Transmembrane Potential and Ionic Content of Rat Alveolar Macrophages

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ABSTRACT The cell volume, cell water, intracellular ionic concentrations, and transmembrane potential of rat alveolar macrophages were determined. The measurements were made on cells which had been separated from the medium by centrifugation through dibutyl phthalate in order to greatly reduce the trapped extracellular space. The mean cell volume of the alveolar macrophages is 1,525 cubic microns and 72% of this volume is water. The intracellular fluid is high in Na^+ (97 mM) and lower in K^+ (50 mM) and the intracellular Cl^- concentration is 64 mM. The transmembrane potential, as measured from the equilibrium distribution of tritiated triphenylmethyl phosphonium and by using the fluorescent probe, Di-S-C_3(5), is approximately −37 millivolts. Neither Na^+, K^+, nor Cl^- is distributed at equilibrium. However, the K^+ permeability of alveolar macrophage membranes appears to be greater than Na^+ permeability.

Alveolar macrophages are free cells found in the small airways and alveoli of the lungs. They are scavenger cells and their major role is to protect the lungs by phagocytizing foreign particles and bacteria which enter the respiratory tract. In addition to engulfment of foreign material, exposure of alveolar macrophages to particles leads to release of antibacterial substances from the cells. These antibacterial substances are various reactive forms of oxygen. For example, particle exposure in phagocytic cells leads to the production of superoxide anion (Drath and Karnovsky, '75), hydrogen peroxide (Gee et al., '70; Klebanoff and Hamon, '76), and possibly singlet oxygen and hydroxyl radical (Miles et al., '78). Thus, the process of phagocytosis and the release of antibacterial substances are important functions of alveolar macrophages.

The cellular events which precede, or act as signals for, phagocytosis and release of antibacterial substances are as yet unknown. Recently, it has been suggested that changes in transmembrane potential play an important role in the function of phagocytic cells. This suggestion is based on some observed changes in transmembrane potential which occur during phagocytosis. For example, Gallin and Gallin ('77) have reported hyperpolarization of human macrophages obtained from blood in response to chemotactic factors. In human polymorphonuclear leukocytes which have been treated with concanavalin A, a substance which binds to glycoproteins on cell membranes, a triphasic change in transmembrane potential has been reported (Korchak and Weissmann, '78). Thus, changes in membrane potential may be an important part of the phagocytotic process, including the release of antibacterial substances. However, very little is known about the transmembrane potential, ionic content, and membrane transport properties of alveolar macrophages.

The objective of this investigation was to determine the transmembrane potential and ionic contents of rat alveolar macrophages at rest, i.e., in the absence of phagocytosis. The following measurements were made: transmembrane potential, cell volume, cell water content, and the intracellular concentrations of sodium, potassium, and chloride ions. The determinations were made by using cell samples which had been centrifuged through dibutyl phthalate, so that there was a mini-

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mal amount of trapped extracellular space to interfere with the measurements. A preliminary report of these results has appeared previously (Castranova et al., '79).

MATERIALS AND METHODS

Preparation of cells

Alveolar macrophages were harvested from male Long-Evans hooded rats (230-300 gm) by tracheal lavage according to the method of Myrvik et al. ('61). Rats were anesthetized by intraperitoneal injection with sodium pentobarbital (0.2 mg/gm body weight) and exsanguinated by cutting the abdominal aorta. The lungs from each rat were lavaged approximately 12 times with a total of 80 ml of ice cold lavage fluid of the following composition: 145 mM NaCl, 5 mM KCl, 9.35 mM Na_2HPO_4, 1.9 mM NaH_2PO_4, and 5 mM glucose (pH = 7.4). The cells were separated from the lavage fluid by centrifugation at 500 × *g* for 5 minutes at 2°C. Cells from different animals were pooled and washed twice by alternate resuspension and centrifugation in ice cold HEPES-buffered medium (140 mM NaCl, 5 mM KCl, 10 mM Na HEPES (N-2-hydroxyethyl piperazine-N'-2-ethane sulfonic acid), and 5 mM glucose (pH = 7.4)). The cells were then resuspended in the HEPES-buffered medium so that the final concentration was approximately 5×10^7 cells per ml.

