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THE DISTRIBUTION OF SODIUM AND POTASSIUM IN AMPHIBIAN EMBRYOS DURING EARLY DEVELOPMENT

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SUMMARY

1. Intracellular and intercellular concentrations of sodium and potassium have been measured in pregastrular embryos of *Xenopus laevis* and *Amblystoma mexicanum*. Calcium and magnesium contents have also been determined.

2. Between egg and gastrula stages of development the potassium concentration is near to 60 m-mole/l. embryo. Up to the blastula stage the sodium concentration is near to 50 m-mole/l. embryo; the amount of sodium in the embryo begins to fall just before gastrulation.

3. Embryo calcium and magnesium concentrations show no significant variations prior to gastrulation. Average calcium concentrations for the different stages range from 5 to 8 m-mole/l. embryo; magnesium concentrations lie between 12·4 and 17 m-mole/l. embryo.

4. The intercellular fluid contains 100 mM sodium and 1 mM potassium; the majority of the sodium is ionically active. As no net uptake of sodium or potassium occurs before gastrulation these cations must have been transferred from the cells to the cavity.

5. At all developmental stages studied, the intracellular potassium concentration is close to 100 m-mole/l. cell water. Intracellular sodium falls steadily from 80 m-mole/l. cell water in eggs to 30 m-mole/l. cell water at the beginning of gastrulation.

6. The intracellular sodium activity, measured with sodium sensitive intracellular micro-electrodes, is relatively constant between egg and blastula stages at about 14 mM. During each cell division cycle the intracellular sodium activity rises transiently by 2–3 mM.

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INTRODUCTION

Little information is at present available on the ionic content of early embryonic cells. Kostellow & Morrill (1968) have looked at sodium and potassium contents of early and late blastulae of *Rana pipiens* embryos whilst Riemann, Muir & Macgregor (1969), Dick & McLaughlin (1969), Century, Fenichel & Horowicz (1970) and Morrill (1965) measured oocyte sodium and potassium levels in a number of amphibian species. Potassium was found at concentrations similar to those in adult cells and organs, but sodium was present at relatively high concentrations (70–100 mM). Variations in sodium and potassium content and concentration during early development have, by contrast, commanded little attention. Kostellow & Morrill's (1968) measurements, on two pre-gastrular stages only, suggested that before gastrulation the intracellular sodium concentration falls, because sodium moves from the intracellular compartment to the intercellular spaces, thus contributing to the developing blastocoel cavity. During this period of development determination of most endodermal and some mesodermal cell types takes place (Holtfreter, 1938*a*; Nieuwkoop, 1969*a*, *b*). Since the relative proportions of sodium and potassium in the cell cytoplasm can influence cell behaviour (Kroeger & Lezzi, 1966; Kuchler, 1967) it seemed worth obtaining more comprehensive information on intracellular sodium and potassium concentrations before gastrulation. Sodium and potassium contents of embryos at a number of early developmental stages have therefore been determined, together with the ionic composition of the intercellular fluid and embryo dry weight and volume, in order to calculate intracellular ionic concentrations. Information on calcium and magnesium levels in pregastrular embryos is also included.

Dick & McLaughlin (1969) showed that a considerable portion of the total intracellular sodium in *Bufo bufo* oocytes was in an ionically inactive form. Similar measurements made during early cleavage of *Xenopus laevis* are also reported in this paper.

METHODS

Embryos were obtained from mature adults of *Xenopus laevis* by injection of chorionic gonadotrophin and staged according to the Normal Table of *X. laevis* (Nieuwkoop & Faber, 1956). *Amblystoma mexicanum* embryos came from spontaneously breeding pairs of axolotls.

Total ion content

Sodium and potassium. Single embryos bathed in Holtfreter solution (60 mM-NaCl; 1 mM-KCl; 0·9 mM-$CaCl_2$; 1 mM-Tris-hydroxymethylamino methane; pH 7·4: Holtfreter, 1943) were cleaned of surrounding jelly with fine forceps, washed three times with double glass-distilled water, then suspended in 6 ml. distilled water and

broken up to release the intracellular contents, either by vigorous shaking, or by freezing and thawing. All washing was done in a room supplied with filtered, conditioned air to prevent atmospheric contamination; the sodium content of the final wash solution was negligible. Sodium and potassium were determined in the flame spectrophotometer. Jelly removed from the embryos and then washed retained negligible amounts of sodium and potassium.

Calcium and magnesium. Six cleaned and washed embryos at the same developmental stage were dispersed in 6 ml. double distilled water and calcium and magnesium concentrations determined in the flame spectrophotometer using the technique of Warren (1965).

The diameter of each embryo was measured before analysis using a calibrated microscope eye-piece graticule and the volume calculated assuming the embryo to be a sphere. This assumption introduced some error into the measurements, but variations in volume with age determined in this way agreed fairly well with estimates based on embryo wet weight, suggesting that the error was small.

Weight measurements

To ensure complete removal of water-retaining jelly, embryos were treated for less than 5 min with Holtfreter solution containing 2% cysteine and 0·1% papain (Dawid, 1965). Each embryo was then transferred to a small weighed aluminium foil cup, excess water removed with a damp filter paper spill and the cup reweighed on a torsion balance before drying to constant weight in an oven at 90° C.

