STUDIES ON CELL METABOLISM AND CELL DIVISION

IV. Combined Action of Substituted Phenols, Cyanide, Carbon Monoxide, and Other Respiratory Inhibitors on Respiration and Cell Division

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In previous papers of this series it has been shown that certain substituted phenols produce a large stimulation of the oxygen consumption and a reversible block to the cell division of marine eggs (1, 2). These results have been confirmed and extended to other eggs by Tyler and Horowitz (4), and Dawson (5). As yet there is no complete understanding of the mechanism of this respiratory stimulation and almost no knowledge of the manner in which the respiratory and division effects of the substituted phenols are related.

The experiments reported in this paper were made to determine: (*a*) the sensitivity of the substituted phenol stimulated respiration to various types of respiratory inhibitors, and (*b*) the possible relationship between the respiratory effects and the division inhibiting effects of the substituted phenols.

The experiments were made with fertilized eggs of the sea urchin, *Arbacia punctulata*.

EXPERIMENTAL METHODS

The measurements of oxygen consumption were made by the direct method of Warburg (6) using the technique previously described (2, 7).

The non-gaseous reagents were dissolved in sea water in a concentration sufficient to give the final concentrations shown in the tables and figures, and then adjusted to the desired pH, which was that of sea water, 8.0, unless otherwise indicated. 0.5 cc. of each of the solutions was placed in the side arm of the Warburg vessel and tipped into the egg suspension at the time indicated. If two reagents were to be added at different times, the first was added directly to the egg suspension before attaching the flasks to the manometers and the second substance was tipped in during the course of the experiments.

The unfertilized eggs of the sea urchin, *Arbacia punctulata*, were shed in the usual way, washed twice with sea water, fertilized in a large volume of sea water, and allowed to settle. They were then adjusted to a volume which would give a final egg concen-

tration of approximately 2 per cent. In the iodoacetic acid experiments, at 5 minutes after fertilization, the normal sea water was replaced with carbonate free sea water at pH 6.

In each case the egg suspension was transferred to the Warburg flasks by means of a volumetric pipette with a wide opening. The volume and concentration of the egg suspension were adjusted to give a total volume of 5.5 cc. of 2 per cent egg suspension in each flask.

At the end of each experiment, a sample of eggs from each flask was fixed in 0.2 per cent formol; from these samples the extent of division was estimated and expressed as previously described (2).

All experiments were run at 20°C.

EXPERIMENTAL RESULTS

Low Oxygen Tension.—The effects of a series of concentrations of 4,6-dinitro-*o*-cresol on oxygen consumption and cell division of fertilized *Arbacia* eggs were determined at each of several partial pressures of oxygen in nitrogen (Fig. 1). There are three points of interest brought out by these results.

First, respiratory stimulation by 4,6-dinitro-*o*-cresol decreased and finally disappeared as the oxygen tension was progressively lowered.

Second, the degree of inhibition of cell division by each concentration of 4,6-dinitro-*o*-cresol increased as the oxygen tension was lowered. This result supports the conclusion previously reached (2), that the division inhibition produced by the 4,6-dinitro-*o*-cresol is not the result of overstimulation of oxidation by this reagent.

Third, when the oxygen consumption, reduced by exposure of the eggs to 2 per cent oxygen, was raised to the normal level in air by using 4,6-dinitro-*o*-cresol with the 2 per cent oxygen (Fig. 1, IC), there was no corresponding reversal of the inhibition of division associated with this low oxygen tension. On the contrary, the combination of 4,6-dinitro-*o*-cresol with low oxygen tension produced an additive inhibition of division.

Carbon Monoxide.—The effects of a series of concentrations of 4,6-dinitro-*o*-cresol were determined at each of two partial pressures of oxygen in carbon monoxide (Fig. 1). It is particularly interesting to note: (*a*) that at 4,6-dinitro-*o*-cresol concentrations of 10^{-6}M, 2×10^{-6}M, and 4×10^{-6}M with 6 per cent oxygen–94 per cent carbon monoxide, the absolute rate of oxygen consumption was less than that in the presence of the carbon monoxide control, and (*b*) that the percentage reduction of 4,6-dinitro-*o*-cresol stimulated respiration produced by including the carbon monoxide was only 51 per cent in the presence of 3.2×10^{-5}M 4,6-dinitro-*o*-cresol and 66 per cent at 3×10^{-6}M 4,6-dinitro-*o*-cresol (Table I), although the rates of

oxygen consumption were the same for the two concentrations of this reagent when the carbon monoxide was absent.

