IODINE COMPOUNDS AND FERTILISATION

IV. CAPACITY FOR FERTILISATION IN WASHED AND UNRIPE EGGS OF *ECHINUS ESCULENTUS* AND *ECHINUS MILIARIS*

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(*Received 16th December*, 1931.)

WASHED EGGS.

It has been shown (Carter, 1931 *b*) that eggs of *Echinus esculentus* and *E. miliaris* which are being washed in a current of sea-water remain fertilisable for a longer time if thyroxine is present in the water of the current. In his investigation of the egg secretions and the part they play in the fertilisation of the egg, Lillie (1914) showed that the rapid decay of echinoderm eggs exposed to a current of sea-water is due to the diffusion of the secretions from the surface of the eggs, and particularly to the loss of one component of the secretions which is essential to the activation of the eggs ("fertilizin"). In view of his work, the results of the previous paper strongly suggest that the manner in which thyroxine acts in prolonging the life of washed eggs is by penetrating the surface of the eggs and causing a change in the cytoplasm by means of which the supply of fertilizin within the egg is maintained for a longer time. The probability that it acts in this way is increased by the evidence given in the earlier papers of this series (1930, 1931 *a*), where it was shown that the egg secretions and thyroxine act so similarly in other phenomena that it is probable that thyroxine is chemically related to some substance present in the secretions. If this conclusion is accepted, and if the substance to which thyroxine is related is fertilizin itself (and further evidence that this is so will be given in later papers), it is not improbable that thyroxine may definitely replace the fertilizin in the cytoplasm, presumably by being itself transformed into this substance by the activity of the protoplasm.

In the discussion of the subject in the previous paper, it was concluded that this view is probably correct, but an alternative explanation of the results was also suggested. The prolongation of the fertilisable life of the egg might result from a lowering of the permeability of the surface to the secretions. By this means the loss of the secretions would be retarded, and the life of the egg consequently prolonged. If it were conceivable that the presence of thyroxine in the surrounding medium

might cause a decrease in the permeability of the surface of the egg, it would be unnecessary to postulate any action of the drug in the cytoplasm. But there is no evidence in favour of the idea that thyroxine acts in this way, and on general grounds any such action appears to be very improbable. Thus, it was concluded that it is unlikely that this explanation is the true one, but the possibility of its truth was not strictly excluded.

The following experiments were carried out with the object of discriminating between these alternative explanations. Woodward (1918) and Glaser (1921) have shown that eggs of *Echinarachnius* and *Arbacia* which have to some extent lost their capacity for fertilisation in a current of sea-water improve if they are later treated with sea-water containing the egg secretions. They concluded that the secretions diffuse into the egg and renew the supply of fertilizin in the cytoplasm. This work was criticised by Lillie (1924, p. 492), who suggested that the effects were due to inaccuracy in the control of the conditions of the surrounding medium, and especially of its *p*H; but, apart from these possible sources of error, it seems impossible to question their conclusion. And, if results similar to theirs can be obtained with the eggs of *Echinus*, we have here the means of answering our question concerning the manner of action of thyroxine on the egg. If thyroxine prolongs the life of washed eggs by replacing the fertilizin as it is lost, it should also renew the supply of this substance in eggs which have already lost it, and thus treatment with thyroxine should make such eggs more fertilisable. On the other hand, if its only action is on the permeability of the surface, it should have no power to replace the lost secretions and no favourable effect on the capacity of the eggs for fertilisation. This argument assumes that the action of both thyroxine and the secretions is on the egg and not on the sperm. This assumption will be considered later in the paper (p. 242) and reasons will be given for believing that it is justified whenever ripe sperm is used.

