Johri[11,12] stressed that the expulsion of larvae were probably due to increased histamine levels in the intestine. Complete larval migration from alimentary tract occurred at 30 h in group C and within 24 h in group D. Larval migration to heart, lung and muscles commenced within 4 h after the 2nd dose in experimental groups C and D whereas, but not in control groups A and B.

The complete elimination of larval burden from alimentary tract in groups C and D indicates that the initial high dose of 1000 and 2000 larvae have imparted severe resistance due to rapid sensitization which become intolerable for the larvae, resulting in their immobilization, death and ultimately expulsion. Murray et al.[13,14] reported vasoactive amines causing the expulsion of *N. brasiliensis* from infected mice. Other pathophysiological changes in intestine due to infection may also act as an effective barrier.

The experiment suggests that the initial low dose of 250 and 500 larvae took some time to induce sufficient resistance in the host for maximum expulsion and the immune response is much greater in high repeated dose than in low repeated doses.

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2 Agarwal, R.K., Vyas, M., Vardhani, V., and Johri, G.N., Bioresearch *3* (1979) 45.
3 Agarwal, R.K., and Johri, G.N., J. Helminth. *54* (1980) 109.
4 Foster, A.O., and Cross, S.K., Am. J. trop. Med. *14* (1934) 565.
5 Nicholas, R.L., J. Parasit. *42* (1958) 363.
6 Kono, M., and Sawada, T., Kitakanto Igaku *11* (1961) 431.
7 Bhopale, M.K., and Johri, G.N., J. Helminth. *49* (1975) 179.
8 Sasada, T., Keio-Igaku *17* (1937) 491.
9 Sen, H.G., Joshi, U.N., and Seth, D., Trans. R. Soc. trop. Med. Hyg. *59* (1965) 684.
10 Scott, J.A., Am. J. Hyg. *8* (1928) 158.
11 Vardhani, V., and Johri, G.N., Indian J. Parasit. *2* (1978) 55.
12 Vardhani, V., and Johri, G.N., Experientia *34* (1978) 122.
13 Murray, M., Miller, H.R.P., Stanford, J., and Jarrett, W.F.H., Int. Archs Allergy appl. Immun. *40* (1971) 236.
14 Murray, M., Jarrett, W.F.H., and Jennings, F.W., Immunology *21* (1971) 17.

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Transmembrane potential of J774.2 mouse macrophage cells measured by microelectrode and ion distribution methods[1]

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Summary. The transmembrane potential (E_m) of J774.2 macrophage cells measured by microelectrodes was -24.1 ± 0.7 mV (mean ± SEM). E_m measured by lipophilic ion distribution was -35 ± 2 mV or -40 ± 2 mV, using a cation or anion, respectively. By any method, colchicine reduced E_m by ~ 3 mV.

Transmembrane potential (E_m) of macrophage cells has been measured by intracellular recording with microelectrodes[3-6], or calculated from lipophilic ion distribution[7], but comparison of these methods in a single cell line has not been made. Therefore, in this study, the E_m of cultured J774.2 mouse macrophages has been determined both by impalement with microelectrodes and by distribution of a lipophilic cation triphenyl methyl phosphonium ($TPMP^+$) or a lipophilic anion, thiocyanate. The effects of the microtubule disrupting agent colchicine have been examined, since this agent alters cell volume and electrolyte composition of J774.2 cells[8] and might therefore modify E_m.

Methods. Cells of the J774.2 mouse macrophage line[9] were grown in Dulbecco's modified Eagle's minimal essential medium, supplemented with 20% horse serum, as previously described[10]. Cells, cultured in monolayer and mounted in 2 ml culture medium (at room temperature or 33–37 °C, containing 10 mM HEPES buffer), were impaled with microelectrodes (3 M KCl-filled, resistance 50–70 MΩ) and voltage signals were recorded using standard techniques.

