# ACTION OF NITRO- AND HALOPHENOLS UPON OXYGEN CONSUMPTION AND PHOSPHORYLATION BY A CELL-FREE PARTICULATE SYSTEM FROM ARBACIA EGGS

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It was first shown 15 years ago that certain nitro- and halophenols could produce two concurrent, but not necessarily interdependent, effects on eggs of *Arbacia punctulata* (1–3). At low concentrations each such agent gave rise to a two or three hundred per cent increase in oxygen consumption. As the concentration of a given substituted phenol was made progressively larger, the rate of oxygen consumption passed through an optimum and then declined to or below the normal rate; parallel to the decline of oxygen consumption there was a reversible block to the cell division of the fertilized eggs.

In 1948 Loomis and Lipmann (4) found that 2,4-dinitrophenol increased the oxygen consumption of a cell-free granule preparation from kidney and, in slightly higher concentrations, blocked the aerobic phosphorylation which was coupled with oxidation of glutamate by the kidney preparation. Similar observations on cell-free systems from mammalian tissues were made by Cross, Taggart, Covo, and Green (5).

It was deemed of considerable importance to extend such observations to the sea urchin egg because of the following considerations: First, it has been suggested that a considerable fraction of the energy derivable from oxidation of foodstuffs is made available for synthesis and function *via* oxidative phosphorylation processes (6); second, the observations of Loomis and Lipmann indicate that 2,4-dinitrophenol can block such energy-yielding processes; and, third, the same and related substances produce a block to one function of the sea urchin egg, namely, cleavage. If a strong parallel between the effects of the substituted phenols upon phosphorylation and upon cleavage could be established, this would provide evidence that the energy for cleavage is made available *via* phosphorylation processes.

In the summer of 1948 (7) it was shown that substituted phenols can increase the oxygen consumption of a cell-free particulate system from *Arbacia* eggs.

The present paper deals with the effects of substituted phenols on oxidative phosphorylation by the cell-free *Arbacia* particulate system described in the preceding paper (8), and with the relation of these effects to those of the same agents upon cleavage of the fertilized *Arbacia* egg.

## EXPERIMENTAL RESULTS

Nine substituted phenols have been investigated with respect to their effects on oxygen consumption and phosphorylation by the cell-free particulate system of unfertilized *Arbacia* eggs (Table I). Each agent was included in the contents of the Warburg flasks in the amount required to give the final concentrations shown in Table I. The order of addition of reagents was the same as that specified in the previous paper (8). Phosphorylation was measured in terms of the

TABLE I

*Effect of Substituted Phenols upon Oxygen Consumption and Inorganic Phosphate Uptake by a Cell-Free Particulate System from Unfertilized Eggs of Arbacia punctulata.*

All experiments were carried out at 20°C., with 0.01 M α-ketoglutarate as substrate. The values represent, respectively, oxygen uptake and inorganic phosphate disappearance per 0.1 ml. packed eggs per hour. See reference 8 for detailed experimental methods; pH values were determined for each flask at the end of the incubation period.

