FERTILIZATION AND THE TEMPERATURE COEFFICIENTS
OF OXYGEN CONSUMPTION IN EGGS OF
ARBACIA PUNCTULATA*

BY B. B. RUBENSTEIN AND R. W. GERARD
(From the Marine Biological Laboratory, Woods Hole)
(Accepted for publication, December 22, 1933)

Since Warburg's (19, 20) pioneer work on Arbacia pustulosa extended by Loeb and Wasteneys to Arbacia punctulata, it has been accepted that there is a several-fold increase in respiratory rate on fertilization. Recent work on the latter form (16, 17, 21) has set this increase as about fivefold. The early experiments showed further that the respiratory increase could not be equated to the morphogenetic increase, for one could be obtained independently of the other. It was not, consequently, so revolutionary when other eggs were found which showed no respiratory change on fertilization (for details, see Needham (12)), or even manifested a decrease (21). The findings we present are, in a sense, another step in the same direction; for it appears that in Arbacia itself the magnitude or even presence of an increase of respiration on fertilization depends on the experimental temperature chosen. The observation that the temperature coefficient of unfertilized egg respiration drops to a value less than half as great on fertilizing (or cytolyzing) has led us, further, to conclusions regarding the catalytic respiration system of these eggs which turn out to be in full harmony with those reached by Runnström (14) from entirely different evidence.

Method

The eggs were prepared and evaluated as described in the preceding paper (7). Each lot was then divided into three portions. One was left untouched; the eggs

---

* This work was supported in part by a grant from The Rockefeller Foundation to the University of Chicago.

1 Needham (12, p. 659) mentions work of Fauré-Fremiet hinting at a similar situation in Sabellaria.
TABLE I

Sample Protocol

August 7, 1933. Oxygen consumption of Arbacia eggs at various temperatures.
Each vessel contains 1.0 cc. of egg suspension and 0.2 cc. of NaOH. Allowed
to come to temperature equilibrium before readings were taken—30 min. 54
round trip shakes per min., arc 5.5 cm.

Values in table are corrected manometric pressure differences in mm. Brodie
fluid, per hour.

The fertilized eggs did not divide before 110 min. at 14.3°C.

10³/cc. Egg diameter average 73.8 μ.

<table>
<thead>
<tr>
<th>Time</th>
<th>Fertilized K = 0.878</th>
<th>Resting K = 0.838</th>
<th>Cytolyzed K = 0.953</th>
<th>Temperature K = 0.763</th>
<th>Temperature K = 0.902</th>
<th>Temperature K = 0.929</th>
</tr>
</thead>
<tbody>
<tr>
<td>min.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–20</td>
<td>14.3 14.2</td>
<td>1.8 2.6</td>
<td>1.8 1.0</td>
<td>12.0</td>
<td>30.2</td>
<td></td>
</tr>
<tr>
<td>21–40</td>
<td>14.5 15.0</td>
<td>3.9 2.7</td>
<td>0.7 1.1</td>
<td>11.8</td>
<td>30.3</td>
<td></td>
</tr>
<tr>
<td>41–60</td>
<td>14.0 14.1</td>
<td>3.0 3.1</td>
<td>1.0 0.9</td>
<td>11.8</td>
<td>30.5</td>
<td></td>
</tr>
</tbody>
</table>

(ΔQ₀₀) . . . 65.4 62.8 8.6 9.2 9.2 8.6 14.3 41.9 416 24.0

61–90 | 13.4 13.3            | 2.6 2.2          | 1.8 1.2             | 10.2                 | 30.2                 |
| 91–120| 14.2 13.6            | 2.4 2.5          | 1.2 1.2             | 10.1                 | 29.9                 |
| (ΔQ₀₀) . . . 63.1 58.2 9.3 7.9 11.7 10.3 14.3 35.6 139 24.0 |

0–20 | 27.9 27.2            | 10.6 11.0        | 3.8 3.7             | 10.9                 | 29.9                 |
| 21–40| 28.1 27.5            | 11.1 10.9        | 3.7 3.7             | 10.9                 | 30.0                 |
| 41–60| 28.0 27.5            | 11.0 11.3        | 3.5 3.5             | 11.0                 | 29.8                 |
| (ΔQ₀₀) . . . 128 120 40.6 36.6 28.0 30.9 21.1 38.9 135 24.0 |