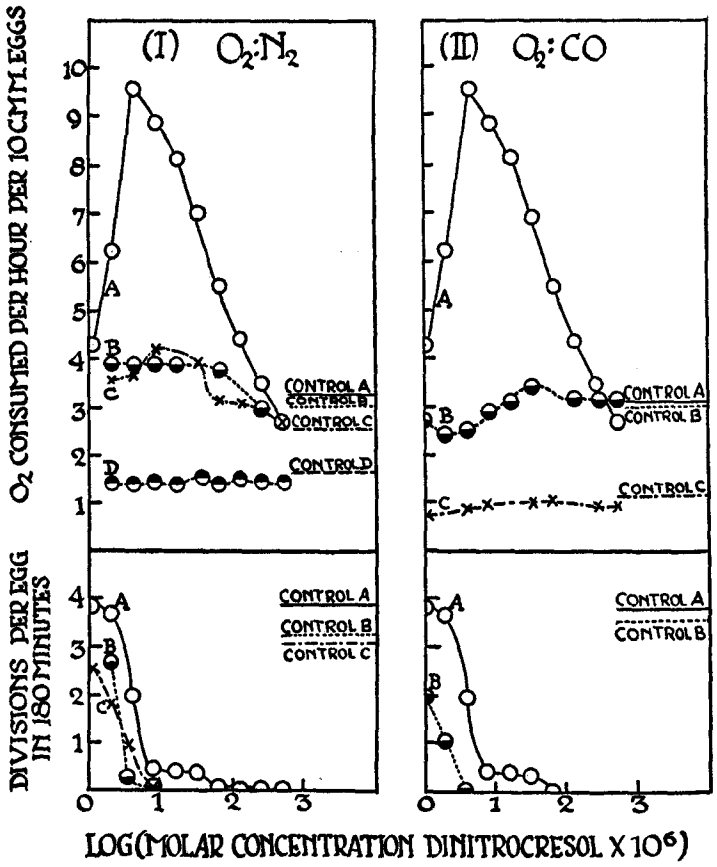

FIG. 1. Effect of various concentrations of 4,6-dinitro-*o*-cresol on oxygen consumption and cell division of fertilized *Arbacia* eggs: I*A* and II*A*, in air; I*B*, in 3 per cent O_2-97 per cent N_2, I*C*, 2 per cent O_2-98 per cent N_2; I*D*, in 0.5 per cent O_2-99.5 per cent N_2; II*B*, in 6 per cent O_2-94 per cent CO; II*C*, in 1.5 per cent O_2-98.5 per cent CO.

In Figs. 1–4 the horizontal lines marked control *A*, etc., are the control levels of oxygen consumption, or division, for the similarly marked curves. Where a division curve corresponding to an oxidation curve is not given, all cleavage was stopped.

All reagents were added 20–30 minutes after fertilization. Temperature, 20°C.

This result would be accounted for if, in greater than optimum concentrations of 4,6-dinitro-*o*-cresol, part of the oxygen consumption passed through carbon monoxide insensitive respiratory catalysts; it would also be explained if high concentrations of substituted phenol produced a decrease in the affinity of the respiratory catalytic system for carbon monoxide.

From the results with cyanide given below, this latter explanation appears to be the correct one.

In 1.5 per cent oxygen–98.5 per cent carbon monoxide, which reduced the oxygen consumption of the eggs to 36 per cent of the control value, the oxygen consumption was not stimulated by any concentration of 4,6-dinitro-*o*-cresol up to 5.12×10^{-4} M.

TABLE I

Percentage Reduction of 4,6-Dinitro-o-Cresol Stimulated Respiration by Respiratory Inhibitors at Various Levels of Oxygen Consumption on the Rising (R) and Falling (F) Segments of the Stimulation Curve

Oxygen consumption	Concentration 4,6-dinitro-*o*-cresol	Segment of curves used	Percentage reduction of stimulated respiration in various reagents					
			2 per cent O_2, 98 per cent N_2	6 per cent O_2, 94 per cent CO	8×10^{-5} M KCN at pH 7.5	10^{-3} M Phenyl-urethane at pH 7.5	10^{-3} M 5-Isoamyl-5-ethyl barbituric acid at pH 7.5	10^{-3} M Malonic acid at pH 7.5
c.mm. per 10 c.mm. eggs	*moles per l. × 10^4*							
3.2	0		22	5	59	29	5	5
5.0	1.4	R	30	50	78	44	20	0
	83.	F	38	34	52	27	0	10
6.0	1.8	R	42	60	80	48	8	0
	51.	F	38	45	60	30	7	11
7.0	2.3	R	49	66	83	54	10	10
	32.	F	44	51	68	26	13	20
8.0	3.0	R	56	70	84	60	3	18
	16.	F	49	61	83	33	18	21
9.0	3.9	R	60	72	86	63	10	23
	6.8	F	53	66	86	56	16	23

As in the experiments with low oxygen tension, prevention of 4,6-dinitro-*o*-cresol respiratory stimulation by carbon monoxide produced no reversal of the 4,6-dinitro-*o*-cresol division block; on the contrary, the division inhibiting effects of the two treatments were additive.

