

IODINE COMPOUNDS AND FERTILISATION

IX. FERTILISATION IN THE STARFISH,
ASTERIAS RUBENS L.

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(With Four Text-figures.)

IN the earlier papers of this series (1931-2) the action of iodine compounds on the fertilisation reactions of the two common British species of sea-urchin, *Echinus esculentus* and *E. miliaris*, has been discussed, and a theory of the changes which occur at fertilisation in this genus has been founded on the results of the discussion. Before this theory can be accepted as true of fertilisation in general, it is clear that gametes of other types must be investigated. Up to the present the only experiments discussed in these papers which have been concerned with animals other than the sea-urchins have been those on the sperm of the rabbit which formed the subject of the preceding paper (1932*e*).

Extension of the investigation to other forms is the more necessary, since, in some of its characters, the sea-urchin egg lies at the extreme of the range of variation that is found among the eggs of marine invertebrates. For instance, in the sea-urchin egg, fertilisation is immediately followed by a very great rise in the oxygen consumption of the egg. Whitaker (1931, 1933) has shown that the extent of the rise of oxygen consumption which occurs at fertilisation is very variable among the marine invertebrates. It is never much greater than in the sea-urchin egg, but in other forms it may be smaller, absent or replaced by a fall. The sea-urchin egg is also unusual in the very long period during which it is fertilisable after it has been laid.

The egg of the common starfish (*Asterias rubens*) lies at the opposite extreme of the range of variation. It shows no rise in its oxygen consumption at fertilisation, and it becomes unfertilisable within an hour and a half, or at most two hours, after it has been laid. These two types of egg differ no less clearly at the beginning of the fertilisable period. The sea-urchin egg matures in the ovary, and is fertilisable as soon as it is laid, whereas the starfish egg hardly ever matures until it has left the animal¹, and fertilisation gives the best results with it if it is carried out not less than 40 min. after the egg has been placed in sea-water. The starfish egg is therefore almost as different from the sea-urchin egg as it is possible for the egg of a marine invertebrate to be.

¹ Gemmill (1914) states that *Asterias* eggs may occasionally mature in the ovary.

Since the object of the following experiments was to observe the action of iodine compounds on gametes which differ in type from the gametes of the sea-urchin, it seemed that gametes of the starfish would form a suitable subject for the experiments. Starfish gametes can be obtained in considerable quantity and, for this reason also, they are convenient as the subject of an investigation of this type. They are, however, by no means so easy a subject of investigation as the gametes of the sea-urchin. The latter, if it is ripe, can be induced to lay its eggs or sperm at the genital pores after it has been opened, but no means of bringing this about in the starfish is known. Starfish eggs and sperm can be secured only by cutting up the gonads in sea-water, and a much less pure sample of the ripe gametes is obtained in this way. Also, the starfish egg is much more sensitive to unfavourable conditions than the sea-urchin egg, and fertilisation is therefore less certain. And the short fertilisable life of the starfish egg introduces difficulties into experiments on the measurement of its oxygen consumption before and after fertilisation. For these reasons the following experiments are by no means so complete as the corresponding experiments on the sea-urchin gametes.

SPERMATOOZOA.

It has previously been noted (1932*b*, p. 251) that the sperm of *A. rubens* agglutinates in the presence of thyroxine. This agglutination is a hetero-agglutination of precisely the same type as the agglutination of the sperm of *Echinus* described in the same paper. It occurs in sea-water containing 1 part (by weight) of thyroxine in 5×10^4 or 10^5 parts of water, and, weakly, in sea-water containing 1 part in 2×10^5 .

It was found (1931*a*) that the modifications which thyroxine causes in the activity of the sperm are different in the two species of *Echinus*. Perfectly ripe¹ sperm of either species is unaffected. The effect of the presence of thyroxine on the unripe sperm is to prolong the active life of the sperm of *Echinus miliaris*, and to cause immediate activation of the sperm of *E. esculentus*, which does not activate immediately after dilution in sea-water unless it is perfectly ripe.