0–20 | 45.8 45.1            | 30.2 28.9        | 6.0 6.1             | 11.0                 | 30.2                 |
| 21–40| 44.8 44.8            | 29.8 30.0        | 6.0 5.9             | 10.8                 | 29.9                 |
| 41–60| 45.9 44.6            | 28.6 29.5        | 5.9 5.8             | 10.7                 | 29.6                 |
| (ΔQ₀₀) . . . 196 185 132 115 47.3 48.2 27.6 38.4 136 24.0 |

in a second were fertilized by adding freshly shed sperm (the excess was washed
off after 15 minutes); and those in the third were cytolyzed by adding three
volumes of distilled water. Cytolysis was complete within half an hour. The
oxygen consumption of each portion was then determined manometrically.
Shaking was at the rate of 54 per minute, arc 5.5 cm., which controls proved
adequate to insure oxygen equilibrium without injury (tested by fertilization
after 5 hours of shaking). Readings were made every 20 minutes, care being taken that the manometers were in temperature equilibrium before starting.

Aliquots of each portion were subjected simultaneously to two temperatures; and one set was subsequently (after 2 hours, usually) run at one, often two, more temperatures. Tests showed that the order of exposure to high or low temperatures was immaterial, and in practice the order was mixed. The actual values were generally chosen over a wide range, favoring an accurate determina-

**Table II**

<table>
<thead>
<tr>
<th>Date</th>
<th>Lower temperature</th>
<th>( Q_{10} )</th>
<th>Higher temperature</th>
<th>( Q_{10} )</th>
<th>( Q_{10} )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Resting</td>
<td>Fertilized</td>
<td>Cyto-lysed</td>
<td>Resting</td>
<td>Fertilized</td>
</tr>
<tr>
<td>July</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>16.4</td>
<td>14.0</td>
<td>80.0</td>
<td>9.2</td>
<td>18.2</td>
</tr>
<tr>
<td>29</td>
<td>21.7</td>
<td>12.5</td>
<td>30.6</td>
<td>84.5</td>
<td>10.9</td>
</tr>
<tr>
<td>25</td>
<td>21.7</td>
<td>12.6</td>
<td>10.4</td>
<td>28.1</td>
<td>18.0</td>
</tr>
<tr>
<td>27</td>
<td>17.4</td>
<td>12.6</td>
<td>96.2</td>
<td>28.1</td>
<td>18.0</td>
</tr>
<tr>
<td>August</td>
<td>1</td>
<td>21.0</td>
<td>13.2</td>
<td>28.1</td>
<td>17.1</td>
</tr>
<tr>
<td>2</td>
<td>13.0</td>
<td>8.8</td>
<td>77.8</td>
<td>24.0</td>
<td>17.3</td>
</tr>
<tr>
<td>3</td>
<td>15.5</td>
<td>12.0</td>
<td>79.9</td>
<td>29.3</td>
<td>17.3</td>
</tr>
<tr>
<td>5</td>
<td>15.0</td>
<td>11.7</td>
<td>70.8</td>
<td>38.5</td>
<td>15.5</td>
</tr>
<tr>
<td>7</td>
<td>14.3</td>
<td>8.6</td>
<td>65.4</td>
<td>41.9</td>
<td>146</td>
</tr>
<tr>
<td>7</td>
<td>21.1</td>
<td>40.2</td>
<td>125</td>
<td>27.6</td>
<td>120</td>
</tr>
<tr>
<td>8</td>
<td>13.0</td>
<td>9.1</td>
<td>83.5</td>
<td>42.2</td>
<td>135</td>
</tr>
<tr>
<td>8</td>
<td>19.9</td>
<td>39.6</td>
<td>123</td>
<td>29.9</td>
<td>139</td>
</tr>
<tr>
<td>9</td>
<td>14.6</td>
<td>14.2</td>
<td>101</td>
<td>24.3</td>
<td>55.7</td>
</tr>
<tr>
<td>9</td>
<td>28.5</td>
<td>115</td>
<td>235</td>
<td>20.3</td>
<td>29.2</td>
</tr>
<tr>
<td>10</td>
<td>11.0</td>
<td>7.0</td>
<td>68.1</td>
<td>28.5</td>
<td>115</td>
</tr>
<tr>
<td>11</td>
<td>21.8</td>
<td>18.9</td>
<td>94.2</td>
<td>28.5</td>
<td>115</td>
</tr>
<tr>
<td>12</td>
<td>29.0</td>
<td>53.6</td>
<td>111</td>
<td>28.5</td>
<td>115</td>
</tr>
</tbody>
</table>

