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Na FLUXES IN SINGLE TOAD OOCYTES WITH SPECIAL REFERENCE TO THE EFFECT OF EXTERNAL AND INTERNAL Na CONCENTRATION ON Na EFFLUX

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The object of the present experiments was to study the flux of sodium across the cell membrane in single cells of well-defined geometrical form so as to allow the maximum possible precision in correlating the morphological or physical characteristics of the cell with the type of kinetics found. In particular the advantages of an isolated spherical cell are, that the rate of diffusion in the extracellular phase may be expected to be equal to or at least very close to that in free solution, that the volume of any surface phase of extracellular material is reduced to a minimum, and lastly that, from a simple measurement of diameter, the volume and surface area of the cell may be calculated with considerable accuracy. For these purposes the immature oocyte of the common toad, *Bufo bufo*, was chosen both as being sufficiently large to contain enough radiosodium to make possible reasonably accurate measurements of the radioactivity of single cells and as satisfying the above criteria of geometrical regularity. Under suitable conditions it was thus possible both to measure the total radiosodium in the cell and to collect and measure the radiosodium escaping from the cell in a given interval. It will be seen that a considerable increase in the precision of kinetic analysis was made possible by this technique.

A previous study of sodium fluxes in oocytes of the frog was made by Abelson & Duryee (1949). Preliminary reports of the present studies have been read to the Physiological Society (Dick & Lea, 1961, 1962).

METHODS

Preparation of material. Immature primary oocytes of *Bufo bufo* were obtained by treating the ovaries of young females in good condition for 1 hr at 30° C with 0·5 % trypsin dissolved in Ca-free Ringer's solution and adjusted to pH 8·0 with NaOH (solution B, Table 1). By shaking the ovary after treatment oocytes of all sizes were liberated and those within the preferred size range (see below) were selected. All manipulations of oocytes were performed by means of a breaking pipette (Holter, 1943). Although the average maturity of the oocytes in the ovary varied considerably with age and season it was always possible by selecting animals of suitable age according to the season to obtain ovaries containing at least some oocytes of the preferred size. In addition to selection by size, only oocytes free

from blebs, distortion, or a visible follicular cell covering were chosen. All selected oocytes were exactly or very nearly spherical.

The condition of the cell membrane of oocytes, selected according to the above procedure and thus subjected to the action of trypsin, was examined by electron microscopy. Electron micrographs showing the microvilli on the surface of cells before and after treatment with trypsin and isolation are shown in Pl. 1*a*, *b*. Although some displacement of the microvilli occurs, they do not appear to be seriously damaged by the process of isolating the cell (cf. Kemp, 1956).

The sodium content of oocytes thus obtained (estimated from the radioactivity as described below) was found to be very variable, particularly between batches of cells from different toads. For many purposes cells with low sodium content were specially selected. Low-sodium cells were best obtained by ensuring that toads were fresh and in good condition; refrigerated animals were found to be quite useless.

The diameter of selected cells was restricted to the range 600–900 μ for several reasons (1) smaller cells contained insufficient sodium, (2) in larger cells radiation losses due to self-absorption of β-particles became inconveniently large, (3) cells in this range were sufficiently immature to avoid complications due to development of animal and vegetal poles in the oocyte, e.g. yolk granules were still uniformly distributed (see Wittek, 1952; Kemp, 1956).

Solutions. The composition of the various solutions used is shown in Table 1. ^{24}NaCl was obtained as 0·9 % solution (Code SGSI, Radiochemical Centre, Amersham) with specific activity of 130–200 mc/g Na (at 'Monday midnight'). Radioactive Ringer's solution was made by adding to 0·7 ml. of 0·9 % ^{24}NaCl, 0·3 ml. of a concentrated mixture of the other constituents required to make up solution A, including a small calculated amount of inactive NaCl. In some cases, however, the ^{24}NaCl solution was evaporated to dryness before use. All chemicals used were 'Analar' grade, or 'Laboratory Reagent' grade (British Drug Houses) where Analar grade was not available. The pH of all non-radioactive solutions (other than the trypsin solution) was adjusted to 7·4 ± 0·1 (measured by a Pye portable potentiometric pH meter) with 100 mM-NaOH (or 100 mM-KOH in the case of solution C). Radioactive solutions were not adjusted; their pH values lay between 6 and 7.

TABLE 1. All concentrations in mM except for glucose (g/l.)

Solution	NaCl	LiCl	$MgCl_2$	Na_2HPO_4	NaH_2PO_4	KH_2PO_4	KCl	$CaCl_2$	Glucose
A	111	—	—	0·4	0·1	—	2·5	0·5	1
B	111	—	—	0·4	0·1	—	2·5	—	1
C	—	111	—	—	—	0·5	2·5	0·5	1
D	111	—	1·0	0·4	0·1	—	2·5	2·0	1
E	—	111	1·0	—	—	0·5	2·5	2·0	1

Measurement of water content. Oocytes were divided according to size into groups of between thirteen and forty-five cells and the cell diameters measured with a filar eye-piece micrometer. A weighing bottle was dried in the oven, cooled in a desiccator over silica gel and weighed correct to ± 0·02 mg. Each group of oocytes was washed briefly in glass-distilled water to remove external salts (see p. 57) then, placed in the weighing bottle, evaporated to dryness in the oven at 90° C and then left overnight at 105° C. The weighing bottle and oocytes were then cooled in a desiccator over silica gel and rapidly re-weighed. Dry weights were in the range 0·5–4·0 mg. The percentage (wt./vol.) of dry matter was readily calculated and the percentage (wt./wt.) from the density of the oocyte which was measured as described below. The percentage (wt./wt.) of water was obtained by difference and the percentage (vol./vol.) of water calculated again from the density of the oocyte.

The density of oocytes was determined by immersion in solutions of raffinose, which was sufficiently soluble and had a sufficiently high molecular weight to give solutions of adequate density without greatly exceeding the osmotic pressure of the Ringer's solution normally used (213 m-osmoles). Approximately 14 % (wt./vol.) solutions (230–240 mM) were used to

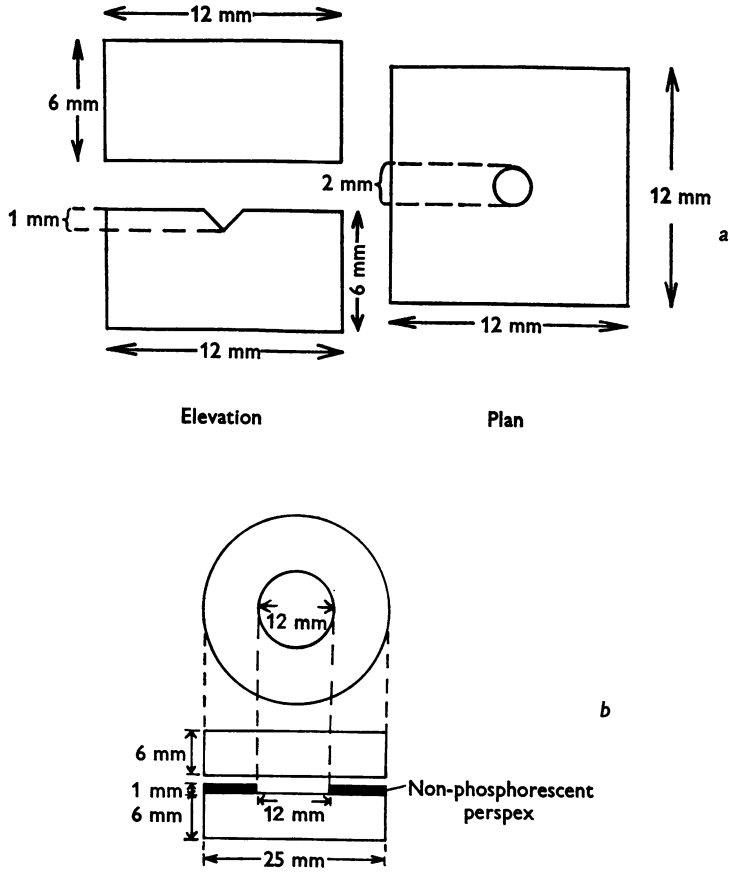
give solutions of densities 1·046 and 1·050 g/ml. measured by means of a relative density bottle. Of ten cells tested all sank in the former solution and of these eight floated in the latter solution, while two cells only just sank. In subsequent calculations a density of 1·05 g/ml. was assumed for all cells.

Flame photometry measurements. The diameters of one or more oocytes were measured and they were then washed by rapid transfer by means of a braking pipette (Holter, 1943) through four salt-cellars each containing 2–3 ml. of double glass-distilled water. The oocytes were placed in a small dry weighed polythene sample tube with a tight-fitting cap and 0·6–0·8 ml. of distilled water added, the exact amount being determined by re-weighing. The sodium and/or potassium content of the sample was determined after allowing osmotic rupture by standing for 24 hr and thorough mixing, by means of an EEL flame photometer with standards of 0·4, 1 and 4 mg Na/l. and 1, 2 and 10 mg K/l. both as chlorides. No interference by K in Na measurements was found nor by Na in K measurements. Measurements at higher concentrations were reproducible to within ±2%, but at lower concentrations, especially of Na, the scale deflexion was low and the estimated error was 5–10%. *All Na and K concentrations have been expressed as m-mole/l. of cell water (represented as mM)* (Possible loss of Na and K during washing of oocytes in distilled water was tested for by comparing the total Na and K contents, measured as above, of three samples of ten oocytes each washed in distilled water with those of three similar samples washed in isotonic choline chloride solution. The water-washed samples gave mean values 4% higher than the choline-washed samples, a difference not significant in relation to the sample variation. Since the choline chloride solution gave significant amounts of Na and K in the blank controls, as contrasted with distilled water which gave blanks of practically zero Na and K content, distilled-water washing was preferred. These results suggest that no serious loss of dry matter occurred during the even briefer washing in distilled water used in the water content measurements described above although loss of solids other than cations was not tested for.)

Radioactivity measurements. β-radiation from ^{24}Na was measured by placing the oocyte in Ringer's solution in a small well in a sandwich of scintillation phosphor, NE 102 (Nuclear Enterprises Ltd.) (Text-fig. 1*a*) and counting in a scintillation counter (Counter SC-LP, Panax Equipment Ltd.). Owing to the favourable geometry of the phosphor the efficiency for ^{24}Na β-particles is approximately 80%, the remaining 20% of activity being lost by self-absorption in the oocyte and in the small amount of solution in the well. In order to reduce the background count the total volume of the phosphor sandwich was restricted to that of a cube of side 12 mm so that the radius from the well was just equal to the maximum range of ^{24}Na β-particles (6 mm of phosphor for maximum energy of 1·39 MeV), so that while the β-particles were efficiently absorbed a minimum of background radiation was intercepted. The background count so obtained was 0·3 counts/sec. Owing to the high energy of the β-particles being detected the amplifier of the counter was set low and the discriminator bias high so that phosphorescence from exposure of the phosphor to light decayed instantly below the bias level after placing in the counter: consequently it was not necessary to darken the counting room. The maximum efficiency of counting so attained was 0·1 counts/sec, pM Na (corrected to 'Monday midnight', i.e. approximately 18 hr after removal from the pile). After counting, the whole phosphor sandwich was immersed in a beaker of the appropriate experimental solution when the two halves easily floated apart and the oocyte was recovered intact for further measurements. Samples (50 μl) of the radioactive solutions were dried down on planchets and counted under a G-M tube (Mullard MX 123) with mica end window of diameter 24 mm and weight 1·5–2·5 mg/cm^2. The relative efficiency of the scintillation counter and the G-M tube was assessed by measuring the radioactivity of living oocytes containing ^{24}Na in the scintillation counter and then rapidly transferring to planchets and drying for measurement with the G-M counter. The relative efficiency was 8·09 ± 0·09 (S.E. of mean of four measurements).

