The Efflux of Potassium from Electroplaques of Electric Eels

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ABSTRACT 1. The movement of labeled potassium ions has been measured across the innervated membranes of single isolated electroplaques, obtained from the organ of Sachs of *Electrophorus electricus*, mounted in an apparatus which allowed a separate washing of the two membranes.

2. Equations have been derived for a 3 compartment system in series in which tracer from a large pool in one outer compartment is collected in the other outer compartment. The amount of unlabeled ion in the middle compartment may be calculated and also the fluxes across the two membranes.

3. The flux of potassium across the innervated membranes of resting cells in a steady state was between 700 to 1000 $\mu\mu$moles/cm.^{2}/sec. and was unaffected by *d*-tubocurarine.

4. Direct stimulation of electroplaques with external electrodes caused an increase in the efflux of potassium from the innervated membrane of 5 to 8 $\mu\mu$moles/cm.^{2}/impulse, which was unaffected by *d*-tubocurarine; no change occurred in the efflux across the non-innervated membrane.

5. It is concluded that the discharge of electroplaques is accompanied by a small outward movement of potassium ions across the innervated membrane of the same order of magnitude as that found on excitation of squid giant axons. The data show a basic similarity of potassium movements across these two entirely different types of conducting membranes and suggest that this phenomenon may be a general feature of bioelectric currents propagating an action potential.

INTRODUCTION

The electrical characteristics of electroplaques of electric fish have long been thought to be similar in nature to those of nerve and muscle fibers. More

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detailed analysis of the bioelectrical potentials became possible by the use of isolated pieces of electric tissue and Auger and Fessard were the first to use such preparations obtained from electric organs of torpedo and ray in the 1930's (see *e.g.* Fessard (1)). During the last decade isolated pieces of *Electrophorus electricus* have been used in Chagas' and Nachmansohn's laboratories and much new information was obtained about the electrical properties of the electroplax and some underlying chemical reactions (Albe-Fessard *et al.* (2), Keynes and Martins-Ferreira (3), Altamirano *et al.* (4, 5). The most significant electrical change during conduction is the reversal of membrane potential, first observed in the squid axon (Curtis and Cole (6), Hodgkin and Huxley (7)), and later in the electroplax. These multicellular preparations of electroplaques, however, were not suitable for studies of ion movements due to the presence of many structural barriers, large extracellular spaces, and the impossibility of studying separately fluxes across conducting and non-conducting membranes.

The rapid reversal of membrane potential during activity in electroplaques raises the problem of whether it is accompanied by shifts of ions similar to those found to occur during stimulation of cephalopod giant axons (8; 8 *a*). Activity in these fibers is accompanied by a small, rapid inward movement of sodium ions and by an outward movement of potassium ions. These movements of ions are down the concentration gradients, which provide the immediate source of energy for conduction. The reversal in membrane potential is satisfactorily explained on the assumption that the membrane becomes momentarily selectively permeable to sodium ions; the return to the resting potential is due to a return to a low sodium permeability which coincides with a small transient increase in potassium permeability (9). The chemical change in the membrane which allows sodium to enter the cell for less than 1 msec. must be a kind of rapidly reversible trigger mechanism, in which, according to Nachmansohn (10, 11), acetylcholine plays an essential role. During recovery the movements are reversed by different processes requiring metabolic energy.

The previous difficulties for the study of ion movements in electroplaques have been overcome by the method of Schoffeniels ((12); Schoffeniels and Nachmansohn (13)), in which a single isolated electroplax separates two pools of fluid. Ions can move from one pool to the other only by crossing the electroplax, and the fluxes across the conducting and non-conducting membranes can be measured separately. A few exploratory experiments on ion movements with this method were made by Schoffeniels himself (14). The present paper reports quantitative measurements of the efflux of potassium across the conducting membrane in rest and during activity.

METHODS

DISSECTION Slices about 2.5 cm. thick were cut from the posterior end of the eel as the large, widely separated and well defined electroplaques from the organ of Sachs were required as previously described (13). A single row of electroplaques was separated from each slice by cutting with thin scissors through the two rows of electroplaques on each side of the chosen row. The isolated row was then tied tautly with cotton thread to a rectangular glass frame, placed in a lucite dish, and covered with Ringer's solution. Individual electroplax, about 10 to 15 mm. long, 1–2 mm. wide, and with papillae about 200 μ long projecting in the anterior direction, could be seen under a dissecting microscope.

Single electroplax, with their extracellular compartment, were isolated by cutting with fine scissors between the innervated membrane of one cell and the extracellular compartment of the next one all along the row. The electroplaques were then separated by cutting through the connective tissue at the ends. A separation of the innervated membrane from most of the connective tissue of the adjacent electroplax can thus be made, as pointed out by Schoffeniels (12). The units obtained (weight 30 to 40 mg.) were single cells with a fairly flat innervated membrane on one side and a non-innervated membrane on the other which enclosed the papillae extending into the extracellular compartment. Only undamaged electroplaques with no visible cuts in the innervated membrane have been used in the experiments.

APPARATUS A single electroplax unit was mounted between two pools of Ringer's solution in the chamber apparatus (13). The innervated membrane was always firmly held against a window (about 8 mm. long and 0.4 mm. wide) in a sheet of nylon, which separated two lucite chambers containing oxygenated Ringer's solution to bathe the two sides of the cell separately. Two types of apparatus have been used. First, the cell was washed continuously on each side by passing Ringer's solution through two chambers (about 1 ml. capacity) fitted with inlet and outlet holes. Second, the cell was mounted with a static pool of Ringer's solution on one side (about 6 ml.) and washed on the other side. The rate of flow of Ringer's solution for washing was kept constant between 5 to 8 ml./min. and was controlled by a simple, home-made leveling device that regulated the rate by adjustment of a difference in hydrostatic pressure.

