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ION AND MACROMOLECULAR TRANSPORT IN THE ALVEOLAR MACROPHAGE

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SUMMARY

1. Some aspects of Na^+, K^+, Cl^- and macromolecular transport have been studied in isolated rabbit alveolar macrophages. Steady-state intracellular concentrations in Ringer's solution (pH 7.4) were as follows: Na^+, 83 ± 7.1 (S.E.); K^+, 75 ± 13.2; Cl, 59 ± 4.1 mequiv/kg cell water. Cl^- is rapidly and completely lost from the cell in Cl^--free media suggesting rapid thermodynamic equilibrium between intracellular and extracellular phases.

2. Na^+ efflux has two rate constants (rapid phase 636 ± 302 (S.D.); slow phase 329 ± 125 mequiv/kg cell water per h). It appears that this cell has high permeability for Na^+ and the high leak down the electrochemical gradient requires a high rate of active transport. Thermodynamic considerations suggest that the major energy source for active Na^+ transport is derived from oxidative phosphorylation.

3. Classical relatively high molecular weight extracellular markers are excluded from cell water. However, ferritin (mol. wt. 465000) influx is rapid with intracellular/extracellular concentrations greater than 1.0 within 3 min. Ferritin efflux is exceedingly slow so that there is essentially unidirectional transport. Simultaneous exposure of cells to both ferritin and dextran leads to no measurable increase in cellular dextran uptake suggesting high specificity for ferritin uptake.

INTRODUCTION

The alveolar macrophage is an important cell involved in pulmonary defense mechanisms. Several considerations suggest that it represents an unusually appropriate cell for the study of transport mechanisms. It can be obtained in isolated fashion as a more or less pure cell type in a relatively simple fashion. In the case of the erythrocyte, the ability to obtain isolated viable cells has led to substantial information concerning transport in general. It might be expected that this would be true with respect to the alveolar macrophage. Pinocytosis and phagocytosis

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represent important segments of normal macrophage function which can be quantitatively investigated in isolated cellular preparations.

In the present studies, some aspects of Na^+, K^+, and Cl^- transport have been investigated. In addition, the mechanisms for the transport of several macromolecular species in solution have been studied.

MATERIALS AND METHODS

Rabbit alveolar macrophages were obtained by pulmonary lavage[1]. The rabbits were anaesthetized and quickly killed by exsanguination. This minimized red cell contamination. After removal of the lungs, the trachea was intubated and the lungs were distended with 40 ml of a modified Ringer's solution buffered with phosphate (pH 7.4). (NaCl, 110 mM; Na_2HPO_4, 4.22 mM; KH_2PO_4, 3.53 mM; Na_2SO_4, 19.5 mM; K_2SO_4, 2.57 mM; $CaCl_2 \cdot 2H_2O$, 2.50 mM; $MgSO_4 \cdot 7H_2O$, 0.81 mM; glucose, 10 mM when appropriate.) The lungs were manipulated so that they were evenly distended and the fluid was then drained. This was repeated 5 times. Approx. 100 mg of cellular material was obtained from each individual animal from the washing fluid by centrifugation. It consisted mainly of macrophages. There were less than 2 % polymorphonuclear leukocytes or other white cells and a small number of red cells. As judged by the eosin viability test[2], 98 % of the macrophages were viable at the end of 60 min at 40°.

Separation into intracellular and extracellular phase

Since alveolar macrophages are capable of taking up high molecular weight substances and even particulates, it was necessary to demonstrate that classical extracellular markers could be used to define the extracellular phase of tissue samples. This was particularly important because of the relatively small size of cellular pellet available and because of the large amount of adsorbed and trapped liquid in cellular samples.

For this purpose, studies were performed using sucrose (mol. wt. 324) and dextran of various molecular weights (38000, 75000 and 500000). Cells were washed twice with Ringer's solution containing 1 % or 2 % (m/v) of the given compound and incubated (100 mg cells in 8 ml Ringer's containing the given marker) in a Dubnoff metabolic shaker at 40°. At varying time intervals, aliquots were obtained and supernatant and cells were separated by centrifugation. Both phases were analyzed for sucrose or dextran by the anthrone method[3]. Analysis of cells was also performed after exposure to these compounds followed by washing the cell samples twice with Ringer's solution containing none of the presumed extracellular marker. The results of these experiments will be reported in detail below (see RESULTS) but it was concluded that there is no substantial penetration of cell water by these compounds for the following reasons: (a) There was no progressive increase in concentrations in the cellular mixture of all four markers with time; (b) the magnitude of tissue content did not increase progressively with decreasing molecular weight; (c) the magnitude of calculated trap varied from 17 to 56 %, values consistent with adsorbed and trapped liquid in other systems; (d) washing the cells with Ringer's solution containing no marker resulted in sharp reductions of the concentrations of the marker in the cellular mixture.

