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Changes in Starch-gel Electrophoretic Pattern of Serum Proteins of a Freshwater Teleost *Channa punctatus* (Bloch), Exposed to Sublethal and Chronic Levels of Three Organophosphorus Insecticides

by

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(With one text figure and one plate)

INTRODUCTION

Electrophoretic analysis of serum proteins is extensively utilized in monitoring the physio-pathological conditions in living organisms, prior to the onset of external symptoms. Electrophoresis has, therefore, become the most effective technique known for locating and recovering physiologically active substances (Strickland, 1970).

A number of workers have observed that the electrophoretic pattern of serum proteins of fishes is sensitive to various intrinsic and extrinsic factors. Booke (1964) has critically reviewed the earlier literature on alterations in fish serum proteins in relation to age, sex, seasonal variations, starvation, and fungal and viral diseases. Factors such as oxygen depletion (Bouck and Ball, 1965), physical stress (Thurston, 1967; Yamashita, 1968) and temperature (Umminger, 1970) have also been shown to affect the serum protein pattern in fishes.

Fujiya (1961) successfully employed paper electrophoretic technique to detect alterations in serum proteins in fishes exposed to sublethal concentrations of pulp mill wastes and inorganic chemicals. Bouck and Ball (1965) proposed that electrophoretic technique can be gainfully employed in the evaluation of sublethal effects of pollutants. They further suggested (Bouck and Ball, 1967) that the amount of low mobility proteins in the blood of a species may generally be associated with general pollution tolerance.

The anti-cholinesterase organophosphorus insecticides are known to be highly toxic to fish (Pimentel, 1971). Recent reviews on the effects of agricultural toxins on fresh water fisheries have given coverage to the pertinent literature (Newsom, 1967; Johnson, 1968; National Technical Advisory Committee, 1968; Katz *et al.*, 1969, 1970, 1972; Sprague, 1969, 1970, 1971; Cope, 1971). However, little is known about sublethal and chronic effects of organophosphates. It has recently been observed that extremely low levels of these insecticides can cause deleterious changes in the normal haematological parameters (Anees, 1973*a*), and may exert tissue damaging effects during prolonged exposures (Anees, 1973*b*, 1973*c*.).

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The low-level insecticidal pollution of aquatic ecosystems poses a potential hazard to fresh water fishery resources. Therefore, the importance of detection and evaluation of sub-lethal and chronic levels of insecticidal pollutants cannot be over-emphasized.

Little work has appeared on the utilization of electrophoretic technique for monitoring physiological alterations in fishes exposed to low levels of insecticides. A few authors, e. g. Yasuda (1965), Ishihara *et al.* (1967), Eisler (1967), Komarovskiy (1970), and Grant and Mehrle (1973) have studied the effects of certain organochlorine insecticides and herbicides on fish serum proteins. However, in case of organophosphorous insecticides, only two reports have become available so far (Ishihara *et al.* 1967; Eisler, 1967).

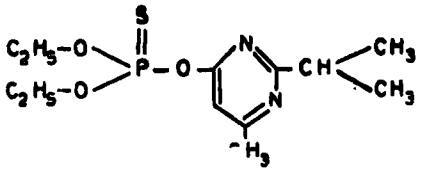
The present study is part of a programme on the evaluation of sublethal and chronic effects of insecticides on fish and presents the results of a qualitative study of electrophoretic pattern of serum proteins of a fresh water teleost *Channa punctatus* (Bloch), exposed to three organophosphates.

MATERIAL AND METHODS

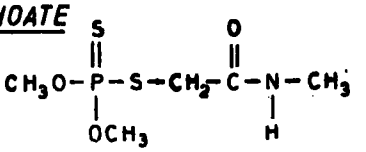
Acquisition, care, maintenance and insecticide treatment of *C. punctatus* have been described (Anees, 1973*d*). Chemical formula of the insecticides are shown in Fig. I. Sublethal and chronic levels of the three insecticides and the respective periods of exposure are also given (Table I).

CHEMICAL FORMULA OF INSECTICIDES*
(FIG. 1)

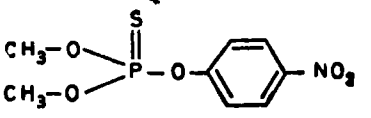
DIAZINON



DIMETHOATE



METHYL PARATHION



* Benoit (1969), Hayes (1971)

Electrophoresis

Blood sampling was done at the end of every exposure period by means of puncture at the point of the caudal peduncle, and the blood was collected from the caudal artery (Klontz and Smith, 1968) into capillary tubes pre-rinsed with the anti-coagulant sodium citrate. Blood was immediately stored at -20° C in sterile stoppered tubes. No quantitative or qualitative changes in serum fractions were envisaged as the deep-freezing of fish serum does not affect its electrophoretic pattern (Goswami and Barua, 1959; Eisler, 1967). At thawing after 24 hours, enough quantity of serum was obtained. In certain cases, however, to get the serum clear of particulate matter, the samples were centrifuged at 3,000 rpm for 10 minutes. The supernatant was used as the sample. Serum samples were used from male members only. Controls were also processed in a similar way.