ELECTRICAL RECORDING The electrical activity of the electroplax was measured with external silver electrodes connected through a cathode follower to a D.C. preamplifier model P6 (Grass Instrument Co.). On each side of the cell was a stimulating and recording electrode, and on one side was also a third electrode connected to ground. The cell was excited by rectangular monophasic pulses of 0.1 msec. duration and of controlled intensity, and the response was observed on a cathode ray oscillograph. The height of the action potential and the voltage threshold for excitation were noted. The passage of stimulating current from non-innervated to innervated side gave a potential characteristic for a direct response.

SOLUTIONS AND CHEMICALS The composition of the Ringer's solution, pH 7.2, is shown in Table I and is based on the analysis of serum of *Electrophorus electricus* L. (14 *a*).

TABLE I

THE COMPOSITION OF *ELECTROPHORUS* RINGER'S SOLUTION

	mM
NaCl	160
KCl	5
$CaCl_2$	2
$MgCl_2$	2
Na_2HPO_4	1.1
NaH_2PO_4	0.4
Glucose	10

Radioactive potassium carbonate ($K^{42}CO_3$) was obtained from the Brookhaven National Laboratory, dissolved in water, and converted to potassium chloride by the addition of 1N–HCl until the pH was 7.2. It was added to potassium-free Ringer's solution to give a final potassium concentration of 5mM. The radioactivity of diluted Ringer's solution and of radioactive washings from the electroplax was measured in an M6 (20th Century Electronics Ltd., Southend, England) liquid counter and corrections were made for isotope decay and background counts. All experiments were made at room temperature (20 to 22°).

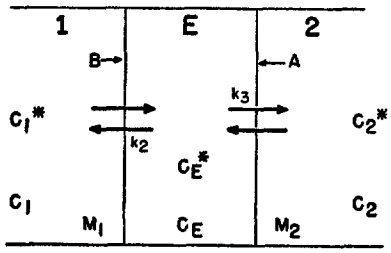

FIGURE 1. Schematic representation of an electroplax, *E*, separating two pools of Ringer's solution, 1 and 2.

THEORETICAL

The system to be analyzed is represented schematically in Fig. 1. The electroplax, *E*, separates two pools of Ringer's solution, 1 and 2, and ions can pass from one pool to the other only *via* the electroplax.

Assumptions

1. The ionic concentration in each compartment is constant.
2. The tracer passes across the membranes at the same rate as the unlabeled ion.
3. In each compartment both the labelled and unlabeled ions are uniformly distributed.

Meaning of Symbols

Symbol	Meaning	Units
A	Area of innervated membrane between E and 2	length^2
B	Area of non-innervated membrane between E and 1	length^2
C_n	Amount of cation in compartment n	mass
C_n^*	Amount of tracer in compartment n	disintegration rate
$X_n = \frac{C_n^*}{C_n}$	Specific activity of tracer in compartment n	disintegration rate/mass
M_1	Rate of exchange of ion across non-innervated membrane	mass/unit area/unit time
M_2	Rate of exchange of ion across innervated membrane	mass/unit area/unit time

Equations

1. First consider the electroplax loaded with tracer and washed on each side with unlabeled Ringer's solution at a rate which does not permit reentry of the tracer into the cell.
It follows that

$$\frac{dC_1^*}{dt} = M_1 B X_E \tag{1}$$

and

$$\frac{dC_2^*}{dt} = M_2 A X_E \tag{2}$$

Therefore,

$$M_1 B / M_2 A = \frac{dC_1^*}{dt} \Big/ \frac{dC_2^*}{dt} = r \tag{3}$$

2. Now consider an unlabeled electroplax mounted between a pool of radioactive Ringer's solution in 1, and unlabeled Ringer's solution in 2 which is made to flow through the chamber. The tracer is measured in washings from 2, and X_1 is known. The increase of tracer in E will be equal to the rate of entry from 1 minus the rates of exit into 1 and 2. X_1 is assumed to remain constant.
Therefore,

$$\frac{dC_E^*}{dt} = M_1 B X_1 - X_E(M_1 B + M_2 A) \tag{4}$$

and,

$$\frac{dC_E^*}{dt} + C_E^* \left(\frac{M_1 B + M_2 A}{C_E} \right) - M_1 B X_1 = 0 \tag{5}$$

At $t = 0$, $C_E^* = 0$ and therefore, solving (5),

$$C_E^* = \frac{M_1BX_1C_E}{M_1B + M_2A}\left\{1 - \exp - \left(\frac{M_1B + M_2A}{C_E}\right)t\right\} \tag{6}$$

In the steady state, at $t = \infty$

$$C_{E_\infty}^* = \frac{M_1BX_1C_E}{M_1B + M_2A}. \tag{7}$$

and

$$X_{E_\infty} = \frac{r}{1 + r}X_1. \tag{8}$$

It follows that at $t = \infty$

$$M_2 = \left(\frac{dC_2^*}{dt}\right)_\infty \Big/ AX_{E_\infty}. \tag{9}$$

From (6) and (7)

$$X_E = X_{E_\infty}\left\{1 - \exp - \left(\frac{M_1B + M_2A}{C_E}\right)t\right\}$$

and, substituting from (9), the slope of a plot of

$$\ln\left\{1 - \frac{\frac{dC_2^*}{dt}}{\left(\frac{dC_2^*}{dt}\right)_\infty}\right\} \text{ against } t \text{ is } -\left(\frac{M_1B + M_2A}{C_E}\right)$$

Therefore,

$$C_E = \frac{M_1B + M_2A}{-\text{ slope}} \tag{10}$$

M_1B and M_2A are known from (3) and (9), and C_E can be calculated.

By a measurement of the ratio of the tracer washed out across the two membranes, and, in another experiment, of the rate of appearance of tracer in 2 from a pool of radioactive Ringer's solution in 1, it is possible to calculate the exchange rates of the ion across the two membranes and the amount of ion in the cell.

