STUDIES ON CELL METABOLISM AND CELL DIVISION

II. Stimulation of Cellular Oxidation and Reversible Inhibition of Cell Division by Dihalo and Trihalophenols

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Two types of reagents have previously been known to produce large increases in the rate of oxygen consumption by living cells. These are, first, certain oxidation-reduction indicators (2), and, secondly, certain nitro and dinitro derivatives of phenols (7). (See also Krahl and Clowes (6) and Alwall (1) for numerous other references.) In this paper it will be shown that certain dihalo and trihalophenols produce, in fertilized sea urchin eggs (*Arbacia punctulata*), a rise in oxygen consumption and a reversible block to cell division comparable to those effected by nitro and dinitrophenols and differing from those obtainable with the oxidation-reduction indicators of the type of methylene blue.

On the basis of experimental results presented in a previous paper (4), it was tentatively concluded that the metabolism stimulating and reversible division blocking properties of the nitrophenols were not the consequence of reduction or reversible oxidation of the nitrophenol molecule. Since the dihalo and trihalophenols display the same oxidation promoting quality and nearly the same reversible division suppressing ability as the nitro and dinitrophenols, it is reasonable to believe that all these phenol derivatives have a common mode of action on the cell.

In a dihalo or trihalophenol molecule there are no substituent groups capable of reduction, or reversible oxidation-reduction, and the entire halogen substituted phenol molecule is oxidized only irreversibly and at very high positive potentials (5). Hence the present experiments provide further confirmation of the hypothesis previously advanced.

According to this view, the dinitrophenols and the dihalophenols derive their intense biological activity from the presence of the phenolic OH group, as modified by suitable substitution in the benzene ring.

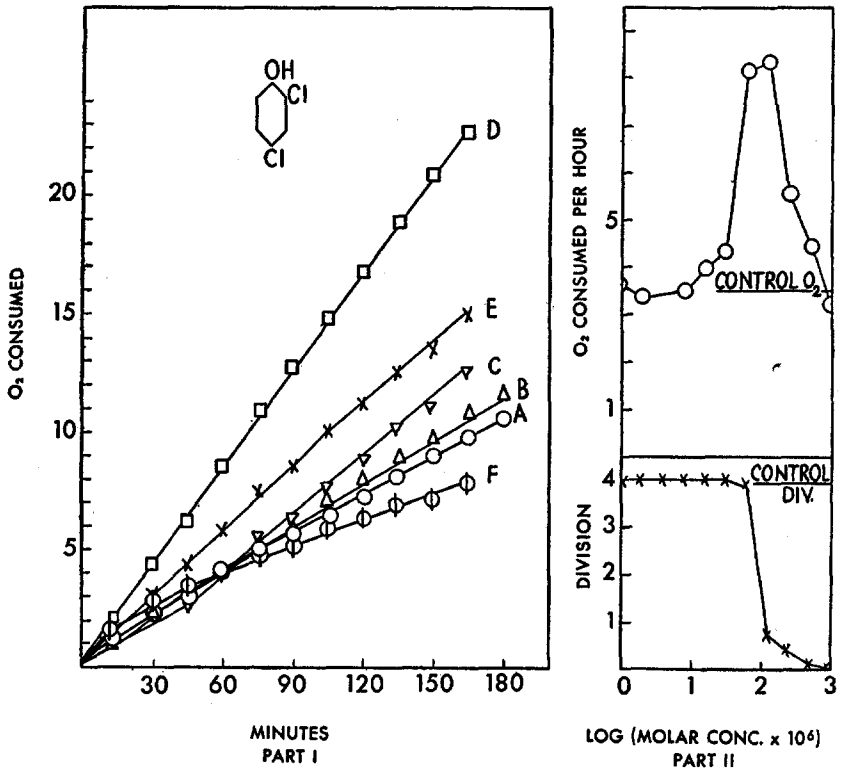

FIG. 1. Part I. Oxygen consumption, at 20°C., of fertilized *Arbacia* eggs for various periods of time in the following molar concentrations of 2,4-dichlorophenol: *A*, none-control; *B*, 1.6×10^{-5}; *C*, 3.2×10^{-5}; *D*, 1.28×10^{-4}; *E*, 2.56×10^{-4}; *F*, 1.024×10^{-3}.