In any event, complete recovery of the eggs in these experiments is unlikely. The changes which take place in the egg as it becomes unfertilisable are undoubtedly complex. The loss of fertilizin almost certainly leads to other damaging changes and some of these will probably be irreversible. Such changes would remain even if the fertilizin itself were completely replaced, and would prevent the complete recovery of the egg. Thus, the expectation of positive results from these experiments must be limited to the extent to which the changes which take place in the egg as it becomes unfertilisable can be reversed. Woodward and Glaser's results indicate that some improvement in the development of the eggs is to be expected, but it would not be surprising if the improvement were slight. The truth of these conclusions is emphasised by the fact that Glaser (1921) found that eggs of *Arbacia* which had become completely unfertilisable showed no recovery on treatment with the secretions. They were then irreversibly damaged. His positive results were obtained only with eggs which were in process of becoming unfertilisable.

An apparatus similar to that described in a previous paper (1931 *b*, pp. 195–6) was used for these experiments. It was modified from the apparatus there described only in the larger bulk of sea-water which was used in it (about 5 litres). This

modification was possible since the eggs were not to be treated with thyroxine during the washing, and there was therefore not the same need as in the earlier experiments to restrict the volume of water used in the apparatus. The eggs were tested for ripeness at the beginning of the experiment and were used only if they gave approximately 100 per cent. of normal fertilisations in sea-water. They were left in the apparatus until it was found that their capacity for fertilisation in sea-water was reduced. The period of washing required for this was found to be very variable. It was clearly controlled partly by the number of eggs in the apparatus and the consequent variations in the efficiency of the washing, but probably also by variations in the condition of the eggs. For a reduction of the capacity for fertilisation to 50 per cent. the appropriate time was sometimes found to be as long as 24 hours and sometimes as short as 6 hours, but it was usually between 10 and 15 hours.

After being washed, the eggs were taken out of the apparatus and placed in various media, such as sea-water, egg-water diluted with sea-water to varying extents, and sea-water containing thyroxine in varying concentrations. In most of the experiments the eggs were fertilised in these media and either allowed to develop in the media or later transferred to sea-water, but in a few experiments they were fertilised and allowed to develop in sea-water after they had been left in the media for various times. The percentages and regularity of the development of the different groups of eggs were compared up to the stage of the blastula. Never more than a few hundred eggs were used in each medium, and the same relatively large amount of the medium (30 c.c.) was always used. Thus, the conditions were kept as nearly as possible the same in all the media, and were favourable to development so far as crowding of the eggs was concerned.

Throughout the experiments the hydrogen-ion concentration was controlled as accurately as possible in all the media. At the start of the experiment it was always adjusted to that of the sea-water at the time (about *p*H 8·2). This necessitated the addition of a small amount of alkali to the egg-water, which is almost always slightly less alkaline than sea-water.

The solutions of thyroxine were made by preparing a $1/10^5$ solution in sea-water by the method previously described (1931 *a*, p. 179) and diluting this solution with sea-water. The precipitate which forms when thyroxine is added to sea-water is very slight at a concentration of $1/10^5$, but it is probable that the concentration of the stronger solutions of thyroxine were actually slightly less than the figures given below on account of the formation of this precipitate.

The slight change in osmotic pressure caused by adding the concentrated solution of thyroxine in *N*/10 NaOH and a similar amount of *N*/10 HCl for neutralisation of the alkali (about 0·6 per cent. of the osmotic pressure of sea-water in all) was controlled by adding the same proportion of distilled water to the sea-water used in the other media. In a few control experiments it was shown that dilution of the sea-water to this extent had no noticeable effect on the results of fertilisation.

The egg-water was made by aerating a large quantity of eggs in a comparatively small quantity of sea-water for 6–12 hours. No attempt was made to estimate the

strength of the undiluted egg-water. Its strength in different experiments cannot therefore be taken to be equivalent.

Each of the recorded experiments is an example of a series of the same type, all of which gave similar results. Eggs of both species were used in experiments of every type, and always behaved similarly. All the results may be considered true of the eggs of both species.

