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STUDIES ON THE OSMOTIC PROPERTIES OF THE EGGS AND LARVÆ OF A BRACKISH-WATER POLYCHÆTE, *MARPHYSA GRAVELYI* SOUTHERN

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INTRODUCTION

WHILE the osmotic behaviour of marine polychætes has been studied by several authors (Schlieper, 1929; Ellis, 1933, 1937; Beadle, 1934, 1937, 1943; Bethe, 1934; and Adolph, 1936), the osmotic properties of their eggs and larvæ have received no attention. McClendon (1910 *a*, *b*), R. S. Lillie (1916, 18), Runnstrom (1925), Luke and McCutcheon (1925–26), Bialaszewicz (1927), Needham (1930), Euphrussi and Rapkine (1928), Needham and Needham (1930 *a*, *b*), Ranzi (1930) and Northrop (1926–27) have performed experiments on the osmotic properties of the eggs of sea urchins and of Sepia among the invertebrates, but have not investigated the behaviour of the larvæ and adults of these forms. An investigation of the osmotic properties of the eggs, of larvæ and of the adult of the same species may throw light on the adaptations of species to different habitats during their whole life cycle. In the present paper an account of the behaviour of eggs and larvæ of the brackish water polychæte, *Marphysa gravelyi* Southern is given and will be followed later by a study of the osmotic regulations of the adult.

MATERIAL AND METHODS

North of the Zoology Laboratory (Madras University) where the present investigations were carried on is the River Cooum. Except in the monsoons the river does not flow into the sea because of the formation of a sand bar at its mouth. By virtue of the formation of the sand bar at the mouth, about 100 yards up the river, the bed of the river is sandy and beyond it, it becomes gradually soft and clayey. The salinity of the waters at the mouth is almost that of seawater and decreases as one travels up the river into the interior. In the vicinity of the clayey region the salinity of the water ranges between 20 $^{o}/_{oo}$ and 34 $^{o}/_{oo}$ in the different months of the year, it being the minimum during the rainy season and highest in the hotter months. Thus there are fluctuations in the degrees of salinity all the year round. Here

may be seen during February to September, and in lesser numbers in the months of December to February (Aiyar, 1931), numerous pear-shaped masses of jelly which are the egg-cases of *Marphysa gravelyi*. Embedded in this jelly are large numbers of eggs, distributed evenly throughout the spawn. These egg-cases are firmly rooted in the soft mud by long stalks. Since the soil is soft, they can, however, be dug out easily. Such egg-cases were collected carefully and brought to the Laboratory along with the water. They were left in glass tanks and samples of eggs were removed from the cocoon from time to time for study. An account of the development has already been given by Aiyar (1931). As an adaptation to its habitat, the *trochophore* stage in the development of *Marphysa* is eliminated and even the earlier stages of development are undergone within the jelly. It is only when a stage which corresponds to the initial *metatrochophore* stage is reached, after a period of $3\frac{1}{2}$ days, that the larvæ come out of the jelly and swim about in the medium. Such larvæ were collected for experimentation.

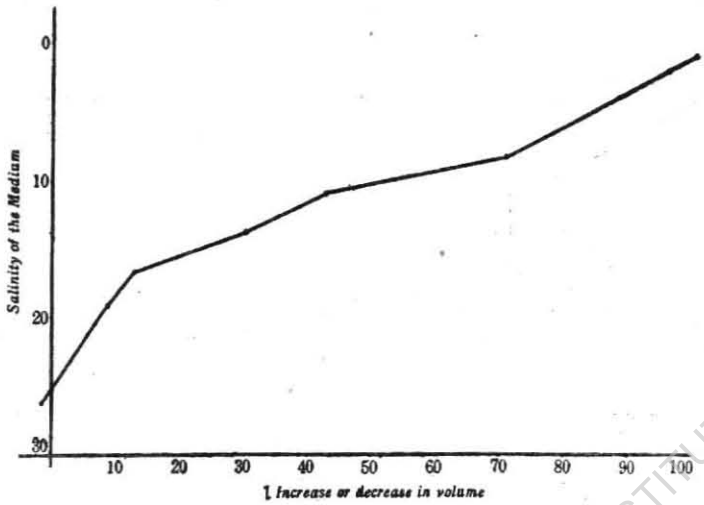
The object of the present investigation being the determination of the effects of different concentrations on the volume changes in the eggs and larvæ, it was necessary to measure their volumes. The method suggested by Weil and Pantin (1931 *a*, *b*) was followed in determining the diameter and area of the eggs and larvæ respectively. The diameter was measured direct by making use of an ordinary ocular-micrometer. Instead of a ghost-micrometer a net-micrometer was used in the eye-piece and the areas of the larvæ were directly read off. For every such determination eye-piece $\times 5$ and objective $\times 40$ were used and the magnification kept constant. Since the eggs are spherical, the volumes of the eggs were calculated by making use of the formula $1/6\ 22/7\ d^3$ as suggested by Krogh (1939). The volume of each egg and larva recorded in this paper represents the mean volume of six readings. For experimentation the eggs and larvæ from a single spawn were used.

