Project No. P-PU/BIO(93)

PAKISTAN SCIENCE FOUNDATION

FINAL TECHNICAL REPORT

(August 1980 —— November 1983)

MORPHOLOGICAL AND METABOLIC HAZARDS OF CHLORINATED INSECTICIDES ON SMALL MAMMALS IN PAKISTAN

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PAKISTAN

FINAL TECHNICAL REPORT

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4.	Project No.	: P-PU/Bio(93)
5.	Project Title	: "MORPHOLOGICAL AND METABOLIC HAZARDS OF CHLORINATED INSECTICIDES ON SMALL MAMMALS IN PAKISTAN".
6.	Research period covered by the report.	: August 1980 - November 1983

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PREFACE

The present report is a tribute to thousands of workers in the Agricultural sector, who constantly expose themselves to the danger of unintentional and accidental exposure to insecticides and other pesticides. They undergo this unnoticed process just to increase the agricultural produce by saving the crops, vegetables, fruits etc., from the pest attack. The present research project was aimed at assessing the visible and or invisible damage done to non target organisms by chlorinated insecticides. Rats were used as experimental (model) animals and the results obtained and reported in this document can be extrapolated to human system, despite all the arguments for and against such extrapolations. The research project MORPHOLOGICAL AND METABOLIC HAZARDS OF CHLORINATED INSECTICIDES ON SMALL MAMMALS IN PAKISTAN was initiated in August, 1980 and the experimental work was completed in September, 1985. Maintenance of a large rat colony for long term experiments in which animals were fed with insecticides continuously for two years for 4 different insecticides was the major problem. This had to be done in the face of acute heat during summer in Lahore, tremendous financial constraints and quite a few unforeseen mishaps. The results being reported in this document are completed according to the approved plan of work of the project. The report has been divided into five Chapters. The first four Chapters deals with one insecticide each viz. Dieldrin, aldrin, rBHC and DDT, while the fifth Chapter interrelates the major findings reported in the first four Chapters. The main conclusions, various recommendation and future line of action with regard to

research activities in this field have also been included in the 5th Chapter. In each Chapter the effects of insecticides have been described with reference to effect on body weight and liver weight, haematology, biochemical analysis of blood, and biochemical and histological analysis of liver. The data has adequately been tabulated and illustrated. This work has solely been done in the Cell Biology Labs. of the Department of Zoology, which is fully equipped to do this type of biochemical work. For a part of this work during initial stages laboratory facilities at Nuclear Institute of Agriculture & Biology, Faisalabad and Quaid-i-Azam University, Islamabad were also used, which is gratefully acknowledged.

Syed Shahid Ali, M.Sc. has been working as a Research Officer in the project and has therefore been the main technical help. The data presented in this report will form a major part of the thesis which Mr. Ali is preparing for his Ph.D. degree.

Thanks are due to Pakistan Science Foundation for financial support, without which this work would not have been possible.

Lahore, Pakistan
10th June, 1986.

A.R.SHAKOORI

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SUMMARY

Adult albino, sprague Dawley rats, were fed on different doses of chlorinated insecticides for different periods of time. The chlorinated insecticides and their time schedule for which they were fed to the rats is as follows:

Insecticide	Dose(mg/kg body wt./day)administered for		
	48 hours	15 days	18 months
1. DDT	100	20	10
2. Gamma BHC (=Lindane)	30	18	9
3. Aldrin	20	8	2.5
4. Dieldrin	40	12	.6

At the end of stipulated period, the animals were slaughtered, their blood samples collected from Inferior Vena Cava and livers taken out. The blood was then processed for haematological and biochemical studies, while the livers were used for histological and biochemical analyses. Most of the biochemical studies of blood and liver were carried out with an object to observe changes in the liver function after exposure to insecticides. In that context the blood serum was analyzed for the estimation of various enzymatic activities including enzymes of liver function tests viz. Amylase, alkaline phosphatase (AP), acid phosphatase (AcP), serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), lactate dehydrogenase (LDH), isocitrate dehydrogenase (ICDH), creatine phosphokinase (CPK) and Cholinesterase (ChE); and several other biochemical components like Bilirubin, serum protein, urea, cholesterol, free amino acids (FAA) and glucose. The liver, on the other hand, was also used for estimation of various enzymatic activities like AP, GOT, GPT, LDH and ICDH; and several other biochemical components such as cholesterol, glucose, FAA, total protein, soluble protein, DNA and RNA contents.

Haemoglobin content, RBC count, WBC count and packed cell volume (PCV) were generally taken as haematological parameters. For histological changes the morphometric studies on the hepatic tissue sections were performed with special reference to record changes in the number and size of hepatic cells, their nuclei and nucleoli. The

various effects of different insecticides are summarized below:

I. EFFECTS OF DIELDRIN

1. Haematological parameters

The haemaglobin content, RBC count and PCV decreased under all experimental conditions. The WBC count was considerably increased.

2. Blood Chemistry

All the blood serum enzymes was raised after dieldrin treatment under all experimental conditions except for amylase, which was unchanged in 48 hour experiment. Besides that CPK and ChE were unaffected in 15 day and 18 month feeding experiment. The two phosphatases and LDH were prominently increased. From amongst the other blood serum components, cholesterol and FAA content exhibit well defined decrease after dieldrin feeding, while the proteins were significantly increased. Bilirubin, urea and glucose did not show any consistent behaviour.

3. Liver Chemistry

Hepatic GPT, AP and LDH activities were raised prominently under all experimental conditions. GOT activity, which generally was not much altered in short term experiment, shown a significant increase of 26% after 18 months of dieldrin feeding. The ICDH activity behaved almost the same way as GOT. Although 17% decrease was noted after 48 hours of dieldrin treatment, the ICDH activity showed 65% increase after 18 months of continuous feeding.

Hepatic cholesterol and FAA content decreased after dieldrin feeding. The glucose content showed significant decrease in the short term experiment, but got considerably increased after 18 months of feeding. The soluble proteins increased while the total proteins did not show any definite pattern.

The DNA content decreased 63% after 48 hours of dieldrin feeding, while 15 days and 18 months of feeding did not cause any change. The RNA content showed almost the same pattern. It decreased 47% after 48 hours of treatment and 12% after 18 months of dieldrin feeding.

4. Histological Changes

The histological changeswere prominently marked by hypertrophy of hepatic cell, its nucleus and nucleolus. The number of nucleoli/nucleus also increased insecticide administration.

II. EFFECTS OF ALDRIN

1. Haematological Changes:

Like a typical insecticidal response, the haemoglobin content, RBC count and PCV decreased, while WBC count increased significantly after feeding aldrin to rats. The WBC response was much more prominent in 15 day treatment experiment and comparatively less so in 48 hours and 18 months feeding.

2. Blood Chemistry

All blood serum enzymes showed increased activities after aldrin feeding. In 48 hour feeding experiment SGPT activity was not affected, while in 15 day feeding this enzyme activity was raised and showed 197% increase after 18 months of feeding (2.5 mg/kg/day). ICDH activity, which was not significantly altered after dieldrin feeding was raised after aldrin feeding for 18 months. The CPK enzymes though showed non significant changes in most of the earlier part of aldrin feeding, showed significant increase, when insecticide was administered for 18 months. The ChE showed 88% increase after 15 day treatment (20mg/kg/day) but did not show any significant change in other treatments.

The bilirubin, urea and the protein contents increased after aldrin treatment. This increase was 64% and 18% in bilirubin content after 15 days and 18 months of feeding, respectively. The protein content likewise showed 26%, 31% and 24% increase after 48 hours, 15 days and 18 months of feeding, respectively. The urea content increased 70% and 45% after 15 days and 18 months of feeding, respectively.

The FAA content decreased significantly throughout under all experimental conditions, while the cholesterol showed 29% decrease in 48 hour treatment experiment and almost no change in the other long term feeding experiments. The glucose content likewise was unaffected in 18 months feeding group, although 28% increase was recorded after 48 hours and 26% decrease after 15 days of feeding.

3. Liver Chemistry

All hepatic enzymes tested were elevated after 48 hours of feeding, except for AP, which increased even after 24 hours and ICDH, which was not affected by this short term treatment. The GPT and LDH enzymes were not significantly altered after 15 days of feeding (8mg/kg/day), while GOT, AP and ICDH activities showed 171%, 172% and 100% increase during this feeding. The long term feeding for 18 months raised all the enzymatic activities.

The hepatic cholesterol content decreased 29%, 72% and 8% respectively after 48 hours, 15 days and 18 months of feeding. Total proteins did not show any significant deviation in 48 hours feeding group, although 33% and 6% increase was observed after 15 days and 18 months of feeding. The soluble proteins showed significant increase after 48 hours and 18 months of feeding, but no significant change was recorded in the 15 days experiment. The FAA content showed 57% increase during the same experimental period. The DNA remained unaltered, while RNA showed no change after 48 hour feeding, 113% increase after 15 days of feeding and 51% decrease after 18 months of feeding.

4. Histological changes

Histologically the cellular morphology was marked by hypertrophied cells, their nuclei and nucleoli. The number of nucleoli/nucleus increased 25% after 15 days of feeding, while the number of nuclei/cell remained unaltered.

III. EFFECTS OF GAMMA BHC (=Lindane)

1. Haematological Changes

The haemoglobin, RBC count and PCV decreased after lindane feeding 7%, 11% and 7% after 48 hours feeding, 8%, 16% and 9% after 15 days feeding, respectively. In long term feeding group the RBC count decreased 10%, but the haemoglobin and PCV did not alter. The WBC count, on the other hand, increased 46% after 48 hour feeding and 24% after 15 day and 18 months of lindane feeding.

2. Blood Chemistry

Like other insecticide treatments, lindane feeding also resulted in increased enzymatic activities. Amylase activity was however unaffected after 48 hour of feeding. SGPT and ChE are not significantly deviated after 15 days of feeding, while SGPT, SGOT and LDH activities are not affected even after 18 months of feeding.

The bilirubin content increase 46%, 69% and 30% after 48 hours, 15 days and 18 months of lindane feeding. Protein

and cholesterol, which were significantly increased after 48 hours of feeding, were not affected in other treatments. FAA content increased 21% after 48 hours feeding, but decreased 19% when lindane was administered for 15 days. Urea content were unchanged, except for 18 months feeding group in which it showed 18% decrease.

3. Liver Chemistry

All hepatic enzymes were significantly increased after lindane feeding. ICDH activity, however, remained unaffected in 48 Hour feeding group, showed 66% increase after 15 days of lindane feeding, and was decreased 27% after 18 months of feeding. All other enzymes behaved uniformly except for AP and GOT activities, which did not show any significant deviation after 18 months of lindane feeding. The hepatic cholesterol and FAA contents decreased, both in 48 hours and 15 days of feeding, while these remained unaffected after 18 months of feeding. Total proteins did not show any significant variation, while the soluble proteins showed 30% increase after 48 hours and 18 months of feeding.

The DNA content did not alter during 48 hours of feeding, but were increased 62% and 55% after 15 days and 18 months of feeding, respectively. The RNA content, on the other hand, decreased during these periods.

4. Histological Changes

The morphological changes in liver were marked by increased size of hepatic cell, its nucleus and nucleolus.

IV. EFFECT OF DDT

1. Haematological Changes

The various haematological parameters generally followed the same pattern, as shown in other insecticides. Haemoglobin content decreased 17% after 48 hours of feeding, while 15 days and 18 months did not cause any change. RBC count decreased 16%, 17% and 14%, respectively, after 48 hours, 15 days and 18 months of feeding. The WBC count is raised 48%, 64% and 29% during the same experimental duration.

2. Blood Chemistry

The various blood serum enzymes reacted sharply to the administration of DDT. All enzymes increased significantly after 48 hours of feeding. In 15 days feeding experiment ChE remained unaffected, while all other enzymes were sharply increased. When DDT was

administered for 18 months, Amylase, AP, AcP, ICDH and CPK increased significantly, while GOT and GPT activities decreased and ChE remained unaltered.

The bilirubin content generally remained unchanged, except for 15 days treatment, when it showed 45% increase. The cholesterol content decreased 40% after 48 hours of feeding but remained unchanged in other feeding groups. FAA content decreased 52% after 15 months of feeding, but did not show any effect in other groups. No definite pattern emerged in urea and glucose content.

3. Liver Chemistry

The hepatic enzymes although showed a general increase after DDT treatment, the effects were not as uniform as in other insecticides. AP and ICDH activity remained unchange after 48 hours of feeding, while AP and GPT showed the same behaviour in 15 day feeding experiment. The ICDH activity increased 85% in this group. After 18 months of feeding, the AP activity increased 39% and GPTactivity by 86%. The ICDH and GOT activities were unaltered or regained their normal values once again.

The hepatic cholesterol and FAA content showed significant decrease in all groups. The glucose content decrease 21% and 31% in 48 hour and 18 months feeding group, but showed 88% increase in 15 day feeding experiment. The total proteins decreased 21% in 15 day group and increased 21% in 18 months feeding group. The soluble proteins on the other hand decreased throughout in 15 day and 18 months group.

The DNA content remained unaffected in 48 hour feeding groups while DNA showed 40% increase after 15 days of feeding, when RNA remained unaltered. Both these content, however decreased after 18 months of DDT feeding.

4. Histological Changes

Except for the increase in hepatic cells, the size of nuclei and nucleoli were not so prominent except for increase in nuclear size in 15 day and 18 month groups and increase in nucleolar size in 48 hour group only. The number of nuclei and nucleoli remained unaffected.

CHAPTER - 1

EFFECTS OF DIELDRIN

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1. INTRODUCTION

The chlorinated hydrocarbon insecticides, including dieldrin, continue to remain a topic of lively debate in the recent years because of their toxic effects on the non-target organisms especially fish, birds and mammals (Rudd and Genelly, 1956; Harrington and Bidlingmayer, 1958; Hayes Jr.,1959; Fitzhugh et al.,1964; Hathway,1965; Stickel et al., 1969; Walker et al.,1969,1973; Stickel, 1973; Thorpe and Walker,1973; Argyle et al.,1975; Kan and Tuinstra,1975,1976; Kan, 1978; Blus, 1978; Radakovic et al.,1980; Beyer and Gish,1980; Griesbach et al.,1982; Mckenzie et al.,1982). In spite of the fact that majority of the American and European States have posed restrictions on their use, these compounds are still quite extensively used in developing third world countries.

Dieldrin is an immense source of danger due to its persistent nature (Bann et al.,1956; Buck and Van, 1968; Deichmann et al., 1971; Korschgen, 1971) and residual effects in the vertebrate systems especially in adipose tissue, lactating organs, brain, muscle, liver and blood (Moss and Hathway, 1964; Klassen and Plaa, 1967; Deichmann et al., 1968; Mick et al., 1971; Harr et al., 1970; Mathews et al., 1974; Stacey and Thomas, 1975; Wassermann, 1976; Driver et al., 1976; Vreman et al., 1976; Klemmer et al., 1977; Polishuk et al., 1977; Wedberg et al., 1977; Ceylan, 1977; Kutz, 1977; Shannon, 1977; Albert et al.,1980; Eckenhausen et al., 1981; Alomar et al., 1985; Abbot et al., 1985). Due to unplanned and indiscriminate use, insecticidal residues.

are also found in atmosphere, aquatic food chain, food crops and dairy products (Graham, 1970; Hill et al., 1973; Balayannis, 1974; Manske and Corneliussen, 1974; Rudd, 1975; Rosenberg, 1975; Downey et al., 1975; Suzuki et al., 1976; Yang et al., 1976; Furr et al., 1976; Hashemy-Tonkabony and Soleimani-Amiri, 1976; Hashemy-Tonkabony and Langaroodi, 1976; Baldwin et al., 1977; Luck and Dyk, 1978; Coway, 1981).

Most of the organochlorine compounds are quite stable but in the soil and living systems they are converted into different metabolites which are not less toxic and in some cases, even more toxic than the parent compound. Toxicological effects are associated either with the direct ingestion of insecticide through drinks, food (fatty meat, dairy products, poultry, eggs) or through occupational exposure. There are indisputable evidences that dietary intake of dieldrin has produced harmful effects in animal systems (e.g. Kadous and Matsumura, 1982; Vrochinskii et al., 1976; Kutz, 1977; Bhatnagar et al., 1980; Reeves et al., 1981; Maliwal and Guthrie, 1981; Lawton et al., 1985; Guzelian, 1985).

The studies on the effects of dieldrin has been undertaken by different laboratories of the world. Many reports exist on the absorption, distribution metabolism, metabolic fate and excretion in vertebrates (Hayes Jr., 1959; Lindstrom et al., 1974,1975,1976; Mathews et al., 1974; Walker and Zorgani, 1974; Iatropoulos et al., 1975; Lay et al., 1975; Reddy and Khan, 1975, 1978; Muller et al., 1975,1979; Hutson, 1976; Virgo and Bellward, 1977; Sell et al., 1977; Somasundaram et al., 1978; Morgan and Lin, 1978; Davidson, 1979; Tanaka et al., 1980; Sudershan and Khan, 1980). After ingestion through intestine the insecticide is taken into the liver through the portal blood where it is reported to induce number of changes (Skalsky and Guthrie, 1975; Kontek et al., 1976; Morgan and Lin, 1978). Moss and Hathway (1964) have reported the preferential localisation of dieldrin in the erythrocytes and plasma. Lone and Javaid (1976) and Hamilton et al.(1978) concluded that

dieldrin causes anaemia in fish and man, which is evident from the low RBC count. Skalsky and Guthrie (1975, 1978) have shown the binding of dieldrin with lipoprotein fraction of the blood in cockroach, rat and man. Mick et al. (1971) studied that greater amount of dieldrin is bound to the alpha-lipoprotein complex. Ishikawa et al. (1978) had shown drastic effects on the total cholesterol and plasma triglycerides in rats. Hurkat and his group has demonstrated effect of dieldrin feeding on the physiological, behavioral, liver weight, body weight, O_2 consumption and several histological and biochemical changes in liver of rabbit (Hurkat and Joshi, 1977; Hurkat et al., 1977; Hurkat, 1978) and histological and histochemical effects on albino rats (Hurkat and Nath, 1975; Hurkat, 1977 a,b,c). There are several studies which correlate dieldrin with neurotoxicity (e.g. Sharma, 1976; Kohli et al., 1977 b; Joy et al., 1980; Bowyer et al., 1980; Joy, 1982; Shankland, 1982; Hermanowicz et al., 1982; Swanson and Woolley, 1983).

Agarwal et al. (1981) have also reported the effect of dieldrin on lipid metabolism of rhesus monkeys. Changes in serum proteins and free amino acids are observed by Wassermann et al. (1973) and Shakoori et al. (1976).

Microsomal electron transport components have been shown to increase after dieldrin treatment from several laboratories (Street, 1969; Sell and Davison, 1973; Den et al., 1974; Kohli et al., 1975, 1977a; Krample and Hladka, 1975; Bellward et al., 1975; Ford et al., 1976; Stevens et al., 1977). Virgo and Bellward (1975) reported increase in cytochrome P450 and microsomal proteins. Bellward et al. (1975) and Vainio and Parkki (1976) have reported increase in several microsomal enzymes. That the dieldrin induces hepatic microsomal monoxygenase system has also been shown by Hutson and Wright (1980).

Many laboratories have reported the effects of dieldrin on carbohydrate metabolism (Bhatia et al., 1972a,b, 1973; Costella and Virgo, 1980). Dieldrin feeding resulted in hyperglycemia, lactic acidemia, lowered glucose tolerance and elevated plasma non-esterified fatty acids (NEFA) concentration. Bhatia et al. (1973) concluded that dieldrin induce the hepatic glycogen accumulation by a stimulation of synthetic pathway coupled with the decreased break down of polysaccharides. However, Hurkat (1977a) showed decrease in liver glycogen in rabbits which according to him may be due to the destruction of glucose-6-phesphatase in the membranes of the endoplasmic reticulum.

Blood also, is a reliable and sensitive indicator of liver malfunctioning due to insecticide treatment. Most of the enzymes in blood, including liver function, have been reported to show increased activities after insecticide feeding (Knox and Greengard, 1965; Jager, 1970; Kagan et al., 1970; Gertig et al., 1971 a, b; Bhatia et al., 1972 a, 1972 b, 1973; Hunter et al., 1972; Krample and Hladka, 1975). Ishikawa et al., (1978) has, however, shown that liver function tests remain unaffected after dieldrin treatment. Total liver DNA in mice show increase after dieldrin feeding which was proportional to hepatic cell hyperplasia (Tennekes et al., 1979).

Wright et al. (1972, 1978) published two informative reports that dealt with the effect of dieldrin on the subcellular structure and function of mammalian liver cells and on the prolonged ingestion of dieldrin on the liver of male rhesus monkeys.

Characteristic changes in liver of laboratory animals are induced by dieldrin and variety of other xenobiotic compounds. These changes include, liver enlargment, liver cell hypertrophy, liver cell hyperplasia, induction of drug metabolising enzymes and proliferation of smooth endoplasmic reticulum. Degenerative changes in liver and kidney parenchymatous cells are also reported in chronic

studies in dogs and rats (Kitselman, 1953; Cleveland, 1966; Mathur, 1975; Reuber, 1975, 1976, 1978 b; Cabral et al., 1979; Reuber, 1980; Ruebner et al., 1980). According to different workers these changes do not cause liver damage and are reversible upon withdrawal or elimination of the compound (Schulte-Hermann et al., 1971,*1974; Wright et al., 1972,1977). These changes in liver are regarded as adaptive responses of the organ to increase functional demands. The chronic exposure of various strains of mice to dieldrin may lead to development of liver tumor (Davis and Fitzhugh, 1962; Walker et al.,1973; Thorpe and Walker, 1973; Epstein, 1975a,b; Hutson,1976; Stevenson et al., 1976; Reuber,1977; Tennekes et al., 1982). Tennekes et al.(1979) concluded that dieldrin itself is not carcinogen and acts by facilitating the expression of pre-existing oncogenic potential only in mouse liver probably by inducing hyperplasia in this organ. Experiments in rats and other animals reveal that dieldrin does not produce any carcinogenic changes in these animals (NCI Report, 1978).

(*Schulte-Hermann)

Although dieldrin has been the focus of attention of several workers, who have studied and reported histopathological changes (e.g.Kitselman,1953;Mathur,1975; Hurkat,1977a,b,c; Reuber,1980,1976,1978), ultrastructural changes (e.g.Kimbrough et al.,1971;Roux et al.,1974; Kohli et al.,1977), teratogenic effect (Ottolenghi et al., 1973; Dix et al.,1977c),carcinogenic effects (Davis and Fitzhugh,1962; Thorpe and Walker,1973; Reuber,1976,1977, 1978; Reubner et al.,1980; Tennekes et al.,1982 etc.and several others) and several biochemical changes in blood liver and kidney (see previous pages in Introduction) no systematic study has ever been undertaken except for a few scattered reports(Shakoori et al.,1982,84) to ascertain the effects of dieldrin feeding on the various liver function tests and the corresponding biochemical components in the liver. These studies have been necessitated due to frequent accidental exposure of farmers, factory workers, or other persons who work with these insecticides and hence get affected by direct exposure or are affected indirectly through contaminated food or water. The possible changes caused by these insecticide must be known in order to plan a remedy and cure for the detrimentaleffects of these persons. In the present study liver function tests havebeen taken as indicators of dieldrin toxicity.

The objective of present study is, therefore, to evaluate the dieldrin toxicity at haematological,biochemical and histological levels in rats. These findings, then can be extrapolated to human beings to augment the argument for potential hazards of dieldrin treatment through insecticidal sprays.

2. MATERIALS AND METHODS

2.1. ANIMALS

A colony of albino rats 'Sprague Dawley strain' was raised from a few animals obtained from National Institute of Health, Islamabad. They were housed in the animal house of Department of Zoology, in groups of 5-6 in each cage and allowed food and water ad libitum.

The rats used were as follows:

a) For short term experiments: Two groups of female rats weighing about 120-180 grams and five to six months of age. One group was used for feeding insecticide for 48 hours, while the second was used for feeding insecticide for 15 days.

b) For long term experiment: Male rats weighing about 70-90 grams and about three months of age.

2.2. PREPARATION OF FEED

The rat feed was prepared in the lab, by mixing the following constituents:

1. Poultry feed ..... 5 Kg
2. Fish Meal ..... 1 Kg
3. Wheat Flour ..... 2 Kg
4. Molasses .....100 gm
5. Water ..... 3 lit.(approx.)

Poultry feed, fish meal and wheat flour were first mixed thoroughly and then mixed in water containing molasses into semi-solid feed cakes. The feed cakes were air dried before presenting them to the animals.

2.3. INSECTICIDE USED

An organochlorine insecticide of cyclodiene group i.e. dieldrin (1,2,3,4,10, 10-hexachloro-6, 7-epoxy-1,4,4a,5,6,7,8,

8a-octahydro-1, 4-endo-exo-5, 8-dimethano-naphthalene =HEOD), was used for this study. This was obtained from Plant Protection Division of Agriculture Department as 20% EC formulation.

2.4. ADMINISTRATION OF INSECTICIDE

Dieldrin was administered to rats as strong and weak doses as follows:

a) Strong dose. For short term experiments, two levels of strong doses were administered. In one group of rats a strong dose of 12 mg dieldrin per kg body weight per day was administered for a total period of 15 days. In the second group 40 mg dieldrin per kg body weight per day was administered for a total period of 48 hours.

b) Weak dose. At the rate of 6 mg/kg body weight/day was administered to another group of rats for long term experiment.

2.4.1. SHORT TERM EXPERIMENTS

For short term experiments, in which the total duration was 48 hours in one case and 15 days in the other case, the insecticide was administered as follows:

a) For 48 hours experiment, the insecticide mixed diet was prepared by mixing 1.66 ml of 20% dieldrin in small amount of water, which was then thoroughly mixed with 1 kg of ingredients of rat feed. Each experimental rat consumed 30 gm of rat feed daily. That way the rats consumed 40mg of dieldrin/kg body weight/day.

b) For 15 day experiment the insecticide mixed diet was prepared by mixing 0.5 ml of 20% dieldrin in small amount of water which was then thoroughly mixed with 1 kg of ingredients of rat feed.

Each experimental rat consumed thirty grams of feed daily. That way the rats took 12 mg of dieldrin/kg body weight/day.

2.4.2. LONG TERM EXPERIMENT

For long term experiment, the total duration of which was 18 months, the insecticide mixed diet was prepared by adding 0.5 ml of 20% dieldrin to 2 kg of ingredient-mixed diet in the same way as above. In this way the experimental animals consumed dieldrin at the rate of 6 mg/kg body weight/ day.

2.5. EXPERIMENTAL PROCEDURE

a) SHORT TERM EXPERIMENTS

Two short term experiments were set up. In one case 8 animals were selected. They were weighed and then fed on dieldrin mixed diet, prepared for this purpose, for 48 hours. A group of 4 rats were weighed, anaesthetized and slaughtered every 24 hours. The blood samples were collected and livers taken out for various analyses. A group of control animals, each of 4 rats, was proceeded exactly in the same manner, except for the dieldrin treatment. For the second short term experiment another group of 20 rats was selected. They were weighed initially and fed on dieldrin-mixed diet regularly for a total period of 15 days. A group of three rats were weighed, anaesthetized and slaughtered regularly every third day. The blood samples were collected and livers taken out for various analyses. A group of control animals was proceeded exactly in the same manner except for the dieldrin treatment.

b) LONG TERM EXPERIMENT

For long term study, a group of 9-10 animals was fed regularly on dieldrin mixed diet,prepared for this

purpose, for a total period of 18 months. Every six months 3-4 rats were weighed, anaesthetized and slaughtered. Their blood samples and livers were collected for various analyses. A group of control rats, each of 3-4 rats, fed on dieldrin free diet, were slaughtered each time and used as control of the long term experiment.

2.5.1. COLLECTION OF BLOOD

Blood specimens were collected from the inferior vena cava with the help of 10 ml sterilized syringe and transferred gently to a blood centrifuge tube after removing the needles from the syringe. It was allowed to clot and centrifuged at a speed of 1,000 rpm to obtain a clear serum which was afterwards used for different biochemical studies. Small quantity of blood was collected in the tube containing EDTA as anticoagulant and was used for various haematological studies. The amount of EDTA used was 15 mg/ml of blood, and it was mixed gently by rotation of tube.

2.5.2. LIVER PROCESSING

The liver was taken out, weighed and then processed for histological and biochemical studies.

Saline extract was prepared by homogenising a piece of liver in 0.89% NaCl solution in a glass homogenizer. The homogenate was centrifuged at 15,000 rpm to obtain clear supernatant, which was then used for different biochemical studies.

A portion of liver was weighed and processed for nucleic acids (DNA and RNA) and total protein content.

For cholesterol estimation ethanol extract was prepared.

A small piece of liver was also fixed in Bouin's fixative for histological studies.

2.5.3. HAEMATOLOGICAL STUDIES

Anticoagulent (EDTA) containing blood was used for studies which involved the estimation of haemoglobin content according to Van-Kampan and Zijlstra (1961), packed cell volume (PCV) according to microhaematocrit method of Strumia et al. (1954), red blood cell (RBC) count, and total leukocyte count (TLC) according to routine clinical methods. These values were then utilized for calculating mean corpuscular haemoglobin (MCH), mean cell volume (MCV) and mean corpuscular haemoglobin concentration (MCHC), as described below:

$$\text{MCH} : \frac{\text{Haemoglobin/l}}{\text{RBC}/\mu\text{l}}$$

$$\text{MCV} : \frac{\text{PCV/l}}{\text{RBC}/\mu\text{l}}$$

$$\text{MCHC} : \frac{\text{Haemoglobin/dl}}{\text{PCV}}$$

2.5.4. BIOCHEMICAL ANALYSIS OF BLOOD

The extensive analysis of blood serum was carried out for evaluating liver dysfunctioning and other metabolic disorders which involved the estimation of acid phosphatase (AcP) and alkaline phosphatase (AP) activities according to King and King (1954), amylase activity according to Wootton (1964), cholinesterase (ChE) activity according to Rappaport et al. (1959), creatine phosphokinase (CPK) activity according to Okinaka et al. (1961), isocitrate dehydrogenase (ICDH) activity according to Bell and Baron (1960), lactate dehydrogenase (LDH) activity according to Cabaud and Wroblewski (1958), serum glutamate oxaloacetate

transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) activities according to Reitman and Frankel (1957). In addition some other biochemical contents i.e. bilirubin according to Jendrassik and Grof (1938), cholesterol according to Liebermann and Burchard reaction of Henry (1964), free amino acids (FAA) according to Moore and Stein (1954), protein according to Lowry et al. (1951) and urea according to DAM method of Netelson et al. (1951)were also estimated.

2.5.5. BIOCHEMICAL STUDIES OF LIVER

Aqueous liver extract (in saline) was used for the estimations of AP activity, GOT activity, GPT activity, ICDH activity, LDH activity and FAA, glucose and protein (Total and Soluble) contents. Cholesterol was estimated from ethanol prepared extract. Total protein content was estimated from the tissue processed for nucleic acid estimation. For this purpose the pellet obtained after extraction of DNA and RNA, was mixed with 2.5 ml of 0.5 N NaOH to solublize the protein fraction for estimation with Lowry's method (Lowry et al., 1951).

Nucleic acids content of liver was extracted by the method described in Shakoori and Ahmed (1973). Weighed amount of liver was crushed in boiling ethanol for 3 minutes. Three washings in ethanol were done, followed by 2-3 washings in methanol:ether (3:1) mixture. The crushed liver was then desiccated over dry NaOH under vacuum. RNA was extracted in 10% PCA at 4°C for 18 hours, while DNA was extracted in 10% PCA at 65°C for 30 minutes.

DNA estimation was based on Diphenylamine method and RNA estimation on Orcinol method. Both these methods follow Schmidt and Thannhauser procedure described by Schneider (1957).

2.5.6. HISTOLOGICAL STUDIES

Histological sections of liver, after fixation in Bouin's fluid, were prepared according to routine section cutting technique. 8 μ thick sections were cut after wax-embedding and stained with hematoxylin and eosin. These sections were then studied to note various histopathological alterations. Morphometric studies were conducted which included the following parameters:-

i. Number of cells/microscopic field.
ii. Number of nuclei/cell.
iii. Number of nucleoli/nucleus.
iv. Size of hepatic cells.
v. Size of nuclei.
vi. Size of nucleoli.

The measurements were made with the help of an ocular micrometer which was calibrated with stage micrometer. The parameters from (i) to (iii) were determined at magnification of 500X, while the rest of the studies were performed at a magnification of 1250X. The number of observations recorded for each parameter has been mentioned as a foot-note in each relevant table.

3. RESULTS

3.1. EFFECT OF DIELDRIN MIXED DIET (40 mg/kg body weight/day) ADMINISTERED FOR 48 HOURS

3.1.1. HAEMATOLOGICAL STUDIES

The dieldrin administered to rats through feed at a dose of 40 mg/kg body weight/day resulted in drastic changes in the various haematological parameters of albino rats (Fig.1 and Table I). A control rat contains haemoglobin 13.27±0.14 g/dl which after dieldrin treatment is reduced significantly, 6% after 24 hours and 4% after 48 hours of insecticide feeding. The RBC count and packed cell vol (PCV) are also likewise affected. The RBC count is decreased 16% and 22%, while the PCV is reduced 8 and 11%, respectively, 24 and 48 hours after dieldrin feeding (Fig.1, Table I). The WBC count, on the other hand, show drastic increase after dieldrin administration. The increase after 24 hours is about 34%, while it is 55% after 48 hours of feeding.

The other haematological parameters like MCV and MCH show significant increases at both points of observation, while MCHC show significant change only after 24 hours of feeding (Table I, Fig.1). The RBC count/WBC count hold a ratio of 1.07 in the control animal. This ratio in 24 hours and 48 hour group, respectively, 0.67 & 0.54.

3.1.2. BIOCHEMICAL ANALYSIS OF BLOOD SERUM

The blood serum of control and dieldrin fed rats were analyzed for numerous enzymatic activities, like phosphatases (acidic and alkaline), transaminases (GOT and GPT), dehydrogenases (lactate and isocitrate; LDH, ICDH), creatine phosphokinase (CPK), Cholinesterase (ChE) and amylase (Table II, Fig.2). Besides that

C = CONTROL
24 = 24 HOURS DIELDRIN FEEDING
48 = 48 HOURS DIELDRIN FEEDING

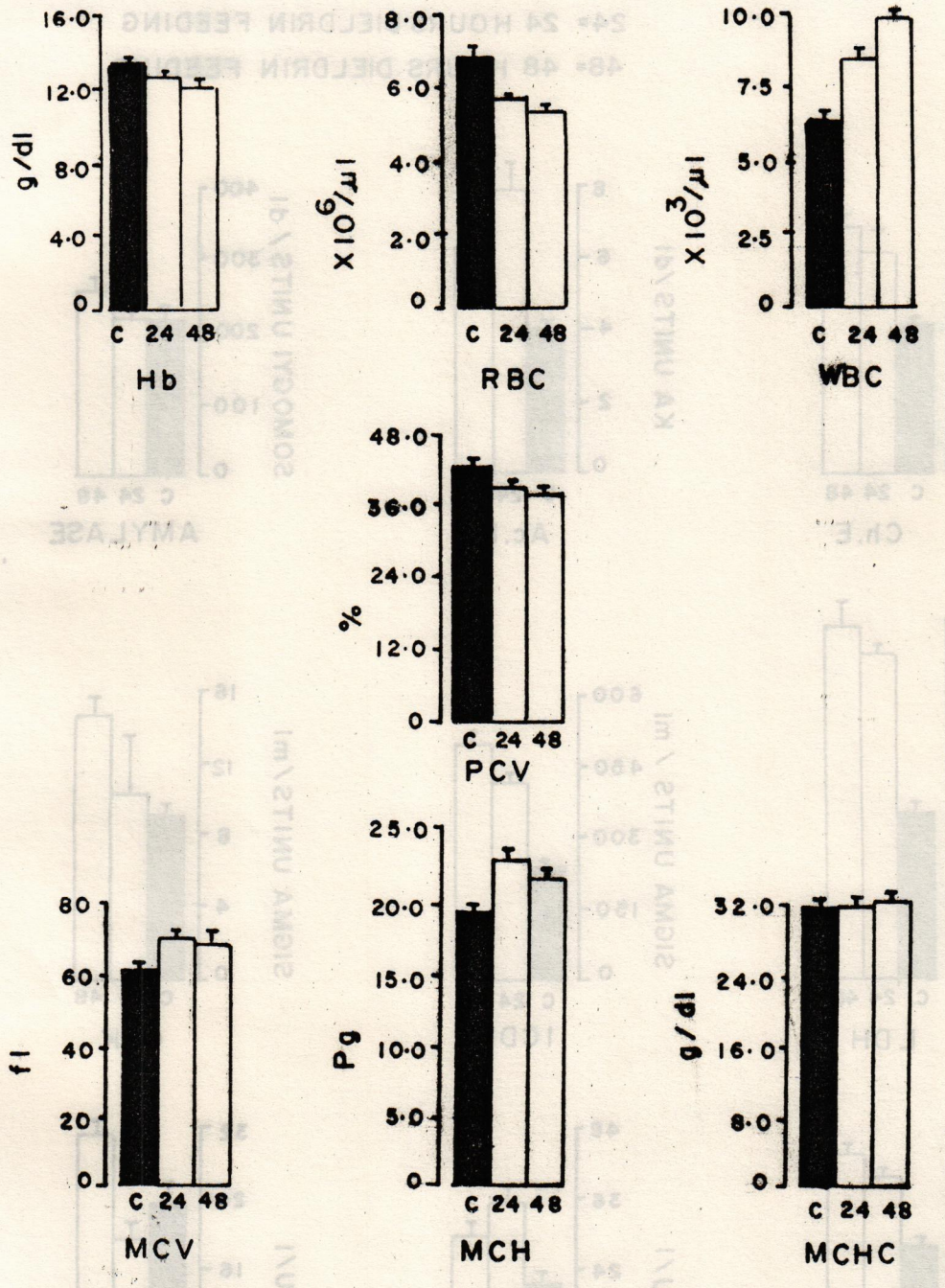


FIG. 1: Effect of feeding dieldrin mixed diet (40 mg/kg body weight/day) for 48 hours on the various haematological parameters of albino rats.

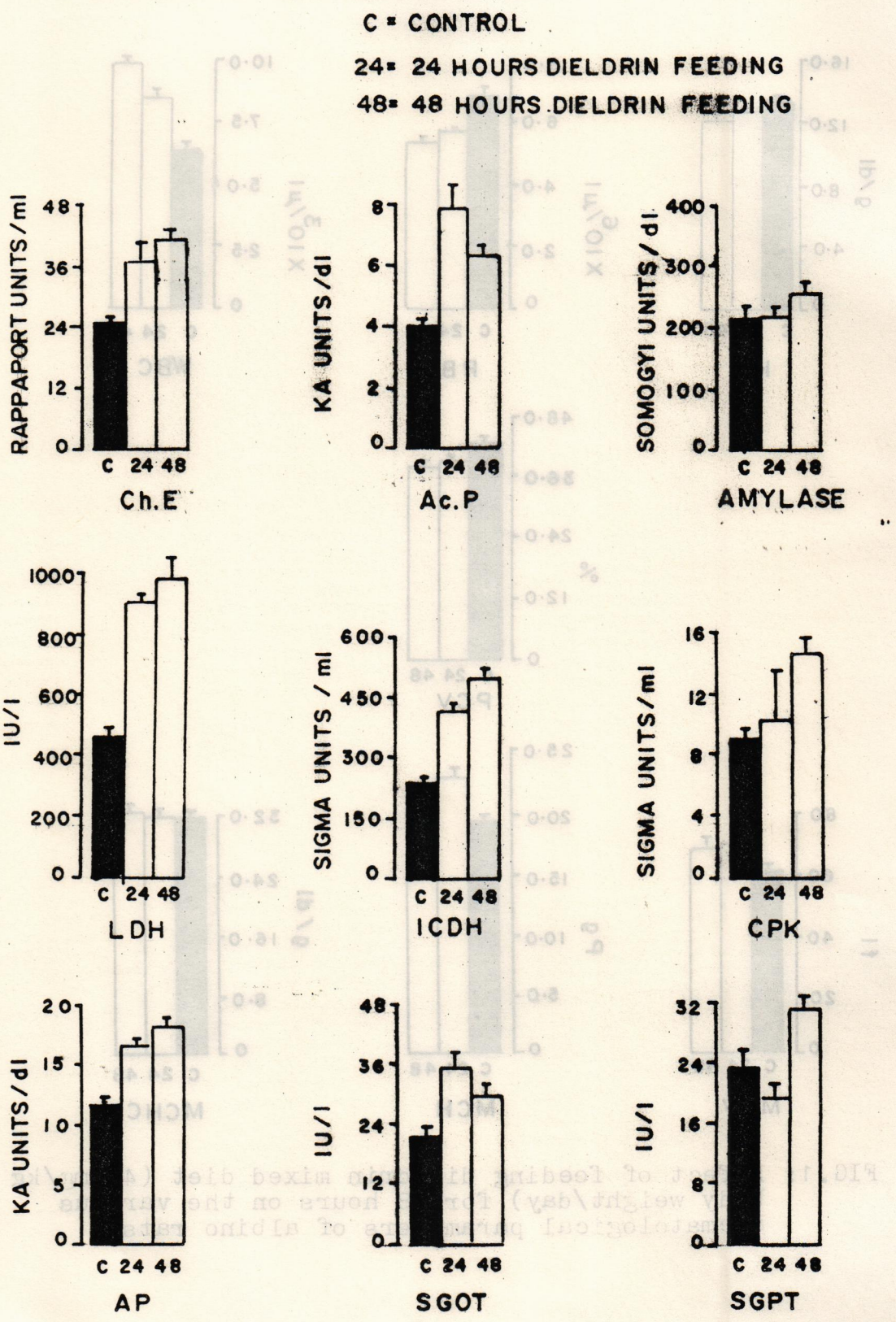


FIG.2: Effect of feeding dieldrin mixed diet (40 mg/kg body weight/day) for 48 hours on the various enzymatic activities of rat blood serum.

