THE EFFECTS OF CAFFEINE ON OXYGEN CONSUMPTION AND
CELL DIVISION IN THE FERTILIZED EGG OF THE SEA
URCHIN, ARBACIA PUNCTULATA

By RALPH HOLT CHENEY

(From the Marine Biological Laboratory, Woods Hole, and the Department of
Biology, Long Island University, Brooklyn)

(Received for publication, August 1, 1945)

INTRODUCTION

As a phase of the general study of variation produced by caffeine upon the
reproductive phenomena in animals, outlined by Cheney (1944), the inhibitory
influence of this purine compound upon the oxidative processes associated with
development is of interest. The data presented are intended to show the relative
(not absolute) effects of several caffeine concentrations in sea water upon
the relative $O_2$ consumption values for fertilized Arbacia eggs and its correlation
with the effect upon cleavage rate.

Progressive development following fertilization is determined by a chain of
respiratory reactions, a system upon which cell division depends and the “ac-
tivity” phase of which is responsible for a considerable (70 to 90 per cent) per-
centage of the total $O_2$ consumption of the fertilized egg. The remainder (10 to
30 per cent) of the $O_2$ consumption involves the “resting” or “basal” system.
Ball and Meyerhof (1940) indicated the presence of an oxidative enzyme upon
which respiratory inhibitors act in unfertilized Arbacia eggs. Krahl, Keltch,
Neubeck, and Clowes (1941) reported a cytochrome oxidase enzyme, in the
fertilized Arbacia egg, which can oxidize reduced cytochrome C. These authors
demonstrated that this enzyme, when acting with cytochrome C as a substrate,
is inhibited completely by sodium cyanide or sodium azide, just as these agents
depress cell division in the fertilized living Arbacia egg. In contrast, the total
respiration can be inhibited only to a maximum of 70 to 80 per cent by cyanide
and only about 50 per cent by azide. Oxygen consumption is also inhibited by
carbon monoxide and hydrogen sulfite. Data by Fisher, Henry, and Low (1944)
indicate that the $O_2$ inhibition effect (55 per cent) of sulfanilamide, resulting in
complete suspension of cell division, is exerted on the main or activity system.
They also state that the inhibition of $O_2$ uptake seems to be the way in which
this agent interferes with cell division in the fertilized sea urchin egg. These
authors, in a study of the cyanide inhibition of $O_2$ uptake by the unfertilized and
fertilized sea urchin eggs, feel that it is difficult to escape the conclusion that
fertilization introduces a respiratory system upon which cell division depends
and which is responsible for 40 to 50 per cent of the $O_2$ consumption of the fer-
tilized egg. They assume apparently that the pathways of oxidation of the
substrate are different in unfertilized and fertilized eggs. Ball (1942), in discussing oxidative mechanisms in animal tissues, has pointed out that the evidence derived from cyanide inhibition of O₂ uptake in a fertilized egg and the absence of its inhibitory effect upon the unfertilized egg (*i.e.*, upon fertilization the egg consumes O₂ at a greatly increased rate and the additional O₂ consumed is cyanide-sensitive) *merely indicates* the possible existence in the unfertilized eggs of a *system alternate* to the cytochrome system. Ball suggests that it is possible that a reaction can occur *directly* between cytochrome oxidase and some flavoprotein in the *Arbacia* egg. This may be true also in the mechanism of biological oxidations in the animal body in its general energy relationships. The chief process by which foodstuffs appear to be oxidized in the living cell has been schematized by Ball (1944) to indicate the relationship of the cytochrome oxidase enzyme to the process of respiratory metabolism as follows: foodstuff—H₂—pyridine nucleotides—H₂—flavoprotein—cytochrome B—C—A—cytochrome oxidase—½O₂ + H₂ → H₂O. This scheme applies also to the chain of reactions in the fertilized sea urchin egg metabolism in the experiments reported here. Caffeine would seem, by inference derived from the data in this paper, to act upon the activity phase of that system. The activity system normally accounts for the greater part of the total O₂ consumption by the fertilized sea urchin egg but this activity phase is inactive in the resting cell according to Fisher and Henry (1944).

