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OXYGEN AND PHOSPHATE METABOLISM OF BLUEGILL LIVER MITOCHONDRIA IN THE PRESENCE OF SOME INSECTICIDES

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ABSTRACT.--The effects of aldrin, carbofuran, chlordane, DDE, dasanit, diazinon, endrin, heptachlor, kepone, lindane, malathion, methoxychlor, parathion, thimet, sevin, and toxaphene on the oxidative phosphorylation by bluegill, Lepomis macrochirus, liver mitochondria in the presence of succinate and alpha-ketoglutarate as substrates were investigated. In the presence of succinate, chlordane, diazinon, heptachlor, kepone, malathion, parathion, thimet and toxaphene, severely inhibited oxygen uptake (57 percent malathion; 100 percent diazinon). The other insecticides did not alter oxygen uptake as severely (lindane 25 percent, endrin 49 percent) except dieldrin and carbofuran which did not affect oxygen uptake. All the insecticides inhibited oxygen uptake less in the presence of alpha-ketoglutarate than in the presence of succinate. Phosphate uptake did not appear to be altered by any of the insecticides. The data suggests a specific interaction of each insecticide with each bluegill liver oxidative enzyme complex.

Insecticides have been widely used for many years, and their effects have been extensively investigated; however, the modes of toxic action of most of the insecticides have not been elucidated (O'Brien 1967). Considerable data indicate that organochloro, organophosphate, and carbamate insecticides affect the nervous system (Metcalf 1957, O'Brien 1967). It is of interest, however, that while DDT has been shown to affect fish and bird reproduction in addition to having toxic affects on fish and birds, only recently has a mechanism of toxic action been suggested which can explain the diverse effects caused by DDT (Hiltibran 1971a).

We have been investigating the effects of possible pollutants on energy production by bluegill liver mitochondria, and have found that many derivatives of 2,4-dichlorophenoxyacetic acid (2,4-D) affected oxygen and phosphate uptake by bluegill liver mitochondria (Hiltibran 1969a, 1969b). Further, we have found that cadmium and zinc altered oxygen uptake, whereas manganese and calcium altered phosphate metabolism (Hiltibran 1971b).

Recently we investigated the effect of some insecticides on the oxygen and phosphate uptake by bluegill liver mitochondria and found that some insecticides severely inhibited oxygen uptake. We also investigated the effects of

insecticides on the hydrolysis of ATP (Hiltibran 1974b).

METHODS

Native wild bluegill from various bodies of water in central Illinois were held approximately two weeks at 25° C in aerated laboratory aquaria prior to use. Procedures for the preparation of the liver mitochondria, estimating the rate of oxygen and phosphate metabolism by conventional Warburg and spectrophotometric techniques, respectively, estimating the rate of release of inorganic phosphate from ATP and estimating the nitrogen content of the mitochondrial preparations have been reported (Hiltibran et al. 1965). Oxygen data were converted to microliter of O_2 per hour per milligram of tissue nitrogen (µl O_2/hr/mg N), and all phosphate data were converted to micromoles of PO_4 per hour per milligram of tissue nitrogen (micromoles PO_4/hr/mg N).

All values, corrected for endogenous enzyme activity, are the average values from three or more experiments. The standard deviation and range of values are also given. The insecticides used in this study were obtained from various sources and some were gifts from the manufacturers. Redistilled acetone or ethyl alcohol was used as a solvent. The effects of the insecticides were estimated at 10^{-4}, 10^{-6}, and 10^{-8} gram of insecticide per ml of reaction medium.

RESULTS

The effects of the various insecticides on the succinate oxidase and alpha-ketoglutarate oxidase are summarized in Tables 1 and 2 respectively.

The organochloro insecticide dieldrin and the carbamate insecticide, carbofuran, did not alter the oxygen or phosphate uptake in the presence of either substrate.

The remaining eight organochloro insecticides at a level of 10^{-4} g/ml altered oxygen uptake to various extents ranging from 25 percent inhibition in the presence of aldrin, to an inhibition of 87 percent in the presence of heptachlor. The organochloro insecticides did not alter phosphate uptake in the presence of succinate (Table 1).