TABLE - I

EFFECT OF FEEDING DIELDRIN MIXED DIET(40 mg/kg body weight/day) FOR 48 HOURS ON THE VARIOUS HAEMATOLOGICAL PARAMETERS OF ALBINO RATS

	Control (n=8)	Dieldrin fed	
		24 hours (n = 4)	48 hours (n = 4)
Haemoglobin (g/dl)	13.27±0.14[a]	12.42±0.12**	12.13±0.15***
RBC count ($X10^5/\mu l$)	68.37±2.53	57.17±1.77**	53.45±1.62***
WBC count ($X10^2/\mu l$)	63.64±2.60	85.00±3.37***	98.62±2.44***
PCV (%)	42.09±0.34	38.74±0.42***	37.47±0.49***
MCV (fl)	61.56±0.17	70.22±1.23***	68.50±2.42*
MCH (Pg)	19.38±0.08	22.73±0.85**	21.76±0.49**
MCHC (g/dl)	31.52±0.09	32.36±0.17**	31.76±0.19

[a] Mean±SEM, Student's 't' test;

*P< 0.05; **P< 0.01; ***P< 0.001

TABLE - II

EFFECT OF DIELDRIN FEEDING MIXED DIET (40 mg/kg body weight/day) FOR 48 HOURS ON SOME ENZYME ACTIVITIES OF RAT BLOOD SERUM

	Control (n = 8)	Dieldrin feeding	
		24 hours (n = 4)	48 hours (n = 4)
AcP (KAU/dl)	4.23±0.20	7.91±0.68***	6.30±0.32***
AP (KAU/dl)	11.67±0.16	16.64±0.75***	18.24±0.77***
Amylase (Somogyi U/dl)	216.77±18.02	219.77±12.93	258.13±15.71
ChE (Rapp-aport U/ml)	24.37±1.42	36.56±4.04*	38.19±3.83*
CPK (Sigma U/ml)	9.01±0.63	10.25±3.27	14.65±0.95***
ICDH (Sigma U/ml)	243.18±14.80	415.73±21.73***	499.61±23.21***
LDH (IU/l)	465.66±26.29	908.40±23.89***	987.60±72.19***
SGOT (IU/l)	21.73±1.65	35.19±3.27**	29.58±2.51*
SGPT (IU/l)	23.05±2.40	19.18±1.93	31.08±1.51*

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

several other biochemical components of sera were also tested e.g. bilirubin, cholesterol, proteins, free amino acids (FAA), glucose and urea (Table III, Fig. 3).

Off all the enzymes tested, amylase activity remains undisturbed. All other enzymes are significantly elevated after dieldrin treatment (Table II, Fig.2). The control blood serum contains alkaline phosphatase (AP) activity of 11.67±0.16 KAU/dl, while the acid phosphatase (AcP) activity is 4.23 KAU/dl. The AP activity increases 43% and 56%, while the AcP activity is increased 87% and 49% respectively, 24 hours and 48 hours after dieldrin treatment. Both the transaminases are also elevated. The SGOT appears to be more sensitive, as it is elevated 62% and 36%, after 24 hours & 48 hours of dieldrin treatment. The SGPT, on the other hand, does not show any significant daviation from the normal, after 24 hrs. of treatment, but is increased 35% after 48 hours exposure to insecticide. Both LDH and ICDH activities are drastically increased. The control blood serum contains 465.66±26.29 IU/l as LDH activity and 243.18±14.80 sigma units/l as ICDH activity. These enzymatic activities increase almost 2 times after dieldrin treatment. The LDH activity shows 1.95X and 2.12X, while ICDH activity shows 1.71X and 2.05X increase, after 24 hours & 48 hours of dieldrin treatment (Table II, Fig.2). The activity of creatinine phosphokinase (CPK) does not show any appreciable increase after 24 hours, but increases significantly (63%), after 48 hrs. of insecticide treatment. Cholinesterase activity also increases 50 and 57% respectively, after 24 hours & 48 hours of insecticide treatment.

Table III and Figure 3 show the effect the dieldrin on the various biochemical components of blood serum. The bilirubin, protein, urea and glucose content increase, while cholesterol and free amino acids decrease

TABLE - III

EFFECT OF FEEDING DIELDRIN MIXED DIET (40 mg/kg body weight/day) FOR 48 HOURS ON THE VARIOUS BIOCHEMICAL PARAMETERS OF RAT BLOOD SERUM

Parameters	Control (n = 8)	Dieldrin feeding	
		24 hours (n = 4)	48 hours (n = 4)
Bilirubin (mg/dl)	0.72±0.07[a]	0.69±0.04	0.95±0.08*
Cholesterol (mg/dl)	195.64±6.94	131.94±9.24***	140.00±8.64***
Free amino acids(mg/dl)	7.26±0.19	6.32±0.29*	5.13±0.38***
Glucose (mg/dl)	107.37±3.36	125.71±6.70*	131.15±9.34*
Proteins (g/dl)	7.30±0.13	8.32±0.14***	8.51±0.49*
Urea (mg/dl)	35.49±0.96	34.67±1.09	49.91±1.72***

[a] Mean±SEM, Student's 't' test;

*P< 0.05; **P< 0.01; ***P< 0.001

C = CONTROL
24 = 24 HOURS DIELDRIN FEEDING
48 = 48 HOURS DIELDRIN FEEDING

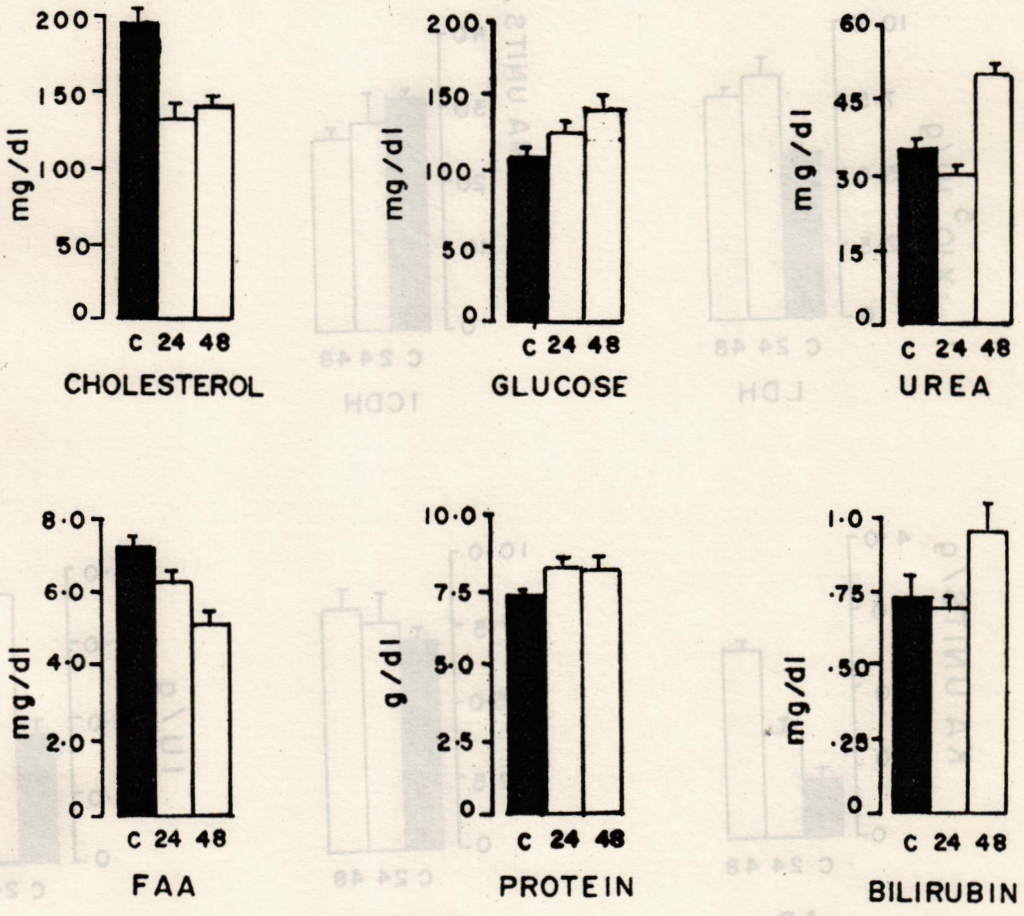


FIG. 3: Effect of feeding dieldrin mixed diet (40 mg/kg body weight/day) for 48 hours on the various biochemical components of albino rat blood serum.

C= CONTROL

24=24 HOURS DIELDRIN FEEDING

48= 48 HOURS DIELDRIN FEEDING

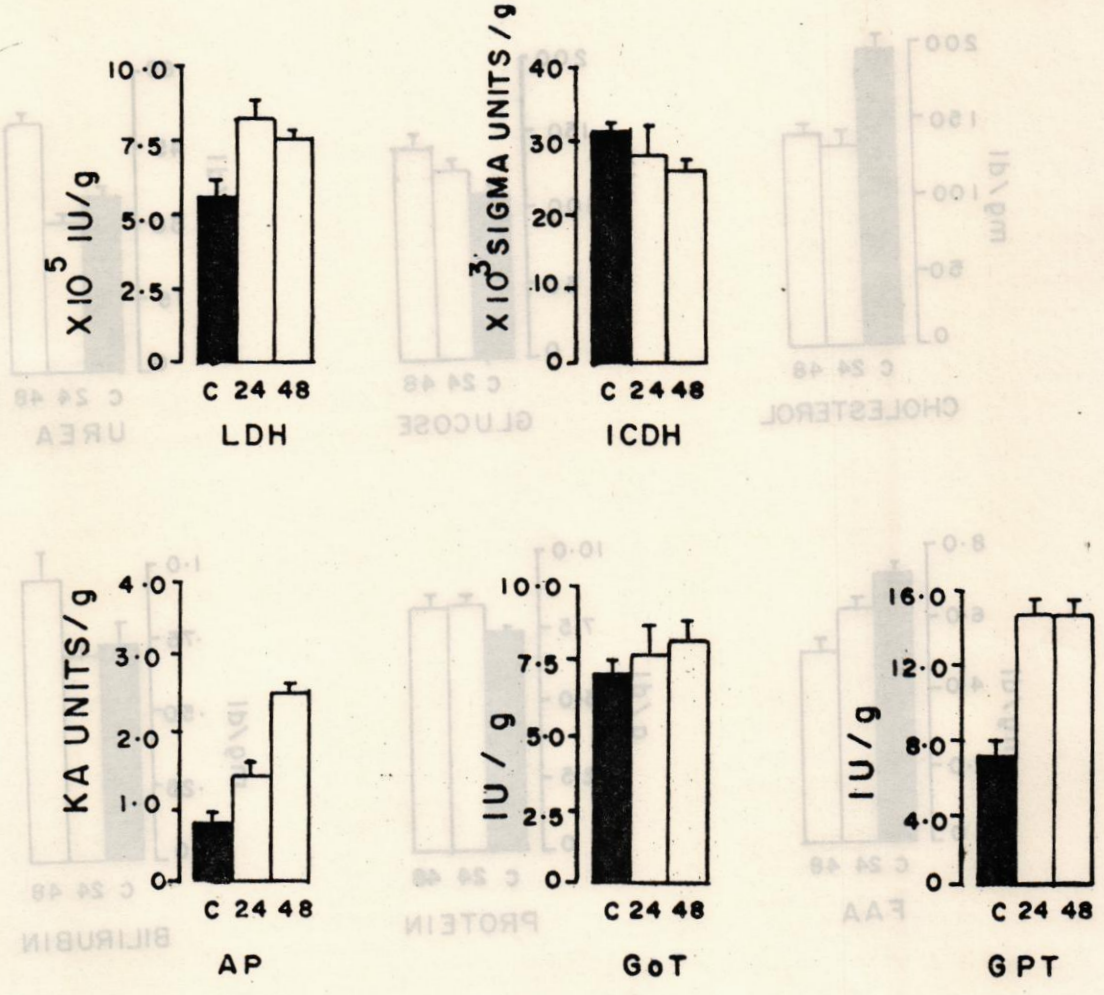


FIG. 4: Effect of feeding dieldrin mixed diet (40 mg/kg body weight/day) for 48 hours on the activities of some hepatic enzymes.

TABLE - IV

EFFECT OF FEEDING DIELDRIN MIXED DIET (40 mg/kg body weight/day) FOR 48 HOURS ON ACTIVITIES OF SOME ENZYMES OF RAT LIVER

Parameters	Control (n = 5)	Dieldrin feeding	
		24 hours (n=4)	48 hours (n = 4)
AP (KAU/g)	0.81±0.16[a]	1.43±0.20*	2.52±0.14***
GOT (IU/g)	7.11±0.38	7.75±0.91	8.21±0.64
GPT (IU/g)	7.32±0.68	14.72±2.52*	14.60±0.88***
ICDH ($X10^3$ Sigma U/g)	31.39±0.78	28.19±3.93	26.79±1.48*
LDH ($X10^4$ IU/g)	56.57±4.43	83.20±5.23**	75.55±3.08**

[a]Mean±SEM, Student's 't' test;

*P < 0.05; **P < 0.01; ***P < 0.001

TABLE - V

EFFECT OF FEEDING DIELDRIN MIXED DIET (40 mg/kg body weight/day) FOR 48 HOURS ON THE VARIOUS BIOCHEMICAL COMPONENTS OF RAT LIVER

Parameters	Control (n = 5)	Dieldrin feeding	
		24 hours (n = 4)	48 hours (n = 4)
Cholesterol (mg/g)	7.62±0.22[a]	5.40±0.36**	5.76±0.53*
Free amino acids(µg/g)	399.21±18.13	223.26±13.57***	239.66±29.20**
Glucose (mg/g)	20.14±0.53	12.35±1.25***	13.12±0.86***
Soluble Protein (mg/g)	111.18±5.08	135.65±4.47**	155.34±15.67*
Total protein (mg/g)	199.33±6.11	250.02±11.82**	191.34±32.06
DNA (mg/g)	3.84±0.44	2.58±0.17*	1.63±0.31**
RNA (mg/g)	9.53±0.55	7.45±0.36*	5.07±1.27*

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

after dieldrin feeding.

The blood serum of control rats contain 0.72± 0.07 mg/dl bilirubin, 7.30±0.13 g/dl proteins, 35.49± 0.96 mg/dl urea and 107.37±3.36 mg/dl glucose. The bilirubin content increases 32%, while urea increases 42%, 48 hours after dieldrin treatment; proteins and glucose content are affected even after 24 hours (Table III, Fig. 3).

3.1.3. BIOCHEMICAL ANALYSIS OF LIVER

The liver was analyzed for activities of some hepatic enzymes like AP, GOT, GPT, ICDH, and LDH (Table IV, Fig.4). A few other biochemical components like cholesterol, free amino acids, glucose, soluble proteins, total proteins, DNA and RNA were also analyzed (Table V, Fig.5).

Table IV and Figure 4 shows the effect of dieldrin on various enzymatic activities. The GOT activity remains undisturbed, while the GPT activity shows 100% increase, both in the 24 and 48 hour group. The AP activity in control liver is 0.81±0.16 KAU/g, which increases 1.78X and 3.13X, after 24 hours & 48 hours of dieldrin treatment. The LDH activity is increased 47 and 36%, after 24 and 48 hours of insecticide treatment. The ICDH activity is not much altered within 24 hours after dieldrin treatment, while it shows 17% decrease during the next 24 hours of insecticide administration.

Majority of the hepatic biochemical components decrease after dieldrin feeding (Table V, Fig.5). The control liver contains 7.62±0.22 mg/g of cholesterol, 20.14 ± 0.53 mg/g of glucose and 399.21±18.13 µg/g of Free amino acids. The cholesterol content decrease 29 and 24%, after 24 hours and 48 hours of insecticide feeding;

while the glucose shows, respectively, 39 and 35% decrease and FAA 44 and 40% decrease. The total and soluble proteins content, on the other hand, show significant increase (Table V, Fig.5). The soluble protein increases 22 and 40%, after 24 & 48 hours of dieldrin feeding, while the total protein increases 25% within 24 hours of exposure to insecticide. The DNA and RNA content also decrease significantly. The control rat liver contains 3.84±0.44 mg/g DNA and 9.53±0.55 mg/g RNA. The RNA content decrease 22% after 24 hours and 47% after 48 hours of dieldrin feeding at a dose of 40 mg/kg body wt./day. The DNA content follow almost the same path. There is 33% decrease after 24 hours and 63% decrease after 48 hours of dieldrin feeding.

3.1.4. HISTOLOGICAL STRUCTURE OF LIVER

Figures 6-8 show histological structure of liver in control rats, while Figures 9-12 show histological changes after 24 hours of feeding. Figures 13-14 demonstrate changes in liver structure after 48 hours of feeding insecticide. The size of hepatic cell is distinctly increased and so are the nuclei and nucleoli. Although the hepatolobular architecture is generally maintained, but the nuclei become vesicular, the number of nucleoli increase and the sinusoidal space increases (Figs.11,12).

Table VI and Figure 15 shows changes in various morphometric parameters. The size of cell, nucleus and nucleolus increase. Hepatic cell, on the average, measures 260.09±8.29 μ^2, which increases 37% and 46%, after 24 hours and 48 hours of dieldrin treatment. The size of nucleus and nucleoli in control rats is respectively 40.27±2.51 μ^2 and 2.44±0.42 μ^2. Both these cellular organelle hypertrophy. The nuclear size increases 53% after 24 hours and 62% after 48 hours of insecticide treatment. The nucleolar size is

C= CONTROL
24= 24 HOURS DIELDRIN FEEDING
48= 48 HOURS DIELDRIN FEEDING

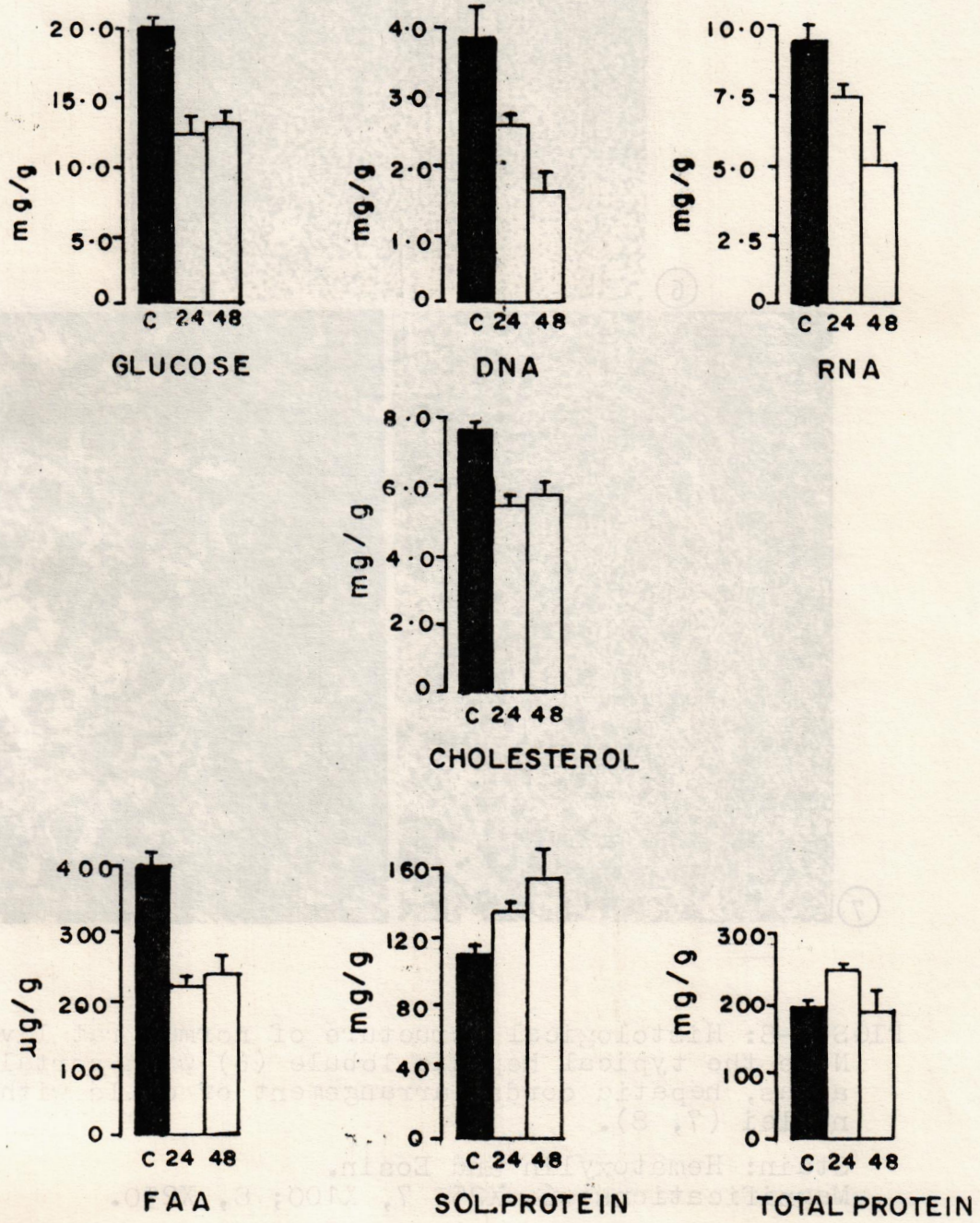
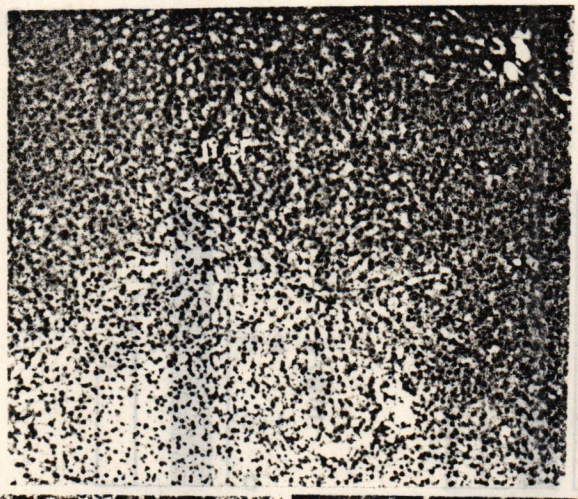


FIG.5: Effect of feeding dieldrin mixed diet (40 mg/kg body weight/day) for 48 hours on the various biochemical components of rat liver.



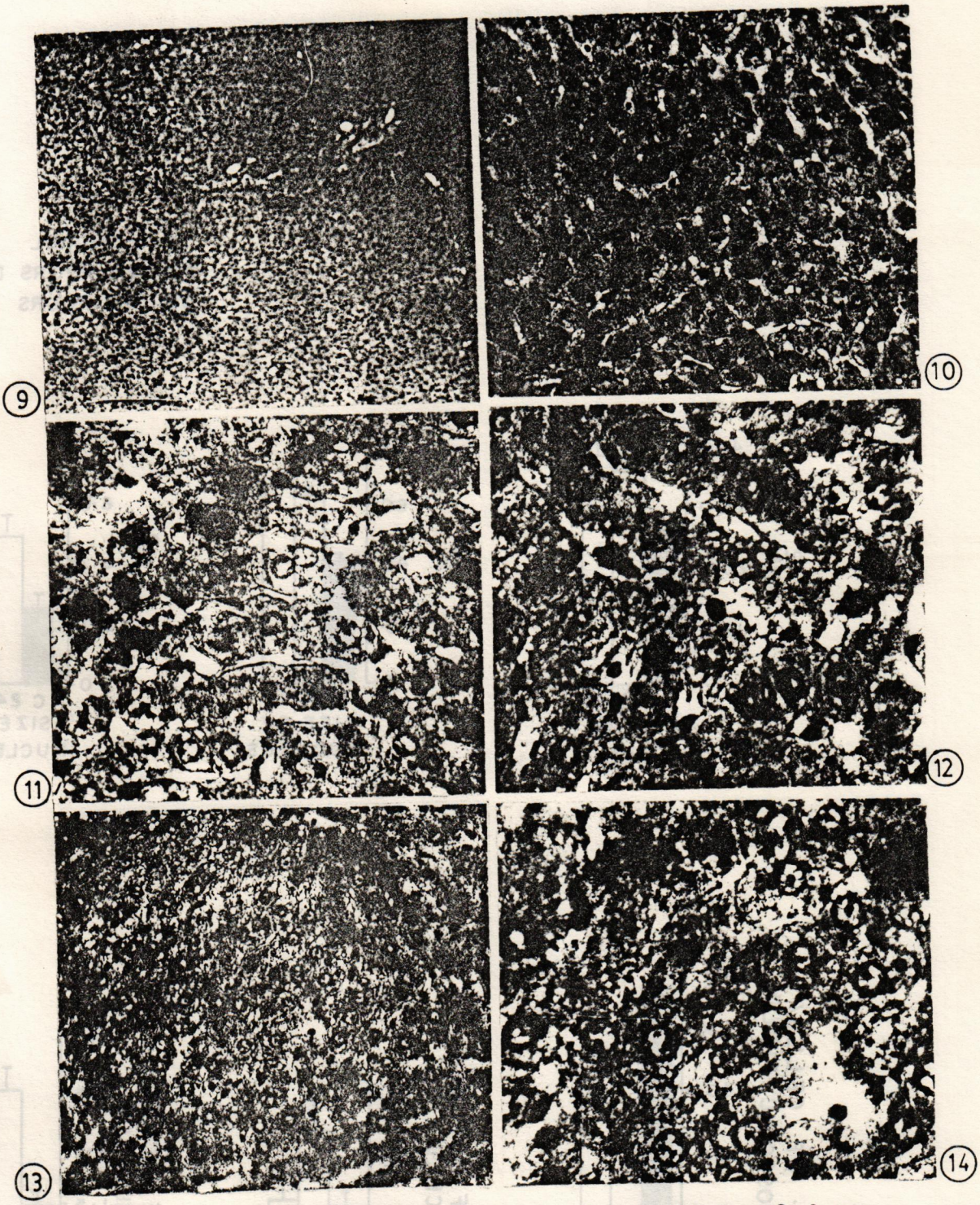
(6)

(7)

(8)

FIGS.6-8: Histological structure of normal rat liver. Note the typical hepatic lobule (6) with portal areas, hepatic cords, arrangement of cells with nuclei (7, 8).

Stain: Hematoxylin and Eosin.
Magnifications: 6, X25; 7, X100; 8, X250.



FIGS.9-14: Histological structure of rat liver fed on dieldrin-mixed diet for 24 hours (9-12) and 48 hours (13-14). Note disturbed lobular structure (9,10), increased sinusoidal spaces (11,12), slight cytoplasmic vacuolation with many clear areas (13-14), hypertrophied cells (10,11, 14) and nuclei (13-14).

Stain: Hematoxylin and Eosin.
Magnifications: 9, X25; 10,13, X100; 11,12,14, X250.

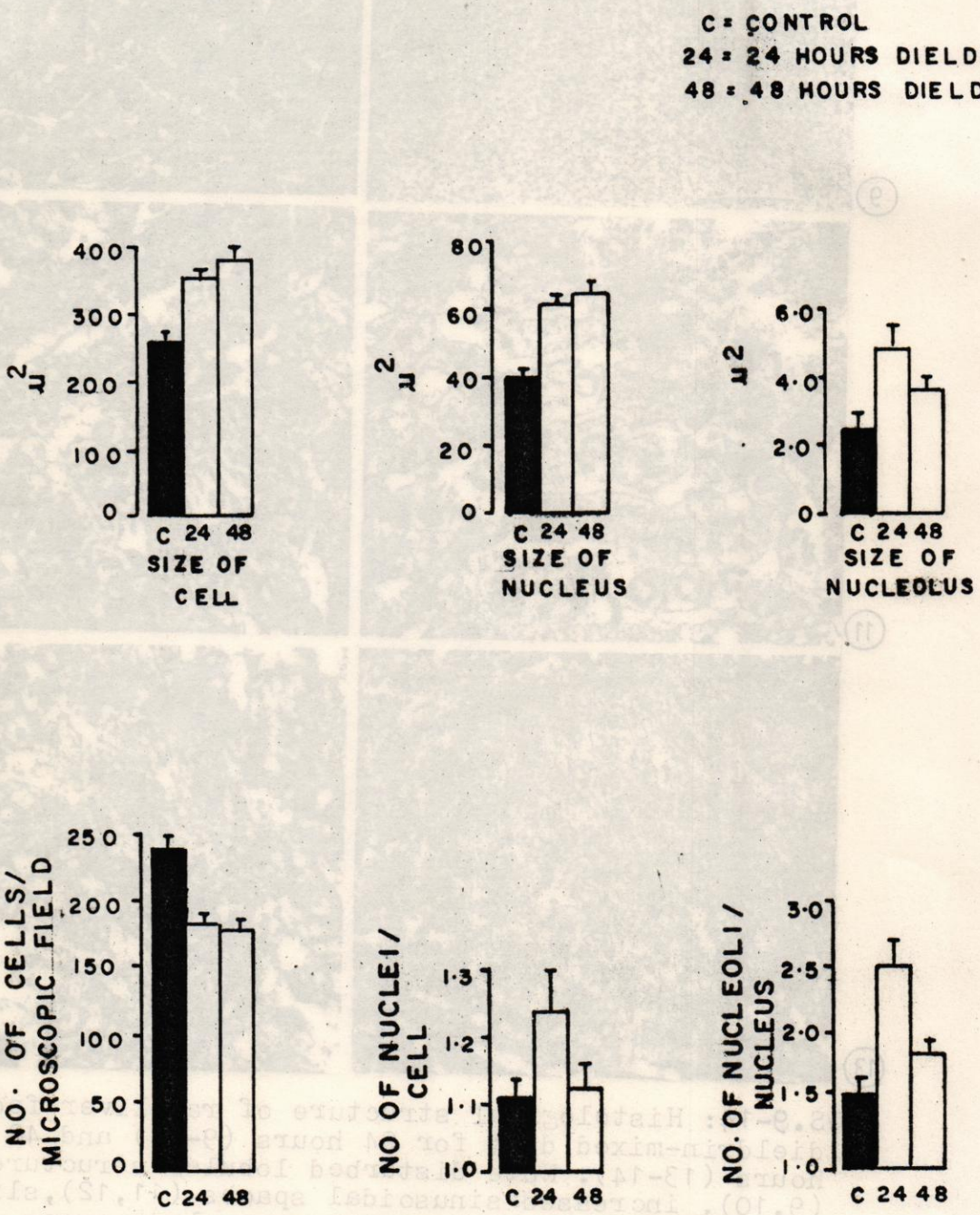


FIG.15: Effect of feeding dieldrin (40 mg/kg body weight/day) for a period of 48 hours on the various histological parameters of rat liver.

TABLE - VI

EFFECT OF FEEDING DIELDRIN MIXED DIET (40 mg/kg body weight/day) FOR 48 HOURS ON THE VARIOUS MORPHOMETRIC PARAMETERS OF RAT LIVER

Parameters	Control (n = 90)	Dieldrin feeding	
		24 hours (n = 90)	48 hours (n = 90)
No.of cells/ field	239.62±9.14	183.55±8.18***	181.44±5.10***
No. of Nuclei/cell	1.11±0.03	1.24±0.06	1.12±0.03
No. of Nucleoli/ Nucleus	1.53±0.12	2.52±0.17***	1.86±0.10*
Size of cell (μ^2)	260.09±8.29	356.46±7.31***	381.23±13.59***
Size of Nucleus(μ^2)	40.27±2.51	61.50±3.19***	65.22±4.85***
Size of Nucleolus(μ^2)	2.44±0.42	4.92±0.55**	3.61±0.22*

*P < 0.05; **P < 0.01; ***P < 0.001

likewise increased 47-100% after dieldrin treatment. The increase in size has led to decreased number of cell per microscopical field (Table VI, Fig.15). The number of nuclei/nucleus did not deviate from the normal liver pattern, while number of nucleoli/nucleus increased significantly after dieldrin feeding.

3.2. EFFECT OF DIELDRIN MIXED DIET (12 mg/kg body weight/day) ADMINISTERED FOR 15 DAYS

3.2.1. BODY WEIGHT AND LIVER WEIGHT

Short term dieldrin feeding at a dose of 12 mg/kg body weight/day has an inhibitory effect on the growth of rats. Control animals, on the average, have 0.47±0.04% (n=4) gain in body weight/day during the 15 days of experimental period but after the dieldrin administration this gain is continuously reduced (Table VII, Fig. 16). The body growth rate on day 9, 12 and 15 is reduced significantly by 69, 70 and 83%, respectively.

During the course of 15 days of experimental period when body weight was considered in relation to liver weight, the body weight/liver weight ratio was significantly altered. The control animals hold the ratio of 32.41±0.23. The ratio increased on day 3,6 and 9 after continuous dieldrin feeding by 17%, 7.5% and 10%, respectively (Table VII, Fig. 16). During subsequent period the ratio levels off with the control ratio.

When liver weight as percent of the body weight was considered the reverse data was obtained (Table VII, Fig. 16).

(n = 4)		
3 day (n = 3)	184.33±11.46 **	185.33±13.38
6 day (n = 3)	183.00±4.72 **	186.00± 4.00 *
9 day (n = 3)	173.00± 7.21 **	175.66± 8.12
12 day (n = 3)	139.00± 6.23	141.33± 6.98
15 day (n = 3)	119.66± 9.21	120.33± 9.28

[a]Mean ±SEM; Student's 't' test; *P < 0

	Control (n = 5)	3 days (n = 3)
Haemoglobin(g/dl)	13.51±0.31[a]	13.42±0.31
RBC ($X10^6$/Ul)	6.37±0.04	6.13±0.13
WBC ($X10^3$/ul)	6.67±0.09	6.96±0.20
PCV (%)	40.63±0.28	40.20±0.32
MCV (fl)	63.76±0.55	65.62±1.77
MCH (pg)	21.20±0.24	21.88±0.35
MCHC (g/dl)	33.25±0.22	33.38±0.86

[a]Mean ± SEM, Student's 't' test; *P 0.0

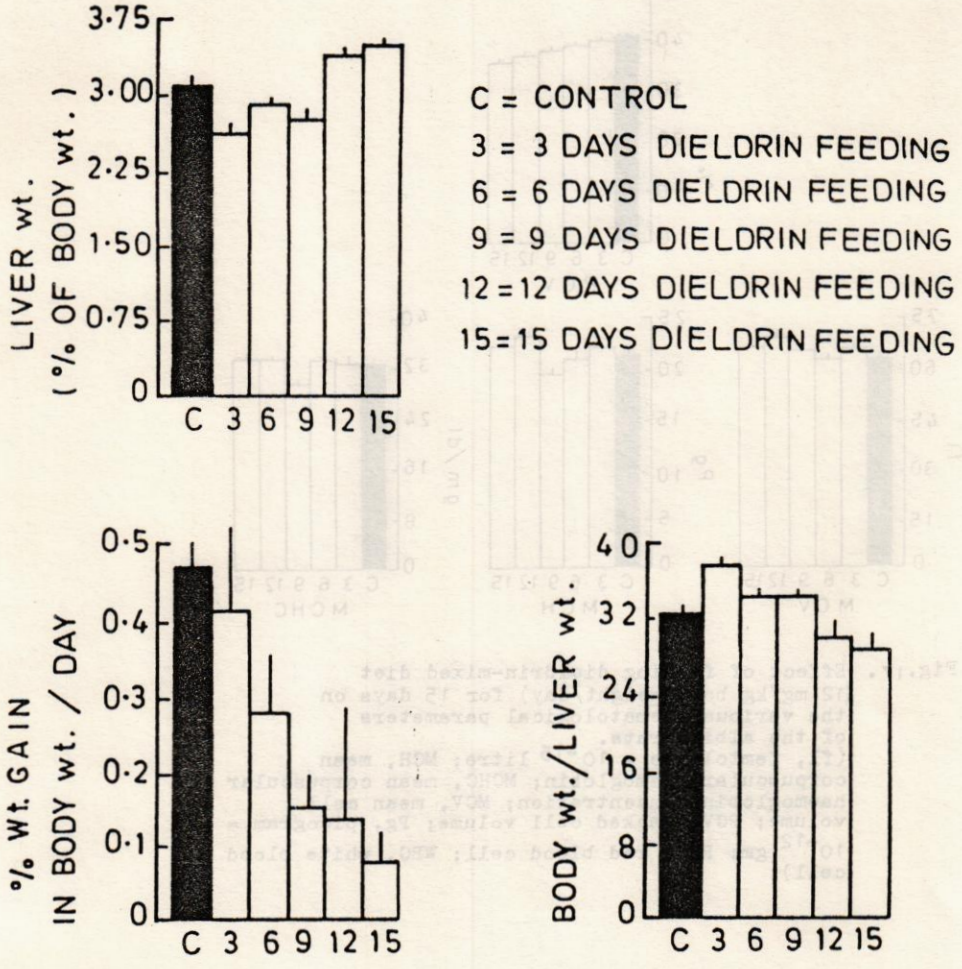


Fig. 16. Effect of oral feeding of dieldrin mixed-diet (12 mg/kg body weight/day) for 15 days on the total body and liver weights of albino rats.

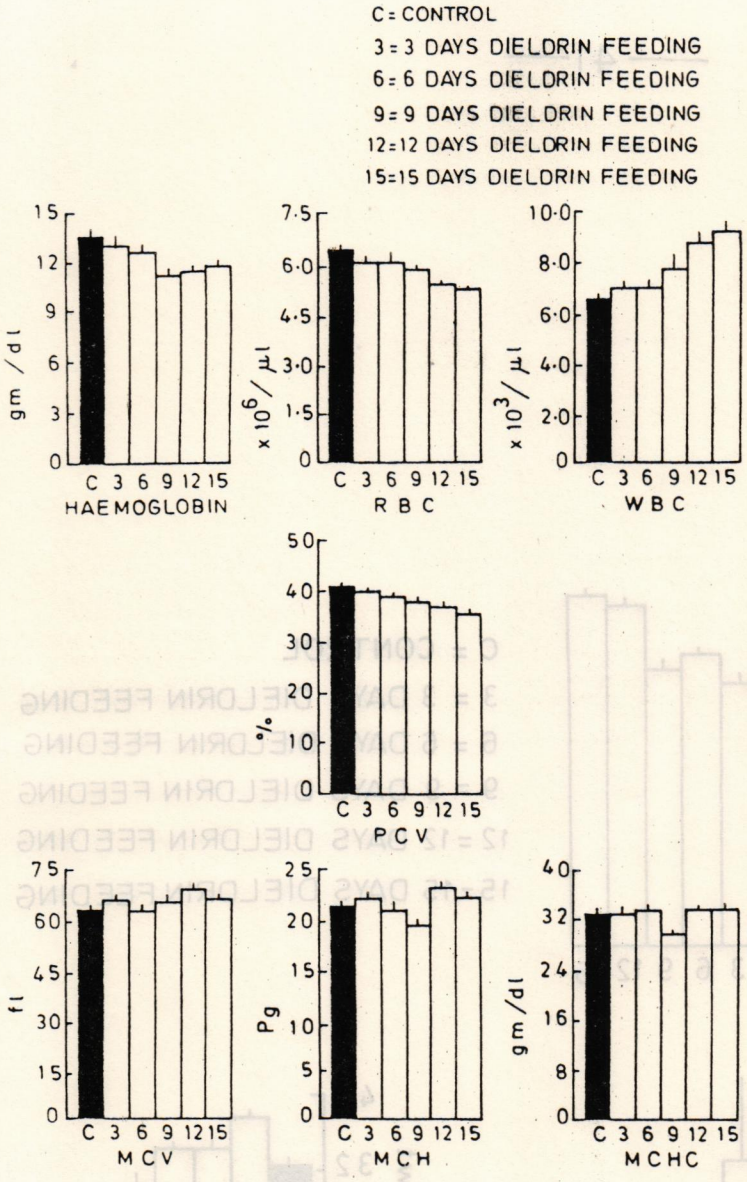


Fig.17. Effect of feeding dieldrin-mixed diet (12 mg/kg body weight/day) for 15 days on the various haematological parameters of the albino rats.
(fl, femtolitre = 10^{-15} litre; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean cell volume; PCV, packed cell volume; Pg, picogram = 10^{-12} gm; RBC, red blood cell; WBC, white blood cell).

3.2.2. HAEMATOLOGICAL STUDIES

Feeding of dieldrin mixed diet at a dose of 12 mg/kg body weight/day for 15 days caused a decrease in haemoglobin content, RBC count and PCV (Table VIII, Fig.17). The haemoglobin content remained unchanged during first 6 days of feeding, but then showed significant decrease during the subsequent period. A decrease of 15, 16 &11% was observed on day 19, 12 and 15, respectively. The RBC count followed exactly the same pattern. The control rats have $6.37 \pm 0.04 \times 10^6$ cells/ul of blood, which decrease 8, 15 and 16%, respectively, on days 9, 12 and 15. The WBC count remains unaltered during the first 9 days of insecticide feeding, but significant increase of 35% and 36% was recorded on day 12 and 15 of dieldrin feeding, respectively. The PCV appears to be a more sensitive indicator of toxicity, as it is affected right from the very beginning. The maximum decrease of 13%, when compared with the control, was observed on day 15.

The MCV remained unaffected till day 12, when it got elevated significantly on day 12 and 15 the MCV values increase, respectively, 9 and 5% (Table VIII, Fig. 17). The MCH shows 7.5% decrease on day 9 of dieldrin feeding from the control value of 21.2 ± 0.24 pg(n=5). During the subsequent experimental period, the values increased in all groups. The MCHC, on the other hand, is not changed significantly in any of the groups except for 9 days observation, when this shows about 11% increase over the control value of 33.25 ± 0.22 g/dl (n=5).