In all subsequent studies, dextran was used as an extracellular marker.

Steady state $[Na^+]$, $[K^+]$ *and* $[Cl^-]$

The cells were washed twice with 2 % dextran (mol. wt. 500000) in Ringer's solution, and packed by centrifugation. A weighed portion of the packed cells was analyzed for dextran in order to estimate the volume of adsorbed and trapped supernatant. The remainder of the cells were weighed and dried at 100° for 24 h to determine the water content. The weight of cell water was obtained by subtracting the weight of trapped supernatant from the value for total water. The dried cells were extracted with 0.75 M nitric acid. Appropriate dilutions were analyzed for sodium and potassium with a Coleman flame photometer, and for chloride by electrometric titration[4]. The supernatant was analyzed and the results corrected for the amount of supernatant trapped.

Simi-quantitative estimate of Cl^- *efflux*

In six experiments, approx. 100 mg alveolar macrophages were added to 20 ml of 0.29 M sucrose containing no Cl^-. The cells were rapidly mixed with the suspending solution, rapidly centrifuged and the supernatant removed. This process required less than 1 min. The cells were then analyzed for Cl^-, as described above.

Na^+ *efflux*

Measurements of Na^+ efflux from alveolar macrophages are difficult because the supernatant trapped in packed cells contributes about 30 % of the total radioactivity measured. Preliminary experiments showed that 50–70 % of the internal Na^+ diffuses very rapidly so that the accuracy of the determination cannot be improved by washing the cells free of contaminating supernatant with non-labelled Ringer's solution.

Approx. 200 mg of cells were resuspended in 10 ml of rabit Ringer's solution (with glucose) and placed in a 25 ml erlenmeyer flask and 5 μC ^{22}Na were added to the suspension. The erlenmeyer flask was then placed in a Dubnoff metabolic shaker and incubated for 1 h at 40°. This allowed sufficient time for "labelling" of the alveolar macrophages. At the end of 1 h, the suspension was centrifuged and the "hot" supernatant discarded. At zero time, the cells were resuspended in "cold" Ringer's solution containing 1 % dextran (mol. wt. 500000) without glucose. The cell suspension was then divided into two equal aliquots, which were placed into separate 25-ml erlenmeyer flasks. One flask (control) contained macrophages suspended in rabbit Ringer's dextran while the other flask contained macrophages, rabbit Ringer's dextran and ouabain octahydrate 10^{-3} M. These 2 flasks were then incubated at 40° as before. At 1, 5 and 30 min, aliquots were removed and rapidly centrifuged. The supernatant was decanted and used for radioactive counting, Na^+ analysis and dextran analysis. From the cells, an aliquot was removed and placed in a pre-weighed tube for dextran analysis. The remainder of the cells was placed into another pre-weighed tube, and used for radioactive counting and determination of ^{23}Na.

The cell sample and 1 ml of the supernatant were counted for radioactivity on a gamma spectrometer. The cells were then dried at 100° to a constant weight and the tube was then again reweighed. From this, the amount of preparation water was calculated. 0.75 M HNO_3 was added to the cell mixture and digested for 48 h. Sodium determinations were carried out on the liquid phase using a flame photometer. Dextran analyses were performed and from this, the amount of trapped extracellular fluid was calculated. Cell radioactive counts and $[Na^+]_i$ were then calcu-

lated and corrected for the amount of trapped counts and extracellular ^{23}Na. All radioactive counts were expressed as counts/min per g cell water and plotted against time on semi-log paper. From this plot, the $t_{0.5}$ (the time for loss of 50 % of the cell radioactivity) was calculated. Na^+ efflux was calculated in a conventional fashion assuming uniform distribution of intracellular Na^+ without correction for back diffusion and expressed as mequiv/kg cell water per h. There was considerable random variation in the calculated $[Na^+]_i$ values and the results are presented as both $t_{0.5}$ (independent of $[Na^+]_i$ determinations) and as absolute Na^+ efflux.