TABLE 1

Sublethal and Chronic Exposure of *Channa punctatus* to Three Organophosphorus Insecticides.

Insecticides	Period of Treatment		
	24 *hours*	96 *hours*	14 *days*
	Dose (ppm)		
Diazinon	0.37	0.28	0.15
Dimethoate	10.00	8.00	5.00
Methyl Parathion	1.15	1.05	0.90

The serum samples from experimentals and controls were electrophorsed on starch-gel. The methods for the starch-gel electrophoretic separation of serum proteins, as described by Smithies (1955, 1959) were generally followed. Gels were stained by the general protein stain (saturated solution of Amido Black + a mixture of 50 ml Methanol, 50 ml water and 10 ml acetic acid). The washed gels were kept in 5% glacial acetic acid till photographed. Electrophoreses were carried out in the laboratories of the University of Maryland/Pakistan Medical Research Center, Lahore.

RESULTS

Starch-gel electrophoretograms of serum proteins of *C. punctatus* after exposure to the various sublethal and chronic levels of Diazinon, Dimethoate and Methyl Parathion are shown in Plate 1, Figs. 2-4. The control usually had a consistent pattern of separation of slow and fast fractions.

Samples for 24-hour Diazinon exposure showed little change in the mobility of the different fractions. However, the 96-hour group showed significant inconsistencies. Traces of an extra band could be detected in the fast fraction zone. No significant departure from the controls was exhibited after 14-day exposure to Diazinon.

In the case of 24-hour Methyl Parathion exposure, the fractions were separated with an overall resemblance with the respective Diazinon group. However, only trace fractions were present in sample number 5. Samples for 96-hour exposure were usually with indistinctly separated fastest fractions. The pattern of separation for 14-day exposure was almost similar to the controls except for little variability of the central bands.

The 24-hour exposure to Dimethoate resulted in the absence of various fractions and only traces of fast fractions could be detected. On the other hand, overall separation after 96-hour exposure presented the characteristic fractions but in a relatively indistinct manner. The pattern after 14-day exposure was very similar to that of Diazinon and Methyl Parathion but for some variability in the mobility of fast fractions.

DISCUSSION

The results of a qualitative study of the electrophoretic pattern of *C. punctatus* have provided preliminary evidence that the three organophosphorus insecticides, at both sublethal and chronic levels, may interfere with the distribution of serum proteins.

Ishihara *et al.* (1967) have reported that the organophosphate Sumithion (Fenitrothion or M. E. P.) at a level of 4 ppm caused an increase in alpha-globulins in carp serum, while a 45-day exposure of the Northern puffers (*Sphaeroides maculatus*) to 20,000 ppb of Methyl Parathion was seen to significantly decrease the total serum protein contents (Eisler, 1967).

Some recent studies indicate that the insecticides may disturb protein metabolism in fish serum. Mehrle *et al.* (1971), utilizing gas-liquid chromatography, have found that DDT and Dieldrin inhibited the serum amino acid metabolism in the rainbow trout (*Salmo gairdneri*). In the same species, various doses of Endrin were found to interact with the distribution of serum proteins, as revealed by cellulose acetate microzone electrophoresis (Grant and Mehrle, 1973). In their study on the production of antibodies against DDT and Malathion, Centeno *et al.* (1970) have shown that the coupling of the pesticide molecules with proteins may occur *via* their metabolites.

The electrophoretic variability due to age, sex and other factors was kept at the minimum, and the experiments for the controls and experimentals were carried out in an identical manner. Therefore, it is tempting to assume that the formation of pesticide-protein conjugates and the subsequent disturbances of protein metabolism were responsible for the alterations in the distribution of serum proteins in *C. punctatus* after exposure to organophosphorus insecticides. The present study indicates that the electrophoretic technique, combined with suitable staining procedures and quantitative analyses, may prove to be a promising detector of sublethal pesticidal pollution.

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EXPLANATION OF PLATE

PLATE I

FIG. 2 Starch-gel electrophoretogram of serum proteins of *Channa punctatus* after 24-hour exposure to three insecticides. (pH 8.00).
A. Control; *B*. Diazinon; *C*. Methy Parath on; *D*. Dimethoate.