Average: ........................................................................ 4.1 1.8 1.9

The temperature of the water bath could be changed rapidly by the addition of steam or of ice. The desired temperature was maintained constant within 0.1°C. during a run.

**RESULTS**

The data of a typical experiment are presented in Table I. It is immediately evident that at different temperatures the respiration of
fertilized, resting, and cytolyzed eggs are in different ratios to one another.

Table II summarizes all the data obtained. A day-by-day tabulation is necessary, since the absolute respiration varies greatly from batch to batch of eggs. It is apparent that the temperature coefficient of the resting eggs (average 4.1) is more than double that of the fertilized (1.8) or cytolyzed (1.9) eggs. The ratio of the rate of oxygen con-
sumption of fertilized to resting eggs is consequently variable. This ratio is plotted against temperature in Fig. 1 and is seen to vary from 8 at 11°C. to 2 at 30°C, or, by extrapolation, 1 at 32°C. Although a single straight line can be fitted to the observed points, two straight lines intersecting at about 21°C., as plotted, afford much better agreement. That such a break is real, is further supported by its appearance at the same temperature in the plot of $\mu$ for fertilized eggs (see Fig. 2).

The critical thermal increment is, perhaps, a more rational expression of temperature relations than the simple $Q_{10}$ (see Crozier (3, 4); and Navez (11)). It is obtained from the van't Hoff-Arrhenius equation

$$V_s = V_0 e^{-\mu \left(\frac{1}{T_s} - \frac{1}{T_s} \right)}$$

where $V$ is the velocity at temperature $T$, $e$ and $R$ have the usual significance, and $\mu$ is the critical thermal increment. The equation is
solved graphically, as in Fig. 2, by plotting the logarithm of velocity against the reciprocal of absolute temperature; the slope of the resultant curve is then \(-\frac{\mu}{R}\). The numerical value of \(\mu\), according to the original theory used in deriving the equation, is a measure of the energy (in calories per mole) required to raise a reactant to its critical level for activity. It has been urged, however, (5) that this reactant may be the catalyst of a particular reaction, or at least a particular catalyst-substrate system (2), which may then be identified by the value of \(\mu\). For a complicated reaction chain, as in cell oxidations, the \(\mu\) value might be that of the slowest (master) reaction. The values found in these experiments are familiar ones. Those of fertilized eggs, 6,500 and 10,800, have, for example, been found for H\(^+\) catalyses; that of resting eggs, 12,500, for iron catalyses (3). The value for cytolyzed eggs is not significantly different from the 6,500 of fertilized eggs.

DISCUSSION

When Arbacia eggs are fertilized the respiration may be increased eight times, at 11\(^\circ\), or doubled, at 29.9\(^\circ\). Extrapolation indicates that no increase would occur at 32\(^\circ\)C. Since other eggs are now known which do not alter, or actually decrease, their respiration when fertilized, the interesting question presents itself whether similar temperature relations hold for them. It is not impossible that, for each egg, particular temperatures may be found at which fertilization increases, decreases, or does not alter the resting respiration rate. At least it is clear that temperature as well as species must be considered in any future generalizations concerning fertilization and respiration; and further exploration of temperature coefficients in other species and conditions is to be awaited.

It remains to consider the significance of the sharp change in temperature coefficient on fertilizing or cytolyzing the resting egg. This change seems of deeper significance than any alteration of the respiratory rate, since this latter is variable and is partly a consequence of the coefficient change. Also a change in \(\mu\) indicates a shift in the catalytic system which would not be necessitated by a mere changing of the rate. This shift, moreover, involves the original egg system since great
changes occur in 2 to 3 minutes after fertilization, when the sperm is still external to the egg membrane (15). Also, according to Loeb (9), parthenogenetic activation leads to typical changes of fertilization; and our own findings show that cytolysis leads to the same change of coefficient as does fertilization. A particular contribution of enzyme (or substrate) by the sperm is thus, apparently, excluded, so the significant change is to be sought in the egg proper.