| Concentration of reagent | 2,4-Dinitrophenol pH 7.2 | | 4,6-Dinitrocresol pH 7.0 | | 4,6-Dinitrocarvacrol pH 7.1 | | 2,4-Dinitrothymol pH 7.1 | | *p*-Nitrophenol pH 7.2 | | *o*-Nitrophenol pH 7.2 | | Picric acid pH 7.4 | | 2,4,5-Trichlorophenol pH 7.1 | | 2,6-Dinitro-4-chlorophenol pH 7.4 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $O_2$ Use | P Loss | $O_2$ Use | P Loss | $O_2$ Use | P Loss | $O_2$ Use | P Loss | $O_2$ Use | P Loss | $O_2$ Use | P Loss | $O_2$ Use | P Loss | $O_2$ Use | P Loss | $O_2$ Use | P Loss |
| *moles per l.* | *c. mm.* | *μg.* | *c. mm.* | *μg.* | *c. mm.* | *μg.* | *c. mm.* | *μg.* | *c. mm.* | *μg.* | *c. mm.* | *μg.* | *c. mm.* | *μg.* | *c. mm.* | *μg.* | *c. mm.* | *μg.* |
| 0 | 36 | 126 | 34 | 131 | 28 | 104 | 38 | 122 | 30 | 122 | 30 | 122 | 37 | 102 | 31 | 102 | 37 | 102 |
| $3.12 \times 10^{-8}$ | .. | .. | .. | .. | .. | .. | 37 | 113 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| $6.25 \times 10^{-8}$ | .. | .. | .. | .. | .. | .. | 35 | 98 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| $1.25 \times 10^{-7}$ | 36 | 117 | 34 | 119 | 28 | 93 | 34 | 67 | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. |
| $2.5 \times 10^{-7}$ | .. | .. | 34 | 112 | 30 | 87 | 24 | 1 | .. | .. | .. | .. | .. | .. | 32 | 107 | .. | .. |
| $5.0 \times 10^{-7}$ | 39 | 117 | 38 | 68 | 32 | 67 | 25 | −22 | .. | .. | .. | .. | .. | .. | 34 | 104 | .. | .. |
| $1 \times 10^{-6}$ | 43 | 108 | 42 | 54 | 32 | 32 | 21 | −30 | .. | .. | .. | .. | .. | .. | 36 | 104 | .. | .. |
| $2 \times 10^{-6}$ | 42 | 77 | 40 | −9 | 30 | −19 | 21 | −34 | 34 | 117 | 33 | 118 | .. | .. | 34 | 99 | 38 | 108 |
| $4 \times 10^{-6}$ | 39 | 39 | 34 | −22 | 26 | −21 | 20 | −30 | .. | .. | .. | .. | 34 | 108 | 36 | 77 | .. | .. |
| $8 \times 10^{-6}$ | .. | .. | 36 | −8 | 30 | −17 | .. | .. | 34 | 115 | 34 | 112 | .. | .. | 37 | 9 | 39 | 86 |
| $1.6 \times 10^{-5}$ | 38 | −4 | 31 | −4 | 26 | −20 | .. | .. | 38 | 105 | 34 | 112 | 34 | 104 | 32 | −18 | 41 | 8 |
| $3.2 \times 10^{-5}$ | .. | .. | .. | .. | .. | .. | .. | .. | 38 | 65 | 35 | 115 | .. | .. | 30 | −17 | 32 | −10 |
| $6.4 \times 10^{-5}$ | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 37 | 106 | .. | .. | .. | .. |
| $1.28 \times 10^{-4}$ | .. | .. | .. | .. | .. | .. | .. | .. | 30 | −3 | 33 | 112 | .. | .. | .. | .. | 22 | −16 |
| $2.56 \times 10^{-4}$ | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 34 | 70 | .. | .. | .. | .. |
| $5.12 \times 10^{-4}$ | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | .. | 16 | −17 |

inorganic phosphorus disappearing from the medium in each flask and is referred to in Table I as P loss.

In confirmation and extension of the results of Crane and Keltch (7), the oxygen consumption was slightly, but consistently, increased by low concentrations of 2,4-dinitrophenol, 4,6-dinitrocresol, 4,6-dinitrocarvacrol, *p*-nitrophenol, 2,4,5-trichlorophenol, and 2,6-dinitro-4-chlorophenol. At higher concentrations of each of these substances, the oxygen consumption was reduced to or

below the normal level. 2,4-Dinitrothymol produced only an inhibition. *o*-Nitrophenol and 2,4,6-trinitrophenol produced neither a stimulation nor a significant depression of oxygen consumption at any concentration tried.