3.2.3. BIOCHEMICAL ANALYSIS OF BLOOD SERUM

Table IX and Figure 18 shows the effect of dieldrin mixed diet (12 mg/kg body weight/day) for 15 days on some enzymatic activities in blood serum. Almost

all the enzymes tested, except for ChE which remains unaffected, are elevated at different times during 15 days of insecticidal feeding. Out of the two phosphatases tested AP is more sensitive to this treatment. The normal blood serum shows AP activity 6.80±0.70 KAU/dl (n=5), which is increased 2.0, 2.6, 3.5, 3.6 and 3.9 fold on day 3, 6, 9, 12 and 15 of experimental period. The AcP activity, on the other hand, shows significant increase during first 6 days of treatment, 47% after 3 days and 43% after 6 days of treatment. During the subsequent period AcP activity did not deviate from the normal level (Table IX, Fig.18). The amylase activity was not much altered during the first week of dieldrin feeding, but there was a marked elevation during the second week. The control animals show amylase activity 216.86±12.84 Somogyi units/dl of blood serum, which increased 29, 40 and 63% on day 9, 12 and 15, respectively. The CPK activity also showed 2.7 and 2.1 fold increase in day 3 and day 6 after dieldrin feeding, which normalizes during the subsequent period. Out of the two dehydrogenases tested ICDH was found to be more sensitive. The normal blood serum shows 291.56±3.53 sigma units/ml LDH activity, which increases 21%, 10.5%, 12%, 10%, 10.5%, after 3, 6, 9, 12 and 15 days of dieldrin feeding. The LDH activity is significantly increased from day 9 onward. The increase is 1.8, 1.9 and 2.2 fold on day 9, 12 and 15, respectively. From amongst transaminases the SGPT activity is affected from the very first day of observation, while the SGOT activity is significantly modified on day 9 and afterwards. The blood serum in control rat shows SGOT activity 31.80±2.91 IU/l (n=5), which increases 32, 49 and 71%, respectively, on day 9, 12 and 15. The SGPT activity showed 55.4% increase within first 3 days of dieldrin feeding, which was maintained as such till the end of experimental period.

TABLE - IX

EFFECT OF FEEDING DIELDRIN MIXED DIET (12 mg/kg body weight/day) FOR 15 DAYS ON SOME ENZYME ACTIVITIES OF RAT BLOOD SERUM.

Parameters	Control (n = 5)	Dieldrin feeding				
		3 days (n=3)	6 days (n=3)	9 days (n=3)	12days (n=3)	15days (n=3)
AcP activity (KAU/dl)	1.86[a] ± 0.24	2.73* ± 0.40	2.65* ± 0.16	2.23 ± 0.32	1.94 ± 0.07	1.90 ± 0.06
AP activity (KAU/dl)	6.80 ± 0.70	14.73** ± 1.37	17.70*** ± 0.61	24.14*** ± 0.76	24.46*** ± 2.38	26.26*** ± 1.48
Amylase activity (Somogyi U/dl)	216.86 ±12.84	240.00 ±23.79	243.80 ±16.92	278.61* ±16.86	303.81*** ± 2.52	354.28*** ± 7.19
ChE activity (RU/ml)	42.38 ± 1.98	44.16 ± 1.45	43.50 ± 2.77	43.33 ± 4.56	39.17 ± 2.68	39.66 ± 3.09
CPK activity (Sigma U/ml)	6.66 ± 0.71	18.16*** ± 1.30	14.00** ± 1.53	7.16 ± 1.09	6.83 ± 1.09	5.16 ± 0.60
ICDH activity (Sigma U/ml)	291.56 ± 3.53	352.83** ±11.62	322.02** ± 7.45	328.21** ± 8.84	320.06* ± 6.96	322.05** ± 7.20
LDH activity (IU/l)	448.40 ±19.47	543.49 ±73.83	559.47 ±46.15	799.24*** ±39.69	885.20*** ±53.44	977.48*** ±70.47
SGOT activity (IU/l)	31.80 ± 2.91	36.50 ± 4.07	40.66 ± 2.73	42.00* ± 2.31	47.50* ± 4.27	54.33** ± 3.18
SGPT activity (IU/l)	14.16 ± 0.62	22.00* ± 2.52	18.00** ± 0.50	21.00* ± 2.64	20.00* ± 1.50	21.83* ± 2.49

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

	(n = 5)	3 days (n=3)
Bilirubin content (mg/dl)	0.43±0.03[a]	0.88±0.11**
Cholesterol content (mg/dl)	148.78±7.41	132.43±8.86
Free amino acids (mg/dl)	9.71±0.90	7.94±0.25
Glucose content (mg/dl)	139.48±4.62	111.33±8.74*
Protein content (mg/dl)	7.08±0.51	8.26±0.66
Urea content (mg/dl)	31.09±1.18	46.83±4.80*

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

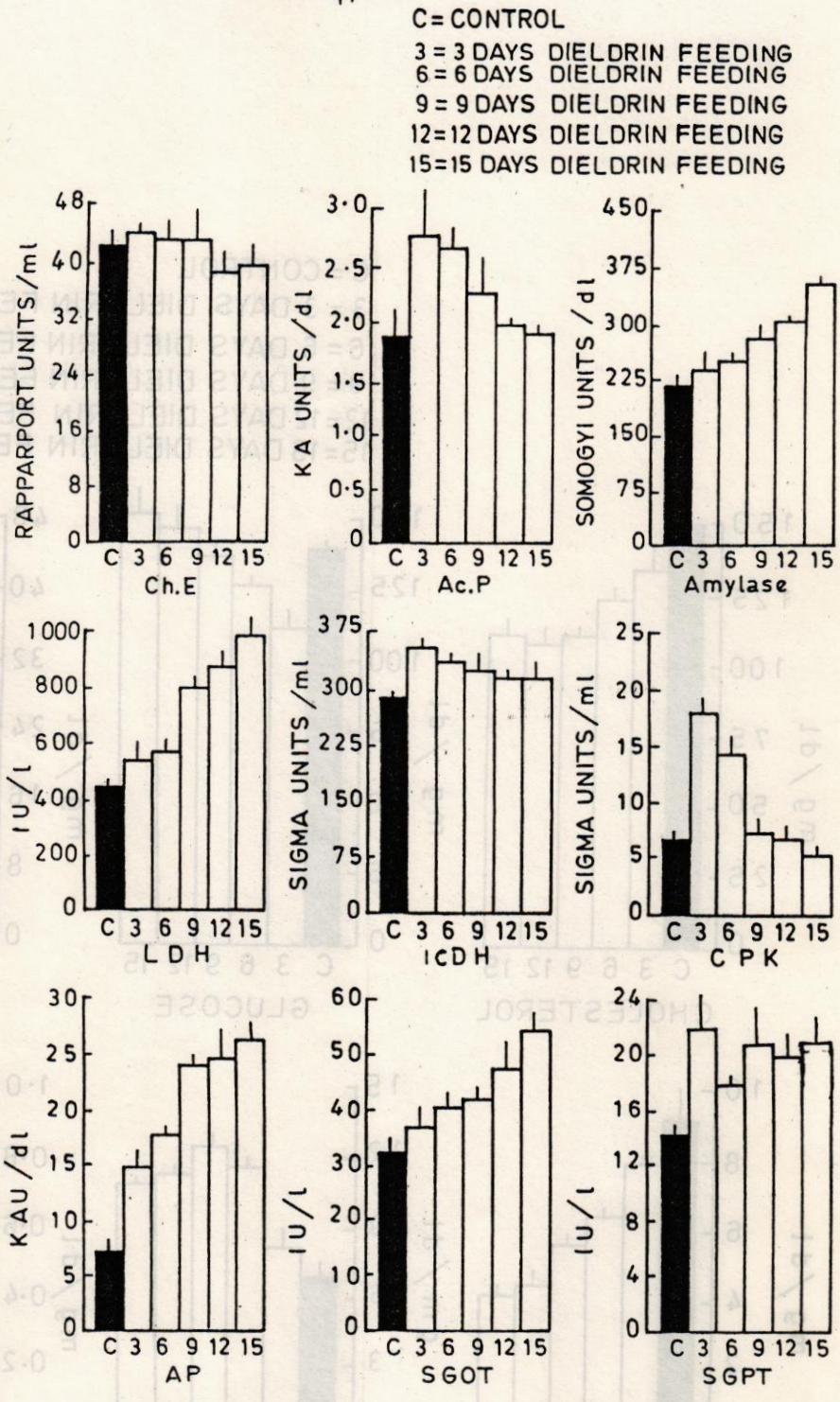


Fig. 18. Effect of feeding dieldrin-mixed diet (12 mg/kg body weight/day) for 15 days on some enzymes activities of rat blood serum.
(AcP, acid phosphatase; AP, alkaline phosphatase; ChE, cholinesterase; CPK, creatine phosphokinase; IDH, isocitrate dehydrogenase; LDH, lactate dehydrogenase; SGOT, serum glutamate pyruvate transaminase; KAU, King Armstrong units).

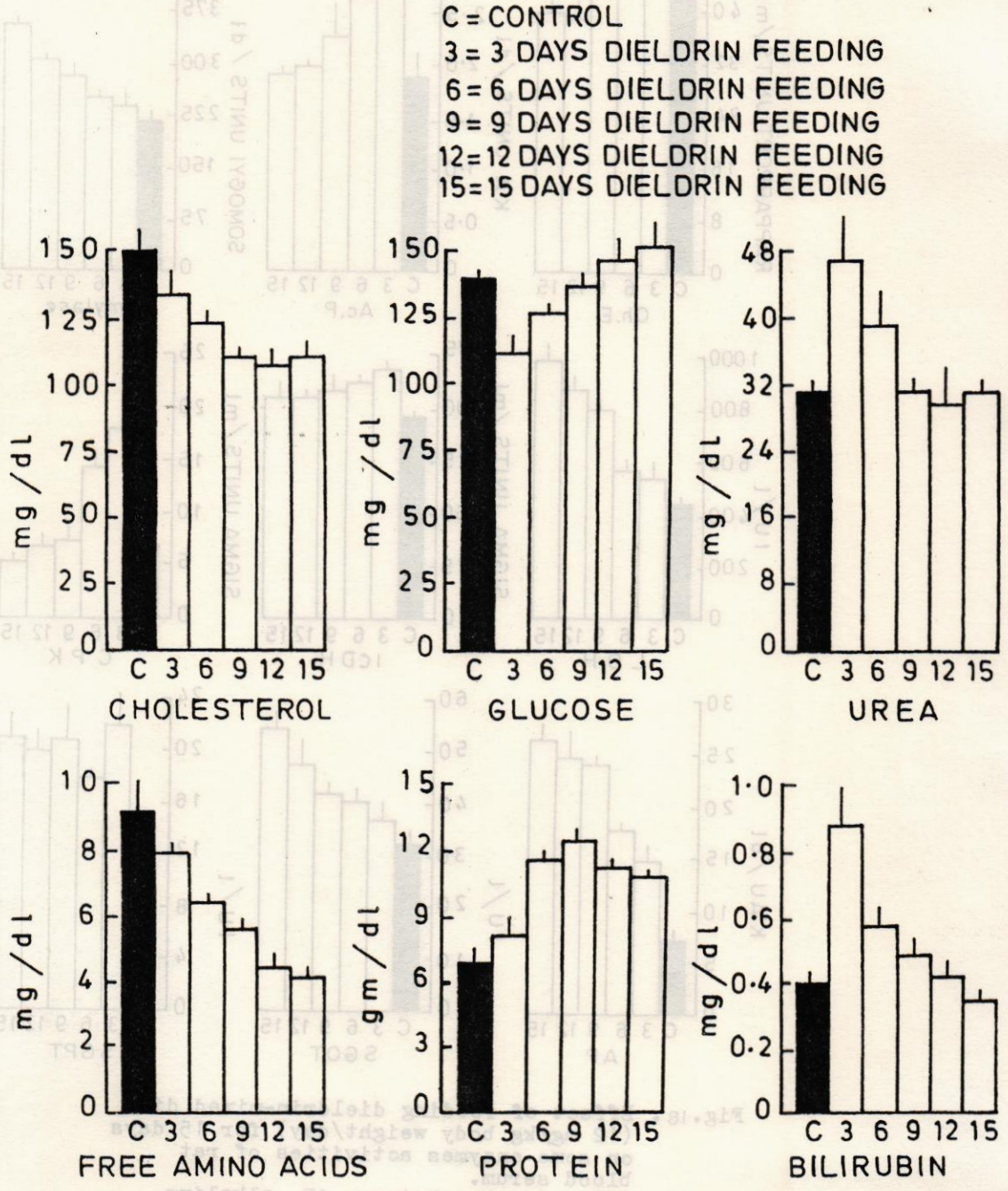


Fig. 19. Effect of feeding dieldrin-mixed diet (12 mg/kg body weight/day) for 15 days on some biochemical contents of rat blood serum.

Table X and Figure 19 shows the effect of feeding dieldrin - mixed diet (12 mg/kg body weight/day) on some other biochemical components of blood serum like Bilirubin, Cholesterol, Free amino acids, Glucose, Protein and Urea. Bilirubin content increase two fold during the initial 3 days. After this preliminary increase, some kind of readjustment sets in and bilirubin content normalize after day 9. The cholesterol content, on the other hand, decrease after dieldrin feeding. Although the 3 day group still has the normal level of cholesterol, the feeding for another 3 days and beyond results in about 27% decrease, which is maintained till the end of experimental period.

The serum protein content increase during 15 days feeding. The control blood serum contains 7.08±0.51 gm protein/dl of blood, which is raised 77% on day 9. A slight decline was noticed on day 12 and 15 but the values were still significantly higher when compared with those of control blood serum. In concomitant with these changes, there is significant decrease in free amino acids, which was first observed in 6 day old group. A maximum decrease of 53% was observed on day 15 (Table X, Fig.19). The nitrogen metabolism apparently is not much affected by dieldrin treatment. The urea content, except for that of 3 day group remains unaltered. A 50% significant rise was observed after 3 days of feeding. The glucose content also did not follow any definite response. Except for 20% decrease within 3 days of feeding, all other experimental groups showed non significant alterations.

3.2.4. BIOCHEMICAL ANALYSIS OF LIVER

Biochemical toxicity of dieldrin in liver was evaluated as described earlier by estimating some hepatic

enzymes and other biochemical components (Figs. 20,21; Tables XI, XII).

Of all the hepatic enzymes tested, ICDH activity remained uneffected during the present experimental conditions. The LDH activity, however, was considerably affected. Within three days an increase of 21% was recorded, which reached 26% on day 15. From amongst the two hepatic transaminases, GPT was found to be more sensitive. A significant increase of 24%, 44%, and 55% was observed on day 6, 9 and 15. The GOT activity, on the other hand, is not significantly affected until day 12, when the increase is 43%. The AP activity was also elevated. The control animals showed 0.64 KAU AP activity/g of liver, which although showed regular increase, but significant values were observed only at day 9, 12 and 15. The maximum increase of 89% was recorded on day 15.

Table XII and Fig. 21 show the effect of dieldrin mixed diet (12 mg/kg body weight/day) for 15 days on some of the biochemical components like cholesterol. Free amino acids, glucose, soluble protein, total protein, DNA and RNA of rat liver. The total cholesterol content of control rabbit liver is 15.72±0.35 mg/g, which after dieldrin feeding is decreased 24 , 42 and 19% on day 3, 6 and 9, respectively, and is then normalized afterwards. The free amino acids are the most sensitive components, which are adversely affected by insecticide treatment. The FAA content decrease 0.8%, 12%, 21%, 25%, 26%, respectively, on day 3, 6, 9, 12 and 15. The total hepatic proteins, on the other hand, after dieldrin treatment showed 16 and 15% decrease on day 3 and 6, while the soluble protein showed about 30% increase after 15 days of continuous dieldrin feeding. The glucose content decreased

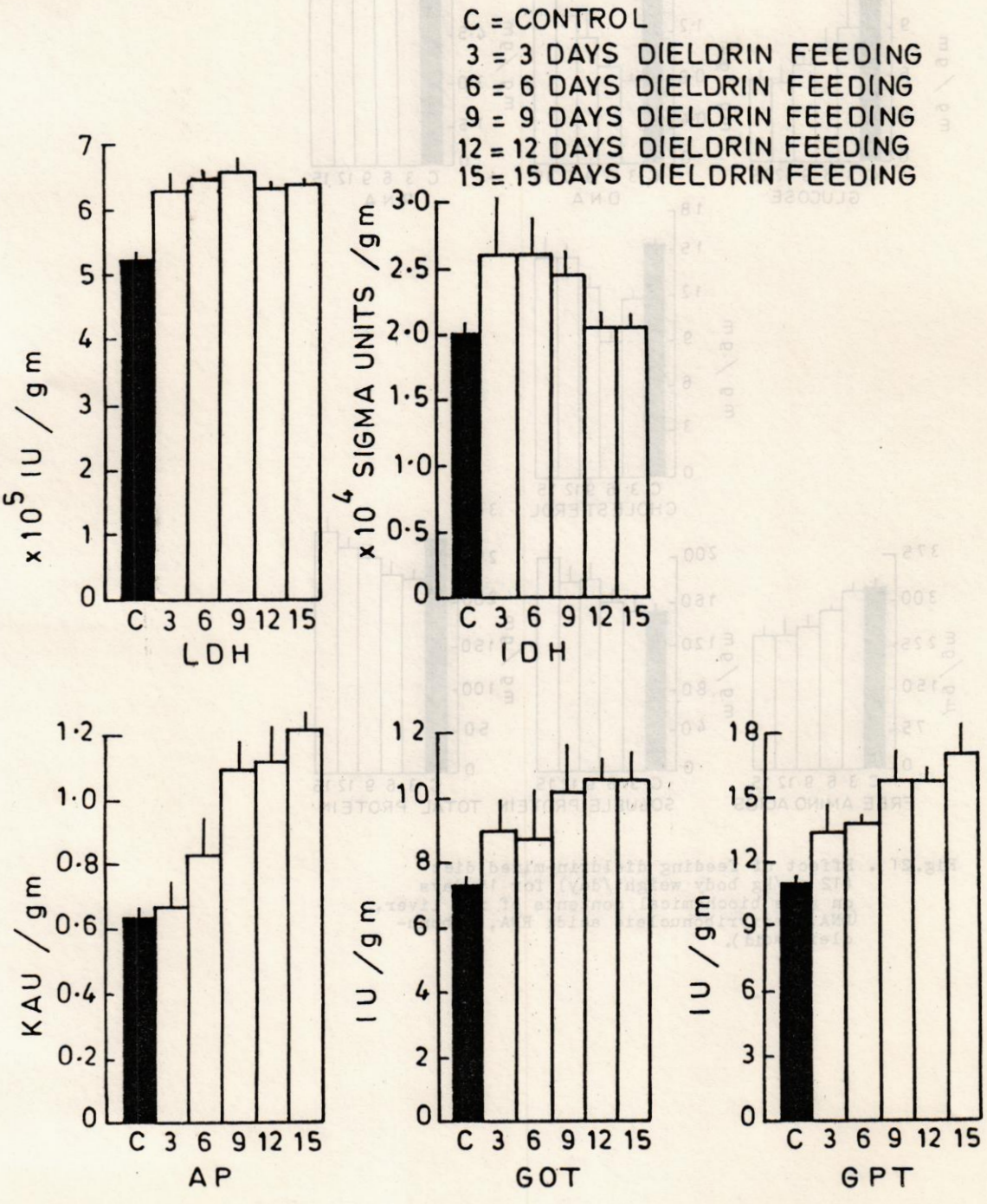


Fig. 20. Effect of feeding dieldrin-mixed diet (12 mg/kg body weight/day) for 15 days on some enzyme activities of rat liver. For abbreviation see Fig. 5.

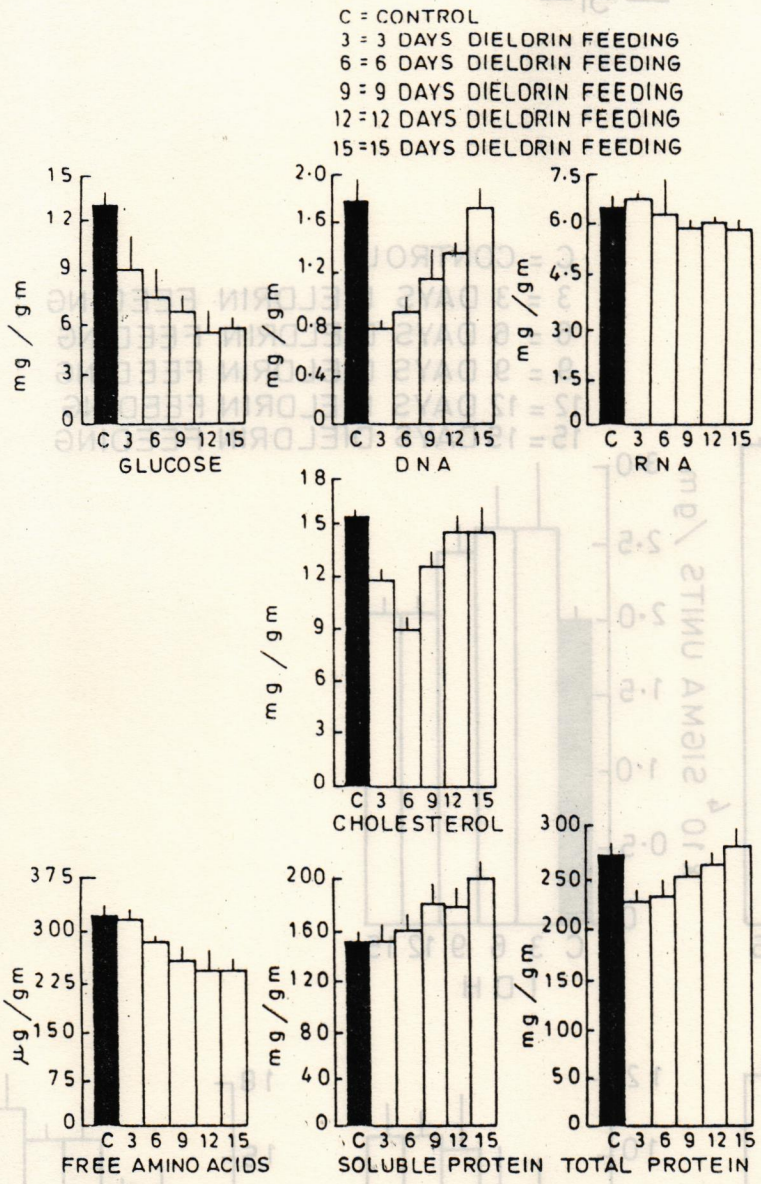


Fig. 21. Effect of feeding dieldrin-mixed diet (12 mg/kg body weight/day) for 15 days on some biochemical contents of rat liver. (DNA, deoxyribonucleic acid; RNA, ribonucleic acid).

AP activity (KAU/gm)	0.64±0.02[a]	0.67±0.08
GOT activity (IU/gm)	7.34±0.17	9.01±0.85
GPT activity (IU/gm)	11.07±0.25	13.63±1.25
ICDH activity ($X10^{-4}$ Sigma U/gm)	2.01±0.05	2.59±1.06
LDH activity ($X10^{5}$ U/gm)	5.19±0.12	6.28±0.22**

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

	(n = 3)	3 days (n = 3)
Cholesterol (mg/gm)	15.82±0.35[a]	12.01±0.68**
Free amino acid(Ug/gm)	319.95±12.68	317.36±12.24
Glucose (mg/gm)	12.93±0.61	8.92±1.91
Soluble protein(mg/gm)	152.99±5.62	151.69±12.24
Total protein(mg/gm)	268.58±8.88	225.01±6.71**
DNA (mg/gm)	1.74±0.18	0.75±0.05**
RNA (mg/gm)	6.05±0.29	6.77±0.07

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

significantly throughout the experimental period. The decrease was 31, 42, 49, 56 and 54%, respectively on day 3, 6, 9, 12 and 15.

Both the nucleic acids are affected during the initial stages of dieldrin treatment. The DNA content is decreased 57% after 3 days, while during subsequent period, the contents level off with the control values. The DNA content show about 12% increase, but no significant alteration was observed.

3.2.5. HISTOLOGICAL STRUCTURE OF LIVER

Dieldrin feeding at a dose of 12 mg/kg body weight/day for the total duration of 15 days, proved moderately toxic as far as the liver structure is concerned.

3.2.5.1. 3 DAYS FEEDING

There was marked increase in the sinusoidal area, (Figs. 25, 26), slight margination (Figs. 25, 26), disruption of definite hepatic cords (Fig. 26), condensed nuclei (Figs. 26, 27), and slight increase in the degree of vacoulation after 3 days of regular dieldrin feeding (Figs. 26, 27). These changes are in contrast to the typical normal hepatic structure (Figs. 22-24). Morphometric studies indicated significant increase in cell size and number of nucleoli per nucleus and decrease in number of cells per microscopic field, which revealed nucleolar and cellular hypertrophy (Table XIII, Fig.45).

3.2.5.2. 6 DAYS FEEDING

Regular dieldrin feeding for 6 days caused further increase in the sinusoidal spaces and vacuolation (Figs. 28-31). Darkly stained condensed nuclei were

also observed (Figs. 29, 30) but definite hepatolobular structure was retained (Figs. 28, 29). There was significant increase in the size of liver cell, nucleolus, number of nucleoli/nucleus and decrease in the number of cells/microscopic field (Table XIII, Fig.45).

3.2.5.3. 9 DAYS FEEDING

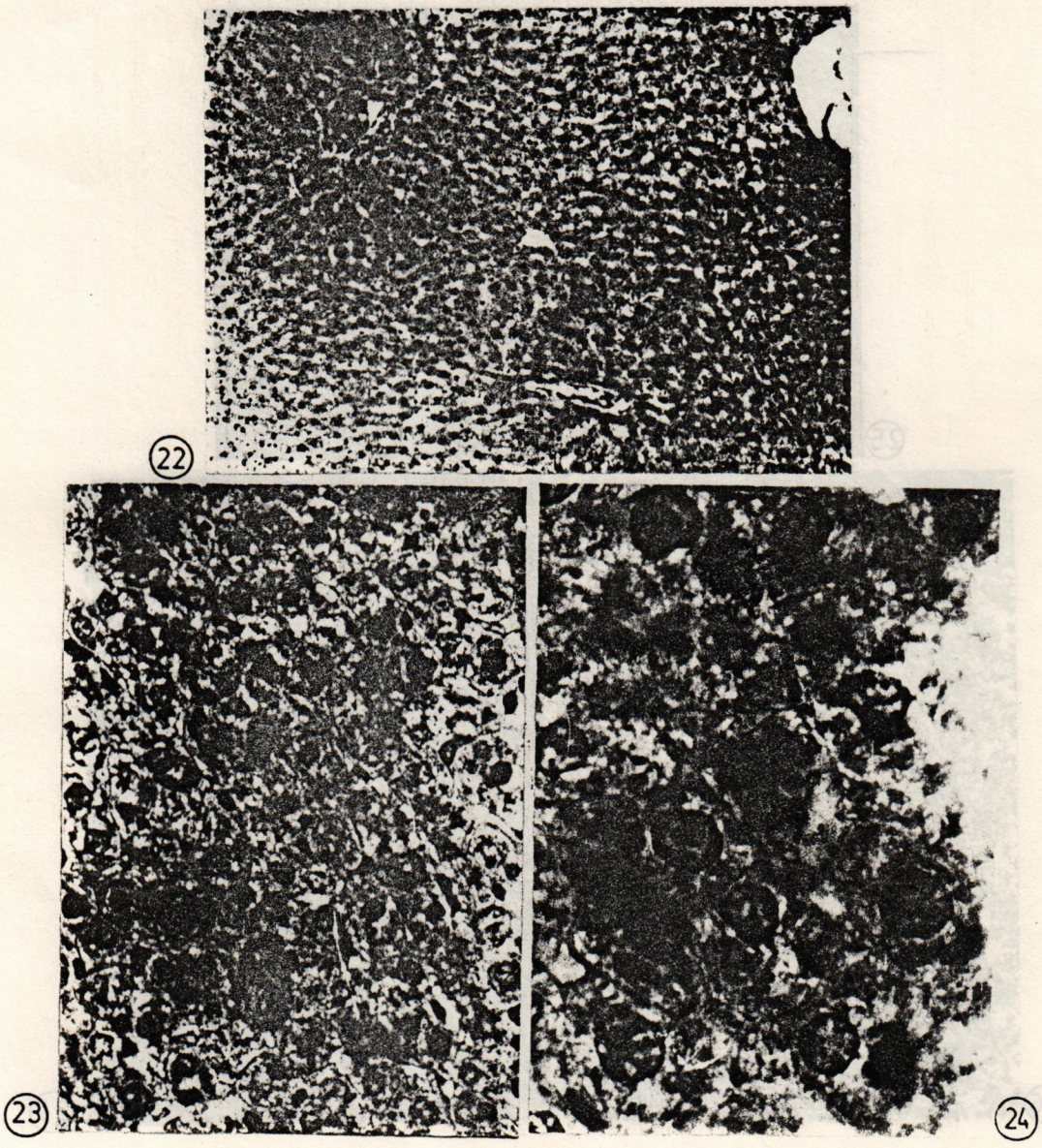
In 9 day dieldrin feeding, the disruption of double cell hepatic cord structure was evident (Figs.34, 35). Excessive cytoplasmic and slight nuclear vacuolation was also noticed (Figs. 32-36) with increase in cellular and nuclear size (Table XIII, Figs. 34-36).

3.2.5.4. 12 DAYS FEEDING

Continuous feeding of dieldrin-mixed diet for 12 days revealed cytoplasmic vacuolation whose intensity is greater near the centre of the hepatic lobule (Figs.39,40). Although generally the nuclear vacuolation was evident, the darkly stained condensed nuclei were also present (Figs. 38-40). Hypertrophy of hepatic cells and nuclei was also revealed by morphometric data (Table XIII, Fig.45).

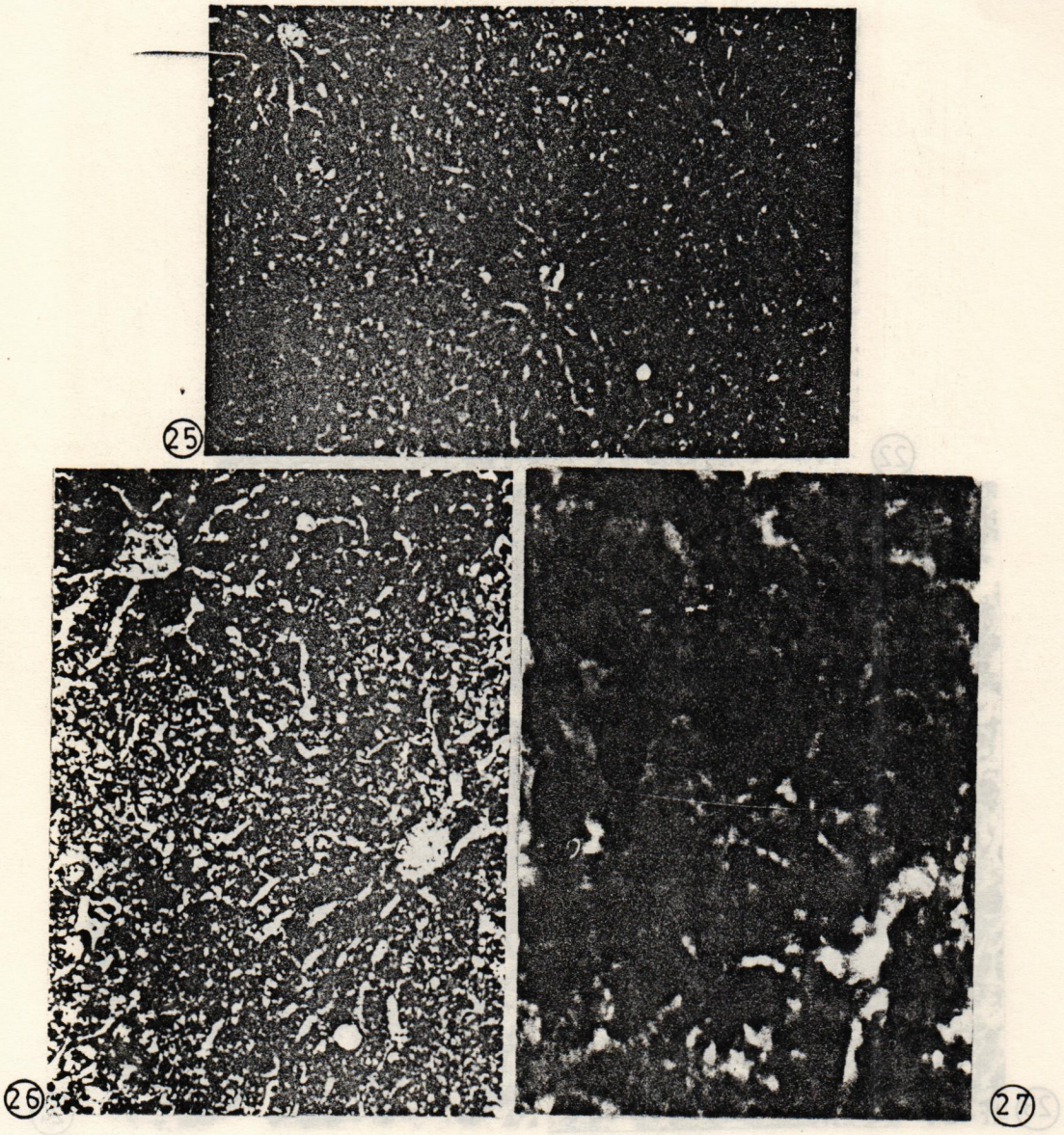
3.2.5.5. 15 DAYS FEEDING

In 15 days dieldrin treated animals the sinusoidal area is increased with the disruption of linear hepatic cord structure (Figs.41,43). Clear rounded areas were observed but not so frequently (Figs.41, 43). Significant increase in the cellular, nuclear and nucleolar areas was indicative of hypertrophy of these organelles (Table XIII, Fig. 45).



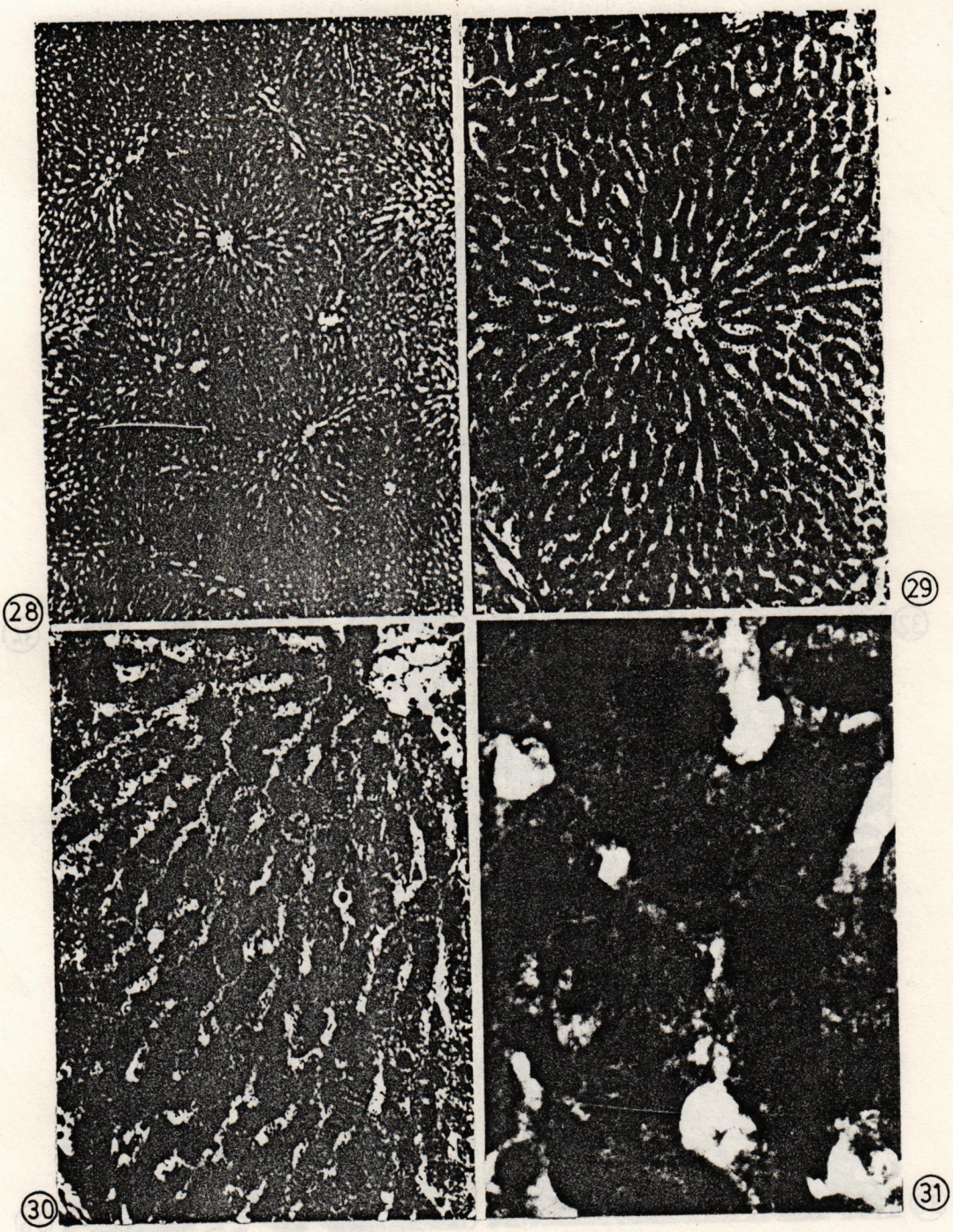
FIGS.22-24: Histological structure of the normal rat liver. Note the typical hepatic lobule (22) with portal areas, arrangement of cells and rounded nuclei (23-24).

Stain: Hematoxylin and Eosin.
Magnifications: 22, X50; 23, X200; 24, X500.



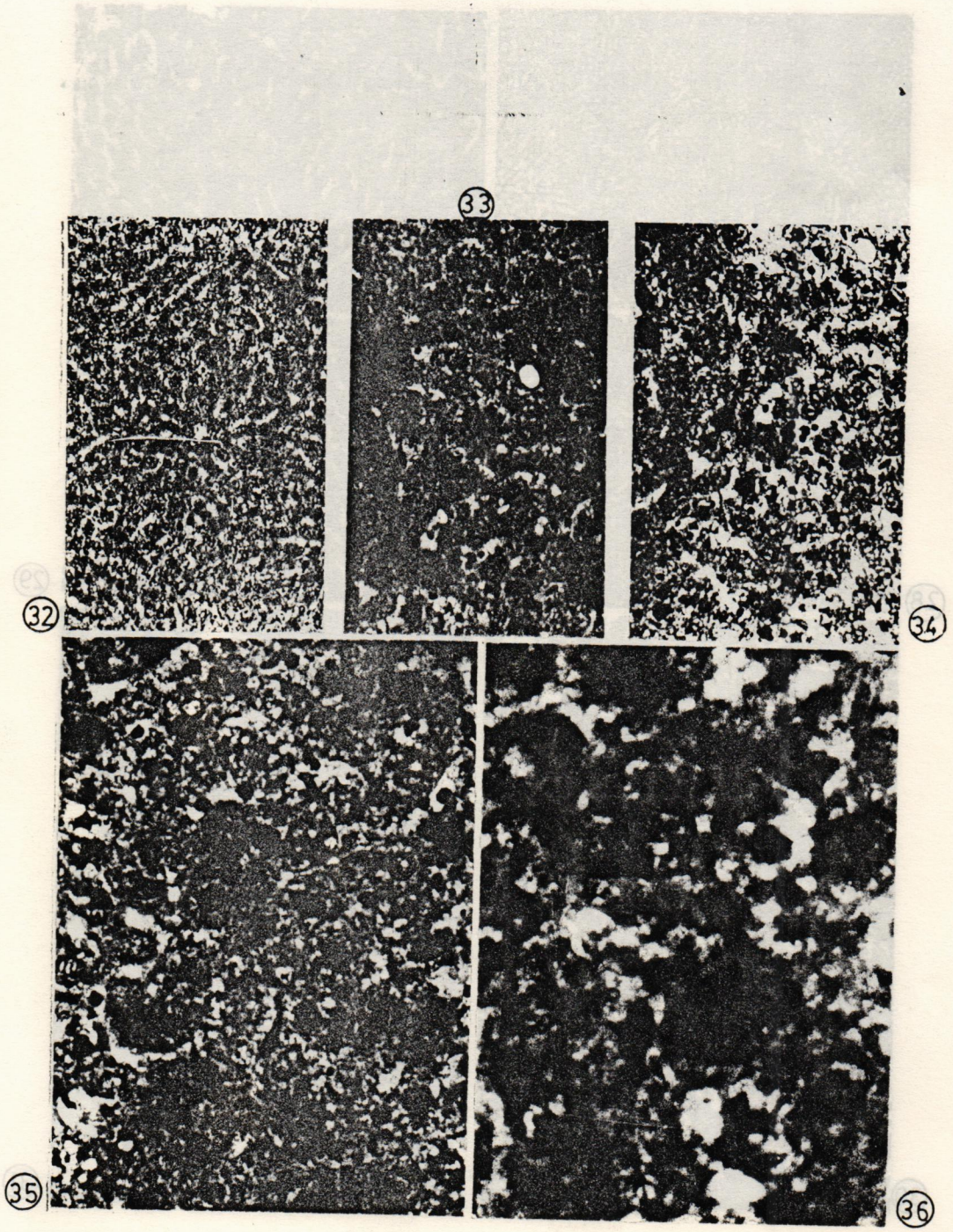
FIGS.25-27: Histological structure of liver of rat fed on dieldrin-mixed diet for 3 days. Note increased sinusoidal areas (25,26) and hypertrophied nuclei and nucleoli (27).

Stain: Hematoxylin and Eosin.
Magnifications: 25, X50; 26, X100; 27, X500.



