Some Ionic and Bioelectric Properties of the Ameba *Chaos chaos*

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ABSTRACT Ionic relationships in the giant ameba *Chaos chaos* were studied by analyzing bulk preparations of ground cytoplasm for K, Na, and Cl. Ion levels under normal conditions were compared with the levels in cells exposed to varying concentrations of different ions, for varying times and at different temperatures. By standard intracellular electrode techniques, the bioelectric potential, electrical resistance, and rectifying properties of the plasmalemma were studied on intact cells in media of different composition. The results obtained, when related to evidence from other studies on ion fluxes and osmotic relationships, suggest the following concept of ionic regulation in *Chaos chaos*. In the absence of active membrane uptake, the plasmalemma is essentially impermeable to anions but permeable to both K and Na, which enter passively. In the cold the cell does not discriminate between K and Na, the cytoplasmic level of K + Na is determined by a Donnan distribution, and osmotic imbalance leads to slow swelling. At normal temperatures active processes are added: Na and water are pumped out by the contractile vacuole system; Cl is accumulated, along with the colloid components of the cytoplasm, only during feeding and growth, which depend upon membrane uptake and intracellular membrane transformations. There is no evidence for active transport of any ion species directly across the plasmalemma.

INTRODUCTION

The giant fresh-water ameba *Chaos chaos* has been studied extensively from other viewpoints, but relatively little is known about ionic relationships between the cell and its environment, or about ionic relationships within the cell. Interest in these matters has been sharpened by studies on pinocytosis and phagocytosis, which have shown that *C. chaos* takes up by membrane engulfment measurable quantities of ions, as well as of other solutes, particulates, and water (25, 17). It has been found that substances taken into the cell by this route are not simply released into the cytoplasm, as was suggested earlier (6), but are separately partitioned within the cell by an intricate and

dynamic process of membrane transformations. Uptake by membrane engulfment has been termed "bulk transport," to distinguish it from the supposedly more specific molecular mechanisms which are thought to regulate ion movements across cell membranes. However, the lack of detailed quantitative information regarding ion regulation in *C. chaos* has limited severely all inquiry into the relationship in this organism between bulk transport and ion transport mechanisms operating directly across the plasmalemma.

Recent reports by Riddle (30) on membrane potentials in *C. chaos*, and by Chapman-Andresen and Dick (15) on ion fluxes, include analytical data for the potassium and sodium content of whole organisms. These reports, as well as the studies of Bingley and Thompson (9) and Bingley (7, 8) on potentials in *Amoeba proteus*, provide some useful information. However, there have been no published data on the ionic compositions of the ground cytoplasm of *C. chaos* or of any similar species. Without such information it is difficult to interpret even the few bioelectrical and permeability experiments which have been reported, or to relate these to what is known about transport by phagocytosis and pinocytosis.

Recent advances in culturing methods have made available *C. chaos* in amounts which permit the isolation of the ground cytoplasm for chemical analysis. The aim of the work here described was to determine the concentrations of potassium, sodium, and chloride in the ground cytoplasm under different conditions and to measure the bioelectrical potentials, the electrical resistance, and the rectifying properties of the plasmalemma under varying conditions of external ion concentration and composition. The results permit some conclusions regarding the relative permeabilities of the plasmalemma to different ion species, and the mechanisms by which different ions affect the potential and resistance. The results suggest, when considered in relation to other evidence available, a general concept of ion regulation in *C. chaos*.

MATERIALS AND METHODS

A. *Culture of Amebae* Specimens of the giant ameba *Chaos chaos* were taken from mass cultures which were maintained in active growth by daily feeding on *Paramecium aurelia*. The medium in which the cells were grown consisted of: 0.5 mM $CaCl_2$, 0.05 mM $MgSO_4$, 0.16 mM K_2HPO_4, 0.11 mM KH_2PO_4. The pH was 6.9 to 7.0. The medium was prepared with reagent grade salts and with deionized water. No sodium salts were used, but repeated analyses by flame photometry showed Na to be present at a concentration of 0.05 to 0.1 mM. This solution will be referred to as "medium" and a 1 to 1 dilution with deionized water as "half-strength medium."

B. *Harvest of Cells and Isolation of Cytoplasm* Approximately 20 gm of amebae were harvested twice weekly from a growing culture. For most experiments, the cells were washed in medium and starved 24 hours before use. The cells were concentrated by successive washing and decantation in medium, either at room temperature (22 to 26°C) or at lower temperatures as the experiment dictated. For experiments done to

determine the effects of exposing cells to different external ion concentrations, the cells were treated with the appropriate solution for a measured time at the temperature designated.

Following these procedures, the cells were rapidly separated from the external solution by centrifugation at 3000 × *g* for 1 minute. The tubes were inverted and drained briefly. At this stage the amebae formed a semisolid, well packed mass in each tube, but the cells were not damaged and showed normal behavior if removed from the mass.

The tubes were transferred immediately to the preparative ultracentrifuge (Spinco model L, SW 39 rotor) and spun at 35,000 RPM (R_{av} = 100,000 × *g*) for 30 minutes. The temperature was either 2 to 5°C, or 22 to 26°C, depending on the experiment.

Preliminary investigation showed that, during centrifugation, the individual ameba in such a packed mass first underwent elongation and internal stratification in a manner very like that of isolated cells (1). At a force of 5,000 to 10,000 × *g*, the plasmalemma ruptured at both poles of the cell as the denser particulates (crystals, heavy bodies, nuclei, mitochondria, etc.) were forced centrifugally, and the organelles less dense than the ground cytoplasm (lipid droplets, contractile vacuoles) centripetally. Following rupture of the plasmalemma envelopes of the individual cells, the particulates stratified in successive layers at the bottom of the centrifuge tube. The ruptured cell envelopes sedimented more slowly. Finally, under stronger centrifugal force, the vesicular and cisternal components of the cytoplasm sedimented, and lipid droplets were forced towards the meniscus. This process of sorting out left a clear intermediate zone of cytoplasm, faintly yellow in color and only slightly opalescent, which was removed at the end of the run by a fine pipet. The clear cytoplasmic zone amounted to between 80 and 85 per cent of the total volume in each tube. Samples for analysis were withdrawn from the central part of this zone, so that the regions near the pellet and near the upper zone of lipid were not disturbed.

To determine the amount of interstitial fluid remaining after the packing step (which might dilute the cytoplasmic sample during the following ultracentrifugation step), cells were packed from medium containing a known level of glucose. From Chapman-Andresen's and Holter's study (16) with C^{14}-labeled glucose, it is known that *C. chaos* is impermeable to this solute. After packing, the volume was measured, the cells resuspended in a known volume of medium free of glucose, and repacked. The supernatant solution was then analyzed for glucose by the glucose oxidase method. In a second experiment the reverse procedure was used. Glucose was deleted from the initial packing medium, but added to the medium used to resuspend the cells. The interstitial volume, calculated from both methods, was 8 per cent of the total packed volume.

Further study revealed that the actual contamination of the cytoplasmic sample by the interstitial fluid might be lower than 8 per cent. After the cells were broken and their contents stratified *en masse* at 100,000 × *g*, each tube had a water-clear stratum at the meniscus, just above the more opaque stratum of cytoplasm. The volume was 5 to 10 per cent of the total, and analysis showed that this stratum consisted of interstitial fluid which had been displaced upward as the denser cytoplasm accumulated within the tube. This finding suggested that little mixing had occurred between the

interstitial fluid and the cytoplasm during ultracentrifugation. For a quantitative estimate, glucose determinations were done on cytoplasm isolated from cells packed in medium containing 50 mg per cent glucose. A control specimen, isolated in the absence of glucose, was also analyzed. The result indicated that cytoplasmic samples, withdrawn carefully from the middle region of the tube, were contaminated by less than 2 per cent by volume of interstitial fluid. The value of 2 per cent obtained with glucose might not apply in the case of a more rapidly diffusing ionic solute. It should therefore be considered a minimal value. By the same reasoning, the figure of 8 per cent interstitial trapping represents a maximum. The quantitative effects of the dilution error on the analytical results vary according to the relative compositions of the interstitial fluid and the cytoplasm. The data as reported are not corrected for this error, but its effects are considered in the Discussion.