All the organophosphate insecticides used in this investigation inhibited oxygen uptake. Basanit inhibited oxygen uptake 38 percent, whereas diazinon completely inhibited oxygen uptake. The inhibition of the oxgyen uptake by bluegill liver mitochondria was more severe in the presence of the organophospate insecticides than in the presence of the organochloro insecticides. Phosphate uptake in the presence of succinate was not altered in the presence of the organophosphate insecticides.

The carbamate insecticide sevin inhibited oxygen uptake 35 percent and was the only carbamate insecticide to alter either oxygen or phosphate uptake in the presence of either substrate.

The organochloro insecticides, chlordane and heptachlor, inhibited oxygen uptake by bluegill liver mitochondria 39 and 29 percent, respectively in the presence of alpha-ketoglutarate (Table 2) and did not appear to have any effect on phosphate uptake. Lindane did not alter oxygen uptake but decreased the

TABLE 1. Effects of Insecticides on O_2 and PO_4 Uptake by Bluegill Liver Mitochondria in the Presence of Succinic Acid.

Insecticide	g/ml of reaction medium	Average change and standard deviation µl O_2/hr/mg N	µmoles PO_4/hr/mg N
		Organochloro	
Aldrin	3.7×10^{-4}	(-)58 ± 1.27 (14-106)	(±)12 ± 1.27 (6-17)
Chlordane	4.0×10^{-4}	(-)161 ± 1.38 (111-252)	(+)11 ± 1.20 (0-22)
DDE	3.2×10^{-4}	(-)81 ± 1.34 (8-170)	(±)18 ± 1.51 (3-38)
Endrin	3.8×10^{-4}	(-)100 ± 1.42 (23-148)	(+)8 ± 1.76 (0-34)
Heptachlor	3.7×10^{-4}	(-)233 ± 1.07 (125-326)	(±)18 ± 1.13 (11-29)
Kepone	4.9×10^{-4}	(-)216 ± 1.48 (106-459)	(±)14 ± 1.13 (7-23)
Lindane	2.9×10^{-4}	(-)76 ± 1.41 (41-129)	(±)32 ± 1.49 (3-105)
Methoxychlor	3.5×10^{-4}	(-)88 ± 1.44 (77-127)	(±)19 ± 1.44 (11-36)
Toxaphene	4.1×10^{-4}	(-)184 ± 1.15 (110-326)	(±)20 ± 1.12 (5-41)
		Organophosphate	
Dasanit	3.1×10^{-4}	(-)105 ± 1.21 (22-181)	(±)20 ± 1.42 (14-29)
Diazinon	2.9×10^{-4}	(-)260 ± 1.10 (194-344)	(±)44 ± 0.71 (35-53)
Malathion	3.3×10^{-4}	(-)141 ± 1.05 (74-214)	(+)24 ± 1.09 (2-41)
Parathion	2.9×10^{-4}	(-)208 ± 1.05 (106-301)	(+) 13 ± 1.15 (1-33)
Thimet	2.6×10^{-4}	(-)279 ± 1.04 (79-454)	(±)82 ± 1.35 (51-123)
		Carbamate	
Sevin	2.0×10^{-4}	(-)72 ± 1.31 (31-104)	(±)25 ± 1.24 (3-50)
Control		268 ± 1.56 (205-325)	43 ± 1.80 (29-82)

TABLE 2. The Effects of Insecticides on the O_2 and PO_4 Metabolism in the Presence of Alpha-ketoglutarate.

Insecticide	g/ml of reaction medium	Average change and standard deviation μl O_2/hr/mg N	μmoles PO_4/hr/mg N
		Organochloro	
Chlordane	4.0×10^{-4}	(-)35 ± 1.38 (14-66)	(±)15 ± 1.21 (2-32)
Heptachlor	3.7×10^{-4}	(-)31 ± 1.13 (3-50)	(±)8 ± 1.04 (1-15)
Lindane	2.9×10^{-4}	(±)19 ± 1.25 (14-26)	(+)7 ± 1.44 (0-20)
Methoxychlor	3.5×10^{-4}	(+)47 ± 1.50 (8-62)	(±)32 ± 1.28 (6-50)
		Organophosphate	
Diazinon	2.9×10^{-4}	(-)86 ± 1.19 (25-138)	(±)37 ± 1.49 (19-75)
Malathion	3.3×10^{-4}	(-)86 ± 1.28 (28-138)	(±)18 ± 1.35 (3-37)
Thimet	2.6×10^{-4}	(-)78 ± 1.44 (8-196)	(±)23 ± 1.16 (7-38)
Control		96 ± 1.39 (73-129)	27 ± 1.12 (12-41)

uptake of phosphate, whereas, methoxychlor increased oxygen uptake. The remaining organochloro insecticides did not alter either oxygen or phosphate uptake by the bluegill liver mitochondria.