Part II. The hourly oxygen consumption and the cell division, at 20°C., of fertilized *Arbacia* eggs in various concentrations of 2,4-dichlorophenol. Reagent added 25 minutes after fertilization.

Although this concept can be made to explain all of the experimental facts available, and although it has led to the realization that the dihalophenols should and do act as cellular oxidative stimulants, further elaboration of the idea will be reserved until the theoretical

consequences of such a concept can be tested by further experiments.

The present experiments were performed and the results expressed by the use of the methods described in a previous paper (4). Fertilized eggs of the sea urchin (*Arbacia punctulata*) were employed.

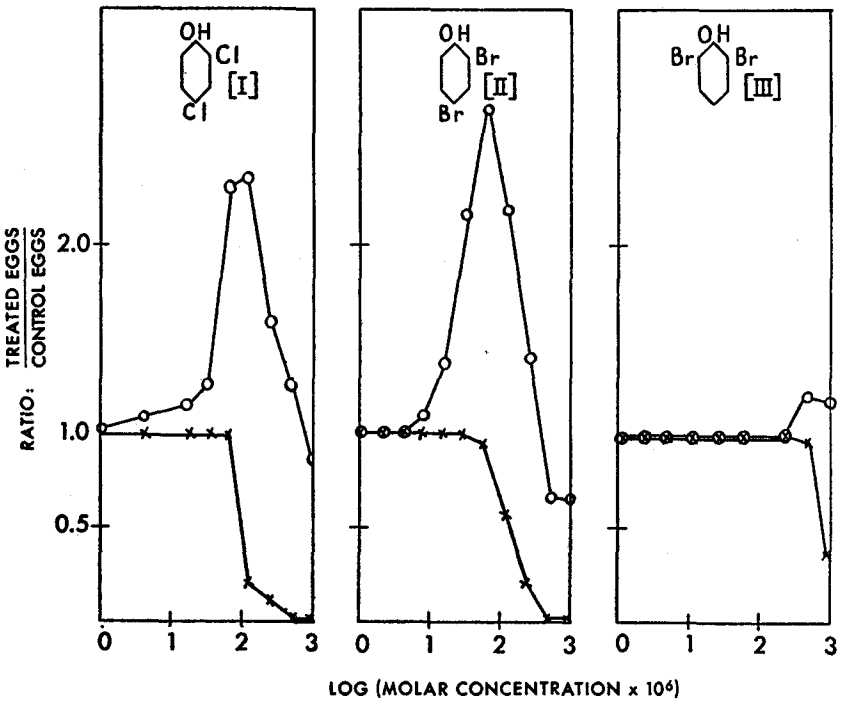

FIG. 2. Stimulation of oxygen consumption and block to cell division of fertilized *Arbacia* eggs produced by various concentrations of 2,4-dichlorophenol (I), 2,4-dibromophenol (II), and 2,6-dibromophenol (III) at 20°C. Reagent added 15 minutes after fertilization.

In Figs. 2–6

$$\text{O} - \text{O} = \frac{\text{O}_2 \text{ consumed in treated eggs}}{\text{O}_2 \text{ consumed in control eggs}}$$

$$\times - \times = \frac{\text{Cell division in treated eggs}}{\text{Cell division in control eggs}}$$

EXPERIMENTAL RESULTS

To illustrate the behavior of the dihalo and trihalophenols, the results with 2,4-dichlorophenol may be cited in detail. When, at a

given time after fertilization, various concentrations of this reagent are added to samples of fertilized and developing eggs of *Arbacia*, the following results are obtained.

Within a short time after the addition of a suitable single stimulating concentration of the reagent, the rate of oxygen consumption by the eggs is increased to more than twice the normal value. This increased rate is maintained for at least 3 hours (Fig. 1).