Ferritin transport

Ferritin influx was determined on washed macrophages prepared in the usual way. Approx. 200 mg of cells were suspended in 20 ml of rabbit Ringer's. 2 ml of the suspension were removed and used for determination of quench. The remaining 18 ml were transferred to a 25-ml flask and approx. 25 μC of [^{3}H]ferritin* added and incubated at 40°. 2-ml aliquots were removed at 3, 5, 30, 60, 120 and 180 min and the cells and supernatant separated by centrifugation. Measured volumes of the supernatant and a weighed cell sample were added to 5 ml of (POPOP) and radioactivity determined in a Tricarb liquid scintillation counter. Quench corrections were obtained in standard fashion. Cell water was determined by drying to a constant weight on separate aliquots. Radioactivity was expressed as counts/min per g supernatant and cell water, respectively. Ferritin efflux was determined on cells that were prelabelled by exposing 200 mg of macrophages to approx. 25 ml of Ringer's for approx. 75 min. Following this, the cells were separated and washed 3 times in non-isotope containing rabbit Ringer's to remove extracellular radioactivity. The cells were then resuspended in 20 ml of Ringer's and aliquots obtained at 3, 5, 30, 60, 120 and 180 min intervals. Each aliquot was processed as described above and cell and supernatant radioactivity determined.

Effect of ferritin uptake on dextran uptake

To determine whether intracellular uptake of ferritin leads to a corresponding uptake of dextran, five studies were performed as follows: Approx. 100-mg aliquots of cells were exposed to 10 ml of Ringer's containing 1 % (w/v) of dextran (mol. wt. 75000). 25 μC of [^{3}H]ferritin were added to one flask and the other served as a control. The suspensions were incubated at 40° for 60 min. Cells and supernatant were separated and the cell mixture rapidly washed twice with Ringer's solution containing no dextran. The cells were analyzed for radioactivity (to insure that cellular uptake of ferritin had occurred) and analyzed for dextran. Uptake of dextran during ferritin exposure would have been indicated by significant increases in intracellular dextran concentration.

RESULTS

Extracellular markers

The results concerning cellular uptake of sucrose and the various dextrans are summarized in Table I. Calculated ratios between the cellular phase and extracellular

* [^{3}H]Ferritin with a specific activity of 17 μC/mg was used. This material was dialyzed for 48 h (Visking tubing) to remove any loosely bound tag.

TABLE I

PERCENTAGE OF "EXTRACELLULAR SPACE" IN MACROPHAGE SAMPLES WITH VARIOUS EXTRACELLULAR MARKERS

Values calculated as $\left(\frac{\text{mg marker/g cell water}}{\text{mg marker/g supernatant water}}\right) \times 100$.

U. = unwashed cells; W. = cells washed twice in Ringers containing none of the marker.

Expt. No.	*Substance*	*Mol. wt.*	*Time (min)*		
			3	*30*	*60*
	Sucrose	324			
1	U.		46	44	56
	W.		1	0	0
2	U.			36	36
3	W.			2	5
	U.			24	23
	Dextran	38 000			
1	U.			35	35
	W.			3	5
2	U.			33	36
	W.			3	4
3	U.			32	29
	W.			2	4
	Dextran	75 000			
1	U.			33	34
	W.			5	5
2	U.			24	29
	W.			3	3
3	U.			29	35
	W.			3	4
4	U.			20	31
	W.			0	0
5	W.			4	4
6	W.			6	5
	Dextran	500 000			
1	U.		22	30	30
	W.		0	3	0
2	U.		31	17	29
	W.		0	0	0
3	U.		47	27	51
	W.		3	3	3
4	U.		27	44	34
5	U.		30	32	30

phase varied from 0.17 to 0.56 in cells in which the final cell mixture was not washed in Ringer's without marker. This variation occurred in a random fashion with respect to time. It may be noted that following washing, cellular ratios fall to levels ranging from 0 to 0.05. There appears to be no significant penetration of cell water by any of these compounds.

