PUNJAB UNIVERSITY

JOURNAL OF ZOOLOGY

(FORMERLY BULLETIN, DEPARTMENT OF ZOOLOGY)
UNIVERSITY OF THE PUNJAB (NEW SERIES)

Volume 8

1993

CONTENTS

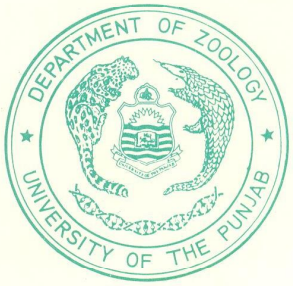
CODEN: PUZBAR PK ISSN 0079-8045

PUNJAB UNIVERSITY

JOURNAL OF ZOOLOGY

(FORMERLY BULLETIN, DEPARTMENT OF ZOOLOGY)
UNIVERSITY OF THE PUNJAB (NEW SERIES)

VOLUME 8
YEAR 1993



DEPARTMENT OF ZOOLOGY
UNIVERSITY OF THE PUNJAB
LAHORE-PAKISTAN

Punjab Univ. J. Zool., Vol. 8, pp.01-13, 1993.

SHORT-TERM TOXICITY OF ENDRIN IN SPRAGUE DAWLEY RATS: BIOCHEMICAL AND HISTOLOGICAL CHANGES IN LIVER*!

SYED SHAHID ALI AND ABDUL RAUF SHAKOORI

Department of Zoology, University of the Punjab, Lahore-54590, Pakistan

Abstract: An organochlorine insecti-cide, endrin (20% EC), was administered alongwith feed, to two groups of rats @ 8.2mg/kg body weight/day for 48 hours, to evaluate the biochemical and histological changes in the liver. Endrin at this dose and duration did not produce any change in body weight and relative liver weight (RLW) of rats. Hepatic alkaline phosphatase (AP), glutamate oxaloacetate transaminase (GOT), and glutamate pyruvate transaminase (GPT) activities were increased significantly by 48% and 69%,82 and 97%, 55 and 71% after 24 and 48 hours treatments, respectively. Isocitrate dehydrogenase (ICDH) activity incresed (65%) only at 48 hours, while lactate dehydrogenase (LDH) activity remained uneffected. Amongst the hepatic metabolites, cholesterol showed 27 and 35% rise at 24 and 48 hours, while saline soluble proteins increased by 35% only at 48 hours. The glucose, free amino acids (FAA) and total protein contents decreased by 40 and 52%, 34 and 40% and 13 and 15% at 24 and 48 hour endrin feeding, respectively. Hepatic DNA and RNA contents remained undisturbed. In morphometric studies, number.of cells/microscopic field decreased by 12 and 19% while size of cell, nucleus and nucleolus increased by 15 and 19%, 29 and 35%, and 48 and 64%, respectively at 24 and 48 hours. Major histological changes in liver were, hypertrophy, fatty infilteration, vacuolation, degeneration, necrosis of hepatic cells/ tissues, and dilation of sinusoidle spaces.

Key words: Organochlorine insecticides, endrin, rat, liver, biochemistry enzymes, histopathology.

INTRODUCTION

Although restrictions have been imposed on the use of organochlorine insecticides in many developed countries but these compounds are still in use in most of the developing and poor, third world countries, including Pakistan and India, primarily against the agricultural insect pests. These insecticides are also used against the insect pests of stored grains, veterinary and house hold importance (Parveen and Masud, 1988; Abdul Jabbar *et al.*, 1991; Lodha and Saxena, 1991). One very significant characteristics responsible for wide spread contamination of these insecticides, is their stability in nature which is due to very slow degradation of their molecules. As these compounds have been extensively applied for plant protection purposes in the vicinity of human environment through manual means or aerial sprays, their residues are quite wide-spread in nature, which have been reported from soil, air water, animals and their food stuff and plants (Hill *et al.*, 1973; Anderson and David, 1980; Atuma, 1985; Schmidt *et al.*, 1985; Ober *et al.*, 1987; Radulescu *et al.*, 1990; Calero *et al.*, 1992; Miller *et al.*, 1992; Bhatnagar *et al.*, 1992; Ferrer *et al.*, 1992; Chandra *et al.*, 1992). Reasonably large quantities of residues are recovered from various parts of the world where these compounds had never been used or where their use has been stopped since several years.

---

*Part of work submitted by the first author to the University of the Punjab, Lahore, Pakistan, for the award of Ph. D. degree.

!The work was supported by Pakistan Science Foundation, grant No. P. PU/BIO (93).

Metabolism of these insecticides, including fate of metabolites which accumulate in various components of the environment and organisms, and their excretion, is well documented (Cole *et al.*, 1970; Bedford *et al.*, 1975a,b; Petrella *et al.*, 1975; Anderson and David, 1980; Schmidt *et al.*, 1985; Grahm *et al.*, 1991). These pesticides are also played very important role in the maintenance of animal and human health by controlling the various vector-borne diseases. At the same time a number of reports indicate the development of several undesirable toxic effects in the non-target animals and other systems of the environment (Main, 1978; Chernoff *et al.*, 1979; Butijn and Koeman, 1983; Shakoori *et al.*, 1984, 1988; Casteel and Cook, 1985; Datta and Ghose, 1985; Spann *et al.*, 1986; Ali *et al.*, 1988; Ali and Shakoori, 1988,1990; Shahida and Solangi, 1990) which are mainly due to their indiscriminate use, poor pesticide management programmes and illiteracy, the factors which are very common in the developing third world countries.

The aim of the present study is to evaluate the toxic effects of endrin on the morphology and biochemistry of the liver. Short term effects of this insecticide on haematology and blood biochemistry has already been reported (Ali *et al.*, 1988).

MATERIALS AND METHODS

Experimental animals

Sixteen healthy female rats (*Rattus norvegicus,* Sprague Dawley strain) with average body weight 153g, were used for insecticide administration. They were maintained in the animal house of the Zoology Department as mentioned in Ali and Shakoori (1988).

Administration of insecticide

A cyclodiene, organochlorine insecticide, endrin (1,2,3,4,10,10-hexachloro-6,7-epoxy-1,4,4a,5,6,7,8, 8a,octahydro-1,4-endo-endo-5,8 dimethanonaphthalene), 20 EC, was used for this study. The insecticide was administered to two groups of rats (4 animals in each), alongwith the feed @ 8.2 mg/kg body weight/day, and its effects were observed for 48 hours. The insecticide-mixed feed was prepared by adding 0.25 ml of endrin (20%EC) to sufficient amount of water which was thoroughly mixed with one kg of rat feed and offered to rats *ad libitum.*

Experimental procedure

Four insecticide fed rats were weighed, anaesthetized and dissected quickly to collect the liver samples at the specified durations of 24 and 48 hours. Another group of four rats was also processed alongwith each group, exactly in the same way, except insecticide treatment which was used as control for the experiment.

The total liver weight of each animal was also taken and used for evaluation of relative liver weight (RLW = liver weight/body weight X 100).

All other procedures for various biochemical and histological analyses of liver havee already been mentioned in Ali and Shakoori (1990).

RESULTS

Body and liver weight

Administration of endrin-mixed diet, at the above mentioned dose level, did not produce any significant change in body and liver weights and RLW of rats at 24 and 48 hours experiment (Table I).

Biochemical analysis of liver

Endrin caused significant changes in almost all the hepatic enzyme activities tested (Table II). The AP activity increased 48% and 69% at 24 and 48 hours of insecticide feeding, respectively. Hepatic transaminase (GOT and GPT) activities exhibited sharp rise during 24 and 48 hours experimental duration which was 82 and 97% in case of GOT and 55 and 71% in case of GPT, respectively. The rise in ICDH activity at 24 hours was not significant while at 48 hours the change (65% rise) was quite significant.

Table I. EFFECT OF FEEDING ENDRIN MIXED DIET FOR 48 HOURS ON THE BODY WEIGHT AND LIVER WEIGHT OF ALBINO RATS.

Parameters	Control (n=4)	Endrin Feeding 24 hours (n=4)	Endrin Feeding 48 hours (n=4)
Weight gain(g% per day)	0.390[a] ±0.075	0.413±0.086	0.382±0.032
Relative liver wight	2.90±0.07	2.93±0.09	2.97±0.12

[a]Mean±SEM.

TABLE II. EFFECT OF FEEDING ENDRIN MIXED DIET FOR 48 HOURS ON THE HEPATIC ENZYMES IN ALBINO RATS.

Parameters[b]	Control (n=4)	Endrin Feeding 24 hours (n=4)	Endrin Feeding 48 hours (n=4)
AP (KAU/g)	0.71[a] ±0.06	1.05** ±0.05	1.20** ±0.09
GOT (IU/g)	6.81 ±0.32	12.42* ±1.57	13.44*** ±0.50
GPT (IU/g)	7.54 ±0.81	11.69* ±1.15	12.89** ±0.24
ICDH ($X10^3$ SU/g)	41.51 ±6.76	65.13 ±7.25	68.69* ±5.15
LDH ($X10^4$ IU/g)	57.94 ±3.94	66.51 ±7.33	69.03 ±6.11

[a]Mean±SEM, Student's `t' test; *P<0.05; **P<0.01 ***P<0.001.

[b]**Abbreviations used**: AP, alkaline phosphatase; GOT, glutamate oxaloacetate transaminase; GPT, glutamate pyruvate transaminase; ICDH, isocitrate dehydrogenase; LDH, lactate dehydrogenase. KAU (King Armstrong Unit), liberation of 1 mg of phenol in 15 minutes under the test conditions; IU (International Unit), transformation of 1 micromole of substrate/minute under the test conditions; SU (Sigma Unit), amount of enzyme that will produce 1 nanomole of NADPH in 1 hour under the test conditions.

TABLE III. EFFECT OF FEEDING ENDRIN MIXED DIET FOR 48 HOURS ON SOME HEPATIC BIOCHEMICAL COMPONENTS OF ALBINO RATS.

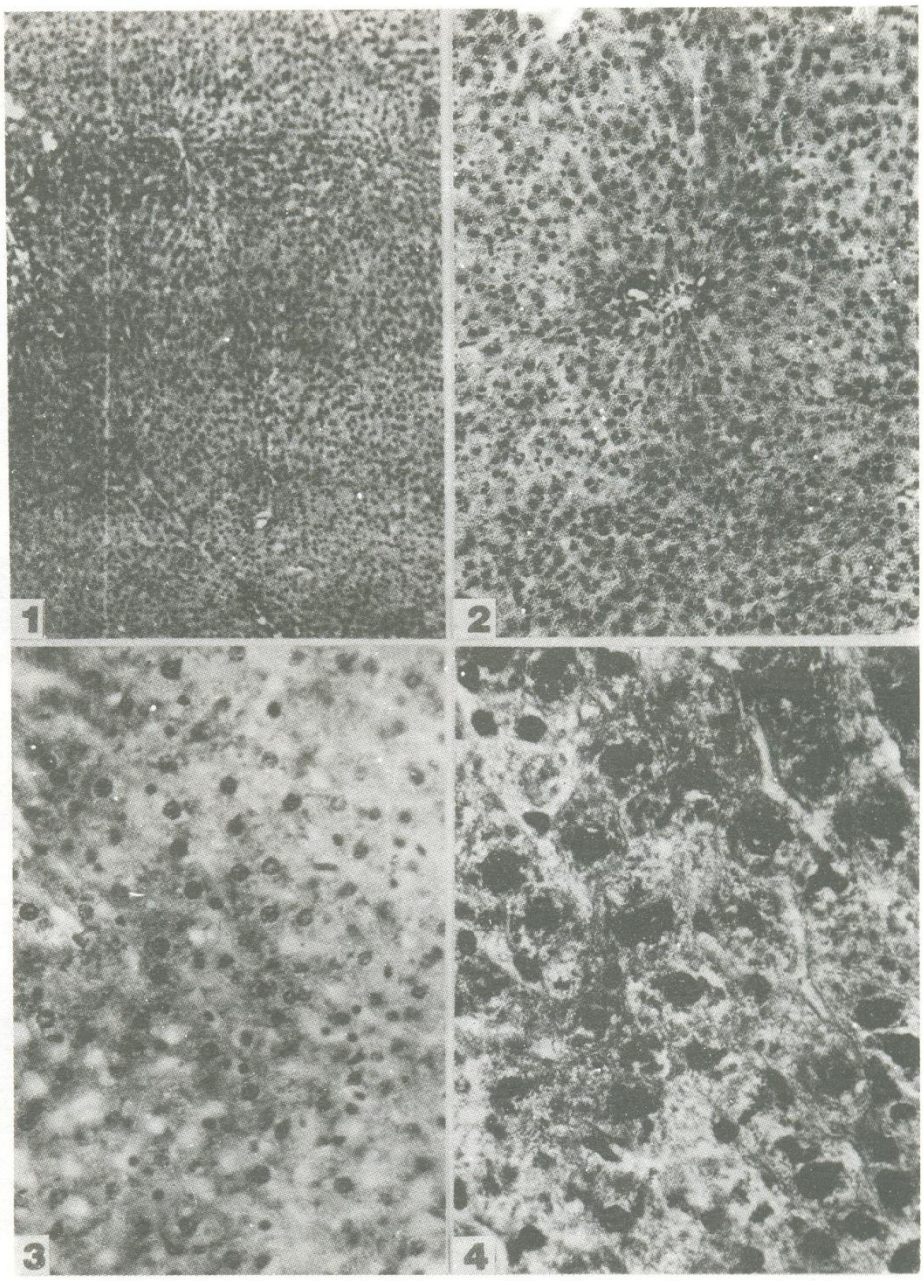
Parameters	Control (n=4)	Endrin Feeding	
		24 hours (n=4)	48 hours (n=4)
Cholesterol (mg/g)	5.95[a] ±0.19	7.56* ±0.62	8.05* ±0.75
Free amino acid (μg/g)	278.01 ±9.78	184.44*** ±7.67	166.24*** ±7.09
Glucose (mg/g)	27.86 ±1.77	16.55** ±0.63	13.78*** ±0.78
Soluble proteins (mg/g)	75.25 ±8.03	76.95 ±6.36	101.71* ±3.82
Total proteins (mg/g)	237.50 ±6.20	209.35* ±8.14	200.91* ±11.43
DNA (mg/g)	3.15 ±0.35	2.68 ±0.17	2.41 ±0.37
RNA (mg/g)	9.33 ±0.91	8.11 ±0.31	9.52 ±0.85

[a]Mean±SEM, Student's `t' test; *P<0.05; **P<0.01; ***P<0.001

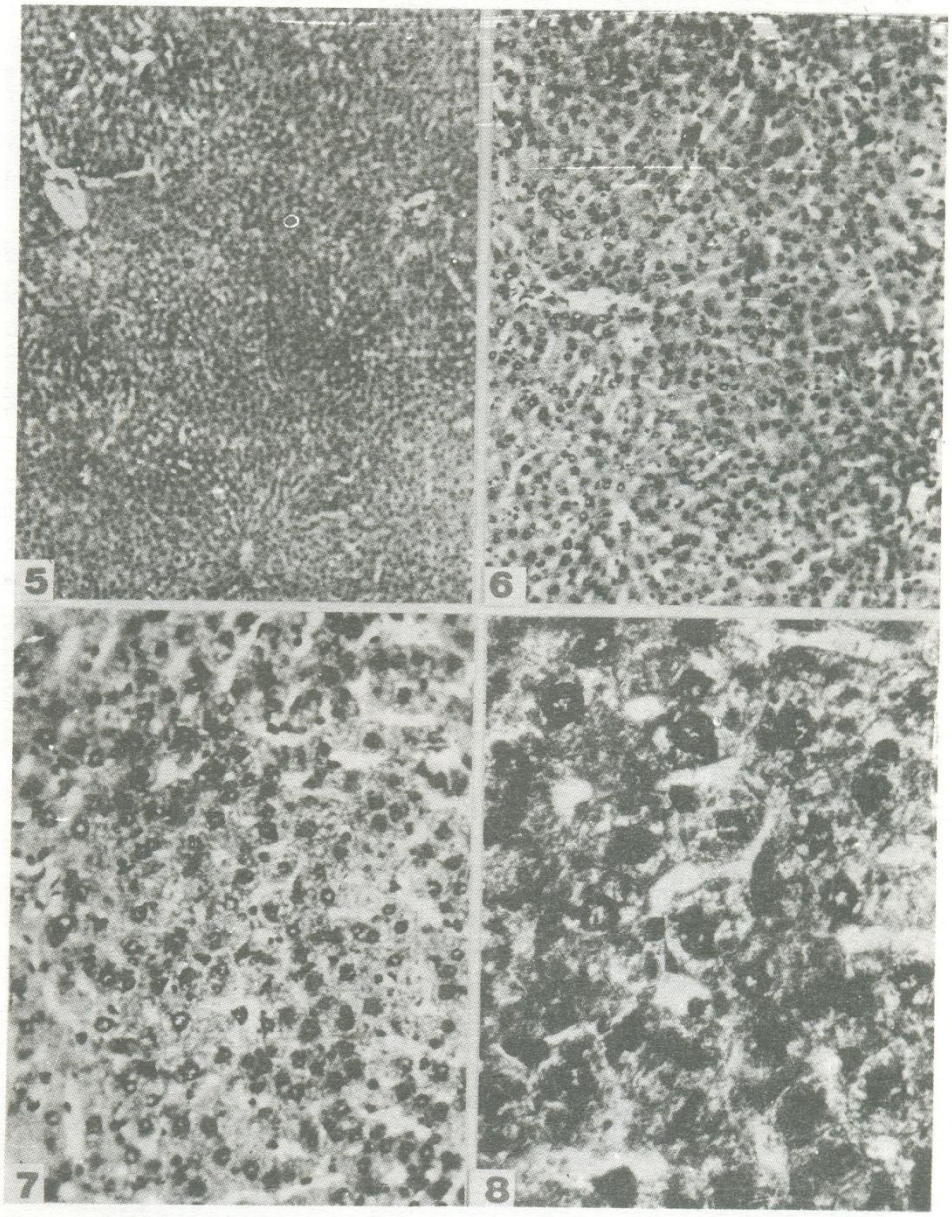
Table III shows the effect of feeding endrin-mixed diet for 48 hours on various biochemical components other than enzymes. Hepatic saline-soluble proteins and cholesterol contents exhibited significant increase after feeding this organochlorine compound. The rise was 27% and 35% at 24 and 48 hour of toxicant feeding, respectively, in case of cholesterol. The soluble proteins showed 35% significant increase until 48 hours of endrin feeding. On the other hand, significant reduction was found in glucose, total proteins and FAA contents during this 48 hour endrin treatment. The decrease in these contents was 41, 13 and 34% at 24 hours and 52, 15 and 40% at 48 hours of insecticide administration, respectively. The changes in DNA and RNA contents were statistically non-significant (Table III).

Histological structure of liver

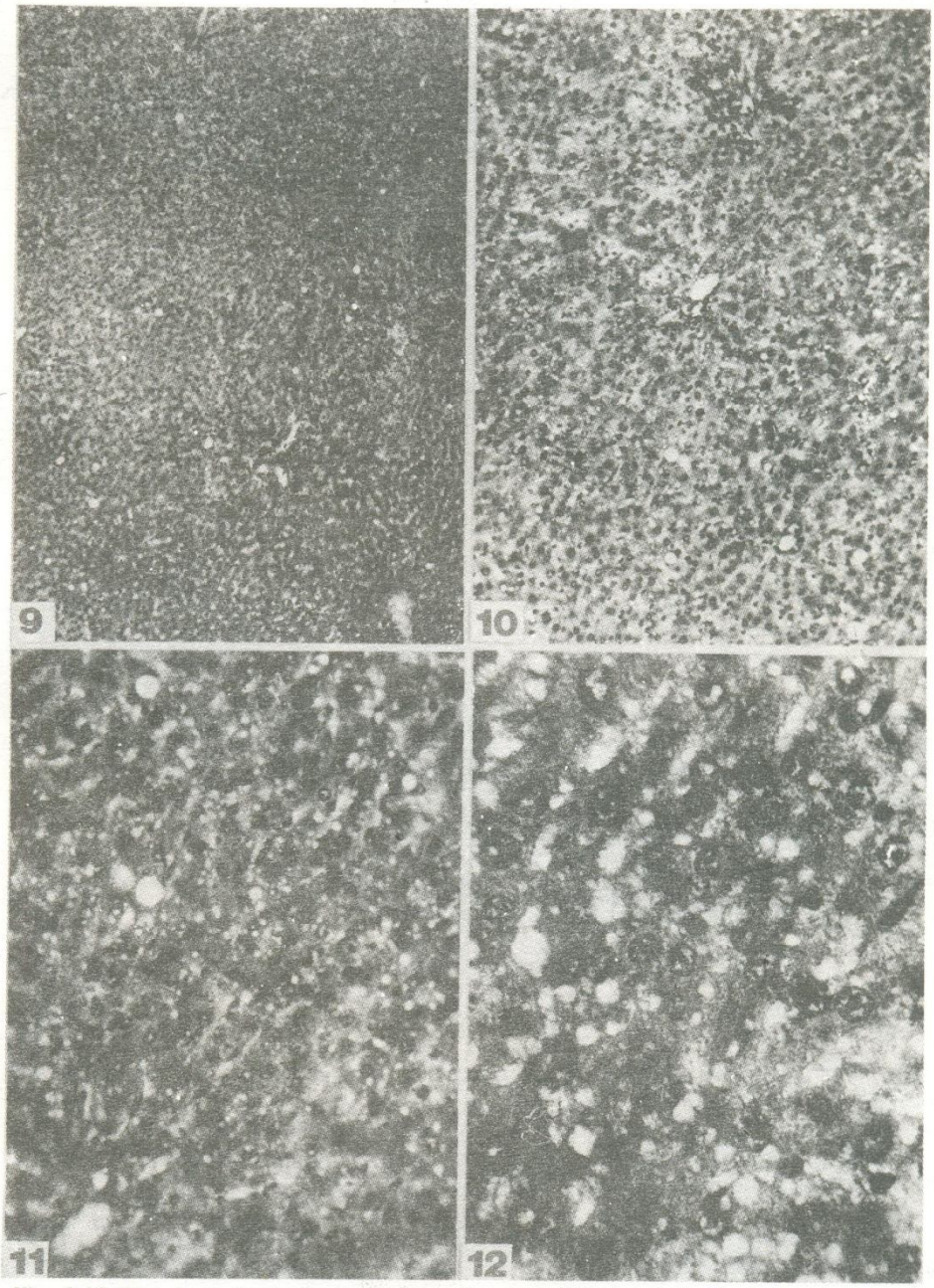
Endrin exposure at a dose of 8.2 mg/kg body weight/day for 48 hours produced significant structural alterations in rat liver. Table IV represents results of some morphometric studies. The important feature was hypertrophy of hepatic cells which was further confirmed by decrease in the number of cells/field (12 and 19%) and corresponding increase in cell size (15 and 19%) at 24 and 48 hours of feeding endrin-mixed diet, respectively.



Figs. 1-4. Histological structure of normal rat liver. Note the uniform hepatic lobular structure (1) with portal areas (2), hepatic cords, arrangement of hepatic cells and nuclei (3-4). Stain: Hematoxylin and Eosin. Magnifications: 1, X25; 2, X50; 3, X100; 4, X250.



Figs. 5-8. Histological structure of rat liver fed on endrin-mixed diet for 24 hours. Note the dilation of sinusoidal spaces (5-6), irregular arrangement of hepatic cells and cords (5-8), numerous irregular clear areas, and hypertrophied cells (7-8). Stain: Hematoxylin and Eosin. Magnifications: 5, X25; 6, X50; 7, X100; 8, X 250.



