ON THE RATE OF OXYGEN CONSUMPTION BY FERTILIZED AND UNFERTILIZED EGGS

V. COMPARISONS AND INTERPRETATION

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(Accepted for publication, September 13, 1932)

In 1895 Loeb (25) found that the segmentation of fertilized sea urchin eggs is inhibited when oxygen is displaced from the medium with hydrogen. He later found the same effect on a variety of eggs, and further that suppression of oxygen consumption by KCN caused the same result. If the suppression of oxidation by either method was not too prolonged the inhibition was reversible. When immature starfish eggs are placed in sea water maturation takes place, before fertilization, and Loeb (28) found that this nuclear activity was also reversibly inhibited either by oxygen lack or by KCN. These facts led to the conclusion that oxidation, or more strictly oxygen utilization, is necessary for the following cell activities: any protracted nuclear activity, cell division, and development. Oxidation and these activities go together. Loeb (26, 29) proposed that the essential feature or one of the essential features of fertilization, the initiation of these activities, is an increase in the rate of oxidations of the resting egg cell.¹ A large body of evidence from the sea urchin egg, some of which will be considered presently, supported this view and led to its elaboration. The point which I wish to emphasize is that this involves the supposition

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¹ Loeb in 1916 (29, page 117) says “These conclusions have been since amply confirmed by the measurements of O. Warburg as well as those of Loeb and Wasteney, both showing that the entrance of the spermatozoön into the egg raises the rate of oxidation from 400 to 600 per cent. . . . .”
that the inhibition of these activities in the unfertilized egg is due
to low oxygen utilization (oxidative) rate as a limiting factor (as it
evidently is in fertilized eggs deprived of oxygen). Loeb was per-
haps right in supposing this for the sea urchin egg. At least by im-
plication (29) he applied this explanation to fertilization in general,
and it is the generalization which I believe to be entirely refuted by
my data from Chaetopterus (60) and Cumingia (58), in which the rate
of oxygen consumption drops sharply at fertilization. The generaliza-
tion has already been seriously questioned (20, 13) on the basis of
measurements on other eggs which show little if any increase follow-
ning fertilization. Further, the general relations between oxidation
rate and development, and oxidation and anesthesia (inhibition) do
not favor increased oxidation as in general a causal factor in the initia-
tion of development.

In 1906 Loeb (27) found that either removal of oxygen or addition
of KCN prevented the rapid disintegration of sea urchin eggs which
follows a number of treatments which induce artificial membrane
formation. This suggested a connection between the disintegration
of the cortex, which also occurs to a lesser extent in the normal fer-
tilization of a number of eggs including the sea urchin, and increased
oxidation rate. He found further that the fertilized sea urchin eggs
produce more acid than the unfertilized eggs. These relations, in
conjunction with the suppression of development by oxygen lack, led
him to suppose that the rate of oxidations in the egg is increased when
the egg is activated, and he expressed the view (26) already quoted
that this is the essential feature or one of the essential features of fer-
tilization. In the decade to follow, Loeb and Loeb and Wasteneys
published an extended series of papers giving the results of many
experiments especially with parthenogenetic agents and the effects of
these upon the oxidative metabolism. Warburg (50) was the first to
prove directly that the oxygen-consumption rate of the sea urchin egg
increases greatly at fertilization. By the Winkler method he found at
Naples that the increase in Arbacia pustulosa, within the 1st hour

Loeb uses the general term "oxidation" in these connections when using data
which measure oxygen consumption. See also paragraph 14 in the Summary.

3 It is important however that such an anesthetic as chloral hydrate has the
same effect without appreciably reducing the oxidation rate.
after fertilization is six- or sevenfold. Loeb and Wasteneys (30) later found an increase of nearly fourfold in the American Arbacia punctulata, and an increase of four- to sixfold in the California sea urchin, Strongylocentrotus purpuratus (33). Increases of the same order of magnitude have been measured in Echinus miliaris by Shearer (46) and by Gray (13), in E. microtuberculatus by Shearer (45), in Paracentrotus lividus by Warburg (55) and by Runnström (44), and recently again in the Arbacia punctulata by Tang (47) and by myself (60).

Warburg, and Loeb and Wasteneys, found that hypertonic solutions increase the rate of oxygen consumption by unfertilized sea urchin eggs toward or to the rate of fertilized eggs. Runnström (44) has shown that hypotonic solutions as well have this effect. In short, it was shown by Warburg and, with certain disagreements, confirmed by Loeb and Wasteneys, that hypertonic solutions and bases increase the rate of oxygen consumption by unfertilized eggs. Warburg (41) also showed that this is true of certain metals which act as parthenogenetic agents. However, to conclude that activation of the egg by these agents, mostly cytolytic, is brought about because they have increased the rate of oxidation involves some danger of reasoning in a circle so long as the oxidation-increasing effect of these agents is determined only on the unfertilized sea urchin egg. It does not follow except by arbitrary assumption which is cause and which is effect. Perhaps these agents increase the rate of oxidation only because they have (otherwise) activated the egg. The effect of the cytolytic agents on the rate of oxygen consumption by fertilized eggs or other organisms gives evidence which is free from this objection. Warburg (51) did find that hypertonic solutions increase the rate of oxygen consumption by fertilized eggs some threefold, but Loeb and Wasteneys (33) found that in S. purpuratus hypertonic solutions increase the rate of oxidation only in unfertilized eggs, not in fertilized eggs. They concluded that the oxidation is increased only when formation of the fertilization membrane is brought about. Complete cytolysis of the

4 And in a way which may effectively activate other species of eggs without causing an increase in the rate of oxygen consumption.

5 It was later necessary to modify this to whenever the condition is brought about which normally gives rise to the fertilization membrane.
unfertilized eggs by saponin increased the rate of oxygen consumption to the level of fertilized eggs, but no further. Warburg (54) also found that complete destruction of the fertilized egg cells caused a drop in the rate of oxygen consumption toward the prefertilization rate, and Runnström (44) finds this effect by both hypertonic and hypotonic solutions on the fertilized eggs of *P. lividus*. There is thus some disagreement as to the effect of cytolysis on the fertilized eggs, possibly due in part to different degrees of destruction.

