(by weight) of gases: (i) 10 percent CO_2, 90 percent N_2; (ii) 1 percent CO_2, 99 percent N_2; and (iii) air. Initial *p*H of the solutions ranged from 7.2 to 10.5; however, because of buffering by the partial pressure of CO_2 and the solid phase, the final *p*H was more restricted in range.

Effects of halogens on the formation of apatite and OCP were determined by the addition of 0.1*M*, 0.01*M*, or 0.001*M* fluoride or chloride to the solution; the results are summarized in Fig. 1. Under a gaseous mixture containing 10 percent CO_2, only OCP formed in solutions containing the chloride ion or lacking any halogens. In the fluoride solution, however, apatite formed in the same *p*H range; under these conditions, the presence of fluoride enlarged the field of formation of apatite.

In a system aerated with 1 percent CO_2 the effect was similar. In a solution at *p*H ~ 7.34, OCP formed in the absence of halogens, while apatite formed at *p*H ≧ 7.74. With solutions bearing 0.01*M* chloride, the boundary between the fields of formation of apatite and OCP is at *p*H 7.58 ± 0.03.

The data do not establish whether or not chloride ions affect the field of formation of apatite. Solutions bearing 0.1*M* and 0.01*M* fluoride ions (190 ppm F) have enlarged the field of formation of apatite by more than 0.6 *p*H units. Furthermore, in the 1-percent CO_2 atmosphere and under acid conditions, apatite can form only from a fluoride solution.

Under an atmosphere of air, apatite formed in systems with or without halogens over a wide *p*H range. Only in a sample formed in a 0.1*M* chloride–bearing system at *p*H ~ 7.1 was OCP found; this fact indicates that the presence of chloride is detrimental to formation of apatite at near-neutral *p*H. Under atmospheres enriched in CO_2 relative to air, the presence of fluoride ions markedly enlarged the field of formation of apatite.

X-ray diffractograms of apatite, formed at low temperatures, characteristically give poor resolution. This phenomenon may result from the crystal size or from some type of disorder in the structure. When all samples included in Fig. 1 were x-rayed, patterns from apatite formed in fluoride-bearing systems consistently showed markedly better resolution than did patterns from apatite formed in the presence of chloride or in the absence of halogens.

In summary, the effect of high partial pressures of CO_2 is to enhance the formation of OCP at the expense of apatite; whereas, under the same conditions, addition of fluoride ions enlarges the field of formation of apatite, an effect that may be a major cause of the beneficial effect of fluoridation on teeth.

The results of this study do not demonstrate that OCP has a true stability field. However, the question of stability has no bearing on conclusions concerning the effect of halogens or of the partial pressure of CO_2 on the formation of apatite and OCP, because, once OCP forms in an oral environment, it has its characteristic physical and chemical properties relative to attack.

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Lactate Dehydrogenase Activity: Effect in vitro of Some Pesticidal Chemicals

Abstract. *The effect of chlorinated hydrocarbon insecticides upon rabbit-muscle lactate dehydrogenase was determined by both the direct determination of diphosphopyridine nucleotide reduction and a tetrazolium salt reduction method. A comparison of the two assay procedures revealed that the pronounced stimulation of lactate dehydrogenase LDH activity was noted with the tetrazolium salt method but could not be detected with a direct photometric determination method. Incubation of lactate dehydrogenase with DDT or heptachlor prior to the addition of either diphosphopyridine nucleotide or substrate resulted in a complete inhibition of enzyme activity. The inclusion of either diphosphopyridine nucleotide or substrate in the prior incubation mixtures afforded no protection to the enzyme.*

Experiments on the mode of action of pesticides suggest that these agents function, in most cases, as inhibitors of specific enzyme systems (*1*). The group of compounds commonly referred to as "chlorinated hydrocarbons" has, however, been very difficult to associate with specific enzyme inhibition either in vivo or in vitro. Studies by Judah (*2*), who used DDT as a potential inhibitor, failed to demonstrate inhibition of aldolase, adenosine triphosphatase, glutamic dehydrogenase, choline oxidase, hexokinase, or succinoxidase. Sactor (*3*) presented evidence that DDT could partially inhibit cytochrome oxidase. This evidence was supported by Johnston (*4*), who found that both DDT and DDE inhibited rat-heart succinoxidase and cytochrome oxidase. Succinic dehydrogenase activity was not affected by the presence of either DDT or DDE. Nachlas *et al.* (*5*) described a colorimetric method for the measurement of lactate dehydrogenase (LDH) activity. They used phenazine methosulfate as an intermediate carrier for electron transfer from reduced diphosphopyridine nucleotide (DPNH) to a tetrazolium salt.

Evidence presented by O'Brien and Matsumura (*6*) indicates that DDT forms "charge-transfer complexes" with a component of the nerve axon. These authors suggest that this complex formation is the primary mode of action of DDT and perhaps other chlorinated hydrocarbons.

Table 1. Comparison of DPN and tetrazolium methods for LDH activity. Pesticide concentration is 1×10^{-4} *M* in all cases, except where otherwise indicated. (−), Inhibition of LDH activity. One hundred percent activity was determined by running the reactions with pesticide omitted.