C. *Analytical Techniques* The ground cytoplasm, isolated as described, was analyzed directly as a liquid sample. In some experiments, the pellet fraction, which included all the particulate and membranous components of the cells, was also recovered, ashed at 500°C for 2 hours, dissolved, and analyzed.

Chloride was determined by amperometric titration, the reproducibility being 0.1 mM/liter. Sodium and potassium were determined by flame photometry, using a lithium internal standard, with a Raymond flame photometer modified to permit the analysis of samples of low sodium content. The reproducibility of the determinations was, for sodium, 0.05 mM and for potassium, 0.1 mM in the range of concentrations encountered in these experiments. All determinations were done on replicate samples, and the results reported are mean values of 2 to 4 such determinations.

D. *Electrical Recording Techniques* Potentials were recorded by the use of glass capillary microelectrodes filled with 3 M KCl. Each microelectrode was tested prior to use, and only those having impedances of 20 to 45 megohms were used. Tip potentials were measured in the various solutions employed, as well as in a bulk sample of ground cytoplasm. The values obtained were from 3 to 12 mv. In each experiment, the tip potential was balanced out immediately before penetrating the cell. To determine whether this procedure was reliable, microelectrodes of different tip potentials were used in succession to measure the potential of the same cell. In each instance the electrode tip was inserted into the same central region of the cytoplasm. The agreement was within 2 mv in all cases. Single electrodes, during as many as 6 successive penetrations of the same cell, were found to give potential readings within a range of 2 mv. Electrodes tested for impedance after penetration of as many as 5 cells gave the same value as before the first penetration.

The amebae were examined in a glass chamber with silver wire built in as the external reference electrode. The chamber was filled with 3 ml of the appropriate solution; then 6 or 8 amebae suspended in no more than 0.1 ml of medium were transferred with a braking pipet to the chamber. Within 2 minutes, the amebae adhered to the glass bottom of the chamber, and the microelectrode penetrations and potential measurements were then begun. In the experiments to be described the electrode tip was advanced into the central region of the cell, in which the cytoplasm has a relatively more fluid consistency. At a time limit of 10 minutes, the experiment was stopped. The chamber was emptied and refilled with fresh solution, and fresh

amebae were introduced. A satisfactory measurement was defined as one in which, after penetration, the potential trace dropped promptly to a stable value, and in which the potential returned to base line promptly upon withdrawal of the electrode. The chamber was held on a microscope stage and the electrodes (either one or two) were mounted on a Leitz micromanipulator assembly.

In the earlier studies, the circuit was from the microelectrode, through a cathode follower stage to a Grass P6 DC preamplifier, to a Tektronix 502 oscilloscope. When the double electrode studies began, each electrode was led to a Bioelectronics negative capacity feedback amplifier, and each of these to one channel of a two channel Sanborn model 297 recorder. For the resistance studies, only the recording electrode amplifier was necessary, the Sanborn recorder preamplifier providing satisfactory amplification for the channel monitoring the stimulus signal. At the time of transition from one amplifying system to the other, 40 amebae in normal medium were each measured, first with one system then with the other, to ensure that no difference was introduced by the change in instrumentation. This having been satisfactorily demonstrated, the circuit for the resistance studies was constructed. The DC source was a 45 volt battery. Its output passed first through a potentiometer, then through a 500 megohm resistor, then through the microelectrode into the cell. Since the ameba was in series with the much larger fixed resistor, changes in the ameba resistance would not appreciably alter the current flow. The external electrode was common to both stimulating and recording circuits. Between its terminal and the battery was a 1.2 megohm resistor. The potential across this resistor was monitored, and from the values so obtained the current flowing in the circuit could be derived. Brief (0.3 to 0.5 second) pulses were applied by depression of a key microswitch. The potentials in all cases returned to base line before application of a subsequent pulse. All measurements of potential were done at room temperatures between 22 and 26°C.

RESULTS

A. *Ion Concentrations in Ground Cytoplasm*

1. A series of 6 preliminary experiments were done to determine whether ion concentrations in the cytoplasm varied with the degree of starvation or with the temperature at which the cytoplasm was isolated. When the isolation procedure was carried out in normal medium, the levels of Na, K, and Cl were not altered by 1 day of starvation, nor were significant differences noted when specimens isolated at 25 and at 3°C were compared. The mean values and ranges for the series were: Na 0.35 mM (0.30 to 0.40); K 28.3 mM (27.0 to 29.0); and Cl 16.5 mM (15.6 to 17.7).

To determine whether the ion levels in the ground cytoplasm differed from those of the particulate and membranous fraction, the pellet formed during ultracentrifugal separation was ashed and analyzed. In 4 determinations, the pellet was found to contain 33.0 to 34.5 mM K, and 2.3 to 2.9 mM Na. The mean values were 34.2 and 2.7, respectively.

2. The effect of exposing cells to increased external concentrations of po-

tassium chloride was studied. Three preparations of amebae, starved for 1 day, were kept at 25°C in normal medium to which additional KCl had been added, for periods of 25, 40, and 90 minutes. Each batch of cells was packed directly from the modified medium and then centrifuged at 100,000 × *g*

TABLE I

ION CONCENTRATIONS IN CYTOPLASM FOLLOWING EXPOSURE OF CELLS TO INCREASED KCl CONCENTRATIONS AT 25°C

KCl added to medium	Duration of exposure	Ion concentrations		
		Na	K	Cl
mM	*min.*	*mM*	*mM*	*mM*
6.5	25	0.3	31.0	19.0
11.0	40	0.1	31.5	18.8
11.0	95	0.1	31.0	18.7

TABLE II

ION CONCENTRATIONS IN CYTOPLASM FOLLOWING EXPOSURE OF CELLS TO VARIOUS MEDIA FOR 20 MINUTES AT 3°C

Ion concentrations in media				Ion concentrations in cytoplasm		
Na	K	Cl	Condition of cells	Na	K	Cl
mM	*mM*	*mM*		*mM*	*mM*	*mM*
0.1	0.2	0.1	Fed	0.4	32.1	19.9
0.05	0.1	0.1	Starved	0.3	30.8	21.4
0.05	0.4	0.9	Fed	0.3	31.0	20.8
0.05	0.5	0.8	Starved	0.35	29.2	19.0
0.05	0.5	0.7	Starved	0.2	27.1	16.6
0.05	23.5	17	Fed	0.15	34.4	—
0.05	26.6	20	Starved	0.1	32.8	—
0.05	27.1	20	Fed	0.2	34.9	—
0.1	24.7	18	Fed	0.3	34.8	19.8
0.05	20.0	15	Starved	0.05	32.0	18.9
0.15	19.2	14	Fed	0.35	35.0	19.3
0.1	22.3	16	Fed	0.4	35.0	20.6
0.1	23.8	18	Starved	0.2	31.8	19.6

to separate the cytoplasmic sample. The results (Table I) indicate that slightly higher cytoplasmic concentrations of both K and Cl resulted.

3. The effect of diluting with ion-free water the normal medium used in preparing and packing the cells was investigated, and the effect of still higher external concentrations of K and Cl, at 3°C rather than at 25°C, was explored. The results of both types of treatment are summarized in Table II. There were no consistent differences between the results for cells prepared

in cold medium and those for cells prepared in dilutions of the same. Cells treated at 3°C with higher concentrations of potassium (for a period of 20 minutes) had slightly higher values for cytoplasmic [K]. The difference observed is no greater than would be expected to result from mixing of the cytoplasmic sample with interstitial fluid of a higher KCl content during the isolation procedure.