The results of an experiment on the eggs of *E. esculentus* are given in Table I. In this experiment the eggs were left in the different media for 30 min. after they had been taken from the apparatus in which they had been under circulation for 14 hours, and were then fertilised in the media. They were allowed to develop in the media in which they had been fertilised. The egg-water used was that of *E. esculentus*.

Table I. *E. esculentus*.

Medium	Membranes (%)	Divisions (%)	Morulae (%)	Blastulae	
				Total (%)	Regular (%)
Sea-water	0	53 a–c	30	23	9
Thyroxine 1×10^{-5}	0	11 b–c	8	6	4
,, $\frac{1}{2} \times 10^{-5}$	1	40 a–c	38	27	13
,, $\frac{1}{10} \times 10^{-5}$	**2**	**65** a–b	**53**	**55**	**30**
,, $\frac{1}{20} \times 10^{-5}$	0	53 a–c	38	35	20
Egg-water $\times$ 1	1	**81** a–b	**52**	**51**	**36**
,, $\times \frac{1}{2}$	0	60 a–b	30	30	16
,, $\times \frac{1}{10}$	0	68 a–b	28	25	15
,, $\times \frac{1}{20}$	0	48 a–c	25	22	12
Sea-water	0	49 a–c	25	15	7

The figures given in this and the following tables are percentages, estimated by taking a sample from the eggs in each medium, after they had been well stirred, and counting 100–200 of them. The sampling was repeated in some experiments and these tests showed that the percentages were accurate to within 5 per cent. All eggs in which there were clear indications of division of the cytoplasm were counted as having divided, although the division might be incomplete, and all larvae which had a recognisable central cavity were counted as blastulae. Only *perfectly* regular blastulae were counted as regular. The percentages of blastulae and morulae are occasionally slightly higher than those of the earlier stages of development. This is due to delayed development, which occurred after the earlier counts had been made. The letters "a" to "d" are used in the tables to indicate the regularity of the fertilisations and divisions, perfectly regular development being classed as "a". The meaning of these letters is more accurately defined in a previous paper (1931 *b*, p. 196).

It will be seen that thyroxine and the egg secretions in appropriate concentrations cause a distinct and approximately equal improvement in the results of fertilisation, and that this improvement is to be observed at all stages of the development. It is, however, greater in the later stages, so much so that in some experiments the improvement in membrane formation was too small to be recognised

with certainty. That in the number and regularity of the blastulae was always evident.

In most of the experiments, but not noticeably in the one here recorded, the improvement was greater in the percentage of regular blastulae than in the total number of eggs which formed blastulae. In addition, it should be noted that the figures given in the last column of the tables give less than a complete conception of the improvement in the regularity of the blastulae. The most obvious difference between the various cultures was the greater regularity of the blastulae in the optimum cultures, and this was due not only to the greater percentage of perfectly regular blastulae present, but also to the much greater percentage of blastulae which were almost regular. Since only perfectly regular blastulae were included in the percentages given in the tables, these almost regular blastulae had no effect on the figures, which would have shown still more striking differences if they had been included.

In Table II the results of an experiment are given in which the eggs were transferred to sea-water after being 30 min. in the solutions of thyroxine and in the egg-water. They were fertilised in the sea-water immediately after they had been transferred to it. Both eggs and egg-water were those of *E. esculentus*. The eggs were under circulation for 14 hours before the experiment.

Table II. *E. esculentus*.

Medium	Membranes (%)	Divisions (%)	Morulae (%)	Blastulae	
				Total (%)	Regular (%)
Sea-water	0	22 b–c	29	30	21
Thyroxine 1×10^{-5}	2	21 a–c	20	20	17
,, $\frac{1}{2} \times 10^{-5}$	3	**49** a–c	**49**	**53**	**41**
,, $\frac{1}{16} \times 10^{-5}$	0	42 a–c	38	33	27
,, $\frac{1}{64} \times 10^{-5}$	1	35 a–c	35	34	28
Egg-water × 1	2	45 b–c	**45**	**45**	**31**
,, × $\frac{1}{2}$	0	**48** a–c	37	32	21
,, × $\frac{1}{16}$	1	40 b–c	36	30	20
,, × $\frac{1}{64}$	0	37 b–c	30	30	22
Sea-water	0	20 b–c	23	24	18

In several experiments of this type it was found that the development of the eggs was always improved by a short treatment with thyroxine or egg-water before fertilisation, but in most of the experiments the improvement was not so great as that in eggs which were fertilised in the solutions of thyroxine and in egg-water and allowed to develop in these solutions. There is, however, less than the usual difference in this respect between the two experiments of which the results are given here.