Intercellular fluid analysis

After careful removal of the vitelline membrane, with fine forceps, a Stage 9 embryo was supported in a paraffin wax depression in a Petri dish filled with 120 m-osmolar sucrose solution buffered at pH 7·4 and topped by a layer of mineral oil. A Pyrex glass sampling pipette (tip diameter 7 μm), held in a micromanipulator, was connected to a 1 ml. glass syringe by silicone rubber tubing and the whole system filled with mineral oil. The pipette tip was pushed between the animal pole cells into the blastocoel and a small quantity of fluid aspirated from the cavity. Slight positive pressure applied to the syringe plunger during pipette withdrawal prevented contamination with intracellular fluid or sucrose; the sample was sealed from the air by taking up mineral oil. It was then expelled into an oil chamber to measure drop diameter, diluted, divided and stored in silica pipettes before analysis for sodium and potassium in the integrating flame photometer (Bosher & Warren, 1968). To test for mixing of intercellular and extra-embryonic fluids the bathing solution was sometimes coloured with dye after insertion of the pipette, but before aspiration; no dye appeared in the subsequent sample.

Sodium activity determinations

Micro-electrodes of sodium-sensitive glass tubing (Corning Code NAS 11–18), with tip diameters between 2 and 8 μm and filled with 0·1 M sodium chloride, were made according to the methods of Hinke (1961) and Thomas (1969, 1970). The electrode potential was measured in solutions of differing sodium concentration (sodium chloride/potassium chloride mixtures; total cation concentration kept constant at 0·1 M) and used to construct graphs relating electrode potential to sodium activity, making appropriate allowance for the activity coefficient of sodium chloride at an ionic strength of 0·1 M, for each electrode. The slope of this graph was always close to 58 mV for a tenfold change in sodium concentration and the small potassium sensitivity of the sodium sensitive glass was, therefore, ignored.

The sodium micro-electrode was either pushed between the animal pole cells into the blastocoel cavity or inserted into a cell along with a conventional glass micro-electrode (KCl-filled 20–60 MΩ resistance, low tip potential) which monitored the resting potential. The resting potential was subtracted from the potential recorded by the sodium electrode to give the sodium potential, E_{Na}. The sodium activity was then calculated according to the relation

$$E_{Na} = 58 \text{ mV} \log \frac{a^{\circ}_{Na}}{a^{u}_{Na}},$$

where E_{Na} is the change in potential on the electrode, a°_{Na} the activity in the bathing solution and a^{u}_{Na} the activity to be determined.

TABLE 1. Sodium and potassium contents of pregastrular stages of *Xenopus laevis*

Stage (Nieuwkoop)	Na content (m-mole/l. embryo)		K content (m-mole/l. embryo)		Na/K ratio		Volume ($\times 10^{-6}$ l.)		n
	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	Mean	S.E.M.	
Infertile egg	56·4	± 5·1	69·1	± 6·6	0·83	± 0·05	1·29	± 0·03	21
Egg 1	60·7	± 2·6	58·5	± 2·0	1·04	± 0·03	1·15	± 0·02	22
2 cell 2	50·6	± 2·2	62·6	± 3·3	0·82	± 0·02	1·21	± 0·02	20
4 cell 3	47·1	± 3·2	56·5	± 2·9	0·83	± 0·03	1·23	± 0·02	22
8 cell 4	55·2	± 3·9	68·9	± 4·5	0·80	± 0·02	1·28	± 0·02	25
16 cell 5	56·4	± 3·3	71·5	± 4·1	0·80	± 0·03	1·34	± 0·02	22
32 cell 6	56·4	± 4·8	65·9	± 4·3	0·84	± 0·04	1·39	± 0·02	20
64 cell 6½	46·4	± 3·6	56·5	± 4·1	0·83	± 0·03	1·33	± 0·03	19
Stage 7	45·3	± 2·8	56·4	± 2·3	0·81	± 0·03	1·33	± 0·02	20
Stage 8	48·3	± 2·7	58·9	± 1·9	0·81	± 0·04	1·29	± 0·03	24
Stage 9	39·7	± 2·7	55·9	± 3·0	0·72	± 0·03	1·41	± 0·02	20
Stage 10	38·2	± 2·3	51·3	± 2·4	0·76	± 0·04	1·50	± 0·03	19

RESULTS

Sodium and potassium contents of pregastrular stages of *Xenopus* are detailed in Table 1. The ratio of sodium content to potassium content and the mean volumes of the embryos analysed are also included.

Considerable variability in sodium content, potassium content and embryo diameter, both from batch to batch and within the same batch, made the detection of small differences in total ion content between each developmental stage impossible. Since the majority of embryos develop normally, it seems likely that within wide limits total ionic concentration is not a major factor determining normal development.

Fertile eggs contained approximately 60 m-mole/l. embryo sodium and potassium. After first cleavage the potassium content had not changed significantly, but the mean sodium content had fallen by about 10 m-mole/l.

embryo, bringing the sodium/potassium ratio down to 0·8. The Na/K ratio remained at about 0·8 up to the late blastula stage, although both Na and K contents rose slightly at 8, 16 and 32 cell stages. With the approach of gastrulation the sodium concentration and sodium/potassium ratio began to fall together. Kostellow & Morrill (1968) found the total sodium content of *Rana pipiens* embryos to increase slightly between the 64 cell stage and Shumway stage 9½ (late blastula, Shumway, 1940); the sodium and potassium concentrations and sodium/potassium ratios were similar to those given in Table 1. The mean embryo volume increased by about 30% between fertilization and gastrulation (Table 1), reflecting the growth of the intercellular cavity (cf. Tuft, 1962). Measurements made on *Amblystoma mexicanum* embryos showed the sodium and potassium contents to be similar to those found in *Xenopus*.