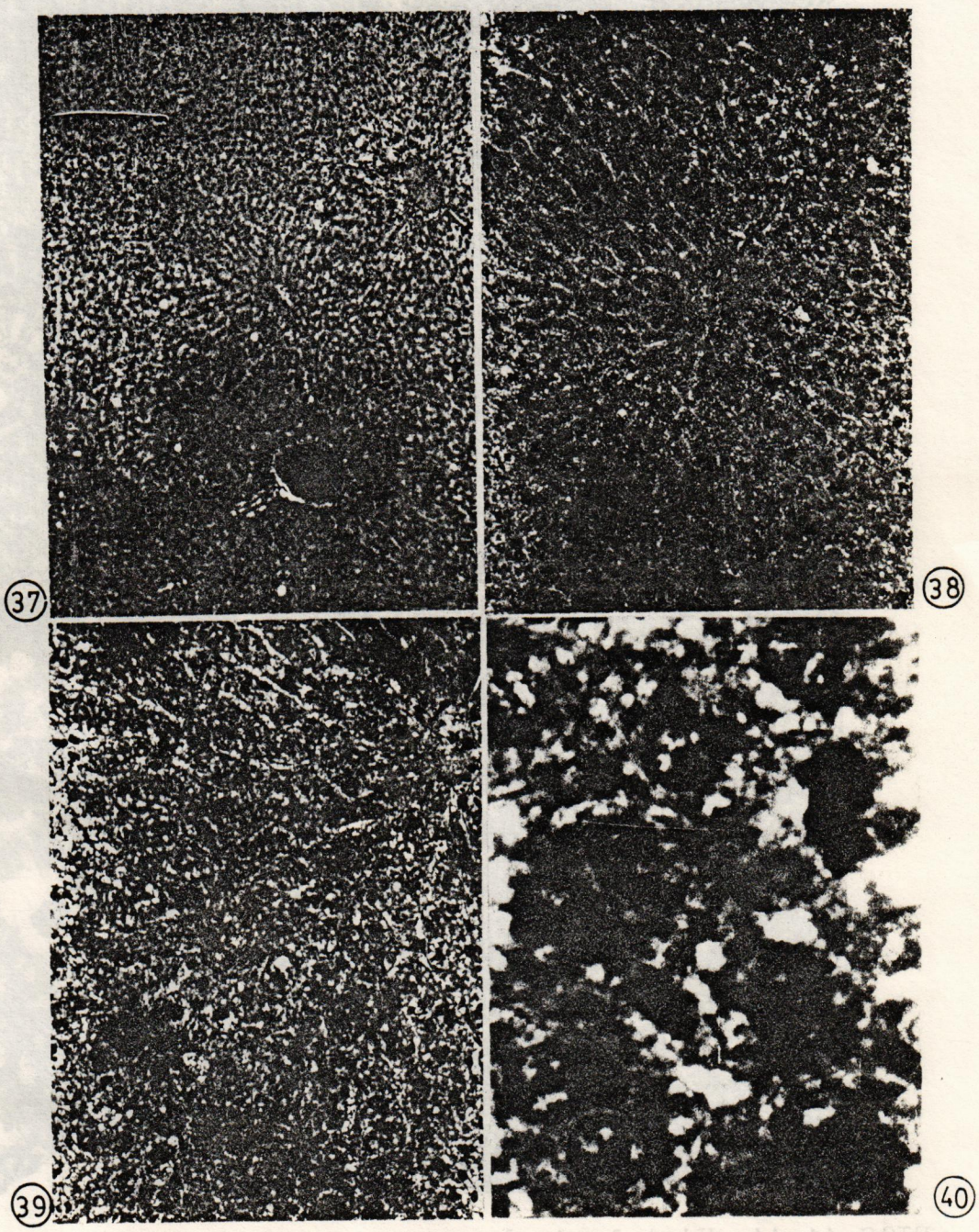
FIGS.28-31: Histological structure of rat liver fed on dieldrin-mixed diet for 6 days. Note increased sinusoidal areas, vacuolation (28,31) and hypertrophied cells (30, 31).

Stain: Hematoxylin and Eosin.
Magnifications: 28, X20; 29, X50; 30, X100; 31, X500.



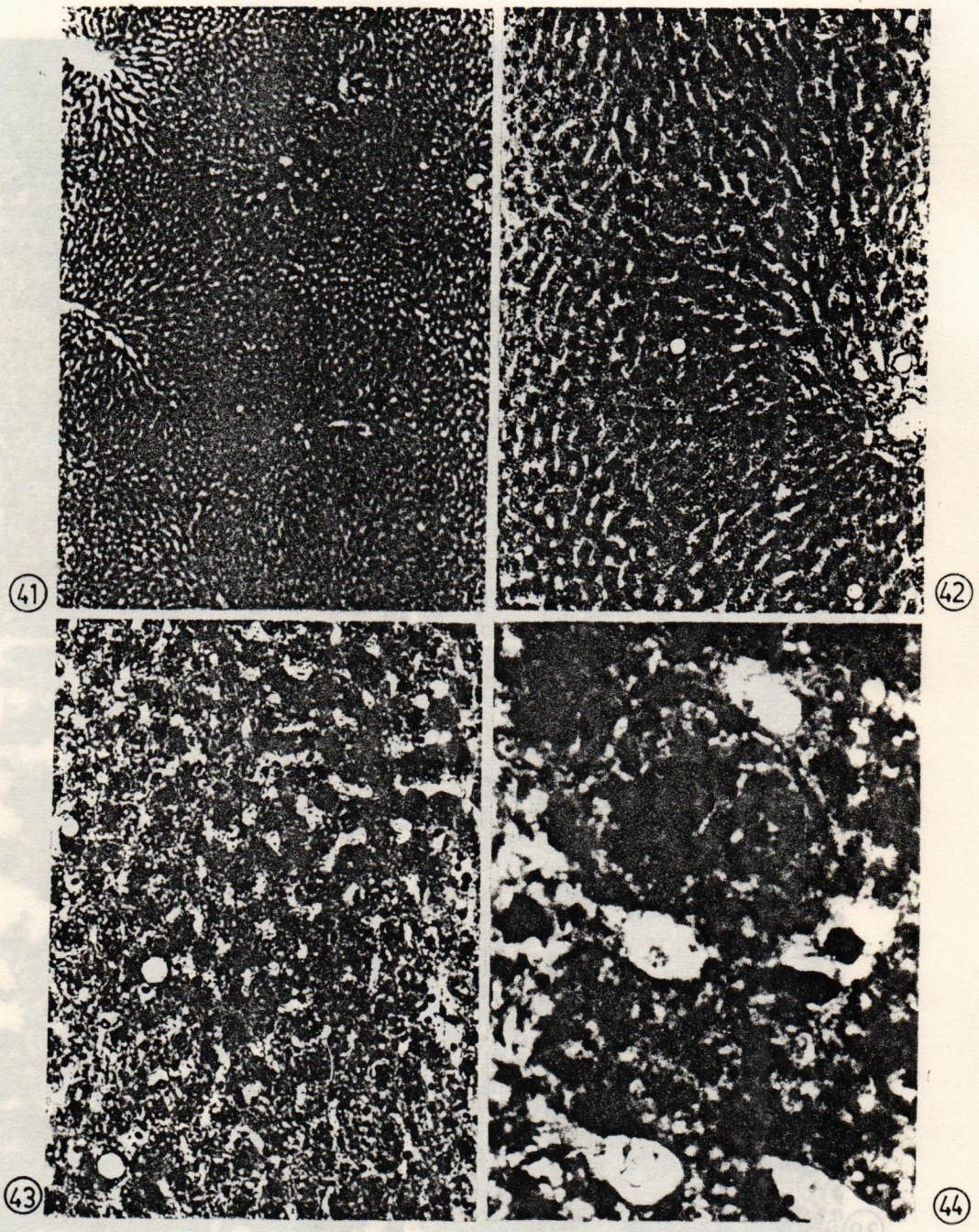
FIGS. 32-36: Histological structure of rat liver fed on dieldrin-mixed diet for 9 days. Note hepatic cord disruption (34, 35) cytoplasmic and nuclear vacuolation (32, 36).

Stain: Hematoxylin and Eosin.
Magnifications: 32, X50; 33, X100; 34, X100; 35, X200; 36, X500.



FIGS. 37-40: Histological structure of rat liver fed on dieldrin-mixed diet for 12 days. Note cytoplasmic and nuclear vacuolation (38,-40) and many rounded clear areas (37).

Stain: Hematoxylin and Eosin.
Magnifications: 37, X20; 38, X50; 39, X100; 40, X500.



FIGS. 41-44: Histological structure of rat liver fed on dieldrin-mixed diet for 15 days. Note disruption of hepatic cords (41,-43), many rounded clear areas and hypertrophied cells (44).

Stain: Hematoxylin and Eosin.
Magnifications: 41, X20; 42, X50; 43, X100; 44, X500.

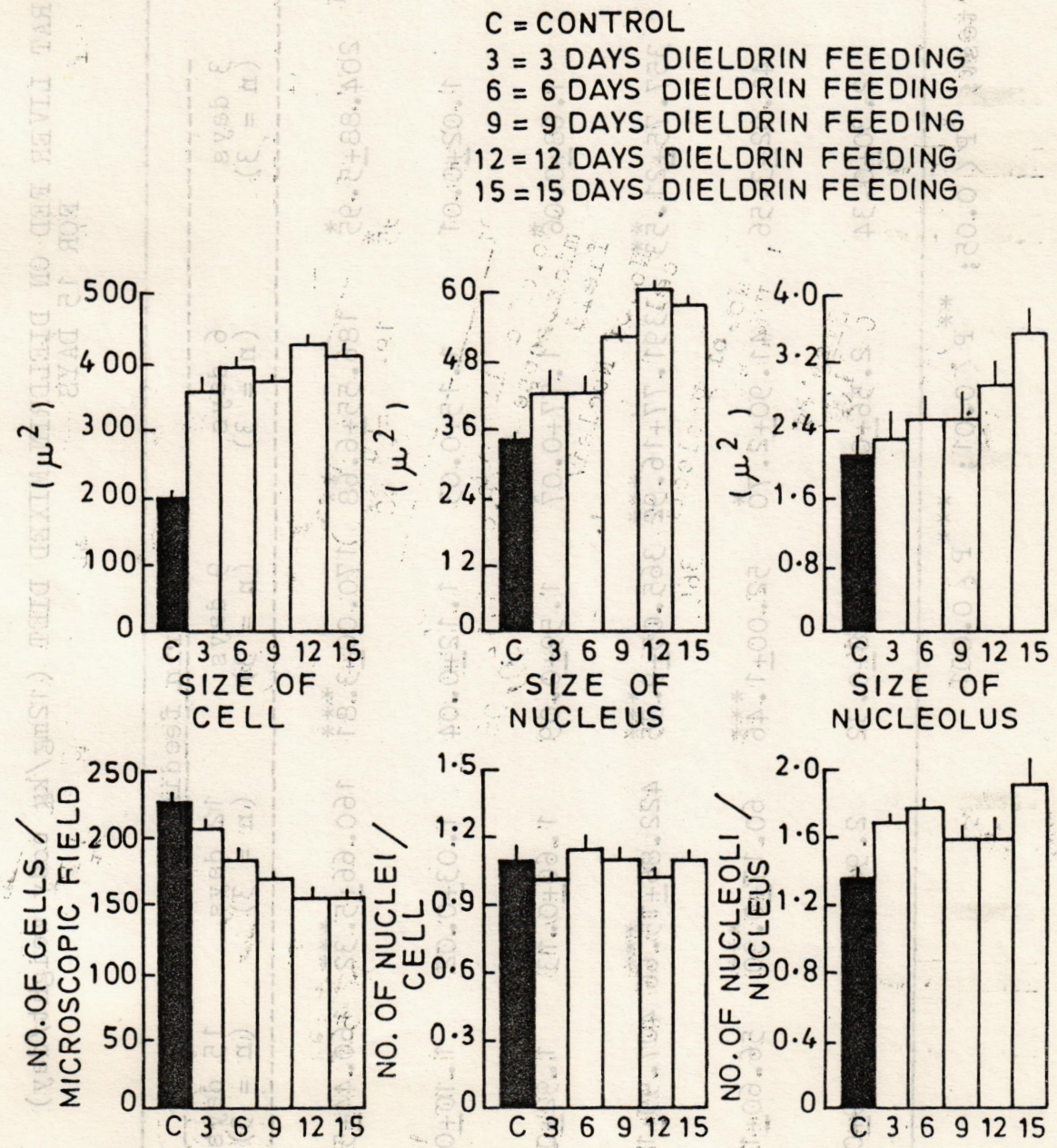


Fig. 45. Morphometric studies on rat liver fed on dieldrin-mixed diet (12 mg/kg body weight/ day) for 15 days.

No.of cells/ microscope field	227.88±5.66[a]	204.88±5.95*	18
No.of Nuclei/ cell.	1.09±0.06	1.02±0.01	
No.of Nucleoli/ Nucleus	1.38±0.07	1.68±0.06*	
Size of Cell(U^2)	199.39±9.52	357.75±21.53**	39
Size of Nucleus(U^2)	33.70±1.03	41.92±3.56	4
Size of Nucleolus(U^2)	2.16±0.22	2.30±0.34	

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

3.3. EFFECT OF DIELDRIN MIXED DIET (6 mg/kg body weight/day) ADMINISTERED FOR 6-18 MONTHS

3.3.1. BODY AND LIVER WEIGHT

The dieldrin feeding apparantly does not cause any significant effect on the body growth rate over a period of 6-18 months. The body/liver weight ratios are however significantly decreased. After 6 months feeding this ratio decreases 19%, the same percent decrease was recorded even after 18 months of feeding. The liver weight which has been reported on the basis of percent of total body weight is increased after dieldrin feeding. This increase in 6 month feeding is 23% and 6% in 18 months feeding experiment (Table XIV).

3.3.2. HAEMATOLOGICAL STUDIES

Table XV and Figure 46 show effect of feeding of dieldrin mixed diet for a total period of 18 months on the various haematological parameters of rat. The RBC count is significantly decreased after dieldrin treatment, while the WBC count is conversely increased during this period. The amount of haemoglobin is also decreased. Control rats usually contain an average of 70.15×10^5 RBC/µl, 64.79×10^2 WBC/µl and 13.32 g haemoglobin/100 ml of blood. All these parameters are drastically affected after dieldrin feeding. The packed cell volume (PCV) is likewise decreased after long term feeding for six and more months. The MCV is not significantly altered in the 6 months feeding group, but is significantly increased in the 12 and 18 month groups. MCH is significantly decreased in the 6 month group and is increased in 12 and 18 months groups. The MCHC, on the other hand, is decreased in all the experimental groups.

Parameters	6 months dieldrin feeding experiment	
	Control (n = 6)	Dieldrin f (n = 3)
Body wt./ liver wt. ratio.	39.27±0.73	31.88±0.27***
Liver wt. (% body weight)	2.55±0.05	3.14±0.03***

***P < 0.001

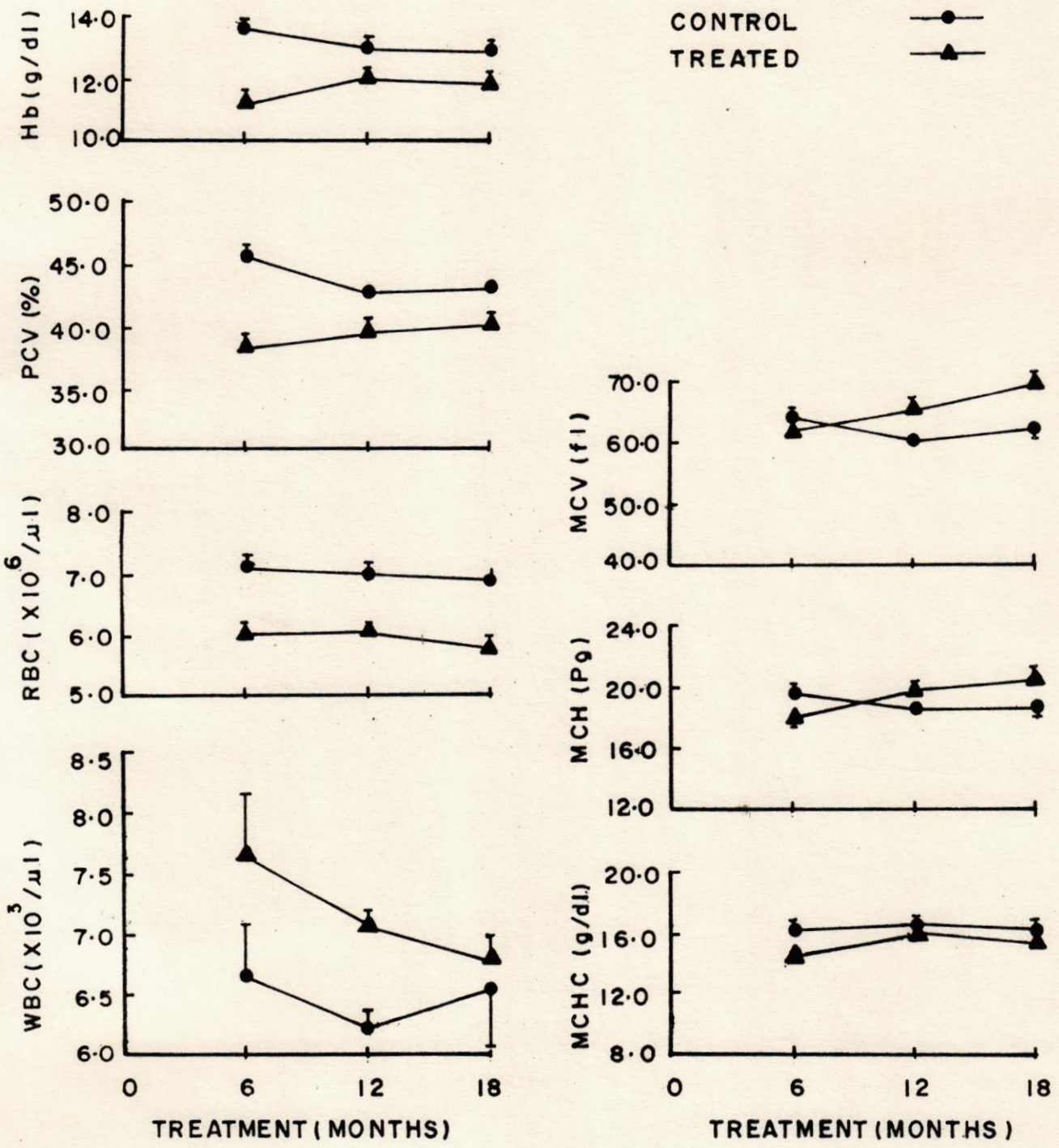


FIG. 46: Effect of feeding dieldrin mixed diet(6 mg/kg body weight/day) for a period of 6-18 months on the various haematological parameters of albino rat.

TABLE - XV

EFFECT OF FEEDING DIELDRIN MIXED DIET (6 mg/kg body weight/day) FOR A PERIOD OF 6-18 MONTHS ON THE VARIOUS HAEMATOLOGICAL PARAMETERS OF ALBINO RAT.

Parameters.	6 months dieldrin feeding exp.		12 months dieldrin feeding exp.		18 months dieldrin feeding exp.	
	Control (n=)	Dieldrin fed (n=)	Control (n=)	Dieldrin fed (n=)	Control (n=)	Dieldrin fed (n=)
Hb (g/dl)	13.79[a] ±0.32	11.21*** ±0.30	13.14 ±0.23	12.07* ±0.22	13.04 ±0.16	11.89*** ±0.11
RBC ($X10^5/\mu l$)	70.87 ±1.26	60.57*** ±1.10	70.48 ±1.28	60.78** ±1.43	69.10 ±1.20	57.60*** ±1.50
WBC ($X10^2/\mu l$)	66.50 ±3.99	76.50 ±5.06	62.38 ±1.34	70.75** ±1.04	65.50 ±5.20	67.50 ±2.10
PCV (%)	45.92 ±0.44	38.32*** ±0.46	42.89 ±0.19	39.61*** ±0.55	43.28 ±0.40	40.23*** ±0.13
MCV (fl)	64.38 ±0.65	62.83 ±0.58	60.91 ±0.66	65.24* ±0.97	62.64 ±0.46	69.85** ±1.60
MCH (Pg)	19.45 ±0.33	18.18 ±0.17	18.64 ±0.06	19.87 ±0.26	18.88 ±0.28	20.65* ±0.58
MCHC (g/dl)	30.24 ±0.65	28.94* ±0.07	30.62 ±0.41	30,46 ±0.21	30.15 ±0.36	29.56 ±0.27

[a] Mean ± SEM, Student's 't' test

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

TABLE - XVI

EFFECT OF FEEDING DIELDRIN MIXED DIET (6 mg/kg body weight/day) FOR A PERIOD OF 6-18 MONTHS ON ENZYME ACTIVITIES IN RAT BLOOD SERUM.

Parameters	6 months Dieldrin feeding exp.		12 months Dieldrin feeding exp.		18 months Dieldrin feeding exp.	
	Control (n=6)	Dieldrin fed (n=3)	Control (n=4)	Dieldrin fed (n=4)	Control (n=6)	Dieldrin fed (n=3)
AcP (KAU/dl)	5.458[a] ±0.48	10.71*** ±0.49	4.48 ±0.25	7.91** ±0.37	5.34 ±0.59	7.27 ±0.54
AP (KAU/dl)	9.568 ±0.40	22.65*** ±0.72	9.46 ±0.68	15.71** ±1.28	10.21 ±0.92	15.15** ±0.38
Amylase (Somogi u/dl)	242.57 ±16.74	235.00 ±16.07	210.29 ±10.57	317.64* ±28.31	202.03 ±12.22	238.45 ±15.90
ChE(Rapport u/ml)	31.80 ±3.76	23.33 ±2.60	26.00 ±1.85	25.94 ±1.12	31.08 ±1.82	30.50 ±2.31
CPK(Sigma units/ml)	10.30 ±0.77	8.03 ±0.58	7.57 ±0.61	9.87 ±1.08	8.75 ±0.97	6.83 ±0.20
ICDH(Sigma units/ml)	391.86 ±28.33	522.10* ±43.71	395.22 ±10.33	541.09* ±52.55	462.53 ±24.13	493.53 ±25.12
LDH (IU/l)	401.44 ±10.87	704.00*** ±38.93	442.20 ±49.62	629.11* ±13.01	509.49 ±27.82	429.60* ±7.71
SGOT (IU/l)	21.02 ±1.46	34.17*** ±1.80	28.98 ±2.73	46.53** ±1.35	35.66 ±2.55	56.66*** ±0.88
SGPT (IU/l)	17.72 ±1.40	17.68 ±0.78	14.82 ±0.99	19.72* ±2.24	18.75 ±1.87	15.83 ±1.73

*P < 0.05; **P < 0.01; ***P < 0.001

3.3.3. BIOCHEMICAL ANALYSIS OF BLOOD SERUM

The activities of several enzymes in blood serum are raised after dieldrin feeding (6 mg/kg body weight/day) for a total period of 18 months (Fig.47, Table XVI). The blood serum of control rat has alkaline phosphatase activity of 9.75 KAU/100 ml, and acid phosphatase activity of 5.13 KAU/100 ml, which are raised 137%, 66% and 48%, and 96%, 76% and 36%, respectively, on 6, 12 and 18 month groups. From amongst two transaminases tested, SGOT exhibits very prominent and distinct increase, which is ranged between 59 and 63% over a period of 18 months. The SGPT activity, on the other hand, is apparently not significantly altered and so is the case with CPK enzyme (Fig. 47, Table XVI). The two dehydrogenases (LDH and ICDH) are also significantly increased after dieldrin feeding. A control rat, on the average, shows LDH activity of 451.04 IU/l in the blood serum. After 6, 12 and 18 months of dieldrin feeding, the LDH activity is increased 75, 42 and 17%, respectively. The ICDH activity is likewise increased 33, 37 and 7%, respectively. The cholinesterase activity remains unaffected, while the amylase activity shows an increase of 51% and 18%, respectively, after 12 and 18 months of dieldrin feeding (Table XVI, Fig. 47).

Figure 48 and Table XVII show the effect of dieldrin feeding on the various biochemical components of blood serum, other than the enzymes.

Bilirubin and Cholesterol content are affected only in the 18 month group. The control rat blood serum contains 0.55 mg bilirubin/100 ml of blood serum and 180.12 mg cholesterol/100 ml of blood serum in the control

TABLE - XVII

EFFECT OF FEEDING DIELDRIN MIXED DIET (6 mg/kg body weight/day) FOR A PERIOD OF 6-18 MONTHS ON THE VARIOUS BIOCHEMICAL COMPONENTS OF ALBINO RAT SERUM.

Parameters	6 months Dieldrin feeding exp.		12 months Dieldrin feeding exp.		18 months Dieldrin feeding exp.	
	Control (n =)	Dieldrin fed (n =)	Control (n =)	Dieldrin fed (n =)	Control (n =)	Dieldrin fed (n =)
Bilirubin (mg/dl)	0.74[a] ±0.05	0.45 ±0.03	0.63 ±0.03	0.66 ±0.05	0.55 ±0.04	0.92** ±0.06
Cholesterol (mg/dl)	182.95 ±6.25	165.19 ±8.29	177.19 ±5.21	159.21 ±10.48	180.12 ±9.80	148.51* ±5.96
Free amino acids (mg/dl)	9.27 ±0.34	7.33* ±0.45	9.00 ±0.36	9.10 ±0.42	11.09 ±1.33	10.90 ±0.42
Glucose (mg/dl)	114.76 ±8.52	98.64 ±4.13	107.45 ±7.26	118.29 ±8.99	150.57 ±3.67	121.71** ±5.84
Protein (g/dl)	6.90 ±0.04	8.05*** ±0.16	7.68 ±0.21	8.17 ±0.47	8.58 ±0.45	9.09 ±0.05
Urea (mg/dl)	36.34 ±2.20	40.44 ±2.70	23.55 ±6.84	30.94 ±4.71	41.15 ±1.98	36.37 ±1.10

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

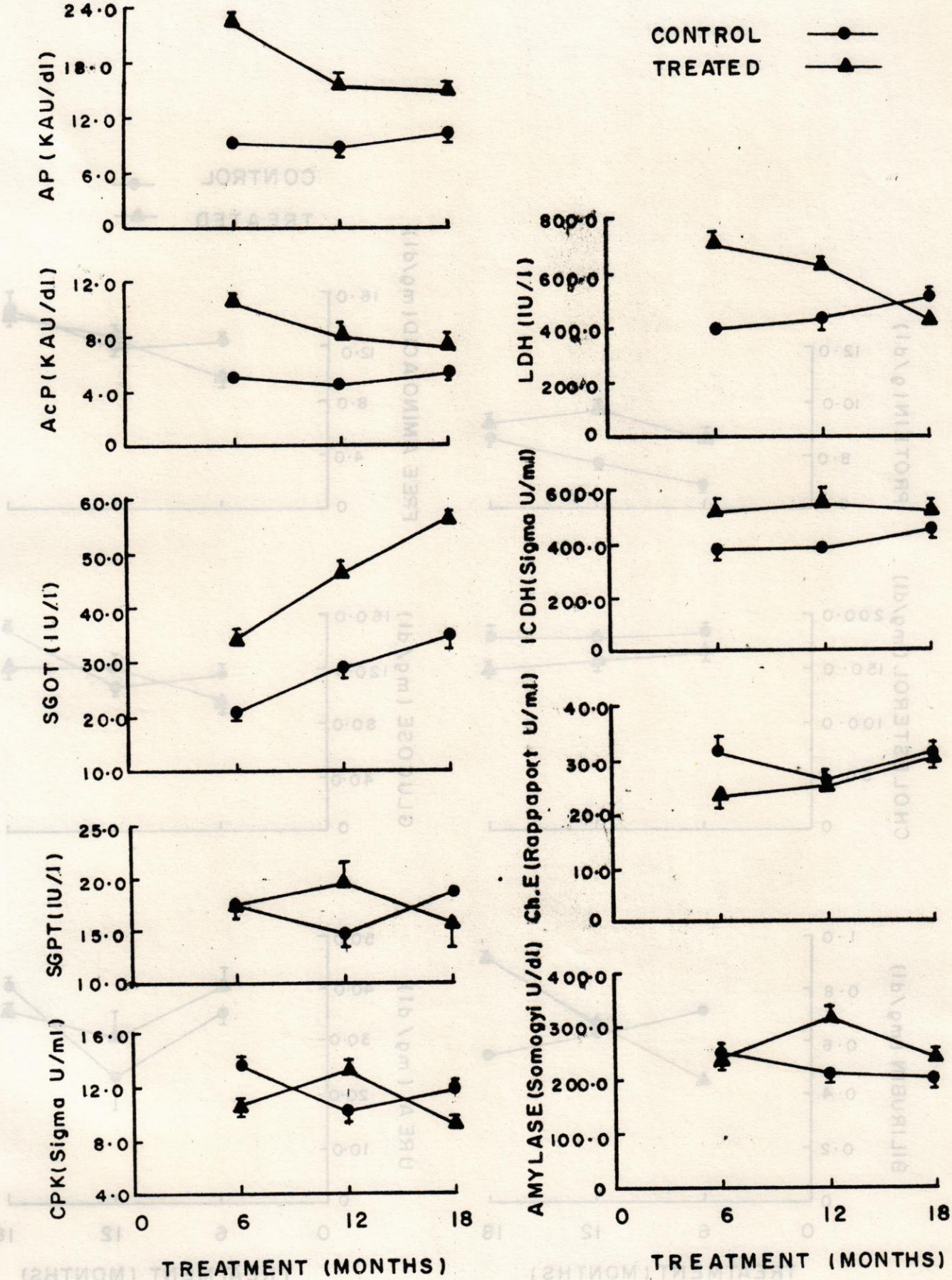


FIG.47: Effect of feeding dieldrin mixed diet (6 mg/kg body weight/day) for a period of 6-18 months on the various enzymatic activities of albino rat blood serum.

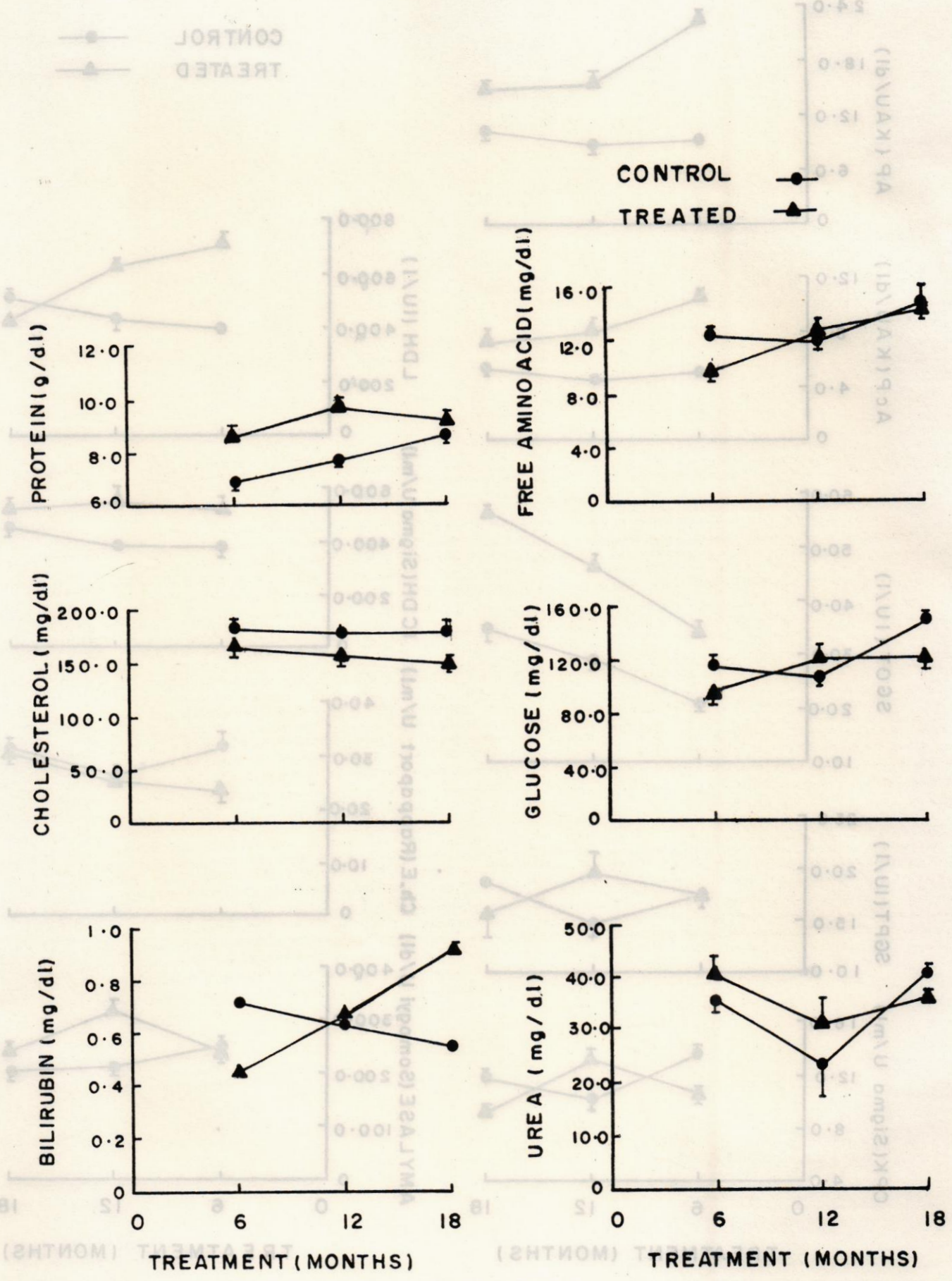


FIG.48: Effect of feeding dieldrin mixed diet (6 mg/kg body weight/day) for a period of 6-18 months on the various biochemical components of rat blood serum.

rats. After 18 month of dieldrin feeding the bilirubin content increase 67%, while the cholesterol content decrease 18%. Just like cholesterol the blood glucose content are also decreased (19%) after 18 months of dieldrin feeding. The blood serum proteins and the free amino acids show 17 and 21% decrease, respectively, during first six months of feeding. During the rest of the experimental period, both these content remain undisturbed. The urea content are also not affected throughout 18 months of experimental period (Fig.48, Table XVII).

3.3.4. BIOCHEMICAL ANALYSIS OF LIVER

All enzyme activities examined in liver are raised after dieldrin feeding (Fig.49, Table XVIII).

The AP activity in control rat liver varies between 0.74±0.05 KAU/g liver weight to 1.12±0.07 KAU/g. This activity increases 122%, 37% and 45% after 6, 12 and 18 months of feeding. The transaminases (GOT and GPT) also show elevated activities after dieldrin feeding. The GOT activity is increased 13, 8 and 26%, while GPT activity is increased 74, 130 and 56% in 6, 12 and 18 month group of animals. The GPT activity is apparently more sensitive, as it is affected significantly even after 6 month feeding, while GOT activity is significantly altered only after 18 months of feeding. Out of the dehydrogenases viz. ICDH and LDH, the former is significantly raised throughout the experimental period, while the later is only significantly changed in 12 month group. The ICDH activity is raised 35%, 31% and 65%, respectively in 6, 12 and 18 month rat group; while LDH is affected generally during later part of the

study (Table XVIII).

Table XIX and Fig. 50 shows the effect of dieldrin feeding on some of the other biochemical components of liver. The total proteins are not affected till 12 months of feeding and show only 20% significant increase after 18 months of dieldrin administration. The soluble protein component, however, show increase throughout insecticidal feeding period, while the free amino acid content show distinct decrease in the rat liver (Fig.50, Table XIX). The hepatic glucose content increase after insecticide administration. This increase in 6, 12 and 18 months groups is, respectively, 39, 45 and 61%. Cholesterol, on the other hand, show reverse behaviour. No significant change was observed after 6 months of feeding, but 37 and 60% increase was recorded after feeding dieldrin mixed diet to rats for 12 and 18 months, respectively (Table XIX, Fig. 50).

The control liver contains an average of 2.9 mg DNA/gm liver weight, which remained unaltered after 6 and 18 months of feeding, but showed about 35% increase after 12 months of feeding. The RNA content, on the other hand, do not show any significant change in the 6 and 12 month feeding group, but show a 12% significant decrease after 18 months of dieldrin feeding (Table XIX, Fig. 50).

3.3.5. HISTOLOGICAL STRUCTURE OF LIVER

Figure 51 and Table XX shows the effect of dieldrin feeding on the various histological parameters of rat. The hepatic cells, their nuclei and nucleoli hypertrophy after dieldrin feeding. The increase in cell

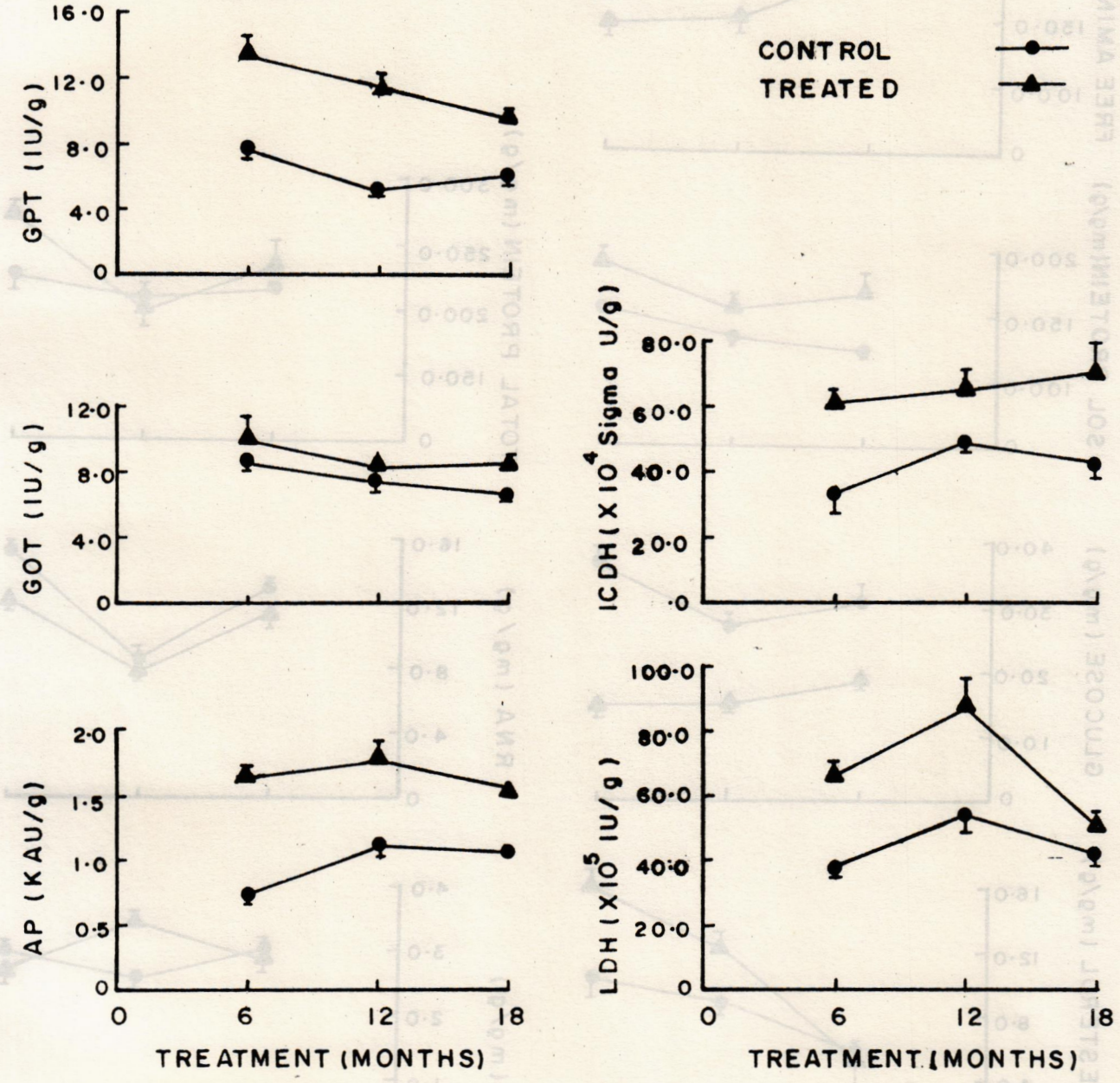


FIG.49: Effect of feeding dieldrin mixed diet (6 mg/kg body weight/day) for a period of 6-18 months on the activities of various hepatic enzymes of albino rats.