3. Now consider an unlabeled electroplax mounted between a pool of labeled Ringer's solution in 2, and unlabeled Ringer's solution in 1 which is made to flow through the chamber. Since,

$$M_2AX_2 = M_1BX_{E_\infty} + M_2AX_{E_\infty} \tag{11}$$

$$X_{E_\infty} = \frac{1}{1 + r}X_2 \tag{12}$$

From (11),

$$M_2 = \left(\frac{dC_1^*}{dt}\right)_\infty \Big/ A(X_2 - X_{E_\infty}) \tag{13}$$

Hence, knowing r and A, the flux, M_2, may also be calculated by measuring the appearance of tracer in 1 with a pool of labeled Ringer's solution in 2.

A method for the determination of the exchange rate in a three compartment, steady-state, closed system has been given by Robertson, Tosteson, and Gamble (15) but their thorough analysis does not apply to the open system discussed above.

RESULTS

The Efflux of Potassium across Both Membranes of Resting and Stimulated Cells

A complete quantitative analysis of the results obtained by measuring the radioactivity collected on washing both sides of a radioactive electroplax has not been undertaken, but a comparison of the rates of efflux across the two membranes has been made to obtain a value for the ratio, r (equation (3)). In five experiments, a cell was soaked in oxygenated radioactive Ringer's solution for 2 to 3 hours and then washed on both sides in the chamber apparatus. The mean value of the ratio, r, of the counts found in the washings from the non-innervated side to those from the innervated side, was 8.4. A semilogarithmic plot against time of the counts in the washings per minute of collection gave parallel straight lines (Fig. 2) when the cell was in the resting condition—as is to be expected for a cell losing radioactivity exponentially with different rates through the two surfaces. Thus, about 8 times as much radioactive potassium was lost across the non-innervated membrane as across the innervated membrane placed against the window. This is probably a reflection of the much greater surface area available for exchange on the non-innervated side.

To find the effect of direct stimulation of electroplaques on the efflux of potassium, the cells were stimulated from 30 to 75 sec.$^{-1}$ and the radioactivity was measured in the washings. This very quickly became too low to measure accurately from the innervated side and the results in Fig. 3 are taken from two of three different experiments which gave similar results. If the loss of radioactivity through the innervated side during stimulation appreciably decreased the radioactivity in the cell, a drop in the efflux from the non-innervated side would be expected; this did not happen and the radioactivity in the cell could not have markedly fallen. The lower line in Fig. 3 was obtained from a cell that was stimulated as soon as possible (about 8 minutes) after the start of washing and shows that excitation caused an increase in the loss of radioactive potassium across the innervated membrane. The upper

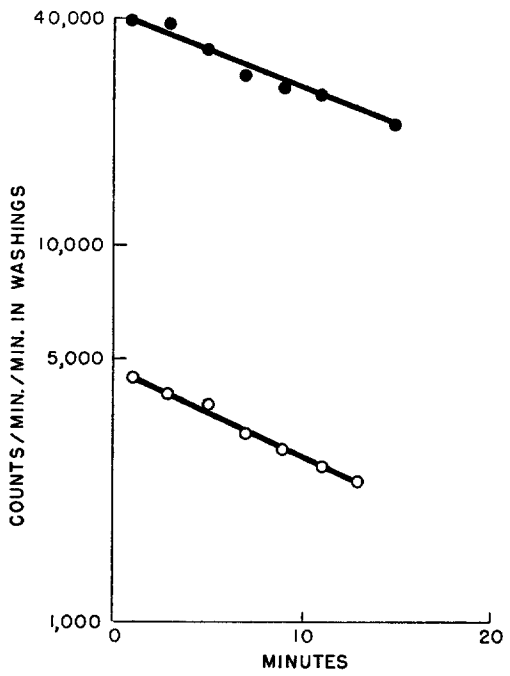

FIGURE 2. The radioactivity found in the washings from the non-innervated (●) and innervated (○) sides of an electroplax, which had previously been soaked for about 4 hours in oxygenated Ringer's solution at room temperature.

line shows that no change in the rate of loss of radioactivity from the non-innervated membrane of the cell occurred during the periods of stimulation. The results show qualitatively that stimulation caused an increased efflux of potassium across the innervated membrane and that no change occurred across the non-innervated membrane.

The Flux of Potassium across the Innervated Membranes of Resting Electroplaques

WASHING THE INNERVATED MEMBRANE—THE RESTING FLUX In order to measure the efflux of potassium across the innervated membrane of the resting electroplax, a cell was mounted with a pool of radioactive Ringer's solution on the non-innervated side and the innervated side was washed continuously with unlabeled Ringer's solution. The amount of tracer in the washings will depend on the permeabilities of the two membranes to potassium as was shown in the theoretical section.

The assumption that tracer ions do not reenter the cell after leaving it, is based on the effect of a variation in the rate of flow of the washing Ringer's solution on the radioactivity collected. Table II shows that the radioactivity in the washing solution was low with low rates of flow, but reached a con-

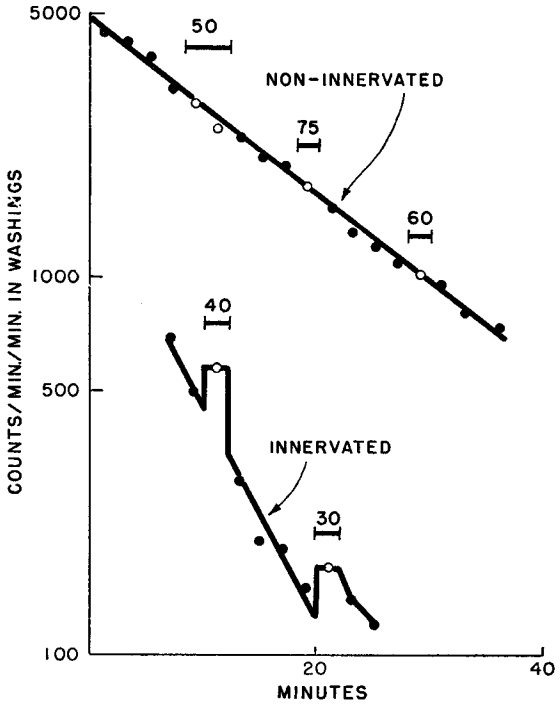

FIGURE 3. The radioactivity found in the washings from the non-innervated and innervated sides of electroplaques during rest and direct stimulation. The results were obtained with separate electroplaques, which had previously been soaked for about 4 hours in oxygenated Ringer's solution at room temperature. The periods of stimulation are indicated by horizontal straight lines and the rates of stimulation are given per second.