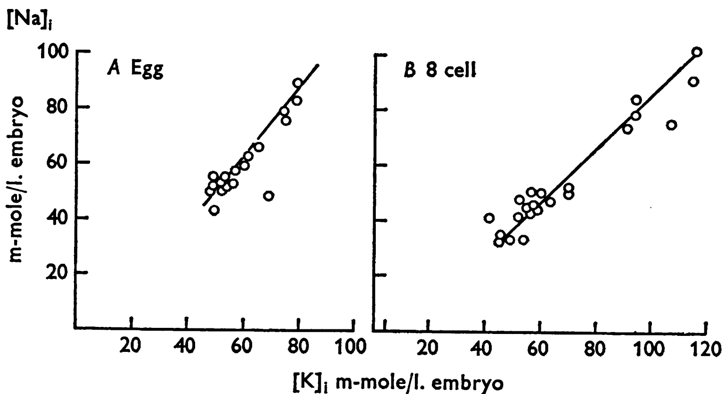

Fig. 1. Relation between sodium and potassium contents of eggs (*A*) and embryos at the 8 cell stage (*B*). Ordinates: concentration of sodium (m-mole/l. embryo). Abscissae: concentration of potassium (m-mole/l. embryo).

An unexpected feature was the good correlation between the sodium and potassium content of each embryo. Fig. 1 plots the sodium concentration (m-mole/l. embryo) as a function of potassium concentration (m-mole/l. embryo) for eggs (Fig. 1*A*) and embryos at the 8 cell stage (Fig. 1*B*). The sodium concentration increases linearly as the potassium concentration rises and the two values are significantly correlated (eggs: $r = +0.78$, $P < 0.001$; 8 cell: $r = +0.75$, $P < 0.001$). The basis of this correlation is not clear. Cannon & Dick (1970) found the sodium and potassium concentrations in *Bufo bufo* oocytes to be *inversely* related: when the sodium concentration was high the oocyte contained little potassium, so that the sum of the intracellular sodium and potassium concentrations remained the same. In their experiments the sodium/potassium ratio depended on

oocyte size and reflected different passive sodium permeabilities between small and large oocytes. In the present measurements embryo size was not the determining factor.

The possibility that the high sodium and potassium contents of some embryos were the result of contamination from another source cannot be ruled out, although it seems unlikely. Atmospheric contaminants are generally sodium rather than potassium salts, and on occasion sodium contamination, reflected in an abnormally high ratio between the sodium content and potassium content of that particular embryo, did occur. When *both* sodium and potassium contents were high, the ratio between the concentrations of these two ions fell within the same range as for embryos containing little sodium and potassium. This is illustrated in Fig. 1 by the linear relation between sodium and potassium concentrations; the slope reflects the average sodium/potassium ratio for that particular stage of development.

TABLE 2. Calcium and magnesium concentrations in pre-gastrular *Xenopus* embryos

Stage (Nieuwkoop)	$[Ca^{2+}]$ m-mole/l. embryo* mean ± S.E. of mean	[Ca] m-mole/l. cell H_2O	$[Mg^{2+}]$ m-mole/l. embryo mean ± S.E. of mean	[Mg] m-mole/l. cell H_2O	Ca/Mg ratio
Egg	7·4 ± 1·23	11·06	15·90 ± 0·69	23·73	0·46 ± 0·07
2 cell	5·3 ± 0·37	7·69	14·04 ± 0·64	21·6	0·38 ± 0·03
4 cell	5·5 ± 0·96	8·84	13·60 ± 0·82	21·8	0·40 ± 0·050
8/16 cell	8·0 ± 0·80	12·97	14·14 ± 0·34	22·8	0·57 ± 0·07
32/64 cell	7·3 ± 0·96	11·74	16·66 ± 1·26	26·87	0·44 ± 0·06
Stage 7	7·9 ± 1·81	13·6	15·20 ± 0·96	26·21	0·41 ± 0·08
Stage 8	7·5 ± 0·68	14·15	14·90 ± 1·15	28·1	0·51 ± 0·03
Stage 9	5·7 ± 0·98	10·91	14·40 ± 0·97	27·4	9·43 ± 0·09
Stage 10	5·4 ± 0·95	11·78	11·86 ± 0·69	25·7	0·47 ± 0·09

* Embryo volume calculated from embryo diameter assuming embryo to be a sphere.

Infertile eggs were significantly larger than those which had been fertilized, yet the sodium and potassium concentrations were high, suggesting that the sodium and potassium permeability of the ovulated egg does not greatly increase if fertilization does not ensue. The higher sodium/potassium ratio in fertile eggs may be the consequence of a change in membrane properties on fertilization (cf. Maeno, 1959; Morrill, 1965).

Calcium and magnesium contents of pregastrular *Xenopus* embryos are given in Table 2. Each value is based on analysis of forty embryos. Intracellular concentrations of calcium and magnesium, determined as for sodium and potassium (see below), are also detailed. The concentration of calcium in the blastocoel fluid was taken to be negligible on the basis of

Stableford's (1967) measurements in *Amblystoma* embryos; magnesium was assumed to be similarly distributed.

Both divalent cations were present at high concentrations in all pre-gastrular stages. Intracellular calcium was in the region of 7 m-mole/l. embryo as found by Morrill (1965) for unfertilized, ovulated eggs of *Rana pipiens*, which may be compared with a total $[Ca]_i$ of between 0·2 and 0·92 m-mole/kg axoplasm for the squid axon (Keynes & Lewis, 1956; Blaustein & Hodgkin, 1969). Intracellular calcium fell at first division, but rose again at the 8/16 cell stage before declining at the approach of gastrulation. Magnesium showed no significant variation. The inherent variability between embryos was considerable: the calcium concentration ranged from 2·8 to 24 m-mole/l. embryo.

The intercellular fluid

The blastocoel cavity becomes sufficiently large to allow aspiration of intercellular fluid by the late blastula stage (Stage 9). Fluid withdrawn from the cavity was viscous, as if it contained a high molecular weight compound, which fits Kalt's (1971 *a*, *b*) finding that the blastocoel contains glycogen. Intercellular sodium was $104 \pm 3{\cdot}6$ mM (mean $\pm$ S.D.) and potassium $1{\cdot}1 \pm 0{\cdot}24$ mM (mean $\pm$ S.D.), closely similar to the values for adult *Xenopus* plasma (Conway, 1957, quoted by Deyrup, 1964).

