THYROXINE AND THE OXYGEN CONSUMPTION OF THE SPERMATOZOA OF *ECHINUS MILIARIS*

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(With Four Text-figures.)

In 1917 Miss Woodward and Miss Hague (5) published an account of experiments in which a solution of iodine in sea-water was found to be effective in producing artificial parthenogenesis in the eggs of *Arbacia*. The results were remarkable in that the percentages of the eggs which developed were not noticeably increased by treatment with hypertonic sea-water after activation by the parthenogenetic agent. In this respect iodine differs from all the better known means of producing artificial parthenogenesis in the echinoderm egg, and resembles only fertilisation by the spermatozoon, and the activation of the frog's egg by pricking its surface.

The results of these experiments suggest that iodine may have some closer relation to the changes which occur in the egg during activation than have the other parthenogenetic agents. Elsewhere the most definite effect which iodine compounds are known to produce in the physiology of animals is the rise in the rate of the basal metabolism, which follows the injection of thyroxine in the vertebrate. In the egg there is also an increase in the chemical activity of the protoplasm during activation, but this increase is far greater in proportion to the previous activity of the protoplasm than is that in the vertebrate. Yet the changes are, in both cases, increases in the general chemical activity of the cells, and may thus, perhaps, be of a similar nature.

In view of these considerations, it seemed worth while to investigate the effects produced in the gametes of echinoderms by the presence of thyroxine. The changes which occur at fertilisation in the egg are very various, and all experiments upon the egg at that time are made difficult, and their results obscured, by the complexity of these changes. In the sperm before fertilisation fewer changes occur, and it is therefore a far more favourable subject for investigation. Also, Gray (3) has recently shown that the activity of the spermatozoa of *Echinus miliaris* (as reflected in their consumption of oxygen) is modified by the presence of egg secretions in the water in which they are active. He found that in clean sea-water the activity of the sperm begins to decrease immediately after it is put into the water, but that in the presence of egg secretions the initial high rate of activity is continued for 2–3 hours without decrease. Although these effects may appear very different from the increase of the activity of the egg after fertilisation, it is at least possible, if iodine compounds are concerned in the latter change, that they are also concerned in these variations in the activity of the spermatozoon. The following experiments were therefore performed to test the effects of the presence of thyroxine upon the oxygen consumption, and therefore the chemical activity, of the spermatozoa of this species, and to compare any effects which it was found to produce with those caused by egg secretions. Further, the experiments also gave an opportunity of observing the effects of the presence of thyroxine on the oxidations of an homogeneous type of cells.

In Figs. 1 and 2 are given the results of two of a series of experiments in which the oxygen consumption of similar suspensions of spermatozoa in clean sea-water and in sea-water containing thyroxine and egg secretions are compared. The sea-water containing egg secretion was prepared by leaving the eggs for 5–6 hours in sea-water stirred by a current of air. The solution of thyroxine was prepared from the synthetic drug supplied by the British Drug Houses. A 1/3 per cent. solution was made up in *N*/30 sodium hydrate, the drug being only very slightly soluble in neutral water. This was added in the correct proportion to sea-water, and the hydrogen-ion concentration adjusted as accurately as possible to that of the sea-water (*p*H 8·2) with *N*/10 hydrochloric acid. The oxygen consumption in the different media was compared in similar Barcroft manometers, immersed in a large water-bath, in which the temperature did not vary more than 0·5° C. during the experiment, and always lay between 12° and 15° C. The hydrogen-ion concentration of the water in the bottles was compared before the addition of the spermatozoa and at the end of the experiment, to insure that differences in the oxygen consumption could not be due to differences in this property of the medium. The suspensions were always less alkaline at the end of the experiment (*p*H 7·4–7·6) than the sea-water at the beginning (*p*H 8·2), but no differences were observed between the bottles containing the suspensions at any one time. Presumably this decrease in the alkalinity was due to incomplete diffusion of the carbon dioxide produced. The "dry" sperm was prepared by pressing the testes of urchins, which appeared to be ripe, through fine bolting silk, and 0·2 c.c. of this concentrated suspension of spermatozoa was added to the water (4 c.c.) in one bottle of each instrument. Great care was taken to avoid temperature changes during the addition of the spermatozoa. The amount of the spermatozoa present in each suspension was estimated by determinations of the total nitrogen by Cole's micro-Kjeldahl method. There was found to be 2–3 mg. N in the 4 c.c. of the suspension in each bottle (0·5–0·75 mg. per c.c.). Gray (3) has shown that the oxygen consumption of suspensions of the spermatozoa of this species is independent of the concentration of the spermatozoa, when the suspension contains between 0·3 and 1·0 mg. per c.c. The curves have therefore not been corrected for this factor. The results of other experiments with lower concentrations of sperm, when the consumption is proportional to the amount of sperm present, showed that the conclusions to be drawn from the results were the same whatever the concentration used.