In other experiments a larger sandwich phosphor was used (Text-fig. 1*b*) capable of containing an oocyte in 100 μl. of Ringer's solution so that after removal of the oocyte the ^{24}Na

effluent could be measured directly. The relative efficiency of this type of phosphor, measured by disintegrating in it previously assayed living oocytes, was 0·81 of that of smaller phosphor shown in Text-fig. 1*a*. Since the side wall of the chamber was of non-phosphorescent plastic the degree of contact between the contents and the wall was immaterial to the efficiency.



Text-fig. 1. (*a*) Small well-type plastic phosphor sandwich for measuring radioactivity in single oocytes. The half-thickness of the sandwich (6 mm) is equal to the maximum range of ^{24}Na particles in the phosphor. (*b*) Larger well-type phosphor sandwich with capacity of 100 μl. for measuring ^{24}Na efflux from single oocytes.

Measurement of sodium influx. Oocytes were placed in a Ringer's solution containing $^{24}NaCl$, solution A, Table 1 (solution D was used in later experiments). At intervals of approximately 2 hr four oocytes were removed, their diameters measured with a filar micrometer eye-piece, their radioactivity measured separately and then each was made up separately as a sample for flame photometry. In other experiments a single oocyte was soaked in $^{24}NaCl$-Ringer's solution. It was then removed, washed by transfer through five salt-cellars each containing 2–3 ml. of inactive Ringer's solution (this took less than 2 min) its radioactivity measured for 1–5 min and finally returned to $^{24}NaCl$-Ringer's

solution. This operation was repeated at intervals of ½ hr. Finally, the oocyte was transferred to inactive Ringer's solution for measurement of ^{24}Na efflux. These and all other experiments were conducted at room temperature, 17–23° C.

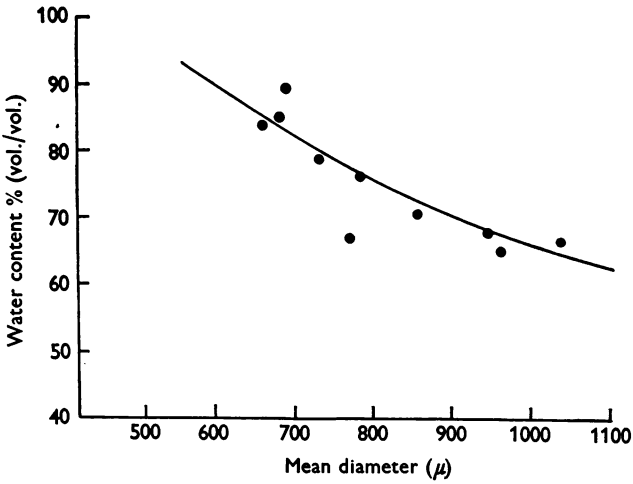
Measurement of sodium efflux. The diameter of a single oocyte was measured with a filar micrometer eye-piece. It was then removed from $^{24}NaCl$-Ringer's solution, washed by transfer through five salt cellars of inactive Ringer's solution as above, its radioactivity measured for between 50 and 400 sec (see below), and then it was returned to fresh inactive Ringer's solution. Its radioactivity was measured at ½ hr or 1 hr intervals with a wash in fresh inactive Ringer's solution immediately before each measurement. The sodium content of each oocyte was estimated from the first radioactivity measurement using Text-fig. 5 to estimate the specific activity. Oocytes estimated to contain more than 50 mM Na were rejected.

In experiments in which the ^{24}Na effluent was measured directly in a large sandwich phosphor (Text-fig. 1*b*), 100 µl. of inactive Ringer's solution was placed in the cavity of the phosphor and the oocyte added in 2 µl. of Ringer's solution with a braking pipette. After a measured interval of time the solution was stirred, the oocyte removed with a braking pipette, again in 2 µl. of solution, the upper part of the phosphor put in place, and the whole counted in the scintillation counter.

RESULTS

Water content of oocytes

The water content of oocytes was found to vary with the size of the oocyte even within the restricted range of sizes employed in the present experiments, and the relation found is represented in Text-fig. 2. The reduction of the water content is presumably due to increase in the yolk content as the oocyte becomes more mature.

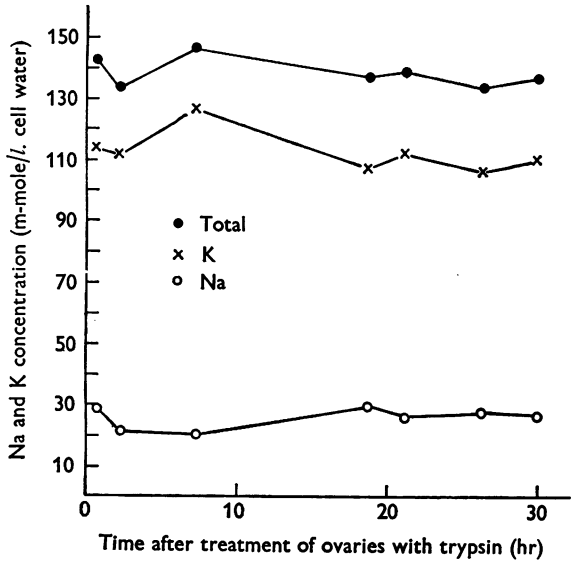


Text-fig. 2. Relation between water content and mean size of groups of oocytes.

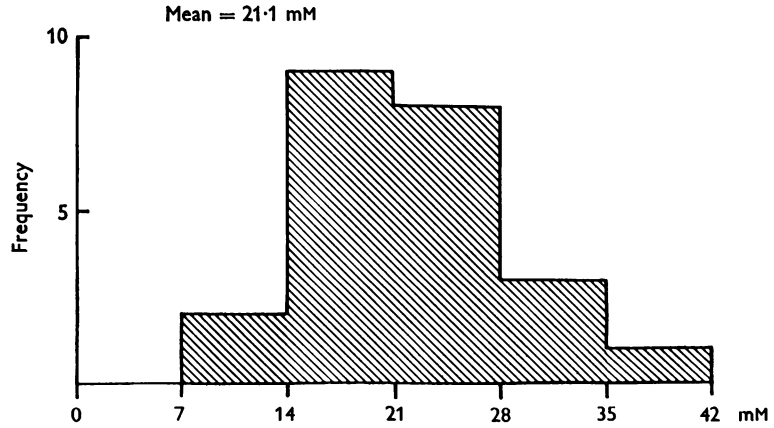
Sodium and potassium content of oocytes after treatment with trypsin

After normal treatment with trypsin for 1 hr at 30° C as described in the Methods section, oocytes were thoroughly washed and placed in Ringer's solution. Samples of from four to eight oocytes were taken at intervals

and analysed by flame photometry (see Methods) for Na and K content. The results of such an experiment are illustrated in Text-fig. 3. Sodium concentration is at first high at 29 mM (this did not occur in every experiment), but drops after 2 hr to 20–21 mM at which it remains for at least 8 hr. Between 18 and 30 hr the sodium concentration has risen to 26–30 mM. The initial fall may be considered as due to recovery of the sodium



Text-fig. 3. Mean Na and K concentration of groups of seven oocytes measured by flame photometry. ●, Na+K; ×, K; ○, Na.



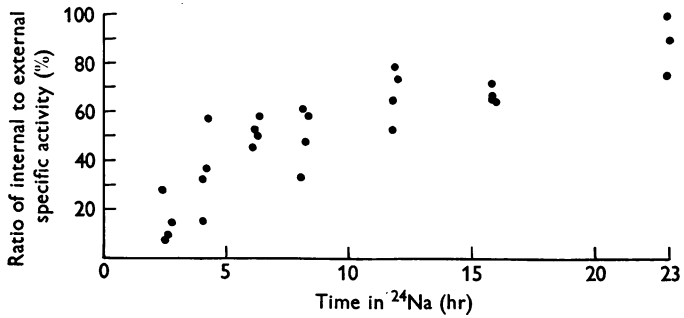
Text-fig. 4. Histogram of distribution of mean Na concentration measured by flame photometry, of groups of oocytes between 1½ and 8 hr after treatment of ovary with trypsin. Mean value, 21·1 mM.

pump from a temporary partial inhibition caused by trypsin. Potassium concentration was initially 112–127 mM and tended to fall to 108–113 mM after 18 hr. Total sodium and potassium concentration was approximately constant at 134–147 mM.

The distribution of the sodium content of twenty-three samples of oocytes taken between $1\frac{1}{2}$ and 8 hr after treatment with trypsin is shown in Text-fig. 4. The mean sodium concentration is 21·1 mM, but the modal value is rather lower as a few very high values tend to influence the mean unduly.

Kinetics of sodium influx from radioactive Ringer's solution

The rise of the specific activity of sodium within oocytes expressed as a percentage of the external specific activity is shown in Text-fig. 5. Each point represents a separate oocyte. It is seen that the internal sodium is



Text-fig. 5. Rise of specific activity in single oocytes on exposure to Ringer's solution containing ^{24}Na.

probably completely replaced. If it is assumed that sodium is fully replaceable and the unreplaced sodium is then plotted against the time on semi-logarithmic paper, the data are found to be compatible with a linear relation although there is some scatter of the points (Text-fig. 6). Thus uptake probably follows the equation

$$[\mathrm{Na}] = [\mathrm{Na}]_{\infty}\,(1-\mathrm{e}^{-kt}). \qquad (1)$$

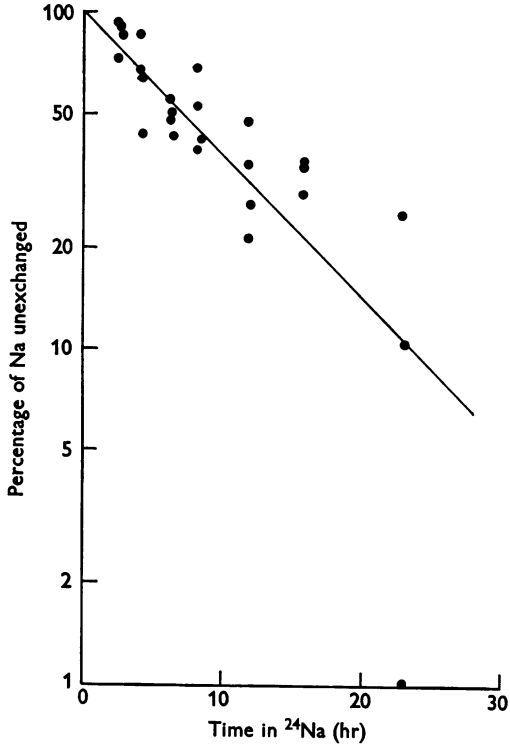
The rate constant estimated graphically from the data of Text-figs. 5 and 6 on the basis of equation (1) was 0·0016 min^{-1}.

Relation between internal Na concentration and Na efflux into Na-free solutions

These experiments are described before those performed in Ringer's solution, first, because they provide a simple case for theoretical interpretation since Na influx is entirely eliminated, and, secondly, because the

conclusions reached in these experiments are essential for interpreting the more complex phenomena found in Ringer's solution.

Single cells whose diameters had been measured were transferred to inactive Na-free Ringer's solution (solution C, Table 1; solution E was used in later experiments) and repeated measurements of their radioactivity made at intervals as for ordinary measurements of Na efflux.



Text-fig. 6. Rate of replacement of Na in single oocytes with ^{24}Na in Ringer's solution. The straight line has been drawn by eye according to the equation

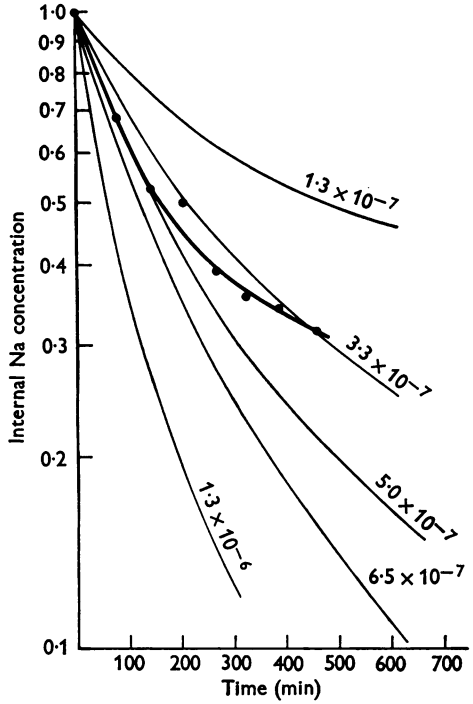
$$[Na] = [Na]_i(1-e^{-kt}).$$

In this experiment k has the value 0·0016 min^{-1}.