A. *Experiments on Eggs*

Experiment I.—*Hypotonic Media and its Effect on Volume Changes in the Eggs*

(*a*) *Increase in Volume in Different Hypotonic Media.*—Six eggs of a single spawn were isolated from the jelly and the diameter was first measured and the volume calculated. They were transferred to petri dishes containing sea water of a particular strength for 30 minutes. At the end of which the diameter of each egg was read and the volume calculated.

In a similar way the experiment was repeated with different concentrations of the medium. Graph 1 indicates that the increase in volume is



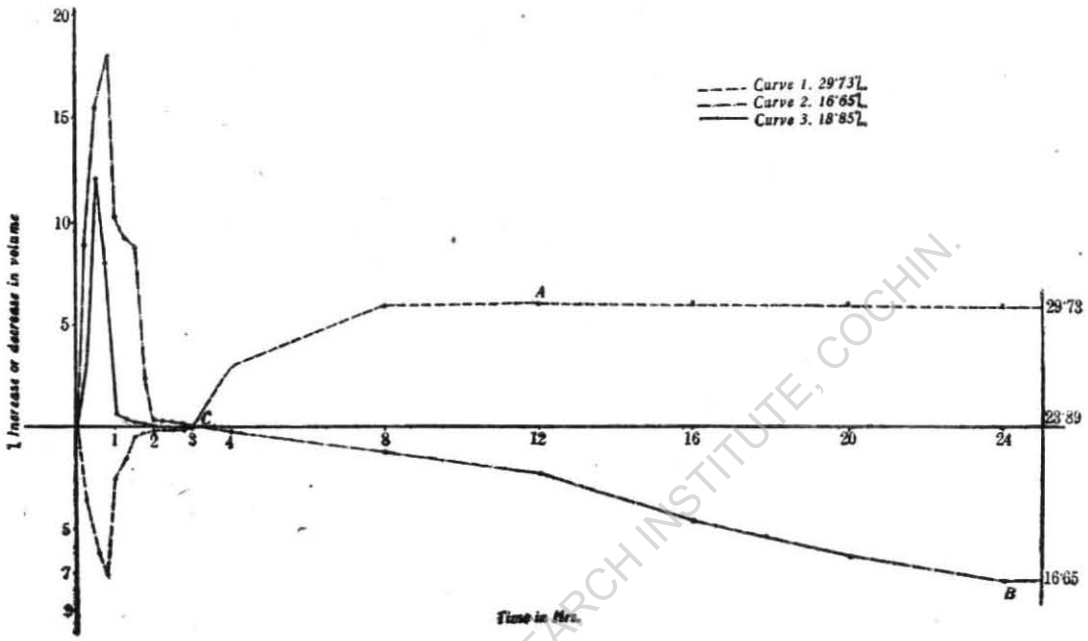
GRAPH 1. Increas in volume in different Hypotonic Media

proportional to the dilution of the medium used. This observation agrees with the results of Lillie (1916) and Runnstrom (1925) on the eggs of *Arbacia* and *Paracentrotus lividus* respectively.

(*b*) *Volume Changes in Hypotonic Media during Different Intervals.*— 400 Eggs taken from a single spawn were left in a petri-dish containing hypotonic medium of a known concentration. The volumes of six eggs were determined for a period of 3½ hours at intervals of every 15 minutes. The figures recorded indicate that the volume increases in about 30 to 45 minutes and decreases subsequently (Curve 3, Graph 2). The eggs reach their original volumes irrespective of the strength of the medium within 3½ hours.