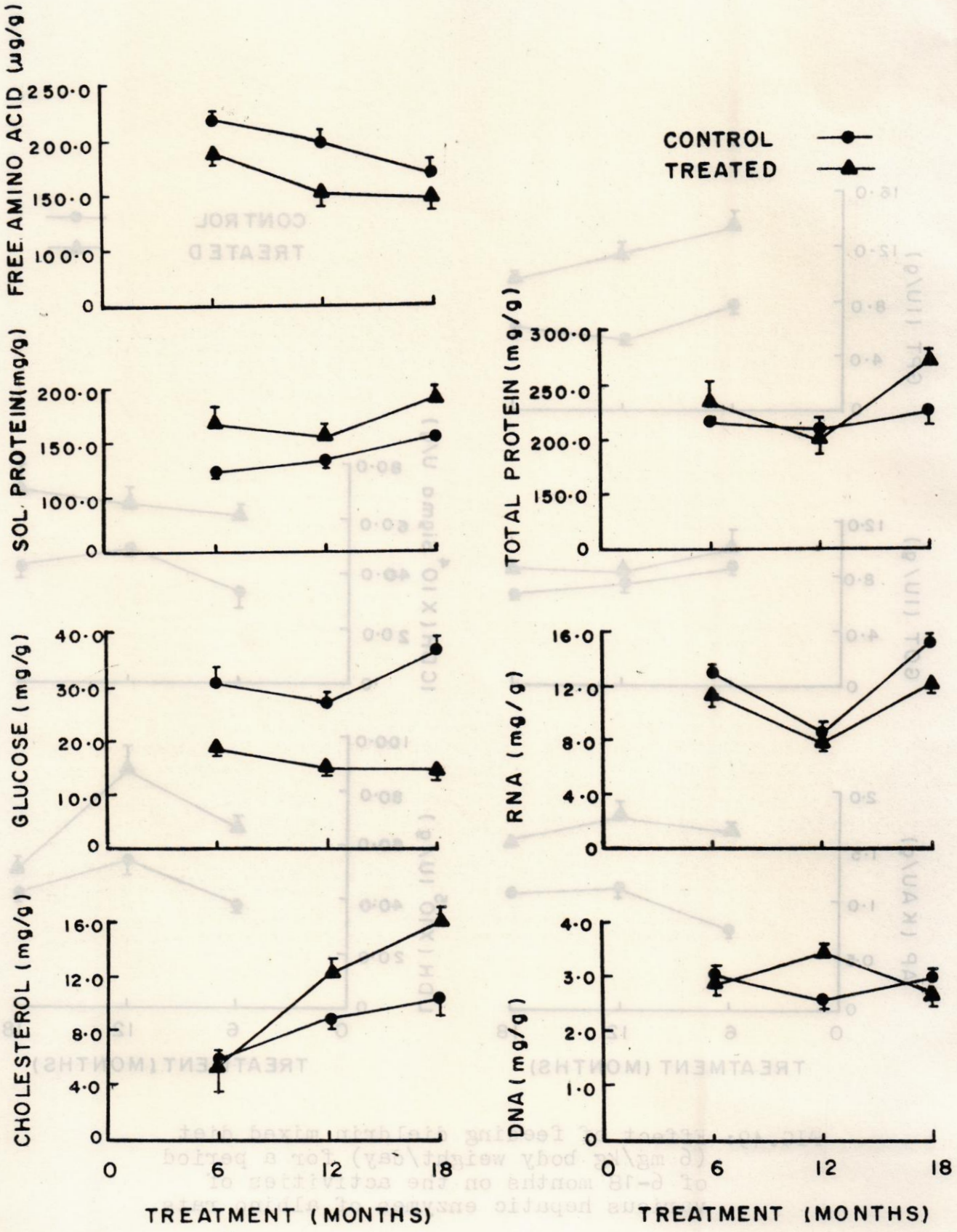


FIG. 50: Effect of feeding dieldrin mixed diet (6 mg/kg body weight/day) for a period of 6-18 months on the various biochemical components of rat liver.

TABLE - XVIII

EFFECT OF FEEDING DIELDRIN MIXED DIET (^{6} mg/kg body weight/day) FOR A PERIOD OF 6-18 MONTHS ON THE ACTIVITIES OF VARIOUS HEPATIC ENZYMES OF ALBINO RATS.

Parameters	6 months Dieldrin feeding exp.		12 months Dieldrin feeding exp.		18 months Dieldrin feeding exp.	
	Control (n = 6)	Dieldrin fed (n = 3)	Control (n = 4)	Dieldrin fed (n = 4)	Control (n = 6)	Dieldrin fed (n =3)
AP (KAU/g)	0.74 ±0.05	1.65*** ±0.05	1.12 ±0.07	1.53* ±0.14	1.08 ±0.08	1.57** ±0.07
GOT (IU/g)	8.66 ±0.54	9.81 ±1.16	7.56 ±0.46	8.19 ±0.36	6.79 ±0.23	8.56*** ±0.20
GPT (IU/g)	7.71 ±0.68	13.43** ±0.85	5.02 ±0.54	11.53** ±1.24	6.05 ±0.60	9.42* ±0.89
ICDH$_3$ ($X10^3$ Sigma U/g)	33.29 ±6.08	61.51** ±2.26	49.49 ±2.52	64.69* ±5.63	42.50 ±5.70	70.10* ±10.30
LDH ($X10^4$IU/g)	58.29 ±2.99	66.51 ±5.60	54.25 ±5.37	86.86* ±9.62	44.60 ±3.00	50.10 ±1.30

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

TABLE - XIX

EFFECT OF FEEDING DIELDRIN MIXED DIET (6 mg/kg body weight/day) FOR A PERIOD OF 6-18 MONTHS ON BIOCHEMICAL COMPONENTS OF ALBINO RAT LIVER.

Parameters	6 months Dieldrin feeding exp.		12 months Dieldrin feeding exp.		18 months Dieldrin feeding exp.	
	Control (n=6)	Dieldrin fed (n=3)	Control (n=4)	Dieldrin fed (n=4)	Control (n=6)	Dieldrin fed (n=3)
Cholesterol (mg/g)	5.97[a] ±0.29	5.41 ±0.61	9.01 ±0.32	12.33** ±0.75	10.50 ±1.21	16.76* ±1.11
Free amino acid(ug/g)	217.27 ±7.70	188.47* ±9.11	197.98 ±14.18	151.26* ±8.65	170.12 ±11.59	145.19 ±7.62
Glucose (mg/g)	30.87 ±2.32	18.71** ±1.68	27.12 ±1.37	15.05*** ±1.06	38.32 ±3.38	14.82*** ±0.86
Soluble protein (mg/g)	124.92 ±4.67	167.86* ±13.87	135.22 ±9.96	152.14 ±7.68	159.00 ±2.58	191.44* ±11.59
Total protein (mg/g)	216.96 ±13.05	232.93 ±17.01	208.48 ±6.7	202.75 ±2.64	227.60 ±11.88	273.69* ±9.45
DNA (mg/g)	3.05 ±0.11	2.89 ±0.20	2.54 ±0.14	3.43*** ±0.01	3.06 ±0.12	2.71 ±0.14
RNA (mg/g)	9.68 ±0.62	11.55 ±0.83	6.39 ±0.31	6.01 ±0.16	11.30 ±0.49	9.26* ±0.58

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

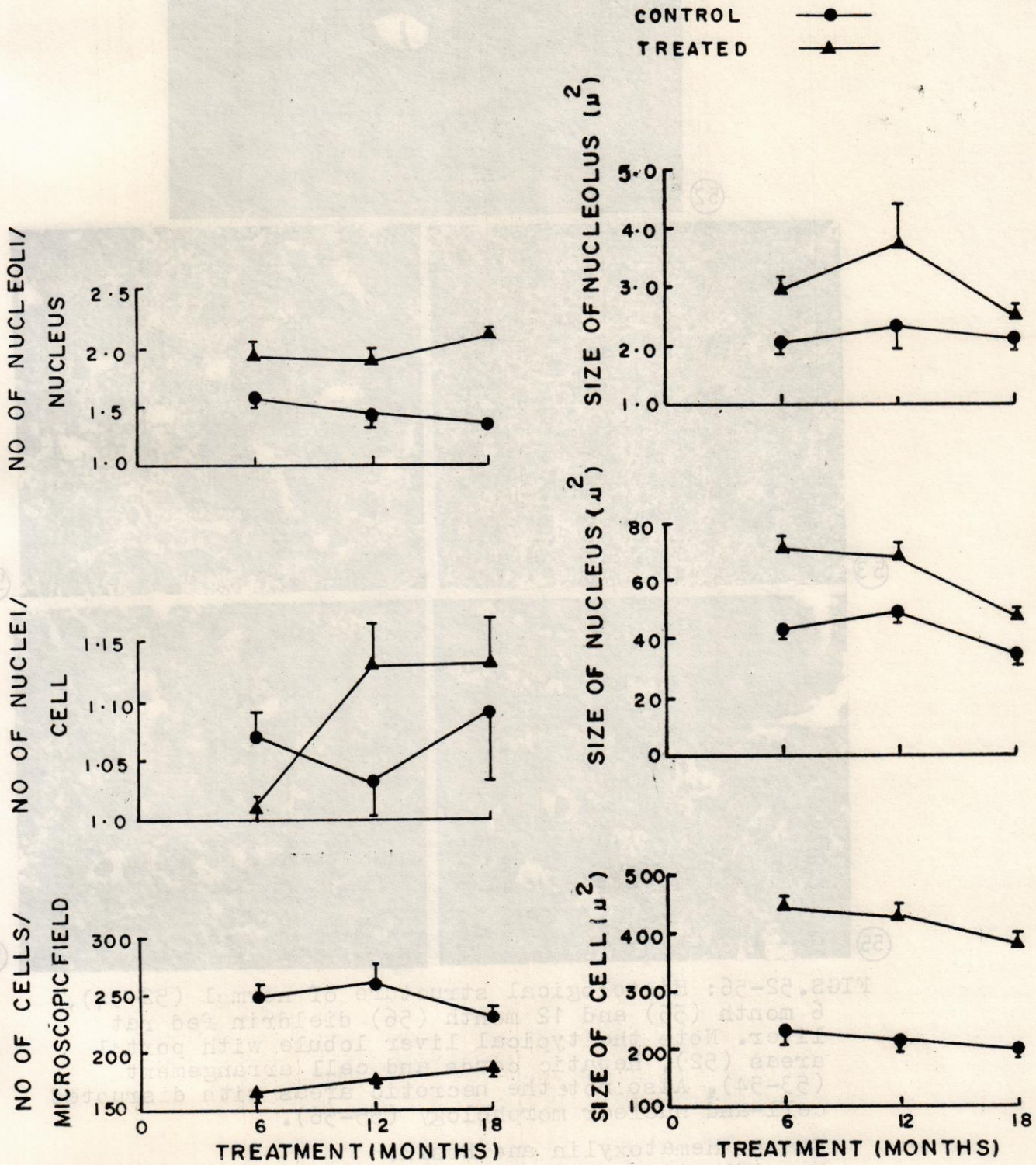
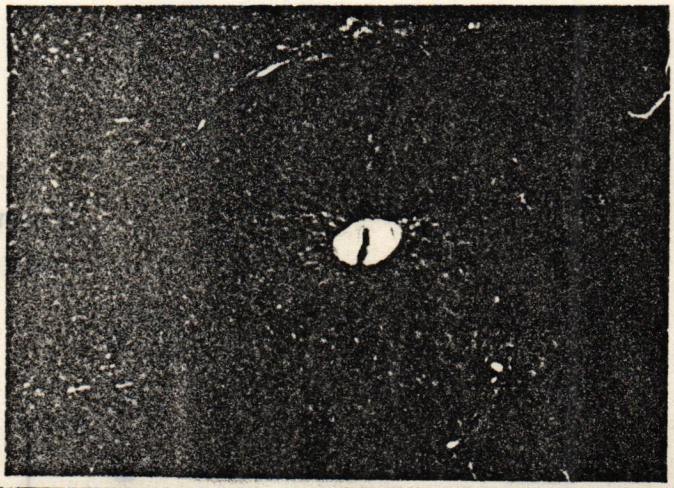


FIG.51: Effect of feeding dieldrin (6 mg/kg body weight/day) for 6-18 months on the various histological parameters of albino rat liver.



(52)

(53)

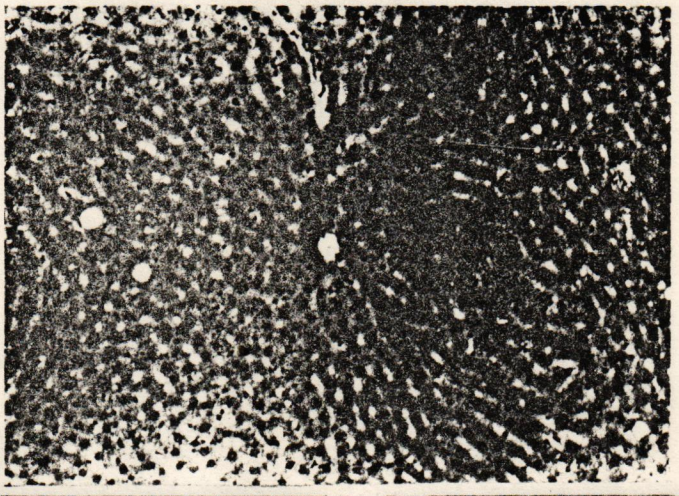
(54)

(55)

(56)

FIGS.52-56: Histological structure of normal (52-54), 6 month (55) and 12 month (56) dieldrin fed rat liver. Note the typical liver lobule with portal areas (52), hepatic cords and cell arrangement (53-54). Also note the necrotic areas with disrupted cell and nuclear morphology (55-56).

Stain: Hematoxylin and Eosin.
Magnifications: 52, X25; 53, X100; 54-56, X250.



57

58

59

FIGS. 57-59: Histological structure of rat liver fed on dieldrin-mixed diet for 18 months. Note disturbed hepatic cord morphology (57,58), vacuoles (57,59) hypertrophied hepatic cells and vesicular nuclei (58,59).

Stain: Hematoxylin and Eosin.
Magnifications: 57, X50; 58, X200; 59, X500.

TABLE - XX

EFFECT OF FEEDING DIELDRIN MIXED DIET (6 mg/kg body weight/day) FOR A PERIOD OF 6-18 MONTHS ON THE VARIOUS MORPHOMETRIC PARAMETERS OF ALBINO RAT LIVER.

	6 months Dieldrin feeding exp.		12 months Dieldrin feeding exp.		18 months Dieldrin feeding exp.	
	Control (n=4)	Dieldrin fed (n=3)	Control (n=4)	Dieldrin fed (n=3)	Control (n=4)	Dieldrin fed (n=3)
No.of cells/field	248.76[a] ±12.05	165.63*** ±9.01	262.20 ±16.75	176.29*** ±9.04	227.88 ±5.66	185.33*** ±5.53
No.of Nuclei/ cell	1.07 ±0.02	1.01* ±0.01	1.03 ±0.04	1.13 ±0.04	1.09 ±0.06	1.13 ±0.04
No.of Nucleoli/ Nucleus	1.59 ±0.09	1.93* ±0.11	1.47 ±0.10	1.85* ±0.12	1.38 ±0.07	2.09*** ±0.08
Size of cell(μ)^2	231.55 ±21.25	446.10*** ±14.06	218.51 ±17.53	429.55*** ±8.79	199.39 ±9.52	372.59*** ±13.24
Size of Nucleus (μ^2)	43.72 ±2.86	71.25*** ±1.97	49.05 ±2.65	68.41*** ±2.21	33.70 ±1.03	46.84*** ±1.12
Size of Nucleolus (μ^2)	2.07 ±0.21	2.95** ±0.18	2.34 ±0.63	3.78 ±0.86	2.16 ±0.22	2.47 ±0.13

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

size is respectively 93%, 97% and 87% in 6, 12 and 18 month group. The increased hepatic cell will result in decreased number of cells/microscopical field. This decrease is, respectively, 33.4, 33 and 18% after 6, 12 and 18 months of dieldrin feeding.

The nuclear size is increased 63, 39 and 39%, while the nucleolar size show an increase of 43, 62 and 14% during the long term feeding experiments for 6, 12 and 18 months respectively. The number of nuclei show significant decrease during the first 6 months but these do not show any statistically significant deviation from the normal for the rest of the period. The number of nucleoli however show very prominent increase during dieldrin feeding (Fig. 51, Table XX).

That the hepatic cells and their nuclei and nucleoli hypertrophy after dieldrin feeding is evident from histological sections shown in Figures 52-59. Figures 56 and 59 show necrotic regions, where typical hepatic cells are not discernable, instead the numerous nuclei are embedded in a mass of broken cells. The hypertrophied cells with distinct nuclear vacuoles are seen in Figure 59.

4. DISCUSSION

The effects of dieldrin on albino rats have been determined on the basis of three doses, viz (a) 40 mg/kg body weight/day for 48 hours (b) 12 mg/kg body weight/day for a total period of 15 days, and (c) 6 mg/kg body weight/day for a total period of 18 months. The first two doses have been designated as short term experiments, while third dose has been called as long term, depending upon the duration of administration of insecticide.

Body Weight

The dieldrin treatment for 15 days and then for 18 months result in a decrease in the total body weight as well as in % weight gain/day. Besides that the body weight/liver weight ratio are also altered after dieldrin administration. This ratio increases 8-17% during the first nine days of dieldrin administration at a dose of 12 mg/kg body weight/day, but is returned to normal level during subsequent administration till day 15. On the other hand when weaker dose of dieldrin (i.e. 6 mg/kg body weight/day) was administered for 18 months, the body weight/liver weight ratio decreases 19%. This decrease may be attributed to decrease in the total body weight without affecting the liver weight or liver weight decrease does not commensurate with the decrease in the total body weight or in fact the liver is hypertrophied in response to dieldrin toxicity. In the short term experiment of 15 days the body weight/liver weight ratio increases during the first 9 days, which may be because the total body weight is not drastically decreasing, while the liver may be. There are conflicting reports in the literature on the effect of dieldrin on body weight. Blus (1978) reported loss of weight in

the short tailed shrew after dieldrin treatment. Shakoori et al. (1984) also reported decrease in total body weight of albino rats, after feeding them dieldrin mixed diet for 6 months. There are, however, several reports which show the reverse (Fitzhugh et al., 1964; Hodge et al., 1967; Walker et al., 1973; Hurkat et al., 1977). Dix et al., (1977) reported reduced body weight gains in pregnant female mice after feeding dieldrin and dimethylsulphoxide. It follows that the feeding of dieldrin for a period upto 8 days does not cause any percepible change in body weight. The loss of body weight in the present study should be ascribed to chronic intoxication with dieldrin.

Haematological Parameters

Dieldrin treatment at different dose levels has caused almost same type of effects on the various haematological parameters. In all these cases the haemoglobin content, the RBC count and PCV has significantly decreased, while the WBC count increased prominently. The decreased haemoglobin is apparently due to decrease in RBC count, which in turn is responsible for decreased PCV. The RBC count is decreased, because perhaps the dieldrin inhibit the RBC production and or haemoglobin synthesis. Not only that the RBC s also disintegrate more rapidly in the presence of dieldrin, which contribute towards decrease in haemoglobin content, RBC cell count and packed cell volume. Like a typical insecticidal toxicity (or pathological) response, the number of WBC increase under all circumstances after treatment with dieldrin. This is perhaps a typical defensive response of the animal against a toxic invasion. Malik et al. (1974) has also reported increase in leukocytic count after dieldrin administration at 25 mg/kg body weight in buffalo calves.

In short term experiments the effect of insecticide is intensified along with the duration of administration. When administered at a dose of 40 mg/kg body weight/day the haemoglobin content decrease 6 and 9%, RBC count 16 and 22% and PCV 8 and 11%, respectively after 24 and 48 hours of dieldrin administration. In the second short term experiment, where dieldrin dose of 12 mg/kg body weight/day was administered, the haemoglobin and RBC count remains unaffected till day 9, when a decrease of 15% was recorded for haemoglobin and 8% for RBC count. During subsequent period the haemoglobin loss is maintained almost to the same level, while the RBC count decreases further. The PCV is significantly changed after 15 days of feeding dieldrin. The WBC count is the most sensitive count. At a dose of 40 mg/kg body weight/day the WBC count increases 34% and 55%, respectively, after 24 and 48 hours of dieldrin administration. The WBC count in the second short term experiment is not significantly affected until day 12, when the WBC count show a 35% and 36% increase after 12 and 15 days of dieldrin feeding. The other parameters like MCV, MCH and MCHC follow almost the same pattern. The long term experiments in which dieldrin was administered at a dose of 6 mg/kg body weight/day although showed the same trends, but behaved slightly differently when compared with short term experiment. Out of the three experimental groups examined for long term treatment, the six month group showed maximum deleterious effect. The animals seem to have adjusted themselves to continuous administration of insecticide. For example haemoglobin, RBC count and PCV show 18, 15 and 17% decrease after 6 months of feeding, but in 18 months group, the decrease is respectively 9, 17 and 7%. The WBC count increases,

but this increase is not very much significant, when compared with the treatments in short term experiment. Probably the amount of dieldrin which is being administered alongwith food is not sufficient enough to bring about any drastic effects to the tune of short term experiments.

The RBC count/WBC count ratio decreases 50 and 37% respectively after 24 and 48 hours of dieldrin treatment at a dose of 40 mg/kg body weight/day, while in long term experiment, this ratio is reduced 26%, 24% and 19%, respectively after 6, 12 and 18 months of feeding.

The decreased PCV is because of decrease in RBC count and decrease in consistency of plasma/serum due to accumulation of water (perhaps) in the blood.

Serum Biochemistry

Although dieldrin is known to affect various metabolic processes associated with lipids, carbohydrates (Bhatia et al., 1972 a,b; 1973; Kohli et al., 1975) and proteins (Wassermann et al., 1973; Skalsky and Guthrie, 1975, 1978; Ishikawa et al., 1978), but the changes in enzyme systems, specially for carbohydrate metabolism, microsomal and liver function have received special attention. Under pathological conditions the parenchymal cells of hepatic lobules fail to carry out vital functions, which may result in disturbed intermediary metabolism. Several enzymes like AP, GOT, GPT, LDH etc leach out into the serum (Kohli et al., 1975; Bhatia et al., 1972a; Gertig et al., 1971 a,b; Shakoori et al., 1982), thus raising their levels in the blood serum. The increase in SGOT, SGPT and LDH accompanied by a

decrease in cholesterol and protein level in the blood serum is an indication of liver damage. The extent of the damage inflicted and hence the increase in enzymatic activity in the blood serum are proportional to the amount and duration of dieldrin feeding. Several enzymes including those of conventional liver function tests (LFT) were tested. Almost all the enzymes got elevated after dieldrin treatment. Dieldrin appears to have either led to necrosis of cells, which ultimately result in increased activities of these enzymes in the serum or perhaps has induced the synthesis of these enzymes. The possibility, that both these processes may be in operation, is very strong. In short term experiments, when dieldrin is administered at a dose of 40 mg/kg body weight, AP, AcP, SGOT, LDH and ICDH activities are raised within 24 hours of dieldrin treatment. The SGPT and CPK activities are not affected until 48 hours of insecticide exposure. The amylase is the only enzyme which is not significantly affected after 48 hours of dieldrin treatment. The two dehydrogenases viz. LDH and ICDH have been found to the most sensitive enzymes. The LDH activity increases 1.98 and 2.12 fold, while ICDH activity increases 1.71 and 2.05 fold after 24 and 48 hours of dieldrin treatment. The AP activity increases 43 and 56%, AcP activity increases 87 and 49%, SGOT 62% and 36%, and ChE 50% and 57% respectively after 24 and 48 hours of dieldrin feeding. The SGPT and CPK activities increase 35% and 63%, respectively after 48 hours of insecticide administration.

In the second short term experiment, in which rats were fed dieldrin mixed diet at a dose of 12 mg/kg body weight/day, the AP, CPK and ChE activities

increased considerably after 3 days of dieldrin administration. The CPK activity increased 2.7 X and 2.1X after 3 and 6 days of feeding, respectively. The CPK activity later on got normalized inspite of persistent feeding for 15 days. The ChE activity likewise got elevated 2 fold after 3 days of insecticide feeding, and was later normalized inspite of extended feeding, AP activity is the one, which goes on increasing, with the increasing duration. This activity increases 2, 2.6, 3.5, 3.6 and 3.9 fold, respectively after 3,6,9,12 and 15 days of feeding. The AcP activity shows 45% increase during first weak of dieldrin administration, and is normalized during the subsequent insecticide feeding. The SGPT activity increases 55% within 3 days. This raised activity is maintained for the entire experimental period of 15 days. The SGOT activity, however, is not affected until day 9, when this activity increases 32%, 49% and 71% after 9, 12 and 15 days of dieldrin feeding. Just like SGOT activity the LDH activity is also not affected until day 9. This activity increases 1.8X, 1.9X and 2.2X after 9, 12 and 15 days of feeding. The ICDH activity is however affected right from the very beginning. This enzyme is increased 21% after 3 days of feeding, and is then maintained at 10-11% increase during the rest of the period despite continuous feeding. The ChE activity is not affected at this dose, while the amylase activity is increased 29, 40 and 63% after 9, 12 and 15 days of dieldrin feeding.

In long term experiments in which dieldrin was administered to rat for a period of 6-18 months at a dose of 6 mg/kg body weight/day, the animal system appears to have got itself adjusted to the continuous feeding and the intensity of effect of dieldrin, which is

normally expected does not appear. The SGPT, CPK and ChE activities do not show any appreciable deviation. The amylase activity is significantly affected in 12 and 18 months group. The AP and AcP, SGOT, LDH and ICDH activities are increased after six months of dieldrin feeding. In the case of AP activity this increase is 137%, 66% and 48%, while for AcP activity it is 96%, 76% and 36% after 6, 12 and 18 months of feeding. The SGOT activity show about 59% increase after 6 months and 63% increase after 18 months of feeding. The LDH activity increases 75%, 42% and 17%, while ICDH activity increases 33%, 37% and 7% after 6, 12 and 18 months.

In addition to several enzymes, some other biochemical components of blood serum were also tested to ascertain the toxic effects of dieldrin. The cholesterol and FAA decreased under all experimental contitions. In short term experiment the cholesterol content decrease 33% and 28% after 24 and 48 hours of dieldrin treatment, while FAA decrease 13 and 29%, respectively. In the second short term experiment the cholesterol content show uniform decrease of 27%, while the FAA content decrease 53% at the end of 15 days of experimental period. In the long term experiment, however, when dieldrin was administered at a dose of 6 mg/kg body wt./ day for a total period of 6-18 months, the cholesterol content decreased 19% at the end of experimental period, while FAA content decreased 21% after 6 months of feeding. No effect on FAA content was observed after prolonged feeding. The biluribin , protein, urea and glucose content increase after dieldrin administration for 48 hours. The biluribin content and urea content increase respectively 32% and 42% after 48 hours of dieldrin

feeding. The protein content show 14 and 17% increase, while glucose content show 17% and 23% increase after 24 and 48 hours, respectively. When dieldrin was administered at a dose of 12 mg/kg body weight/day for 15 days the biluribin and urea showed 2 fold and 50% increase during the first 3 days. No change was observed during the subsequent period. The glucose content showed 20% decrease during the same period. The protein content increase 77% after 9 days of feeding.

During long term feeding for 6-18 months at a dose of 6 mg/kg body weight/day the biluribin content increase 67%, while glucose and cholesterol content decrease 21% after 18 months of feeding. The urea content remain unchanged, while the protein, and FAA content decrease, respectively, 17% and 21% after 6 months of feeding.

A cursory glance on the above data will show that all the liver function tests are abnormal and deviate significantly from their respective control values. The short term experiments with 40 mg dose and 12 mg dose for 48 hours and 15 days, respectively, apparently cause much greater effects than long term experiments. All liver function enzymes (AP, SGOT, SGPT, LDH) are increased. The biluribin and urea content increase, while cholesterol and FAA content decrease significantly. All these changes manifest liver malfunction and are indicative of structural and metabolic disturbance under the specified conditions of dieldrin administration. Onikienko (1963) have also demonstrated an increased AP activity in rats chronically exposed to dieldrin. However, Harkat (1972 a) was unable to detect any change in the phosphatases (AP as well as AcP) activity after dieldrin treatment. Ishikawa et al. (1978) likewise

maintained that liver function tests remain unaffected after dieldrin treatment. Elevation of the aminotransferases activity after single dose of dieldrin was also reported by Bhatia et al. (1973) and Gertig et al. (1971 a,b). This increase was attributed to enhanced gluconeogenases (Bhatia et al., 1973). Elevation of serum LDH activity after dieldrin treatment was also reported by Bhatia et al. (1972 a). On the other hand, Hendrickson and Bowden (1976) and Meany and Pocker(1979) showed an in vitro inhibition of the LDH activity after treatment with dieldrin or other organochlorinated insecticides. The raised enzymatic level in the blood serum may be attributed to extensive hepatic necrosis, as a result of which the enzymes leach out into the blood serum or conversely the synthesis of these enzymes is induced with concomitant leaking out of these enzymes into the blood serum and thus raising their activities beyond normal limit. Raised bilirubin level is indicative of enormous RBC breakdown and disintegration of haemoglobin molecules, while decreased level of FAA and cholesterol is because dieldrin has interefered with the process of synthesis in the liver. The increased protein content of blood serum after dieldrin treatment in short term experiment is perhaps due to raised levels of different enzymes.

Raised level of amylase activity in the blood serum after 12 mg dose and that of ChE in 40 mg dose experiment is indicative of accumulation of these enzymes after cellular necrosis.

In long term experiments in which dieldrin was administered at a dose of 6 mg/kg body weight/day for 6-18 months, all the major liver function tests became

abnormal after 6, 12 and 18 months of dieldrin feeding. SGPT, CPK and ChE remain undeviated. Plasma cholinesterase activity of male rats have also been reported to be unaffected by other acute or chronic dieldrin administration. Although the general trend of events in serum in long term experiment is generally the same as in short term experiment, the data suggests adjustment of various metabolic processes to dieldrin feeding as a result of which the various biochemical changes will not be magnified, as one would have expected from a chlorinated insecticides, which are known for their low biodegradibility and comulative effects.

Shakoori et al. (1984) have also reported increase in almost all the enzymes of LET viz. AP, GOT, GPT and LDH after treatment with Dieldrin at a dose of 2 mg/kg body weight/day for a total period of 6 months. In their studies, cholesterol and protein levels, however, did not change appreciably. Morgan and Roan (1974), however, did not report any appreciable change in the serum enzyme activity in the dieldrin exposed workers.

Call and Call (1974) reported significant increase in total lipids, but no effect was seen in serum Ca^{++} total protein, inorganic phosphate or cholesterol levels in Japanese quails fed on dieldrin. Bhatnagar et al. (1980) have shown below normal values of cholinesterase and high serum cholesterol, phospholipid and GOT levels in pesticide factory workers.

Liver Biochemistry

The changes in blood serum were corraborated by recording changes in the various enzymatic activities of

liver and other hepatic components.

In short term experiment in which a dose of 40 mg/kg body weight/day was administered for 48 hours, all the LFT enzymes except for GOT were elevated. Hepatic GPT got raised 100% after 48 hours of dieldrin treatment, while AP showed 1.78 fold and 3.13 fold increase after 24 and 48 hours of dieldrin treatment. The LDH activity increased 47% and 36% during the same period. The hepatic ICDH activity remained unaffected after 24 hours, but was reduced 17% after 48 hours of dieldrin administration. In the second short term experiment in which dieldrin was administered at a dose of 12 mg/kg body weight/day for 15 days the ICDH activity is not significantly deviated. The GOT activity is not affected until day 15 of dieldrin feeding, when this activity increases 43%. The GPT and AP activitiesare also not affected until day 9 of feeding when the enzymatic activities continue to increase with increasing duration of insecticide administration. LDH is the only enzyme, which is affected from the very beginning. This enzyme shows 21% increase on day 3 and 26% on day 15 of the feeding experiment.

In long term experiment, in which dieldrin has been fed for a period ranging between 6 months and 18 months at a dose of 6 mg/kg body weight/day, all the hepatic enzymes tested showed elevated activities. GOT activity was, however, significantly elevated only in 18 month feeding group. The hepatic GPT activity shows raised enzymatic values 74%, 130% and 56% after 6, 12 and 18 months of dieldrin feeding. The AP activity likewise is raised 122%, 37% and 45%, respectively. The LDH activity is raised 14%, 60% and 12% after 6, 12 and 18 months of feeding, while the ICDH activity is raised

35%, 31% and 65%, respectively in the same group.

The increase in enzymatic activity in blood serum after dieldrin treatment may be because of (i) enzyme induction, followed by leakage into the blood serum, and or (ii) leaching out of the enzymes in the blood stream after destruction of pathological cells formed under the influence of dieldrin treatment. This leaking/ leaching out of enzymes may be beaause of pathological condition in liver or some other organs of the body. For example GOT activity is not much affected in liver, if at all, it is after prolonged feeding. Apparently liver biochemical milieu could not have contributed towards enhanced GOT levels in serum. This is definitely because of some non hepatic source. The hepatic GPT is increased after dieldrin treatment, but the increase of this enzyme in serum is comparatively low. This is perhaps because hepatic cells do not allow movement of SGPT enzymes that freely as it does in the case of other enzymes. The AP activity in the liver increases tremendously but its leakage outside into blood serum almost tally to a large extent. This should be kept in mind that there are several other organs in the body, which contribute towards increase of AP and other enzyme activities in the blood serum. So is true of LDH and ICDH activity, the hepatic ICDH activity is not much affected in short term experiment, but significant increase in blood serum is obviously because of/in some organ other than liver. In long term experiment all enzymatic activities increase, which eventually contribute towards raised blood serum enzymes.

The raised enzymatic levels in blood are attributable to liver damage under pathological conditions,

while their low levels in blood could either be because of great regenerative power of liver, as a result of which leaking out of the enzymes in blood serum becomes minimal, or due to the biosynthetic activity which implies routing of all the biochemical components towards this activity (Rosen and Nichol, 1963; Knox and Greengard, 1965; Bhatia et al., 1972 b, 1973) in liver. The raised enzymatic activity in the liver, on the other hand, may be because of induction of enzyme synthesis (Street, 1969; Kimbrough et al., 1971; Krample and Hladka, 1975), while their low levels could either be because of enzymatic inhibition (Hendrickson and Bowden, 1976; Meany and Pocker, 1979) or due to liver damage without any regeneration.

Dieldrin is also known to affect several other enzyme systems, other than LFT. It has been reported by Kohli et al. (1977a) that mixed functions hydroxylases, microsomal NADPH oxidase and phosphatidylcholine increase, but enzymes of lipid peroxidation decreases. Hepatic aminopyrine N-demethylase increase in horses, cattle and sheep (Ford et al., 1976).

Shakoori et al. (1982) have reported increase in hepatic GOT and AP activity after treatment of rat into dieldrin at a dose of 2 mg/kg body weight/day for 6 months. The GPT activity remained unchanged.

DDT, dieldrin and lindane also produce change in the lysosomal enzyme activity (Roux et al., 1974), and plasma membrane enzymes (Bandyopadhyay et al., 1982; Bandyopadhyay and Tewari, 1982). Verma et al. (1978) have reported inhibition of total ATPase and Mg^{++}, Na^{+} and K^{+} dependent ATPase.

In addition to enzymes, some other biochemical components have been considered as indicators of dieldrin toxicity. In short term experiment, at a dose of 40 mg/kg body weight/day cholesterol, FAA and glucose content decrease. The same trend is observed, when a dose of 12 mg/kg body weight/day was administered for 15 days. When a weaker dose of 6 mg/kg body weight was administered for 6-18 months, the FAA and cholesterol content decreased, but the glucose content in these long term experiments showed significant increase.

The hepatic soluble protein content increase under all circumstances. The total protein on the other hand increase 25% after administration of 40 mg dieldrin/kg body weight for 24 hours. When dieldrin was administered at a dose of 12 mg/kg body weight the soluble protein content increase 30% after 15 days of feeding. Six and 12 months of dieldrin feeding at a dose of 6 mg/kg body weight/day does not make any difference on the total proteins, but 20% increase was recorded in the 18 month feeding group.

Shakoori et al. (1982) have reported decrease in bilirubin, cholesterol and total protein content after long term feeding of albino rats with dieldrin at a dose of 2 mg/kg body weight/day for 6 months. The urea and glucose content did not show any change after insecticide treatment.

Somasundaram et al. (1978) have reported increased liver RNA, protein and glycogen content when higher concentration of dieldrin was coated on the skin of guinea pig.

Hepatic nucleic acids content

A strong dose of 40 mg/kg body weight/day administered for 48 hours reduce the RNA and DNA content of liver. The DNA content decrease 33 and 63% after 24 and 48 hours of dieldrin feeding, while the RNA content show 22 and 47% decrease during the same time. In second short term experiment the DNA content show a decrease of 57% after 3 days of dieldrin feeding, which is recovered to the normal level during prolonged feeding. The RNA content remain unchanged. In long term experiment the DNA content increase 35% after 12 months of dieldrin feeding, while the RNA content decline in quantity (12%) after 18 months of feeding.

Bhatia et al. (1973), who administered dieldrin to rats for shorter period of time, have not shown different results from the ones reported here. There are, however, some reports in the literature contrary to these (Annau, 1953; Ahmed et al., 1977) in tissue culture system. Bergen et al. (1974) did not find any effect in brain RNA synthesis and synthesis of DNA in brain and liver of suckling rats after dieldrin treatment. Shakoori et al. (1982) have also reported 4.38 times increase in the hepatic RNA content, while no change was seen in DNA content, when rats were fed on dieldrin mixed diet at a dose of 2 mg/kg body weight/day for 6 months.

Walker et al. (1973) have reported raised RNA and DNA contents in malignant tumors of mouse induced by dieldrin treatment.

Histological structure of liver

Amongst conflicting reports of carcinogenicity of

dieldrin, the chlorinated insecticides have overwhelmingly been reported to be carcinogenic to mice (Davis and Fitzhugh, 1962; Song and Harville, 1964; Innes et al., 1969; Walker et al., 1973; Tomatis et al., 1973; Epstein, 1975; Reuber, 1976, 1978 a,b, 1980; Deichmann and Macdonald, 1977; Sternberg, 1979; Sugar et al., 1979; David, 1979; Axelson, 1980; Ruebner et al., 1980; Meierhenry et al.,1981,1983; Maslansky and Williams,1981). In the present studies the dieldrin treatment has resulted in hepatotoxicity as evidenced by typical histological changes, but no indication of carcinogenicity was noticed. Liver show typical toxic effects. The appearance of vacuoles within cytoplasm, hypertrophy of hepatic cells, their nuclei and nucleoli are typical indicators of chemical toxicity. The general hepatolobular architecture generally remains unaffected, except for a slight disturbance in the cords of hepatic cells, which were no longer 2 to 3 cell thick and marked by greater sinusoidal spaces.

Microscopical structure of rat liver is considerably modified after dieldrin treatment. Some of the typical cellular responses to toxic chemicals are shown. The hepatic cells, their nuclei and nucleoli hypertrophy. Although the number of nuclei/cell generally remain unchanged, but sometimes binucleated nuclei were seen in the treated liver sections. The number of nucleoli/ nucleus, however, increase significantly. In addition to increase in the size of cell, the sinusoidal spaces usually increase in area, while the cytoplasm is marked by numerous vacuoles.

That the dieldrin is a potent compound, which besides disturbing biochemical millieu can also bring

about vast histopathological changes in liver (Hurkat, 1977; Kiselman, 1953; Mathur, 1975; Shakoori et al., 1982) and kidney (Reuber, 1980; Reddy and Khan, 1975, 1978).

Several ultrastructural changes caused by dieldrin feeding have also been described (Kimbrough et al.,1971; Kohli et al., 1977c; Roux et al., 1974). Lysosomes usually get stabilized and increase in number after dieldrin treatment. The AcP activity has been reported to disappear (Roux et al., 1974).

General Observations

Effects of dieldrin in the non target organisms is marked by quite diverse types of results from different labs. Inspite of several controversial and diverse findings in connection with carcinogenic effects (e.g. Davis and Fitzhugh, 1962; Innes et al., 1969; Epstein 1975 a, b; Reuber, 1976, 1978a,b; 1980; Maslansky and Williams, 1981 and several others, see paragraphs on page 102), teratogenic effects (Dix et al., 1977; Ottolenghi et al., 1973), mutagenic effects (Majumdar et al., 1977; Marshall et al., 1976; Dean et al., 1975) due to administration of dieldrin, the several histopathological changes produced in different tissues/ organs mainly liver, kidney and blood are generally uniform , which are manifested in terms of typical effects of toxicity. That the LFT are disturbed has abundantly been made clear in the present studies. The dieldrin spray has therefore a great potential hazard for human health and animal life (Allsopp, 1978; Babcock and Flickinger, 1977) and unless proper care is taken at the time of insecticidal spray, this insecticide will cause

ecological and human and wildlife problems inspite of the controvernal effects mentioned above. Dieldrin is a metabolite of aldrin, which is produced as a result of microsomal epoxidation. Aldrin, therefore, besides inflicting its own pathological changes, may be more harmful through its metabolite - dieldrin, which is produced by microsomal epoxidation (Ghiasuddin and Menzer, 1976; Kulkarni and Hodgson, 1984; Kurihara et al., 1984; Buck and Van, 1968).

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CHAPTER - II

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1. INTRODUCTION

Aldrin is a widely used chlorinated compound of cyclodiene group. It is one of the most active, general contact and stomach insecticide and has been used against the crop insects, and the termites in the soil (Hassall, 1969; Korschgen, 1970,1971; Lichtenstein et al., 1970). Like other compounds of the group aldrin is highly lipophillic and is a central nervous system stimulant. Aldrin is readily converted into its epoxide, dieldrin, in the soil and in the biological systems such as fat, muscle and liver (Bann et al., 1956; Buck and Van, 1968; Lichtenstein et al., 1970; Korschgen, 1971; Corbett, 1974; Ghiasuddin and Menzer, 1976), where it is stored almost unchanged.

Remarkable amounts of residues are reported from the plants and food stuffs (Correia, 1972; Balayannis, 1974). In literature several cases regarding contamination of aquatic and terrestrial food chains with insecticide residues are quoted (Graham, 1970; Korschgen, 1970; Rudd, 1975; Hashemy-Tonkabony and Langaroodi, 1976). Deichmann et al. (1971a) fed aldrin to pure-bred beagles for 10 months which resulted in a constantly increasing concentrations of dieldrin in blood and body fat. Discontinuation of aldrin administration resulted in the gradual decline in dieldrin fat concentrations from 75 ppm to 25 ppm after 12 additional months (Deichmann et al., 1971a).