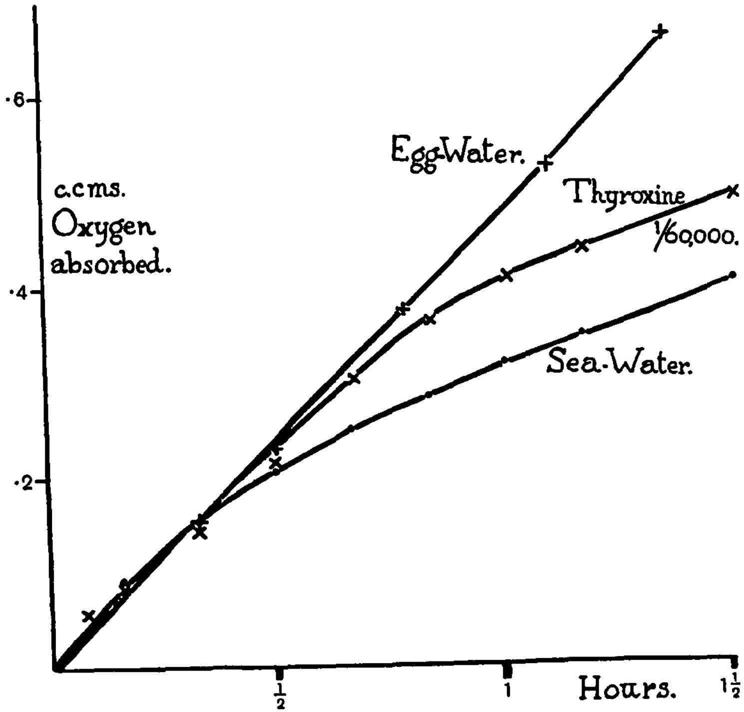

Fig 1.

