THE RELATION BETWEEN THE ELECTRICAL CONDUCTIVITY OF THE EXTERNAL MEDIUM AND THE RATE OF CELL DIVISION IN SEA URCHIN EGGS.

By R. S. LILLIE and WARE CATTELL.

(From the Marine Biological Laboratory, Woods Hole, and the Nela Research Laboratories, Cleveland.)

(Received for publication, April 3, 1923.)

Our chief purpose in the following experiments has been to determine whether in the case of dividing cells immersed in physiologically indifferent or balanced media of varying salt concentrations there is any definite relation between the rate of cell division and the electrical conductivity (or total ionic concentration) of the medium. For the normal activity of most living cells an essential condition appears to be that the chief ions of the external medium should be present in certain approximately constant (physiologically balanced) proportions. Within a certain range of concentrations the proportions of the salts are apparently of more importance than their absolute concentrations, provided the osmotic equilibrium is not injuriously disturbed; for example, muscle and nerve live almost as long in Ringer's solution diluted with an equal volume (or more) of isotonic sugar solution as in the undiluted medium.[1] In pure sugar solutions these tissues soon lose irritability and transmissivity, but regain these properties on return to Ringer or other appropriate salt solution;[1] these facts show that a certain minimal concentration of salt in the external medium is required for normal activity; the need of a certain minimal electrical conductivity is thus indicated.

The rate of transmission in muscle and nerve in balanced media has recently been found in several instances to vary in a definite manner with the concentration of salts, showing in fact a close paral-

[1] Overton, E., *Arch. ges. Physiol.*, 1902, xcii, 346.

lelism with the electrical conductivity of the medium.[2] This relation is consistent with the view that in these tissues electrical currents traversing the media are an essential factor in transmission.[3] Conceivably the same may be true for other forms of protoplasmic transmission or correlation; thus it might be expected that the rate of such a process as cell division, which apparently also involves transmission (*i.e.*, the coordination of several spatially separate processes), would show a similar dependence in the electrical conductivity of the medium. In the eggs of some marine animals this does not appear to be the case; *e.g.*, the *Fundulus* egg cleaves normally both in sea water and in distilled water.[4] In this case, however, the presence of a water-proof chorion may be a factor—*e.g.*, the egg may be thus insulated from the surrounding medium—and the electrolytes required for the activity of its cleaving portion (blastodisc) may be furnished by the yolk. In other marine eggs, of the small totally cleaving type, *e.g.* sea urchin eggs, cleavage ceases on transfer to isotonic non-electrolyte solutions and is resumed on return to sea water. This parallel with the conditions of activity of muscle and nerve suggests the possibility that a similar correlation between the rate of cleavage and the external conductivity may exist in these eggs, and our experiments have been designed to test this possibility.

The experiments were performed in the summer of 1921 at the Marine Biological Laboratory at Woods Hole. The eggs of the common sea urchin *Arbacia* were used. The eggs were transferred, 10 minutes after fertilization in normal sea water, to mixtures of isotonic sugar solution and sea water, and the intervals between fertilization and cleavage and between first and second cleavages were then determined by direct observation. Standard sugar solutions were prepared (using glass-distilled water) from good commercial preparations of crystallized cane-sugar free from chloride (rock-candy, Domino loaf sugar); the concentration of cane-sugar isotonic

[2] Lillie, R. S., *Am. J. Physiol.*, 1916, xli, 126. Mayer, A. G., *Am. J. Physiol.*, 1915–16, xxxix, 375; 1916–17, xlii, 469; 1917, xliv, 591. Pond, S. E., *J. Gen. Physiol.*, 1920–21, iii, 807.

[3] For a discussion of the part played by electric factors in transmission, *cf.* Lillie, R. S., *Physiol. Rev.*, 1922, ii, 1.

[4] Loeb, J., *Am. J. Physiol.*, 1900, iii, 383.

with the Woods Hole sea water ($\Delta = 1.81°$) is 0.73 M, and this solution was used throughout. The solutions were mixed in glass stoppered graduates and thoroughly aerated. A large volume of solution (250 to 300 cc.) was used in each experiment; the eggs were transferred from the sea water to the solution by pipette, together with a minimal volume of sea water (about 1 cc.), and stirred gently to distribute. Observations were made in watch-glasses under a low power of the microscope.

TABLE I.

Approximate Time (after Fertilization) in Minutes Required for 50 Per Cent of Arbacia Eggs to Reach 2-Cell and 4-Cell Stages in Normal Sea Water and in Sea Water Diluted with Isotonic Sugar Solution (0.73 M).