Aliquots (150 μl) of this cell suspension were added to 400-μl microcentrifuge tubes over a 100-μl cushion of dibutyl phthalate doped with mineral oil. The density of the dibutyl phthalate-mineral oil mixture was 1.025 grams per ml. The alveolar macrophages were separated from the medium by centrifugation for 1 minute through this cushion using a microcentrifuge (Eppendorf Model 5412. Brinkman Instrument Co., Westbury, New York). The cell pellets, obtained in this manner, were used for the following measurements: cell volume, cell count, trapped extracellular space, intracellular ion content, cell H_2O content, and transmembrane potential. Viability of the cells in these pellets was determined by assessing the ability of the alveolar macrophages to exclude trypan blue dye with light microscopy (Phillips, '73).

Determination of cell volume and cell count

After centrifugation, the supernatant and dibutyl phthalate layers were aspirated and the cell pellet was resuspended in an isotonic medium (Isoton, Curtin Matheson, Scientific, Cleveland, Ohio). The number of alveolar macrophages in the sample and the cell volume were determined by using a Coulter Model Z_B electronic cell counter interfaced with a Channelyzer II cell sizing attachment (Coulter Instrument Co., Hialeah, Florida).

Measurement of trapped extracellular space

The volume of medium present in the cell pellet after centrifugation through the dibutyl phthalate cushion was determined by using tritiated inulin. In these experiments, 5 μCi of tritiated inulin (New England Nuclear Corp., Boston, Massachusetts) was added to the cell suspension. This suspension was then layered on a dibutyl cushion and centrifuged as described above. A sample of the supernatant was taken, mixed with 10% trichloroacetic acid (TCA), added to a fluor (Aquasol, New England Nuclear Corp., Boston, Massachusetts), and the dpm per ml of medium determined using a liquid scintillation counter with automatic quench correction (Model 544, Packard Instrument Co., Downers Grove, Illinois). The remainder of the supernatant was aspirated and the centrifuge tube rinsed twice with incubation medium to remove any isotope due to residual supernatant. This fluid and the dibutyl phthalate layer were aspirated. The cell pellet was dissolved in 100 μl of 10% TCA to precipitate the protein. This mixture was centrifuged, an aliquot of the resultant supernatant added to Aquasol, and the dpm in the cell sample determined. From these data, the volume of extracellular fluid trapped in the cell pellet was determined by dividing the dpm in the cell pellet by the dpm per ml of medium.

Measurement of cell water content

Cell pellets were weighed immediately after aspiration of the supernatant and dibutyl phthalate (wet weight). These cell pellets were dried overnight in an oven which was maintained at 80°C and then weighed again (dry weight). The cell H_2O (per-cent) was calculated from these wet weight-dry weight measurements after correcting for trapped extracellular space.

Measurement of intracellular concentrations of Na^+, K^+, and Cl^-

Cell pellets were obtained by centrifugation through dibutyl phthalate, the supernatant aspirated, and the tubes rinsed with incubation medium to remove residual supernatant.

This fluid and the dibutyl phthalate layer were then aspirated and the cell pellet was dissolved in 500 μl of 0.1% Triton × 100. For determinations of Na^+ and K^+, $LiNO_3$ (final concentration = 15 mM) was added to this solution and Na^+ and K^+ levels were determined by using a flame photometer with an internal Li standard (Model 143, Instrumentation Laboratory, Inc., Lexington, Massachusetts). For determination of intracellular Cl^-, cell pellets were dissolved in 0.1% Triton × 100 as described above. Aliquots were taken, diluted with 0.1 N HNO_3 plus 10% acetic acid, and Cl^- determined by using a chloridometer (Buchler-Cotlove, Buchler Instruments, Inc., Fort Lee, New Jersey). The values for intracellular Na^+, K^+, and Cl^- were corrected for trapped extracellular space as measured by tritiated inulin and expressed as millimoles per liter of cell water.