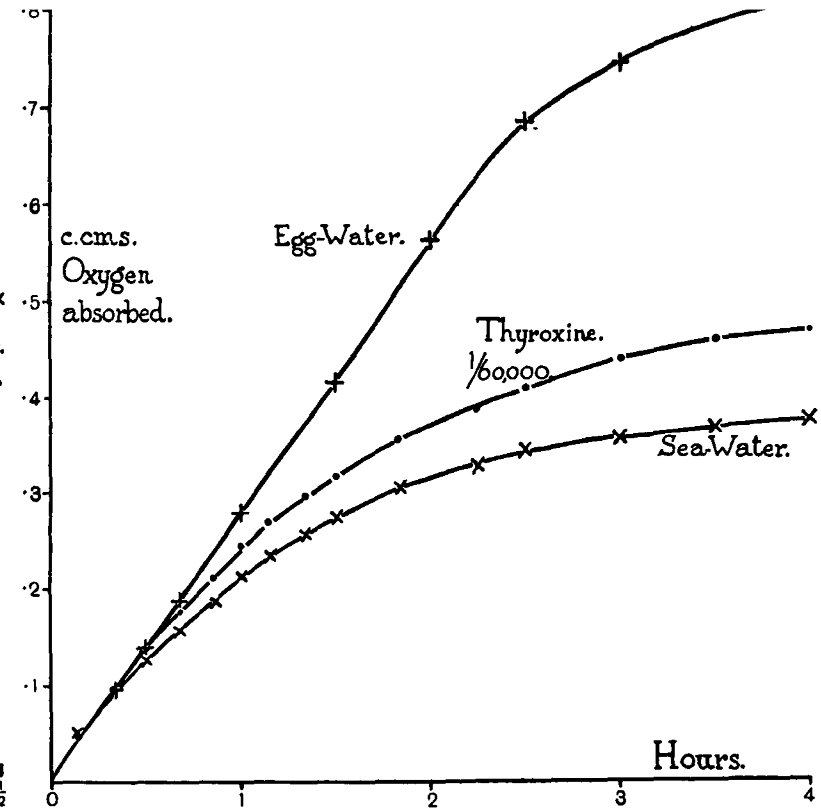

Fig. 2.

Figs. 1 and 2. Curves showing the total oxygen consumption of similar suspensions of spermatozoa of *Echinus miliaris* in sea-water, in sea-water with 1/60,000 thyroxine by weight, and in sea-water containing egg secretions.

The curves giving the oxygen consumption in the presence of egg secretions are in agreement with those obtained by Gray (3). The secretions produce no alteration in the amount of the initial consumption of oxygen. In the presence of the secretions, the initial rate is continued for 2–3 hours, but the consumption falls off rapidly after that time. In clean sea-water there is a rapid fall in the consumption from the first few minutes of the experiment.

It will be seen that the presence of thyroxine also causes no alteration in the initial rate of consumption. In this respect the effects of thyroxine and of egg secretions are the same. There is no appreciable fall in the rate of oxygen consumption in the presence of the drug for 30 minutes after the beginning of the experiment, but the decrease starts after that time. At the end of 1–3 hours the suspension in the sea-water containing thyroxine is respiring at the same rate as that in clean sea-water. This is also true of the suspension in egg-water in most experiments at the end of 5–6 hours from the beginning of the experiment (Gray (3)), but the experiments of which the results are given in these figures were not prolonged sufficiently to show this[1].

In the whole series of the experiments the proportional rate of the decrease in the oxygen consumption in each of the media was found to vary greatly in different samples of sperm, probably with its ripeness. Differences in this respect can be seen between Figs. 1 and 2, and are clearer in Fig. 3, in which the rates of oxygen consumption in the three media in two other experiments are expressed as percentages of the initial consumption. In the experiment from which the upper curves of this figure were derived, the decrease was much slower than in that to which the lower curves refer. But in all experiments the curves were of the same type. The two experiments of Fig. 3 express the extremes of the variation in this respect.

In all the experiments the curve of the consumption in the presence of thyroxine lay between those of the consumption in clean sea-water and in sea-water containing the egg secretions[2]. It appeared also that the changes produced by the presence of thyroxine were of the same nature as those caused by the egg secretions, although different in extent. It has been stated above that there was an initial period of about 30 minutes, when thyroxine was present, in which the consumption did not decrease appreciably. This may be compared with the period of 2–3 hours, in which there is no decrease, when egg secretions are present. That the forms of the curves expressing the decrease in the three media are very similar is shown most clearly in Fig. 3, and especially in the lower of the two experiments of that figure. Here the curves are almost identical in form, and differ only in the length of the initial period of high consumption. In the experiment of the upper curves of this figure, in addition to the slow decrease of the consumption in all the media, the reaction due to the presence of the egg secretions was not complete,

[1] It is better shown in the experiment recorded on the lower part of Fig. 3, in which the suspensions in sea-water and egg-water were respiring equally after 3 hours.

[2] With two exceptions, in which the presence of thyroxine appeared to have no effect. least one of these the urchin from which the spermatozoa were derived was not completely ripe.

either on account of weakness of the egg-water or of unripeness of the sperm, and the consumption in the presence of the secretions fell slightly in the first hour. But in this experiment, as in all the others, the three curves are very similar in form.