(*c*) *Volume Changes in Hypotonic Media during Different Intervals.*— Five to six hundred eggs were immersed in a medium of a particular concentration (16·65‰) and the volumes of six eggs were determined after an interval of every 15 minutes for a period of 3 hours and later after intervals of 4 hours for a period of 40 hours. Curve 2 in Graph (2) shows the results of such an experiment. They indicate that the eggs increase in volume within the first half hour and then begin to decrease in volume. The volume shrinks to the original level in 3½ hours but the decrease continues for about 24 hours till the egg is 92·6% of the original size. There is no shrinkage beyond this upto a period of 40 hours.

During this decrease in volume obviously due to loss of salts, nearly 41·2% of the eggs died within 24 hours and about 50% died in 40 hours. It is quite probable that the initial increase in volume was due to the higher concentration of the egg and later due to loss of salts as well as water there



GRAPH 2. Volume Changes in Hypotonic and Hypertonic Media during different intervals

was shrinkage of volume. The differences in the rate and amounts in loss of salts essential for normal well being, probably account for the mortality of 50% of the eggs—for by 96 hours all the eggs died (*vide infra*).

(*d*) *Volume Changes in Hypertonic Media during Different Intervals.*— 200 Eggs were immersed in a hypertonic medium of 29·73‰ and the volumes of six eggs were determined after an interval of every 15 minutes for a period of 3 hours and later after intervals of 4 hours for a period of 32 hours. Curve 1, Graph (2) shows the results of such an experiment. The readings recorded indicate that the volumes decrease at first and reach the maximum in 45 minutes and subsequently the volumes increase. The volume increases to the original level in 3 hours and the increase continues for about 12 hours till the egg is 106·2% of the original size. There is no increase in volume beyond this upto a period of 32 hours.

During this increase in volume, obviously due to uptake of salts and water, nearly 41·6% of the eggs died within 12 hours and about 75% died in 32 hours. It is quite probable that due to the higher concentration of the external medium, there is an initial decrease in volume and later due to uptake of water as well as salts from the medium there is an increase in volume. The differences in the rates of diffusion of salts and water into the egg probably account for the different percentages of mortality and also the final volume being greater than the original.

A comparison of the Curves 1 and 2 in Graph (2) suggests that as at A and B the eggs may be isotonic with the two different media used, it is probable that C gives the osmotic concentration of the egg.

EXPERIMENT II.—*Effect of Hypotonic Media on the Development : Eggs*

(*a*) *On Eggs without Jelly.*—Numerous eggs removed from the jelly were exposed to different salinities. After every 24 hours of such exposure, 100 eggs from each lot were taken and the number of eggs that were dead* were counted. Column A in Table I gives the results of such a series of experiments. The figures indicate a rise in mortality correlated with a prolongation

TABLE I

Effect of Hypotonic Media on Development—On Eggs without jelly, with jelly and larvæ

Concentration of the medium ‰	A				B			C			
	Egg—Without Jelly				Egg—With Jelly			Larva			
	24 hrs.	48 hrs.	72 hrs.	96 hrs.	24 hrs.	48 hrs.	72 hrs.	24 hrs.	48 hrs.	72 hrs.	96 hrs.
Distilled water	..	..	..	..	5%	7%	nil	..	..	..	..
11·40	..	..	..	..	3%	6%	nil	..	..	..	..
14·45	..	..	..	..	..	..	..	100%	100%	100%	100%
15·39	..	..	..	..	2%	5%	nil	..	..	..	..
15·65	..	..	..	..	..	..	..	53%	51%	53%	53%
16·82	..	..	..	..	..	..	..	24%	22%	22%	23%
17·50	..	..	..	..	..	..	..	2%	nil	nil	nil
17·78	33·3%	86·6%	88·3%	100%	..	..	..	..	..	..	..
17·86	..	..	..	..	6%	7%	nil	..	..	..	..
18·42	10·0%	83·3%	84·3%	100%	..	..	..	..	..	..	..
20·63	..	..	..	..	3%	3%	nil	..	..	..	..
20·65	..	..	..	..	..	..	..	2%	nil	nil	nil
21·88	..	..	..	..	..	..	..	2%	nil	nil	nil
22·18	..	..	..	..	..	..	..	1%	nil	nil	nil
24·33	..	..	..	..	4%	3%	nil	..	..	..	..
30·12	6·6%	66·6%	100·0%	100%	..	..	..	..	..	..	..
31·05	..	..	..	..	..	..	..	1%	nil	nil	nil
31·11	..	..	..	..	nil	nil	nil	..	..	..	..
31·30	..	..	..	..	..	..	..	nil	nil	nil	nil
33·42	..	..	..	..	..	..	..	nil	nil	nil	nil