Measurements of sodium activity in the blastocoel cavity gave further evidence for a high intercellular sodium concentration. The activity of sodium, given by the change in potential on a sodium sensitive microelectrode on moving from Holtfreter solution into the blastocoel cavity, assuming the intrinsic blastocoel potential to be negligible (Slack & Warner, 1973), fell between 80 and 95 mM in four embryos; this puts the activity coefficient for sodium in the intercellular fluid close to that of an 0·1 M sodium chloride solution. The cavity fluid is at least 200 m-osmolar (Tuft, 1962) making a monovalent sodium salt a major constituent of the blastocoel fluid.

The present findings show the intercellular solution to have a higher sodium concentration than has hitherto been used for the isolation and culture of amphibian embryonic cells (60 mM-Na: Holtfreter, 1943; Jones & Elsdale, 1963; Curtis, 1957; 80 mM-Na: Barth & Barth, 1959) and suggest that a culture medium similar to Ringer solution (120 mM-Na) might be more suitable for isolated cell preparations. We have found cells prepared in Ringer solution to be more robust and have higher membrane potentials and intracellular potassium concentrations than those prepared in a low sodium medium (C. Slack & A. E. Warner, unpublished). Such cells survive well and differentiate in long term culture (P. A. Jackson & A. E. Warner, unpublished).

Intracellular ion concentrations

To convert the information given in Table 1 into intracellular ionic concentrations the relative proportions of water in the cells and intercellular fluid must be known. Because neither the cell volume (Tuft, 1962) nor the protein content (cf. Brachet, 1960) alter significantly before gastrulation the dry and wet weight of embryos of different ages (Table 3), will yield this information. As expected, the dry weight altered little before gastrulation and the wet weight increased approximately linearly from the two-cell

TABLE 3. Dry and wet weights of *Xenopus laevis* embryos during development

Stage (Nieuwkoop)	Dry wt. (mg) Mean	Dry wt. (mg) ± S.E. of mean	Wet wt. (mg) Mean	Wet wt. (mg) ± S.E. of mean	Dry/Wet wt. (%) Mean	Dry/Wet wt. (%) ± S.E. of mean	*n*	Blastocoel (%)
Egg	0·56	0·01	1·71	0·05	33·2	0·64	33	—
2 cell	0·52	0·01	1·45	0·05	36·0	1·7	25	—
4 cell	0·52	0·02	1·48	0·04	36·3	1·3	37	1·7
8 cell	0·53	0·02	1·57	0·05	33·8	1·3	35	3·0
16 cell	0·53	0·02	1·49	0·04	33·5	0·79	40	4·6
32 cell	0·46	0·03	1·48	0·06	32·2	1·4	21	5·8
48 cell	0·60	0·02	1·55	0·05	33·0	1·3	28	7·1
Stage 7	0·52	0·02	1·68	0·08	32·0	1·7	20	9·9
Stage 8	0·55	0·02	1·62	0·04	34·2	0·8	47	12·8
Stage 9	0·49	0·01	1·71	0·05	29·2	1·1	23	18·5
Stage 10	0·57	0·01	1·88	0·06	30·4	0·7	25	23·6

Embryos staged according to Normal Table of *Xenopus laevis* (Nieuwkoop & Faber, 1956).

stage onwards. The rise in wet weight (Table 3) and the corresponding increase in embryo volume (Table 1) are linked to the increasing size of the blastocoel cavity (Tuft, 1962), which can be identified even at the two cell stage (Kalt, 1971*a*). The proportionate volume occupied by the blastocoel, determined by plotting wet weight against age and assuming the blastocoel volume to be negligible at the two cell stage, is given in Table 3. By Stage 10 this had risen to 23·6%. The mean embryo volume was then $1{\cdot}5 \times 10^{-6}$ l. (Table 1), making the blastocoel volume 350×10^{-9} l.; the maximum amount of fluid which could be aspirated from the cavity at Stage 9 was 300×10^{-9} l. The ratio of the dry to the wet weight gives the fraction of the embryo occupied by solid material and the remainder is made up by the intracellular water.

The mean volume of eggs was slightly less than in embryos which had cleaved once (Table 1). The wet weights given in Table 3, however, show eggs to be nearly as heavy as embryos at Stage 9. The eggs used for the weight measurements happened

to include an unusually high proportion of large diameter cells. A plot of wet weight against volume showed that for eggs of the same volume as those used for ion content analysis the wet weight was a little less than that found for two-cell embryos.

Intracellular concentrations for each ion were calculated, with allowance for intercellular volume and ion content, using the formula (cf. Desmedt, 1953):

$$C_0 = \frac{C_{tot} - \text{fraction intercellular space} \times C_{intercell}}{\text{fraction cell water}},$$

where

C_0 = concentration in the intracellular water,

C_{tot} = total concentration in embryo,

$C_{intercell}$ = concentration in the intercellular water.

