PROTECTIVE ROLE OF VITAMIN B_{12} AGAINST METHYL-PARATHION TOXICITY IN *OPHIOCEPHALUS (CHANNA) PUNCTATUS*

A THESIS

submitted in fulfilment of the

requirements for the award of the degree

of

DOCTOR OF PHILOSOPHY

By

MS. SANDHYA GOEL

DEPARTMENT OF BIOSCIENCES AND BIOTECHNOLOGY
UNIVERSITY OF ROORKEE
ROORKEE-247 667 (INDIA)

JUNE, 1992

DEDICATED TO MY RESPECTED MOTHER

CANDIDATE'S DECLARATION

I hereby certify that the work which is being presented in the thesis entitled **"PROTECTIVE ROLE OF VITAMIN B_{12} AGAINST METHYL-PARATHION TOXICITY IN OPHIOCEPHALUS (CHANNA) PUNCTATUS"** in fulfilment of the requirement for the award of the Degree of Doctor of Philosophy and submitted in the Department of Biosciences & Biotechnology of the University is an authentic record of my own work carried out during a period from July 1987 to June 1992 under the supervision of Dr. V.P.Agrawal and Prof. C.B. Sharma.

The matter presented in this thesis has not been submitted by me for the award of any other degree of this or any other University.

Sandhya

Signature of the Candidate

This is to certify that the above statement made by the candidate is correct to the best of my (our) knowledge.

V.P. Agr

Date: 30.6.92 Signature of Supervisor

C. B. Sharma

Signature of Supervisor

The Ph.D. Viva-Voce examination of Ms. Sandhya Goel ..... Research Scholar, has been held on March 5, 1993

C. B. Sharma

C. B. Sharma

5.3.93

V.P. Agr 5/3/93

Signature of Supervisor(s) Signature of H.O.D. Signature of External Examiner

ABSTRACT

"PROTECTIVE ROLE OF VITAMIN B_{12} AGAINST METHYL-PARATHION TOXICITY IN OPHIOCEPHALUS (CHANNA) PUNCTATUS"

Experimental studies were conducted to investigate the role of vitamin B_{12} against toxicity induced by methyl-parathion intoxication in a teleost fish, O.punctatus. To study the effect of methyl-parathion (O-O-dimethyl O-p-nitrophenyl-phosphorothionate) on behavioural, morphological, histopathological, haematological, histoenzymological and biochemical parameters in the liver, kidney and blood of fish, the healthy, living specimens of O.punctatus were collected measuring about 15 to 18 cm in length and 40-50 gm in weight.

Bioassays have been conducted to gain preliminary information about the toxicity of MP on fish. The LC_{50} values for 24, 48, 72 and 96h have been determined and were found to be 0.034 ml/l., 0.0056 ml/l., 0.0031 ml/l and 0.0015 ml/l of MP. For acute exposure, two sublethal concentrations of MP i.e., $1/5^{th}$ and $1/10^{th}$ fractions of LC_{50} were selected. The results indicate that fishes exposed to high concentration (0.06 ml/l) of MP exhibit a significant degree of mortality after 24h exposure but there is a decline in mortality when the fishes are exposed to lower concentration (0.005 ml/l). Mortality rate decreases with the increase of exposure. The data was collected at the four time intervals i.e., after 24, 48, 72 and 96h. Controls were also conducted simultaneously.

The behavioural response of fish towards toxicant was grossly dependent on concentration and period of exposure. After introducing the fish into test solution, the secretion of mucus from the body, hyperexcitability, increased aerial excursions and opercular movements were observed. Some fishes were characterized by the development of tremors and convulsions.

Quantitative estimation of MP in tissue (liver, kidney) were done by HPLC. The absorption of MP concentration by the liver and kidney increased with the duration of the exposure time. After 96h exposure, the pesticide is completely absorbed by the tissues.

Histopathologically, a number of degenerative changes were observed in the histology of the liver as nuclear pycnosis, nuclear mitosis, clumping of nuclei and hypertrophy of hepatocytes. The liver damage was more severe after 96h exposure. The structure of the treated kidney show remarkable changes as loss of haemopoitic tissue, vacuolation, swelling of renal tubules, glomerular shrinkage and nuclear pycnosis in the renal tubules. After 96h exposure, the kidney damage was more severe due to MP intoxication. Irregular distribution of reticulin fibres and collagen fibres was also observed which show progression of tissue injury.

Further, haematological investigation revealed a decrease in the haemoglobin percentage, haematocrit values and RBC counts, reflecting the anaemic state of fish, while

there was a slight elevation in the WBC counts. Other absolute values (MCV, MCH and MCHC) also altered in response to the changes in the above parameters. However, no significant alteration is found in all the acutely treated fish. Glucose, urea and cholesterol level increased significantly. Serum glutamic-oxalic transaminase, serum glutamic pyruvic transaminase and alkaline phosphatase increased while the acid phosphatase decreased in all the acutely treated fish.

Biochemical observations include measurements of moisture contents, total proteins, lipids and carbohydrates. Moisture contents and carbohydrates increased significantly. The tissue protein depleted maximally in the kidney while the tissue lipids depleted maximally in the liver after 96h intoxication. The enzymological alterations revealed significant inhibition in the alkaline and acid phosphatase activity and elevation in the lipase activity at all the time intervals both in the liver and kidney. The biochemical alterations in the enzymes were parallel to histochemical results.

The effect of MP on liver, kidney and blood of fish is more severe after 96h exposure. Vitamin B_{12} (dose of 0.25 ml) was injected in the intoxicated fish intramuscularly on each alternate day for two weeks. Administration of vitamin B_{12}, shows loss of excessive secretion and deposition of the mucus on the body surface. Loss of lesions like hepatocytic necrosis, vacuolation and

normal hepatocytes was observed after vitamin B_{12} injection as evidenced by histopathological examination in the liver. No necrosis was observed in the kidney by vitamin B_{12} administration. A regular distribution of reticulin fibres was noticed in the liver while in kidney, intertubular accumulation of reticulin fibres was observed after vitamin B_{12} administration. No topographical differences in the distribution of collagen fibres was observed either in the liver or in the kidney. Anaemia, hyperglycemia and hypercholestremia was cured by vitamin B_{12} administration. MP induced glyconeogenesis in tissues is prevented by injecting vitamin B_{12}. It is indicated that vitamin B_{12} provides protection to the fish against MP toxicity.

ACKNOWLEDGEMENT

I am unable to find words of adequate beauty and meaning that could even fractionally express my deep and sincere sense of gratitude and indebtedness to Dr. V.P.Agrawal, M.Sc., Ph.D., F.L.S., F.Z.S. (London), Department of Biosciences and Biotechnology, University of Roorkee, Roorkee, for his expert guidance and valuable suggestions given from time to time in going through the manuscript and offering criticism and giving constant encouragement with a great interest during the whole period of research.

It is a matter of great pleasure for me to express my sincere gratitude and indebtedness to Dr. C.B.Sharma, Ph.D. (Texas), Professor and Head of Department of Biosciences and Biotechnology, University of Roorkee, Roorkee, whose constant supervision, helping attitude and untiring working always encouraged me to complete my work.

I express my sincere thanks to Dr. B.M.J. Preiera, Lecturer, and Dr. Reena Kumar, Pool Scientist, Department of Biosciences and Biotechnology, University of Roorkee, Roorkee and Dr. Ajay Kumar, Head, Department of Zoology, Chinmay degree college BHEL, Hardwar who have been ever ready to help me whenever the need has so arisen.

I welcome this opportunity to express my deep sense of gratitude to Dr. R.N.Manikvasagam, Reader and Dr. S.Balakrishnan, Lecturer, Department of Earth Sciences, University of Roorkee, Roorkee for their care and help during my research work.

I express my sincere thanks to Santosh Typing Institute for typing this manuscript, to Mr. V.K.Sharma for preparing nice drawing and to Mr. J.P.Garg for excellent photography. I also appreciate the cooperation of the ministerial and technical staff of the Department of Biosciences and Biotechnology.

I fall short of words to reflect my sense of gratitude to my family and friends, who have been my side all through, encouraging me all the time and providing the much needed moral support.

I duly acknowledge the U.G.C. (University Grants Commission), India, for the financial assistance.

Let me finally dedicate this whole effort to my father Late Sh. Hari Prakash Goel Ji.

(SANDHYA)

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Acetylcholinesterase	AChE
Acid phosphatase	AcPase
Alkaline phosphatase	AlPase
Blood vessel	BV
Bovine serum albumin	BSA
Central vein	CV
Clumping	C
Collecting tubule	Ct
Control	Cont.
Dissolved oxygen	DO
Dividing nuclei	dn
Figures	Fig.
Glomerulus	gl
Glutamic-oxaloacetic transaminase	GOT
Glutamic-pyruvic transaminase	GPT
Haemoglobin percentage	Hb%
Haematocrit value (Packed cell volume)	PCV
Hepatic nuclei	hn
Hepatocyte	hc
High performance liquid chromatography	HPLC
Hour (s)	h
Lumen	l
Mean cell haemoglobin	MCH
Mean cell volume	MCV
Mean corpuscular haemoglobin concentration	MCHC
Median lethal concentration	LC_{50}

Median lethal dose	LD_{50}
Median tolerance limits	TLm
Methyl parathion	MP
Nuclei	n
Optical density	OD
Organophosphorus pesticides	OP
Ist proximal tubule	PI
IInd proximal tubule	PII
Ruptured haemopoitic tissue	rht
Ruptured tubule	rt
Red blood corpuscles	RBCs
Shrunken glomeruli	sgl
Sinusoids	S
Space	s
Standard deviation	SD
Stanadard error	SE
Succinate dehydrogenase	SDH
Tabular epithelium	te
Vacuolation	V
Vitamin B_{12}	Vit. B_{12}
White blood corpuscles	WBCs

INTRODUCTION 1.0

The biocidal agricultural chemicals collectively known as pesticides, are without any doubt, the largest group of poisonous substances that are widely broadcast today. They play an important role in the development of agricultural productivity, in public health to control vector borne diseases and for eradication of pests and insects. Extensive use of pesticides in modern land and water management has posed potential health hazard to non-target organisms in aquatic habitat especially fishes. Pesticides are introduced into ecosystem through spraying from land based or aerial sprayers. In ideal application, the chemical should fall exactly on the target and should be biodegraded completely to non-toxic compounds. But in practise, it does not happen and most of the pesticides drift into the environment. Some toxic chemicals find their way into the streams and lakes with the run off from the agricultural, forest, municipal and industrial areas. Water serves as a transport medium. Residues of pesticides are found in various living and non-living systems for prolonged periods of their life span and are responsible for a variety of known and unknown toxic effects. Aquatic flora and fauna absorb the residues which enter the food chain through crustaceans, molluscs and fishes and ultimately find their way to man at the top of the food chain. The acute poisoning by pesticides have presented serious water pollution problems resulting in incidence of poisoning of fish and in some instances of human beings, cattle and other form of aquatic life and

their long term effects in the environment which are essential to maintain ecological balance in many industrialized countries like U.S.A., U.K. and Germany. In a developing country like India, we are going through agricultural revolution and the use of pesticides is essential. The pesticides directly or indirectly effect the human population. Nevertheless, we can not afford to avoid their use. Hence we have to look to some other sources which may minimise the harmful effect of the pesticides. The pesticides can be organised as insecticides, herbicides, rodenticides, fumigants, fungicides, molluscicides, acaricides and nematocides. Insecticides are divided into four categories namely organochlorine, organophosphorus, carbamate and botanical insecticides. Due to persistence nature of organochlorine pesticides, organophosphorus (OP) pesticides are favoured and used in large quantity in agricultural pest control.

The use of OP pesticides in agriculture and public health is gaining momentum since the cancellation of environmental persistant chlorinated pesticides. Organophosphates degrade more rapidly than the chlorinated hydrocarbons but are more acutely toxic as they inhibit the enzyme cholinesterase in invertebrates, insects and vertebrates. Since OP pesticides have only limited persistence, more than one application is often required in order to control the same pests. Additional applications result in greater consumption of the pesticide. Continuous exposure to OP pesticides is envisaged to be a great hazard to water

quality affecting aquatic fauna especially fishes as they are quite sensitive to a wide variety of toxicants in water and are mostly used as pollution indicators. Pesticides have been found to be highly toxic not only to fishes but also to fish food organisms. Toxic residues accumulate to a minimum concentration in their body when compared to other organisms in the aquatic environment. Thus, it is important to examine the toxic effects of OP pesticides on fish as they form an important part of the human food.

One of the present day OP pesticide,methyl-parathion (MP) is widely used in modern land and water management. MP (O, O-dimethyl O-p-nitrophenyl phosphorothionate) procured from Bayer India Ltd., Bombay; marketed under the trade name 'Metacide 50 (50% wt/wt)'. The toxicant is a tan to brown liquid with a pungent garlic-like odor. MP is more effective than parathion against aphids and beetles and is found to be toxic to non-target organisms especially fish. Various metabolic activities and physiological response of fresh water fish are adversely affected by methyl parathion.

In recent years, incidences of fish mortality due to pesticides, industrial effluents and sewage pollution have increased. A number of reports have appeared on the uptake and tissue distribution of pesticides in a number of fish. The protective role of vitamin C against pesticides and heavy metal toxicity has been reported. The complex relationship of vitamin C to enzymes and drugs in liver function has been studied and limited protective action

against a major group of pesticides has been reported. However, there is no report on the role of vitamins against MP toxicity in fishes. Vitamin B_{12} plays a curative role against MP toxicity and may be used to protect living beings against MP toxicity. To fill up this gap, a fresh water teleost fish, Ophiocephalus (Channa) punctatus belonging to the order Ophiocephalidae has been selected for the present study because of its easy availability, easy for handling in the laboratory and high food value.

Bioassays have been conducted to gain preliminary comparable information about the toxicity of MP on Ophiocephalus (Channa) punctatus. The dose response of MP on the target organs, liver and kidney of O.punctatus employing histological, biochemical, histoenzymological and haematological parameters has been studied. Estimation of MP in O.punctatus by high performance liquid chromatography (HPLC) has been done. Role of vitamin B_{12} against MP in the tissues of O.punctatus has also been investigated. So as to suggest some remedial measures. The following are the objectives of the present experimental work:

(1) **To obtain LC_{50} values of MP on O.punctatus employing bioassays technique.**

(2) **Quantitative estimation of MP in tissues (liver, kidney) of O.punctatus by HPLC.**

(3) **The dose response study of MP on the liver and kidney of O.punctatus employing histological, biochemical and histoenzymological parameters in**

addition to the haematological approach.

(4) Antitoxicant effect of vitamin B_{12} against MP toxicity in tissues of O.punctatus.

LITERATURE REVIEW 2.0

HISTORICAL BACKGROUND 2.1

The environmental pollution by pesticides has been increasing day by day due to their extensive usage for eradication of various pests and insects and to protect the agricultural crops (Matsumura et.al., 1962; Muirhead-Thomsan, 1971). Most of the pesticides that drift into the environment are liable to affect the fresh water fauna in general and fish in particular (Holden, 1973; Edwards,1973). The persistence of these toxic chemicals in aquatic environment may be dangerous for the survival of fish (Jhonson, 1968; Mawdesley-Thomas, 1971). The hazards of the pesticides to fish are of great concern. Incidences of fish mortality due to pesticides, industrial effluents, and sewage pollution have been reported by a number of workers (Katz et.al., 1972; Coppage, 1976). The effect of OP pesticides on fishes have been extensively studied and reviewed by many workers (Konar, 1969; Holden, 1973; Mckim et.al., 1976; Gupta, 1986; Chakraborty and Banerjee, 1988).

The scientific information on the toxicity of various pesticides has been reported by Hayes (1975), Anees (1975) and Dalela et.al. (1978). The nature of the effects vary but include structural and functional modification, at both cellular and sub-cellular level in a variety of organisms (Verma et.al., 1978; 1981). Several pesticides are known to reduce growth, survival reproduction of fish and other aquatic animals (Jhonson, 1968; Mckim et.al.,

1975; Pal and Konar, 1985) large scale fish mortality (Konar, 1975) and also induce histopathological lesions in various organs of fish (Couch, 1975; Anees, 1978). Several other studies have shown that exposure of fish to pesticides leads to a number of disturbed pathological and biochemical processes (Anees, 1978; Sastry and Sharma, 1978). Some work has been focussed on the pathological changes induced by pesticides.

In recent years, the use of pesticides has increased manifold in the different parts of the world. It has been shown to produce hazardous effects on the fish population which in turn forms parts of the human food. Toxicity of insecticides to fish, plankton and worm has been reported by Pal and Konar (1986) and it helps in evaluating the safe concentration of the pesticides in aquatic life. Studies on the effect of malathion on teleost fish have also been reported by Singh and Sahai (1984) and Jyoti et.al. (1989). Pathak et.al. (1989) have studied season and environment related variation in body contents. The toxicity of endosulfan and MP were compared by Bashamohideen et.al. (1988) in Catla catla. Rao et.al. (1984; 1985) have reported the effect of MP on the contents of lipids and their derivatives in O.mossambicus and respiratory parameters in T.mossambica. Influence of MP in Hetropneustes fossilis was reported by Chakraborty et.al. (1989) on the AchE activity of brain and olfactory organs.

Histopathological observations were made by Desai

et.al. (1984) in T.mossambica by using monocrotophos and by Gill et.al. (1988) in P.conchonius after exposure to carbaryl and dimethoate and by Jayasundaramma et.al. (1990) in Cyprinus carpio by using MP. Sastry and Siddiqui (1984) have studied some hematological, biochemical and enzymological parameters of a fresh water teleost fish, Channa punctatus, exposed to sublethal concentrations of quinalphos.

TOXICITY OF ORGANOPHOSPHORUS PESTICIDES IN FISHES 2.2

The study of toxicity in organism is an essential step concerning the evaluation of pesticide impact on the fresh water environment specially to determine the safe concentration and to formulate the safe application rate of the insecticide concerned (Sprague, 1969; 1970). Information about the toxicity of OP insecticides on Indian fishes are few (Sreenivasan and Swaminathan, 1967; Konar, 1969 c,d; Arora et.al., 1971 a,b; Pandey et.al., 1976; Mohammed et.al., 1979).

The toxic effect of various insecticides have been observed by a number of workers viz, (Panwar et.al., 1976; Dalela et.al., 1978). The tolerance of toxicity of OP pesticide such as malathion has been observed by Chambers (1976). The effect of pesticides on fishes have been extensively studied and reviewed (Sprague, 1971; Holden, 1973; Mckim et.al., 1976).

Toor and Kaur (1974) studied the toxicity of pesticides on Cyprinus carpio communis (Linn). Toxicity of

malathion on T.mossambica and of sumithion and sevin on S.mossambicus was observed by Kabeer and Ramana Rao (1980) and Koundinya and Ramamurthy (1980) respectively. Verma et.al. (1981) observed the acute toxicity of three formulated pesticides on Mystus vittatus. Choudhuri et.al. (1984) observed the toxicity of two OP insecticides malathion and phosphamidon on Channa striatus.

The toxicity of insecticides phosphamidon, MP and mixture of three OP pesticides namely DDVP, phosphamidon, MP to fish, plankton and worm to evaluate the safe concentrations of the pesticides for aquatic life was determined by Pal and Konar (1986).

DETERMINATION OF LD_{50} TEST 2.2.1

The concept of median lethal dose (LD_{50}) test for biological standardization of potent drugs was first introduced by Trevan (1927). The LD_{50} test has been used for years to evaluate the acute toxicity of a wide variety of chemicals including food, drugs, pesticides, cosmetics, feed additives and industrial chemicals.

A large number of animals are required in LD_{50} test for exact assessment of acute toxicity of chemical substances. Miller and Tainter (1944) and Morrison et.al. (1968) made major efforts to improve acquisition and statistical treatment of the bioassay data. Efforts were then made to reduce the number of animals used in such tests. Based on the relationship between dose and survival time, Molinengo (1979) proposed a new method for the

determination of LD_{50} with fewer animals. Schutz and Fuchs (1982) and DePass (1989) proposed alternative approach for LD_{50} testing with minimum number of animals.

TLm VALUES OF PESTICIDES IN THE FISHES AS TEST ORGANISM EMPLOYING BIOASSAYS TECHNIQUES 2.2.2

For the protection of aquatic life, bioassays which are primarily oriented towards water quality criteria and can be used to develop water quality standards are essential. A series of experiments have been conducted by employing the bioassay techniques to establish the median tolerance limits (TLm) of the toxic chemicals that are generally used in agriculture and other industrial operations. TLm values of some modern pesticides to the fresh water fish, Puntius puckelli have been reported by Shivaji Rao et.al. (1967).

Bioassays studies of some commercial organic insecticides have been reported by Arora et.al. (1971 a,b). Bioassay measures the relative degree of toxicity in terms of TLm which is the concentration of tested material in water in which 50% of the test animals are able to survive for a specific period of exposure (APHA et.al., 1980). Verma et.al. (1979) have studied the acute toxicity of twenty three pesticides to a fresh water fish, Saccobranchus fossilis to evaluate the TLm values both by graphical interpolation and probit analysis methods for a period of 24, 48, 72 and 96 hour (h).

A simple bioassay method for estimating safe disposal of the insecticide to protect fish, plankton and worm

has been reported by Konar and Ghosh (1980). Singh et.al. (1981) observed the toxicity of three biocides under continuous flow system in fresh water fish, Cyprinus carpio communis (Linn.) to determine LC_{50}, relative toxicity and safe concentrations of biocides.

Ruparelia et.al. (1984) reported various 96h LC_{50} values of the pesticides, as a measure of acute toxicity to fresh water fishes. The use of static bioassays procedure to evaluate 24, 48, 72 and 96h TLm for six pesticides of 12 species of fishes have been reported by Verma et.al. (1984). Short term bioassays experiments were done on a fresh water fish, Channa punctatus by using 96h LC_{50} value of an OP pesticide malathion by Jyoti et.al. (1989).

MORTALITY AND BEHAVIOURAL RESPONSE OF FISHES AFTER EXPOSURE TO PESTICIDES 2.2.3

Choudhary et.al. (1981) have studied the effect of malathion, an OP pesticide, on behaviour, growth and body composition of an air breathing fish, Heteropneustes fossilis. The effect of different concentrations of malathion on the mortality and behaviour of two fresh water teleosts was studied by Singh and Sahai (1984). Goel and Agrawal (1989) reported the effect of MP, and OP pesticide on the behaviour and mortality of a fresh water teleost fish, O.punctatus.

BEHAVIOURAL RESPONSE AND OTHER ACTIVITIES EFFECTED BY PESTICIDES IN FISHES 2.2.4

Pal and Konar (1987) evaluated the effects of sublethal levels of OP insecticide MP on respiratory rate, behaviour,

feeding, survival, growth and reproduction of the fish, T.mossambica so as to estimate the desirable amount of the insecticide for its safe disposal.

The respiratory parameters of a fresh water fish, T.mossambica (Peters), were studied under sublethal intoxication of MP by Rao et.al. (1985). Chakraborty et.al. (1989) have studied the lethal and sublethal effects of an extensively used OP pesticide, the commercial grade MP (50% wt/wt), on the behavioural responses, and on the acetylcholinesterase (AChE) activity of the brain and olfactory organs, after different levels of exposure.

EFFECT OF PESTICIDES AND THEIR COMBINATIONS ON BODY TISSUES OF FISHES 2.2.5

Verma et.al. (1980) reported the effect of thiotox, an organochlorine and malathion, an OP pesticide and their combinations on the body composition of S.fossilis and Mystus vittatus. The effect of sublethal concentrations of thiotox, malathion and their two combinations on tissues of a fresh water fish, Notopterus notopterus were studied by Verma et.al. (1983).

ANALYTICAL TECHNIQUES FOR THE SEPARATION OF ORGANOPHOSPHORUS PESTICIDES 2.3

Several analytical techniques are available for the separation of OP pesticides including gas/liquid chromatography (Webster et.al., 1976; Bushway et.al., 1988), thin layer chromatography (Srivastava et.al., 1982) and more recently, high performance liquid chromatography (Carbas et.al., 1979;

Rosenberg and Nakatsugany, 1984). However, only few HPLC methods are available for the quantitative determination of some OP pesticides.

Two methods have been compared for the analysis of azinphos-methyl, AChE (in vitro) and HPLC. It was found that the enzymic method was faster but that several metabolites could be assayed by HPLC simultaneously (Sherma and Zweig, 1985). A study has been made on the use of HPLC for the analysis of parathion, chlorpyrifos, and chlorpyrifos-methyl from rat tissues (Sultatos et.al., 1982). Simultaneous quantitative determination of six OP pesticides by reversed phase HPLC was reported by Kumar R (1989).

HISTOPATHOLOGICAL LESIONS IN THE FRESH WATER TELEOST FISHES 2.4

The toxic effects of OP pesticides on fishes have been examined by many researchers. Several studies have shown that exposure of fish to pesticides leads to a number of disturbed pathological changes (Robert, 1978; Anees, 1978; Sastry and Sharma, 1978; Rashatwar and Ilyas, 1984). Other studies have emphasized the effect of pesticides on histopathological changes in fish (Chambers and Yarbrough, 1974; Sastry and Sharma, 1981; Sastry and Malik, 1982). It has frequently been observed that acute or chronic treatment of pesticides cause histopathological changes and biochemical alterations in organs involved in detoxication mechanism.

PATHOLOGICAL OBSERVATIONS ON THE LIVER 2.4.1

A number of workers have examined the toxic effects of OP

pesticides on fishes. Dubale and Shah (1979) studied the histopathological changes induced by malathion in the liver of Channa punctatus. The histological changes in the liver of Tilapia mossambica after exposure to a sublethal concentration of monocrotophos were studied by Desai et.al.(1984). Jayasundaramma et.al. (1990) studied the histopathological lesions in the hepatopancreas of the fresh water teleost, Cyprinus carpio exposed to MP.

RENAL PATHOLOGY AFTER PESTICIDE EXPOSURE 2.4.2

Except for scattered references, detailed studies on the histopathology of kidney in fresh water fish are scanty. Kumar and Pant (1985) reported the pathological changes in kidney after exposing the teleost, Puntius conchonius to acutely lethal and sublethal concentration of monocrotophos, an OP insecticide.

HAEMATOLOGICAL PARAMETERS OF FISHES 2.5

Blood takes part directly or indirectly in almost all the activities of fish and thus it can be a good indicator of stress conditions (Verma et.al., 1979; Bansal et.al.,1979). Tomar (1984) studied the hematology of a few fresh water teleost.

ALTERATIONS IN THE BLOOD CHEMISTRY FOLLOWING EXPOSURE TO PESTICIDES 2.5.1

Changes in the blood parameters or in some haematological values following the exposure of fishes to various types of pesticides or pollutants have been studied by few workers (Raizada and Mahashwari, 1977; Anees, 1978). Joshi et.al.

(1979) observed changes in the blood cell composition of a fish, Heteropneustes fossilis following its exposure to folidol at low temperature. Verma et.al. (1979a), Bansal et.al. (1979) and Gupta (1980) found that the pesticides induced hematological alterations in the fresh water fish, Saccobranchus fossilis.

In the past few years many chemicals used for high agricultural procurements have been reported to cause marked changes in the blood parameters of fresh water fishes (Pandey et.al., 1979; Koundinya and Ramamurthi, 1979). Sastry and Sharma (1981) studied the chemical composition of the blood of a fresh water teleost, Ophiocephalus (Channa) punctatus, exposed to the LC_{50} for 96h to a sublethal concentration for 15 and 30 days respectively of an OP insecticide diazinon. Verma et.al. (1981) studied the effect of ascorbic acid and sublethal concentrations of thiotox and malathion on certain hematological parameters of Saccobranchus fossilis.

Many workers have reported on the variations of blood parameters in teleosts due to toxic effects of pesticides (Pandey et.al., 1976; Joshi, 1982). Dabral and Chaturvedi (1983) reported the folidol induced changes in some hematologic values of H.fossilis at low temperature. Chakrabarty and Banerjee (1987) evaluated the toxic effect of three OP pesticides at their sublethal concentrations, on the peripheral hemogram of Channa punctatus (Bloch).

The effect of 48h-LC_{50} concentration of three

pesticides, namely phorate, phenthoate and diazinon on the peripheral hemogram of Channa punctatus (Bloch) has been studied by Chakrabarty and Banerjee (1988). Anees (1978) examined the concentration of hemoglobin in the blood of fish exposed to OP pesticides. Sastry and Siddiqui (1984) examined the hematological, biochemical and enzymological parameters of Channa punctatus exposed to sublethal concentrations of quinalphos, an OP insecticide. Srivastava and Srivastava (1988) studied the effect of the organochlorine insecticide aldrin and OP insecticide malathion on blood chloride levels of H.fossilis following both acute (96h) and chronic (15-70 days) exposure to subacute and sublethal concentrations, respectively of these pesticides.

IMPACT OF PESTICIDES ON SOME SELECTED PARAMETERS OF OXIDATIVE METABOLISM AND HAEMATOLOGY 2.5.2

Srivasthawa and Singh (1981) and Singh and Srivasthawa (1981) investigated the effects of MP and formathion on oxidative metabolism of Heteropneustes fossilis. Verma and Tonk (1984) studied the fish respiration, blood parameters and few enzymes as the indicator of the sublethal pesticides contamination of water. Pandey et.al. (1984) studied the effect of some pesticides on the oxidative metabolism and hematology of Clarias batrachus (Bloch). Natrajan (1984) observed the effect of sublethal concentration of metasystox on selected oxidative enzymes, tissue respiration, and hematology of Channa striatus (Bleeker). An attempt has been made to evaluate the comparative toxicity of endosulfan

and MP on some selected parameters of oxidative metabolism in the nutritionally valuable fish, Catla catla at different sublethal exposure periods by Bashamohideen et.al. (1988).

BIOCHEMICAL ALTERATIONS 2.6

Various chemicals entering the aquatic ecosystem through man's activities, either accidently or by design, may cause adverse effects on the aquatic biota, including deleterious changes which disrupt the metabolic activity at a biochemical level (Verma et.al., 1979 a,b,c). Mukhopadhyay et.al. (1980) observed the toxicological effects including the biochemical changes resulting from exposure of the air breathing fish, Clarias batrachus to subacute toxic doses of OP insecticides malathion. Sastry et.al. (1982) examined the alteration in some biochemical and enzymological parameters in Channa punctatus exposed chronically to quinalphos, an OP pesticide.

ENZYMOLOGICAL CHANGES IN FISH TISSUE AFTER EXPOSURE TO PESTICIDES 2.6.1

Verma et.al. (1981) have studied in vivo the enzymative alterations in certain tissues of S.fossilis following exposure to sublethal concentrations of four toxic substances. The effect of exposure for 96h to LC_{50} and for 15 and 30 days to the sublethal concentration of diazinon has been examined by Sastry and Malik (1981) on the activities of some enzymes in certain tissues of Channa punctatus (Bloch). Sastry and Malik (1982) observed the effect of exposure to LC_{50} for 96h and to a sublethal

concentration of diazinon for 15 and 30 days with the enzymological changes in the digestive system of H.fossilis.

ENZYMATIC ALTERATIONS IN THE ACTIVITIES OF PHOSPHATASES, DEHYDROGENASES AND ESTERASES 2.6.2

Koundinya and Ramamurthi (1978) observed the effects of sumithion (fenitrothion) on some selected enzyme systems in Tilapia mossambica. Effect of various sublethal concentrations of pesticides and their combinations (Verma et.al., 1980) on the activities of three serum phosphatases of Mystus vittatus has been described by Verma et.al. (1984). The alterations in the activities of alkaline phosphatase (AlPase), acid phosphatase, (AcPase), glucose-6-phosphatase, lactic dehydrogenase, pyruvic dehydrogenase, succinic dehydrogenase and cholinesterase were observed by Sastry and Sharma (1980). Verma et.al. (1982) studied the effects of sublethal concentration of pesticides on three phosphatases of S.fossilis.

The effect of acute exposure for 96h LC_{50} concentration of phosphamidon on the biochemical alterations in the liver and kidney of Nemachelius denisonii (Day) has been studied by Rashatwar and Ilyas (1984). In vitro inhibitory effects of dieldrin and MP on succinate dehydrogenase (SDH) activity in Tilapia mossambica have been observed by Manirathnam Reddy et.al. (1985).

INFLUENCE OF VITAMINS IN ANIMALS 2.7

ROLE OF VITAMIN C AGAINST TOXICITY 2.7.1

The protective role of vitamin C against pesticides and

heavy metal toxicity was studied by Fox and Fry (1970) and Agrawal et.al. (1978) respectively. The complex relationship of vit. C to the enzymes and drugs in liver function has been reviewed and protective action against a major group of pesticides was reported by Street and Chadwick (1975). The influence of vit. C in a fish against the pesticide toxicity was studied by Verma et.al. (1981).

EFFECT OF VITAMIN B_{12} AGAINST TOXICITY 2.7.2

A comparative study on the protective effects of vit. B_{12} and other substances in rat liver has been reported by Rana and Tayal (1981). Eymard Poydock et.al. (1982) reported the effect of vitamins on the longevity of mice bearing ascites tumors.

MATERIALS AND METHODS 3.0

MATERIALS 3.1

DESCRIPTION OF TEST FISHES 3.1.1

Fishes are widely used as test animals to evaluate the toxicity of wastes and water pollutants; probably due to their adaptability to laboratory conditions; availability and varying degrees of sensitivity to toxic substances. For the present experimental work Ophiocephalus (Channa) punctatus belonging to the family Ophiocephalidae; order Ophiocephaliformes, commonly known as 'Sauli' in vernacular language has been studied. It is commercially important fish found in fresh water habitats, including the confined and slow running muddy shallow waters. Being carnivorous, they are a menance to organized fish culture. It is esteemed as food because of its invigorating, animating, nourishing and medical qualities. It could survive in laboratory aquaria for long periods. Due to the presence of accessory respiratory organs, it does not easily suffer from asphyxiation and can be kept out of water for some times for experimental purposes. They can be easily transported alive and commonly known as 'Jiolmachh' or live fishes. Healthy living specimens of O.punctatus, irrespective of sex were procured from local fish market, conveyed to laboratory in aerated polythene containers having water and were maintained in the laboratory aquaria. Fishes were placed in dilute bath of 0.1 mg/l $Kmno_4$ solution for about 5 to 10 minutes to eliminate any dermal infection and then rinsed with fresh water. Prior to experimentation, fishes

were allowed to acclimatize to laboratory conditions atleast for one week at natural photoperiod and temperature.

TOXICANT 3.1.2

The widely used agricultural pesticide; MP (O-O-dimethyl, O-p-nitrophenyl phosphorothionate), has been selected which is the product of Bayer India Ltd., Bombay; marketed under the trade name 'Metacide 50 (50% wt/wt)', the synonyms are metaphos, dimethyl parathion, metron, folidol M, fosfero M50, parataf, methyl niran, nitron 80, partron-M, pencap-M, wofatox. The toxicant is a tan to brown liquid with a pungent garlic-like odor.

ANTITOXICANT 3.1.3

Vitamin B_{12} (each ml contained cyanocobalamin anhydrous (as cyanocobalamin IP) 1000 mcg phenol IP (preservative) 0.5% W/V), has been selected as a antitoxicant for intoxicated fishes, the product of Glaxo India Ltd., Bombay; marketed under the trade name 'Macrabin®-'1000'.

METHODS 3.2

BIOASSAY DETERMINATION 3.2.1

Bioassays were conducted employing the technique of static bioassays (with renewal design). This method generally provide exploratory information in terms of relative toxicity of various compounds and is widely adopted by Public health authorities in many countries for evaluating toxicity of chemicals in relation to fish tolerance.

Bioassay measure the relative degree of toxicity in terms of TLm which is the concentration of the tested

material (toxicant), in the medium under which 50% of the animals are able to survive for a specific period of exposure (APHA, 1976).

Bioassay encompasses three main phases: acclimation of fish, exploratory tests and final tests from which estimation of the TLm are determined.

ACCLIMATION 3.2.1.1

Fishes were transferred to the acclimatizing aquaria immediately after procuring from market to laboratory to lessen the high mortality rate during the first few days in the laboratory because of shock and mechanical damages which results due to fungal and bacterial infection (Lewis, 1963). The transfer was done with a small mesh nylon dip net. During acclimation, the fishes were fed with artificial diet. Filteration and aeration of the aquarium water were necessary to provide an acceptable biological environment during the acclimation period. Feeding was stopped two days prior to the start of the test as feeding would increase the rate of metabolism and the excretory substances may influence the toxicants of the test solution. Any dead fish was removed immediately from the container, to avoid change in concentration of the dissolved oxygen (D.O.) which affect static bioassay.

EXPLORATORY TESTS 3.2.1.2

A series of two-step exploratory tests were performed to determine the approximate range of test concentration (in the range, lower than the test range) at which all fish

survived for 24h and the lowest concentration (in the range higher than the test range) at which all or most fish died in the same period. The mortality of the fish in test concentration was recorded. The exploratory tests were conducted with five test animals per concentration.

FINAL TEST 3.2.1.3

The purpose of the final test was to determine the TLm of O.punctatus to MP toxicity for the period of 24, 48, 72 and 96h.

Estimation of the TLm was made by interpolation and plotting of the data on graph paper with the log values of selected toxicant (MP) on the semilogarithmic scale and mortality percentage on arithmatic scale (Anees, 1975). The point at which the straight line thus found crosses the 50% mortality level has been taken as the median lethal concentration (LC_{50}).

SELECTION OF THE TOXICANT CONCENTRATION 3.2.2

After static bioassay tests (with renewal design) the LC_{50} for the toxicant MP for model fish was determined. Two sublethal concentrations of MP i.e., $1/5^{th}$ and $1/10^{th}$ fractions of LC_{50} for acute exposure were selected.

MODE OF TREATMENT 3.2.3

Acclimatized, healthy, uninjured fishes, measuring about 15 to 18 cm in length and 40-50 gm in weight were selected for the present study.

ACUTE EXPOSURE 3.2.4

For acute exposure, the fish were treated with sublethal concentrations ($1/5^{th}$ and $1/10^{th}$ fractions of LC_{50}) of MP. The data was collected at four time intervals i.e., after 24, 48, 72 and 96h. Control experiments were also conducted simultaneously, keeping identical conditions. Test solution in the aquaria was renewed regularly after 24h so as to remove the debris and to maintain the concentration of MP constant during the periods of exposure.

QUANTITATIVE DETERMINATION OF PESTICIDE EMPLOYING HPLC 3.2.5

EXPERIMENTAL 3.2.5.1

MP of HPLC grade was prepared in methanol and diluted as required with the mobile phase.

APPARATUS 3.2.5.2

For HPLC, LC-4A chromatograph (Shimadzu, TOKYO, Japan) with an SPD-2AS variable wave length UV detector was used. The column was a ZORBEX ODS (25x0.26 cm. ID); analysis were performed at room temperature.

CHROMATOGRAPHY 3.2.5.3

Elution was performed with the mobile phase, methanol-water (80:20) at a flow rate of 1 ml/min. The best wave length for quantitative determination was found to be at 254 nm. A standard curve was also constructed simultaneously.

EXTRACTION PROCEDURE 3.2.5.4

Take 2 gm of tissue (liver, kidney) add 4 gm sodium sulphate (from merck) with 20 ml acetonitrile (HPLC grade), a homogenate was prepared using a Potter-Elvehjem homogenizer.

The extract was filtered through cheese cloth into a round bottom flask. This extraction procedure was repeated two times with 10 ml acetonitrile. The extract was then dried by passing it through a rotatory evaporator at 40°C and the elute was collected in a round bottom flask. The residue was dissolved in 1.5 ml of methanol and collected in a culture tube for analysis by HPLC.

HISTOPATHOLOGY 3.2.6

For histopathological studies, fish from both control and treated groups were dissected after 24, 48, 72 and 96h. Tissue samples of liver and kidney were removed carefully using freshly prepared alcoholic Bouin's solution as the fixative and processed for embedding in paraffin wax (m.p 58°C) for routine histological examination. After preparing blocks, sections were cut on a rotatory microtome to a thickness of 5-7 micron (μ). The double stain method was followed by using haematoxylin and eosin. Silver impregnation technique (Gorden and Sweets, 1936) and Van Gieson's picric acid fuschin (Pearse, 1971) were also employed.

HAEMATOLOGY 3.2.7

COLLECTION OF BLOOD 3.2.7.1

At the end of the experiment; fishes were sacrificed after 24, 48, 72 and 96h. Blood was drawn by severing caudal peduncle with a heparinised syringe and was analysed. Haemoglobin, RBC and WBC counts and haematocrit or PCV values were determined in whole blood. For the estimation

of other components, blood was allowed to clot and then centrifuged immediately at 3000 r.p.m. for 15 min and the supernatant serum removed with a clean rubber bulb pipette.

BLOOD ANALYSIS 3.2.7.2

IN WHOLE BLOOD 3.2.7.2.1

Haemoglobin percentage (Hb%) of blood was determined by Sahli's haemometer (Germany). Haematocrit value (Packed cell volume; PCV) was calculated by Wintrobe method (3000 rcv/min for one hour) as described by Dacie and Lewis (1975). RBC and WBC counts were determined by Neubauer's double chambered haemocytometer (U.S.A.) using Hayem's and Tuerk's diluting fluid, respectively. Mean cell volume (MCV), mean cell haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated according to the formulae given in 'Practical haematology' by Dacie and Lewis (1975).

IN BLOOD SERUM 3.2.7.2.2

SERUM UREA 3.2.7.2.2.1

Serum urea was determined by adopting the method of King (1946).

SERUM GLUCOSE 3.2.7.2.2.2

Serum glucose was analysed by the method of Marks (1959).

SERUM CHOLESTEROL 3.2.7.2.2.3

Serum cholesterol was estimated by using the method given by Zlatkis et.al. (1953).

ENZYMES 3.2.7.2.2.4

TOTAL PROTEIN 3.2.7.2.2.4.1

In blood serum, the total protein was determined using Bovine serum albumin (BSA) as standard (Lowry et.al., 1951).

PHOSPHATASES 3.2.7.2.2.4.2

AlPase and AcPase were estimated in blood serum according to the method described in Bergmayer (1974).

TRANSAMINASES 3.2.7.2.2.4.3

Glutamic-oxaloacetic transaminase (GOT) and Glutamic-pyruvic transaminase (GPT) were determined adopting the method of Bergmayer (1974).

HISTOCHEMISTRY 3.2.8

Since histochemistry is considered as a link between morphology and chemistry, its parameters were chiefly adopted in the present study. Specific histochemical techniques for a few enzymes viz, AlPase, AcPase and lipase were employed in both the tissues. The tissues from the autopsied fishes were removed carefully and fixed in the respective fixatives (Table-1). Paraffin wax of low m.p (52-54°C) was used for tissue embedding 5-7 μ thick sections were processed for localization techniques. The methods employed for the histochemical studies are given in Table-1. To check the validity of each reaction, suitable controls were also set simultaneously.

