PHYSIOLOGICAL STUDIES OF DIFFERENTIATION IN MYTILUS EDULIS. I. THE OXYGEN UPTAKE OF ISOLATED BLASTOMERES AND POLAR LOBES[1]

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The classic experiments of Wilson (1904), Conklin (1905) and others have demonstrated that the eggs and embryos of many invertebrate animals contain regions of cytoplasm which are determined as to their subsequent fate. A precocious differentiation may even precede cleavage of the eggs, as in some annelids, molluscs, and ascidians. The cytoplasmic regions of these so-called mosiac eggs have been described variously as containing "organ forming" substances or of being chemo-differentiated, but as yet there has been slight progress in defining these mosaic regions in terms of specific physicochemical differences.

Spek (1934) attempted to demonstrate regional cytoplasmic differences by means of vital dyes and concluded on the basis of differential vital staining that a pH gradient existed along the polar axis of various mosaic eggs. Ries (1937, 1939) compared mosaic and regulative eggs by the use of cytochemical techniques. Tests for ascorbic acid, glutathione, benzidine peroxidase, and indophenol oxidase were used, in addition to measurements of rH_2 by means of oxidation-reduction dyes. In some mosaic eggs a localization of these substances or a difference in redox potential could be demonstrated, although the significance of these in the process of differentiation was unclear. Similar investigations on mosaic eggs have been carried out and extended by Reverberi and Pitotti (1940), Pitotti (1947) and Urbani and Mistruzzi (1947). Cytochemical tests have also been used to demonstrate localization of enzymes in mosaic eggs by Lehmann (1948) and Raven (1948).

Although cytochemical techniques have yielded valuable information regarding the chemical heterogeneity of mosaic eggs, they have the disadvantages of being limited in scope as well as being mainly qualitative. Also considerable controversy exists as to the validity of some of the cytochemical techniques (Brachet, 1947). It is apparent that the most desirable approach to this problem would be to carry out direct microchemical analyses and physiological studies of the various cytoplasmic regions. It was with this intention that the present investigation was undertaken.

Owing to the small size of mosaic eggs, it would be difficult to obtain, by cutting the egg into fragments, sufficient amounts of cytoplasm for microchemical studies. It is known, however, that in several mosaic eggs the first cleavage plane normally separates cytoplasmic regions with different presumptive fates. This is particularly striking in the eggs which form polar lobes such as those of Dentalium, Ilyanassa

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and Myzostoma. It seemed that a profitable approach to the problem of cytoplasmic localization would be to take advantage of this natural segregation of cytoplasmic areas and to develop methods for large scale separation of blastomeres in such a form.

The eggs and embryos of *Mytilus edulis* have proven to be particularly suitable for this purpose. Field (1921) has described the early development of *M. edulis* in detail. The first cleavages are similar to those of Dentalium (Wilson, 1904); a polar lobe forms at the vegetal pole of the fertilized egg and is subsequently incorporated into the CD blastomere. Prior to the next cleavage a second polar lobe forms which fuses with the D cell. The first cleavage blastomeres are unequal in size and therefore may be readily recognized.

Investigations on the development of isolated blastomeres and eggs lacking the polar lobes indicate that a typical cytoplasmic localization exists in the Mytilus egg. The polar lobe contains factors which are necessary for the normal development of the larval form and particularly for an apical tuft (to be published).

As a prerequisite for physiological and microchemical studies, it was necessary to develop methods for separating the blastomeres of early cleavage stages of Mytilus. Even on a small scale this is ordinarily difficult, owing to a tough protective membrane surrounding the eggs and an intercellular cement binding the blastomeres together. Fortunately it was discovered that sperm extracts contain lysins which dissolve the enclosing membranes as well as the intercellular cement (Berg, 1950). The use of these lysins permits large scale separation of the blastomeres which subsequently may be used for physiological studies. A method has also been discovered for obtaining large numbers of isolated polar lobes which permits investigations to be carried out directly on the polar lobe itself.

The present report, dealing with the respiration of isolated blastomeres and polar lobes of Mytilus, was undertaken in view of the many attempts in the past to correlate embryonic differentiation with metabolic gradients. Child (1941), for example, has for many years postulated physiological gradients or "respiratory gradients" as controlling factors of differentiation. Ries (1942) also suggests that there is a coupling of oxidation intensity and the oxidase content of regions of mosiac eggs. These conclusions, however, have been based on the results of cytochemical studies, whereas the present techniques permit direct quantitative measurements of the respiratory intensity of different regions of the egg. A preliminary report of this work has recently appeared (Berg and Kutsky, 1950).

Methods

Mytilus edulis were collected from the San Francisco Bay and stored "dry" in a refrigerator at 4° C. Eggs and sperm were obtained by placing animals in separate bowls of sea water and allowing natural spawning to occur. Although spawning occurs up to one week after storage of the animals at low temperatures, as a rule they were used within the first few days to avoid a progressive deterioration of the eggs.

The procedure for separation of the first cleavage blastomeres is based upon the discovery of lysins in sperm extracts. As previously reported (Berg, 1950), sperm extracts prepared by freezing and thawing of a concentrated sperm suspension contain lysins which dissolve the membrane enclosing the egg and also the inter-

cellular cement binding the blastomeres together. For the present experiments it was convenient to prepare sperm extracts from the testes of *M. californianus* since considerably greater amounts of sperm may be obtained from this species. Several liters of a concentrated sperm suspension were prepared by homogenizing the testes of several dozen males. This was frozen, centrifuged, and the supernatant measured into 10-ml. vials which were stored at −20° C.