TABLE II

THE EFFECT OF THE RATE OF FLOW OF RINGER'S SOLUTION PAST THE INNERVATED MEMBRANE ON THE RADIOACTIVITY FOUND IN THE WASHINGS

The electroplax was mounted between a pool of K^{42}-labeled Ringer's solution on the non-innervated side and a stream of inactive Ringer's solution on the innervated side. The electroplax was first washed with Ringer's solution flowing at a constant rate (about 6 ml./min.) until the radioactivity collected was constant, and the radioactivity was then measured in the washings obtained with different rates of flow.

Rate of flow	Time of collection	Radioactivity in Ringer's solution
ml./min.	*min.*	*c.p.m.*[2]
0.95	13	319
1.30	13	560
2.20	6	490
3.30	5	1130
5.35	3	1370
6.75	2	1350
7.85	2	1330

stant value with a rate above 5 ml./min. showing that all the tracer emerging from the cell was then collected in the Ringer's solution.

Fig. 4 shows that the radioactivity in the washings (C_t or (dC_2^*/dt)) increased to a maximum (C_{max} or $(dC_2^*/dt)_\infty$) in a way that gave a straight line when the logarithm of $(1 - C_t/C_{max})$ was plotted against time. From the value of C_{max}, the area of the window that the innervated membrane was

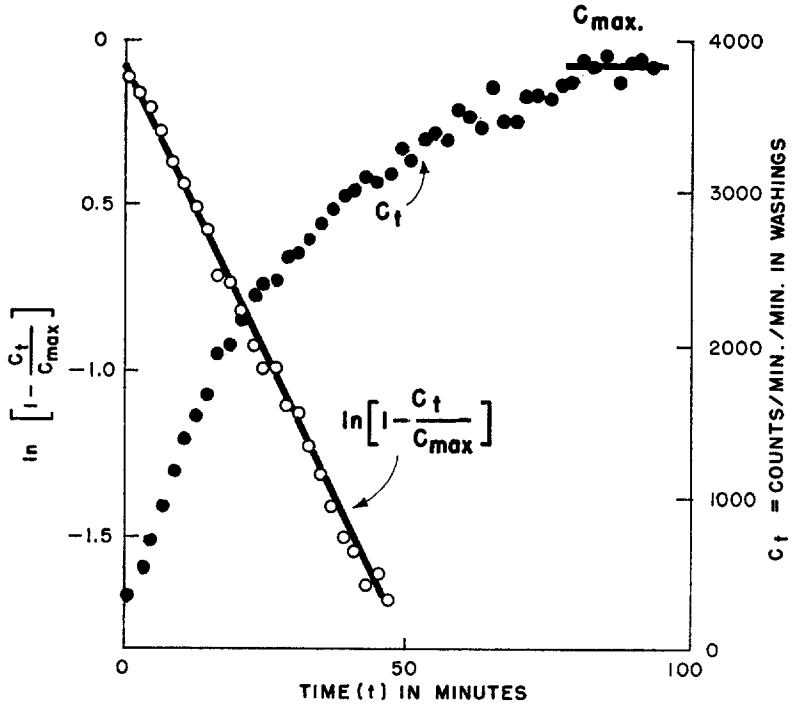

FIGURE 4. Radioactivity in the washings from the innervated side of an electroplax, which was bathed on the non-innervated side with a pool of K^{42}-labeled Ringer's solution. The right-hand ordinate gives the counts/min./min. in the washings and the left-hand ordinate, the natural logarithm of 1 minus the counts at time t divided by the maximum counts found when there was no further change in the radioactivity in the washings.

placed against, A, (3.3 mm.^{2}), the ratio r, and the specific activity of the Ringer's solution on the non-innervated side, X_1, the efflux of potassium, M_2, may be calculated from equations (8) and (9). The mean value of r was obtained with different cells and separate values undoubtedly vary with the extent of invagination of the non-innervated membrane. However, since r was not determined for each cell, the mean value was substituted in (8) to give a value of $X_{E\infty}$, the specific activity of potassium in the cell when C_{max} was reached.

Values for the resting efflux across the innervated membrane are shown in Table III and the mean of 20 values was 990 $\mu\mu$moles/cm.^{2}/sec. (S.D. = 268, S.E. = 67). *d*-Tubocurarine hydrochloride was added to the washing Ringer's solution to abolish the indirect spike, to test whether this compound affected the resting efflux. The results in three experiments showed that the resting efflux in the presence of curare was the same as that found in its absence.

The values found with different electroplaques obtained from the same eel on the same day agreed fairly closely (within 10 per cent) as shown in experiments 21*a* and *b* (950 and 860 $\mu\mu$moles/cm.^{2}/sec.) and in 37*a* and *b* (1030 and 1060 $\mu\mu$moles/cm.^{2}/sec.). A systematic study of the efflux of potassium from electroplaques from different positions in the organ of Sachs was not made, but with 2 electroplaques (Experiments 22 and 22 *c*) taken at an interval of 6 days, one from the posterior, the other from the anterior end of the organ, the resting efflux was the same, though the exact agreement is probably fortuitous.

The electroplaques usually remained excitable for at least 6 hours after dissection, when they were soaked in oxygenated Ringer's solution at room temperature, and one electroplax (Experiment 11 *a*) was still excitable after being soaked overnight (20 hours) in the radioactive Ringer's solution.