As shown in a previous paper (7) the inhibition of oxygen consumption by carbon monoxide in fertilized eggs of *Arbacia punctulata* was almost completely reversed by light. Likewise, the carbon monoxide block to respiratory stimulation by 4,6-dinitro-*o*-cresol, and the additive effect of

the carbon monoxide on cell division were in large part reversed by light of sufficient intensity (Table II), the respiration and cell division being then approximately the same as if the carbon monoxide were replaced by nitrogen. Bodine and Boell (8) have observed that the carbon monoxide inhibition of respiratory stimulation by 2,4-dinitrophenol in grasshopper embryos is reversed by light.

Potassium Cyanide.—The effects of each of ten concentrations of 4,6-dinitro-*o*-cresol were determined at each of five concentrations of potassium

TABLE II

Oxygen Consumption (C.Mm. Per Hour Per 10 C.Mm. Eggs) and Cell Division of Fertilized Eggs of Arbacia punctulata in 2.1 Per Cent Oxygen–97.9 Per Cent Carbon Monoxide with and without 4 × 10^{-6}M 4,6-Dinitro-o-Cresol. The Eggs Were First Exposed to Reagents 25 Minutes after Fertilization. Temperature, 20°C.

Treatment	50–110 min. after fertilization		125–185 min. after fertilization		200–260 min. after fertilization		Divisions per egg at 260 min.
	Light*	O_2	Light	O_2	Light	O_2	
None-control	Off	2.7	Off	2.4	Off	2.4	4.77
None-control	Off	2.5	On	2.3	Off	2.4	4.62
CO	Off	1.9	Off	1.6	Off	1.3	1.41
CO	Off	1.8	On	2.7	Off	1.2	2.08
4,6-Dinitro-*o*-cresol	Off	6.2	Off	5.6	Off	5.2	3.01
4,6-Dinitro-*o*-cresol	Off	6.2	On	5.2	Off	5.3	3.16
CO and 4,6-dinitro-*o*-cresol	Off	1.9	Off	1.7	Off	1.2	0.03
CO and 4,6-dinitro-*o*-cresol	Off	2.2	On	4.0	Off	1.9	0.95

* All samples were kept in the dark for the first 50 minutes after fertilization. During the 110–125 minute and 185–200 minute periods the condition of illumination for each period was the same as for the immediately following period.

cyanide (Table III). Both the absolute and relative reduction of respiration produced by each concentration of cyanide depended on the concentration of 4,6-dinitro-*o*-cresol simultaneously employed. In concentrations of 4,6-dinitro-*o*-cresol smaller than that producing the optimum respiratory stimulation without cyanide, the percentage reduction by cyanide was invariably greater than in a concentration of the substituted phenol larger than the respiratory optimum, even if the absolute rates of oxygen consumption in the two respective concentrations of 4,6-dinitro-*o*-cresol were the same in the absence of cyanide. For example (Table I), at points of equal oxygen consumption on the ascending and descending segments of the curve of respiratory stimulation by 4,6-dinitro-*o*-cresol, at which the oxygen consumption was 7.0 c.mm. per hour per 10 c.mm. eggs, 8 × 10^{-5}M KCN

produced an 83 per cent reduction on the rising segment of the curve and only a 68 per cent reduction on the falling segment.

Similar experiments were performed with 2,4,5-trichlorophenol as the stimulating agent. As in the case of 4,6-dinitro-*o*-cresol, the addition of cyanide, at points of equal oxygen consumption on the rising and falling segments of the curve, produced a greater reduction on the rising than on the descending segment.

The increase in the optimum stimulating concentration of 4,6-dinitro-*o*-cresol (Figs. 1 and 2, Table III) or 2,4,5-trichlorophenol (Table IV) with increasing concentrations of cyanide or carbon monoxide deserves comment. At first sight, this appears to indicate that the substituted phenols, in high concentrations, stimulate a cyanide insensitive form of respiration. This, however, cannot be the case, since the absolute level of residual oxygen consumption under maximum cyanide inhibition was nearly the same at concentrations of 4,6-dinitro-*o*-cresol (Table V) or of 2,4,5-trichlorophenol (Table VI) less than or greater than the concentration producing optimum stimulation in air. The least unsatisfactory explanation of the data therefore appears to be that, in the higher range of 4,6-dinitro-*o*-cresol concentrations, the affinity of the respiratory system of the fertilized *Arbacia* egg for cyanide or carbon monoxide is less than that in lower concentrations of the substituted phenol. This is apparently not due to competition of the substituted phenol and the cyanide for the iron component of the enzyme system because 4,6-dinitro-*o*-cresol has, in cell free systems, no inhibiting effect on the activity of the respiratory enzymes poisoned by cyanide. The cyanide sensitive systems upon which the 4,6-dinitro-*o*-cresol, in concentrations up to 1000 times the physiological concentrations, had no effect are: indophenol oxidase, catalase, polyphenol oxidase, hemin, pyridine and nicotine hemochromogens, iron and copper as catalysts for cysteine oxidation, and iron and copper as catalysts for ascorbic acid oxidation (9).