Pesticide	Stimulation of enzyme activity (%)	
	DPN reduction	Tetrazolium
Aldrin	(−) 13	29
Dieldrin (1×10^{-3}*M*)	(−) 19	37
DDT	(−) 19	51
Endrin	(−) 16	59
Heptachlor	(−) 16	42
Ceresan M		(−) 20
Thiram		84

Table 2. Effect of prior incubation upon LDH activity, as judged by the tetrazolium and DPN reduction methods. The indicated reaction components were incubated at 37°C for 8 minutes before addition of the remaining components. Control reactions were assayed with no prior incubation. Concentration of both DDT and heptachlor was 1×10^{-4} mole/liter.

Prior incubation mixture	Inhibition (%)	
	Tetrazolium	DPN
Enzyme + DPN + DDT	100	100
Enzyme + substrate + DDT	100	100
DPN + DDT	0	0
Substrate + DDT	0	0
Enzyme + DDT	100	100
Enzyme + DPN + heptachlor	100	100
Enzyme + substrate + heptachlor	100	100
DPN + heptachlor	0	0
Substrate + heptachlor	0	0
Enzyme + heptachlor	100	100

When the effect of chlorinated hydrocarbons upon lactate dehydrogenase was investigated, certain anomalies were noted, and I now report on their effect upon the data obtained from enzyme reactions in the presence of various pesticidal chemicals.

Lactate dehydrogenase activity was determined by the tetrazolium salt reduction method (*7*) and by the direct photometric measurement of DPN reduction at 340 mμ. Stock solutions of all pesticides (*8*) were prepared in isopropyl alcohol.

Except where otherwise noted, all reactions were carried out in a water bath at 37°C. After 20 minutes, the reactions were stopped by the addition of 0.5 ml of 0.5*N* HCl, and the optical density at 540 mμ was measured with a Bausch and Lomb Spectronic 20 colorimeter. In the control tubes the enzyme was added immediately before addition of the HCl.

The stimulation of LDH activity by the various chlorinated hydrocarbon compounds is observed when the tetrazolium reduction assay is used (Table 1). However, when the reaction is assayed by direct measurement of DPN reduction, a small inhibition of the reaction is noted.

Ceresan M inhibits the activity of LDH. The Ceresan M inhibition can be prevented by the addition of 2-mercaptoethanol to the reaction mixture. This, as well as the composition of the compound, suggests that the inhibition is due to the reaction of essential sulfhydryl groups with Ceresan M (*9*).

Although Table 1 indicates either a pronounced stimulation or a slight inhibition of LDH activity, depending upon assay method, Table 2 shows that prior incubation of either DDT or heptachlor with the enzyme results in severe inhibition. In addition, the inclusion of either substrate or DPN in the prior incubation mixture had no effect upon the observed inhibition.

These data indicate that the apparent stimulation of LDH activity by DDT and heptachlor when assayed by tetrazolium reduction is a result of interference with the mechanism of formazan production rather than a result of stimulation of enzyme activity. When the reaction is assayed by DPN reduction, there is no stimulation by either DDT or heptachlor. On the other hand, when DDT or heptachlor is incubated with LDH prior to addition of substrate, there is a complete inhibition of enzyme activity regardless of the method of assay. Thus DDT and heptachlor are capable of inhibiting the activity of LDH, although this is not apparent when the tetrazolium reduction assay is used without modification through prior incubation with an inhibitor.

The stimulation, attributed to an artifact, observed with the tetrazolium reduction method was further investigated by allowing the LDH reaction to proceed for 20 minutes in the presence of $1 \times 10^{-4}M$ DDT. The reactions were stopped as usual with HCl, and the DDT was removed by extraction with petroleum ether. The DDT in the extract was determined by means of gas chromatography (*10*) with an electron-capture detector. Only 60 percent of the added DDT could be recovered in the extracts. Control extractions were carried out by analysis of water solutions of DDT in the same manner. The controls showed 100 percent recovery of DDT. These data suggest the possibility that DDT forms a complex with a component of the reaction. Furthermore, when the concentration of iodophenylnitrophenyl tetrazolium chloride (INT) was doubled, only 40 percent of the added DDT could be recovered. While such data are not definitive, these results suggest the possibility of complex formation, perhaps with the INT.

The inhibition of various enzyme systems by chlorinated hydrocarbon compounds has been investigated frequently, and the results of such investigations have often been inconsistent and have led to considerable confusion. Therefore, the methods used for the determination of enzyme activity should be thoroughly evaluated before use in assay of enzymatic reactions involving chlorinated hydrocarbons.

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Nuclei from Rat Liver: Isolation Method That Combines Purity with High Yield

Abstract. *A procedure is described that gives almost quantitative separation of clean nuclei from a homogenate of rat liver. It is a modification of methods that use concentrated sucrose solutions rather than citric acid or detergents and, therefore, permits further quantitative fractionation of cytoplasmic components.*

A recent review (*1*) has compared various methods for the preparation of nuclei from rat liver, which has usually been done by a series of differential centrifugations in isotonic sucrose. Approximately 100 percent of nuclei in the homogenate were recovered, but preparations also contained 12 to 14 percent of cellular RNA (*2*). Introduction of more concentrated sucrose solutions (2.2*M*) by Chauveau *et al.* (*3*) permitted recovery