Measurement of transmembrane potential

The transmembrane potential of alveolar macrophages was measured with two different techniques: (1) determination of the equilibrium distribution ratio of the lipophilic cation, tritiated triphenylmethyl phosphonium bromide ($TPMP^+$), and (2) by using the fluorescent probe Di-S-C_3(5). The measurements with $TPMP^+$ (New England Nuclear Corp., Boston, Massachusetts) were obtained by using a method similar to that of Schuldiner and Kaback ('75). In these experiments, 5 μCi of $TPMP^+$ was added to 150-μl aliquots of the cell suspension and the suspensions were incubated at 37°C. At various times after addition of isotope, the cell suspension (150 μl) was layered on to a 100-μl cushion of dibutyl phthalate and the cell pellet was obtained as described previously. A sample of the supernatant was taken, mixed with 10% TCA, added to Aquasol, and the dpm per ml of medium determined by liquid scintillation counting. The remainder of the supernatant was aspirated and isotope due to residual supernatant removed by rinsing the centrifuge tube twice with incubation medium. This fluid and the dibutyl phthalate cushion were then aspirated. The cell pellet was dissolved in 100-μl of 10% TCA. This mixture was centrifuged and an aliquot of the supernatant was added to Aquasol. The dpm in the cell sample were determined and corrected for trapped extracellular space. The time course for equilibration of $TPMP^+$ was determined in this manner. It was found that net uptake is complete after 30 minutes of incubation at 37°C. Therefore, membrane potential was calculated from the distribution of $TPMP^+$ after 30 minutes of incubation by using the Nernst equation as follows:

$$E_m = -61 \log \frac{[TPMP^+]_i}{[TPMP^+]_o}$$

where $[TPMP^+]_i$ is dpm per ml cell water and $[TPMP^+]_o$ is dpm per ml of extracellular fluid.

Membrane potential was also measured by using the fluorescent dye, Di-S-C_3(5) (Hoffman and Laris, '74). Fluorescence was measured with a fluorescence spectrophotometer (Model MPF-3L, Perkin-Elmer Corp., Norwalk, Connecticut) fitted with a magnetic stirrer. Excitation and emission wavelengths were set at 622 and 665 nm, respectively. For these experiments, alveolar macrophages were harvested by tracheal lavage as described above. The cells were then washed twice in ice cold incubation medium (140 mM NaCl, 1 mM KCl, 10 mM Na HEPES, and 5 mM glucose at pH = 7.4). Then 4 × 10^6 cells were resuspended in 3 ml of incubation media of various potassium concentrations, i.e., NaCl replaced with KCl. Di-S-C_3(5) (0.66 μg/ml) was added to each suspension and equilibrated at 22°C until the fluorescence level was steady. The fluorescence level of each suspension was recorded and changes in fluorescence were initiated by the addition of the K^+ ionophore, valinomycin (final concentration = 10^{-6} M). (Stock solutions of fluorescent dye and valinomycin were made in ethanol.) In this manner, the null point for K^+, i.e., the extracellular concentration of K^+ at which there is no change in fluorescence upon the addition of valinomycin, was determined (Parker et al., '77). At the K^+ null point, the membrane potential can be estimated from the Nernst potential for K^+ as follows:

$$E_m = -58 \log \frac{[K^+]_i}{[K^+]_o^*}$$

where $[K^+]_i$ is the intracellular concentration of K^+ in millimoles per liter of cell H_2O and $[K^+]_o^*$ is the extracellular K^+ concentration at the null point in millimoles per liter of medium. Intracellular K^+ concentrations and cell H_2O content were measured as described above.

There are several lines of evidence which indicate that Di-S-C_3(5) is not toxic to cells. Parker et al. ('77) have reported that the membrane permeability of dog red blood cells is not affected by this fluorescent probe. Fur-