Figs.9-12. Histological structure of rat liver fed on endrin-mixed diet for 48 hours. Note the disruption of hepatic lobular and cord pattern, darkly stained lumps of necrotic cells (9-10), numerous clear areas giving the tissue granular appearance (10-12). Stain: Hematoxylin and Eosin. Magnifications: 9, X25; 10, X50; 11, X100; 12, X250.

TABLE IV. EFFECT OF FEEDING ENDRIN MIXED DIET FOR 48 HOURS ON VARIOUS MORPHOMETRIC PARAMETERS OF ALBINO RATS.

Parameters	Control	Endrin Feeding	
		24 hours	48 hours
No. of cells/field (n=9)	269.34[a] ±9.17	236.54* ±11.24	219.29** ±8.57
No. of nuclei/cell (n=90)	1.12 ±0.13	1.14 ±0.09	1.15 ±0.07
No. of nucleoli/nucleus (n=90)	1.64 ±0.15	1.92 ±0.12	1.82 ±0.14
Size of cell (μ^2; n=90)	271.87 ±12.31	311.78* ±10.29	324.61** ±9.58
Size of nucleus (μ2; n=90)	44.07 ±3.16	56.88** ±3.42	59.67** ±2.97
Size of nucleolus (μ2; n=90)	2.57 ±0.21	3.80*** ±0.24	4.22*** ±0.18

[a]Mean±SEM, Student's `t' test; *P<0.05; **P<0.01; ***P<0.001.

Figures 5-8 showed the effect of endrin feeding for 24 hours on liver when compared with normal liver (Figs. 1-4). Dilation of sinusoidal spaces (Figs. 6-8) alongwith disorganized and degenerated zones is clearly visible. The linear hepatic cord structure was also disturbed (Figs. 7,8 and 10, 12). The hepatocytes and their nuclei were also hypertrophied. This finding was further supported by morphometric data (Table IV). In 48 hour endrin treatment, vacuolation and fatty infiltration of hepatic cells indicates the endrin toxicity (Figs. 10-12). The darkly stained clusters of dead cells indicate the degenerative changes in the liver tissue (Figs. 7 and 10).

DISCUSSION

Biochemical studies

The liver plays a key role in maintenance of body metabolism. Moreover, it is an important site for biotransformation and degradation of various xenobiotics. All the toxic compounds are likely to be metabolized in this organ. The various hepatic enzymes and other biochemical constituents can best be used as indicators of sublethal exposure to toxic compounds such as insecticides.

Almost all the tested hepatic enzymes, with few exceptions, respond severely to endrin administration. AP alongwith both transaminase (GOT and GPT) activities exhibit consistent and significant elevation in both 24 and 48 hour experiments. The effect on ICDH was delayed until 48 hours. The LDH activity remained unchanged. As already mentioned, these enzymes showed increased activities in the rat blood serum following endrin treatment (Ali *et al.*, 1988). Increased enzyme activities in liver

reflect the possibility that synthesis of these enzymes was stimulated. It looks reasonable argument if hepatic regeneration is underway following cell injury or necrosis by this insecticide. Similar findings have also been reported after administering other chlorinated insecticides to various other experimental animals. (Dikshith *et al.*, 1978; Sastry and Sharma, 1978, 1979a,b; Lopez-Aparicio *et al.*, 1989; Numan *et al.*, 1990; Bagchi *et al.*, 1992; Hassoun *et al.*, 1993). Elevation of AP and LDH activities was also reported in rats fed on gamma BHC for 3 hours to 1 week. The GPT activity in the liver was significantly reduced while GOT remained unaltered during the same period (Shivanandappa and Krishnakumari, 1981). Simultaneous rise in both hepatic transaminases was reported by Bhatia *et al.* (1973). The decrease in FAA and glucose may indicate decreased intestinal absorption or these components are being utilized by the body to cope with the toxic insult. Increase in both hepatic transaminase activities gives some clue about the stimulation of gluconeogenesis. Early observations showing increased LDH, GOT and GPT activities in the serum and liver of pesticide administered monkeys also go in favour of elevated gluconeogenesis (Dudeja *et al.*, 1980). Increase in liver ICDH activity indicates the rapid oxidation of nutrient compounds through citric acid cycle to balance increased energy demands, most probably using glucose and FAA as a fuel by transamination so reducing their amount in the liver. Furthermore these amino acids may be utilized for protein (enzyme) synthesis as several workers have reported the induction of enzymes by the pesticides (Bhatia *et al.*, 1973).

The increased synthesis of soluble hepatic enzymes may be responsible for rise in soluble hepatic (microsomal) protein contents at 48 hours duration. It has been shown by Bhatia *et al.* (1973) that *in vivo* incorporation of C^{14} leucine into microsomal proteins was significantly elevated in dieldrin treatment, suggesting the induction of hepatic enzymes by the insecticide. The decrease in total hepatic proteins in strong dose (short term) experiment reflects the degenerative changes in different tissues (Bakthavathsalam and Srinivasa Reddy, 1982). Similar change in hepatic total proteins was observed by Bell and Mehendale, (1987) in rats treated with chlordecone. Hepatic cholesterol content exhibits a significant rise when endrin was administered as strong dose for 48 hours. In another similar study, strong dose of DDT induces cholesterol biosynthesis, which was evident from the incorporation of C^{14} labelled acetate into free cholesterol (Mahmood *et al.*, 1980). This is also in agreement with the present studies and with the findings of the Shivanandappa and Krishnakumari (1981) in rats using dietary BHC. However, Borady *et al.* (1983) did not notice any change in hepatic cholesterol content in rats after endrin administration for 24 hours.

Among the nucleic acid contents, DNA was quite resistant to endrin when different doses for variable durations were administered. Earlier reports from this laboratory (Shakoori and Haq, 1987; Shakoori *et al.*,1982,1988; Ali and Shakoori, 1988,1990) and from other laboratories (Wright *et al.*, 1978; Tayyaba *et al.*, 1981; Bell and Mehendale, 1987) also confirm these findings, working with different chlorinated insecticides. The RNA content also remained unchanged during the study which is difficult to explain even though, there is considerable rise in enzyme activities and saline-soluble protein fraction. Some other complicated factors may be responsible for this molecular behaviour. However, Wright *et al.* (1978) and Dudeja *et al.* (1980) did not find any significant deviation in DNA and RNA content in DDT fed monkeys.

Histological studies

In the present histological studies the number of nucleoli and nuclei/cell remained unchanged, however, they exhibit significant hypertrophy. In biochemical studies, the amount of DNA and RNA did not show any change. This pattern indicates that increase in size of nucleus and nucleolus was not related to rise in DNA and RNA content, respectively, but it may be due to changes in fluid contents of the nucleus which in-turn indicates changes in permeability along the nuclear membrane.

Liver is one of the target organs for the toxic action of insecticides and other xenobiotics. Endrin treatment produced marked structural alterations in liver. The immediate response in almost all treatments was hypertrophy of hepatic parenchyma cells. The degeneration of hepatic tissue was also a notable change induced by the toxicant. These degenerative changes in hepatocytes may lead to necrosis, which is evident from our results. The changes in cell size may be due to the proliferation of smooth endoplasmic reticulum and induction of hepatic mixed function oxidases by chlorinated insecticides (Kohli *et al.*, 1977; Wright *et al.*, 1978; Mikol *et al.*, 1980; Kurihara *et al.* 1984). These histopathological changes, according to some workers, may be adaptive responses which are reversible after the removal of inducing factors (Ocampo, 1976; Dikshith *et al.*, 1980).

REFERENCES

ABDUL JABBAR, AKHTAR, M.S. AND ALI, M., 1991. Soil and aquifer pollution by agrochemicals in Pakistan. *Progress. Farm.*, **11:** 40-44.

ALI, S.S. AND SHAKOORI, A.R., 1988. Gamma-BHC induced haematological and biochemical changes in the blood of albino rats. *Proc. Pakistan Congr. Zool.*, **8**: 61-76.

ALI, S.S. AND SHAKOORI, A.R., 1990. Toxicology of aldrin in rats. *Punjab Univ. J. Zool.*, **5:** 1-56.

ALI, S.S., ALI, N.S. AND SHAKOORI, A.R., 1988. Biochemical alterations induced by short term feeding of endrin on various blood components of albino rats. *Proc. Pakistan Congr. Zool.*, **8:** 101-112.

ANDERSON, R.L. AND DAVID, L.D., 1980. Toxicity and bioaccumulation of endrin and methoxychlor in aquatic invertebrates and fish. *Environ. Pollut. Ser. A., Ecol. Biol.*, **22:** 111-122.

ATUMA, S.S., 1985. Residues of organochlorine pesticides in some Nigerian food materials. *Bull. environ. Contam. Toxicol.*, **35:** 735-738.

BAGCHI, M., HASSOUN, E.A., BAGCHI, D. AND STOHS, S.J., 1992. Endrin-induced increases in hepatic lipid peroxidation, membrane micro-viscosity and DNA damage in rats. *Arch. environ. Contam. Toxicol.*, **23:** 1-5.

BAKTHAVATHSALAM, R. AND SRINIVASA REDDY, Y., 1982. Intoxication effects of lindane (gamma-BHC) on the activities of aspartate- and alanine- aminotransferases of *Anabas testudineus* (Bloch). *Pestic. Biochem. Physiol.*, **18:** 226-234.

BAYER, H., MITTERER, M. AND SCHINNER, F., 1982. The influence of insecticides on microbial processes in an agricultural soil. *Pedo-Biologia*, **23:** 311-319.

BEDFORD, C.T., HARROD, R.K., HOADLEY, E.C. AND HUTSON, D.H., 1975a. The metabolic fate

of endrin in the rabbit. *Xenobiotica,* **5:** 485-500.

BEDFORD, C.T., HUSTON, D.H. AND NATOFF, I.L., 1975b. The acute toxicity of endrin and its metabolites to rats. *Toxicol. Appl. Pharmacol.,* **33:** 115-121.

BELL, A.C., AND MEHENDALE, H.M., 1987. Comparative changes in hepatic DNA, RNA, protein, lipid and glycogen, induced by a subtoxic dose of CCl_4 in chlordecone, mirex and phenobarbital pretreated rat. *Toxicol. Lett.,* **35:**191-200.

BHATIA, S.C., SHARMA, S.C. AND VENKITASUBRAMANIAN, T.A., 1973. Effect of dieldrin on the hepatic carbohydrate metabolism and protein biosynthesis, *in vitro. Toxicol. Appl. Pharmacol.,* **24:** 216-229.

BHATNAGAR, V.K., PATEL, J.S., VARIYA, M.R., VENKAIAH, K., SHAH, M.P. AND KASHYAP, S.K., 1992. Levels of organochlorine insecticides in human blood from Ahmedabad (rural), India. *Bull. environ. Contam. Toxicol.,* **48:** 302-307.

BORADY, A.M.A., TAHANI, A., MIKHAIL, R. AND AWADALLAH, K.A., 1983. Effect of some insecticides on fat metabolism and blood enzymes in rats. *Egypt. J. Anim. Prod.,* **23:** 33-44.

BUTIJN, G.D., KOEMAN, J.B., 1983. Evaluation of the possible impact of aldrin, dieldrin and endrin on the aquatic environment. *Gov. Rep. Announce. Index* (U.S.)., **83:** 4439.

Calero, S., Fomsgaard, I., Lacayo, M.L., Martinez, V. AND Rugama, R., 1992. Preliminary study of 15 organochlorine pesticides in lake Xolotlan, Nicaragua. Chemosphere, 24: 1413-1419.

CASTEEL, S.W. AND COOK, W.O., 1985. Endrin toxicosis in cat. *J. Am. Vet. Med. Assn.,* **186:** 988-989.

CHANDRA, H., PANGTEY, B.S., MODAK, D.P., SINGH, K.P., GUPTA, B.N., BHARTI, R.S. AND SRIVASTAVA, S.P., 1992. Biological monitoring of chlorinated pesticides among exposed workers of mango orchards: A case study in tropical climate. *Bull. environ. Contam. Toxicol.,* **48:** 295-301.

CHERNOFF, N., KAVLOCK, R.J., HANISCH, R.C., WHITEHOUSE, D.A., GRAY, J.A., GRAY, L.E. AND SOVOCOOL, G.W., 1979. Perinatal toxicity of endrin in rodents, I. Feto-toxic effects of prenatal exposure in hamsters. *Toxicology,* **13:** 155-166.

COLE, J.F., KLEVAY, L.M. AND ZAVON, M.R., 1970. Endrin and dieldrin: a comparison of hepatic excretion in rat. *Toxicol. Appl. Pharmacol.,* **16:** 547-555.

DATTA, S.K. AND GHOSE, K.C., 1985. Toxic effect of endrin on the hepato-pancreas of a teleost, *Cyprinus carpio. Indian Biol.,* **17:** 37-41.

DIKSHITH, T.S.S., DATTA, K.K. AND RAIZADA, R.B., 1980. Response of carbon tetrachloride treated male rats to benzene hexachloride and quinalphos. *Indian J. exp. Biol.,* **18:** 1267-1272.

DIKSHITH, T.S.S., DATTA, K.K., KUSHWAH, H.S. AND RAIZADA, R.B., 1978. Histopathological and biochemical changes in guinea pigs after repeated dermal exposure to benzene hexachloride. *Toxicology,* **10:** 55-66.

DUDEJA, P.K., SANYAL, S.N., AGARWAL, N., RAO, T.N., SUBRAHMANYAM, D. AND KHULLER, G.K., 1980. Acute exposure of rhesus monkeys to DDT: Effect on carbohydrate metabolism. *Chem. Biol. Interact.,,* **31:** 203-208.

FERRER, A., BONA, M.A., CASTELLANO, M., TOFIGUERAS, J. AND BRUNET, M., 1992. Organochlorine residues in human adipose tissue of population of Zera-Goza (Spain). *Bull. environ. Contam. Toxicol.,* **48:** 561-566.

GRAHAM, M.J., WILLIAMS, F.M. AND RAWLINS, M.D., 1991. Metabolism of aldrin to dielrin by rat skin following topical application. *Food. Chem. Toxicol.*, **29:** 707-712.

HARGRAVE, B.T., HARDING, G.C., VASS, W.P., ERICKSON, P.E., FOWLER, B.R. AND SCOTT, V., 1992. Organochlorine pesticides and polychlorinated biphenyls in the Arctic ocean food web. *Arch. environ. Contam. Toxicol.* **22:** 41-54.

HASSOUN, E., BAGCHI, M., BAGCHI, D. AND STOHS, S.T., 1993. Comparative studies on lipid peroxidation and DNA single stranded breaks induced by lindane, DDT, chlordane and endrin in rats. *Comp. Biochem. Physiol. C*, **104**: 427-431.

HILL, E.G., FISHWICK, F.B. AND THOMPSON, R.M., 1973. Pesticide residues in food stuffs in Great Britain: organochlorine residues in imported cereals, nuts, pulses and animals food stuffs. *Pestic. Sci.*, **4:** 33-39.

KOHLI, K.K., MAGGON, K.K. AND VENKITASUBRAMANIAN, T.A., 1977. Induction of mixed-function oxidases on oral administration of dieldrin. *Chem. Biol. Interact.*, **17:** 249-256.

KURIHARA, N., HNORI, N. AND ICHINOSE, R., 1984. cytochromep-450 content and aldrin epoxidation to dieldrin in isolated rat hepatocytes. *Pestic. Biochem. Physiol.*, **21:** 63-72.

LODHA, R.M. AND SAXENA, V., 1991. *Pesticide and Environmental Pollution.* Himanshu Publications Udaipur,India, pp. 87-90.

LOPEZ-APARICIO, P., RECIO, M.N. GUTIERREZ-OCANA, M.T. AND PEREZ-ALBARSANZ, M.A., 1989. *In vitro* alterations in rat kidney lipogenesis induced by lindane *Pestic. Biochem. Physiol.*, **35:** 223-230.

MAHMOOD, A., AGARWAL, N., DUDEJA, P.K., SANYAL, S., MAHMOOD, R. AND SUBRAHMANYAM, D., 1980. Effect of a single oral dose of DDT on intestinal uptake of nutrients and brush border enzymes in protein calorie-malnourished monkeys, *Macaca mulatta. Pestic. Biochem. Physiol.*, **15:** 143-148.

MAIN, D.C., 1978,. Endrin toxicity in cage birds. *Australian Vet. J.*, **54:** 198-199.

MIKOL, Y.B., ROUX, F., DECLOITRE, F. AND FOURNIER, E.P., 1980. Liver enzyme induction in lindane and captae treated rats. *Food Cosmet. Toxicol.*, **18:** 337-382.

Miller, M.A. Madenjian, C.P. AND Masnado, R.G. 1992. Patterns of organochlorine contami-nation in lake trout from Wisconsin water of the Great Lakes. *J. Gr. Lakes. Res.*, **18**: 742-754

NUMAN, I.T., HASSAN, M.Q. AND STOHS, S.J., 1990. Protective effects of antioxidants against endrin-induced lipid peroxidation, glutathion depletion and lethality in rats. *Arch. environ. Contam. Toxicol.*, **19:** 302-306.

OBER, A., VALDIVIA, M. AND MARIA, I.S., 1987. Organochlorine pesticide residues in Chilean fish and shell-fish species. *Bull. environ. Contam. Toxicol.*, **38:** 528-533.

OCAMPRO, C.L., 1976. Chronic toxicity trials of hexachlorobenzene in lambs. *Vet. Mexico*, **7:** 132-135.

PARVEEN Z., MASUD, S.Z., 1988. Organochlorine pesticide residues in cattle drinking water. *Pakistan J. Sci. Ind. Res.*, *Sci. Ind. Res.*, **31:** 53-56.

PETRELLA, V.J., FOX, J.P. AND WEBB, R.E., 1975. Endrin metabolism in endrin-susceptible and resistant strains of pine mice. *Toxicol. Appl. Pharmacol.*, **34:** 283-291.

Radulescu, V., Rauta, C., Pere, N. AND Chiribelea, C., 1990. The dynamics of organochlorine insecticide residues in soil and their plant uptake. *An. Inst. Cercet. Pedol. Agrochim.*, **50**: 255-270.

SASTRY, K.V. AND SHARMA, S.K., 1978. The effect of *in vivo* exposure of endrin to the activities of

acid, alkaline and glucose-6-phosphatase in liver and kidney of *Ophiocephalus (Channa) punctatus*. *Bull. environ. Contam. Toxicol.*, **20**: 456-460.

SASTRY, K.V. AND SHARMA, S.K., 1979a. *In vivo* effects of endrin on three phosphatases in kidney and liver of the fish, *Ophiocephalus punctatus*. *Bull. environ. Contam. Toxicol.*, **21:** 125-130.

SASTRY, K.V. AND SHARMA, S.K., 1979b. Endrin toxicosis on few enzymes in liver and kidney of *Channa punctatus* (Bloch). *Bull. environ. Contam. Toxicol.*, **22:** 4-8.

SCHMIDT, C.J., ZAJICEK, J.L. AND RIBICK, M.A., 1985. National pesticide monitoring programme. Residues of organochlorine chemicals in fresh water fish. 1980-81. *Arch. environ. Contam. Toxicol.*, **14:** 225-260.

SHAHIDA, A. AND SOLANGI, A., 1990. Effects of pesticides on non-target soil micro-organisms: a review. *Progress. Farm.* **10:** 25-29.

SHAKOORI, A.R. AND HAQ. R., 1987. Effect of heptachlor on the liver of starved rabbits. *Folia Biol., (Krakow),* **35:** 23-32.

SHAKOORI, A.R., ALI, S.S. AND SALEEM, M.A., 1988. Effect of six months feeding of cypermethrin on the blood and liver of albino rats. *J. biochem. Toxicol.*, **3:** 59-71.

SHAKOORI, A.R., RASUL, Y.G. AND ALI, S.S., 1984. The effect of long term administration of dieldrin on biochemical components in blood serum of albino rats. *Folia Biol. (Krakow),* **32:** 213-222.

SHIVANANDAPPA, T. AND KRISHNAKUMARI, M.K., 1981. Histo- chemical and biochemical changes in rats fed dietary benzene hexachloride. *Indian J. exp. Biol.*, **19:** 1163-1169.

SPANN, J.W., HEINZ, G.H. AND HULSE, C.S., 1986. Reproduction and health of mallards fed endrin. *Environ. Toxicol. Chem.*, **5:** 755-759.

SURINDER, V. KAMAIDEEP, K. SATINDERPAL, K. 1987. Histopathological and biochemical changes induced by endrin and carbaryl in the stomach, intestine and liver of *Mystus tengara*. *Indian J. Ecol.*, **14:** 14-20.

SUZUKI, M., YAMATO, Y. AND WATANABE, T., 1976. Organochlorine insecticide residues in vegetables of the Kitakyushu District, Japan, 1971-1974. *Pestic. Monit. J.*, **10:** 35-40.

TAYYABA, K., HASAN, M., ISLAM, F. AND KHAN, N.H., 1981. Organophosphorous pesticide metasystox-induced regional alterations in brain nucleic acid metabolism. *Indian J. exp. Biol.*, **19:** 688-690.

WRIGHT. A.S., DONNINGER, C., GREENLAND, R.D., STEMMER, K.L. AND ZAVON, M.R., 1978. The effect of prolonged ingestion of dieldrin on the liver of male rhesus monkey. *Ecotoxicol. environ. Saf.*, **1:** 477-502.

(Received: 10 February, 1993)

Punjab Univ. J. Zool., Vol. 8, pp. 15-19, 1993.

STRUCTURE OF THE RESPIRATORY ORGANS OF A HILL-STREAM LOACH *NOEMACHEILUS RUPICOLA* (MCCLELLAND), COBITIDAE

NARAYAN C. ROOJ

Department of Zoology, S. P. College, Dumka, Pin-814101, Bihar, India.

Abstract: Morphological and histological structure of the respiratory organs with special reference to mouth, gill-openings, paired and unpaired fins , gills have been studied in detail. Four pairs of gills are not much developed. However, the region of the gill-head is provided with many taste buds forming a layer which are chemical detectors for food and water. Mucous gland cells also have formed a well developed middle layer, and the inner layer has formed by well developed abductor muscles. Primary lamellae are pointed at their tips, provided with two rows of moderately developed secondary lamellae in which thicker water blood diffusion barrier (2.25 μm) in comparison to other denizen, *Garra lamta* (1.75μm) are present.

Key Words: *Noemacheilus*, respiratory organs, histology, hill stream fish.

INTRODUCTION

Fishes inhabit a variety of aquatic environments. If marine fishes are kept apart, some live in normoxic and lentic water of the ponds and lakes, lotic water of rivers, some inhabit hypoxic water of the hill-streams, which are characterized by rocky, zig-zag water beds, high current of water, and plenty of oxygen. Hence the fishes which inhabit such aquatic bodies, undergo a variety of morphological adaptations to live in, and to withstand swift water current (Rooj, 1984). Hora (1982) made some preliminary observations on the structural modifications in some Indian hill-stream fishes with special reference to respiratory system also. However, litle information is available regarding the respiratory apparatus (Robotham, 1978; Sharma *et al.*, 1982; Ojha *et al.*, 1982; Rooj and Ojha, 1985). Recently some studies have been conducted on the respiratory apparatus of some hill-stream fishes with the help of modern instruments and appliances (Rooj, 1984; Ojha and Singh, 1986; Ojha, 1987; Ojha and Singh, 1992).

In the present work the morphological and histological structure of the respiratory organs of a hill-stream loach, *Noemacheilus rupicola* (McClelland) has been studied.