Throughout this series, those cells which had been recently fed gave slightly higher values of [K] than did cells starved for 1 day. The difference (1 to 3 mM) was not larger than the variation encountered within each group, but the consistency of the result suggests that a larger series of determinations might show it to be significant. There may be a small and transient rise in the concentrations of all three ions during the peak period of digestion and assimilation of the prey. Analyses of the ion content of the prey paramecia (unpublished), considered in relation to the known rate of feeding by amebae during normal growth (17, 25) indicate that a considerable uptake of Na, K, and Cl occurs by this route.

The experiments summarized in Table II indicate that starving *C. chaos* maintains the cytoplasmic levels of K and Cl for 20 minutes even at 3°C in media in which [K] varies between the limits of 0.1 and 27.1 mM, and [Cl] between the limits of 0.1 and 20 mM. Since at 3°C any active transport process should be suppressed, the results suggest that neither K nor Cl is actively transported at a rate detectable under these conditions.

4. The concentration of sodium ion in the cytoplasm was in the range of 0.1 to 0.4 mM, in the results described above. The concentration in normal medium was 0.05 to 0.1 mM. To determine the effects of increased sodium levels in the external solution, 10 experiments were done by adding different concentrations of NaCl to the normal medium. The time of exposure and the temperature were also varied, the limits of time being 12 and 50 minutes, and the temperature of exposure to the solution being either 25 or 2°. The results (Table III) show that at 25°C the cytoplasmic level of Na increased as the external concentration was raised and the time of exposure lengthened. The results at 2°C were similar. No consistent pattern of change in [K] or [Cl] was seen.

In 6 additional experiments, higher concentrations of sodium in the external solution were used (16.7 to 18 mM). The cells were treated with a simple solution of NaCl for a fixed period of 30 minutes, either at 5 or at 14°C. In 3 instances, $CaCl_2$ was added to a concentration of 0.4 mM, which is approximately the concentration in normal medium and in the experiments described above. In the other 3, calcium was deleted and potassium phosphate buffer added to adjust the pH to 7.0. The results (Table IV) show that, in the absence of calcium (and in the presence of 6.8 mM K) the sodium level in

the cytoplasm rose to much higher values. In the 3 experiments done at 5°C, the increase in [Na] was accompanied by a fall in [K].

B. *Bioelectric Measurements under Varying Conditions*

1. CONTROL STUDIES In normal medium, 70 observations on 40 different cells gave a mean potential of 87.2 mv. The standard error was 1.6 mv. In all cases, as in the results to be described later, the cell interior was negative with respect to the exterior, and the minus sign will therefore be assumed. In half-strength medium 24 cells had a mean potential of 90.4 ± 2.2 mv. Both groups of cells were taken directly from actively feeding cultures, washed, and used within a period of 3 hours. To determine whether the potential

TABLE III

ION CONCENTRATIONS IN CYTOPLASM FOLLOWING EXPOSURE OF CELLS TO INCREASED NaCl CONCENTRATIONS AT 25 AND 2°C

NaCl added to normal medium	Duration of exposure	Temperature	Ion concentrations		
			Na	K	Cl
mM	*min.*	°C	*mM*	*mM*	*mM*
7.4	12	25	1.3	28.5	17.6
7.7	15	25	1.3	28.0	17.5
7.7	14	25	1.5	31.0	19.4
8.9	19	25	1.9	30.4	19.4
12.0	30	25	3.0	29.0	19.1
12.0	50	25	2.8	29.0	18.9
8.5	11	2	0.7	28.5	18.8
8.6	11	2	0.6	27.6	18.3
8.8	22	2	1.3	30.4	20.8
8.9	22	2	1.7	29.3	20.2

changed during starvation, a group of 10 cells were studied after 4 days without food. In half-strength medium, the mean potential was 89.5 ± 2.1 mv.

Since the potential was not significantly altered by starvation, cells to be used in the experiments to follow were selected as routine from actively growing cultures and used within 24 hours. Because the method used for varying the ionic composition of the environment was to mix equal parts of medium and of an aqueous solution of the salt to be studied, the value of 90.4 mv, in half-strength medium, was taken as the control figure.

To determine whether osmotic effects might influence the potential, a series of measurements were done on cells in half-strength medium with glucose added to the levels of 50 and 100 mM. The internal osmolarity of *C. chaos* is estimated to be in the range of 80 to 107 milliosmols (24) and the cell is nearly impermeable to glucose. The results were as follows: 14 cells,

in 50 mM glucose, had a mean potential of 96.8 ± 4.4 mv; 15 cells, in 100 mM glucose, had a mean potential of 92.6 ± 4.0 mv.

Throughout the control studies on cells in normal and half-strength media, as well as in the experiments to be described on cells in various media, the recorded potentials were stable. No spike potentials were observed. These results differ from those of Tasaki and Kamiya (33), who reported transients lasting for 1 to 20 seconds in *C. chaos* and *A. proteus*, when the cells were confined in a special capillary chamber. In the work here reported the cells were free of restraint and normally adherent to the glass substratum.

TABLE IV

ION CONCENTRATIONS IN CYTOPLASM FOLLOWING EXPOSURE OF CELLS TO HIGHER NaCl CONCENTRATIONS FOR 30 MINUTES AT 14 AND 5°C

Ion concentrations in media					Ion concentrations in cytoplasm		
Na	K	Cl	Ca	Temperature	Na	K	Cl
mM	*mM*	*mM*	*mM*	°C	*mM*	*mM*	*mM*
18.8	6.8	20.2	0	5	8.0	22.5	20.7
17.0	6.9	19.9	0	5	8.4	24.1	21.6
16.7	6.9	17.9	0	14	5.1	26.7	21.5
18.5	0	20.1	0.4	14	3.4	28.0	21.0
18.3	0	20.8	0.4	14	2.3	33.6	22.3
18.3	0	20.4	0.4	5	2.4	24.2	20.1

2. EFFECTS ON POTENTIAL OF VARYING EXTERNAL ION CONCENTRATIONS

The effect of $[K]_{out}$ was studied by preparing solutions of KCl in half-strength medium at 12 concentrations, between 0.50 mM and 11.0 mM. At each concentration 9 to 13 cells were examined. The results are plotted in Fig. 1, in which each point represents the mean potential of one such group. The standard error for each point fell within the range of 1.1 to 3.1 mv. Plotted also, as the first point of the curve, is the mean potential of 90.4 mv, determined on 24 cells in half-strength medium (0.25 mM K).

Table V lists the results obtained when other anions were substituted for Cl. At 2.7 and 5.2 mM concentrations of the K salts, there were no significant differences between the potentials determined in the presence of Cl, NO_3, or SO_4. The results indicate that the effect of K on the potential is independent of the nature of the anion.

To determine the effect of $[Na]_{out}$ the potential was measured in half-strength medium to which NaCl was added. At Na concentrations of 4.7, 6.3, and 8.0 mM, in half-strength medium, the mean potentials were 85.0, 84.6, and 86.5 mv respectively. These values were only slightly lower than the control value of 90.4 mv, for cells in half-strength medium.

Tables VI, VII, and VIII summarize 3 series of studies aimed at detecting interacting effects of Na and K on membrane potentials. $[Na]_{out}$ was maintained constant within each series (as NaCl) and $[K]_{out}$ was varied as the chloride. Calcium, magnesium, and pH were constant at the values for half-strength medium.

When the values of potential obtained in each series were plotted against $[K]_{out}$ inflections occurred in each curve. In an attempt to find correlations

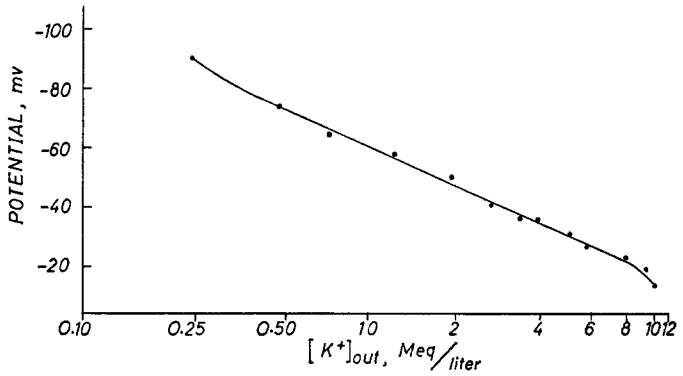

FIGURE 1. Potentials in half-strength medium with increasing concentrations of KC added.