The rate of oxygen consumption (which is here expressed as the hourly average of a 2 hour observation) depends on the concentration of reagent. As this concentration is increased from zero, the rate of oxygen consumption by the treated eggs increases until an optimum rate is attained. At still higher concentrations the rate of oxygen consumption is less than at this optimum.

If a concentration of 2,4-dichlorophenol slightly greater than the optimum for respiration is added within 30 minutes after fertilization, at 20°C., the eggs do not divide while the untreated control eggs proceed to the 16 cell stage.

90 to 100 per cent of all fertilized eggs which have been completely blocked in division by 2,4-dichlorophenol resume their mitotic activity immediately after return to sea water and develop to swimming larvae. This holds true for exposures of 3 hours and concentrations up to 10^{-3} molar, the latter concentration being ten to twenty times the optimum for respiration. This reversibility of the division blocking effect in concentrations greater than the respiratory optimum deserves particular emphasis because it is displayed by the respiratory stimulating nitro compounds and is not shown by any of the respiratory stimulating oxidation-reduction dyes which have so far been investigated (4).

A second general question of interest, in view of the results previously obtained with nitro compounds (3, 4) is that of variation in intensity of block to cell division obtainable by varying the time after fertilization at which a suitable fixed concentration of reagent is added to a sample of eggs. As in the case of the nitro compounds, it is found that the division block is greatest when the reagent is added within thirty minutes after fertilization. If the reagent is added at subsequent times during the first mitotic cycle, the eggs can complete the first division but do not perform the second division (Table I). This

cyclic variation in sensitivity to 2,4-dichlorophenol is repeated in the second mitotic cycle of fertilized *Arbacia* eggs.

An experimental survey of a number of other dihalophenols and their derivatives brings out the following data concerning the dependence of biological activity on the type and arrangement of the substituent groups in the phenol molecule.

First, the introduction of a methyl group into dichlorophenol does not markedly affect the activity, since 4,6-dichloro-*o*-cresol is approximately equal to 2,4-dichlorophenol in regard to its effective concen-

TABLE I

The Effect of 10^{-4} Molar 2,4-Dichlorophenol on Division in Fertilized Eggs of Arbacia punctulata at Various Times after Fertilization. Temperature 19°C., and pH 8.2

Time of addition after fertilization	Divisions per egg at addition of reagent	Divisions per egg 180 min. after fertilization
min.		
10	0	0.02
20	0	0.12
30	0	0.14
40	0	0.43
50	0	0.83
60	0.05	0.90
65	0.30	0.94
70	0.90	0.95
75	0.97	1.00
No addition	—	4.00

tration and in regard to the percentage effect produced (Fig. 3). The presence of aliphatic side chains in addition to the methyl group lowers the aqueous solubility of the halogenated phenols and, perhaps partially as a result of this lower solubility, lowers the activity. For instance, 2,4-dibromothymol and 4,6-dibromocarvacrol (Fig. 4) are much less active than 2,4-dichlorophenol or 2,4-dibromophenol.

Certain types of enlargement of the central ring nucleus also decrease the aqueous solubility and lower the activity of the dihalophenols. This may be seen by comparing the results obtained with 2,4-dichloro-α-naphthol (Fig. 3) and 5,7-dibromo-8-hydroxyquino-

line (Fig. 4) with the results obtained with 2,4-dichloro and 2,4-dibromophenol.

Second, with a fixed arrangement of halogen and alkyl substituents in relation to the phenol group, the dibromo substituted molecule appears to be slightly more effective than the dichloro compound.