The mechanism involved is of significance to all research on growing cell metabolism including postfertilization phenomena. Fertilized sea urchin eggs are excellent material for studies on the effect of a substance upon the respiration of a cell because they depend so completely upon O₂ uptake for the utilization of nutrients, since they possess no anaerobic metabolic activity. Caffeine has a stimulatory effect upon the O₂ consumption in some animal tissues. Saslow (1937) reported an increase in O₂ consumption in unstimulated caffeinized striated muscle of *Rana pipiens* Schreber. He cites the average O₂ consumption of muscles in Ringer solution of 32 mm.₃ per gm. (wet) per hour in contrast with the average O₂ consumption of muscles in 0.037 to 0.042 per cent caffeine-Ringer of 201 mm.₃ per gm. (wet) per hour. Saslow also states that no lactic acid accumulates in caffeinized muscles in oxygen. Wortis (1935), however, in the case of the brain tissue of the rat, presented evidence that caffeine sodiobenzoate in high concentrations (0.5 per cent) diminishes the O₂ consumption. Thus the literature indicates a distinct variation with regard to the effect of caffeine upon the O₂ uptake phase of the respiratory metabolism of animal cells. At least divergent results have been reported for different tissues. This situation is not necessarily disturbing in view of the fact that certain tissues, such as striated muscle, possess oxidation mechanisms upon which caffeine might act *as in a trigger release fashion*; whereas, the fertilized sea urchin egg has no such mechanism. In the current data presented here, caffeine in the higher concen-
trations has been found to be inhibitory on the $O_2$ uptake by fertilized sea urchin eggs.

A fractionation of normal respiration into an activity and a resting portion might explain some cases of inhibitory action, i.e. one phase of the system might be more sensitive than the other to the agent used; or, lower concentrations of caffeine may merely be ineffective. For the sea urchin egg, Korr (1937) and Ballentine (1940) and, for the frog muscle, Stannard (1941) have reported fractionations of oxygen consumption similar to that described for narcotics. The relationship of caffeine inhibition of $O_2$ consumption to the concentration of this agent is expressed by Fig. 2 which is derived from a typical experiment, No. 10, of the series. It will be noted later that the degree of inhibition of cell division with increasing concentrations of caffeine approximates this same curve.

Materials and Experimental Method

Eggs and sperm of *Arbacia punctulata* were collected as described by Just (1939), exercising care to avoid contamination with body fluids and to utilize only egg batches in which 95 per cent or better fertilization had occurred. This was determined by microscopic examination at 100 × for the presence of the fertilization membrane. The eggs were concentrated by low speed centrifugation and only 35 per cent suspensions in sea water or caffeine-in-sea-water were employed. A total volume of 2 cc. for each Warburg vessel was maintained. Each flask contained 0.5 cc. of the 35 per cent suspension of fertilized eggs, thereby assuring an equal number (volume) of eggs per vessel. The pure alkaloid, caffeine U.S.P. Merck, was used. The caffeine-in-sea-water concentrations ranged in percentages and approximate molarities as follows: 0.002 per cent (M/10,000), 0.004 per cent (M/5,000), 0.02 per cent (M/1,000), 0.10 per cent (M/200), 0.2 per cent (M/100), 0.5 per cent (M/40), 2.0 per cent (M/10).

The manometric determinations of $O_2$ consumption were made with the Warburg-Barcroft apparatus using the Warburg direct method technique described by Dixon (1934). All experiments were performed at 25°C. and a shaker rate of 83 oscillations per minute with an 8 cm. amplitude. The pH of the sea water in the laboratory system at Woods Hole during the period of these experiments was 7.94. The annual average pH for open sea water at Woods Hole is 8.2 (Harvey, 1932). The addition of caffeine resulted in making the caffeine-in-sea-water 0.05 more alkaline, pH 7.99. This alkalinity increase is not significant since Smith and Clowes (1924) showed that the cell division rate in *Arbacia* is quite independent of a pH range between 6.0 and 8.3.

Controls (in nearly all cases, duplicate controls were run and showed a difference of less than 2 per cent) and thermobarometric checks (blanks) were recorded for all readings. Results were observed at 15 minute intervals over a 3 hour period. At the termination of each experiment, cell division was stopped in all of the solutions by the addition of formalin to 1 cc. of the contents of each flask to make its concentration equivalent to 0.1 per cent in sea water. The remainder of the flask contents was examined also microscopically for the condition of the eggs without formalin. The
EFFECTS OF CAFFEINE ON FERTILIZED SEA URCHIN EGG

cleavage stage reached by the eggs in each suspension was compared with the controls. The rate of cell division is expressed as a percentage of the normal (control) rate.

EXPERIMENTAL RESULTS

The 15 experiments reported here showed an average oxygen consumption of 66 mm.\textsuperscript{3} \textsubscript{O}_2 per hour for the normal, non-caffeine-treated, uninhibited, fertilized sea urchin egg respiration during the 3 hour duration of the experiments.\textsuperscript{1} Plotting the data of the oxygen consumption of the controls and the experimental series of seven different concentrations of caffeine-in-sea-water as an \textsubscript{O}_2 consumption-time relationship curve (Fig. 1) reveals an increasing degree (average) of inhibition of \textsubscript{O}_2 consumption from 23.4 per cent with 0.1 per cent (m/200) to 61 per cent with 2 per cent (m/10) concentrations of caffeine.