Bases also act as parthenogenetic agents. Warburg (52) found that strong bases increase the rate of oxygen consumption of fertilized as well as of unfertilized eggs. Loeb and Wasteneys (35) found that both weak penetrating and strong bases increase the rate of oxygen consumption by both unfertilized and fertilized eggs. More recent work on many organisms has established that bases do tend in general to increase the rate of cell oxidation, quite apart from the activation of eggs, and at least in some cases cytolysis does also (Shearer (46)). It is reasonable to suppose that agents which tend in general to increase respiratory rate may be acting parthenogenetically because of this effect. However, fatty acids and fat solvent anesthetics which act as parthenogenetic agents tend in general to suppress oxidation. It is true that anesthetics may increase the rate of oxygen consumption by the unfertilized eggs (having activated them) but to a lower level than is achieved by fertilization. Anesthetics decrease the rate of respiration of fertilized eggs. In other words they activate unfertilized sea urchin eggs and at the same time suppress the new rate of oxygen consumption. Loeb (41) found that the presence of oxygen is not necessary for the parthenogenetic action of fatty acids and fat solvent anesthetics. Lyon (37) found that prolonged exposure to KCN activates the eggs of a Mediterranean sea urchin. Therefore even for the sea urchin the case is not so very strongly in favor of the oxidation-increasing properties of parthenogenetic agents, acting directly as such, as the principal causal factor in the activation of the egg.

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6 It would be of value to know if cytolysis as such increases the rate of oxygen consumption by the unfertilized *Chaetopterus* egg since activation of this egg results in a decrease (60).

7 See also Ralph Lillie (24) on aerobic and anaerobic phases of activation in the starfish egg.
Loeb’s hypothesis as outlined in his book, Artificial parthenogenesis and fertilization, 1913 (28), and more briefly in his last somewhat extended review of the subject in 1916 in his book, The organism as a whole (29), may be briefly abstracted as follows: Parthenogenetic agents are in general cytolytic agents. Their effect is achieved by a superficial cytolysis of the egg cortex. Cytolysis as such, and perhaps especially superficial cytolysis of the cortex, increases the rate of cell oxidation. This increase in the rate of oxidation in parthenogenesis as in normal fertilization is the essential or one of the essential features of fertilization (activation), increased oxidation rate permitting or causing the developmental activities. Probably the important feature of the cortical cytolysis is the alteration or destruction of a lipoid film, and the increase in rate of oxygen consumption which follows may be due, for example, to the liberation thereby of some oxidative catalyst from the film. This hypothesis of Loeb’s is supported in some of its steps by a wealth of experimental data, concisely reviewed in 1916 (29), and accounts well for some of the extensive and diverse results of experiments on parthenogenesis of the sea urchin egg. But even for parthenogenesis in this egg, the case does not appear to be entirely conclusive, for reasons just set forth.

Loeb and Wasteneys (32) were the first to measure the rate of oxygen consumption before and after fertilization by any egg other than that of the sea urchin. In five measurements on eggs of starfish, two of which had more than 50 per cent fertilization, they found very little change and concluded that there is no change at fertilization in this egg. Since the essential feature of fertilization is entirely present here, i.e. the transformation of the egg from a comparatively resting condition to a state in which growth and differentiation are initiated, this appears at once to prevent generalization of Loeb’s interpretation devised for the sea urchin egg as Frank Lillie (20) points out. Loeb and Wasteneys anticipated this objection at the time of their measurements on the starfish egg however by pointing out that the starfish egg is already active in maturation before fertilization and is there-

8 This tentatively proposed mechanism for the increase in rate of oxygen consumption is not a necessary part of the hypothesis. The liberated catalyst could as well be in the cell interior. See Runnström (44) for an analysis of the mechanism of increase.
fore (supposedly) already at the high rate before fertilization. But even though there is nuclear activity before fertilization in this egg, there is still a very radical further increase in activity of the egg at fertilization, with no accompanying increase of respiratory rate, so it is difficult to bring this egg into a generalization of Loeb's hypothesis. Further, since no absolute measurements were made in terms of volume, weight, or number of cells no more proof was actually presented to show that the rate of the starfish egg is high throughout than that it is low, although they were probably right in supposing it to be high compared with the unfertilized sea urchin egg. Recently Tang (48) has made a few measurements on the same egg (*Asterias*). He also finds no appreciable change at fertilization. He gives his results in absolute units in terms of the number of eggs (160 μ in diameter). When converted to volume units, mm.³ O₂ per hour per 10 mm.³ eggs, at 23°C. the value becomes about 0.8 which (compare Fig. 2) is relatively low but still about twice as high as Shearer's, Warburg's, and my own measurements on the unfertilized eggs of as many species of sea urchins. Tang's measurements were made with a rapid rate of manometer shaking which would seriously damage most kinds of eggs. He finds that when compared with his own values [which are considerably higher than mine (60)] for *Arbacia* eggs, the two unfertilized eggs consume oxygen at about the same rate per unit surface. The fertilized eggs therefore of course do not. On this basis of comparison his results directly oppose Loeb and Wasteney's assumption that the starfish egg operates at a high rate before fertilization.

Measurements of the rate of oxygen consumption by the eggs of other invertebrates have in no instance revealed such a rise following fertilization as that which occurs in the sea urchin egg. Fauré-Fremiet (9) found only a slight increase in the eggs of the polychaete *Sabellaria alveolata*, of the order of 12 per cent at 20°C. My own measurements using the eggs of the brown alga *Fucus vesiculosus* (57) show an increase of about 90 per cent, and in *Cumingia* (58) and *Chaetopterus* a drop following fertilization. I found the egg of *Nereis* (59) to increase its rate of oxygen consumption about 35-45 per cent following fertilization.⁹

⁹ Barron and Tyler measured the change in this egg before I did. Barron (3) concludes that there is an increase of about 25 per cent immediately after fertiliza-
Among the eggs of vertebrates there is also no uniform behavior. Several investigators have found an increase following fertilization in amphibian eggs (see Needham's text (40)). Gray (13) says, "A sudden increase of respiration following fertilization is not shown by other eggs than those of the sea urchin," and in 1919 Lillie (20, pages 145-146) in effect predicts that eggs may be found which like the starfish do not change much, or which may even decrease, as has turned out to be the case for Chaetopterus (60) and Cumingia (58).

The question then arises, does the change in rate of oxygen consumption at fertilization, when present, have any direct relation to fertilization as such, or is it as chaotic a relationship as the divergent results might suggest? In the first place so many eggs change abruptly in one direction or another at exactly this time, that it seems highly probable that there is some definite relationship. On general physiological grounds it is to be expected that if efficiency remains constant an increased rate of oxygen utilization is required in aerobic systems if growth and development are speeded up, but it is well established that the rate of development is not ordinarily limited by the rate of oxidation. Cleavage rate is certainly not ordinarily limited by the rate of oxygen consumption in a number of forms, e.g. Amberson (2) found that the rate of cleavage in Arbacia eggs is not retarded, when the oxygen pressure is diminished, until the pressure has been so greatly reduced as to decrease the rate of oxygen consumption to about half normal. Loeb and Wasteneys (31) showed that the temperature coefficient of cleavage in Arbacia is entirely different from...
the temperature coefficient of oxygen-consumption rate. It is quite possible that the rate of oxygen consumption is a direct part of the limiting factor which inhibits the unfertilized eggs of some species, and not of others. An egg so inhibited might be expected to have a relatively low rate of oxygen consumption before fertilization, as does the sea urchin egg. Runnström (44) concludes from experiments in which he analyzes by steps the ability of both the unfertilized and the fertilized sea urchin egg to carry out the steps in the oxidation process, that the oxygen-activating ferment or "Atmungsferment" is not fully in contact with its substrate in the unfertilized egg, but becomes so at fertilization, in conjunction with colloidal changes in the protoplasm. In this case the inhibition to development may be directly due to the oxidation limitations. This situation in the sea urchin egg would agree essentially with Loeb's hypothesis, while supplying a more modern and exact mechanism for the increase in rate of oxygen consumption at fertilization. The results of similar experiments on the eggs of Chaetopterus would be interesting.