The considerable improvement produced by treatment with thyroxine or the secretions in experiments such as that of Table II almost certainly excludes the possibility that the improvement is due to some action of the dissolved substances on the sperm and not on the eggs. Care was taken that only a very small proportion of the solution was carried over with the eggs, certainly not as much as 1 per cent.

of the sea-water into which the eggs were put. Thus, in experiments of this type the optimum effect was obtained when the sperm was in contact with not more, and probably much less, than $\frac{1}{4} \times 10^{-7}$ of thyroxine, and this concentration is low enough to make it very improbable that the sperm was affected. But another argument leading to the same conclusion can be brought forward and is more conclusive. There was no clear difference between the extent of the improvement in experiments such as that of Table II and others in which the eggs were transferred to sea-water immediately after they had been fertilised in the presence of thyroxine or of the secretions. In these last experiments the sperm was in contact with the higher concentrations of the dissolved substances before and during fertilisation, but did not produce an increased effect. Thus, the greater improvement in eggs which were fertilised in the presence of the dissolved substances and allowed to develop in the solutions must have been due to action of the substances on the eggs and must have continued during the course of development. The action is therefore not on the sperm, and not only on the unfertilised egg or on the immediate reactions of the egg to fertilisation.

These conclusions differ from those which Fuchs (1915) drew from the results of his work on the fertilisation of ascidians and *Strongylocentrotus*. He found evidence that in his experiments the action of the secretions and egg extracts in improving the results of fertilisation was on the sperm and not on the eggs. It is not difficult to find a reason for this difference between his conclusions and those of this paper. That unripe sperm should be improved by treatment with the egg secretions is to be expected from the results given in a previous paper (1931 *a*, p. 193), but there should be no similar improvement of ripe sperm. The sperm used in the experiments of this paper was always ripe. This was shown, for the sperm of *E. esculentus*, by its immediate activation in sea water, and, for the sperm of both species, by the perfect fertilisations which it gave with ripe eggs. On the other hand, the eggs had been washed and were not in perfect condition. If in Fuchs' experiments the eggs were, on the whole, ripe but the sperm unripe, the difference in the conclusions is intelligible. In both sets of experiments the effect would be, as it should be on the theory proposed in these papers, on the gamete which was in the less perfect condition.

In the experiments so far discussed, the eggs had been washed until they were unable to form membranes in sea-water. The results given in Table III show that the development of eggs which had not progressed so far in loss of their capacity for fertilisation can be improved by treatment with the secretions or thyroxine. In this experiment eggs of *E. miliaris* were used, and the egg-water was also of that species. The eggs were washed in sea-water for 12 hours before the experiment.

Finally the results given in Table IV show that the improvement may be produced by foreign egg-water. In this experiment eggs of *E. miliaris* were treated with egg-water of *E. esculentus*. The eggs were washed for 10 hours before the experiment. As in all the other experiments, thyroxine and the secretions produced approximately equal improvement in the development of the eggs.