Steady-state electrolyte concentrations

Values for alveolar macrophages and extracellular $[Na^+]$, $[K^+]$, $[Cl^-]$ and intracellular/extracellular ratios and calculated percentage of cell water are listed in

Table II. The alveolar macrophages may be classified as an "intermediate Na^+, intermediate K^+ cell" resembling the electrolyte pattern found in leukocytes[5] and glial cells[6]. Assuming thermodynamic equilibrium for Cl^- (see below) the transmembrane potential calculated from the Nernst relationship averaged 16 mV, the inside of the cell being electrically negative with respect to the outside. The distribution ratio for Na^+ is too high and the distribution ratio of K^+ is too low to fit a Donnan distribution, suggesting active transport of these ions.

TABLE II

INTRACELLULAR ION CONCENTRATIONS IN ALVEOLAR MACROPHAGES

Internal ion expressed as mequiv/kg cell water; external ion expressed as mequiv/kg medium water. $R_{[Cl^-]}$ = ratio of intracellular to extracellular ion concentration; $R_{[Na^+]}$ = ratio of extracellular to intracellular ion concentration; $R_{[K^+]}$ = ratio of extracellular to intracellular ion concentration.

Number of studies	7
$[Cl^-]_{internal}$	59 ± 4.1 (S.E.)
$[Cl^-]_{external}$	116
$R_{[Cl^-]}$	0.51
$[Na^+]_{internal}$	83 ± 7.1 (S.E.)
$[Na^+]_{external}$	156
$R_{[Na^+]}$	1.88
$[K^+]_{internal}$	75 ± 13.2 (S.E.)
$[K^+]_{external}$	5.9
$R_{[K^+]}$	0.08
% Cell water	80.0 ± 0.46 (S.E.)

Semi-quantitative aspects of Cl^- movement

The results of all six experiments in which macrophages were placed in Cl^--free medium and separated in less than 1 min were similar. In each experiment, $[Cl^-]_i$ fell to levels of less than 2 mequiv/kg cell water per h. In four of the experiments, $[Cl^-]_i$ was too low to measure. These studies indicate that Cl^- movements are exceedingly rapid and that essentially all of intracellular Cl^- is available for diffusion.

Na^+ efflux

Na^+ efflux involves at least 2 constants as shown in Fig. 1, which pools all of the values obtained in 13 experiments. There is a rapid component with a $t_{0.5}$* of 10.2 ± 6.4 (S.D.) min and a slow component with a $t_{0.5}$ of 24.3 ± 9.2 min. The absolute efflux of the rapid phase averaged 636 ± 302 mequiv/kg cell water per h, and the slow phase averaged 329 ± 125 mequiv/kg cell water per h.

As indicated in Table III, there was no significant difference in the rapid phase between untreated cells and those treated with 10^{-3} M ouabain. However, during the slow phase, efflux was significantly slower in ouabain-treated as compared with control cells. The percent reduction in efflux with ouabain inhibition averaged 60 % and in terms of absolute reduction amounted to 133 mequiv/kg cell water per h. This finding is consistent with the high activity of $(Na^+$–$K^+)$-dependent ATPase which has been reported in this cell[7].

* $t_{0.5}$, time required for 50 % equilibrium between extra- and intracellular phases.

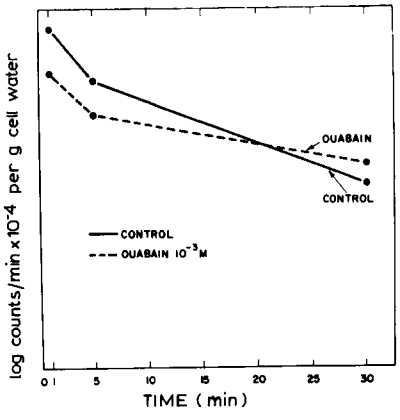

Fig. 1. Semi-log plot of loss of ^{22}Na from pre-labelled alveolar macrophages under control conditions and during exposure to 10^{-3} M ouabain octahydrate. Each point represents the mean of 13 studies.