MATERIALS AND METHODS

Live specimens of *N. rupicola*, were collected from Jonha Fall (near Ranchi) with the help of local tribals. Morphological details were studied in living conditions and operculum was removed from one side after anesthetizing with MS-222, dissolved in water, and all pieces were fixed in Bouin's fixative, dehydrated inside laboratory with graded ethanol, embedded in molten paraffin wax and horizontal sections (7μm) were obtained with the microtome and stained with haematoxylin, counterstained with eosin. The microphotographs were obtained from different parts of the gill to show histological structures, after mounting in DPX.

RESULTS

Mouth (also the incurrent aperture for water) is situated as a small, simple and more or less crescentic aperture on the antero-ventral side of the snout, margin of which forms lip like boundary and a ring like sucker. Pectoral fins are transverse in position and small fan like structures.

All the fins are very thin and are highly vascularized. The gill openings or opercular apertures (the excurrent apertures for water) are laterally placed and are reduced in size. Water is seen to be retained for a longer period in side opercular chamber. The branchiostegal rays and membranes are greatly reduced. Gills (4 pairs), are small and slender structures, gill filaments are small, not compactly arranged and are borne over epi-and cerato-branchial parts of the gill arch.

On histological examination, the outer surface of the gill arch is seen to be evenly provided with outer epithelial layer (E) middle taste bud (T) layer, and mucous gland (M) layers and inner striped muscle (M) layers, (abductor muscles) which together constitute a well developed gill head (Fig. 1). At some places short gill rakers (R) originate as elevations from the gill arch epithelium (Fig. 2).

The inner surface of the gill arch develops two rows of primary lamellae (PL) which are short, pointed towards tips, and are provided with two rows of oppositely arranged. secondary lamellae (SL) and a central filamentar axis (A) (Fig. 3 and 4). lamellar spaces are more or less uniform (Fig. 5). Each secondary lamella consists of an outer layer of squamous epithelium (EP) a middle layer of basement membrane and inner core of blood channels (BC) separated by series of pillar cells (PC). Chloride cells could not be observed.

Water blood diffusion barrier have been measured to vary from 2.20-2.25 μm.

Fig. 1. Horizontal section (H.S.) of the gill of *N. rupicola* (x100) showing epithelial layer (E) Taste bud layer (T) Mucous gland land layer (M) and striped muscle fibre layer (S).

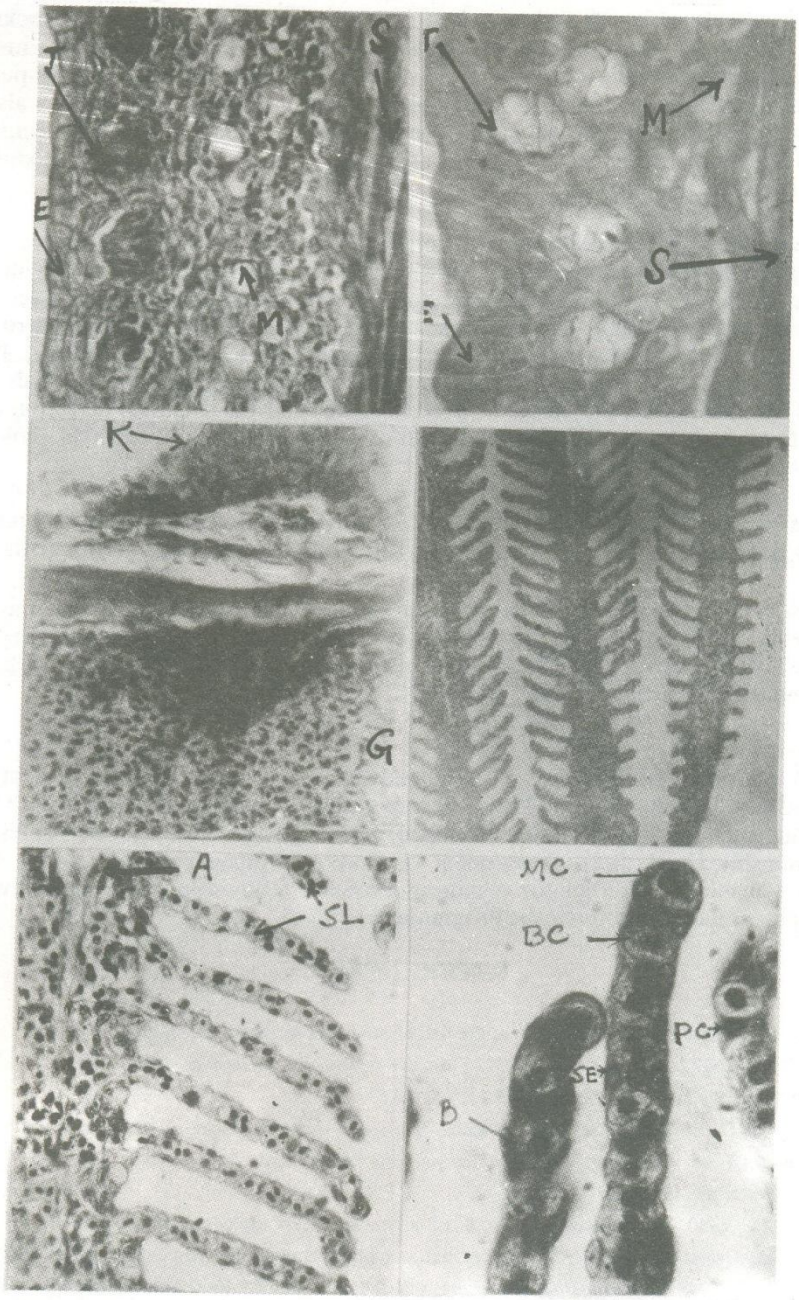
Fig. 2. Enlarged Fig. 1, (x450)

Fig. 3. H. S. of the part of the gill (x100) of *N. rupicola* showing gill arch (G) and gill raker (R).

Fig. 4. H. S. of the part of gill (x100) of *N. rupicola* showing primary lamellae (tip-region).

Fig. 5. Part of Fig. 4 (x450) showing secondary lamellae (SL) and filametar axis (A).

Fig. 6. Enlarged Fig. 4 (x1000), showing S.L. containing squamous epithelium (SE) Basement membrane (B) Pillar cell (PC) Blood channel (BC) and Marginal channel (MC).


T
S
E
M
T
M
S
E
K
G
A
SL
MC
BC
PC
B

DISCUSSION

With the employment of under surface for the purpose of adhesion to rocks and stones of the hill-streams, the gill openings are restricted to the sides. The inspired water is therefore retained for a longer period inside opercular chamber for the purpose of better gas exchange. Probability of the use of the fins of hill-stream fishes, also for respiration, was observed earlier (Hora, 1992). High vascularization, thin membrane like structures with capillary circulation, and their constant remaining in undulating condition in water, supports also the earlier observations.

Gills are not much developed, as intestine in loaches also acts as accessory respiratory organ (Moitra and Singh, 1987). Highly developed gill-head with taste buds, mucous glands, with small and stumpy gill rakers, and abductor muscles show resemblance with a catfish, *Chaca chaca* (Ojha *et al.*, 1989). Such type of well developed abductor muscles are also seen in *Macrognathus aculeatum* (Ojha, 1975), stumpy gill-rakers are indicative of the carnivorous feeding habit of the fish, well developed taste bud system signifies its greater capacity of the chemical detection of the nature of food, and surrounding water. Presence of large number of mucous glands are of great value in various purposes.

Structures like tertiary lamellae (Munsji, 1960, Hughes and Mittal, 1980) and inter filamentar fusions (Rooj, 1984; Singh *et al.*, 1992) have not observed in this fish.

The water blood diffusion barrier in *Garra lamta* (1.75μm) a denizen of the same environment, signifies the lesser adaptability of *N. rupicola* for respiration, specially by gills (Rooj, 1984). Striped muscle layers constitute abductor muscles of the gills, was observed earlier (Ojha and Munshi, 1976).

Acknowledgements

Author wishes to thank professor J. Ojha under whose supervision, the present work was carried out for the award of Ph.D. degree under University of Bhagalpur. Thanks are also due to Dr. G. C. Kapri, Ex-Principal, S. P. College, Dumka, and Professor J. S. Dattamunshi, Head, P.G. Department of Zoology, Bhagalpur University, whose cooperation enabled the author for obtaining a teacher fellowship under the University of Bhagalpur (Faculty improvement Programme).

REFERENCES

HORA, S.L., 1992. Structural modification in the fish of mountain torrents. *Rec. Indian Mus.* XXIV: 31-61.

HUGHES, G.M. AND MITTAL, A.K., 1980. Structure of the gills of *Barbus sophore* (Ham.) a cyprinid with tertiary lamellae. *J. Fish Biol.* **16**: 461-467.

MOITRA, A. AND SINGH, O.N., 1987. Nature of mucous secreting cells of the digestive tube of an intestinal air breathing fish, *Lepidoecphalychthys guntea*. *Proc. 74th. Indian Sci. Congr.* Abstract, pp. 121.

MUNSHI, J.S.D., 1960. The structure of the gills of certain fresh water teleosts. *Indian J. Zool.*, **1**:1-40.

OJHA, J., 1975. Cytochemical differentiation of red and white fibres in the respiratory muscles of a fresh water mud eel, *Macrognathus aculeatum* (Bloch). *Z. mikrosk, Anat, Forsch, Leip, 3. G89*: 143-

160.

OJHA, J. AND MUNSHI, J.S.D., 1976. Succinic dehydrogenase in the gill-filament muscles of certain freshwater teleosts. *Zoologische Beitrage*, **29**: 447-754.

OJHA, J., ROOJ, N.C. AND MUNSHI, J.S.D., 1982. Dimensions of the gills of an Indian hill-stream cyprinid fish, *Garra lamta, Jap. J. Ichth.* **29**: 272-278.

OJHA, J., AND SINGH, S.K., 1986. Scanning electron microscopy of the gills of a hill-stream fish, *Danio dangilla* (Ham.) *Arch. Biol.* (Bruxelles), **97**: 455-467.

OJHA, J., 1987. Scanning electron microscopy of the gills of hill-stream fish, *Danio dangilla* (Ham.). *Proc. 74 Indian Sci. Congr.* Abstracts, pp.84.

OJHA, J., DAS, A.K. AND SINGH, B., 1989. Morphometrics, micromorphology, microanatomy and cytochemistry of the gills of a swamp catfish, *Chaca chaca* (Ham.), *Proc. Indian Acad. Sci.*, **98**: 291-303.

OJHA, J. and SINGH, S.K., 1992. Functional morphology of the anchorage system and food scrapers of a hill-stream fish, *Garra lamta* (Ham.) (Cyprinidae, Cypriniformes), *J. Fish. Biol.*, **41**: 159-161.

ROBOTHAM, P.W.J., 1978. The dimensions of the gills of two species of loach, *Noemacheillus barbatulus* and *Cobitis taenia. J. exp. Biol.*, **76**: 181-184.

ROOJ, N.C., 1984. *Structure and function of the respiratory organs of certain hill-stream fishes of Chhotanagar Division.* Ph. D. Thesis. Bhagalpur University.

ROOJ, N.C. AND OJHA, J. 1985. Structure of the gills of two hill-stream fishes, *Garra lamta* and *Noemacheillus rupicola. Proc. 72nd. Indian Sci. Congr.* Abstract, pp.60.

SHARMA, S.N., 1980. *Respiratory adaptation in a hill-stream fish.* Ph. D. Thesis, University of Bihar, India.

SINGH. B., KUMAR, B. AND OJHA, J., 1992. Effect of branchial parasites on the efficacy of fish gills. *National Symp. Mod. Trend. Fresh Water Biol. Res.* Abstract, pp. 18.

(Received: April 23, 1993)

Punjab Univ. J. Zool., Vol. 8, pp. 21-36, 1993.

HISTOLOGICAL STUDIES OF THE THORACIC GANGLIA OF *PIERIS BRASSICAE* DURING METAMORPHOSIS (PIERIDAE: LEPIDOPTERA)

FIRDAUSIA AZAM ALI

Department of Zoology, University of the Punjab, Lahore, 54590, Pakistan.

Abstract: A pair of peripheral nerves is given off from the prothoracic ganglion of the different developmental stages and the adult. In the larvae the most abundant among the neurones are the medium-sized motor neurones, while in the late pupae and adult the large motor neurones are the most prominent and numerous. The cellular contents, on the whole, are more abundant as compared to the abdominal ganglia. The neuropile is also that of the late pupae and adult when compared to the early stages or the abdominal ganglia. Thirty two fibre tracts in the larval prothoracic ganglia and 39 in that of the adult could be traced fully. These are given off by paired groups of neurones.

Key Words: Thoracic ganglia, Pieridae, neuropile.

INTRODUCTION

Arthropod bodies are specialized into functional regions or taga. The function of locomotion has been taken over almost exclusively by three body segments, collectively referred to as thorax. These are called pro —, meso — and metathorax respectively. Most of the immature insect forms bear paired appendages on these segments. Each segment is usually well sclerotized to maintain a rigid position so as to prevent the body wall from flexing during movement of the appendages (Elzinga, 1988). Each of these appendages receives a pair of peripheral nerves from the corresponding segment. The coordination among the appendages is due to the nervous ganglia via the synaptic junctions. Medulla or the neuropile which is surrounded by the cellular cortex is the seat of these synapsis (Richard and Davies, 1977; Blum, 1985).

Polypod lepidopteran larvae and the adults also have the same general structure where each thoracic segment has a pair of legs, each receiving a pair of peripheral nerves from the ganglion pf its segment. The present work on the histology of the prothoracic ganglia of *Pieris brassicae* was undertaken to provide the basic structure of these ganglia so as to get an initial understanding of the involvement of nervous system in locomotion.

MATERIAL AND METHODS

The different developmental stages used during the present work were taken from a colony. They were fed on cabbage leaves and kept constantly at 20-23 °C. Various larvae were killed for dissection and histological treatment in the middle of the instar. The ventral nerve cord was removed by dissection in all cases except in the 1st and 2nd instar larvae where the size was too small to do this satisfactorily. Material was fixed in Bouin's, Zenker's or Gilson's fixatives and stained in Heidenhain's iron Haematoxylin or Mallory's Triple Stain. It was embedded in paraffin wax and serially sectioned at 5-6 μm. Wigglesworth's (1957, 1959) method of osmium tetraoxide fixation followed by ethyl gallate treatment was used to study the finer histology. These sections were

mounted in D.P.X.

RESULTS AND DISCUSSION

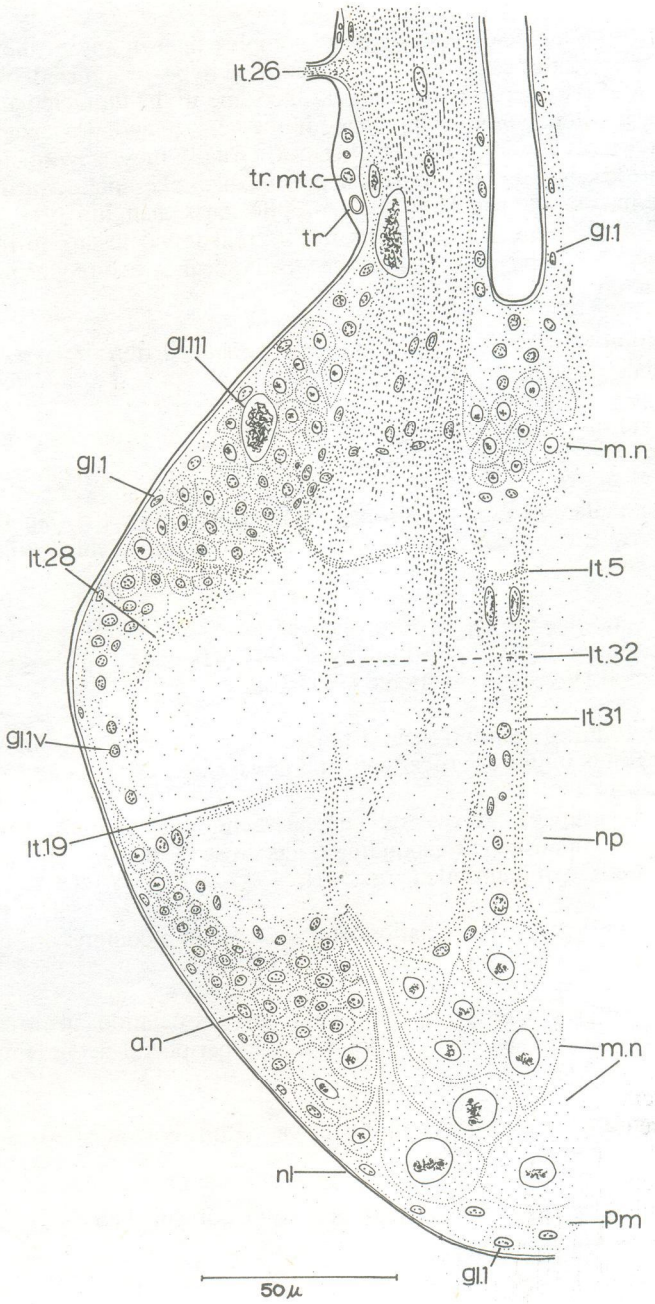
Peripheral nerves

Two main peripheral nerves are given off from each ganglion of the larvae. While in the abdominal ganglia both nerves come from the main body of the ganglion (Ali, 1991), in the thorax the case is somewhat different. The anterior or transverse nerve is very feebly developed and comes from the interganglionic connective just in front of the ganglion. Histological sections (Fig. 1; 1t. 26) show, however, that these fibre tracts originate in the main body of the ganglion, though it is not always possible to specify the neurones in a more anteriorly placed ganglion. In the late pupal stages and the adult two peripheral nerves are given off from the prothoracic ganglion. One is at the extreme anterior end and the other at the extreme posterior end, actually arising from thr mesothoracic part of the connective.

Cellular composition

The cells comprising the cortex of the prothoracic ganglion are of the same kind as described for the abdominal ganglia (Ali, 1993). The distribution of the different types of glial cells and the size of their nuclei is also similar. In the thoracic region a ventral diaphragm is not developed. The number of the type III glial cells is about 10-12 in the prothoracic ganglion instead of 7-8 as in the third or more posterior abdominal ganglia. Similarly the number of association nrurones is also much greater as compared to the abdominal ganglia (Ali, 1980). They are mostly in paired groups, one on each side of the ganglion. These groups give rise to many of the fibre tracts which run in the neuropile or are given off to the peripheral nerves. There are about 10 groups of these neurones on the prothoracic ganglion. In addition, association neurones are scattered among the motor neurones at the anterior and posterior end of the ganglion. The number of large motor neurones is also much greater compared with the number found in the abdominal ganglia and the number also increases during the pupal stage. But the most abundant among the neurones are the medium-sized motor neurones in the larva but in the adult the large ones are the more prominent and numerous. These groups of cells are described in detail in relation to their respective fibre tracts in the next section.

Fig. I. Frontal section through prothoracic ganglion of 5th instar larva shoeing neurones with their fibre tracts and other features. Cells with dark nuclei and dotted cytoplasm represent large motor neurones; cells with dark nuclei and white cytoplasm represent medium sized neurones; cells with dark cytoplasm represent association neurones; small clusters of circles represent axons cut teansversely and heavily shaded area represent glomerular bodies.


lt.26
tr. mt.c
tr
gl.1
gl.111
gl.1
m.n
lt.28
lt.5
lt.32
lt.31
gl.1v
lt.19
np
a.n
m.n
nl
pm
gl.1
50 μ

Neuropile and axonal tracts

The neuropile of the thoracic centre is more complex than in any of the abdominal ganglia, though it is of the same structured stratified type recognized by Maynard (1962). The pattern of the neuropile is generally the same in the thoracic ganglia of the 5th instar larva but it is much more complicated in the adult. There are some new tracts in the adult which are not found in the larval stages. Usually these new tracts are given off by motor neurones whose differentiation presumably takes place during the late pupal stages of the insect. The pattern of the neuropile starts changing after 48 hours of pupal life. Figs. 2-4 show the various tracts in different larval instars in the thoracic ganglia, while Figs. 5-9 and 10-11 show the histological structure during different pupal periods and adult respectively.

The differenciation of motor neurones and their associated fibre tracts is correlated with the development of the power of flight in the adult.

The following are the paired tracts and other features of the prothoracic ganglion in the 5th instar larvae:

Tract It.1: From dorsolateral motor neurones at anterior end of the ganglion. Runs ventrally around periphery of neuropile. Forms fine fibres along which glial IV cells are scattered (Fig. 2A).

Tract It.2: From ventrolateral motor neurones at anterior end of ganglion. Runs dorsally around periphery of neuropile forming a sort of boundary for it. Forms fine fibres along which glial IV cells are scattered (Fig. 2A).

Tract It. 3: From mid-dorsal and mid-ventral motor and association neurones. Forms a median partition of fibres running vertically up and down (Fig. 2A).

Tract It. 4: From a group of dorsolateral and lateral motor neurones. Runs across middle of ganglion, two opposite corresponding tracts cross in mid-dorsal line and then pass along dorsal boundary of neuropile (Fig. 2 A).

Tract It. 5: From lateral group of association neurones. From a commissure just below the chiasma formed by tract It.4 (Fig. 2A).

Tract It. 6: From ventrolateral group of motor and association neurones. Runs dorsalwords, curves towards outer side and joins second peripheral nerve (Fig. 2B and C)

Tract It. 7: From ventral group of association neurones. Runs dorsomedially and passes midline in the centre (Fig. 2B).

Tract It. 8: From ventral group of motor neurones. Runs dorsomedially close to tract It 7 and forms a commissure (Fig. 2C).

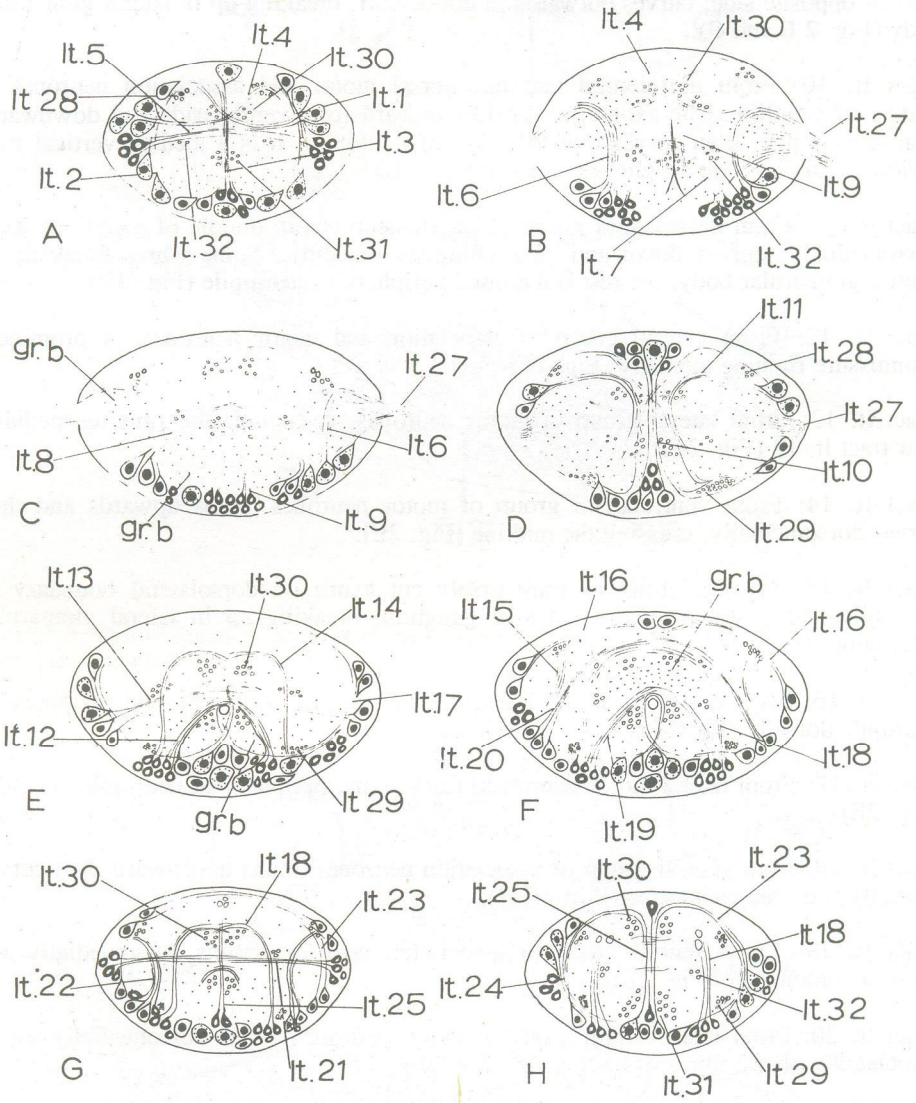


Fig. 2. Diagrammatic serial drawings of transverse sections of prothoracic ganglion of 5th instar larva showing the major fibre tracts with neurones. (Explanation same as in Fig. 1).