Starfish sperm reacts to thyroxine in the same manner as the sperm of *E. esculentus*. Ripe sperm activates at once in sea-water, unripe sperm only after an interval which is greater the less ripe the sperm is. The presence of thyroxine in the medium has no effect upon the activity of the ripe sperm but causes immediate activation of unripe sperm. This is shown in Fig. 1, in which the oxygen consumptions of similar suspensions of slightly unripe sperm in sea-water (*C*), sea-water containing $1/10^5$ of thyroxine (*A*) and sea-water containing $1/5 \times 10^5$ of thyroxine (*B*) are compared. This experiment was one of a series which gave similar results. The experiments were carried out in Barcroft manometers fitted with side-tubes, by which the sperm could be introduced without opening the respirometer bottles. The method of experimentation was that previously described (1931*a*, p. 177), except that the amount of sperm present was estimated by counting them at the end of the

¹ See 1931*a*, p. 180. The term "ripe" is used to describe the condition of the sperm as it is normally laid by the animal.

experiment by means of a haemocytometer, and that the curves are drawn to give the consumption of 10^9 sperm instead of the consumption per mg. N present in the sperm.

The almost immediate activation of the sperm in the presence of the higher concentration of thyroxine is shown clearly by the form of curve *A* of Fig. 1. Visual observation of similar suspensions under the microscope confirmed the immediate activation of the sperm in the presence of thyroxine and the much slower activation in sea-water. It will be observed from the shape of the curves that the maximum rate of oxygen consumption, and therefore the maximum degree of activity, of the sperm is the same in all the suspensions. This is also true of the sperm of *E. esculentus*.

In the experiment of Fig. 1 the smaller concentration of thyroxine ($1/5 \times 10^5$) gave a recognisable but incomplete effect. In other experiments a concentration of

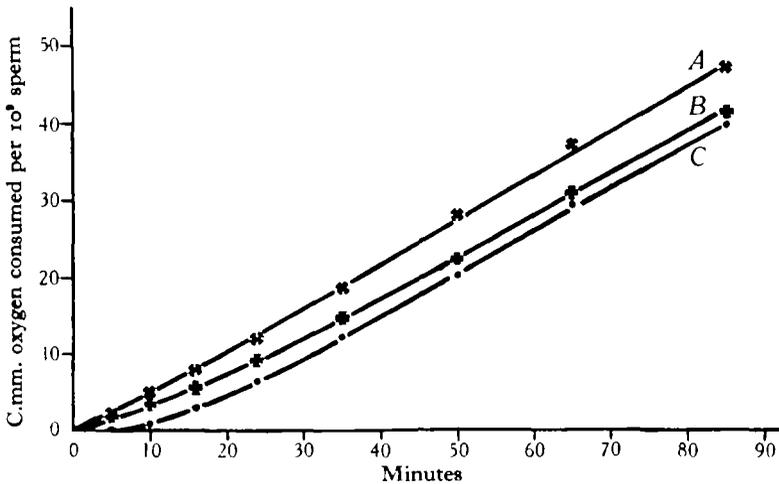


Fig. 1. Oxygen consumption of similar suspensions of sperm of *Asterias rubens* in: *A*, sea-water containing $1/2 \times 10^5$ of thyroxine by weight; *B*, sea-water containing $1/10^5$ of thyroxine; and *C*, sea-water. All at pH 8.3 at the beginning of the experiment and pH 8.45 at the end.

$1/10^6$ was found to be ineffective. These concentrations are less than those that are necessary to produce activation in *Echinus* sperm (minimum $1/10^5$). It has been shown (1931*a*, p. 185) that the effective concentration of thyroxine in experiments such as these varies with the density of the sperm suspension, but this consideration does not apply here, for the suspensions of *Echinus* and *Asterias* sperm used in the two sets of experiments were of approximately the same density. It must therefore be concluded that *Asterias* sperm is slightly more sensitive to thyroxine than *Echinus* sperm.

The minimum effective concentrations of thyroxine in the experiments on the sperm of *Asterias* were considerably higher than those that were found to give the optimum effects in experiments on the egg which are to be discussed below. This was equally true of the experiments on the eggs and sperm of *Echinus*. This difference has been discussed previously (1932*a*, pp. 246-7). It is almost certainly due to the greater concentration of active protoplasm in the sperm suspensions.

THE EGG.