TABLE III-A

CONTROL MEASUREMENTS OF $t_{0.5}$ AND Na^+ EFFLUX

No.	*Rapid phase*		*Slow phase*	
	$t_{0.5}$ *(min)*	Na^+ *efflux (mequiv/kg cell water per h)*	$t_{0.5}$ *(min)*	Na^+ *efflux (mequiv/kg cell water per h)*
1	4.8	426.6	13	570.2
2	3.5	962	13	382.2
3	17	955.9	32.5	360.3
4	9.8	228.4	23	113.6
5	9.5	634.7	29.5	316.2
6	8.5	232.3	39	204
7	8.5	954.3	27.3	238.4
8	28	747.2	40	268.1
9	13.3	273.7	12.3	282.5
10	9.8	375	26	520.6
11	3.3	627.4	20.5	221
12	11	679.2	13.5	462.6
13	5	1177.6	26.5	333.8
Mean ± S.D.	10.2 ± 6.4	636.5 ± 302.5	24.3 ± 9.2	328.7 ± 125.4

TABLE III-B

THE EFFECT OF OUABAIN ON $t_{0.5}$ AND Na^+ EFFLUX

N.S., difference of means not significantly different.

No.	*Rapid phase* $t_{0.5}$ *(min)*		*Na^+ efflux (mequiv/kg cell water per h)*		*Slow phase* $t_{0.5}$ *(min)*		*Na^+ efflux (mequiv/kg cell water per h)*	
	Control	*Ouabain*	*Control*	*Ouabain*	*Control*	*Ouabain*	*Control*	*Ouabain*
1	9.5	11.8	747.2	288.3	29.5	40	307.8	146.6
2	8.5	20.5	273.7	517.9	27.3	32.5	238.4	47.6
3	28	17.5	375	503.2	40	45	268.1	239.8
4	13.3	11	627.4	250	12.3	45	282.5	245.6
5	9.8	21.5	679.2	250.9	26	36	520.6	182.7
6	3.3	11.3	1177.6	333.8	20.5	28	221	186.5
7	11	23	954.3	1209.9	13.5	46	426.6	196.2
8	5	6.2			26.5	33.6	333.8	324.7
Mean ± S.D.	11.1 ± 7.1	15.4 ± 5.7	690.6 ± 289.7	479.1 ± 315.8	24.5 ± 8.4	38.3 ± 6.3	329.4 ± 100.5	196.2 ± 75.6
	N.S.		N.S.		$0.025 < P > 0.01$		$0.025 < P > 0.01$	

Ferritin influx and efflux

Ferritin influx is rapid and within 3 min intracellular ferritin concentrations greatly exceed extracellular concentrations. Following this initial phase, intracellular concentrations rise at a slower rate (Fig. 2). Ferritin efflux from pre-labelled cells is exceedingly slow (Fig. 3). The $t_{0.5}$ (four studies) averaged 40.0 ± 10.4 h.

Data relevant to the effect of ferritin uptake on dextran (mol. wt. 75000) uptake are summarized in Table IV. Intracellular/extracellular ratios of ferritin are greater

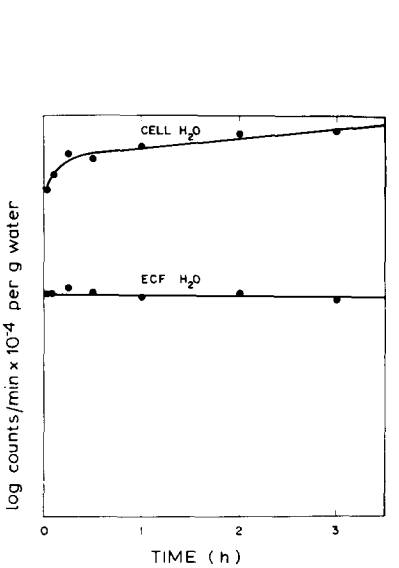

Fig. 2. Semi-log plot of a typical study of [^{3}H]ferritin uptake by alveolar macrophages. Note that intracellular/extracellular radioactivity is greater than 1.0 even by 3 min. ECF = extracellular fluid.