Table III. *E. miliaris.*

Medium	Membranes (%)	Blastulae	
		Total (%)	Regular (%)
Sea-water	28 b–d	67	25
Thyroxine 1×10^{-5}	29 b–d	70	29
,, $\frac{1}{4} \times 10^{-5}$	27 b–d	**80**	**48**
,, $\frac{1}{10} \times 10^{-5}$	36 b–c	76	42
,, $\frac{1}{64} \times 10^{-5}$	**47** a–b	76	31
,, $\frac{1}{256} \times 10^{-5}$	45 a–b	70	27
Egg-water × 1	12 c–d	61	28
,, × $\frac{1}{4}$	23 b–d	78	**43**
,, × $\frac{1}{10}$	**40** b–d	**83**	37
,, × $\frac{1}{64}$	34 b–d	70	32
,, × $\frac{1}{256}$	29 b–d	70	25
Sea-water	32 b–d	71	24

Table IV. *E. miliaris.*

Medium	Membranes (%)	Blastulae	
		Total (%)	Regular (%)
Sea-water	81 a–b	75	62
Thyroxine 1×10^{-5}	81 a–b	70	60
,, $\frac{1}{4} \times 10^{-5}$	—	78	64
,, $\frac{1}{10} \times 10^{-5}$	84 a–b	81	68
,, $\frac{1}{64} \times 10^{-5}$	**85** a–b	**88**	**76**
,, $\frac{1}{256} \times 10^{-5}$	80 a–b	78	59
Egg-water × 1	74 a–b	75	55
,, × $\frac{1}{4}$	88 a–b	**94**	78
,, × $\frac{1}{10}$	**98** a	92	**79**
,, × $\frac{1}{64}$	95 a	85	75
,, × $\frac{1}{256}$	89 a–b	81	62
Sea-water	82 a–b	72	62

II. UNRIPE EGGS.

Lillie (1914) showed that unripe echinoderm eggs do not form active egg-water, and he therefore concluded that fertilizin does not diffuse from the surface of unripe eggs. On these grounds he believes that this substance is only present in ripe eggs, and that it is formed in the last stages of the ripening of the eggs. Further, he believes that its formation is an essential part of the process of ripening.

If he is right in this, it might be possible to hasten the ripening of slightly unripe eggs by allowing fertilizin to diffuse into them from the outside. Thus, treatment of such eggs with egg-water should improve their capacity for fertilisation. And, if thyroxine can serve as a source of fertilizin to the eggs, it should also be able to improve the capacity of slightly unripe eggs for fertilisation.

The following experiments were intended to put these possibilities to the test. Eggs were shown to be unripe by fertilisation in sea-water and were immediately treated with different concentrations of the egg secretions and thyroxine. They were then fertilised either in these solutions or in sea-water and their development

was watched. The control of *p*H and osmotic pressure described above was maintained throughout.

In Table V the results of an experiment are given in which eggs of *E. miliaris* and egg-water of *E. esculentus* were used. The egg-water was therefore foreign to the eggs, but in these experiments, as in those on washed eggs, it was found that foreign egg-water was as effective as that of the same species as the eggs. The eggs were allowed to develop in the presence of thyroxine and the egg secretions.

Table V. *E. miliaris.*

Medium	Membranes (%)	Divisions (%)	Blastulae	
			Total (%)	Regular (%)
Sea-water	76 a	79 a	78	61
Thyroxine $\frac{1}{2} \times 10^{-5}$	80 a	79 a	83	66
,, $\frac{1}{8} \times 10^{-5}$	83 a	84 a	87	80
,, $\frac{1}{32} \times 10^{-5}$	**84** a	**83** a	**88**	**84**
,, $\frac{1}{128} \times 10^{-5}$	78 a	77 a	77	68
Egg-water × 1	**87** a	**86** a	**87**	67
,, × $\frac{1}{4}$	86 a	83 a	84	78
,, × $\frac{1}{16}$	85 a	81 a	86	**81**
,, × $\frac{1}{64}$	81 a	79 a	82	77

In the experiment of Table VI, both eggs and egg-water were those of *E. esculentus*, and the eggs were transferred to sea-water 45 min. after fertilisation.