BIOCHEMISTRY 3.2.9

The biochemical mechanism responsible for the insecticide injury are little understood, a quantitative approach to

the liver and kidney was made with MP. The biochemical analyses of the tissues included the estimation of some important organic constituents (Proteins, lipids and carbohydrates) inorganic constituent (moisture) and a few enzymes like AlPase, AcPase and lipase.

PROTEIN DETERMINATION 3.2.9.1

Protein was determined by the procedure developed by Lowry et.al. (1951) using Bovine serum albumin (BSA) as standard.

LIPIDS 3.2.9.2

Lipids were extracted with chloroform in a soxhlet apparatus. The flask, containing chloroform was heated in a water bath until the chloroform in the fractionating column became colourless. The chloroform extract was evaporated to dryness and the lipid re-extracted with hot petroleum ether. This extract contained all the tissue lipids (Colowick and Kaplan, 1963).

CARBOHYDRATES 3.2.9.3

Total carbohydrates were estimated employing orcinol-reagent (Frank-Consolazio and Iacono, 1963). The weighed tissue after adding acid was centrifuged, filtered and diluted. Filtered solution after adding acid and orcinol was heated and the optical density (O.D.) of solution was noted at 520 μ.

MOISTURE 3.2.9.4

The tissues (liver and kidney) after recording the fresh weight were dried in a low temperature drying oven at 50°C.

Drying, cooling and weighing continued until the weight became constant. The difference in the weight of the fresh and dehydrated tissue is the amount of moisture present in the total tissue. The dried tissue was powdered and utilized for the estimation of organic and inorganic components.

ENZYME ASSAY 3.2.9.5

PREPARATION OF TISSUE HOMOGENATES 3.2.9.5.1

For the quantitative determination of few enzymes, control as well as treated fishes were sacrificed and the liver and kidney were removed, washed, weighed and immediately frozen at 4°C. 10% (w/v) homogenates were prepared separately in 0.25 M ice cold sucrose solution using a Potter Elvehjem homogenizer and spundown in a cooled centrifuge and supernatants were used for enzyme study.

PHOSPHATASES AND LIPASE 3.2.9.5.2

The activity of AlPase and AcPase was determined by the method of Bodansky (1933). The substrate used was 0.16 M sodium β-glycerophosphate at 9.3 and 5.0 pH for AlPase and AcPase respectively. Lipase activity was determined by the method of Bier (1955) with Tween 20 as substrate.

STATISTICAL EVALUATION OF STATISTICAL DATA 3.2.10

The student t-test described by Fisher (1950) was employed to calculate the significance of difference between control and experimental value.

ROUTE OF ADMINISTRATION AND DOSAGE SCHEDULE OF VITAMIN B_{12} 3.2.11

Vit. B_{12} was injected in the intoxicated fish intramuscularly. In the intoxicated fish, vit. B_{12} administered at the dosage of 0.25 ml on each alternate day for two weeks. The dose levels were selected after primary toxicological tests such as LD_{50}.

TABLE-1 : HISTOCHEMICAL TECHNIQUES EMPLOYED FOR THE LOCALIZATION OF ENZYMES

Constituents	Fixative used	Fixation time	Substrate	Test applied
Alkaline phosphatase	Chilled Acetone	Overnight(24h)	Sodium- β -glycero-phosphate	Ca-cobalt method of Gomori (1952)
Acid phosphatase	Chilled Acetone	Overnight(24h)	Sodium- β -glycero-phsophate	Lead nitrate method of Gomori (1952)
Lipase	Chilled Acetone	24h	Tween-60	Tween's method of Martin (Pearse, 1968)

RESULTS 4.0

TLm VALUES (LC_{50} VALUES) 4.1

The toxicity caused by the pollutants accumulated can be measured in terms of mortality. Keeping this in mind, bioassays were conducted to evaluate the toxicity of the MP with respect to O.punctatus. TLm values (LC_{50} values) for MP were found to be 0.034 ml/l, 0.0056 ml/l, 0.0031 ml/l and 0.0015 ml/l at 24, 48, 72 and 96h interval respectively (Figs. 1, 2, 3 & 4). Two sublethal concentrations of MP i.e., $1/5^{th}$ and $1/10^{th}$ fractions of LC_{50} for acute exposure were selected. The sublethal concentrations selected were 0.0068 ml/l, 0.0034 ml/l, 0.00112 ml/l, 0.00056 ml/l, 0.00062 ml/l, 0.0031 ml/l, 0.003 ml/l and 0.00015 ml/l. Fishes treated with higher concentration (0.06 ml/l) show significant rate of mortality during 24 h exposure, but there was decline in the rate of mortality when fishes were exposed to lower concentrations for the same duration (Fig.1). When fishes were exposed to the concentration of 0.005 ml/l to 0.02 ml/l, the rate of mortality remained constant. However, there was sudden increase in the rate of mortality when the concentration was increased.

Higher TL_{50} values are suggestive of low toxicity where as lower TL_{50} values indicate high toxicity. There was no mortality of the fish in control test containers. This clearly indicates that no other factor was responsible for the mortality of the experimental fish except the chemical stress of the toxicant used.

During acute toxicity experiments, changes in behaviour and external signs of poisoning have been studied. Histological, haematological, histochemical and biochemical alterations under the stress of each concentration have also been studied during acute exposure.

BEHAVIOURL CHANGES 4.2

Observations on the behavioural response of the fishes under the stress of different concentrations of MP was carefully studied. The behavioural response of the test fish, O.punctatus was grossely dependent on the concentration and the duration of exposure. In lethal and higher concentrations, the fish showed some symptoms of poisoning, such as increased excitability and occasionally non coordinated swimming movements with relatively reduced respiratory activity as revealed by the decrease in opercular movements. Fishes were often observed swimming with jerky movements on the surface of the water and tried to jump out of aquaria. Longer exposure to low and gradual increasing concentrations of MP, showed relatively enhanced activity in the beginning and reduced activity later. Some of the fishes frequently dashed against the walls of the container suggesting impairment of sense of balance. With the decreased concentration of the MP, the loss of the equilibrium was regained and the movements became normal. Subsequently, the fish became progressively lethargic and lost their balances. Some of the fishes were characterized by the development of tremors and convulsions. Ultimately, the fish sank to the

Fig.1 Illustrating LC_{50} (Median lethal concentration) of methyl-parathion (MP) for O.punctatus after 24h exposure.

Fig.2 Illustrating LC_{50} (Median lethal concentration) of methyl-paration (MP) for O.punctatus after 48h exposure.

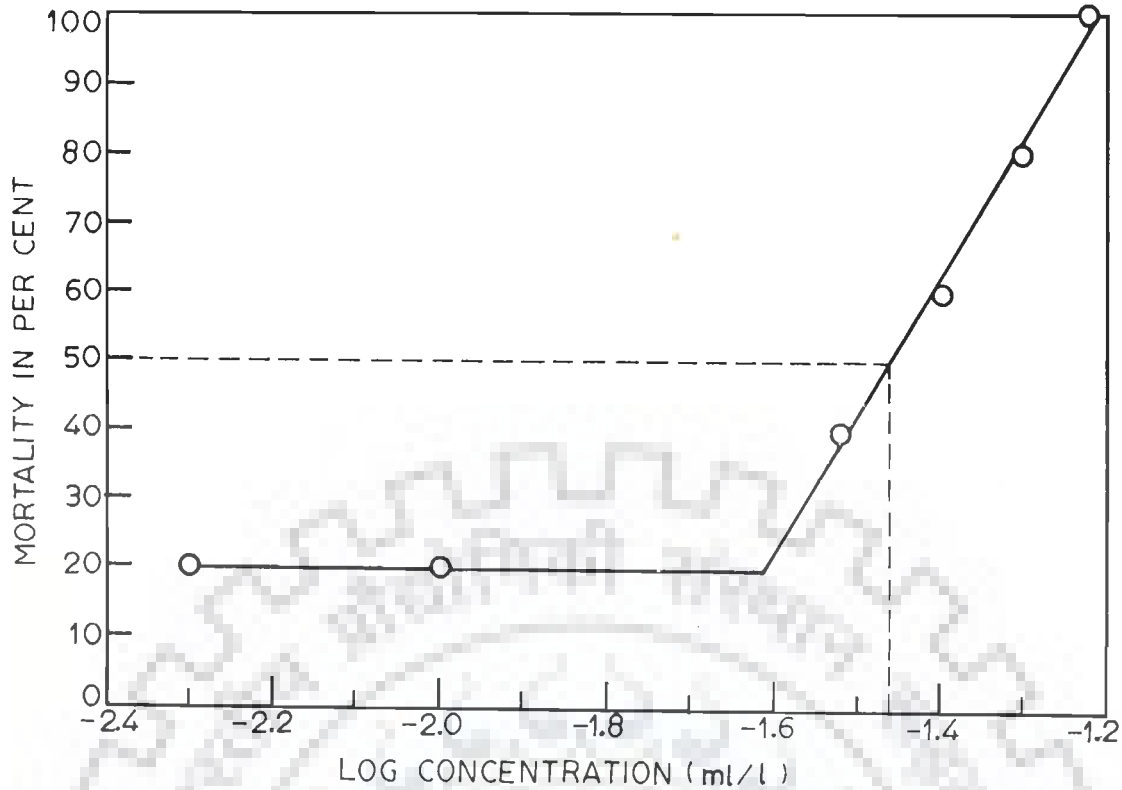
MORTALITY IN PER CENT
100
90
80
70
60
50
40
30
20
10
0
-2.4
-2.2
-2.0
-1.8
-1.6
-1.4
-1.2
LOG CONCENTRATION (ml/l)

Fig.1

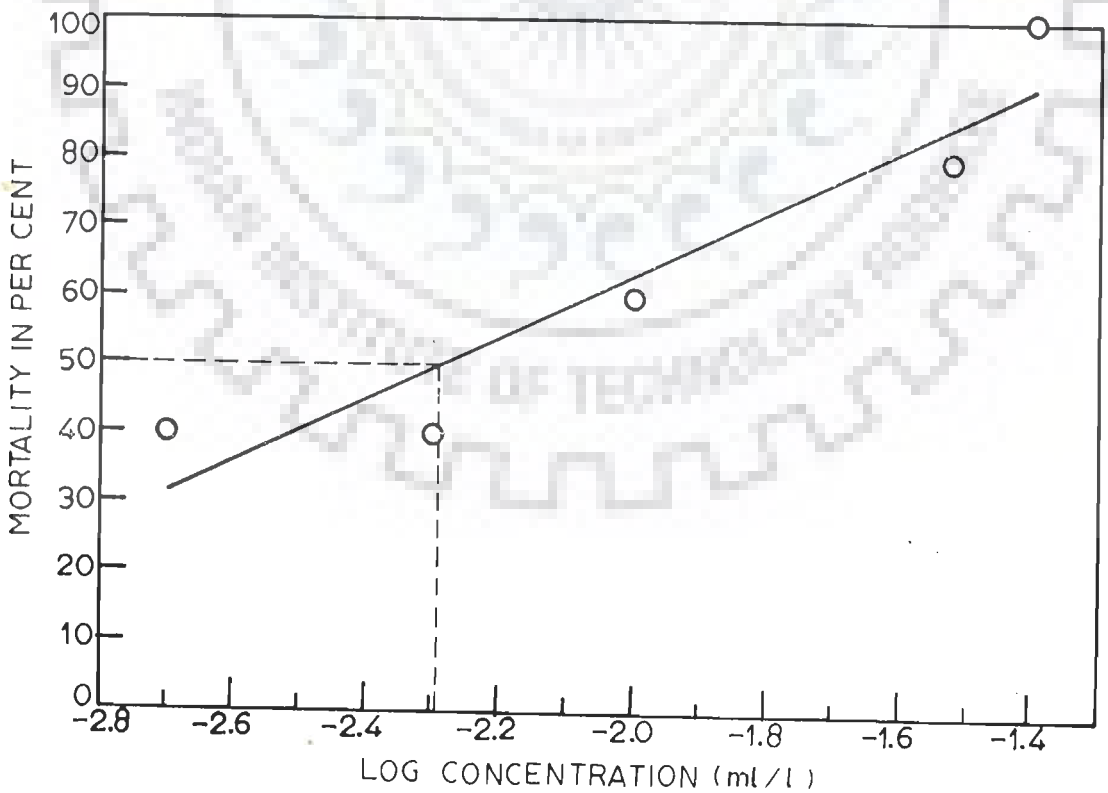
MORTALITY IN PER CENT
100
90
80
70
60
50
40
30
20
10
0
-2.8
-2.6
-2.4
-2.2
-2.0
-1.8
-1.6
-1.4
LOG CONCENTRATION (ml/l)

Fig. 2

Fig.3 Illustrating LC_{50} (Median lethal concentration) of methyl-parathion (MP) for O.punctatus after 72h exposure.

Fig.4 Illustrating LC_{50} (Median lethal concentration) of methyl-parathion (MP) for O.punctatus after 96h exposure.

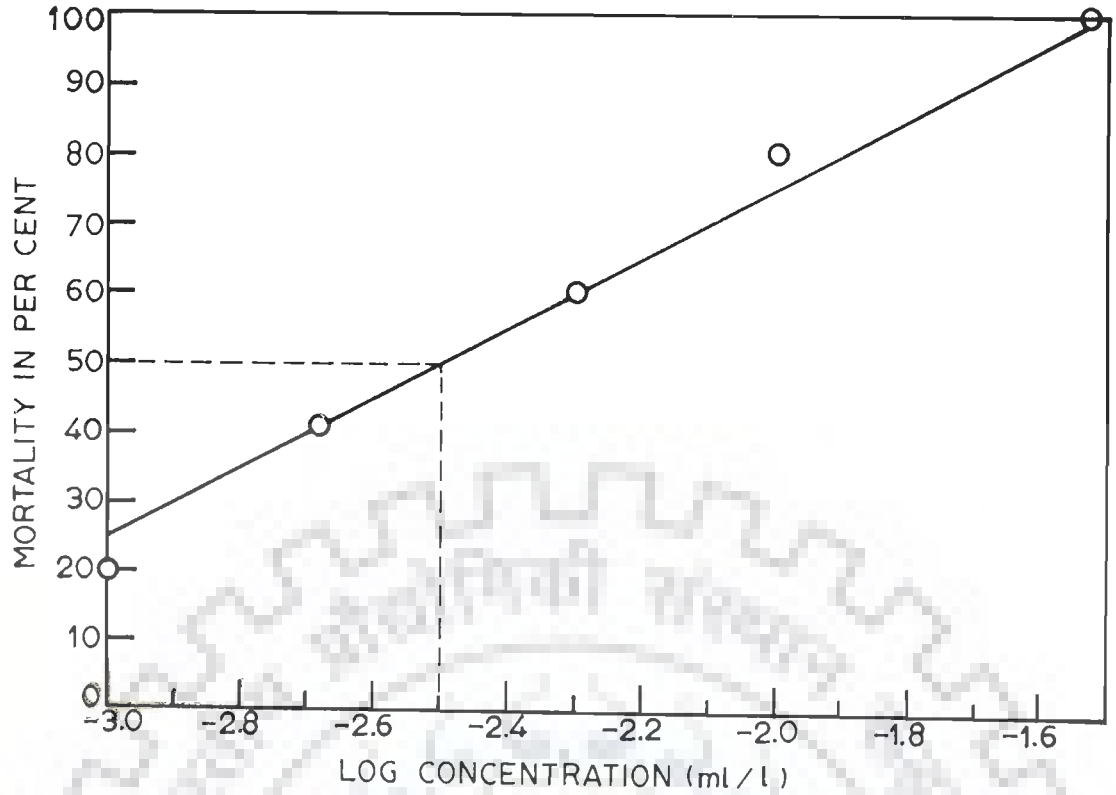
MORTALITY IN PER CENT
100
90
80
70
60
50
40
30
20
10
0
-3.0
-2.8
-2.6
-2.4
-2.2
-2.0
-1.8
-1.6
LOG CONCENTRATION (ml/l)

Fig.3

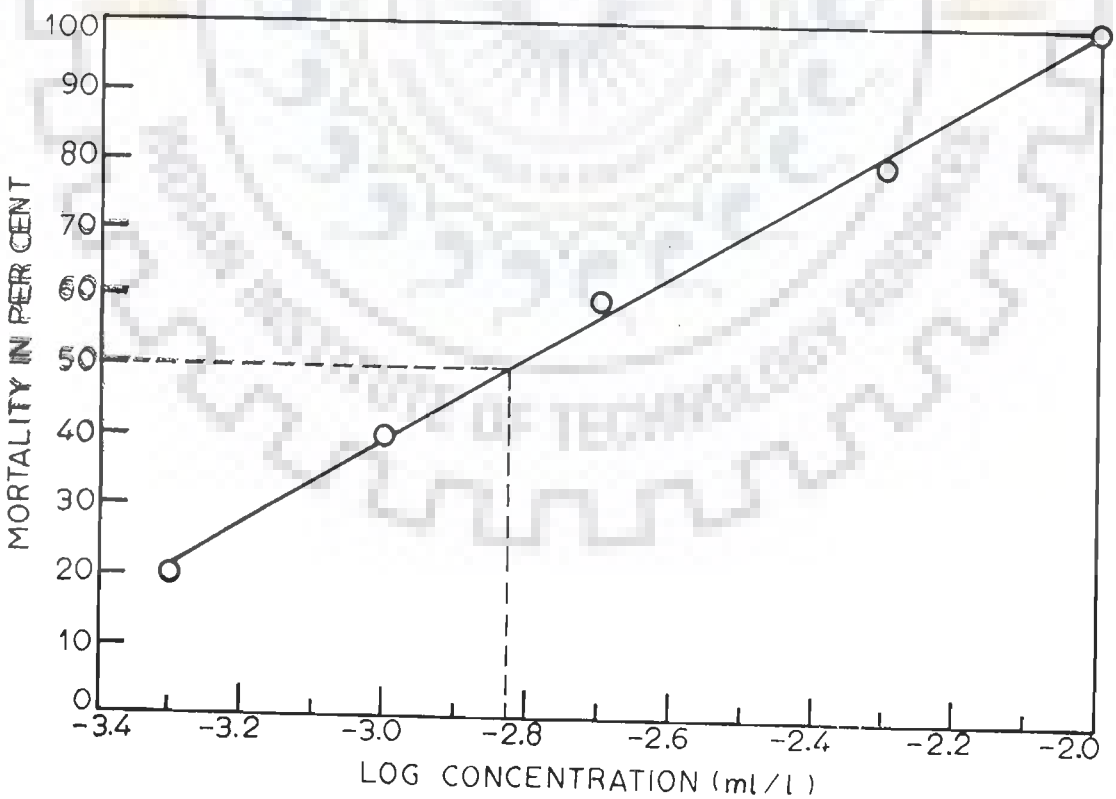
MORTALITY IN PER CENT
100
90
80
70
60
50
40
30
20
10
0
-3.4
-3.2
-3.0
-2.8
-2.6
-2.4
-2.2
-2.0
LOG CONCENTRATION (ml/l)

Fig.4

bottom of the containers and died. A fish was considered dead when it gave no response on a slight probe with a glass rod. Dead fishes were removed from the experimental containers because such mortality in the static bioassays might deplete the D.O. for other fish (Schreck and Broucha, 1975). The dead fish exhibited open opercula, sometimes bent body, and whitish gills with copious mucus, occasionally with signs of petechial bleeding.

CHANGES IN EXTERNAL MORPHOLOGY 4.3

Fishes exposed to different concentrations of MP showed excessive secretion and deposition of mucus over the entire surface of the body. The colour of the body became dull. Extreme signs of restlessness such as muscle spasm, body torsion and coughing (air bubbles coming out from the mouth) were observed in the treated fish. The blood clots were often seen on the sides of mouth. Some wounds were also observed near the buccal and head region because of its jerky body movements. Fishes protected with vit.B_{12} after 96h exposure, shows loss of excessive secretion and deposition of the mucus on the body surface. The colour of the body slightly come to the normal colour. Subsequently, the fish became active with co-ordinated movements.

QUANTITATIVE ESTIMATION OF MP IN FISH TISSUE BY HPLC 4.4

Fig. (5, 6) shows the elution profiles of MP residues in fish tissues (liver and kidney) treated with MP. Pure MP shows a sharp single peak with retention time of 2.715 with 99.18% concentration [Fig. 5(A),6(A)]. In case of liver,

the concentration of the MP absorbed by the tissue increases as the time duration is increased. At 24h exposure, the concentration of MP absorbed by the liver is 58.86% and 57.61% (for $1/5^{th}$ and $1/10^{th}$ fractions of LC_{50}), respectively but when the exposure time is increased, the concentration of MP is increased [Fig. 5(B), 6(B)]. At 96h exposure, the concentration of MP absorbed by the tissue is highest i.e., 99.45% and 96.13% (for $1/5^{th}$ and $1/10^{th}$ fractions of LC_{50}), respectively [Fig. 5(D), 6(D)].

In case of kidney tissue, the pattern is same i.e., the concentration of MP is increased with the duration of exposure time. At 24h exposure, the concentration of MP absorbed by the tissue is 47.73% and 47.63% (for $1/5^{th}$ and $1/10^{th}$ fraction of LC_{50}), respectively [Fig. 5(C), 6(C)] but as the time increased the MP is completely absorbed by the tissues and gives the highest concentration of 95.98% and 95.84% for 96h exposure (for $1/5^{th}$ and $1/10^{th}$ fractions of LC_{50}), respectively [Fig. 5(E), 6(E)]. After 96h exposure, the parent toxicant (MP) is completely absorbed by the tissue (both in liver and kidney).

It clearly shows that the absorption of MP concentration by the tissues increased with the duration of the exposure time (Tables 2, 3).

The main purpose of this study is to see, how much quantity is absorbed by the tissue after pesticide treatment and to confirm that the abnormalities in fish is only due to the presence of this pesticide (MP) in tissues.

Fig.5 HPLC Chromatograms of methyl-parathion (MP) in the liver (B,D) and kidney (C,E) of O.punctatus after 24 and 96h exposure (Sublethal concentration : 1/5th fraction of LC_{50}), respectively.

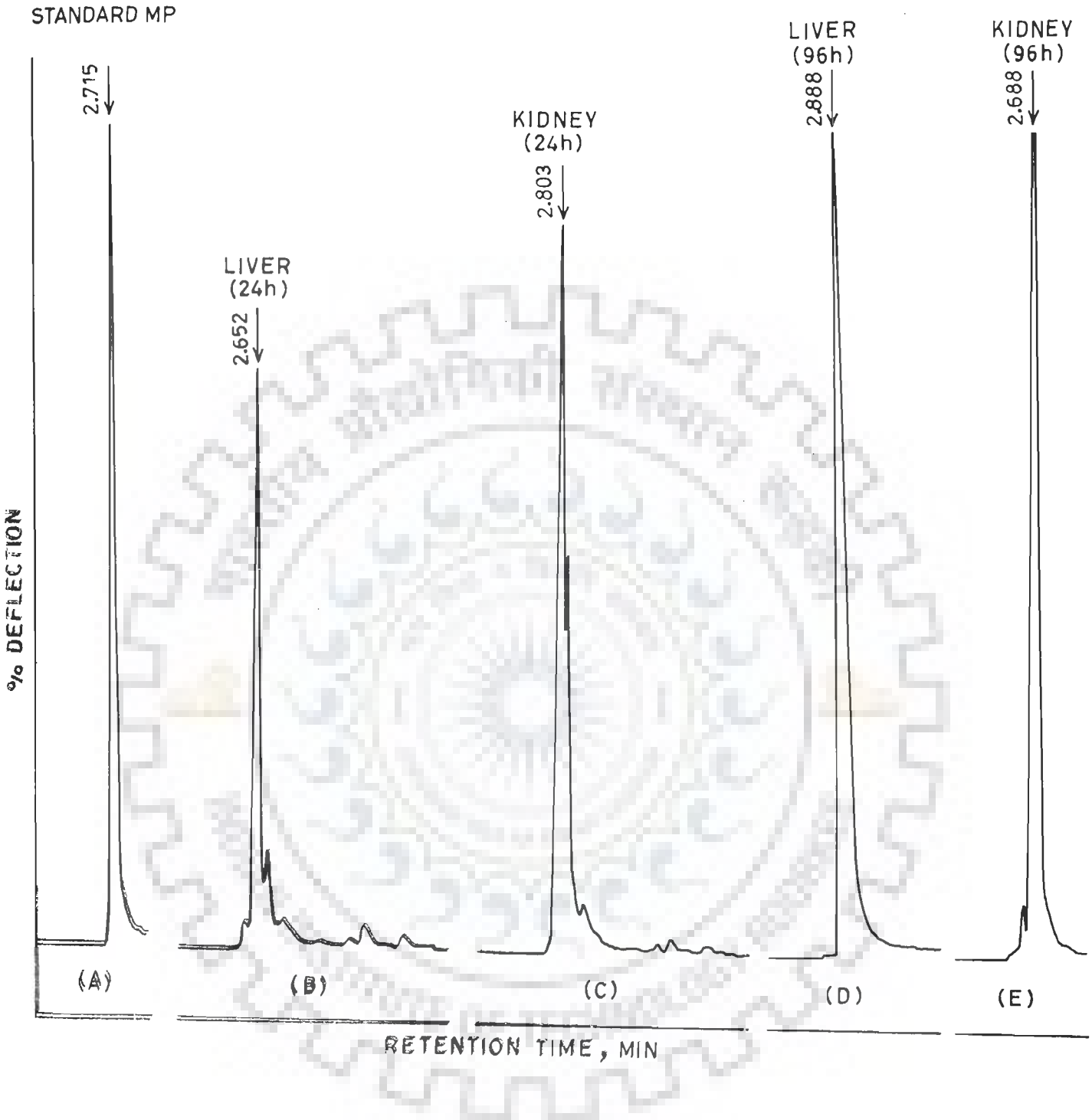
STANDARD MP
2.715
LIVER
(24h)
2.652
KIDNEY
(24h)
2.803
LIVER
(96h)
2.888
KIDNEY
(96h)
2.688
% DEFLECTION
(A)
(B)
(C)
(D)
(E)
RETENTION TIME, MIN

Fig. 5

Fig.6 HPLC Chromatograms of methyl-parathion (MP) in the liver (B,D) and kidney of (C,E) of O.punctatus after 24 and 96h exposure (Sublethal concentration : $1/10^{th}$ fraction of LC_{50}), respectively.

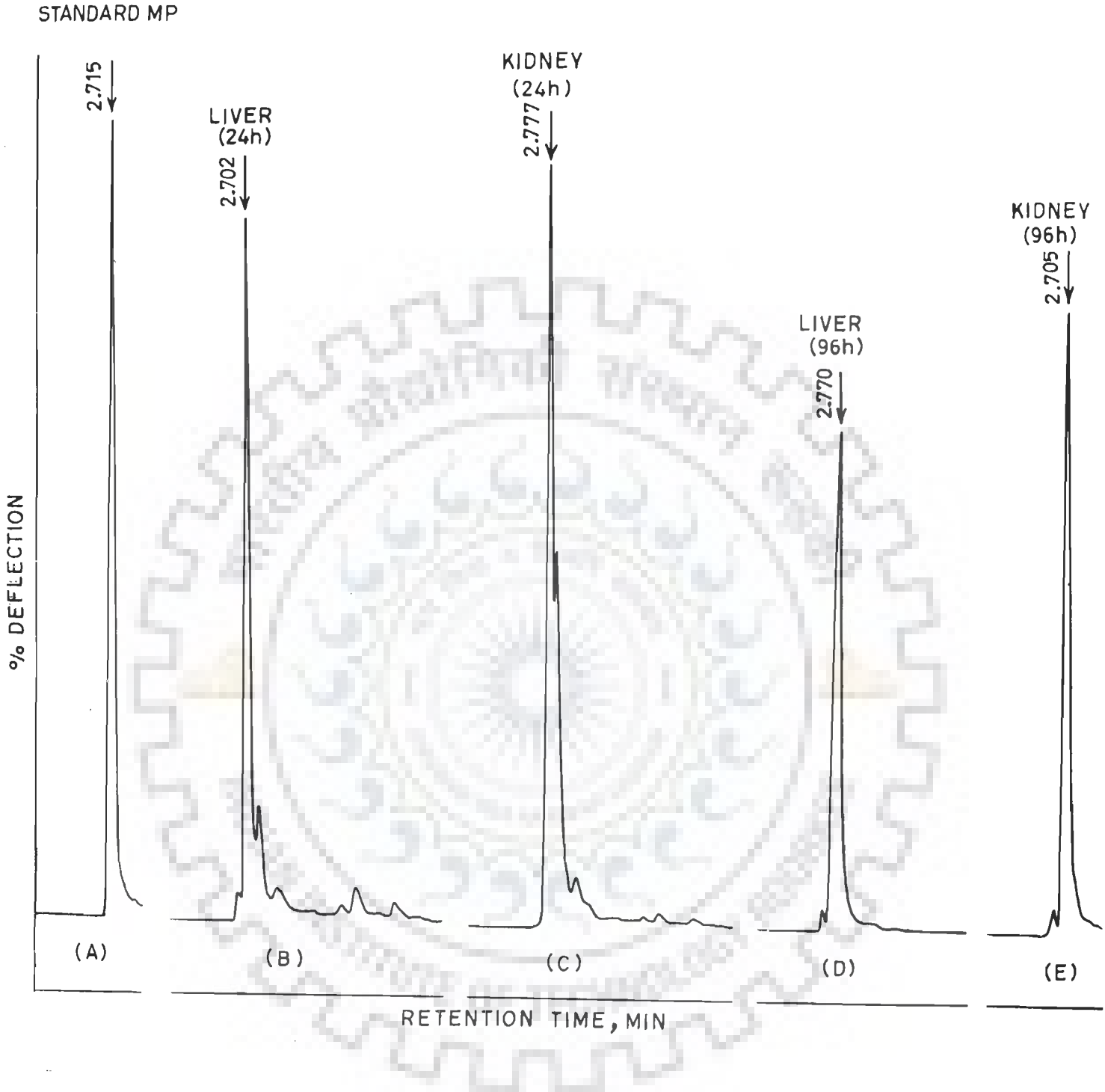
STANDARD MP
2.715
LIVER
(24h)
2.702
KIDNEY
(24h)
2.777
LIVER
(96h)
2.770
KIDNEY
(96h)
2.705
% DEFLECTION
(A)
(B)
(C)
(D)
(E)
RETENTION TIME, MIN

Fig. 6

TABLE-2 : ABSORBTION OF MP IN THE LIVER AND KIDNEY OF O.PUNCTATUS FOR 24, 48, 72 AND 96h INTERVALS (SUBLETHAL CONC: $1/5^{th}$ FRACTION OF LC_{50})

Time of administration	Conc. (%) in liver	Conc.(%) in kidney
24 h	58.86	47.73
48 h	94.41	90.89
72 h	95.56	95.49
96 h	99.45	95.98

TABLE-3 : ABSORBTION OF MP IN THE LIVER AND KIDNEY OF O.PUNCTATUS FOR 24, 48, 72 AND 96h INTERVALS (SUBLETHAL CONC:1/10th FRACTION OF LC_{50})

Time of administration	Conc.(%) in liver	Conc.(%) in kidney
24h	57.61	47.63
48h	91.26	83.93
72h	95.10	95.47
96h	96.13	95.84

HISTOPATHOLOGICAL OBSERVATIONS 4.5

Histology gives useful data concerning the tissue degeneracy prior to external manifestation of the abnormalities. Thus the microscopic examination of some important tissues (liver and kidney) of test fish before and after the toxicant exposure (MP) reveals reveals histopathological lesions. Multiple fixation and staining procedures ranging from hematoxylin/eosin to silver impregnation technique were employed. The results thus obtained are described below.

HISTOLOGY OF CONTROL TISSUE (LIVER) 4.5.1

The liver of the O.punctatus is a distinct bilobed gland, dark brownish-red in colour. In normal liver, hepatocytes which form the homogenous mass are arranged radially around the branches of the hepatic vein in the form of hepatic cords. It is however, less organized than in the mammals and other higher vertebrates. The hepatic cords are formed by the liver parenchyma which encloses the blood sinusoids, lymphatic channels containing bile pigments and the lymphocytes etc. The so formed hepatic cords confirm the rich supply of the vascular system to each hepatocyte which is polygonal in shape, containing a distinct centrally placed nucleus with densely stained chromatin (Fig.7.1).

HISTOPATHOLOGY OF TESTED TISSUE (LIVER) UNDER THE STRESS OF MP AND VITAMIN B_{12} 4.5.1.1

LIVER (SUBLETHAL CONC. : $1/5^{th}$ FRACTION OF LC_{50}) 4.5.1.1.1

After 24 h exposure to MP, a number of degenerative changes

were observed in the histological structure of the liver as evidenced by the degeneration of both cytoplasmic and nuclear material of parenchymal cells throughout the liver lobules. A few of them were completely vacuolated with nuclear pycnosis (Fig. 7.2). Liver of fish exposed to MP for 48h showed nuclear mitosis particularly in the centro-lobular area (Fig. 7.3). After 72h exposure, the liver showed centrolobular necrosis. The liver cord membrane of most of the hepatocytes were ruptured and cell counts were scattered within the sinusoids (Fig.7.4). The liver damage was more severe in the fish exposed for 96h and resulted in the formation of clusters of vacuolated cells, spread throughout the tissue (Fig.7.5). Hepatocytic necrosis and vacuolation were seen all over. The liver cord arrangement was highly disturbed and the parenchymal cells could be distinguished only by scattered and pycnotic nuclei surrounded by small masses of vacuolated cytoplasm. Administration of vit. B_{12} in fishes exposed to MP for 96h restored the lesions as evidenced by histopathological examination. Loss of lesions like hepatocytic necrosis and vacuolation by vit. B_{12} in O.punctatus previously exposed to MP. (Fig. 7.6).

LIVER (SUBLETHAL CONC. : $1/10^{th}$ FRACTION OF LC_{50}) 4.5.1.1.2

After 24 and 48h exposure, the liver showed increased mitosis and clumping of nuclei. The degeneration of both cytoplasmic and nuclear material of parenchymal cells was also seen throughout the liver lobules.

EXPLANATIONS TO THE FIGURE.7

Photomicrograph of T.S. of control and treated liver (Sublethal conc.: $1/5^{th}$ fraction of LC_{50}) of *O.punctatus*.

7.1 T.S. of control liver HE x 400

7.2 after 24h exposure to MP. HE x 500

7.3 after 48h exposure to MP. HE x 400

7.4 after 72h exposure to MP. HE x 400

7.5 after 96h exposure to MP. HE x 500

7.6 after 15d treated with vit. B_{12} HE x 200

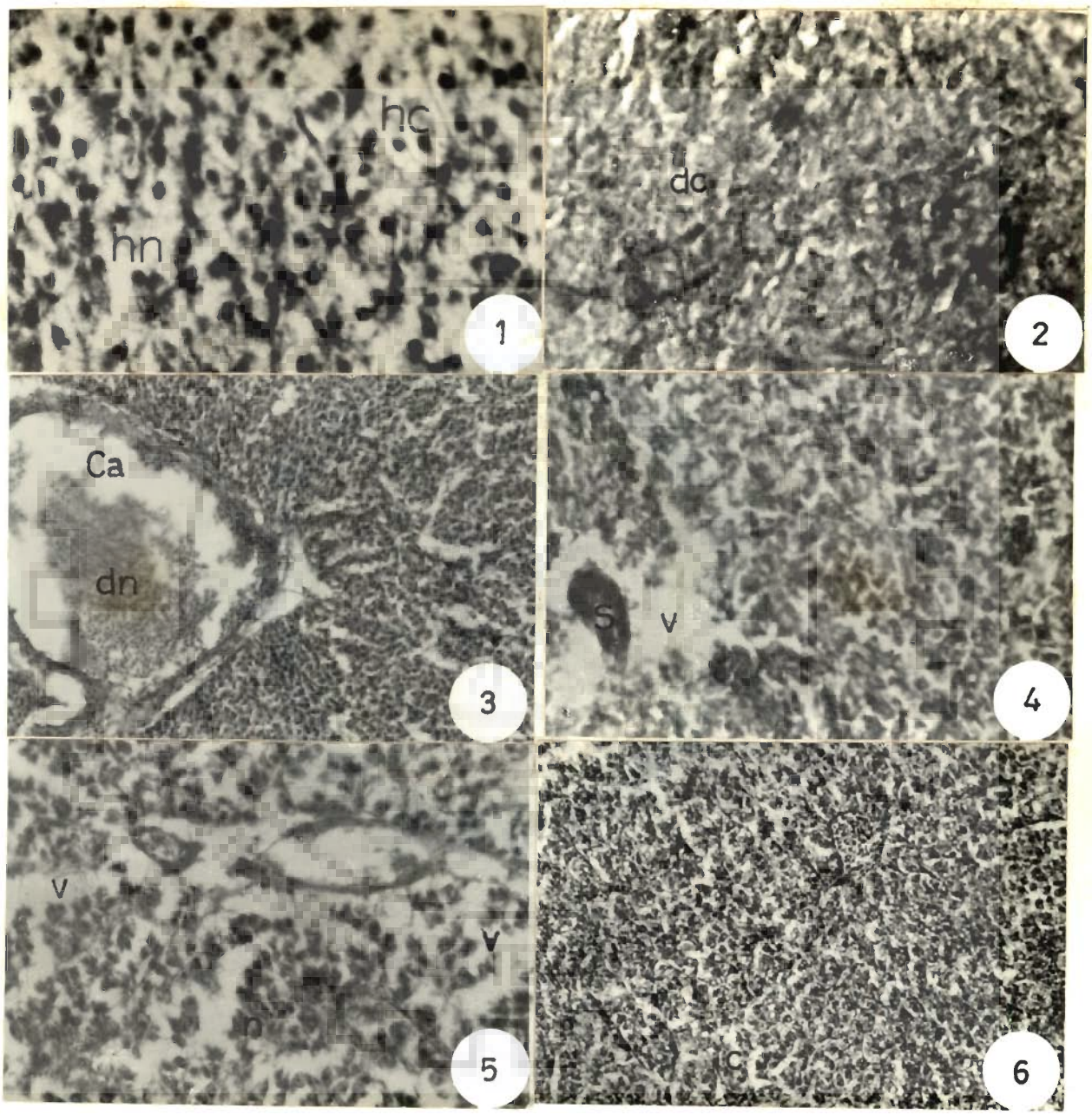

Fig.7

EXPLANATIONS TO THE FIGURE.8

Photomicrograph of T.S. of treated liver (Sublethal conc. : $1/10^{th}$ fraction of LC_{50}) of O.punctatus. (contd.)

8.1 after 24 & 48h exposure to MP HE x 625

8.2 after 72h exposure to MP HE x 500

8.3 after 96h exposure to MP HE x 625

8.4 after 15d treated with vit. B_{12} HE x 200

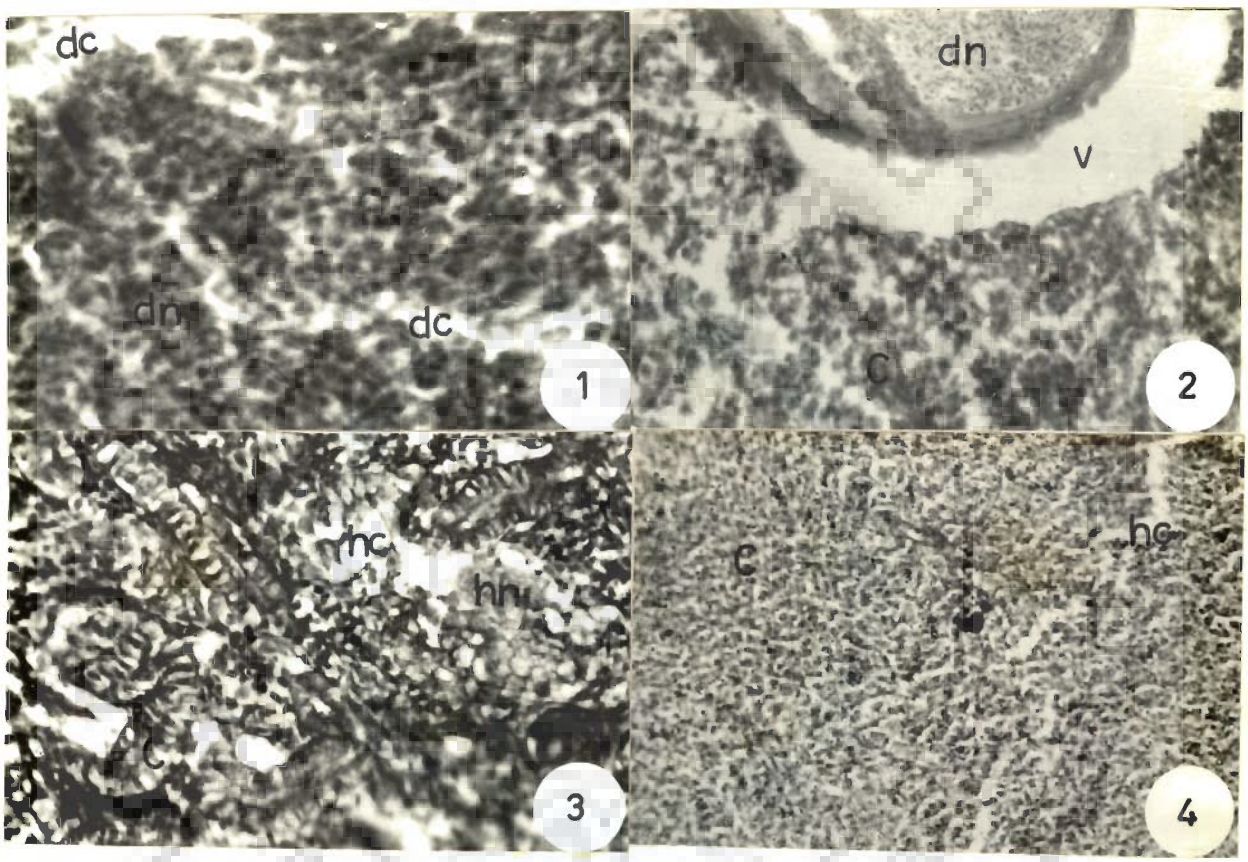

Fig. 8

(Fig. 8.1). Nuclear mitosis in the centrolobular area and degeneration of hepatocytes was observed in the liver of fish after exposure to MP for 72h (Fig. 8.2). The liver damage was more severe in the fish exposed for 96h, hypertrophy of hepatocytes with nuclear clumping was seen (Fig. 8.3). Normal hepatocytes was observed after injection of vit. B_{12}. However, nuclear clumping still persist even after vit. B_{12} treatment (Fig. 8.4).

HISTOLOGY OF CONTROL TISSUE (KIDNEY) 4.5.2

The histology of kidney in control fish shows a large number of loosely packed functional units known as uriniferous tubules (nephrons). The renal corpuscle comprises of Bowman's capsule and glomerulus. The Bowman's capsule is an oval and rounded structure, formed of double layer of squamous epithelium. The glomerulus is a lobulated tuft of thin walled blood capillaries within the Bowman's capsule. The neck segment is a thin and short connection between the Bowman's capsule and the proximal tubule. Intermediate segment is the peculiarity of the fresh water teleosts and is made up of cuboidal epithelial cells. The collecting tubules are lined with cuboidal and goblet cells. It constitutes the last part of the nephron and consists of wider lumen (Fig. 9.1).