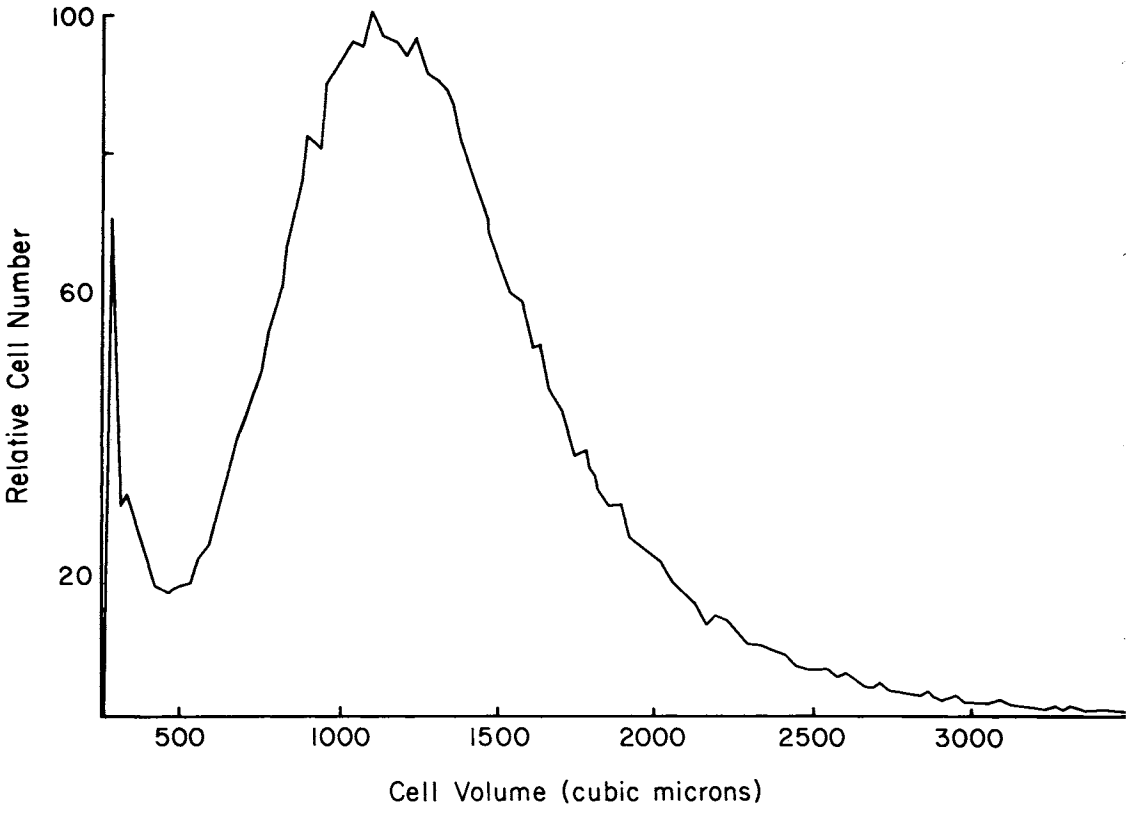

Fig. 1 Distribution of cell volumes for alveolar macrophages from one rat obtained by using a Coulter Model Z_B electronic cell counter with a cell sizing unit. Similar plots were obtained with cells from other animals. The mean value ± the standard error of the mean for mean cell volume from ten experiments is 1,525 (±55) cubic microns.

TABLE 1

Characteristics of cell pellets after centrifugation through dibutyl phthalate

Cell count ($\times 10^{-6}$ cells/pellet)	Volume of pellet (μl)	Trapped extracellular space (% pellet volume)	Cell viability (%)
7.1±0.5	10.9±0.8	1.3±0.2	84±2

These values shown are mean values ± the standard errors of the means for ten experiments (cell count, volume of pellet, and trapped extracellular space) or three experiments (cell viability).

thermore, we find that the ionic content (Na^+, K^+, and Cl^-) and cell volume of rat alveolar macrophages are unaffected by incubation with dye for 10 minutes at 22°C, i.e., conditions similar to those used to measure membrane potential. Therefore, Di-S-C_3(5) can be used to measure E_m in rat alveolar macrophages.

RESULTS

Most of the data presented in this paper were obtained by using a microcentrifuge to spin the alveolar macrophages through a dibutyl phthalate cushion. The general characteristics of the cell pellets obtained with this procedure are given in table 1. The cell pellets have a workable volume of about 11 μl and contain approximately 7×10^6 alveolar macrophages. The cell ets are almost free of trapped extracellular fluid; i.e., only about 1.3% of the pellet volume is extracellular space. These data also indicate that the cell viability, which is approximately 84%, is not greatly affected by this procedure. Therefore, with this technique for cell sampling a relatively small number of rat alveolar macro-