It is more difficult to imagine the relation between the high rates of oxygen consumption by the unfertilized eggs of Chaetopterus and Cumingia and their state of inhibition. Both of these, like the starfish egg, undergo maturation activities in the sea water before fertilization, but only as far as the metaphase of the first polar spindle. In Chaetopterus this activity is over in 15 minutes at 21°C, and thereafter the egg is morphologically at rest. Even so, the high metabolic rate continues uniformly for at least 8 hours. Evidently, some condition other than limitations imposed by the rate of oxygen utilization must be responsible for inhibiting the development of the egg, since when it is fertilized and developmental activity is initiated, it proceeds with a much reduced rate of oxygen consumption.

When the absolute rates of oxygen consumption by the several species of eggs, in mm.³ O₂ per hour per 10 mm.³ eggs at 21°C., are compared it is found that the rates are much closer to one another after fertilization than before. Chaetopterus, Cumingia, and Nereis are strikingly close, while Arbacia is somewhat higher. Thus the range of these four eggs before fertilization is 0.4 or 0.5 to 3.1, a range of more than sixfold, while after fertilization it is only 1.3 to 2.0 (Fig. 1). Comparison with certain other growing animal cells of roughly com-
Fig. 1. Absolute rates of oxygen consumption, before and after fertilization at 21°C, except Fucus which is at 18°C.
parable size, and which are not actively motile, shows a number of comparable values, e.g. Emerson's (8) measurement on *Amoeba proteus* at 20°C., for which the quotient is 1.6. I have also converted as many measurements on the eggs of marine invertebrates as I have been able to find in the literature together with sufficient data to make the conversions, into volume units for comparison. Since a variety of

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**Fig. 2.** Absolute rates of oxygen consumption, per unit volume, compiled. Where temperature is preceded by the symbol @, a temperature correction has been made. (See appendix.)

units have been used by different workers in some cases assumptions, e.g. of density, have been necessary for these approximate conversions. For temperature corrections the coefficient $Q_{10} = 2$ has been assumed. The assumptions and the sources of data are given in the appendix. The compilation, given graphically in Fig. 2 shows that a number of fertilized eggs of several phyla fall approximately within this same
range, 1.3–2.0 mm$^3$ O$_2$ per hour per 10 mm$^3$ eggs at 21°C. The values for the *Arbacia punctulata* of Tang (47) and Tang and Gerard (49), unlike my own (60) are outside this general range. The relative similarity of the rates of oxygen consumption per unit volume by these various fertilized eggs, some of which increase to a greater or lesser extent, and some of which decrease the rate of oxygen consumption following fertilization suggest a certain orderly basis for the directions and magnitudes of the changes. It is as if the change at fertilization is in the direction, and of such magnitude, as to bring the rate of the fertilized eggs to the approximate rate 1.3–2.0 mm$^3$ O$_2$ per hour per 10 mm$^3$ eggs at 21°C. Eggs which have a very low prefertilization rate (notably the sea urchins) increase greatly, while those with a very high rate (*Chaetopterus* and *Cumingia*) decrease. Those in the middle range (*Nereis* and *Sabellaria*) change little.$^{18}$

The wide differences in rate of oxygen consumption by the various unfertilized eggs is still unaccounted for. It is not difficult to asso-

$^{18}$ It remains to be seen how widespread this relation will be, but too many cases fit in for it to be purely a chance assortment. It is by no means to be expected that all or even most fertilized eggs will fit closely into this narrow range on a volume basis. Not only are there probably different characteristic concentrations in different species of eggs of the substances which determine the rate of oxygen consumption, but different proportions of inert materials occur for which it would be difficult to make accurate allowance. Especially, larger eggs which contain proportionally more yolk must be expected to respire at a lesser rate per unit volume, and this is the case for the absolute rates of the eggs of the frog (5) the Plaice egg (7) and for *Fundulus* (6). My own measurements on the eggs of the brown alga *Fucus* (57) indicate a rate entirely too high to fit into this system. This is probably in part due to the relatively small volume of water and inert storage in the *Fucus* eggs, which although very small settle so much more rapidly in sea water than animal eggs as to indicate a considerably greater density. When the *Fucus* egg is centrifuged, the inclusions come to occupy a relatively small part of the volume of the egg. Fig. 1 shows that the *Fucus* egg has a respiratory rate of the same order of magnitude as several small-celled algae (8) which appear in general to respire at a higher rate than immotile animal cells. The eight eggs and the *Amoeba* in Fig. 2 are all small cells which probably have a roughly comparable volume of active protoplasm per unit volume of cells. Tang's (48) absolute value for the eggs of the starfish *Asterias* when converted to volume units indicates a comparatively low rate for this fairly large and comparatively yolky egg (160μ in diameter). Both before and after fertilization it is about 0.8 mm$^3$ O$_2$ per hour per 10 mm$^3$ eggs at 23°C.
ciate the low rate of the unfertilized sea urchin egg with the inhibited resting condition of the cell. It is more surprising to find the high rates of *Cumingia* and *Chaetopterus* when the eggs are in the inhibited resting state. It should be emphasized that the inhibited unfertilized egg is a cell in a very unusual condition. It is young; it contains all of the substances necessary for rapid growth, yet it halts suddenly in a state of suspended animation until its condition is in some way changed by any one of a variety of external stimulating agents. As soon as activation has taken place it again behaves as an ordinary cell, with division and growth, like a protozoon or somatic cell when conditions are favorable. It is after the return to this ordinary state that the rate of oxygen consumption by the different eggs are similar, and similar also to the rates of *Amoeba* and the frog’s skin. The relations seem to me to suggest that the diverse rates of oxygen consumption, like the cell division and growth phenomena, are in an extraordinary state before fertilization, being related thereto in a manner which is in most cases at present obscure, and that fertilization is a termination of this unusual condition. This is not wholly a new idea. Fertilization has often been regarded as the termination of an inhibition. I wish to point out that these oxygen consumption relations conform to this point of view. The eventual generalized interpretation of fertilization may well resolve itself into an analysis of the nature of the inhibition of the unfertilized egg.