(1) *The fertilisable life of the egg.*

The rapidity with which the starfish egg becomes fertilisable after it has been laid (or taken from the gonad) varies with the ripeness of the egg. Eggs which have been laid naturally by the starfish, and are therefore, presumably, perfectly ripe, are fertilisable to some extent immediately after they are laid (Gemmill, 1914), but even such eggs give better results if fertilisation is carried out in the middle of the fertilisable period (about 40 min. after the egg is laid). The optimum period for fertilisation is approximately the same for all eggs, but the less ripe the egg appears (from the condition of the gonad) to be, the poorer the results of fertilisation before the optimum time. In spite of this, many samples of moderately ripe eggs may give a few successful fertilisations as soon as the eggs are placed in sea-water (Table I). It is also true that the less ripe is the egg, the sooner it becomes unfertilisable after the optimum period. Thus, the fertilisable life of the egg is lengthened at both ends as the egg ripens.

The fertilisability of the starfish egg seems to bear little relation to the progress of maturation. The egg certainly becomes unfertilisable before maturation is complete. In most samples of the eggs maturation is not complete until the end of the second hour after the egg is laid or taken from the ovary. Fertilisation is possible in very few eggs after they have been kept in sea-water for more than one and a half hours.

The results of the previous experiments on the *Echinus* egg suggest two questions concerning the action of thyroxine on the fertilisable life of the starfish egg. It was found (1931 *b*) that the fertilisable life of the sea-urchin egg is greatly prolonged if thyroxine is present in the medium surrounding it, especially if the egg is washed in a considerable body of the medium. The question, therefore, arises whether thyroxine prolongs the fertilisable life of the starfish egg. Secondly, treatment with thyroxine has been shown to improve the fertilisability of slightly unripe sea-urchin eggs (1932 *a*). It may be asked whether thyroxine has this effect on unripe starfish eggs.

The answer to the first of these questions is that thyroxine almost certainly does not prolong the fertilisable life of starfish eggs. Eggs were placed, immediately after they had been taken from the gonad, in sea-water containing thyroxine at various concentrations between 1 in 10^5 and 1 in 10^7 , and kept in these media for various times. They were then fertilised in sea-water. In a few of these experiments the eggs kept in sea-water containing thyroxine were fertilisable for a slightly longer time (up to 30 min. longer). But it was not clear that this was a true prolongation of the fertilisable life of the same kind as that which thyroxine causes in the sea-urchin egg. The effect was most obvious in experiments in which the eggs were somewhat unripe, and it seemed more probable that it was a result of the general improvement of the fertilisability of the unripe egg which we shall see that thyroxine produces. It has been noted that the riper is the starfish egg, the longer it remains fertilisable.

It is, perhaps, not surprising that thyroxine fails to prolong the fertilisable life of the starfish egg. It is known that the sea-urchin egg becomes unfertilisable owing to the loss of its egg secretions, and it has been suggested (1932*d*) that the prolongation of the fertilisable life which is produced by thyroxine is due to maintenance of the supply of these secretions for a longer time. The shortness of the fertilisable life of the starfish egg makes it very unlikely that it loses its fertilisability for the same reason. If it becomes unfertilisable from some other cause, we have no reason to expect that thyroxine would prolong the fertilisable life of the egg.

On the other hand, thyroxine has the same action on unripe starfish eggs as it has on unripe sea-urchin eggs. The fertilisability of the unripe eggs is definitely improved by treatment with thyroxine. Several experiments were carried out in which

Table I. *Fertilisability of unripe starfish eggs treated with thyroxine.*

Period of treatment. min.	Sea-water containing thyroxine at a concentration of			Sea-water %
	$1/10^6$ %	$1/4 \times 10^6$ %	$1/10^7$ %	
0 Membranes Divisions Blastulae Total Regular	4 b-c* 18 b-c*			
	18 0			
5 Membranes Divisions Blastulae Total Regular	32 b 81 a-c	29 b 66 b-c	32 b 67 b-c	32 b 46 b-c
	67 7	64 4	41 2	46 0
10 Membranes Divisions Blastulae Total Regular	47 b 78 b	46 b 75 b	61 a-b 78 b	42 b 56 b
	68 6	69 2	64 9	46 4
20 Membranes Divisions Blastulae Total Regular	85 a-b 87 a	78 a-b 82 a	78 a-b 81 a	65 a-b 77 a-b
	86 64	86 55	90 68	78 38
30 Membranes Divisions Blastulae Total Regular	85 a 85 a	85 a-b 86 a	93 a 86 a	86 a-b 82 a
	81 69	81 71	89 81	84 71
45 Membranes Divisions Blastulae Total Regular	80 a 80 a	84 a 84 a	83 a 84 a	84 a 75 a
	75 68	88 77	80 75	76 69