Tract It. 9: From ventral group of association neurones.Runs parallel to corresponding tract of opposite side; curves outwards in dorsal part, breaking up in lateral glomerular body (Fig. 2 B and C).

Tract It. 10: From mid-ventral and mid-dorsal motor and association neurones in middle of ganglion. The axons run dorsally upward from ventral side and downwards from dorsal side, then curves towards sides of neuropile. It is a median vertical tract dividing ganglion into a right and left half (fig. 2D).

Tract It. 11: From dorsolateral group of motor neurones in middle of ganglion. Runs dorsomedially, curves down and runs obliquely outwards. Some fibres break up in ventral glomerular body, the rest run around periphery of neuropile (Fig. 2D)

Tract It. 12: From ventral group of association and motor neurones. A prominent commissure running medially (Fig. 2E).

Tract It. 13: From lateral group of motor neurones. A commissure running medially near tract It. 12 (Fig. 2E).

Tract It. 14: From ventrolateral group of motor neurones. Runs upwards and then curves dorsomedially, crossing the midline (Fig. 2E).

Tract It. 15: From a group of transversely cut axons on dorsolateral boundary of neuropile. Runs downwards at sides of ganglion, breaking up in lateral glomarular body (Fig. 2F).

Tract It. 16: From dorsolateral and lateral motor neurones. Runs around periphery of neuropile dorsally (Fig. 2E).

Tract It. 17: From lateral motor neurones. Runs round periphery of neuropile ventrally (Fig. 2E).

Tract It. 18: from ventral group of association neurones. Runs dorsalward then curves medially and crosses midline (Fig. 2F-H).

Tract It. 19: From ventral group of association neurones. Runs dorsomedially and forms a commissure (Fig. 2F).

Tract It. 20: From lateroventral group of motor neurones. Runs dorsomedially and is associated with the fibres of tract It. 18 (Fig. 2F).

Tract It. 21: From lateral group of association neurones. Forms a median commissure (Fig. 2G).

Tract It. 22: From ventrolateral motor and association neurones. Runs upward and then curves outwards. Probably continues in the preceeding interganglionic connective and gives fibres to the anterior peripheral nerve of mesothoracic ganglion (Fig. 2G).

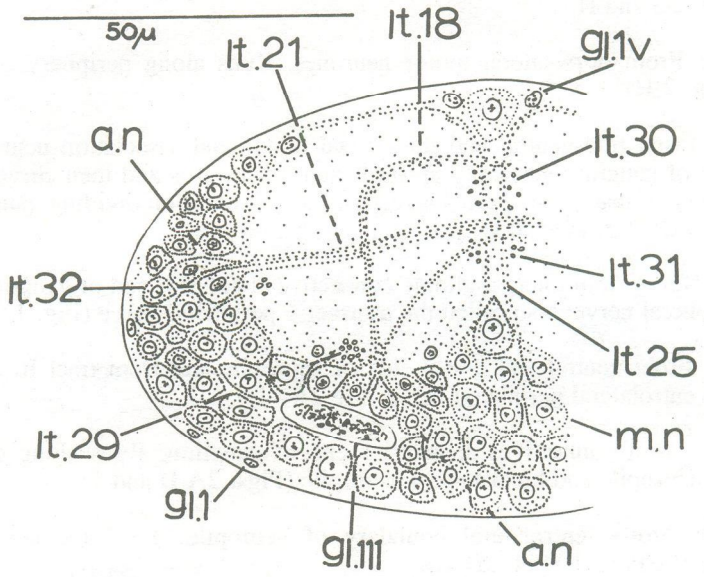


Fig. 3. Cross-section through prothoracic ganglion of 1st instar larva showing neurones, fibre tracts and glial cells.

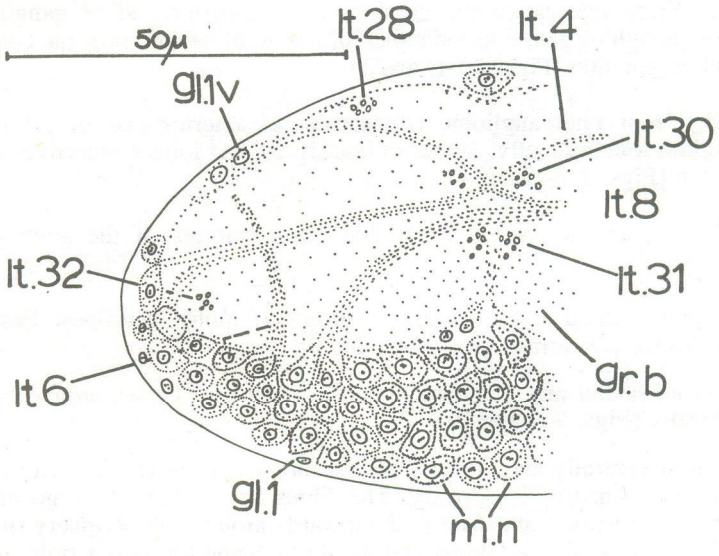


Fig. 4. Cross-section through prothoracic ganglion of 1st instar larva showing same features as in Fig. 3.

Tract It. 23: From lateroventral motor neurones. Runs along periphery of neuropile dorsally (Fig. 2G and H.

Tract It. 24: From dorsolateral motor neurones. Runs along periphery of neuropile ventrally (Fig. 2 H).

Tract It 25: From mid-ventral and mid-dorsal motor and association neurones. Runs along middle of ganglion vertically upwards and downwards and then curves outwards along periphery of neuropile. Forms a vertical median partition dividing ganglion into a right and left half (fig. 2G and H).

Tract It. 26: Nerve from interganglionic connective at anterior end of ganglion. It is the anterior peripheral nerve; also called the transverse peripheral nerve (Fig. 12).

Tract It. 27: From ventral and dorsal side of neuropile and from tract It. 6. It is the posterior or ventrolateral peripheral nerve (Fig 2A - D).

Tract It. 28: From connectives at anterior end of ganglion. Runs along dorsolateral periphery of neuropile and breaks up in neuropile (Figs. 2A-D and 1).

Tract It. 29: From ventrolateral boundary of neuropile. Joins the connectives at posterior end of ganglion (Fig. 2D-H).

Tract It. 30: From connectives at anterior end of ganglion. Runs through ganglion longitudinally and joins the connectives at posterior end of ganglion. The two opposite corresponding tracts run parallel to each other (Fig. 2A-H).

Tract It. 31: From interganglionic connectives at anterior end of ganglion. Passes through whole length of ganglion, on either side of midline and joins the connectives at posterior end of ganglion (Figs. 2A-H and 1).

Tract It. 32: From interganglionic connectives at anterior end of ganglion. Runs through ganglion longitudinally, lateral to tract It. 31 and joins connectives at posterior end of ganglion (Figs. 2 A-H and 1).

The following are the paired tracts and other features of the adult prothoracic ganglion:

Tract t. 1: From lateral and dorsolateral group of motor neurones. Passes around boundary of neuropile ventrally (Figs. 7 A and B).

Tract t. 2: From lateral and dorsolateral motor neurones. Passes around boundary of neuropile dorsally (Figs. 7 A and B).

Tract t. 3: From ventrally and dorsally placed motor and association neurones. Passes around boundary of neuropile dorsally. The fibres from ventral side go up vertically forming a median partition and then pass outwards around the periphery of neuropile. The fibres from dorsal side go down and divide the ganglion into a right and left half (Fig. 7 A and B).

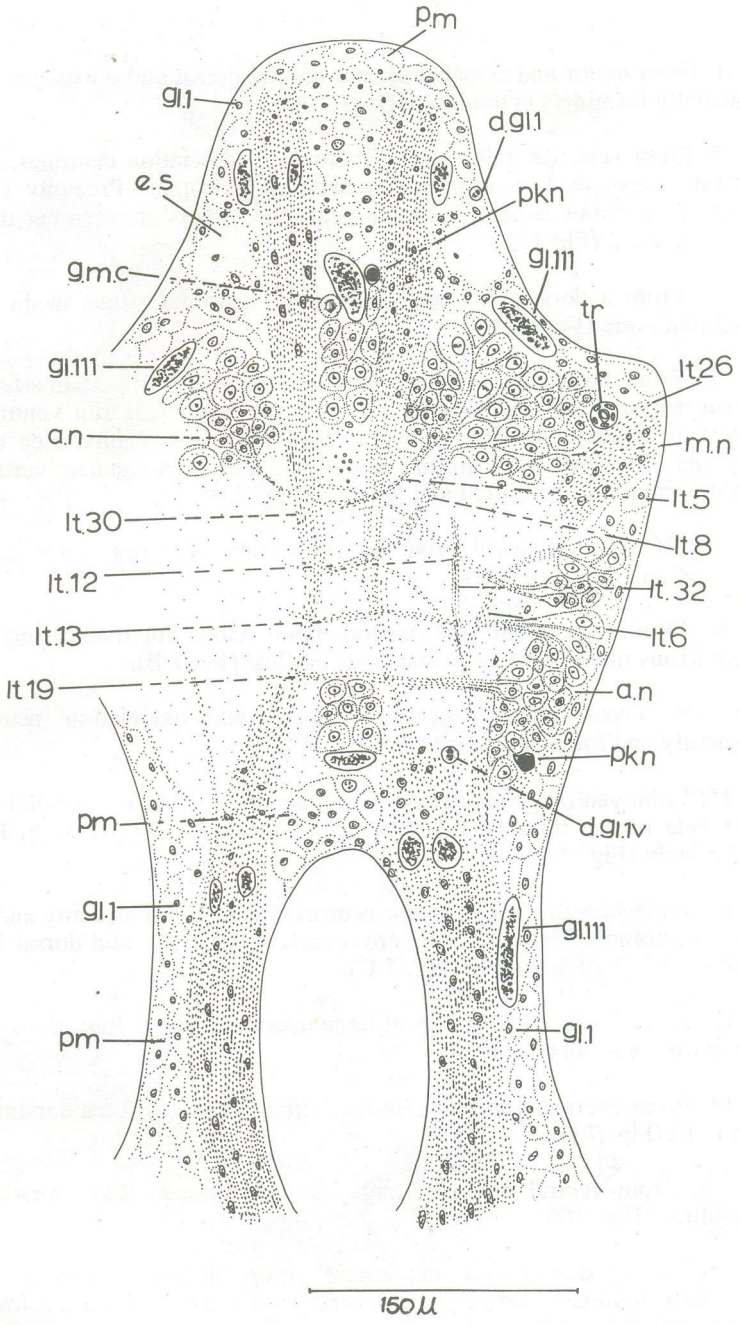


Fig. 5. Frontal section through prothoracic ganglion of 48 hours pupa showing neurones, fibre tracts, pyknosis, mitosis and other features.

Tract t. 4: From motor and association neurones on dorsal and dorsolateral side. Passes ventromedially forming a commissure (Fig. 7 A).

Tract t. 5: From ventrolateral group of motor and association neurones. Runs upward and outwards towards dorsolateral boundary of neuropile. Probably contributes to peripheral nerve. Some fibres are lost in neuropile, others are seen cut transversely in the sections studied (Fig 7A).

Tract t. 6: From a dorsolateral group of motor neurones. Runs medioventrally and forms a commissure (Fig 11B).

Tract t. 7: From large motor neurones on dorsal side and median-sized motor and association neurones on ventral side. Fibres from dorsal side run ventrally and then pass outwards around periphery of neuropile. Frbres from ventral side run vertically upwards and then outwards around neuropile. Forms a median vertical partition dividing the ganglion into a right and left half (Fig. 7 B).

Tract t. 8: From ventrolateral group of association neurones. Runs dorsomedially, forming a commissure (Fig 11B).

Tract t. 9: From a group of ventrolateral motor axons cut transversely in sections. Prominent axons running medially and cross midline (Fig 7 B).

Tract t. 10: From some dorsolateral motor and association neurones. Runs ventromedially and forms a commissure (Fig, 7 C).

Tract t. 11: From ventral group of association neurones. Both run parallel to each other on either side of the midline and then curve outwards and break up in the lateral glomerular body (Fig. 7 c).

Tract t. 12: From laterally placed motor neurones. Runs dorsomedially and some of the fibres of correspinding opposite tract cross each other in the mid-dorsal line and pass the dorsal boundary of neuropile (Fig. 7 C).

Tract t. 13: From lateroventral group of large motor neurones. Runs dorsomedially and crosses midline near dorsal side (Fig. 7 D).

Tract t. 14: From lateroventrally placed association neurones. Runs dorsimedially close to tract t. 13 (Fig. 7 D).

Tract t. 15: From ventral group of large motor neurones. Runs dorsomedially and crosses midline (Fig. 7D).

Tract t. 16: From dorsolateral and lateral group of large motor neurones. Runs medially, near midline, curves inwards and runs ventrally, finally lost in ventral glomerular body (Fig. 7 D).

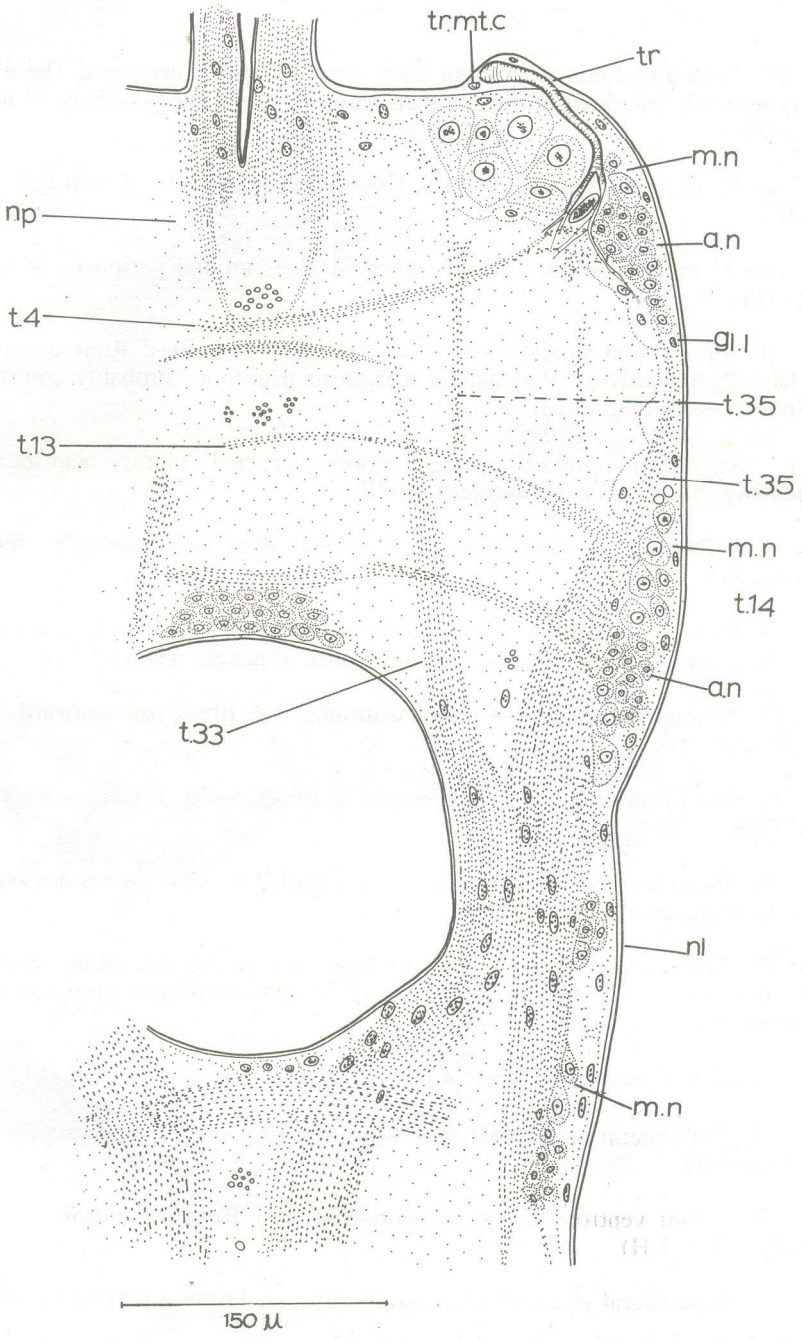


Fig. 6. Frontal section through adult prothoracic ganglion, as in Fig. 1.

Tract t. 17: From a mid-dorsal group of association and motor neurones. The fibres run vertically upwards and downwards and then outwards along the periphery of neuropile (Fig. 7 and E).

Tract t. 18: From lateral motor neurones. Goes around periphery of neuropile dorsally (Fig. 7 D).

Tract t. 19: From lateroventral motor neurones. Goes around periphery pf neuropile ventrally (Fig. 7 E).

Tract t. 20: From ventrolateral motor and association neurones. Runs upwards then curves laterally towards the dorsolateral margin of neuropile. Probably contributes to the peripheral nerve (Fig. 7 F).

Tract t. 21: From ventrally placed association and motor neurones. Runs dorsomedially, forming a commissure (Fig. 7E).

Tract t. 22: From ventrolateral motor neurones. Runs dorsomedially, forming a commissure (Fig. 7 E).

Tract t. 23: From ventral group of association and possibly some motor neurones. Runs dorsomedially and forms a commissure in the centre of ganglion (Fig. 7 E).

Tract t. 24: Ventrally placed large motor neurones. The fibres pass outwards and join tract t. 20 (Fig. 7 f).

Tract t. 25: From ventral group of large motor neurones. From a tract similar to tract t. 21 (Fig. 7 F).

Tract t. 26: From lateroventral group of large motor neurones. Passes dorsomedially, forming a commissure (Fig. 7 F).

Tract t. 27: From a ventrolateral group of large motor neurones. Runs upwards and then curves outwards and turns to run longitudinally, so forming a group of axons cut transversely (Fig. 7 G and H).

Tract t. 28: From lateral motor neurones. Runs around neuropile dorsally (Fig. 7 G).

Tract t. 29: From lateral motor neurones. Runs around boundary of neuropile ventrally (Fig. 7 G and H).

Tract t. 30: From ventrolateral association neurones. Runs dorsomedially, forming commissure (Fig. 7 H).

Tract t. 31: From lateral group of association neurones. Forms a prominrnt commissure (Fig. 7 H).

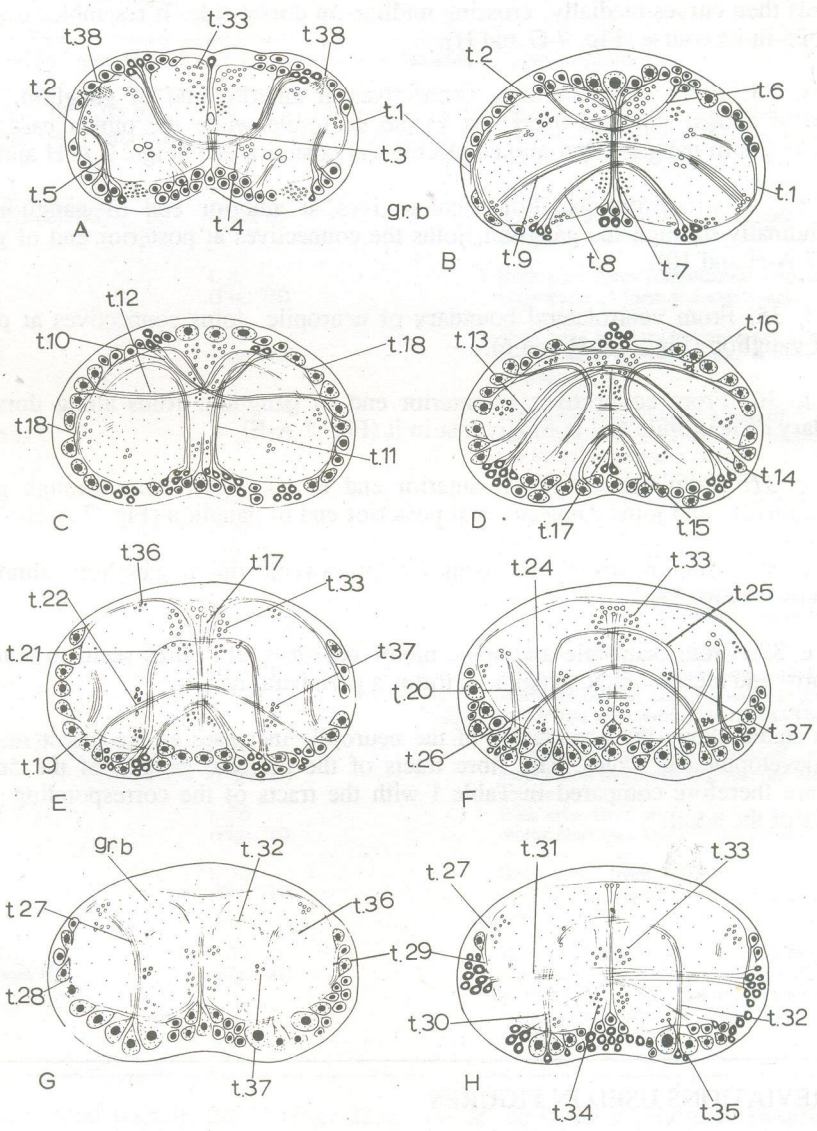


Fig 7. Diagrammatic serial drawings of adult prothoracic ganglion cut transversely with the same explanations as Fig. 1.

Tract t. 32: From lateroventral motor and association neurones. Runs vertically upwards then curves medially, crossing midline on dorsal side. It resembles tracts t. 21 and t. 25 in its course (Fig. 7 G and H).

Tract t. 33: From interganglionic connectives at anterior end of ganglion. A thick bundle of axons, some of them lost in the neuropile while the others pass through whole length of ganglion and join connectives at posterior end (Figs. 2 A-H and 10).

Tract t. 34: From interganglionic connectives at anterior end of ganglion. Runs longitudinally through the ganglion, joins the connectives at posterior end of ganglion (Fig. 7 A-H and 10).

Tract t. 35: From ventrolateral boundary of neuropile. Joins connectives at posterior end of ganglion (Fig. 7 A-H and 6).