Organophosphate insecticides, diazinon, malathion, and thimet inhibited oxygen uptake from 60 percent (thimet) to 88 percent (diazinon). Dasanit and parathion did not alter either oxygen or phosphate uptake. The carbamate insecticides carbofuran and sevin did not alter oxygen or phosphate uptake in the presence of alpha-ketoglutarate.

DISCUSSION

The results of previous investigations of the mode of action of the insecticides discussed by Metcalf (1955) and O'Brien (1967), indicates the organochloro, organophosphate, and carbamate insecticides interfere with nerve function. O'Brien (1967) further suggested that cyclodiene insecticides are not antienzymes, but indicated that definitive data are needed.

Johnson (1968), summarized the effects of insecticides on fishes, as damage to the central nervous system which resulted in instability, respiratory changes, and sluggishness. The chronic effects were residue accumulation, effects on reproduction, reduced growth rate, and gill damage.

Mount (1962) reported that endrin increased the oxygen consumption and ventilation rate of bluntnose minnow, whereas Huner et al. (1967) reported at low levels of endrin, oxygen uptake increased, whereas at higher concentrations, oxygen consumption decreased. DDT inhibited oxygen uptake by rat liver mitochondria (Johnston 1951), insect flight muscle mitochondria (Sacktor 1958, Anderson et al. 1954), and bluegill liver mitochondria (Hiltibran 1971a).

Lindane stimulated respiration and increased the respiratory rate of insects (Harvey et al. 1951). Chlordane, heptachlor, aldrin, dieldrin, and toxaphene increased the respiration rate of insects. Also, most of the increased respiratory activity was due to the hyperactivity after the application of insecticides (Metcalf 1955, O'Brien 1967). Further, it was suggested that death may be due to suffocation caused by the loss of nerve function.

Van Overbeek (1964) has defined a herbicide from physiological considerations as a chemical agent that deranges the physiology of the plant over a period long enough to kill the plant. Most toxic agents are chemicals and it would be reasonable to expect a chemical interaction between a toxic agent and the biochemistry of the organism. Thus, a toxic agent could be defined as a chemical which would alter the biochemistry of an organism to such an extent and for such periods of time that the physiology of the organism is severely altered and the organism dies. Such a definition of a toxic agent would also suffice to explain the chronic effects of toxic agents as well. The relationship between the biochemical effects of some phenoxy compounds, which had been used as herbicides, and their toxicity to bluegill have been discussed (Hiltibran 1969b, 1974a).

Rotenone, which is used as a fish toxicant and as an insecticide, is not a nerve poison (O'Brien 1967), increased respiratory activity in fishes. But, as the poisoning became more severe, it reduced frequency of breathing

rate, reduced oxygen uptake, caused loss of equilibrium, and death (Hiltibran unpublished data). However, fish treated with rotenone and removed to fresh water survived even after loss of equilibrium and reduced breathing rate (Bouck et al. 1965). Rotenone has been shown to reduce oxygen uptake in fishes (Danneel 1933) and grasshoppers (Tischler 1935). Later rotenone was thought to cause destruction of the gill tissue (Danneel 1933, Scheuring et al. 1935) and block the circulation in gill tissue (Hamilton 1941). Oberg (1959) reported that in severe rotenone poisoning, the circulation in the gills was normal and the destruction of the gill tissue was apparently due to secondary changes. Lindahl et al. (1961), studying the effect of rotenone on cellular respiration, reported that rotenone inhibited the uptake of oxygen in the presence of pyruvate and glutamate, but not in the presence of succinate. Similar results had been previously reported by Fukami (1954), Fukami et al. (1956), and Tomizawa et al. (1956).

Rotenone has been found to inhibit the flow of electrons between flavin adenine dinuclectide (FAD) and the cytochrome chain (Oberg 1961), and is the only biochemical action which has been demonstrated for rotenone. This would suggest that the specific biochemical effect described for rotenone may be of greater importance in the toxic action of rotenone than the previously described effects suggested. Rotenone severely inhibited the oxygen uptake by bluegill liver mitochondria (Hiltibran et al. 1965).