These are plotted in Fig. 2 for sodium and potassium. Fertilized eggs have about 90 m-mole/l. cell water of both sodium and potassium. First cleavage produces a fall in intracellular sodium and by the 48-cell stage

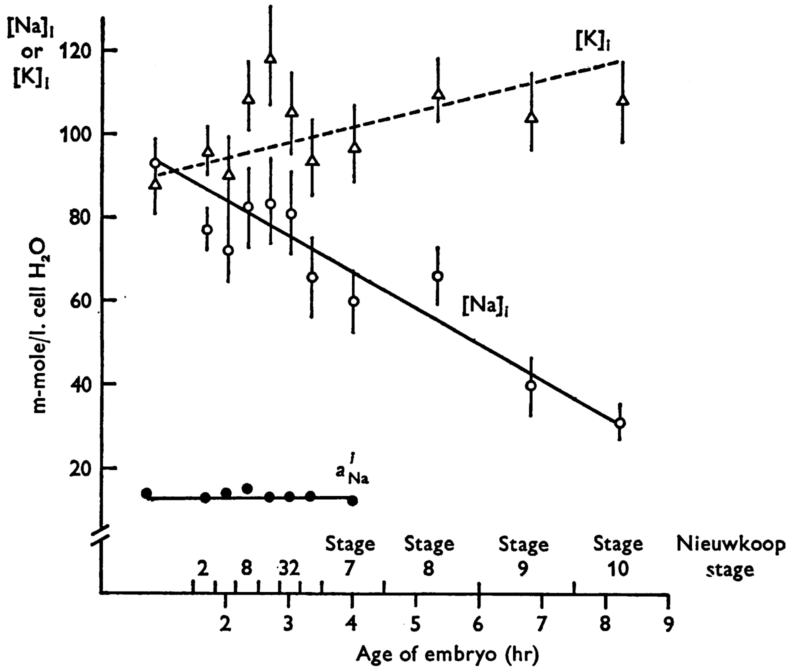

Fig. 2. Intracellular potassium and sodium concentrations and intracellular sodium activities in early *Xenopus laevis* embryos. Ordinate: cation concentration or activity expressed as m-mole/l. cell H_2O. Abscissa: age of embryo expressed as time from laying. Nieuwkoop Stages are also indicated. △: intracellular potassium concentration. ○: intracellular sodium concentration. ●: intracellular sodium activity. Vertical bars give ± 1 S.E. of mean. The S.E. of the activity measurements falls within the dots.

(Stage 6½) the cells have become further depleted of sodium. Intracellular sodium then falls steadily until gastrulation. The decline in cell sodium begins before any fall in the total sodium concentration (see Table 1), inferring that all the sodium found in the intercellular fluid comes from the cells. By contrast, the intracellular potassium concentration gradually increased over this phase of development, starting at a level below that in cells such as nerve and muscle, but ending up close to 120 m-mole/l. cell H_2O (intracellular potassium in frog muscle: 140 m-mole/kg. cell H_2O; Adrian, 1956). Consequently the ratio of sodium to potassium in the cells fell from unity to 0·3.

It should be emphasized that the intracellular concentrations of sodium, potassium, calcium and magnesium given in this paper represent average values for the whole embryo. From the morula stage (forty-eight cells) onwards the presumptive ectoderm cells at the animal pole divide more frequently and have a higher oxygen consumption than the presumptive endoderm cells at the vegetal pole, and it seems possible that the concentrations of inorganic cations may vary similarly. Such gradients in metabolic state between different parts of the early embryo have been invoked as part of the mechanism defining the spatial pattern of differentiation (Child, 1941; Brachet, 1960).

TABLE 4. Intracellular sodium activity coefficients during early development

Stage (Nieuwkoop)	a_{Na} (mM)	$[Na]_i$ (mM)	$a_{Na}/[Na]_i$	a_k (mM)	$[K]_i$ (mM)	a_{Na}/a_k
Egg	14·3 ± 1·2	93·2	0·15	66·4	88·5	0·22
2 cell	13·4 ± 0·8	77·5	0·17	72·2	96·2	0·19
4 cell	14·4 ± 0·7	72·2	0·20	67·9	90·5	0·21
8 cell	15·2 ± 1·2	83	0·18	81·8	109	0·19
16 cell	13·7 ± 1·6	84	0·16	88·5	118	0·15
32 cell	13·7 ± 2·0	81·5	0·17	79·5	106	0·17
Stage 6½	13·6 ± 1·7	66	0·21	70·5	94	0·19
Stage 7	12·7 ± 1·8	60·5	0·21	72·8	97	0·17

Mean ± S.E. of mean.
Each value of a_{Na} based on mean of five measurements.
Values for $[Na]_i$ and $[K]_i$ taken from Fig. 2.
a_k calculated from $[K]_i$ assuming the activity coefficient for K to be 0·75 (Robinson & Stokes, 1959).

Ionic activity of intracellular sodium

Fig. 2 plots the values of a^i_{Na} measured between egg and early blastula stages; the cells then became too small to allow reliable measurement with the sodium sensitive micro-electrodes. The activity is close to 14 mM throughout these stages, by contrast with the total intracellular Na concentration (Fig. 2). The variability from embryo to embryo also was small by comparison with that found in determinations of total ionic

content. The proportion of the intracellular sodium found to be ionically active ranged between 15 and 21 % (Table 4). Dick & Mclaughlin (1969) showed the sodium activity coefficient to be 0·36 in *Bufo bufo* oocytes (a^i_{Na} = 9·3 mM; C_{Na} = 25·8 m-mole/l. cell H_2O). Either the total sodium concentration in *B. bufo* eggs is lower than in *Xenopus laevis* or there is a sharp increase in intracellular sodium at about the time of laying and fertilization.

No measurements of the activity of cytoplasmic potassium have been made in these embryos. Dick & McLaughlin (1969) found the intracellular potassium activity coefficient in *Bufo bufo* oocytes to be closely similar to that of Ringer's solution (0·75; Robinson & Stokes, 1959). Woodward (1968) placed the activity of intracellular potassium in early embryos of *Rana pipiens* at about 90 mM and measurements of the membrane potential at different external potassium concentrations in cells