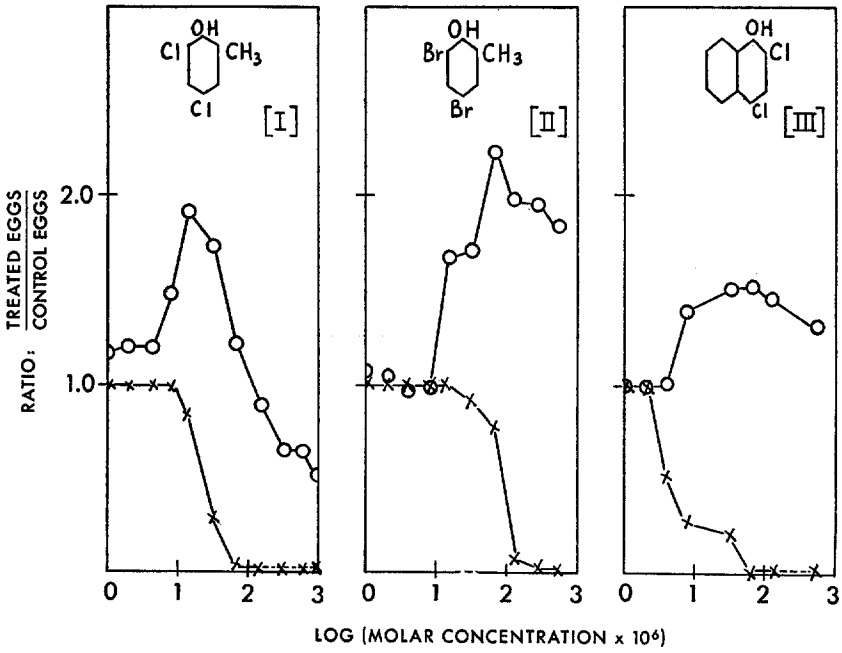

FIG. 3. Stimulation of oxygen consumption and block to cell division of fertilized *Arbacia* eggs produced by various concentrations of 4,6-dichloro-*o*-cresol (I), 4,6-dibromo-*o*-cresol (II), and 2,4-dichloro-α-naphthol (III) at 20°C. The dotted division lines denote irreversible injury. Reagent added 15 minutes after fertilization.

The phenols, cresols, and thymols may be cited as examples (Figs. 2, 3, and 4).

Third, 2,6-dibromophenol is less effective than 2,4-dibromophenol (Fig. 2).

Fourth, 2,4-dichloroaniline and the dichloro benzenes, compounds containing respectively an amino and a hydrogen in place of the OH,