It was early pointed out (1, 18) that in a concatenated reaction chain the speed of the total reaction is controlled by that of the slowest member; and, further, as temperature, concentration of H+ or other substances, etc., are changed, one or another of the individual reactions may become the slowest. Such an interpretation has been successfully applied to many in vitro reactions (13). In the case of the egg, the same total reaction—foodstuff plus oxygen forming carbon dioxide, etc.—continues after fertilization, sometimes at the same rate. But whether the rate be increased on fertilization or decreased on cytolysis or unchanged, the factor limiting this rate is different from that operating in the unfertilized egg. A chemical change is further documented by the amazing morphogenetic changes released by activation.

Further analysis of the reactions involved remains hypothetical pending more experimentation. It is not without interest, however, to consider in this connection some of the facts regarding activation. There is evidence that activation depends in part on surface changes. Agents, like narcotics, which can displace substances from adsorbing surfaces, act as activators. Heat, also effective, may partly disorganize surfaces and adsorbed material; and moderate surface injury, produced by cytolyzing agents (NaOH, hypertonic NaCl), likewise activates. The activator, then, tends to disorganize existing cell surfaces, micellar or membrane, and so liberate for free reaction in solution the partly bound and inactive enzyme or substrate. Complete cytolysis would clearly act in the same way, though complicated by actual destruction of some catalytic material. It will be important to determine, for example, if the respiration of eggs activated with hypertonic saline shows the temperature coefficient of fertilized eggs.

Although non-penetrating acids decrease respiration (20), appropriate treatment with penetrating acids leads to activation. This has been studied especially by Lillie (8) for Asterias, and he has been led
to the view that increased intracellular acidity (possibly just within the membrane) leads to activation. Possibly surface disorganization, with effective increase of reactants, and increased H+ concentration act together in initiating the new events. It may be noted that the μ values of the fertilized egg respiration are numerically those attributed to H+ catalyses (3).

Runnström (14) found that carbon monoxide (and cyanide in low concentration), while barely diminishing the respiration of unfertilized eggs, greatly decreased that of fertilized ones. Urethane actually increased resting respiration while cutting that of fertilized eggs to about the same level. He concluded that a limited contact of respiratory enzyme and substrate determined reaction velocity in the inactive egg, hence that considerable enzyme might be poisoned with no decrease in oxidations. With these substances freed to react by colloidal changes in the active egg, the inhibitors manifest their usual effect by binding enzyme. In exactly the same way, the high temperature coefficient of resting eggs might document the influence of temperature on the state of adsorption, while the lower one, of structurally disorganized eggs, measures the direct effect of temperature on the speed of reaction. Change in colloidal state, e.g. gelation of gelatin, or membrane structure, e.g. in nerve (6), often shows a remarkably high temperature coefficient; and the fraction of free reactants released from the adsorbed bulk would be similarly sensitive to temperature. Further temperature studies should help to check these interpretations.

We wish to thank Dr. Ralph S. Lillie for his kind assistance.

SUMMARY

The eggs of *A. punctulata* have a high temperature coefficient in the resting state: $Q_{10} = 4.1$.

On fertilization and on cytolysis the temperature coefficient falls to less than half the resting value: $Q_{10} = 1.8$ and 1.9 respectively.

The factor by which oxygen consumption increases on fertilization is a variable, its magnitude depending on temperature as well as on egg species. It is nearly ten times greater at 11°C. and only double at 29.9°C. By extrapolating to 32°C. there would be no increase on fertilization.
Critical thermal increments common to many oxidations, 6,500, 10,800, and 12,500, have been found.

The possible significance of these results is discussed in relation to the catalytic mechanisms and structural organization of the egg cell.

BIBLIOGRAPHY

11. Navez, A. E., 1931, Protoplasma, 12, 86.
18. van’t Hoff, H. T., 1884, Etudes de dynamique chimique, Amsterdam, F. Müller & Co.