Tract t. 36: From connectives at anterior end of ganglion. Runs along dorsolateral boundary of neuropile and is finally lost in it (Fig. 7 A-E).

Tract t. 37: From connectives at anterior end of ganglion. Runs through ganglion longitudinally, and joins connectives at posterior end of ganglion (Fig. 7 A-H).

Tract t. 38: From neuropile and comes from extreme tip of ganglion, almost from connectives (Fig. 7A).

Tract t. 39: From neuropile and some motor neurones. Its fibres seem to come from both pro- and mesothoracic ganglia. It forms a peripheral nerve.

As stated above the complexity of the neuropile increases as the insect reaches its final developmental stages. The fibre tracts of the thoracic ganglia of the 5th instar larva are therefore compared in Table I with the tracts of the corresponding thoracic ganglia of the adult.

ABBREVIATIONS USED IN FIGURES

a.n., Association neurone; d.gl., Dividing glial cell; gv.b., Glomerular body g.m.c., Ganglion motor cell; gl.I., Type I glial cell; gl.II., Type II glial cell; gl.III., Type III glial cell; gl.IV., Type IV glial cell; It. 1, It. 2, Larval thoracic fibre tract; m.n., Motor neuron; nl., Neurolamma; np.,Neuropile; pkn., Pyknotic cell; pm., Perineurium; t. 1, t. 2, adult prothoracic ganglion fibre tracts; tr., Trachea; tr. mt., Tracheal matrix; tr. mt. c., Tracheal matrix cell.

Table 1. Comparison of fibre tracts of the prothoracic ganglion of 5th instar larva and adult.

Tracts in the 5th instar larvae	Comparable tracts in the adult	Points of similarity
1. It. 1,2,3,4, 10,16,17,23, and 24 (Fig. 2 A, D and E-H)	t. 1,2,3,12,17,18,19,28 and 29 (Fig. 11 A,B,D,E,G and H)	These arise from corresponding groups of neurones and run the same course in the two stages.
2. It. 2 and 22 (Fig 2 B,C,E, and G)	t. 5 and 27 (Fig 7B)	These arise from corresponding groups of neurones and probably all contribute to the peripheral nerves.
3. It. 7 (Fig. 2B)	t. 8 (Fig. 7B)	Both arise from ventrolateral association neurones and form a commissure.
4. It. 8 (Fig. 2G)	t. 15 (Fig. 7D)	Both arise from motor neurones somewhat ventrolateral in position and run the same course.
5. It. 9 (Fig. 2B and C)	t. 11 (Fig. 7C)	Both arise from ventral group of association neurones and then break up in the lateral glomerular body.
6. It. 11 (Fig 2D)	t. 16 (Fig. 7D)	Both arise from lateral group of motor neruones and run the samse course.
7. It. 12 (Fig. 2E)	t. 23 (Fig. 7E)	Both arise from corresponding groups of neurones and form a commissure.
8. It. 13 (Fig. 2E)	t. 22 (Fig. 7E)	Both arise from lateroventral groups of motor neurones and form a commissure.
9. It. 14 (Fig. 2E)	t. 21 (Fig. 7E)	It. 14 might be comparable to t. 21 but where It. 14 arises from association neurones only, t. 21 originates from both motor and association neurones. The tracts run the same course.
10. It. 21 (Fig. 2G)	t. 31 (Fig. 7H)	Both arise from lateral groups of association neurones and run the same course.
11. It. 20 (Fig. 2F)	t. 26 (Fig. 7F)	Both arise from correspinding groups of motor neurones and run the same course.
12. It. 19 (Fig. 2F)	t. 30 (Fig. 7H)	Both arise from ventrolateral groups of association neurones and run the same course.
13. It. 18 (Fig. 2G and H)	t. 32 (Fig. 7H)	These two might be comparable but It. 18 originates only from association neurones whereas t. 32 arises from both motor and association neurones. Their course is the same in both stages.

The longitudinal tract It. 28-32 (Fig. 22A - H) of the larval prothoracic ganglion are similar to t. 33-37 (Fig. 23A-H) of the adult.

It. 5, 15 and 25 of the larva (Fig. 7A F-H) have no counterparts in the adult protheracic ganglion, while t. 4, 6, 7, 9, 10, 13, 14, 20, 24 and 25 (Fig. 7 A-D and F) have no equivalents in the larval prothoracic ganglion.

REFERENCES

ALI, F. A. 1973. Postembryonic changes in the central nervous system of *Pieris brassicae* (L) (Lepidoptera: Pieridae) *Trans. R. Ent. Soc. Lond,* **124**: 463-498.

ALI, F. A. 1980. Some quantitative aspects of the metamorphosis of the central nervous system of *Pieris brassicae* (L) *Pakistan J. Zool*; **12**: 99-114.

ALI, F. A. 1991. Histological changes occuring in some of the ganglia of the ventral nerve cord of *Pieris brassicae* (Linn.) (Pieridae: Lapidoptera). *Punjab Univ. J. Zool*; **6**: 19-29.

SHANKLAND, D. L. AND FRAZIER, J. L. 1985. In: Fundamentals of Insect Physiology ED. (M. S. Blum). 253-286. John Wily and Sons.

ELZINGA, R. A. 1988. Fundamentals of Entomology. Prentice-Hall, New Jersey. pp. 442.

MAYNARD, D. M. 1962. Organization of neuropile. *Am. Zoologist,* **2**: 79-96.

RICHARDS, O. W, AND DAVIES, R. G. 1977. Imm's General Textbook of Entomology. Vol. 1. (10th Ed.) Chapman & Hall Ltd. London.

WIGGLESWORTH, V. B. 1957. The use of Osmium in the fixation and staining of tissues. *Proc. Royl. Soc. Lond.* **147**: 185-199.

WIGGLESWORTH, V. B. 1959. The histology of the nervous system of an insect, (Hemiptera). i. The peripheral nervous system. ii. The central ganglia. *Q. J. Microse, Sci.*, **100**: 285-296; 299-313.

(Received: January 16, 1993)

Punjab Univ. J. Zool., Vol. 8, pp. 37-40, 1993.

A CONTRIBUTION TO THE FISHES OF THE KURRAM AGENCY, PAKISTAN

M. R. MIRZA, ISLAM ALI AND M. N. JAVED

Department of Zoology, Government College Lahore, Pakistan

Abstract: The fish fauna of the river Kurram and its tributaries in the Kurram Agency has been discussed. *Labeo calbasu* (Hamilton), *Racoma labiata* McClelland, *Shistura punjabensis* (Hora), *Glyptothorax kashmirensis* Hora and *G. naziri* Mirza and Naik have been recorded for the first time from this river. *Tor putitora* (Hamilton) and *Cyprinion watsoni* (Day), previously recorded from Kurram Agency, have not been collected.

INTRODUCTION

The Kurram Agency lies at an altitude of 3000 feet at Chappary and 7830 feet at Trymangal. These mountains include Koh-i-Siah and Charmaghaz. Parachinar, the headquarter of Kurram Agency, is situated at a distance of 116 miles from Kohat.

The Kurram river originates in the Koh-i-Siah in Afghanistan. It enters the Kurram Agency at Kharlachi and leaves at Chappary. The current of water is rapid and the bed is rocky and stony. The water temperature usually is between 11-17 °C and pH 6.5. Water is almost clear but it becomes muddy during rains.

The collection of specimens was done from the Kurram river. The following places were selected for collection.

Kharlachi: December 1991, January and March 1992, temperature 12 °C; pH 6.5

Shangak: December 1991, January, March and April 1992, temperature 12 °C; pH 6.5

Ahmadzai: March and April 1992, temperature 11 °C

Agrah: October and November 1992, temperature 11 °C

Sultan: November and December 1992, temperature 13 °C

Lower Alizai: March and April 1992, temperature 17 °C

SYSTEMATIC ACCOUNT

Cohort	:	EUTELEOSTEI
Superorder	:	OSTARIOPHYSI
Order	:	CYPRINIFORMES
Family	:	CYPRINIDAE

1. *Aspidoparia morar* (Hamilton)

Only one specimen of this species, 72mm long, was captured from Kharlachi.

2. *Barilius pakistanicus* Mirza and Sadiq

It is a small-sized laterally compressed fish. The description of this fish is also based on the single specimen 59.5 mm long from Kurram river at Kharlachi.

3. *Garra gotyla* (Gray)

This is a small-sized fish of fast streams. Characteristic feature is the presence of a deep, transverse groove in front of nostrils, spiny tubercles and a vestibule and sucker along the oral side of head for the attachment to rocks. Five specimens were captured from the Kurram river at Agrah, the longest being 130 mm.

4. *Crossocheilus diplocheilus* (Heckel)

It is a small-sized fish in which upper lip forms a vestibulum. It is common throughout Pakistan and Azad Kashmir. Four specimens including the longest 132.25 mm were collected from Kurram river at Agrah.

5. *Schizocypris brucei* Regan

There are three specimens of this species from Agrah. The longest specimen measures 11.5 cm in total length and 10.2 cm in standard length.

6. *Schizothorax plagiostomus* Heckel

It is a medium-sized fish with long cylindrical body. Lower lip in this fish forms a papillated plate. This fish is common in fast moving waters. Eleven specimens are present in our collection captured from Kurram river at Sultan and Shangak. The maximum length of the specimen is 172 mm.

7. *Racoma labiata* McCelland

R. labiata is a fish with streamlined body and is an active swimmer. lower lip in this fish forms a continuous fold with trilobed edges. Nine specimens were captured at Shangak and Sultan with maximum length of 221 mm.

Family: NOEMACHEILIDAE

8. *Schistura curtistigma* Mirza and Nalbant

It is also a small-sized fish with incomplete lateral line and visible scales. This fish is endemic to Pakistan. Only two specimens were captured from Kurram river at Shangak. The largest specimen is 81.5 mm long.

9. *Schistura punjabensis* (Hora)

This small-sized fish is of no food value. Only one female fish 67mm long is present in our collection which was captured at Sultan. It is a new record from Kurram river.

Order: SILURIFORMES
Family: SISORIDAE

10. *Glyptothorax naziri* Mirza and Naik

A mall fish with a U-shaped thoracic sucker. Three specimens were captured from Kurram river at Sultan village. Of these the longest specimen is 94 mm. It is a new record from the river Kurram.

11. *Glyptothorax stocki* Mirza and Nijssen

This fish is endemic to Pakistan and Azad Kashmir. It is characterized by the presence of an oval sucker. The longest specimen in our collection is 100.25 mm.

12. *Glyptothorax kashmirensis* Hora

Four specimens with maximum length of 136.5 mm were captured from Kurram river at village Sultan. This is the first record of this species from Northwest Frontier Province. It is also being recorded for the first time from Pakistan.

13. *Gagata cenia* (Hamilton)
Four specimens with maximum length of 81 mm were captured at Sultan village and Ahmadzai.

DISCUSSION

The fishes of the river Kurram were listed by Mirza *et al.* (1989). The present report includes some new records, *i.e. Racoma labiata, Shistura punjabensis, Glyptothorax naziri* and *Glyptothorax kashmirensis* from the Kurram river. In addition one specimen of *Labeo calbasu* was captured from Kurram Garhi near Bannu. It is also a new record for the river Kurram. This specimen (365 mm long) is dirty brown in colour, quite different from the normal slaty colour of this species.

The following species previously recorded from the Kurram Agency are not represented in our collection:

1. *Tor putitora* (Hamilton)
2. *Cyprinion watsoni* (Day)

The mahseer (*Tor putitora*) was widely distributed in the river Kurram in the past. Its absence from the present collection is alarming. This is a game fish of great economic importance. The causes of its disappearance from the Kurram Agency should

be studied. The other species was recorded from Parachinar by Ahmad and Mirza (1963). Its absence is also noteworthy.

Acknowledgements

We are indebted to Dr. Azizullah, Head of Zoology Department, Government College, Lahore for providing facilities during this research work. We are also obliged to the Fishery Officer of Bannu for helping in collection.

REFERENCES

AHMAD, N. AND MIRZA, M.R., 1964. Some fishes from the Kurram river near Parachinar. *Pakistan J. scient. res.*, **16**: 44-46.

MIRZA, M.R., BAKR, K.A. AND SAEED, T.B., 1989. Fishes of the river Kurram in Afghanistan and Pakistan. *Sci. Int.* (Lahore), **1**: 376-377.

(Received: December 27, 1992)

Punjab Univ. J. Zool., Vol. 8, pp. 41-44, 1993.

EFFICACY OF DIAZINON AGAINST MANGE IN SHEEP

MAQBOOL AHMAD, SHABBIR AHMAD AND FIRDAUSIA AZAM ALI

Department of Clinical Medicine and Surgery, University of Agriculture, Faisalabad, (M.A.) Veterinary Research Institute, Lahore (A.S.), Department of Zoology, University of the Punjab, Lahore (F A.A.), Pakistan

Abstract: Efficacy of diazinon (Neocidol) was evaluated through spray application of 0.15% aqueous solution against naturally infested sarcoptic, psoroptic and chorioptic mange in 46 sheep. It cured 44, 87.5 and 100% cases of chorioptic mange after first, second and third applications, respectively. Efficacy against psoroptic mange was 40, 80 and 93% percent respectively while the efficacy against sarcoptic mange was found to be 33 and 100% after two consecutive applications. No side effects were observed after treatment with diazinon. The untreated control group remained positive for mange throughout the course of treatment.

INTRODUCTION

Sheep is an economically important animal to a vast number of landless rural population and marginal farmers of Pakistan. Out of 1.100 million sheep in the world, 35.41 million sheep are located in Pakistan (FAO, 1990). Among the many diseases to which these animals are prone to suffer, mange is the commonest. It is a dermatological problem caused by mites belonging to order Astigmata of class Arachnida. *Sarcoptes scabiei* (Family: Sarcoptidiae), *Psoroptes ovis* and *Chorioptes ovis* (Family: Psoroptidae) are the mites mostly responsible. (Sweatman, 1958; Service, 1980). These parasites pierce the skin to suck lymph and may also feed on the young epidermal cells. The iching and scratching causes inflammation of the skin and is accompanied by an exudate which coagulates and forms crusts. Excessive keratinization and proliferation of connective tissue thickens and wrinkles the skin also causing the hair to fall out. This disease is prevalent in cold weather but spreads slowly during summer months (Rathore and Lodha, 1973). The impact of the mange in sheep is on their general health, growth and productivity. It is known to cause 30 percent loss in weight (Kirkwood, 1980) besides the loss in wool. Keeping in view the importance of this malady, the affected sheep were treated with 0.15% solution of diazinon (Neocidol) in order to test its efficacy.

MATERIALS AND METHODS

A total of 46 sheep naturally infested with Psoroptic, Chorioptic and Sarcoptic mange at Muridke and Sadoke near Lahore were used in this study. Of these, 13 animals were kept as untreated control. All these animals were kept under similar feeding and environmental conditions throughout the course of the treatment. The study was conducted during late winter months.

Collection and examination

Besides the clinical signs, skin scrapings collected in 10 percent potassium hydroxide were examined for mites and their eggs. The mites were identified from their

characteristic morphology (Maharat and Ruesel, 1978; Soulsby, 1982).

Psorotic mange

Fifteen animals (6 months to 5 years) naturally infested with *Psoroptic ovis* were used in this study. In 12 animals lesions were distributed all over the woolly parts of the body. These animals were divided randomly into two groups *i.e.* A1 and A2. First group (A1) contained 12 animals while second group (A2) comprised of 3 animals which served as untreated control.

Chorioptic mange

Sixteen animals naturally infested with *Chorioptic ovis* ranging between 7 months to 4 1/2 years were used in this study. Lesions were distributed on the hind legs between toes and on the scrotum of rams. These animals were randomly divided into two groups *i.e.* B1 and B2. Group B1 comprised of 11 animals while group B2 consisted of 5 animals which acted as untreated control.

Sarcoptic mange

Fifteen animals with age ranging between 6 months to 5 years were used in this study. These animals were naturally infested with Sarcoptic mange. Lesions were distributed on non-woolly parts of the body especially head and face. These were divided randomly into two groups C1 and C2. Group C1 comprised of 10 animals while group C2 consisted of 5 animals which served as unntreated control.

Acaricide used

Diazinon (*Neocidol-R*, Ciba Geigy) at a concentration of 0.15 percent in water was used. Three spray applications were given at an interval of 7 days on each occasion. Building and bedding were also sprayed with the same concentration of diazinon for making the surroundings free from mites. General safety instructions were also followed as desribed by USDA (1980).

Assessment criteria

All the mange infested sheep in group A1, B1 and C1 were sprayed with diazinon at a concentration of 0.15 percent and were constantly observed daily for clinical improvement and for frequency of rubbing or scratching of the body. The skin scrapings were examined on 7th, 14th and 21st day of treatment. The scrapings were processed as per technique used by Magee (1974). Negative skin scrapings, subsidense of lesions, stopping of itching and smoothing of skin surface were taken as criteria to evaluate the efficacy of diazinon.

RESULTS AND DISCUSSION

The results of the skin scrapings observed microscopically at different days before and after treatment with diazinon are presented in Table-1.

Table 1: Efficacy of diazinon against Mange

Type of mange	Animals cured (%) after treatment		
	1st (n=15)	2nd (n=15)	3rd (n=15)
Psoroptic	40	80	93
Chorioptic	44	87.5	100
Sarcoptic	33	100	-

The lesions showed signs of healing within 7-15 days. Thereafter, rubbing and scratching of the body stopped completely, while mild lesions were noticed in one animal treated for Psoroptic mange. On day 21 no live mites or eggs were present in the skin scrapings except in one animal. No side effects were observed after spraying the animals infested with Psoroptic, Chorioptic and Sarcoptic mange with 0.15 percent watery solution of diazinon. The lesions in the untreated sheep (A2, B2, C2) become more extensive, itchiness continued unabated annd they remained positive for mange throughout the course of study.

The present results on the efficacy of diazinon against Psoroptic, Chorioptic and Sarcoptic mange of sheep confirmed the earlier observations (Kirkwood and Quick, 1981; Milic *et al.*, 1985; Rosa and Lukovick, 1970; Blanchflowewr *et al.*, 1990). Thus diazinon can be recommended for safe treatment of mange in sheep with no side effects.

REFERENCES

BLANCHFLOWER, W.J., MCCRACKEN, R.J., RICE, D.A. AND CLEMENT, A., 1990. Survey of levels of Propetamphos and Diazinon used to control sheep scab in Northern Ireland. *Vet. Rec.*, **126**: 263-265.

FAO (1990): *FAO yearbook: Production*. Vol. 44. Statistics Series No. 99. Rome, 1991.

KIRKWOOD, A.C., 1980. Effect of *Psoroptes ovis* on the weight of sheep. *Vet. Rec.*, **107**: 469-470.

KIRKWOOD, A.C. AND QUICK, M.P., 1981. Diazinon for the control of sheep scab. *Vet. Rec.*, **108**: 279-280.

MAGEE, J.C., 1974. *Biology and control of the itching mites Sarcoptes scabiei in swine. Ph.D. Dissertation*. Iowa State Univ. Ames, USA.

MAHARAT, W.S. AND RUSSEL, L.D., 1978. *Clinical Parasitology*, 5th ed. The Iowa State Univ. Press, Iowa.

MILIC, L., SAJTI, G., MUATOV, L., ODRI, S. AND DERMANOVIC, D., 1985. Results of using Ektanon (Diazinon) for controlling sheep scab. *Veterinarski Glasnik*, **39**: 1055-1059.

RATHORE, M.S. AND LODHA, K.R., 1973. observations on Sarcoptic mange in camel in Rajasthan.

Incidence and intensity. *Indian Vet. J.* **50**: 1083-1088.

ROSA, W.A.J. AND LUKOVICH, R., 1970. Response of different *Psoroptes ovis* to dips containing Lindane or Diazinon. *Revta. Med Vet.*, **51**:127-129.

SERVICE, M.W., 1980. *A guide to Medical Entomology,* The MacMillan Press Ltd. London, pp. 226.

SOULSBY, E.J.L., 1982. *Helminths, Arthropods and Protozoa of Domesticated Animals*. 7th ed. Baillier Lindall. London.

SWEATMAN, G.K., 1958. On the life history and validity of the species in Psoroptes. *Can. J. Zool.*, **36**:110.

USDA 1980. Guideline to the control of insects and mites, pest for toad fibres, feed ornamental, Livestock, households, forests and forest products. *Agric. Handbook*. No. 571.

(Received: December 11, 1992)

Punjab Univ. J. Zool., Vol. 8, pp. 45-52, 1993.

GRAM NEGATIVE BACTERIUM CARRYING TRANSFERABLE IRON RESISTANT MARKER AND SOME FACTORS AFFECTING TRANSFER FREQUENCY

SHAHIDA HASNAIN AND ANJUM NASIM SABRI

Department of Botany, University of the Punjab, Quaid-e-Azam Campus, Lahore-54590, Pakistan.

Abstract: From the effluents of Comfort Chemicals, a bacterium, AnFe-6, which could deter $FeCl_3$ up.to 2500 μg ml^{-1} was isolated. It had circular, convex, entire, off-white opaque colonies. Cells of AnFe-6 were Gram-negative, motile, pleomorphic, rods. It confers resistance to antibiotics, ampicillin (Ap) and chloramphenicol (Cm) and other metallic salts (Ba^{+2}, Zn^{+2}, Ni^{+2}, Co^{+2}, Cu^{+2}, Hg^{+2}, Mn^{+2} and Sn^{+2}). However it was sensitive to kanamycin (Km), tetracycline (Tc) and streptomycin (Sm) antibiotics and Hg^{+2} salt. It favours neutral to alkaline pH (7.0 - 7.5) for its growth. It could respire anaerobically, had oxidase and urease activity. AnFe-6 could produce acid from arabinose, glucose and rhamnose. The isolate gave positive results for ONPG, Lysine decarboxylase, gelatin hydrolysis, ornithine decarboxylase and arginine dihydrolase tests. On the basis of these characters the isolate could be affiliated to family Vibrionaceae. By the gel electrophoresis of total cell lysate, no plasmid band was detected in this strain, but conjugation experiments revealed the presence of transferable iron resistance marker, genetic determinant for which has to be established. Minimum of 8 hours were required to establish mating aggregates. Conjugal transfer of genetic element carrying iron resistant marker was affected by donor and recipient proportion, temperature as well as pH of mating mixture. 25 °C inconcomitant with pH8 showed synergistic response for conjugal transfer.

INTRODUCTION

Ever proliferating industrialization is resulting in the augmented organic and inorganic waste deposits in the environment, which are detrimental to biotic life (Gilberg, 1974). Iron is one of the routinely used raw material in many industries, which in excessive amount is carcinogenic and mutagenic to animals and human (Lehninger, 1982). In microorganisms it has dual role. It behaves as an essential micronutrient (Neilands, 1981; Holmes and Russells, 1983) but it also has certain inhibitory effects, *i.e.* inhibition of pigment production in *Pseudomonas aeruginosa* (Palumbo, 1972), repression of phenolic acid synthesizing enzyme in *Bacillus subtilis* (Downer *et al.*, 1970), diphtheria toxin production in *Corynebacterium* diptheriae (Edward and Seamer, 1960), inhibition of extracellular protein synthesis in *Aeromonas hydrophilia* (Pansare *et al.*, 1985) and inhibition of protein synthesis in *E. coli* (Klebba *et al.*, 1982). It is also required for the synthesis of porphyrin proteins (Lehninger, 1982).