Table VI. *E. esculentus.*

Medium	Membranes (%)	Divisions (%)	Blastulae	
			Total (%)	Regular (%)
Sea-water	86 a	77 a–b	79	71
Thyroxine 1×10^{-5}	84 a	77 a–b	85	82
,, $\frac{1}{4} \times 10^{-5}$	**92** a	81 a–b	85	81
,, $\frac{1}{16} \times 10^{-5}$	87 a	**88** a–b	**87**	**83**
,, $\frac{1}{64} \times 10^{-5}$	87 a	79 a–b	79	74
Egg-water × 1	32 a	85 a–b	84	80
,, × $\frac{1}{2}$	71 a	87 a–b	84	**81**
,, × $\frac{1}{4}$	86 a	**89** a–b	**85**	79
,, × $\frac{1}{8}$	**90** a	76 a–b	72	60
Sea-water	87 a	74 a–b	71	63

An improvement in the development of the eggs is evident in both experiments, and the effects of thyroxine and the secretions are, as in the previous experiments, approximately equal.

Some improvement may be observed in eggs which are so nearly ripe as to give 100 per cent. of good membranes on fertilisation in sea-water. This is shown in the results of the following experiment. The eggs were fertilised in the solutions of thyroxine and in the egg-water. Some were allowed to develop in these media and others were transferred to sea-water 1 hour after fertilisation. The egg-water, as well as the eggs, was of *E. esculentus.*

Table VII. *E. esculentus*.

Medium	Membranes (%)	Later development in			
		Thyroxine, sea-water or egg-water		Sea-water	
		Blastulae		Blastulae	
		Total (%)	Regular (%)	Total (%)	Regular (%)
Sea-water	100 a	100	93	100	91
Thyroxine 1×10^{-5}	,,	,,	84	,,	99
,, $\frac{1}{4} \times 10^{-5}$	,,	,,	99	,,	97
,, $\frac{1}{16} \times 10^{-5}$	,,	,,	97	,,	96
,, $\frac{1}{64} \times 10^{-5}$	,,	,,	96	,,	95
Egg-water $\times$ 1	96 a	,,	84	,,	64
,, $\times \frac{1}{4}$	100 a	,,	97	,,	96
,, $\times \frac{1}{16}$	,,	,,	96	,,	90
,, $\times \frac{1}{64}$	,,	,,	95	,,	92
Sea-water	99 a	,,	91	,,	90

DISCUSSION.

The experiments discussed in this paper have shown that:

1. The development of washed and over-ripe eggs of either of the two species of *Echinus* is improved by treatment of the eggs before fertilisation with thyroxine or the egg secretions in appropriate dilution.

2. Thyroxine and the secretions improve the development of such eggs to about the same extent.

3. In most of the experiments the improvement is slightly greater in eggs which are allowed to develop in the presence of the secretions or thyroxine than in eggs which are treated with these substances only for a short time before fertilisation.

4. The improvement may be produced by egg-water of another species.

5. These effects are due to some action of the substances on the egg and not on the sperm, and this action continues during development to the blastula stage.

6. The development of slightly unripe eggs is improved by treatment with thyroxine and the secretions. This effect also is produced by foreign egg-water.

7. These effects are not due to variations in *p*H or osmotic pressure, which were controlled throughout the experiments.

It has been shown that thyroxine is effective in producing the improvement in very low concentrations. In fact, the lowness of the effective concentrations is perhaps the most striking characteristic of these experiments. In the experiments in which the eggs were allowed to develop in the presence of thyroxine, the optimum concentration was most often $\frac{1}{16} \times 10^{-5}$, but in some experiments concentrations of $\frac{1}{4} \times 10^{-5}$ or $\frac{1}{64} \times 10^{-5}$ gave better results. It is not surprising that the optimum concentration was slightly higher when the treatment was short and the eggs were made to develop in sea-water, but the difference is not large (Tables I and II). For some undetermined reason the optimum concentration

for membrane formation was sometimes lower than that for the later development of the eggs (Tables III, VI, egg-water).

