period it has also been found that if Na^+ concentration is reduced to 90 mM or less the level of [$^{14}C_6$]*myo*-inositol present in the cells at 90–120 min is less than that found at 60 min. This effect may be due to some deterioration of the cell membrane after prolonged exposure to a Na^+-free medium.

These data suggest that either the ratio of K_2/K_1 is not the only factor determining the "exchangeability" of a transport system or that the difference between K_1 and K_2 in this system is so large that even a 10-fold change in K_1 is not sufficient to make the two K_m values of the same order of magnitude. At present sufficient information is not available to decide in favour of either of these alternatives.

In summary, data have been presented to show that Ehrlich ascites cells are capable of transporting and accumulating inositol by an energy, Na^+- and K^+-dependent process which does not appear to be capable of undergoing exchange diffusion.

The financial assistance of the National Cancer Institute of Canada and the Medical Research Council of Canada is gratefully acknowledged.

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Received June 26th, 1967