The area of membrane being washed by the Ringer's solution has been assumed to be equal to the area of the nylon window in the calculation of the results given in Table III. But it is certainly greater than this due to foldings of the membrane near the nerve endings and the occasional papilla. An accurate assessment of the true area is not possible, and, although on the basis of electron micrographs of Luft (16), the area of membrane might be markedly higher than the area of the window, it is unlikely to be more than 50 per cent higher. The value for the efflux will be correspondingly lowered. It may be concluded therefore that the real efflux of potassium lies between 990 and 660 $\mu\mu$moles/cm.^{2}/sec., these being values respectively for a flat membrane and one that is 50 per cent greater than the area of the flat surface.

WASHING THE NON-INNERVATED MEMBRANE—THE RESTING FLUX To check whether the resting flux across the innervated membrane was the same when measured in a different way, a cell was first soaked in labeled Ringer's solution for about 4 hours and then mounted between a pool of radioactive Ringer's solution on the innervated side and a washing stream of inactive Ringer's solution on the non-innervated side. Fig. 5 shows that the counts in the washings (C_t or (dC_1^*/dt)) fell to a constant value (C_{min} or $dC_1^*/dt)_\infty$), due, first, to the removal of K^{42} from the extracellular compartment and, second, to the development of an equilibrium state in the passage of the tracer from one side of the electroplax to the other. The absence of a straight line in

the appropriate semilogarithmic plot of the radioactivity in the washings $\left(\frac{C_t - C_{\min}}{C_{t-1} - C_{\min}}\right)$ against time is to be expected for a diffusion of K^{42} through the extracellular compartment, in contrast to the straight line found in a similar

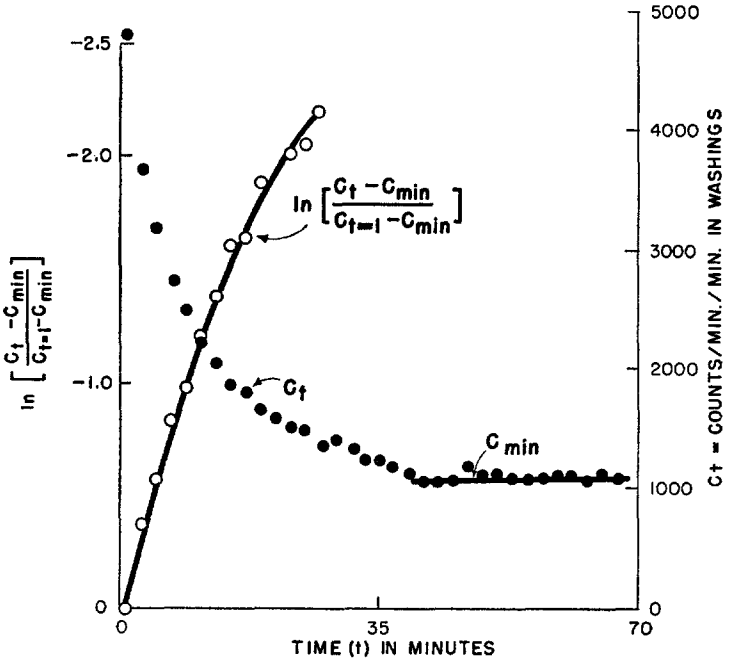

FIGURE 5. Radioactivity in the washings from the non-innervated side of an electroplax. An electroplax had been previously soaked for about 4 hours in oxygenated Ringer's solution and then bathed on the innervated side with a pool of K^{42}-Ringer's solution. The right-hand ordinate gives the counts/min./min. in the washings and the curve (●) shows the fall in radio-activity in the washings until a constant minimum (C_{min}) was reached. The left-hand ordinate is the natural logarithim of the function which should give a straight line if the appearance of the counts in the washings was determined by a first-order process.

C_t = counts/min./min. at time t

C_{t-1} is counts/min./min. at t = 1 min. The plot of $\ln\left[\frac{C_t - C_{\min}}{C_{t-1} - C_{\min}}\right]$ is shown by the open circles and is not a straight line.

plot of the washings from the innervated side where there was practically no extracellular compartment. The occurrence of a small net leakage might partly explain the deviation from linearity in Fig. 5.

The flux, M_2, across the innervated membrane has been calculated from equation (13) to be 830 $\mu\mu$moles/cm.^{2}/sec., which is in reasonable agreement with the mean value of 990 $\mu\mu$moles/cm.^{2}/sec. found by washing the inner-

vated membrane. The similarity of the two values seems to justify the assumption that significant net changes in the potassium concentration of the cell did not occur during the time of the experiment but it does not exclude a small leakage.

Potassium Content of an Isolated Electroplax

This can be calculated from the slope of the semilogarithmic plot in Fig. 4 (Equation 10). Knowing M_2A, and hence, from r, the value of M_1B, the value of C_E, the potassium content of the electroplax has been calculated to be 0.45 μmole. Unfortunately this cannot be turned into a concentration as the volume of the cell is unknown, although the water content is known (17).

Half-Times for the Exchange of Intracellular Potassium

It may be calculated from the previous mean results that $M_2A = 31.4$ and $M_1B = 264$ $\mu\mu$moles/sec. Now, knowing the potassium content of the cell, C_E, the rate constants for the outward movement of intracellular potassium across the two membranes can be found, since

$$M_1B = k_2C_E$$

and

$$M_2A = K_3C_E,$$

where k_2 and k_3 are the rate constants for the efflux of potassium across the non-innervated and innervated membranes respectively. k_2 equals 2.0 hr.$^{-1}$ and k_3, 0.25 hr.$^{-1}$, which means that the half-times for the exchange across the non-innervated and innervated membranes are 0.34 and 2.8 hours respectively.

