Action of Cyclodiene Pesticides on Oxidative Metabolism in the Yeast *Saccharomyces cerevisiae*

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The chlorinated pesticide chlordane inhibits cell growth of *Saccharomyces cerevisiae* when nonfermentable substrates (glycerol, lactate, or ethanol) are provided as the energy source, but not when fermentable sugars (glucose, galactose, or fructose) are the energy sources. This suggests that chlordane, as well as several other cyclodienes, inhibits yeast growth by interfering specifically with oxidative metabolism. Chlordane does not induce respiratory deficient mutants.

With the exception of the organophosphate compounds, little is known about the mechanisms of toxic action of most pesticides. Limited evidence indicates that certain chlorinated hydrocarbon pesticides exert their toxic action on the nervous system (Ecobichon and Saschenbrecker, 1969; Naraahashi and Haas, 1967). However, in view of the high concentrations of DDT needed to induce alterations in electrical impulses in nerve fibers (Naraahashi and Haas, 1967), the question is unresolved whether the primary limiting action *in vivo* involves impulse conduction *per se* or the production of energy required for generation of axon potentials.

We have been concerned with the possibility that certain pesticides and pesticidal synergists act by interfering with energy metabolism (Nelson, 1970a). As part of this program, we have attempted to determine the action of these agents on oxidative metabolism both *in vitro* and *in vivo*. The *in vivo* action of environmental agents has been determined by measuring growth-inhibition of *Saccharomyces cerevisiae* supplied with either fermentable (glucose, galactose, or fructose) or nonfermentable (glycerol, lactate, or ethanol) energy sources. Agents which act specifically on mitochondrial oxidation will inhibit growth when nonfermentable compounds are the sole energy source, but have no effect on growth supported by fermentable substrates. This simple technique has been useful for evaluating antibiotic effects on mitochondrial biogenesis and function (Davey *et al.*, 1970; Kovac *et al.*, 1967; Linnane *et al.*, 1968).

The data reported in this paper show that certain cyclodiene pesticides inhibit yeast cell growth by interfering with oxidative metabolism. Their action cannot be explained on the basis of induction of respiratory deficient (RD) cells.

MATERIALS AND METHODS

Studies were carried out using a haploid strain (D273-10B) of *S. cerevisiae* supplied by Gottfried Schatz. Cells were grown in the liquid medium described by Wallace *et al.* (1968) containing, in g per l.: Difco Yeast extract 10 g/l. (Linnane *et al.*, 1968), NaCl (0.5), $MgCl_26H_2O$ (0.7), $(NH_4)_2SO_4$ (1.2), $CaCl_2$ (0.1), KH_2PO_4 (1.0), and $FeCl_3$ (0.005). This basic medium was supplemented with 1% glucose, galactose, or fructose, or 3% glycerol, 2% lactate, or 3% ethanol as the energy source. Growth was carried out in 100 ml of media in 250-ml Erlenmeyer flasks with continuous agitation on a rotary shaker at 26° C. The medium was inoculated with early log phase cells (4 hr) to give a final cell concentration of 2 or 5 × 10^4 cells per ml, depending upon the energy source. Pesticides were added immediately after inoculation in 1 ml of a 0.01 *M* pesticide solution in dimethyl sulfoxide (DMSO). Control cultures received DMSO with no pesticide. Incubation was continued for 20 hr at which time growth was quantitated by counting cells in a hemacytometer or by optical density measurements at 540 mμ (Table II). Aliquots were then removed for respiration, plating efficiency, or respiratory deficiency determinations.

RESULTS AND DISCUSSION

The data in Figure 1 show that chlordane (10^{-4} *M*) completely abolished growth when glycerol and ethanol were supplied as the energy source, but had little or no effect with glucose as the energy source. Comparisons using galactose or fructose as the fermentable substrate and glycerol, lactate, or ethanol as the nonfermentable energy source produced similar results. Figure 2 shows the effects of chlordane and the phosphorylation uncoupling agent, 2,4-dinitrophenol (DNP), on glucose and lactate-supported growth. As expected, both compounds inhibited growth on lactate but had little or no effect on cell growth in the presence of glucose. These results show that chlordane inhibits cell growth only when nonfermentable energy sources are supplied and, in this respect, mimics the action of DNP.