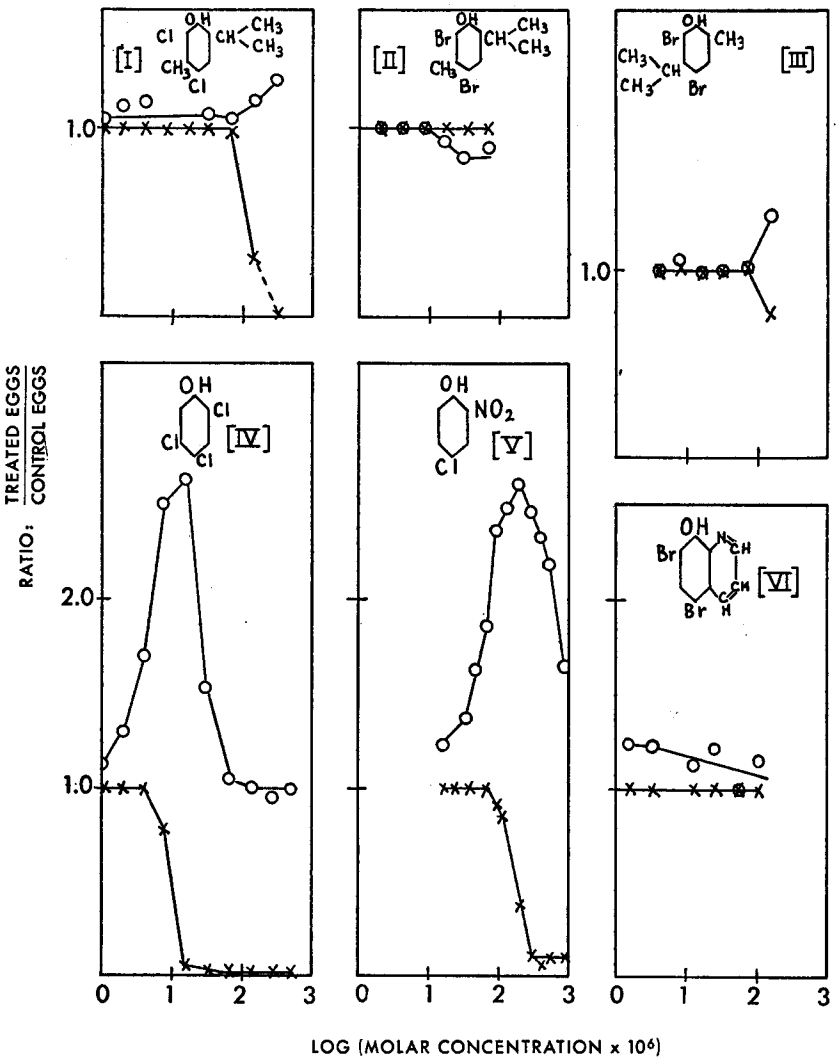

FIG. 4. Stimulation of oxygen consumption and block to cell division of fertilized *Arbacia* eggs produced by various concentrations of 2,4-dichlorothymol (I), 2,4-dibromothymol (II), 4,6-dibromocarvacrol (III), 2,4,5-trichlorophenol (IV), *o*-nitro-*p*-chlorophenol (V), and 5,7-dibromo-8-hydroxyquinoline (VI) at 20°C. Dotted division lines denote irreversible injury. Reagents added 15 minutes after fertilization.

have no ability to stimulate oxidation or block division in fertilized *Arbacia* eggs.

Since *p*-chlorophenol and *p*-chlorothymol were found to possess no respiratory stimulating or reversible division blocking ability, no other monohalophenols were investigated.

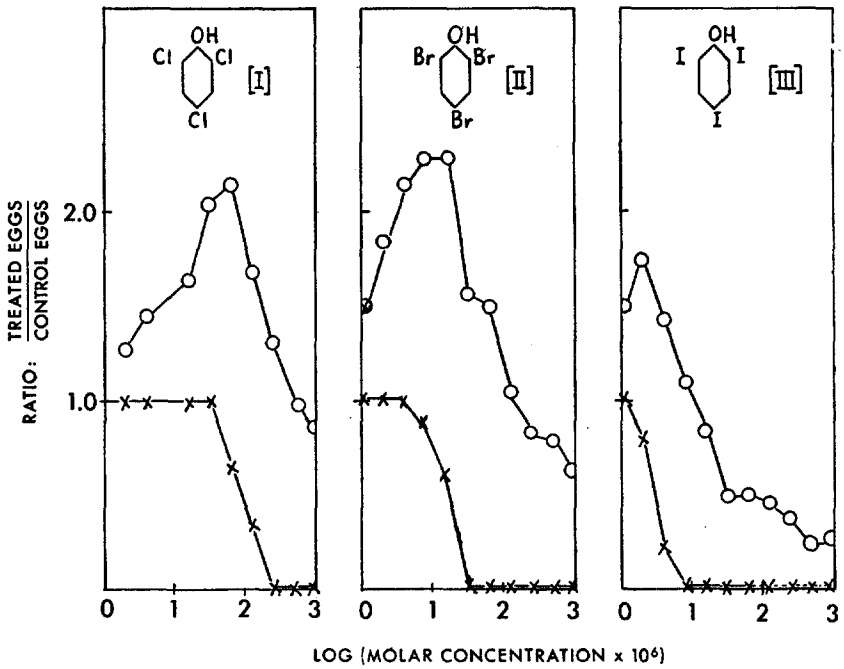

FIG. 5. Stimulation of oxygen consumption and block to cell division of fertilized *Arbacia* eggs produced by various concentrations of 2,4,6-trichlorophenol (I), 2,4,6-tribromophenol (II), 2,4,6-triiodophenol (III) at 20°C. Dotted division lines denote irreversible injury. Reagent added 15 minutes after fertilization.

Of the trihalophenols investigated 2,4,5-trichlorophenol gives the highest percentage rise in oxygen consumption. It also produces a reversible block to cell division at very great dilution. According to both of these criteria, 2,4,5-trichlorophenol compares favorably with any of the nitrophenols (Fig. 4).