A semi-logarithmic plot of data so obtained (Text-fig. 7) showed at once that the Na* efflux under these conditions was certainly not an exponential process, i.e. not in direct proportion to the internal Na* concentration. In an attempt to account for non-exponential loss the relation between the Na efflux rate and the internal Na concentration, $[Na]_i$, was investigated. The Na efflux rate was measured in two ways:

(1) from the first differences of the repeated measurements of the total internal Na* concentration (since there is no external Na to reduce it, the specific activity remains constant throughout so that $[Na^*] \propto [Na]$):

(2) by direct measurements of the sodium escaping from a single oocyte during measured intervals of time as described in the Methods section, such measurements being carried out in parallel with repeated measurements of total $[Na^*]_i$ as in the first method; thus $[Na^*]_i$ and $d[Na^*]_i/dt$ were measured independently.



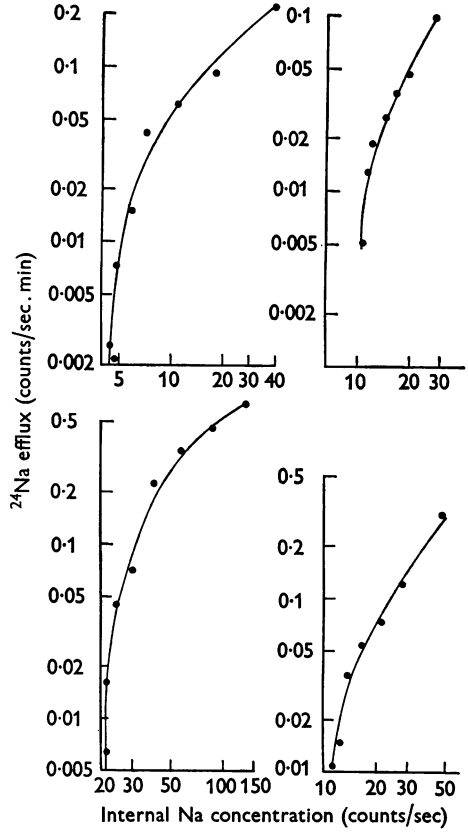
Text-fig. 7. Efflux of ^{24}Na from a single oocyte in Na-free Ringer's solution. Abscissa: time after transfer to inactive solution. Ordinate: internal concentration of ^{24}Na as a fraction of the concentration at time of transfer (log plot). Thick line drawn by eye through experimental points (Expt. 113/2). Thin lines—values computed assuming complete membrane permeability to Na and various values of the diffusion coefficient for Na in the oocyte interior in cm^2/sec shown on each line.

Such data were used to test five possible mechanisms for non-exponential sodium efflux. In each case $d[Na^*]_i/dt$ was plotted against the mean $[Na^*]_i$ over the interval of measurement.

(1) That Na is carried through the cell membrane on a multisite carrier with n sites, as suggested by Keynes & Swan (1959*a*). This implies that Na efflux is proportional not to $[Na]_i$ but to $[Na]_i^n$. This conclusion may be tested by plotting $\log d[Na^*]_i/dt$ against $\log[Na^*]_i$ (Text-fig. 8). It is immediately apparent that the data depart from this theory in two ways: (*a*) The graph is curved instead of straight. This could be accounted for

by supposing that saturation of the carrier occurred at high $[Na]_i$, (evidence for this is given later) so that a modified Michaelis–Menten equation might be used,

$$v = \frac{-d[Na]_i}{dt} = \frac{V[Na]_i^n}{K_m + [Na]_i^n}, \quad (2)$$



Text-fig. 8. ^{24}Na efflux from four single oocytes into Na-free Ringer's solution plotted against internal ^{24}Na content. Abscissae: internal ^{24}Na (counts/sec) (log plot). Ordinates: ^{24}Na efflux (counts/sec/min.) (log plot).

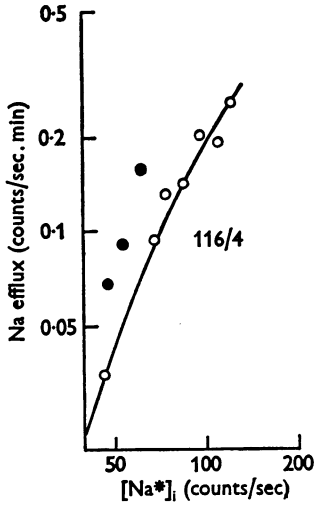
where v, V and K_m have the usual significance. (*b*) At low $[Na]_i$, equation (2) reduces to

$$v = \frac{V}{K_m}[Na]_i^n, \quad (3)$$

i.e. the gradient of the double logarithmic plot should increase from 0 when the carrier is saturated at high $[Na]_i$ to a maximum value of n at low $[Na]_i$. However, the final gradient in Text-fig. 8 has become approximately 13 or more, much larger than the value of 3 obtained by Keynes & Swan (1959*a*).

It seems improbable that a multisite carrier, with the supposed property of being transferred only when all sites are occupied, could have as many as 13 sites.

As a final test cells whose Na-efflux rate had been measured in Li Ringer's solution (solution C) were transferred to Na-free Ringer's solution containing an additional 200mM of sucrose, i.e. with 1·97 times the normal osmotic pressure (Text-fig. 9). The cell volume was reduced to 0·75



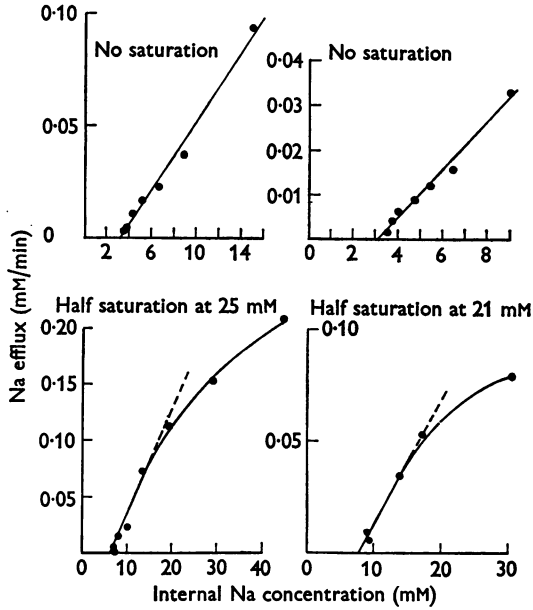
Text-fig. 9. Effect on ^{24}Na efflux of increasing internal ^{24}Na concn. in a single oocyte by shrinkage in hypertonic Na-free Ringer's solution containing an added 200 mM sucrose (i.e. with 1·97 times normal osmotic pressure). ○, in isotonic solution; ●, in hypertonic solution. In hypertonic solution when the internal ^{24}Na concentration is approximately doubled, ^{24}Na efflux is doubled also.

of the original (thus implying a non-solvent volume of 0·50). At the same time Na efflux was raised 2·1 times between 7 and 42 min after transfer. During two further periods of measurement up to 2·3 hr after transfer the efflux was raised in both periods by 1·8 times. Comparison has been made in each case with the efflux rate interpolated for the known [Na*], by means of the data obtained for the cell in the non-shrunken state. Since water efflux during osmotic shrinkage has been found to continue for approximately 30 min in these cells, it seems possible that the first Na efflux value may have been raised slightly by water-sodium interaction as described by Dick (1961). Subsequent values should be quite unaffected by water movement. Since $[Na^*]_i$ has been raised by 1·97 times while Na efflux rises between 1·8 and 2·1 times, this experiment seems to imply clearly a single site carrier.

(2) That a fraction of the sodium is bound in such a way as not to be exchangeable with Li. This implies an equation of the form

$$\frac{d[Na^*]_I}{dt} = k([Na^*]_I - [Na^*]_B) \quad (4)$$

where $[Na^*]_B$ is the concentration of 'bound' Na*. Data may be fitted to this equation. Straight lines are obtained (Text-fig. 10) cutting the abscissa at points which indicate the value of $[Na^*]_B$, the quantity of



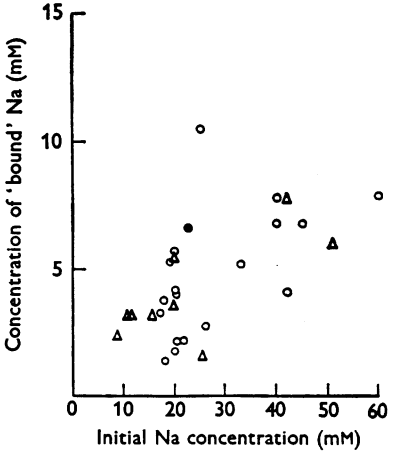
Text-fig. 10. Relation between Na efflux into Na-free solution and Na content of four single oocytes. Abscissae, Na content of oocyte (mM). Ordinates, Na efflux (m-mole/l. cell water per min). At low Na concentration there is a linear relation between Na efflux and internal Na concentration. The intercept on the X-axis gives the concentration of 'bound' Na in the oocyte. In two experiments saturation of the Na carrier apparently occurs at high internal Na concentration.

'bound' Na*. No positive evidence was obtained for slow replacement of the remaining 'bound' Na by Li, such as described by Keynes & Swan (1959*b*); however, it is quite possible that such a replacement occurs at a very low rate. It will be seen later that equation (4) is merely a limiting case of a more general mathematical solution to the problem of Na efflux which is presented in the discussion.

Values for the 'bound' sodium content of many cells were obtained by regression analysis on data similar to that of Text-fig. 10, and these values are shown in Table 2 and also plotted (Text-fig. 11) against the estimated initial total Na_I of the cell (calculated from the initial radioactivity with a

TABLE 2

Expt. no.	Cell diam. (μ)	Initial Na concn. (mM)	'Bound' Na concn. (mM)	Rate const. for loss of 'free' Na(k_{21}) (min^{-1}) $\times 10^3$	Na efflux (pM/cm^2, sec)
90/2	829	60	7·9 ± 0·3	2·0 ± 0·4	18
90/3	701	33	5·2 ± 0·1	3·4 ± 0·4	14
90/4	740	45	6·8 ± 0·1	4·8 ± 0·4	28
90/5	749	40	6·8 ± 0·1	3·1 ± 0·4	16
90/7	663	42	4·1 ± 0·03	7·8 ± 0·3	41
90/8	774	40	7·8 ± 0·2	4·5 ± 0·8	23
92/1	778	25	10·5 ± 0·1	5·4 ± 1·3	12
96/1	799	26	2·8 ± 0·04	9·0 ± 0·5	34
96/2	736	18	1·4 ± 0·04	7·0 ± 0·5	18
96/3	748	20	1·8 ± 0·05	9·7 ± 0·7	27
96/4	693	21	2·2 ± 0·1	6·9 ± 1·3	19
96/5	731	21	2·2 ± 0·1	6·2 ± 1·2	18
97/1	735	18	3·8 ± 0·5	13·0 ± 1·4	28
97/2	744	20	5·6 ± 0·4	14·0 ± 1·0	30
97/3	774	20	4·2 ± 0·02	12·1 ± 0·6	31
97/4	782	20	4·1 ± 0·07	9·5 ± 1·4	25
97/5	782	17	3·3 ± 0·04	12·5 ± 1·2	28
97/6	753	19	5·3 ± 0·02	15·6 ± 0·8	34
98/1	801	23	6·6 ± 0·8	6·7 ± 1·3	18
113/1	705	20	3·6 ± 0·02	7·8 ± 0·3	18
113/2	722	11	3·2 ± 0·01	5·4 ± 0·3	7
113/3	756	51	6·0 ± 0·03	9·2 ± 0·6	65
115/1	722	26	1·6 ± 0·03	6·1 ± 0·3	22
116/2	778	16	3·3 ± 0·04	4·3 ± 0·4	9
116/3	804	11	3·2 ± 0·04	5·3 ± 0·9	7
116/4	748	9	2·4 ± 0·02	2·8 ± 0·3	3
117/2	752	20	5·5 ± 0·02	5·3 ± 0·2	12
117/3	804	42	7·8 ± 0·02	5·6 ± 0·3	32



Text-fig. 11. Relation between internal concentration of Na not replaceable by Li ('bound Na) and total concentration ○, efflux measured by difference, with ^{24}Na; △, efflux measured directly, with ^{24}Na; ●, efflux measured by difference, with flame photometer.

value for the specific activity estimated from Text-fig. 5). It is seen that the 'bound' Na concentration is correlated with the initial total Na concentration ($r = 0{\cdot}58$, corresponding to $0{\cdot}005 > P > 0{\cdot}001$). This suggests that the Law of Mass Action applies at least approximately to the 'binding' reaction, so that Na transfer between the two compartments is reversible.