Separation of the first cleavage blastomeres was carried out using sperm extract alone or by a combination of sperm extract and calcium-free sea water. With the former method the eggs were fertilized and approximately five minutes before cleavage the excess sea water was pipetted off and 5–10 ml. of sperm extract added. Dissolution of the egg membranes occurred in a few minutes and, as described previously (Berg, 1950), on completion of the first cleavage the blastomeres became partially or completely separated. It was usually necessary to draw the eggs gently in and out of a pipet to increase the percentage of complete separation. Calcium-free sea water also dissolves or prevents the formation of intercellular cement and may be used for blastomere separation. With this method the membranes were removed by means of the sperm extract shortly after fertilization and the eggs were then transferred through several dishes of calcium-free sea water.

The use of sperm extract alone is preferred in some instances since the blastomeres in calcium-free sea water are more fragile and the agitation necessary to ensure complete separation often causes cytolysis. Both methods, however, have been used satisfactorily. After separation, the blastomeres were transferred through several dishes of normal sea water.

Isolated polar lobes were obtained by subjecting membraneless eggs to $MgCl_2$ solutions at the time of polar lobe formation, a procedure which causes a number of the polar lobes to constrict off rather than fuse with the CD cells. Moderate agitation increases the percentage of isolated polar lobes. Since there is considerable variability in the success of this method and the factors involved have not been studied in detail, a fuller report of this method will be published at a later date.

The oxygen uptake of whole eggs, isolated blastomeres, and polar lobes was measured with the Cartesian diver ultramicrorespirometer. Divers were constructed from Pyrex glass according to a design described by Zeuthen (1950), in which the part of the diver containing the biological material is plugged with a hollow glass stopper. Air volumes of the divers ranged from 0.4 μl. to 1.1 μl. and the liquid charge amounted to about two-thirds the size of the air bubble. In paired experiments divers with approximately the same volume were nearly always used since there was a tendency for larger divers to give slightly higher oxygen uptake values. As reported by Zeuthen, these divers are practically gas tight. Blank tests in which pure oxygen or nitrogen was introduced as the bubble resulted in a drift of the equilibrium volume of about 0.1 cm. manometer pressure per hour (after an initial equilibration period).

The flotation vessels were filled with alkaline sodium chloride solution isotonic with sea water. Equilibrium pressures, as determined by raising the diver slightly off the bottom of the flotation chamber, were measured every 10 to 20 minutes depending upon the type of experiment. In nearly all experiments the number of cells introduced into the diver was chosen so as to cause a manometer change of 10–15 cm. per hour.

Immediately after placing a loaded diver in the flotation chamber, the equilibrium pressure decreases rapidly at first and then more slowly. This is probably due to equilibration taking place between the liquid and gas phases of the diver charge. Accordingly actual measurements of oxygen uptake were not begun until after a half hour equilibration period. The error due to a residual drift becomes negligible (less than 0.5 cm. per hour) after about 30 minutes. All experiments were carried out at 20° C.

Oxygen uptake was calculated in the usual manner from the volume of the bubble at equilibrium pressure, the change of the equilibrium pressure per unit time, and the number of cells. Corrections were made for the decrease in oxygen tension in the liquid charge and also for the manometer pressure during the period of measurement.

The Oxygen Uptake of Isolated Blastomeres

AB and CD cells were separated from one another by either of the previously described methods and then transferred through several dishes of sea water. Since AB cells average about 41 μ in diameter and CD cells about 51 μ, they can be distinguished under a low power microscope. The dish from which the blastomeres were to be counted was kept at 10°–15° C. in order to delay cleavage, as it is somewhat easier to distinguish the two types of cells prior to their first cleavage. In each experiment 75 CD cells were selected under the microscope with a braking pipet and introduced into the diver by inserting the tip of the pipet into the diver and slowly expelling the cells so that they settled to the bottom. Loading of the diver was done under a dissecting microscope with the diver held in a fixed position on a small glass plate. One hundred fifty AB cells were selected and loaded similarly into another diver. Air bubbles were measured with a calibrated braking pipet and introduced into the respective divers. The sea water above the air bubbles was then displaced with an alkaline sodium chloride solution, the divers stoppered, placed in the flotation chambers and allowed to equilibrate for approximately 30 minutes.

After loading the divers, the diameters of representative samples of AB and CD cells (usually about 20 to 30 each) were determined with an ocular micrometer. The measured blastomeres were selected from the same batch as that used for the respiration experiment.

The time necessary for counting the cells, loading the divers, etc., caused a delay such that actual oxygen uptake measurements could not begin until nearly three hours after fertilization. At this time the blastomeres had undergone cleavage and were all at least in the two-cell stage. Respiration was measured for an 80-minute period in which time several more cleavages occurred and the embryos consisted of eight or more cells. It might be pointed out here that the cleavage rates of the AB and CD cells are nearly identical during their early development (Fig. 4).

In initial experiments it became apparent that due to biological variability and experimental errors, it would be necessary to carry out a number of experiments and apply a statistical test. To facilitate the statistical analysis paired experiments were carried out, *i.e.*, AB and CD cells were obtained from the same batch of eggs and the oxygen uptake of these determined simultaneously. The data for 12 paired experiments are listed in Table I. Oxygen uptake was first calculated, using the equilibrium volume of the bubble, change in manometer pressure for the period,

number of cells, etc., and with the appropriate corrections expressed as μl. O_2 uptake per hour per embryo. Since the amount of cytoplasm in the AB and CD embryo differs considerably, it was decided to express oxygen uptake in terms of unit amount of cytoplasm. The above figures were therefore divided by the average initial volume of the cells for the particular experiment and final comparative values could then be expressed as μl. oxygen uptake/hr./mm.^{3} cytoplasm.