TABLE V

POTENTIALS IN HALF-STRENGTH MEDIUM, VARYING [K] AS KCl, KNO_3, AND K_2SO_4

[K]	Anion	No. of cells	Mean potential	SE
mM			*mv*	
2.72	Cl	10	41.5	2.2
2.72	NO_3	10	42.4	1.5
2.72	SO_4	9	40.4	0.7
5.20	Cl	9	31.1	1.6
5.20	NO_3	9	31.3	2.5
5.20	SO_4	10	32.8	1.8

which might explain these, the data were plotted as a function of ionic strength, the square root of ionic strength, and the ratios of [Na]/[Ca], [Na + K]/[Ca], and [Na]/[K]. Only the ratio of [Na]/[K] gave a correlation which appeared consistent for the 3 series. The result is given in Fig. 2, in which the potential is plotted against the [Na]/[K] ratio. The inflections in each curve occur with a maximum at a ratio of about 1.1, and a minimum at a ratio of about 1.6.

3. ELECTRICAL RESISTANCE OF THE CELL MEMBRANE The resistance of the cell to hyperpolarizing and depolarizing current was measured in differ-

TABLE VI

POTENTIALS IN HALF-STRENGTH MEDIUM, VARYING [K] AS KCl, WITH 4.7 mM NaCl IN ALL SOLUTIONS

[K]	No. of cells	Mean potential	SE	[Na]/[K]
mM		*mv*		
0.25	10	85.0	3.6	18.80
0.62	10	73.5	4.4	7.58
0.87	10	63.5	1.9	5.40
1.12	10	57.5	2.0	4.20
1.25	10	58.0	3.0	3.76
2.12	17	46.5	1.5	2.22
2.72	10	35.5	1.4	1.73
2.82	10	34.0	1.0	1.67
3.27	10	36.5	1.0	1.44
3.47	10	38.0	0.8	1.35
4.12	17	40.3	1.3	1.14
5.00	15	26.6	1.7	0.94
6.47	14	13.2	0.6	0.73
8.50	12	10.8	1.0	0.55
10.42	10	17.0	1.1	0.45
12.92	10	15.0	1.0	0.36

TABLE VII

POTENTIALS IN HALF-STRENGTH MEDIUM, VARYING [K] AS KCl, WITH 6.25 mM NaCl IN ALL SOLUTIONS

[K]	No. of cells	Mean potential	SE	[Na]/[K]
mM		*mv*		
0.25	13	84.6	4.8	25.00
0.62	10	69.5	2.4	10.08
0.87	10	66.0	1.4	7.18
1.12	10	61.0	1.4	5.58
2.12	10	45.0	1.5	2.95
2.74	10	38.0	0.8	2.28
3.27	13	33.1	1.8	1.91
3.47	12	31.7	3.3	1.80
4.12	15	32.7	2.5	1.52
5.12	10	37.5	2.0	1.22
5.55	10	42.0	2.6	1.13
6.58	10	26.0	1.6	0.94
8.18	10	19.5	1.6	0.76
10.68	10	14.0	1.2	0.58

ent media. The external solutions were prepared as in the previous studies of potential by varying the concentrations of different ions in half-strength medium.

First, a sample of ground cytoplasm was prepared by ultracentrifugation, and the resistance was determined with an AC (1000 CPS) conductivity bridge. At 25°C, the specific resistance was 256 ohm cm. As will be seen, the total

TABLE VIII

POTENTIALS IN HALF-STRENGTH MEDIUM, VARYING [K] AS KCl, WITH 8.0 mM NaCl IN ALL SOLUTIONS

[K]	No. of cells	Mean potential	SE	[Na]/[K]
mM		*mv*		
0.25	10	86.5	4.0	32.00
0.62	10	71.0	2.2	12.90
0.87	10	67.5	3.0	9.20
1.12	10	59.0	1.6	7.14
1.74	10	46.5	2.1	4.60
2.12	16	39.1	1.4	3.77
2.72	10	33.5	1.8	2.94
2.82	10	31.0	1.0	2.84
3.27	16	27.8	1.3	2.45
3.47	22	20.9	0.8	2.31
4.12	10	20.5	1.4	1.94
4.22	10	15.5	0.9	1.90
5.00	10	13.5	1.3	1.60
6.47	10	19.5	1.6	1.24
8.50	10	27.5	3.1	0.94
10.42	10	17.0	1.1	0.77
12.92	10	15.0	1.0	0.62

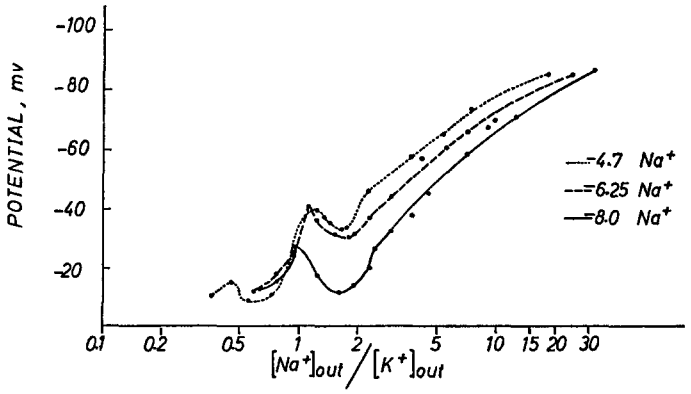

FIGURE 2. The effects of varying both $[Na]_{out}$ and $[K]_{out}$, expressed as plots of mean potential *vs.* the ratio [Na]/[K] in the solutions.

cell resistance was found to be so high that essentially all the resistance must be attributed to the plasmalemma.

To obtain cells of uniform size, amebae were cooled and the container tapped repeatedly until the cells rounded up to a spherical shape. The cell diameters were quickly measured using a microscope with a calibrated image-splitting eyepiece (Cooke, Troughton, and Simms, Ltd.). In this manner, cells having an average diameter of 400 μ ($\pm$20 μ) were selected. The surface

area of a sphere of 400 μ diameter is 5.0×10^{-3} cm^2. From other studies in this laboratory, it is estimated that upon resuming the less regular form, such cells increase their surface area about 20 per cent. The average surface area of the cells used in the resistance studies was therefore taken as 6×10^{-3} cm^2.

Two microelectrodes were placed in the central plasmasol region of each cell being studied. The potential of this region referable to the external solution was continuously monitored with one electrode while DC pulses of each polarity were applied through the other. Preliminary studies were done in which the distance between microelectrode tips was varied from 50 to 150 microns. Variations in spacing were found not to change the results, but in subsequent studies an effort was made to maintain a standard interelectrode separation of approximately 75 microns. Three to five different intensity pulses were applied at each polarity to each cell studied. In no case did any response resembling an action potential appear in the tracing. The tracing in all cases returned promptly to base line upon cessation of the polarizing current.

Because the passage of a current through the cell during measurements of membrane resistance requires the electrophoretic injection of K or Cl ions, it was necessary to determine whether a significant change in the internal concentration of these ions might occur during the measurements. Experimentally, it was observed that the zero-current potentials did not change even after repeated pulses of current. Calculation showed that the maximum amount of K injected during a single depolarizing pulse of 0.5 second duration was about 10^{-13} moles. From the known cell volume (33×10^{-6} μ^3) and internal K concentration (30 mM) the cell contained 10^{-9} moles of cytoplasmic K. It can be seen that even many pulses should not significantly affect the internal levels of K or Cl in a cell as large as *C. chaos*.

From 6 to 10 cells were studied in each of 9 different solutions. Current-voltage curves were plotted for each cell. From these curves, a series of mean values were calculated for the group of cells in each of the 9 solutions used. Curves constructed from these mean values are shown in Figs. 3 to 5. The mean zero-current potential for each group of cells is indicated adjacent to the curve for that group. The mean potentials are in good agreement with the values found in the earlier studies of potential.