These concentrations are not lower than those in which other drugs act upon living tissues, but they show either that the eggs must be able to absorb the drug very actively, or that the effects must be produced by a very minute amount of the drug. And the fact that they are so low increases the probability that thyroxine is somehow intimately connected with the chemical processes going on in the egg.

It is also striking that the concentrations which were effective in these experiments are much lower than those which were found to be necessary to produce the effects of the drug upon the oxygen consumption of the sperm (1931 *a*). This is perhaps not surprising. In the present experiments a few eggs were used in a relatively large quantity of solution, and by far the greater part of the egg consists of inactive yolk. In the experiments on the sperm it was necessary to use a somewhat dense suspension in order that the changes in oxygen consumption might be large enough to be measurable. It is clear that the active protoplasm of the eggs was much smaller in proportion to the amount of thyroxine in the solution than that of the sperm.

On the argument given at the beginning of this paper, these results show that the effects of thyroxine upon the egg are not confined to a lowering of the permeability of the surface, and it seems that the only possible explanation of the results is that thyroxine can replace fertilizin, at least functionally, in the cytoplasm of the egg. If a functional replacement is denied, thyroxine must produce in the egg some other modification which makes fertilisation and development more successful, and this must be so in the experiments of both this and the last paper. But this seems very improbable. The exact parallel between the action of thyroxine and the secretions in the other phenomena dealt with in the papers of this series makes it hard to doubt that this similarity of effect is the result of similarity in the manner of action. And the probability of this conclusion will be increased by results to be given in later papers, in which the list of phenomena in which thyroxine and the secretions act similarly will be extended. If they act in the same way in these phenomena, it is very probable that they act in the same way in their action on the development of the egg.

If a functional replacement is admitted, it is again hard to doubt that the substance fertilizin is itself directly formed from thyroxine in the cytoplasm. Thyroxine and the secretions have been shown to act similarly in several very distinct phenomena, and the varied character of these phenomena makes it extremely probable that the similarity of action is the result of chemical relatedness. With regard to the experiments of the present paper, the control of pH and osmotic pressure which has been maintained throughout has excluded the possibility that the results are due to inaccurate control of the medium. This is at least true of these two conditions of the medium, and it is very improbable that any other condition of the medium was responsible for the results by its variation. Lillie's work, if this is admitted, forces us to conclude that the effects of the secretions are due to diffusion of fertilizin into the egg, and therefore that thyroxine, which we have concluded

to act on the egg in the same manner as the secretions, must also renew the supply of fertilizin in the egg. And, since thyroxine is chemically related to some component of the secretions, it seems clear that the component to which it is related is fertilizin, and that the renewal of the supply of fertilizin in the cytoplasm comes about by the transformation of thyroxine into this substance.

The fact that foreign egg-water may improve the development of both washed and unripe eggs suggests that the renewal of fertilizin in the cytoplasm is a more complex process than the simple diffusion into the egg of the iso-agglutinin, which Lillie believes to be identical with it. The iso-agglutinin is specific, and it seems either that the iso-agglutinin of one species can be transformed in the cytoplasm into that of another, or that some non-specific component of the secretions can be transformed into the iso-agglutinin. This point will be discussed further in a later paper. The author is indebted to the Government Grant Committee of the Royal Society for a grant by which part of the expenses of the research recorded in all the papers of this series was defrayed. He also wishes to express his thanks to the staff of the Millport Laboratory for continual help in the course of the research.

SUMMARY.

1. The development of eggs of *E. esculentus* or *E. miliaris* which have been washed in a current of sea-water until they are becoming unfertilisable is improved by later treatment with egg-water or solutions of thyroxine in sea-water. The secretions and thyroxine improve the development of the eggs to about the same extent.

2. The improvement may be produced by egg-water of the opposite species.

3. These effects are due to action on the egg and not on the sperm.

4. The development of unripe eggs is also improved by treatment with egg-water or thyroxine.

5. None of these effects are due to variations of the hydrogen-ion concentration or osmotic pressure.

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