However some bacteria play an important role in regulating iron concentration with the help of high affinity uptake system (Kloepper *et al.*, 1980; Scher and Baker, 1982; Schroth and Hancock, 1982). They also form chelating agent, which when associated with plant roots increase their yield (O'Sullivan and Gara, 1982). Due to presence of intracellular traps (in bacteria) for iron removal, bacteria cut down the toxic effect of metal. Resistance to heavy metal toxicities is genetically controlled (Mergeay, 1991). Hence it is of interest to study the metal resistant bacteria in general and genetic mechanisms involved in the resistance in particular. In the work described here iron

resistance bacterium which harbour either conjugative megaplasmid/s or insertion sequences is being reported.

MATERIALS AND METHODS

Water sample was obtained from the effluent of Comfort Chemicals. It was blackish in colour, odourless with pH 7 and had electric conductivity 0.65mS/m. It also contains metals in the following proportions, Cu, 2.75; Zn, 0.03; Mn, 0.00; Na, 250; K, 540; Cd, 0.24; Fe, 1.00; Ca, 80Ig ml^{-1}). 50μl of water sample was plated on selective plates of nutrient-agar (25μg ml^{-1}, $FeCl^3$) as well as on mineral agar plates (Nies *et al.*, 1987). Bacterial growth was obtained after 24 hours of incubation at 37°C. Colonies were purified and subjected to elevated level of $FeCl^3$ *i.e.*, up to 2500μg ml^{-1}. Gerhardt *et al.*, (1981) was followed for morphological, physiological and few biochemical tests (oxidation, fermentation urease, catalase, methyl red tests). Further, 21 biochemical tests were performed by using QTS-20 and cytochrome oxidase strips (Desto Laboratories, Karachi). AnFe-6 was also checked on agar medium (nutrient agar - Gerhardt *et al.*, 1981) for its sensitivity behaviour against antibiotics such as Ap (300 μg ml^{-1}), Km (50 μg ml^{-1}), Cm (5 μg ml^{-1}), Tc (20 μg ml^{-1}) and Sm (500 μg ml^{-1}). The susceptibility/resistance to different metallic salts ($HgCl^2$, $NiCl^2$, $SnCl^2$,$BaCl^2$, $CoCl^2$ and $ZnCl^4$) was checked on different agar-plates containing 25 μg ml^{-1} - 600 μg ml^{-1} of each above metal salts separately. Antibiotics and metallic salts were aseptically added in the agar medium after autoclaving. Spore-forming ability was authenticated by the method of Moir (1981). Total cell lysate method of Thomas (1984) was used for the detection of plasmid. Willett's (1984) broth mating technique was used for conjugation experiment. *E. coli* K-12 strain CSR603 [*recA1 pharl* derivative of AB1886 (*thr-1 leu-6 lacY1 galK2 ara-14 xyl-5 mtl-1 proA His-4 str-31 tsx-33 sup-37 uvxa6*)] was used as recipient. The varying effect of time (1,2,4,6,18,24 hours), donor to recipient ratio (1-10), temperature (25, 28, 32 and 37°C), pH (6.5 - 8.5) and combined effect of temperature and pH, on conjugation efficiency of a plasmid was also studied. Transconjugants were scored by plating conjugation on double selective plates (Sm and $FeCl^3$, 500μg ml^{-1}). For studying different pHs, overnight cultures of recipients and donors, grown in LB (Sambrook, 1989) with different pH (6.5 - 8.5) were used for preparing conjugation mixture. Transfer frequency was calculated by plating 10^3 dilution of recipient on nutrient agar plates containing Sm 500μg ml^{-1}. Transfer frequency was converted to log scale.

RESULTS AND DISCUSSION

A strain AnFe-6, which could deter $FeCl^3$ up to 2500μg ml^{-1} in the medium, was isolated from Comfort Chemicals. It had light yellow, opaque, circular and entire colonies, which ranged from 3 - 3.5 mm in size. It had gram negative, pleomorphic, motile cells. With the increased concentration of iron, the growth rate as well as cell size decreased. Which is due to decrease in cell division, elongation and metabolism (Zevenhuizen *et al.*, 1979). Less survival at higher concentrations may be due to presence of genetic adaptation (Brook, 1978). Kaneko *et al.*, (1987) also observed inferior bacterial growth (*Streptococcus lactis*) in the presence of Cu, Co, Mn, Mo, Fe, Al and Zn ions. The AnFe-6 could also grow on media other than nutrient agar, *i.e.*, L agar (Sambrook, 1989), potato dextrose, yeast extract-Fe-acetate media (Henert, 1986).

But it showed very weak growth on McConkey's agar. It could endure other metals such as $BaCl^2$ and $CoCl^2$ (100μg ml^{-1}); $ZnSO^4$ (500μg ml^{-1}) $NiCl^2$ and $SnCl^2$ (25μg ml^{-1}); $CuSO^4$ and $MnSO^4$ (200μg ml^{-1}), but showed sensitive behaviour toward $HgCl^2$ (25μgml^{-1}). This showed that AnFe-6 could tolerate lower levels of other metal in the media. These resistance markers *i.e.* Cu, Zn, Co, Ni, Sn, Ba, Mn, and Fe may be existed on the same genetic determinant or they may be present on different DNA molecules. AnFe-6 showed different tolerance level for different metallic salts, variation in resistance to different metallic salts may be due to differences in their chemical forms, specification, reactivity, solubility, pH and complex formation (Hughes and Poole, 1991). Previously we have reported plasmid bearing iron resistant strains from industrial effluents (Malik *et al.,* 1991). Those strains also showed pleotropism for metal resistance. AnFe-6 was sensitive to Km, Sm, and Tc, but resistant to Ap and Cm. Here our results are inconcomitant with the previously isolated strains AnFe-3, AnFe-4 which were Km and Sm sensitive (Malik *et al.,* 1991). All the isolates have one character common that they were sensitive to Sm. Previously isolates from hospital patients showed resistance to both heavy metals and antibiotics (Silver, 1985; Ahmad and Yadava, 1988), while Silver (1985) have described isolates from industrial effluents (*E. coli*) which showed only antibiotic resistance. Both resistances *i.e.*, antibiotic and metal resistances, have been reported either to be chromosomally coded (Wang *et al.,* 1989) or plasmid encoded (Summer and Silver, 1978; Laddaga *et al.,* 1987; Silver and Mirsa, 1988). In case of AnFe-6 two different type of resistances (antibiotic or metal) are conferred by the same or different genetic elements remained to be determined. The optimum pH range for the growth of this strain was 7-7.5. It could also tolerate alkaline pH (8-8.5) but under acidic conditions (6-6.5) rather poor growth was observed. pH is an important factor in determining growth of bacterial strain because reactivity of chemical is pH dependent. Fe-salts are more soluble under acidic conditions as compared to neutral and alkaline conditions (Neiland, 1982). The most plausible explanation for the poor growth of this strain under acidic conditions might be due to the toxicity of iron in dissolved state. The AnFe-6 had urease and oxidase activity, could respire anaerobically (OF positive) and hydrolyze gelatin. It had the ability to produce acid from arabinose, rhamnose and glucose. It gave positive results for lysine decarboxylase. ONPG (W+), arginine hydrolase (W+) and ornithine decarboxylase (W+). While for sodium citrate, sodium malonate, H^2S production, urea hydrolysis, tryptophan deaminase, indole, acetoin (VP), nitrate reduction, acid from maltose, sucrose, manitol, sorbitol and inositol, catalase, tetrazolium, methyl red tests it gave negative results. On the basis of above biochemical test it could be affiliated with Vibrionaceae.

By gel electrophoresis of total cell lysate no plasmid band was detected. But positive results with conjugation experiments revealed the presence of an autonomous genetic element in AnFe-6, independent of the main host chromosome or the existence of transposons. Megaplasmids (cointegrate plasmids) cannot be detected by total cell lysate method (Thomas, 1984). Transposons, cointegrate plasmids as well as megaplasmids can mediate their transposition (transposons) or promote their own transfer (plasmids) (Bennett *et al.,* 1988). Both plasmids conferring resistance to metals (Mergeay, 1991) and transposons-governed resistance to mercury (Diels *et al.,* 1987) have been reported previously. Conjugation mixture was plated after different time intervals for the selection of iron-resistant marker of donor and Sm marker of recipient. Results of

conjugation, *i.e.*, transconjugants were obtained after 8 hours of mating, thus minimum of 8 hours were required for the transfer of either megaplasmid from the donor or iron-resistant marker of AnFe-6 strain. Maximum transconjugants were scored after 18 hours of mating after which decrease in transfer frequency was recorded (Fig. 1a). It is in accordance with our previous reports of iron-resistant bacteria, *i.e.*, AnFe-3 and AnFe-4 (Malik *et al.*, 1991) where plasmid transferred after 8 and 6 hours of mating respectively. Malik *et al.*, (1991) have also reported transfer of iron-resistant plasmids after two hours of mating in two different strains. The transfer of plasmid not only depends upon the environmental factor but also upon the nature of plasmid. Different plasmids transfer their marker at different times (Rochelle *et al.*, 1989; Adiga *et al.*, 1961). Combination of time and temperature are important in determining the transfer frequency (Rochelle *et al.*, 1989; Fontaine and Hoadley, 1976). For studying the effect of other factors on conjugal transfer of plasmid/cointegrate plasmid/ transposon, mating mixture was plated after 18 hours. As regards the donor to recipient ratio, maximal transfer of genetic determinant carrying iron-resistant marker was at ratio 2-5 (Fig. 1b) suggesting that these were the best ratios for making aggregants. With the further increase in the proportion of donor a slight decrease in the transfer frequency was observed. A conjugation efficiency was very low at donor to recipient proportion 1:1, which means for making mating aggregates more donor bacteria/recipient bacteria are required. According to Rochelle *et al.*, (1989) it again depends upon the type of plasmid, because their results showed that PQMI, PQM3 and RPI transferred at donor: recipient 0.4-30. While Gauthier *et al.*, (1985) reported that Hg-resistant bacteria transferred maximal at donor: recipient ratio 1. Malik *et al.*, (1991) have described varying results for the transfer of different iron-resistance plasmids. pSH1223, pSH1224, pSH1225 could transfer at wide range of this ratio with maximum frequency at donor: recipient ratio 10. While pSH1222 showed maximal transfer at 1 and 10 ratio. Temperature and pH of the medium plays an important role in the transfer of any plasmid. Genetic determinant in AnFe-6 promote its maximal transfer at 25°C and with the increase in temperature (28°C) an abrupt decrease in this ability was observed but with further rise in temperature, transfer efficiency was increased, which was almost the same at 30 and 37°C (Fig. 1c). Malik *et al.*, (1991) have also observed maximal transfer of two plasmids at 25°C. As far as pH is concerned, iron resistant marker determinant in AnFe-6 was transferred with highest frequency at 7.5 (a bit alkaline medium, Fig. 1d) and this is the pH where this strain showed best growth. In addition to strains, nature of plasmid and pH of the medium can also affect conjugal transfer. Optimum pH for plasmid transfer under antibiotic and heavy metal stress ranged from 6.5 to 7.5 (Rochelle *et al.*, 1989; Gauthier *et al.*, 1985; Singleton and Anson, 1983).

Since temperature and pH have synergistic effect on plasmid transfer (Rochelle *et al.*, 1989), combined effects of varying pH and different temperature on conjugal transfer of this genetic determinant was also investigated (Fig. 1e). In this, pH8 along with temperature 25°C and 32°C; 28°C with pH 6.5; 37°C in conjucture with pH 7.5 showed maximal transfer. Maximum synergism in transfer frequency was recorded at 25°C with pH8 (Fig. 1e). While synergistic effects of temperature and pH on conjugal transfer of iron-resistant marker were also observed in other cases. At 32°C with pH8, and 37°C along with pH7.5, increase in transfer frequency of this plasmid from the overall transfer frequency, at these factors, of this genetic determinant was observed (Fig. 1e). Thus pH of the medium and temperature for mating exhibited synergism in

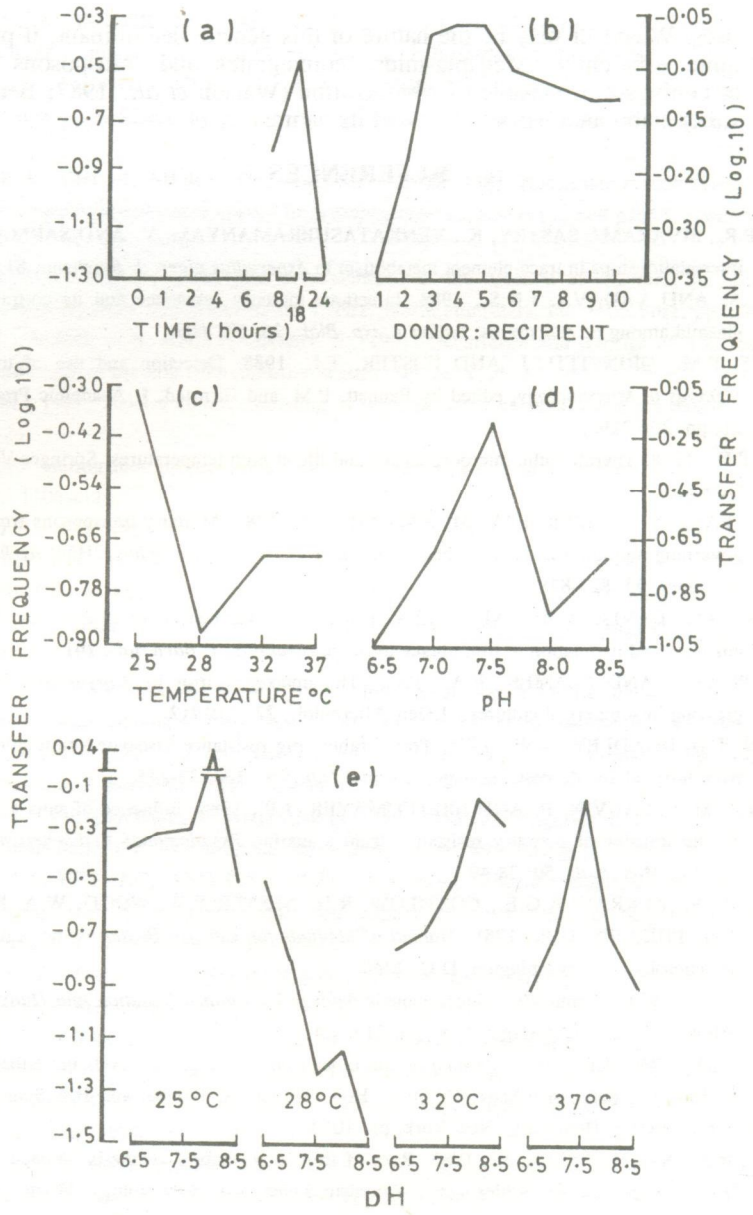


Fig. 1: Effects of various factors on the conjugal transfer of iron-resistant genetic determinants of AnFe-6 to *E. coli* K12 strain. a) mating time; b) donor to recipient ratio; c) different temperatures; d) varying pH; e) combined effects of pH and temperature.

most of cases. Whatever may be the nature of this genetic determinant, it promoted its transfer quite efficiently. Megaplasmids, cointegrates and transposons have been reported to conjugate or capable of transposition (Watson *et al.*, 1987; Bennett *et al.*, 1988). Further elaborated work will reveal its nature.

REFERENCES

ADIGA, P.R., SIVARAMA SASTRY, K., VENKATASUBRAMANYAM, V. AND SARMA, P.S., 1961. Interrelationships in trace-element metabolism in *Aspergillus niger*. *J. Biochem.*, **81**: 545-550.

AHMAD, S. AND YADAVA, J.N.S., 1988. Infectious mercury resistance and its co-transfer with R-plasmid among *E. coli* strains. *Indian J. exp. Biol.*, **26**: 601-605.

BENNETT, P.M., GRINSTED, J. AND FOSTER, T.J., 1988. Detection and use of transposons. I: *Methods in Microbiology*, edited by Bennett, P.M. and Grinsted, J. Academic Press, U.K. Vol. 21, pp. 206-229.

BROOK, T.D., 1978. Thermophilic microorganisms and life at high temperatures, Springer Verlag: Heidel, Germany.

DIELS, L. FAELEN, N., MERGEAY, M. AND NIES, D., 1987. Mercury transposons from R-plasmids governing multiple resistance to heavy metals in *Alcaligenes eutrophus* CH34. *Arch. Int. Physiol. Biochem.*, **93**: 827-828.

DOWNER, D.N. DAVIS, W.B. AND BYERS, B.R., 1970. Repression of phenolic acid synthesizing enzymes and its relation to iron uptake in *Bacillus subtilis*. *J. Bacteriol.*, **101**: 181-187.

EDWARDS, D.C. AND SEAMER, P.A., 1960. The uptake of iron by *Corynebacterium diphtheria* growing in submerged cultures., J.Gen. Microbiol., **22**: 705-712.

FONTAIN, T.D. HOADLEY, A.W., 1976. Transferable drug resistance associated with coliforms isolated from hospital and domestic sewage. *Hospital Lab. Sci.*, **13**: 232-245.

GAUTHIER, M.J., CAUVIN, F. AND BREITTIMAYER, J.P., 1985. Influence of salts and temperature on the transfer of mercury resistance from a marine *Pseudomonas* to *Escherichia coli*. *Appl. environ. Microbiol.*, **50**: 38-40.

GERHARDT, P. MURRAY, R.G.E., COSTILOW, R.N. NESTER E.W. WOOD, W.A. KRIEG, N.R.. AND PHILLIPS, G.B., 1981. *Manual of Methods for General Bacteriology*. American Society for Microbiology, Washington, D.C. 2006.

GILBERG, B.O., 1974. Chemically induced genetic damage In: *Against Pollution and Hunger*. (ed. A.M. Hilton), Universitets forlaget, U.K. pp. 213-214.

HENERT, H.H., 1986. The genus *Siderocapsa* and other iron or manganese oxidizing eubacteria, In: *The Prokaryotes*, (eds. M.P.Starr, H. Stolp, H.G. Truper, A. Balous, and H.G.Schlegel), Springer Verlag, Berlin, Hedelberg, New York, pp. 1052.

HOLMES, R.K. AND RUSSELL, L., 1983. Role of iron in regulation synthesis of bacterial toxins. In: *Microbiology*, (ed. D. Schlesinger), American Society for Microbiology, Washington, D.C. pp. 359-362.

HUGHES, M.N. AND POOLE, R.P., 1991. Metal specification and microbial growth - the hard (and soft) facts. *J. Gen. Microbiol.*, **137**: 725-743.

KANEKO, T. SUZUKI, H. AND TAKAHASHI, T., 1987. The effect of metal ions on diacetyl production

by *Streptococcus lactis* sub sp. *diacetylactis* 3022. *Agric. biol. Chem.*, **51**(9): 2315-2320.

KLEBBA, P.E., MCINTOSH, M.A. AND NEILANDS, J.B., 1982. Kinetics of biosynthesis of iron-regulated membrane protein in *Escherichia coli*. *J. Bacteriol.*, **149**: 880-888.

KLOEPPER, J.W., LEONG, J., TEINTZE, M. AND SCHROTH, M.N., 1980. Enhanced plant growth by siderophores produced by plant growth promoting rhizobacteria. *Nature* (London)., **227**: 885-886.

LADDAGA, R.A., CHU, L. MISRA, T.K. AND SILVER, S., 1987. Nucleotide sequence and expression of the mercurial-resistance operon from *Staphylococcus aureus* plasmid p1258. *Proc. Natl. Acad. Sci. USA* 24: 5106-5110.

LEHNINGER, A.L., 1982. Vitamins and trace elements in the function of enzymes, In: *Principles of Biochemistry*, (eds. S. Anderson, and J. Fox, Worth Publishers, Inc. New York, pp. 270.

MALIK, A.N., SABRI, A.N. AND HASNAIN, S., 1991. Occurrence and transfer of iron resistance in Gram-negative bacteria from industrial wastes. *Sci. Int.* (Lahore). **3**: 321-326.

MERGEAY, M., 1991. Toward an understanding of the genetics of bacterial metal resistance. *Tibtech.*, **9**: 17-24.

MOIR, A., 1981. Germination properties of a spore coat-defective mutant of *Bacillus subtilis*. *J. Bacteriol.*, 146: 1106-1116.

NEILANDS, J.B., 1981. Microbial iron compounds. *Ann. Rev. Biochem.*, **50**: 715-721.

NEILANDS, J.B., 1982. Microbial envelope proteins related to iron. *Ann. Rev. Microbiol.*, **36**: 285-309.

NIES, D., MERGEAY, M., FRIEDRICH, B., SCHLEGEL, H.G., 1987. Cloning of plasmid genes encoding resistance to cadmium, zinc and cobalt in *Alcaligenes eutrophus* CH34. *J. Bacteriol.*, **169**: 4865-4868.

O'SULLIVAN, D.J. AND O'GARA, F., 1990. Iron regulation of ferric uptake in a flourescent Pseudomonad: cloning of a regulatory gene. *Mol. Plant Microbe Interaction.*, **3**: 86-93.

PALUMBO, S.A., 1972. Role of iron and sulphur in pigment and slime formation by *Pseudomonas aeruginosa*. *J. Bacteriol.*, **111**: 430-436.

PANSARE, A.C., VENUGOPAL, V. AND LEWIS, N.F., 1985. Fe^{+2} inhibition of extracellular protease synthesis in *Aeromonas hydrophila*. *Agric. biol. Chem.*, **49**: 2451-2452.

ROCHELLE, P.A. FRY, J.C. AND DAY, M.J., 1989. factors affecting conjugal transfer of plasmids encoding mercury resistance from pure cultures and mixed natural suspensions of epilithic bacteria. *J. Gen. Microbiol.*, **135**: 409-424.

SAMBROOK, J., FRITSCH, E.F. AND MANIATIS, T., 1989. In: *Molecular Cloning*. Cold Spring Harbor Laboratory Press, USA.

SCHER, F.M. AND BAKER, R., 1982. Effect of *Pseudomonas putida* and a synthetic iron chelator on induction of soil suppressiveness to *Fusarium* with pathogens. *Phytopathology*, **72**: 1567-1573.

SCROTH, M.N AND HANCOCK, J.C., 1982. Disease suppressive soil and root colonizing bacteria. *Science*, **216**: 1376-1381.

SILVER, S., 1985. Bacterial Transformation and Resistance, In: *Environmental and Inorganic Chemistry*, (eds. K.J. Irgolic, and A.E.Martell), VCH Publishers. Inc. Deerfield Beach, Florida, pp. 513-539.

SILVER, S. AND MISHRA T.K., 1988. Plasmid-mediated heavy metal resistance. *Ann. Rev. Microbiol.*, **42**: 717-743.

SINGLETON, P. AND ANSON, A.E., 1983. Effect of pH on conjugal transfer at low temperatures. *Appl environ. Microbiol.*, **46**: 291-292.

SUMMER, A.O. AND SILVER, S., 1978. Microbial transformations of metals. *Ann. Rev. Microbiol.*, **32**: 637-672.

THOMAS, C.M., 1984. Analysis of clones. In: *Methods in Microbiology,* (eds. P.M. Bennett, and J. Grinsted), Vol. 17, Academic Press, U.K. pp. 163-195.

WANG, Y., MOORE, M., LEVINSON, H.S., SILVER, S., WALSH, C. AND MAHLERS, I., 1989. Nucleotide sequence of a chromosomal mercury resistance determinant from *Bacillus* sp. with broad spectrum, mercury resistant. *J. Bacteriol.*, **171**: 83-92.