Part of the division inhibition produced by 2×10^{-5}M KCN was reversed by either 4,6-dinitro-*o*-cresol or 2,4,5-trichlorophenol (Tables III and IV). The 1×10^{-6}M 4,6-dinitro-*o*-cresol, for example, raised the oxygen consumption from 2.1 to 2.7 (*i.e.* from 68 to 87 per cent of the untreated control) and the division from 2.56 to 3.25 (*i.e.* from 69 to 88 per cent of the untreated control). Repetition of this type of experiment during the seasons of 1936 and 1939 has shown that the reversal of the cyanide division inhibition by substituted phenols can be obtained only when the division in the cyanide alone is about 65–75 per cent of that in the control and when suboptimum respiratory concentrations of the substituted phenol are used.

TABLE III

Oxygen Consumption (C.Mm. Per Hour Per 10 C.Mm. Eggs) and Cell Division (Divisions Per Egg in 180 Minutes) of Fertilized Eggs of Arbacia punctulata in Various Combined Concentrations of Potassium Cyanide and 4,6-Dinitro-o-Cresol. Both Reagents Were Added 30 Minutes after Fertilization. The pH Was 8.0. Temperature, 20°C.

Concentration of 4,6-dinitro-*o*-cresol	No KCN		1×10^{-5} M KCN		2×10^{-5} M KCN		4×10^{-5} M KCN		8×10^{-5} M KCN		1.6×10^{-4} M KCN	
	O_2	Division	O_2	Division	O_2	Division	O_2	Division	O_2	Division	O_2	Division
moles per l. $\times 10^6$												
0	3.2	3.79	2.7	3.23	2.1	2.56	1.9	0.37	1.3	0	1.1	0
1	4.5	3.81	3.4	3.14	2.7	3.25	1.8	0.33	1.1	0	1.0	0
2	6.0	3.66	3.3	1.81	2.2	3.32	2.1	0.11	—		1.2	0
4	9.4	1.97	3.8	0.97	2.4	0.24	2.3	0	1.4	0	1.4	0
8	8.3	0.43	4.0	0.09	2.7	0	2.6	0	1.4	0	1.3	0
16	7.8	0.45	4.4	0.10	3.4	0	2.8	0	1.5	0	1.4	0
32	6.6	0.38	4.3	0.10	3.2	0	2.9	0	2.3	0	1.8	0
64	5.6	0.10	4.2	0	3.0	0	3.0	0	2.3	0	1.7	0
128	4.8	0	3.6	0	2.5	0	2.5	0	2.1	0	1.8	0
256	3.8	0	2.7	0	2.3	0	2.9	0	2.4	0	1.5	0
512	3.0	0	2.7	0	1.9	0	2.6	0	2.1	0	1.8	0

TABLE IV

Oxygen Consumption (C.Mm. Per Hour Per 10 C.Mm. Eggs) and Cell Division (Divisions Per Egg in 180 Minutes) of Fertilized Eggs of Arbacia punctulata in Various Combined Concentrations of Potassium Cyanide and 2,4,5-Trichlorophenol. Both Reagents Were Added 30 Minutes after Fertilization. The pH Was 7.5. Temperature, 20°C.

Concentration of 2,4,5-trichlorophenol	No KCN		2×10^{-5} M KCN		1.6×10^{-4} M KCN	
	O_2	Division	O_2	Division	O_2	Division
moles per l. $\times 10^6$						
0	3.1	3.48	2.5	2.36	0.9	0
1	3.5	3.52	2.5	2.93	0.7	0
2	3.2	3.47	2.5	2.77	0.9	0
4	3.9	3.37	2.5	1.85	1.1	0
8	4.3	2.93	2.8	1.53	1.1	0
16	7.5	0.75	2.8	0.70	1.1	0
32	9.2	0.14	3.5	0	1.4	0
64	4.1	0	3.2	0	1.7	0
128	3.0	0	2.4	0	1.6	0
256	2.3	0	2.7	0	1.8	0
512	2.4	0	2.5	0	1.8	0

These two groups of experiments, taken in conjunction with those on the joint effects of 4,6-dinitro-*o*-cresol and carbon monoxide, appear to afford

TABLE V

Oxygen Consumption (C.Mm. Per Hour Per 10 C.Mm. Eggs) of Fertilized Eggs of Arbacia punctulata in 4,6-Dinitro-o-Cresol with and without Various Concentrations of Potassium Cyanide. The pH Was 8.0. Temperature, 20°C.