In Fig. 3, "control" designates the curve for normal cells in half-strength medium. Under these conditions, *C. chaos* showed a high resistance and marked rectifying properties, the resistance to depolarizing current being definitely higher than that to hyperpolarizing current. The effect of increasing the potassium concentration of the medium to 2 and 5 mM is seen to be a lowering of resistance and a diminution of rectification. At the level of 5 mM KCl, there is essentially no rectification.

Fig. 4 gives the results of 4 different experiments. Cells in half-strength

medium containing 5 mM NaCl showed a current-voltage (I-V) curve similar to the control curve of Fig. 3, but with somewhat less rectification. Increasing $[Na]_{out}$ had therefore no effect on the resistance. It was previously shown to

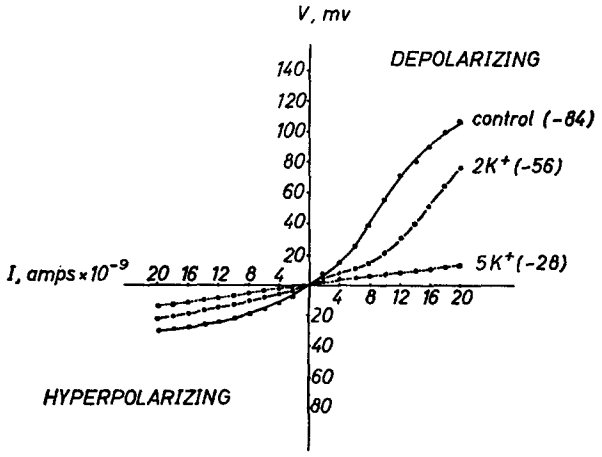

FIGURE 3. Abscissa, polarizing current. Ordinate, millivolt response of membrane potential. Figures in parentheses are mean potentials of cells before current pulse.

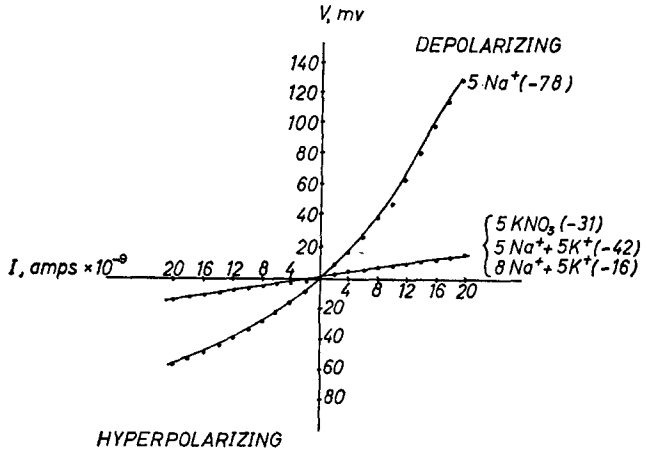

FIGURE 4. Description same as for Fig. 3.

affect the potential only slightly. With respect to both resistance and potential, Na and K differ markedly in their actions.

5 mM KCl (Fig. 6) and 5 mM KNO_3 (Fig. 4) gave identical I-V curves. Furthermore, the I-V curve was not changed when 5 mM NaCl or 8 mM NaCl was added to the 5 mM KCl solution in half-strength medium (Figs. 3 and 4). These concentrations of NaCl were chosen because they gave [Na]/[K]

ratios which had been found previously to affect the potential. The results confirmed the earlier findings regarding the effect of the [Na]/[K] ratio on the potential: the mean potential was 16 mv at the higher Na concentration ([Na]/[K] = 1.6) and 42 mv at the lower Na level ([Na]/[K] = 1.0). Despite the large difference in potential, the I-V curves did not differ, and the resistance was therefore the same. In sum, the I-V curve and therefore the resistance was the same for all 4 solutions containing 5 mM K regardless of the other ions present.

The effect of calcium concentration upon resistance was examined. Half-strength medium was prepared to contain, in one case, 2 mM $CaCl_2$. A second solution of half-strength medium was prepared to contain 2.25 mM sodium EDTA, pH 7.0. Since half-strength medium contains 0.25 mM Ca the excess of EDTA was 2.0 mM. Eight cells were studied in each of the two solutions.

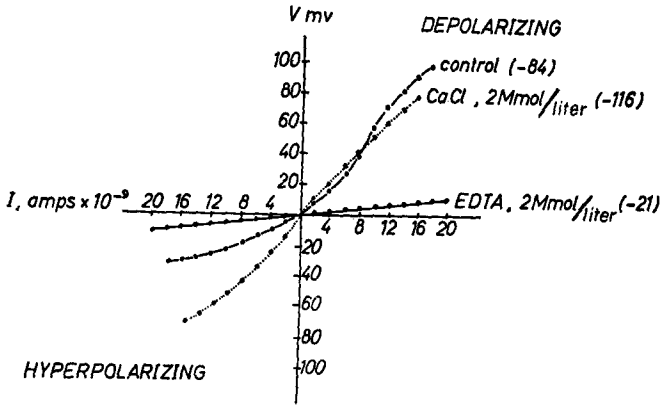

FIGURE 5. Description same as for Fig. 3.

The results are shown in Fig. 5, with the control curve included for comparison. The mean potential of each group is given in parentheses. Increasing the calcium concentration from 0.25 mM to 2.0 mM resulted in an increase in mean potential from 84 mv to 116 mv.

Resistance values may be calculated by taking the slopes of the curves in Figs. 3 to 5 at zero current. The following values in megohms per cell were obtained: 2 mM $CaCl_2$, 5.0; controls and 5 mM NaCl, 4.0; 2 mM KCl, 1.75; 5 mM of potassium as the chloride or nitrate, with or without NaCl, 0.75; 2 mM EDTA, 0.60. Three of these values (the control, 2 mM KCl, and 5 KCl) involved only a variation in concentration of KCl in the medium. The resistance values obtained from these three curves were plotted against external potassium concentration and were found to vary as a linear function of log $[K]_{out}$.

DISCUSSION

1. *General Features of Regulation in Chaos chaos* *C. chaos* has no rigid envelope to protect the cell mechanically or to oppose osmotic swelling, but has instead a structurally complex coat (28, 12, 11) about 1000 A thick, composed of an acidic mucopolysaccharide (25–27). The plasmalemma, including the negatively charged surface coat, is a composite structure which is actively consumed by the cell during the process of feeding and drinking, and as actively reformed from the cytoplasm so long as feeding and growth continue. The rates at which the plasmalemma is taken in and replaced under different conditions of activity have been estimated, as have the volumes of fluid and the amounts of food taken in under standardized conditions of growth (25).

For the purpose of this investigation, which was concerned with the permeability characteristics of the membrane in the absence of pinocytosis or phagocytosis, it should be noted that membrane uptake occurs only at a very low rate in starving cells maintained in normal medium (about 1 per cent of the cell surface is taken up per hour). The addition of various salts to the medium stimulates uptake (10, 13, 14) but the low concentrations used in these studies and the time intervals involved make it unlikely that any of the experiments here reported were seriously complicated by pinocytosis. If pinocytosis in bulk occurred in amounts sufficiently large to affect the internal ion concentrations in a given experiment, it should be detectable by three features in the results: it should occur in those instances in which the external medium contained the highest concentrations of KCl or NaCl; it should be manifest at normal temperatures but completely suppressed at low temperatures; and it should cause a parallel shift in both cation and anion concentrations, depending on the salt used in the experiment. The data reported in Tables I through IV do not suggest any such effect.