WATSON, J.D., HOPKINS, N.H., ROBERTS, J.W., STEITZ, J.A. AND WEINER, A.M., 1987. Recombination at Molecular Levels. In: *Molecular Biology of the Gene*: 4th edition Benjamin/Cummings Publishers, Inc. California 94025. Vol., pp. 332-338.

WILLETTS, N., 1984. Conjugation. In: *Methods in Microbiology*, (eds. P.M. Bennett, and J. Grinsted), Vol. 17. Academic Press, U.K. Vol. 17, pp. 33-59.

ZEVENHUIZEN, L.P., DOLFING, J., ESHUIS, E.J. AND SCHLOTEN-KOERSELMAN, I.J., 1979. Inhibitory effects of copper on bacteria related to free ion concentration. *Microb. Ecol.*, **5**: 139-146.

(Received: April 28, 1992)

Punjab Univ. J. Zool., Vol. 8, pp. 53-61. 1993.

EMBRYOTOXICITY AND TERATOGENICITY OF MALATHION IN MICE

ASMATULLAH, SHAHZAD A. MUFTI, ABDUL MAJEED CHEEMA AND JAVED IQBAL

Department of Zoology, University of the Punjab, Quaid-e-Azam Campus, Lahore-54590. Director General, Pakistan Museum of natural History, F-7 Markaz, Islamabad (SAM).

Abstract: An organophosphorus insecticide, malathion was tested for its embryotoxic and teratogenic properties in mice embryos. Following relatively high doses (125, 250 and 500 mg/g body wt.) of the insecticide, it was observed that alongwith a decrease in body weight and crown rump (CR) length, the embryos showed a significant lag in the development of main body parts such as brain, snout, external pinnae, fore and hind-limbs and tail while a significant increase in uncovered area of eye-ball was also noticed. It is, therefore, concluded that malathion is toxic to mouse embryos, especially in the quantities used in this study.

INTRODUCTION

In recent years, considerable interest has been generated in the studies of relationship between adverse effects on the mother caused by exposure to chemicals during pregnancy and possible indirect effects on the conceptus. A series of papers by Khera (1984, 1985, 1987a,b) has provided much of the impetus for the renewed interest in "maternal effects". It is now well established that deleterious influences resulting from maternal toxicity can indeed affect the conceptus.

There is an obvious decrease in the use of the chlorinated hydrocarbons all over the world due to their non-degradable and thus accumulative nature resulting into all kinds of general toxicities and eventually lethal effects (Niimi, 1983; Barron and Adelman, 1984). With the diminished utilization of chlorinated hydrocarbons, organophosphorus insecticides such as malathion, Parathion, Diazinon etc., have started being used quite heavily now (Davis and Richardson, 1980). These insecticides are considered relatively safe, especially in the sense of these being biodegradable and thus non-cumulative. Unfortunately, however, these compounds have also been seen to be quite harmful to the non-target organisms (Durham and Williams, 1972; Jennings *et al.*, 1975; Harbison, 1975). A survey by WHO tested about hundred organophosphorus insecticides and found "acute toxicity for (non-target) experimental animals" (WHO report, 1986). The harmful effects of these compounds have been primarily attributed to their acetylcholinesterase (AChE) inhibition properties (Harbison, 1975; Richardson, 1983). Banerjee *et al.* (1991) have also stated that pure and commercial organophosphates are able to significantly alter the acetylcholinesterase activity. Acetylcholinesterase has been tested *in vitro* as a predictor of the toxic potential of pesticides like organophosphate compounds in white rats (Chin *et al.*, 1980) and goats (Guhathakurta and Bhattacharya, 1989). Ishikawa *et al.*, (1975) reported that acetylcholine induced cardiac anomalies in nine of 23 chick embryos at a total dose of 20 mg given for a period of 3h 20 min. The anomalies induced ventricular septal defect, atrial septal defect and double aortic arch.

Many other studies have further shown that these insecticides may also be teratogenic. The harmful effects of these compounds especially to avian embryos have been shown quite convincingly (Khera, 1966; Khera and Bedok, 1967; Meiniel *et al.*, 1970; Fishbein, 1975; Meiniel, 1976; Sternberg, 1979; Wyttenbach and Thompson, 1985). In most of these studies it has been shown that even very small quantities of organophosphates induced gross embryonic malfunctions which included microcephaly, eye cataracts, ascites, hepatic degeneration, micromelia, ectrosyndactyly and many other musculo-skeletal abnormalities. Malathion, malaoxon, parathion and paraoxon caused dose-dependent development defects, such as abnormal pigmentation, abnormal gut development, notochordal defects and reduced growth (Snawder and Chambers, 1989) in African clawed frog. Greenberg and LaHam (1969) found that malathion caused shortening of hindlimbs, shortening of plumage and beak defects in chick embryos. They dubbed these defects as "malathion Syndrome". Some other studies have also shown that many of the commonly used organophosphorus insecticides are embryotoxic in birds. For example, it was discovered that Phosphamidon not only caused brain defects, dwarfism and stunted growth in chick embryos (Mufti and Dad, 1977), but many internal organs such as heart and kidneys were also adversely affected (Mufti and Nasim, 1987).

As far as the effects of organophosphorus insecticides on mammalian development are concerned, there are relatively very few studies present in literature. In most of these studies it has been generally observed that although these insecticides are relatively less toxic to mammalian fetuses than avian embryos, they can still cause many adverse effects (Tanimura *et al.*, 1967; Kimbrough and Gaines, 1968; Budreau and Singh, 1973; Lechner and Abdul-Rehman, 1984; Machin and McBride, 1989). For example, Karlow and Marton (1961) observed reduced growth rate and increased mortality of youngs following treatment of adult rat females, both prior to or during pregnancy, with malathion. Dobbins (1967) found decreased fetal weight, increased incidence in external hemorrhagic spots on the fetuses of rats following an administration of 50mg/kg of malathion. The teratogenicity was also found in rat fetuses following the treatments of dams with either diazinon or parathion (Dobbins, 1967; Kimbrough and Gaines, 1968). Budreau and Singh (1973) showed that demeton, administered as a single IP dose of 7-10 mg/kg, between days 7 and 12 of gestation in rats, proved embryotoxic with some teratogenic effects. More recently, however, Machin and McBride (1989) have shown that a dose of 100mg/kg, given on day 7 to 12 of gestation in rabbits, did not cause noticeable abnormalities in most of the fetuses, although there was seen incidence of acrania, microphthalmia and microcardia in at least one of the fetuses.

In some related studies it has been shown that malathion caused many malformations in mice embryos (Mufti and Nazir, 1988; Riaz, 1988). In these studies it has been discovered that even a small dose of 5μg/g body weight (BW) produces gross neural defects such as microcephaly and spina bifida with myeloschisis. The present study is designed as a step to evaluate embryotoxicity of malathion in mice.

MATERIALS AND METHODS

In the present series of experiments, white laboratory mice *(Mus musculus)* were used. These were maintained in standard animal room facilities with 12 ± 1h light/dark cycle. Estrus females were caged with males for overnight mating. Vaginal plug and/or sperms in the vaginal tract in the morning confirmed mating and marked day 0 of gestation.

These impregnated females were then isolated and were administered concentrations of 125, 250 and 500μg/g BW of organophosphorus insecticide, malathion. These concentrations were prepared by dilution of malathion in corn oil in such a way that 0.1 ml of solution contained the desired concentration of the insecticide. The route of the administration was oral. The pregnant mice were force fed the solution with the help of 1ml syringe attached with a capillary rubber tubing. This tubing ensured complete ingestion of the dose by the mice. The control and vehicle control groups were also maintained by applying no treatment and only corn oil, respectively.

On day 15 of gestation the pregnant mothers were anaesthetized and uteri, bearing the fetuses were dissected out. The fetuses were taken out from these gravid uteri and were fixed in Bouin's fixative for 48 hours. These were then washed in 70% alcohol and preserved in 80% alcohol for further studies. The preserved fetuses were examined under dissecting microscope. For morphometric studies brain, snout, eye, ear, fore-limb, hind-limb and tail were considered for extent of development. These organs were measured (Fig. 2) under dissecting microscope equipped with ocular micrometer. Individual fetuses per litter were studied for each organ. Mean values obtained for every experimental group were then analyzed by applying student T-test.

RESULTS

Although a reduction in the overall size of the treated fetuses was observed which obviously signified tendency toward dwarfism, it was decided to quantify this reduction. When the fetuses were studied more closely it was discovered that the ones obtained after 500 μg/g BW did not show any distinguishable developmental difference as compared to control. On a closer examination of the fetuses, following an administration of 500 μg/g BW dose, it was noted that the three main parts of the brain showed quite distinct bulges, which is a primitive condition of brain development. Other craniofacial organs such as eyes, ears, snout and vibrissae lines were far less developed. Both fore and hind limbs and tail were also noted underdeveloped as compared to the control (Fig. 3). Decrease in brain size was significantly different ($P < 0.001$) from control. A decrease in length of snout was noted following different concentrations of the insecticide, which was maximum at the dose of 500 μg/g BW (Fig. 4) and was significantly ($P < 0.001$) different from controls.

Eye development was noted by examining the lens covered by eyelids. Eye lens was found mostly covered with eyelids in control group, indicating an advanced state of development. Lens was found quite uncovered by the eyelids in fetuses recovered from treated mothers. A maximum increase in eye-lens open area was noted at dose level of 500 μg/g BW which was significantly ($P < 0.001$) different from controls (Fig. 5,6).

Pinna size showed decrease in the groups exposed to different concentrations of the insecticide. (Fig. 8). The decrease, however, was dose dependent and significantly different from control group.

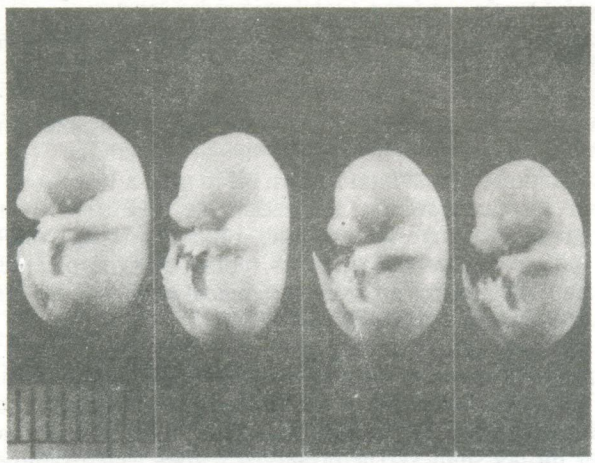


Fig. 1

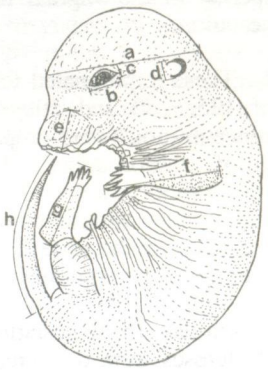


Fig. 2

Fig. 1: Composite photograph of 15-day old fetuses, recovered from pregnant mice after oral administrations of malathion at day 6 of gestation (Left to right, control, 125, 250 and 500 μg/g BW).

Fig. 2: A sketch of the 15-day old fetus showing points of morphometric studies: a, brain; b, eye length; c, eye width; d, pinna size; e, snout length; f, fore-limb length; g, hind limb length and h, tail length.

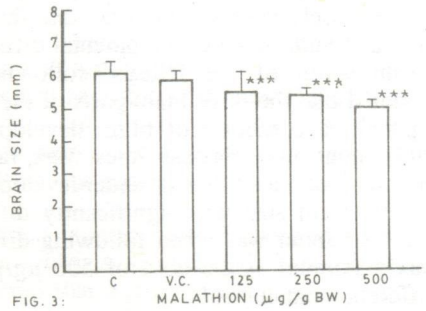


FIG. 3:

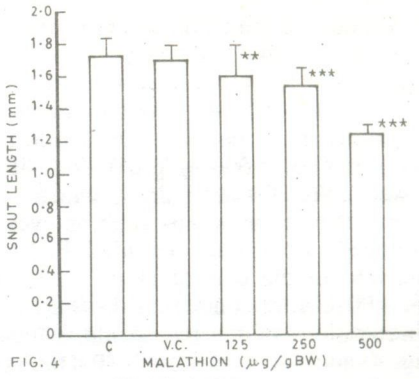


FIG. 4

Fig. 3: Histograms showing relationship of brain size of fetuses with the dose of insecticide administered to pregnant mice at day 6 of gestation.. (*=P<0.05; **=P<0.01; ***=P<0.001; c=control; v.c=vehicle control).

Fig. 4: Histograms showing relationship of snout length of fetuses with the dose of insecticide administered to pregnant mice at day 6 of gestation. (*=P<0.05; **=Ptl<0.01; ***=P<0.001; c=control; v.c=vehicle control).

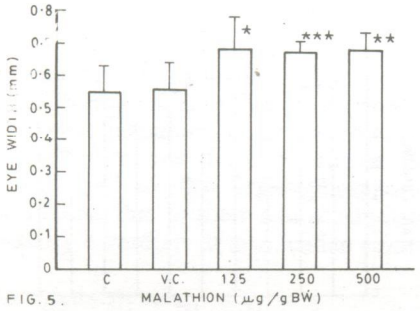


FIG. 5.

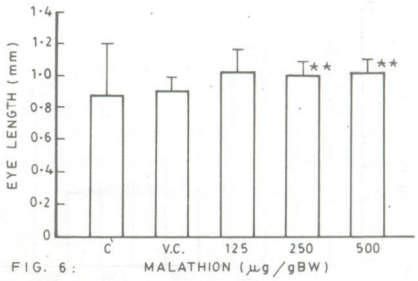


FIG. 6:

Fig. 5: Histograms showing relationship of eye width of fetuses with the dose of insecticide administered to pregnant mice at day 6 of gestation. (*=P<0.05; **=P<0.01; ***=P<0.001; c=control; v.c=vehicle control).

Fig. 6: Histograms showing relationship of eye length of fetuses with the dose of insecticide administered to pregnant mice at day 6 of gestation. (*=P<0.05; **=P<0.01; ***=P<0.001; c=control; v.c=vehicle control).

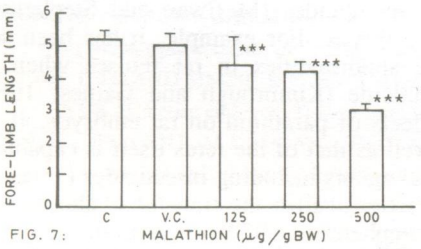


FIG. 7:

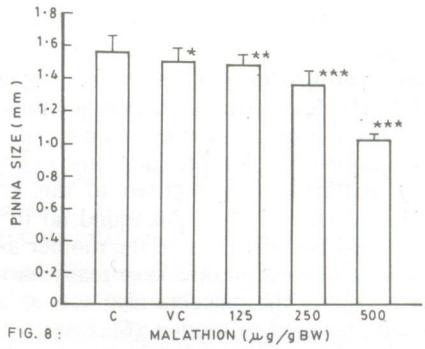


FIG. 8:

Fig. 7: Histogram showing relationship of fore-limb length of fetuses with the dose of insecticide administered to pregnant mice at day 6 of gestation. (*=P<0.05; **=P<0.01; ***=P<0.001; c=control; v.c=vehicle control).

Fig. 8: Histogram showing relationship of pinna size of fetuses with the dose of insecticide administered to pregnant mice at day 6 of gestation. (*=P<0.05; **=P<0.01; ***=P<0.001; c=control; v.c=vehicle control).

The fore-limb size was found to be decreased (Fig. 7) significantly from the controls ($P<0.001$). A significant ($P<0.001$) decrease in hind-limb size was also observed in treated group of fetuses which was dose dependent (Fig. 9). An overall decrease in tail length was observed, which was also highly significant ($P<0.001$) at high doses (250 to 500 μg/g BW when compared with control group (Fig. 10).

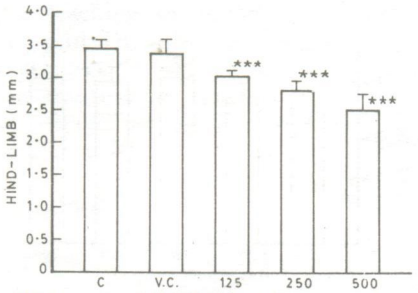


FIG. 9:

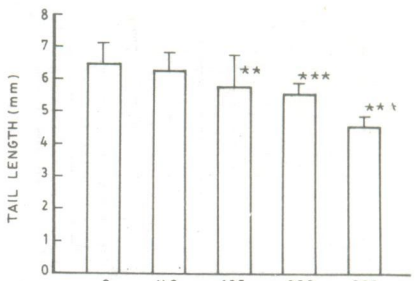


FIG. 10:

Fig. 9: Histogram showing relationship of hind-limb length of fetuses with the dose of insecticide administered to pregnant mice at day 6 of gestation. (*=P<0.05; **=P<0.01; ***=P<0.001; c=control; v.c=vehicle control).

Fig. 10: Histogram showing relationship of tail length of fetuses with the dose of insecticide administered to pregnant mice at day 6 of gestation. (*=P<0.05; **=P<0.01; ***=P<0.001; c=control; v.c=vehicle control).

DISCUSSION

It has been generally observed that organophosphorus insecticides such as malathion are relatively less toxic than organochlorine insecticides (McEwan and Stephenson, 1979). This is especially true of mammalian embryos. For example, it has been seen that malathion did not produce any definite abnormalities in rat fetuses when the pregnant mothers were exposed to this insecticide (Kimbrough and Gaines, 1968). Khera and Trinett (1978) also found no ill effects of parathion on rat embryos. It has been assumed that the liver of the mother as well as that of the fetus itself is capable of detoxifying the harmful effects of many harmful agents including insecticides (Frias and Thomas, 1988). The placenta also acts as a barrier against the transfer of the harmful agents administered to mother (McEwen and Stephenson, 1979). In spite of these facts if the chemical concerned is in high enough quantity, it may pass through the placenta and can cause damage. This is exactly what apparently happened during present studies.

Morphometric studies of fetuses recovered following different concentrations of malathion showed reduction in body weight and CR length, which was basically dose dependent. Detailed studies of different organs such as brain, snout, pinnae, eyes, fore-limbs, hind-limbs and tail also showed reduced development of these organs at high concentrations of the insecticide.

Our results are supported by some other studies as well. For example, it was observed that malathion caused decreased fetal weight, an increased incidence of fetal resorption and other anomalies such as hydronephrosis and hydroureter in rat embryos (Dobbins, 1967). Karlow and Martin (1961) also found that a continuous administration of malathion for a 10 week period to rats before and during pregnancy also resulted into dwarfism and increased mortality. more recently, Machin and McBrige (1989) also studied the effects of a 100mg/kg dose of malathion in rabbits, given on day 7-12 of gestation and found that although this dose produced no½

significant fetal abnormalities, still at least one case of fetal resorption and one case of fetal malformation was recorded. Some studies carried out in our laboratory also showed that malathion in a high dose of 1 to 3 mg/gBW to pregnant mice caused embryolethality and fetal resorption (Mufti and Nazir, 1988). All these studies obviously indicate that organophosphorus insecticides, which are relatively safe for adult animals due to their non-accumulative and biodegradable properties, can still be potentially hazardous to mammalian embryos.

Acknowledgements

We thank Dr. Inaam, Incharge, Animal Breeding Section, Veterinary Research Institute, Lahore, for providing mice. This research was supported by a National Scientific Research and Development Board (NSRDB), research grant # UN-LHR-04(9)/03.

REFERENCES

BANERJEE, J., GOSH, P., MITRA, S., GOSH, N. and BHATTACHARYA, S., 1991. Inhibition of human fetal brain acetylcholinesterase: Marker effect on neurotoxicity. *J. Toxicol. and environ. Hlth.*, **33**: 283-290.

BARRON, M. G. AND ADELMAN, J.R., 1984. Neurological disruption prolonged in hens by two organophosphate esters, *Br. J. Pharmacol.*, **23**: 295-304.

BURDEAU, C.H. AND SINGH, R.P., 1973. Teratogenicity and embryotoxicity of Demeton and Fenthion in CF #1 mouse embryos. *Toxicol. App. Pharmacol.*, **24**: 324 332.

CHIN, B.H., TALLANT, M.J., DUANE, W.C. AND SULLIWAL, L.J., 1980. Anticholinesterase effects of carbamate insecticide thiofanox and its metabolites in rats. J. Agric. Food Chem., **28**: 1327-1330.

DAVIS, C.S. AND RICHARDSON, R.J., 1980. *Organophosphorus Compounds in Experimental and Clinical Neurotoxicology* (eds. P.S. Spencer and H.H. Schaumburg) Williams and Wilkins Co., Baltimore, pp. 517-544.

DOBBINS, P.K., 1967. Organic phosphate insecticides as teratogens in the rat. *J. Fla. Med. Assoc.*, **54**: 452-456.

DURHAM, W.F. AND WILLIUMS, C.H., 1972. Mutagenic teratogenic and carcinogenic properties of pesticides. *Ann. Rev. Entomol.*, **17**: 123-148.

FISHBEIN, L.., 1975. Teratogenic, mutagenic and carcinogenic effects of insecticides. In: *Insecticide Biochemistry and Physiology* (ed. C. Wilkinson). Plenum press, New York.

FRIAS, J.L. AND THOMAS, I.T., 1988. *Teratogens and teratogenisis: General principles of clinical teratology*, Vol. 2, Institute for Clinical Sciences, spp. 174-179,.

GREENBERG, J. AND LAHAM, Q.N., 1969. Malathion-induced teratisms in the developing chick. *Can. J. Zool.*, **47**: 539-542.

GUHATHAKURTA, S. AND BHATTACHARYA, S., 1989. *In vitro* inhibition of goat brain acetylcholinesterase by pure and commercial anticholinesterase pesticides. *Ecotoxicol. environ. Safety.*, **17**: 16-20.

HARBISON, R.D., 1975. Comparative toxicity of some selected pesticides in neonatal and adult rats.

Toxicol. Appl. Pharmacol., **32**: 443-446.

ISHIKAWA, S., KAWAMURA, T., TAKAO, A., ANDO, M., MIWA, H. AND ANDOKAI, O., 1975. Cardiovascular malformations following acetylcholine chloride administration to chick embryos (abstract). *Teratology,* **12**: 198.

JENNINGS, D.M., BUNYAN, P.J., BROWN, P.M., STERNLY, P.I. AND JONES, F.J.S., 1975. Organophosphorus poisoning: A comparative study of toxicity of carbophenothion to the Canada Goose, the pigeon and the Japanese quail. *Pestic. Sci.,* **6**: 246-257.

KARLOW, W. AND MARTON, A., 1961. Second generation toxicity of malathion in rats. *Nature (London)*, **192**: 464-465.

KHERA, K.S. AND BEDOK, B., 1967. Effects of Thiophosphates on mitochondrial and vertebral morphogenesis in duck and duck embryos. *Food Cosmet. Toxicol.*, **5**: 359-365.

KHERA, K.S., AND TRINETT, G.., 1978. Teratogenicity studies on linuron and methoxychlor in rats. *J. Toxicol. Appl. Pharmacol.*, **45**:435-444.

KHERA, K.S., 1966. Toxic and teratogenic effects of insecticide in duck and chick embryos. *Toxicol. Appl. Pharmacol.*, **8**: 345.

KHERA, K.S., 1984. Maternal toxicity - A possible factor in fetal malformations in mice. *Teratology*, **29**: 411-416.

KHERA, K.S., 1985. Maternal toxicity. A possible etiological factor in embryo-fetal deaths and fetal malformations of rodent-rabit species. Teratology, **31**: 129-153.