These residues pose a severe threat to our ecosystem because of their greater stability. They are very slowly metabolized and excreted by mammalian system in milk and other dairy products (Buck and Van, 1968; Downey et al., 1975; Vreman et al., 1976; Kodric-Smit et al., 1980). As far as vertebrates are concerned, aldrin is a highly toxic compound. The toxicity may be due to direct ingestion through food, through inhalation, and through industrial and occupational exposure for example during agriculture and public health programmes. Hayes (1963) has reported that the effects of aldrin and dieldrin are similar, both qualitatively and quantitatively in animals and appeared to be true in man also. Cleveland (1966) did not observe any mortality or decrease in growth

rate in rats after feeding aldrin-mixed diet for 2 years at the concentrations of 2.5, 12.5 and 25 ppm. However, at 12.5 and 25 ppm dose, the animals showed increased liver weights. Oral administration of aldrin to beagles, whose diet already included a fixed oral dosage of DDT resulted in a dramatic rise in the concentrations of DDT, DDE and DDD in the blood and fat (Deichmann et al., 1971b).

Aldrin affects the blood components (e.g. Moss and Hathway, 1964 ; Deichmann et al., 1971a ; Mick et al.,1971 ; Mahajan and Juneja ,1979) as it is the first target and carrier of insecticide inside the body and the liver in fish, beagle dog and man because it has a key role in body metabolism. There are few scattered reports regarding the effect of aldrin in biochemical aspects of vertebrates. Gertig et al. (1971a,b) has reported the effect of aldrin on transaminase (aspartate amino transferase and alanine amino transferase) and phosphatase (alkaline and acidic) activities. Aldrin, like other xenobiotics, also induce microsomal enzymes, the extent of which, according to Krample and Hladka (1975), is dose dependent.

Aldrin also affects the carbohydrate metabolism in Indian cat fish (Srivastava and Singh, 1981).

Reproductive function and development in animals is also altered by aldrin (Kitselman, 1953; Deichmann et al., 1971b; Ottolenghi et al., 1973), besides known neurotoxic effects (Gupta, 1975).

Exposure of animals to aldrin resulted in histopathological changes in liver, kidney and other tissues, which include parenchymatous cell degeneration, hypertrophy and necrosis (Kitselman, 1953; Cleveland, 1966; Reuber, 1976, 1980). Corelations between accumulation of organochlorine insecticides and pathologies of liver, carcinoma, premature birth, lung cancer, leukemia malignant neoplasia, aplastic anemia, atrophy of the bone marrow, neurological syndromes and kidney diseases have been made (Vrochinskii et al., 1976). Aldrin has also been shown to affect the development and course of the cardiac pathological processes in rats and rabbits (Kagan et al., 1974).

There are conflicting reports about the carcinogenic potential of aldrin in the literature (for example Cleveland, 1966; David, 1979).

However, National Cancer Institute (NCI) report 1978, conclude that there was no convincing evidence that aldrin or dieldrin were carcinogenic. Although, increase in liver lesions called hepatoma, was observed in mice. This report is based on the previous work of Song and Harville (1964); Fitzhugh et al. (1964); Deichmann et al. (1977); Walker et al. (1968); Stevenson et al. (1976); Cleveland (1966); Davis and Fitzhugh (1962); and Thorpe and Walker (1973).

Gillespie et al. (1979) have made an assessment of carcinogenic risks in the United States and Great Britain due to aldrin and dieldrin.

The present studies aim at evaluating the effect of long term feeding of aldrin to rats. These effects have been assessed in terms of haematological, biochemical and histopathological changes in liver of aldrin treated rats.

2. MATERIALS AND METHODS

2.1. ANIMALS

A colony of sprague Dawley albino rats raised as described in the first chapter of this report was used for the present studies. The rats used were as follows:-

a) For short term experiments, two groups of female rats, weighing about 160-250 g and 6-8 months of age were used. One group was used for feeding insecticide for 48 hours, while the second was used for feeding insecticide for 15 days.

b) For long term experiments,male rats about 50-70 grams and 2-3 months of age were used.

2.2. PREPARATION OF FEED

The rat feed was prepared in the same way as described in Chapter I of this report.

2.3. INSECTICIDE USED

Aldrin (1,2,3,4,10, 10-hexachloro-1,4,4a,5,8, 8a-hexahydro-1, 4-endo-exo-5, 8-dimethanonaphthalene) a chlorinated insecticide of cyclodiene group (20% EC) was obtained from Entomology Department, University of Agriculture, Faisalabad, and administered to the animals orally along with feed.

2.4. ADMINISTRATION OF INSECTICIDE

Aldrin was administered to rats as strong and weak doses as follows:-

a) Strong dose

For short term experiments, two levels of strong doses were administered. In one group of rats a strong dose of 8 mg aldrin/kg body wt/day was administered for a total period of 15 days. In the second group, 20 mg aldrin/kg body weight/ day was administered for a total period of 48 hours.

b) Weak dose

A weak dose at a rate of 2.5 mg/kg body weight/ day was administered to another group of rats for 18 months.

2.4.1. SHORT TERM EXPERIMENTS

For short term experiments, in which the total duration is 48 hours in one case and 15 days in the other, the insecticide was administered as follows:-

a) For 48 hour experiment the insecticide mixed diet was prepared by mixing 0.8 ml of 20% EC Aldrin in 1 kg of rat feed. Since each experimental rat on the average consumed 30 g of feed daily, it will get 20 mg aldrin/kg body wt/day.

b) For 15 day experiment, the insecticide mixed diet was prepared by mixing 0.32 ml of 20% EC Aldrin in 1 Kg of rat feed. The rats in this way got 8 mg aldrin/kg body weight/day.

2.4.2. LONG TERM EXPERIMENT

The insecticide-mixed diet was prepared by adding 0.1 ml of 20% aldrin EC in small amount of water and then that insecticide-mixed water was thoroughly mixed with 1 kg of ingredient mixed feed.

That way the rats consumed 2.5 mg aldrin (a.i.)/ kg body weight/day.

2.5. PROCEDURE ADOPTED

Experimental procedure adopted for the two short term and one long term experiments is the same as described in Chapter I for dieldrin experiments. The procedures adopted for collection of blood, liver processing, haematological studies, biochemical analysis of blood, biochemical analysis of liver and histological studies were the same as described in Chapter I.

3. RESULTS

3.1. EFFECT OF ALDRIN MIXED DIET (20 mg/kg body weight/day) ADMINISTERED FOR 48 HOURS

3.1.1. HAEMATOLOGICAL STUDIES:

Aldrin administered at 20 mg/kg body weight/day produced typical reaction in the haemoglobin content, RBC counts and WBC counts (Fig. 1, Table I). A control rat has 13.27±0.14 g haemoglobin/100 ml, 6.84 X 10^6±0.25 RBC/µl and 6.36 X 10^3 WBC/µl. The haemoglobin content decrease 11 and 12% respectively after 24 and 48 hours of insecticide feeding. The RBC count is likewise decreased 14% after insecticide administration. The WBC count is conversely increased 27 and 25% respectively after 24 and 48 hours (Fig. 1, Table I). The PCV in control rats is 42.09 ± 0.34%, which remains unaffected after aldrin treatment. The MCV and MCH is significantly increased after insecticide treatment while the MCHC is decreased 8-9% after insecticide treatment for 48 hours (Fig.1, Table I).

3.1.2. BIOCHEMICAL ANALYSIS OF BLOOD

Figure 2 and Table II show the effect of aldrin fed for 48 hours on the various enzymatic activities of rat blood serum. Almost all the enzymes except for GPT activity are significantly altered. The control rat blood serum shows AP activity 11.67 ± 0.16 KAU/100 ml and AcP activity as 4.23±0.2 KAU/100 ml. The AP activity is comparatively more drastically changed, as compared with AcP. After 24 and 48 hours of aldrin administration, the increase in AP activity is respectively, 51 and 141%, while the increase in AcP activity is 26 and 15%. The GOT activity is one of those enzymes, which is most drastically affected after aldrin treatment. The activity

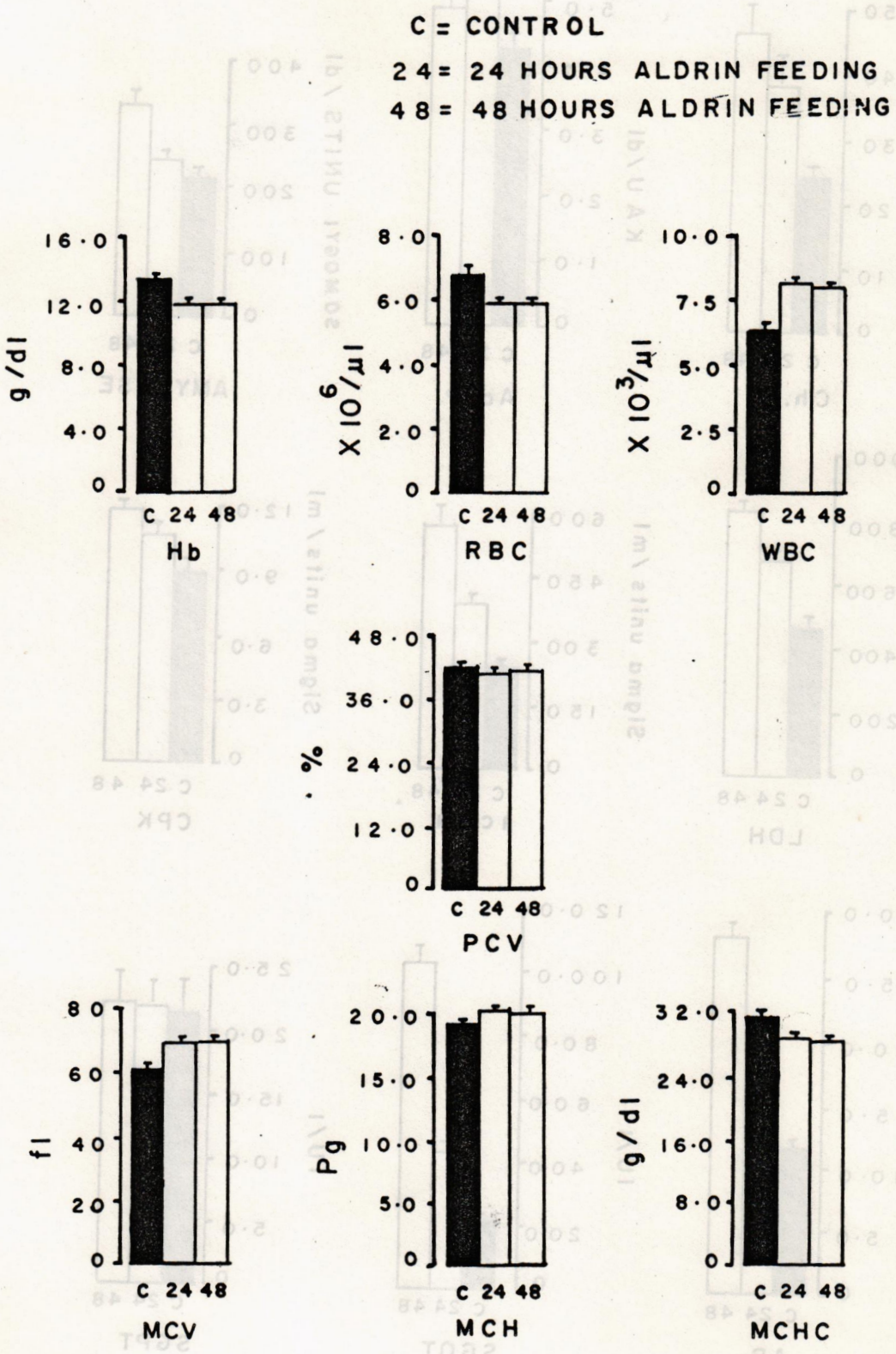


FIG.1. Effect of feeding aldrin mixed diet (20 mg/kg body weight/day) for a period of 48 hours on the haematological parameters of albino rats.

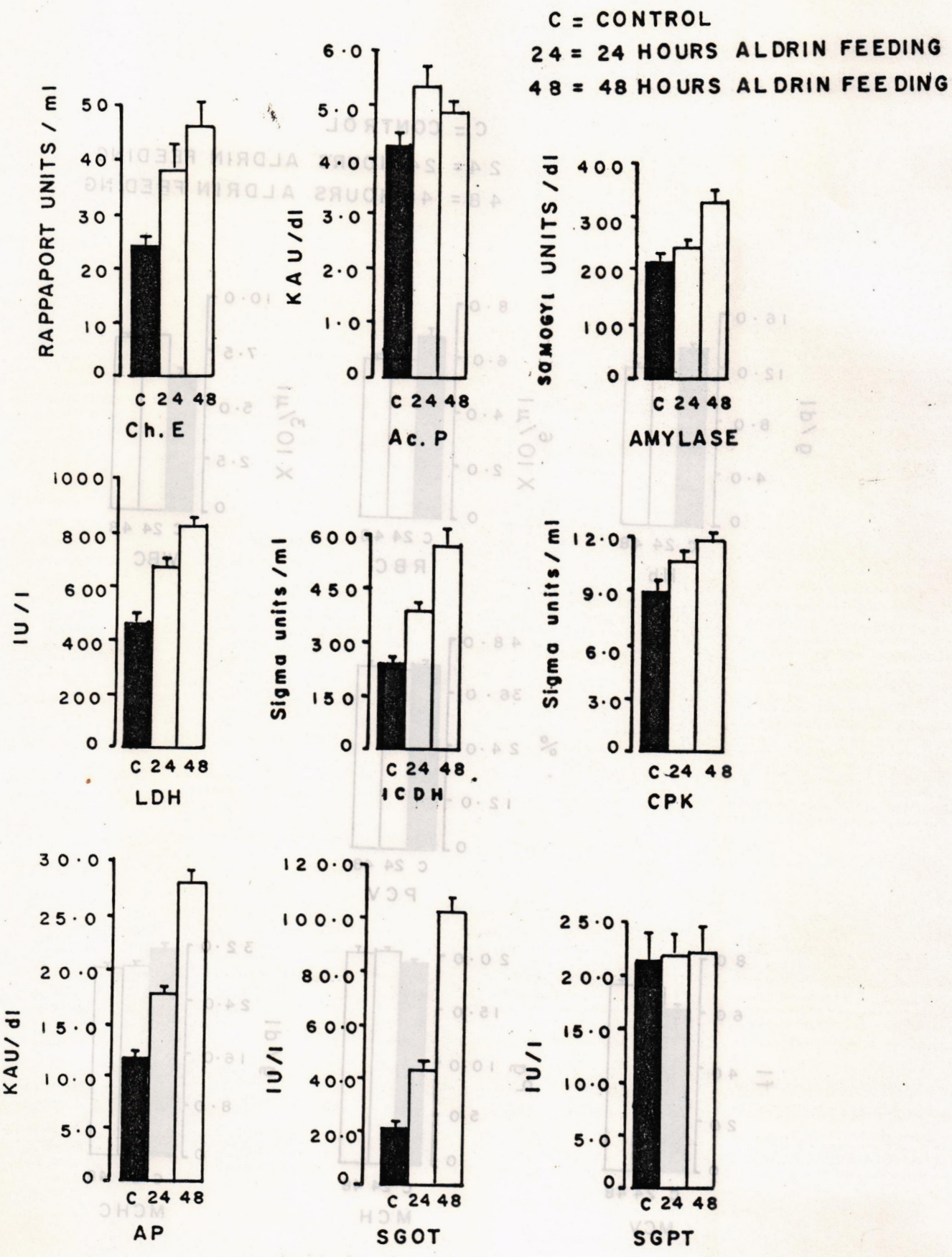


FIG.2. Effect of feeding aldrin mixed diet (20 mg/kg body weight/day) for 48 hours on activities of some enzymes of albino rat blood serum.

TABLE - I

EFFECT OF FEEDING ALDRIN MIXED DIET (20 mg/kg body weight/day) FOR A PERIOD OF 48 HOURS ON THE MAEMATOLOGICAL PARAMETERS OF ALBINO RATS.

Parameters	Control (n = 7)	Aldrin feeding	
		24 hours (n = 4)	48 hours (n = 4)
Hb (g/dl)	13.27±0.14[a]	11.77±0.19***	11.74±0.23***
RBC ($X10^6$ cells/µl)	6.84±0.25	5.87±0.06**	5.89±0.12**
WBC ($X10^3$ cells/µl)	6.36±0.26	8.11±0.13***	7.98±0.09***
PCV (%)	42.09±0.34	40.77±0.63	41.00±0.57
MCV (fl)	61.56±0.17	69.51±0.39***	69.70±0.46***
MCH (Pg)	19.38±0.08	20.07±0.13**	19.95±0.15**
MCHC (g/dl)	31.52±0.09	28.88±0.24***	28.61±0.23***

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

TABLE - II

EFFECT OF FEEDING ALDRIN MIXED DIET (20 mg/kg body weight/day) FOR 48 HOURS ON ACTIVITIES OF SOME ENZYMES OF ALBINO RAT BLOOD SERUM.

Parameters	Control (n = 8)	Aldrin feeding	
		24 hours (n = 4)	48 hours (n = 4)
Ap (KAU/dl)	11.67±0.16[a]	17.62±0.72***	28.11±1.12***
AcP (KAU/dl)	4.23±0.20	5.31±0.34*	4.85±0.16*
Amylase (Somogyi units/dl)	216.77±8.02	238.82±10.76	327.27±16.82***
ChE (Rappaport units/ml)	24.37±1.42	38.40±4.34*	45.75±4.77**
CPK (Sigma units/ml)	9.01±0.63	10.70±0.64	11.82±0.32**
GOT (IU/l)	21.73±1.65	43.49±2.40***	101.99±5.31***
GPT (IU/l)	23.05±2.40	23.16±1.81	23.67±2.21
ICDH (Sigma units/ml)	243.18±14.80	392.48±23.05***	570.26±44.04***
LDH (IU/l)	465.66±26.29	678.12±21.10***	827.64±25.55***

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

increase 100% and 369% from a control value of 21.73±1.65 IU/l after 24 and 48 hours of insecticide feeding. The LDH activity and ICDH activity also show increase after insecticide treatment. The control blood serum shows LDH activity 465.66±26.29 IU/l and ICDH activity 243.18±14.80, which increase 46 and 78% after 24 and 48 hours (for LDH) and 61 and 135% (for ICDH activity). The CPK activity is affected only after 48 hours of insecticide exposure, when it is increased 31% and is not significantly affected after 24 hours of aldrin administration. Amylase, just like CPK, is also not affected at 24 hour observation time, but shows an increase of 60% over the control activity. The control serum shows ChE activity of 24.37±1.42 Rappaport units/ml, which is increased 58 and 88% after 24 and 48 hours, respectively (Table II, Fig.2).

The blood serum protein increase after aldrin treatment, while the free amino acids (FAA) decrease during the same period (Table III, Fig.3). The blood serum proteins increase 13 and 26%, while the FAA content decrease 14 and 18% after 24 and 48 hours of aldrin treatment. The bilirubin, cholesterol and urea decrease during the same period after insecticide treatment. The bilirubin decrease 35 and 29% from control level of 0.72±0.07 mg/100 ml, while the cholesterol content decrease 31 and 29% after 24 and 48 hours of aldrin treatment. The urea content, on the other hand, remain unaffected after 24 hours treatment and shows 31% decrease after 48 hours of aldrin treatment. The glucose content likewise decrease 12 and 28% after 24 and 48 hours of aldrin administration (Table III, Fig. 3).

TABLE - III

EFFECT OF FEEDING ALDRIN MIXED DIET (20 mg/kg body weight/day) FOR 48 HOURS ON SOME BIOCHEMICAL COMPONENTS OF RAT BLOOD SERUM.

Parameters	Control (n = 8)	Aldrin feeding	
		24 hours (n = 4)	48 hours (n = 4)
Bilirubin (mg/dl)	0.72±0.07[a]	0.47±0.03**	0.51±0.07
Cholesterol (mg/dl)	195.64±6.94	135.34±6.78***	139.06±5.33***
Free amino acids (mg/dl)	7.26±0.19	6.21±0.05***	5.93±0.28**
Glucose (mg/dl)	107.37±3.36	119.99±5.94	137.74±4.76***
Protein (g/dl)	7.30±0.13	8.28±0.23**	9.18±0.40**
Urea (mg/dl)	35.49±0.96	33.63±1.03	24.46±1.54***

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

C = CONTROL
24 = 24 HOURS ALDRIN FEEDING
48 = 48 HOURS ALDRIN FEEDING

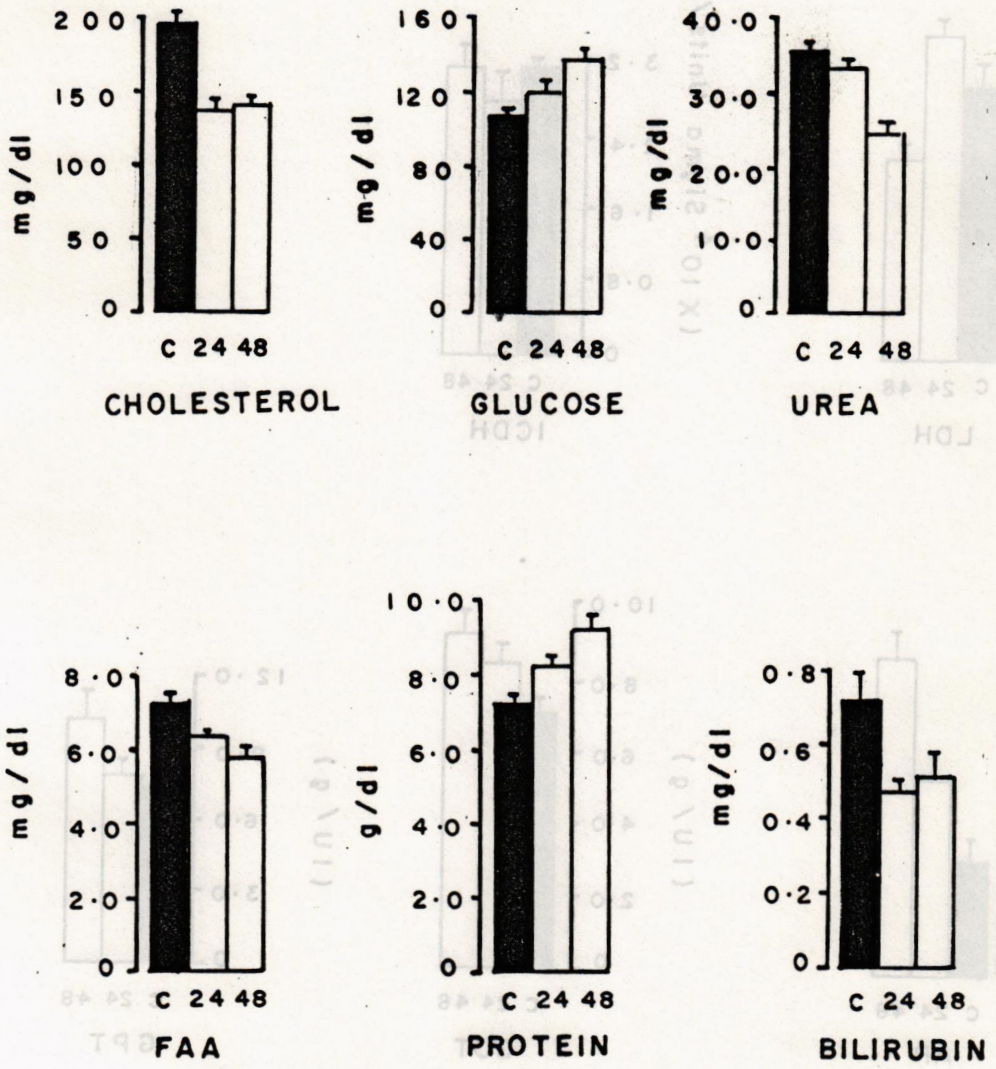


FIG.3. Effect of feeding aldrin mixed diet (20 mg/kg body weight/day) for 48 hours on some biochemical components of rat blood serum.

C = CONTROL
24 = 24 HOURS ALDRIN FEEDING
48 = 48 HOURS ALDRIN FEEDING

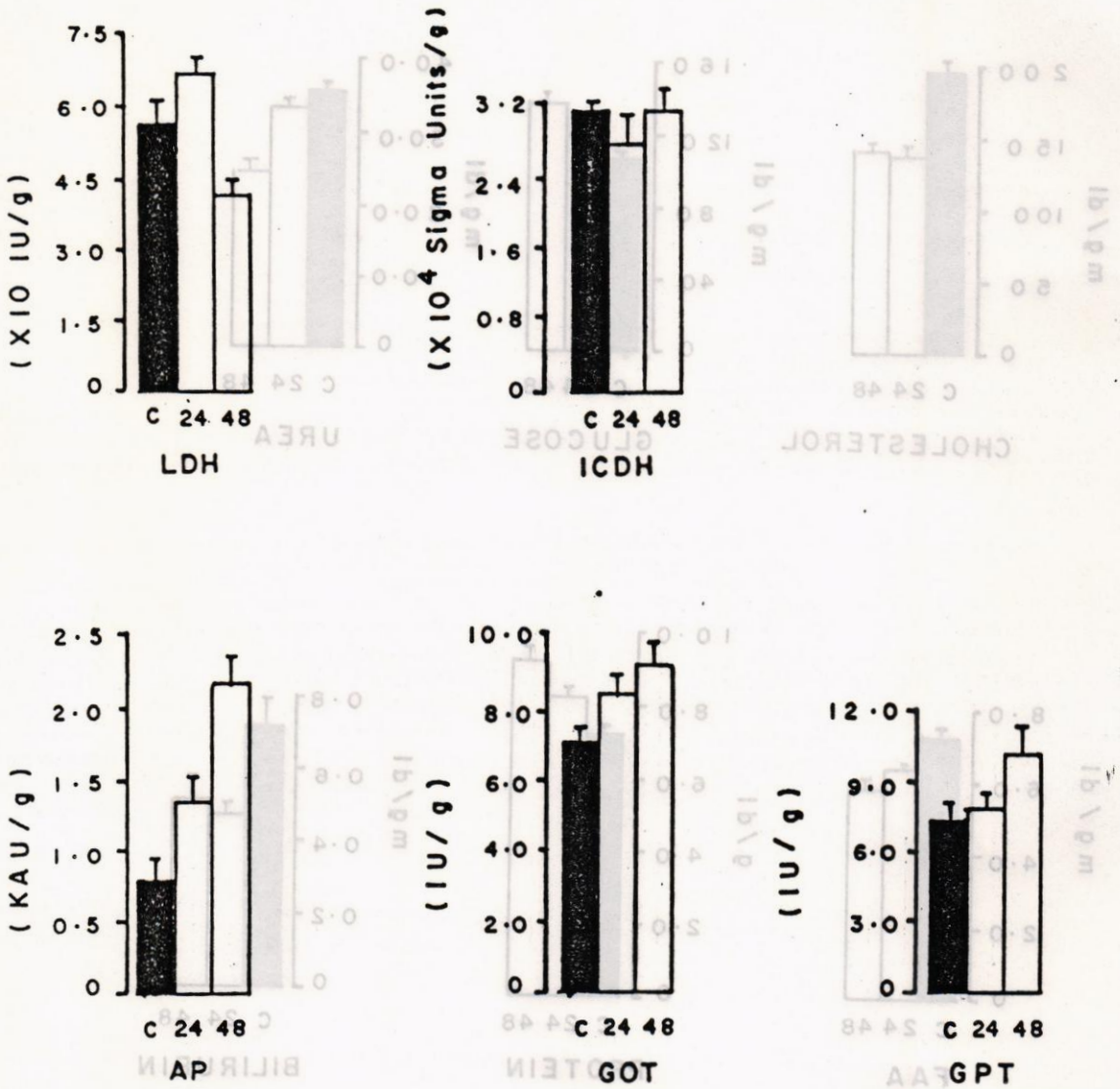


FIG.4. Effect of feeding aldrin mixed diet (20 mg/kg body weight/day) for a period of 48 hours on the activities of hepatic enzymes of albino rats.

3.1.3. BIOCHEMICAL ANALYSIS OF LIVER

The hepatic enzymes are not affected to that extent, as they were in blood serum after aldrin treatment. In fact GPT and ICDH activities are not affected at all, while the LDH activity and GOT activities are significantly altered only after 48 hours of aldrin feeding (Table IV, Fig.4). The control liver shows GOT activity 7.11±0.38 IU/g, which increases 18 and 28%, 24 and 48 hours after aldrin administration. The LDH activity, on the other hand, decreases 26% after 48 hours of aldrin treatment. The AP activity is, however, drastically affected. The control liver shows AP activity 0.80±0.16 KAU/g, which increases 69% and 171% after 24 and 48 hours of aldrin feeding.

Figure 5 and Table V shows the effect of aldrin feeding on the various biochemical components of liver other than enzymes. The nucleic acids (both DNA and RNA) do not show any significant change after aldrin treatment, while the total proteins show 19 and 13% increase during the same period i.e. 24 and 48 hours after aldrin treatment. The soluble protein likewise show increase of 13 and 18%, but conversely the FAA content decrease 68 and 37% after 24 and 48 hours , respectively. The cholesterol content in control rat liver is 7.62±0.22 mg/g, while the glucose content are 20.14±0.53 mg/g. The Cholesterol content decrease 29% after aldrin treatment, while the glucose content is significantly (43%) decreased after 48 hours of aldrin treatment (Table V, Fig.5).

TABLE - IV

EFFECT OF FEEDING ALDRIN MIXED DIET (20 mg/kg body weight/day) FOR A PERIOD OF 48 HOURS ON THE ACTIVITIES OF HEPATIC ENZYMES OF ALBINO RATS.

Parameters	Control (n = 5)	Aldrin feeding	
		24 hours (n = 4)	48 hours (n = 4)
AP (KAU/g)	0.80±0.16[a]	1.36±0.17*	2.18±0.09***
GOT (IU/g)	7.11±0.38	8.40±0.51	9.11±0.56*
GPT (IU/g)	7.32±0.68	7.88±0.55	10.21±1.22
ICDH ($X10^3$ Sigma units/g)	31.39±0.78	27.71±3.16	31.20±2.46
LDH ($X10^4$ IU/g)	56.57±4.43	66.24±3.32	41.83±2.76*

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

C = CONTROL
24 = 24 HOURS ALDRIN FEEDING
48 = 48 HOURS ALDRIN FEEDING

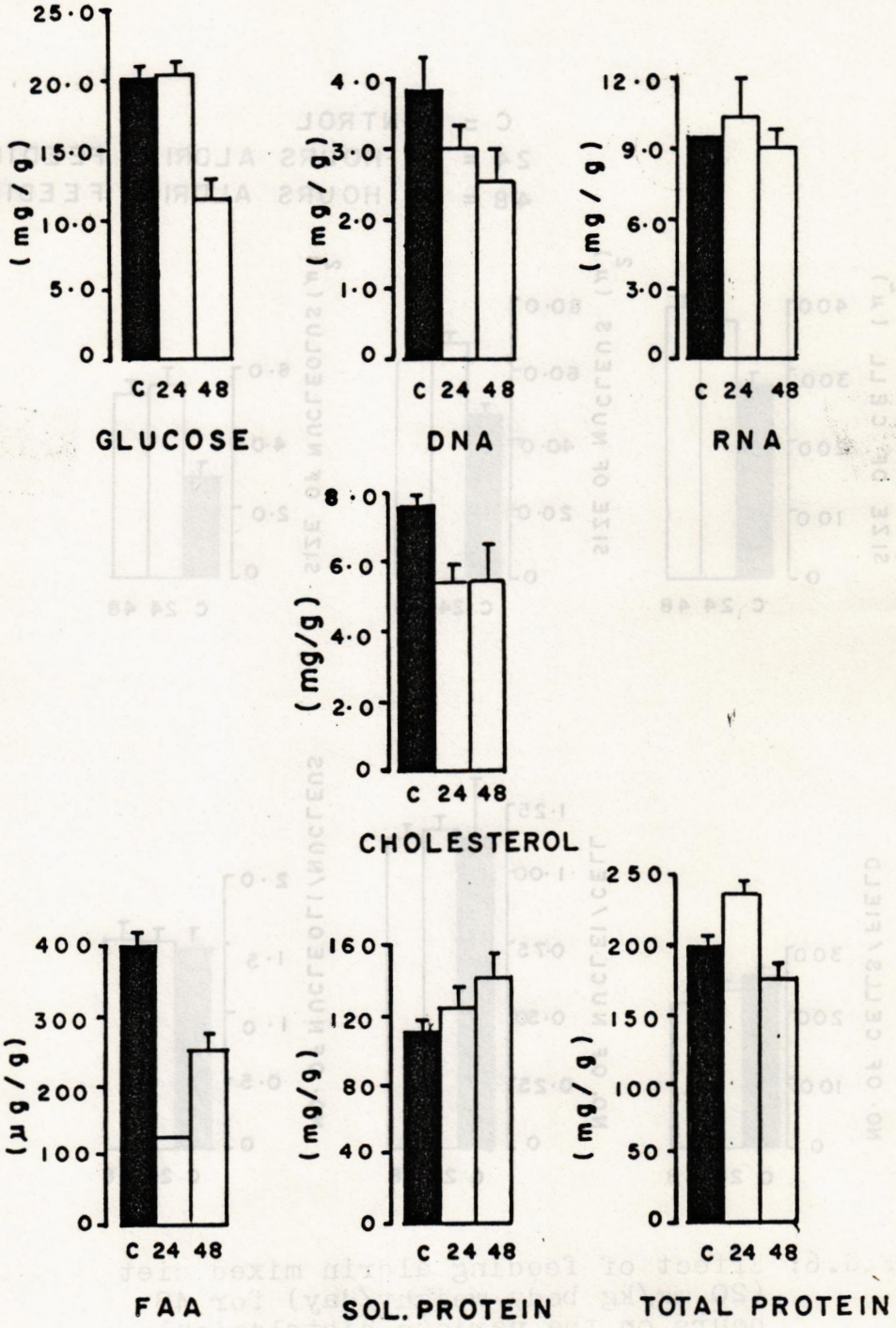


FIG.5. Effect of feeding aldrin mixed diet (20 mg/kg body weight/day) for 48 hours on the various biochemical components of albino rat liver.

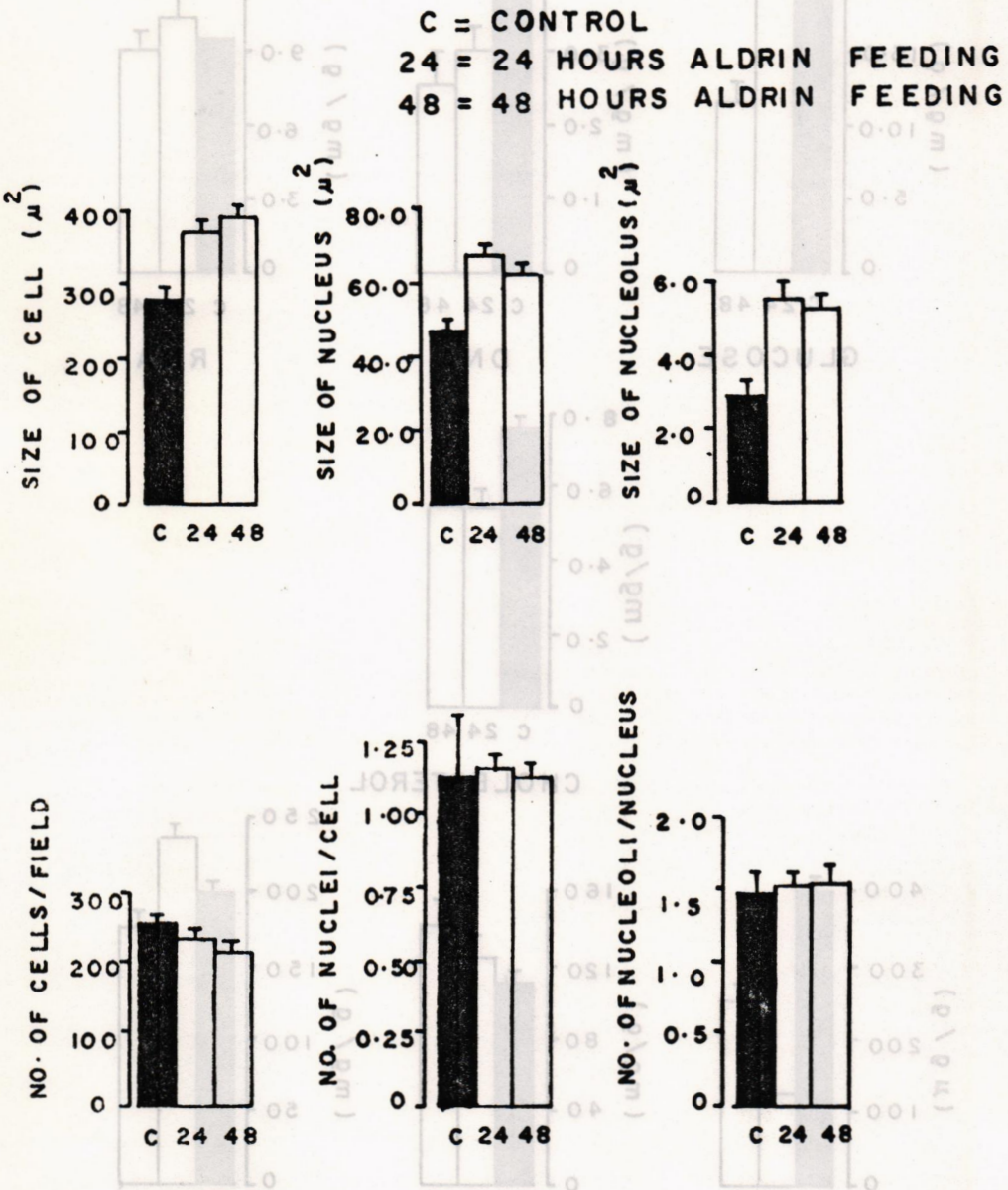


FIG.6: Effect of feeding aldrin mixed diet (20 mg/kg body weight/day) for 48 hours on the various histological parameters of albino rat-liver.

Table - V

EFFECT OF FEEDING ALDRIN MIXED DIET (20 mg/kg body weight/day) FOR 48 HOURS ON THE VARIOUS BIOCHEMICAL COMPONENTS OF ALBINO RAT LIVER.

Parameters	Control (n = 5)	Aldrin feeding	
		24 hours (n = 4)	48 hours (n = 4)
Cholesterol (mg/g)	7.62±0.22[a]	5.35±0.50**	5.42±1.06
FAA (ug/g)	399.21±18.13	126.24±0.51***	250.23±20.27***
Glucose (mg/g)	20.14±0.53	20.44±0.76	11.57±1.29***
Soluble Protein (mg/g)	111.18±5.08	125.41±10.25	142.17±12.01*
Total protein (mg/g)	199.33±6.11	237.38±8.21**	174.02±10.58
DNA (mg/g)	3.84±0.44	2.99±0.33	2.54±0.45
RNA (mg/g)	9.53±0.55	10.44±1.72	9.04±0.75

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

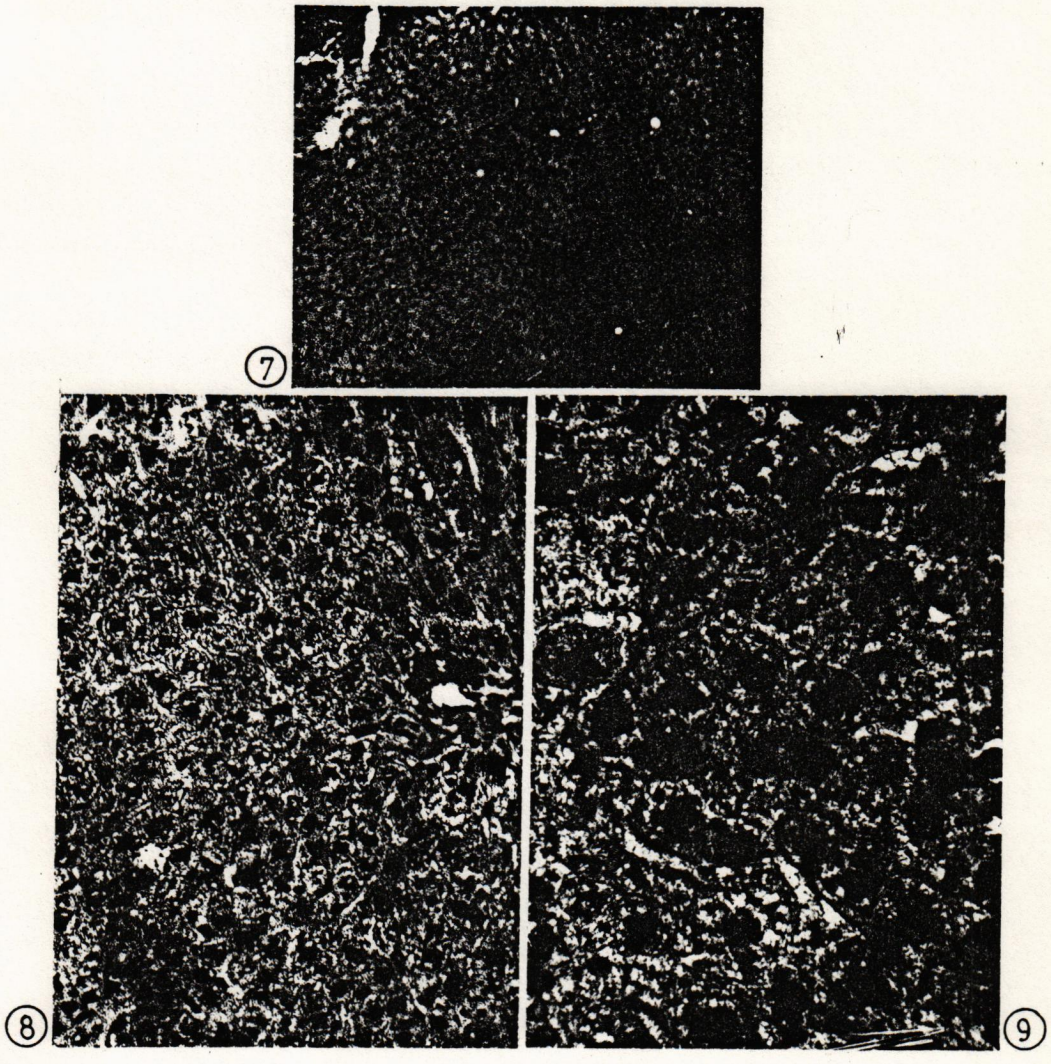
TABLE-VI

EFFECT OF FEEDING ALDRIN MIXED DIET (20 mg/kg body wt./ day) FOR 48 HOURS ON THE VARIOUS HISTOLOGICAL PARAMETERS OF RAT LIVER.