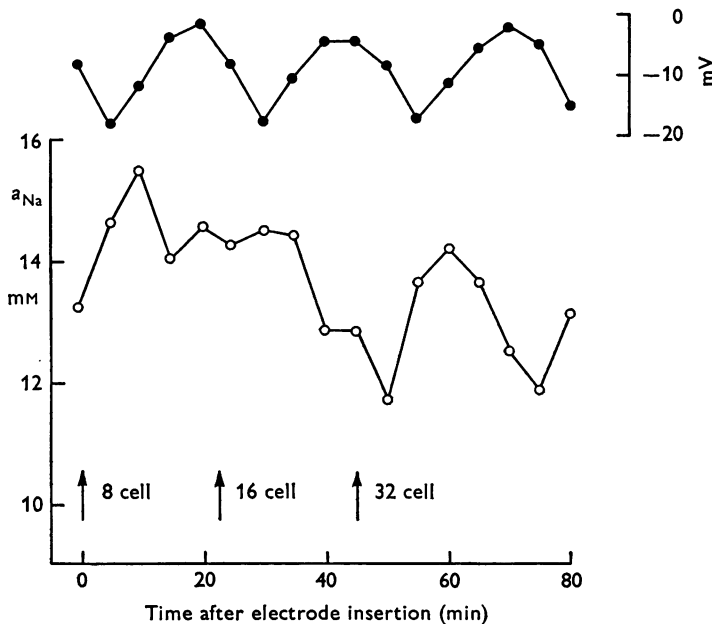

Fig. 3. Membrane potential and sodium activity recorded simultaneously in one cell of an embryo during cleavage from four cells to thirty cells. Ordinates: upper, membrane potential (mV); lower, intracellular sodium activity (mM). Abscissa: time in minutes. Arrows mark first appearance of division furrows.

isolated from *Xenopus laevis* blastulae (C. Slack & A. E. Warner, unpublished) indicate a similar activity of intracellular potassium at late cleavage stages. It therefore seems reasonable to suppose that in the cells of pregastrular *Xenopus* embryos potassium has a similar activity coefficient to that of the bathing solution.

On occasion the sodium sensitive micro-electrode was left in one cell for some time. Such experiments usually revealed a small, transient increase in

intracellular sodium activity during the cell division cycle. The variations in membrane potential and intracellular sodium activity in an animal pole cell of an embryo cleaving from four cells to thirty-two cells are shown in Fig. 3. At the beginning of 3rd cleavage the intracellular Na activity was 13·3 mM. It rose transiently to 15·5 mM about halfway through the cycle. During the early part of the subsequent cycle the sodium activity remained close to 14 mM, but then dropped back reaching 11·7 mM shortly after the start of division to thirty-two cells. Another transient rise in sodium activity took place during this cycle and the impaled cell began to cleave again just after the end of the record, by which time furrowing was already in progress in some animal pole cells. The upper graph shows the membrane potential of this cell recorded concurrently. This embryo happened to show the transient hyperpolarization which sometimes occurs at about the middle of the cell cycle; it fell close to the time of increase in intracellular sodium activity. Since nuclear and cytoplasmic divisions are one cycle out of phase in the early amphibian embryo (see Brachet, 1960) mitosis for the subsequent cell division must then be under way.

DISCUSSION

Amphibian oocytes have been reported to contain high intracellular concentrations of sodium relative to potassium (Naora, Naora, Izawa, Allfrey & Mirskey, 1962; Morrill, 1965; Kostellow & Morrill, 1968; Century *et al.* 1970) in contrast to most vertebrate and invertebrate cells. The present measurements show that this situation persists in the developing embryo until shortly before gastrulation. It seems likely that sodium initially found in the egg becomes divided between the cells and the intercellular fluid as cleavage proceeds, with the embryo neither gaining, nor losing, much sodium until the late blastula stage. Kostellow & Morrill (1968) proposed that sodium was actively taken up by the amphibian embryo from the bathing medium to an extent related to the increasing size of the blastocoel cavity. This was based on their finding of a greater uptake of ^{22}Na from a half-strength Ringer solution (55 m-equiv Na/l.) just before gastrulation than at the early blastula stage. The results given in this paper do not support this conclusion, but another explanation for Kostellow & Morill's (1968) findings seems possible. During the corresponding period of development in *Xenopus laevis* and *Triturus pyrrhogaster* embryos the intracellular membrane potential becomes more negative (Ito & Hori, 1966; Palmer & Slack, 1970) providing an additional inward driving force for all cations and in *Xenopus* embryos the intracellular sodium concentration falls from about 60 m-mole/l. cell water to 30 m-mole/l. cell H_2O. If the same situation existed in *Rana pipiens* embryos

then net entry of Na from half-strength Ringer fluid into embryos at Shumway stages 9½–10 (early gastrula) might result from an inwardly directed concentration gradient for sodium ions, coupled with an increased driving force imposed by the membrane potential.

The activity coefficient for sodium in the egg is about 0·15, which is low compared with the activity coefficient of an 0·1 M sodium chloride solution (0·75: Robinson & Stokes, 1959), suggesting that a large proportion of the intracellular sodium is held in an ionically inactive form. The level of ionically active sodium is regulated at about 14 mM during early cleavage stages, while total intracellular sodium falls (Fig. 2), so that 'bound' and 'free' sodium within the cell must be in dynamic equilibrium. Presumably sodium is continuously released from an intracellular store to keep pace with the movement of sodium from the cells to the intercellular spaces. The present experiments give no information about the location of this 'bound' sodium. In addition to the nucleus, embryonic cells contain numerous yolk platelets and lipid droplets which could all act as sites for the sequestering of sodium. There have been a number of estimates of nuclear sodium concentration in amphibian oocytes, giving values ranging from 15 m-equiv. Na/l. H_2O (Century *et al.* 1970) to 281 m-equiv. Na/l. H_2O (Naora *et al.* 1962). There is no comparable information on sodium distribution in the cells of the early embryo. The present measurements show an increase in cytoplasmic sodium activity of 2–3 mM at about the time of nuclear membrane break-down before nuclear division (see Brachet, 1960), which would be consistent with a high nuclear sodium concentration. However, Century *et al.* (1970) have argued strongly in favour of their finding of a low concentration of rapidly exchanging sodium in oocyte nuclei and this has recently been supported by Muir & Whitley (1972). If this situation existed in the early *Xenopus* embryo then nuclear and cytoplasmic sodium activities would be closely similar. The small increase in a^i_{Na} during the division cycle would then reflect a cyclic release of sodium from some as yet unidentified cytoplasmic store.