High concentrations of chlordane (10^{-4} *M*) were used in the previous experiments to insure maximum inhibition of cell growth in nonfermentable substrates. However, lower concentration of chlordane also produces significant inhibition of cell growth (as shown in Figure 3) for cells supplied with lactate. The toxicity threshold of chlordane lies at approximately 5 × 10^{-7} *M* (0.2 μg per ml) to 1 × 10^{-6} *M* (0.4 μg per ml). Chlordane concentrations higher than 5 × 10^{-6} *M* to 1 × 10^{-5} *M* (2.0 to 4.0 μg per ml) are completely inhibitory. The significance of these values are, however, open to speculation, since the concentration of chlordane inside the cell, or more importantly, in the mitochondria, is not known. It is possible that penetration of chlordane through the cell wall is limited, and the effective concentration within the mitochondria is very small. Moreover, we have not determined whether yeast can metabolize and detoxify chlordane, as in the case of DDT (Chacko *et al.*, 1966; Kallman and Andrews, 1963). Thus, the precise threshold inhibitory concentration of chlordane bound to mitochondrial targets may be only a fraction of that indicated above.

Studies were also undertaken to determine if chlordane inhibits cell growth by inducing mutants deficient in some com-

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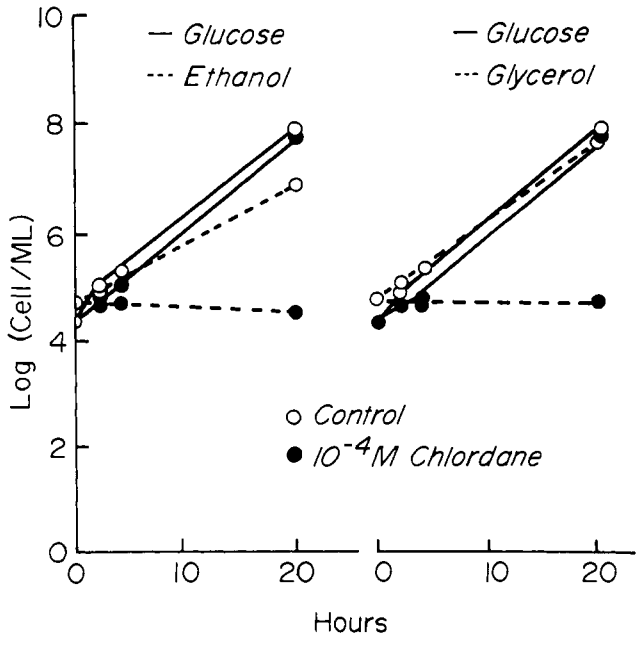

Figure 1. Differential effects of chlordane on *Saccharomyces cerevisiae* growth in glucose, glycerol, and ethanol growth media

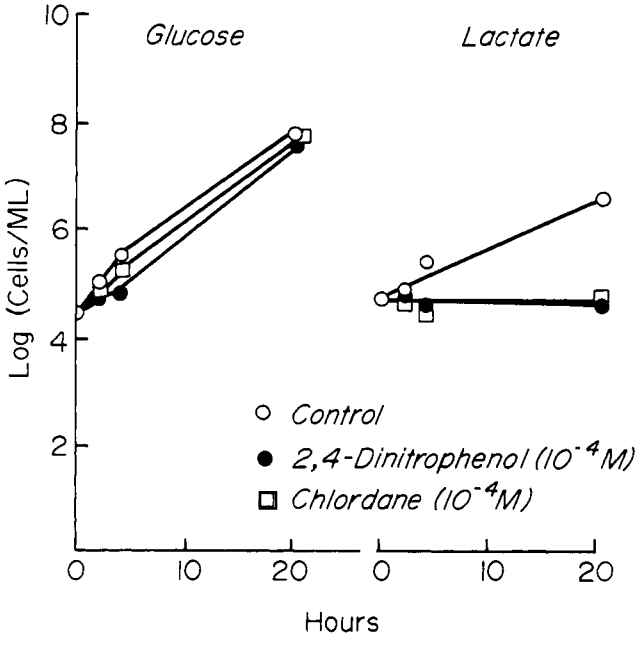

Figure 2. Comparison of the effects of chlordane and 2,4-dinitrophenol (DNP) on yeast cell growth in glucose and lactate growth media