KHERA, K.S., 1987a. Maternal toxicity of drugs and metabolic disorders - A possible etiologic factor in the intrauterine death and congenital malformations: A critique on human data. *CRC Crit. Rev. Toxicol.*, **17**: 345-375.

KHERA, K.S., 1987b., Maternal toxicity in humans and animals: effects on fetal development and criteria for detection. *Teratogen. Carcinogen. Mutagen.*, **7**:287-295.

KIMBROUGH, R.D. AND GAINES, R.B., 1968. Effects of organic phosphorus compounds and alkylating agents on the rat fetus. *Arch. environ. Hlth.*, **16**: 805-808.

LECHNER, D.M.. AND ABDEL-RAHMAN, M.S., 1984. A teratogenicity study of carbaryl and malathion mixtures in rat. *J. Toxicol. environ. Hlth.*, **14**: 267-278.

MACHIN, M.G.A. AND McBRIDE, W.G., 1989. Teratological study of malathion in the rabbit. *J. Toxicol. Environ. Hlth.*, **26**: 249-253.

McEWAN, F.L. AND STEPHENSON, G.R., 1969. *The use and significance of pesticides in the environment.* John Wiley and Sons, New York.

MEINIEL, R. 1976. Pluralite dans be determinisms des effects teratogenes des composes organophosphores. *Experimentia,* **32**: 920-921.

MEINIEL, R., LUTZ-OSTERTAG, T. AND LUTZ, H. 1970. Effects teratogenes due parathion sure le squelette embryonnaire dela caille japonaise (*Cortunix coturnix-japonica*). *Arch. Anat. Microse. Morphol. Exp.*, **59**: 167-183.

MUFTI, S.A. AND DAD, K., 1977. Preliminary observations on the effects of Phosphamidon on chick embryo. *Pakistan J. Zool.*, **9**: 245-246.

MUFTI, S.A. AND NASIM, R. 1987. Avian embryotoxicity of Dimicron, a commonly used insecticide. *Biologia,* **33**: 109-120.

MUFTI, S.A. AND NAZIR, N., 1988. Embryotoxic effects of malathion in mice. *Proc. Pakistan Congr.*

Zool., **8**: 15-19.

NIIMI, A.J., 1983. Biological and toxicological effects of environmental contaminants in fish and their eggs. *Can. J. Fish. Aquat. Sci.*, **40**: 306-312.

RIAZ, R., 1988. *Effects of malathion on the embryogenesis in mice*. M. Sc.. Thesis, Punjab University, Lahore.

RICHARDSON, R.J., 1983. Neurotoxic Esterase: Research trends and prospects. *Neurotoxicology*, **4**: 157-162.

SNAWDER, J.E. AND CHAMBERS, J.E., 1989. Toxic and developmental effects of organophosphorus insecticides in embryos of the South African clawed frog. *J. environ. Sci. Hlth.*, Part B. Pestic. Food Contam. Agric. Wastes, **24**: 205-218.

STERNBERG, S.S., 1979. The carcinogenesis, mutagenesis and teratogenesis of insecticides. Review of studies in animals and man. *Pharmac. Ther.*, **6**: 147-166.

TANIMURA, T., KATSYUA, T. AND NISHIMURA, H., 1967. Embryotoxicity of acute exposure to methyl parathion in rats and mice. *Arch. environ. Hlth.*, **15**: 609-613.

WHO REPORT, 1986. Environment Health Criteria No. 63. *Organophosphorus Insecticides: A general Introduction*. World Health Organization, Geneva, Switzerland.

WYTTENBACH, C.R. AND THOMPSON, S.C., 1985. The effects of the organophosphate insecticide malathion on very young chick embryos: malformations detected by histological examination. *Am. J. Anat.*, **174**: 187-202.

(Received: September 16, 1992)

Punjab Univ. J. Zool. Vol. 8, pp. 63-68. 1993.

LABORATORY EVALUATION OF DIELDRIN AND LORSBAN IN PROTECTING WOODEN BLOCKS FROM TERMITE (ISOPTERA) ATTACK

MUHAMMAD SAEED AKHTAR, MUHAMMAD IRSHAD AND AMJAD FAROOQ

Department of Zoology, University of the Punjab, Quaid-e-Azam Campus, Lahore-54590, Pakistan

Abstract: Wooden blocks of *Magnifera indica* were treated with different concentrations (0.1%, 0.2%, 0.4%, .8%) of dieldrin and Lorsban. Blocks treated with each insecticide were segregated into two groups, *i.e.* leached and unleached. Lorsban was found more effective than dieldrin in protecting wooden blocks from attack of *Microcerotermes championi* Snyder, during short term laboratory experiments.

INTRODUCTION

Scientists all over the world are trying to find out insecticides, other than chlorinated compounds for protecting wooden structures from termite attack (Beal and Smith, 1972; Beal and Miller, 1980; Howick and Creffield, 1981; Rose *et al.*, 1984; Kard *et al.*, 1989; Akhtar and Sarwar, 1991.

In the present paper efficacy of Lorsban and dieldrin in protecting wooden blocks from attack of *Microcerotermes championi* is discussed.

MATERIALS AND METHODS

Laboratory evaluation of two insecticides applied to wooden blocks

For laboratory evaluation of insecticides by leaching and unleaching method, the procedure used by Tsundo and Nishimoto (1985) was adopted. Two insecticides involved were Lorsban and Dieldrin. The concentrations of the insecticides used were 0.1%, 0.2%, 0.4% and 0.8%.

Termite test

Termite test was done according to Japan Wood Preservation Association standard, Anon 11(1) 1981.

Wood specimen

Wood specimens, measuring 10(R)$\pm$0.5 x 10(T)$\pm$0.5 x 20(L)$\pm$0.5 mm, were prepared from the sound sapwood of *Magnifera indica*.

Treatment and conditioning of test wooden blocks

Treatment was done by brushing @ 110$\pm$10 g/m^2 of treating solution. After treatment, the wooden blocks were dried in the ambient temperatures for at least 20

days before leaching or termite test. Twenty four blocks treated with different concentrations of Dieldrin solution were then assigned to two groups, leached and unleached groups. So that 12 of treated-leached and treated-unleached blocks were prepared. In the same way, blocks treated with different concentrations of Lorsban were also assigned to two groups. Untreated-unleached wooden blocks were also subjected to termite test as control.

Leaching procedure

Leaching procedure consisted of ten times repeated wet and dry cycles. wooden blocks were dipped in non-running water for 30 seconds and then kept in a desiccator with water at the bottom for 4 hours at 26±2 °C. After the wet cycle, the blocks were transferred into an oven and kept at 40±2 °C for 20 hours. All the wooden blocks were dried at 60±2 °C before termite test for determining the oven-dried weights of them. Then oven dried weights (W_1) were measured by analytical balance to the nearest 0.001g.

Petri dishes (90mm x 15mm) were used as test container. One each of treated or untreated wooden block was placed at the center of petri dish. One hundred and fifty (150) termites were introduced into each petri dish. All the petri dishes were then stored at 28±2 °C in the dark for 15 days. After the test period, all the blocks were cleaned and dried at 60±2 °C for two days and then reweighed (w_2). Weight loss of wood blocks were then calculated from the following equation:

$$\text{Weight loss (\%)} = \frac{W1 - W2}{W1} \times 100$$

Number of dead termites were recorded at the end of the test so that mortality of termites could be determined as follows:

$$\text{Termite mortality (\%)} = \frac{\text{No. of dead termites}}{150} \times 100$$

Besides, regular observations were made to count dead termites.

RESULTS

Laboratory evaluation of two insecticides applied to wooden blocks

Table No. 1 shows the percentage weight loss of treated blocks caused by termite attack. As a candidate chemical can be considered as "effective" when it succeeds in limiting termite attack to below 3% weight loss of treated blocks even after leaching according to the qualitative standards (Japan Wood Preserving Association Standard), Lorsban tested was satisfactorily effective. Percentage weight loss of the blocks treated at 0.2, 0.4 and 0.8% was less than 3%, though moderate termite attack was found at 0.1% in leached Lorsban-treated wood blocks.

Percent weight loss was higher than 3% at 0.1, 0.2 and 0.4% in treated leached wood blocks with dieldrin. At 0.8% dieldrin was effective in limiting termite attack below 3%, as is shown in Table 1. Termite mortality change with time has been shown in Fig. 1 and Fig. 2.

Table 1: Percent Weight Loss of Wooden Blocks and Termite Mortality

Chemical (Solvent)	Treating conc. %	Leaching	Weight loss (%) Min. - Max.	Mean	Mortality (%) Min. - Max.	Mean
Dieldrin	0.1	Yes	6.4 - 7.9	7.03	3 -7	5
		No	1.1 - 1.4	1.23	30 - 42	35
	0.2	Yes	5.2 - 6.1	5.6	6 - 11	9
		No	0.4 - 0.9	0.66	53 - 85	70
	0.4	Yes	3.9 - 4.7	4.3	11 - 15	13
		No	0.1 - 0.3	0.16	69 - 91	82
	0.8	Yes	0.4 - 1.1	0.7	24 - 38	34
		No	0.0 - 0.1	0.03	100 - 100	100
Lorsban	0.1	Yes	2.7 - 3.3	3.03	40 - 53	45
		No	0.3 - 1.0	0.6	51 - 79	64
	0.2	Yes	1.5 - 2.4	1.93	57 - 98	74
		No	1.0 - 3.0	0.2	87 - 100	95
	0.4	Yes	0.1 - 0.7	0.4	98 - 100	99
		No	0.0 - 0.1	0.06	100 - 100	100
	0.8	Yes	0.0 - 0.2	0.1	100 - 100	100
		No	0.0 - 0.0	0.0	100 - 100	100
	Untreated control		12.4 - 22.6	17.3	0.0	0

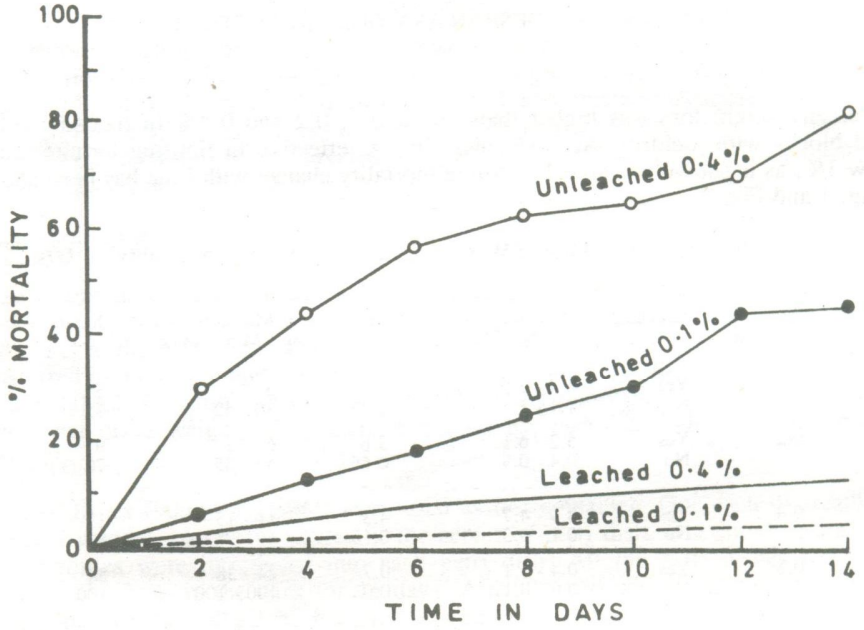


Fig. 1. Percent mortality of *Microcerotermes championi* exposed to wooden blocks treated with aldrin.

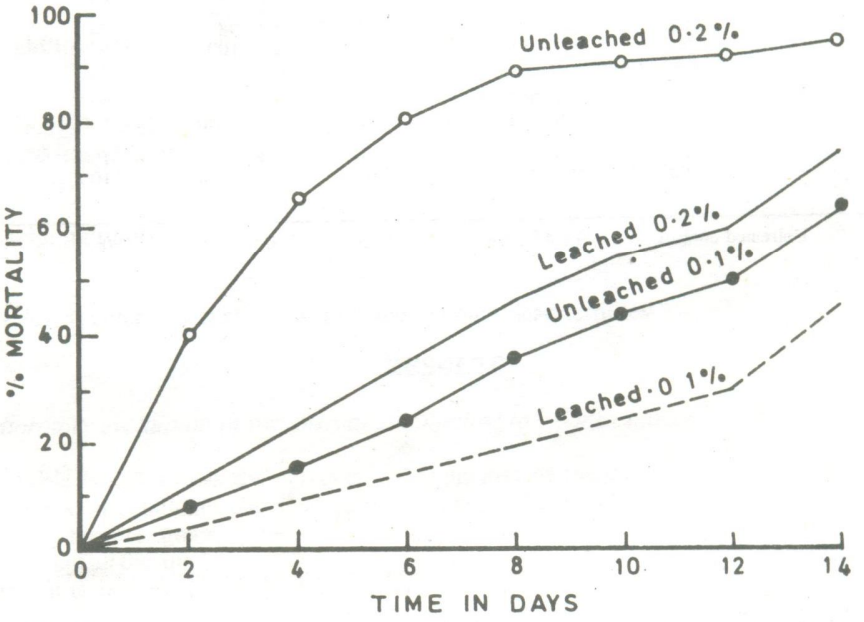


Fig. 2. Percent mortality of *Microcerotermes championi* exposed to wooden blocks treated with Lorsban.

DISCUSSION

Toxic properties of an insecticide may vary in different climatic zones, depending upon the pH and percent base saturation of soil (Beal, 1980).

Hydrocarbons provided decades of termite control at United states test sites, and organophosphates and pyrethroids did not last as long, though they were effective for 5 or more years at same rates (Kard *et al.* 1989). But as regards chloropyrifos (an organophosphate), Su *et al.* 1987 have reported that this insecticide exhibited its effect more rapidly than chlordane. Tamashiro *et al.*, 1989 have also shown Dursban TC insecticide as a preventive treatment for Formosan termite in Hawaii. According to them chlorpyrifos, the active ingredient in Dursban TC insecticide, forms an effective barrier in sand and clay to prevent infestation of Formosan subterranean termite in most locations in Hawaii for 5 years or more. Jones (1989) have carried out studies with *Reticulitermes flavipes* and *Coptotermes formosanus* regarding toxicity and repellency of chlordane, chlorpyrifos and permeth.'n. He has reported that during contact toxicity tests workers of both the species were knocked down within a few hours. But permethrin was more repellent than other two insecticides tested. Besides, borate preservatives have been reported to offer effective protection for building timber and other above ground uses of wood products with less hazard to users and the environment (Curtis, 1990).

In Pakistan soil toxicity and repellency of some insecticides have been studied against *Microtermes championi* Snyder and *Bifiditermes beesoni* (Gardner). Akhtar and Irshad (1990) compared toxicity of dieldrin, Lorsban, Bestox and heptachlor at different concentrations 6.25, 12.5, 25, 50, 100, and 200 ppm. They have reported that heptachlor showed greater toxicity against *M. championi* than other insecticides. Next to heptachlor was Bestox. Akhtar and Sarwar (1991) have also compared toxicity and repellency of dieldrin, Lorsban, Bestox and Decis-D against *Bifiditermes beesoni*. Bestox showed greater toxicity against *Bifiditermes beesoni* than dieldrin at 200, 100, and 50 ppm. Next to Bestox in toxicity was Decis-D.

Tsunoda and Nishimoto (1985) have also studied efficacy of organophosphates in giving protection to wooden blocks. They have reported that of five organophosphates applied to wooden blocks, chlorpyrifos and phoxim were the most effective against *Coptotermes formosanus* even after leaching (weathering). Present studies with *Microcerotermes championi* have also shown that lorsban was more effective than dieldrin in protecting wooden blocks after leaching (weathering), during short term laboratory experiments.

REFERENCES

AKHTAR, M.S. AND IRSHAD, M., 1990. Toxicity of some insecticides against *Microtermes championi* Snyder. *Proc. Pakistan Congr. Zool.* **10**: 33-41.

AKHTAR, M.S. AND SARWAR, G., 1991. Comparison of toxicity and repellency of Dieldrin with other insecticides used against *Bifiditermes beesoni* (Gardner) (Isoptera). *Pakistan J. Zool.* **23**: 269-271.

ANONYMOUS, 1981. Japan wood preserving association standard 11(1). Method for testing effectiveness of surface treatments (Brushing, Spraying and Dipping) with termicides against termites. Laboratory tests.

BEAL, R.H., 1980. New methods for termite control. *Pest Control*, **48**: 46-54.

BEAL, R.H. AND MILLER, W.O., 1980. Termite control investigation with broad spectrum insecticides. *Down to Earth*, **37**(1): 16-21.

BEAL, R.H. AND SMITH V.K., 1972 Carbonate and phosphate insecticides for subterranean termite control. *Pest Control*, **40**(7):20-43.

CURTIS, A.B. AND WILLIAMS, L.H., 1990. Borates offer effective protection with less hazard to the environment. Cooperative Forestry Utilization Report RB-UR-6., pp. 1-4.

HOWICK C.D. AND CREFFIELD, J.W., 1981. Laboratory bioassay to compare the efficacy of Chlorpyrifos and Dieldrin in protecting wood from termites. *Int. Pest Control*, March/April: 40-42.

JONES, S.C., 1989. How toxic and repellent are soil insecticides to subterranean termites. *Pest Management*, **8**(2):16-19.

KARD, B.M. MAULDIN, J.K. AND JONES, S.C., 1989. Evaluation of soil termicides for control of subterranean termites. *Sociobiology* **15**: 285-297.

ROSE, K., KOZMA, J. AND SPARROW, P., 1984. *Evaluation of Chlorpyrifos as an insecticidal component of a wood preservation.* The Int. Res. Group on Wood Preserv. Document No. IRG/WP/3301.

SU, N.Y., TAMASHIRO, M., YATES, J.R. AND HAVERTY, M.I., 1987. Characterization of slow-acting insecticides for the remedial control of the Formosan subterranean termites (Isoptera: Rhinotermitidae) *J. Econ. Entomol.*, **80**: 1-4.

TAMASHIRO, M., YATES, J.R., EBESU, R.H., YAMAMOTO, R.T., SU, N.Y. AND BEAN, J.N., 1989. Dursban Tc insecticide as preservative treatment for formosanus subterranean termites in Hawaii. Down to Earth, **45**: 1-5.

TSUNODA, K. AND NISHIMOTO, K., 1985. Laboratory evaluation of organophosphates as termicides. Paper prepared for the 16th Annual meeting, Brazil. Document No. IRG/WP/3330.

(Received February 27, 1992)

Short Communication

Punjab Univ. J. Zool., Vol. 8, pp. 69-70, 1993.

EFFECT OF ROOT - KNOT NEMATODE, *MELOIDOGYNE JAVANICA* ON NODULATION AND ROOT GROWTH OF CHICKPEA

Chickpea (*Cicer arietinum* L.) is an important grain legume crop in Pakistan. It occupies about 1.05 million ha, producing 456,000 tons of grain, contributing about 81% to the total production of pulses in the country (*Agric. Stat. of Pakistan, Ministry of Food Agric. and Co-operat. Islamabad,* pp. 289, 1989). Its yield per unit area is very low. Many factors contribute to low yield of chickpea in Pakistan. The most important of these factors is the occurance of different diseases. The root-knot disease caused by *Meloidogyne* spp. has been studied on chickpea (Srivastava *et. al, Indian J. Nematol.*, **4**:248-251, 1974). It causes considerable damage to chickpea crop. in Pakistan (Maqbool *et. al, U.S.-Pakistan Internat. Workshop, Plant Nematol., Univ. Karachi, Karachi*, pp. 229-240, 1988). Root-knot nematodes are widely distributed in our soils and cause considerable damage to various crops including pulses. The annual yield losses due to *Meloidogyne* spp. are 16.9% and in pulses these are 39% (Handa and Mishra, *Indian J. Pulses Res.* **2**:152-155, 1989). These parasites not only infect the root system and cause direct damage but also interfere with the formation and functioning of rhizobial nodules. The present investigation was, therefore, made to study the effect of *M. javanica* on nodulation and root growth of chickpea.

Materials and Methods

Surface sterilized seeds of five cultivars of gram were sown in plastic pots of (12cm diameter) containing sterilized sandy loam soil. The seeds were treated with *Rhizobium* spp. Pots were completely randomized under field conditions. Each variety had six replications and two treatments *i.e.* treated with nematodes and non-treated, uninoculated plants were kept as check. Culture of *M. javanica* was continuously maintained on tomato cv. money maker. Extraction and estimation of second stage larvae from heavily infested roots of tomato were done by modified Whitehead and Hemming trays method (Whitehead and Hemming, *Ann. Appl. Biol.* **55**: 25-38, 1965). Clean hand pump water was used to irrigate young seedlings throughout the period of studies.

Ten days old healthy seedlings of chickpea were inoculated with 4,000 larvae of *M. javanica* per pot. The uninoculated chickpea plants with soil were gently removed from pots and their roots were carefully washed in running water. Data were recorded on the basis of number of nodules, root length, fresh and dry weight of root. The data were analyzed statistically by using factorial design.

Results and Discussion

The results of present investigation (Table. 1) revealed that there was significant reduction in number of nodules of chickpea by the application of *Meloidogyne javanica* in all the cultivars. The number of nodules was more in non-treated as compared to treated with nematodes. The reduction in nodulation has been explained to be more due

to nutritional interferences, particularly carbohydrates or physiological changes brought about by nematode infection rather than due to competition for infection sites. Similar observations have been reported by (Taha and Raski, *Indian J. Nematol.*, **1**:201-211, 1969; Raut and Sethi, *Indian J. Nematol.*, **10**:166-176, 1980).

The root length was also more in non-treated as compared to treated with nematodes. Due to the development of galls on the root system the root weight was not adversely affected. In fact, It was increased which could be due to severe galling on root system as was also observed by (Sethi, *Thesis D.I.C Imperial College, Univ. of London, London*, 1966).

Table 1. **Effect of *Meloidogyne javanica* treatment on nodulation and root growth of chick pea (Each value is mean of six replicates).**

Varieties	Nodules Numbers		Root Length (cm)		Fresh Root Weight (g)		Dry Root Weight (g)	
	Non Treated	Treated	Non Treated	Treated	Non Treated	Treated	Non Treated	Treated
CM72	35.33	24.16b	34.33a	25.66b	4.60a	5.53b	0.43a	0.73b
918	42.5a	22.50b	38.16a	23.66b	2.80a	5.48b	0.43a	0.78b
1435	32.83a	23.00b	31.00a	21.83b	2.41a	3.90b	0.35a	0.71b
C727	43.33a	31.83b	57.50a	29.83b	4.05a	6.81b	0.60a	0.75b
1430	38.66a	33.83b	35.16a	26.83b	4.15a	5.58b	0.60a	0.73b
S.E.	0.52		0.47		0.15		0.04	

- Any two means in rows in each column sharing different letters differ significantly.
- Ducan's Multiple range test at 0.05 probability.

Ahmad Ali Shahid
Riaz Ahmad Chohan

Department of Plant Pathology,
Univetrsity of Agriculture,
Faisalabad, Pakistan.

PUNJAB UNIVERSITY

JOURNAL OF ZOOLOGY

(FORMERLY BULLETIN, DEPARTMENT OF ZOOLOGY)
UNIVERSITY OF THE PUNJAB (NEW SERIES)

Volume 8 1993

CONTENTS

PUNJAB UNIVERSITY

JOURNAL OF ZOOLOGY

(FORMERLY BULLETIN, DEPARTMENT OF ZOOLOGY)
UNIVERSITY OF THE PUNJAB (NEW SERIES)

Volume 8

1993

CONTENTS