The average of 12 experiments on the respiration of AB embryos, as shown in Table I, is 0.90 ± .032 μl. O_2/hr./mm.^{3} cytoplasm, as compared with 0.78 ± .027 for

TABLE I

Comparison of oxygen uptake of isolated AB and CD embryos for an 80-minute interval three hours after fertilization

Experiment No.	Oxygen uptake of AB embryos 10^{-5} μl. O_2/hr./AB embryo	Average volume of AB cells 10^{-5} mm.^{3}	Oxygen uptake of AB embryos 10^{-1} μl. O_2/hr./mm.^{3} AB cytoplasm	Oxygen uptake of CD embryos 10^{-5} μl. O_2/hr./CD embryo	Average volume of CD cells 10^{-5} mm.^{3}	Oxygen uptake of CD embryos 10^{-1} μl. O_2/hr./mm.^{3} CD cytoplasm
1	3.1	3.95	7.8	4.8	7.54	6.4
3	2.9	3.27	8.9	4.9	6.62	7.4
4	3.0	3.46	8.7	5.2	7.10	7.3
6	3.2	3.40	9.4	5.5	6.62	8.3
8	3.5	4.24	8.3	6.7	8.52	7.9
9	3.0	3.82	7.9	5.4	7.62	7.1
10	3.0	3.58	8.4	4.8	7.35	6.5
11	3.5	3.66*	9.6	6.9	7.35*	9.4
12	2.8	3.66*	7.7	5.8	7.35*	7.9
13	3.8	3.95	9.6	6.0	7.54	8.0
14	3.5	3.52	9.9	5.9	7.10	8.3
15	4.0	3.46	11.5	6.3	6.80	9.3
Average			9.0 ± .32 S.E.			7.8 ± .27 S.E. (13% lower than respiratory rate of AB embryos). t = 5.6, sig. at 1% level

* Diameters not measured. Average of all experiments used.

the corresponding CD embryos. The difference of 13 per cent is significant at the 1 per cent level, and it may be concluded from this that the respiration of CD cytoplasm is significantly lower than that of AB cytoplasm.

In addition to the above experiments three others were carried out which were not paired, *i.e.*, the AB and CD cells did not come from eggs of the same female. If these are included with the data of Table I, the rate of O_2 uptake of the CD embryos is found to be 15 per cent less than that of AB embryos. Statistical treatment of this unpaired series indicates a difference significant at the 1 per cent level.

THE OXYGEN UPTAKE OF ISOLATED POLAR LOBES

The polar lobe is incorporated into the CD cell at the close of the first cleavage and one might reasonably suspect that any physiological differences existing between

the first cleavage blastomeres might be due to the polar lobe. Thus, the lower respiratory rate of the CD cells might be owing to the presence of the polar lobe material.

Polar lobes were isolated by the use of sperm extracts and $MgCl_2$ solutions, as described previously, and transferred through several dishes of normal sea water. In nine experiments 425–475 lobes were drawn up into a fine braking pipet and loaded into a diver in the same manner as described for AB and CD cells. A sample (30 to 40) of polar lobes was removed from the same dish and their diameters measured. The average diameter of isolated polar lobes is almost 30 microns which is about 10 microns less than the AB cells. AB cells were also present in the dish

TABLE II

Comparison of oxygen uptake of isolated polar lobes and whole eggs

Oxygen uptake of whole eggs during time of polar lobe formation 10^{-5} µl./hr./egg	Average volume of eggs 10^{-5} mm.^{3}	Oxygen uptake of eggs during time of polar lobe formation 10^{-1} µl. O_2/hr./mm.^{3} egg cytoplasm	Oxygen uptake of isolated polar lobes 10^{-6} µl./hr./polar lobe	Average volume of polar lobes 10^{-5} mm.^{3}	Oxygen uptake of polar lobes 10^{-1} µl. O_2/hr./mm.^{3} polar lobe cytoplasm
6.7*	12.3	5.4	5.6	1.39	4.0
6.8*	13.7	5.0	6.8	1.71	4.0
6.9*	12.6	5.5	4.8	1.56‡	3.1
6.6	12.5	5.3	8.7	1.84	4.7
7.3	12.4	5.9	6.7	1.50	4.5
7.5	12.0	6.3	5.2	1.22	4.3
6.8	12.2‡	5.6	6.3	1.56‡	4.0
6.8	11.8	5.8	4.9	1.39	3.5
6.3	12.2‡	5.2	8.1	1.41	5.7
Average		5.6 ± .13 S.E.			4.2 ± .25 S.E. (25% lower than respiratory rate of whole egg cytoplasm. t = 4.9, sig. at 1% level)

* Average of two or more experiments on the same batch of eggs.
‡ Diameters not determined. Average of all experiments used.

with isolated polar lobes and it was sometimes difficult to distinguish between the largest of the polar lobes and the smallest of the AB cells, particularly when several hundred polar lobes had to be selected rather rapidly. In almost all experiments a few AB cells were thus unavoidably included in the diver with the polar lobes. These served a useful purpose, however, since the presence of swimming AB embryos in the diver after the respiration measurements served as an indication of the validity of the test. In calculating the respiration of the polar lobes a correction was made for the respiration of these AB cells.

Actual oxygen consumption measurements were carried out for a period of at least three hours. The polar lobes were examined afterwards, and a few tests were discarded because of a marked clumping of the lobes. This, coupled with the absence of swimming AB embryos, suggested that alkali had crept past the air bubble and mixed with the sea water.