ponent(s) of the electron transport chain. For this, cells were grown in liquid media containing 1% glucose in the presence of 10^{-4} *M* chlordane, as described above. After 20 hr of growth, appropriate dilutions were made and 150 to 200 cells were spread on solid plates containing basic growth medium, 1% glucose, and 2% agar. Colonies appearing on the plate after 3 to 4 days of incubation at 26° C were overlayed with triphenyltetrazolium chloride (Ogur *et al.*, 1957). Cells containing a normal electron transport chain give rise to colonies which reduce the tetrazolium and turn a dark red. Mutants containing a partial respiratory chain give rise to colonies which do not reduce tetrazolium. These colonies remain white. The results show that induction of RD mutants by chlordane was very low. In most experiments, no increase above control values (<1%) was observed, but occasionally as many as 10% of the cells on a single plate were shown by this technique to be respiratory deficient. Thus, the effect of the highest concentration of chlordane tested (40 μg per ml) is insignificant relative to that of a classical inducer of respiratory deficiency such as acriflavin (Roodyn and Wilkie, 1968), which in our hands produced 100% respiratory deficiency at 1 μg per ml. These experiments do not eliminate the possibility that mutants other than RD mutants are formed in the presence of chlordane, since several mutants of oxidative phosphorylation which reduce tetrazolium salts have been described (Beck *et al.*, 1968; Kovac *et al.*, 1967; Parker and Mattoon, 1969). However, inasmuch as reduction of tetrazolium was not impaired in glucose-grown, chlordane-treated cells, it would appear that the effect of chlordane is on oxidative phosphorylation rather than on the respiratory chain *per se*. Future studies will be made to determine whether inhibition is due to a direct action of chlordane on some mitochondrial component(s) involved in oxidative phosphorylation or on the genetic determinants responsible for synthesis of these components. Data (Nelson, 1970b) regarding the *in vitro* effects of chlordane on isolated rat liver mitochondria provide evidence that chlordane interacts directly with some component(s) of the oxidative phosphorylation system, suggesting that the first alternative is the more probable.

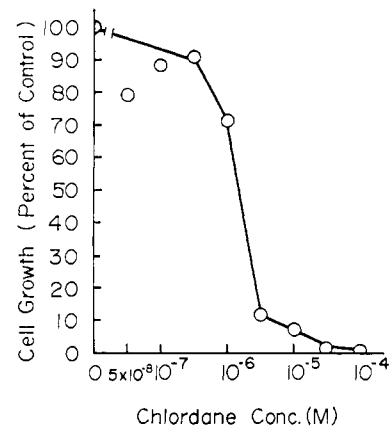

Figure 3. Inhibition of yeast cell growth on lactate as a function of chlordane concentration

In accordance with the lack of an effect of chlordane on the respiratory chain, as shown by the tetrazolium overlay technique, respiration in glucose-grown cells after 20 hr of exposure to 10^{-4} *M* chlordane was also unaltered (Table I). However, respiration was depressed in cells grown on the nonfermentable substrate lactate, but not those grown on glycerol. In contrast, respiration was inhibited during the first 30 min of exposure to 10^{-4} *M* chlordane, regardless of the substrate used (Figure 4). The reason for this apparent discrepancy is not clear. However, it suggests that some compensatory respiratory adaptation may occur in glucose and glycerol cells during the 20 hr growth period.

In addition to chlordane, a number of other chlorinated hydrocarbons have been tested for growth inhibition on lactate (Table II). All are cyclodienes, with the exception of Mirex. Endrin and Mirex were the only compounds tested that produced no differential inhibition of growth on lactate. The results agree with unpublished findings from this laboratory using isolated rat liver mitochondria, which show chlordane, heptachlor, heptachlor epoxide, aldrin, and dieldrin to be nearly equally effective as inhibitors of oxidative phosphorylation *in vitro*, and endrin and Mirex to be ineffective.