Parameters	Control (n = 90)	Aldrin feeding	
		24 hours (n = 90)	48 hours (n = 90)
No.of cells/ field.	252.91±8.54[a]	230.41±6.24	212.68±11.64*
No.of nuclei/ cell.	1.13±0.19	1.16±0.04	1.13±0.04
No.of nucleoli/ Nucleus.	1.47±0.11	1.52±0.08	1.53±0.09
Size of cell (μ^2)	279.79±14.24	373.26±12.68**	391.92±9.84***
Size of nucleus (μ^2)	47.29±2.01	68.01±1.93***	62.05±1.83***
Size of Nucleolus(μ^2)	2.94±0.26	5.52±0.35***	5.26±0.32***

[a]Mean±SEM, Student's 't' test;

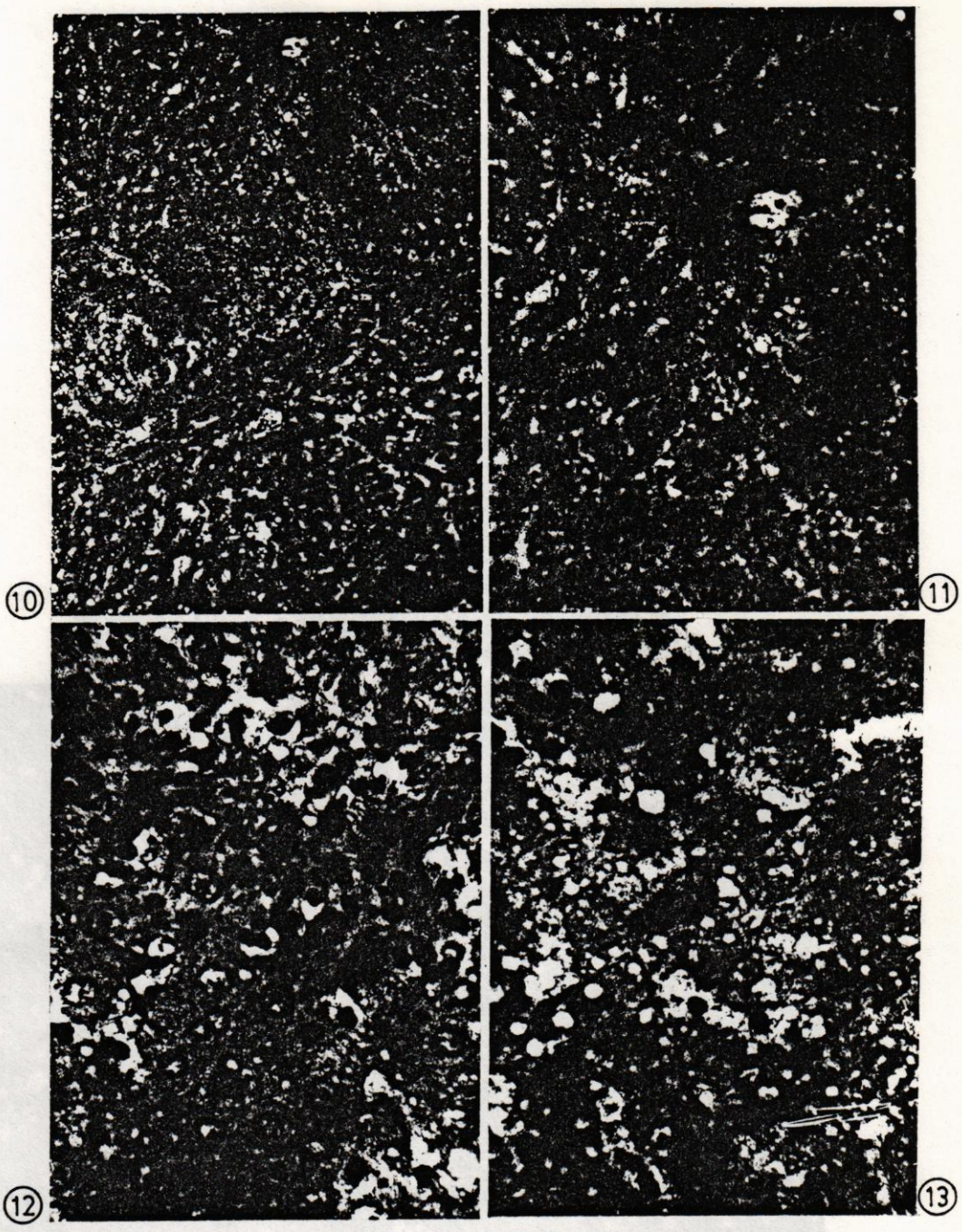
*P < 0.05; **P < 0.01; ***P < 0.001.



FIGS.7-9: Histological structure of normal rat liver. Note the general lobul structure (7) with many portal areas, almost linear hepatic cords (8) and arrangement of nuclei and cells (8, 9).

Stain: Hematoxylin and Eosin.

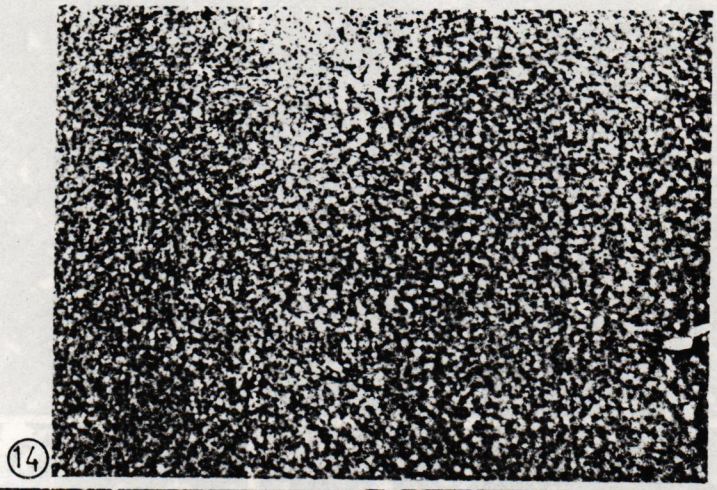
Magnifications: 7, x25; 8, x100; 9, x250.

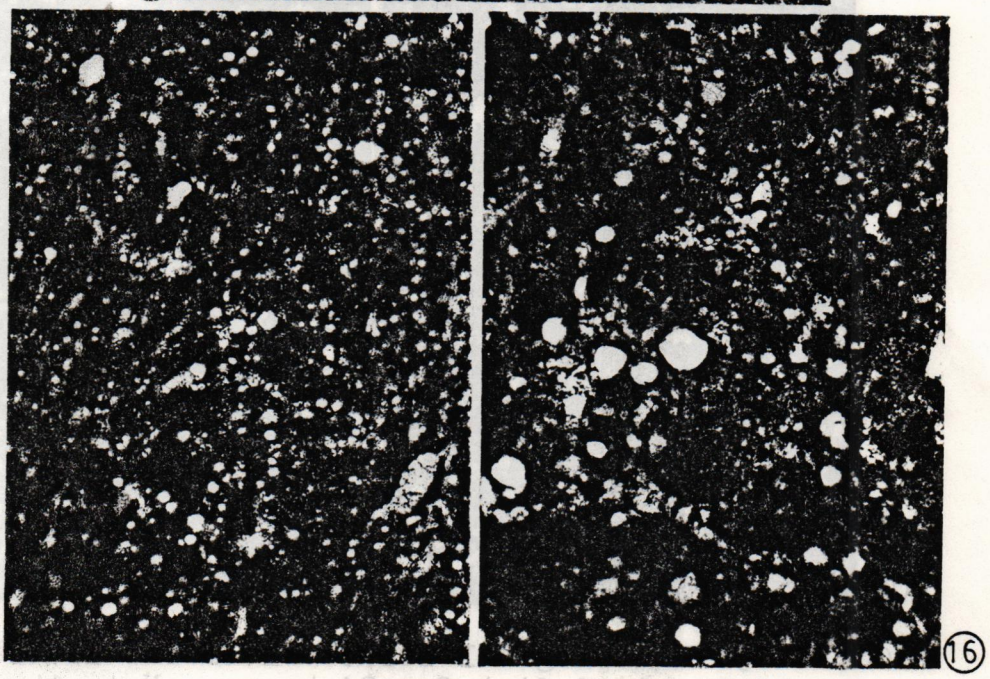


FIGS.10-13: Histological structure of rat liver fed on aldrin-mixed diet for 24 hours. Note the disturbed lobular structure (10) necrotic areas (10-12), extensive vacuolation (10-13) and hypertrophied cells and nuclei (12-13).

Stain: Hematoxylin and Eosin.

Magnifications: 10, x50; 11, x100; 12-13, x250.





FIGS.14-16: Histological structure of rat liver fed on aldrin-mixed diet for 48 hours. Note the alteration in hepatic lobular structure (14), hypertrophied cells, large number of vacuoles, certain clear areas and many large rounded nuclei (15-16).

Stain: Hematoxylin and Eosin.

Magnifications: 10, x50; 11, x100; 12-13, x250.

3.1.4. HISTOLOGICAL STRUCTURE OF LIVER

Table VI and Figure 6 show the effect of aldrin feeding on the various histological parameters of male albino rat livers. The size of hepatic cell, its nucleus and nucleolus increases tremendously after insecticide treatment for a total period of 48 hours. A typical hepatic cell measures $279.79 \pm 14.24\ \mu^2$ (n = 90), while its nuclear size is $47.29 \pm 2.01\ \mu^2$ (n = 90) and nucleolar size is $2.94 \pm 0.26\ \mu^2$ (n = 90). After aldrin treatment for 24 hours and 48 hours, the hepatic cell size increases 33 and 41%, respectively, the nuclear size increases 44 and 31% respectively, while the nucleolus shows 88 and 79% increase (Table VI, Fig. 6). The number of nuclei/cell and number of nucleoli/nucleus remain unaltered.

That the hepatic cells and their nuclei and nucleoli hypertrophy after insecticide treatment is obvious from Figures 10-16. The insecticide exposure for 48 hours has much more prominent and drastic effect as compared to 24 hours treatment, comparison of Figures 9 (control) with 13 (24 hours) and 16 (48 hours) highlight the effect of insecticide. Nuclei are prominently vesicular with well defined nucleoli. The entire hepatic lobule is marked by extensive vacuolation, which is a typical sign of toxicity. In certain cases a part of the liver tissue was seen to develop signs of necrosis (Figs. 10, 11).

3.2. EFFECT OF ALDRIN MIXED DIET (8 mg/kg body weight/day ADMINISTERED FOR 15 DAYS

3.2.1. BODY WEIGHT AND LIVER WEIGHT

The total body weight of rabbit decreases after administration of aldrin at a dose of 8 mg/kg body weight

	0-day	Before Slaughtering
Control (n=6)	185.00±11.86[a]	214.42±12.1
3 day (n=4)	237.37±13.56	252.75±15.50
6 day (n=4)	168.75±6.84	188.37±7.03
9 day (n=4)	146.75±6.05	162.37±6.53
12 day (n=4)	161.75±12.31	183.37±13.78
15 day (n=4)	164.00±9.61	191.50±11.08

[a]Mean±SEM, Student's 't' test; *P< 0.

per day. Control rabbits generally show 1.99±0.15% gain in body weight per day. After three days of aldrin feeding the percent weight gain is 2.13±0.18 per day which decreases to 1.12% per day after 15 days of insecticide administration (Table VII). The body weight/liver weight ratio is 36.32±0.62 (n = 6), which decreases to 26.78±1.33 (n = 4) after three days of insecticide administration. The ratio further decreases to 26.27 after 6 days but starts increasing during prolonged aldrin feeding (Table VII). The liver wieght in terms of % body weight increases after insecticide treatment (Table VII).

3.2.2. HAEMATOLOGICAL STUDIES

Aldrin administered at a dose of 8 mg/kg body weight causes significant decrease in the haemoglobin content, RBC count, and PCV (Table VIII, Fig. 17). The haemoglobin content decrease from 13.04 ±0.16 g/dl (n = 6) to 12.06±0.19 g/dl (n = 4) within 6 days of aldrin treatment. It continues to do so till day 15, when the haemoglobin content are decreased 12%. The RBC count is affected within three days of aldrin feeding, when it shows 10% decrease. This count gradually decreases till day 15, when it is reduced by 22%. The PCV is likewise affected, but only after 9 days of aldrin feeding and continues to do so till day 15 (Table VIII, Fig. 17). The WBC on the other hand is conversely affected. A control rat contains 65.50± 5.20 X 10^2 cells/µl (n = 6), which increase 20% within three days of aldrin treatment. The WBC continues to increase till day 15, when it is 45% more than the control. The various other haematological parameters such as MCV and MCH are also significantly increased after

aldrin treatment for 15 days. The MCHC on the other hand decreases during this treatment. The decrease affected is generally 6-7% (Table VIII, Fig. 17).

3.2.3. BIOCHEMICAL ANALYSIS OF BLOOD

Table IX and Figure 18 show the effect of aldrin administered at a dose of 8 mg/kg body weight/day for 15 days on the various enzymatic activities of rat blood serum.

Activities of most of the enzymes tested such as AcP, Ap, amylase, SGOT, SGPT, LDH and CPK are increased after aldrin feeding for 15 days. Usually the increase in activity is accelerated with increasing duration of insecticide administration. Most of these enzymes are drastically affected within three days of aldrin feeding. The AP activity increases 94, 129, 240, 278 and 368% after 3, 6, 9, 12 and 15 days of insecticide feeding. The AcP activity likewise is increased 115, 194, 256, 142 and 105% after 3, 6, 9, 12 and 15 days of feeding. The control rats show 27.80±1.48 IU/l of SGOT and 22.39±1.60 IU/l of SGPT activity. After aldrin feeding the former activity shows an increase of 136, 143 and 195% after 9, 12 and 15 days of feeding, while the latter enzyme shows an increase of 88, 117, 73, 75 and 23% on day 3, 6, 9, 12 and 15 (Table IX, Fig. 18). From amongst dehydrogenases LDH activity increases 39, 49, 96, 103 and 115% after 3, 6, 9, 12 and 15 days of aldrin feeding while the ICDH activity is conversely decreased 41, 40, 11, 23 and 22% during the same observation periods. The CPK activity is also increased significantly after 9 days of aldrin feeding, when it shows 83% increase over the control activity (Table IX

C = CONTROL
3 = 3 DAYS ALDRIN FEEDING
6 = 6 DAYS ALDRIN FEEDING
9 = 9 DAYS ALDRIN FEEDING
12 = 12 DAYS ALDRIN FEEDING
15 = 15 DAYS ALDRIN FEEDING

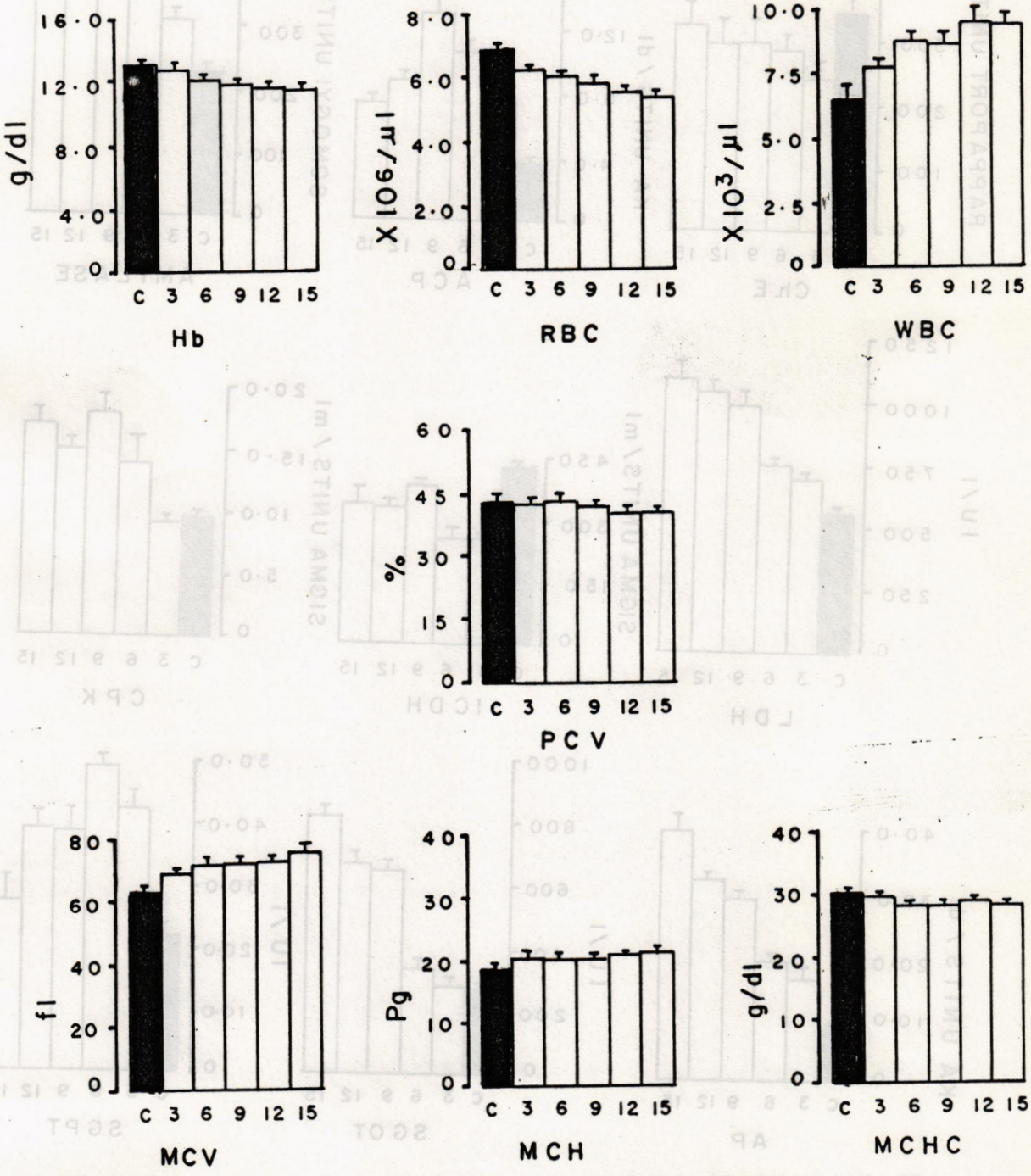


FIG. 17. Effect of feeding aldrin mixed diet (8 mg/kg body weight/day) for 15 days on the various haematological parameters of albino rats.

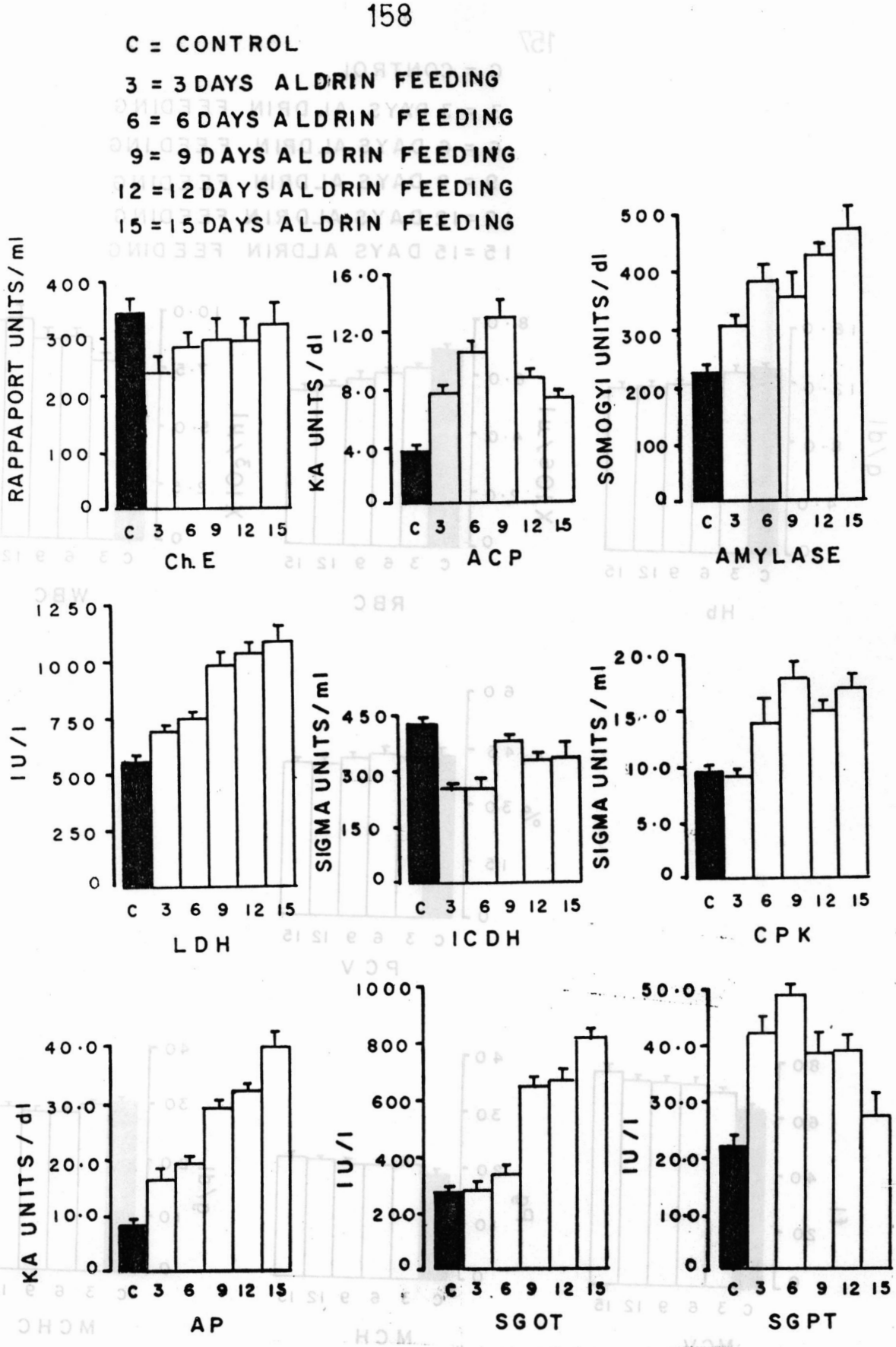


FIG.18. Effect of feeding aldrin mixed diet (8 mg/kg body weight/day) for 15 days on the activities of various enzymes of rat blood serum.

TABLE - VIII

EFFECT OF FEEDING ALDRIN MIXED DIET (8 mg/kg body weight/day) FOR 15 DAYS ON THE VARIOUS HAEMATOLOGICAL PARAMETERS OF ALBINO RATS.

Parameters	Control (n=6)	Aldrin feeding				
		3 days (n = 4)	6 days (n = 4)	9 days. (n = 4)	12 days (n = 4)	15 days (n = 4)
Hb (g/dl)	13.04±0.16[a]	12.71±0.19	12.06±0.19**	11.78±0.18***	11.63±0.33**	11.48±0.19***
RBC ($X10^5$ cells/µl)	69.10±1.20	62.38±0.95**	60.18±1.62**	58.25±1.37***	55.42±1.08***	53.75±19.01***
WBC ($X10^2$ cells/µl)	65.50±5.20	78.00±2.30.	88.50±3.23**	87.25±3.37**	96.00±4.87**	94.92±4.43**
PCV (%)	43.28±0.40	42.84±0.35	42.97±0.25	41.93±0.33*	40.17±0.43***	40.61±0.44**
MCV (fl)	62.64±0.46	68.70±0.47***	71.49±1.47***	72.07±1.15***	72.51±0.83***	75.70±1.94***
MCH (Pg)	18.88±0.28	20.29±0.04**	20.06±0.22*	20.23±0.18**	20.97±0.31**	21.39±0.34***
MCHC (g/dl)	30.15±0.36	29.54±0.23	28.06±0.29*	28.09±0.22**	28.94±0.58	28.27±0.23**

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

TABLE - IX

EFFECT OF FEEDING ALDRIN MIXED DIET (8 mg/kg body weight/day) FOR 15 DAYS ON THE ACTIVITIES OF VARIOUS ENZYMES OF RAT BLOOD SERUM

Parameters	Control (n = 7)	Aldrin feeding 3days (n=4)	6days (n=4)	9days (n=4)	12days (n=4)	15days (n=4)
AcP (KAU/dl)	3.63[a] ± 0.26	7.82*** ± 0.38	10.68*** ± 0.71	12.92*** ± 1.08	8.77*** ± 0.49	7.45*** ± 0.28
AP (KAU/dl)	8.57 ± 0.39	16.64** ± 2.14	19.58*** ± 1.60	29.15*** ± 1.32	32.36*** ± 2.59	40.09*** ± 2.78
Amylase (Somogyi u/dl)	226.76 ±14.45	309.23** ±18.86	384.61*** ±28.11	354.28* ±39.03	429.62*** ±21.80	473.91*** ±41.74
SGOT (IU/l)	27.80 ± 1.48	28.74 ± 1.65	34.12 ± 3.83	65.74*** ± 2.87	67.49*** ± 2.63	81.99*** ± 2.87
SGPT (IU/l)	22.39 ± 1.60	41.99*** ± 3.16	48.59*** ± 1.87	38.66** ± 4.06	39.11** ± 2.91	27.47 ± 3.89
LDH (IU/l)	506.56 ±21.91	702.48*** ±17.53	754.32*** ±25.85	990.96*** ±50.55	1029.24*** ±38.51	1090.20*** ±56.24
ICDH (Sigma units/ml)	428.76 ±12.93	254.36*** ± 9.25	256.18*** ±18.18	380.18* ±15.48	328.66*** ±15.76	335.96* ±34.69
CPK (Sigma units/ml)	9 9.66 ± 0.60	9.12 ± 0.55	13.81 ± 2.37	17.67*** ± 1.10	14.76** ± 1.15	11.87 ± 1.11
ChE (Rappaport U/ml)	34.77 ± 2.34	24.00* ± 3.06	28.72 ± 2.56	29.62 ± 3.74	29.25 ± 4.17	32.21 ± 4.59

[a] Mean±SEM; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

C = CONTROL
3 = 3 DAYS ALDRIN FEEDING
6 = 6 DAYS ALDRIN FEEDING
9 = 9 DAYS ALDRIN FEEDING
12 = 12 DAYS ALDRIN FEEDING
15 = 15 DAYS ALDRIN FEEDING

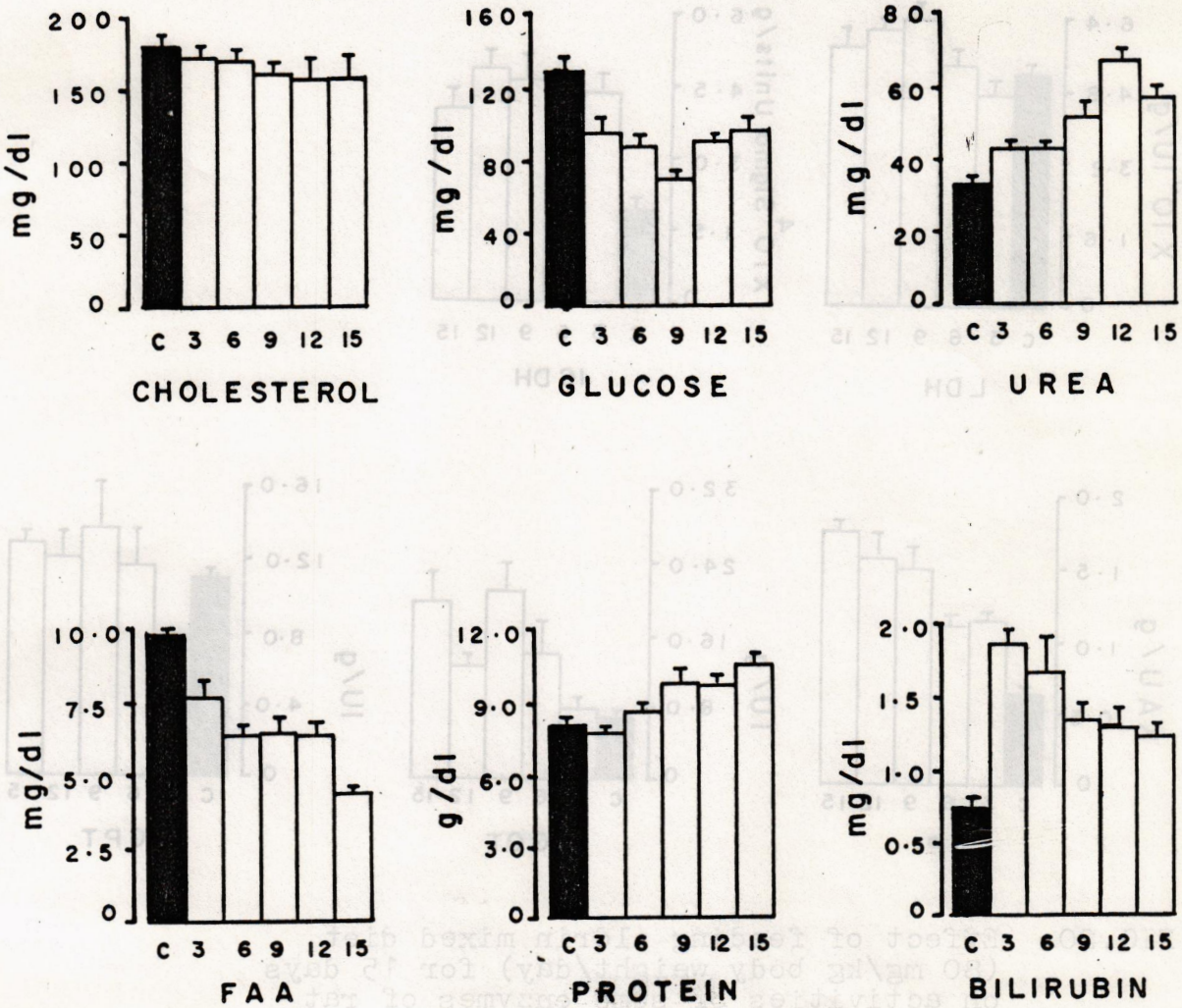


FIG.19. Effect of feeding aldrin mixed diet (80 mg/kg body weight/day) for 15 days on the various biochemical parameters of rat blood serum.

C = CONTROL
3 = 3 DAYS ALDRIN FEEDING
6 = 6 DAYS ALDRIN FEEDING
9 = 9 DAYS ALDRIN FEEDING
12 = 12 DAYS ALDRIN FEEDING
15 = 15 DAYS ALDRIN FEEDING

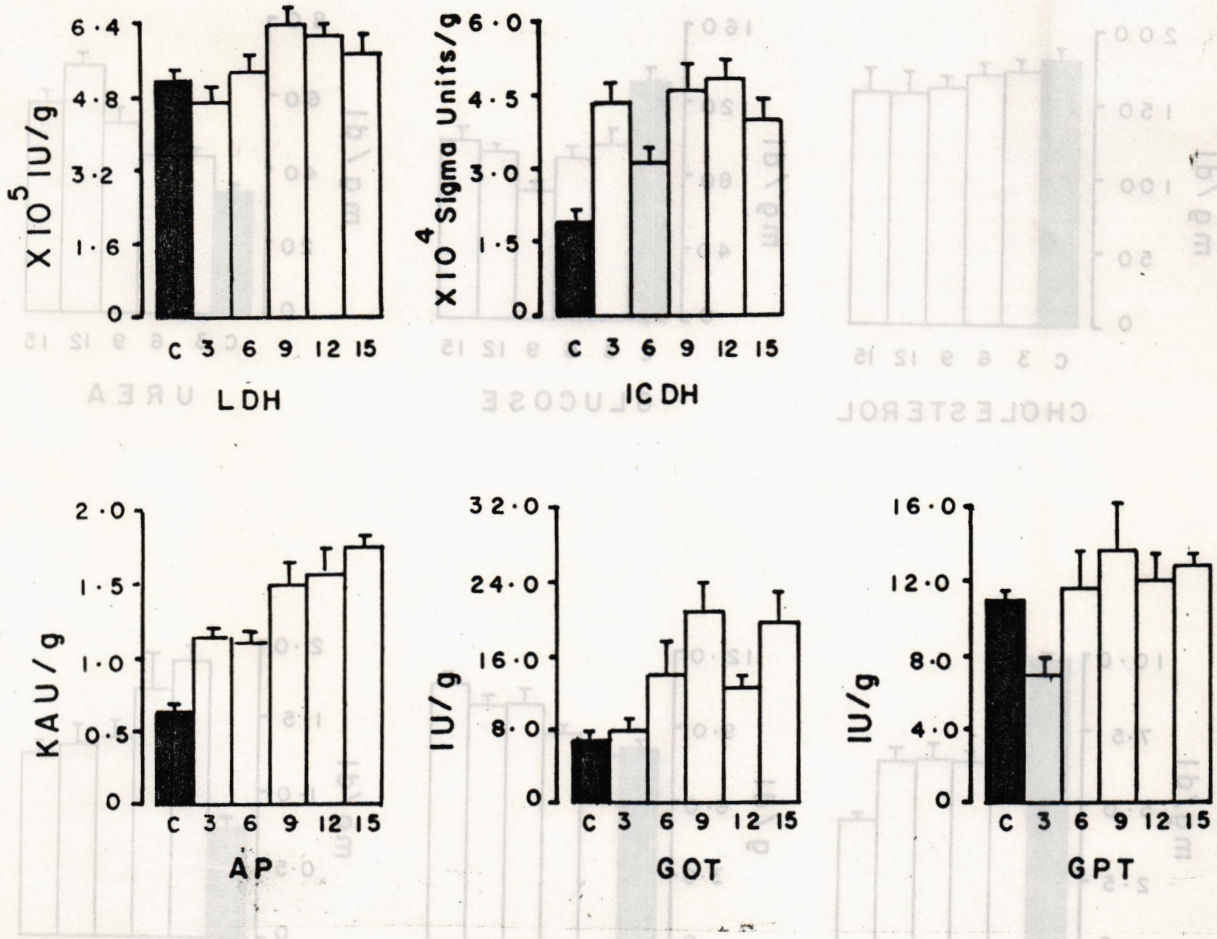


FIG.20. Effect of feeding aldrin mixed diet (80 mg/kg body weight/day) for 15 days on activities of some enzymes of rat liver.

Bilirubin (mg/dl)	0.76±0.04[a]	1.87±0.11***	
Cholesterol (mg/dl)	180.73±7.16	172.38±7.37	1
Protein (g/dl)	8.04±0.21	7.64±0.16	
FAA (mg/dl)	9.81±0.14	7.68±0.53**	
Glucose (mg/dl)	129.60±7.08	94.61±8.85*	
Urea (mg/dl)	33.56±1.19	42.99±2.36***	

[a]Mean ± SEM, Student's 't' test; *P< 0.

Fig.18). The amylase activity is likewise increased, which is affected within three days of insecticide feeding. The increase is 36, 70, 56, 90 and 109% after 3, 6, 9, 12 and 15 days of insecticide feeding.

Cholinesterase is the only enzyme, which is least affected by aldrin feeding. Except for first 3 day observation, when ChE activity is decreased 31%, all other deviations were non significant.

Table X and Figure 19 shows the effect of aldrin feeding on the various biochemical components of blood serum other than enzymes. A control rat shows 0.76±0.04 mg/100 ml (n = 7) bilirubin content, which increase 147, 121, 77, 71 and 64% after 3, 6, 9, 12 and 15 days of insecticide treatment. The urea content are likewise increased. The increase is 28, 27, 53, 102 and 70% during the same observation period. The blood serum proteins increase, but only after 9 days of aldrin feeding, when it is 22% more than the control. After 15 days of aldrin feeding the protein content increased 31%. The FAA content are highly sensitive and decrease 22, 36, 34, 35 and 56% after 3, 6, 9, 12 and 15 days of aldrin treatment. The glucose content also decrease (Table X, Fig. 19). The cholesterol content, however, remain unaltered.

3.2.4. BIOCHEMICAL ANALYSIS OF LIVER

Most of the hepatic enzymatic activities (Fig.20, Table XI) and other biochemical components (Fig. 21, Table XII) are significantly affected after oral administration of aldrin at a dose of 8 mg/kg body weight/ day for 15 day.

AP (KAU/g)	0.64±0.02[a]	1.15±0.05***	1
GOT (IU/g)	7.34±0.17	8.34±0.78	14
GPT (IU/g)	11.07±0.25	6.98±0.94**	11
ICDH ($X10^3$ sigma u/g)	20.10±0.50	44.33±3.17***	31
LDH ($X10^4$ IU/g)	51.90±1.20	47.21±2.77	53

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

From amongst enzymes, the AP and ICDH activities are increased within three days of insecticide feeding. The hepatic AP activity shows an increase of 80, 73, 133, 144 and 172%, while the ICDH activity shows an increase of 121, 56, 132, 142 and 100% after 3, 6, 9, 12, and 15 days of aldrin feeding in both cases. The LDH activity is affected only after 9 days of aldrin feeding, when the activity is 23% more than the control activity. The GOT activity is likewise significantly affected after 9 days of feeding. It shows 186, 76 and 171% increase after 9, 12 and 15 days of insecticide feeding. The GPT activity however, generally remains unaffected except for 3 day feeding observation time, when this activity show about 37% increase over the control values (Table XI, Fig. 20).

Table XII and Fig. 21 show effect of aldrin feeding on the various biochemical components of liver other than enzymes. The hepatic cholesterol content decrease significantly. The decrease after 3, 6, 9, 12 and 15 days of insecticide feeding is 66, 49, 65, 64 and 72%, respectively. The glucose content, on the other hand, increase after aldrin feeding. The increase is 52, 78, 80, 98 and 143% after 3, 6, 9, 12 and 15 days of insecticide feeding.

The soluble proteins do not show any significant change after insecticide treatment. A 14-16% increase after 15 days of treatment is statistically non-significant. The total proteins, on the other hand, after initial decrease (19%) during the first three days gradually increase to 9, 19, 28 and 33% on day 6, 9,12, and 15 of insecticide feeding. The FAA do not show any change until day 9, when they increase 53%. After 15

C = CONTROL
3 = 3 DAYS ALDRIN FEEDING
6 = 6 DAYS ALDRIN FEEDING
9 = 9 DAYS ALDRIN FEEDING
12 = 12 DAYS ALDRIN FEEDING
15 = 15 DAYS ALDRIN FEEDING

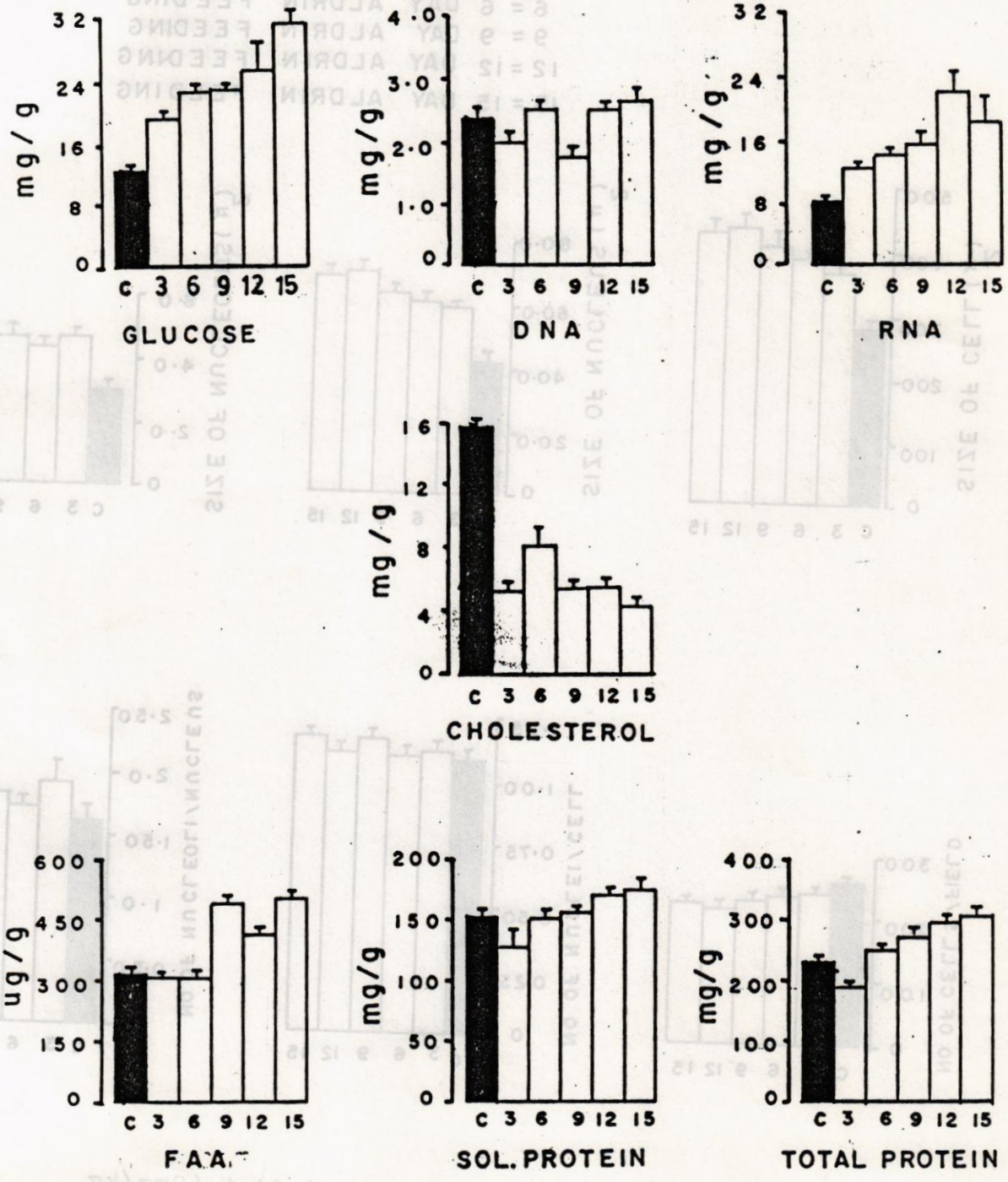


FIG.21. Effect of feeding aldrin mixed diet (8 mg/kg body weight/day) for 15 days on the various biochemical components of rat liver.