HISTOPATHOLOGY OF TREATED TISSUE (KIDNEY) UNDER THE STRESS OF MP AND VITAMIN B_{12} 4.5.2.1

KIDNEY (SUBLETHAL CONC. : $1/5^{th}$ FRACTION OF LC_{50}) 4.5.2.1.1

A number of striking histopathological changes develop in

the structure of the treated kidney. After 24h exposure to MP, few renal tubules show necrosis. Some glomerular shrinkage is also seen (Fig. 9.2). Kidney of the fish exposed to MP for 48h showed marked loss of haemopoitic tissue, vacuolation and disintegration in the cells of the renal tubules (Fig. 9.3). After 72h exposure, a marked swelling of the renal tubule and clumping of nuclei in renal tubules develops (Fig. 9.4). The kidney damage was more severe in the fish exposed for 96h. In this case, there was loss of haemopoitic tissue, shrinkage of glomeruli and there were some instances of degenerate glomeruli due to intoxication. The ruptured epithelial cells in renal tubules also showed considerable damage (Fig. 9.5). After vit. B_{12} treatment, no necrosis was observed in kidney previously exposed to MP for 96h (Fig. 9.6).

KIDNEY (SUBLETHAL CONC.: 1/10th FRACTION OF LC_{50}) 4.5.2.1.2

After 24h exposure to MP, there was glomerular shrinkage and nuclear pycnosis in the renal tubule (Fig.10.1). Kidney of the fish exposed to MP for 48h showed hydropic dequamation of the renal tubule and vacuolation (Fig.10.2). After 72h exposure, tubular necrosis and nuclear necrosis was seen (Fig. 10.3). The kidney damage was more severe in the fish treated for 96h as shown by the clumping of nuclei in renal tubules at some places where as at other places renal tubules became necrosed (Fig. 10.4). Vit. B_{12} failed to restore nuclear necrosis at some places in fishes exposed to MP for 96h. However, nuclear clumping was noticed throughout the renal tissue (Fig.10.5).

EXPLANATIONS TO THE FIGURE.9

Photomicrograph of T.S. of control and treated kidney (Sublethal conc. : $1/5^{th}$ fraction of LC_{50}) of O.punctatus.

9.1 T.S. of control kidney HE x 400

9.2 after 24h exposure to MP. HE x 400

9.3 after 48h exposure to MP. HE x 400

9.4 after 72h exposure to MP. HE x 500

9.5 after 96h exposure to MP. HE x 500

9.6 after 15d treated with vit. B_{12} HE x 400

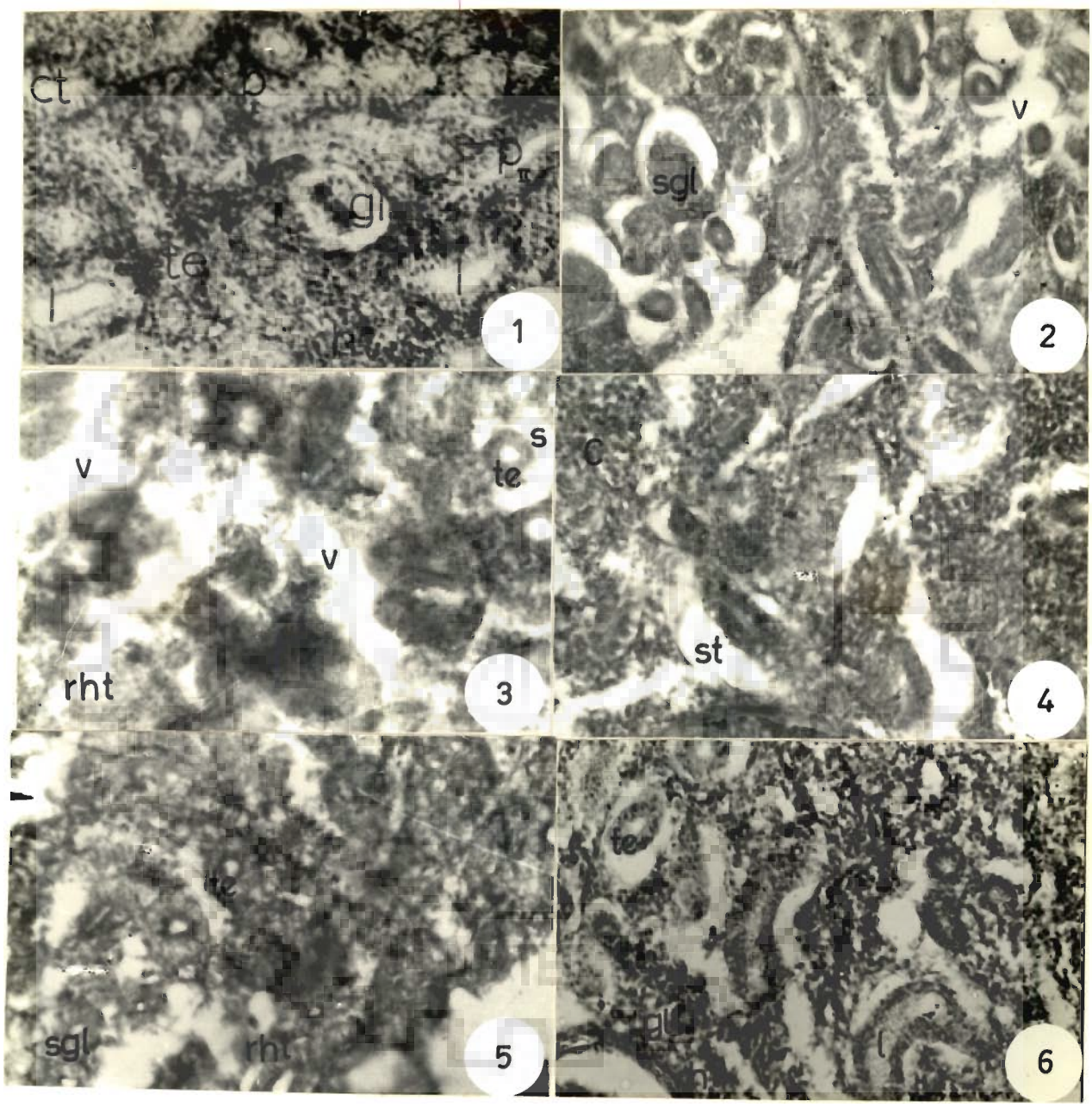

Fig. 9

EXPLANATIONS TO THE FIGURE.10

Photomicrograph of T.S. of treated kidney (Sublethal conc.: $1/10^{th}$ fraction of LC_{50}) of O.punctatus (contd.).

10.1 after 24h exposure to MP. HE x 500

10.2 after 48h exposure to MP. HE x 400

10.3 after 72h exposure to MP. HE x 500

10.4 after 96h exposure to MP. HE x 500

10.5 after 15d treated with vit. B_{12} HE x 400

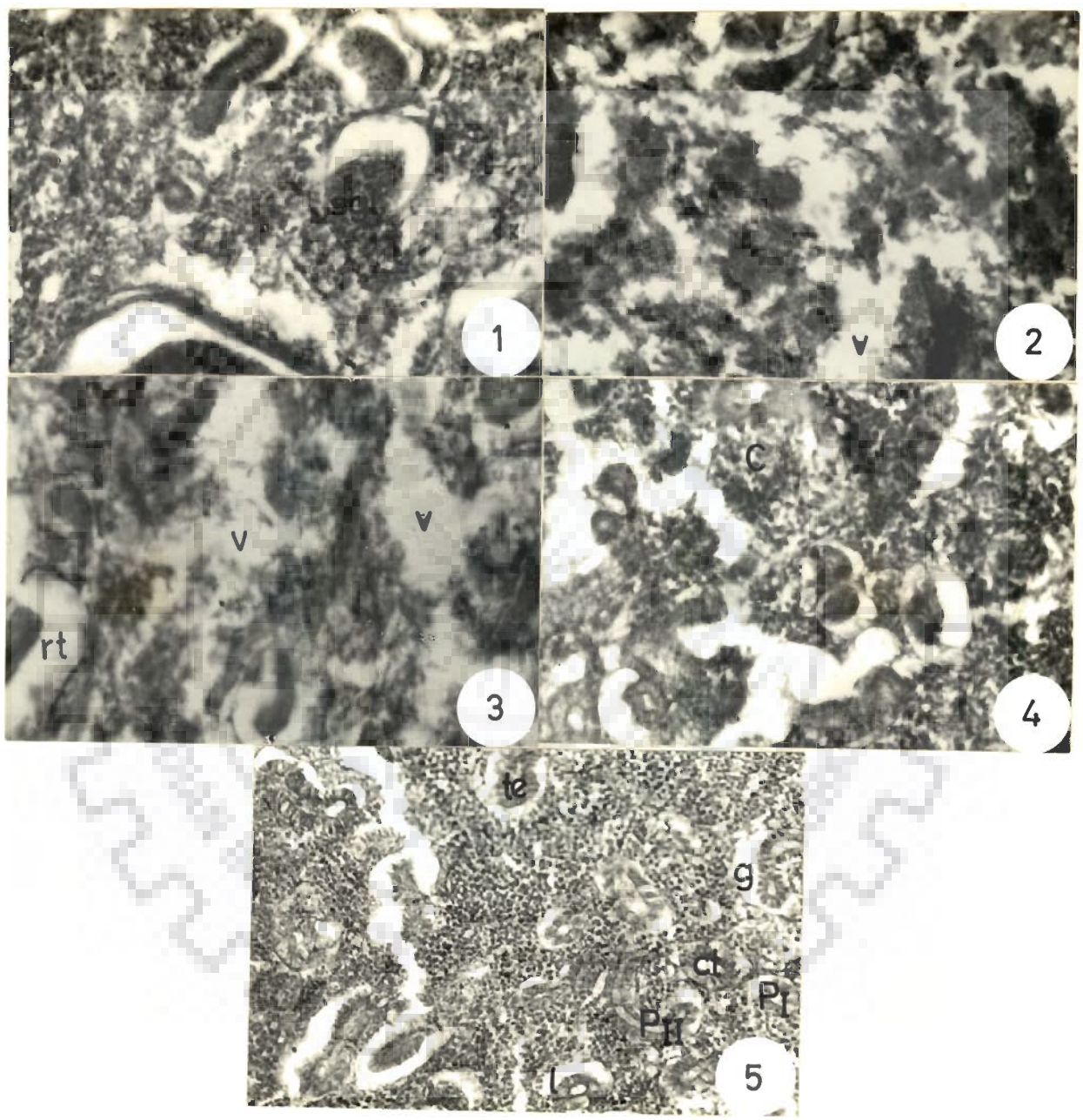

Fig. 10

CONNECTIVE TISSUE 4.5.3

Since liver and kidney both possess a network of fibrous connective tissue, an attempt was made to study their arrangement following acute ingestion of the MP.

RETICULIN 4.5.3.1

Reticular connective tissue fibres or reticulin consists of fine branching fibres that provide a fine supporting frame work to the tissues. Silver impregnation technique differentiates reticulin fibres specially imparting them brown to black colour.

CONTROL LIVER 4.5.3.1.1

In control liver, a regular distribution of reticular fibres binds the sinusoids throughout the liver lobules (Fig.11.1).

LIVER UNDER THE STRESS OF MP AND VITAMIN B_{12} (SUBLETHAL CONC.: 1/5th FRACTION OF LC_{50}) 4.5.3.1.1.1

Liver of the fish treated for 24h showed very thin reticulin fibre encircled by hepatic cells (Fig.11.2). Whereas fishes treated for 48h did not exhibit any significant changes in the distribution of the reticulin fibres (Fig.11.3). A marked change in the distribution was observed in the liver of the fish exposed for 72h as evidenced by very thick reticulin fibre encircled by very thick reticulin fibre encircled by the central vein (Fig. 11.4). After 96h treatment, the fish liver showed comparatively thick reticulin fibres particularly around the central vein and bicanaliculi (Fig. 11.5). After

injection of vit. B_{12}, thick reticulin fibres particularly at the centrolobular zone exhibited by the liver of fish previously exposed to MP for 96h (Fig.11.6).

LIVER UNDER THE STRESS OF MP AND VITAMIN B_{12} (SUBLETHAL CONC. : 1/10th FRACTION OF LC_{50}) 4.5.3.1.1.2

Fishes treated for 24h exposure did not show any marked change in the reticulin fibre (Fig.12.1). After 48h exposure to MP, very thin reticulin fibre was found around the bile canaliculi (Fig.12.2). An irregular distribution of the reticulin fibres was seen around the bile canaliculi (Fig.12.3). After 96h treatment, the liver of the fish showed comparatively thick aggregation of reticulin fibres throughout the liver parenchyma (Fig.12.4). Regular distribution of reticulin fibres was observed in the liver of fish after vit. B_{12} administration (Fig.12.5).

CONTROL KIDNEY 4.5.3.1.2

In control kidney, there is a regular distribution of the reticular fibres throughout the renal tubules (Fig.13.1).

KIDNEY UNDER THE STRESS OF MP AND VITAMIN B_{12} (SUBLETHAL CONC. : 1/5th FRACTION OF LC_{50}) 4.5.3.1.2.1

In the kidney of the fish treated for 24h, there was irregular distribution of the reticulin fibres, (Fig.13.2). Whereas in the fishes treated for 48h, show thicky reticulin fibres (Fig.13.3). A marked change in the distribution was observed in the kidney of the fish exposed for 72h as evidenced by thin reticulin fibres encircling the renal

EXPLANATIONS TO THE FIGURE.11

Photomicrograph of T.S. of control and treated liver (Sublethal conc. : 1/5th fraction of LC_{50}) of O.punctatus showing arrangement of reticulin fibres.

11.1 T.S. of control liver x 500

11.2 after 24h exposure to MP. x 400

11.3 after 48h exposure to MP. x 80

11.4 after 72h exposure to MP. x 80

11.5 after 96h exposure to MP. x 400

11.6 after 15d treated with vit. B_{12} x 200

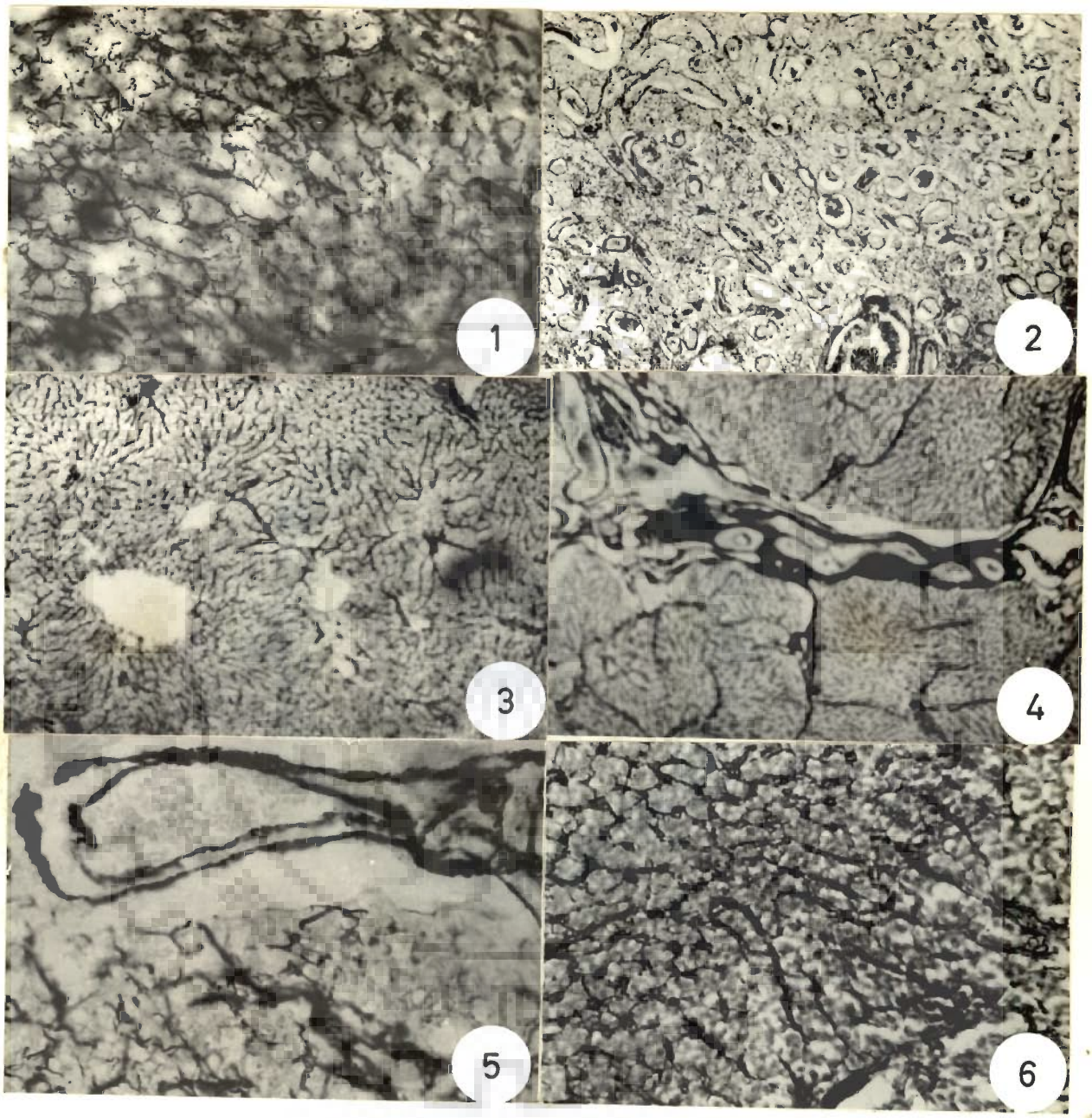

Fig.11

EXPLANATIONS TO THE FIGURE.12

Photomicrograph of T.S. of treated liver (Sublethal conc. : $1/10^{th}$ fraction of LC_{50}) of *O.punctatus* showing arrangement of reticulin fibres (contd.).

12.1 after 24h exposure to MP. x 80

12.2 after 48h exposure to MP. x 80

12.3 after 72h exposure to MP. x 80

12.4 after 96h exposure to MP. x 400

12.5 after 15d treated with vit. B_{12} x 200

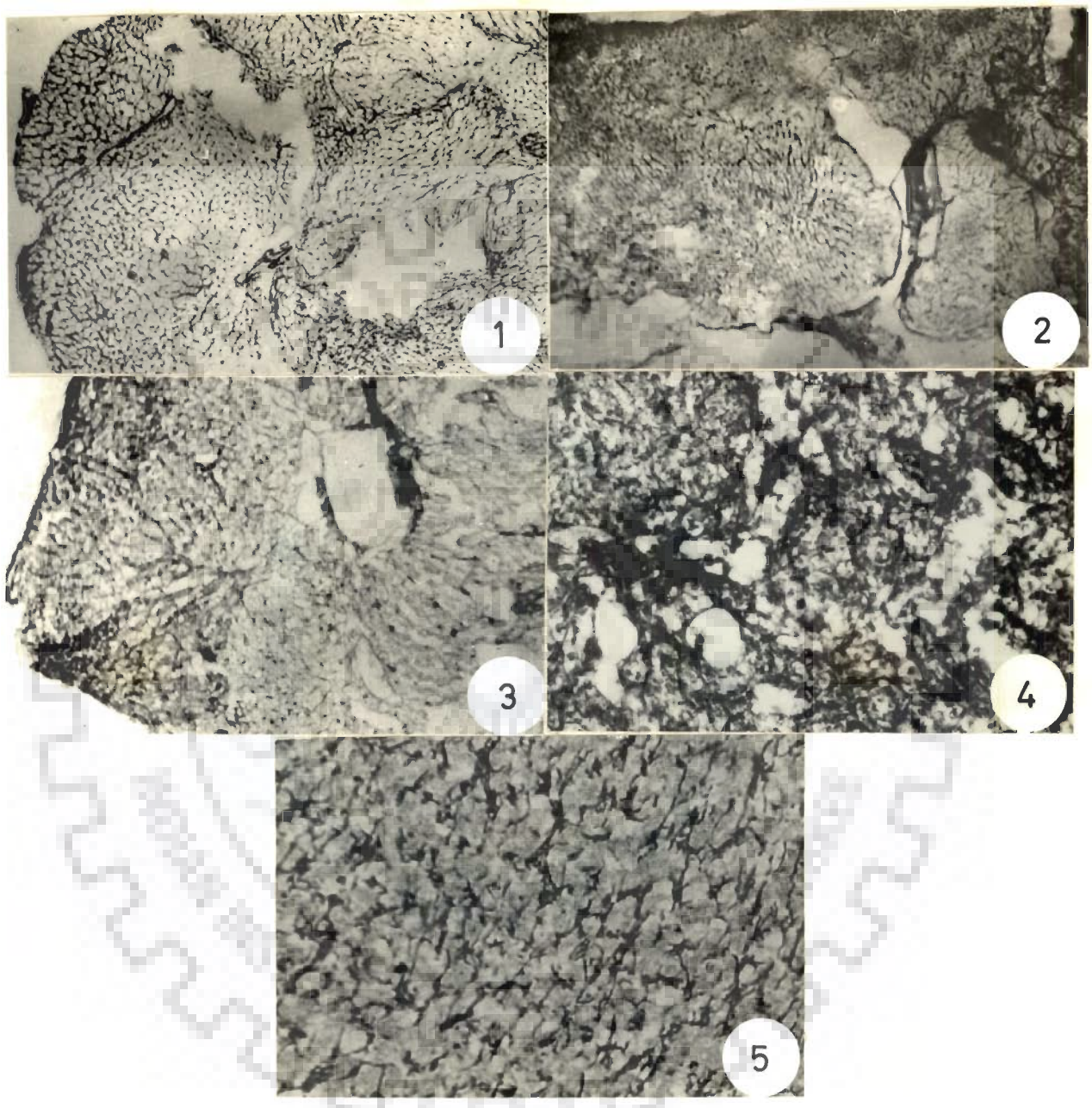

Fig. 12

EXPLANATIONS TO THE FIGURE.13

Photomicrograph of T.S. of control and treated kidney (Sublethal conc. : $1/5^{th}$ fraction of LC_{50}) of O.punctatus showing arrangement of reticulin fibres.

13.1 T.S. of control kidney x 400

13.2 after 29h exposure to MP. x 400

13.3 after 48h exposure to MP. x 400

13.4 after 72h exposure to MP. x 625

13.5 after 96h exposure to MP. x 500

13.6 after 15d treated with vit. B_{12} x 400

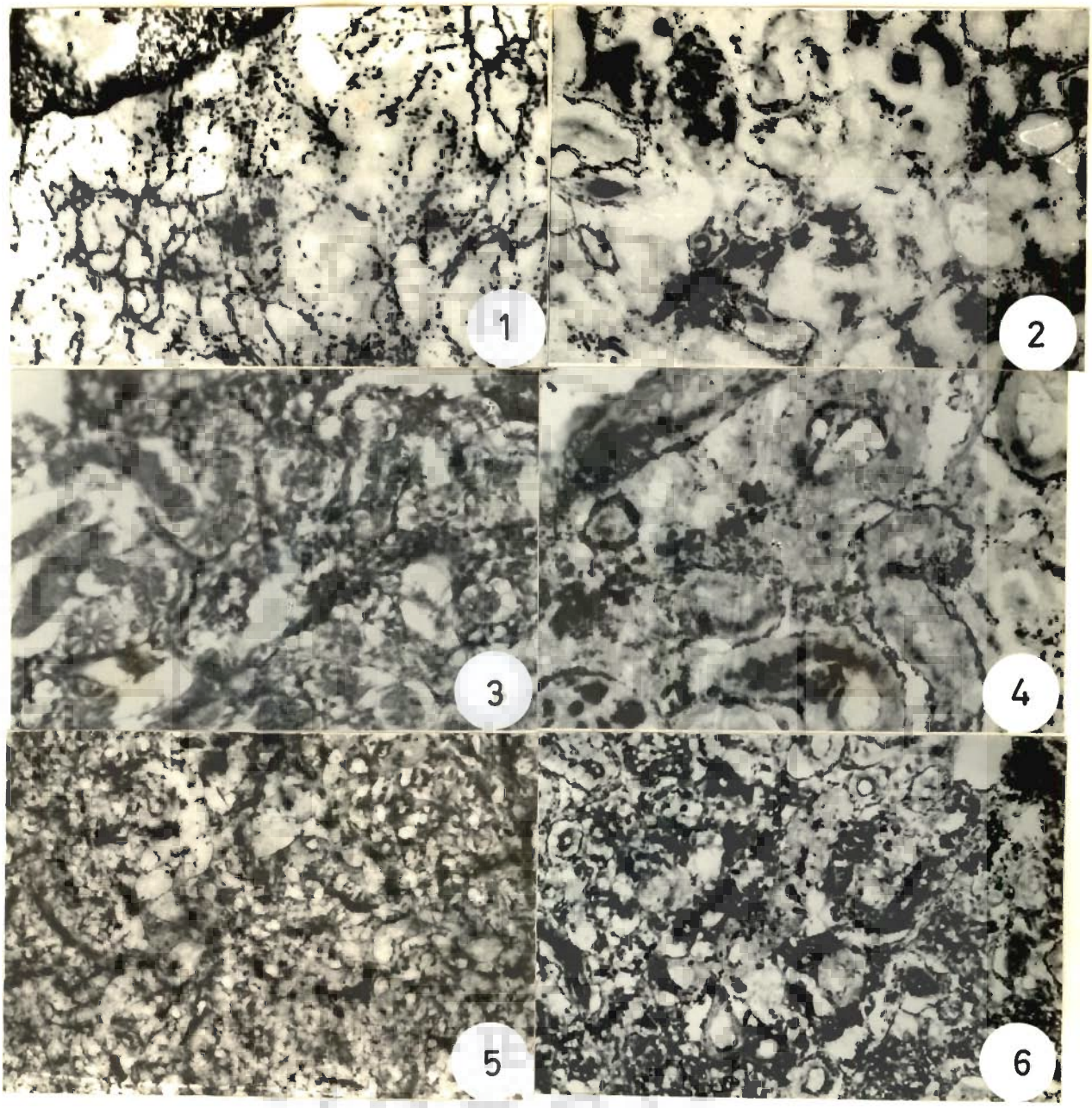

Fig. 13

EXPLANATIONS TO THE FIGURE.14

Photomicrograph of T.S. of treated kidney (Sublethal conc. : 1/5th fraction of LC_{50}) of O.punctatus showing arrangement of reticulin fibres (contd.).

14.1 after 24h exposure to MP. x 400

14.2 after 48h exposure to MP. x 625

14.3 after 72h exposure to MP. x 500

14.4 after 96h exposure to MP. x 625

14.5 after 15d treated with vit. B_{12} x 400

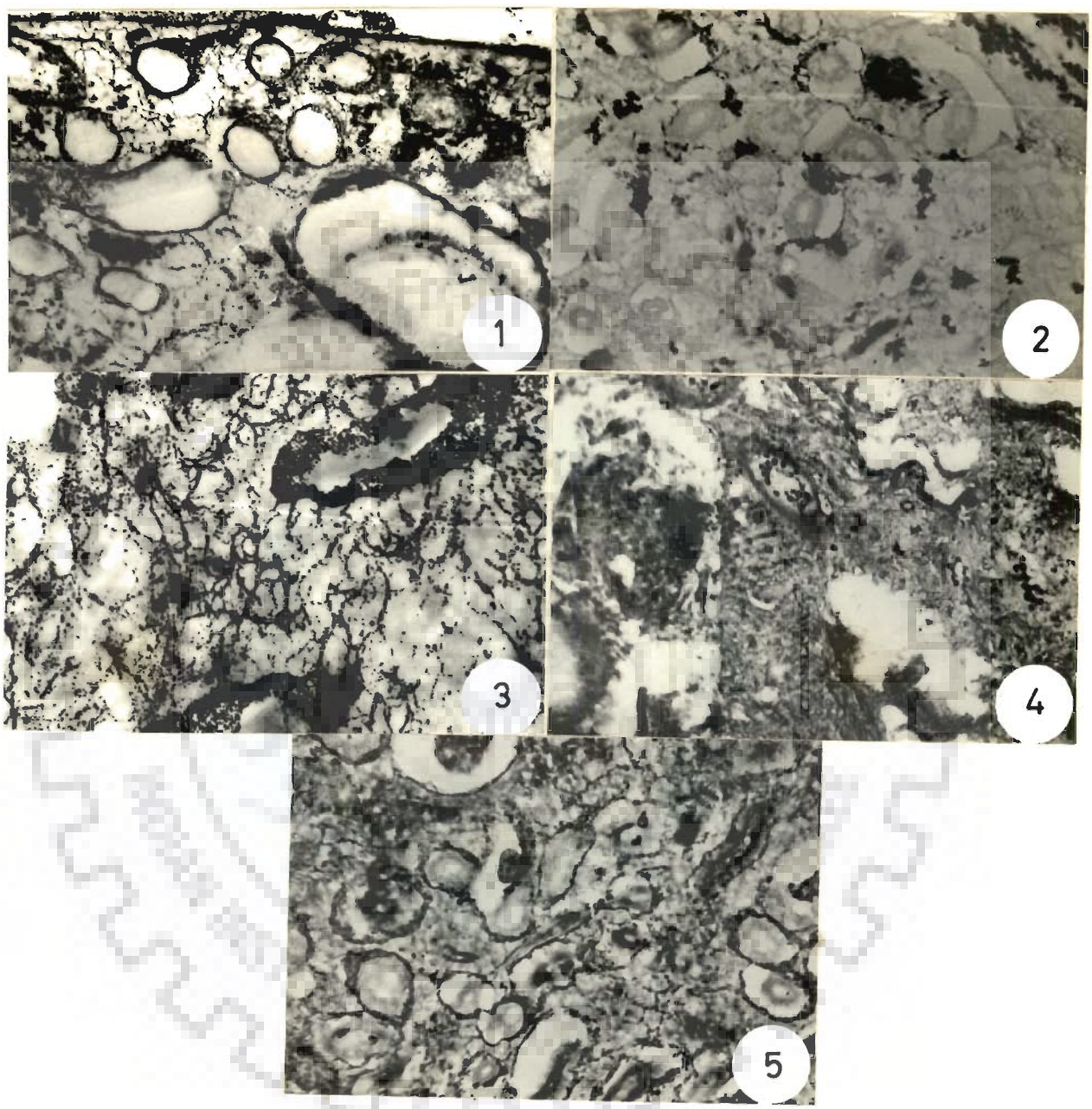

Fig.14

tubules (Fig. 13.4). After 96h treatment, the fish kidney showed comparatively thick reticulin fibres deposited in between renal tubules (Fig.13.5). Administration of vit.B_{12} induced intertubular accumulation of reticular fibres in the kidney of fish (Fig.13.6).

KIDNEY UNDER THE STRESS OF MP AND VITAMIN B_{12} (SUBLETHAL CONC. : $1/10^{th}$ FRACTION OF LC_{50}) 4.5.3.1.2.2

In the kidney of the fish treated with MP for 24h, thin reticulin fibres were found around the renal tubules (Fig.14.1). Whereas fishes treated for 48h, very thin reticular fibres were found deposited near the renal tubules (Fig.14.2). In the fish treated for 72h, comparatively thick reticulin fibres were observed near the renal tubules (Fig.14.3). A marked change was observed in the kidney of the fish after exposure for 96h as evidenced by thin reticulin fibre around the blood vessel (Fig. 14.4). Fishes treated with vit. B_{12} after 96h exposure to MP showed thin reticular fibres encircled the renal tubules (Fig.14.5).

COLLAGEN 4.5.3.2

Collagen fibres were studied after the reaction with Van-Gieson's picric acid-fuchsin stained red.

CONTROL LIVER 4.5.3.2.1

In control liver, collagen remains mainly confined to the central vein (Fig.15.1).

LIVER UNDER THE STRESS OF MP AND VITAMIN B_{12} (SUBLETHAL CONC. : 1/5th FRACTION OF LC_{50}) 4.5.3.2.1.1

After 24h exposure to MP, collagen fibres are found around the central vein (Fig.15.2). Whereas after 48h exposure, collagen fibres are confined only at the periphery (Fig.15.3). In the fishes treated for 72h, a bunch of the collagen fibres were visible particularly at the central zone area (Fig.15.4). A comparatively thick collagen fibres were found around the central vein after 96h treatment (Fig.15.5). After vit.B_{12} administration, comparatively thin collagen fibres was seen encircling the central vein (Fig.15.6).

LIVER UNDER THE STRESS OF MP AND VITAMIN B_{12} (SUBLETHAL CONC. : 1/10th FRACTION OF LC_{50}) 4.5.3.2.1.2

After 24h treatment the collagen fibres are seen encircling the bile canaliculi and also distributed along the periphery (Fig.16.1). Whereas in fishes treated for 48h, collagen fibres are confined only at the periphery (Fig.15.3). Fine collagen fibres are found around the blood vessels after 72h treatment (Fig.16.2). After 96h of exposure, very thick collagen fibres are found around the central vein. (Fig.16.3). No topographical differences in the distribution of collagen fibres was noticed after vit.B_{12} administration in fishes. However, comparatively thin collagen fibres was seen encircling the central vein in the liver (Fig.15.6).

CONTROL KIDNEY 4.5.3.2.2

In normal kidney, the collagen is mainly confined to the capsular region and blood vessels (Fig.17.1).

EXPLANATIONS TO THE FIGURE.15

Photomicrograph of T.S. of control and treated liver (Sublethal conc.: $1/5^{th}$ fraction of LC_{50}) of O.punctatus showing arrangement of collagen fibres.

15.1 T.S. ofcontrol liver x 200

15.2 after 24h exposure to MP. x 80

15.3 after 48h exposure to MP. x 80

15.4 after 72h exposure to MP. x 80

15.5 after 96h exposure to MP. x 400

15.6 after 15d treated with vit. B_{12} x 200

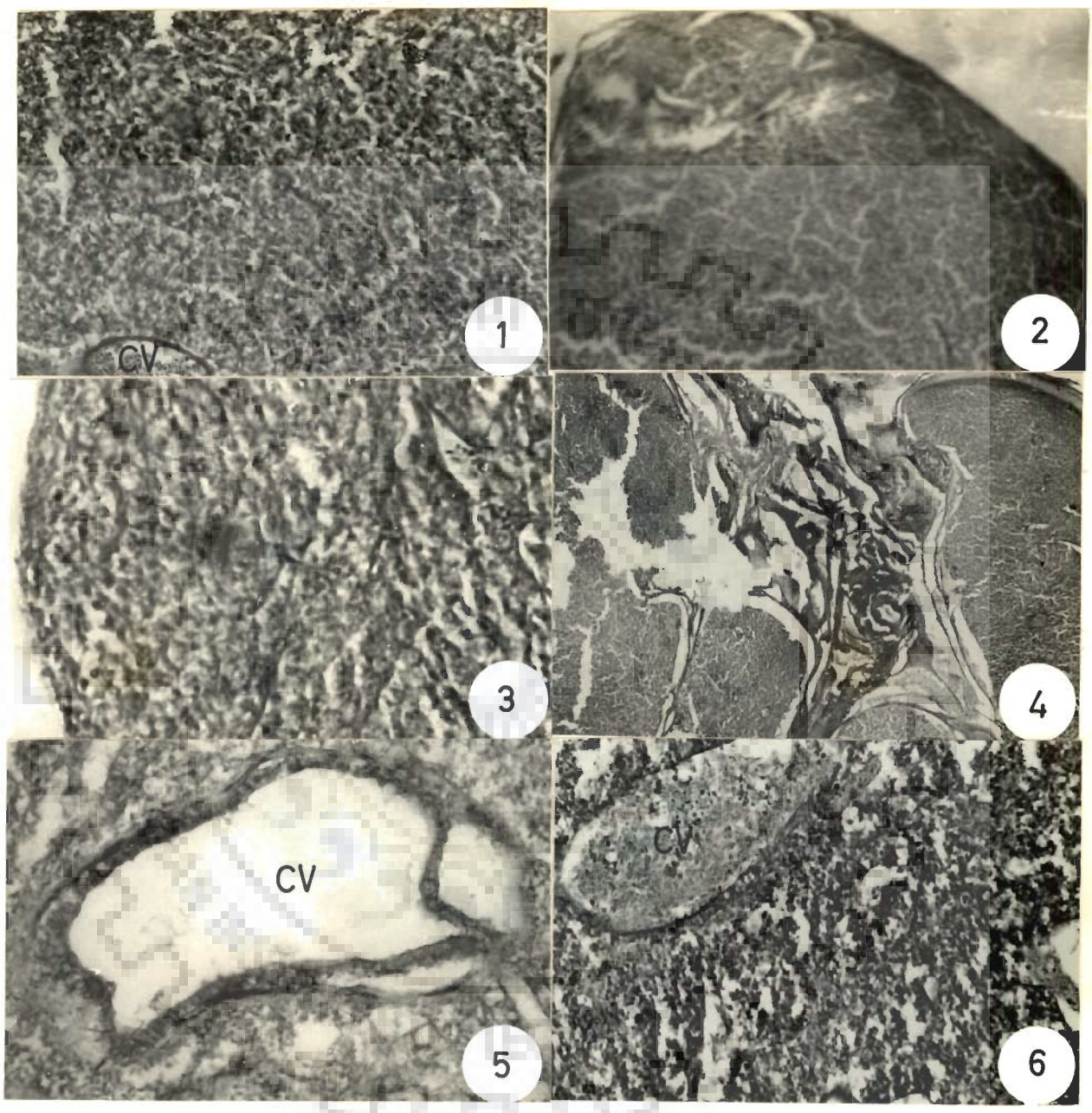

Fig. 15

EXPLANATIONS TO THE FIGURE.16

Photomicrograph of T.S. of treated liver (Sublethal conc. : $1/10^{th}$ fraction of LC_{50}) of O.punctatus showing arrangement of collagen fibres (contd.).

16.1 after 24h exposure to MP. x 80

16.2 after 72h exposure to MP. x 80

16.3 after 96h exposure to MP. x 80

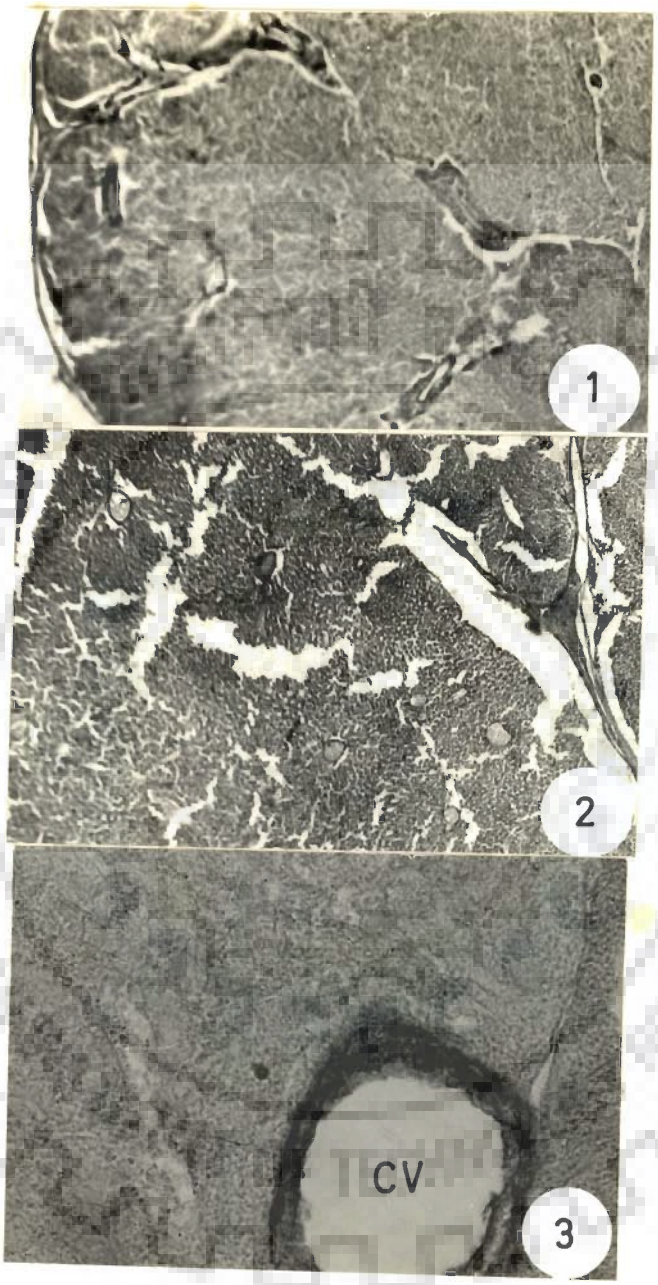

Fig. 16

EXPLANATIONS TO THE FIGURE.17

Photomicrograph of T.S. of control and treated kidney (Sublethal conc.: $1/5^{th}$ fraction of LC_{50}) of O.punctatus showing arrangement of collagen fibres.