The 2,4,6-trichlorophenol produces a smaller optimum percentage rise in oxygen consumption and, in order to produce this rise and to

block cell division, requires a higher concentration than its 2,4,5 isomer (Fig. 5). The 2,4,6-tribromophenol and 2,4,6-triiodophenol yield a respiratory stimulation and cell division block in smaller concentrations than those required for the two trichlorophenols first mentioned, but with the iodo compound the increase in oxygen consumption is small (Fig. 5).

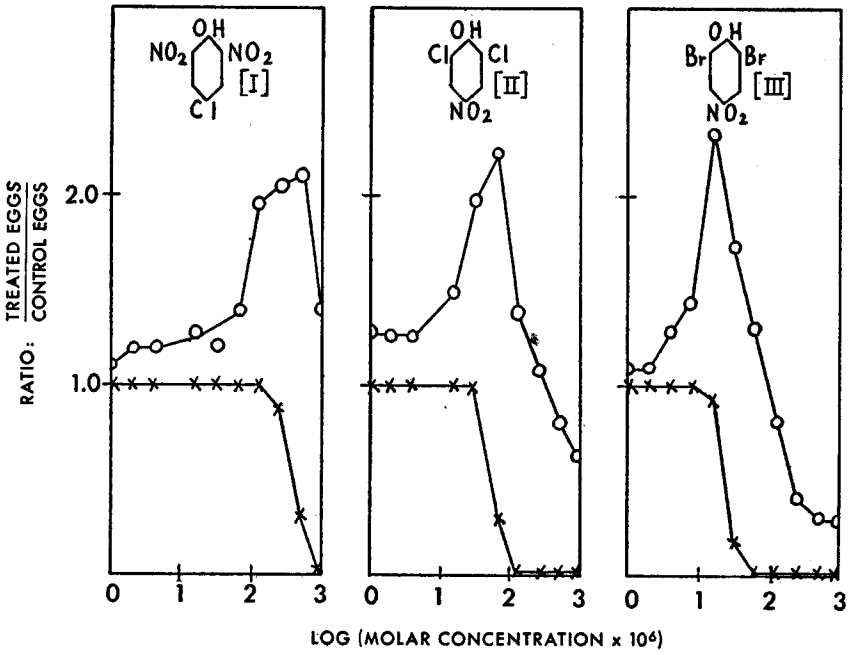

FIG. 6. Stimulation of oxygen consumption and block to cell division of fertilized *Arbacia* eggs produced by various concentrations of 2,6-dinitro-4-chlorophenol (I), 2,6-dichloro-4-nitrophenol (II), 2,6-dibromo-4-nitrophenol (III) at 20°C. Reagents added 25 minutes after fertilization.

High concentration of 2,4,6-tribromo and 2,4,6-triiodophenol, and, to a lesser extent, of 2,4-dibromophenol and 2,4-dichloro-α-naphthol produces some cytolysis of fertilized *Arbacia* eggs. For this reason, eggs blocked in division by high concentration of these agents show a relatively low percentage of recovery when returned to sea water.

In general, phenols which contain both nitro and halo substituents

have respiratory stimulating and division blocking properties very similar to those of the phenols containing only nitro groups and those of the phenols containing only halo groups. An example having one nitro and one chloro group is *o*-nitro-*p*-chlorophenol. This compound (Fig. 4) is much more active than either *o*-nitro or *p*-chlorophenol, somewhat more active than 2,4-dichlorophenol, almost equal in activity to *p*-nitrophenol, and slightly less active than 2,4-dinitrophenol.

A phenol containing two nitro substituents and one halogen, or a phenol containing two halogen substituents and one nitro has nearly the same respiratory stimulating and division blocking effects as the trihalophenols (Fig. 6).