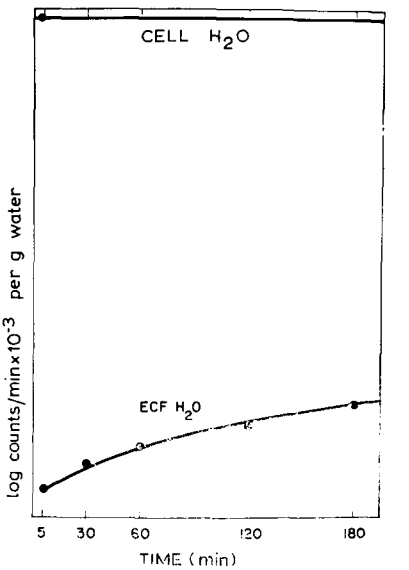

Fig. 3. Semi-log plot of a typical study of loss of radioactivity from alveolar macrophages pre-labelled with [^{3}H]ferritin. ECF = extracellular fluid.

TABLE IV

THE EFFECT OF FERRITIN INFLUX ON UPTAKE OF DEXTRAN (mol. wt. 75000) BY ALVEOLAR MACROPHAGES

$$R_{dextran} = \frac{\text{mg dextran/g cell water}}{\text{mg dextran/g extracellular fluid water}}$$

$$R_{ferritin} = \frac{\text{counts/min per g cell water}}{\text{counts/min per g extracellular fluid water}}$$

Expt. No.	$R_{dextran}$		$R_{ferritin}$
	Without ferritin	*With ferritin*	
1	0.050	0.055	14
2	0.034	0.034	17
3	0.038	0.038	12
4	0.029	0.025	8
5	0.039	0.029	8
Mean	0.038	0.036	

than 1.0 in all studies. Dextran concentrations in the cellular mixture are essentially the same in cells not exposed to ferritin as in the exposed cells. Moreover, the ratio $[\text{dextran}]_i/[\text{dextran}]_e$ are all below 0.06, suggesting no penetration.

DISCUSSION

Data concerning ion transport in alveolar macrophages are lacking. However, there have been a number of studies in leukocytes. In the early 1900's, HAMBURGER AND VAN DER SCHROEFF[8], using pus cells from septic abscesses and lymphocytes from minced lymph nodes, were able to demonstrate rapid exchanges of intracellular Cl^- for bicarbonate, sulfate or nitrate from the medium. Mixed horse leukocytes were reported by FLEISCHMANN[9] to be permeable to the anion iodide, thiocyanate and salicylate but not to the cations barium and calcium. The high permeability of the leukocyte to water was emphasized by several workers[10,11] who pointed out that the distensible, gelatinous wall of this cell (in contrast to the relatively inelastic erythrocyte plasma membrane) permitted extensive swelling in water, without rupture, thus demonstrating osmometer-like qualities.

WILSON AND MANERY[5] performed careful measurements of inorganic ion composition and ionic permeability of rabbit leukocytes obtained from sterile peritoneal exudates. In particular, they employed dye tests to insure the viability of the preparation. After 45 min in an aerated, phosphate buffered, Ringer–Dale solution (pH 7.5) containing glucose; Na^+, 167 mequiv; Cl^-, 145 mequiv; and K^+, 4.0 mequiv per l, the leukocyte contained 79.2 % water, 80 mequiv Na^+, 106 mequiv of K^+ and 93 mequiv of Cl^- per l of cell water. These values do not differ substantially from the values for alveolar macrophages found in the present study. Moreover, $[Cl^-]_i$ bore a linear relationship to $[Cl^-]_e$, Cl^- appearing to exchange for sulfate. The loss of Na^+ and K^+ in low Na^+ or K^+ medium and the rapid uptake of radioactive Na^+ or K^+ suggested high permeability to these ions. In spite of this, rabbit leukocytes maintained substantial ionic gradients between cell water and the external medium.

The present studies indicate that ion transport in the alveolar macrophage resembles that in leukocytes generally. As described above, the steady-state composition of this type of cell can be classified as "intermediate Na^+, intermediate K^+", with concentration values of these ions in cell water falling between the Na^+–K^+ concentrations in low Na^+ erythrocytes like those of rabbit[12] or human[13] and the high Na^+ erythrocyte like that of the cat[14], dog[15] and seal[16].

Na^+ permeability* in the macrophage is impressively high as compared to the erythrocyte and the macrophage demonstates an impressive rate of active Na^+ transport as shown by the ouabain inhibition studies. The ouabain sensitive efflux averaged 133 mequiv/kg cell water per h as compared with a value of 1.6 mequiv/l cells per h in the human erythrocyte[13]. In a number of systems the absolute rate of active Na^+ transport correlates with intracellular (Na^+–K^+)-ATPase activity and this is true of the alveolar macrophages, high ATPase activities in the alveolar macrophages having been reported[7].