Where present, the increase in oxygen consumption was small as compared to the effects of the same agents upon oxygen consumption of intact unfertilized or

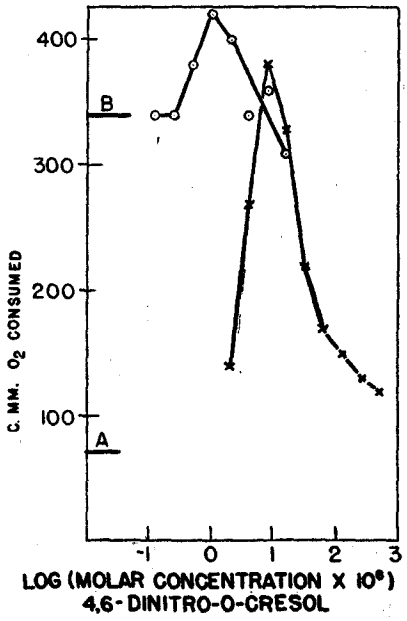


FIG. 1. Effect of 4,6-dinitrocresol upon oxygen consumption at 20°C. of intact unfertilized *Arbacia* eggs and of the cell-free particulate system derived from the same quantity of unfertilized eggs. The pH of the medium for unfertilized eggs was 7.9, that of the homogenate, 7.0. The values of oxygen consumption on the ordinate are expressed as cubic millimeters per hour per gram wet eggs. The control level, without added dinitrocresol, for the intact eggs, is denoted by the line labeled *A*, that for the particulate system by *B*. O————O, cell-free particulate system. ×————×, intact unfertilized *Arbacia* eggs.

fertilized eggs (Figs. 1–4). This seems to be due in part to the fact that the basal oxygen consumption of the homogenate is relatively very high, on the average 340 c.mm. per hour for the cell-free material derived from 1 gm. of eggs, as compared with 70 and 310 c.mm. per hour for the same quantity of *intact* unfertilized and fertilized eggs, respectively. The high rate for the homogenate is in part due to the fact that the inorganic phosphate required for the phosphate uptake measurements produces some increase in oxygen consumption of the

same type as that induced by the substituted phenol and perhaps to the fact that undefined factors, possibly associated with compartmentation, controlling oxygen consumption are partially lost by homogenization.

The phosphorylation was blocked by the same agents as were active in increasing the oxygen consumption (Table I), the concentration required being in each instance slightly larger than that producing the optimum oxygen uptake; 2,4-dinitrothymol produced an inhibition of both oxygen consumption and phosphorylation. The *o*-nitrophenol and picric acid had no effect on either oxygen consumption or phosphorylation at concentrations up to $10^{-3}$ M. There

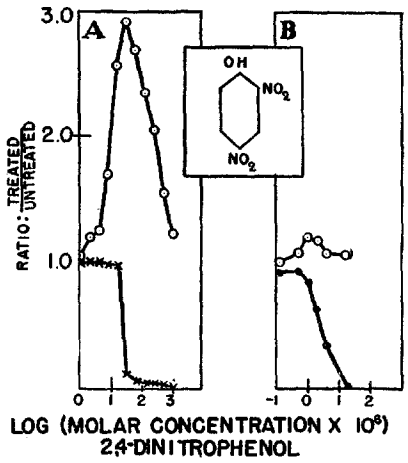


FIG. 2. Effect of 2,4-dinitrophenol upon oxygen consumption and cell division of fertilized *Arbacia* eggs (*A*), and upon oxygen consumption and phosphorylation by a cell-free particulate system from unfertilized *Arbacia* eggs (*B*). All measurements were made at 20°C. O————O, oxygen consumption. ×————×, cell division. ●————●, phosphorylation.

are five striking parallels between the effects on the cell-free oxidative phosphorylating system and the effects on the intact, cleaving eggs.

1. The block to both cleavage and phosphorylation is produced by concentrations just larger than those required for optimum oxygen consumption, as illustrated by the data for dinitrophenol (Fig. 2).

2. Dinitrocarvacol produces an increase in oxygen consumption in both the intact eggs and the particulate system, but blocks both cleavage and phosphorylation; on the other hand, its isomer, dinitrothymol, inhibits oxygen consumption in both systems and blocks both cleavage and phosphorylation (Fig. 3).