17.1 T.S. of control kidney x 100

17.2 after 24h exposure to MP. x 400

17.3 after 48h exposure to MP. x 400

17.4 after 72h exposure to MP. x 80

17.5 after 96h exposure to MP. x 400

17.6 after 15d treated with vit. B_{12} x 400

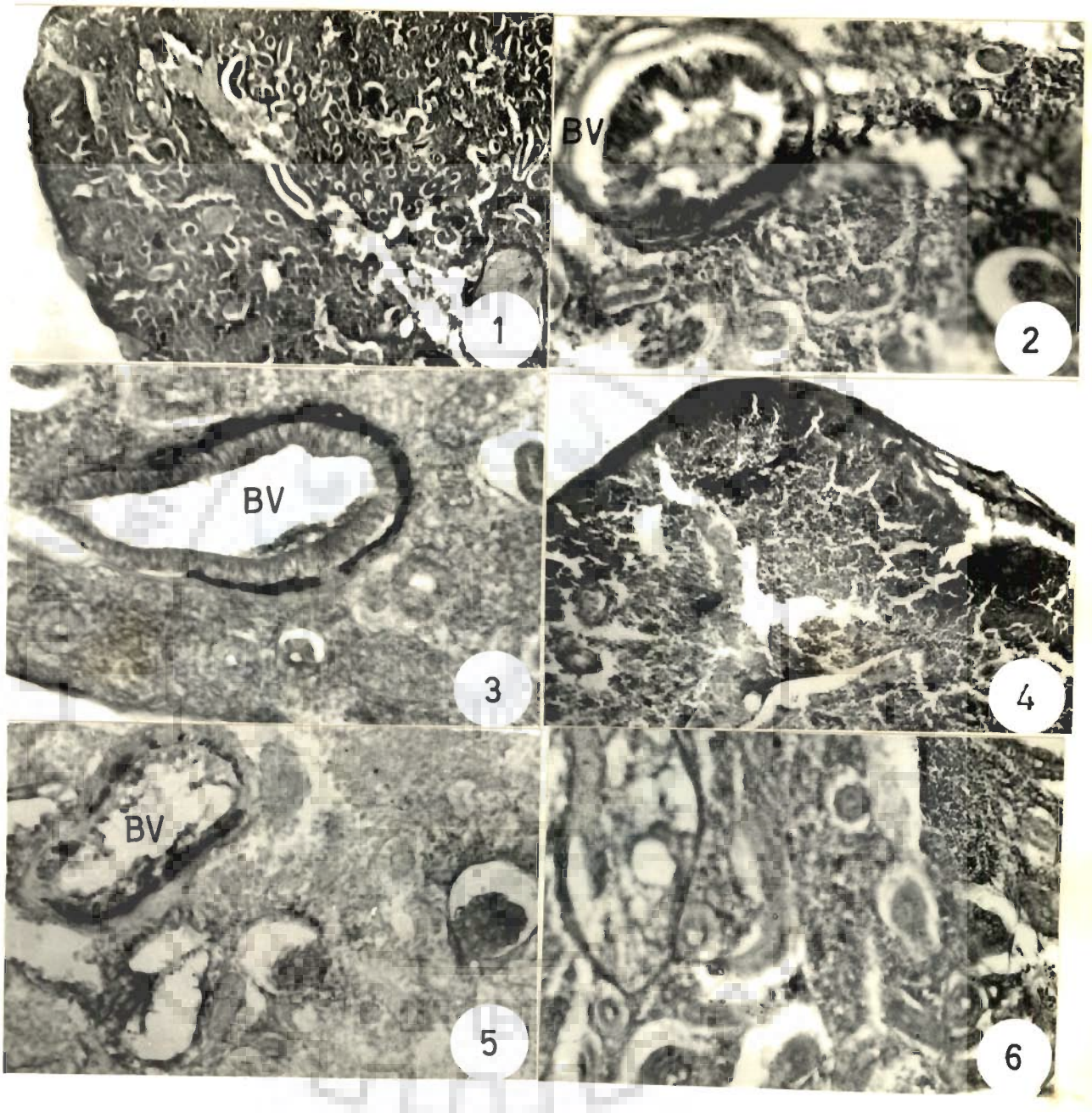

Fig.17

EXPLANATIONS TO THE FIGURE.18

Photomicrograph of T.S. of treated kidney (Sublethal conc.: $1/10^{th}$ fraction of LC_{50}) of O.punctatus showing arrangement of collagen fibres (contd.).

18.1 after 24h exposure to MP. x 80

18.2 after 48h exposure to MP. x 80

18.3 after 72 & 96h exposure to MP. x 200

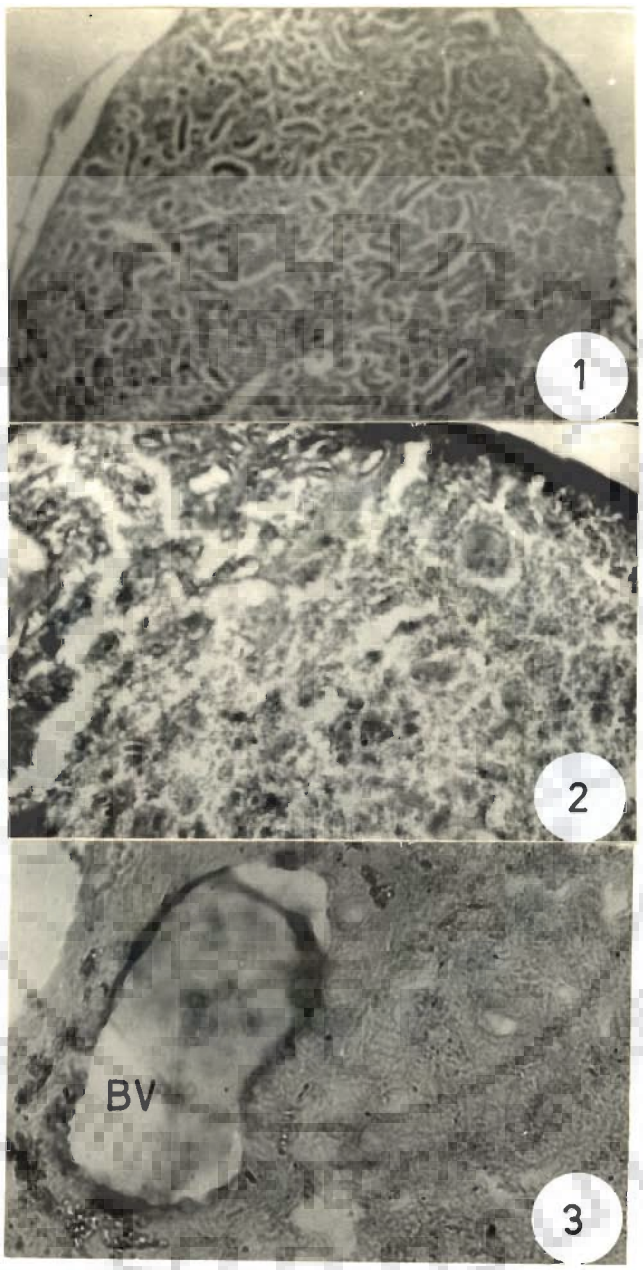

Fig.18

KIDNEY UNDER THE STRESS OF MP AND VITAMIN B_{12} (SUBLETHAL CONC. : 1/5th FRACTION OF LC_{50}) 4.5.3.2.2.1

After 24h exposure to MP, collagen fibres are found distributed around the blood vessels (Fig.17.2). Whereas, fishes treated for 48h showed collagen fibres near the blood vessels (Fig. 17.3). A thick collagen fibres was observed at the capsular region after 72h exposure to MP (Fig.17.4). After 96h treatment, the fish kidney showed comparatively thin collagen fibres near the blood vessels and the glomerular region (Fig.17.5). No topographical differences in the distribution of collagen fibres was noticed after vit.B_{12} administration in fishes (Fig.17.6).

KIDNEY UNDER THE STRESS OF MP AND VITAMIN B_{12} (SUBLETHAL CONC. : 1/10th FRACTION OF LC_{50}) 4.5.3.2.2.2

After 24h exposure to MP, there was no significant change (Fig. 18.1). Whereas fishes treated for 48h exhibited collagen fibres encircling the capsular region (Fig. 18.2). After 72 and 96h of exposure, collagen fibres are confined only around the blood vessel region (Fig.18.3). No topographical differences in the distribution of collagen fibres was noticed after vit. B_{12} administration in fishes. However, comparatively thin collagen fibres was seen encircling the blood vessel in the kidney (Fig.17.6).

HAEMATOLOGICAL OBSERVATIONS 4.6

The changes produced in the blood parameters after exposure to MP and vit. B_{12} administration for 24, 48, 72 and 96h intervals have been summarised in the Tables (4, 5) and Fig.(19-20).

EFFECT OF MP AND VITAMIN B_{12} (SUBLETHAL CONC. : $1/5^{th}$ FRACTION OF LC_{50}) 4.6.1

HAEMOGLOBIN PERCENTAGE (Hb%) 4.6.1.1

Hb% decreased significantly after 96h exposure. Maximum significant fall (19.13%) is found at P<0.001 level. Fishes protected with vit. B_{12} after 96h exposure show decrease in Hb% to 1.74%.

HAEMATOCRIT VALUES (PCV) 4.6.1.2

The haematocrit values in the treated fish decreased (20%) in PCV values is at P<0.001 level. However, after vit.B_{12} administration, the PCV is reduced to 1.76%.

RED BLOOD CORPUSCLES (RBCs) 4.6.1.3

RBC counts decreased significantly only after 96h exposure. The significant fall (P<0.001) in RBC counts is found to be 18.90%. Fishes protected with vit. B_{12} show that the RBC counts is reduced to 0.99%.

WHITE BLOOD CORPUSCLES (WBCs) 4.6.1.4

The total WBC counts increased insignificantly at all the time intervals. The maximum increase was found to be 10.02%. After vit.B_{12} administration there is slight incline in WBC counts.

MEAN CELL VOLUME (MCV) 4.6.1.5

The absolute value, MCV increased significantly (P<0.001) after 24 and 48h exposure, while decreased significantly after 72 (at P<0.05 level) and 96h (at P<0.001 level) exposure. After vit. B_{12} administration the decrease in MCV is found to be 0.78%.

Fig.19 Effect of methyl-parathion (MP) and vitamin B_{12} on Hb, PCV, RBC and WBC in fish (O.punctatus). Bars represent mean ±SE (n = 5). Asterisks denote means significantly different from controls (0) at *P<0.05; ***P<0.001.

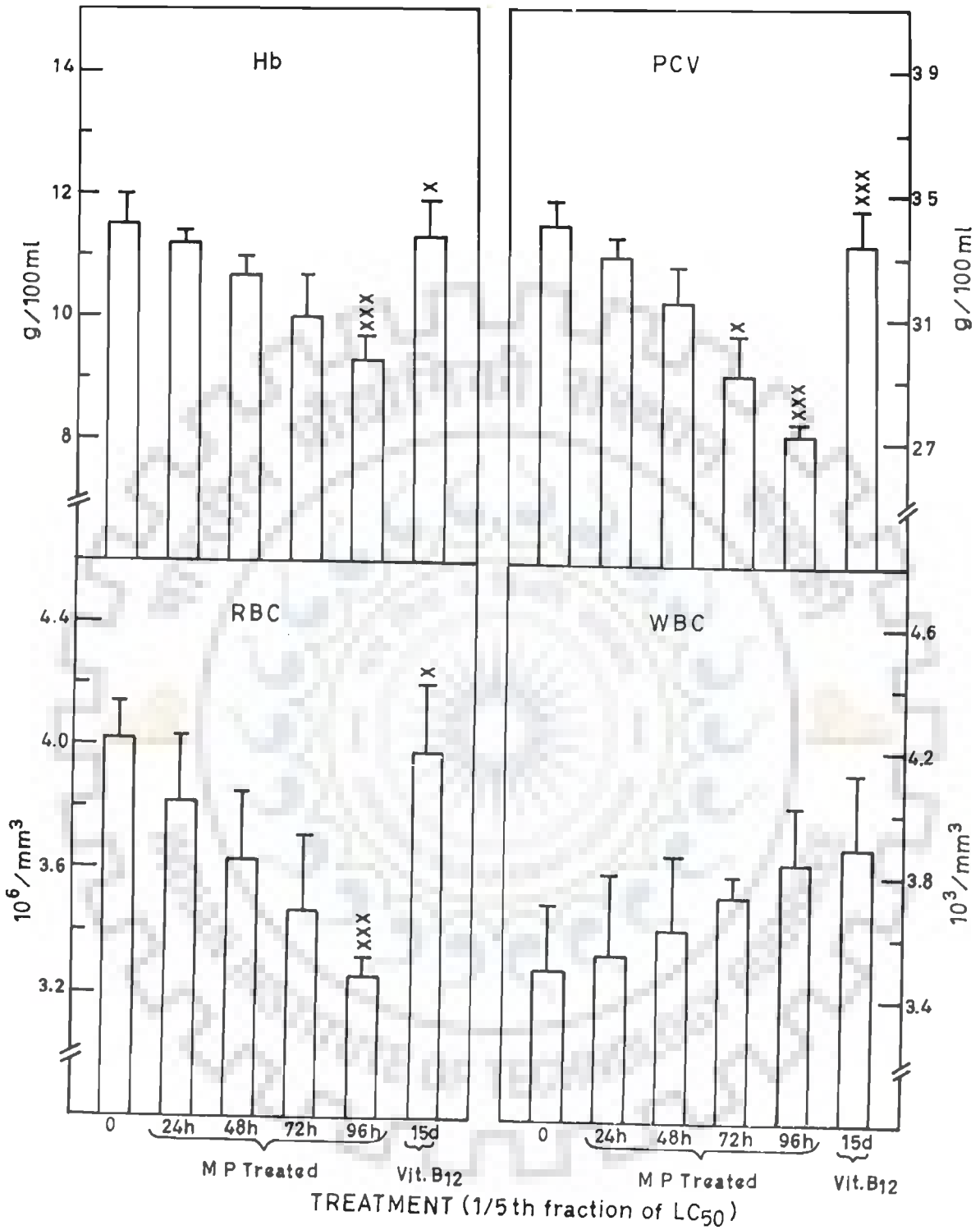
Hb
PCV
RBC
WBC
g/100ml
g/100ml
$10^6/mm^3$
$10^3/mm^3$
14
12
10
8
39
35
31
27
4.4
4.0
3.6
3.2
4.6
4.2
3.8
3.4
XXX
X
0
24h
48h
72h
96h
15d
M P Treated
Vit. B_{12}
TREATMENT (1/5th fraction of LC_{50})

Fig . 19

Fig.20 Alterations in the mean cell volume (MCV), mean cell haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and total plasma protein under the stress of methyl-parathion (MP) and vitamin B_{12}. Bars represent mean $\pm$SE (n = 5). Asterisks denote means significantly different from controls (0) at *P<0.05; **P<0.01; ***<0.001.

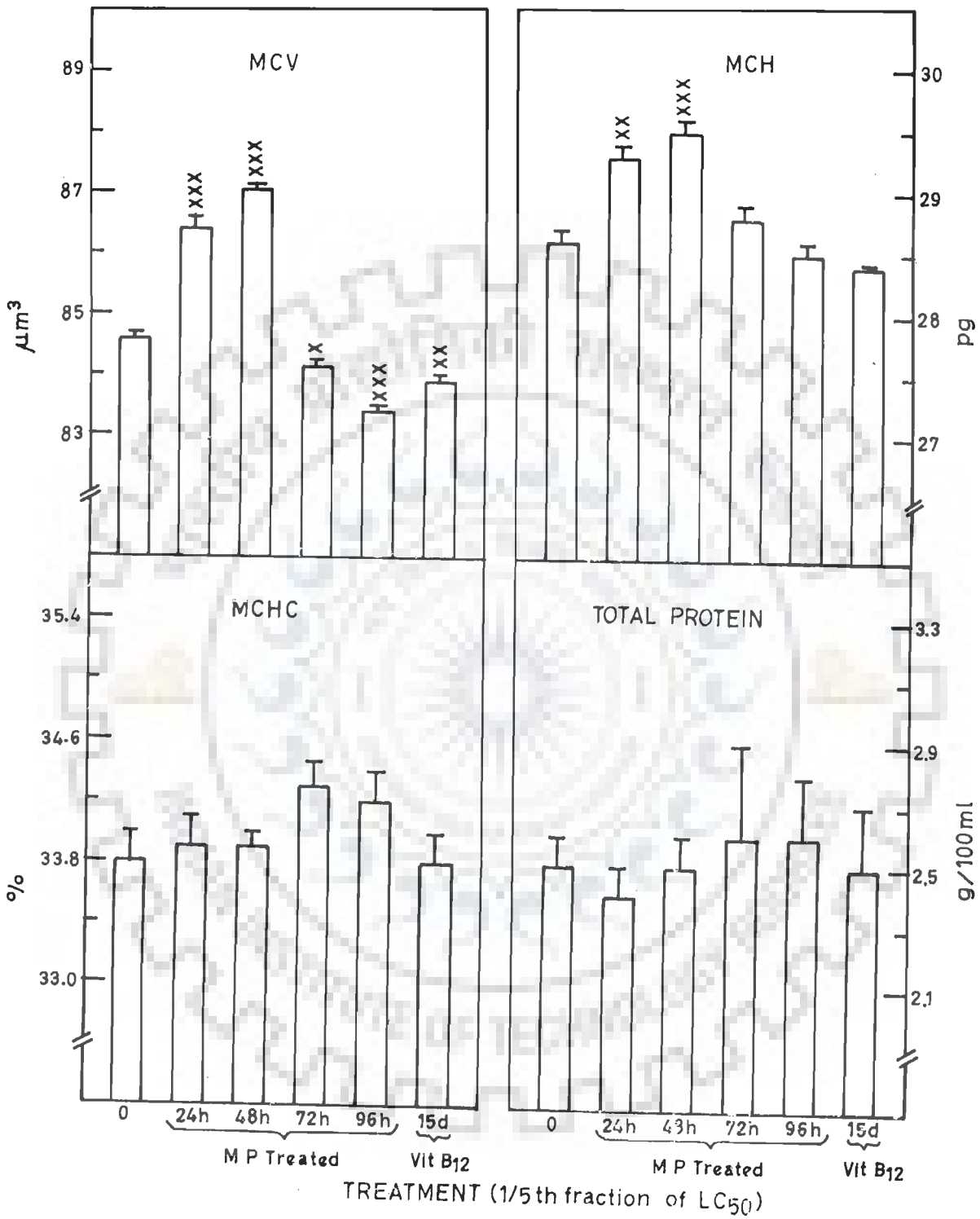
MCV
MCH
MCHC
TOTAL PROTEIN
89
87
85
83
30
29
28
27
35.4
34.6
33.8
33.0
3.3
2.9
2.5
2.1
µm³
pg
%
g/100ml
XXX
XXX
X
XXX
XX
XX
XXX
0
24h
48h
72h
96h
15d
M P Treated
Vit B12
TREATMENT (1/5th fraction of LC50)

Fig. 20

MEAN CELL HAEMOGLOBIN (MCH) 4.6.1.6

The absolute value, MCH increased after 24h, significant at P<0.01 and 48h (significant at P<0.001) exposure. Fishes protected with vit. B_{12}, show decrease in MCV to 0.73%.

MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION (MCHC) 4.6.1.7

The absolute value, MCHC increased insignificantly at all the time intervals. Increase in MCHC is found to be 0.03% after vit. B_{12} administration.

TOTAL PLASMA PROTEIN 4.6.1.8

No significant alteration is found in the total plasma protein in all the acutely treated fishes, which indicates that there is no change in the blood volume. After vitamin B_{12} administration total plasma proteins remain almost constant.

BLOOD GLUCOSE 4.6.1.9

The blood glucose level increased significantly after 48, 72 and 96h exposure. Maximum increase (41.55%) is found to be significant at P<0.001 level after 96h interval. Fishes protected with vit. B_{12} after 96h exposure, the increase caused due to intoxication in the level of the blood glucose.

BLOOD UREA 4.6.1.10

The blood urea level in treated fish increased significantly at 72 and 96h intervals. The maximum increase (26.60%) significant at P<0.001 level is found to be after 96h exposure. Fishes protected with vit. B_{12} after 96h exposure,

show that the increase in the blood urea level is reduced to 1.83%.

BLOOD CHOLESTEROL 4.6.1.11

The level of the blood cholesterol increased significantly only after 96h exposure. Maximum increase significant at $P<0.001$ is found to be 9.70%. The blood cholesterol level is reduced to only 0.37% after vit. B_{12} administration.

SERUM GLUTAMIC-OXALIC TRANSAMINASE (GOT) 4.6.1.12

The activities of GOT increased significantly after 48, 72 and 96h exposures. Maximum increase (21.25%) is found to be significant at $P<0.05$ after 96h exposure. After vit.B_{12} administration, the level of GOT activity is reduced to 3.28%.

SERUM GLUTAMIC-PYRUVIC TRANSAMINASE (GPT) 4.6.1.13

The activities of the GPT increased significantly only after 96h exposure. Maximum significant ($P<0.05$) increase is found to be 16.62%. Fishes protected with vit. B_{12}, show reduced GPT activity to 0.52%.

ALKALINE PHOSPHATASE (AlPase) 4.6.1.14

The activities of AlPase increased significantly after 96h exposure. Maximum increase (9.74%) is found to be significant at $P<0.05$ level. The activity of AlPase is reduced to 0.86% after vit. B_{12} administration.

ACID PHOSPHATASE (AcPase) 4.6.1.15

The activities of AcPase decreased significantly after 72 and 96h exposure. Maximum significant ($P<0.05$) fall is

Fig.21 Blood glucose, urea and cholesterol level in methyl-parathion (MP) and vitamin B_{12} treated fish (O.punctatus). Bars represent mean ±SE (n = 5). Asterisks indicate significant (*P<0.05; ***P<0.001) difference from control (0) fish.

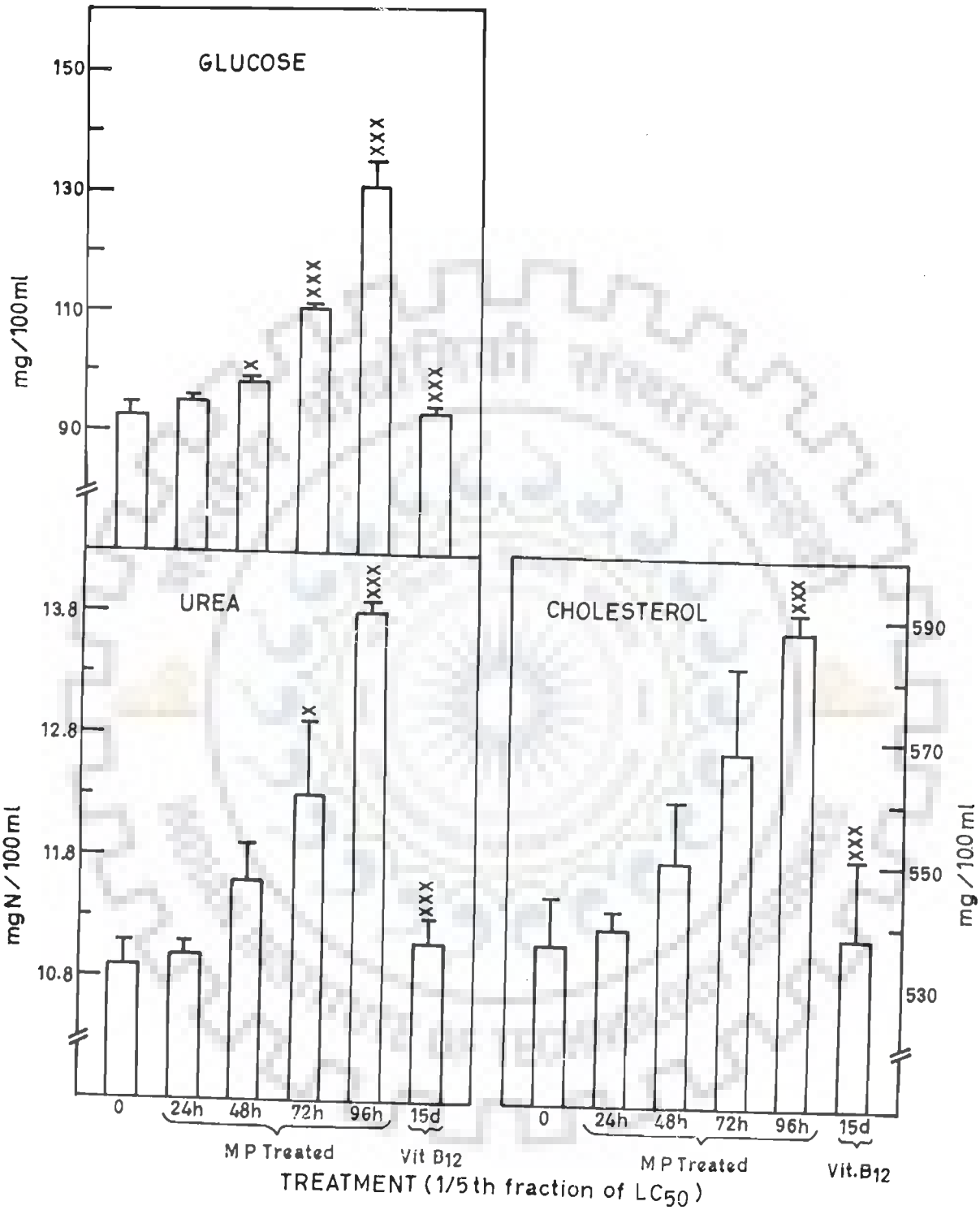
GLUCOSE
150
130
110
90
mg/100ml
x
xxx
xxx
xxx
UREA
13.8
12.8
11.8
10.8
mgN/100ml
x
xxx
xxx
CHOLESTEROL
590
570
550
530
mg/100ml
xxx
xxx
0
24h
48h
72h
96h
15d
M P Treated
Vit B12
0
24h
48h
72h
96h
15d
M P Treated
Vit.B12
TREATMENT (1/5th fraction of LC50)

Fig.21

Fig.22 Serum GOT, GPT, AlPase and AcPase activity in fish (O.punctatus) under the stress of methyl-parathion (MP). Bars represent mean ±SE (n = 5). Asterisks denote means significantly different from controls (0) at *P<0.05; **P<0.01.

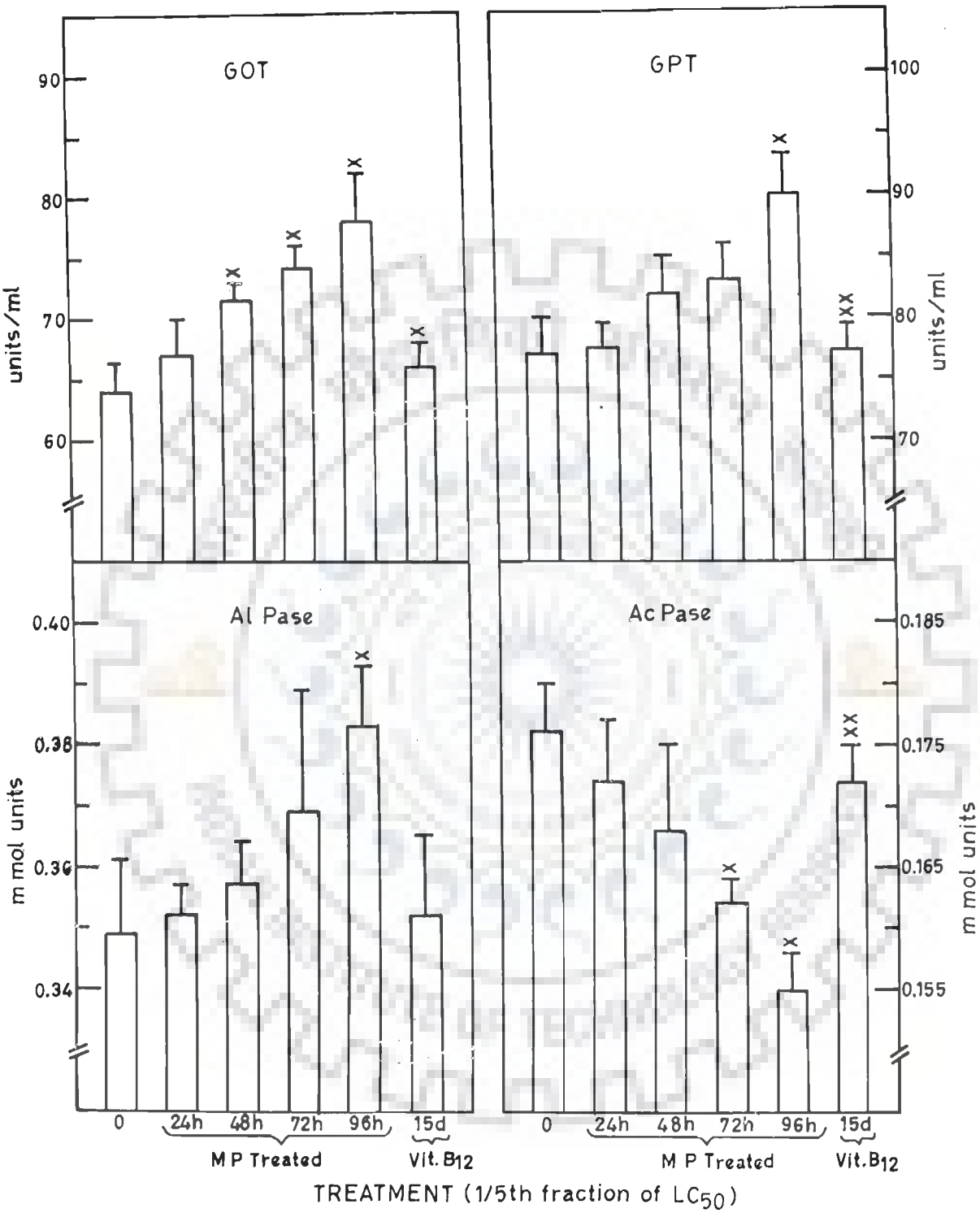
GOT
GPT
Al Pase
Ac Pase
units/ml
m mol units
90
80
70
60
100
0.40
0.38
0.36
0.34
0.185
0.175
0.165
0.155
0
24h
48h
72h
96h
15d
M P Treated
Vit. B12
TREATMENT (1/5th fraction of LC50)

Fig.22

TABLE-4 : CHANGES IN BLOOD OF CONTROL, MP TREATED AND AFTER VITAMIN B_{12} ADMINISTRATION FISHES FOR 24, 48, 72 and 96h EXPOSURE (SUBLETHAL CONC. : $1/5^{th}$ FRACTION OF LC_{50})

Biological values in blood	Control	Treatment				
		MP Treated				Vit. B_{12}
		24h	48h	72h	96h	15d
Haemoglobin (g/100 ml)	11.5±0.5	11.2±0.2 (-2.60)	10.7±0.3 (-6.96)	10.0±0.7 (-13.04)	9.3±0.4*** (-19.13)	11.3±0.6* (-1.74)
Haematocrit (g/100 ml)	34.0±0.8	33.0±0.6 (-2.94)	31.6±1.1 (-7.06)	29.2±1.3* (-14.11)	27.2±0.4*** (-20.0)	33.4±1.1*** (-1.76)
RBC(10^6/mm^3)	4.02±0.12	3.82±0.21 (-4.97)	3.63±0.22 (-9.70)	3.47±0.24 (-13.68)	3.26±0.06*** (-18.90)	3.98±0.22* (-0.99)
WBC (10^3/mm^3)	3.49±0.21	3.54±0.26 (+1.43)	3.62±0.24 (+3.72)	3.73±0.07 (+6.88)	3.84±0.18 (+10.02)	3.89±0.24 (+11.46)
MCV/ μm^3	84.58±0.12	86.39±0.17*** (+2.14)	87.05±0.11*** (+2.92)	84.15±0.09* (-0.50)	83.43±0.09*** (-1.36)	83.92±0.07** (-0.78)
MCH (pg)	28.60±0.09	29.32±0.12** (+2.52)	29.48±0.14*** (+3.08)	28.82±0.08 (+0.77)	28.53±0.07 (-0.24)	28.39±0.03 (-0.73)
MCHC (%)	33.82±0.21	33.94±0.22 (+0.35)	33.86±0.14 (+0.12)	34.25±0.15 (+1.27)	34.19±0.20 (+1.09)	33.83±0.16 (+0.03)
Total plasma protein (g/100 ml)	2.5±0.1	2.4±0.1 (-4.0)	2.5±0.1 (00)	2.6±0.3 (+4.0)	2.6±0.2 (+4.0)	2.5±0.2 (00)

Contd...

Glucose (mg/100 ml)	92.4±2.2	94.9±1.3 (+2.70)	98.2±1.2* (+6.28)	110.4±0.4*** (+19.48)	130.8±4.2*** (+41.55)	92.7±0.7*** (+0.32)
Urea (mg N/100 ml)	10.9±0.2	11.0±0.1 (+0.92)	11.6±0.3 (+6.42)	12.3±0.6* (+12.84)	13.8±0.1*** (+26.60)	11.1±0.2*** (+1.83)
Cholesterol (mg/100 ml)	536.0±8.0	539.0±3.0 (+0.56)	550.0±10.0 (+2.61)	568.0±14.0 (+5.97)	588.0±3.0*** (+9.70)	538.0±13.0** (+0.37)
GOT (units/ml serum)	64.0±2.4	66.9±3.2 (+4.53)	71.4±1.6* (+11.56)	74.0±2.0* (+15.62)	77.6±4.2* (+21.25)	66.1±2.1* (+3.28)
GPT (units/ml serum)	77.0±2.9	77.5±2.1 (+0.65)	81.7±3.3 (+6.10)	83.0±2.7 (+7.79)	89.8±3.4* (+16.62)	77.4±2.3** (+0.52)
Alkaline phosphatase (mmol units)	0.349±0.012	0.352±0.005 (+0.86)	0.357±0.007 (+2.29)	0.369±0.020 (+5.73)	0.383±0.010* (+9.74)	0.352±0.013 (+0.86)
Acid phosphatase (mmol units)	0.176±0.004	0.172±0.005 (-2.27)	0.168±0.007 (-4.54)	0.162±0.002* (-7.95)	0.155±0.003* (-11.93)	0.172±0.003** (-2.27)

All values are mean ± SE percentage alterations in parenthesis, (+)% stimulation, (-)% inhibition.

Values are significant at *P<0.05, **P<0.01, ***P<0.001 (Fisher's t-test).

found to be 11.93%. In the fishes protected with vit. B_{12}, the decrease in the AcPase activity is reduced to 2.27%.

EFFECT OF MP AND VITAMIN B_{12} (SUBLETHAL CONC. : 1/10th FRACTION OF LC_{50}) 4.6.2

HAEMOGLOBIN PERCENTAGE (Hb%) 4.6.2.1

Hb% decreased significantly after 72 and 96h exposure. Maximum decrease (15.65%) is found to be significant at $P<0.05$ level. This decrease in the Hb% is reduced to only 0.87% after vit.B_{12} administration.

HAEMATOCRIT VALUE (PCV) 4.6.2.2

PCV values decreased significantly ($P < 0.001$) only after 96h exposure. Maximum decrease is found to be 16.76%. After vit. B_{12} administration, this decrease in PCV value is reduced to only 0.59%.

RED BLOOD CORPUSCLES (RBCs) 4.6.2.3.

RBC counts decreased significantly after 96h exposure. Maximum significant fall was 15.92% at $P < 0.01$ level. Fishes protected with vit. B_{12}, show that this decrease in RBC counts is reduced only to 0.25%.

WHITE BLOOD CORPUSCLES (WBCs) 4.6.2.4

WBC counts increased insignificantly after all the time intervals. Maximum increase is 8.59%. There is slight increase in the WBC counts after vit. B_{12} administration.

MEAN CELL VOLUME (MCV) 4.6.2.5

The absolute value, MCV increased significantly ($P < 0.001$) after 48 and 72h exposure. However, there was significant

decrease after 24h (at $P < 0.05$ level) and 96h (at $P < 0.001$) level. Decrease in MCV is reduced to 0.35% after vit. B_{12} administration.

MEAN CELL HAEMOGLOBIN (MCH) 4.6.2.6

The absolute value, MCH increased significantly after 48 and 72h exposure. Maximum increase (3.29%) is found to be significant at $P < 0.001$ level. Fishes protected with vit. B_{12} show that the decrease in MCH is only 0.59%.

MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION (MCHC) 4.6.2.7

The absolute value, MCHC increased insignificantly at all the time intervals. Maximum increase is 1.33% after 96h exposure. After vit. B_{12} administration the decrease in MCHC is found to only 0.26%.

TOTAL PLASMA PROTEIN 4.6.2.8

There is significant alteration in the total plasma protein in all the acutely treated fishes, which indicates that there is no change in the blood volume. The total plasma proteins remain almost constant after vit. B_{12} administration.

BLOOD GLUCOSE 4.6.2.9

The blood glucose level increased significantly after 72 and 96h exposure. Maximum increase (30.08%) is found to be significant at $P < 0.001$ level after 96h exposure. Increase in the blood glucose level is reduced to 0.32% after vit. B_{12} treatment.

Fig.23 Effect of methyl-parathion (MP) and vitamin B_{12} on Hb, PCV, RBC and WBC in fish (O.punctatus). Bars represent mean ±SE (n = 5). Asterisks denote means significantly different from controls (0) at *P<0.05; **P<0.01; ***P<0.001.

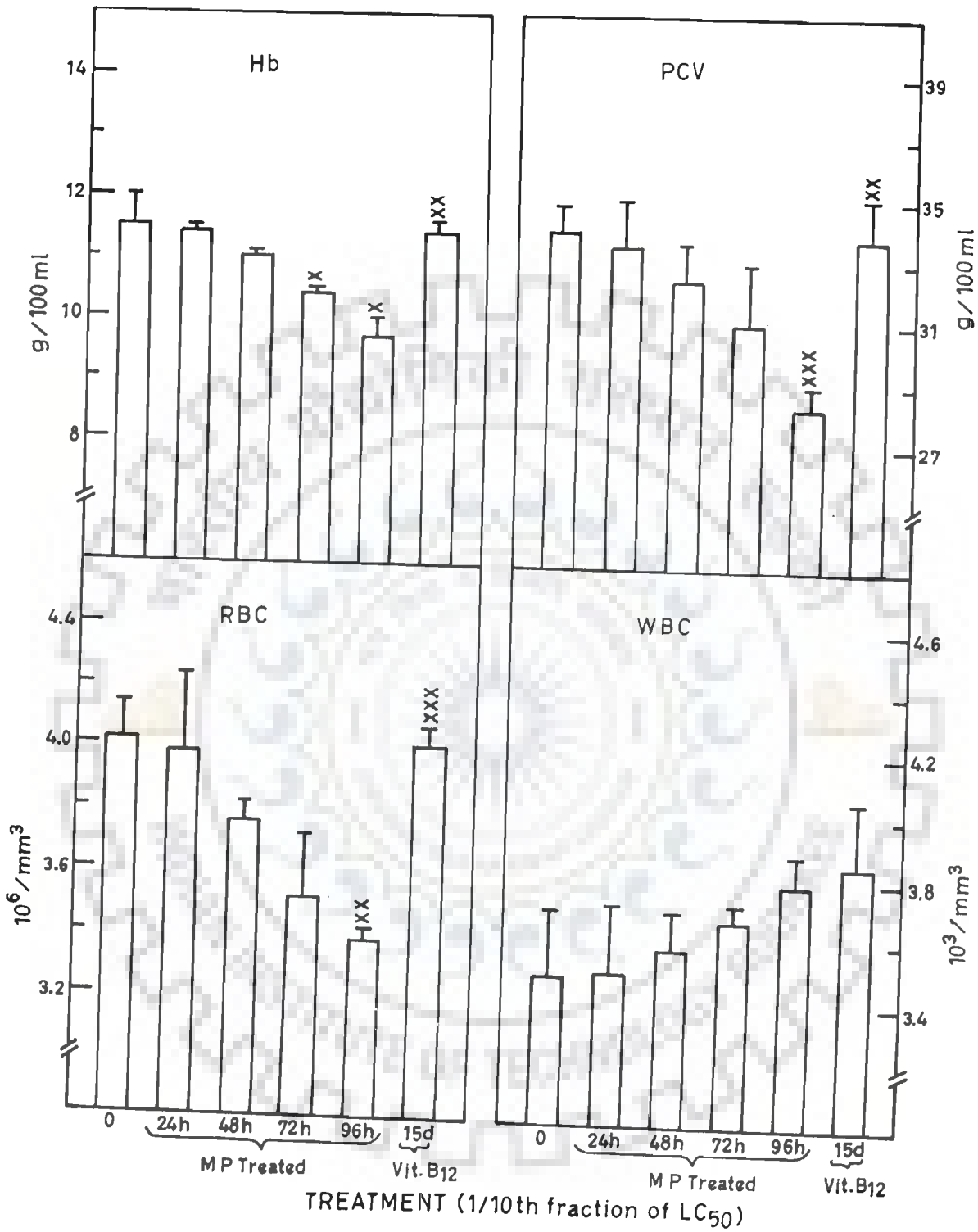
Hb
PCV
RBC
WBC
g/100 ml
g/100 ml
10^6/mm^3
10^3/mm^3
14
12
10
8
39
35
31
27
4.4
4.0
3.6
3.2
4.6
4.2
3.8
3.4
x
xx
xx
xxx
xxx
xx
0
24h
48h
72h
96h
15d
M P Treated
Vit. B12
TREATMENT (1/10th fraction of LC_{50})

Fig.23

Fig.24 Changes in the mean cell volume (MCV), mean cell haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and total plasma protein after methyl-parathion (MP) and vitamin B_{12} treatment. Bars represent mean $\pm$SE (n = 5). Asterisks denote means significantly different from controls (0) at *P<0.05; **P<0.01; ***P<0.001.

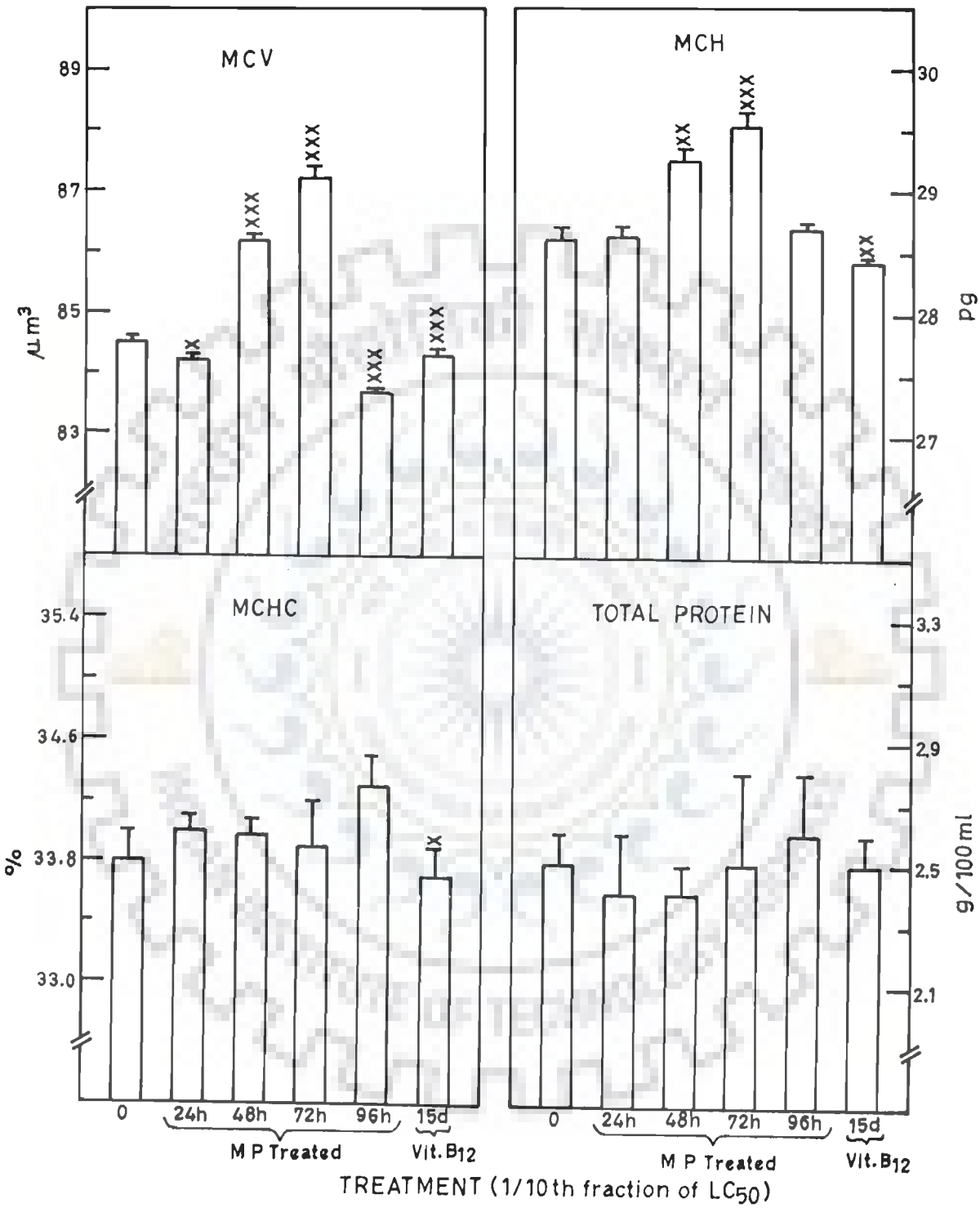
MCV
MCH
MCHC
TOTAL PROTEIN
89
87
85
83
30
29
28
27
35.4
34.6
33.8
33.0
3.3
2.9
2.5
2.1
µm³
pg
%
g/100ml
XXX
XX
X
0
24h
48h
72h
96h
15d
M P Treated
Vit. B12
TREATMENT (1/10th fraction of LC50)

Fig.24

BLOOD UREA 4.6.2.10

The level of the blood urea is increased significantly after 72 and 96h intervals. Maximum significant fall (at $P < 0.001$ level) is found to be 18.34% after 96h treatment. Fishes protected with vit. B_{12}, the increase in the level of blood urea is reduced to 0.92%.