*The Inhibition of the Unfertilized Egg*

The behavior of the unfertilized egg suggests certain resemblances between its condition and that of a cell under the influence of an anesthetic. The resemblance may be partly superficial but it is probably not entirely so. It is of course not to be supposed that the unfertilized egg is actually subjected to an anesthetic substance from without. Rather it may be that the unfertilized egg cell, upon reaching its “resting stage,” has spontaneously assumed the condition, whether it be related to the structure and composition of the surface

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11 Surprising in so far as we expect the organism to be efficient. It is not justifiable, however, either philosophically or biologically, to suppose that the organism will necessarily adjust its fuel consumption to a low level when apparent useful work is at a minimum.
film, or to the degree of dispersion and the nature of the adsorptions on
the plasma colloids, similar to that which anesthetics impose on cells. The failure of cutting to activate eggs may favor the supposition that
the condition resides in the plasma rather than exclusively in the cell
surface film, although disruption of the lipoid surface film is supposed
by Loeb (29) and by Gray (11) (with different explanations of how it
is brought about in normal fertilization) to result in activation of the
egg.

If the inhibition or autoanesthesia of the unfertilized sea urchin
egg is caused by the very low rate of oxygen consumption as a limit-
ing factor, this inhibition is then an "asphyxiation anesthesia." While as has been stated, this explanation is tenable for the sea urchin
egg, it is not to be expected from the general relations between anes-
thesia and oxidation that inhibitions will generally be "asphyxiation-
anesthesia." Loeb and Wasteneys (34), among others, have shown
that while anesthesia, caused by narcotics, tends to suppress the rate
of oxygen consumption it does not do so at all sufficiently for this to
be the limiting factor. Lowering the temperature a few degrees will
lessen the oxygen consumption to the same extent while by no means
causing anesthesia. They conclude that anesthesia is not ordinarily
an "asphyxiation" phenomenon. Loeb and Wasteneys (31) have also
shown that the rate of development of the fertilized sea urchin egg is
not directly dependent upon the rate of oxygen consumption; i.e.,
that "oxidation is not the independent variable" in development.
They showed that the temperature coefficient of development is
variable over a temperature range and decidedly not the same as the
temperature coefficient ($Q_10 = 2$) of oxygen consump-
tion, as it would be if development were a direct dependent variable.
It is thus quite in conformity with these relationships to find eggs
which do not have a low rate of oxygen consumption, responsible for
the inhibition, in the unfertilized condition. Examples are known in
which mild anesthesia is actually accompanied by an increased rate of
respiration (38, 42).

Heilbrunn (15) has shown that the apparent viscosity or consistency
of the protoplasm of the eggs of both Arbacia and Cumingia changes
greatly at or soon after fertilization so that the degree of gelation is
increased. Before fertilization, in the inhibited condition, the proto-
plasm is comparatively fluid. Anesthetics cause the protoplasm of these and other cells to liquefy. If the fertilized egg is inhibited by means of an anesthetic it becomes less gelated, as it was before fertilization. Heilbrunn believes that the colloidal condition of the protoplasm, as revealed by the consistency, is causally related to anesthesia, or on the other hand to stimulation and increased activity. It is notable here that release from inhibition (fertilization) in Arbacia and in Cumingia are accompanied by the same types of changes in consistency (correlating with liberation from anesthesia), while the changes in rate of oxygen consumption run in the opposite direction and must therefore be eliminated from the correlation.

Ralph Lillie (21, 22, 23) has shown that the permeability to water of the Arbacia egg increases following fertilization or activation, and he draws parallels on this basis between activation of the egg and stimulation in general. In Arbacia the increase in water permeability is of about the same order of magnitude as the increase in oxygen consumption rate, while in the starfish egg (23), in which the rate of oxygen consumption does not change at fertilization, neither does the permeability to water change at fertilization. It is therefore interesting to note that the correlation between permeability and rate of respiration breaks down when the Cumingia egg is considered, as Heilbrunn (14) has found that the permeability to water of the Cumingia egg increases slightly following fertilization while as I have shown (58) the rate of oxygen consumption decreases. No data are available on the effects of fertilization upon the permeability of the Chaetopterus egg. Decreased permeability to water is in some cases associated with anesthesia and increased permeability with stimulation and increased activity.

This is probably interrelated with the more or less parallel changes in

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12 The unfertilized Nereis egg is comparatively highly gelated, as is the immature sea urchin egg, due perhaps to the large amount of water held at this time in the large immature nucleus.

13 Hobson (17) also finds an increase following fertilization in Psammechinus miliaris.

14 Depending however as Lucké (36) has shown in the case of the unfertilized Arbacia egg upon the ions in the medium, etc.
protoplasmic consistency. As far as the data go with respect to oxygen-consumption rate, viscosity, and permeability, the unfertilized egg resembles in a general way a cell in a state of anesthesia, although the evidence from the rates of oxygen consumption is of the negative sort that inhibition does not appear to be caused by low oxygen-consumption rate as a limiting factor except perhaps in the case of the sea urchin egg.

There is possibly some danger of word play in distinguishing between release from inhibition on the one hand and stimulation on the other, but it seems to me profitable to make the distinction here, considering the former the result of the latter, and that it is necessary to regard the young resting egg cell as positively inhibited. Moderate anesthesia raises the threshold for stimulation but it does not abolish responsiveness. The immediate response of the unfertilized egg to the stimulation of the spermatozoon or a variety of agents is to abolish the condition of inhibition and transform to a normal (i.e., growing) cell. Specificity in fertilization, and related complications have to do with the nature of the stimulation itself, and the mechanism of activation; i.e., the release from inhibition. I shall not attempt to bring this into the discussion.

Wasteneys (56) used Loeb’s methods for inducing what is regarded as a reversal of activation of the Arbacia egg, and measured the effects on the rate of oxygen consumption. When eggs have been given preliminary parthenogenetic treatment, the development which ordinarily ensues when they are placed in sea water is prevented by NaCN or by chloral hydrate. Eggs so inhibited are now subject either to fertilization, or to artificial reactivation and will develop. Wasteneys shows that when eggs are “reversed” with NaCN, the oxygen-consumption rate reverts to the low prefertilization rate, and that when the eggs are

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15 Hobson (17) finds a changing permeability to water between fertilization and the first cleavage in the sea urchin egg which has interesting parallels in part to Helbrunn’s (15) corresponding consistency curve.

16 Ralph Lillie (24) gives an excellent brief theoretical discussion of the nature of activation in the starfish egg.

17 Frank Lillie (20, page 165) is of the opinion that this is an inhibitory effect rather than a true complete reversal of activation.
reactivated, it again rises to the rate of fertilized eggs. However, and this is important, the same developmental effects are caused by chloral hydrate, although this anesthetic does not lower the rate of oxygen consumption nearly to the same extent, nor nearly to the low level of the unfertilized eggs. Wasteneys concludes that the main factor concerned in bringing about the reversion is a suppression or inhibition of the developmental processes, and that lowering the rate of oxidation (NaCN) is merely one means of bringing this about. It is perhaps the means naturally operating in the unfertilized sea urchin egg. There is no reason to expect it to be the means in eggs generally.