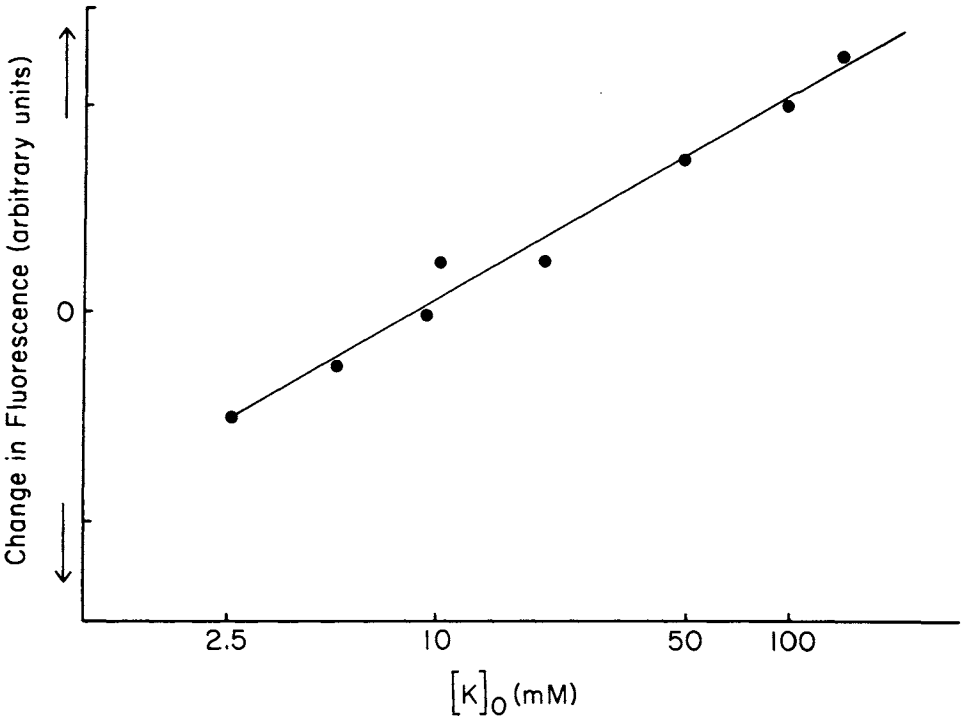

Fig. 2 Changes in the fluorescence of Di-S-C_3(5) as a function of the external K^+ concentration. Rat alveolar macrophages (4×10^6 cells) were added to 3 ml of HEPES-buffered media of various K^+ concentrations. Di-S-C_3(5) was added to these cell suspensions at 0.66 μg/ml final concentration and the fluorescence level recorded using an excitation wavelength of 622 nm and an emission wavelength of 665 nm. Changes in fluorescence intensity were measured after the addition of valinomycin (1×10^{-6} M), a K^+ ionophore. ↑ indicates an increase in fluorescence while ↓ indicates a decrease in fluorescence. From this graph the external K^+ concentration at the null point, i.e., 0 change in fluorescence, was determined. The transmembrane potential of the alveolar macrophages was calculated with the Nernst equation by using this external K^+ concentration and the internal K^+ concentration measured in this study. This figure contains data from a typical experiment. The value for E_m calculated from this experiment is about −41 mV.

TABLE 2

Intracellular water content and ionic concentrations of rat alveolar macrophages

H_2O (% cell weight)	$[Na]_i$ (mmol/1 cell H_2O)	$[K^+]_i$ (mmol/1 cell H_2O)	$[Cl^-]_i$ (mmol/1 cell H_2O)
72±1	97±7	50±4	64±5

The values shown are mean values ± the standard errors of the means for ten experiments.

phages can be used, rapid cell sampling is allowed, and a viable cell pellet which contains a minimal amount of trapped extracellular space is obtained.

The distribution of cell volumes for rat alveolar macrophages obtained with this technique is shown in figure 1. The size of the alveolar macrophages at the peak of the distribution is about 1,000-1,100 cubic microns. However, the distribution is skewed to the right, so that the mean cell volume is actually

TABLE 3

Transmembrane potential (E_m) of rat alveolar macrophages

E_m determined with $TPMP^+$	E_m determined with Di-S-C_3(5)
−34±4 mV	−40±2 mV

Transmembrane potential was determined from the equilibrium distribution of tritiated triphenylmethyl phosphonium ($TPMP^+$) and by using the fluorescent probe, Di-S-C_3(5). The values shown are mean values ± the standard errors of the means for ten experiments ($TPMP^+$) or three experiments (Di-S-C_3(5)).