### TABLE I

| Variable Data for \textsubscript{O}_2 Consumption by Fertilized Arbacia Eggs in Lower Caffeine Concentrations |
|---|---|---|---|
| No. of experiment | Per cent concentration | Molarity | Average increase in \textsubscript{O}_2 uptake | Average decrease in \textsubscript{O}_2 uptake |
| 10 | 0.02 | m/1,000 | 0 | 10.6 |
| 5 | " | " | 6.2 | 0 |
| 7 | 0.004 | m/5,000 | 0 | 6.2 |
| 8 | " | " | 6.1 | 0 |
| 12 | 0.002 | m/10,000 | 0.44 | 0 |
| 3 | " | " | 0.70 | 0 |

The inhibition of \textsubscript{O}_2 uptake may be correlated with the effect on the cleavage rate or complete suppression of the cleavage process in the higher percentages. See Table II for the condition of the eggs at the termination of the 3 hour period in the Warburg flasks approximately 3½ hours after fertilization.

It may be observed from Fig. 3 that the degree of inhibition of the cleavage process approximately parallels the \textsubscript{O}_2 inhibition curve seen in Fig. 2. A distinct plateau showing a maximum depression of \textsubscript{O}_2 consumption and a complete inhibition of cell division is indicated by these curves in the instance of all caffeine percentages of 0.5 per cent or higher.

It will be noted in Fig. 1 that a progressive sequence of inhibition occurred in the experimental eggs from 0.1 to 2.0 per cent caffeine-in-sea-water inclusive. 2 per cent approaches the limits (2.2 per cent) of solubility of caffeine-in-sea-water. The effects noted in the remaining lower percentages were variable,—

\textsuperscript{1} To prevent the possibility of mechanical cytolysis and to avoid the danger, emphasized by Whitaker (1933), of cortical injury which would abnormally increase the rate of respiration, no attempt was made to concentrate the eggs to a maximum.
see Table I. In five experiments the data for 0.02 per cent suggest stimulation instead of the inhibition indicated by the average of all data for 0.02 per cent in the series. This variability in the effect of lower percentages causes one to consider whether or not there is some sort of competitive action for control of the oxidative steps in the metabolism.

Fig. 1. O₂ consumption-time relationship graph.

However, changes of less than 10 per cent, whether inhibitory or stimulatory, were considered not significant in biological material of this sort. All the variable data occurred only in experiments involving a concentration of less than 0.10 per cent caffeine. The lower concentrations are either ineffective or possibly act upon a different phase of the respiratory metabolism. The writer is of the opinion, however, that the evidence indicates that the caffeine affects only a single site on the main pathway (activity phase).

Most inhibitors, sodium cyanide in particular, have their action upon the main pathway in cellular respiration. Cyanide probably has its action at the
FIG. 2. Relationship of caffeine concentration to inhibition of O₂ consumption.

FIG. 3. Relationship of caffeine concentration to the degree of cleavage inhibition.
cytochrome oxidase point from the evidence of Krahl, Keltch, Neubeck, and Clowes (1941) and others. I have no direct evidence that caffeine acts specifi-

<table>
<thead>
<tr>
<th>Composition of experimental medium</th>
<th>Egg suspension used 35 per cent in sea water</th>
<th>Caffeine-in-seawater added</th>
<th>Sea water added</th>
<th>Alkali (10 per cent KOH) added to center well</th>
<th>Condition of eggs in Warburg vessel at end of experiment 4 hrs. after fertilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.5</td>
<td>0</td>
<td>1.3</td>
<td>0.2</td>
<td>Normal 64 and 32 celled late cleavage stages</td>
</tr>
<tr>
<td>0.002 per cent CSW*</td>
<td>“</td>
<td>“</td>
<td>1.3</td>
<td>0.2</td>
<td>Similar to controls</td>
</tr>
<tr>
<td>0.004 “ “ “</td>
<td>“</td>
<td>“</td>
<td>“</td>
<td>“</td>
<td>Mostly late cleavages. Few showed tendency to clump.</td>
</tr>
<tr>
<td>0.02 “ “ “ “</td>
<td>“</td>
<td>“</td>
<td>“</td>
<td>“</td>
<td>Rarely red echinochrome pigment was clumped in egg</td>
</tr>
<tr>
<td>0.10 “ “ “ “</td>
<td>“</td>
<td>“</td>
<td>“</td>
<td>“</td>
<td>32 celled mostly. Few 64 celled. 5 per cent with</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>tendency for pigment to clump. ±1 per cent with</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Very few 32. 40 per cent with tendency to clump.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Actual clumping in a few</td>
</tr>
<tr>
<td>0.50 “ “ “ “</td>
<td>“</td>
<td>“</td>
<td>“</td>
<td>“</td>
<td>No cleavage but 50 per cent “appeared” normal</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>microscopically. In 45 per cent, tendency to clump.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>In 5 per cent, pigment was clumped</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Red echinochrome concentrated in one area in 100</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>per cent of eggs, usually in center, sometimes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>eccentric but always localized</td>
</tr>
</tbody>
</table>