Chaos chaos can survive and remain active for many hours in distilled water, without food or other sources of ions. In various culture media containing calcium and traces of other ions, survival may be prolonged to 3 weeks or more. Under such conditions the cells decrease in reduced weight by 2 to 8 per cent per day (35, 20), but it appears possible that the internal ionic and osmotic properties of the cytoplasm are maintained with little change. Ion concentrations in the cytoplasm may decline during prolonged starvation in normal medium (36) but such changes as occur are probably gradual and balanced. Further studies on ionic changes in the cytoplasm during starvation are desirable, but the general implication of the results obtained during the first days of starvation is clear. *C. chaos* has the ability to maintain the levels of the principal ions of the cytoplasm under varying environmental conditions, and to discriminate between potassium and sodium.

Taking into account both osmotic effects (to be discussed further) and the error resulting from the dilution of the cytoplasmic sample during the isolation procedure, the normal level of potassium in cells starved for 1 day is 30 to 32 mM and of chloride 19 to 20 mM (Tables I and II). The level of sodium in the cytoplasm under normal conditions is 0.3 mM, a value which is lower both absolutely and in relation to the potassium level than that in any other cell for which comparable data have been found.

In addition to the three ion species which are dealt with in this study, the cytoplasm isolated at 3°C is known to contain 4 to 5 mM inorganic phosphate (36). Divalent cations have not been determined nor have HCO_3 or other anions, but the evidence from conductivity and osmotic pressure measurements suggests that there are no other ion species present in amounts comparable to K and Cl. If this is true, the total cations amount to between 30 and 35 mM and the total inorganic anions to between 25 and 30 mM. The net anionic charge contributed by the colloidal polyelectrolytes of the cytoplasm must then be 5 to 10 mM.

2. *Osmotic Regulation in C. chaos* Observations on the contractile vacuole system in *C. chaos* were summarized by Andresen (2) and these were related to similar systems in other protozoa by Kitching (22). The contractile vacuole is known to bail out water, and presumably some salts and wastes as well. Although no direct analysis has been done of the vacuolar fluid excreted by *C. chaos*, Schmidt-Neilsen and Schrauger (31) found that in *Amoeba proteus*, which is in many respects similar to the larger ameba, the fluid osmolarity was 32 milliosmols. The osmotic pressure of a comparable cytoplasmic sample was 101 milliosmols.

Belda found that the rate of output of the contractile vacuole system in *C. chaos* was 3.8 per cent of cell volume per hour (3–5). Løvtrup and Pigon, from measurements of the osmolarity of the cytoplasm and from isotopic exchange, concluded that the net osmotic uptake of water through the plasmalemma was 2 to 4 per cent of cell volume per hour (24). The figures for net influx of water and for contractile vacuole output are in reasonable agreement, and indicate the magnitude of the net flow of water through the membrane and through the cytoplasm under basal conditions; *i.e.*, in the absence of feeding or overt drinking and in normal media of low osmolarity. The total influx of water through the plasmalemma is considerably greater than the net osmotic flow. According to the data of Løvtrup and Pigon, it amounts to 13 times the volume of the cell per hour. The permeability coefficient for the plasmalemma of *C. chaos* was 0.011 μ^3/μ^2/atm/min., a value far lower than that of most cell membranes. Comparison of the permeability to water measured osmotically with that measured by isotope exchange has been done (29) and the theoretical significance of the two measurements has been discussed (23).

Because more recent work suggests that these cells maintain a very low level of pinocytic activity even under basal conditions, it has been necessary to examine the interpretations of Løvtrup and Pigon to determine whether all or part of the uptake of water might be by pinocytosis rather than by permeation of the external cell membrane. Our calculations indicate that pinocytic uptake in normal medium is too low to account for the observed rate of D_2O entry. We conclude that the plasmalemma is permeable to water, though the permeability is at least an order of magnitude lower than that of most cell membranes, that the net osmotic uptake by diffusion through the membrane is larger than the uptake by pinocytosis in the basal state, and that the combined uptake by the two routes is consistent with the contractile vacuole output.

In a recent report (33) Tasaki and Kamiya argued that the osmotic coefficient of the ions in the cytoplasm of fresh-water amebae must be very low since the cells do not swell and burst in distilled water. Their treatment of the problem did not take into account the functions of the contractile vacuole or the considerable body of experimental evidence available on this subject, and must therefore be discounted.

3. *The Influence of Osmotic Changes and Dilution Errors on the Experimental Results* In those experiments done to determine the effects of varying ion concentrations on the potential and on membrane resistance and rectification, the observations were completed in less than 10 minutes at 22 to 26°C, and the osmolarity of the external solutions varied from 1 to 26 milliosmols. From the known osmotic characteristics of *C. chaos*, the effect on internal ion levels and membrane characteristics of the variations in external osmolarity should be negligible. In confirmation of this prediction, no significant effects on potential were observed in the control experiments in which the osmolarity of the medium was varied by the addition of glucose.

The circumstances were different in the analytical studies on ion concentrations in the cytoplasm. Here the interpretation of the results of each experiment required consideration of the time of exposure, the osmolarity of different solutions used, and the temperature. The times of exposure to the solutions varied between the limits of 11 and 95 minutes, the osmolarity of the medium varied between the limits of 1 and 54 milliosmols, and the temperature between 2 and 25°C. It is known from other studies that the contractile vacuole is inactive at temperatures of 5°C or below, and that *C. chaos* swells or shrinks at such temperatures according to the external osmolarity (36). In normal medium (2 to 3 milliosmolar) at 3°C, the ameba swells at a rate of 1 per cent of cell volume per hour. In hypertonic sucrose medium (160 milliosmolar) at 3°C the cell shrinks at the same rate. The internal osmolarity is therefore approximately 80, a value in satisfactory argreement with that deduced by Løvtrup and Pigon from other evidence. It is possible to estimate

from this information the osmotic effect on cell volume and therefore on internal ion concentrations in each experiment done in this study at low temperatures. Calculation on this basis indicates that in none of the experiments should the osmotic effect cause a significant difference in the Na, K, and Cl levels of the cytoplasm.

For experiments done at temperatures at which the contractile vacuole is active the osmotic effect could not be so calculated, but it was found by direct measurement of packed cell volumes that cells exposed at 25°C to 150 milliosmolar sucrose in medium lost only 4 to 5 per cent of cell volume per hour. Taking into account the time of exposure and the external salt concentration in each experiment, it may be concluded that osmotic effects on cell volume should not alter the analytical results sufficiently to affect our interpretations.

In all experiments done to determine ion concentrations in the cytoplasm by direct analysis, the principal source of error appeared to be the mixing of the cytoplasmic sample with the interstitial fluid remaining after the cells were packed. For the reasons cited under Methods, the limits of this error should be 2 to 8 per cent of total cell volume. The analytical results summarized in Tables I through IV were not corrected for this dilution factor. In general, it can be seen that the effect of mixing the cytoplasm during the isolation procedure with less than 10 per cent by volume of the medium from which the cells were isolated should cause errors which in most experiments should be less than the variations encountered in replicate experiments. In those experiments in which the external concentration of potassium, for example, was approximately that of the cytoplasm, the dilution error should clearly be least. In instances of low external K levels, the analytical result should be low by no more than 8 per cent; *i.e.*, about 2 mM K. Experiments in which the external concentration of a given ion exceeded the internal should give results in which the error is in the opposite direction. The high external levels of Na in Table IV, for example, would be expected to cause a positive error in the analytical results for cytoplasmic Na. Even in such instances, however, it can be seen that the calculated error (less than 1 mM) is not sufficient to affect the interpretation.

4. *Ion Concentration, Potentials, and Membrane Permeability* Riddle (30) first described the logarithmic relation between the membrane potential of *C. chaos* and the external concentration of K. He reported a 40 mv change of potential for a tenfold change in $[K]_{out}$. Our studies yield a slightly higher value of 44 mv (Fig. 1). In the past some significance has been attached to this value. Our results show, however, that it is not independent of the concentrations of Na and Ca in the medium in which the potassium dilution series is done.

Riddle found the K content of whole cells to be 34.5 ± 5.0 mM, and assumed that this value represented $(K)_{in}$. Our results show that the concen-

tration in the cytoplasm proper is 30 to 32 mM and in the pellet fraction 34 mM. Taken together these results are in agreement with Riddle's value for the whole cell as well as with the value of 33 mM (also for the whole cell) reported by Chapman-Andresen and Dick (15).