* These letters refer to the condition of the membranes and divisions. Those marked "a" were perfectly regular and normal; when marked "b," the membranes were narrow and slightly irregular and the divisions somewhat irregular; and when marked "c," the membranes were irregular and often partial, and the divisions often incomplete.

slightly unripe eggs were placed in solutions of thyroxine immediately after they had been taken from the gonad, and were fertilised in sea-water after short periods in these solutions. The results of a typical experiment of this type are given in Table I. It will be seen that there is a distinct improvement in the fertilisability of the eggs as a result of treatment with thyroxine. This improvement is much more marked in the later stages of the development of the egg than in the formation of the fertilisation membranes. It is most marked in the improved regularity of the blastulae which develop from the eggs. In these respects it agrees with the improvement which thyroxine was found to cause in the development of the unripe sea-urchin egg (1932*a*).

The improvement is shown in the better results of fertilisation throughout the fertilisable life of the egg. It is less in eggs which are allowed to reach the optimum period before they are fertilised, probably because the eggs which have not been treated with thyroxine have by that time ripened naturally, but it is recognisable even at the optimum period (Table I). In other experiments, it was observed to some extent after the optimum period.

It may be concluded that treatment with thyroxine results in a general improvement in the fertilisability of unripe starfish eggs.

The concentrations of thyroxine which were effective in these experiments were even lower than those which were found to be effective in the corresponding experiments on the sea-urchin egg (1 part in 6×10^6). The minimum effective concentration in the experiments on the starfish egg was 1 part in 5×10^7 . This represents a somewhat lower concentration of iodine than that in mammalian blood (15 γ per 100 c.c. or 1 part in 7×10^6). It represents an increase of about 100 per cent. in the iodine content of the medium, since sea-water contains about 20 γ of iodine per litre or 1 part in 5×10^7 . The effective concentrations are very small, but there is no reason to think it unlikely that concentrations as small as this could be effective in producing the results which we have found them to produce. It is clear that the starfish egg is more sensitive to thyroxine than the sea-urchin egg, just as we have found starfish sperm to be more sensitive than sea-urchin sperm.

(2) *Oxygen consumption of the egg before and after fertilisation.*

The results which have just been given show that treatment of the unripe egg with thyroxine improves its fertilisability. In the hope of obtaining some light on the nature of this change in the egg, the oxygen consumption of eggs treated with thyroxine was compared with that of normal eggs.

The experiments were carried out in Barcroft manometers of the same type as those in which the oxygen consumption of the sperm had been measured (p. 109 above). Immediately after the eggs were taken from the animal, they were passed through bolting silk and concentrated on a hand centrifuge, the smallest possible number of turns being used. They were then washed on the centrifuge several times with sea-water. By these means most of the fragments of ovarian tissue that were always present in the sample, and a large proportion of the smaller and more unripe eggs, were removed. 0.5 or 0.6 c.c. of this purified suspension of eggs (in as

concentrated a form as possible) was placed in the bottle of each instrument with 5.5 c.c. of the medium. The pH of the medium was controlled to pH 8.3—that of the sea-water at the time—before each experiment. The instruments were set up and allowed to come into temperature equilibrium with the bath, and then the measurement of the oxygen consumption of the eggs was commenced.

These preliminary processes necessarily consumed a considerable amount of time. It was found impossible to start the measurements of oxygen consumption earlier than 25–40 min. after the eggs had been taken from the animal. This was unfortunate, especially in view of the short fertilisable life of the starfish egg, but it was unavoidable.