BLOOD CHOLESTEROL 4.6.2.11

The blood cholesterol level increased significantly only after 96h exposure. It is found to be 6.90% significant at $P < 0.05$ level. After vit. B_{12} administration, the increase in blood cholesterol level is reduced to 0.19%.

SERUM GLUTAMIC-OXALIC TRANSMINASE (GOT) 4.6.2.12

The activities of GOT increased significantly after 72 and 96h treatment. Maximum increase (17.34%) is found to be significant at $P < 0.001$ level after 96h exposure. The activity of GOT is reduced to 1.41% after vit. B_{12} administration.

SERUM GLUTAMIC-PYRUVIC TRANSAMINASE (GPT) 4.6.2.13

The activities of the GPT increased significantly only after 96h exposure. Maximum significant ($P < 0.05$) increase is found to be 12.07%. Increase in the activity of GPT is reduced to 0.26% after vit. B_{12} administration.

ALKALINE PHOSPHATASE (AlPase) 4.6.2.14

The activities of AlPase increased insignificantly at all the time intervals. Fishes protected with vit. B_{12} show that the increase in the AlPase activity is reduced to 0.86%.

Fig.25 Blood glucose, urea and cholesterol level under the stress of methyl-parathion (MP) and vitamin B_{12}. Bars represent mean $\pm$SE (n = 5). Asterisks indicate significant (*P<0.05; ***P<0.001) difference from control (0) fish.

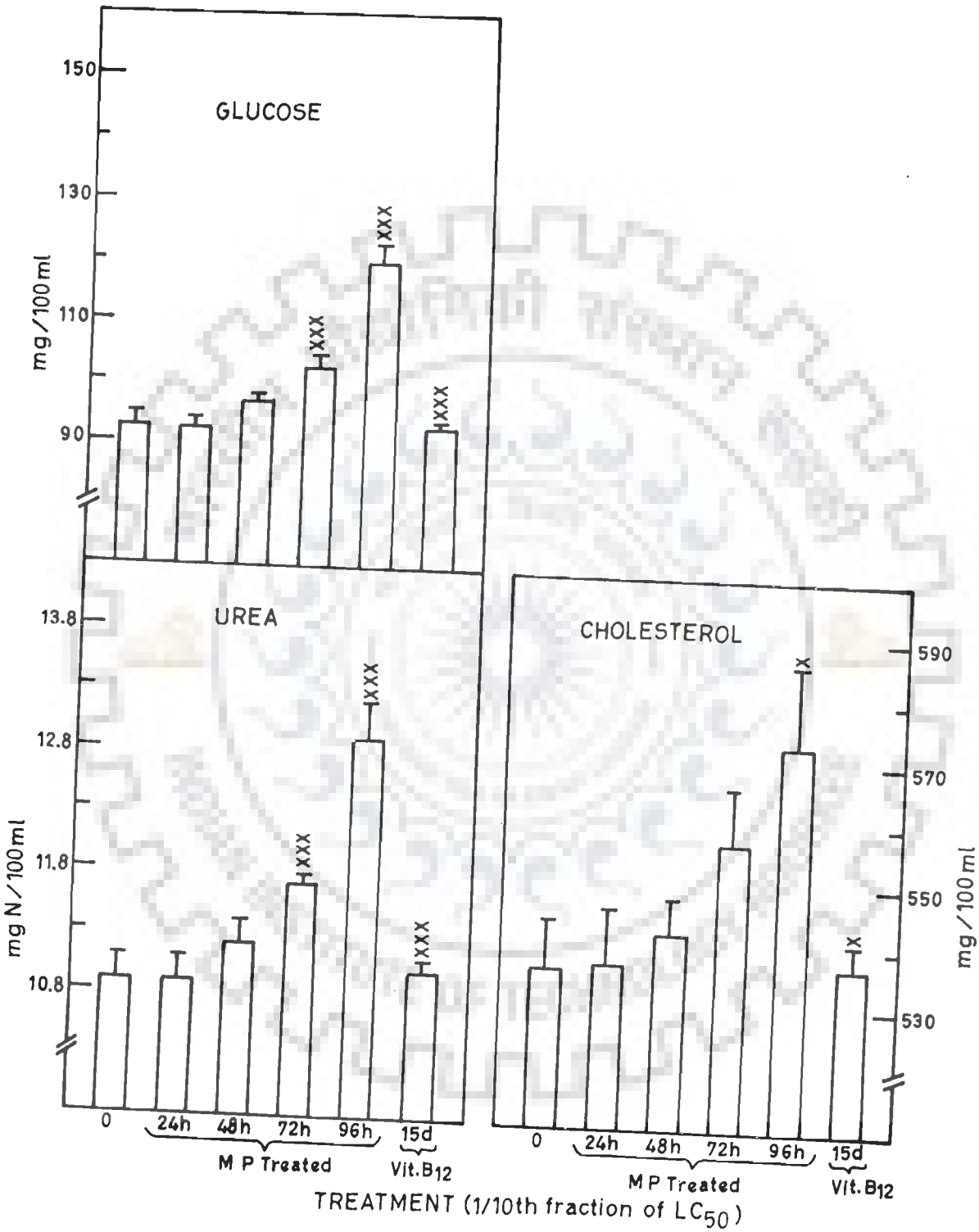
GLUCOSE
mg/100ml
150
130
110
90
XXX
XXX
XXX
UREA
mg N/100ml
13.8
12.8
11.8
10.8
XXX
XXX
XXX
CHOLESTEROL
mg/100ml
590
570
550
530
X
X
0
24h
48h
72h
96h
15d
M P Treated
Vit.B12
TREATMENT (1/10th fraction of LC50)

Fig.25

Fig.26 Serum GOT, GPT, AlPase and AcPase activity in methyl parathion (MP) and vitamin B_{12} treated fish (O.punctatus). Bars represent mean ±SE (n = 5). Asterisks denote means significantly different from controls (0) at *P<0.05; **P<0.01; ***P<0.001.

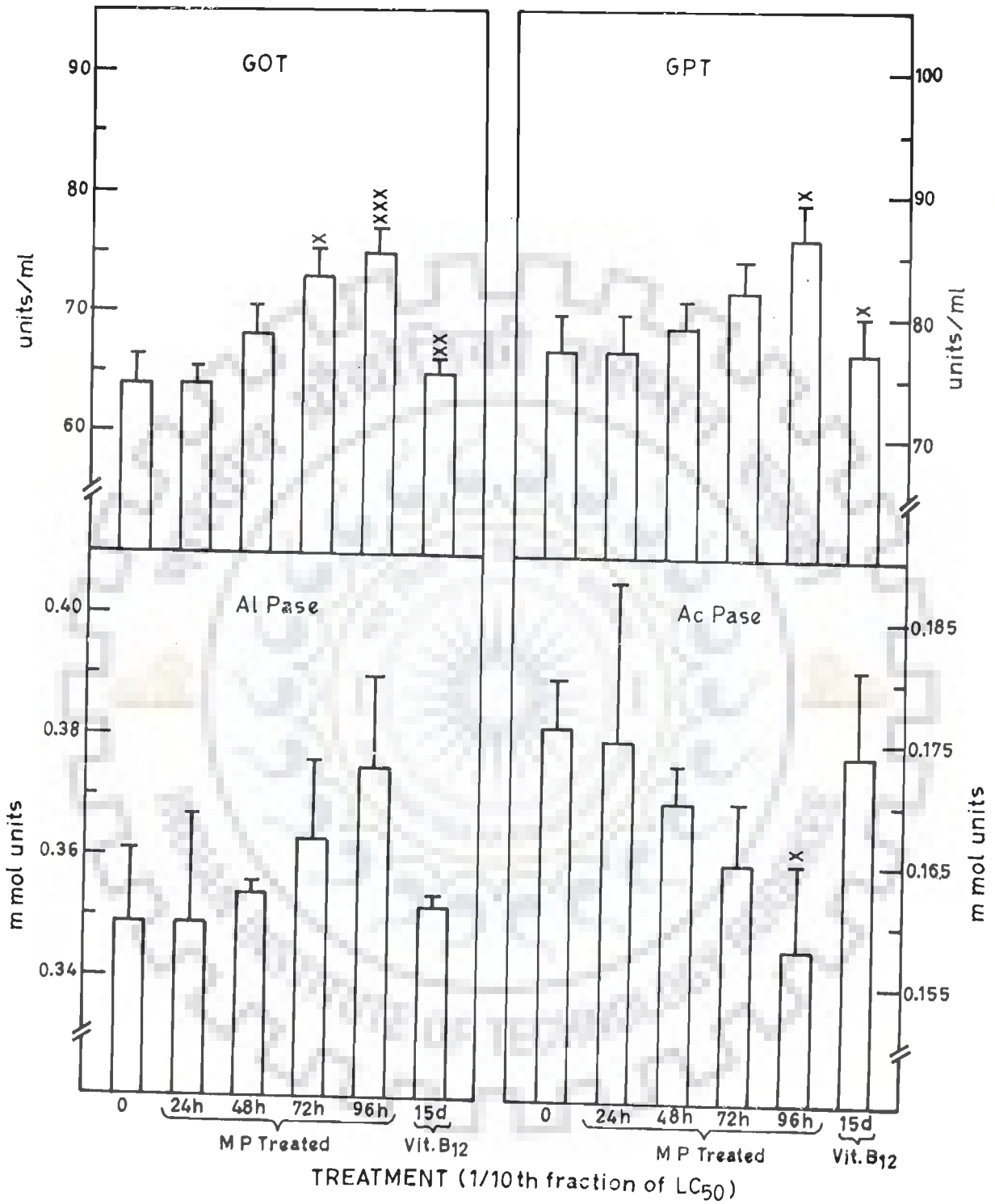
GOT
GPT
units/ml
90
80
70
60
100
90
80
70
X
XXX
XX
Al Pase
Ac Pase
m mol units
0.40
0.38
0.36
0.34
0.185
0.175
0.165
0.155
0
24h
48h
72h
96h
15d
M P Treated
Vit. B12
TREATMENT (1/10th fraction of LC50)

Fig. 26

TABLE-5 : CHANGES IN BLOOD OF CONTROL, MP TREATED AND AFTER VITAMIN B_{12} ADMINISTRATION FISHES FOR 24, 48, 72 AND 96h EXPOSURE (SUBLETHAL CONC.: $1/10^{th}$ FRACTION OF LC_{50})

Biological values in blood	Control	Treatment				
		MP Treated				Vit. B_{12}
		24h	48h	72h	96h	15d
Haemoglobin (g/100 ml)	11.5±0.5	11.4±0.12 (-0.87)	11.0±0.1 (-4.35)	10.4±0.1* (-9.56)	9.7±0.3* (-15.65)	11.4±0.2** (-0.87)
Haematocrit (g/100 ml)	34.0±0.8	33.5±1.5 (-1.47)	32.4±1.2 (-4.70)	30.7±2.1 (-9.70)	28.3±0.7*** (-16.76)	33.8±1.3** (-0.59)
RBC ($10^6/mm^3$)	4.02±0.12	3.98±0.26 (-0.99)	3.76±0.06 (-6.47)	3.52±0.21 (-12.43)	3.38±0.04** (-15.92)	4.01±0.06*** (-0.25)
WBC ($10^3/mm^3$)	3.49±0.21	3.50±0.22 (+0.29)	3.58±0.12 (+2.58)	3.67±0.05 (+5.16)	3.79±0.09 (+8.59)	3.85±0.21 (+10.31)
MCV/ μm^3	84.58±0.12	84.17±0.13* (-0.48)	86.17±0.07*** (+1.88)	87.21±0.19*** (+3.10)	83.73±0.05*** (-1.00)	84.28±0.09*** (-0.35)
MCH (pg)	28.60±0.09	28.64±0.07 (+0.14)	29.25±0.11** (+2.27)	29.54±0.12*** (+3.29)	28.69±0.09 (+0.31)	28.43±0.03** (-0.59)
MCHC(%)	33.82±0.21	34.03±0.13 (+0.62)	33.95±0.13 (+0.38)	33.88±0.26 (+0.18)	34.27±0.18 (+1.33)	33.73±0.17* (-0.26)

Contd....

Total plasma protein (g/100 ml)	2.5±0.1	2.4±0.2 (-4.0)	2.4±0.1 (-4.0)	2.5±0.3 (00)	2.6±0.2 (+4.0)	2.5±0.1 (00)
Glucose (mg/100 ml)	92.4±2.2	92.5±1.5 (+0.10)	96.7±0.7 (+4.65)	102.5±1.9*** (+10.93)	120.2±2.8*** (+30.08)	92.7±0.8*** (+0.32)
Urea (mg N/100 ml)	10.9±0.2	10.9±0.2 (00)	11.2±0.2 (+2.75)	11.7±0.12*** (+7.34)	12.9±0.3*** (+18.34)	11.0±0.12*** (+0.92)
Cholesterol (mg/100 ml)	536.0±8.0	537.0±9.0 (+0.19)	542.0±6.0 (+1.12)	557.0±9.0 (+3.92)	573.0±13.0* (+6.90)	5.37±4.0* (+0.19)
GOT (units/ml serum)	64.0±2.4	64.2±1.6 (+0.31)	68.3±2.1 (+6.72)	72.9±2.3* (+13.90)	75.1±2.1*** (+17.34)	64.9±1.3** (+1.41)
GPT (units/ml serum)	77.0±2.9	77.0±2.9 (00)	79.2±2.3 (+2.86)	81.9±2.5 (+6.36)	86.3±3.1* (+12.07)	77.2±2.7* (+0.26)
Alkaline phosphatase (mmol units)	0.349±0.012	0.349±0.018 (00)	0.354±0.002 (+1.43)	0.363±0.013 (+4.01)	0.375±0.015 (+7.45)	0.352±0.002 (+0.86)
Acid phosphatasc (mmol units)	0.176±0.004	0.175±0.013 (-0.57)	0.170±0.003 (-3.40)	0.165±0.005 (-6.25)	0.158±0.007* (-10.22)	0.174±0.007 (-1.14)

All values are mean ± SE, percentage alterations in parenthesis, (+)% stimulation, (-)% inhibition.

Values are significant at *P<0.05, **P<0.01, ***P<0.001 (Fisher's t-test).

ACID PHOSPHATASE (AcPase) 4.6.2.15

AcPase activities decreased significantly only after 96h exposure. Maximum decrease (10.22%) is found to be significant at $P < 0.05$ level. After vit. B_{12} administration, the decrease in the activity of AcPase is reduced to 1.14%.

HISTOCHEMICAL OBSERVATIONS 4.7

Histochemistry is a sister science of biochemistry and chemistry. The successful use of the histochemical studies for different cells in body tissue is directly responsible for initiating biochemical studies. The preference of the histochemical studies is due to the fact that it demonstrates the gross chemical nature of the cell or tissue. According to de Duve (1967) enzyme histochemistry is not simply a link between descriptive morphology and biochemistry but a separate discipline with its own objectives, rules and methods being capable of making unique contributions which neither biochemistry nor morphology could possibly achieve within their respective boundaries. In the present study, histochemical localization of some important enzymes has been made to correlate and support the biochemical results.

HISTOCHEMICAL LOCALIZATION OF ALKALINE PHOSPHATASE 4.7.1

Calcium cobalt method of Gomori (1952) was employed in the present investigation that impaired brown to black deposites which indicates the presence of alkaline phosphatase.

NORMAL TISSUE (LIVER) 4.7.1.1

Intense enzyme activity was observed in the central part of the cytoplasm of polyhedral hepatic cells in control liver. Nuclei, nucleoli, bile capillaries and the blood corpuscles exhibited strong AlPase activity. The cytoplasm gave the granular appearance showing diffused distribution of AlPase activity (Fig.27.1).

TREATED TISSUE (SUBLETHAL CONC. : $1/5^{th}$ FRACTION OF LC_{50}) 4.7.1.1.1

The liver of the fish after 24h exposure to MP gave histochemical AlPase reaction throughout the hepatic parenchyma (Fig. 27.2). Whereas only a few cells show positive reaction for AlPase activity at the centrolobular region after 48h exposure to MP (Fig.27.3). After 72 and 96h treatment, a decreased activity for AlPase was observed in the liver of fish (Fig.27.4).

TREATED TISSUE (SUBLETHAL CONC. : $1/10^{th}$ FRACTION OF LC_{50}) 4.7.1.1.2

Intense reaction for AlPase was observed throughout the liver parenchyma in fishes after 24h exposure to MP (Fig.27.5). After 48h of treatment, only perilobular region showed stimulated reaction for AlPase activity (Fig.27.6). Whereas a moderate reaction for AlPase activity was observed throughout the liver parenchyma after 72h exposure to MP (Fig.27.7). After 96h exposure, a decrease AlPase activity was observed throughout the hepatic parenchyma (Fig.27.8).

EXPLANATIONS TO THE FIGURE.27

Photomicrograph of histochemical localization of alkaline phosphatase in control and treated (Sublethal conc.: $1/5^{th}$ and $1/10^{th}$ fraction of LC_{50}) liver of O.punctatus.

27.1 T.S. of control liver x 160

27.2 after 24h exposure to MP. x 160

27.3 after 48h exposure to MP. x 160

27.4 after 72 & 96h exposure to MP. x 160

27.5 after 24h exposure to MP. x 160

27.6 after 48h exposure to MP. x 160

27.7 after 72h exposure to MP. x 160

27.8 after 96h exposure to MP. x 160

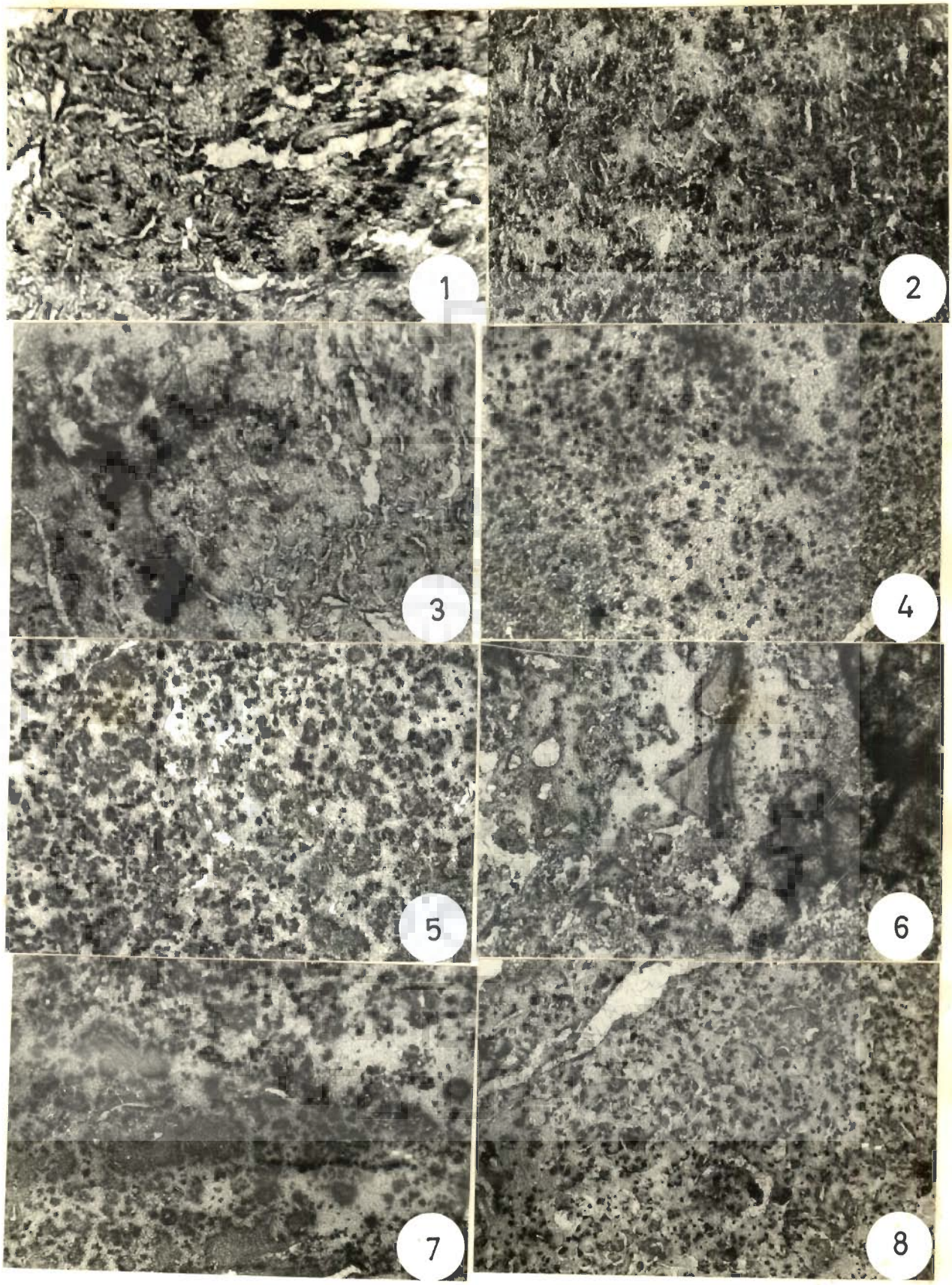

Fig.27

EXPLANATIONS TO THE FIGURE.28

Photomicrograph of histochemical localization of alkaline phosphatase in control and treated kidney (Sublethal conc.: $1/5^{th}$ fraction of LC_{50}) of O.punctatus.

28.1 T.S. of control kidney x 160

28.2 after 24h exposure to MP. x 160

28.3 after 48h exposure to MP. x 160

28.4 after 72h exposure to MP. x 160

28.5 after 96h exposure to MP. x 160

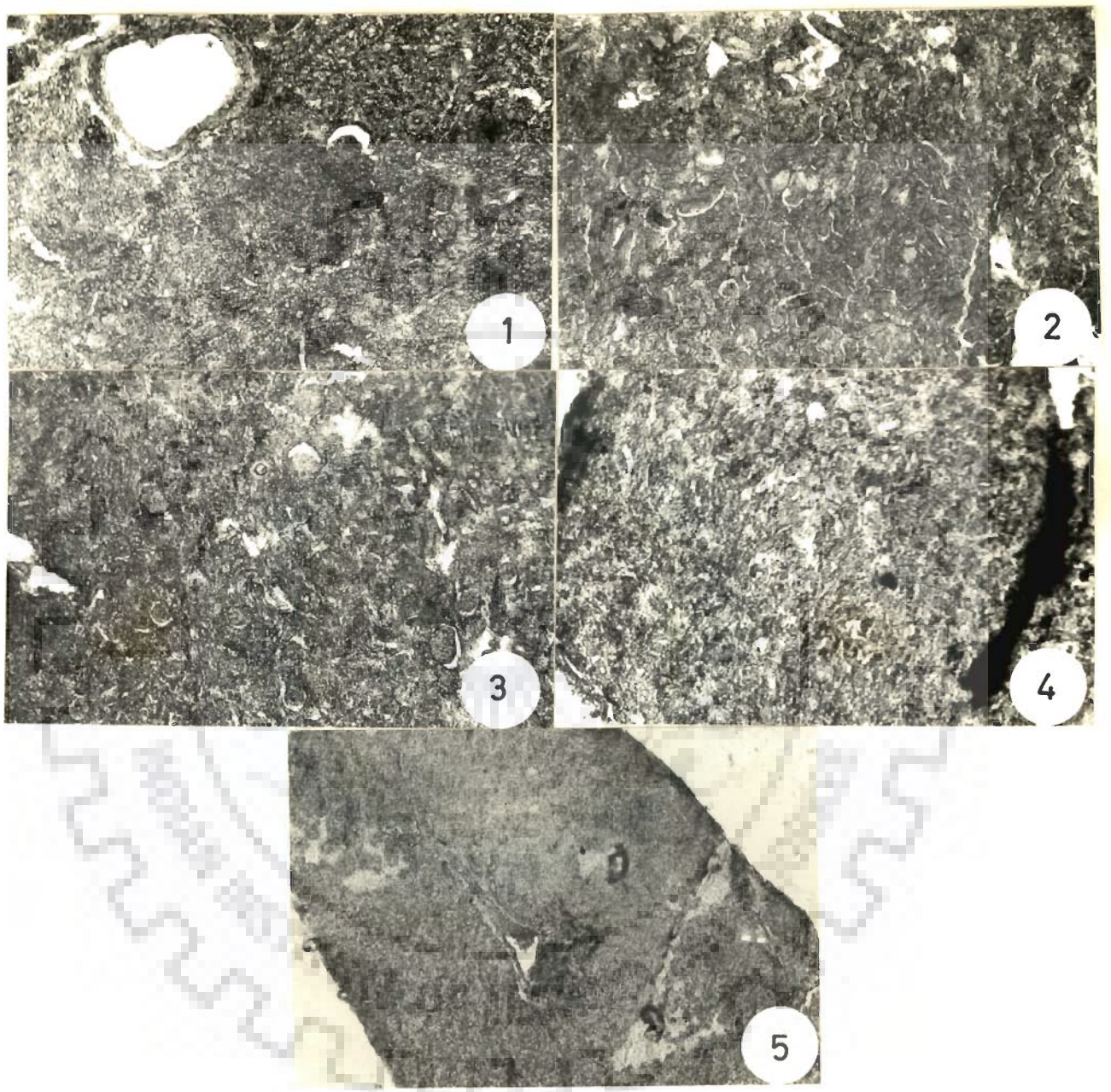

Fig. 28

EXPLANATIONS TO THE FIGURE.29

Photomicrograph of histochemical localization of alkaline phosphatase in treated kidney (Sublethal conc.: $1/10^{th}$ fraction of LC_{50}) of O.punctatus (contd.).

29.1 after 24h exposure to MP. x 160

29.2 after 48h exposure to MP. x 160

29.3 after 72h exposure to MP. x 160

29.4 after 96h exposure to MP. x 160

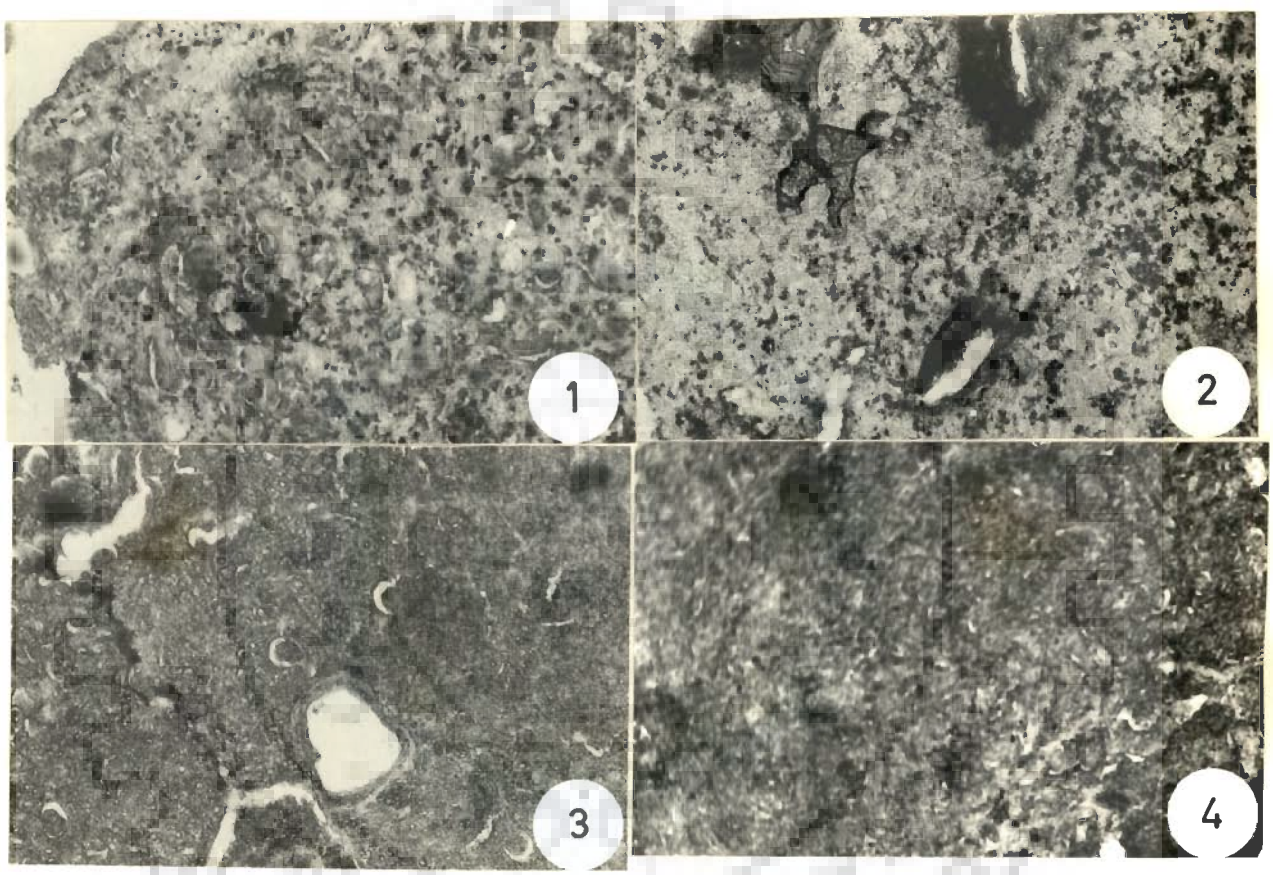

Fig.29

NORMAL TISSUE (KIDNEY) 4.7.1.2

The kidney of the control fish exhibited a strong histochemical AlPase activity. The renal tubules, interstitial tissue and glomeruli gave strongly positive reaction for AlPase activity. Glomerular tufts, however gave a weak histochemical localization of AlPase activity (Fig. 28.1).

TREATED TISSUE (SUBLETHAL CONC. : 1/5th FRACTION OF LC_{50}) 4.7.1.2.1

Treatment of MP to the fish after 24h exposure caused a marked decreased in the AlPase activity especially in the renal tubules (Fig.28.2). Whereas after 48h exposure, only renal tubules adjacent to capsular region showed a positive reaction (Fig. 28.3). A diffused and moderate reaction for AlPase activity was observed after 72h of treatment (Fig.28.4). Whereas after 96h of exposure, AlPase activity was decreased throughout the renal tissue (Fig.28.5).

TREATED TISSUE (SUBLETHAL CONC. : 1/10th FRACTION OF LC_{50}) 4.7.1.2.2

Intense reaction for AlPase was observed after 24h exposure to MP (Fig. 29.1). A strongly positive reaction for AlPase activity was observed after 48h of treatment (Fig.29.2). After 72h exposure a decreased AlPase activity was observed (Fig.29.3). 96h exposure to MP caused a decreased AlPase reaction in the epithelial cells of the renal tubules (Fig.29.4).

HISTOCHEMICAL LOCALIZATION OF ACID PHOSPHATASE 4.7.2

Lead-nitrate method was employed to localize the acid phosphatase activity in the present investigation. Sites of AcPase activity are shown by black deposits of lead sulphide.

NORMAL TISSUE (LIVER) 4.7.2.1

In the control liver the AcPase was localized in the blood spaces and bile capillaries of liver. In the cytoplasm of the liver cells the enzyme activity was diffusely distributed (Fig.30.1).

TREATED TISSUE (SUBLETHAL CONC. : $1/5^{th}$ FRACTION OF LC_{50}) 4.7.2.1.1

The liver of the fish after 24h exposure to MP gave a moderate AcPase reaction throughout the liver parenchyma (Fig.30.2). After 48h exposure, the hepatic cells of the intermediary zone could restore the AcPase activity (Fig.30.3). After 72 and 96h intoxication, a decreased reaction of AcPase activity was observed throughout the liver parenchyma (Fig.30.4) and (Fig.30.5).

TREATED TISSUE (SUBLETHAL CONC. : $1/10^{th}$ FRACTION OF LC_{50}) 4.7.2.1.2

A diffused but strong reaction for AcPase was observed in the liver parenchyma after 24h exposure to MP (Fig.31.1). The liver of the fish after 48, 72 and 96h exposure to MP show a decrease reaction for AcPase activity (Fig.31.2).

EXPLANATIONS TO THE FIGURE.30

Photomicrograph of histochemical localization of acid phosphatase in control and treated liver (Sublethal conc.: $1/5^{th}$ fraction of LC_{50}) of O.punctatus.

30.1 T.S. of control liver x 160

30.2 after 24h exposure to MP. x 160

30.3 after 48h exposure to MP. x 160

30.4 after 72h exposure to MP. x 160

30.5 after 96h exposure to MP. x 160

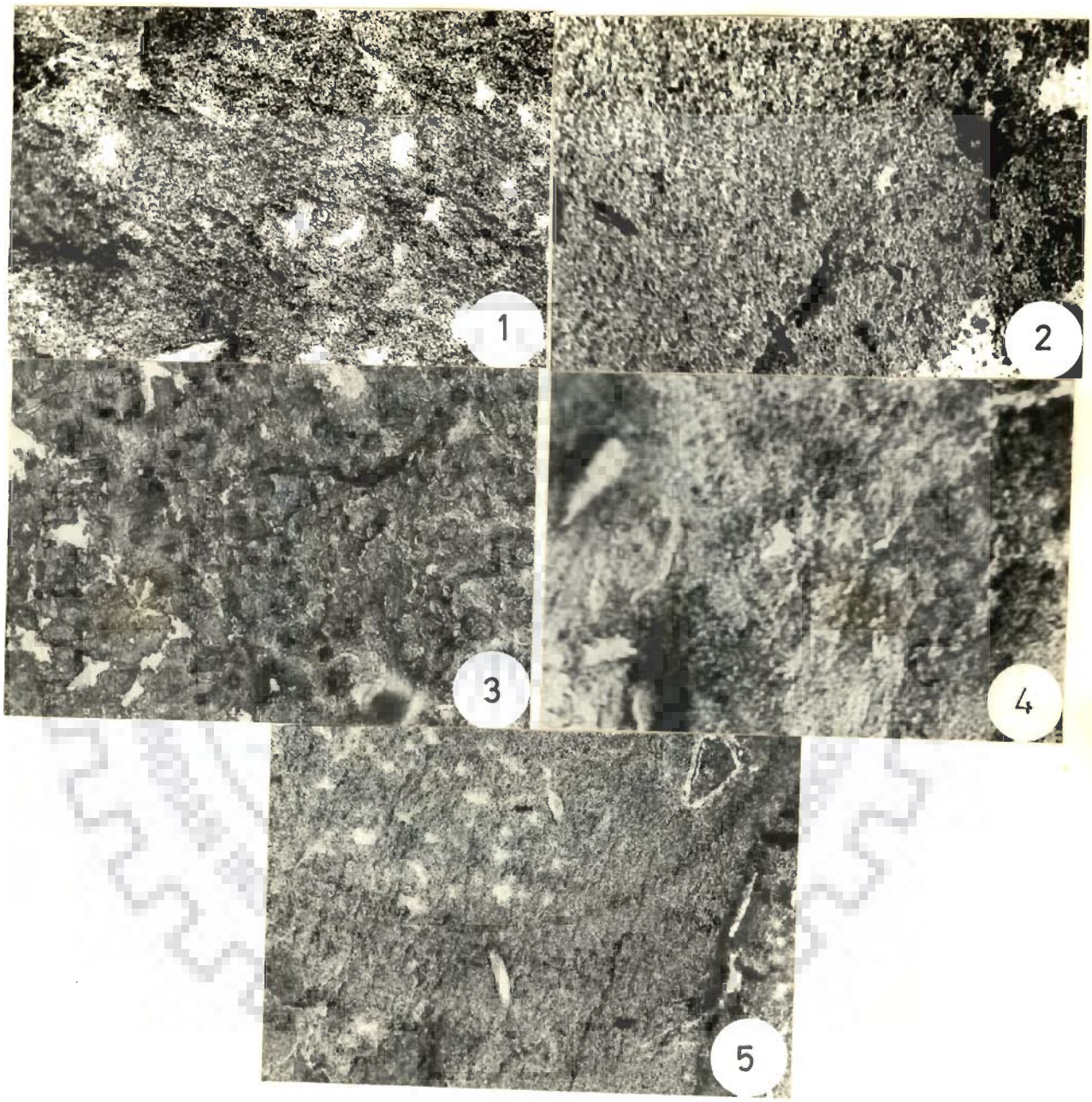

Fig.30

EXPLANATIONS TO THE FIGURE.31

Photomicrograph of histochemical localization of acid phosphatase in treated liver (Sublethal conc.: $1/10^{th}$ fraction of LC_{50}) of O.punctatus (contd.).

31.1 after 24h exposure to MP. x 160

31.2 after 48, 72 and 96h exposure to MP. x 160

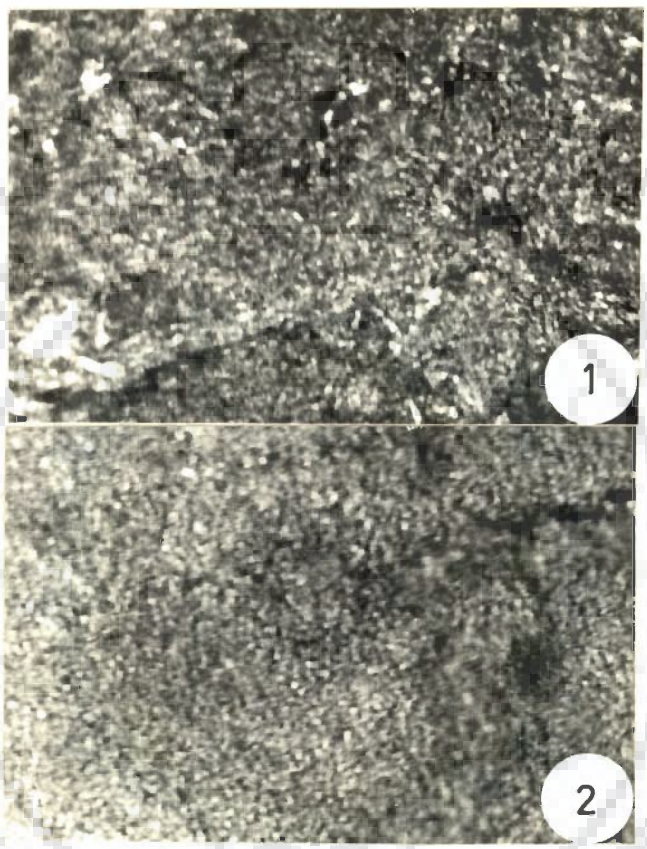

Fig. 31

EXPLANATIONS TO THE FIGURE.32

Photomicrograph of histochemical localization of acid phosphatase in control and treated kidney (Sublethal conc.: $1/5^{th}$ fraction of LC_{50}) of O.punctatus.

32.1 T.S. of control kidney x 160

32.2 after 24h exposure to MP. x 160

32.3 after 48 & 72h exposure to MP. x 160

32.4 after 96h exposure to MP. x 160

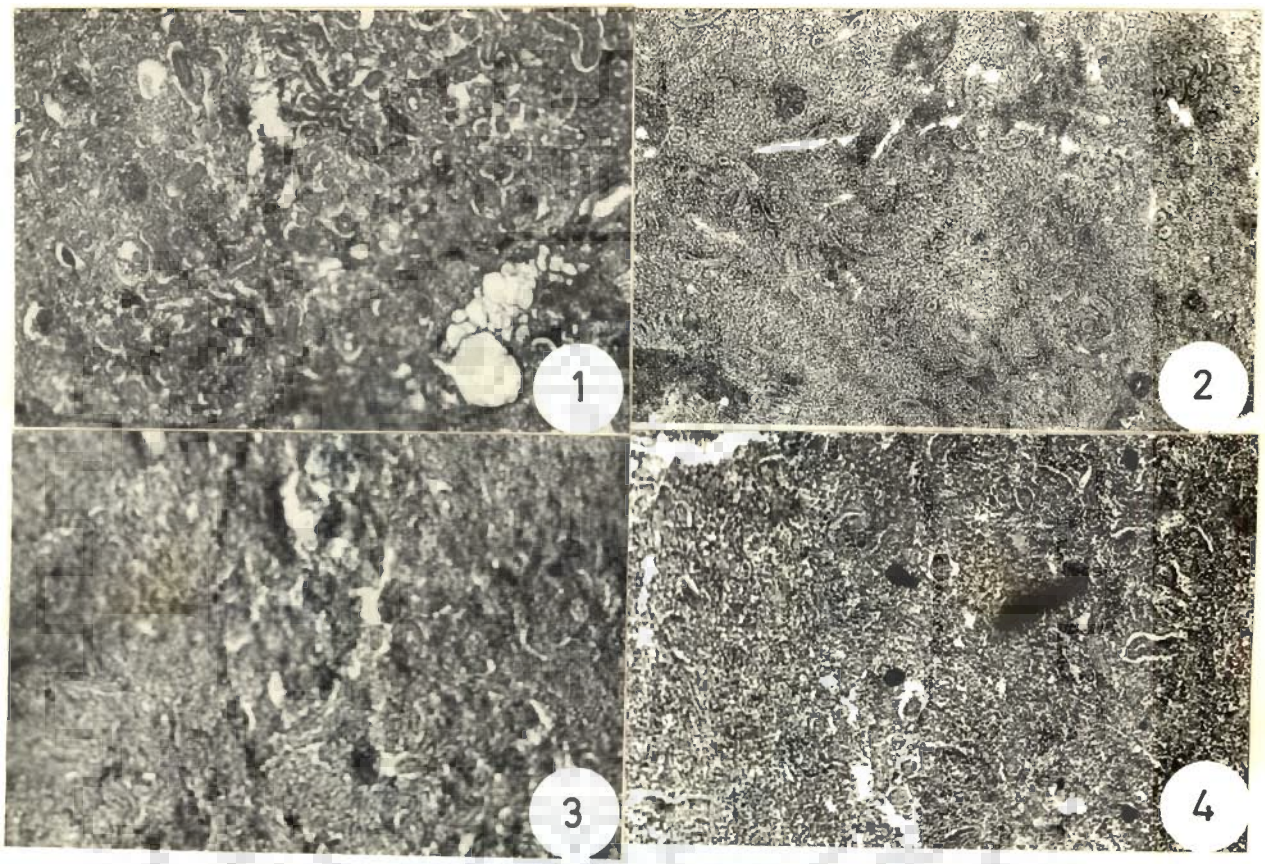

Fig. 32

EXPLANATIONS TO THE FIGURE.33

Photomicrograph of histochemical localization of acid phosphatase in treated kidney (Sublethal conc.: $1/10^{th}$ fraction of LC_{50}) of O.punctatus (contd.).