The Effect of Direct Stimulation on the Efflux of Potassium

To measure the effect of direct stimulation on the efflux of potassium from the innervated membrane, cells were mounted in the chamber apparatus with the radioactive solution on the non-innervated side and then washed on the innervated side until the counts in the washings were constant in successive samples. The electroplaques were stimulated directly by passing current pulses from the non-innervated to the innervated sides. Fig. 6 shows first that continuous stimulation at successively 25, 40, and 60/second caused an increase in the radioactivity in the washings that was roughly proportional to the frequency of stimulation, and second that after stimulation the radio-

activity fell to the original level. Fig. 6 also shows that during continuous stimulation at 25 and 40/sec., the efflux first rose and then fell back although the height of the spike remained unaltered. This might arise from a small fall in X_E due to the increased loss of radioactivity in raising M_2. Fig. 7 shows a similar experiment where the electroplax was allowed to rest between the periods of stimulation at different frequencies. During the resting intervals the radioactivity in the washings returned from the higher levels reached dur-

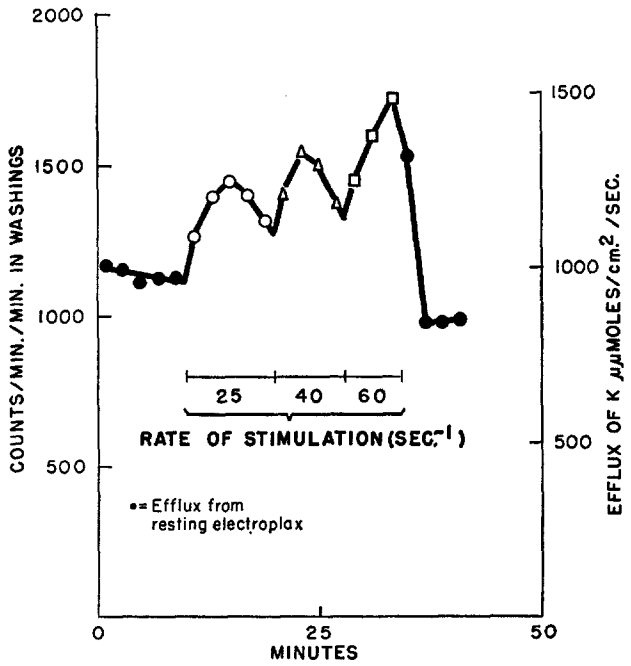

FIGURE 6. The effect of continuous direct stimulation of an electroplax on the efflux of potassium from the innervated membrane.

ing stimulation to the low level characteristic of the unexcited cells. The right-hand ordinate of the graphs gives the efflux of potassium, calculated from equation (9), from which the extra efflux due to stimulation has been calculated. The whole of the radioactivity found above the resting value has been taken to be due to stimulation, even though part of it was found in the sample of washing Ringer's solution collected after the end of the period of stimulation. The results are given in Table III and show that the mean of twenty values for the extra efflux of potassium across the innervated membrane was 8.0 $\mu\mu$moles/cm.^{2} impulse. The S.D. is high (3.0) and the result should, therefore, be regarded as approximate only. The addition of *d*-tubocurarine to block the indirect spike (Experiments 23 *a* and 23 *b*) did not

significantly affect the efflux of potassium due to an impulse (7.0 and 7.3 $\mu\mu$moles/cm.^{2}/impulse) when the cell was stimulated directly.

The same consideration regarding the true area of membrane that was discussed in connection with the resting flux applies also to the value for the extra efflux of potassium per impulse, and the true value is probably between 5 and 8 $\mu\mu$moles/cm.^{2}/impulse. A possible error in the determination of this value could arise if the first assumption in the theoretical section no longer

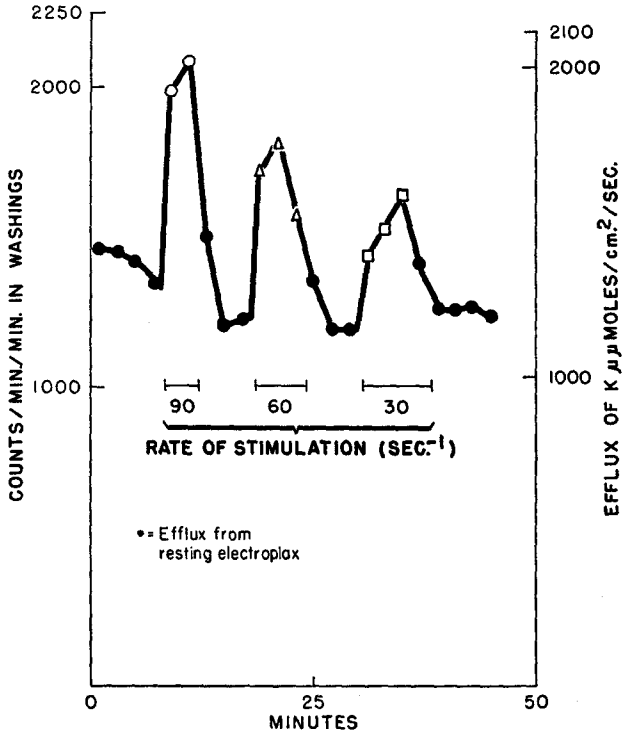

FIGURE 7. The effect of direct stimulation of an electroplax on the efflux of potassium

applied, as might happen if enough potassium left the cell during stimulation to reduce substantially the potassium concentration in the cell. This possibility can be excluded, however, since the maximum amount of potassium that would leave the cell during the longest single period of stimulation was about 3.30 mμmoles (in Experiment 15); *i.e.*, less than 1 per cent of the potassium in the cell (0.45 μmole). Another error might arise from an unstirred layer of fluid adjacent to the membrane which offers diffusion resistance to the movement of tracer emerging from the cell. This possibility is discussed later. Measurements of the effect of stimulation on the influx of potassium have not been made, and it is possible that the net efflux of potas-

TABLE III

THE EFFLUX OF POTASSIUM FROM THE INNERVATED MEMBRANES OF ISOLATED SINGLE ELECTROPLAQUES DURING REST AND DIRECT STIMULATION

Isolated single electroplaques were mounted with the innervated membrane against a window in a sheet of nylon, which separated two pools of Ringer's solution in the apparatus described in Methods. Radioactive (K^{42}) Ringer's solution was placed in the compartment containing the cell and to wash the innervated membrane, a constant flow of non-radioactive Ringer's solution (5 to 8 ml./min.) was maintained through the other compartment. Samples were collected continuously for successive 2 minute periods. The radioactivity in the washings when the cell was at rest and during direct stimulation was measured and the efflux of K calculated.