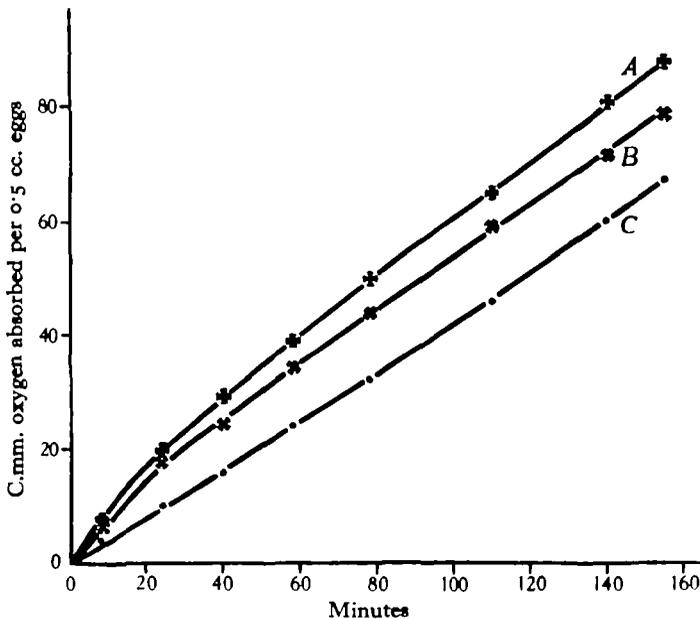


Fig. 2. Oxygen consumption of similar suspensions of unfertilised eggs of *Asterias rubens* in: A, sea-water containing $1/2 \times 10^6$ of thyroxine; B, sea-water containing $1/10^7$ of thyroxine; C, sea-water. All at pH 8.3 at the beginning of the experiment, 8.4 at the end. Start of the experiment 26 min. after the eggs were placed in the bottles of the instruments and 40 min. after they were taken from the animal.

In the experiments in which the eggs were to be fertilised, respirometer bottles with side-tubes were used and a drop of dilute sperm suspension was introduced from the side-tubes during the experiment. The suspension of sperm was always so dilute that the respiration of the sperm introduced into the bottle was too low to make any appreciable effect on the respiration of the whole suspension.

At the end of the experiment the pH of the medium was again estimated, and any experiment in which there were appreciable differences in pH between the media in the different instruments was discarded. Also, at the end of the experiment, the volumes of the eggs in the instruments were determined more accurately by centrifuging them to constant volume on the hand centrifuge. The curves of

the figures have been corrected for the small inequalities which were found in these determinations of the volumes of the eggs in the different instruments. In all the figures, the curves give the oxygen consumption of 0.5 c.c. of eggs.

Fig. 2 gives the results of one of several experiments in which the eggs were not fertilised. The eggs used in this experiment came from gonads which were not completely ripe. It will be seen that the presence of thyroxine has a considerable effect on the oxygen consumption of the eggs. In sea-water (*C*) they give a constant consumption which continues unaltered for long after they have ceased to be fertilisable. In the presence of thyroxine (*A*, *B*) they show a distinctly higher consumption at the beginning of the experiment, but their consumption later falls to