For comparison, the optimum levels of respiratory stimulation produced in fertilized *Arbacia* eggs by representative nitro and halophenols may be expressed in the following order as percentages of normal: *p*-nitrophenol, 342; 4,6-dinitro-*o*-cresol, 300; 2,4-dinitrophenol, 292; 2,4,5-trichlorophenol, 264; 2,4-dichlorophenol, 236; 2,6-dichloro-4-nitrophenol, 232; 2,4,6-tribromophenol, 227; 2,6-dibromo-4-nitrophenol, 222; 2,4,6-trichlorophenol, 214; 2,6-dinitro-4-chlorophenol, 205; 2,4,6-triiodophenol, 175; 2,4,6-trinitrophenol, none.

The molar concentrations of these reagents required to produce the optimum respiratory rise and initial division block fall in the following order: 2,4,6-triiodophenol, 2×10^{-6}; 4,6-dinitro-*o*-cresol, 8×10^{-6}; 2,4,6-tribromophenol, 10^{-5}; 2,4,5-trichlorophenol, 2×10^{-5}; 2,6-dibromo-4-nitrophenol, 2×10^{-5}; 2,4-dinitrophenol, 3×10^{-5}; 2,4,6-trichlorophenol, 6×10^{-5}; 2,6-dichloro-4-nitrophenol, 6×10^{-5}; *p*-nitrophenol, 10^{-4}; 2,4-dichlorophenol, 10^{-4}; 2,6-dinitro-4-chlorophenol, 3×10^{-4}. Using these values as a key, other relationships may be found by referring to the figures in this and a previous paper (4).

In having a high division blocking activity associated with a small stimulation of respiration 2,4,6-triiodophenol is similar to 2,4-dinitrothymol.

It was obviously desirable to determine whether the dihalo and trihalophenols produced respiratory effects in mammals similar to those described here for single cells. From experiments carried out in

collaboration with Dr. K. K. Chen, it appears that injection or oral administration of 2,4-dichlorophenol, 2,4,5-trichlorophenol, and other halophenols does not produce significant increases in the body temperature or the respiratory rate of rats or dogs.

DISCUSSION AND SUMMARY

The dihalo and trihalophenols, and phenols containing both halo and nitro substituents in the same molecule, produce, in fertilized eggs of *Arbacia punctulata*, a rise in rate of oxygen consumption and a reversible block to cell division. To define the conditions which affect the degree of this activity, the following factors have been varied: the arrangement of substituents in the molecule, the concentration of reagent, and the time after fertilization at which the reagent is added.

The stimulation of oxygen consumption and reversible block to cell division produced by the dihalophenols are qualitatively the same as those previously produced in fertilized *Arbacia* eggs by certain dinitrophenols. To yield optimum respiratory effect and maximum division block, it usually requires a higher concentration of dihalo than of the corresponding dinitrophenol. For example, with fertilized *Arbacia* eggs at 20°C., 2,4-dinitrophenol, in optimum concentration of 3×10^{-5} molar, raises oxygen consumption to 292 per cent of normal (4). The corresponding values for two dihalo analogues are: 2,4-dichlorophenol, 10^{-4} molar and 236 per cent; 2,4-dibromophenol, 6×10^{-5} molar and 282 per cent.

The halophenols differ from the nitrophenols in two interesting respects: (*a*) The monohalophenols produce little or no oxidative stimulation or division block in fertilized *Arbacia* eggs; *p*-nitrophenol is very active in both respects. (*b*) The symmetrical trihalophenols have an appreciable ability to stimulate oxygen consumption and block division; symmetrical trinitrophenol is inactive in both respects (4).

The increases in oxygen consumption produced in fertilized *Arbacia* eggs by 2,4-dichloro and 2,4-dinitrophenol are larger than the percentage increases given by methylene blue and *o*-cresol indophenol under the same experimental conditions. The dihalo and dinitrophenols produce a reversible block to the cell division of fertilized

marine eggs. The oxidation-reduction indicators, in contrast to the dihalo and dinitrophenols, block cell division irreversibly and fertilized eggs of *Arbacia* do not recover from optimum respiratory stimulating concentrations of these oxidation-reduction dyes.

The present experiments with halophenols are in harmony with and lend considerable support to the hypothesis (4) that nitro and similarly substituted phenols derive their biological activity from the presence and properties of the phenolic OH group, as modified by proper substitution in the phenolic benzene ring.

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