The successful substitution of chloral hydrate and other anesthetics for cyanide or oxygen lack, to produce the same effect in a number of situations (e.g. see footnote 3), suggests that in them it is not the changed oxidation rate as such which is effective, but rather entrance into or liberation from a state of inhibition (whatever structure or condition of the protoplasm this state may imply) and that an induced “asphyxiation” anesthesia is merely one way of bringing about the anesthesia.

Energy Relations

The rate of oxygen consumption is by no means a complete measure of the rate of oxidations in the egg. Anaerobic oxidations may in some cases play an appreciable part in the metabolic energy source. Loeb (28) found evidences of hydrolytic processes in the sea urchin egg, and Barron (4) has shown not only that the *Nereis* egg has a high tolerance for anaerobiosis but also (3) that its metabolic reaction to certain dyes supports the view that it carries out carbohydrate fermentations. The calorific quotients obtained with sea urchin eggs before and after fertilization by Meyerhof (41) and by Shearer (46) do not indicate any anaerobic source of heat nor any change at fertilization, and Runnström (44) has shown that the anaerobic reduction of methylene blue occurs with the same rapidity in fertilized and unfertilized eggs. But especially in an egg such as that of *Chaetopterus* which reduces its aerobic oxidation rate at fertilization, it is possible that anaerobic oxidations increase at fertilization so that the total oxidations may not decrease at this time when energy requirements would appear to be increased. Measurements of heat and metabolite
production may yet demonstrate this to be the case, and if so, using "oxidation" in the broad sense, generalization of Loeb’s hypothesis may turn out to hold. But in view of the very high absolute rates of aerobic oxidation of the inhibited Chaetopterus and Cumingia eggs, together with the relations shown in Fig. 2, there is no basis for supposing so at the present time.

The Rate of Oxygen Consumption Per Unit Cell Surface

Many eggs undergo a change in diameter, and therefore of cell surface, following fertilization. This has been best measured in the Arbacia egg, and the change in surface is very slight compared with the change in rate of oxygen consumption, which can in no wise be explained as due to or proportional to the change in surface area. In Arbacia the change in diameter is so slight that there has been dispute as to its direction. According to Glaser (10) the diameter decreases from 74 to 71.7 microns. Gray (12) has shown that cell division, with its sudden increase of surface, is not accompanied by any change in rate of oxygen consumption, and Warburg (52) has shown that the suppression of cell division by urethane does not appreciably effect the rate of respiration. Gray (13) has shown that in the growing trout embryo the rate of oxygen consumption is proportional to the wet weight of living embryo. The change in rate of oxygen consumption at fertilization, in those eggs in which the change is great, can be explained only by assuming a change in the arrangement or condition of the substances in the egg.

A comparison of the rates of oxygen consumption by the fertilized eggs of the several animal species shows that they are somewhat more nearly the same per unit volume of egg material than per unit cell surface. The unfertilized eggs differ widely on either basis. It is no doubt to be expected that, other things being equal, the rate of respiration per unit weight or volume will be greater when cells are small and have relatively more surface. The fertilized egg of Arbacia punctulata has a comparatively high rate per unit volume (Fig. 2) and is a comparatively small egg (74 microns diameter). But other factors enter, as the still smaller fertilized Cumingia egg (about 66 microns diameter) has a considerably lower rate (Fig. 2).
The eggs of Chaetopterus and of Cumingia, which I have found to decrease the rate of oxygen consumption following fertilization, both respire at a comparatively high rate in the resting stage before fertilization. They also both rest in the same nuclear stage of maturation, the metaphase of the first polar spindle. This stage (the metaphase) is an especially stable one in the mitotic cycle, and the possibility of a correlation between this stage and a high respiratory rate must be considered. The general evidence from most work which bears on the effect of the mitotic stage in the cleavage cycle is against any relationship (see Needham (40, vol. 2, page 641)), although it is possible that an effect might be of such brief duration during mitosis that it has not been detected, whereas the effect would cover an indefinite period of time in eggs which rest in this stage. I attempted to make measurements on another egg which rests in this same stage (Cerebratulus) but was unable to obtain satisfactory measurements because of the large amounts of jelly adhering to these eggs. However Runnström (44) reports that a few measurements on the eggs of Ciona intestinalis indicate a rise following fertilization. Morgan (39, page 62) states that this egg rests before fertilization in the metaphase of the first polar spindle. There is not therefore a consistent relation between this resting stage and a decrease in the rate of oxygen consumption following fertilization.

The relation between increase in rate of oxygen consumption and the cortical breakdown which attends fertilization and membrane formation in a number of eggs has entered into most attempts to explain the changes in the egg at fertilization, beginning with Loeb's early hypothesis. However, as Just points out, there is great variation among eggs in the extent to which this breakdown or disintegration takes place. Just (18, 19) relates this breakdown in Arbacia to the peak of heat production found immediately after fertilization by Rogers and Cole (43), and to the greater peak in oxygen-consumption rate reported in Echinus by Shearer (45). Just states (private communication) that the cortical changes at fertilization in Cumingia, Chaetopterus, and Asterias are rather slight compared with the changes
in Arbacia and Nereis. A certain amount of correlation therefore exists between the degree of cortical change and the increase in respiratory rate at fertilization, but it is not striking since the Nereis egg, which has the greatest and especially the longest duration of cortical change, increases its respiratory rate only very moderately, and then not to the high absolute rate (either per unit volume or per unit cell surface) of the resting unfertilized eggs of Cumingia and Chaetopterus. This correlation, like the comparison of surface and volume rates, and comparisons of the nuclear stages of resting eggs and the change in rate of respiration at fertilization, can be more profitably attempted when accurate absolute data are available from a greater variety of eggs.