For purposes of comparison the respiration of whole eggs was determined during the time of polar lobe formation. A period of 20 minutes was chosen, beginning 60 minutes after fertilization which is approximately the time at which the polar bulge first appears. Fertilized eggs were first treated to remove the membranes and then washed with sea water. In each experiment 50 to 75 of these were loaded into a diver as rapidly as possible, so as to allow sufficient time for the diver to equilibrate. The diameters of representative eggs were then determined during the equilibration period.

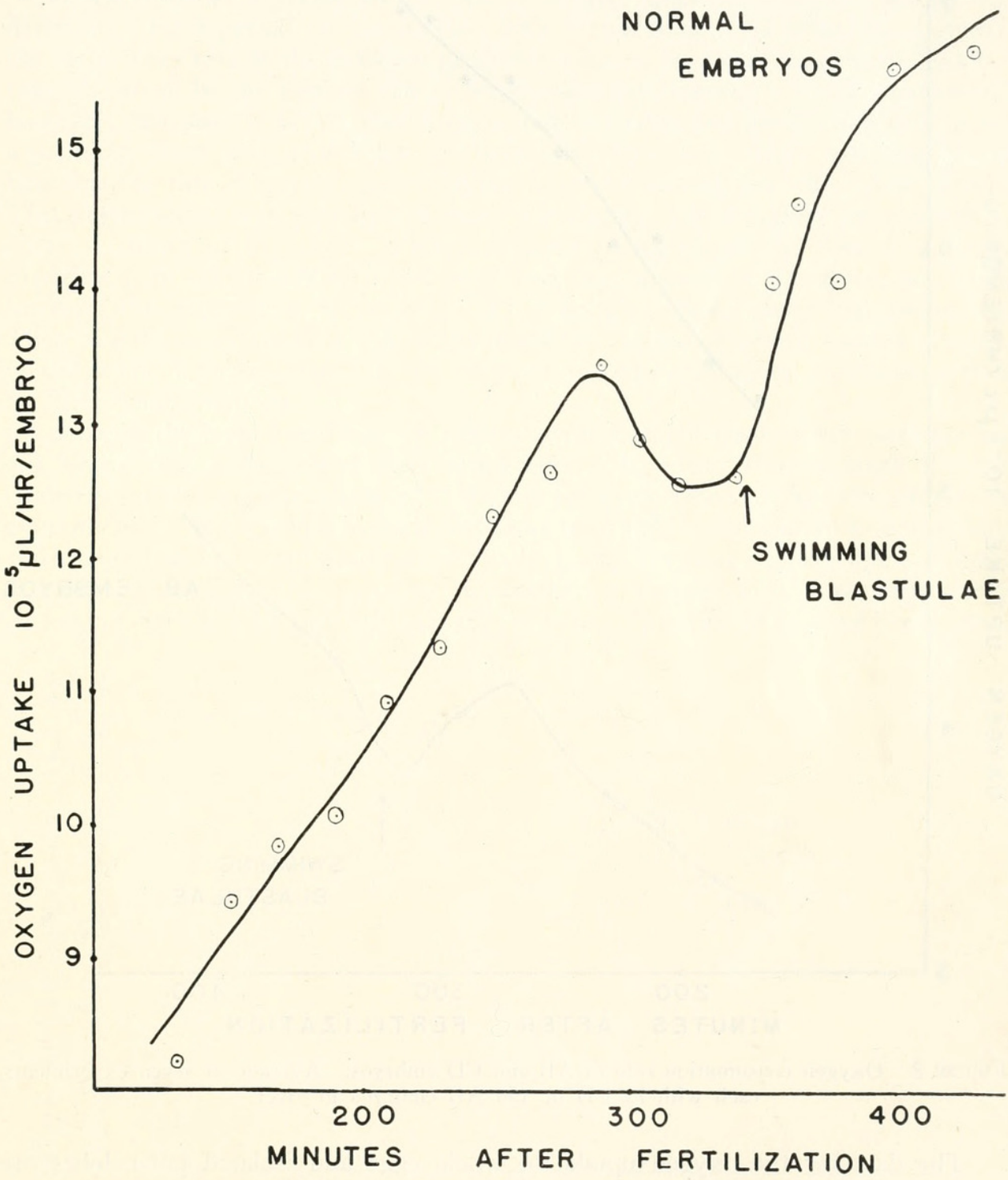

FIGURE 1. Oxygen consumption rate of normal *Mytilus edulis* embryos. 250 embryos in Cartesian diver.