Concentration 4,6-dinitro-*o*-cresol	Concentration KCN	O_2
moles per l. × 10^6	*moles per l.* × 10^6	
0	0	3.2
0	32	0.9
0	64	0.9
0	128	1.4
2	0	4.1
2	32	0.9
2	64	0.9
2	128	0.8
128	0	6.1
128	32	1.4
128	64	1.1
128	128	0.8

TABLE VI

Oxygen Consumption (C.Mm. Per Hour Per 10 C.Mm. Eggs) of Fertilized Eggs of Arbacia punctulata in 2,4,5-Trichlorophenol with and without Various Concentrations of Potassium Cyanide. The pH Was 8.0. Temperature, 20°C.

Concentration 2,4,5-trichlorophenol	Concentration KCN	O_2
moles per l. × 10^6	*moles per l.* × 10^6	
0	0	3.4
0	32	0.5
0	64	0.5
0	128	0.6
10	0	6.3
10	32	0.4
10	64	0.3
10	128	0.4
50	0	3.0
50	32	0.7
50	64	0.7
50	128	0.4

an important clue to the systems involved in respiratory stimulation and division inhibition by the substituted phenols.

In concentrations below the optimum, the respiratory increase produced by the substituted phenols is completely cyanide and carbon monoxide sensitive. As the concentration of the substituted phenol is progressively increased beyond the optimum, these reagents themselves inhibit more and more of the cyanide and carbon monoxide sensitive respiration so that the percentage inhibition by cyanide or carbon monoxide again approaches that in eggs not treated with the substituted phenol. This result appears to be very significant for the interpretation of the effects of the phenols on cell division. It has been shown in previous papers (2, 3) that the minimum concentrations of substituted phenols required to inhibit division completely are invariably the concentrations just larger than those which produce optimum respiratory stimulation. Hence it appears that the substituted phenols begin to inhibit division in those concentrations which produce the initial inhibition of the cyanide sensitive oxidative systems. This suggests that inhibition of cell division by the substituted phenols is to be associated not with over-stimulation of oxidative processes but with the inhibition of a particular type of oxidative process, even though the total oxygen uptake of the eggs may be greatly in excess of the control value. This tentative conclusion is given further support by the fact that the division effects of *greater than optimum* concentrations of the substituted phenols are always additive and never antagonistic to those of cyanide or carbon monoxide.

Phenylurethane, 5-Isoamyl-5-Ethyl Barbituric Acid, and Malonic Acid.— These three agents produce a marked inhibition of the activity of several dehydrogenases (11) but, in contrast to cyanide and carbon monoxide, have little or no effect on the iron containing respiratory pigments (12).

As pointed out by Warburg (13) and emphasized in a previous paper of this series (7), phenylurethane produced, at 10^{-3}M, a reversible block to the cell division of fertilized sea urchin eggs. At this concentration, which reduced the oxygen consumption of the eggs untreated with substituted phenol to 71 per cent of the control value, this reagent prevented maximum respiratory stimulation by 4,6-dinitro-*o*-cresol but was much less effective in this respect than cyanide or carbon monoxide in concentrations giving the same degree of inhibition of respiration in a series untreated with substituted phenol (Fig. 2 and Table I). The inhibition of division by phenylurethane was not antagonized by any concentration of 4,6-dinitro-*o*-cresol.

In a concentration of 10^{-3}M, which produced little or no reduction of respiration in a control series, 5-isoamyl-5-ethyl barbituric acid produced a very slight reduction of respiratory stimulation by concentrations of 4,6-dinitro-*o*-cresol less than that required for the respiratory optimum and

a much larger reduction of respiratory stimulation by concentrations of 4,6-dinitro-*o*-cresol greater than the respiratory optimum (Fig. 3 and Table I). This is the reverse of the picture obtained when cyanide or carbon monoxide were used as inhibitors. At 2 × 10^{-3}M 5-isoamyl-5-