Discrepancies in Measurements on the Eggs of Arbacia punctulata, and the Relation of These to the Calorific Quotient

The absolute rates of oxygen consumption per unit volume by eggs of the four sea urchins: Arbacia pustulosa, Arbacia punctulata, Echinus miliaris, and Paracentrotus lividus, as determined by Warburg, Shearer, Runnström, and myself agree quite closely (Fig. 2), although the large egg of E. miliaris (Shearer) with relatively less surface and probably more yolk in proportion respires at a slightly lower rate per unit volume than the small egg of A. punctulata. Tang's (47) values for Arbacia punctulata however are about three times as high as mine for the same species (Fig. 2). It is difficult to examine into the probable correctness of Tang's values because he gives very little data bearing on the question. His immediate interest is the rate of oxygen consumption by the unfertilized eggs as a function of the oxygen tension. Twenty-four measurements made on the unfertilized eggs in equilibrium with air show a range of threefold in absolute determination. He gives no data on the increase at fertilization, but states that, "the experiments showed fertilized eggs to have a respiration 5 times that of the unfertilized ones, confirming Warburg and Loeb and Wasteneys; . . . ." There is no indication whether the fivefold increase was observed from the lower, average, or higher level of the threefold span of rates of unfertilized eggs. Tang states that the rate of shaking was demonstrated adequate for maintaining gas equilibrium, but he does
not say what it was nor indicate that the other danger, namely that
too rapid shaking abnormally increases the rate of respiration was
realized or guarded against. In his work on the starfish egg (48) he
used a rate of shaking so rapid (seventy complete oscillations per min-
ute with 15 cm. amplitude) that in my experience it inevitably would
have seriously damaged Arbacia eggs. It is not quite clear whether
Tang used ½ cc. of egg suspension, or ½ cc. of eggs in 3 cc. suspension
per manometer vessel. If the latter is the case the high concentration
of eggs, requiring high shaking rate, would have tended to increase
cortical damage.

Tang and Gerard (49) have more recently obtained absolute meas-
urements of the rate of oxygen consumption by Arbacia eggs 95 per
cent or more fertilized before being placed in the manometer vessels.
The fertilized eggs are probably less subject to increased respiration
rate by cortical damage than unfertilized eggs (see discussion earlier
in this paper), and Tang and Gerard in this case find the average rate
to be 3.5 times the average rate for unfertilized eggs found by Tang,
instead of five times. In volume units this means a rate of 4.2 in-
stead of 6.1 for the fertilized eggs (Fig. 2). They explain this dis-
crepancy by stating that it is not entirely safe to compare values from
one season to another. The methods were the same as in the pre-
ceding work. Eggs which exuded from a large number of gonads were
filtered through cheese cloth. This is a dangerous procedure, be-
cause the respiratory rate of the Arbacia eggs may be altered when
damage is not great enough to prevent fertilization and cleavage. It
is by no means necessary, at least in the case of the unfertilized eggs,
to cause complete cytolysis before greatly increasing the respiratory
rate. Tang and Gerard used a rapid shaking rate, sixty-five complete
oscillations per minute, with 10 cm. amplitude, which they state was
just short of the rate which caused the fertilized eggs to be largely
cytolized at the end of an experiment. They also found that oxygen-
consumption rate varied with changed rate of shaking, although this
could not be due to incomplete oxygen equilibrium because substitut-
ing oxygen for air did not increase the observed rate.

The precautions which I took in guarding against damage to the
eggs on the one hand and inadequate gas exchange on the other, have
been discussed in the fourth paper of this series (60) in conjunction
with my results on *Arbacia*. By way of summarizing, it can be said that the higher values of Tang, and of Tang and Gerard, especially the former, could be adequately accounted for in magnitude by the elevation of respiratory rate which a number of investigators have found to be caused in the sea urchin egg by even moderate cortical damage. If on the other hand their values, or one of them, is correct, my lower value would have to be explained by assuming inadequate gas exchange due to slower shaking. To the evidence earlier presented against this it should be added that in the same vessels, with the same depth of solution, the same rate of shaking, and even greater gas-exchange rate, gas equilibrium was adequately maintained when *Chaetopterus* eggs were used. The adequacy of gas exchange could be more certainly determined with the *Chaetopterus* egg due to its superior properties for the purpose.

So far as I have been able to determine, the numerous measurements of the rate of oxygen consumption which Loeb and Wasteneys made on this egg were always in relative terms, so that no absolute measurements are afforded for comparison. The only other absolute measurements which directly correlate with the oxygen-consumption measurements are the heat-production measurements by Rogers and Cole (43). They give the rate of heat production per million eggs per hour, before fertilization as 0.08 calories, and after fertilization when the eggs have reached the two-cell stage as 0.52 calories. But since in their paper they do not state the temperature at which the eggs produced heat at this rate, the values are indeterminate. Dr. Cole informs me (private correspondence) that the measurements were made at the temperature of the running sea water in the laboratory, which he believes to have been in the neighborhood of 16–18°C.

If the gram calories of heat produced per unit amount of eggs per unit time is divided by the milligrams of oxygen consumed, the caloric quotient is obtained. Needham (40, page 651) gives the approximate theoretical caloric quotients for carbohydrate, fat, and protein metabolism respectively as 3.5, 3.3, and 3.2. Meyerhof's (41) values for the caloric quotient of *Paracentrotus lividus* eggs, unfertilized and at various stages of development, lie between 2.53 and 2.9, all lying under the theoretical. Shearer's (46) determinations on *Echinus miliaris* are somewhat higher: 3.07 before fertilization and 3.2 for the
The rate of oxygen consumption by eggs. V

first hour after fertilization. Relatively more heat was produced and
the calorific quotients approximate more closely to the theoretical.
If the calorific quotients are calculated, using Rogers and Cole's values
for the heat production in Arbacia punctulata, and the oxygen-con-
sumption measurements for the same species by Tang, Tang and
Gerard, and myself, assuming Rogers and Cole's temperature to have
been 17°C. and correcting all temperatures to 21°C. on the assumption
that \( Q_10 = 2 \), the results are rather anomalous. For the heat pro-
duction I have taken the rate which holds constantly after the two-
cell stage is reached. Before this stage the heat-production rate is
considerably greater, and also therefore is the calorific quotient.
Tang's values give a calorific quotient before fertilization of 2.9,
after fertilization of 3.8. Tang and Gerard's later value for fertilized
eggs gives a calorific quotient of 5.5. My values give a calorific
quotient before fertilization of between 7 and 8.7 and after fertiliza-
tion of 11.4. In other words, the rate of heat production is much too
high to give a theoretical quotient with any of the oxygen measure-
ments except Tang's high values for unfertilized eggs, which are almost
certainly too high. My own values, which agree well with those ob-
tained with a variety of sea urchin and other eggs, fit the least well.
An aerobic metabolism could account for a calorific quotient above
the theoretical for the complete oxidation of carbohydrate, fat, or
protein, but it is improbable that it could account for such great
elevation of the quotient. It therefore seems to me probable that
Rogers and Cole's values for heat production are too high. Most
of the factors which can so readily cause an increased rate of oxygen
consumption, notably cortical damage, also cause increased heat pro-
duction (Shearer (46)). If anaerobic metabolism is involved the ef-
ccts might be greater in the case of heat. There is also the possibil-
ity, when measurements of heat production are made in a thermostat
below room temperature, of slight influx of heat into the experimental
vessel. Even with a differential method of calorimetry this is possible
when the system is not entirely symmetric; i.e., when the apparatus
or treatment attached to the two Dewar flasks differs.