Experiment No.	Temperature.	Dilution (volumes of sea water in 100 volumes of mixture).						
		100 (control).	80	60	50	40	30	20
	°C.							
1	22	2-cell: 56	56	56		58		58
		4-cell: 83	83	83		85		About 90.
2	About 21.	2-cell: 59			66	62	62	
		4-cell: 95			105	102	103	
3	23	2-cell: 55			60	60	60	
		4-cell: 84			90	89	85	
4	23	2-cell: 54				58	62	62
		4-cell: 79				83	83	87

The results are best summarized in the form of tables. Table I gives the results of four typical experiments showing the time (in minutes) required for an estimated proportion of 50 per cent of all eggs to reach the 2-cell stage in sea water and in mixtures of sea water and sugar solution ranging between 80 and 20 volumes per cent sea water. The temperature of the solutions in the finger-bowls containing the eggs is given in all cases within half a degree. There is always a variation of some minutes in the time at which different eggs in the same solution begin to cleave, and the cleavage process itself occupies 2 or 3 minutes. These conditions make it somewhat difficult to determine the exact time at which a constant proportion

of eggs have reached a definite stage. The completion of the cleavage furrow was the stage chosen for measurement; from our experience we estimate that the time at which half of the eggs are completely divided, while the rest are still in process of division or undivided, can be determined with a probable error of not more than 2 or 3 minutes. The variability becomes greater as the dilution increases.

It will be seen that as dilution increases the rate of cleavage is little, if at all, affected until the concentration of the salts is reduced to about 30 per cent of the normal. Evidently, therefore, no relation exists between conductivity and rate of cell division within this range of dilutions. With further dilution the rate of cleavage decreases progressively, and more rapidly after 20 per cent dilution is reached than before. Within the range between 20 per cent and 5 per cent dilution decrease of electrolyte content is quite definitely associated with decrease in rate of cleavage, but no constant proportionality between the two is indicated by our experiments. In mixtures containing 10 per cent sea water (Table II) the rate of cleavage is approximately from three-quarters to two-thirds that in normal sea water, and in 6 per cent sea water the rate is reduced to about one-half the normal. Many eggs fail to cleave when this dilution is reached and the proportion of non-cleaving eggs increases rapidly with further dilution. In 2 per cent sea water and in pure sugar solution the eggs never cleave, although they remain living for some hours and will resume cleavage if returned to sea water.

Table II summarizes the results of eight typical experiments with the lower concentrations of sea water (10 to 2 volumes per cent), carried out between July 19 and July 29, 1921. The eggs were normal and the range of temperature was 21–24°C. Six other similar experiments not cited in the table yielded results of the same general character, but the rate of cleavage was somewhat slower than normal and in three experiments the temperature was below 20°C. The eight experiments cited are those in which the conditions and the controls were most nearly uniform. Pure sugar solution and 2 per cent sea water were also used in all experiments, but no cleavage occurred in these solutions.

This region of dilution (from 20 to 4 volumes per cent sea water) shows most distinctly the effect of varying the concentration of salts. In the more dilute solutions, from 6 volumes per cent down, there arises the difficulty of a greatly increased variability in the cleavage rates of different eggs; many eggs fail entirely to cleave, or the cleavage is so retarded that the exact time ceases to have significance. In this series we found it more satisfactory to measure the time required for 25 per cent (instead of 50 per cent) of the eggs to reach

TABLE II.

Minutes between Fertilization and Completion of First Cleavage.

Experiment No.	Temperature.	Dilution (volumes of sea water in 100 volumes of mixture).					
		100 (control).	10	8	6	5	4
	°C.						
1	24	51	74	83	100		20 per cent cleaved after 180 min.
2	24	54	63	85	93		10 per cent cleaved after 145 min.
3	21	54	75	94	108		10 per cent cleaved after 150 min.
4	21	56	79	90	107	12 per cent cleaved after 150 min.	5 per cent cleaved after 150 min.
5	21	59	89	105	16 per cent cleaved after 160 min.	1 per cent cleaved after 165 min.	0.3 per cent cleaved after 165 min.
6	22.5	52	85	95	106	114	5 per cent cleaved after 165 min.
7	23	50	82	125	About 3 per cent cleaved after 170 min.	About 3 per cent cleaved after 170 min.	1 per cent cleaved after 170 min.
8	23	50		109	10 per cent cleaved after 170 min.	8 per cent cleaved after 173 min.	1 per cent cleaved after 175 min.

the 2-cell stage. At dilutions of 4 and 5 per cent, relatively few eggs (usually from 5 to 10 per cent) cleave within 2 or $2\frac{1}{2}$ hours; and at a dilution of 2 per cent cleavage fails entirely. Apparently the lowest concentration of electrolytes at which cleavage is possible is one corresponding to about 4 per cent sea water. Below this concentration the rate of some of the processes concerned in cell division appears to be decreased to such a degree that cleavage is unable to

complete itself. There is evidence that the cytoplasmic rather than the nuclear processes are primarily affected by deficiency of electrolytes in the medium.[5]