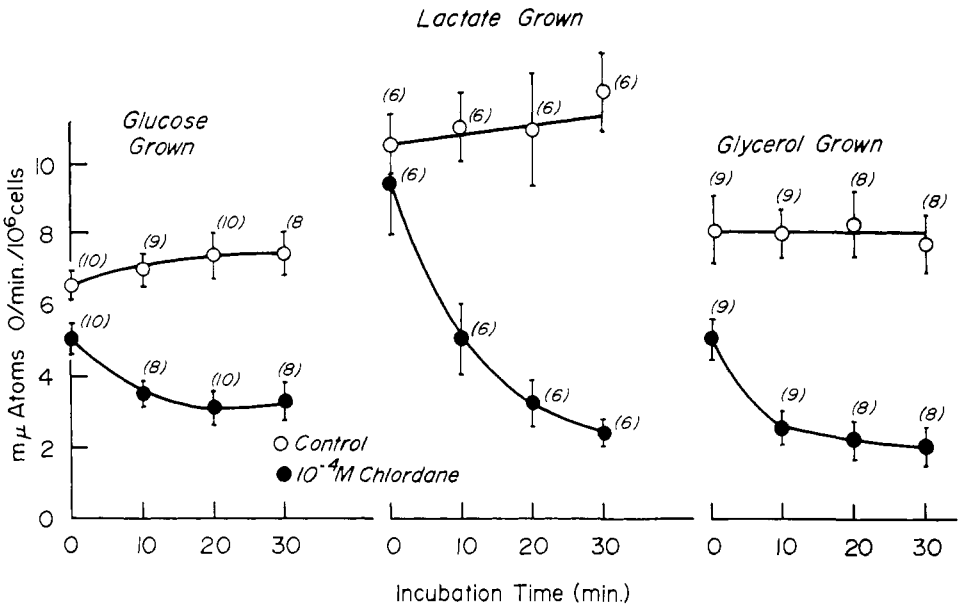

Figure 4. Inhibition of yeast cell respiration during the first 30 min of exposure to chlordane. Cells with no previous history of exposure to chlordane were isolated after 4 hr of growth on chlordane-free media containing glycerol, lactate, or glucose as the energy source. They were resuspended in 5 ml of fresh chlordane-free media to give a cell concentration of 24 × 10^6 cells per ml. Two 2.5 ml samples were then equilibrated at 30° C for 3 min and 25 μl of 0.01 *M* chlordane was added to one. At the indicated time intervals, 0.5 ml aliquots were added to an oxygen electrode vessel containing 1.2 ml of fresh media at 30° C. Oxygen consumption was measured over a 3–5 min period. Exposure to chlordane and the respiratory measurements was always carried out in media containing the same substrates on which the cells were originally grown. Each point is expressed as the $\bar{x} \pm$ S.E. The numbers in parentheses are the numbers of individual experiments

Table I. Respiration in Yeast Cells Grown for 20 hr in the Presence of Chlordane

Substrate	n	Respiration rate (mμg Atoms O/min/10^6 cells) Control	10^{-4} *M* Chlordane
Glucose	19	4.98 ± 0.33	4.56 + 0.39
Glycerol	13	10.68 ± 1.33	9.58 ± 0.96
Lactate	6	7.77 ± 1.40	4.54 ± 0.76

After 20 hr of exposure to 10^{-4} *M* chlordane, cells were isolated by centrifugation, washed, and suspended in chlordane-free media. Respiratory determinations were carried out as described in Figure 4, but in the absence of the pesticide. The media used for respiratory measurements always contained the same energy source as was present during exposure to chlordane. Values expressed as $\bar{x} \pm$ S.E. n = number of experiments.

Table II. Comparison of the Effects of Several Cyclodiene Pesticides on Cell Growth in Glucose and Lactate-Containing Growth Media

Compound	% Inhibition Glucose	Lactate
Chlordane	37	100
Heptachlor	13	100
Heptachlor epoxide	16	79
Aldrin	20	78
Dieldrin	17	40
Endrin	17	6
Mirex	2	0

Cells were grown in the presence of the inhibitors for 20 hr. Cell growth was measured spectrophotometrically at 540 μm. All inhibitors were present at 10^{-4}*M*.

This paper provides evidence that chlordane, as well as several other cyclodiene pesticides, inhibits growth of *S. cerevisiae* by interfering with oxidative metabolism. The fact that chlordane inhibited growth on all of the nonfermentable substrates tested, but had no effect on growth supported by fermentable substrates, tends to eliminate the possibility that chlordane merely inhibits transport of substrate into the cell, and strengthens the interpretation that the primary site of chlordane action is oxidative metabolism. Whether chlordane inhibits by a direct action on the components of oxidative phosphorylation or by genetically altering the synthesis of these components remains to be determined.

It would be premature to generalize regarding the toxic action of cyclodienes in all organisms. However, it is well known that the principle manifestation of chlorinated hydrocarbon toxicity in higher organisms is a neural response (Ecobichon and Saschenbrecker, 1969), and it is possible that disruption of oxidative metabolism in yeast cells is a secondary action of the cyclodienes. On the other hand, the present results are also compatible with the corollary, *i.e.*, that the neural response in mammals is due not to a direct action of chlorinated hydrocarbons on nerve impulse transmission (Naraahashi and Haas, 1967), but rather to loss of energy supply resulting from mitochondrial inhibition.

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Received for review August 21, 1970. Accepted November 3, 1970.