It may be visualized that the high permeability leads to a large inward leak

* The term, "permeability", is used since studies on leukocytes[5] and preliminary studies in our laboratory on the alveolar macrophages suggest that ^{23}Na movement is exceedingly rapid.

of Na^+ down its electrochemical gradient requiring a high rate of active transport to maintain constant intracellular Na^+ activity and prevent intracellular edema.

The energetic aspects of active Na^+ transport in this cell are of considerable interest. The minimal work of active Na^+ transport may be calculated on the basis of relatively simple thermodynamic considerations as follows[17]:

$$W = n\left(RT \ln \frac{[Na^+]_i}{[Na^+]_e} + F(\psi_i - \psi_0)\right)$$

where W = work in cal/kg water per h; n = moles of Na^+ activity transported/kg water per h; R = universal gas constant in cal/degrees; T = absolute temperature; F = Faraday in cal/V; $\psi_i - \psi_0$ = transmembrane potential. Assuming that Cl^- is in thermodynamic equilibrium, $(\psi_i - \psi_0)$ can be calculated from $[Cl^-]_i/[Cl^-]_0$. If n = moles of Na^+ that are inhibited by ouabain and thus represents the fraction of transport that is endergonic, it may be calculated that approx. 56 cal/kg water per h represents the minimal work cost. Similar calculation of minimal work cost of Na^+ transport in human erythrocytes indicate that approx. 6 cal/kg water per h are required for Na^+ transport. The high energy requirement of Na^+ transport in the macrophage suggests that Na^+ transport should be quite sensitive to ATP limitation.

The basal rate of lactate generation in the alveolar macrophages may be estimated as approx. 3.0 mmoles/kg water per h (see ref. 18). Assuming an energy yield of 14 cal/mole per h in ATP equivalents, it would appear that anaerobic glycolysis would provide only 42 cal/kg water per h, suggesting that a substantial portion of the energy for Na^+ transport must be derived from oxidative phosphorylation.

Cl^- permeability appears to be substantially higher than Na^+ permeability. The data are consistent with (although not rigorously establishing) rapid thermodynamic equilibrium between intra- and extracellular phases of this ion.

An important aspect of macrophage function involves transport of relatively large molecules. On one hand, the cell membrane appears to resemble the plasma membrane of most cells since a wide variety of uncharged molecular species like sucrose and dextran are excluded from cell water. In addition, high molecular anionic dyes like eosin y (mol. wt. 691000) are excluded from the cytoplasm of most living cells including the macrophage but rapidly penetrate the cytoplasm after cell death.

On the other hand, unlike most cells, specific mechanisms are available in the macrophage for the transport of other macromolecular species. In the present studies, the transport of ferritin (mol. wt. 465000) has been investigated.

The uptake of macromolecular species present in solution is usually classified as pinocytosis and this term may be used to describe the uptake of ferritin by macrophages. This is not entirely satisfactory since there are no precise physiological criteria for describing the process of pinocytosis.

The uptake of ferritin involves at least 2 rate constants, one of them being rapid, so that even by 3 min intracellular/extracellular concentration ratios are greatly in excess of 1. Anatomically, uptake seems to involve 2 components: (a) diffuse cytoplasmic deposition, and (b) sequestration by membrane-bound intracellular (? lysozomal) vacuoles. Sequential studies suggest that with time most of the cytoplasmic ferritin becomes incorporated into membrane-bound vesicles[19]. Intracellular sequestration is quite tight, leading to very slow efflux. Once having entered the cell, ferritin is essentially unavailable to the extracellular phase. Aside from

the rapidity and largely unidirectional nature of the transport process, the degree of chemical specificity is surprising. Uptake of ferritin occurs without substantial increase in dextran space. This finding implies a highly discrete and specific process capable of distinguishing between ferritin and dextran. Moreover, electron microscopy suggests that ferritin uptake requires a living cell, ferritin being largely excluded from apparently dead macrophages[19]. Thus, the living cell excludes eosin and takes up ferritin (chemical species of approximately the same molecular weight) whereas dead cells take up eosin and exclude ferritin. This also suggests great specificity for the transport processes involved.

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