33.1 after 24h exposure to MP. x 160

33.2 after 48 & 72h exposure to MP. x 160

33.3 after 96h exposure to MP. x 160

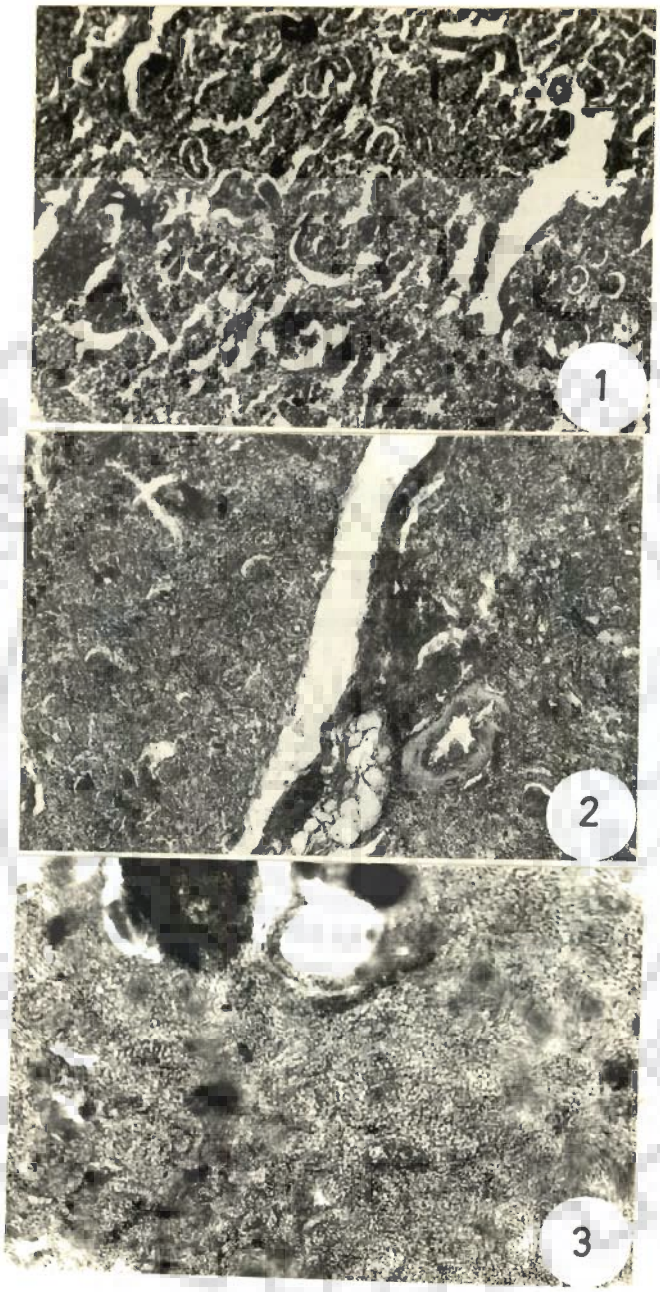

Fig. 33

NORMAL TISSUE (KIDNEY) 4.7.2.2

AcPase activity was found in the cytoplasm of the tubular epithelial cells, nuclei intertubular tissue and in the renal corpuscles. Haemopoietic tissue, too, was strongly positive for enzyme (Fig.32.1).

TREATED TISSUE (SUBLETHAL CONC. : 1/5th FRACTION OF LC_{50}) 4.7.2.2.1

A moderate reaction for AcPase activity was seen throughout the renal tubules after 24h exposure (Fig.32.2).After 48 and 72h of exposure, a dull AcPase activity was found in the cytoplasm of the tubular epithelial cells (Fig.32.3). A diffused reaction for AcPase activity was observed after 96h treatment (Fig.32.4).

TREATED TISSUE (SUBLETHAL CONC. : 1/10th FRACTION OF LC_{50}) 4.7.2.2.2

After 24h exposure a strong positive reaction for AcPase activity was observed in the kidney tissue (Fig.33.1). A moderate reaction for AcPase activity was found in the epithelial cells of the renal tubules after 48 and 72h of exposure (Fig. 33.2). After 96h of treatment, a decreased reaction for AcPase was observed in the cytoplasm of the renal tissue (Fig. 33.3).

HISTOCHEMICAL LOCALIZATION OF LIPASE 4.7.3

During present investigation, Tween's method was employed that formed brownish - black precipitate indicating the presence of lipase activity.

NORMAL TISSUE (LIVER) 4.7.3.1

Lipase activity was localized in the cytoplasm and around the cell membrane of hepatocytes. Blood vessels and connective tissue do not show enzyme activity. In hepatic cells, the enzyme is more concentrated in the central part of the cytoplasm around the nucleus than towards the periphery (Fig.34.1).

TREATED TISSUE (SUBLETHAL CONC. : 1/5th FRACTION OF LC_{50}) 4.7.3.1.1

A dull reaction throughout the liver parenchyma was found after 24h exposure to MP (Fig.34.2). After 48 exposure, a moderate reaction for lipase activity was observed throughout the hepatic parenchyma (Fig.34.3). After 72h exposure, only few cells showed positive rfeaction for lipase activity (Fig.34.4). A strong reaction for lipase activity was found throughout the hepatic parenchyma after 96h exposure (Fig.34.5).

TREATED TISSUE (SUBLETHAL CONC. : 1/10th FRACTION OF LC_{50}) 4.7.3.1.2

After 24h exposure to MP, dull reaction for lipase activity at the intermediary zone was observed (Fig.34.6). After 48 and 72h of exposure, only few cell at the centrolobular zone showed positive reaction for lipase activity (Fig.34.7). Intense reaction for lipase activity was observed throughout the liver parenchyma after 96h of treatment (Fig.34.8).

EXPLANATIONS TO THE FIGURE.34

Photomicrograph of histochemical localization of lipase in control and treated liver (Sublethal conc. : $1/5^{th}$ and $1/10^{th}$ fraction of LC_{50}) of O.punctatus.

34.1 T.S. of control liver x 160

34.2 after 24h exposure to MP. x 160

34.3 after 48h exposure to MP. x 160

34.4 after 72h exposure to MP. x 160

34.5 after 96h exposure to MP. x 160

34.6 after 24h exposure to MP. x 160

34.7 after 48 & 72h exposure to MP. x 160

34.8 after 96h exposure to MP. x 160

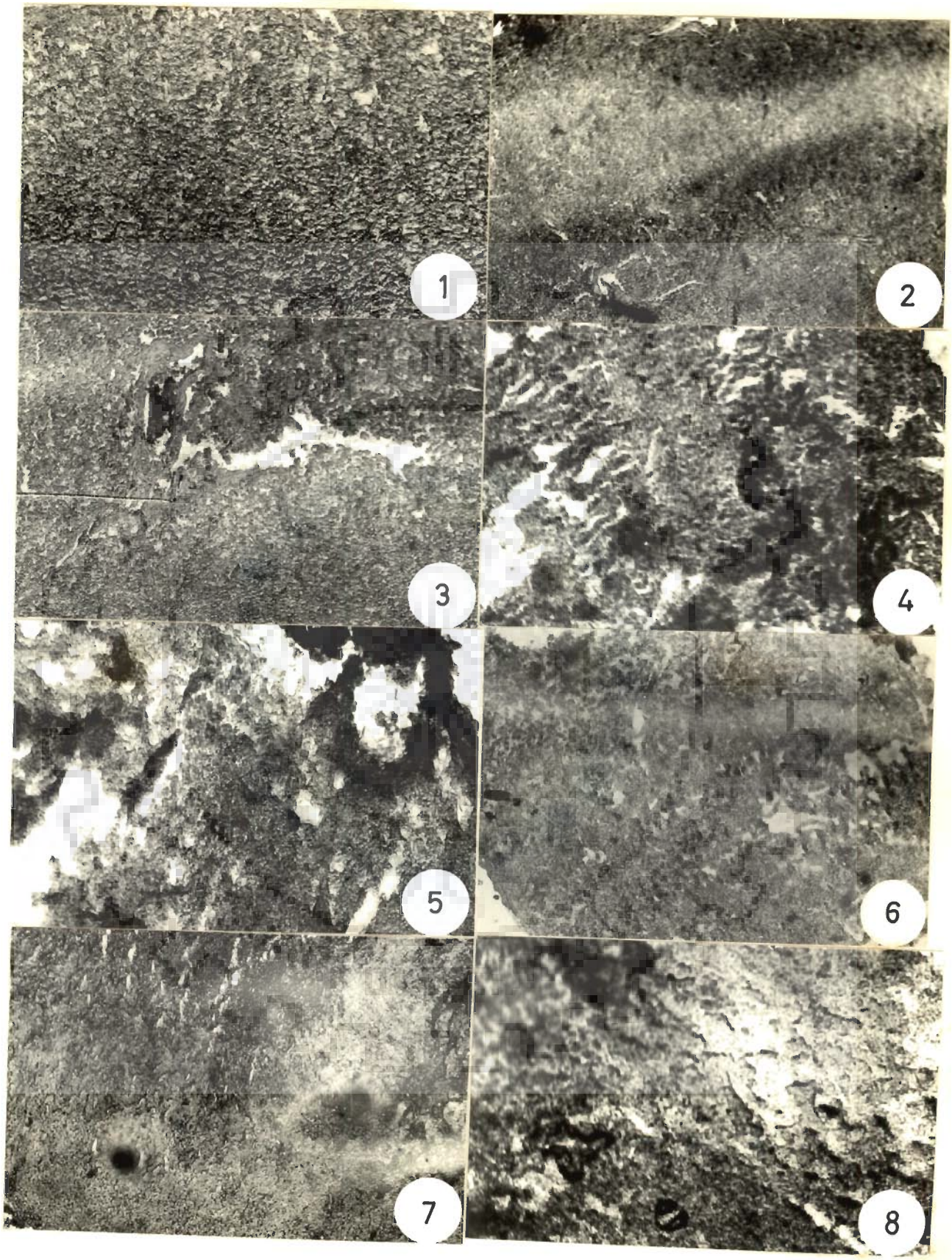

Fig. 34

EXPLANATIONS TO THE FIGURE.35

Photomicrograph of histochemical localization of lipase in control and treated kidney (Sublethal conc.: $1/5^{th}$ and $1/10^{th}$ fraction of LC_{50}) of O.punctatus.

35.1 T.S. of control kidney x 160

35.2 after 24h exposure to MP. x 160

35.3 after 48 & 72h exposure to MP. x 160

35.4 after 96h exposure to MP. x 160

35.5 after 24h exposure to MP. x 160

35.6 after 48h exposure to MP. x 160

35.7 after 72h exposure to MP. x 160

35.8 after 96h exposure to MP. x 160

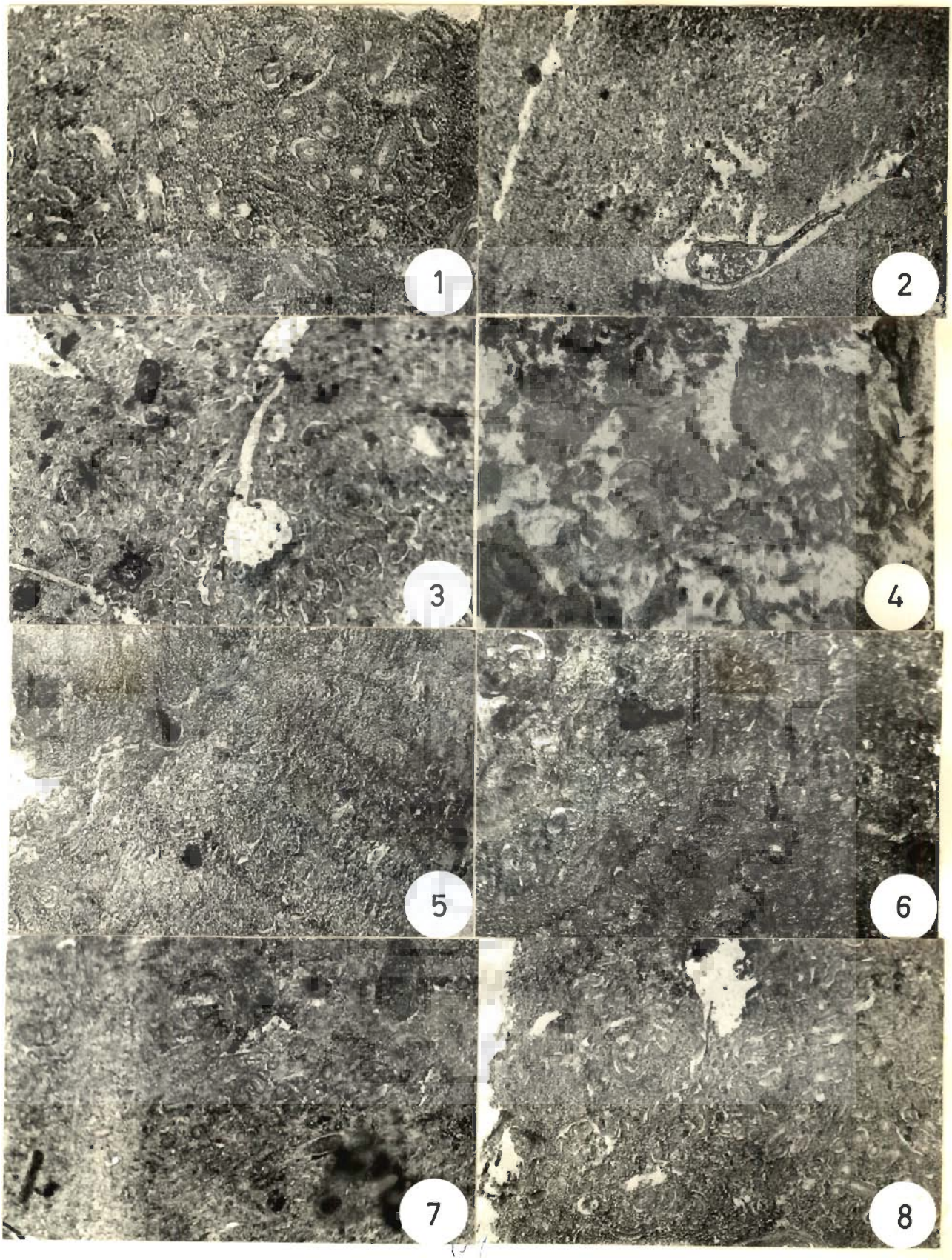

Fig. 35


CENTRAL LIBRARY
ROORKEE
UNIV. OF ROORKEE

NORMAL TISSUE (KIDNEY) 4.7.3.2

Moderate lipase activity was localized in the cytoplasm of tubular cells of proximal and distal tubules. Glomerulus shows mild activity of this enzyme. Other portion of kidney is lipase negative (Fig.35.1).

TREATED TISSUE (SUBLETHAL CONC. : $1/5^{th}$ FRACTION OF LC_{50}) 4.7.3.2.1

After 24h exposure, the kidney show diffused reaction for lipase activity (Fig.35.2).A diffused and moderate reaction for lipase activity was observed after 48 and 72h exposure to MP (Fig.35.3). Intense reaction of lipase activity in renal tubules and glomerulus was shown after 96h of exposure to MP (Fig.35.4).

TREATED TISSUE (SUBLETHAL CONC. : $1/10^{th}$ FRACTION OF LC_{50}) 4.7.3.2.2

Treatment of MP to the kidney after 24h exposure show diffused and moderate reaction of lipase activity (Fig.35.5). A strong lipase activity was observed throughout the renal epithelium after 48h of exposure (Fig.35.6). After 72h of exposure, strong lipase activity was observed at the peripheral region (Fig.35.7). After 96h treatment the lipase activity was stimulated throughout the renal tubules (Fig.35.8).

BIOCHEMICAL OBSERVATIONS 4.8

Morphological study tells only about a part of the tissue whereas the biochemical information, refers to the

composition of the million of cells. With this point in view the changes induced by the pesticide in the state of macromolecules of the liver and kidney have been determined after applying these parameters. The available literature shows that no such attempt has been made so far therefore, the present observation show some of the important physico-chemical activities exhibited in the liver and kidney of the treated fish.

The results of the experiments conducted to determine the biochemical constituents and enzymes in the liver and kidney of control, MP toxicated and vitamin B_{12} injected fish for 24, 48, 72 and 96h intervals have been summarised in the Tables (6,7,8,9) and Fig. (36,37,38).

EFFECT OF MP AND VITAMIN B_{12} (SUBLETHAL CONC. : 1/5th FRACTION OF LC_{50}) 4.8.1

MOISTURE 4.8.1.1

The preliminary observations made on the moisture contents indicated that there is a significant increase during all the time intervals of MP intoxication. Maximum increase (8.21%) was found in the liver after 96h exposure at $P<0.001$ level. In the kidney also, it was 8.21% after 96h exposure, the change was significant at $P<0.001$ level. Fishes protected with vit. B_{12}, the increase in the moisture content is reduced by 0.36% in liver and 0.49% in the kidney.

The percentage of proteins and lipids is diminished

Fig.36 Percentage of moisture in the liver and kidney of O.punctatus treated with methyl-parathion (MP) and vitamin B_{12}. Bars represent mean ±SE (n = 5). Asterisks denote means significantly different from controls (0) at *P<0.05; **P<0.01; ***P<0.001.

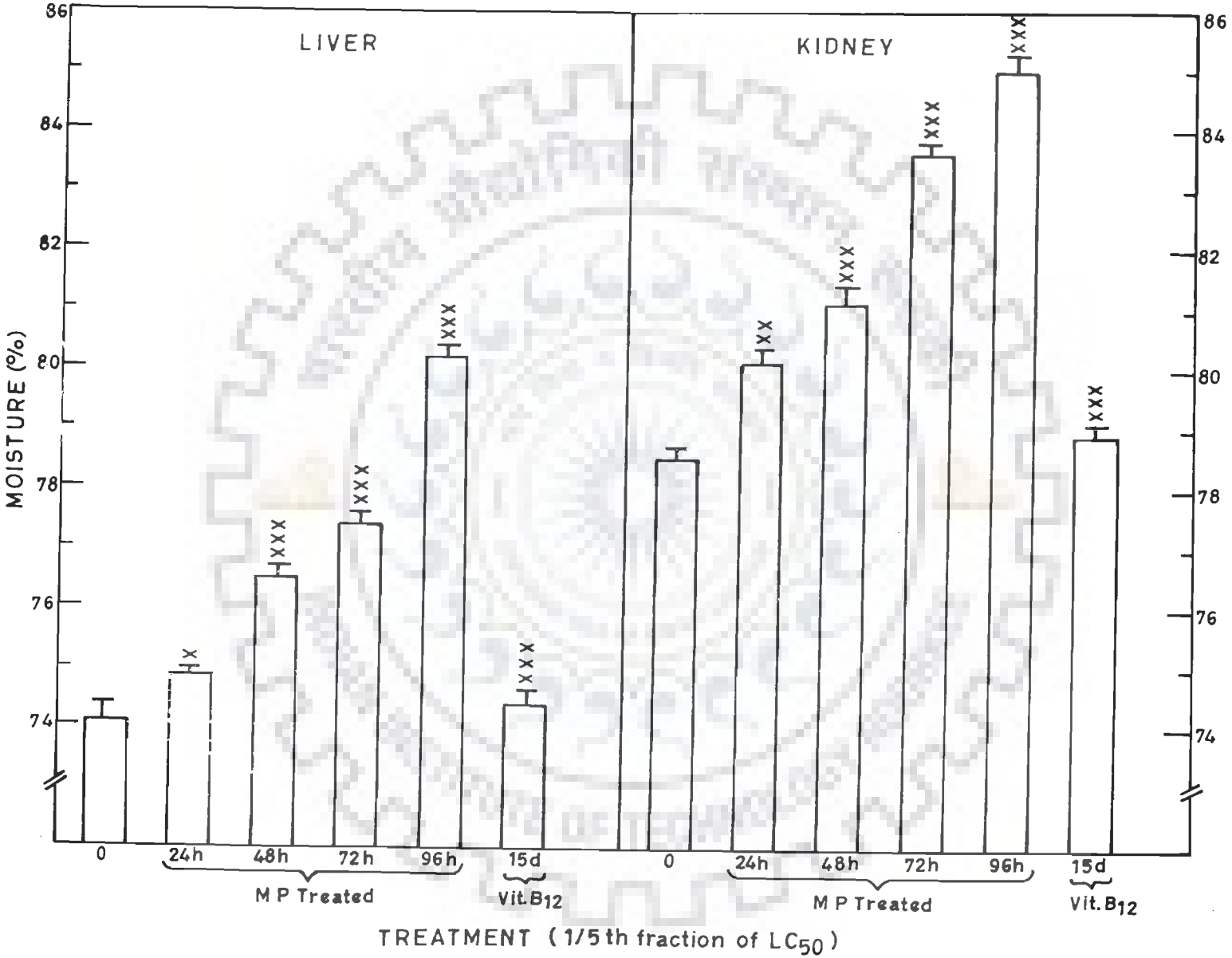
LIVER
KIDNEY
MOISTURE (%)
86
84
82
80
78
76
74
x
xxx
xx
0
24h
48h
72h
96h
15d
M P Treated
Vit.B12
TREATMENT (1/5 th fraction of LC50)

Fig.36

both in the liver and the kidney at all the time intervals of MP intoxication. A significant increase is found in the carbohydrate percentage both in the liver and kidney during all the time intervals of the treated fish.

PROTEINS 4.8.1.2

Quantitative data on total proteins in both the tissues and its comparison with controls revealed that protein contents decrease in all the experimental fish exposed to MP. The maximum decrease was found after 96h exposure. Significant depletion in the protein content was by 42.91% in the liver and 47.27% in the kidney at $P<0.001$ level. Fishes protected with vit. B_{12} show that the decrease in the protein content is reduced to 1.79% in liver and 1.92% in kidney.

LIPIDS 4.8.1.3

Maximum decrease (42.31%) significant at $P<0.001$ is found in liver of MP toxicated fishes after 96h treatment. In the kidney, the maximum decrease of 38.51% ($P<0.001$) is found after 96h exposure. It is reduced to 2.06% in liver and 1.44% in kidney after vit. B_{12} administration.

CARBOHYDRATES 4.8.1.4

Percentage of carbohydrates in the liver of fishes after MP treatment was found to be significantly increased at all the time intervals. The maximum increase (42.59%) significant at $P<0.001$ was found after 96h exposure in the liver. It is reduced to 2.03% when protected with vit. B_{12}. In kidney, the maximum increase was found to be 44.41% ($P<0.001$) after 96h treatment and reduced to 2.23% after

TABLE-6 : PERCENTAGE OF MOISTURE CONTENTS, TOTAL PROTEINS, LIPIDS AND CARBOHYDRATES IN THE LIVER OF CONTROL, MP TREATED AND AFTER VITAMIN B_{12} ADMINISTRATION FISHES FOR 24, 48, 72 AND 96h EXPOSURE (SUBLETHAL CONC.: $1/5^{th}$ FRACTION OF LC_{50})

Components	Control	Treatment				
		MP Treated				Vit. B_{12}
		24h	48h	72h	96h	15d
% Moisture	74.08±0.26	74.94±0.13* (+1.16)	76.45±0.23*** (+3.19)	77.38±0.21*** (+4.45)	80.16±0.24*** (+8.21)	74.35±0.26*** (+0.36)
% Total Proteins	7.83±0.12	7.23±0.03** (-7.66)	6.12±0.04*** (-21.84)	5.27±0.07*** (-32.69)	4.47±0.03*** (-42.91)	7.69±0.06*** (-1.79)
% Total Lipids	10.21±0.17	8.96±0.12*** (-12.24)	7.91±0.07*** (-22.53)	6.98±0.13*** (-31.63)	5.89±0.07*** (-42.31)	10.0±0.13*** (-2.06)
% Total Carbohydrates	4.93±0.09	5.19±0.07* (+5.27)	5.61±0.11** (+13.79)	6.30±0.08*** (+27.79)	7.03±0.12*** (+42.59)	5.03±0.07*** (+2.03)

All values are mean ± SE, percentage alterations in parenthesis, (+)% stimulation, (-)% inhibition. Values are significant at *P<0.05, **P<0.01, ***P<0.001 (Fisher's t-test).

TABLE-7 : PERCENTAGE OF MOISTURE CONTENTS, TOTAL PROTEINS, LIPIDS AND CARBOHYDRATES IN THE KIDNEY OF CONTROL, MP TREATED AND AFTER VITAMIN B_{12} ADMINISTRATION FISHES FOR 24, 48, 72 AND 96h EXPOSURE (SUBLETHAL CONC. : $1/5^{th}$ FRACTION OF LC_{50})

Components	Control	Treatment				
		MP Treated				Vit. B_{12}
		24h	48h	72h	96h	15d
%Moisture	78.54±0.24	80.06±0.26** (+1.93)	81.11±0.29*** (+3.27)	83.63±0.22*** (+6.48)	84.98±0.32*** (+6.93)	78.93±0.19*** (+0.49)
% Total Proteins	6.24±0.06	5.75±0.09** (-7.85)	4.84±0.06*** (-22.43)	3.92±0.12*** (-37.18)	3.29±0.03*** (-47.27)	6.12±0.08*** (-1.92)
% Total Lipids	8.31±0.09	8.01±0.04* (-3.61)	7.19±0.07*** (-13.48)	6.33±0.04*** (-23.83)	5.11+0.07*** (-38.51)	8.19±0.03*** (-1.44)
% Total Carbohydrates	3.58±0.12	3.80±0.07 (+6.14)	4.13±0.09** (+15.36)	4.71±0.06*** (+31.56)	5.17±0.23*** (+44.41)	3.66±0.17*** (+2.23)

All values are mean ± SE, percentage alterations in parenthesis, (+)% stimulation, (-)% inhibition. Values are significant at *P<0.05, **P<0.01, ***P<0.001 (Fisher's t-test)

Fig.37 Percentage of total proteins, lipids and carbohydrates in the liver and kidney of O.punctatus under the stress of methyl-parathion (MP) and vitamin B_{12}. Bars represent mean ±SE (n = 5). Asterisks denote means significantly different from controls (0) at *P<0.05; **P<0.01; ***P<0.001.

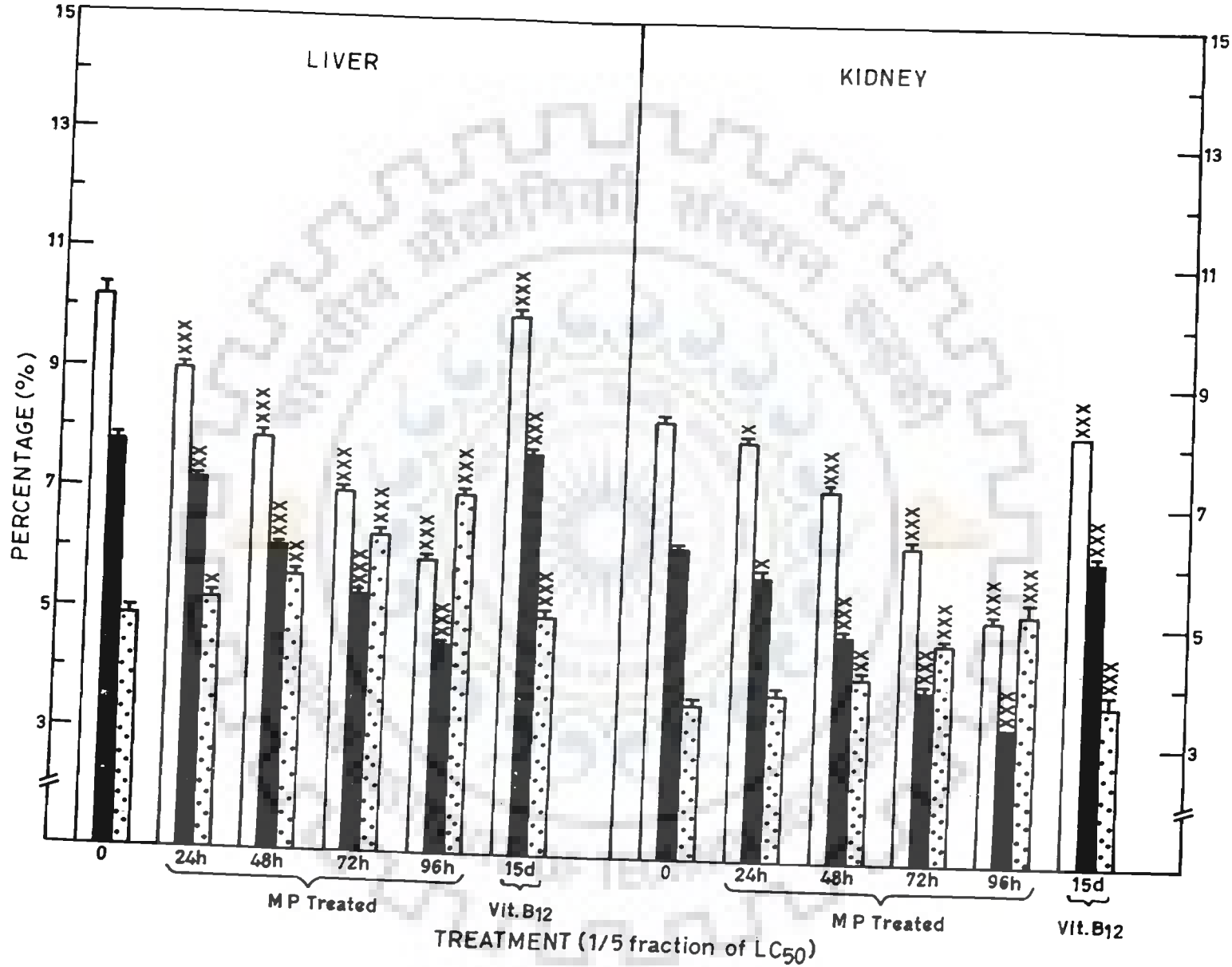
LIVER
KIDNEY
PERCENTAGE (%)
15
13
11
9
7
5
3
0
24h
48h
72h
96h
15d
M P Treated
Vit.B12
TREATMENT (1/5 fraction of LC50)

Fig. 37

Fig.38 Alterations in the activity of alkaline and acid phosphatase and lipase under the stress of methyl-parathion (MP) and vitamin B_{12}. Bars represent mean $\pm$SE (n = 5). Asterisks indicate significant (*P<0.05; **P<0.01; ***P<0.001) difference from control (0) fish.

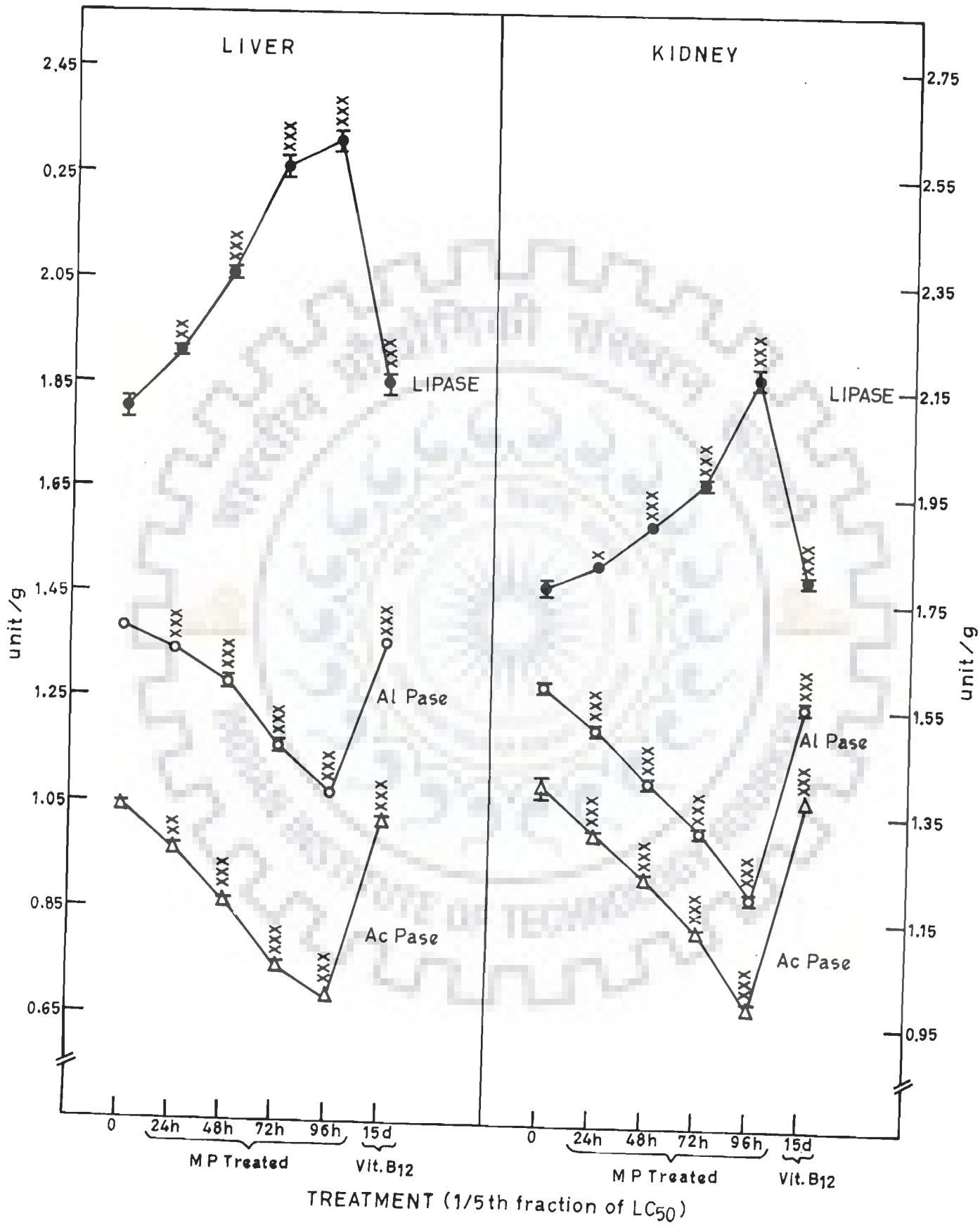
LIVER
KIDNEY
LIPASE
Al Pase
Ac Pase
unit/g
2.45
0.25
2.05
1.85
1.65
1.45
1.25
1.05
0.85
0.65
2.75
2.55
2.35
2.15
1.95
1.75
1.55
1.35
1.15
0.95
0
24h
48h
72h
96h
15d
M P Treated
Vit. B12
TREATMENT (1/5th fraction of LC50)

Fig.38

vit. B_{12} administration.

ENZYMES 4.8.1.5

ALKALINE PHOSPHATASE 4.8.1.5.1

There is significant inhibition in the AlPase activity both in liver and kidney at all the time intervals after MP intoxication. Maximum inhibition is 22.16% ($P<0.001$) in the liver and 24.94% ($P<0.001$) in the kidney. Fishes protected with vit. B_{12}, the inhibition in the AlPase activity is reduced to 1.31% in liver and 2.21% in kidney.

ACID PHOSPHATASE 4.8.1.5.2

The activity of AcPase under the stress of MP showed decrease in liver and kidney after all the time intervals. Maximum suppresion was recorded after 96h, by 34.58% ($P<0.001$) in liver and 29.52% ($P<0.001$) in kidney. After vit. B_{12} administration. It is found to be reduced to 1.83% and 1.51% in liver and kidney, respectively.

LIPASE 4.8.1.5.3

MP intoxication resulted in significant increased activity of the lipase in liver and kidney after all the time intervals. The lipase activity increased by 27.88% ($P<0.001$) in liver and 23.17% ($P<0.001$) in kidney after 96h treatment. It is found to be reduced to 2.33% and 1.13% in liver and kidney, respectively after vit. B_{12} administration.

EFFECT OF MP AND VITAMIN B_{12} (SUBLETHAL CONC. : $1/10^{th}$ FRACTION OF LC_{50}) 4.8.2

The results of the experiments conducted to determine the biochemical constitutents and enzymes in the liver and

TABLE-8 : ENZYMOLOGICAL ALTERATIONS IN THE LIVER OF CONTROL, MP TREATED AND AFTER VITAMIN B_{12} ADMINISTRATION FISHES FOR 24, 48, 72 AND 96h EXPOSURE (SUB-LETHAL CONC. : $1/5^{th}$ FRACTION OF LC_{50})

Enzymes	Control	Treatment				
		MP Treated				Vit. B_{12}
		24h	48h	72h	96h	15d
AlPase Bodansky unit/g	1.376±0.003	1.344±0.001*** (-2.32)	1.283±0.010*** (-6.76)	1.157±0.011*** (-15.91)	1.071±0.007*** (-22.16)	1.358±0.009*** (-1.31)
AcPase Bodansky unit/g	1.038±0.012	0.963±0.009** (-7.22)	0.857±0.012*** (-17.44)	0.743±0.013*** (-28.42)	0.679±0.003*** (-34.58)	1.019±0.013*** (-1.83)
Lipase unit/g	1.804±0.021	1.907±0.012** (+5.71)	2.063±0.013*** (+14.36)	2.257±0.024*** (+25.11)	2.307±0.021*** (+27.88)	1.846±0.021*** (+2.23)

All values are mean ± SE, percentage alterations in parenthesis,(+)% stimulation,(-)% inhibition.

Values are significant at **P<0.01 and ***P<0.001 (Fisher's t-test).

TABLE-9 : ENZYMOLOGICAL ALTERATIONS IN THE KIDNEY OF CONTROL, MP TREATED AND AFTER VITAMIN B_{12} ADMINISTRATION FISHES FOR 24, 48, 72 AND 96h EXPOSURE (SUB-LETHAL CONC. : $1/5^{th}$ FRACTION OF LC_{50})

Enzymes	Control	Treatment				
		MP Treated				Vit. B_{12}
		24h	48h	72h	96h	15d
AlPase Bodansky unit/g	1.584±0.010	1.501±0.011*** (-5.24)	1.401±0.012*** (-11.55)	1.309±0.009*** (-17.36)	1.189±0.013*** (-24.94)	1.549±0.016*** (-2.21)
AcPase Bodansky unit/g	1.389±0.021	1.302±0.006** (-6.26)	1.219±0.009*** (-12.24)	1.117±0.007*** (-19.58)	0.979±0.013*** (-29.52)	1.368±0.004*** (-1.51)
Lipase unit/g	1.765±0.015	1.807±0.003* (+2.38)	1.891±0.009*** (+7.14)	1.971±0.012*** (+11.67)	2.174±0.022*** (+23.17)	1.785±0.013*** (+1.13)

All values are mean ± SE, percentage alterations in parenthesis, (+)% stimulation, (-)% inhibition. Values are significant at *P<0.05, **P<0.01 and ***P<0.001 (Fisher's t-test).

kidney of control, MP toxicated and vit. B_{12} injected fish for 24, 48, 72 and 96h intervals have been summarised in the Tables (10,11,12,13) and Fig.(39, 40, 41).

MOISTURE 4.8.2.1

The moisture contents increased at all the time intervals both in the liver and the kidney. Maximum increase (6.53%) significant at P<0.001 level was found in the liver after 96h exposure, whereas the moisture contents increased in significantly after 24h exposure. In kidney, the maximum increase in moisture contents 7.01% (P<0.001) was found after 24h exposure. Fishes protected with vit. B_{12} show reduction in the moisture contents. It was by 0.27% in liver and 0.10% in kidney.

PROTEINS 4.8.2.2

Significant depletion was recorded in the liver after 48, 72 and 96h exposure, whereas in kidney, protein contents decreased significantly at all the time intervals. Protein was depleted by 37.04% (P<0.001) in liver and 46.15% (P<0.001) in kidney after 96h of exposure. Vitamin B_{12} administration reduced the decrease in protein contents by (0.38%) in liver and 0.80% in kidney.

LIPIDS 4.8.2.3

Total lipid contents decreased significantly after all the time intervals in the liver, whereas the significant depletion was recorded after 48, 72 and 96h exposure in kidney of MP toxicated fish. The maximum depletion was

Fig.39. Percentage of moisture in the liver and kidney of O.punctatus treated with methyl-parathion (MP) and vitamin B_{12}. Bars represent mean ±SE (n = 5). Asterisks indicate significant (*P<0.05; ***P<0.001) difference from control (0) fish.