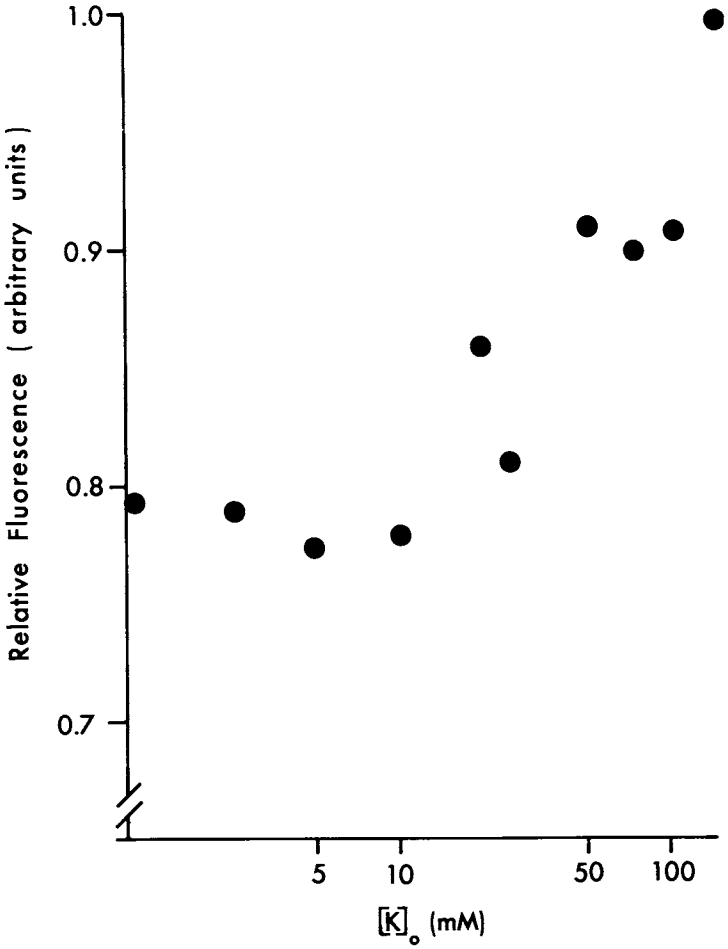

Fig. 3 Relative changes in baseline fluorescence intensity of Di-S-C_3(5) as a function of the external K^+ concentration. This figure contains data from a typical experiment.

1,525 (±55) cubic microns. If one assumes that macrophages are spherical when in suspension, the mean diameter of these cells is approximately 14 μ. This value for the diameter of alveolar macrophages is similar to that reported by Weibel ('73). The initial peak in figure 1 is electronic noise and the second, smaller peak is caused by contaminating cells which represent less than 5% of the cell sample. The identity of these smaller cells has not been positively determined. They may be lymphocytes or immature alveolar macrophages. The relative homogeneity of cells obtained from tracheal lavage in rats is much different from cells harvested from dog lungs where a great deal of heterogeneity has been reported (Lee et al., '78).

The cell water content and the intracellular concentrations of sodium, potassium, and chloride ions in rat alveolar macrophages were measured. The results are shown in table 2. The cell water content, which is approximately 72%, is similar to that found in most cells. Rat alveolar macrophages contain approximately 97 mM Na^+, 50 mM K^+ and 64 mM Cl^-. The cation content is very unusual. Most cells in the rat contain a large amount of K^+ and a small amount of Na^+. In fact, this is the case for most mammalian cells. Thus, rat alveolar macrophages differ from most mammalian cells in that they contain more Na^+ than K^+.

The transmembrane potential (E_m) of rat alveolar macrophages was estimated from the

TABLE 4

Ionic concentrations and equilibrium potentials for Na^+, K^+, and Cl^- in rat alveolar macrophages

Ion	Extracellular concentration (millimoles/1)	Intracellular concentration (millimoles/1 cell H_2O)	Equilibrium potential (millivolts)
Na^+	150	96	+12
K^+	5	50	−61
Cl^-	145	64	−22

equilibrium distribution of the radioactive lipophilic cation, tritiated triphenylmethyl phosphonium ($TPMP^+$). At equilibrium, the membrane potential can be calculated from the Nernst potential for $TPMP^+$. The results of these experiments are shown in table 3. These data indicate that rat alveolar macrophages possess a sizable transmembrane potential of −34 millivolts with the cytoplasm being negative with respect to the extracellular fluid.