of exposure, till all the eggs died after 96 hours. The table also indicates that the mortality increases in media of lower salinity.

(*b*) *On Eggs with Jelly.*—A single, complete and uninjured egg case was allowed to develop in each of the different media of known salinities. After

* Complete cessation of the setting movement of the yolk indicated the outset of death.

a duration of every 24 hours the number of eggs that were dead were counted. It is evident from Column B in Table (I) that the rate of mortality is comparatively low and at the end of 72 hours all the eggs hatched out into larvæ irrespective of the salinity of the outside media in which they were left.

B. *Experiments on Larvæ (Initial Metatrochophore)*

EXPERIMENT I.—*Hypotonic Media and Its Effect on Volume Changes*

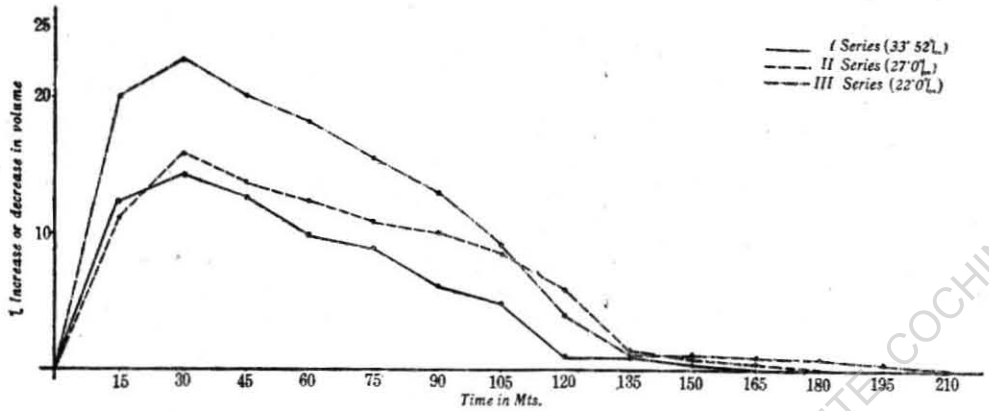
(*a*) *Increase in Volume in Different Hypotonic Media.*—The volumes of the larvæ hatched in the laboratory were first determined. Six of them were left in each of the seven petri dishes containing different concentrations of brackish water of the natural habitat of the worm. The volumes of these larvæ were measured after 30 minutes in each case so as to determine the effect of the different media on the volumes of the larvæ. Table II shows that in and beyond a salinity of 14·43 °/₀₀ the larvæ swell and disintegrate.

Concentration of the medium ‰	Laivae Initial mean volume 72C μ V	%
Distilled water	DISINTEGRATES	
7·43 ..	..	..
9·43 ..	DISINTEGRATES	
10·83 ..	..	..
13·31 ..	..	..
14·43 ..	DISINTEGRATES	
16·72 ..	..	..
17·76 ..	91·33	24·0
19·20 ..	..	..
21·10 ..	88·33	22·7
26·01 ..	..	..
26·10 ..	84·50	17·4
34·14 ..	81·66	13·4

(*b*) *Volume Changes in Hypotonic Media during Different Intervals.*—A number of larvæ were exposed to hypotonic media of different concentrations graded from 33·52 °/₀₀ to 22 °/₀₀ in order to determine their behaviour at different intervals. They were exposed for 3½ hours and the volume measured at an interval of every 15 minutes. Graph (3) shows that in all cases the original volume was regained after a duration of 3½ hours and that the larvæ are capable of reaching osmotic equilibrium in the different concentrations of the media.