The ratio between the intracellular concentrations of sodium and potassium has been invoked as a factor controlling cell behaviour; for example, a reduction in the cytoplasmic sodium/potassium concentration ratio in *Drosophila* salivary gland explants induces the normal sequence of chromosome puffing (Kroeger & Lezzi, 1966). In *Xenopus* embryos the ratio of cytoplasmic sodium and potassium concentrations falls considerably between first cleavage and gastrulation and this could influence the processes governing cell specification and differentiation known to occur prior to gastrulation (Holtfreter, 1938*a*, *b*; Nieuwkoop, 1969*a*, *b*), a possibility also considered by Kostellow & Morrill (1968). However, if the ratio of cytoplasmic sodium and potassium *activities*, which seem to be maintained at a

relatively constant level (Table 4) is more relevant for the control of cell behaviour, then the 2–3 mM cyclic increase in intracellular sodium activity seen at the time of nuclear division could assume greater importance.

The present results show the early embryo to contain large amounts of calcium and magnesium but give no information on the levels of ionically active divalent cation. The concentration of ionized calcium in the squid axon is about 3×10^{-7} M (Baker, Hodgkin & Ridgeway, 1971); a similar level of ionic calcium probably pertains in resting crab muscle (Portzehl, Caldwell & Ruegg, 1964). Free calcium in the cells of the early amphibian embryo could be of the same order since only 10^{-6} M ionic calcium is necessary to maintain normal cell cleavage (Baker & Warner, 1972). Neither the location, nor function of the large amount of calcium and magnesium found in these cells is known, although the possibility that variations in the ionic concentrations of these divalent cations may play a part in the control of cellular specification and differentiation must not be overlooked.

The osmotic load imposed on the amphibian embryo by the environment is considerable. Development normally takes place in pond water where the forces driving water into the cells, and the intracellular contents out, must be high. The total intracellular inorganic cation concentration is about 200 m-equiv/l. cell H_2O. Little information on the anion complement of the intracellular contents is available, although preliminary measurements put the intracellular chloride concentration in the region of 100 m-mole/l. cell H_2O. In order to resist the osmotic driving forces the external cell membrane is relatively impermeable to both water and ions. A finite permeability of the external membrane to sodium ions and water does, however, exist, for there is net entry of water into the embryo prior to gastrulation and some sensitivity of the egg membrane potential to variations in extracellular sodium concentration can be demonstrated (Slack & Warner, 1973). The rate of formation of the blastocoel cavity sets a lower limit of 9×10^{-8} cm/sec to the water permeability. This is considerably below the values quoted for body cavity *Xenopus* eggs (1×10^{-4} cm/sec; Stein, 1968), suggesting that the permeability to water falls precipitately on laying, when the egg is exposed to a low ionic strength medium for the first time.

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REFERENCES

ADRIAN, R. H. (1956). The effect of internal and external potassium concentration on the membrane potential of frog muscle. *J. Physiol.* **133**, 631–658.

BAKER, P. F. & WARNER, ANNE E. (1972). Intracellular calcium and cell cleavage in early embryos of *Xenopus laevis*. *J. cell Biol.* **53**, 579–581.

BAKER, P. F., HODGKIN, A. L. & RIDGEWAY, E. B. (1971). Depolarization and calcium entry in squid giant axons. *J. Physiol.* **218**, 709–755.

BARTH, L. G. & BARTH, LUCENA J. (1959). Differentiation of the *Rana pipiens* gastrula in unconditioned medium. *J. Embryol. exp. Morph.* **7**, 210–222.

BLAUSTEIN, M. P. & HODGKIN, A. L. (1969). The effect of cyanide on the efflux of calcium from squid axons. *J. Physiol.* **200**, 497–528.

BOSHER, S. K. & WARREN, R. L. (1968). Observations on the electrochemistry of the cochlear endolymph of the rat: a quantitative study of its electrical potential and ionic composition as determined by flame spectrophotometry. *Proc. R. Soc.* B **171**, 227–247.

BRACHET, J. (1960). *The Biochemistry of Development*. Oxford: Pergamon Press.

CANNON, J. D. & DICK, D. A. T. (1970). The effect of cell size on the sodium and potassium concentrations in single oocytes of *Bufo bufo*. *J. Physiol.* **211**, 51–53*P*.

CENTURY, T. J., FENICHEL, I. R. & HOROWICZ, S. B. (1970). The concentrations of water, sodium and potassium in the nucleus and cytoplasm of amphibian oocytes. *J. cell Sci.* **7**, 5–13.

CHILD, C. M. (1941). *Pattern and Problems of Development*. Chicago: University of Chicago Press.

CURTIS, A. S. G. (1957). Role of calcium in aggregation of *Xenopus* embryos. *Proc. R. Phys. Soc. Ed.* **26**, 25–32.

DAWID, I. B. (1965). Deoxyribonucleic acid in amphibian eggs. *J. molec. Biol.* **12**, 581–599.

DESMEDT, J. E. (1953). Electrical activity and intracellular sodium concentration in frog muscle. *J. Physiol.* **121**, 191–205.

DEYTRUP, I. J. (1964). *Physiology of the Amphibia*, ed. MOORE, E. New York: Academic Press.

DICK, D. A. T. & MCLAUGHLIN, S. A. (1969). The activities and concentrations of sodium and potassium in toad oocytes. *J. Physiol.* **205**, 61–79.