In order to estimate $TPMP^+$ uptake, cell suspensions ($1-3 \times 10^6$ cells ml^{-1}) were incubated at 37 °C in 5% CO_2 with tritiated $TPMP^+$ (0.1 μCi ml^{-1}, 2.8×10^{-8} moles l^{-1}). Cell volume was estimated in identically treated samples of the cell suspensions from the distribution of tritiated H_2O and ^{14}C inulin. Cpm were measured in cell pellets obtained by spinning samples through silicone fluid. Cellular $TPMP^+$ uptake was calculated and used to estimate E_m from the equation:

$$E_m = -61 \log \frac{(TPMP^+)\ \text{inside}}{(TPMP^+)\ \text{outside}}$$

Thiocyanate distribution was determined in cell suspensions containing 0.1 μCi ml^{-1} ^{14}C KSCN (1.6×10^{-6} moles l^{-1}), using the same protocol.

$(K^+)_{outside}$ was raised in some experiments by adding KCl to the commercially obtained culture medium. Since osmolarity was changed, control experiments were performed in which osmolarity was increased with sucrose, and E_m was found to be unchanged.

Results. The mean E_m, measured by microelectrode impalement, was -24.1 ± 0.7 mV (mean ± SEM, 558 cells from 26 cultures) and the distribution of E_m values is shown in figure 1. The range of E_m-values was large, but more than 75% of cells were in the range -10 to -35 mV. E_m was the same whether measured at 33–37 °C or at room temperature (~ 20 °C). A small number of cells (< 10%) exhibited hyperpolarization during recording (E_m -60 to -75 mV) but, with continuous observation by phase contrast microscopy, it was found that these cells invariably developed surface blebbing, suggesting irreversible cell damage.

E_m in cells incubated with $TPMP^+$ was -35 ± 2 mV (range -27 to -47 mV, 25 experiments). $TPMP^+$ uptake was slow

but complete by 90 min (fig. 2). Thiocyanate uptake was faster and complete by 20 min; calculated E_m was -40 ± 2 mV (range -32 to -51 mV, 21 experiments).

Raising $(K^+)_{outside}$ caused a concentration-related decrease in E_m. Depolarization was considerably less than predicted for a Nernst relationship e.g. increasing $(K^+)_{outside}$ from 5 to 50×10^{-3} moles l^{-1} changed E_m by $+11$ mV compared to $+61$ mV predicted. The changes in E_m as gauged by the different methods, were in good agreement e.g. $+12 \pm 1.5$ mV and $+10 \pm 2$ mV by microelectrodes and $TPMP^+$ distribution respectively, for a 10-fold increase in $(K^+)_{outside}$. At any $(K^+)_{outside}$ E_m was more negative when measured by ion distribution compared to microelectrodes.

Cells treated with colchicine (10^{-6} moles l^{-1}) developed the protuberant shape associated with this drug within 30 min and underwent a reduction in cell volume averaging 15%, a value similar to that previously described[8]. E_m was consistently reduced by a few mV after colchicine treatment, as estimated by each of the 3 methods. The mean changes in E_m were $+3 \pm 1.1$ mV (3 experiments, microelectrodes); $+3.5 \pm 0.6$ mV (10 experiments, $TPMP^+$ distribution); $+3.4 \pm 0.4$ mV (8 experiments, SCN^- distribution).

Discussion. The E_m measured with microelectrodes is close to that obtained with the same technique in other mouse macrophages[3-6]. A wide range of E_m-values was observed which may reflect differing degrees of cell damage or possibly differences in state of activity or position in cell cycle. Gallin and Livengood[5] described a sub-group of cells distinguished by high E_m. In the present experiments a small number of cells with high E_m was observed also, but these cells exhibited abnormal morphology characterized by surface blebbing.

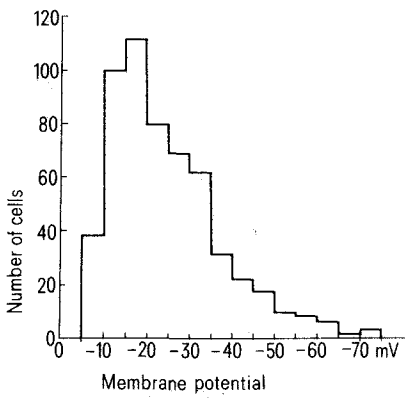

Figure 1. The distribution of individual E_m-values obtained from microelectrode impalement in 558 cells of the J774.2 line.