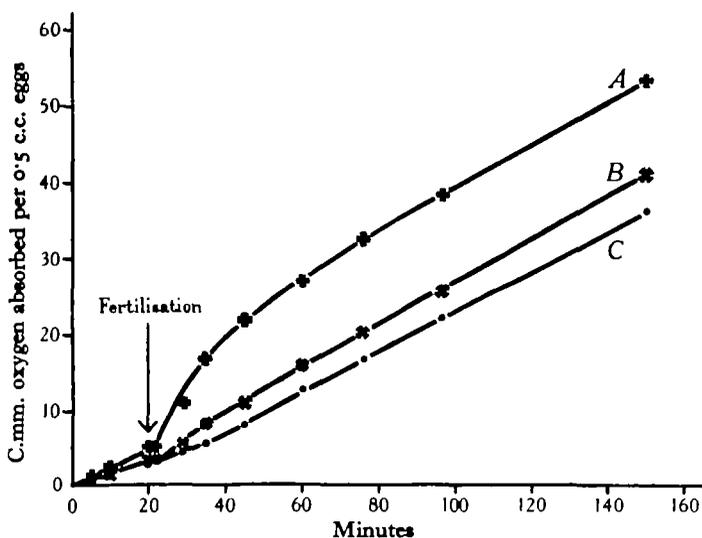


Fig. 3. Oxygen consumption of similar suspensions of eggs of *Asterias rubens* in: *A*, sea-water containing $1/10^7$ of thyroxine; *B*, *C*, sea-water. Fertilisation at 20 min. after the start of the experiment in *A*, *B*. All at pH 8.3 at the beginning of the experiment and 8.3 at the end. Start of experiment 15 min. after the eggs were placed in the bottles of the instruments and 25 min. after they were taken from the animal.

the same value as that of the eggs in sea-water. The initial, increased, consumption is slightly, but not very much, greater in the presence of the higher concentration of thyroxine (*A*).

In this experiment the measurement of the oxygen consumption was started 40 min. after the eggs were taken from the animal. It seems probable that the differences between the curves would have been greater if it had been possible to measure the oxygen consumption earlier.

In other experiments it was shown that the riper the eggs appeared to be (from observation of the condition of the gonad) the smaller was the effect produced by thyroxine. In two experiments in which extremely ripe eggs were used, thyroxine produced no measurable effect. On the other hand, in a few experiments with very

unripe eggs, the eggs equally failed to respond to the presence of thyroxine. There is, presumably, a stage in the ripening of the egg before which the change which thyroxine produces cannot take place.

Figs. 3 and 4 give the results of experiments in which the eggs were fertilised. The eggs used in the experiment of Fig. 3 were less ripe than those of the experiment of Fig. 4 and showed a larger thyroxine effect. It appears from both figures that the increased consumption due to thyroxine continues after fertilisation. It is,

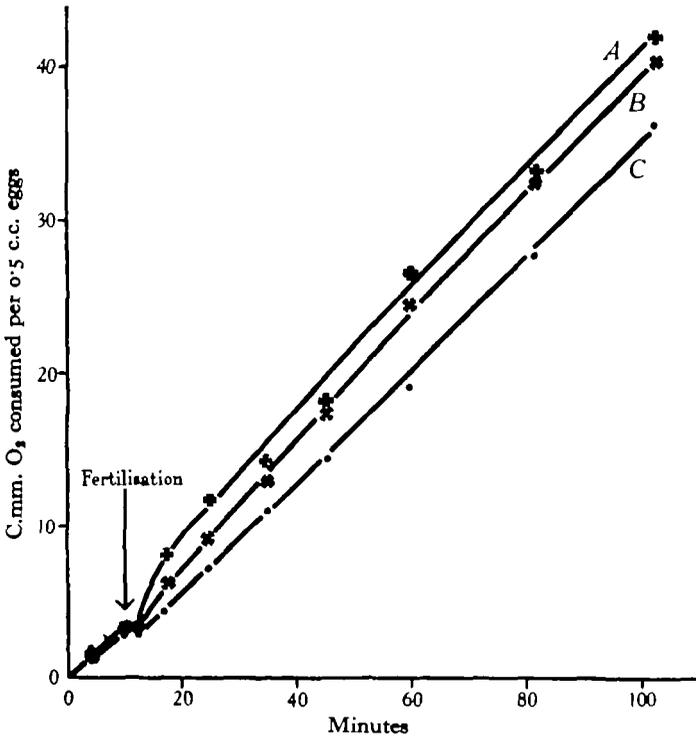


Fig. 4. Oxygen consumption of similar suspensions of eggs of *Asterias rubens* in: *A*, *B*, sea-water containing $1/10^7$ of thyroxine; *C*, sea-water. Fertilisation at 10 min. after the start of the experiment in *A*. All at pH 8.3 at the start of the experiment and 8.4 at the end. Start of the experiment 15 min. after the eggs were placed in the bottles of the instruments and 25 min. after they were taken from the animal.

in fact, considerably greater in some experiments immediately after fertilisation than before it (curve *A*, Fig. 3).

Curves *B* and *C* of Fig. 3 show the oxygen consumption of eggs fertilised in sea-water as compared with that of unfertilised eggs. In this figure the fertilised eggs show a very slightly increased consumption after fertilisation. This increase did not appear in all experiments and it was so small that it was not easy to be sure that it was real. It is certain that it was not large enough to be recognisable in any experiment in which the eggs were nearly ripe.