The experiments therefore support the view that the action of thyroxine upon the oxygen consumption of the sperm is of the same nature as that of egg secretions, but is not so great.

Similar results were obtained with thyroxine in all concentrations between 1/45,000 and 1/75,000. At a concentration of 1/30,000 the drug was found to be toxic, and the consumption fell off rapidly after the first 30 minutes. At a

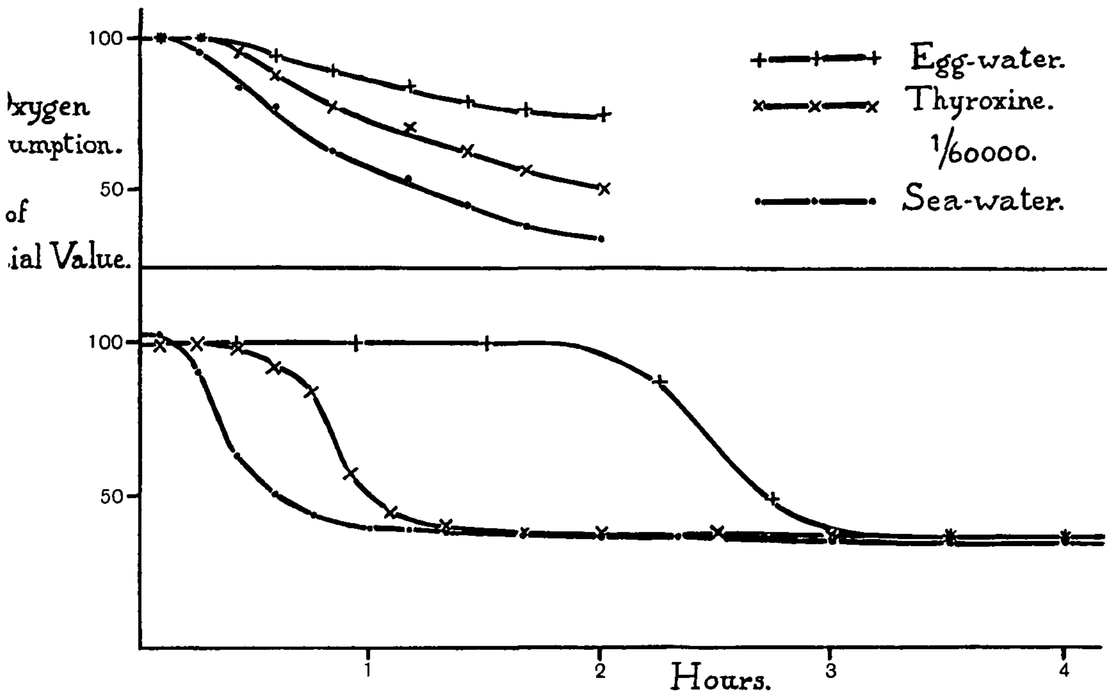

Fig. 3. Two similar experiments to those of Figs. 1 and 2. Rates of consumption of oxygen expressed as percentages of the initial consumption.

concentration of 1/100,000 the effect of the presence of the drug was still noticeable, but not complete.

In Fig. 4 are given the results of experiments in which thyroxine was added to suspensions of spermatozoa after their active life in sea-water had been started. Two instruments were set up with suspensions of the spermatozoa in sea-water, and the consumption read for different periods. To the one suspension there was then added sufficient thyroxine, contained in one drop of *N*/30 sodium hydrate, to give a 1/45,000 solution in the water. One drop of a solution of hydrochloric acid, which had been found by previous experiment to be of the correct strength to neutralise any changes in the hydrogen-ion concentration due to the addition of the alkali, was also added to this suspension. In order to avoid any complications due to the slight change in osmotic pressure, two drops of distilled water were added to the suspension in the other instrument. Care was taken to avoid, as far as possible, any temperature changes in the bottles during these operations.