(3) That Na efflux is proportional to the total internal Na concentration, but that the proportionality constant declines with time. This possibility was tested by estimating the decline of the permeability to Na efflux by making repeated measurements of $[Na]_i$ in a batch of oocytes kept in Ringer's solution by means of the flame photometer, while $[Na]_i$ was simultaneously estimated on a control batch from the same ovary kept in Na-free Ringer's solution by the same method and the rate of Na efflux estimated by difference. Such an experiment is illustrated in Text-fig. 12. In order to test the above hypothesis the following equation was assumed for Na-free solutions

$$-\frac{d[Na]_i}{dt} = v = k(1-qt)[Na]_i, \tag{5}$$

where v is the rate of Na efflux.

From the gradual rise of Na concentration in normal Ringer's solution the fractional rate of decline (q) of the initial Na efflux rate constant k was calculated from the formula (obtained as described in Appendix 2)

$$q = \frac{1}{[Na]_i}\frac{d[Na]_i}{dt}\left(\frac{1}{kt}+1\right) \tag{6}$$

on the assumption that the cell is initially in the steady state and that the rise of $[Na]_i$ is due to a fall of Na efflux while the rate of Na influx remains constant. Assuming an average initial value of $k = 0{\cdot}18\ hr^{-1}$, the value of q was calculated from the data shown in Text-fig. 12 ($d[Na]_i/dt$ being estimated graphically) and was found to have values in the range $0{\cdot}06\ hr^{-1}$ to $0{\cdot}12\ hr^{-1}$. An approximate value of $0{\cdot}09\ hr^{-1}$ was therefore used subsequently.

From equation (5)

$$\frac{v}{(1-qt)[Na]_i} = k. \tag{7}$$

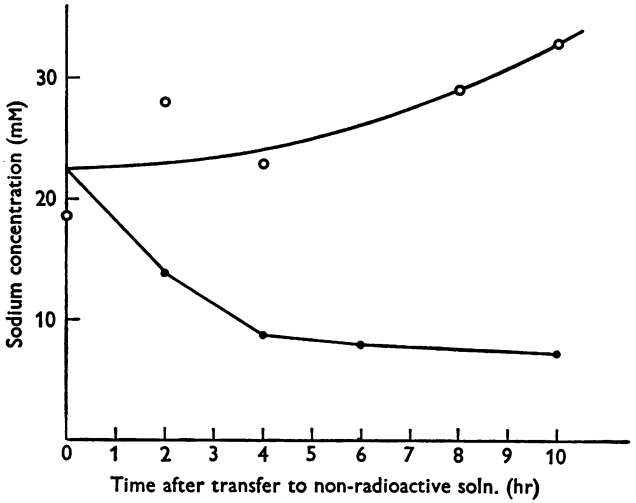
This function is calculated in Table 3 from the data of Text-fig. 12. It is clearly not constant.

On the other hand if the preceding hypothesis is adopted and it is assumed that 6·5 m-mole/l. of the internal Na are not exchangeable for Li then equation (7) becomes

$$\frac{v}{(1-qt)([Na]_i-6{\cdot}5)} = k. \tag{8}$$

This function is shown in column (6) of Table 3 and it is found to be constant within the error of the experimental data.

Thus the value of q, which has been measured, is much too low to account by itself for the type of non-exponential loss of Na found in these experiments. The possibility, however, still remains that the assumption



Text-fig. 12. Change of Na content of groups of oocytes, measured by flame photometry. ○, Cells in Ringer's solution (solution A). ●, Cells in Na-free Ringer's solution (solution C). Expt 98.

TABLE 3

(1) Time internal (hr)	(2) Mean time t (hr)	(3) Mean Na concn. $[Na]_i$(mM)	(4) Mean rate of Na efflux v(mM/hr)	(5) $\frac{v}{(1-qt)[Na]}$	(6) $\frac{v}{(1-qt)[Na]_i-6{\cdot}5)}$
0–2	1	18·2	4·3	0·26	0·40
2–4	3	11·4	2·6	0·32	0·73
4–6	5	8·4	0·4	0·09	0·38
6–10	8	7·6	0·2	0·09	0·65

$q = 0{\cdot}09$ hr^{-1} and $Na_B = 6{\cdot}5$ mM.

of constant Na influx from Ringer's solution may be erroneous. For instance if the Na influx actually declines, the value of q may be seriously under-estimated.

(4) That sodium is lost from two internal compartments *in parallel* with one another but having different rate constants as tentatively suggested by Keynes & Swan (1959*a*) in treating Na flux in frog muscle. This theory supposes that Na* efflux is governed by an equation of the form

$$\frac{-\mathrm{d}[Na^*]_i}{\mathrm{d}t} = k_1[Na^*]_1 + k_2[Na^*]_2, \qquad (9)$$

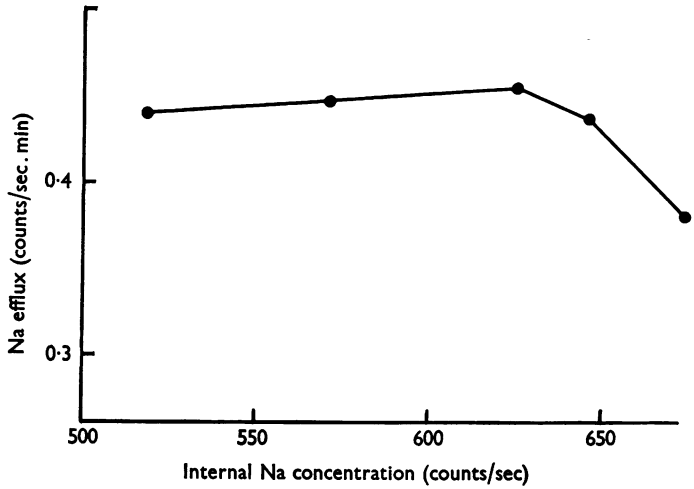
where $[Na^*]_1$ and $[Na^*]_2$ were the Na* contents of the two compartments. If $d[Na^*]/dt$ is plotted against the mean [Na*] during the interval of measurement then the graph obtained should be initially almost straight with slope k_1 and then curve towards the origin to end again as an approximately straight line with slope k_2. Such plots have been already performed in Text-fig. 10 and it may clearly be seen that they lend no support to this hypothesis.

(5) That loss of Na is restricted after a time by slow diffusion of Na in the cell interior, i.e. a gradient of Na concentration is created so that the peripheral concentration is less than the central concentration. This theory is similar to that already employed in the analysis of data on erythrocytes and muscle by Harris & Prankerd (1957) and Harris (1957). The possibility that non-exponential Na efflux from the oocyte could be explained in this way was investigated by calculating efflux curves on the basis of various assumed values of the internal diffusion coefficient and comparing these with curves obtained experimentally. Both calculated curves and data are shown in Text-fig. 7 on a semi-logarithmic plot. The plot of the data has a pronounced curvature and maximum curvature of the calculated lines was obtained by assuming that the membrane permeability was infinitely large. (At all finite values of the permeability coefficient the curvature is reduced until, when the permeability coefficient is very low compared with the coefficient of internal diffusion, the calculated line is straight.) Nevertheless, it is seen that no value of the diffusion coefficient consistent with the rate of Na efflux is capable of giving a graph of sufficient curvature.

The effect of high internal Na concentration on Na efflux into Na-free Ringer's solution

The relation between $[Na]_i$ and Na efflux is different from that previously described when $[Na]_i$ is high. An experiment on a cell with high $[Na]_i$ is illustrated in Text-fig. 13. As the internal sodium concentration (initially 117 mM) falls the sodium efflux rate actually rises at first and then remains practically constant. Such behaviour can only be attributed to saturation of the sodium carrier. The initial rise even suggests that a degree of auto-inhibition may occur.

In the case of Expts. 113/3 and 117/3, illustrated in Text-fig. 10, the half-saturation values of $[Na]_i$ may be estimated as 25 and 21 mM, respectively, but in some other experiments no definite evidence of saturation was apparent even up to 40 mM. Half-saturation values were obtained by fitting the data to a Michaelis–Menten type equation with the use of Lineweaver & Burk's method of analysis.



Text-fig. 13. Relation between Na efflux and internal Na concentration when this is high (initially 117 mM). The comparative constancy of Na efflux indicates saturation of the Na carrier.

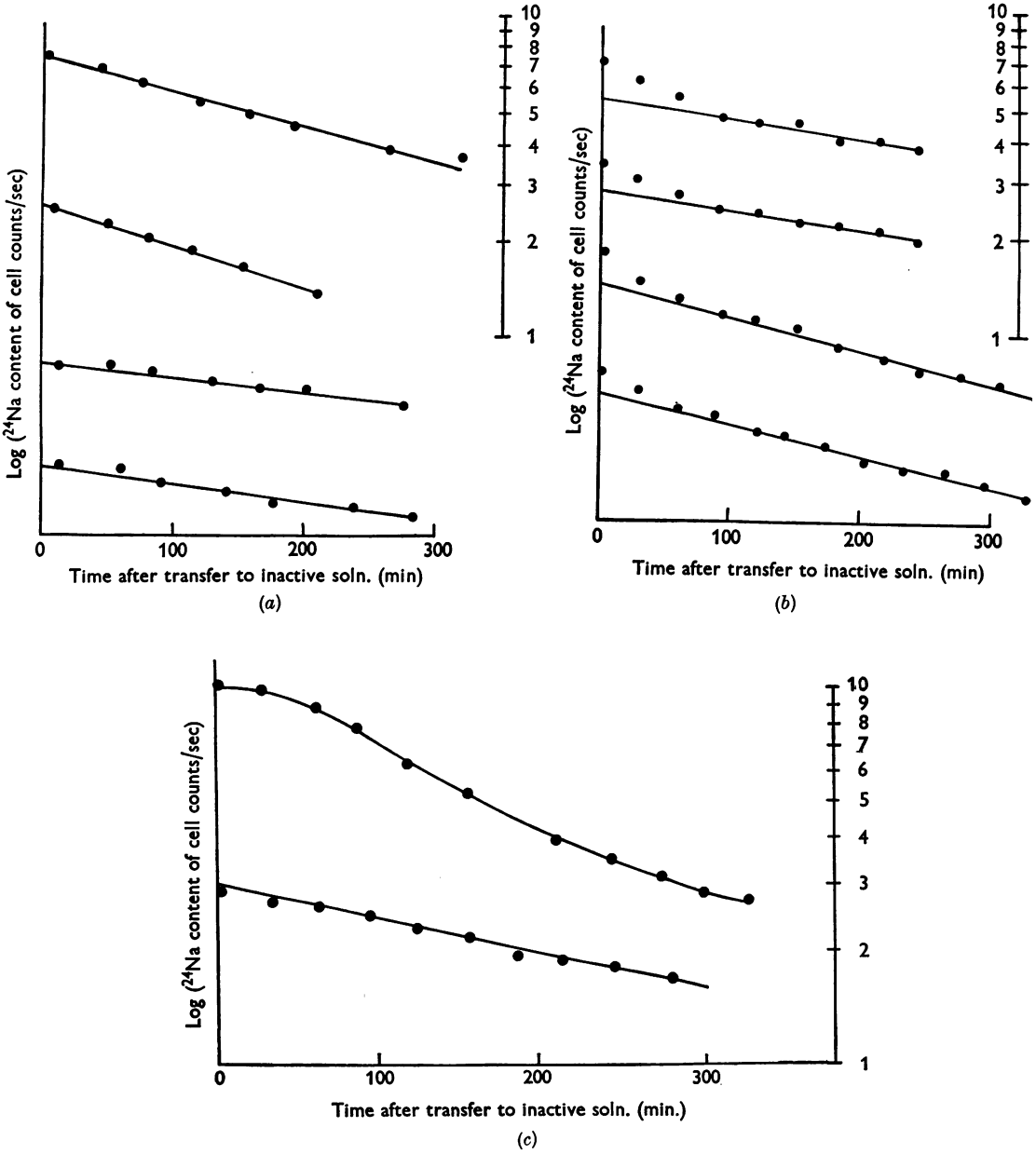
Kinetics of Na efflux into Ringer's solution

The patterns of Na* efflux from single oocytes soaked for 6–17 hr in radioactive Ringer's solution were found to fall into three categories:

Group A, efflux exponential throughout;
Group B, initial fast-moving component apparently present;
Group C, initial slow-moving component apparently present.