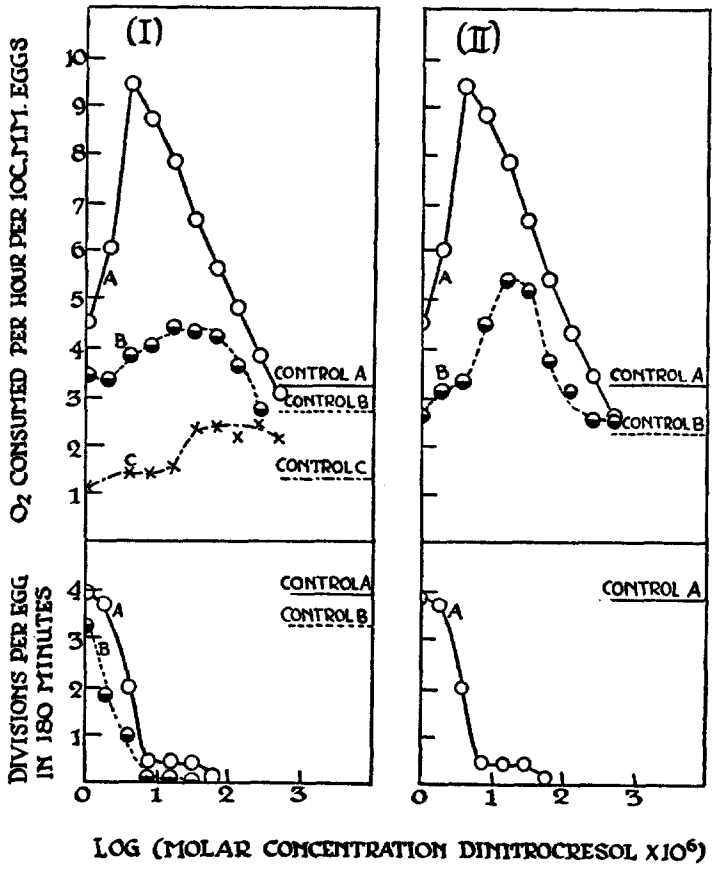

FIG. 2. Effect of various concentrations of 4,6-dinitro-*o*-cresol on oxygen consumption and cell division of *Arbacia* eggs: I*A*, with no other reagent; I*B*, in 10^{-5}M KCN; I*C*, in 8 × 10^{-5}M KCN; II*A*, with no other reagent; II*B*, in 10^{-3}M phenylurethane.

The reagents were added 30 minutes after fertilization. Temperature, 20°C.

ethyl barbituric acid, which reduced the respiration of the untreated eggs to 77 per cent of the control, all respiratory stimulation by 4,6-dinitro-*o*-cresol was prevented. This agent is therefore more efficient than cyanide as an inhibitor of respiratory stimulus by substituted phenols when comparisons are made at concentrations of the two agents which produce equal effects on the respiration of eggs untreated with 4,6-dinitro-*o*-cresol.

The results with malonic acid (Fig. 3 and Table I) were comparable, both in kind and degree, to those obtained with 5-isoamyl-5-ethyl barbituric acid, which contains a substituted malonic acid as part of its structure. At the concentrations used, neither 5-isoamyl-5-ethyl barbituric acid nor malonic

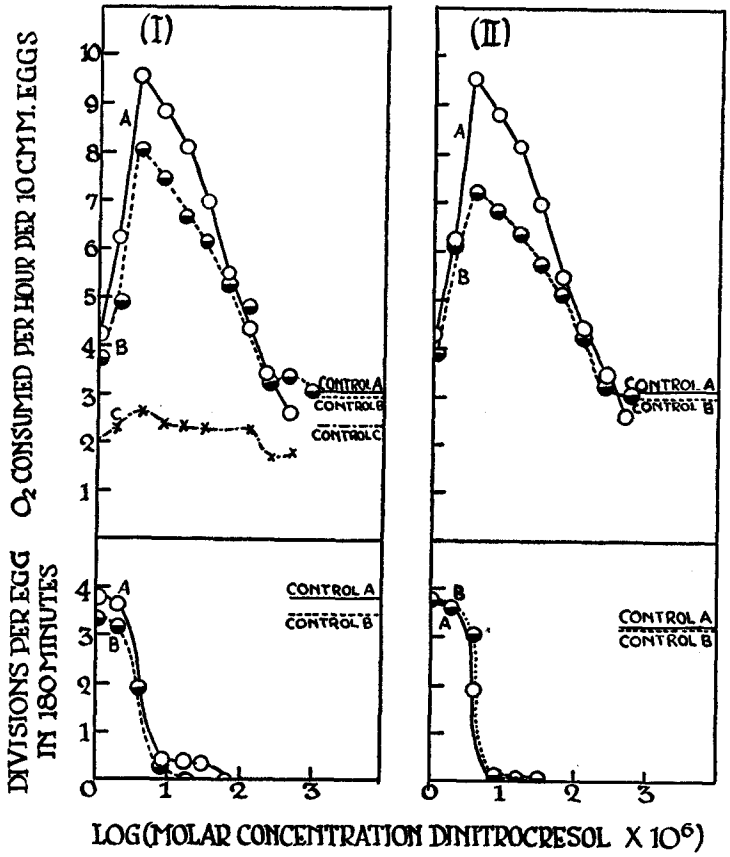

FIG. 3. Effect of various concentrations of 4,6-dinitro-*o*-cresol on oxygen consumption and cell division of *Arbacia* eggs: I*A* and II*A*, with no other reagent; I*B*, in 10^{-3}M 5-isoamyl-5-ethyl barbituric acid; I*C*, in 2×10^{-3}M 5-isoamyl-5-ethyl barbituric acid; II*B*, in 10^{-3}M malonic acid.