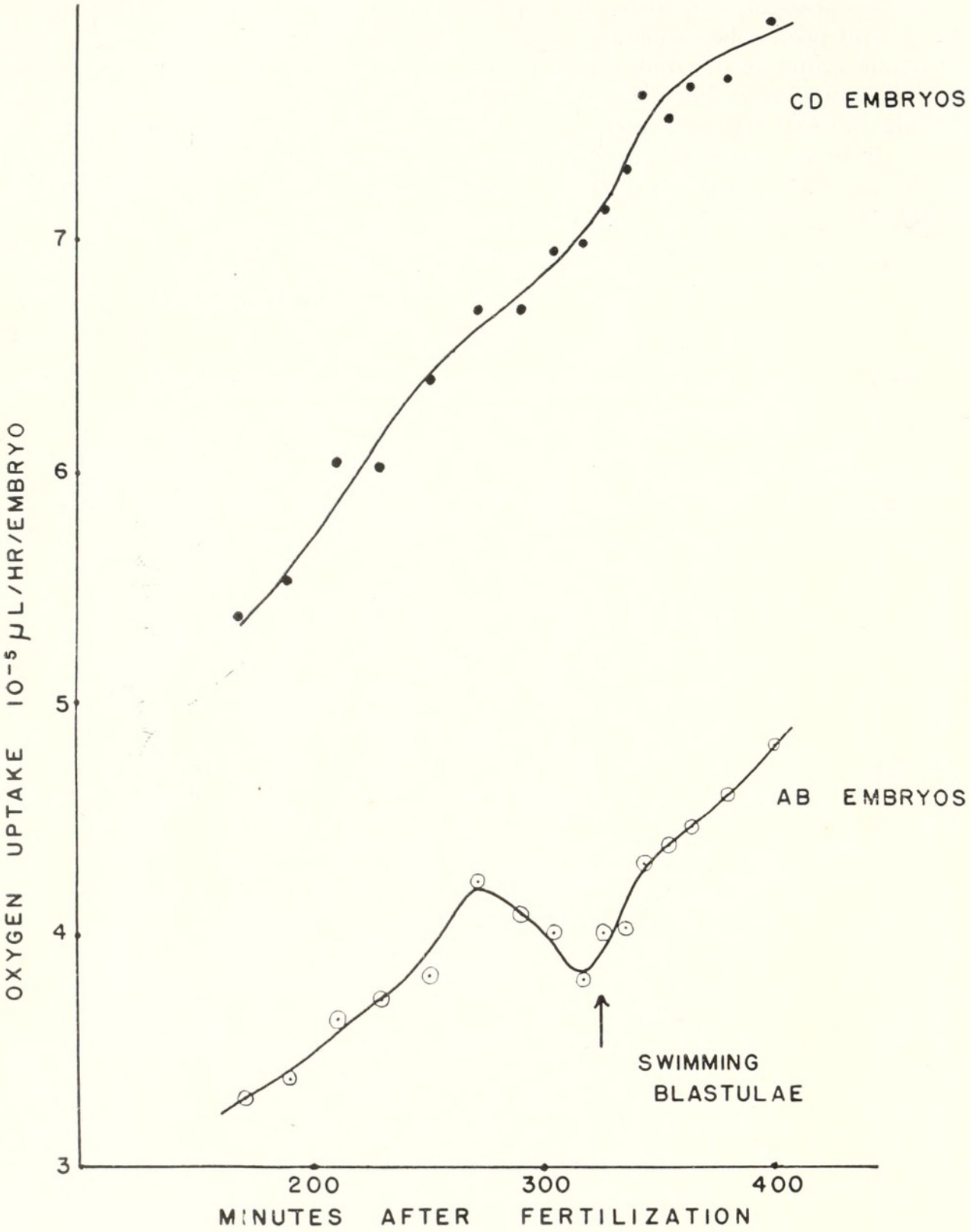

FIGURE 2. Oxygen consumption rate of AB and CD embryos. Average of seven experiments, each with 75 CD or 150 AB embryos in diver.

The data for the oxygen uptake of whole eggs and isolated polar lobes are summarized in Table II. Again, for the purpose of comparison, the oxygen uptake is expressed in terms of oxygen consumed per unit amount of cytoplasm. The average oxygen uptake of whole eggs during the time of polar lobe formation is

0.56 ± 0.013 μl. O_2/hr./mm.^{3} egg cytoplasm as compared to 0.42 ± 0.025 for the polar cytoplasm. The difference of 25 per cent is significant at the 1 per cent level (data treated as an unpaired series). It thus appears that the lower respiration of the CD cells can be explained, at least in part, on the basis of a lower oxygen consumption of the polar lobe cytoplasm.

Rate of Oxygen Uptake During Early Development

In a few initial experiments the rate of oxygen uptake of normal embryos was determined for a period of 7 or 8 hours after fertilization. As would be expected, the respiratory rate of the embryos gradually rises as development proceeds, as has been shown to be the general rule in embryonic development. It was discovered, however, that shortly before the embryos hatched from the membranes and began swimming, there was a decrease in the respiratory rate. After hatching of the blastulae the rate of oxygen consumption increased sharply and then more slowly. This phenomenon was investigated more thoroughly, and a typical oxygen uptake curve is shown in Figure 1. This curve was plotted from the data of a single experiment in which 250 fertilized eggs had been placed in the diver, and it illustrates this interesting decrease in rate of oxygen uptake preceding swimming. There was some variation in the extent of the decrease in different batches of eggs; in the particular curve figured, the decrease is somewhat more pronounced than in other determinations.

Some of the experiments on the respiration of isolated blastomeres were carried out for as long as six hours after fertilization. In these, there seemed to be a tendency for the oxygen uptake of AB embryos to decrease toward the end of the experiment. This was also investigated more thoroughly, and, due to a considerable scattering of points of the respiration curves, it was necessary to average a number of experiments together. Figure 2 illustrates the average of 7 experiments each (a total of 525 CD cells and 1050 AB cells) of the respiration of isolated blastomeres