Some experiments of Groups A, B and C are illustrated in Text-fig. 14*a–c*. The sodium content of each oocyte immediately after removal from radioactive Ringer's solution was calculated using the measured radioactivity and an estimate of the ratio of internal to external specific activity obtained from Text-fig. 5. (The error of such an estimate is, of course, considerable, probably up to 25% as may be judged from the scatter of points in the figure.) The distribution of the initial Na concentration of oocytes in Groups A, B and C is shown in Text-fig. 15. The distribution of Group B is seen to be comparatively restricted with a mean value of 23·6 mM, slightly higher than that of the oocytes which had been treated with trypsin, but not exposed to radiosodium described in a previous section, which had a mean sodium concentration of 21·1 mM. On the other hand the sodium concentration of oocytes in Group A are widely scattered. Those of Group C are too few to allow any conclusion to be formed.

Leaving aside for the moment Groups A and C, the most obvious explanation of the behaviour of oocytes of Group B was that some sodium was retained on the surface of the oocyte after washing and subsequently



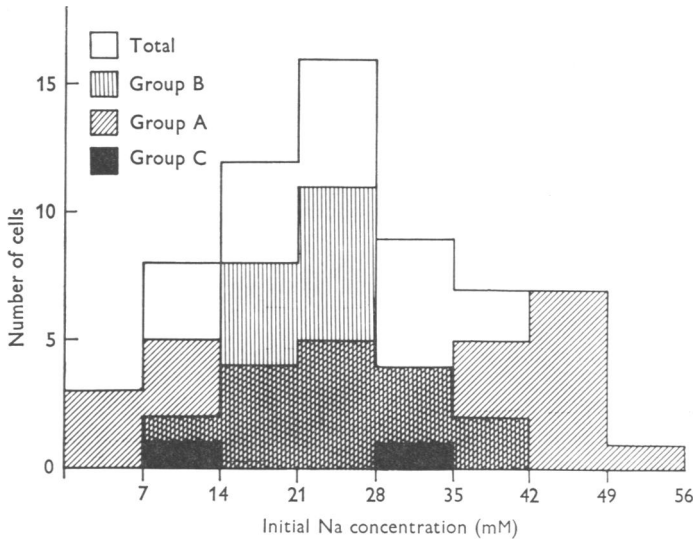
Text-fig. 14. Efflux of ^{24}Na from single oocytes into inactive Ringer's solution. Cells belong to (*a*) Group A, (*b*) Group B, (*c*) Group C (see text).

escaped more rapidly than the rest. However, a number of facts were against this view.

1. The half-life of the fast component is 30–60 min, which is much longer than that expected for loss by a simple diffusion process from a surface layer of sodium.

2. Washing was very thorough as described above, producing a theoretical attenuation of approx. 10^{-11} times in the external concentration of radiosodium.

3. Electron microscopic investigation of the oocyte surface showed that it was partially covered with follicular cells varying from 2 to 0·5 μ or less in thickness (Pl. 2). The volume of these cells can thus be estimated as

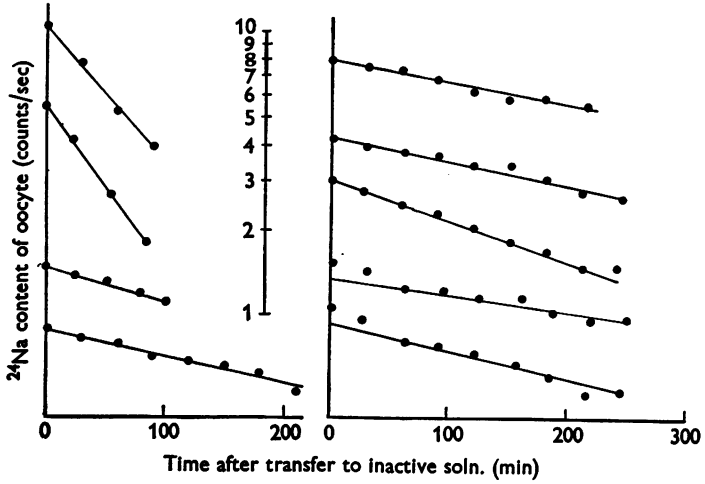


Text-fig. 15. Frequency distribution of internal Na concentration of oocytes with different types of Na efflux. Cells are divided in Groups A, B and C (see text, p. 17). □, total cells; ▨, Group A; ▥, Group B; ■ Group C.

approx. 1 % of the total cell volume. On the supposition that these cells remain functionally intact and that their sodium concentration is the same as that of the oocyte, these cells could thus only contribute a fast component of 1 % of the total cell sodium. (The follicular cell sodium would be expected to move faster merely on account of the high surface/volume ratio of the cell.) Even if the sodium content of the follicular cells were raised to approximately that of Ringer's solution they would still not contain more than 5–6 % of the total cell sodium. This amount is not large enough to account for the apparent fast component seen in Group B, consisting of approximately 20–50 % of total cell sodium.

In order to test the possibility that the apparent fast-moving component

of the radiosodium content of the oocytes of Group B might lie in a surface layer, a further experiment was performed. The initial soaking time in radioactive Ringer's solution was reduced to 2 hr so that it would be expected that the fast component would be approximately 80 % replaced by ^{24}Na in this time, whereas the main slow component would be only approx. 25 % replaced. Thus on placing the cells in inactive Ringer's solution the apparent relative size of the fast-moving component should be increased by a factor of three. The results of such experiments are illustrated in Text-fig. 16. It is seen that only in a few cases is any fast component



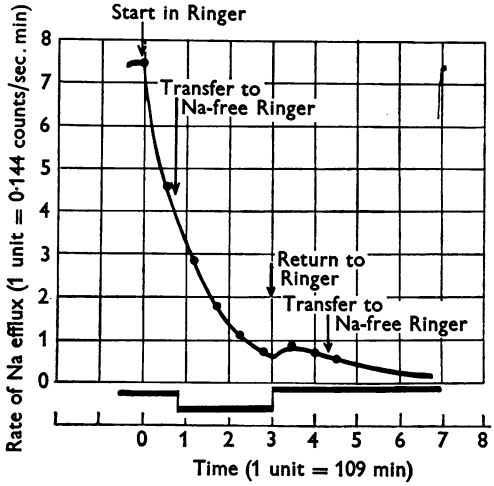
Text-fig. 16. Loss of ^{24}Na from single oocytes loaded with ^{24}Na for only 2 hr. Log plot. Little or no decrease in ^{24}Na efflux rate occurs.

visible at all, while in these cases where it does occur the size is *diminished* to a maximum of only 13 % of the total ^{24}Na content. This behaviour is not compatible with the presence of a fast-moving component of cell sodium at all.

The explanation which was finally adopted and to which no serious objections were found was based on the assumption of a three-compartments-in-series model to account for the fluxes of sodium. The assumption was also made that in cells of Group A total Na influx and efflux were equal, in Group B efflux exceeded influx (see Discussion p. 80), and in Group C influx exceeded efflux. The calculations necessary to demonstrate the consequences of this theory and the degree of fit between these and the data are given in the Discussion and Appendix 1.

The effect of sudden changes in the external Na concentration on Na efflux

An oocyte loaded with radiosodium was placed in inactive Ringer's solution (solution A). Both the total activity of ^{24}Na in the cell and the ^{24}Na escaping from the cell were measured at intervals of approximately 1 hr. At 134 min from the beginning of the experiment the oocyte was transferred to Na-free Ringer's solution (solution C), at 366 min it was returned to Ringer's solution, and finally at 493 min it was transferred for a second time to Na-free Ringer's solution (Text-fig. 17). The striking result is



Text-fig. 17. Effect on Na efflux from a single oocyte of sudden transfer from Ringer's solution to Na-free Ringer's solution, followed by return to Ringer's solution and finally transfer to Na-free Ringer's solution. Abscissa, time from initial transfer to non-radioactive solution. Ordinate, ^{24}Na efflux (counts/sec per min). Dots represent experimental points. Continuous line represents theoretical behaviour computed on the basis of the three-compartments-in-series model as described in Appendix 1. (Zero time shown here is the mean time of the initial period of Na efflux measurement. The experiment was actually begun 40 min earlier.)

that neither on the first or second transfer to Na-free Ringer's solution is there any sudden change of Na efflux. Only on return to Ringer's solution is a comparatively rapid increase of efflux produced. This result is clearly not compatible with any direct linkage of Na influx and efflux. A possible theoretical model which accounts accurately for these changes is presented in the Discussion (p. 76).

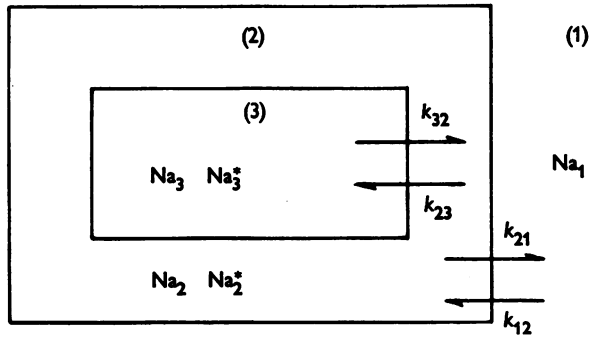
DISCUSSION

Interpretation of kinetics of Na efflux: the three-compartments-in-series model

Compartment (1) represents the external solution.

Compartment (2) contains Na replaceable by Na or Li ('free' Na).

Compartment (3) contains Na replaceable only by Na from compartment (2) ('bound' Na).



k_{12}, k_{21} are rate constants for exchange of sodium between compartments (1) and (2). Asterisks indicate concentrations of labelled sodium.

The exchange between compartments (2) and (3) deserves particular attention.

It is assumed that the Law of Mass Action applies (see p. 68). This leads to the equation

$$Na_2^* + Na_3 \underset{k_{32}}{\overset{k_{23}}{\rightleftharpoons}} Na_2 + Na_3^*. \tag{10}$$

At equilibrium

$$k_{23}[Na^*]_2[Na]_3 = k_{32}[Na]_2[Na^*]_3, \tag{11}$$

$$\frac{k_{23}}{k_{32}} = \frac{[Na^*]_3}{[Na]_3} \Big/ \frac{[Na^*]_2}{[Na]_2} = 1. \tag{12}$$

(Since at equilibrium, specific activities must be equal.)

When there is no radioactivity in the external solution, the equations for exchange between compartments are

$$\frac{d[Na]_2}{dt} = k_{12}[Na]_1 - k_{21}[Na]_2, \tag{13}$$

$$\frac{d[Na]_3}{dt} = 0, \tag{14}$$

$$\frac{d[Na^*]_2}{dt} = -k_{21}[Na^*]_2 - k_{23}[Na^*]_2[Na]_3 + k_{23}[Na^*]_3[Na]_2, \tag{15}$$

$$\frac{d[Na^*]_3}{dt} = k_{23}[Na]_3[Na^*]_2 - k_{23}[Na]_2[Na^*]_3. \tag{16}$$

Note. Equations (13) and (14) refer to net movement of total sodium. The sodium content of compartment (3) remains constant.