The reagents were added 20–30 minutes after fertilization. Temperature, 20°C.

acid had any marked effect on cell division, regardless of whether or not 4,6-dinitro-*o*-cresol was present. Greville (14) has reported that malonic acid limits respiratory stimulation by 4,6-dinitro-*o*-cresol in rat liver.

Iodoacetic Acid.—Iodoacetic acid, when used at a pH sufficiently low to allow its penetration into the eggs, produced an inhibition of respiration of sea urchin eggs as shown by Runnström (15) and confirmed in a previous

paper of this series (7). It also produced a reduction of respiratory stimulation by 4,6-dinitro-*o*-cresol which was comparable in degree to its effect on the respiration of eggs untreated with substituted phenol. Its slight inhibitory effect on cell division was additive to that of the 4,6-dinitro-*o*-cresol (Table VII). Bodine and Boell (8) have observed that iodoacetic acid limits respiratory stimulation by 2,4-dinitrophenol in orthopteran embryos.

TABLE VII

Oxygen Consumption (C.Mm. Per Hour Per 10 C.Mm. Eggs) and Cell Division (Divisions per Egg in 175 Minutes) of Fertilized Eggs of Arbacia punctulata in 8 × 10^{-6} M 4,6-Dinitro-o-Cresol with and without Various Concentrations of Iodoacetic Acid. Iodoacetic Acid Was Added at 25 Minutes after Fertilization, the 4,6-Dinitro-o-Cresol at 55 Minutes. The pH Was Approximately 6.0. Temperature, 20°C.

Concentration 4,6-dinitro-*o*-cresol	Concentration iodoacetic acid	O_2	Division
moles per l. × 10^6	*moles per l.* × 10^4		
0	0	3.0	1.88
0	2.1	2.7	2.20
0	4.2	2.4	2.05
0	8.4	2.4	1.82
0	16.8	2.0	1.05
0	33.6	1.6	0.12
8	0	5.9	0.56
8	2.1	5.0	0.35
8	4.2	5.4	0.30
8	8.4	5.1	0.31
8	16.8	3.6	0.18
8	33.6	1.2	0.08

Non-Stimulating Phenols.—2,4-dinitrothymol and 4-nitrocarvacrol differ from all other substituted phenols so far investigated in that they have been found to produce, in very small concentrations, a reversible inhibition of the cell division of fertilized *Arbacia* eggs without producing, in any concentration, a stimulation of oxygen consumption (2, 10). This appears to afford further evidence that the division inhibition by substituted phenols is associated with a depression and not a stimulation of respiratory processes. Although 2,4-dinitrothymol and 4-nitrocarvacrol produced only a moderate reduction of normal respiration, the former, at 1.6 × 10^{-6}M, produced a complete block of respiratory stimulation by 4,6-dinitro-*o*-cresol or by 2,4,5-trichlorophenol; the latter, at 4 × 10^{-6}M, likewise produced a complete block to respiratory stimulation by 4,6-dinitro-*o*-cresol (Fig. 4).

Another substituted phenol, *o*-nitrophenol, produced no inhibition of cell division and no inhibition of oxygen consumption. This substance in concentrations up to 2×10^{-3}M (*i.e.*, a thousand times the effective con-