FIG. 3 Starch-gel electrophoretogram of serum proteins of *Channa punctatus* after 96-hour exposure to three insecticides. (pH 8.00).
A. Control; *B*. Diazinon; *C*. Methyl Parathion; *D*. Dimethoate.

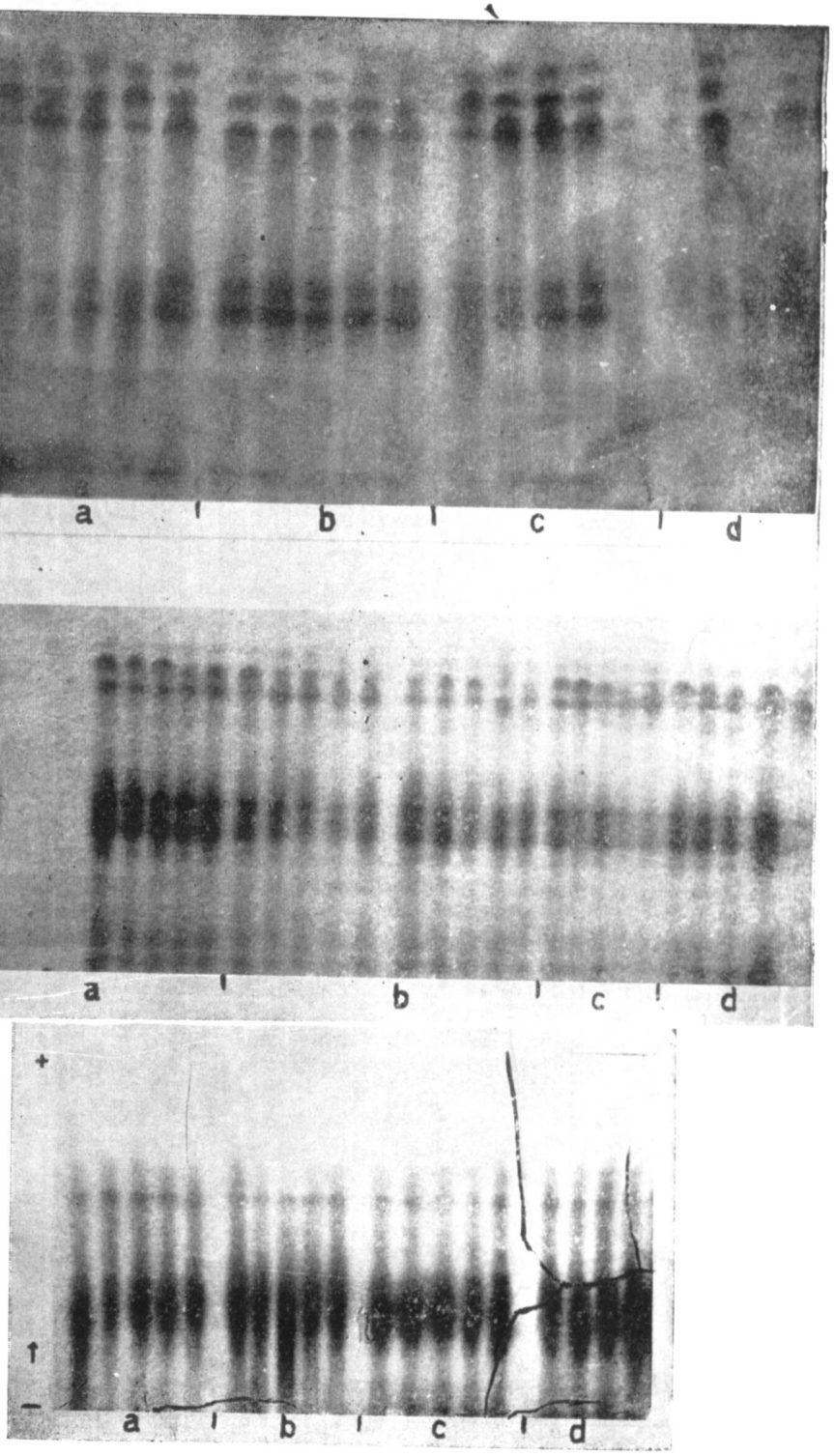
FIG. 4 Starch-gel electrophoretogram of serum proteins of *Channa punctatus* after 14-day exposure to three insecticides. (pH 8.00).
A. Control; *B*. Diazinon; *C*. Methyl Parathion; *D*. Dimethoate.

(MS. received 3.12.73)

FIG. 2

FIG. 3

FIG. 4



Starch-gel electrophoretograms of serum proteins of *Channa punctatus*.