Let the influx of total sodium be x times the efflux, i.e.

$$k_{12}[\mathrm{Na}]_1 = xk_{21}[\mathrm{Na}]_2. \tag{17}$$

Thus equation (13) may be written

$$\frac{\mathrm{d}[\mathrm{Na}]_2}{\mathrm{d}t} = -k_{21}[\mathrm{Na}]_2(1-x) \tag{18}$$

and on integrating

$$[\mathrm{Na}]_2 = [\mathrm{Na}]_{2(0)}\mathrm{e}^{-k_{21}(1-x)t}, \tag{19}$$

where $\mathrm{Na}_{2(0)}$ is the initial sodium content of compartment (2). Substituting for Na_2 in (15) and (16)

$$\frac{\mathrm{d}[\mathrm{Na}^*]_2}{dt} = -(k_{21}+k_{23}[\mathrm{Na}]_3)[\mathrm{Na}^*]_2 + k_{23}[\mathrm{Na}]_{2(0)}\,[\mathrm{Na}^*]_3 e^{-k_{21}(1-x)t}, \tag{20}$$

$$\frac{\mathrm{d}[\mathrm{Na}^*]_3}{dt} = k_{23}[\mathrm{Na}]_3[\mathrm{Na}^*]_2 - k_{23}[\mathrm{Na}]_{2(0)}\,[\mathrm{Na}^*]_3 e^{-k_{21}(1-x)t} \tag{21}$$

Three different cases for efflux of [Na*] are considered:

(1) Efflux of [Na*] into Li-Ringer's solution.

This is described by the solution of (20) and (21) for $x = 0$ and

$$\mathrm{d}[\mathrm{Na}^*]_3/dt = 0$$

(see Text-fig. 18), i.e. from (21)

$$k_{23}[\mathrm{Na}]_3[\mathrm{Na}^*]_2 = k_{23}[\mathrm{Na}]_{2(0)}[\mathrm{Na}^*]_3 e^{-k_{21}(1-x)t} \tag{22}$$

and thus on inserting (22) in (20)

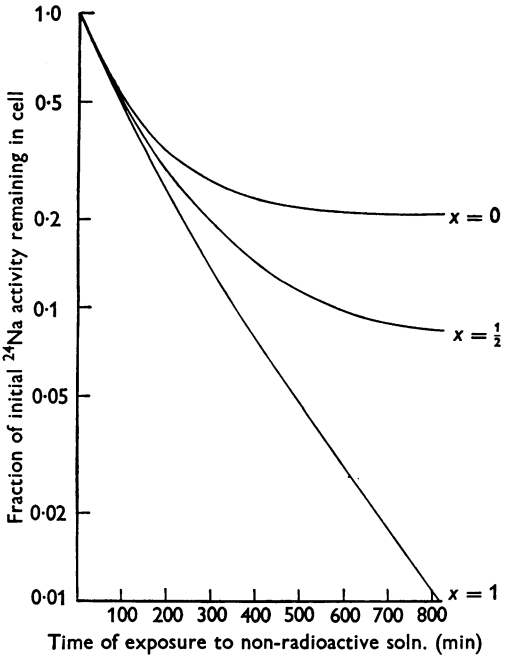
$$\frac{\mathrm{d}[\mathrm{Na}^*]_2}{\mathrm{d}t} = -k_{21}[\mathrm{Na}^*]_2. \tag{23}$$

Since $[\mathrm{Na}^*]_2 = [\mathrm{Na}^*]_1 - [\mathrm{Na}^*]_B$ (in terms of equation (4)), equation (4) is thus equivalent to equation (23) and is merely a special case of equations (20) and (21). Since equation (4) has been found to provide a satisfactory fit to the appropriate data (Text-fig. 10), the three-compartment-in-series model provides an adequate explanation of this case.

(2) Efflux of Na* into Ringer's solution.

This is described by the solution of equations (20) and (21) for $x = 1$ in this case of cells of Group A and with $0 < x < 1$ for Group B. Such solutions were obtained as described in Appendix 2 by means of the Analogue Computer and are illustrated in Text-fig. 18 replotted on semilog graph paper. It is clear that the curves obtained are similar to those in Text-fig. 14*a*, *b*, and that the three-compartments-in-series model provides

an adequate explanation for the data in these cases. (Two points must, however, be made: (i) in theory, since exchange between compartments 2 and 3 has been estimated to be comparatively rapid compared with that between compartment 1 and 2, over-all Na loss should be exponential and the graph for $x = 1$ should be straight; the slight curvature present is due to small errors in the functioning of the computer; (ii) when $x = \frac{1}{2}$ the internal Na concentration will fall gradually so that Na efflux should decrease after a time so that in this case x is not strictly a constant as assumed in the solution of equations (20) and (21); however, the error due to this approximation is likely to be small and the curve shown certainly



Text-fig. 18. Computed rate of loss of ^{24}Na from a cell, calculated as described in Appendix 2 from equations (20) and (21) with $x = 0$, $\frac{1}{2}$, and 1. Curves for $x = 0$ may be compared with that of a cell in Na-free Ringer's solution (Text-fig. 7), and curves for $x = 1$ and $x = \frac{1}{2}$ with those for cells of Groups A and B (Text-fig. 14*a*, *b*) respectively (allowing for variations of efflux rate constant between different cells).

indicates the result qualitatively.) It was not conveniently possible to compute the graph for $x > 1$, simulating the behaviour of Group C, but it may be seen that a curve of increasing gradient, such as that obtained, is to be expected.

(3) The effect of sudden changes in the external Na concentration on Na efflux.

The effect of Na* efflux of changing from Ringer's solution to Na-free Ringer's solution is represented by altering the value of x in the above equation from 1 to 0. The effect of changing from Na-free Ringer's solution back to Ringer's solution is represented by replacing equations (20) and (21) as follows:

From (13)
$$\frac{d[Na]_2}{dt} = k_{12}[Na]_1 - k_{21}[Na]_2. \tag{24}$$

But in the initial steady state of the cell

$$[Na]_2 = [Na]_{2(0)} \quad \text{and} \quad \frac{d[Na]_2}{dt} = 0. \tag{25}$$

Thus
$$k_{12}[Na]_1 = k_{21}[Na]_{2(0)}.$$

Substituting in equation (24) this gives

$$\frac{d[Na]_2}{dt} = k_{21}([Na]_{2(0)} - [Na]_2). \tag{26}$$

On integrating this gives

$$[Na]_2 = [Na]_{2(0)}\left(1 - \frac{[Na]_{2(0)} - [Na]_{2(1)}}{[Na]_{2(0)}} e^{-k_{21}t}\right), \tag{27}$$

where $Na_{2(1)}$ is the sodium content of compartment (2) when the change from Na-free Ringer's solution back to Ringer's solution takes place. Substituting this value for Na_2 in (15) and (16)

$$\frac{d[Na^*]_2}{dt} = (k_{21} + k_{23}Na_3)[Na^*]_2 + k_{23}[Na^*]_3[Na]_{2(0)}\left(1 - \frac{[Na]_{2(0)} - [Na]_{2(1)}}{[Na]_{2(0)}} e^{-k_{21}t}\right) \tag{28}$$

$$\frac{d[Na^*]_3}{dt} = k_{23}[Na]_3[Na^*]_2 - k_{23}[Na]_{2(0)}[Na^*]_3\left(1 - \frac{[Na]_{2(0)} - [Na]_{2(1)}}{[Na]_{2(0)}} e^{-k_{21}t}\right) \tag{29}$$

Equations (28) and (29) were used in place of equations (20) and (21).

Equations (20) and (21), and (28) and (29), were solved by means of the analogue computer, as described in Appendix 1, and the solution shown in Text-fig. 17 superimposed on the data of experiment 119 (2). It is seen that the computed solution correctly predicts the small effect of the first transfer to Na-free Ringer's solution and also the considerable rise in Na-efflux on return to Ringer's solution. It was not possible to simulate on the computer the effect of the second transfer to Na-free Ringer's solution.

The appropriate values of the parameters used in computing the solution for experiment 119 (2) were

$$[Na]_{2(0)} = 30 \text{ V} \quad [Na]_3 = 10 \text{ V},$$

$$\left.\begin{aligned} K_1 &= 1{\cdot}0 \\ K_2 &= 0{\cdot}5 \\ K_3 &= 1{\cdot}5 \\ K_4 &= 0{\cdot}5 \\ K_5 &= 0{\cdot}5 \end{aligned}\right\} \text{(machine units of time)}^{-1},$$

K_1, K_2, K_3, K_4 and K_5 are defined in Appendix 1. Since:

Total initial Na concentration = $[Na]_{2(0)} + [Na]_3 = 18{\cdot}9$ mM $\equiv 40$ V and 1 machine unit of time $\equiv 54{\cdot}5$ min (see Appendix 1), then after changing units and substituting in equations (5′), (6′), (7′), and (8′) (of Appendix 1),

$$\begin{aligned} [Na]_{2(0)} &= 14{\cdot}2 \text{ mM}, \\ [Na]_3 &= 4{\cdot}7 \text{ mM}, \\ [Na]_{2(1)} &= 7{\cdot}1 \text{ mM}, \\ k_{21} &= 0{\cdot}009 \text{ min}^{-1}, \\ k_{23} &= 1{\cdot}90 \text{ M}^{-1} \text{ min}^{-1}. \end{aligned}$$

Evidence that some oocytes create a net sodium efflux

In order to test the theory just applied to cells of Group B, an experiment was performed in which the sodium content of a batch of nine cells was analysed by flame photometry immediately after removal from radioactive Ringer's solution and found to contain 33·8 m-mole/l. of Na. A second batch of the same cells was placed in non-radioactive Ringer's solution for 4 hr and then similarly analysed and found to contain 26·3 m-mole/l. of Na. In this case Na efflux clearly exceeded influx after removal from radioactive Ringer's solution.

Another group of experiments suggested a similar result. The initial rate of uptake of radioactivity by a single oocyte from radioactive Ringer's solution was assessed by measuring the radioactivity at $\frac{1}{2}$ hr intervals for $1\frac{1}{2}$ hr (as described in Methods) and the rate of Na influx calculated from the known specific activity of the solution. The oocyte was then transferred to inactive Ringer's solution and the rate of sodium efflux estimated by measuring the rate constant for loss of radioactivity (as described in Methods) and multiplying this by the volume/surface ratio of the oocyte and by the $[Na]_i$ estimated from the maximum radioactivity of the oocyte at the end of the period of soaking in radioactive Ringer's

solution in conjunction with the data of Text-fig. 5 (the error of this estimate is discussed on p. 71). In cases where non-exponential loss of radioactivity occurred the initial and final rate constants were measured and the corresponding rates of sodium efflux were calculated. Data so obtained are shown in Table 4. It is seen that in three out of four cases the initial rate of sodium efflux immediately after removal from radioactive Ringer's solution exceeded the initial rate of influx, but that subsequently the rate of efflux declined so as to be equal to or less than the initial rate of influx. In one case the rate of efflux remained throughout equal to the rate of influx. While it seems likely that a considerable increase of Na efflux occurs after removal from radioactive solution, it also seems possible that in Expts. 55/1 and 55/2 a decrease of Na influx occurred as well, assuming that the cell was finally in the steady state, so that the final Na influx was equal to the final Na efflux.