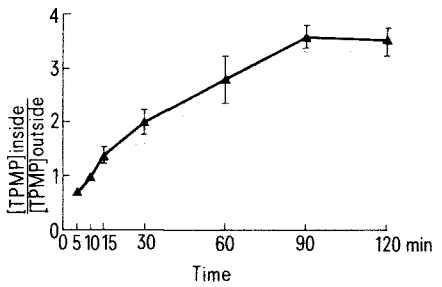

Figure 2. The ratio of internal to external $[TPMP^+]$ in cell suspensions vs period of incubation. Values are the mean of 3-14 observation and bars represent SEM.

A more negative value of E_m was obtained from ion distribution measurements. This value, however, agrees well with the E_m of rat alveolar macrophages estimated by $TPMP^+$ distribution[7]. The smaller E_m measured by microelectrodes is probably due to cell damage upon impalement. Ion distribution measurements are non-invasive and damaged cells (leaky to inulin) are excluded. Cell damage aside, other differences in the 2 methods may be important. Microelectrodes sample E_m in a relatively small number of cells; in contrast a mean E_m for the whole population is obtained using the ion distribution technique. Therefore a difference in estimated E_m could arise if the sample of cells impaled by microelectrodes were not random. Since it is easier to impale large, well-spread cells, E_m measured thus may be representative only of this sub-population of cells. Ion distribution studies are performed in cells in suspension which are typically rounded-up, hence it is interesting to speculate that the difference in E_m is related to the difference in morphology of the cells under the 2 conditions.

The results obtained using thiocyanate distribution correlate well with those using $TPMP^+$ distribution, suggesting that both ions, although oppositely charged, are distributed according to E_m. Binding or compartmentalization of either on the basis of charge would be expected to produce differences in calculated E_m derived from their separate distribution.

The results suggest that K^+ permeability of the cells is low. E_m is far from the equilibrium potential for K^+ (-86 mV, calculated on the basis of previous measurements of (K^+) cells[8]). Raising $(K^+)_{outside}$ causes a far smaller change in E_m than is found in cells freely permeable to K^+, where the Nernst relation holds e.g. skeletal muscle[11].

A slight decrease in E_m was associated with colchicine treatment. Since colchicine increases $(K^+)_{inside}$[8], hyperpolarization might be expected, but interpretation is complicated by changes in the distribution of other ions, such as Na^+ and Cl^-, whose contribution to E_m is not known. The depolarizing effect of colchicine was small, but consistent, and it remains to be shown whether a relationship exists between this and other effects of colchicine on microtubules and membrane function.

In conclusion, although the absolute E_m differed according to the technique used to estimate it, changes in E_m evoked by raising $(K^+)_{outside}$ or colchicine were independent of the measuring technique.

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3 Dos Reis, G.A., and Oliveira-Castro, G.M., Biochim. biophys. Acta *469* (1977) 257.
4 Kowri, J., Noa, M., Diaz, B., and Niubo, E., Nature *283* (1980) 868.
5 Gallin, E.K., and Livengood, D.R., J. cell. Biol. *85* (1980) 160.
6 Gallin, E.K., and Livengood, D.R., Am. J. Physiol. *241* (1981) C9.
7 Castranova, V., Bowman, L., and Miles, P.R., J. cell. physiol. *101* (1979) 471.
8 Melmed, R.N., Karanian, P., and Berlin, R.D., J. cell. Biol. *90* (1981) 761.
9 Ralph, P., and Nakoinz, I., Nature *257* (1975) 393.
10 Walter, R.J., Berlin, R.D., Pfeiffer, J.R., and Oliver, J.M., J. cell. Biol. *86* (1980) 199.
11 Hodgkin, A.L., and Horowicz, P., J. Physiol. *148* (1959) 127.

0014-4754/83/080906-02$1.50 + 0.20/0