Riddle observed that NaCl at 10 mM external concentration caused no change in the potential during a period of 15 minutes. Riddle did not measure $[Na]_{in}$, but Chapman-Andresen and Dick found that the Na content of whole cells was 0.5 mM (the range was 0.2 to 0.6 mM). Our analyses of ground cytoplasm gave values of 0.05 to 0.4 mM, and of the pellet fraction, 2.3 to 2.9 mM. Again, agreement is good if the weighted average of cytoplasm and pellet values is compared to the whole cell value.

From the different effects of the two ion species on the potential, Riddle concluded that the membrane must be permeable to K and that the effective permeability to Na must be low. He noted that the membrane potential was lower than the potassium equilibrium potential calculated from the Nernst equation and concluded that the internal potassium level could not be ascribed to a Donnan equilibrium. He suggested that there must be an active uptake of K, and perhaps an active extrusion of Na, but recognized that no definite conclusions could be drawn from the incomplete evidence available at that time.

Chapman-Andresen and Dick measured the inward and outward fluxes of Na^{22} and Br^{82} in *C. chaos*, as well as the average concentrations of Na and K in whole cells. They reported evidence suggesting that Na is expelled by the contractile vacuole system and discussed the relation between ion fluxes and membrane potential, but their conclusions were also limited by the incomplete scope of the evidence. They did not determine the Cl content of the cytoplasm or of the whole cell, nor did they measure K fluxes or the electrical properties of potential and resistance. They suggested, however, that K might be passively distributed and that Na must be actively transported out of the cell.

From this review of the rather sparse data on electrochemical relations in *C. chaos* it should be evident that few conclusions have been possible. The results here reported are likewise incomplete in some respects. However, they provide information about the concentrations in the cytoplasm proper of the three ion species, K, Cl, and Na. They indicate the changes in internal ion levels which can be produced by varying the external ion concentrations and the temperature. They demonstrate that the potential, although primarily determined by the relative concentrations of K across the membrane, is also affected by changes in the external concentration of Na and Ca. They show the effects of different ions on the resistance of the membrane to inward and outward current flow.

5. *Chloride Ion Regulation* Chapman-Andresen and Dick (15) reported

that Br^{82}, which is assumed to distribute like Cl, is taken up by *C. chaos* at a very slow rate. Inward and outward fluxes were determined to an order of magnitude. The values calculated for the inward and outward permeability coefficients differed by no more than the error in the determinations. Both values were in the range of 10^{-3} to 10^{-4} μ/sec., which is much lower than the values in the literature for erythrocytes and frog muscle. It was suggested by Chapman-Andresen and Dick that Cl might be passively distributed across the membrane, on the assumption that the internal concentration was very low. The basis of that assumption was the evidence that the internal level of Br after 4 hours was only 5 to 10 per cent that of the external medium; since this level was thought to represent equilibrium, it was concluded that the internal level of Cl must be only one-tenth that of the external. Our analyses of the ground cytoplasm show that the actual level of Cl is about 300 times greater than that deduced from the isotope experiments. A review of the data of Chapman-Andresen and Dick suggests that the error lay in the assumption that the isotope had reached equilibrium in 4 hours, since the curves indicate rather a continuing change. Reconsidering their kinetic data in the light of our analyses, we conclude that it might require as long as a week to approach equilibrium in such an experiment, rather than 4 hours.

Since the internal concentration of Cl is 19 to 20 mM and the external in normal medium is 1 mM, the electrochemical gradient is outward. On this basis alone it might be thought that some active transport mechanism is required to maintain the internal level of Cl. There is, however, no evidence of any such "pump" in the results here reported. Changes in the external concentration of Cl (between 0.1 and 20 mM) had no effect on the internal level of Cl. At 3°C, when any active process should be suppressed, the cells did not lose Cl. Variations in the external level of Cl had no effect on the potential or resistance of the membrane, although variations in [K], [Na], and [Ca] were all found to affect the electrical properties in different ways. Substitution of NO_3 or SO_4 in the external medium caused no changes in potential or resistance. The results seem paradoxical but only when the frame of reference is restricted to the starving, basal state.

During active feeding and growth, the total uptake of Cl in the food ingested and in the accompanying fluid is sufficient to supply the entire requirement for Cl (36). This suggests that the active process of Cl accumulation is directly linked to growth, and that the route of Cl entry is by bulk uptake; *i.e.*, by phagocytosis and pinocytosis. Since Cl, PO_4, and the cytoplasmic colloids which contribute the balance of the anionic charges are all accumulated during feeding and growth, there is no need to postulate special pumping mechanisms in the plasmalemma to account for anion accumulation against the electrochemical gradient.

When considered in this larger frame of reference, the results become con-

sistent. In the starving cell, cytoplasmic Cl is not at equilibrium and Cl is not pumped in, but the internal level is nevertheless maintained by the impermeability of the membrane to anions. It may be inferred on the basis of the critique already given of the isotope work that the actual permeability to Cl must be even lower than that calculated by Chapman-Andresen and Dick. It seems likely that the negatively charged mucopolysaccharide layer of the plasmalemma determines the impermeability to anions and controls the relative permeability properties of various cations. The Teorell-Meyer-Sievers model (34) of a charged or ionic membrane should be the most appropriate model for the plasmalemma of the ameba.

6. *Potassium and Sodium Regulation* As Riddle first showed, the potential in *C. chaos* is primarily determined by the ratio $[K]_{in}/[K]_{out}$. Even though isotopic K fluxes have not been measured, the experiments on resistance and current flow reported here support the presumption that the membrane is permeable to K, and that the potential may properly be considered a diffusion potential. Calculations based on the resistance measurements in different external solutions suggest further that the permeability to K is greater than that to Na, which has been measured by isotope techniques.

Chapman-Andresen and Dick (15) reported that the permeability coefficients for Na were comparable to the values reported elsewhere for frog muscle and squid giant axon. In medium containing 0.72 mM Na, the range for efflux was 15 to 41 $\times 10^{-4}$ μ/sec. and for influx 3 to 11 $\times 10^{-4}$ μ/sec. The internal concentration of Na^{22} in their experiments reached a limiting steady state value of 10 to 15 per cent of the external concentration in 4 hours. Other experiments suggested that the explanation of this low steady state value was active secretion of Na by the contractile vacuole system. No conclusions regarding the distribution of Na^{22} within the cell were possible.

Our results (Tables III and IV) obtained by direct analysis of ground cytoplasm are not sufficient to permit kinetic interpretations, but do demonstrate unequivocally that Na enters the cytoplasm proper. At a temperature at which the contractile vacuole is active, in medium containing 12 mM Na, the internal concentration rose to a level about one-fourth that of the external solution in 30 to 50 minutes. The analyses done after exposing amebae to higher concentrations of NaCl at 5 and at 14°C support the idea that Na is normally excreted by an active process, which is presumed to be the contractile vacuole system. That system is shut down at 5°C, reduced in activity at 14°C, and fully active at 25°C. In the 6 experiments reported (Table IV) contractile vacuole activity was suppressed both by cooling and by the increased osmotic pressure of the medium. The principal differences among the six results are attributable to the presence or absence of Ca in the medium. In the absence of Ca, the internal level of Na rose to a maximum of 8.4 mM, a value nearly half that of the external medium. This occurred in the rela-

tively short time of 30 minutes. Considering this result in relation to Chapman-Andresen's and Dick's, it might be expected that exposure in the cold for longer times (2 to 4 hours) would lead to an internal concentration approaching that of the external medium. That the action of Ca is only upon the rate of entry and not upon the final level attained cannot be decided from the data in Table IV, but is suggested by the evidence linking Ca to membrane permeability in the resistance studies.