TABLE 4

Expt. no.	Influx from radio-active Ringer's solution (pM/cm², sec)	Into ordinary Ringer's solution: Initial efflux (pM/cm², sec)	Into ordinary Ringer's solution: Final efflux (pM/cm², sec)
55/1	35·5	53·3	12·0
55/2	17·9	54·0	10·6
58/1	7·0	16·7	6·9
5/2	9·2	9·5	9·5

From Text-fig. 15 it seems probable that the cells of Group B which create a net Na efflux are those with a slightly raised $[Na]_i$, when Na efflux rises in proportion to the raised $[Na]_i$. In cells of Group A where Na efflux approximately equals Na influx, the values of $[Na]_i$ are widely scattered. It may be supposed that actually Group A cells fall into two separate categories, (1) cells with normal $[Na]_i$ which are in a physiological steady state, and (2) cells with a greatly raised $[Na]_i$ in which either the outward Na carrier is wholly or partly saturated or an irreversible increase in Na influx has occurred, i.e. in either case the rise in $[Na]_i$ is irreversible. The fact that in Text-fig. 15 categories (1) and (2) appear to overlap with each other and with Group B is most probably due to error in the estimation of $[Na]_i$ (see p. 71), although it may also be due to individual variation in cell responses to increased $[Na]_i$.

Change of Na fluxes in radioactive Ringer's solution

Since the above results suggest that occasionally a temporary and reversible inhibition of Na efflux or increase of Na influx occurred during exposure to radioactive Ringer's solution, it is necessary to look for possible causes of this. Two possibilities may be mentioned:

(1) High radiation dose to the oocyte. The total dose during the normal

8 hr soaking period in radioactive Ringer's solution was approximately 4000 rad. However, in those experiments which were conducted on Wednesdays (42 hr after removal of ^{24}Na from the pile) and in which the radiation dose was only 1/3 of this, no significant difference was noted so that radiation may not be the significant factor.

(2) Contamination of radioactive solution with heavy metal. This possibility was considered by Hodgkin & Horowicz (1959). However, the effect described by these authors was an increase of sodium influx and was not reversible.

Properties of the amphibian oocyte

The water content of amphibian oocyte was reported by Hunter & De Luque (1959) as $50 \pm 10\%$ (wt./wt.) for *Bufo marinus* and $55 \pm 10\%$ (wt./wt.) for *Hyla labialis*. These values agree reasonably well with those shown in Text-fig. 2 (when corrected for a density of 1·05), since the oocytes used were large (1·2–1·4 μl. in volume or 1300–1400 μ in diameter). The water content obtained by Abelson & Duryee (1949) for frog oocytes, 1760 μ in diam., was 58% (vol./vol.).

The Na and K concentrations in oocytes were found by Hunter & De Luque (1959) to be 68 and 83 mM respectively for *Bufo marinus* (concentrations expressed per l. of cell water). While the sum of the Na and K concentrations is similar to that found now (151 mM as against approx. 140 mM shown in Text-fig. 3) the relatively high Na and low concentrations suggest that some inhibition in Na efflux or stimulation of Na influx had occurred. This may be a consequence of the later stage of development of the oocytes used or of the physiological conditions of the study. Hunter & De Luque's values of 44 mM-Na and 54 mM-K for oocytes of *Hyla labialis* give a sum of Na +K which appears to be rather low.

Abelson & Duryee (1949) also used large oocytes (diam. approx. 1760 μ). The Na concentration obtained by them was 68 mM which is again high and supports the hypothesis that older and larger oocytes have a higher Na concentration.

The relation between internal Na concentration and Na efflux

There is at present disagreement as to the relation between Na efflux and $[Na]_i$ in frog muscle. When the data are shown on a double log plot, Keynes & Swan (1959*a*) and Keynes (1963) find that the gradient is approximately 3 for $[Na]_i$ between 5 and 13 mM, but below 5 mM the gradient decreases to 1 or less ($[Na]_i$ from approximate calculations by present authors). On the other hand Mullins & Frumento (1963), while agreeing that the gradient is 3 between 3 and 8 mM $[Na]_i$ ('corrected for 5 mM-Na which was not exchanged with Na*), show a steadily increasing gradient

at low concentrations. As seen in Text-fig. 8 this latter finding is in agreement with the present experiments on toad oocytes. The cause of the discrepancy from Keynes's findings is not clear. Carmeliet (1964) recently reported a gradient of 2 in cat ventricular muscle.

Mullins & Frumento (1963) also show their data plotted on linear coordinates (their Fig. 8). These may be fitted closely by a straight line comparable to that used in Text-fig. 9. The value of 'bound' [Na] so obtained is 4·5 mM when the initial $[Na]_i$ is 35 mM, thus agreeing quite well with the present data shown in Text-fig. 10, in spite of the dissimilar material.

Keynes (1963) describes an experiment in which the rate of Na efflux in a muscle whose $[Na]_i$ is raised 1·9 times by shrinkage in hypertonic Ringer's solution is increased 3·5 times. This finding is again in disagreement with those of the present experiment (p. 65).

Keynes & Swan (1959*a*) proposed the hypothesis of two compartments in parallel to account for Na efflux into Na-free Ringer's solution, but then abandoned it on two grounds:

(1) that it would be necessary to make the unlikely supposition that in normal Ringer's solution the two compartments have equal rate constants so that the over-all process is exponential, and

(2) that, when a muscle is exposed in turn to Na-free Ringer's solution, Ringer's solution, and Na-free Ringer's solution, the final reduction of Na efflux is the same as the initial reduction while on the two-compartment hypothesis one would have expected that since activity remained principally in the compartment whose Na was not exchangeable for Li, sensitivity to Li should have increased on the second exposure. This effect has also been found in the oocyte (see Text-fig. 17).

Neither of these objections applies to the present hypothesis of two compartments in series, provided it is assumed that exchange of Na between the two components is comparatively rapid (an assumption supported by the correlation found between total $[Na]_i$ and 'bound' [Na]). Keynes & Swan (1959*a*) also discussed the possibility of two Na compartments in series, but concluded that this was unsatisfactory owing to its inability to explain the rapid increase in Na efflux produced by returning to Ringer's solution after long exposure to Na-free Ringer's solution. This rapid increase is also found in the oocyte (see Text-fig. 17). As shown above, however, such an effect may readily be explained by means of the present theory. In physical terms it may be supposed that replenishment of the 'free' Na compartment with external Na occurs rapidly and that the specific activity of this is quickly brought to equilibrium with that of the still radioactive 'bound' Na compartment. The replenished and radioactive 'free' Na compartment thus created quickly gives rise to the increased Na efflux which is found.

Does exchange diffusion of Na occur?

Since exchange diffusion was originally proposed by Ussing (1947) in order to account for Na exchange in muscle, perhaps the most persuasive evidence for its existence so far produced has been that of Keynes & Swan (1959*a*). However, as noted by Mullins & Frumento (1963) exchange diffusion may not always be present in muscle and may even appear to be negative in the squid axon (Hodgkin & Keynes, 1955; Mullins, Adelman & Sjodin, 1962). Mullins & Frumento have presented an extended theory of exchange diffusion to take account of these facts, based on the three site carrier of Keynes & Swan and the apparent saturation of the carrier at high concentrations.

However, the present data are not consistent with the presence of exchange diffusion in the oocyte at all. It may be that amphibian muscles and oocytes possess different Na transport mechanisms. On the other hand it may be noticed that almost all of the data produced by Keynes & Swan (1959) on the effect of Na efflux of sudden removal and restoration of external Na (their Figs. 1, 2, and 3) are in fact practically identical with the present data (Text-fig. 17), (allowing for the difference between linear and log scales), showing a slow falling off of Na efflux on removing external Na and a rapid rise on restoring it. Carmeliet (1964, Fig. 14) found a similar result in cat heart muscle. Keynes & Swan attributed the slow falling off to slow diffusion in the interfibre space of the muscle. However, it seems likely that this effect could be explained by postulating a 'bound' Na fraction in muscle as had been done above for the oocyte. This possibility must throw some doubt on the existence of an exchange diffusion mechanism at all. The absence of apparent exchange diffusion in some muscles and in the squid axon could be attributed to these tissues having a high Na content mainly as 'free' Na, so that the effect of 'bound' Na on Na efflux when the external Na concentration is altered would be relatively insignificant.

The mechanism of Na efflux

The rate constant, k_{21}, calculated above must be considered as representing both active and passive Na efflux. The mean value of k_{21} obtained from the data of Table 2 was $(7{\cdot}3 \pm 0{\cdot}7) \times 10^{-3}$ min^{-1}. The mean rate of Na efflux across the cell membrane was $22{\cdot}8 \pm 5{\cdot}6$ pM/cm^2 sec. Abelson & Duryee (1949) obtained an efflux rate equivalent to 668 pM/cm^2 sec; however, these authors found an internal Na concentration of 68 mM so that the oocytes used by them were probably not in a physiologically comparable state, possibly owing to their greater age and size.

The efflux rate may be compared with the value of approx. 3·5 pM/cm^2

sec obtained by Hodgkin & Horowicz (1959) in single frog-muscle fibres. It seems possible that the higher value in oocytes is due to the presence of microvilli on the cell surface, thus increasing the area available for Na transport. Kemp (1956) has estimated that the surface area might thus be increased 35 times. He also described the development of folds in the cortical layer at a later stage so that the area might be still further increased, thus providing a possible explanation for the findings of Abelson & Duryee (1949).

The high Na efflux rate through the oocyte membrane is concordant with the high membrane conductance of 6 m-mho/cm^2 reported by Bayliss (1960) in frog oocytes. The high conductance is probably due to the presence of microvilli on the oocyte surface.

An intracellular Na fraction exchangeable with Na but not with Li has been described previously by Maizels & Remington (1959) in human erythrocytes and by Goodford & Hermansen (1961) and Goodford (1962) in guinea-pig smooth muscle. Goodford and Hermansen suggested that the fraction might lie in the cell nucleus. The nucleus also seems a very plausible site for the 'bound' Na fraction in the oocyte but no experiments have yet been carried out which demonstrated this.

SUMMARY

1. The water content of toad oocytes decreases with increasing size from 85 % (vol/vol.) to 65 % (vol./vol.) probably due to increasing accumulation of yolk.

2. Normal mean Na and K concentrations of oocytes are 21 and 120 mM, respectively.

3. Oocytes exchange Na with external ^{24}Na approximately exponentially. Probably all internal Na can be exchanged.

4. Only part of the internal Na ('free' Na) appears to give rise to Na efflux into Na-free Ringer's solution (Li replacing Na). The remainder ('bound' Na) appears to be unexchangeable (or very slowly exchangeable) with Li; the quantity of 'bound' Na varies with the total content Na of the cell and is approximately 10–30 % of the total Na. The mean rate constant for efflux of 'free' Na (k_{21}) was 0·0073 min^{-1} S.E. $\pm$ 0·0007.

5. Na efflux into Ringer's solution was found to be exponential in some cells (Group A). In others the rate of Na efflux decreased with time (Group B) or increased with time (Group C). These variations have been explained by the suggestion that only cells of Group A are in the steady state and that in Groups B and C net efflux and influx of Na occurs, respectively.

6. At high internal Na concentration Na efflux into Na-free Ringer's

solution either remains constant or increases with decreasing internal Na concentration, suggesting that either saturation or auto-inhibition of the Na carrier occurs.

7. On sudden replacement of external Na with Li, no sudden change of Na efflux occurs, but only a gradual decline. On restoration of external Na a sudden increase of Na efflux occurs. This behaviour has been interpreted on the basis of the above division of the Na of the system into three compartments (1) external Na (2) 'free' internal Na (3) 'bound' internal Na. It is unnecessary to postulate an exhange diffusion mechanism.

We wish to thank Professor W. D. M. Paton, F.R.S., for the kind loan of a Heathkit D.C. Analogue Computer. The investigation was supported by a grant for expenses from the Medical Research Council. E. J. A. Lea held a Research Scholarship awarded by the Wellcome Trust.

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APPENDIX 1

Equations (20), (21), (28) and (29) were solved using an analogue computer (Heathkit Model E.C. 1) modified by the provision of multiplier and additional amplifiers.

For an account of analogue computing techniques see Korn & Korn *Electronic Analog Computers*, McGraw Hill, 2nd edn., (1956).