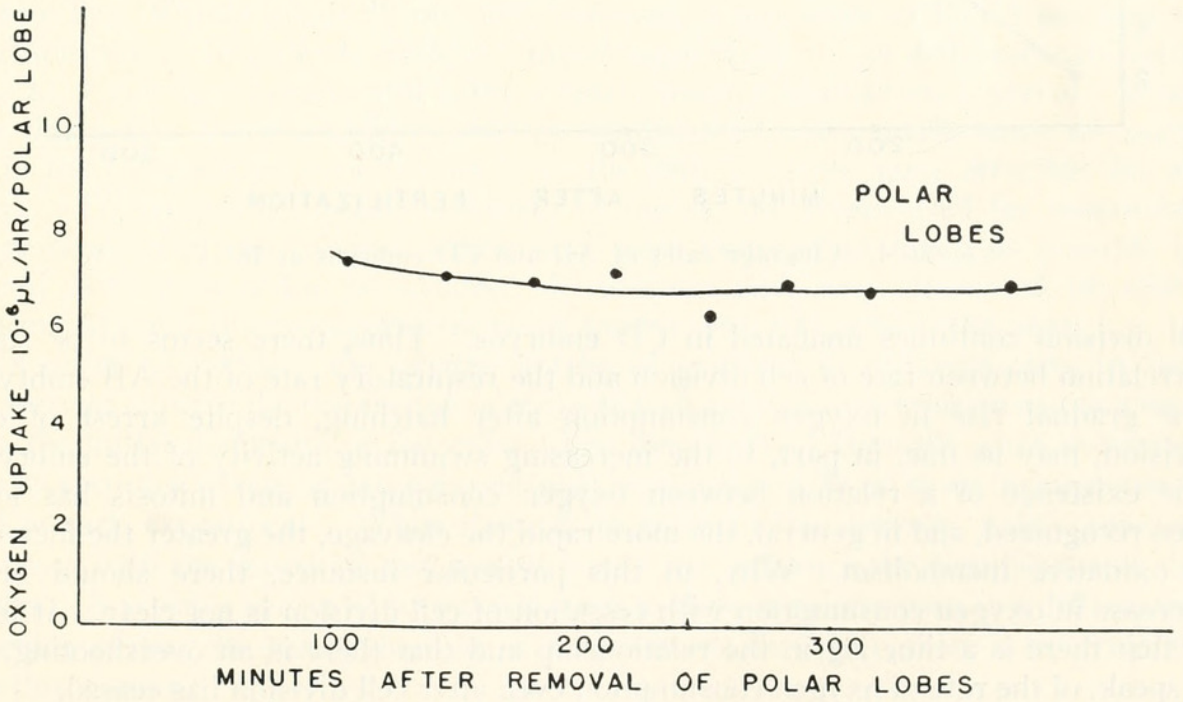

FIGURE 3. Oxygen consumption rate of isolated polar lobes. 467 polar lobes in diver.

up to 7 hours after fertilization. From this, it is clear that the decrease in respiration of whole embryos is due to a decrease in respiration of the derivatives of the AB cells. Whether the oxygen uptake curve of the CD embryos is a straight line or perhaps exhibits a slight decrease prior to hatching is open to question. It may be that these variations in slope are merely due to experimental errors.

It seemed possible that the above difference in respiratory rates of AB and CD embryos might be related to differential rates of cell division. Accordingly, counts were made of the number of cells composing AB and CD embryos at various stages of development (Fig. 4). It is evident that cell division in AB embryos decreases in rate and ceases at about the time the rate of oxygen consumption drops, whereas

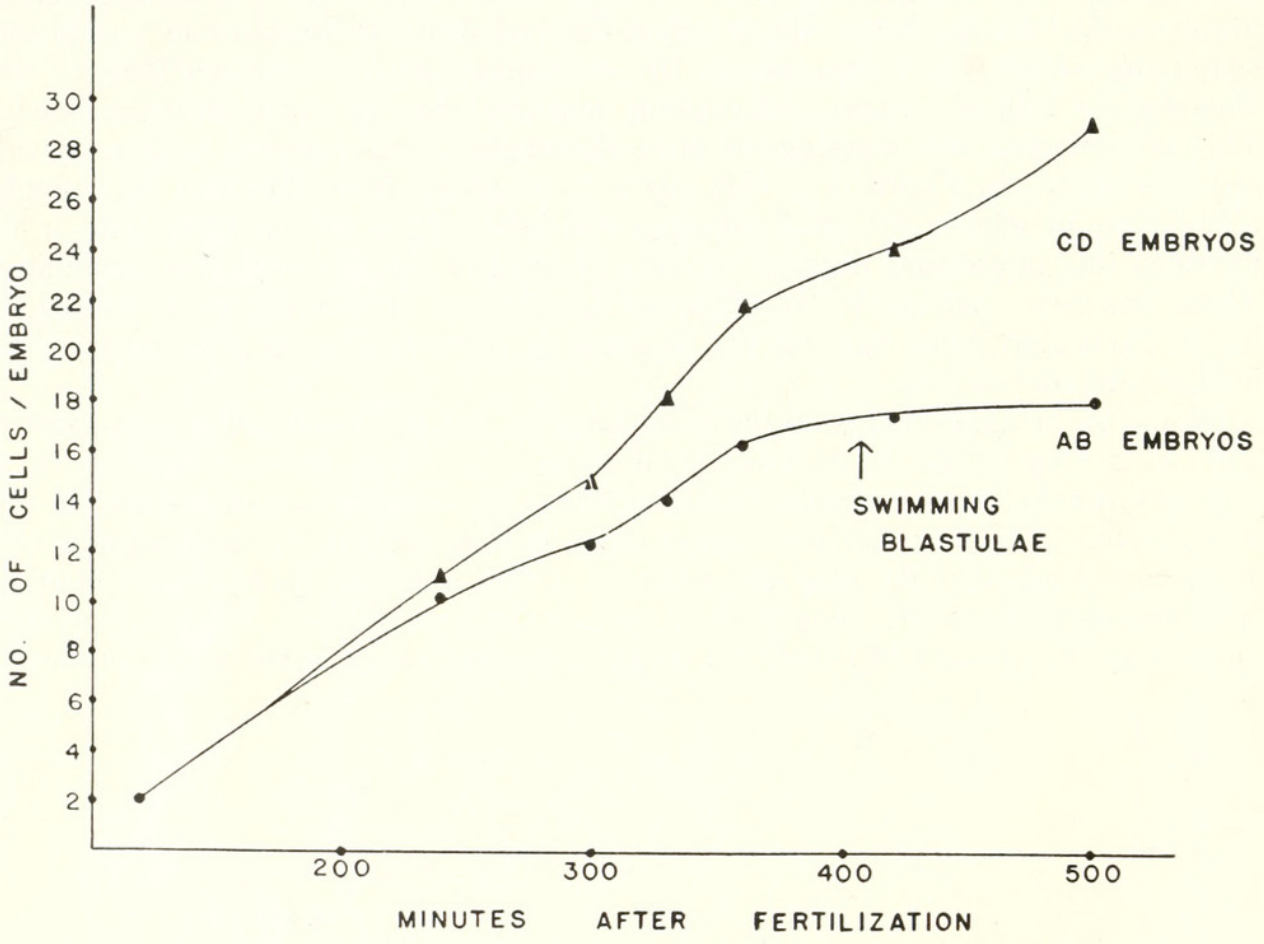

FIGURE 4. Cleavage rates of AB and CD embryos at 18° C.