EXPERIMENT II.—*Hypotonic Media and Their Effects on Development : Larvæ*

A number of larvæ of same age were allowed to continue their development in nine different grades of hypotonic media of known salinities in order to test their effect on the development of the larvæ. Controls were also



GRAPH 3. Volume Changes in Hypotonic Media during different intervals

maintained. After every 24 hours the rate of mortality was calculated and Column C in Table I shows the readings of such a series of experiments. The experiments were continued for a period of 96 hours at the end of which period all the larvæ developed into the next stage. The low rate of mortality bears testimony to the fact that the larvæ continued their development irrespective of the low salinity of the media in which they were allowed to develop. It is further evident that larvæ are not able to tolerate a medium which falls below a salinity of 14·45 °/₀₀.

DISCUSSION

The investigations of Loeb and Westeneys (1915) on the eggs of *Fundulus heteroclitus* are of interest since they found that the eggs of this marine fish can develop and hatch in distilled water. They conclude that the protoplasmic membrane of these eggs is impermeable to salts and almost impermeable to water. Ramult (1925) who studied the influence of salt solutions upon the development of *Daphnia* eggs, Gray (1920, 1932) and Krogh and Ussing (1937) who studied the eggs of *Salmo*, a fresh water trout, have come to a similar conclusion since they found a normal and healthy development in all of them due to the impermeable membrane, conserving and protecting the internal concentration. The envelopes of the eggs of the present form show that they are permeable both to water and salts and exposure for about 24 hours to a hypotonic medium of 17·78 °/₀₀ proves harmful for the normal development of about 33·3% of the eggs. If the exposure is for 48 hours the percentage of mortality of the eggs rises to 86·6%, until at the end of 96 hours all are dead. In a state of nature, however, the eggs are covered with jelly and changes in salinity through long periods of exposure do not affect the development of eggs. Therefore it can be concluded that the

envelop of jelly protects the eggs and serves the same purposes as the non-permeable egg coats of animals without jelly.

In the light of the observations made by August Krogh, Agnes Krogh and Wernstedt (1938) on the osmotic behaviour of the eggs of *Pleuronectes flesus* and *Crenilabrus exoletus*, teleosteon fish, the conclusion that the eggs of the present form are permeable both to salts and water seems to be justifiable. The behaviour of the eggs, which these authors studied, show that they decrease in diameter in water of 25–34 $^{o}/_{oo}$ at first, but increased to the original later. The eggs of the Polychæte, *Marphysa gravelyi*, also decreased in diameter in water of 29·73 $^{o}/_{oo}$ in the first 45 minutes but increased to the original in 3 hours, suggesting that they are permeable both to salts and water. Needham (1930), Ephrussi and Rapkine (1928) who studied the osmotic behaviour of the eggs of *Strongylocentrotus lividus* during their development stress the fact that they absorb large amounts of salts from the surrounding water. Ranzi (1930) observes that the eggs of *Sepia officinalis* increase in weight, ash content and water content during development. But in the present form shrinkage in volume due to loss of water and salts is seen. It is quite probable that due to the loss of salts and water which are quite essential for a normal and healthy development the eggs die and the rise in the percentage of mortality is directly proportional to the length of exposure. But the initial increase in volume in the eggs of the present form is probably due to the fact that the rate of inflow of water is greater than the rate of loss of salts, which indicates that the eggs must have had a greater concentration at the beginning. Similar conclusions were arrived at by Lillie (1916, 1918), Northrop (1926–27) and Runnstrom (1925) who observed an increase in the volumes of the eggs of echinoderms when they were subjected to the effect of different hypotonic media.

The experiments regarding the effect of different hypotonic media on the volume changes of the larvæ suggest that when they are exposed to different diluted media there is an inflow of water through the skin. But when they are exposed for a long time, they show a recovery of the original volume, indicating loss of water probably through the excretory organs. Since nephredia of the protonephridial type are already developed at this stage of the larvæ, it can be supposed that the excretory organs are responsible for osmo-regulation. Westblad (1922) who came to a similar conclusion, emphasises the fact that the flame cell system in the turbellaria is mainly concerned with osmo-regulation. Herfs (1922) by observing the rate of pulsation of flame cell system in rotifers and trematodes in solutions of different osmotic pressure, has obtained more direct evidence of their osmo-regulatory action. In the light of the above observations it is justifiable to

conclude that the recovery of the original volume, through the excretion of the water taken up during swelling, and the maintanance of a constant internal concentration, must be due to the activity of the protonephridial organs in the larvæ.