3. *p*-Nitrophenol stimulates oxygen consumption in both systems and blocks both cleavage and phosphorylation; its isomer, *o*-nitrophenol, is inactive toward

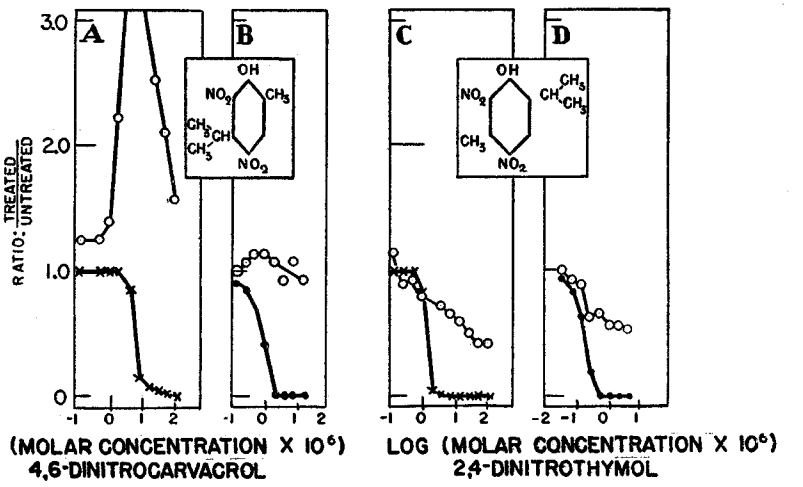


FIG. 3. Effect of dinitrocarvacrol and dinitrothymol upon oxygen consumption and cell division of fertilized *Arbacia* eggs (*A* and *C*), and upon oxygen consumption and phosphorylation by a cell-free particulate system from unfertilized *Arbacia* eggs (*B* and *D*). All measurements were made at 20°C. O————O, oxygen consumption. ×————×, cell division. ●————●, phosphorylation.

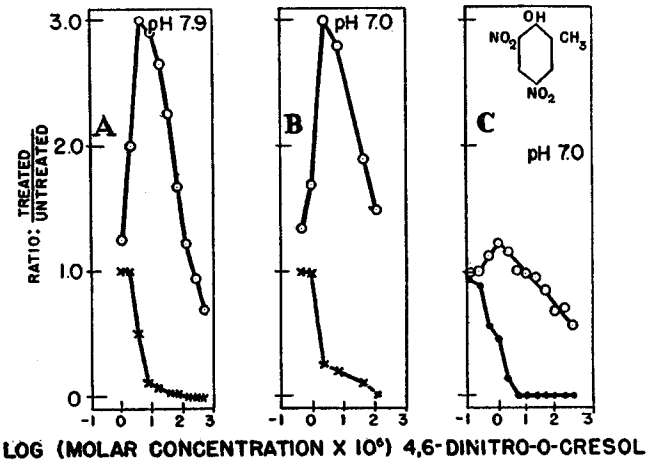


FIG. 4. Effect of dinitrocresol upon oxygen consumption and cell division of fertilized *Arbacia* eggs at pH 7.9 (*A*) and pH 7.0 (*B*), compared with its effect upon oxygen consumption and phosphorylation by a cell-free particulate system from unfertilized *Arbacia* eggs at pH 7.0 (*C*). All measurements were made at 20°C. O————O, oxygen consumption. ×————×, cell division. ●————●, phosphorylation.

oxygen consumption in both systems, and blocks neither cleavage nor phosphorylation.

4. Picric acid is completely inactive in both systems.

5. The concentration of each active agent required to block cleavage at pH 7.0 or to block phosphorylation at pH 6.9–7.4 is smaller than that required to block cleavage of the fertilized eggs in sea water at pH 7.9 (Fig. 4). This is what would be expected if the undissociated acid form of the substituted phenol were the form in which these agents penetrate the egg surface (9).