Since the effects to be observed in these experiments were the changes in the

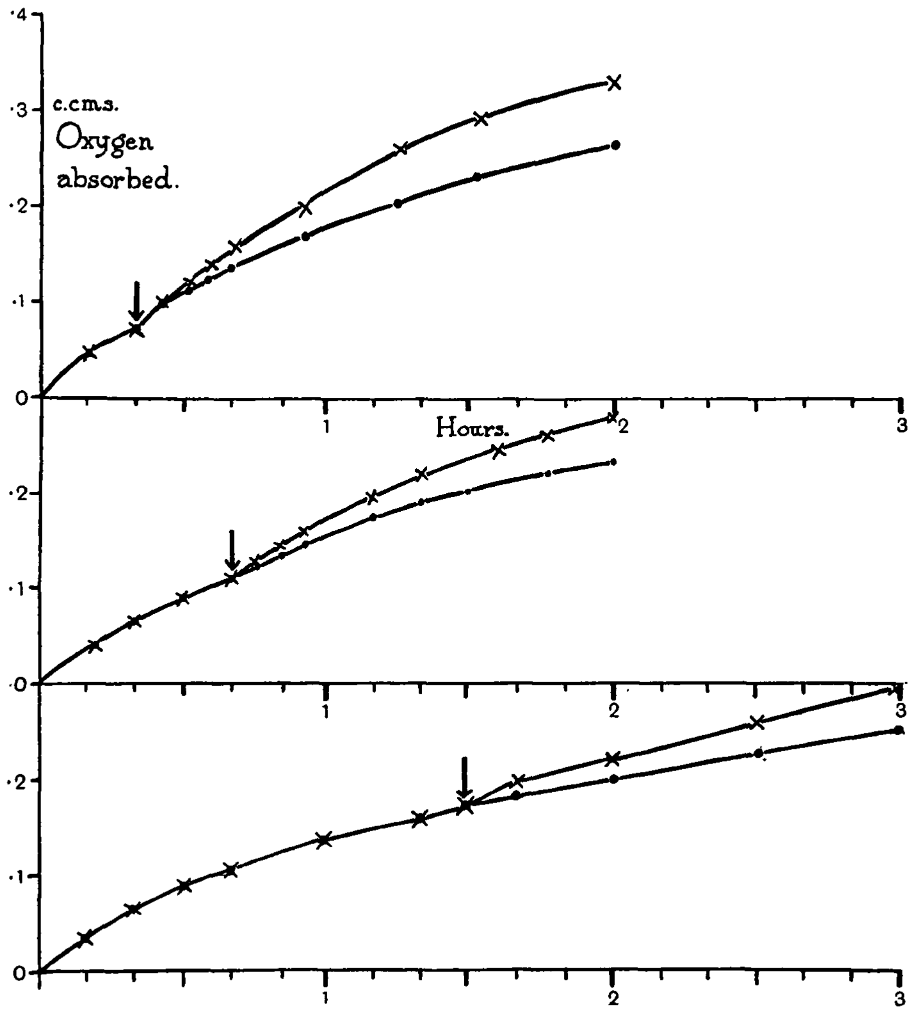

Fig. 4. Curves showing the results of addition of 1/45,000 thyroxine at various times after the start of the active life of suspensions of spermatozoa. In each experiment the thyroxine was added at the time indicated by the arrow.

shapes of the curves due to the addition of thyroxine, and not the absolute amount of the consumption of oxygen, the curves relating to the earlier parts of the experiments have been adjusted in scale so as to fit over each other as accurately as possible in the figure.

It will be seen that in all these experiments there was an increase of 40–50 per cent. in the consumption after the addition of thyroxine. In some of the experiments there is a still higher apparent consumption during the first 5 minutes after the thyroxine was added. This did not occur in all the experiments or only in the instrument to which thyroxine had been added. It was probably due to slight warming of the bottles containing the suspensions during the manipulations necessary in adding the drug and the distilled water. It was found impossible to avoid temperature changes of this size in all the experiments. The effects of these alterations of temperature could not have affected the curves beyond 5 or at most 10 minutes after the manipulations were performed.