cell division continues unabated in CD embryos. Thus, there seems to be some correlation between rate of cell division and the respiratory rate of the AB embryos. The gradual rise in oxygen consumption after hatching, despite arrest of cell division, may be due, in part, to the increasing swimming activity of the embryos. The existence of a relation between oxygen consumption and mitosis has long been recognized, and in general, the more rapid the cleavage, the greater the increase in oxidative metabolism. Why, in this particular instance, there should be a decrease in oxygen consumption with cessation of cell division is not clear. It may be that there is a time lag in the relationship and that there is an overshooting, so to speak, of the rate of oxygen consumption even after cell division has ceased.

The oxygen uptake rate of isolated polar lobes was followed for a period of three to four hours after their constriction from the eggs. A single experiment in which 467 polar lobes were placed in the diver is shown in Figure 3. It is significant that the polar lobe cytoplasm, at least when isolated, does not participate in the general rise in respiratory rate as exhibited by the whole eggs or isolated blastomeres. Although only a single curve is presented, five other experiments also indicated that there is no change in the respiratory rate of the isolated polar lobes.

The fact that polar lobes do not show the gradual increase in oxygen consumption indicates that the increase itself is coupled with nuclear and cytoplasmic divisions rather than being an inherent characteristic of the cytoplasm itself. Although the isolated polar lobes undergo cyclic phases of activity (ameboid activity and forming what appears to be a miniature polar lobe), no changes in oxygen consumption were detected during these periods.

Discussion

The possibility of quantitative differences in the respiration of various regions of eggs or embryos has long been a subject of speculation. Thus, in the present experiments wherein such differences have been demonstrated, it is of importance to analyze the possible sources of errors. Previous to the oxygen uptake measurements, the AB and CD cells were subjected to identical treatment, and it is unlikely that any external factors were operating to change their physiological characteristics. There is no reason to believe that a short treatment of the eggs with sperm extract or calcium-free sea water has any immediate or permanent effect on the rate of respiration.

There are, of course, errors which may be of considerable magnitude in the measurement of respiration with the Cartesian diver. Each diver has a characteristic drift of the equilibrium pressure which depends upon the size of the air volume, temperature at which it is loaded, etc. This drift is considerable at the beginning of an experiment and amounts to as much as 50 per cent of the respiration of the cells. Within about 30 minutes, however, it decreases to 1 to 5 per cent of the respiration. In order to avoid the initial large error due to drift, measurements were as a rule not begun until after a thirty-minute equilibration period. Even after this period there is a slight drift of the divers; however, this tends to cancel out in the comparative experiments since the drift is in the same direction for all divers. To avoid other possible sources of errors, the divers used for measuring the respiration of the isolated blastomeres were matched as much as possible in general dimensions and in air volume. In addition, they were alternated, the diver used for AB cells in one experiment being loaded with CD cells in the next.

In order to compare the oxygen uptake of embryos of different sizes, it was necessary to express the respiration in terms of unit amount of cytoplasm as calculated from the initial diameters of the cells. The diameters of the cells were measured with an accuracy of one or two microns and the average of 20 or more measurements used in each experiment. While there is an error in this average, it is a random error and with the statistical test used it would not influence the significance of the results. A possible source of systematic error in these measurements is a differential flattening of the cells during the time of measurement; however, there is no evidence that this occurs.

The respiratory rate of whole eggs or isolated blastomeres may vary as much as 20 per cent, as indicated in Tables I and II. While it is difficult to distinguish errors of technique from biological variability, the results of duplicate tests suggest that this is mainly due to the latter. Variations of oxygen uptake of considerable magnitude have been observed, for example, in sea urchin eggs (Krahl, 1950).

In the experiments on the respiration of polar lobes, it was suspected that pretreatment with $MgCl_2$ may have caused a decrease in rate of oxygen uptake. This cannot be tested directly since there is no other known method for obtaining polar lobe cytoplasm in sufficient quantity for respiration measurements. However, as an indirect test, a series of experiments were made on whole eggs which had been exposed to $MgCl_2$ solutions much in the same way as for removing the polar lobes. The average oxygen uptake of the treated eggs was not significantly different from the controls. There still remains the rather unlikely possibility that $MgCl_2$ has a differential effect on the respiration of polar lobes as compared to the remaining cytoplasm; however, at present there is no method for conveniently testing this. The fact that the CD cells, which contain the polar lobe cytoplasm, show a low oxygen uptake would tend to confirm the conclusion that the low respiration of the polar lobe cytoplasm is not artificially induced. This also indicates that the lower respiration of the polar lobes is not due to the absence of nuclear material.