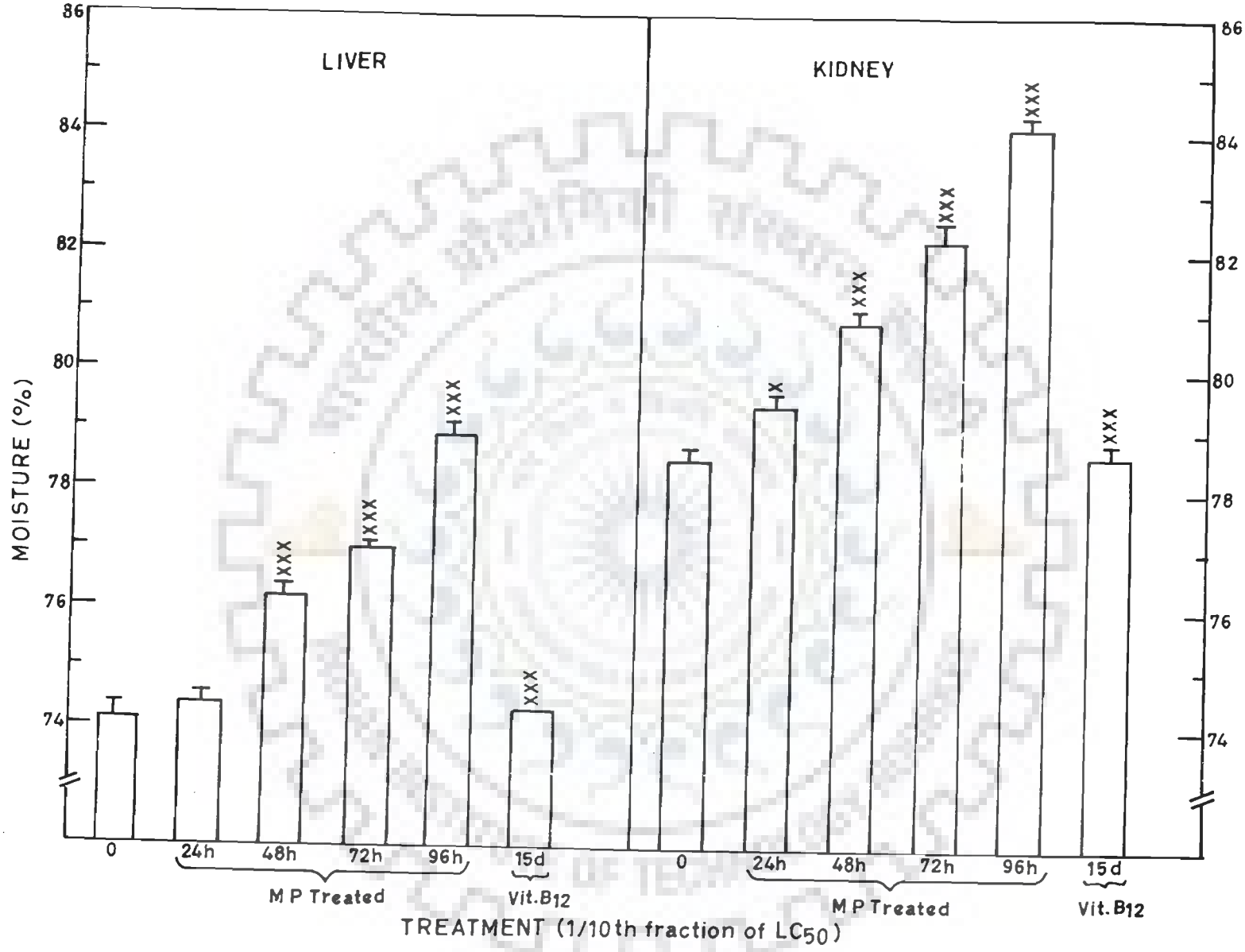
LIVER
KIDNEY
MOISTURE (%)
86
84
82
80
78
76
74
XXX
X
0
24h
48h
72h
96h
15d
M P Treated
Vit.B12
TREATMENT (1/10th fraction of LC50)

Fig.39

Fig.40 Percentage of total proteins, lipids and carbohydrates in the liver and kidney of O.punctatus under the stress of methyl-parathion (MP) and vitamin B_{12}. Bars represent mean ±SE (n = 5). Asterisks indicate significant (**P<0.01; ***P<0.001) difference from control (0) fish.

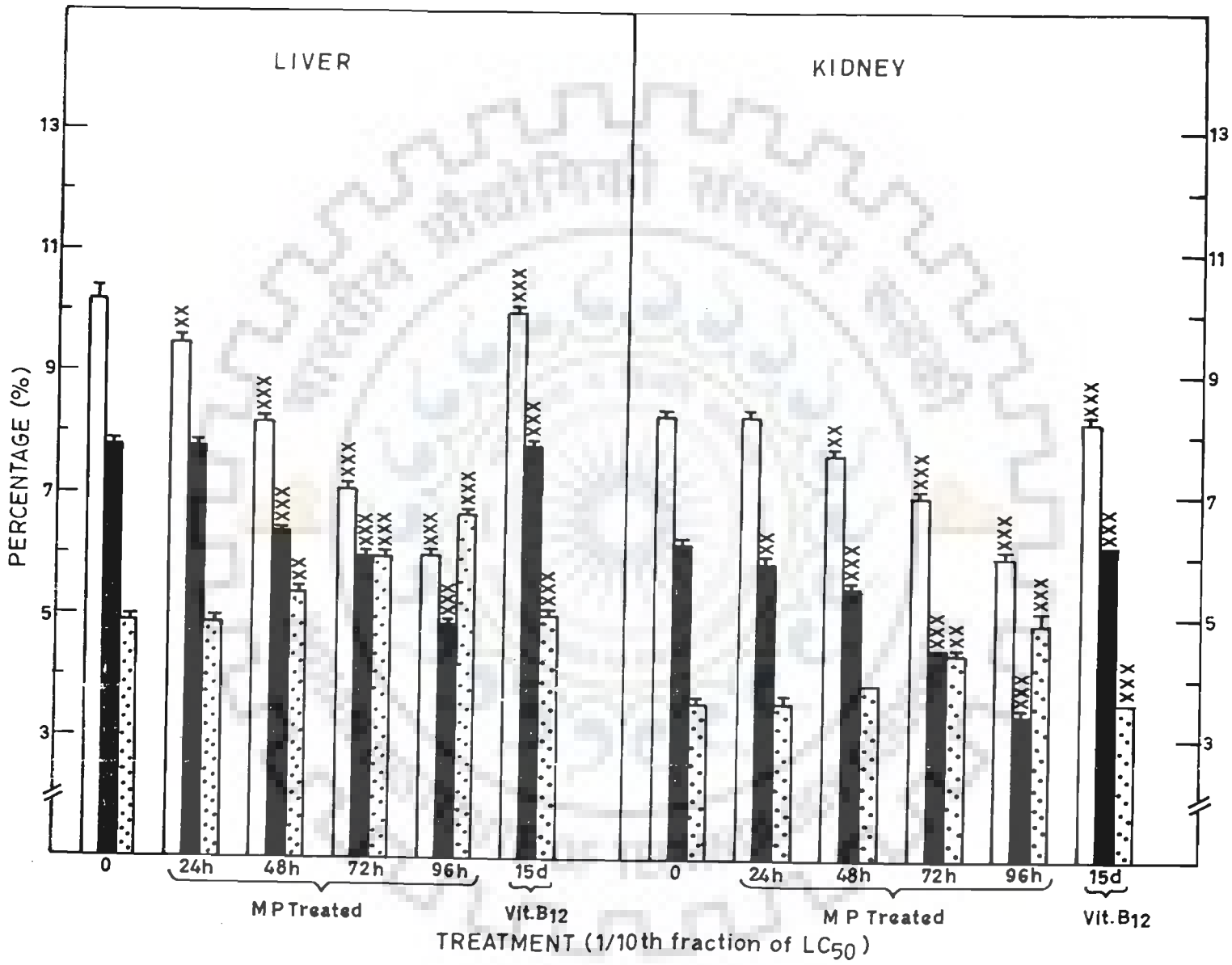
LIVER
KIDNEY
PERCENTAGE (%)
13
11
9
7
5
3
0
24h
48h
72h
96h
15d
M P Treated
Vit.B12
TREATMENT (1/10th fraction of LC50)

Fig.40

TABLE-10 : PERCENTAGE OF MOISTURE CONTENTS, TOTAL PROTEINS, LIPIDS AND CARBOHYDRATES IN THE LIVER OF CONTROL, MP TREATED AND AFTER VITAMIN B_{12} ADMINISTRATION FISHES FOR 24, 48, 72 AND 96h EXPOSURE (SUBLETHAL CONC. :1/10^{th} FRACTION OF LC_{50})

Components	Control	Treatment				
		MP Treated				Vit. B_{12}
		24h	48h	72h	96h	15d
% Moisture	74.08±0.26	74.41±0.19 (+0.44)	76.16±0.19*** (+2.81)	76.98±0.13*** (+3.91)	78.92±0.24*** (+6.53)	74.28±0.03*** (+0.27)
% Total Proteins	7.83±0.12	7.79±0.07 (-0.51)	6.38±0.05*** (-18.52)	5.98±0.09*** (-23.63)	4.93±0.06*** (-37.04)	7.80±0.08*** (-0.38)
% Total Lipids	10.21±0.17	9.46±0.08** (-7.34)	8.21±0.12*** (-19.59)	7.11±0.07*** (-30.36)	6.04±0.08*** (-40.84)	10.03±0.13*** (-1.76)
% Total Carbohydrates	4.93±0.09	4.93±0.07 (00)	5.41±0.09** (+9.74)	6.01±0.11*** (+21.91)	6.71±0.05*** (+36.11)	4.98±0.07*** (+1.01)

All values are mean ± SE, percentage alterations in parenthesis, (+)% stimulation,(-)% inhibition.

Values are significant at *P<0.05, **P<0.01, ***P<0.001 (Fisher's t-test).

TABLE-11 : PERCENTAGE OF MOISTURE CONTENTS, TOTAL PROTEINS, LIPIDS AND CARBOHYDRATES IN THE KIDNEY OF CONTROL, MP TREATED AND AFTER VITAMIN B_{12} ADMINISTRATION FISHES FOR 24, 48, 72 AND 96h EXPOSURE (SUBLETHAL CONC. :1/10th FRACTION OF LC_{50})

Components	Control	Treatment				
		MP Treated				Vit. B_{12}
		24h	48h	72h	96h	15d
% Moisture	78.54±0.24	79.36±0.19* (+1.04)	80.78±0.24*** (+2.85)	82.18±0.28*** (+4.63)	84.05±0.23*** (+7.01)	78.62±0.21*** (+0.10)
% Total Proteins	6.24±0.06	5.90±0.07** (-5.45)	5.53±0.08*** (-11.38)	4.54±0.02*** (-27.24)	3.36±0.06*** (-46.15)	6.19±0.03*** (-0.80)
% Total Lipids	8.31±0.09	8.31±0.11 (00)	7.74±0.09** (-6.86)	6.98±0.07*** (-16.00)	5.97±0.09*** (-28.16)	8.23±0.13*** (-0.96)
% Total Carbohydrates	3.58±0.12	3.58±0.09 (00)	3.92±0.02 (+9.49)	4.39±0.13** (+22.62)	4.86±0.17*** (+35.75)	3.62±0.04*** (+1.12)

All values are mean ± SE, percentage alterations in parenthesis,(+)% stimulation,(-)% inhibition. Values are significant at *P<0.05, **P<0.01 and ***P<0.001 (Fisher's t-test).

40.84% (P<0.001) in liver and 28.16% (P<0.001) in kidney after 96h treatment. Fishes protected with vit. B_{12} show that this depletion is reduced to 1.76% in liver and 0.96% in kidney.

CARBOHYDRATES 4.8.2.4

Total carbohydrate percentage increased significantly after 48, 72 and 96h exposure in liver and kidney. The maximum significant depletion was by 36.11% (P<0.001) in liver and 35.75% (P<0.001) in kidney after 96h treatment. After vit.B_{12} administration, it is found to be reduced by 1.01% in liver and 1.12% in kidney.

ENZYMES 4.8.2.5

ALKALINE PHOSPHATASE 4.8.2.5.1

AlPase activity decreased significantly after 48, 72 and 96h treatment (at P<0.001 level) in liver and in kidney after all the time intervals. The AlPase activity decreased by 20.35% (P<0.001) in liver and 19.76% (P<0.001) in kidney after 96h exposure. After vit. B_{12} administration, decrease in activity is reduced to 0.58% and 0.95% in liver and kidney, respectively.

ACID PHOSPHATASE 4.8.2.5.2

AcPase activity inhibited significantly after 48, 72 and 96h exposure in liver and in kidney at all the time intervals. Maximum inhibition is 32.18% (P<0.001) in liver and 24.19% (P<0.001) in kidney. It is reduced to 1.35% in liver and 1.15% in the kidney after vit. B_{12} administration.

TABLE-12 : ENZYMOLOGICAL ALTERATIONS IN THE LIVER OF CONTROL, MP TREATED AND AFTER VITAMIN B_{12} ADMINISTRATION FISHES FOR 24, 48, 72 AND 96h EXPOSURE (SUB-LETHAL CONC. : $1/10^{th}$ FRACTION OF LC_{50})

Enzymes	Control	Treatment				
		MP Treated				Vit. B_{12}
		24h	48h	72h	96h	15d
AlPase Bodansky unit/g	1.376±0.003	1.367±0.005 (-0.65)	1.302±0.009*** (-5.38)	1.208±0.013*** (-12.21)	1.096±0.009*** (-20.35)	1.368±0.012*** (-0.58)
AcPase Bodansky unit/g	1.038±0.012	1.013±0.003 (-2.41)	0.900±0.001*** (-13.29)	0.805±0.007*** (-22.45)	0.704±0.008*** (-32.18)	1.024±0.009*** (-1.35)
Lipase unit/g	1.804±0.021	1.879±0.009* (+4.16)	1.973±0.017*** (+9.37)	2.104±0.019*** (+16.63)	2.289±0.023*** (+26.88)	1.838±0.013*** (+1.88)

All values are mean ± SE, percentage alterations in parenthesis,(+)% stimulation,(-)% inhibition.

Values are significant at *P<0.05 and ***P<0.001 (Fisher's t-test).

TABLE-13 : ENZYMOLOGICAL ALTERATIONS IN THE KIDNEY OF CONTROL, MP TREATED AND AFTER VITAMIN B_{12} ADMINISTRATION FISHES FOR 24, 48, 72 AND 96h EXPOSURE (SUB-LETHAL CONC. : $1/10^{th}$ FRACTION OF LC_{50})

Enzymes	Control	Treatment				
		MP Treated				Vit. B_{12}
		24h	48h	72h	96h	15d
AlPase Bodansky unit/g	1.584±0.010	1.528±0.008** (-3.53)	1.473±0.013*** (-7.01)	1.377±0.017*** (-13.07)	1.271±0.011*** (-19.76)	1.569±0.021*** (-0.95)
AcPase Bodansky unit/g	1.389±0.021	1.336±0.011* (-3.81)	1.271±0.011** (-8.49)	1.184±0.010*** (-14.76)	1.053±0.009*** (-24.19)	1.373±0.014*** (-1.15)
Lipase unit/g	1.765±0.015	1.789±0.016 (+1.36)	1.876±0.006*** (+6.29)	1.903±0.021*** (+7.82)	2.098±0.018*** (+18.87)	1.772±0.007*** (+0.39)

All values are mean ± SE, percentage alterations in parenthesis,(+)% stimulation,(-)% inhibition.

Values are significant at *P<0.05, **P<0.01 and ***P<0.001 (Fisher's t-test).

LIPASE 4.8.2.5.3

A significant increase in lipase activity was recorded at all the time intervals whereas in kidney lipase activity increased significantly after 48, 72 and 96h exposure. The lipase activity increased by 26.88% ($P<0.001$) in liver and 18.87% ($P<0.001$) in kidney after 96h expoure. When fishes protected with vit. B_{12} increased in activity is reduced to 1.88% and 0.39% in liver and kidney, respectively.

Fig.41 Alterations in the activity of alkaline and acid phosphatase and lipase under the stress of methyl-parathion (MP) and vitamin B_{12}. Bars represent mean $\pm$SE (n = 5). Asterisks denote means significantly different from controls (0) at *P<0.05; **P<0.01; ***P<0.001.

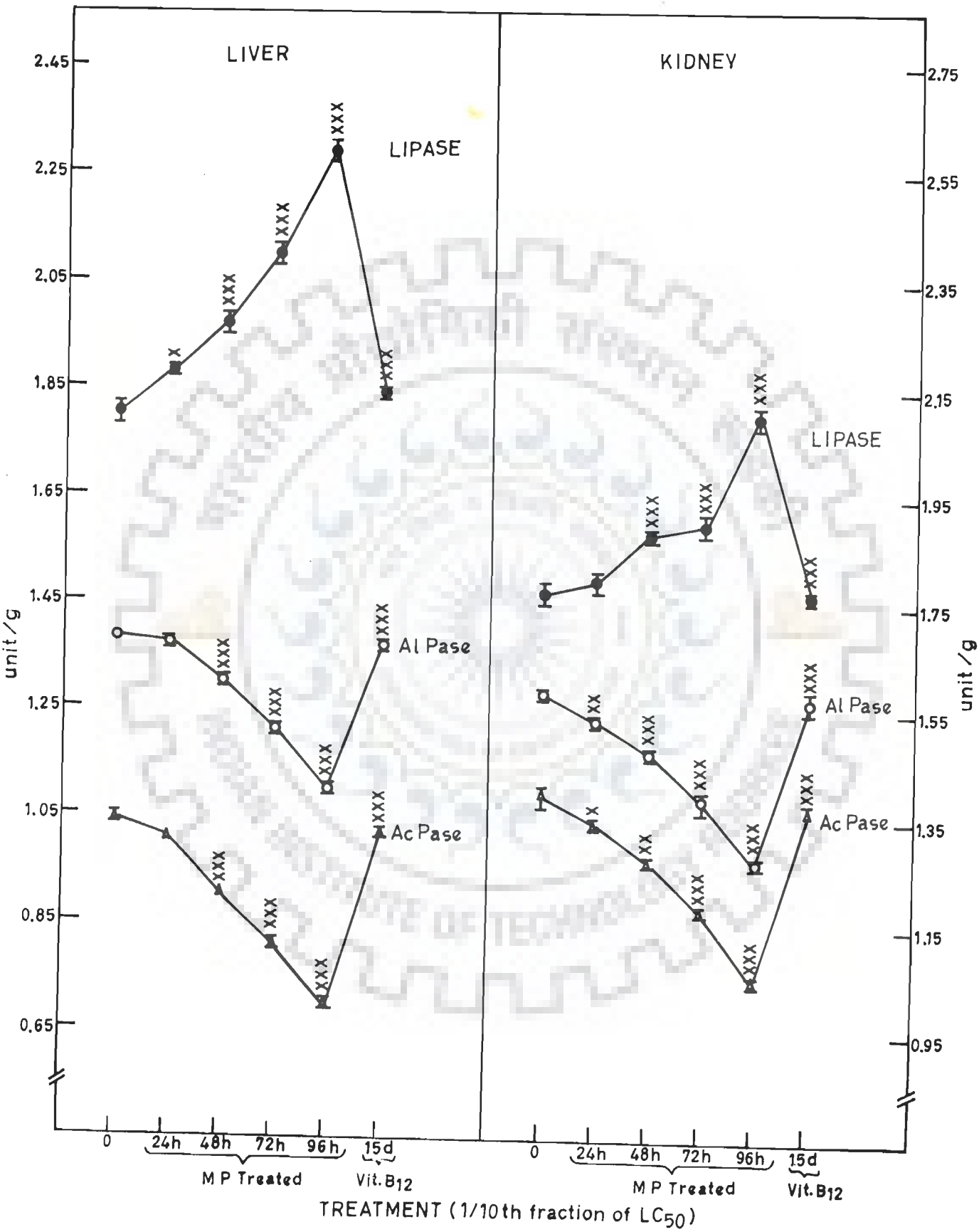
LIVER
KIDNEY
LIPASE
Al Pase
Ac Pase
unit/g
2.45
2.25
2.05
1.85
1.65
1.45
1.25
1.05
0.85
0.65
2.75
2.55
2.35
2.15
1.95
1.75
1.55
1.35
1.15
0.95
0
24h
48h
72h
96h
15d
M P Treated
Vit. B12
TREATMENT (1/10th fraction of LC50)

Fig. 41

DISCUSSION 5.0

Ours is the age of technological advancement. Man has reached height of success through the application of science and technology. But this success is also an era of a huge paradox. The greatest contribution of scientific knowledge to human health comes from the use of chemicals. The concentration of organic chemical pollutants carried in our natural water courses is increasing day by day. New chemical formulation are used for mosquito-control by various public health organizations.

With the modernisation of agricultural operations and rapid growth of industrial activity in our country, there has been a great increase in the manufacture and utilization of pesticides, fertilizers, synthetic drugs and petrochemicals etc. Thus, pesticides occupy a unique position among the many chemicals that man encounters daily. Pesticides the most important groups of environmental pollutants are delibrately excreted into the fresh water bodies like rivers, lakes, ponds and streams along with the run-off from the agricultural and forest lands. At the same time the use of these chemicals in modern land and water management has posed potential health hazards not only to livestock and wild life but also to fishes, birds, mammals and even to human beings. These contaminants enter the body of the fish through a number of routes and creates several deleterious hazards to fish.

As the conservation of these natural water courses

is of utmost importance, an understanding of the effect of these chemicals on fish and their lethal levels of concentrations becomes a pertinent topic of concern to all those intersted in safe water supply and sanitary waste disposal problems of our communities.

The studies on pesticide impact deals in general with the reaction of a living organism in an aquatic environment and according to Muirhead-Thomson (1971) the objective of such investigation is either to evaluate the impact of known pesticide against a particular undesirable fresh water animal or aquatic plant, or is concerned with the undesirable effects of a pesticide contaminating the habitat of fresh water food fish or other protected aquatic forms. The bioassays are generally used to determine the level of toxic agents that produce adverse effects to a specified percentage of the test organism in a short period of time. The most common acute toxicity is the acute mortality test and 50% mortality effect is the most reproducible measurement of the toxicity. Doudoroff et.al. (1951), Henderson (1957), Sprague (1969, 1970) and Macek et.al. (1978) have suggested the utility of bioassay methods in determining the toxicities of the industrial wastes and other chemicals in fish. Acute toxicity data for a large number of pesticides have been reported for quite a number of fish species by various authors (Anees, 1975; Verma et.al., 1977, 1978; Choudhary et.al., 1981; Singh and Sahai, 1984) using static bioassay procedure and pointed

out that it is an important tool for toxicity study. All these studies show that the effect of pesticides on mortality is dose dependent. Since toxicity bioassay is influenced by a number of factors including the technique of bioassay employed, therefore, a comparison of LC_{50} values obtained for several fish species by various authors would not be meaningful.

The results of the present test confined our study to the MP. The TLm (LC_{50} values) recorded for O.punctatus in case of MP are found to be 0.034 ml/l, 0.0056 ml/l, 0.0031 ml/l and 0.0015 ml/l at 24, 48, 72 and 96h interval respectively (Goel and Agrawal, 1989).

Behavioural, morphological, histopathological, haematological, biochemical and histochemical adversities produced in test fish, O.punctatus and its tissue viz, liver, kidney and blood were studied after acute exposure of MP. Hasler (1979) has reviewed certain aspects of chemical ecology of fish and documented the importance of chemical communication in maintaining the behaviour patterns of fish as well as other marine organisms. Todd et.al. (1967) demonstrated that the animals are sensitive to chemical signals at low concentration and may rely extensively on this sensory input to control their attitudes and behaviour. According to Muirhead-Thomson (1971), "there is increasing realization that the effect of pesticides on the reaction of fish other than the easily observable mortality effects, must be taken into account in

evaluating the complete ecological impact of a contaminating substance". The reaction of fish to various organophosphate poisoning can be in general characterized by erratic movements, the muscle tetanus, paralysis and flexed opercula. Secretion of the mucus, lightening of body colour, initial hyperexcitability, restlessness and loss of balance have also been reported by Wildish et.al. (1971) in Salmo salar in response to insecticide intoxication. The subjective observations on behavioural responses of O.punctatus to the lethal and sublethal concentrations of MP are in agreement with those of Puntius puckelli and Channa punctatus exposed to malathion (Shivaji Rao et.al., 1967; Anees, 1975) while on the same species Pandey et.al. (1976) reported decrease in the opercular frequency when exposed to malathion. Toxicity of malathion also induced abnormal behaviour in Rasbara daniconius and Puntius ticto (Singh and Sahai, 1984). Henry and Atchison (1984) observed that the normal aggresive behaviour of the blue gill decreased after MP exposure. The behavioural activity of the fish changed with numerous jerks, partial jerks, fin flicks and repeated opening and closing of mouth as concentrations were increased within 6-8h of exposure to MP in the fish, Tilapia mossambica (Pal and Konar, 1987).

Histopathology envisages histological evidences of damage and changes in the tissue caused by the toxicant. Pathological data as a whole provide sensitive indices to the toxic processes in fish poisoning. The liver of the

fish is a relatively large organ connected with several vital functions such as absorption of digestive food stuffs, detoxification, secretion of bile, excretion of detoxified and harmful substances, synthesis of several components of blood plasma, storage of glycogen, release of glucose and control over general metabolism. Though liver has no direct contact with the pollutants dissolved in the water but since it is the main detoxifying organ in the body, it is susceptible to a number of toxic and metabolic disturbances and it serves as a suitable index to the toxicity of polluted waters. Liver is an important organ of detoxification which helps in the breakdown of the xenobiotics and their metabolic products. This breakdown is carried out by endoplasmic reticulin of hepatic cells. Due to these reasons, the damage in hepatocytes are more than the cells of other organs. The histopathological changes produced in the liver of O.punctatus exposed to MP induced severe liver damage including hepatocyte vacuolation, pycnotic nuclei and damaged connective tissue. There is an acute cytoplasmic vacuolation in the liver of the fish exposed for 96h. However, other conspicuous changes observed in the liver are fibrosis, small and degenerated nuclei and liver enlargement with necrosis. Severity in pathological lesions is found to be dependent on the exposure time. Many pesticides are known to induce liver injury in animals including fish. Eller (1971) and Bhattacharya et.al. (1975) observed rupture and vacuolation in the liver cells of the

fish exposed to endrin; other conspicuous changes in the liver were liver cord disarray connective tissue damage, and enlargement of the liver cells and their nuclei. Similar changes have been observed by Mathur (1962) in the liver of Ophiocephalus punctatus exposed to BHC and lindane. Liver enlargement with necrosis, congestion, and fatty degeneration have been produced by BHC (Rudd and Genelly, 1956). Sastry and Malik (1979) observed liver cord disarray, enlargement of the nuclei, rupture of cell membrane, infiltration of phagocytes into the hepatic lobules and focal necrosis in the liver of O.punctatus exposed to sublethal concentrations of dimecron. Dubale and Shah (1979) reported a variety of liver lesions in Channa punctatus exposed to malathion. In Channa punctatus after exposure to diazinon, rupture of cell membranes and widening of the intercellular spaces were observed in the liver (Sastry and Malik, 1982). Rashatwar and Ilyas (1984) observed vacuolated hepatocytes, necrosis and damage of connective tissue in the liver of Nemachelius denisonii after exposure to phosphamidon. In Cyprinus carpio, after lethal and sublethal exposure of MP, the rupture of the cell boundaries of hepatocytes, congestion of blood vessels and sinusoids, desquamtion of bile duct epithelium and delation of bile duct were observed in the hepatopancreas by Jayasundaramma et.al. (1990).

The histopathological changes in the kidney led to the glomerular shrinkage, loss of haemopoitic tissue,

degenerated glomeruli and ruptured epithelial cells in the renal tubules when subjected to the stress of MP. However, other conspicuous changes observed in the kidney are swelling, necrosis and clumping of nuclei in renal tubules.

Other pesticides in the environment have also been found to produce severe adversities in the histological constitution of the kidney tissues. In widowtetra, Gymnocorymbus ternetzi, renal lesions appeared following exposure to agallol and thiodan (Amminikutty and Rege, 1978). Rudd and Genelly (1956) reported that DDT produced chronic nephritis in gold fish. Diazinon induced histopathological alterations in the kidney of Ophiocephalus punctatus. The most conspicuous changes were the shrinkage of glomerular network and necrosis of proximal tubules. The distal and collecting tubules were ruptured and the nuclei were degenerated and pycnotic (Sastry and Sharma,1981). Rashatwar and Ilyas (1984) reported a marked loss of haemopoitic tissue, degeneration of glomeruli and cloudy swelling of the renal tubule after exposure to phosphamidon in Nemachelius denisonii. The histopathological alterations in the kidney of P.conchonius reveal that monocrotophos is capable of inducing renal dysfunction of high order and the same might have been partly responsible for the death of the fish during acute poisoning by Kumar and Pant (1985). Gill et.al. (1988) reported hypertrophy, vacuolization, nuclear pycnosis and disruption of the absorptive surface. Swollen Bowman's spaces and collapsed glomeruli were also

encountered in P.conchonius after exposure to carbaryl and dimethoate. The disorganization of the tubular epithelial cells and the glomeruli could be due to the damage to the cells permeability barrier resulting in the leakage of the metabolites together with the vital enzymes and coenzymes. It is of imense interest to note that the renal pathologies registered by MP are more severe than those caused by other pesticides.

Blood being overall reflector of the body health is also affected by the MP. Study of the haematological parameters no doubt play an important role in the diagnosis of the disease in the fish. MP in sublethal concentration induced several significant changes in the blood picture of O.punctatus.

Haemoglobin content and RBC counts have long been a routine investigation in detecting the pathologic conditions of the animals. Hb%, PCV and RBC counts decreased significantly after 96h exposure. Significant decrease in Hb%, haematocrit value and RBC counts reflect the anaemic state of the fish. Decrease in Hb% have also been reported in the fishes exposed to malathion (Pandey et.al., 1976). Sumithion and sevin (Kundinya and Ramamurthi, 1979) and folidol (Joshi, 1982; Dabral and Chaturvedi, 1983). The decrease in the RBC number under the pesticides can be attributed to symptoms leading to hypochromic microlytic anaemia which is ascribed to iron deficiency and consequent reduction in the haemoglobin synthesis (Bhai et.al., 1971). The decrease in

RBC number may be due to the disruptive action of the pesticides on the erythropoietic tissue as a result of which the viability of the cells might be affected (Verma et.al., 1982). Mandal and Kulshrestha (1980) observed the degradation of haemopoietic tissue and loss of RBC in Clarias batrachus under the exposure of sumithion. The decreasing trend in the RBC count has also been observed in Channa punctatus exposed to phenthoate and carbaryl (Sambasiva Rao and Ramana Rao, 1986). Bhashamohideen et.al. (1988) observed a significant decrease in the RBC count in Catla catla exposed to endosulfan and MP. Other absolute values (MCV, MCH and MCHC) also altered in response to the changes in above parameters. The increase in the WBC counts in treated fish is probably due to the removal of cellular debris of necrosed tissue at quicker rate as reported by McLeay and Brown (1974) in Oncorhynchus kishush under the chemical stress. Increasing tendency in WBC counts has been reported in Clarias batrachus and Heteropneustes fossilis exposed to folidol (Joshi, 1982; Dabral and Chaturvedi, 1983) and in Channa punctatus exposed to elsan and bazanon (Chakrabarty and Banerjee, 1988). An increase in WBC counts has also been observed here in O.punctatus after exposure to MP. No significant alteration is found in the total plasma protein in all the acutely treated fish, which indicates that there is no change in the blood volume.

Blood glucose level increased significantly in O.punctatus after exposure to MP. The percentage of

stimulation increased with the duration of treatment. An increase in the blood glucose level has also been observed in the fish treated with diazinon (Sastry and Sharma, 1981). According to Gupta (1974) the hyperglycemia induced by malathion, another organophosphate might be explained in part by inhibition of cholinesterase at neuroeffector sites in the adrenal medulla leading to hypersecretion of adrenalin which stimulates the breakdown of glycogen to glucose. An increase in the blood glucose level followed by a decrease in the hepatic glycogen level was reported by Kundinya and Ramamurthi (1979) in Sarotherodon mossambicus treated with sumithion. The increased blood glucose level indicating a hyperglycemic response may be due to the mobilization of glucose from liver to blood. The elevation in blood cholesterol level may be due to the hypermetabolic state of fish or to impaired liver function which is evident from the pathological changes observed in the present study. The increase in serum urea level may be attributed to the kidney damage.

The increase in the activities of the glutamic-oxalic transaminase and glutamic-pyruvic transaminase is indicative of liver damage. According to Gupta and Paul (1978) a possible mechanism for the elevation of amino-transferase due to organophosphates may be tissue damage, particularly in the liver, kidney and heart or increased synthesis or decreased catabolism of aminotransferase. The increase in the serum alkaline phosphatase is also

indicative of liver damage. According to Gupta and Paul (1978) this increase in alkaline phosphatase may possibly be attributed to the leakage of this enzyme to the circulating blood from hepatocytes.

The physiology and the chemical make up of an organism is directly related to the environment and "in some cases even the minutest environmental change is reflected by a measurable physiological change that may influence the results of the experiments" (Klotz and Smith, 1968). The exposure of test fish to MP has led not only to the measurable, but significant alterations in the gross biochemistry of the test tissues. The proteins and lipids decreased in the liver and kidney. Moisture content and carbohydrates however, increased in all these experimental tissues. Himsworth and Glynn (1945) have also reported similar increase of fluid content causing swelling of tissue (intrinsic oedema) in the acute hepatic necrosis, and have shown the existence of an inverse relationship between the moisture and the fat content of the tissue. The present findings are in agreement with that of Hall et.al. (1926) who reported that in the fish, under stressful condition of the toxicants, water content of the body and tissues increased as it is released from the blood and the cells into the surrounding tissue fluid. This results in the increased blood concentration. Thus the water content of the experimental tissues had increased during the present investigation. Choudhary et.al. (1981) reported

increased liver water content in Heteropneustes fossilis exposed to malathion. Who concluded that the hydration of liver may be due to increased metabolic activities of this organ as it caused serious physiological disturbances. Same is the case with the fish exposed to MP. Proteins and lipids, in the form of lipoproteins form the basic unit of any cell organelle and carbohydrates are the energy stores. It is therefore, clear that the toxins induced cellular injury that has led to a significant fall in the total protein, and the lipid stored in the tested tissues. The imbalanced moisture constituents during MP intoxication suggest a definite development of tissue acidosis or alkalosis. The protein content of the liver and kidney are depleted by MP poisoning. Bano (1982) has found depletion in protein in the liver and the serum of C.batrachus exposed to aldrin. A significant fall in protein and RNA contents was observed in the liver, heart and body muscles in Channa punctatus after malathion exposure (Jyoti et.al., 1989). The depletion in the tissue proteins in the present study might have been caused due to impaired protein synthesis by liver disorders or excretion of proteins by kidney disorders (albumineria) or increased breakdown of protein into aminoacids which diffuses out of the cells.

Lipids, the reservoir of potential chemical energy are stored in the tissue, in water free state. They serve as energy depots at the time of limited nutritional availabilities or extra energy demands. This energy source, lipids

was also found to be depleted in all the liver and kidney of the experimental fish after MP intoxication. Rao and Rao (1981) have reported depleted lipids and elevated cholesterol in the methyl parathion poisoning of fish. Goel and Agrawal (1981) however, have observed an increase in the total lipids in the liver of Channa punctatus and Clarias batrachus during tannic acid exposure. The levels of total lipids and phospholipids decreased while the free fatty acids and total cholesterol levels increased under sublethal stress of methyl-parathion in Oreochronius mossambicus (Rao and Rao, 1983).

The total lipid contents progressively decreased throughout the period of investigation. These changes are statistically significant in all the tissue of the fish. Since, extra energy is needed to mitigate any stress condition. The decrease in the total lipid contents could be due to the utilization of these compounds under MP stress conditions and the decrease in the tissue lipid content found during present investigation induced glycone-ogenesis.

The toxicosis of the pesticide, MP is further reflected by the disturbances in the various metabolic processes in test fish as evidenced by the alteration in the activities of important enzymes. Phosphatases are hydrolytic enzymes capable of spliting off phosphate, from organic phosphate esters at different pH. As pointed out by de Duve (1959) hydrolytic enzymes are concentrated in a

small subcellular fraction which sediments between the mitocondrial and microsomal fraction. These hydrolytic enzymes are presumed to be enclosed within a lipoprotein membrane, the lysosomes. Alkaline and acid phosphatase are the lysosomal enzymes. Delamirande et.al. (1967) found these enzymes bound to the lysosomes, endoplasmic reticulum, mitochondria, nuclei and cytoplasm.

The decrease in the AlPase and AcPase activity was noted in the liver and kidney of MP intoxicated fish. The biochemical alterations of AlPase and AcPase in response to MP treatment are also confirmed by the histochemical studies.

AlPase is a brush border enzyme, also found in microsomes, and splits various phosphate esters at an alkaline pH (transphosphorylation) and mediates membrane transport (Gold Fischer et.al., 1964) and relative activity of an enzyme in the tissue under chemical stress. The significant decrease in the activity of AlPase observed after exposure to MP may be due to decrease in the rate of transphosphorylation. Pearse (1968) described that AlPase activity is concerned with the transfer of phosphate and a high activity indicates increased phosphate transfer rather than hydrolysis of phosphate ester. Galdhar et.al. (1978) reported inhibition in this enzyme activity in rats, produced by various insecticides. Significant decrease in the activity of this enzyme in the liver of Ophiocephalus due to exposure to dimecron for 20 days has been reported

by Sastry and Malik (1979) and in fishes treated with endrin (Sastry and Sharma, 1979). The significant fall in the activity of AlPase observed after exposure to diazinon (Sastry and Malik, 1982), thiotox and malathion (Verma et.al., 1982) and phosphamidon (Rashatwar and Ilyas, 1984) may be due to decrease in the rate of transphosphorylation.

Acid phosphatase is a lysosomal enzyme which hydrolyses the ester linkage of phosphate esters and contributes to autolysis of cell after death. Rees and Sinha (1960) believe that the damaged organ might produce an augmented quantity of enzymes. According to Novikoff (1961) and de Duve (1968) the increased lysosomal activity occurs as a part of prenecrotic changes. Increase in the AcPase activity may be due to the necrotic changes occuring in the different parts of the digestive system of Hetero-pneustes (Sastry and Malik, 1982) after exposure tc diazinon and inhibition in AcPase activity was observed in Sacco-branchus fossilis after exposure to the pesticides (Verma et.al., 1982). Inhibition in AcPase activity is observed after MP intoxication.

Lipase is a microsomal enzyme of wide occurence in the liver than in the kidney and hydrolyses emulsified triglycerides of the long chain fatty acids (lipids) into fatty acid and glycerol. The action of lipase is greatly activated by bile salts and bile acids which emulsify fats to minute droplets thus poisoning large surface area for lipolysis. Gomori (1946) emphasized that a decrease in the

lipase activity of liver is invariably followed by the increased lipids. Zimmerman (1976) discussed that liver injury as well as other cellular abnormality is responsible for the lipolytic alteration. Garg (1981) correlated an interrelationship between lipase activity and histological degenerative changes. The lipase activity was inhibited in the fish exposed to diazinon (Sastry and Malik, 1982). The enhanced lipase activity, therefore, might be due to strong toxic effect of MP. The present findings reveal that an interrelationship exists between the increased lipase activity and histological degenerative changes in the liver and kidney.

Thus many identificable morphological, behavioural, pathological and biochemical alterations are produced in the test fish, O.punctatus exposed to MP. Exposure to this toxicant leads the animal to dearranged conditions. This results in a high degree of histological disarchitecture in the liver and kidney. Blood profiles also reflected, the dysfunctioning and the altered biochemistry of test tissues. Apparently, MP at even low concentration is harmful to fish as it spills into water quite frequently which may reduce the fish yield and hamper normal production of fish seed. Such sublethal studies are important as even small physiological disturbances might reduce the chances of the animal being successful in its environment. It must also be emphasised that elimination of aquatic animals by small insidious physiological or behavioural changes can be

regarded as more serious than a massive fish kill, since it is less likely to be observed and corrected.

Finally, it may be emphasized that with the growing industrialization in our country, it is essential to collect large amounts of such data on a variety of pollutants, so that the same can be usefully employed for setting up water quality as standards. The toxic effect of MP is more severe after 96h interval. With a view to determine the antitoxicant effect of vit. B_{12}, it was injected in the MP intoxicated fish intramuscularly. Administration of vit. B_{12}, shows loss of excessive secretion and deposition of the mucus on the body surface and normal behaviour in swimming. Loss of lesions, like hepatocytic necrosis and vacuolation by vit. B_{12} are evidenced by histopathological examination. Normal hepatocytes was observed after vit. B_{12} injection in liver. In the kidney, no necrosis was observed after vit. B_{12} administration. A regular distribution of reticulin fibres was noticed in the liver while in the kidney, the intertubular accumulation of reticulin fibres was observed after vit. B_{12} administration. No topographical differences in the distribution of collagen fibres was observed both in the liver and kidney but thin collagen fibres were found around the central vein and blood vessels both in the liver and kidney. Administration of vit. B_{12} cured anaemia, hyperglycemia and hypercholestremia induced by MP. MP induced glyconeogenesis (increase total lipids) in tissues is prevented by the

administration of vit. B_{12} (formation of lipids from carbohydrate is influenced by vit. B_{12}). Vitamin B_{12} plays a curative role against MP toxicity. Fox and Fry (1970) reported that the administration of ascorbic acid, completely prevented the anaemia produced by the intoxication of Cd and improved the growth rate of the Japanese quail. The complex relationship of vit. C to the enzymes and drugs in the liver function has been reviewed and limited protective action against a major group of pesticides was reported by Street and Chadwick (1975). Agrawal et.al. (1978) observed the protective role of ascorbic acid by studying haematological parameters of a fish, Channa punctatus exposed to organochlorine pesticide, aldrin. Limited protective action against a major group of pesticides has been reported. There is no report on the antitoxicant effect of vit. B_{12} against OP toxicity in fishes. An attempt has been made to see the role of vit.B_{12} against MP toxicity. It can be concluded that vit. B_{12} reduces the toxicity of this pesticide.

The results of this investigation might be helpful in taking immediate remedial measures for pesticide toxicity.

SUMMARY AND CONCLUSIONS 6.0

Water pollution poses a continuous dilemma for the public. Its effects are world wide and well recorded. The coastal waters, rivers, reservoirs and other types of waters have been affected by the pollutants because during the course of daily living, many materials find their way into the water. These include not only domestic wastes but also run off from ground surfaces and industrial wastes. Remarkable increase in the industrial technology along with the explosive advance in the development of pesticides, provides an everlasting source of contaminants in the aquatic ecosystem. The chemicals or such products when enter into the water course either on direct application or as a component of effluent discharge alien to water ecosystem create the risk to the biota of the water ecosystem. These components may be of different types viz, trace metals, pesticides etc. The industrial pollutants discharged in the inland water bodies not only change the quality of water but also adversely affect the inhabiting flora and fauna including fish. With the increase in the world population, fish is one of the best source of rich diet for the human health and it is to be protected from the adverse effects of such pollutants. These pesticides directly or indirectly effect the human population.

In a developing country like India, the increase in the food production through agricultural revolution is essential and the use of pesticides is undertaken on a

large scale, to achieve this. As they are valuable in agriculture (a) for the protection of crops, (b) in the public health, against the control of disease vectors, (c) as protection of material and to prevent attack by insects, (d) in the industry, for the control of hazardous vegetations and fumigation of ships and ware houses, (e) in domestic use, as house hold and garden spray and control of ectoparasites for the pet animals, and (f) as an application to clothings, skin and for the control of ectoparasites. Nevertheless, we can not afford to avoid their use. Hence, we have to look to some other sources which may minimise the harmful effect of the pesticides. With a view to determine the antitoxicant effect of vit. B_{12}, it was injected in the intoxicated fish intramuscularly. It has been found that it plays a curative role against toxicant and may be used to protect biota against MP toxicity. MP is more effective than parathion and cause desecration to aquatic ecosystem and adversely affects the various metabolic activities and physiological responses of the fresh water fish, with the result that a large number of palatable fish are destroyed. The present venture aims to envisage the toxic effects of an OP pesticide, MP and antitoxic effect of vit. B_{12} on a fresh water fish, O.punctatus which is commonly used as food.

With this aim, an attempt has been made to cover the behavioural, morphological, biochemical and other

toxicological effects of MP on O.punctatus. The living, healthy specimens of O.punctatus, irrespective of sex were collected from local fresh water sources, measuring about 15 to 18 cm in length and 40-50 gm in weight. They were acclimatized to the laboratory conditions for about 7 days at natural photoperiod and temperature. During this period, the fish were fed with commercial fish food and pieces of earthworms and freshly killed insects etc.