The increases in the consumption observed in these experiments after the addition of thyroxine were about equal to the difference between the rate of consumption immediately before the thyroxine was added, and the rates at which the suspension would have been respiring, if thyroxine had been present from the beginning of the experiment (as deduced from the forms of the curves in experiments such as those recorded in Figs. 1 and 2). It appeared that the thyroxine immediately removed or decreased the cause of the lowered consumption in sea-water, and that the suspension proceeded thereafter as if thyroxine had been present throughout. Gray (3) has shown that the decrease in the consumption of suspensions in sea-water is not due to any alteration of the medium during the active life of the suspension. The effects of both egg-water and thyroxine in modifying the cause of the decrease must therefore take place within the body of the sperm.

If it may be concluded from the results of these experiments that the effects produced by thyroxine and egg secretions are the same in kind, though different in extent, it seems natural to conclude that the egg secretions contain a body, which, having a similar physiological effect, is probably closely related in chemical structure to thyroxine. Since no concentration of thyroxine produces so marked an effect as that produced by the egg secretions, the experiments undoubtedly show that thyroxine is not itself a constituent of the egg secretions. But it is not impossible that they contain some similar substance, or, more probably, that they contain, uncombined or in some form of combination, some essential constituent of such a substance, which is built up into the active substance, when it is introduced into the body of the spermatozoon.

Such a view is not in conflict with previous work upon the chemical nature of the egg secretions. The known effects of these secretions are:

(1) The agglutination of the spermatozoa.

(2) The lipolytic effect on fats and similar substances.

(3) The increase in the initial activity, and rate of oxygen consumption of the sperm when it is put into sea-water. This is observed in the sperm of some species, but not in that of *E. miliaris* (Gray (3)).

(4) The prolongation of the life of the sperm at the initial high rate of oxygen consumption, the effect with which this paper is concerned.

Previously the nature of the secretions has been studied chiefly with regard to the first and second of these effects. Glaser (1) and Richards and Miss Woodward (4) have produced evidence that both these effects are due to the presence of enzymes in the secretions. What relation the substance which is responsible for the prolonged high rate of oxygen consumption bears to those which cause these other effects must be left for further work to decide. And whether this substance has any function in the activation of the egg, and, if so, what the nature of that function is, are also questions which cannot at the present time be discussed.

Whatever conclusions may be reached on these questions, the results of these experiments show that the effect of thyroxine upon the oxidations of this type of cell is not a general increase in the oxygen consumption in all circumstances, but rather that the drug plays some part in neutralising the effects which produce the decreased oxygen consumption of the cells, when they have been respiring actively for some time; and so, in enabling a high rate of oxygen consumption to be maintained for a longer time than in its absence. By this means a greater oxygen consumption over a given period of time is produced, a result which would equally be produced by a general increase in the oxidations of the cells.

SUMMARY.

1. In the presence of thyroxine in concentrations of 1/45,000 to 1/75,000 the initial rate of oxygen consumption of the spermatozoa of *Echinus miliaris* is the same as in sea-water without the drug, but this rate of oxygen consumption is prolonged for a longer time in the presence of the drug. The rate of oxygen consumption in the presence of the drug falls after this period, and at the end of 2–4 hours is approximately equal to that in clean sea-water.

2. In a concentration of 1/30,000 the drug is toxic, and of 1/100,000 the effect is incomplete.

3. These effects are parallel to, and apparently of the same nature as, the effects of secretions of the eggs of the same species upon the oxygen consumption; but the effects of the egg secretions are much greater in extent.

4. The suggestion is made that the egg secretions contain, in addition to their other known constituents, either a body of which the physiological effect is similar to that of thyroxine, and which is perhaps similar to it in chemical structure, or some constituent from which such a body is built, when it is absorbed by the spermatozoon.

5. In its action upon the spermatozoon the effect of thyroxine is to remove or reduce the cause of the decreasing activity of the spermatozoon in sea-water (as revealed by its oxygen consumption). This effect is immediate and may take place at any period during the life of the spermatozoon at which its oxygen consumption in the presence of thyroxine is greater than that in sea-water.

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