Experiment No.	Height of spike	Frequency of stimulation	Period of stimulation	Extra efflux of potassium during stimulation	Efflux of potassium from resting cell
	mv.	*sec.*$^{-1}$	*min.*	*μμmoles/cm².impulse*	*μμmoles/cm.²/sec.*
11 *a*	41	50	2	4.4*	1000*
14	39	50	4	5.3	1170
		50	4	4.2	
		75	4	4.8	
		75	4	3.9	
15	46	25	10	10.4	1090
		40	8	9.6	
		60	6	11.6	
		25	10	10.7‡	
		40	8		
		60	6		
		60	4	11.7	
15 *a*	35	30	8	5.6	1040
		60	6	7.9	
19 *a*	67	30	8	12.5	1340
		60	8	5.5	
		90	6	6.1	
19 *b*	52	30	6	12.6	1340
		60	6	9.5	
		90	4	10.6	
22	52	50	6	8.7	930
23 *a*	64	60	6	7.0§	1110§
23 *b*	43	50	8	7.3§	1040§
11 *b*	41	—	—	—	770
18 *a*	41	—	—	—	570
18 *b*	29	—	—	—	810
18 *c*	41	—	—	—	850
20	46	—	—	—	1040
21 *a*	46	—	—	—	950
21 *b*	29	—	—	—	860
22 *c*	52	—	—	—	930
37 *a*	52	—	—	—	1030
37 *b*	72	—	—	—	1060
38	58	—	—	—	870§
Mean	47	—	—	8.0	900
No. of observations	20	—	—	20	20
S.D. of mean	—	—	—	3.0	268
S.E. of mean	—	—	—	0.71	67

* This cell was soaked overnight (20 hours) in radioactive Ringer's solution before the measurements were made.

‡ This value is the mean for a period of continuous stimulation at the frequencies indicated.

§ These values were obtained with cells bathed in Ringer's solution containing *d*-tubocurarine chloride (100 μg./ml.) which gave no response to indirect stimulation.

sium may be slightly lower than that measured here, due to a small increase in influx.

Fig. 8 shows the spread of the results and also that the extra efflux per impulse is approximately independent of the rate of stimulation within the range 25 to 90 sec.$^{-1}$. The efflux of potassium returned to the resting level when the spike disappeared even though ineffective pulses were still being passed across the cell. To avoid fatigue, therefore, the frequency used was never greater than 90 sec.$^{-1}$ and a well defined spike was seen during the whole time of stimulation.

Electrical characteristics of electroplaques are also given in Table III. The height of the spike recorded with external electrodes was about 47 mv. (range 29 to 72 mv.).

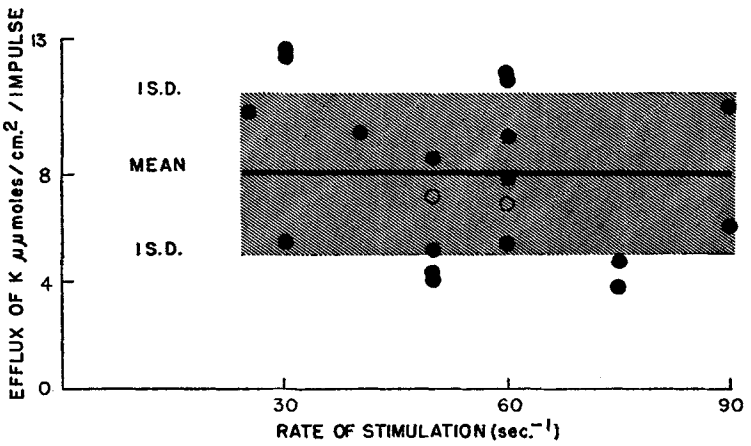

FIGURE 8. The increase in the efflux of potassium from the innervated membrane of electroplaques due to stimulation. This figures shows the spread of the results, and the mean value with S.D. is indicated.

DISCUSSION

Although much information has accumulated suggesting the basic similarity of the electrical properties and the chemical reactions during the activity of electric tissue of *Electrophorus electricus* with those in other excitable tissues, nothing has been known about the ion fluxes across the membranes of electroplaques during activity. Experiments with nerve axons have shown that the energy used in generating the action currents is derived from a net gain of sodium, roughly balanced by a loss of potassium (see reference 9), and the lack of information about such ion movements across the innervated membrane of the electroplax has been a weakness in interpreting the potential changes according to the ionic hypothesis. The isolated single electroplax preparation developed by Schoffeniels has provided a method for the quanti-

tative measurement of ion movements across the conducting membrane and for testing whether these movements, during activity, are similar to those measured on cephalopod giant axons. The desirability and significance of such a study are obvious for, if the ion movements in this type of cell from a vertebrate species are comparable to those in the squid giant axon, they would support the view that such movements are a general feature of the bioelectric currents propagating the spike.

The measurement of movements of labeled potassium has been easier to do than those of labeled sodium, and the theoretical section gives the necessary equations for a quantitative analysis. These apply to conditions where the ionic concentrations remain constant and in principle allow the fluxes across both the non-innervated and innervated membranes to be determined from the radioactivity collected in the washings on one side of an electroplax, which is bathed on the other side with labeled Ringer's solution. However, the flux across the non-innervated membrane has not been calculated because of uncertainty about the area of the membrane, which is extensively folded and invaginated. Some uncertainty in the flux values across the innervated membrane is also inherent because the exact area of the membrane is unknown, but the assumption has been made that the invagination at synapses and the occasional folds seen in the electron microscope (16) increase the surface area of the membrane by between 20 and 40 per cent although not by more than 50 per cent above the area of the corresponding flat surface. An increase of 50 per cent has been taken as the upper limit in the correction of the flux values. The value for the potassium content of an electroplax has been determined indirectly from the radioactive kinetic data. A comparison of this value with the potassium concentration determined by Davson and Lage (18) is not possible because the volume of the single electroplax is unknown. The advantage of the present indirect method for the determination of the ionic content of an electroplax is more evident in the case of sodium, whose intracellular content is much more difficult to determine chemically because of the large approximate corrections for extracellular sodium.