Krogh and Ussing (1937) further observe that the production of impermeable plasma membranes is probably a general mechanism for the protection of eggs in fresh water against osmotic swelling, a protection which can be dispensed with when mechanisms for the excretion of water become functional in the embryo. The observations of Ikeda (1937 *a*) on the eggs of *Oryzias latipes*, a fish living in fresh water and brackish waters of Japan, furnish another example of this kind. Since in the present form there is already a larval kidney (of the type of protonephredia) functioning, the larvæ seem to have dispensed with the protection afforded by the jelly. The almost insignificant low rate of mortality of the larvæ when they are exposed to different hypotonic media further strengthens the conclusion that the larvæ at this stage are able to maintain a steady internal concentration due to the presence of the functional kidneys which begin to actively excrete water.

The different modes of behaviour as exhibited by the eggs and larvæ when they are exposed to the effects of similar hypotonic media, further suggest that the eggs can scarcely be supposed to possess mechanisms specially adapted for invasion of water with fluctuating salinities, whereas the larvæ are endowed with well-developed excretory organs which serve as the osmoregulatory mechanism, as evidenced by the present investigations.

Summary

1. Experiments regarding the effects of the hypotonic and hypertonic media on the volume changes are described and the conclusion that the eggs are passive as far as the transport of the salts and water are concerned is arrived at.

2. The rate of mortality observed when eggs with jelly and without jelly were subjected to hypotonic media is recorded and the conclusion that the envelop of jelly acts in the same way as an impermeable membrane in the case of eggs of other animals is drawn.

3. The effects of hypotonic media on the volume of larvæ is described and the role played by the excretory organs and their importance in osmoregulation is indicated.

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BIBLIOGRAPHY

1. Adolph, E. F. .. "Differential permeability to water and osmotic exchanges in the marine worm *phascolosma*," *J. Cel. Comp. Phisol.*, 1936, **9**, 117–35.

2. Aiyar, R. G. .. "An account of the development and breeding habits of brackish-water polychæte worm of the genus *Marphysa*," *Jour. Lin. Soc. Zool.*, 1931, **37**, 387–403.

3. Beadle, L. C. .. (*a*) "Osmotic regulation in Gunda ulvae," *J. Exp. Biol.*, 1934, **11**, 382–96.

(*b*) "Adaptations to changes of salinity in the Polychætes. I. Control of body volume and body fluid concentration in *Nereis diversicolor*," *Ibid.*, 1937, **14**, 56–70.

(*c*) "Osmotic regulation and the faunas of inland waters," *Biol. Rev.*, 1943, **18**, 172–83.

*4. Bethe, A. .. "Die salz-und Wassendurchlassigkeit der korperoberflachen verschiedener Seetiere in ihrem gegenseitigen Verhaltnis," *Pflug. Arch. ges. Physiol.*, 1934, **234**, 629–44.

*5. Bialaszewicz, K. .. "Contributions a'l'e'tude de la composition minerale des cellules-oeufs," *Pubbl. Staz. Zool. Napoli.*, 1927, **8**, 355–69.

6. Ellis, W. G. .. (*a*) "Calcium and the resistance of Nereis to brackish-water," *Nature*, Lond., 1935, **132**, 748.

(*b*) "The water and electrolyte exchange of *Nereis diversicolor*," *J. Exp. Biol.*, 1937, **14**, 340–50.

7. Euphrussi, B. and Rapkine, L. .. *Ann. Physiol. Physicochim. Biol.*, 1928, **4**, 386.

8. Gray, J. .. (*a*) "The relation of the animal cell to electrolytes. I. A physiological study of the egg of trout," *J. Physiol.*, 1920, **53**, 308–19.

(*b*) "The osmotic properties of the eggs of the trout (*Salmo fario*)," *J. Exp. Biol.*, 1932, **9**, 277–99.