At 5°C and when the external concentration of Na was high enough to cause an increase of about 8 mM in the internal concentration of Na in the 30 minute time interval employed, K was displaced. This finding is of special interest because it suggests that there may be no marked difference between K and Na in their associations with the cytoplasmic colloids at 5°C, at which temperature the cytoplasm is in the "solated" state. The apparent absence of any such effect in the experiments done at low and more nearly equal concentrations of the two ions in the medium (Table II) might suggest that K is preferentially bound, but the experiments were of too short duration to permit conclusions regarding competitive association. It will be necessary to measure the competition between the two at equilibrium, in the cold state, to settle this question, but the results already obtained suggest that the ability of the cell to discriminate between K and Na at normal temperatures depends on an active process of Na extrusion which is suppressed by cooling, and not simply upon equilibrium binding of K to cytoplasmic polyelectrolytes, or upon the active uptake of K, or the selective exclusion of Na at the plasmalemma.

We conclude that in the cold, the ameba appears to be a simple Donnan system, the properties of which depend on a charged membrane which is effectively impermeable to anions but permeable to both K and Na. At 5°C the system is osmotically imbalanced, but if the osmolarity of the medium is increased to about 80 milliosmols by the addition of an impermeant solute, it should be possible to maintain the cell for many hours in an external medium containing very low levels of K, Na, and Ca with little change in the total of cytoplasmic [K] + [Na]. If metabolic processes could be completely suspended in the cold, so that no changes occurred in the colloid components, the intracellular cation level should fall only very slowly over a period of days, as a consequence of the very gradual loss of Cl.

7. *Membrane Resistance, Rectification, and the Effects of K, Ca, and Na* Tasaki and Kamiya reported values of 1.5 to 5 MΩ total resistance in 4 specimens of *C. chaos*, measured in Prescott-James solution (33). In our studies, amebae in half-strength medium had a mean total cell resistance of 4.0 MΩ. For an average surface area of 6×10^{-3} cm², the specific membrane resistance is thus 24 KΩcm². This may be compared with the values for nerve and muscle for nerve and muscle cells from several sources (32), which vary but are in

general much lower, and with the values for plant cells (18). In *Chara australis*, a plant adapted to a pond water environment like that of *C. chaos*, Hope and Walker (21) found the specific resistance to vary inversely as the external concentration of K. Between 0.1 and 1.0 mM $[K]_{out}$, the resistance of *Chara* ranged from about 13 to 5 $K\Omega cm^2$. In *C. chaos*, the specific resistance likewise varies inversely with $[K]_{out}$. It is increased slightly (to 30 $K\Omega cm^2$) by an increase in the concentration of Ca to 2 mM, and is decreased to 3.6 $K\Omega cm^2$ by 2 mM EDTA. Varying $[Na]_{out}$ had no effect on the resistance, and KNO_3 had the same effect as KCl.

The experiments on resistance and rectification permit further interpretations, when analyzed in detail and related to the evidence obtained in other parts of this work. The data support the conclusion already reached on the basis of our analyses and the isotope data of Chapman-Andresen and Dick, that the membrane is essentially impermeable to Cl and to other anions. The observed variations in resistance, particularly at currents approaching zero intensity, are therefore due to changes in the permeability of the membrane to the inward or outward flow of cations, changes which are induced by small variations in the concentrations of the ions in the medium. Another conclusion to be drawn from evidence already reviewed is that in none of the electrical experiments was the duration long enough to permit a significant change in the internal concentration of any ion species. The results are therefore attributable to actions upon the membrane. This conclusion is supported by other observations not reported here, which indicate that the electrical changes occur within seconds after changing the composition of the medium in a flow chamber.

K is the principal current-carrying species in both directions of current flow and in each of the resistance experiments. The other ions present act primarily by effects on the membrane which modify the inward or outward mobility of K. This conclusion stems from a detailed comparison of the effects of ion substitutions and combinations on the resistance, rectification, and current flow. From this it follows that the rectification observed in the control group of amebae in half-strength medium implies a lower resistance to K influx than to K efflux. That rectification is abolished by the addition of 2 mM $CaCl_2$ is interpreted as evidence that Ca binding to the fixed negative charge system within the composite membrane "tightens" the structure which controls the access routes from the outside to the unit membrane. That structure is believed to be the mucopolysaccharide slime coat, and in particular the inner amorphous layer, which is 200 to 300 A thick. At low ionic strength, K is assumed to move through the slime coat by a saltatory or jump process. This interpretation is speculative but attractive because it also serves to explain the increase in resistance at zero current and the increase in potential, which are caused by 2 mM Ca.

The effect of Na on rectification and on the potential can be explained on a similar basis. The effect of 5 mM Na_{out} was to diminish rectification without changing the resistance at zero current. This is interpreted as the result of competition with K ions for anionic sites within the matrix of the slime coat, which results in a decrease in the influx mobility of K, and therefore in a decrease in rectification.

The effects of Na on the potential differ from the effect of Ca. An adequate explanation must account for the effects of Na on potential summarized in Fig. 2. Analysis of the data suggests that two different factors are involved in the action of Na. The first is an increase in the K diffusion potential which results from the decrease in K influx. If monovalent cations move through the slime coat of the plasmalemma by a jump process from one fixed negative site to the next, cooperative processes may be postulated. The mol ratio of [Na]/[K] should then affect K ion mobility in a critical fashion. Such a mechanism might explain the correlation shown in Fig. 2 between the inflections in potential and the Na/K ratio of the medium.

It should be noted that increasing $[Na]_{out}$ had little effect on membrane resistance to the *outward* flow of K, and that the marked reduction in resistance caused by 5 mM K was not affected by the simultaneous addition of 5 or 8 mM Na to the external medium. This suggests that the ion jump mechanism controls K influx only at very low ionic strengths, as might be expected, and that its operation reflects a functional polarity which derives from the asymmetry of the composite plasmalemma.

The second factor involved in the effect of $[Na]_{out}$ on the potential is the independent action of Na as an ion species capable of diffusing into the cytoplasm. From the evidence already discussed regarding the rate of Na entry, it follows that in the time required for electrical recording there could be no significant change in $[Na]_{in}$. Therefore, the ratio $[Na]_{out}/[Na]_{in}$ varied in different experiments between the limits of 0.3 (controls) and 25 (cells in 8 mM Na). The diffusion potential generated by the Na gradient was opposite in direction to that generated by K, and smaller under most circumstances. Its effect would therefore be a diminution of the observed potential, which, if the effects of both K and Na upon the membrane could be ignored, might be expressed as a linear function of

$$\log \frac{p\,[K]_{in} + p\,[Na]_{in}}{p\,[K]_{out} + p\,[Na]_{out}}.$$

Since the first factor discussed—the effect of $[Na]_{out}$ upon the influx permeability of K—enters here as well, values for the permeability coefficients under these conditions are unknown. The coefficients are in fact complex variables. It is therefore not possible to describe the system in quantitative

terms by any derivative of the Goldman equation (19) but it can be deduced that the two factors by which Na acts on the potential are opposed in sign, and that the curves relating E to $[K]_{out}$, in the presence of different concentrations of Na, should be inflected in a manner like that observed (Fig. 2).

In addition to the work here reported, studies have been done on potential differences between different regions of the cytoplasm in active amebae, on the conductivity of the isolated cytoplasm, and on the correlation of the electrochemical data with dynamic changes in cytoplasmic fine structure. These will be reported elsewhere. For the purpose of this communication it is necessary only to stress that the determinations of potential and resistance here described were done with intracellular electrodes inserted into the central "plasmasol" region of the cytoplasm. In the interpretations advanced, no assumptions were made regarding uniformity of ion activity throughout the cytoplasm. Some deductions which might otherwise be drawn from calculations based on both the potential and resistance values have not been attempted. An example is the calculation of flux values through the plasmalemma from the electrical data. Since the potential across the membrane proper is, in *C. chaos*, probably less than that measured between an external electrode and the central plasmasol region, fluxes calculated from the observed potentials and resistances would be erroneously high. This may explain why such calculations, done on other types of cells, have yielded flux values higher than those obtained by isotope methods (18).

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