Antimycin A, used as a fish toxicant, inhibits the flow of electrons from substrate to oxygen, and apparently is the only known biochemical effect for antimycin A. Cyanide also has been used as a fish toxicant, and inhibits the flow of electrons from substrate to oxygen. The effects of antimycin A and cyanide on the uptake of oxygen by bluegill liver mitochondria has been discussed (Hiltibran 1965, 1967). These are the only known biochemical effects which have been shown for these fish toxicants, and would suggest some relationship between their inhibtion of oxygen uptake and their toxic action.

The mechanism of the toxic action of heavy metals to fishes is not known. Previously, the death of fishes was thought to be suffocation caused by coagulation of mucus on gill surfaces or damage to gill tissues (Doudoroff et al. 1953). This hypothesis was modified by Skidmore (1970) who suggested that tissue hypoxia was the cause of death of fishes after exposure to zinc. Data also cited by Doudoroff et al. (1953) indicated that the breathing rates and respiration rates of fishes increased in toxic metal solution, but oxygen consumption fell gradually until death, and the production of carbon dioxide declined. Other data cited by Doudoroff et al. (1953) indicated that some metals, which did not cause mucus to coagulate, may produce harmful effects.

Skidmore (1970) noted the decrease in oxygen utilization, increased gill ventilation volume, and decrease in heart rate, and the decline of PO_2 of dorsal aortic blood. Burton et al. (1972) reported that lactic acid accumulated in fish tissues after exposure to zinc and low oxygen levels, which would support the tissue hypoxia hypothesis suggested by Skidmore (1970). Hiltibran (1971b) reported that cadmium and zinc severely inhibited the uptake of oxygen of bluegill liver mitochondria. Bilinski et al. (1973) reported that cadmium and copper inhibited the oxidation of lactate by gill tissue of the rainbow trout.

The inhibition of oxygen utilization at the cellular level, as indicated

by the data of Bilinski et al. (1973) and Hiltibran (1971b) for cadmium, copper and zinc, would result in the accumulation of lactic acid, since it could not be metabolized via the Krebs tricarboxylic acid cycle due to the blockage of the oxidative systems by the heavy metals. The blockage of the oxidative systems by the heavy metals would reduce oxygen uptake, and decrease the production of carbon dioxide, observations that have previously been noted. The hyperactivity of insects and fishes in the presence of toxic agents would be the initial reaction of these organisms to the toxic agents and result in an increase in oxygen uptake. But, the effect which has been noted as poisoning became more severe, the decline in production of carbon dioxide at the cellular level would also cause a decrease in respiratory activity as indicated by a decrease in breathing rate activity, which also would reduce oxygen consumption. The hyperactivity would reduce the oxygen level of the blood, and what appeared to be tissue hypoxia as suggested by Skidmore (1970) and the data of Burton et al. (1973) would also indicate, would essentially be that oxygen utilization at the cellular level was blocked by the toxic agents such as heavy metals and the fish toxicants discussed above. Thus there is a cellular biochemical explanation of the physiological effects observed.

The cellular biochemical alterations caused by various toxic chemical agents to fishes have not been considered in any hypothesis of the mode of action of toxic agents, although it has been suggested (Hiltibran 1969b, 1971a, 1971b). But the possible cellular biochemical derangements, as indicated by the data in Tables 1 and 2 for the insecticides, could cause many of the physiological responses of fishes upon exposure of fishes to these toxic agents.

The inhibition of oxygen uptake of fishes by the insecticides would result in an increased activity and increased oxgyen utilization. But as the poisoning became more severe, there would be a decrease in oxygen utilization, a decrease in carbon dioxide production, a decrease in respiration rate, and finally death, physiological responses noted by various investigators.

Thus, it has been shown that most of the insecticides can severely alter the oxidative enzymes of bluegill liver tissues, as indicated by their effects on oxygen uptake of bluegill liver mitochondria, and that their toxic action could be mediated by their effects on these systems.

Further, since all the insecticides used interacted with each oxidative enzyme complex to different extents, it would suggest an interaction between each insecticide and each enzyme complex and that insecticides are not general enzyme inhibitors, or activators. Further support of this concept is indicated by the effect of these insecticides on the hydrolysis of ATP (Hiltibran, 1974b).

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