In Table II the oxygen uptake of polar lobes was compared with that of whole eggs. The latter, however, contain the lower respiring polar lobe material and for a comparison between the polar lobe cytoplasm and the remaining cytoplasm, one should include a correction for this. If one adjusts for the amount of polar lobe material actually pinched off (which has a volume of about 11 per cent of that of whole eggs) the correction is negligible. If it is assumed that the volume differences between AB and CD cells are due to polar lobe material, and furthermore that all polar lobe cytoplasm respires at the same low rate, then the correction becomes considerable. Such a calculation indicates that polar lobe cytoplasm has an oxygen consumption per unit volume 35 per cent lower than that of the remaining cytoplasm.

The assumptions made in the above calculation would be difficult to check; however, another calculation lends some support to the above interpretation. The respiration of the polar lobes cannot be compared directly with the AB cytoplasm, since by the time the AB cells can be counted and loaded in the diver, they have continued development and their rate of oxygen uptake increased accordingly. It is possible, however, to extrapolate the oxygen consumption curves of AB embryos (such as in Fig. 2) back to the time of completion of the first cleavage. If these values are expressed in terms of unit amount of cytoplasm and compared with the polar lobes (from Table II), the polar lobes are found to have an oxygen consumption 44 per cent lower than that of AB cytoplasm. While the above calculations are very approximate, they do, however, indicate that the value of 25 per cent obtained from the data of Table II is too low, and that there is probably a greater differential in rate of oxygen consumption between the polar lobe and the remaining cytoplasm than appears on first inspection.

Since the volume of an isolated polar lobe is about one fifth that of a CD cell, the lower respiratory rate of the isolated polar lobe cannot by itself account for the difference in respiratory rates of AB and CD cells. There is reason to believe, however, that not all the polar lobe cytoplasm is isolated by the $MgCl_2$ treatment.

Visual inspection of normally cleaving eggs indicates that the total polar lobe material is appreciably greater than the amount removed. If it is assumed that this remaining material has an equally low respiration as the isolates, then the difference between AB and CD cells can be reasonably accounted for.

Gradients of various types have been suggested as being important factors in embryonic development. The nature of these has not always been made clear; however, several investigators have suggested that they are physiological, or more specifically, metabolic in nature. Child (1941), for example, has proposed on the basis of differential reduction of dyes, a metabolic gradient along the polar axis as a controlling factor in development, particularly of the sea urchin. This has been suggested and often been interpreted as a gradient of oxidative activity. Considerable controversy has existed with regard to this interpretation, and at present there is a tendency to question it, especially in view of the fact that Holter and Lindahl (1940) found no difference in oxygen uptake of animal and vegetal halves of sea urchin embryos.

Although the present data show a regional difference in oxidative metabolism of Mytilus eggs, it cannot be concluded that this difference exists in the form of a gradient. Also, whether this difference is directly coupled with the differentiation of these areas is a point which must await further investigation.

The results of Navez and Harvey (1935), Shapiro (1935), and Ballentine (1940) indicate that oxidative enzymes are attached to submicroscopic granules which can be shifted by centrifugation. The polar lobe cytoplasm is relatively free of granules of the larger visible size and may be lacking to some extent in the smaller ones with attached oxidative enzymes. While there is no proof for this, it is a possible explanation for the lower oxidative rate of the polar lobe cytoplasm.

Ries (1939) has shown by cytochemical methods that specific regions of mosaic eggs are particularly abundant in oxidative enzymes. A striking example of localization of oxidases occurs in the myoplasm of the ascidian egg and one might suppose that this area would have a higher oxidative metabolism. Holter and Zeuthen (1944), however, found no difference between the oxygen uptake of the anterior and posterior blastomeres of the four-cell stages of Ciona.

The present work is the first indication that regions of a mosaic egg may be characterized by different respiratory intensities; however, it might be pointed out that the results are not particularly in accordance with those of cytochemical studies. Pitotti (1947) has suggested that oxidative enzymes tend to be correlated with cytoplasmic areas, the developmental fates of which are to form organs of movement. Thus, the ectoplasm of Beröe eggs, the myoplasm of the ascidian egg, and the polar lobe of Myzostoma are positive with cytochemical tests for oxidative enzymes. While it is not known if the polar lobes of Mytilus exhibit a localization of oxidative enzymes as revealed by cytochemical tests, it is of interest that the respiratory intensity of the polar lobe is lower rather than higher than that of the remaining cytoplasm.

Summary

1. The oxygen consumption of isolated blastomeres and isolated polar lobes of the eggs of *Mytilus edulis* was measured by means of the Cartesian diver microrespirometer. The average oxygen uptake of CD embryos is $0.78 \pm .027$ μl. O_2/hr./mm.^{3} CD cytoplasm. This is 13 per cent lower than the average oxygen

uptake of AB embryos (0.90 ± 0.032) over a comparable period of development.

2. This lower respiratory rate of CD cytoplasm is undoubtedly due to the presence of the polar lobe cytoplasm. Isolated polar lobes have an oxygen uptake of $0.42 \pm .025$ μl. O_2/hr./mm.^{3} cytoplasm, which is 25 per cent lower than the average oxygen uptake of whole eggs during the time of polar lobe formation ($0.56 \pm .013$). Calculations by several methods suggest that the difference in respiratory rate between the polar lobe and the remaining egg cytoplasm may be appreciably greater than the above figure.

3. The rate of oxygen consumption of polar lobes remains constant after isolation from whole eggs whereas those of AB and CD embryos, as well as whole embryos, exhibit a gradual rise during subsequent development. Prior to hatching, whole embryos show a temporary decrease in oxygen consumption. This has been found to be due to a decrease in oxygen uptake of derivatives of the AB cell. Isolated CD embryos exhibit a steady rise in respiratory intensity during this period. This difference in rate of oxygen consumption of AB and CD embryos prior to hatching can, to some extent, be correlated with their rate of cell division.

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