Equations (20), (21), (28), and (29) were written in a more convenient form for analogue computing.

$$\frac{d[Na^*]_2}{dt} = -K_1[Na^*]_2 + K_3[Na^*]_3 e^{-K_2 t}, \tag{1'}$$

$$\frac{d[Na^*]_3}{dt} = K_4[Na^*]_2 - K_3[Na^*]_3 e^{-K_2 t}, \tag{2'}$$

$$\frac{d[Na^*]_2}{dt} = -K_1[Na^*]_2 + K_3(1 - K_5 e^{-K_2 t})[Na^*]_3, \tag{3'}$$

$$\frac{d[Na^*]_3}{dt} = K_4[Na^*]_2 - K_3(1 - K_5 e^{-K_2 t})[Na^*]_3, \tag{4'}$$

where

$$K_1 = k_{21} + k_{23}[Na]_3,$$

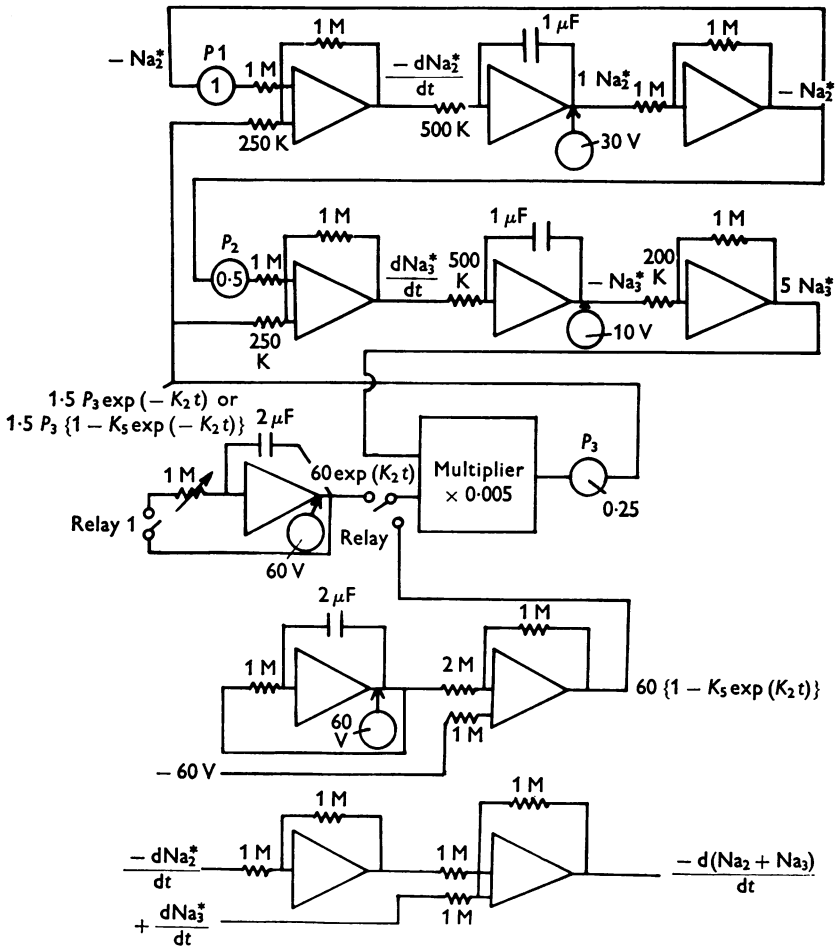
$$K_2 = k_{21}(1 - x),$$

$$K_3 = k_{23}[Na]_{2(0)},$$

$$K_4 = k_{23}[Na]_3,$$

$$K_5 = \frac{[Na]_{2(0)} - [Na]_{2(1)}}{[Na]_{2(0)}}.$$

The variables of the above equations are represented by voltages in the analogue computer. The time course of any of the variables or functions of the variables could be examined simply by measuring the voltage at the



Text-fig. 19. For an explanation of the symbols see Korn & Korn (1956), p. 37. Relay 1 alters the value of the resistance indicated from 1 MΩ to ∞. This represents the change from Ringer's solution to Li-Ringer's solution. Relay 2 replaces the $60\ e^{-K_2 t}$ by $60\ (1 - K_5 e^{-K_2 t})$. This represents the change from Na-free Ringer's solution to Ringer's solution.

Note that the initial condition on the circuit generating $60\ (1 - K_5 e^{-K_2 t})$ is also operated by relay 2.

appropriate part of the computer circuit with an oscilloscope. Records were made by photographing the oscilloscope screen. The time course of the analogue trace was of the order of seconds in comparison with the time of the actual experiment which was of the order of hours.

Cases (1) and (2) are quite straight forward and were carried out using

the circuit shown in Text-fig. 19. The resulting solutions are shown in Text-fig. 18. For case (3) the value of x was altered from 1·0 to 0 by opening relay 1 thus simulating the change from Ringer's solution to Na-free Ringer's solution. The change from Na-free Ringer's solution back to Ringer's solution was simulated by using relay 2 to replace the part of the computer circuit representing the $K_3[Na_3^*]e^{-K_2t}$ of equations 1′ and 2′ by a circuit representing the $K_3[Na_3^*]\ (1-K_5e^{-K_2t})$ of equations 3′ and 4′. The computed solution is shown by the continuous line in Text-fig. 17.

APPENDIX 2

In Ringer's solution containing Na let:

$$\frac{d[Na]_i}{dt} = -k(1-qt)[Na]_i + U \tag{5′}$$

(equation (5) is identical with equation (5′) with $U = 0$), where

U = rate of influx of Na,

k = permeability coefficient,

q = fractional rate of decline of permeability coefficient,

$$q = \frac{k[Na]_i + \frac{d[Na]_i}{dt} - U}{kt[Na]_i}. \tag{6′}$$

But

$$[Na]_i \simeq [Na]_{i(0)} + t\frac{d[Na]_i}{dt} \tag{7′}$$

(assuming for the moment that $d[Na]_i/dt$ is constant to a first approximation).

Then

$$q = \frac{k\left([Na]_{i(0)} + t\frac{d[Na]_i}{dt}\right) + \frac{d[Na]_i}{dt} - U}{kt[Na]_i} \tag{8′}$$

(where $[Na]_{i(0)} = [Na]_i$ when $t = 0$). But if it is assumed that the cell is initially in the steady state, then $k[Na]_{i(0)} = U$.

Thus

$$q = \frac{1}{[Na]_i}\frac{d[Na]_i}{dt}\left(1 + \frac{1}{kt}\right). \tag{9′}$$

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EXPLANATION OF PLATES

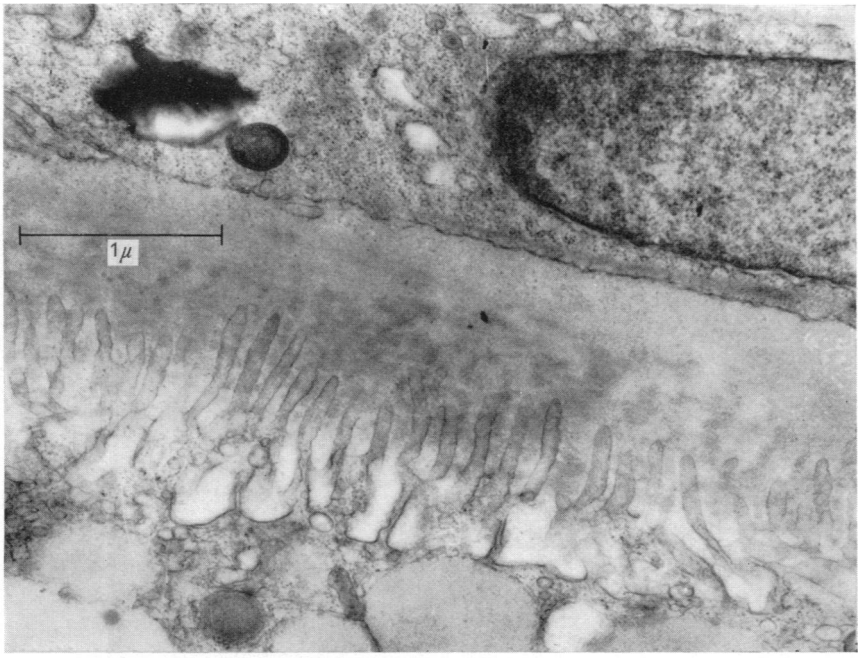
PLATE 1

Electron micrograph of surface of oocyte. (*a*) Before treatment with trypsin. Microvilli are present approx. 1 μ long by 0·05–0·1 μ in diam. (*b*) After treatment with trypsin for 1 hr at 30° C. Microvilli have been distorted by removal of the follicular cells, but are not otherwise changed (see p. 56).

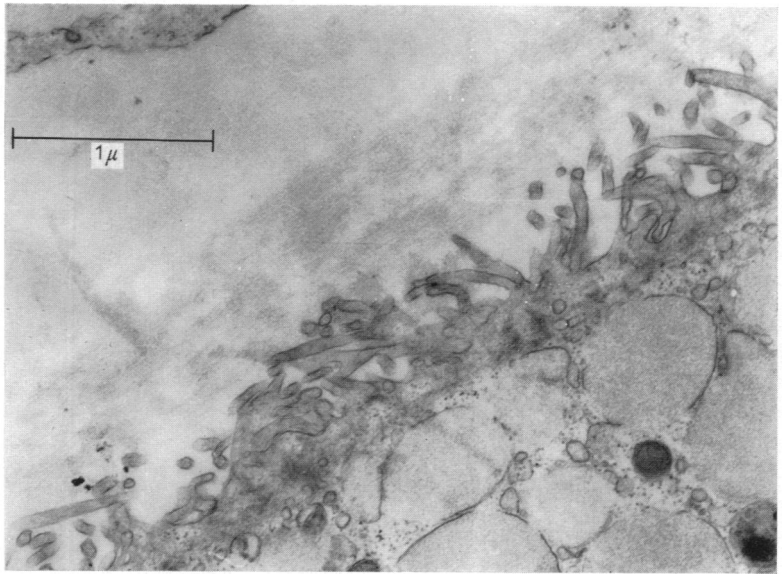
PLATE 2

Low power electron micrograph of oocyte surface after treatment with trypsin for 1 hr at 30° C. The surface is here covered with a layer of follicular cells; this layer only partially covered the oocyte surface (see p. 73).

(*a*)

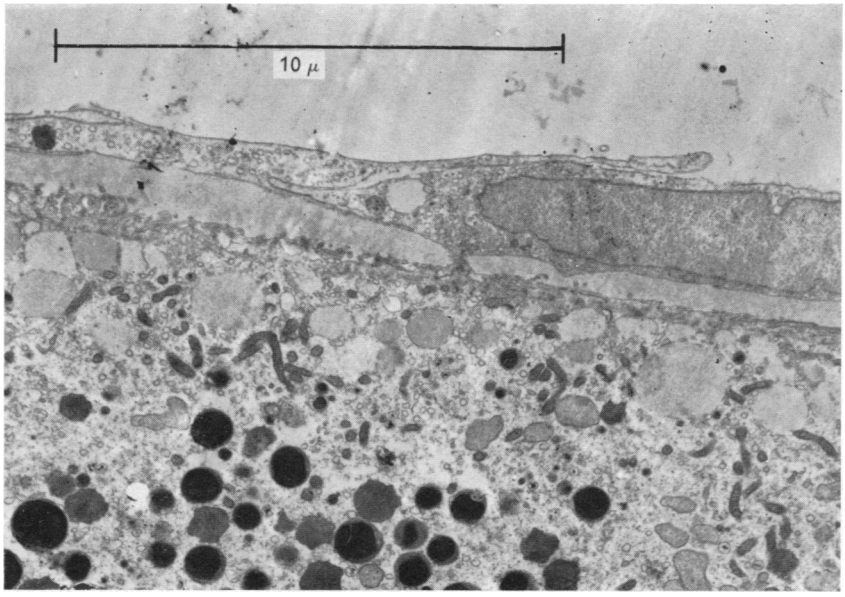


(*b*)



D. A. T. DICK AND E. J. A. LEA

(*Facing p.* 90)



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