* CSW = caffeine-in-sea-water.

cally on the cytochrome oxidase enzyme but it should be noted that the caffeine maximum inhibition of O₂ consumption is above 60 per cent and cyanide maximum inhibition is above 70 per cent. Caffeine and cyanide depression of O₂ uptake in fertilized sea urchin eggs is, therefore, of the same general order of magnitude.
Caffeine would appear to exert its influence then somewhere on the primary (activity) pathway, especially when it is noted that the cell division inhibition seems to parallel the O$_2$ consumption depression. The O$_2$ consumption is undoubtedly the major energy source for the cell division process. Cleavage stages were recorded in all controls and experimental vessels and compared with corresponding percentages in parallel experiments of the series. Table II shows that 2 per cent (m/10) and 0.5 per cent (m/40) caffeine-in-sea-water blocked cleavage completely. An examination of Fig. 1 indicates that the normal uptake, in comparison with the controls, was depressed over 50 per cent. Cleavage rate, however, is not necessarily limited by the rate of O$_2$ consumption per se in a number of forms. For example, Amberson (1928) found the cleavage rate was not retarded seriously in *Arbacia* eggs, when O$_2$ pressure was diminished, until the pressure reduction was sufficient to decrease the O$_2$ consumption to about half normal. My findings agree with this generality in so far as cleavage was not completely inhibited otherwise.

**SUMMARY**

1. By means of the Warburg-Barcroft microrespirometer apparatus and the Warburg direct method, the relative effect of caffeine upon the O$_2$ consumption of the fertilized egg of *Arbacia punctulata* was shown for the following concentrations in sea water: 0.002 per cent (m/10,000), 0.004 per cent (m/5,000), 0.02 per cent (m/1,000), 0.1 per cent (m/200), 0.2 per cent (m/100), 0.5 per cent (m/40), and 2 per cent (m/10).

2. In comparison with the normal eggs (uninhibited, non-caffeine-treated controls), caffeine in concentrations including and greater than 0.1 per cent (m/200) depressed the average uptake from approximately 25 to 61 per cent over the 3 hour period. In a number of instances, as typified by Experiment 10, the effective inhibitory concentration ranged from 0.02 per cent (m/1,000) upward and the degree of depression of the O$_2$ consumption ranged from 10.6 per cent to 60.6 per cent.

3. All caffeine concentrations including and above 0.02 per cent (m/1,000) in the series used, resulted in decreasing the normal rate of cleavage division in the fertilized *Arbacia* eggs.

4. The higher concentrations (0.5 and 2 per cent) produced a complete blockage of the cleavage process.

5. Complete cleavage inhibition was noted only when the O$_2$ uptake had been depressed to 50 per cent or more of the normal controls.

6. O$_2$ consumption–time relationship data indicate an average depression, in O$_2$ consumption over a 3 hour period, ranging from 25 per cent with a caffeine concentration of 0.1 per cent to a 61 per cent inhibition with a concentration of 2 per cent.

7. Concentrations of less than 0.1 per cent (certainly of less than 0.02 per cent) give variable results and indicate no significant effect.
8. It is inferred from the respiration data presented that it is probable that the inhibition of the O₂ consumption in fertilized * Arbacia * eggs is due to the influence of caffeine upon the main (activity or primary) pathway. It will be observed that there are certain similarities of the caffeine data to the degree of inhibition accomplished by sodium cyanide. Moreover, it has been demonstrated that the cyanide probably acts on the cytochrome oxidase step in the cytochrome oxidase-cytochrome chain of reactions constituting the O₂ uptake phase of respiratory metabolism. It is not improbable, therefore, that caffeine also may act upon the cytochrome oxidase enzyme.

9. From the viewpoint of environmental conditions influencing reproductive phenomena, it is of interest that caffeine can affect the normal metabolism of the zygote.

**LITERATURE CITED**


Ball, E. G., 1942, Oxidative mechanisms in animal tissues, in a symposium on respiratory enzymes, Madison, University of Wisconsin Press, 16–33.