DISCUSSION.

The behaviour of starfish sperm has been found to be exactly similar to that of the sperm of *Echinus esculentus* in the characters which have been investigated. We may therefore conclude that the deductions which have been previously drawn from the behaviour of the sea-urchin sperm are also true for the sperm of the starfish.

The experiments on the starfish egg need more discussion.

Whether the egg is ripe or unripe, many of its characters are unaffected by treatment with thyroxine. This is true of the *general* rate of oxygen consumption of the egg before and after fertilisation. Whitaker (1933) has shown that the change in the rate of oxygen consumption of eggs such as that of the sea-urchin at fertilisation is due to their ability to pass into an inactive state before fertilisation, whereas other eggs, such as that of the starfish, do not possess this power. There is no reason to think that iodine compounds are concerned in the control of these phenomena.

The progress of maturation seems to be equally independent of the presence of iodine compounds. It is unaffected by treatment with thyroxine.

In the starfish egg, as in the sea-urchin egg, it is the ripening of the egg, and, as a result of this, the success of the fertilisation reactions, which seem to be controlled by the amount of these compounds in the egg. That this is so is shown by the improvement in the fertilisability of unripe eggs which treatment with thyroxine has been shown to produce. And this conclusion is confirmed by the fact that completely ripe eggs are unaffected in this respect by thyroxine.

The oxygen consumption of unripe eggs has been shown to be increased for a short time when they are treated with thyroxine. The form of the curve of oxygen consumption in the unripe eggs suggests very strongly that this increased consumption is the result of some oxidative reaction in the eggs which is accelerated by the introduction of thyroxine into the egg, and it is hard to resist the conclusion that this reaction is associated with the accelerated ripening of the eggs. Thus, it would seem probable that the improved fertilisability of the eggs is, at least in part, the result of the occurrence of an oxidative reaction in the egg, but it must be emphasised that there may be other changes which also play a part in the improvement. This reaction may take place before fertilisation or may be completed after the egg is fertilised.

There is no increase in the oxygen consumption of ripe eggs when they are treated with thyroxine, and this is parallel to the absence of any change in their fertilisability. We must suppose that the oxidative reaction has already occurred naturally in ripe eggs.

It was the object of these experiments to explore how far the theory of the fertilisation changes which has been put forward on evidence derived from the gametes of the sea-urchin may be true of fertilisation in other types of gametes. We have found evidence that iodine compounds play an important part in the ripening of the gametes of the starfish, as they do in that of the gametes of the sea-urchin. If this is so, it is to be expected that the part these substances play in the ripening of both types of gamete is the same. The evidence which we have discussed in this

paper supports this belief. Investigation of the gametes of the sea-urchin led to the belief that a thyroxine-like substance is formed in the egg and sperm during the last stages of their ripening. All the evidence which has been given in this paper is in accordance with the view that such a substance is formed at that time in the starfish eggs and sperm.

If this is so, it is probable that this substance is closely related to the secretions of the starfish egg, for we have found that the thyroxine-like substance of the sea-urchin egg is related to the secretions of that egg.

The starfish gametes differ very greatly in type from the gametes of the sea-urchin. If these substances play an important part in the fertilisation of both these types of gamete, it seems probable that their importance in fertilisation is general.

SUMMARY.

1. The unripe sperm of the starfish is activated by treatment with thyroxine in the same manner as that of *Echinus esculentus* is activated.
2. The fertilisability of slightly unripe starfish eggs is improved by treatment with thyroxine. Ripe eggs are unaffected.
3. In slightly unripe eggs, treatment with thyroxine causes a temporary increase in the oxygen consumption of the eggs. The oxygen consumption of ripe eggs is unaffected.
4. Thyroxine causes, or more probably, accelerates, an oxidative reaction in the unripe eggs, which is, at least in part, responsible for the improvement in the fertilisability of the eggs.
5. Iodine compounds play a part in the fertilisation of the starfish egg, which is in many respects similar to the part they play in the fertilisation of the sea-urchin egg.

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