Needham (40, pages 652 and 658) has made an interesting calcula-

\(^{18}\) Neither Meyerhof nor Shearer obtained enough heat production to account
for any anaerobic oxidation.
tion of the calorific quotient using Shearer’s data on *Echinus miliaris* for oxygen consumption and Rogers and Cole’s values from *Arbacia punctulata* for heat production. Needham did this in the absence at the time of oxygen-consumption data on *Arbacia punctulata*, and regards the calculation, involving as it does different species, as merely an interesting “feeler.” The interesting thing about this calculation is that the values for the calorific quotient turn out to be 3.51 for the unfertilized eggs, and 3.7 for the fertilized eggs in the two-cell stage. The first of these values is almost exactly theoretical for carbohydrate metabolism (which is expected here from the respiratory quotient values (46)) and the second is not much above. In other words, it is the most perfect fit, compared with Meyerhof’s and Shearer’s determinations. This appears in a sense to afford a rather striking confirmation of Rogers and Cole’s values. However, since Shearer’s measurements were made at 14.5°C. and Rogers and Cole’s at about 17°C, when correction is made for temperature the quotients are reduced. Further, the comparison was on the basis of oxygen and heat per million eggs. One *Echinus miliaris* egg has four times the volume of one *Arbacia punctulata* egg (see appendix). There is therefore no reasonable basis for comparison on the basis of equal number of eggs. If the calorific quotients are calculated with temperature corrections and on the basis of heat and oxygen per unit volume of cells, they become: before fertilization, 12.6, after fertilization in the two-cell stage, 14.4. These are the highest values of all, and in so far as comparison between species is tenable, add to the probability that Rogers and Cole’s heat determinations are too high. Per million eggs, Rogers and Cole’s values for heat production are only somewhat higher than those of Meyerhof and Shearer. But per unit volume of egg material they are more than five times as high as Shearer’s, for eggs in the two-cell stage.

Needham (40, page 658) points out that both Meyerhof and Shearer found an increasing rate of heat production, closely following the increasing rate of oxygen consumption, after fertilization, giving thus a constant calorific quotient, while Rogers and Cole find constant heat production from the two- to the eight-cell stage. An inspection of Table V in the fourth paper of this series (60) shows that the rate of oxygen consumption is already rising in *Arbacia punctulata* within the
2nd hour after fertilization,19 during which time and after which Rogers and Cole find constant heat production. This implies a constantly decreasing calorific quotient between the two- and eight-cell stages which would be very interesting if the absolute value of the quotient were more reasonable.

It is difficult to obtain accurate absolute metabolic rate measurements with the delicate eggs of Arbacia punctulata. Measurements should be repeated with great attention to the condition and the treatment of the eggs, as well as to the method of measurement.

SUMMARY

1. The rate of oxygen consumption by eggs may not merely undergo no change at fertilization, as in the case of the starfish, but it decreases to about half in Chaetopterus and in Cumingia.

2. The absolute rate of oxygen consumption in mm. O₂ per hour per 10 mm.³ eggs differs widely in several species of unfertilized eggs. It is very low in the sea urchin, intermediary in Nereis, and high in Chaetopterus and Cumingia. The range for these eggs is approximately 0.4 to 3.1 mm. O₂ per hour per 10 mm.³ eggs at 21°C., in the ratio of about 1:8.

3. The absolute rates of oxygen consumption by the same fertilized eggs are much more nearly the same. They lie within the range 1.3 to 2.0 mm. O₂ per hour per 10 mm.³ eggs at 21°C., in the ratio of approximately 1:1.5. Within this same range lie the values obtained by a number of investigators using a variety of eggs of invertebrates from several phyla. Amoeba proteus and frog skin also are within this range (see Fig. 2).

4. The changes in rate of oxygen consumption at fertilization by the different species of eggs, differing both in direction and magnitude, appear to be such as to bring the rate, when development is initiated, to about the same rate, which is also the rate of other comparable normally growing cells.

5. The direction and magnitude of the change in rate at fertilization therefore appears in the cases cited to be primarily a function of the absolute rate of oxygen consumption by the unfertilized eggs.

19 This agrees with the measurements of Loeb and Wasteneys (28, page 29).
which are characterized in their peculiar inhibited condition, among other things, by a wide range of respiratory rates.

6. It is not to be supposed that this range of rates will apply at all universally to eggs, especially to eggs of extremes in proportional content of inert materials, such as large yolky eggs. Fish and amphibian eggs for example respire at a much lower rate per unit volume. The effect on surface: volume ratios attending extremes of cell size might also be expected to shift the absolute rate.

7. The absolute rate of oxygen consumption by the eggs of the alga *Fucus vesiculosus* is considerably higher than the rates of the animal eggs measured. It is of the same order of magnitude as the rates of several other small-celled algae, which respire at a greater rate per unit volume than most non-motile animal cells.

8. The comparatively high rates of oxygen consumption by the inhibited (unfertilized) eggs of *Chaetopterus* and *Cumingia* are not directly associated with nuclear or morphological activity of the cell since they continue at the high rate for hours after cessation of the brief initial nuclear activity, which takes place when the eggs are placed in sea water.

9. It is concluded that the rate of oxygen consumption is not necessarily and probably not generally the limiting factor which causes inhibition of the unfertilized egg. Increase in rate of oxygen consumption is not directly related to the initiation of development, in general, nor even necessarily concomitant. It is not improbable that the low rate of oxygen consumption is an immediate part of the cause of inhibition of the unfertilized sea urchin egg, but this is a special case.

10. This thesis, that the rate of oxygen consumption is not necessarily nor ordinarily the limiting factor in the inhibition of the unfertilized egg, and conversely that increase in the rate of oxygen consumption is not usually the essential feature of fertilization, is quite in agreement with the general relations between the rate of oxygen consumption on the one hand and anesthesia, growth, and development on the other in fertilized eggs and other organisms.

11. This conclusion is opposed to Loeb's explanation of the essential feature of fertilization, as an increase in oxidation rate or more strictly to generalization of his hypothesis to include eggs other than those of the sea urchins (or of other similar special cases which may be dis-
It extends to fertilization (the initiation of development) his and Wasteney's well established conclusion that "oxidation is not the independent variable in development."

12. It is suggested that the crux of the problem of fertilization lies in the nature of the inhibition of the unfertilized egg. Certain similarities between this condition, arrived at spontaneously in the case of the egg cell, and the condition of cells in narcosis or anesthesia are pointed out.

13. Although the rate of oxygen consumption by the unfertilized eggs of Chaetopterus and Cumingia cannot be regarded as the limiting factor which causes the inhibition of the eggs, in these and other cases with different absolute rates, it appears highly probable that the rate of oxygen consumption is in some way, at present obscure, tied up with or related to the condition of inhibition. This seems probable especially in view of the sharp change in rate which in most cases immediately attends cessation of the inhibition, but the relationship may be a non-causal one, as in narcosis.