The flux of potassium across the innervated membrane of the resting electroplax was estimated to be 700 to 1000 $\mu\mu$moles/cm.^{2}/sec., the lower value being that for a membrane 50 per cent greater in area than a flat surface, and the greater value that for a flat membrane. The true flux probably lies somewhere between these two values. It is instructive to calculate from the flux and electrical data the component of the total membrane conductance contributed by potassium. The mean value for the resistance of the innervated membrane of electroplaques from the main organ of *Electrophorus electricus*, assuming a flat membrane, is 7.4 ohm cm.^{2} (3), which means that the total membrane conductance is 0.135 mho/cm.^{2}. Now, Hodgkin and Huxley (23) showed that if potassium ions cross the membrane independently of one

another and their movement is affected only by the forces of diffusion and the electric potential gradient, then, for a system in a steady state, the potassium conductance, G_K, is given by

$$G_K = \frac{F^2}{RT} M_K$$

where M_K is the potassium flux and the other letters have their usual significance. A detailed study of the relationship between the influx and efflux of potassium in the electroplax has not been made but the values for the flux measured in two experiments in different ways agreed reasonably and it seems justifiable to assume that the cell was in a steady state. Using a value of 1000 $\mu\mu$moles/cm.^{2}/sec. for M_K, the potassium conductance, G_K, is 0.0038 mho/cm.^{2}.

The 35-fold difference between the total conductance and the smaller potassium conductance is far greater than can be accounted for by experimental errors, and may be explained in two ways. First, potassium ions may carry only a small fraction of the current when the membrane resistance is measured, in which case a change in the external potassium concentration would be expected to produce but a small change in membrane potential. A tenfold increase in the external potassium concentration bathing the innervated membrane has, in fact, been shown to decrease the membrane potential difference by only about 5 mv. (24), in contrast to the change of 58 mv., predicted from the Nernst equation, which is found in *Sepia* giant axons (25) and frog sartorius muscle (26). The small contribution of the potassium conductance to the total membrane conductance thus provides a qualitative explanation for the deviation of the membrane potential from the values predicted by the Nernst equation which appears not to apply to the innervated membrane of electroplaques. This explanation predicts large fluxes of other ions to account for the total conductance, and the measurement of chloride and sodium fluxes is needed since these are the ions most likely to contribute appreciably to the membrane conductance.

Another explanation which might partly explain the discrepancy is that suggested by Keynes (27) to explain a similar discrepancy in the toe muscle of the frog. This is that potassium ions compete amongst themselves instead of passing through the membrane independently, thus vitiating the assumptions on which the calculation of the potassium conductance was based. This kind of interference with potassium movements across the membrane would result in a larger potassium conductance than that calculated, and has been shown to occur in *Sepia* axons (25).

It might be argued that a gelatinous layer of unstirred fluid outside the innervated membrane of the electroplax might offer such high resistance to the diffusion of potassium ions as to reduce substantially the measured potas-

sium conductance, which would then be falsely low as a measure of the membrane potassium conductance. This possibility might therefore account for the discrepancy between the total membrane conductance, calculated from the electrical resistance, and the potassium conductance, calculated from the flux result, and, indeed, might introduce a severe complication in the measurement of fluxes. Such a possibility can be discounted, however, because the thickness of any such layer of unstirred fluid is unlikely to be more than 200 μ. Now, taking the specific resistance of *Electrophorus* Ringer's solution as 50 ohm cm.^{2}, a 200 μ layer would have a resistance of $0.02 \times 50 = 1$ ohm cm.^{2} and a total conductance of 1 mho/cm.^{2}. Since potassium ions in the Ringer's solution (5 mM) would be expected to carry about 1.5 per cent of the current, the potassium conductance of this layer would be 0.015 mho/cm.^{2}. This value is still four times greater than the conductance calculated from the potassium flux and the small value of the latter cannot therefore be wholly due to the diffusion resistance of a layer of unstirred liquid outside the membrane.

The resting flux is about forty times greater than the comparable value in crustacean nerve axons (20 $\mu\mu$moles/cm.^{2}/sec.) (22) and probably reflects the lower electrical resistance of the innervated membrane of the electroplax (7.4 ohm-cm.^{2}) (3) compared with that of crab giant axons of about 7700 ohm-cm.^{2} (19). The resting exchange of potassium in the electroplax may also be compared with the value of 8.8 $\mu\mu$moles potassium/cm.^{2}/sec. found with single fibres of frog semitendinosus muscle (20).

The increase in the efflux of potassium across the innervated membrane of the electroplax on direct stimulation was 5 to 8 $\mu\mu$moles/cm.^{2}/impulse, which is only slightly higher than the values found with giant axons of squid (3.0 $\mu\mu$moles/cm.^{2}/impulse) and *Sepia* (3.6 $\mu\mu$moles/cm.^{2}/impulse) (8) and shows that the impulse is associated with a similar loss of intracellular potassium. Potassium losses during the excitation of muscle are also similar; both frog semitendinosus muscle (20) and rat diaphragm (21) lose about 10 $\mu\mu$moles/cm.^{2}/impulse. Neither the resting flux nor the extra efflux due to excitation was significantly affected by curare, which blocked the response of the electroplax to indirect stimulation.

No measurements of the influx of sodium have been made as yet. However, in view of the loss of potassium on excitation, and the inexcitability of the cell on removal of external sodium (3), it may be assumed that a roughly equivalent amount of sodium enters the electroplax during activity. This assumption, of course, requires experimental verification. A further problem needing solution is the way in which the flux of chloride ions changes during excitation.

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