*9. Herfs, A. .. "Die pulsierende Vakuole der Protozoen, ein Schutzorgan gegen Aussussung. Studien uber Anpassung der Organismen an das Leben im Susswasser," *Arch. Protistenk*, 1922, **44**, 227–60.

10. Ikeda, Y. .. "Effect of sodium and potassium salts on the rate of development in *Oryzias latipes*," *J. Fac. Sci. Tokyo Imp. Univ.*, 1937 *a.*, **4**, 307–12.

11. Krogh, A. .. *Osmotic Regulations in Aquatic Animals*, Camb. Univ. Press, 1939.

12. Krogh, A. and Ussing, H. H. "A note on the permiability of trout eggs to D_2O and H_2O," *J. Exp. Biol.*, 1937, **14**, 35–37.

13. Krogh, A. Agnes Krogh and Wernstedt, C. — "The salt concentration in the tissues of some marine animals," *Skand. Arch. Physiol.*, 1938, **80**, 214–22.

14. Loeb, J. and Westeneys, H. — "Note on the apparent change of the osmotic pressure of cell contents with the osmotic properties of the surrounding solution," *J. Biol. Chem.*, 1915, **23**, 157–62.

15. Luke, B. and McCutcheon — "The effect of hydrogen ion concentration and swelling of cells," *J. Gen. Physiol.*, 1925–26, **9**, 709.

16. Lillie, R. S. .. (*a*) Increase of permeability to water following normal and artificial activation in sea urchin eggs," *Amer. Jour. Physiol.*, 1916, **40**, 249–66.

(*b*) "The increase or permeability to water in fertilised sea urchin eggs and the influence of cyanide and anæsthetics upon this change," *Ibid.*, 1918, **45**, 406–30.

17. McClendon, J. F. .. "Electrolytic experiments showing increase in permeability of the egg to ions at the beginning of development," *Science*, 1910 *a*, **32**, 122–24.

(*b*) "Further proofs of the increase in permeability of the sea urchin egg to electrolytes at the beginning of development," *Ibid.*, 1910 *b*, **32**, 317–18.

18. Needham, J. .. "On the penetration of marine organisms into fresh water," *Biol. Zbl.*, 1930, **50**, 504–09.

19. Needham, J. and Needham Dorothy .. (*a*) "Nitrogen-excretion in Selachian ontogeny," *Brit. J. Exp. Biol.*, 1930, **7**, 7–18.

(*b*) "On phosphorous metabolism in embryonic life. I. Invertebrate eggs," *Ibid.*, 1930, **7**, 317–48.

20. Northrop, J. N. .. "Kinetics of the swelling of cells and tissues," *J. Gen. Physiol.*, 1926–27, **11**, 43.

21. Ramult, M. .. "Development and resisting power of Cladocera embryos in the solutions of certain inorganic salts," *Bull. Inst. Acd. Sci. Cracovie.*, 1925, 135–94.

*22. Ranzi, S. .. "La'ccrescimento dell' embrione dei cefalopodi," *Arch. Entw-Mech. Org.*, 1930, **121**, 345–65.

23. Runnstrom, J. .. "Ueber den Einfluss des Kalimmangels auf das secigelie. Experimentelle Beitrage zur Kenntuiss des Plasmabaues, der Teilung und der Determination des Eies," *Publ. Staz. Zool. Napoli.*, 1925, **6** (1).

24. Schlieper, C. .. "Ueber die Ein wirkung niederer salzkonzentrationen auf marine organismen," *Z. Vergl. Physiol.*, 1929, **9**, 478–514.

25. Weil, E. and Pantin, C. F. A. (*a*) "The adaptation of *Gunda ulvae* to salinity. I. The Environment," *J. Exp. Biol.*, 1931 *a*, **8**, 63–72.

(*b*) "The adaptation of *Gunda ulvae* to salinity. II. The Water exchange," *Ibid.*, 1931 *b*, **8**, 73–81.

*26. Westblad, E. .. "Zur Physiologie der Turbellarien. II. Die Exkretion," *Lunds. Univ. Arsskr.*, 1922, **18**, No. 6.

* Not referred to in the original.