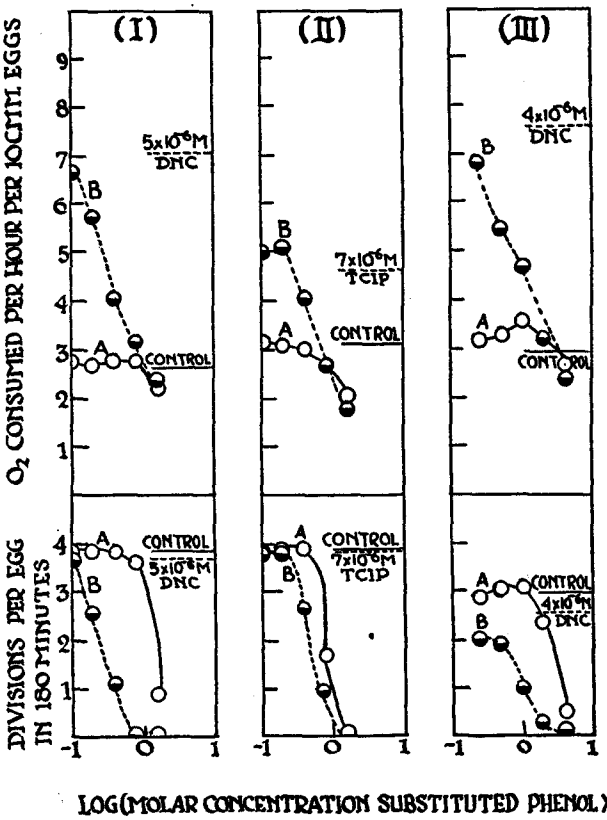

FIG. 4. Effect of various concentrations of non-stimulating phenols on oxygen consumption and cell division of fertilized *Arbacia* eggs with and without a fixed concentration of a stimulating phenol: I*A*, 2,4-dinitrothymol; I*B*, 2,4-dinitrothymol and 5×10^{-6}M 4,6-dinitro-*o*-cresol (DNC) together; II*A*, 2,4-dinitrothymol; II*B*, 2,4-dinitrothymol and 7×10^{-6}M 2,4,5-trichlorophenol (TCLP) together; III*A*, 4-nitrocarvacrol; III*B*, 4-nitrocarvacrol and 4×10^{-6}M 4,6-dinitro-*o*-cresol together.

All reagents were added 20–30 minutes after fertilization. Temperature, 20°C.

centration of 2,4-dinitrothymol) produced only a barely detectable reduction of respiratory stimulation by 4,6-dinitro-*o*-cresol.

The division inhibiting effects of 2,4-dinitrothymol and 4-nitrocarvacrol were additive to those of 4,6-dinitro-*o*-cresol; *o*-nitrophenol, which alone has no inhibiting effect on division, displayed in conjunction with 4,6-dinitro-*o*-cresol a very slight inhibiting effect comparable in degree with the very

small inhibiting effect which it had on 4,6-dinitro-*o*-cresol respiratory stimulation.

DISCUSSION AND SUMMARY

The effects of 4,6-dinitro-*o*-cresol and 2,4,5-trichlorophenol on the respiration and cell division of fertilized eggs of *Arbacia punctulata* have been determined in the presence of each of a number of respiratory inhibitors. The experimental results obtained appear to afford some understanding of the mechanism of action of the substituted phenols on respiration and on cell division.

1. From the fact that the stimulated respiration is completely cyanide and carbon monoxide sensitive, it may be concluded that all of the extra oxygen uptake induced in *Arbacia* eggs by 4,6-dinitro-*o*-cresol passes through the metal containing oxidase system. All of the extra oxygen uptake also passes through oxidative steps which can be poisoned by non-stimulating phenols like 2,4-dinitrothymol and 4-nitrocarvacrol, by phenylurethane, by 5-isoamyl-5-ethyl barbituric acid, by malonic acid, or by iodoacetic acid. To abolish all respiratory stimulation by suboptimum concentrations of 4,6-dinitro-*o*-cresol, each of these inhibitors must be present in a concentration which reduces the normal respiration in the absence of substituted phenols by at least 20–40 per cent.

2. The degree of reduction of the stimulated respiration by a given concentration of carbon monoxide or potassium cyanide depends on the concentration of 4,6-dinitro-*o*-cresol or 2,4,5-trichlorophenol, being most marked in suboptimum concentrations and least marked in greater than optimum concentrations of the substituted phenol. In contrast to this result, the reduction of the stimulated respiration by a given concentration of 5-isoamyl-5-ethyl barbituric acid or malonic acid is least marked in suboptimum concentrations and most marked in greater than optimum concentrations of the substituted phenol.

3. The present experiments appear to indicate that the inhibition of cell division by substituted phenols is not attributable to a direct action of these agents on mitotic processes nor to an overstimulation of any respiratory process. The inhibition of cell division appears to be associated with the inhibition, by the substituted phenols, of some component of the cyanide sensitive respiratory system. This inhibition is of such a type as to allow the overall respiration to proceed at a rate in excess of the control value, even when division is completely suppressed. The dependence of the division mechanism on a respiratory step which is relatively hypersensitive to poisoning by the substituted phenols is comparable to the depend-

ence of the Pasteur reaction in certain normal and tumor tissues on an oxidative step which is specifically poisoned by the substituted phenols (16).

The substituted phenols have no inhibiting effect *in vitro* on the principal metal containing respiratory catalysts or the principal dehydrogenases; they also do not inhibit the fermentative reactions involved in the anaerobic glycolysis of fertilized *Arbacia* eggs. It is therefore suggested that the respiratory inhibiting and division inhibiting effects of the substituted phenols may be attributable to the action of these substances on one or more of the oxidation-reduction or phosphorylating steps which are involved in the transfer of hydrogen from the dehydrogenase systems to the specifically cyanide sensitive oxidase mechanism of the eggs. The identification of the respiratory step poisoned by the substituted phenol would constitute an interesting contribution to the chemistry of cell division and experiments to this end are now in progress.

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