If the undissociated form, rather than the anion, were the active form, the concentration of undissociated molecules required to block egg cleavage might be expected to be very close to that required by the same agent to block phosphorylation. This was found to be the case for each of the five active nitrophenols (Table II). For example, the *total* concentration of dinitrocresol required to reduce phosphorylation by 50 per cent at pH 7.0 was one-tenth the *total* concentration of the same agent required to reduce cleavage by 50 per cent at pH 7.9, but the concentrations of undissociated molecules required in the two systems were virtually identical, $1.6 \times 10^{-9}$ M and $1.3 \times 10^{-9}$ M, respectively. For reasons as yet incompletely understood, this correspondence of the concentrations of undissociated molecules required to cause inhibition in the two systems does not hold for two substituted phenols containing halogens.

TABLE II

*Concentrations of Undissociated Molecules of Various Substituted Phenols Required to Inhibit Cleavage in Intact Fertilized Arbacia Eggs and Phosphorylation by Cell-Free Particulate Systems, as Calculated from the Data of Table I and Previous Papers (2, 3)*

| Compound | pK′ | Total concentration for 50 per cent block in: | | Concentration of undissociated molecules at 50 per cent block of: | |
|---|---|---|---|---|---|
| | | Cell division | Phosphorylation | Cell division | Phosphorylation |
| | | *moles per l.* | *moles per l.* | *moles per l.* | *moles per l.* |
| *p*-Nitrophenol | 7.2 | $8.9 \times 10^{-5}$ | $3.4 \times 10^{-5}$ | $1.49 \times 10^{-5}$ | $1.7 \times 10^{-5}$ |
| 2,4,5-Trichlorophenol | 6.9 | $1.0 \times 10^{-5}$ | $5.6 \times 10^{-6}$ | $9.1 \times 10^{-7}$ | $2.1 \times 10^{-6}$ |
| 4,6-Dinitrocarvacrol | 4.5 | $5.8 \times 10^{-6}$ | $7.9 \times 10^{-7}$ | $2.3 \times 10^{-9}$ | $2.0 \times 10^{-9}$ |
| 4,6-Dinitrocresol | 4.4 | $4.0 \times 10^{-6}$ | $6.3 \times 10^{-7}$ | $1.3 \times 10^{-9}$ | $1.6 \times 10^{-9}$ |
| 2,4-Dinitrophenol | 4.1 | $2.3 \times 10^{-5}$ | $2.5 \times 10^{-6}$ | $3.9 \times 10^{-9}$ | $2.0 \times 10^{-9}$ |
| 2,4-Dinitrothymol | 4.1 | $1.3 \times 10^{-6}$ | $1.6 \times 10^{-7}$ | $2.1 \times 10^{-10}$ | $1.6 \times 10^{-10}$ |
| 2,6-Dinitro-4-chlorophenol | 3.5 | $3.9 \times 10^{-4}$ | $1.1 \times 10^{-5}$ | $1.6 \times 10^{-8}$ | $1.4 \times 10^{-9}$ |

The fact that the effects of the substituted phenols upon cell division parallel those upon oxidative phosphorylation in five critical respects enumerated above suggests that the reversible block to cleavage produced by these agents is due to interruption of oxidative phosphorylation. This, in turn, would imply that the energy for cleavage in the *Arbacia* egg is made available *via* oxidative phosphorylation processes.

It should be remembered, however, that these studies on phosphorylation were made with cell-free particulate preparations from *unfertilized* eggs, and it remains to be determined whether the substituted phenols exert the same effects on oxidative phosphorylating systems obtained from *fertilized* eggs.

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## SUMMARY

1. The ability of 4,6-dinitrocresol and eight other substituted phenols to stimulate oxygen uptake and inhibit phosphorylation by a cell-free particulate system from unfertilized *Arbacia* eggs has been determined.

Five of those agents can produce both stimulation of oxygen consumption and inhibition of phosphorylation; one inhibits both oxygen consumption and phosphorylation; and two have no effect on either oxygen consumption or phosphorylation.

In every case the effects of these substituted phenols upon the cell-free particulate systems parallel those upon oxygen consumption and cleavage in the intact fertilized *Arbacia* eggs.

2. The data suggest that energy for cleavage of the *Arbacia* egg is provided at least in part by oxidative phosphorylation and that substituted phenols may block cleavage by interfering with generation and transfer of high-energy phosphate groups.

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