Membrane potential in rat alveolar macrophages was also determined by using the fluorescent probe, Di-S-C_3(5). Hoffman and Laris ('74) have shown in red blood cells that the fluorescence of this dye decreases as the E_m becomes more negative and increases with E_m changes in the positive direction. The data in figure 2 show that the intensity of fluorescence, and thus the E_m, changes with the K^+ ionophore, valinomycin, is added to macrophages suspended in media of differing K^+ concentrations. At low external K^+ concentrations valinomycin causes membrane hyperpolarization and at high K^+ concentrations depolarization occurs. Thus, addition of valinomycin to alveolar macrophages causes the transmembrane potential to shift toward the equilibrium potential for K^+. At the null point, i.e., at the external K^+ concentration where valinomycin causes no change in fluorescence intensity, the transmembrane potential can be estimated from the Nernst potential for K^+. The transmembrane potential calculated in this manner is shown in table 3. The value of −40 millivolts is very close to the estimate of membrane potential obtained by using $TPMP^+$. Therefore, the data obtained by using these two different techniques indicate that the E_m of rat alveolar macrophages is approximately −37 mV.

The effect of the external K^+ concentration on baseline fluorescence intensity, i.e., the level of fluorescence in the absence of valinomycin, was also studied. The results, which are shown in figure 3, indicate that baseline fluorescence is dependent upon the extracellular K^+ concentration. Baseline fluorescence increases (i.e., membrane potential becomes less negative) as the external K^+ concentration is increased above 5 mM. There is also another line of evidence to suggest that the resting membrane potential of alveolar macrophages is dependent upon extracellular K^+. The equilibrium distribution of $TPMP^+$ was measured in alveolar macrophages which had been incubated for 30 minutes in extracellular fluid containing 150 mM K^+ and in a solution containing 1 mM K^+. E_m was 14 millivolts less negative in 150 mM K^+ than in 1 mM K^+. These results indicate that the permeability of alveolar macrophage membranes to K^+ is greater than the permeability to Na^+.

The equilibrium potentials for Na^+, K^+, and Cl^- are shown in table 4. These values were calculated from the ionic concentrations measured in this study by using the Nernst equation. Since the transmembrane potential of rat alveolar macrophages is −37 millivolts, neither Na^+ nor K^+ is in equilibrium. In fact, there is a net electrochemical gradient favoring Na^+ influx and K^+ efflux. These data suggest that rat alveolar macrophages possess an active mechanism for K^+ influx and Na^+ efflux which maintains the steady state concentrations of these cations. It is also of interest to note that these data suggest that Cl^- is not in equilibrium, i.e., the intracellular concentration of Cl^- is too high.

DISCUSSION

Almost all of the measurements in this study were made on alveolar macrophages which were separated from the extracellular fluid by spinning through a dibutyl phthalate cushion. The advantages of utilizing this technique are that a relatively small number of cells can be used and a cell sample which is almost free of trapped extracellular space is obtained. There are four lines of evidence which indicate that centrifugation through dibutyl phthalate does not cause cell injury or

introduce errors into the measurements: (1) measurement of trypan blue exclusion indicates that cell viability remains high after separation, (2) the volume distribution for alveolar macrophages centrifuged through dibutyl phthalate does not differ from that obtained in cells treated in the conventional manner (data not reported here), (3) the value for E_m determined with the fluorescent probe is similar to that estimated by using $TPMP^+$, even though cells used in the $TPMP^+$ experiments were spun through the cushion while those used for the fluorescent dye measurements were not, and (4) oxygen consumption is the same in alveolar macrophages centrifuged through dibutyl phthalate as in normal cells (data not reported here). Thus, the data reported here are not artifacts of the method.

Measurement of the intracellular concentrations of sodium, potassium, and chloride ions in rat alveolar macrophages indicates that these cells are of the high Na^+-low K^+ type. The values we measured are close to those measured by Robin et al. ('71) in rabbit alveolar macrophages. Reports concerning the ionic composition of other phagocytic cells, such as rabbit polymorphonuclear leukocytes (Naccache et al., '77) human polymorphonuclear leukocytes (Dunham et al., '74), and human lymphocytes (Negendank and Shaller, '79), indicate that these cells are of the high K^+-low Na^+ type. Therefore, the ionic content of alveolar macrophages seems to be different from that of other phagocytic cells.