Although the eggs remain uncleaved in the more dilute solutions and in pure sugar solution, they remain living for at least several hours and capable of resuming cell division and development when returned to sea water. We have not made a detailed study of the maximum length of life in the different solutions, but in all of the experiments of Table II part of the eggs were returned to sea water after 2 to $2\frac{1}{2}$ hours, and the proportion developing to a blastula stage was determined. In the less dilute solutions (6 volumes per cent and stronger) the eggs are only slightly affected by this exposure and the great majority form active blastulæ. In solutions containing 4 per cent sea water, 50 per cent of the eggs formed blastulæ in four out of the eight experiments of Table II after exposures varying from $1\frac{3}{4}$ to $2\frac{1}{2}$ hours, and a varying number reached this stage in the other four experiments. In 2 per cent sea water these proportions were not greatly altered.[6] In the pure sugar solution the rate of deterioration was decidedly more rapid; yet in all but one of the eight experiments a certain proportion of blastulæ (in most cases less than 5 per cent) developed from eggs which had remained for $2\frac{1}{4}$ to $2\frac{1}{2}$ hours in the solutions. In two other experiments in which eggs were transferred to sea water after 98 and 110 minutes (respectively) in the sugar solution, 50 per cent or more formed blastulæ. Allowance has to be made for the small quantity of sea water introduced with the eggs; the experiments with 2 per cent sea water indicate that a slight trace of salt may have a well marked protective influence.

The curve (Fig. 1) shows graphically the general manner in which the rate of division varies with the concentration of salts. Ordi-

[5] Some years ago (Lillie, R. S., *Biol. Bull.*, 1902–03, iv, 164), I observed in *Arbacia* eggs nuclear division without cytoplasmic division in solutions of non-electrolytes.

[6] Loeb (Loeb, J., *Biochem. Z.*, 1910, xxix, 80) found that in a mixture of 49 volumes of 0.75 M dextrose *plus* 1 volume of sea water the great majority of *Arbacia* eggs died in 3 hours if oxygen was present. In absence of oxygen or presence of cyanide life was greatly prolonged.

nates are volumes per cent of sea water in the mixtures, abscissæ the observed intervals between fertilization and the formation of the first cleavage furrow. The points are averages of the observations in the tables; these observations are few, but sufficient to show the general course of the curve; the horizontal direction toward its lower end indicates absence of cleavage in concentrations below 4 per cent.

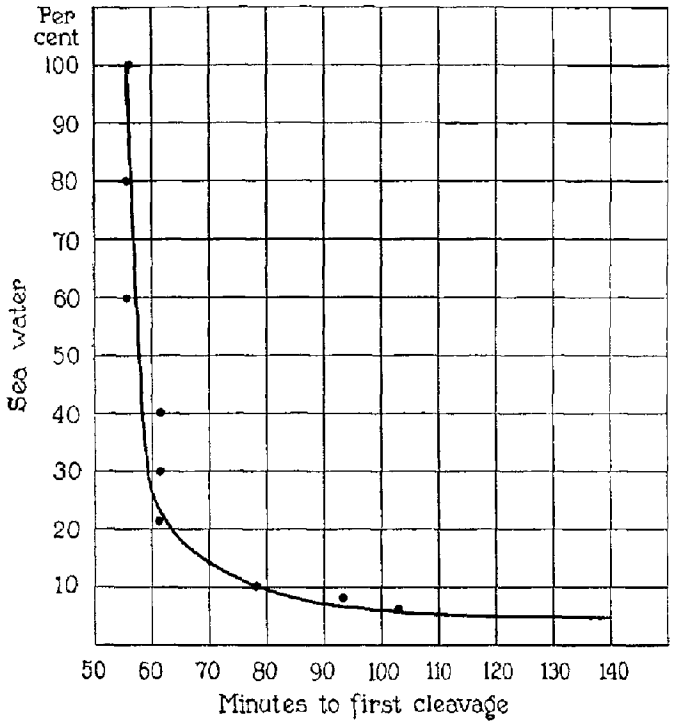

FIG. 1.

We conclude that under normal conditions the rate of the cell division process is determined by factors which are independent of the electrical conductivity of the external medium within a wide range of variation. Conductivity may be reduced to less than one-third of the normal without significantly altering the rate of division. Our experiments suggest, however, that in media of lower salt content electrical conductivity may become a limiting factor; *i.e.*, the

rate possible to the entire division process may in these solutions be controlled by the conductivity. It seems probable that for the other factors to be effective a certain minimal external conductivity is required; when the conductivity falls below this critical value a correlation between the conductivity and the rate of the entire composite process of cell division may then appear. An apparently linear relation between conductivity and rate of transmission is seen in Mayer's[2] and Pond's[2] observations on muscle and nerve. Within a short range of concentrations (10 or 15 per cent to 5 per cent sea water) there appears to be an approximation to this relationship in the above experiments. The part played by bioelectric currents in cell division is, however, still obscure; we can only regard it as probable that if these currents are an essential factor in the transmission of excitation in irritable forms of protoplasm they also play some part in cell division.

SUMMARY.

Dilution of sea water with isotonic sugar solution leaves the rate of cleavage of *Arbacia* eggs almost unchanged until the proportion of sea water is decreased to 20 or 25 volumes per cent. From this point cleavage becomes progressively slower with further dilution. Many eggs fail to cleave at dilutions of 5 to 6 volumes per cent. No cleavage occurs in 2 volumes per cent sea water or in pure sugar solution. Eggs returned from these media to sea water resume cleavage and development.

There is thus no relation between the rate of cleavage and the electrical conductivity of the medium, except possibly within the range of dilutions from 20 to 5 volumes per cent sea water. In this range cleavage rate decreases as conductivity decreases, but the relation is not a linear one.