HINKE, J. (1961). The measurement of sodium and potassium activities in the squid axon by means of cation-selective glass micro-electrodes. *J. Physiol.* **156**, 314–335.

HOLTFRETER, J. (1938*a*). Differenzierungspotenzen isolierter teile der Urodelengastrula. *Wilhelm Roux Arch. EntwMech. Org.* **138**, 522–656.

HOLTFRETER, J. (1938*b*). Differenzierspotenzen isolierter teile der Anurengastrula. *Wilhelm Roux Arch. EntwMech. Org.* **138**, 657–738.

HOLTFRETER, J. (1943). Properties and functions of the surface coat in amphibian embryos. *J. exp. Zool.* **93**, 251–323.

ITO, S. & HORI, N. (1966). Electrical characteristics of *Triturus* egg cells during cleavage. *J. gen. Physiol.* **49**, 1019–1027.

JONES, K. W. & ELSDALE, T. (1963). The culture of small aggregates of amphibian embryonic cells *in vitro*. *J. Embryol. exp. Morph.* **11**, 135–154.

KALT, M. R. (1971*a*). The relationship between cleavage and blastocoel formation in *Xenopus laevis*. I. Light microscope observations. *J. Embryol. exp. Morph.* **26**, 37–49.

KALT, M. R. (1971*b*). The relationship between cleavage and blastocoel formation in *Xenopus laevis*. II. Electron microscope observations. *J. Embryol. exp. Morph.* **26**, 51–66.

KEYNES, R. D. & LEWIS, P. R. (1956). The intracellular calcium contents of some invertebrate nerves. *J. Physiol.* **134**, 399–407.

KOSTELLOW, A. B. & MORRILL, G. A. (1968). Intracellular sodium ion concentration changes in the early amphibian embryo and the influence on nuclear metabolism. *Expl cell Res.* **50**, 639–644.

KROEGER, H. & LEZZI, M. (1966). Regulation of gene action in insect development. *A. Rev. Ent.* **11**, 1–22.

KUCHLER, R. J. (1967). The role of sodium and potassium in regulating amino acid accumulation and protein synthesis in LM-strain mouse fibroblasts. *Biochim. biophys. Acta* **136**, 473–483.

MAENO, T. (1959). Electrical characteristics and activation potential of *Bufo* eggs. *J. gen. Physiol.* **43**, 139–157.

MORRILL, G. A. (1965). Water and electrolyte changes in amphibian eggs at ovulation. *Expl Cell Res.* **40**, 664–667.

MUIR, C. & WHITLEY, J. E. (1972). Variation in the nuclear sodium concentration of newt oocytes during maturation. *J. cell Sci.* **10**, 335–338.

NAORA, H., NAORA, H., IZAURA, M., ALLFREY, V. G. & MIRSKY, A. E. (1962). Some observations on differences in composition between the nucleus and cytoplasm of the frog oocyte. *Proc. natn. Acad. Sci. U.S.A.* **48**, 853–859.

NIEUWKOOP, P. D. (1969*a*). The formation of the mesoderm in Urodelean amphibians. I. Induction by the endoderm. *Wilhelm Roux Arch. EntwMech. Org.* **162**, 341–373.

NIEUWKOOP, P. D. (1969*b*). The formation of the mesoderm in Urodelean amphibians. II. The origin of the dorso-ventral polarity of the mesoderm. *Wilhelm Roux Arch. EntwMech. Org.* **163**, 298–315.

NIEUWKOOP, P. D. & FABER, J. (1956). *Normal Table of Xenopus laevis (Daudin).* Amsterdam: North Holland Publishing Company.

PALMER, J. F. & SLACK, CHRISTINE (1970). Some bioelectric parameters of embryos of *Xenopus laevis. J. Embryol. exp. Morph.* **24**, 535–553.

PORTZEHL, H., CALDWELL, P. C. & RUEGG, J. C. (1964). The dependence of contraction and relaxation of muscle fibres from the crab, *Maia squinado*, on the internal concentration of free calcium ions. *Biochim. biophys. Acta* **79**, 581–591.

REIMANN, W., MUIR, C. & MACGREGOR, H. C. (1969). Sodium and potassium in oocytes of *Triturus cristatus. J. cell Sci.* **4**, 299–304.

ROBINSON, R. H. & STOKES, R. H. (1959). *Electrolyte Solutions.* London: Butterworths.

SHUMWAY, W. (1940). Stages in the normal development of *Rana pipiens. Anat. Rec.* **78**, 139–148.

SLACK, CHRISTINE & WARNER, ANNE E. (1973). Intracellular and intercellular potentials in early amphibian embryos. *J. Physiol.* **232**, 313–330.

STABLEFORD, L. T. (1967). A study of calcium in the early development of the amphibian embryo. *Devl Biol.* **16**, 303–314.

STEIN, W. D. (1968). *The Diffusion of Molecules Across Membranes.* New York: Academic Press.

THOMAS, R. C. (1969). Membrane current and intracellular sodium changes in a snail neurone during extension of injected sodium. *J. Physiol.* **201**, 495–514.

THOMAS, R. C. (1970). A new design for sodium sensitive glass micro-electrodes. *J. Physiol.* **210**, 82*P*.

TUFT, P. (1962). The uptake and distribution of water in the embryos of *Xenopus laevis* (Daudin). *J. exp. Biol.* **39**, 1–19.

TUFT, P. (1965). The state and movement of water in living organisms. In *S.E.B. Symposium no.* XIX, pp. 385–402. Cambridge: Cambridge University Press.

WARREN, R. L. (1965). The determination of Cu^{++} and Mg^{++} in blood serum by high resolution flame spectrophotometry. *Analyst, Lond* **90**, 549–553.

WOODWARD, D. A. (1968). Electrical signs of new membrane production during cleavage of *Rana pipiens* eggs. *J. gen. Physiol.* **52**, 509–531.