Bioassays tests were conducted to evaluate the lethality of the toxicant to the model test fish. The TLm values (LC_{50} values) for 24, 48, 72 and 96h were found to be 0.034 ml/l, 0.0056 ml/l, 0.0031 ml/l and 0.0015 ml/l of MP. Two sublethal concentrations of MP i.e., $1/5^{th}$ and $1/10^{th}$ fractions of LC_{50} for acute exposure were selected. Behavioural, morphological, histopathological, histoenzymological, haematological and biochemical studies were made to study the effects produced by this toxicant (MP) in liver, kidney and blood of O.punctatus.

The observations were recorded after the intervals of 24, 48, 72 and 96h respectively. Control experiments were also set under identical conditions. Morphological and behavioural changes of the toxicant poisoning during acute toxicity experiments include excessive secretion of mucus on the general body surface. The colour of the body of the treated fish became very dull greenish grey or somewhat lighter in comparison to dark green colour of the control fish. Extreme signs of restlessness, muscle spasm, body

torsion and coughing coming out of secretion from mouth was observed. In the treated fish, some wounds and blood clots were often seen on the sides of the mouth or head region. Behavioural response of the fish varied in accordance to the test concentrations of the toxicants. It showed highly excitable conditions, occasionally uncoordinated swimming movements and the fish made frequent visits to the surface of the water to gulp atmospheric air. It also showed relatively reduced respiratory activity during the early hours of exposure to MP, as evidenced by decreased opercular movement. However, this period was followed by hyperexcitability as revealed by increased of erratic and jerkey body movements. Difficulty in the respiration (respiratory stress) increased during the stressful condition. Fish were characterized by highly agitated movements, suggesting the impairment of balancing capacity. Subsequently, the fish became progressively lethargic.

Quantitative estimation of MP in the tissues (liver and kidney) were done by HPLC. The concentration of the MP absorbed in the tissue, increased as the time duration increased. After 96h exposure, MP is completely absorbed by the tissues (liver, kidney). The main purpose of this study is to see, how much quantity is absorbed by the tissue after pesticide treatment and to confirm that the abnormalities in fish is only due to the presence of MP in the tissues.

Histopathologically, the liver exposed to MP

induced severe liver damage including hepatocyte vacuolation, pycnotic nuclei and damaged connective tissue. Mild vacuolation in the cytoplasm of hepatocytes also takes place. A considerable degree of pathological changes occur in the kidney of MP exposed fish. Initial stages of necrosis, swelling, clumping of nuclei in the renal tubules and loss of haemopoitic tissue were seen during acute exposure. Glomerular and tubular shrinkage also takes place. Haemopoitic tissue becomes loose due to the damage of the connective tissue. The renal tubules of the kidney are most affected.

Under the stress of the toxicant, conspicuous alterations were found in the blood. Further, haematological investigation revealed, decrease in the Hb%, PCV and RBC counts reflect the anaemic state of the fish, causing hypochromic microlytic anaemia which is caused due to iron deficiency. Other absolute values (MCV, MCH and MCHC) also altered in response to the changes in the above parameters. Increase in the WBC counts was also noted after the MP exposure to fish. In all the acutely treated fish, no significant alteration is found in the total plasma protein, which indicates that there is no change in the blood volume.

Hyperglycemic condition also appears in the experimental fish due to the depletion in tissue glycogen which results in the elevation of the blood sugar. The decrease in the tissue lipids is associated with

hypercholestremia in the blood of the experimental fish. The increase in the serum urea level may be attributed to the kidney damage.

The enzymological alterations of the experimental tissue revealed increase in AlPase and decrease in AcPase activity of phosphatases. Among transaminases, both GOT and GPT increase steadily with the exposure time of the toxicant, in all the experimental tissues. This increase in the transaminases indicates the liver malfunctioning.

Biochemical analysis included the estimation of some important organic (total proteins, lipids and carbohydrates) and inorganic (moisture) constituents and enzymes (AlPase, AcPase and lipase) in the liver and kidney of the experimental fish in comparison to the controls. A significant decrease of the organic constituents (proteins and lipids) in both the tissues was observed under the stress of the toxicant. The changes depend on the exposure time. The tissue protein depleted maximally in the kidney than in the liver after 96h intoxication of MP. Depletion in protein was by 47.27% and 46.15% at both the sublethal concentrations. Lipids of liver depleted by 42.31% and 40.84% at both the sublethal concentrations of 96h exposure. Similarly, there was depletion in the lipid content of the kidney and the maximum decrease of 38.51% and 28.16% at both the sublethal concentrations after 96h exposure.

The depletion in the tissue (liver, kidney)

proteins is caused due to impaired protein synthesis by liver disorders or excretion of proteins by the kidney disorders (albuminaria) or increased breakdown of protein into aminoacids which diffuses out of the cells. Lipids was also depleted in both liver and kidney of the experimental fish. The decrease in the tissue lipids is associated with the hypercholestremia in the tissues and indicated to induce glyconeogenesis. Moisture content and carbohydrates however, increased in all the experimental tissues.

The enzymological alterations of the experimental tissues revealed significant decreased activity of the phosphatases both in the liver and the kidney of experimental fish. A significant increase in the lipase activity was recorded at all the time intervals both in the liver and kidney. The biochemical alterations in AlPase, AcPase and lipase activity were parallel to histochemical results in all the tested tissues.

It can thus be concluded that MP is lethal to O.punctatus. Under the sublethal concentrations of MP, different morphological, pathological, physiological and other biochemical characteristics of the blood and the whole fish body are affected adversely. Whatsoever, may be the mode of toxic effects, the results show that, an OP pesticide, MP is toxic to the fish population. After 96h interval, the effect of MP is more severe. Vit. B_{12} was injected in the intoxicated fish (after 96h exposure)

intramuscularly on each alternate day for two weeks. Administration of vit. B_{12}, shows loss of excessive secretion and deposition of the mucus on the body surface, loss of lesions like hepatocytic necrosis, vacuolation in the liver and no necrosis was observed in the kidney after vit. B_{12} injection. After vit. B_{12} administration, a regular distribution of reticulin fibres in the liver and intertubular accumulation in the kidney was observed. No topographical differences in the distribution of collagen fibres was observed both in the liver and the kidney. Administration of vit. B_{12} cured anaemia, hyperglycemia and hypercholestremia induced by MP. MP induced glyconeogenesis is prevented by vit. B_{12} administration. It plays a curative role against MP toxicity. The findings might be able to suggest some remedial measures against pesticide toxicity.

REFERENCES 7.0

1. Agrawal, N.K., Juneja, C.J. and Mahajan, C.L. : Protective role of ascorbic acid on haematological parameters of a fish, Channa punctatus exposed to organochlorine pesticide, aldrin.
   Toxicology 19, 369, 1978.

2. Amminikutty, C.K. and Rege, M.S. : Acute and chronic effects of thiodan 35 EC and agallol 3 on kidney, stomach, and intestine of the widowtetra, Gymnocorymbus ternetzi Boulenger.
   Ind. J. Exp. Biol. 16, 202 - 205, 1978.

3. Anees, M.A. : Hepatic pathology in a fresh water teleost, Channa punctatus (Bloch.) exposed to sublethal and chronic levels of three organophosphorus insecticides.
   Bull. Environ. Contam. Toxicol. 19, 524-527, 1978.

4. Anees, M.A. : Haematological abnormalities in a fresh water teleost, Channa punctatus exposed to sublethal and chronic levels of three organophosphorus insecticides.
   Int. J. Ecol. Environ. 4, 53, 1978.

5. Anees, M.A. : Acute toxicity of four organophosphorus insecticides to a fresh water teleost, Channa punctatus (Bloch.)
   Pakistan J. Zool. 7, 135, 1975.

6. APHA, AWWA and WPCF: Standard method, for water and waste water analysis 1976.

7. APHA : Standard methods for examination of water and waste water (15ed.). American Public Health Association, Washington D.C. 1980.

8. Arora, H.C., Sharma, V.P. and Chattopadhya, S.N.: Bioassays studies of some commercial organic insecticides. Trials of malathion with exotic and indigenous carps.
Indian J. Environ. Hlth. 13, 4, 1971 a.

9. Arora, H.C., Shrivastava, S.K. and Seth, A.K. : Bioassays studies of some commercial organic insecticides. Studies with an exotic carp, Puntius sophore (Ham.).
Indian J. Environ. Hlth. 13, 3, 1971 b.

10. Bano, Y. : Effects of aldrin on serum and liver constituents of fresh water catfish, Clarias batrachus L.
Proc. Ind. Acad. Sci. (Animal Science) 91 (1), 27 - 32, 1982.

11. Bansal, S.K. : Toxicological studies with few organic biocides to Labeo rohita and Saccobranchus fossilis and their application for controlling water pollution. Ph.D. Thesis, Meerut University, Meerut, 1979.

12. Bashamohideen, Md., Suneetha, N. And Mallareddy, P.: Impact of endosulfan and methyl parathion on some selected parameters of oxidative matabolism in the fish, Catla catla.
Biol. Mem. 14(1), 43-47, 1988.

13. Bergmeyer, H.U. : "Methods of enzymatic analysis". Vol. III Verlag Chemie, New York, 1974.

14. Bhai, I., Nath, N. and Nath M.C. : Haematological changes in rats injected with acetoacetate (35949). Proc. Soc. Exp. Biol. Med. 138, 597-599, 1971.

15. Bhattacharya, S., Mukherjee, S. and Bhattacharya, S. : Toxic effects of endrin on hepatopancreas of the teleost fish, Clarias batrachus (Linn). Indian J. Exp. Biol. 13, 185, 1975.

16. Bier, M. : In : Methods in Enzymology. Vol II (Colowick, S.P. and Kaplan, N.O. Eds). Academic Press, New York, 1955.

17. Bodansky, A. : In : Hawk's physiological Chemistry (Oser, B.L., Ed) Tata McGraw Hill, Bombay, 1933.

18. Bushway, R.J., Brian Perkins, L. and King, J.M. : Simultaneous determination of diazinon and chlorpyrifos pesticide formulations by liquid chromatography. J. Assoc. Off. Anal. Chem. 71(2), 321-322, 1988.

19. Carbas, P., Meloni, M. and Pirisi, F.M. : Separation of insecticides by reversed-phase high performance liquid chromatography. Journal of Chromatography. 176, 473-477, 1979.

20. Chakrabarty, P. and Banerjee, V. : Effect of sublethal toxicity of three organophosphorus pesticides

on the peripheral hemogram of the fish, Channa punctatus.
Environ. & Ecol. 6, 151-158, 1987.

21. Chakrabarty, P. and Banerjee, V. : Effect of sub-lethal toxicity of three organophosphorus pesticides on the peripheral hemogram of the fish, Channa punctatus.
Environ. & Ecol. 6(1), 151-158, 1988.

22. Chakrabarty, P. and Banerjee, V. : Effect of organo-phosphorus pesticides on the peripheral hemogram of the fish, Channa punctatus.
Environ. & Ecol. 6(2), 390-394, 1988.

23. Chakraborty, P.S. and Banerjee, S. : Methyl parathion as a fish toxicant for eradication of trash fishes from fresh water pond.
Environ. & Ecol.6, 146-150, 1988.

24. Chakraborty, P.S., Mallik, A., Dingal, D.K. and Banerjee, S. : Effect of methyl-parathion on brain and olfactory organ acetylcholinesterase activity of the fish, Heteropneustes fossilis.
Environ. & Ecol. 7(2), 310-314, 1989.

25. Chambers, J.E. and Yarbrough, J.D. : Parathion and methyl-parathion toxicity to insecticide resistant and susceptible mosquito fish (Gambusia affinis).
Bull. Environ. Contam. Toxicol. 11(4), 315-320, 1974.

26. Chambers, J.E. : The tolerance of the toxicity of organophosphates such as malathion.
Pest. Biochem. Physiol. 6, 577, 1976.

27. Choudhary, B.P., Pandey, P.K. and Dubey, N.K. : Effect of malathion on the behaviour and body composition of Heteropneustes fossilis.
J. Environ. Biol. 2(1), 21-36, 1981.

28. Choudhuri, D.K., Sadhu, A.K. and Mukhopadhyay, P.K. : Toxicity of two organophosphorus insecticides malathion and phosphamidon to the fish, Channa striatus.
Environ. & Ecol. 2, 143-147, 1984.

29. Colowick, S.P. and Kaplan, M.O. : Methods in Enzymology. Vol. III, Academic Press, Inc. New York, 303, 1963.

30. Coppage, D.L. and Braidech, T.E. : River pollution by anticholinesterase agents.
Water Res. 10, 19, 1976.

31. Couch, J.A. : Histopathological effects of pesticides and related chemicals on the liver of fishes. In : Ribelin WE, Magaki G (eds) Pathology of Fishes, University of Wisconsin Press, Madison, 1975.

32. Dabral, R. and Chaturvedi, L.D. : Folidol induced changes in some hematologic values of Heteropneustes fossilis at low temperature.
J. Adv. Zool. 4, 92-96, 1983.

33. Dacie, J.V. and Lewis, S.M. : Practical hematology, 5th edition. Churchill Livingstone, Edinburg, UK, 1975.

34. Dalela, R.C., Verma, S.R. and Bhatnagar, M.C. : Biocides in relation to water pollution. Part I : Bioassays studies on the effects of a few biocides on fresh water fish, Channa gachua.
Acta. Hydrochim. Hydrobiol. 6, 15-25, 1978.

35. de Duve : The function of intracellular hydrolases.
Exptl. Cell. Research Suppl. 7, 169-182, 1959.

36. de Duve, C. : [Scient, Am. 208, 64 (1968)] : In Pearse, A.G.E. Histochemistry, Theoretical and Applied Vol. I. Churchill Livingstone, London, 1975.

37. Delamirande, G., Morais, R. and Blackstein, M. : Intracellular distribution of 5' ribonuclease and 5' phosphodiesterase in rat liver.
Arch. Biochem. Biophys. 118, 347, 1967.

38. DePass, L.R. : Alternative approaches in median lethality (LD_{50}) and acute toxicity testing.
Toxlet. 159-170, 1989.

39. Desai, A.K., Joshi, U.M. and Ambadkar, P.M. : Histological observations on the liver of Tilapia mossambica after exposure to monocrotophos, an organophosphorus insecticide.
Toxicology Letters. 21, 325-331, 1984.

40. Doudoroff, P., Anderson, B.G., Burdick, G.E., Galtsoff, P.S., Hart, W.B., Patrick, R., Strong, E.R., Surber, E.W. and Van Horn, W.M. : Bioassay methods for the evaluation of acute toxicity of industrial wastes to fish.
Sewage Ind. Wastes. 23, 130-139, 1951.

41. Dubale, M.S. and Shah, P. : Histopathological lesions induced by malathion in the liver of Channa punctatus.
Indian J. Exp. Biol. 17, 693-697, 1979.

42. Edwards, C.A. (Ed.) : Environmental pollution by pesticides, Plenum, London, 1973.

43. Eller, L.L. : Histopathologic lesions in cutthroat trout, Salmo clarki exposed chronically to the insecticide endrin.
Amer. J. Pathol. 64, 321, 1971.

44. Eymard, P.M., Reikert, D. and Rice, J. : Influence of vitamin C and B_{12} on the survival rate of mice bearing ascites tumor.
Expl. Cell Biol. 50, 88-91, 1982.

45. Fisher, R.A. : "Statistical methods for research workers" 11 ed. Oliver and Boyd. London, 1950.

46. Fox, R.M.S. and Fry, B.E. : Cadmium toxicity decreased by dietary ascorbic acid.
Science. 169, 1970.

47. Frank-Consolazio, C. and Iacono, J.M. : In : Newer methods of nutritional biochemistry. (Albanese, A.A., Ed.), Academic Press, New York, 1963.

48. Galdhar, N.R., Fawde, M.M., Halde, U.K. and Pawar, S.S. : Acid and alkaline phosphatase activities during pesticide intoxication.
Indian J. Biochem. Biophys. 15(2), 78, 1978.

49. Garg, V. : Histopathological and biochemical investigations on a few tissues liver, kidney and blood of Channa punctatus under toxication with bismark brown azodye. Ph.D. Thesis, Meerut University, Meerut, 1981.

50. Gill, T.S., Pant, J.C. and Pant, S. : Gill, liver and kidney lesions associated with experimental exposures to carbaryl and dimethoate in the fish (Puntius conchonius Ham.)
Bull. Environ. Contam. Toxicol. 41, 71-78, 1988.

51. Goel, B.B. and Agrawal, V.P. : Tannic acid induced biochemical changes in the liver of two teleost fishes, Clarias batrachus and Ophiocephalus punctatus.
Ecotoxicology and Environmental Safety. 5, 418-423, 1981.

52. Goel, S. and Agrawal, V.P. : Effect of methyl-parathion on the mortality and behaviour of a fresh water teleost, Ophiocephalus (Channa) punctatus.
Ad. Bios. 8 (II), 191-196, 1989.

53. Goldfisher, S., Esser, E. and Novikoff, A.B. : J. Histochem. Cytochem., 12, 72-95, 1964. In :Hinton, D.E., Kendall, M.W. and Silver, B.B. : Use of histological and histochemical assessment in the prognosis of the effects of aquatic pollutants. Amer. Soc. Test. and Mat. 528, 194-208, 1973.

54. Gomori, G. : Proc. Soc. Exp. Biol. and Med., 58, 362, 1946. (not consulted in original)

55. Gomori, G. : "Microscopic histochemistry", Univ. Chicago Press, Chicago, Illinois, 1952.

56. Gorden, H. and Sweets, H.H. Jr. : In : Carleton's Histological Technique, Oxford, Univ. Press, Toronto, 1936.

57. Gupta, A.K. : Pollutants toxicity on a few fresh water fishes, Clarias batrachus and Cirrhina mrigala with special reference to hematology. Ph.D. thesis submitted to Meerut University, Meerut, 1980.

58. Gupta, P.K. : Malathion induced biochemical changes in rats.
Acta. Pharmacol. Toxicol. 35, 191-194, 1974.

59. Gupta, P.K. : Toxicology of Pesticides : A review of the problem.
Proc. Sem. Eff. Pest. Aq. Fau. 19-36, 1984.

60. Gupta, P.K. : Environmental pollution by pesticides. In pesticides in the Indian environment. Interprint, New Delhi, India, 83-105, 1986.

61. Gupta, R.G. and Paul, B.S. : Influence of malathion on body enzymes in dermal subacute toxicity studies in Bubalus bubalus species.
Bull. Environ. Contam. Toxicol. 20, 811-818, 1978.

62. Hall, F.G., Gray, I.E. and Lepkovsky, S. : The influence of asphyxiation. The blood constituents of certain marine fishes.
J. Biol. Chem. 67, 549-554, 1926.

63. Hasler, A.D. : Chemical ecology of fish, 1979. In : Chemical Ecology, Eds. E. Sondheimer and J.B. Simeone, Academic Press, New York and London.

64. Hayes, W.J. Jr. : Toxicology of Pesticides, The Williams and Wilkins Co., Baltimore, 1975.

65. Henderson, C. : Application factors to be applied to bioassays for the safe disposal of toxic wastes, 1957. In : Biological problems in water pollution 31-37, U.S. Deptt. of Hlth. Education and Welfare, Public Health Service Washington, D.C.

66. Henry, M.G. and Atchison, G.J. : Behavioral effects of methyl parathion on social groups of blue gill (Lepomis macrochirus).
Environ. Toxicol. Chem. 3, 399-408, 1984.

67. Himsworth, H.P. and Glynn, L.E. : The gross chemical changes in the liver in dietic necrosis.
Proc. Roy. Soc. Med. 39, 267-271, 1945.

68. Holden, A.V. : Effect of pesticides on fish, 1973 : In C.A. Edwards, editor. Environmental pollution by pesticides. Plenum Press, New York, USA.

69. Jayasundaramma, B. Nagaratnamma, R. and Ramamurthi, R. : Induced histopathological lesions in the hepatopancreas of the fresh water teleost, Cyprinus carpio exposed to methyl-parathion.
Ad. Bios. 9(II), 75-81, 1990.

70. Johnson, D.W. : Pesticides and Fishes - a review of selected literature.
Trans. Am. Fish. Soc. 97, 398-424, 1968.

71. Joshi, B.D., Chaturvedi, L.D. and Dabral, R. : Changes in the blood cell composition of a fresh Heteropneustes fossilis, following its exposure to folidol water at low temperature.
Life & Toxic Environ. 86-91, 1979.

72. Joshi, B.D. : Changes in some hematological values of Clarias batrachus following folidol treatment at 25 C.
Ind. J. Zool. 23, 93-98, 1982.

73. Jyoti, A., Khan, A.A., Haque, N. Fatima, M. and Barbhuyan, S.I. : Studies on the effect of malathion on a fresh water fish, Channa punctatus (Bloch).
J. Environ. Biol. 10(3), 251-257, 1989.

74. Kabeer, A.S. and Ramana Rao, K.V. : Toxicity of malathion to the fresh water Tilapia mossambica.
Bull. Environ. Contam. Toxicol. 24, 870-874, 1980.

75. Katz, M., Legore, R.S., Weitkamp, K., Cumin, J.M., Anderson, D. and May, D.R. : Water pollution. Effects on fresh water fish.
J. Water Pollut. Cont. Fed. 44, 1226, 1972.

76. King, E.J. : Micro-analysis in Medical biochemistry, Churchill, London, 1946.

77. Klotz, G.M. and Smith, L.S. : Methods of using fish as biological research subjects in method of Animal experimentation III ed. (Ed. Gay, W.I.), Academic Press, N.Y., 323-385, 1968.

78. Konar, S.K. : Lethal effects of the insecticide DDVP on the eggs and hatchlings of the snake head, Channa punctatus (Bl),
Jap. J. Ichthyol, 15, 130-133,1969c.

79. Konar, S.K. : Laboratory studies on two organo-phosphorus insecticides, DDVP and phosphamidon, as selective toxicants.
Trans. Amer. Fish. Soc. 98, 430-437, 1969d.

80. Konar, S.K. : Pesticides and aquatic ecosystem.
Indian J. Fish. 22, 80-85, 1975.

81. Konar, S.K. and Ghosh, T.K. : Safe disposal of the insecticide sevidol to protect fish, plankton and worm.
J. Environ. Biol. 1(1), 15-24, 1980.

82. Koundinya, P.R. and Ramamurthy, R. : Effect of sumithion on some selected enzyme systems on the

fish, Tilapia mossambica (Peters).
Indian J. Exp. Biol. 16(7), 809, 1978.

83. Koundinya, P.R. and Ramamurthy, R. : Toxicity of sumithion and sevin to fresh water fish, Sarotherodon mossambicus.
Current Sci. 49, 875-876, 1980.

84. Kumar, R. : Simultaneous determination of some organophosphorus pesticides by high performance liquid chromatography.
Biomedical Chromatography, 3(5), 1989.

85. Kumar, S. and Pant, S.C. : Renal pathology in fish (Puntius conchonius Ham.) following exposure to acutely lethal and sublethal concentrations of monocrotophos.
Bull. Environ. Contam. Toxicol. 35, 228-233, 1985.

86. Kundinya, R.P. and Rammurthi, R. : Hematological studies in Sarotherodon mossambica (Peters) exposed to lethal ($LC_{50/48}$ hours) concentration of sumithion and sevin.
Curr. Sci. 48, 877, 1979.

87. Lewis, W.M. : Maintaining fishes for experimental and instructional purposes. Southern Illinois Univ. Press, Illinois, 1963.

88. Lowry, O.H., Rosebrough, N.J., Farr, A.I. and Randall, R.J. : Protein measurement with Folin phenol reagent.
J. Biol. Chem., 193, 265-275,1951.

89. Macek, K.J., Birge, W., Mayer, F.L., Buikema, A.L. Jr. and Maki, A.W. : Discussion, session synopsis of the use of aquatic toxicity tests for evaluation of the effects of toxic substances to aquatic life (Ed. by Cairns, J. Jr., Dickson, K.L. and Maki, A.W.) pp. 27-32, 1978, American society for testing and materials. Philadelphia, P.A.

90. Mandal, P.K. and Kulshrestha, A.K. : Histopathological changes induced by the sublethal concnetration of sumithion in Clarias batrachus. Indian J. Exp. Biol., 18, 547-552, 1980.

91. Marks, V. : An improved glucose-oxidase method for determining blood, CSF and urine glucose levels. Clin. Chem. Acta. 4, 395-399, 1959.

92. Mathur, D.S. : Histochemical changes in the liver of certain fishes as induced by BHC and lindane. Proc. Nat. Acad. Sci. India. 32, 429, 1962.

93. Matsumura, F., Boush, G.M. and Misato, T. : In : Environmental toxicology of pesticides. Academic Press, New York and London, 1962.

94. Mawdesley-Thomas, L.E. : Toxic chemicals the risk to fish. New scientist. 49, 74-75, 1971.

95. McKin, J.M., Benoit, D.A., Biesinger, K.E., Brungs, W.A. and Siefert, R.E. : Effects of pollution on fresh water fish. J. Water Pollut. Control. Fed. 47, 1711-1768, 1975.

96. Mckim, J.M., Anderson, R.L., Benoit, D.A., Spehar, R.L. and Stokes, G.N. : Effects of pollution on fresh water fish.
J. Water Pollut. Control. Fed. 48, 1544-1620, 1976.

97. McLeay, D.J. and Brown, D.A. : Growth stimulation and biochemical changes in juvenile coho salmon (Oncorhynchus kishush) exposed to bleached kraft pulpmill effluent for 200 days.
J. Fish. Res. Bd. Can. 31, 1043-1049, 1974.

98. Miller, L.C. and Tainter , M.L. : Estimation of the ED_{50} and its error by means of logarithmic probit graph paper.
Proc. Soc. Exp. Biol. Med. 57, 261-264, 1944.

99. Mohammed, M.P., Gupta, R.A., Nath, D. and Shrivastava, G.N. : Influence of sublethal ethyl parathion on the activity metabolism in the carp, Labeo rohita (Ham).
Proc. Symp. Environ. Biol. 89, 1979.

100. Molinengo, L. : The curve doses Vs survival time in the evaluation of acute toxicity.
J. Pharm. Pharmacol. 31, 343-344, 1979.

101. Morrison, J.K., Quinton, R.M. and Reinert, H. : The purpose and value of LD_{50} determinations. Historical introduction. In : Boyland E, Goulding R. (eds). Modern trends in toxicology Butter Worths, London, 1, 1968.

102. Muirhead-Thomson, R.C. : Pesticides and fresh water fauna. Academic Press, London and New York, 1971.

103. Mukhopadhyay, P.K. and Dehadrai, P.V. : Biochemical changes in the air breathing cat-fish, Clarias batrachus (Linn) exposed to malathion. Environ Pollut. (Ser. A), 22, 149-158, 1980.

104. Munirathnam Reddy, M., Jabeen Begum, S., Indira, S. and Swami, K.S. : In vitro inhibitory effects of dieldrin and methyl parathion on succinate dehydrogenase activity in fish, Tilapia mossambica. Environment & Ecology. 3(1), 76-78, 1985.

105. Natarajan, G.M. : Effect of sublethal concentration of metasystox on selected oxidative enzymes, tissue respiration, and hematology of the fresh water air breathing fish, Channa striatus (Bleeker). Pesticide Biochemistry and Physiology. 21, 194-198, 1984.

106. Novikoff, A.B. : The Cell (Eds. J. Brachet and A.P. Mirsky), New York Academic Press, 423, 1961.

107. Pal, A.K. and Konar, S.K. : Chronic effects of organophosphorus insecticide DDVP on feeding, survival, growth and reproduction of fish. Environ. Ecol. 1, 398-402, 1985.

108. Pal, A.K. and Konar, S.K. : Toxicity of insecticides phosphamidon, methyl-parathion and mixture of DDVP, phosphamidon, methyl-parathion to fish, plankton

and worm.

Environment & Ecology, 4(1), 76-79, 1986.

109. Pal, A.K. and Konar, S.K. : Long term effects of organophosphorus insecticide methyl-parathion on fish.

Environment & Ecology, 5(3), 564-571, 1987.

110. Pandey, B.N., Chanchal, A.K. and Singh, M.P. : Effect of malathion on oxygen consumption and blood of Channa punctatus (Bloch).

Indian J. Zootomy, 16, 95, 1976.

111. Pandey, B.N., Chanchal, A.K. and Singh, M.P. : Effect of malathion on O_2 consumption and blood of Channa punctatus.

Indian J. Zool. 27, 95-100, 1979.

112. Pandey, B.N., Prasad, S.S., Chanchal, A.K. and Singh, S.B. : Effect of some pesticides on the oxidative metabolism and haematology of Clarias batrachus (Bloch).

Proc. Sem. Eff. Pest. Aq. Fau., 13-18, 1984.

113. Panwar, R.S., Kapoor, D., Joshi, H.C. and Gupta, R.K. : Toxicity of insecticides to fish, Trichogaster fasciatus. (Bloch & Schneider).

J. Inland Fish. Soc. India, VIII, 1976.

114. Pathak, P., Pandey, P.K. and Pandey, R. : Season and environment related changes in the body composition of the fish, Channa punctatus.

Environment & Ecology, 7(2), 387-390, 1989.

115. Pearse, A.G.E. : Histochemistry, Theoretical and Applied. Vol. 1. Churchill Livingstone, New York, 648, 1968.

116. Pearse, A.G.E. : Histochemistry theoretical and applied. Churchill Ltd. London, 1971.

117. Raizada, M.N. and Maheshwari, V.K. : Haematological studies of fresh water teleosts.
Curr. Sci. 46, 790-791, 1977.

118. Rana, S.V.S. and Tayal, M.K. : Lipotropic effects of zinc, vitamin B_{12} and glutathione on the fatty liver of rat. A histochemical study.
Mikroskopie (Wien), 38, 294-300, 1981.

119. Rao, K.S.P. and Rao, K.V.R. : Lipid derivatives in the tissues of the fresh water teleost. Saurotherodon mossambicus (alias Tilapia mossambica) Peters effects of methyl parathion.
Proc. Ind. Natl. Sci. Acad. B. 47(1), 53-57, 1981.

120. Rao, K.S.P. and Ramana Rao, K.V. : Toxicity of methyl-parathion to the fresh water teleost, Tilapia mossambica (Peters).
Comp. Physiol. Ecol. 7, 271-274, 1982.

121. Rao, K.S.P. and Ramana Rao, K.V. : Changes in the tissue lipid profiles of fish (Oreochromis mossambicus) during methyl-parathion toxicity-A time course study.
Toxicology Letters, 21, 147-153, 1983.

122. Rao, K.S.P., Ahmmad Sahib, I.K. and Ramana Rao, K.V. : Methyl-parathion (O-O-dimethyl O-4-nitrophenyl thiophosphate) effects on whole-body and tissue respiration in the teleost, Tilapia mossambica (Peters).
Ecotoxicology and Environmental Safety, 9, 339-345, 1985.

123. Rashatwar, S.S. and Ilyas, R. : Effect of phosphamidon in a fresh water teleost fish, Nemachelius denisonii (Day)- Histopathological and biochemical study.
J. Environ. Biol. 5(1), 1-8, 1984.

124. Rees, K.R. and Sinha, K.P. : Blood enzymes in liver injury.
J.Pathol. Bacterial. 80, 297-307, 1960.

125. Robert, R.J. (ed). : Fish Pathology, Bailliers Tindall Cassell Ltd., 1978.

126. Rosenberg, A. and Nakatsugany, I. : Separation of parathion metabolites by reversed phase ion pair high performance liquid chromatography.
J. of Chromatography, 294, 436-441, 1984.

127. Rudd, R.L. and Genelly, R.E. : Pesticides. Their use and toxicity in relation to wild life Calif, Fish Game Bull. 7, 1-309, 1956.

128. Ruparelia, S.G., Verma, Y., Kashyap, S.K. and Chatterjee, B.B. : Status report on acute toxicity

of pesticides in fishes in India.
Proc. Sem. Eff. Pest. Aq. Fau. 107-114, 1984.

129. Sambasiva Rao, K.R.S. and Ramana Rao, K.V. : Independent and combined toxicity of carbaryl and phenthoate on some haematological parameters of a murrel, Channa punctatus (Bloch).
Indian Zool, 10, 29-32, 1986.

130. Sarna, L.P., Howe, G.J., Findlay, G.M. and Webster, G.R.B. : Quantitative gas chromatographic analysis of dimethoate and several metabolites in Rape, Quail, and grass hopper tissues by derivatization.
J. Agric. Food. Chem. 24(5), 1976.

131. Sastry, K.V. and Malik, P.V. : Studies on the effect of dimecron on the digestive system of a fresh water fish, Channa punctatus.
Arch. Environ. Contam. Toxicol. 8, 397-407, 1979.

132. Sastry, K.V. and Sharma, S.K. : The effect of endrin on the histopathological changes in the liver of Channa punctatus.
Bull. Environ. Contam. Toxicol. 20, 674-677, 1978.

133. Sastry, K.V. and Sharma, S.K. : Endrin toxicosts on few enzymes in liver and kidney of Channa punctatus (Bloch).
Bull. Environ. Contam. Toxicol. 22, 004-008, 1979.

134. Sastry, K.V. and Sharma, K. : Diazinon effect on the activities of brain enzymes from Ophicoephalus

(Channa) punctatus.
Bull. Environ. Contam. Toxicol. 24, 326-332, 1980.

135. Sastry, K.V. and Sharma, K. : Diazinon induced histopathological and hematological alterations in a fresh water teleost, Ophiocephalus punctatus.
Ecotoxicology and Environmental Safety, 5, 329-340, 1981.

136. Sastry, K.V. and Malik, P.V. : Acute and chronic effects of diazinon on some enzymes in certain tissues of a fresh water teleost fish, Channa punctatus (Bloch).
J. Environ. Biol. 2(3), 19-28, 1981.

137. Sastry, K.V. and Malik, P.V. : Histopathological and enzymological alterations in the digestive system of a fresh water teleost fish, Heteropneustes fossilis exposed acutely and chronically to diazinon.
Ecotoxicology and Environmental Safety 6, 223-235, 1982.

138. Sastry, K.V., Siddiqui, A.A. and Singh, S.K. : Alteration in some biochemical and enzymological parameters in the snake head fish, Channa punctatus exposed chronically to quinalphos.
Chemosphere, 11, 1211, 1982.

139. Sastry, K.V. and Siddiqui, A.A. : Some hematological, biochemical and enzymological parameters of a fresh

water teleost fish, Channa punctatus, exposed to sublethal concentrations of quinalphos.
Pesticide Biochemistry and Physiology, 22, 8-13, 1984.

140. Schreck, C.B. and Brouha, P. : Dissolved oxygen depletion in static bioassay systems.
Bull. Environ. Contam. Toxicol., 14, 149-152, 1975.

141. Schutz, E. and Fuchs, H. : A new approach to minimizing the number of animals used in acute toxicity testing and optimizing the information of test results.
Arch. Toxicol., 51, 197-228, 1982.

142. Sherma, J. and Zweig, G. : Pesticides.
Analytical Chemistry, 59(12), 18R-31R, 1987.

143. Shivaji Rai, T., Dutt, S. and Mangaiah, K. : TLm values of some modern pesticides to the fresh water fish, Puntius puckelli.
Environmental Health, 9, 103-109, 1967.

144. Singh, D.N., Tyagi, R.K. and Panwar, R.S. : Toxicity of some organic biocides to a fresh water fish, Cyprinus carpio communis (Linn).
J. Environ. Biol. 2(3), 41-46, 1981.

145. Singh, N.N. and Srivasthawa, A.K. : Effect of a paired mixtures of aldrin and formothion on a carbohydrate metabolism in a fish, Heteropneustes fossilis.
Pestic. Biochem. Physiol. 15, 257, 1981.

146. Singh, S. and Sahai, S. : Effect of malathion on the mortality and behaviour of two fresh water teleosts.
J. Environ. Biol. 5(1), 23-28, 1984.

147. Sprague, J.B. : Measurement of pollutant toxicity to fish. I. Bioassay methods for acute toxicity.
Water Res. 3, 793-821, 1969.

148. Sprague, J.B. : Measurement of pollutant toxicity to fish II. Utilizing and applying bioassays results.
Water Res. 4, 3-32, 1970.

149. Sprague, J.B. : Measurement of pollutant toxicity to fish III. Sublethal effects and 'Safe' concentration.
Water Res. 5, 245-266, 1971.

150. Sreenivasan, A. and Swaminathan, G.K. : Toxicity of six organophosphorus insecticides to fish.
Curr. Sci., 36, 397, 1967.

151. Srivasthawa, A.K. and Singh, N.N. : Effect of acute exposure to methyl- parathion on carbohydrate metabolism of Indian cat fish.
Acta. Pharmacol. Toxicol., 48, 26, 1981.

152. Srivastava, S.P. and Reena, K. : TLC Separation of some common organophosphorus pesticides on metal salt as well as on phenol impregnated layers.
Analytical Letters, 15 (A1), 39-46, 1982.

153. Srivastava, A.K. and Srivastava, A.K. : Effects of aldrin and malathion on blood chloride in the Indian cat fish, Heteropneustes fossilis.
J. Environ. Biol., 9 (1 Suppl), 91-95, 1988.

154. Street, J.C. and Chadwick, Ann. N.Y. Acad. Sci., 258, 132, 1975 (not consulted in original).

155. Sultatos, L.G., Costa, L.G., and Murphy, S.D. : Determination of organophosphorus insecticides their oxygen analogues and metabolites by HPLC.
Chromatographia, 15(10), 669-671, 1982.

156. Todd, J.H., Atema, J. and Bardach, J.E. : Chemical communication in social behaviour of a fish, the yellow bull head (Ictalurus natalis).
Science, 158, 672-673, 1967.

157. Tomar, B.S. : Studies on the haematology of a few fresh water teleosts.
Proc. Sem. Eff. Pet. Aq. Fau., 163-167, 1984.

158. Toor, H.S. and Kaur, K. : Toxicity of pesticides to the fish, Cyprinus carpio communis.
Indian J. Exp. Biol., 12, 334-336, 1974.

159. Trevan, J.W. : The error of determination of toxicity.
Proc. R. Soc. (London), Ser. B., 101, 483-514, 1927.

160. Verma, S.R., Bansal, S.K. and Dalela, R.C. : Bioassay trials with a few organic biocides on fresh water fish, Labeo rohita.
Indian J. Environ. Hlth., 19, 107-115, 1977.

161. Verma, S.R., Bansal, S.K. and Daslela, R.C. : Toxicity of selected organic pesticides to a fresh water teleost fish, Saccobranchus fossilis and its application in controlling water pollution.
Arch. Environ. Contam. Toxicol., 7, 317-323, 1978.

162. Verma, S.R., Bansal, S.K., Gupta, A.K., Pal, N., Tyagi, A.K., Bhatnagar, M.C., Kumar, K. and Dalela, R.C. : Acute toxicity of twenty three pesticides to a fresh water teleost, Saccobranchus fossilis.
Proc. Symp. Environ. Biol., 481-497, 1979.

163. Verma, S.R., Bansal, S.K. Gupta, A.K. and Dalela, R.C. : Pesticides induced haematological alterations in a fresh water fish, Saccobranchus fossilus.
Bull. Environ. Contam. Toxicol, 22, 467-474, 1979a.

164. Verma, S.R., Bansal, S.K., Gupta, A.K. and Dalela, R.C. : Sensitivity of AlPase system in certain tissues of Labeo rohita and Saccobranchus fossilis following in vitro treatment with chlordane.
Environ. Res., 14, 1979b.

165. Verma, S.R., Tyagi, A.K., Bhatnagar, M.C. and Dalela, R.C. : Organophosphate poisoning to fresh water teleost, Channa gachua : Acetyl-cholinesterase inhibition.
Bull. Environ. Contam. Toxicol., 21, 502-506, 1979c.

166. Verma, S.R., Tonk, I.P. and Dalela, R.C. : In vivo enzymatic dysfunction induced by some aquatic pollutants in a fish Saccobranchus fossilis.

J. Environ. Biol., 1, 1-8, 1980.

167. Verma, S.R., Tonk, I.P. and Dalela, R.C. : Effect of thiotox and malathion and their combination on the body composition of Saccobranchus fossilis and Mystus vittatus.
Intl. J. Acad. Ichthyol., 1 , 35-43, 1980.

168. Verma, S.R., Kumar, V. and Dalela, R.C. : Acute toxicity of three newly formulated pesticides to fish, Mystus vittatus.
Ind. J. Environ. Hlth., 23, 215-221, 1981.

169. Verma, S.R., Tonk, I.P., Gupta, A.K. and Dalela, R.C. : Role of ascorbic acid in the toxicity of pesticides in a fresh water teleost.
Water, Air, Soil Pollut., 15, 11-18, 1981.

170. Verma, S.R., Tonk, I.P., Gupta, A.K. and Dalela, R.C. : In vivo enzymatic alterations in certain tissues of Saccobranchus fossilis following exposure to four toxic substances.
Environmental Pollution (Series A), 26, 121-127, 1981.

171. Verma, S.R., Rani, S. and Dalela, R.C. : Indicators of stress induced by pesticides in Mystus vittatus haematological parameters.
Indian J. Environ. Hlth., 24, 58-64, 1982.

172. Verma, S.R., Tonk, I.P. and Dalela, R.C. : Effects of a few xenobiotics on three phosphatases of

Saccopranchus fossilis and the role of ascorbic acid in their toxicity.
Toxicology Letters, 10, 287-292, 1982.

173. Verma, S.R., Tonk, I.P. and Kumar, V. : Effects of thiotox, malathion and their two combinations on the tissues of Notopterus notopterus.
J. Environ. Biol. 4(1), 27-33, 1983.

174. Verma, S.R., Kumar, V., Ramesh Chand and Tonk,I.P.: Use of static bioassays procedure for the evaluation of the relative toxicity of pesticides and relative resistance of fishes.
Proc. Sem. Eff. Pest. Aq. Fau., 115-127, 1984.

175. Verma, S.R., Rani, S. and Dalela, R.C. : Effects of pesticides and their combinations on three serum phosphatases of Mystus vittatus.
Water, Air and Soil Pollution, 21, 9-14, 1984.

176. Verma, S.R. and Tonk, I.P. : Biomonitoring of the contamination of water by a sublethal concentration of pesticides-A system analysis approach.
Acta Hydrochim et Hydrobiol., 12(4), 399-409, 1984.

177. Wildish, D.J., Carson, W.G., Gunningham, T. and Lister, N.J. : Toxicological effects of some organophosphate insecticides to Atlantic salmon.
Fish. Res. Bd. Can. Manusc. Rep. Ser., 1157, 1-22, 1971.

178. Wintrobe, M.M. : Clinical haematology, VI ed. (Lea and Febiger, Philadelphia, U.S.A., 1967.

179. Zimmerman, H.J. : Various forms of chemically induced liver injury and their detection by diagnostic procedures.
Environ. Hlth. Perspect, 15, 3, 1976.

180. Zlatkis, A., Zak, B. and Boyle, A.J. : A new method for direct determination of serum cholesterol.
J. Lab. Clin. Mcd., 41, 486-489, 1953.