14. It must be borne in mind that oxygen consumption is not necessarily a complete measure of oxidation, and that other measures such as of heat and metabolite production are necessary before the complete amount of oxidation is known. When these are completely worked out, if free energy relations are known, it is probable that more direct and inclusive relations may be found between oxidation, growth, development, and anesthesia. Generalization of Loeb's hypothesis, using "oxidation in the broad sense might then turn out to hold, with fertilization fitting into the general scheme, but there is no basis for it at the present time.

I am much indebted to Professor W. J. Crozier for advice and criticism, especially in the earlier stages of this investigation, and for reviewing the manuscript.

APPENDIX

Derivation and Conversion of Data Plotted in Fig 2.

The data given in graphical form in Fig. 2 have been taken from a number of sources which often have involved conversion of units and in some cases involve assumptions which are given here. Runnström
(44) gives data for a number of controls in his experiments on *Paracentrotus lividus* which involve the volume of egg suspension used, the per cent by volume of eggs in the suspension as determined by centrifuging in calibrated blood tubes, the temperature, the duration of measurement, and the total cubic millimeters of oxygen consumed. I have taken only data on normal control runs on fertilized eggs from his paper. The measurements which are accompanied with sufficient data for the purpose, when converted to the absolute rate mm.³ O₂ per hour per 10 mm.³ eggs, are as follows:

<table>
<thead>
<tr>
<th>Table 2 a,</th>
<th>°C.</th>
<th>105 minutes measured</th>
<th>52 mm.³ eggs in 2 cc. suspension</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot; 3 c,</td>
<td>23</td>
<td>105 &quot; &quot;</td>
<td>2.91</td>
</tr>
<tr>
<td>&quot; 4 c,</td>
<td>23</td>
<td>75 &quot; &quot;</td>
<td>2.71</td>
</tr>
<tr>
<td>&quot; 11 c,</td>
<td>20.5</td>
<td>120 &quot; &quot;</td>
<td>3.92</td>
</tr>
<tr>
<td>&quot; 13 c,</td>
<td>21</td>
<td>120 &quot; &quot;</td>
<td>1.5</td>
</tr>
<tr>
<td>&quot; 22 a,</td>
<td>20.5</td>
<td>90 &quot; &quot;</td>
<td>2.1</td>
</tr>
<tr>
<td>&quot; 25 a,</td>
<td>25</td>
<td>140 &quot; &quot;</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Before averaging the results I have multiplied the values obtained at 25°C. by 0.75 and those at 23°C. by 0.85 as approximate temperature corrections to 21°C. on the assumption that Q₁₀ = 2. Leaving unchanged the values obtained at 20.5°C., the average value then becomes, as of 21°C., 2.16, which is plotted in Fig. 2. It should be noted that these measurements run for from 75 to 140 minutes, and since the respiratory rate is increasing with time after fertilization, the value taken is undoubtedly somewhat higher than it would be more immediately after fertilization. Considering this, it agrees very well with Warburg’s value for the same species.

Fauré-Fremiet’s (9) determinations of the rate of oxygen consumption by the fertilized and unfertilized eggs of *Sabellaria alveolata*, at 20°C., are expressed in terms of weight of eggs and weight of oxygen. These have been converted to volume units by assuming a density of the eggs of 1.04. The rates then become for fertilized eggs 2.05 mm.³ O₂ per hour per 10 mm.³ eggs, and 1.83 for unfertilized eggs at 20°C.

Shearer (46) gives the rate of oxygen consumption per hour per million eggs, fertilized and unfertilized, of *Echinus miliaris*. Professor J. Gray has kindly referred me to Hobson’s (17) measurements²⁹

²⁹ An earlier paper (16) indicates a smaller size.
of the volume of this egg, and has placed me in communication with Professor Hobson who has been good enough to inform me that there is appreciable variation in the size of the egg, but that on the average the volume of an unfertilized egg is about 850,000 $\mu^3$. This permits conversion of Shearer's data to volume units. Shearer's measurements were made at 14.5°C. and a comparatively large correction is therefore necessary to convert to values corresponding to 21°C. This was done, assuming $Q_{10}$ to equal 2, and the values become for the unfertilized eggs 0.28 mm.$^3$ O$_2$ per hour per 10 mm.$^3$ eggs, and for fertilized eggs 1.59, at 21°C.

The values for *Amoeba proteus* are taken directly from Emerson's paper (8), in which the same units were employed which I have used. I have also taken the value for Adolph's (1) measurements on fresh frog skin from Emerson's paper since here Adolph's values have been converted to volume units from weight units by assuming a density of 1. Warburg (55) states that at 23°C., an amount of unfertilized eggs of *Paracentrotus lividus* which contain 20 mg. egg nitrogen consume 10–14 mm.$^3$ O$_2$ in 20 minutes. In an earlier paper (53) he also states that 1 cc. of centrifuged *Paracentrotus* eggs contain 20 mg. egg nitrogen. Therefore 10 mm.$^3$ unfertilized eggs consume oxygen at the average rate of 0.36 mm.$^3$ per hour at 23°C. Multiplying this by 0.85 the appropriate value for 21°C. becomes 0.31. He also states that the fertilized eggs, beginning 10 minutes after fertilization, consume oxygen at six times this rate, or at about 1.86 mm.$^3$ O$_2$ per hour per 10 mm.$^3$ eggs at 21°C.

In 1908 Warburg (50) found that the unfertilized eggs of the European *Arbacia pustulosa* consume 0.05–0.06 mg. O$_2$ per hour per 28 mg. egg nitrogen at 20.5°C., and that the fertilized eggs consume oxygen at six to seven times this rate. I have converted mg. O$_2$ to mm.$^3$ O$_2$, and then in order to get an approximate value in terms of volume units I have taken some license and assumed that the same ratio exists between egg volume and egg-nitrogen content as in *Paracentrotus*; i.e., that 20 mg. nitrogen represents 1 cc. eggs. If this is true the middle value at 20.5°C. becomes 1.68 mm.$^3$ O$_2$ per hour per 10 mm.$^3$ eggs.

Tang's (47) average value for the rate of oxygen consumption by unfertilized eggs of *Arbacia punctulata*, at 24.7°C. is 33.6 mm.$^3$ O$_2$.
the egg, and multiplying by 0.77 as an approximate temperature correction to 21°C., the converted values become 1.22 and 6.1 mm.\textsuperscript{3} O\textsubscript{2} per hour per 10 mm.\textsuperscript{3} eggs at 21°C.

Tang and Gerard (49) find an average rate, at 25°C., of 118 mm.\textsuperscript{3} O\textsubscript{2} per hour per million fertilized eggs of Arbacia punctulata. Converting to volume units and to 21°C., this becomes 4.2 mm.\textsuperscript{3} O\textsubscript{2} per hour per 10 mm.\textsuperscript{3} eggs.

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