Lichtman and Weed ('69) have shown that leukocytes have a low K^+/Na^+ ratio immediately after isolation of the cells. Then the cells revert to a high K^+/Na^+ ratio after incubation at 37°C. They suggested that the initial low K^+/Na^+ ratio was an artifact and was caused by the trauma of treatment with 150 mM NH_4Cl which was used during the isolation procedure. Then during subsequent incubation, the leukocytes recovered to their normal state, i.e., a high K^+/Na^+ ratio. However, unlike the situation with leukocytes the low K^+/Na^+ ratio reported here for rat alveolar macrophages does not seem to be an artifact caused by the isolation procedure for two reasons: (1) the isolation procedure for alveolar macrophages does not involve harsh treatment of the cells (e.g., exposure to NH_4Cl) as is the case for leukocytes, and (2) cell H_2O content, intracellular Na^+ and K^+ content, and cell volume remain unchanged in alveolar macrophages after one hour of incubation at 37°C.

We have measured a membrane potential of -37 mV across the rat alveolar macrophage membrane. This value is greater than membrane potentials reported for other types of macrophages. For example, Gallin and Gallin ('77) reported a potential of -14.5 mV in human macrophages isolated from blood, while Gallin et al. ('75) and Dos Reis and Oliveira-Castro ('77) reported potentials of -13 mV and -26 mV for peritoneal macrophages from guinea pigs and mice, respectively. Two possibilities exist to explain the differences in these values. First, cells used for these studies differ in species and origin. Second, in all measurements except the ones done in the present study microelectrodes were used to measure E_m, and it is possible that leakage occurs around the microelectrodes. Therefore, one would expect to measure larger potentials with $TPMP^+$ and the fluorescent dye. This explanation has been used by Laris et al. ('76) to interpret differences in the E_m of Ehrlich ascites tumor cells estimated from fluorescent probe and microelectrode techniques. In addition to macrophages, a wide range of values have been reported for the membrane potentials of other phagocytic cells. Holian et al. ('77) used $TPMP^+$ distribution and found an E_m of -73 mV in human lymphocytes. Utsumi et al. ('77) reported an E_m of -70 mV for guinea pig polymorphonuclear leukocytes using the fluorescent probe, while Korchak and Weissmann ('78) used $TPMP^+$ distribution in human polymorphonuclear leukocytes and found the E_m to be -26 mV.

The data in figure 3 suggest that the K^+ permeability of rat alveolar macrophages is greater than the Na^+ permeability. The membrane potentials of other phagocytic cells have also been shown to be K^+ dependent. For example, Utsumi et al. ('77) used a fluorescent dye with guinea pig polymorphonuclear leukocytes and reported K^+-dependent shifts in baseline fluorescence similar to those reported here. In addition, Gallin et al. ('75) reported that E_m is K^+-dependent in guinea pig peritoneal macrophages by using a microelectrode technique, while Korchak and Weissmann ('78) also found K^+ dependence of E_m in human polymorphonuclear leukocytes by using $TPMP^+$ distribution.

Robin et al. ('71) were unable to measure a time course for Cl^- efflux in rabbit alveolar macrophages and concluded that CL^- is dis-

tributed at equilibrium in these cells. We used their data to calculate the Nernst potential for Cl^- and obtained a value of -25 mV. This value is not as large as the membrane potential of rat alveolar macrophages reported in the present study. In addition, our data suggest that intracellular Cl^- is too high for Cl^- to be in equilibrium in rat alveolar macrophages. However, it is possible that we could have overestimated the intracellular Cl^- level in these cells if Cl^- is sequestered into the nucleus. Indeed, this explanation has been suggested by Laris et al. ('76) to resolve a similar discrepancy in Ehrlich ascites tumor cells.

In summary, rat alveolar macrophages differ from other cells in the rat, and from most mammalian cells, in that they contain more Na^+ than K^+. The transmembrane potential of the cells is approximately -37 millivolts. The data also indicate that neither Na^+, K^+, nor Cl^- is distributed at equilibrium but that the K^+ permeability is relatively high. It is possible that the transmembrane potential plays an important role in the function of phagocytic cells. The fluorescent probe may be a useful tool in studying the relationship between transmembrane potential changes and phagocytosis.

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