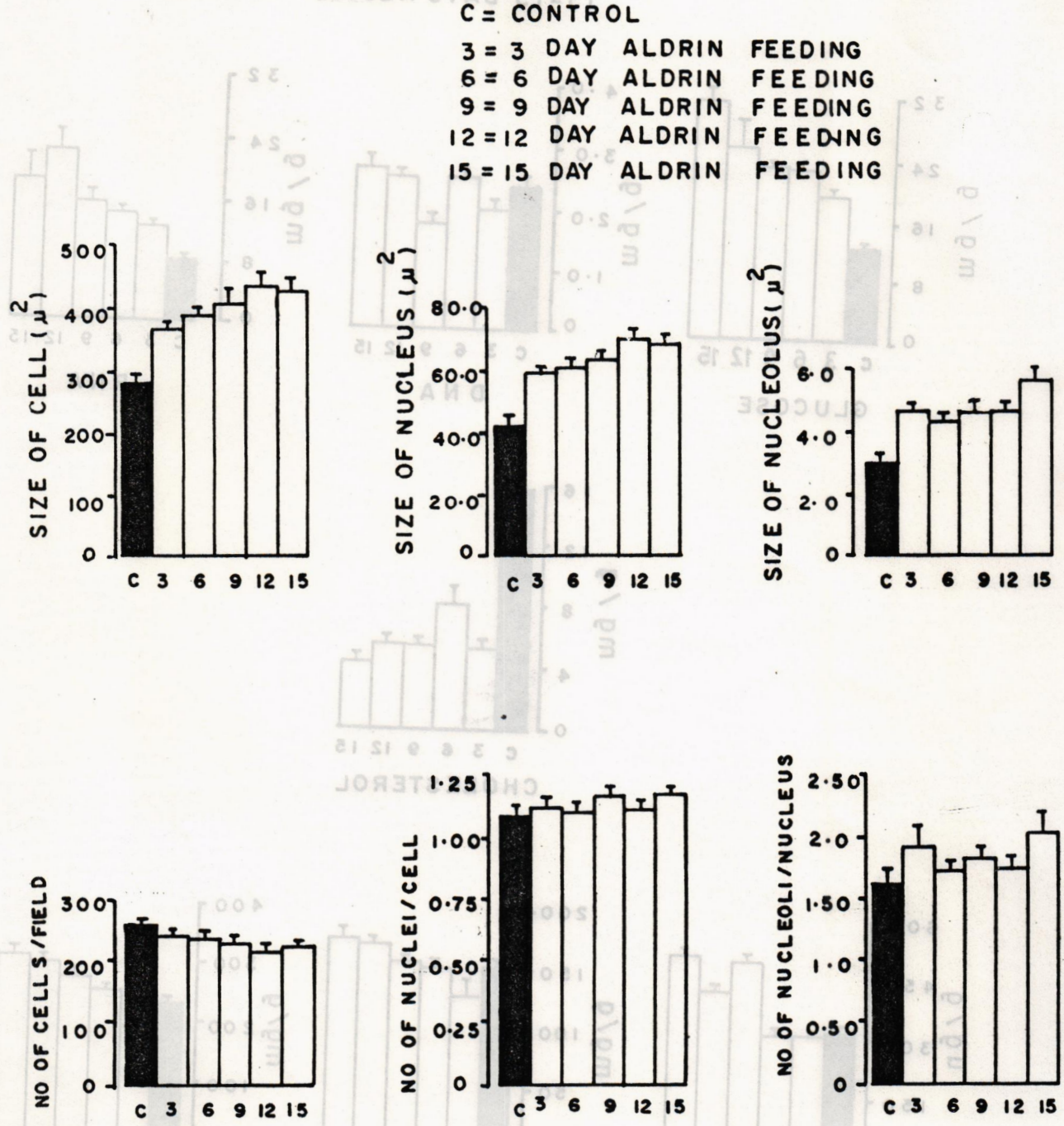


FIG.22. Effect of feeding mixed diet (8mg/kg body weight/day) for 15 days on the various histological parameters of albino rat liver.

TABLE - XII

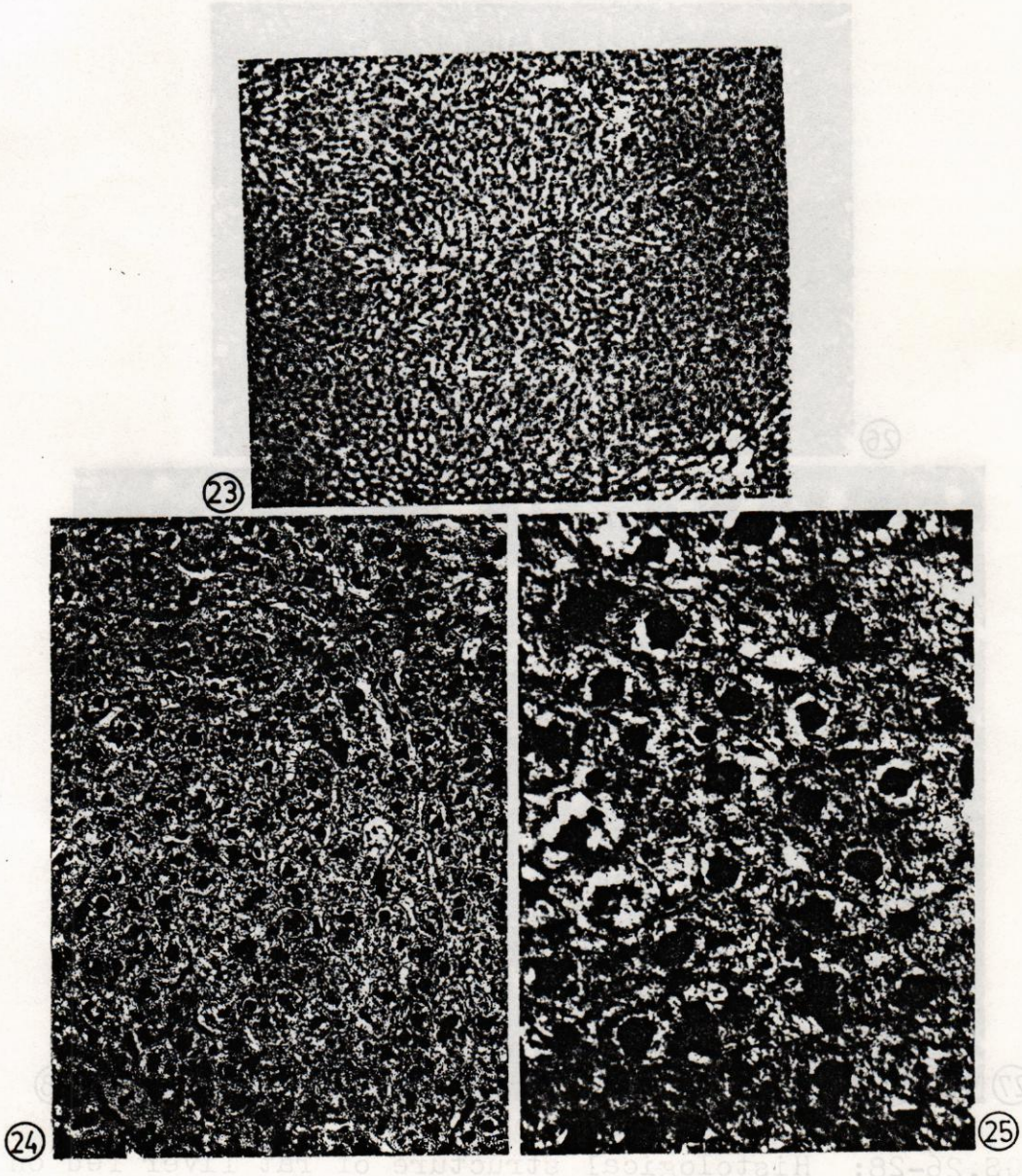
EFFECT OF FEEDING ALDRIN MIXED DIET (8 mg/kg body weight/day) FOR 15 DAYS ON THE VARIOUS BIOCHEMICAL COMPONENTS OF RAT LIVER.

Parameters	Control (n = 5)	Aldrin feeding				
		3 days (n = 4)	6 days (n = 4)	9 days (n = 4)	12 days (n = 4)	15 days (n = 4)
Cholesterol (mg/g)	15.72±0.35[a]	5.32±0.51***	8.09±1.40**	5.44±0.44***	5.63±0.48***	4.41±0.37***
Glucose (mg/g)	12.93±0.61	19.65±1.03***	22.99±0.32***	23.24±0.41***	25.60±3.43	31.41±1.43
FAA (µg/g)	319.95±12.68	312.86±11.61	312.36±13.66	489.37±20.75***	410.71±13.08**	503.18±13.35***
Soluble protein (mg/g)	152.99±5.62	127.52±14.95	151.33±4.85	156.69±3.09	170.62±5.80	174.51±8.40
Total protein (mg/g)	233.67±7.45	190.40±11.36*	253.85±5.74	278.82±7.66**	297.88±12.75**	311.27±11.60***
DNA (mg/gm)	2.42±0.17	1.99±0.16	2.52±0.14	2.47±0.14	2.53±0.06	2.65±0.21
RNA (mg/g)	8.62±0.38	12.93±0.75**	14.41±0.60***	16.52±1.32***	22.24±2.40***	18.42±3.31*

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

No.of cells/ microscopid field	257.54[a] ± 9.78	240.5 ± 8.0
No.of Nuclei/ cell	1.09 ± 0.03	1.1 ± 0.0
No.of nucleoli/ nucleus	1.63 ± 0.10	1.9 ± 0.1
Size of cell (u^2).	281.03 ± 9.62	369.7** ± 7.4
Size of nucleolus(μ^2)	3.09 ± 0.22	4.7* ± 0.3
Size of nucleus(μ^2)	42.51 ± 1.79	59.1** ± 1.7

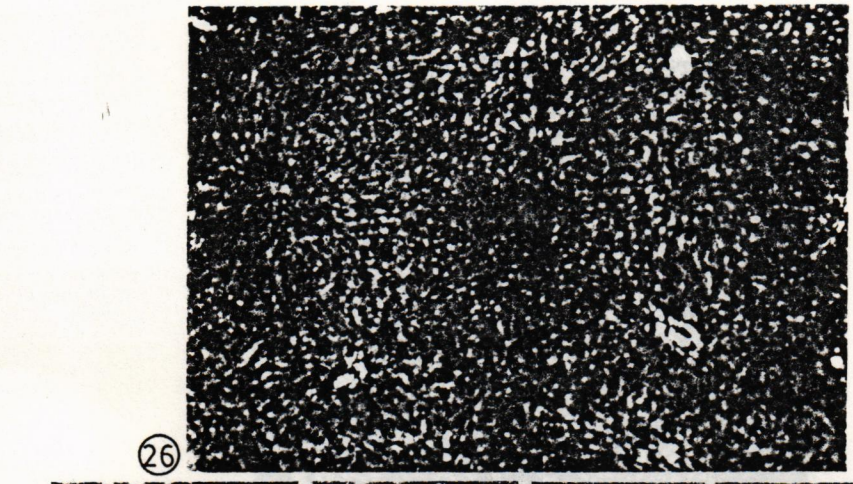
[a] Mean±SEM, Student's 't' test; *



FIGS.23-25:Histological structure of normal rat liver
Note the structure of normal hepatic lobule with portal areas (23),arrangement of hepatic cords (23-24) with cells and nuclei (24-25).

Stain: Hematoxylin and Eosin.

Magnifications: 23, x25; 24, x100; 25, x250.



(26)

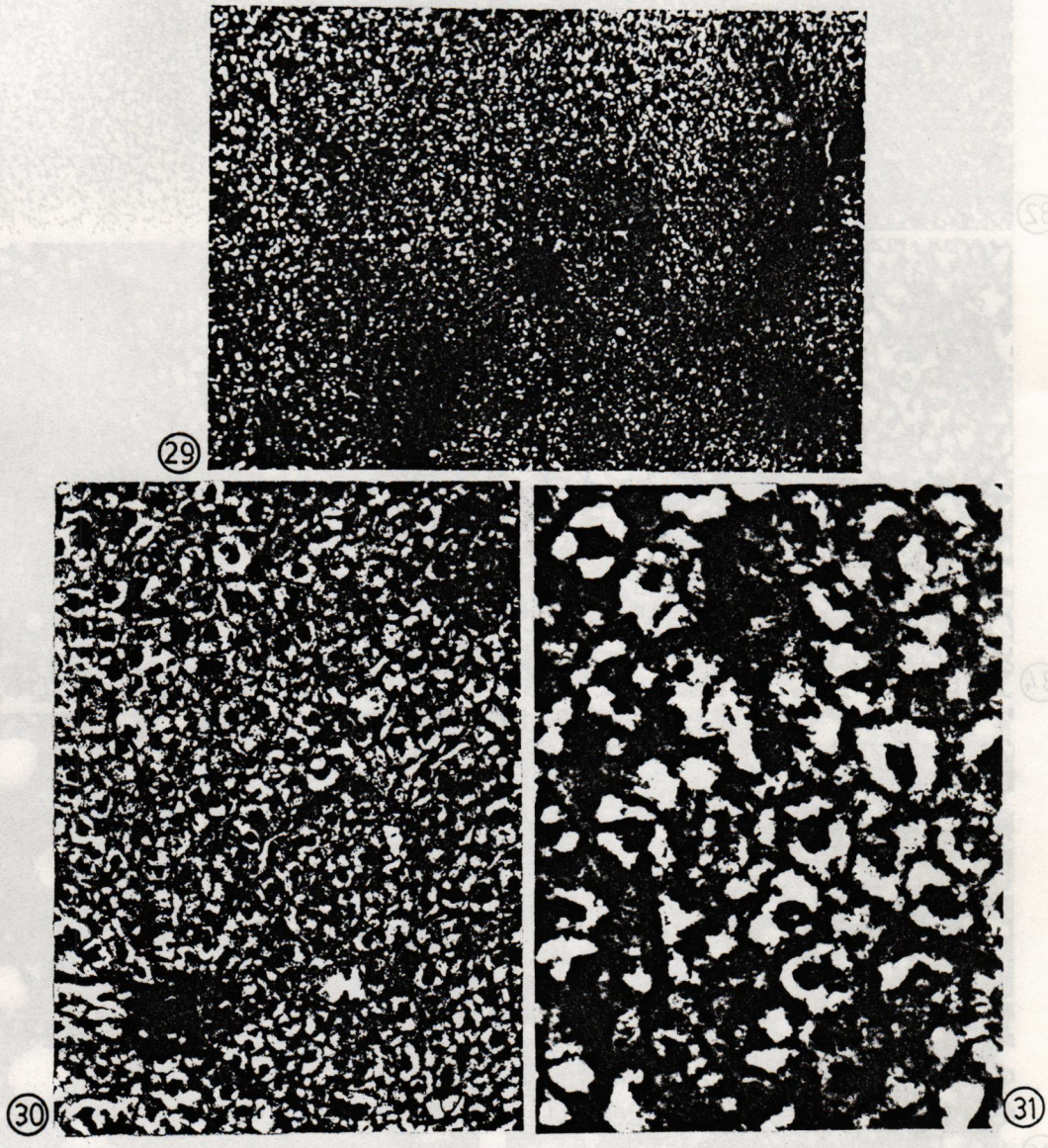
(27)

(28)

FIGS. 26-28: Histological structure of rat liver fed on aldrin-mixed diet for 3 days. Note the lobule with increased simusoidal space,(26) scattered irregular clear areas (27-28) and many binucleated cells(28).

Stain: Hematoxylin and Eosin

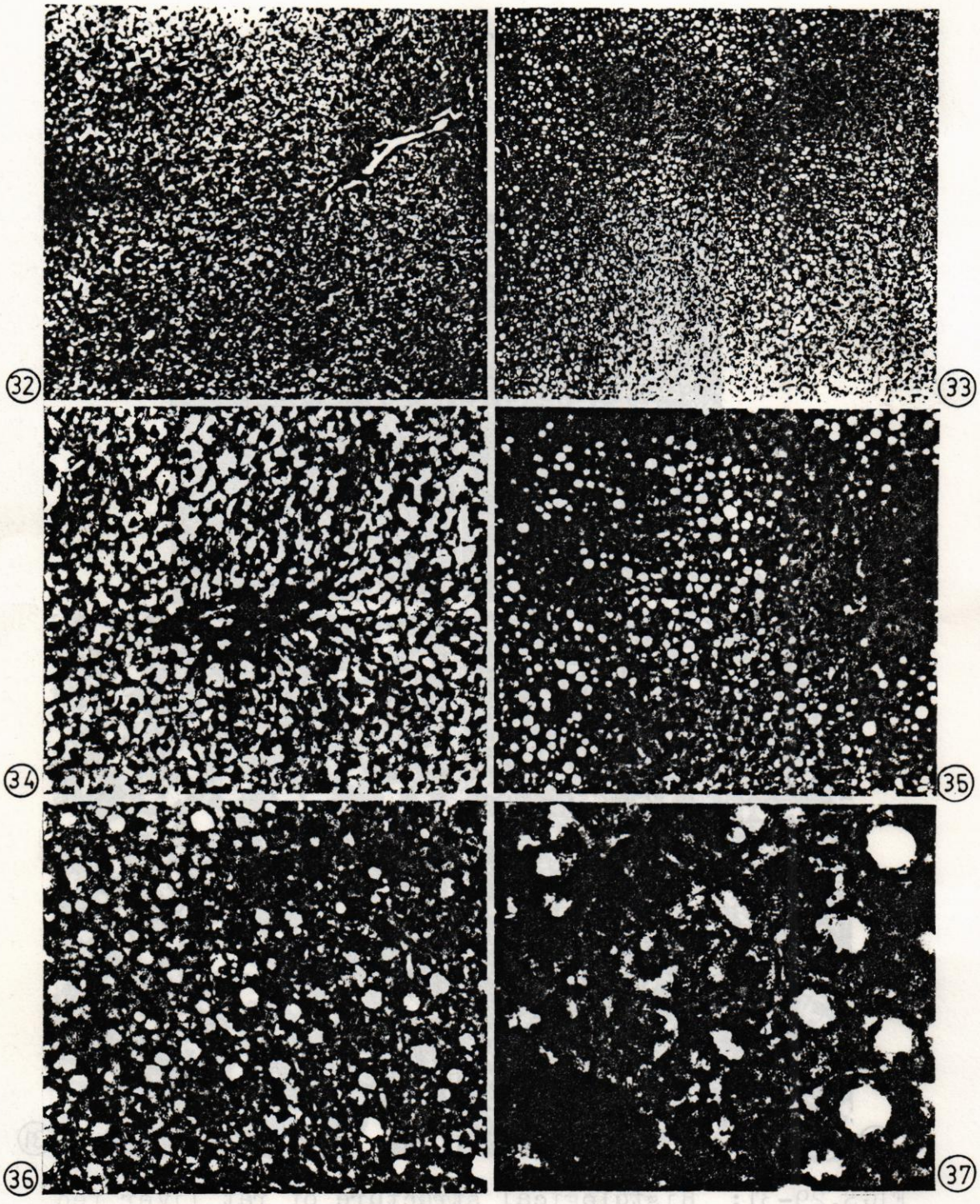
Magnifications: 26, x25; 27, x100; 28, x250.



FIGS.29-31: Histological structure of rat liver fed on aldrin-mixed diet for 6 days. Note extensive cytoplasmic vacuolation, cytoplasmic margination (29-31), irregular shape of nuclei (31) and hypertrophied cells (30-31).

Stain: Hematoxylin and Eosin

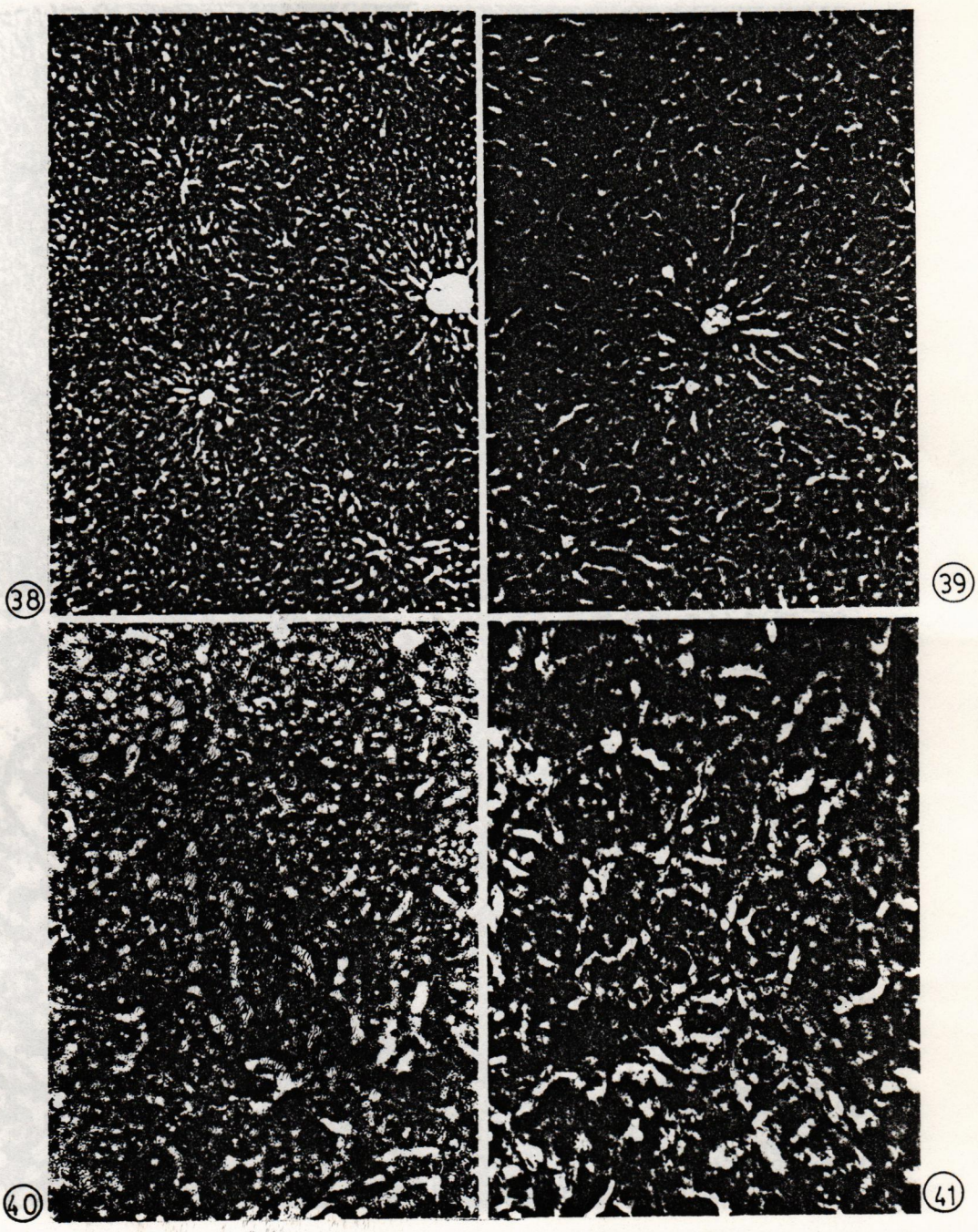
Magnifications: 29, x25; 30, x100; 31, x250.



FIGS. 32-37: Histological structure of rat liver fed on aldrin-mixed diet for 9 days. Note cytoplasmic vacuolation and margination (32,34), large number of rounded clears areas (vacuolation) in the tissue (33,35-37), hypertrophied cells (34, 36-37) and irregular shape nuclei (34).

Stain: Hematoxylin and Eosin

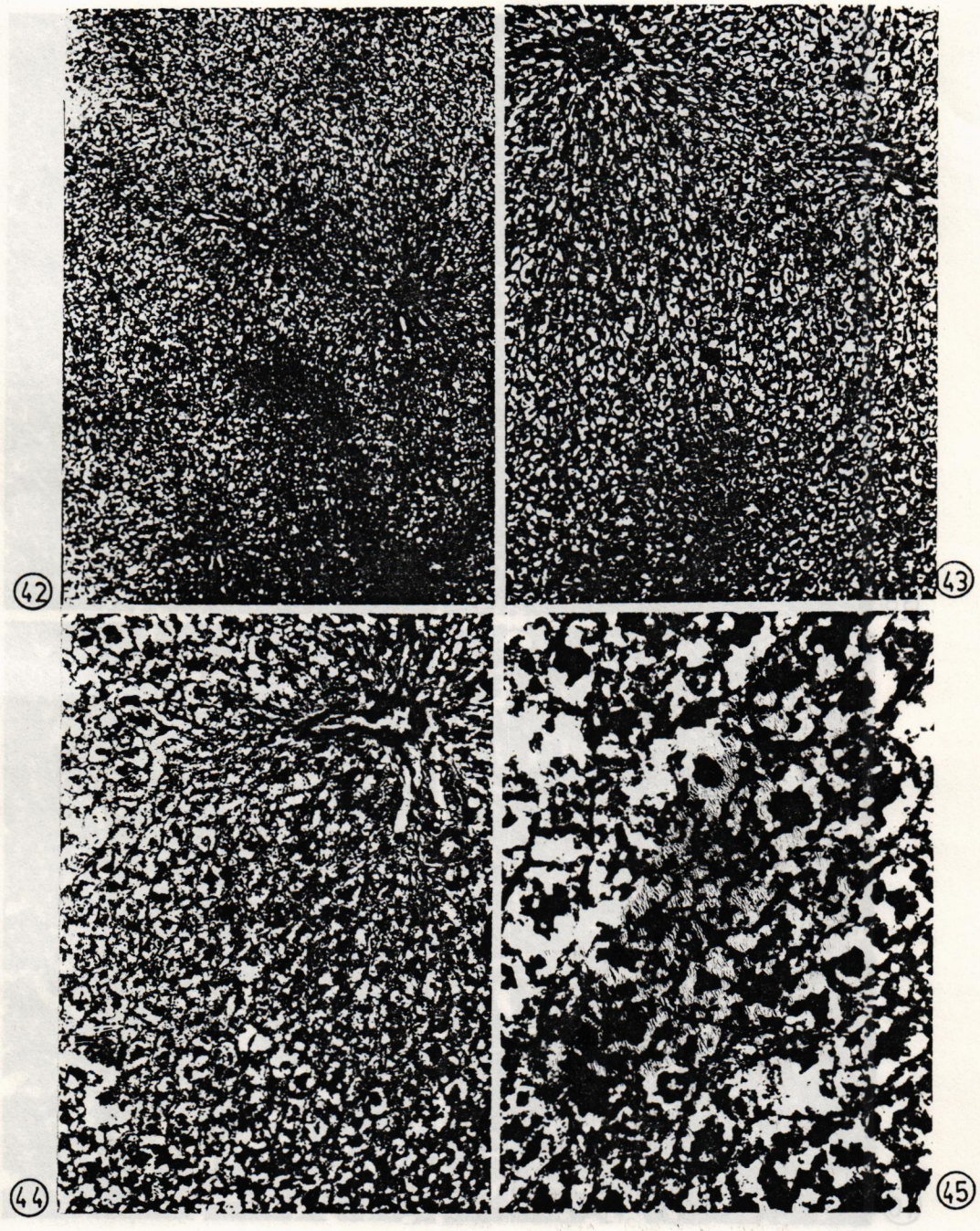
Magnifications: 32-33, x25; 34-35, x50; 36, x100; 37, x 250.



FIGS. 38-41: Histological structure of rat liver fed on aldrin-mixed diet for 12 days. Note the compactness of hepatic tissues with slight increase in sinusoidal spaces (38, 39), irregular and rod shape clear areas, hypertrophied cells and nuclei (40, 41).

Stain: Hematoxylin and Eosin

Magnifications: 38, x25; 39, x50; 40, x100; 41, x250.



FIGS.42-45: Histological structure of rat liver fed on aldrin-mixed diet for 15 days. Note the cytoplasmic vacuolation (42-45), hypertrophied cells and irregular shaped nuclei (43-45).

Stain: Hematoxylin and Eosin.

Magnifications: 42,x25; 43,x50; 44,x100; 45, x250.

days of aldrin feeding the FAA content increase 57%.

The DNA content remain unaltered, while the RNA content increase 50, 67, 92, 158 and 114% after 3, 6, 9, 12 and 15 days of aldrin feeding (Table XII, Fig. 21).

3.2.5. HISTOLOGICAL STRUCTURE OF LIVER

Table XIII and Figure 22 show the effect of aldrin on the various histological parameters of albino rats liver. The hepatic cells, their nuclei and nucleoli are significantly hypertrophied. The degree of hypertrophy increases with the duration of administration of insecticide. The number of hepatic cells/microscopical field are accordingly decreased. The number of nuclei/cell and number of nucleoli/ nucleus remain unchanged under the influence of aldrin feeding except that the number of nucleoli/ nucleus increase 25% after 15 days of aldrin feeding.

That the hepatic cells hypertrophy, and that the hepatic nuclei and nucleoli are also enlarged is abundantly clear from Figures 26-45. The nuclei are vesicular during the first three days of feeding (Fig. 28), but are converted into condensed bodies during prolonged feeding (Fig. 37 and 45). The hepatic structure of 9 day aldrin feeding is marked by extensive vacuolation (Figs. 34-36). Most of the hepatic cells at higher doses have extensive granulation with cytoplasm (Fig. 45).

3.3. EFFECT OF ALDRIN MIXED DIET (2.5 mg/kg body weight/day ADMINISTERED FOR A PERIOD OF 6-18 MONTHS

3.3.1. BODY WEIGHT AND LIVER WEIGHT

The body wt./liver wt. ratio decreases when animals are fed on aldrin mixed diet for a period of 12 months. Eighteen months of feeding results in about 22% decrease in the body weight/liver weight ratio (Table XIV).

The liver weight when considered in terms of % of body weight is increased after insecticide treatment. This increase is 8% after 12 months and 11% after 18 months of aldrin feeding.

3.3.2. HAEMATOLOGICAL STUDIES

Table XV and Figure 46 show the effect of long term feeding of aldrin on the various haematological parameters of male albino rats. The haemoglobin content, RBC count and PCV decreases after long term feeding. The haemoglobin content, decrease from 29-30%, the RBC count decrease from 15-18%, while PCV show 5-9% decrease (Table XV). The WBC count, just as in other typical insecticide treatment exposure, on the other hand increases 5-11% (Table XV; Fig. 46). The figure 46 shows a distinct increase in MCV and MCH and a distinct decrease in MCHC after aldrin treatment. The deviation of MCHC is however not significant (Table XV).

TABLE - XIV

EFFECT OF FEEDING ALDRIN MIXED DIET (2.5 mg/kg body weight/day) FOR A PERIOD 6-18 MONTHS ON THE TOTAL BODY AND LIVER WEIGHT OF ALBINO RAT.

Parameters	6 months aldrin feeding experiment		12 months aldrin feeding experiment		18 months aldrin feeding experiment	
	Control (n = 6)	Aldrin fed (n = 3)	Control (n = 4)	Aldrin fed (n = 3)	Control (n = 6)	Aldrin fed (n = 3)
Body wt/ liver wt. ratio	39.27±0.73[a]	39.21±0.10	37.95±0.92	35.21±0.40	48.21±0.40	38.29±2.87*
Liver weight (% body weight)	2.55±0.05	2.55±0.01	2.64±0.07	2.84±0.03*	2.37±0.08	2.64±0.19

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

TABLE - XV

EFFECT OF FEEDING ALDRIN MIXED DIET (2.5 mg/kg body weight/day) FOR A PERIOD OF 6-18 MONTHS ON THE VARIOUS HAEMATOLOGICAL PARAMETERS OF ALBINO RATS.

Parameters	6 months aldrin feeding exp.		12 months aldrin feeding exp.		18 months aldrin feeding exp.	
	Control (n=6)	Aldrin fed (n=4)	Control (n=4)	Aldrin fed (n=3)	Control (n=6)	Aldrin fed (n=4)
Hb (g/dl)	13.79[a] ± 0.32	11.88** ± 0.29	13.14 ± 0.23	11.90* ± 0.21	13.04 ± 0.16	11.70** ± 0.22
RBC ($X10^6$ cells/µl)	70.87 ± 1.27	57.85*** ±0.98	70.48 ± 1.28	59.40*** ± 1.00	69.10 ± 1.20	58.92*** ± 1.60
WBC ($X10^3$ cells/µl)	66.50 ± 3.99	69.87 ± 1.13	62.38 ± 1.34	69.20* ± 1.80	65.50 ± 5.20	70.25 ± 3.19
PCV (%)	45.92 ± 0.44	41.84*** ± 0.24	42.90 ± 0.20	40.64** ± 0.37	43.28 ± 0.40	40.09*** ± 0.21
MCV (fl)	64.38 ± 0.65	72.37*** ± 0.87	60.91 ± 0.86	68.43*** ± 0.64	62.64 ± 0.46	68.15** ± 1.49
MCH (Pg)	19.45 ± 0.32	20.52* ± 0.15	18.64 ± 0.06	20.04** ± 0.05	18.88 ± 0.28	19.87* ± 0.19
MCHC (g/dl)	30.24 ± 0.65	28.37 ± 0.56	30.62 ± 0.41	29.28 ± 0.26	30.15 ± 0.36	29.18 ± 0.38

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

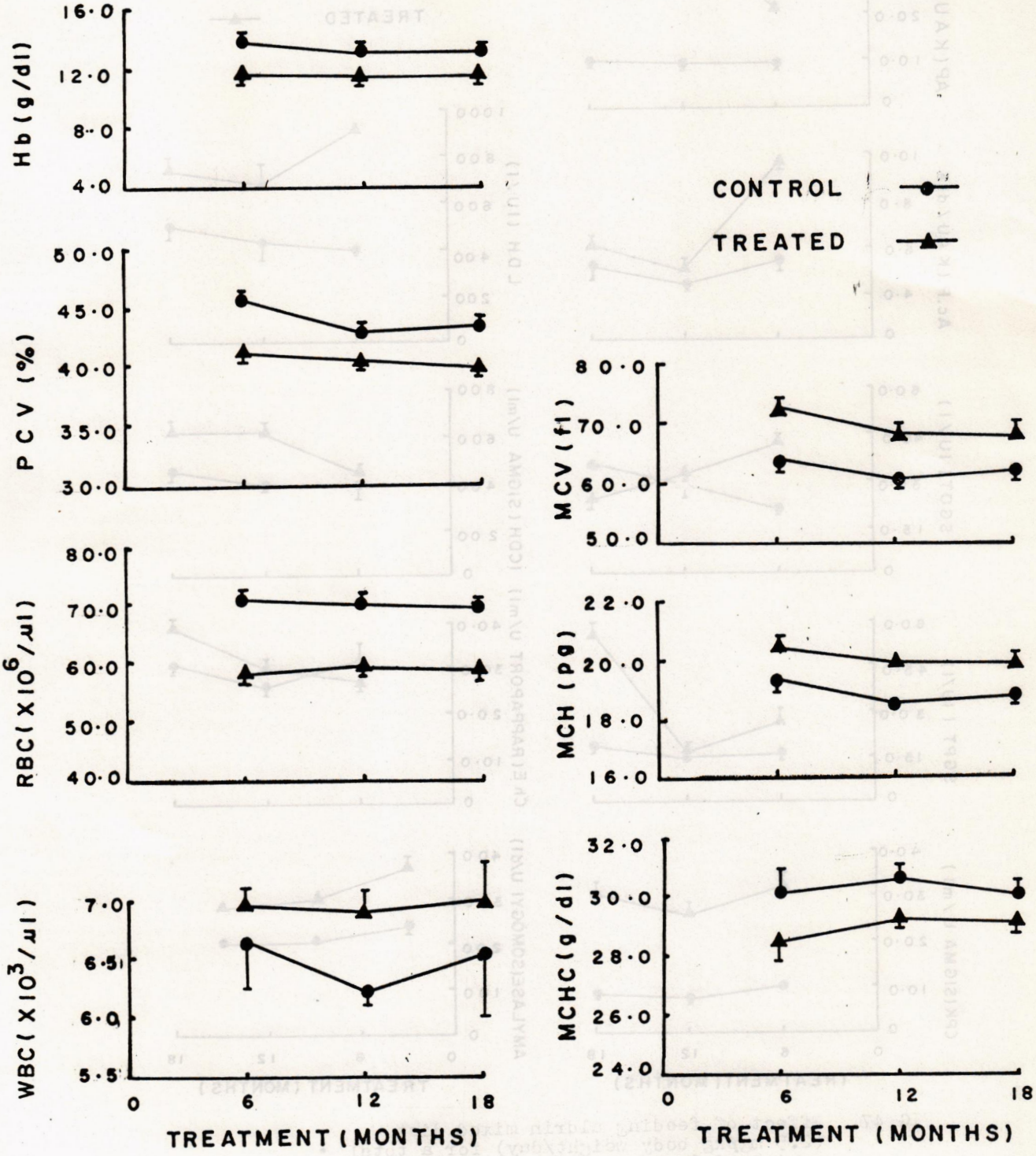


FIG.46. Effect of feeding aldrin mixed diet (2.5 mg/kg body weight/day) for a period of 6-18 months on the various haematological parameters of albino rats.

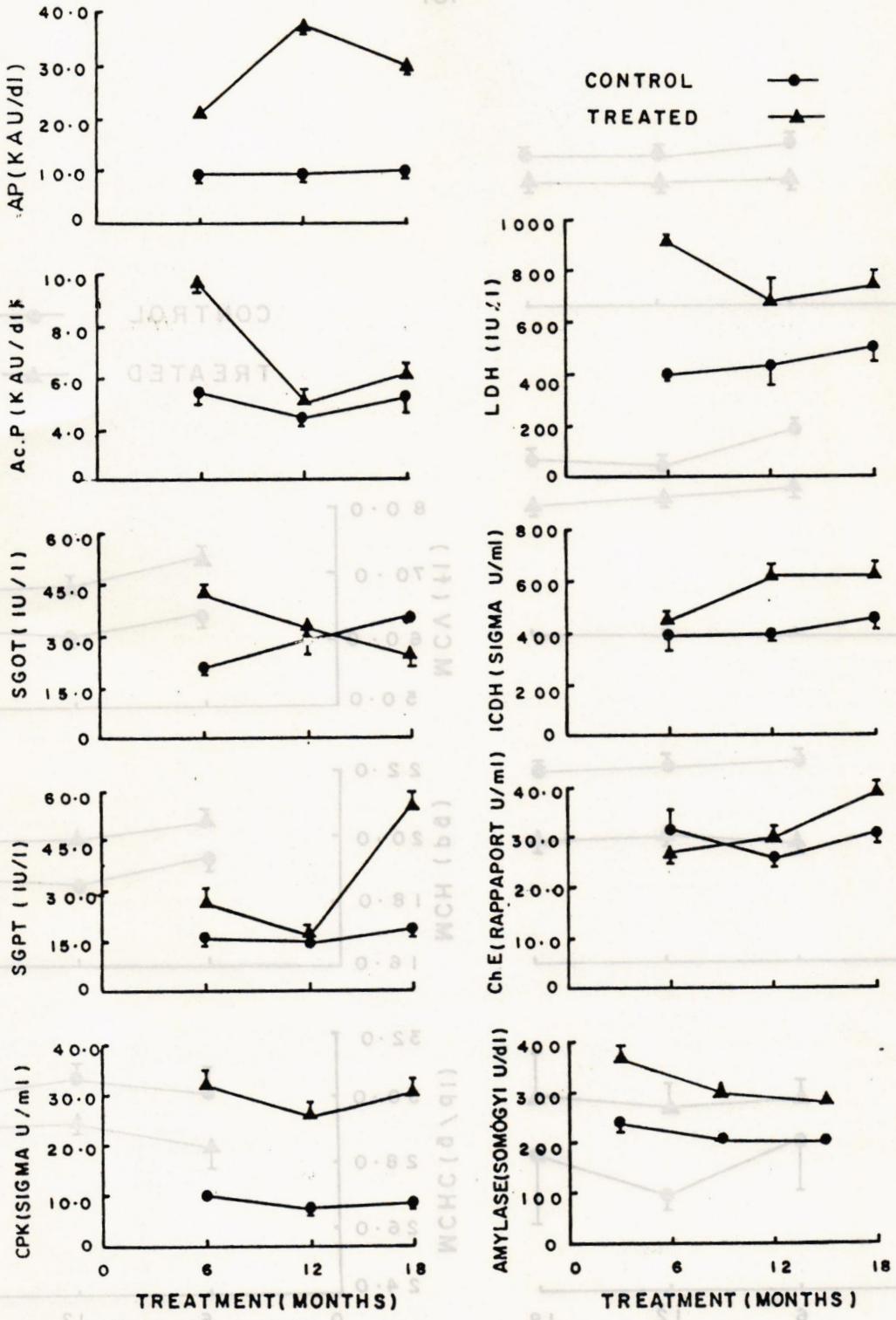


FIG.47. Effect of feeding aldrin mixed diet (2.5 mg/kg body weight/day) for a total period of 6-18 months on the various enzymes activities in albino rat blood serum.

3.3.3. BIOCHEMICAL ANALYSIS OF BLOOD

Table XVI and Figure 47 depict the effect of aldrin mixed diet administered for a period of 6-18 months on the various enzymes in blood. From amongst two phosphatases, AP was found to be the most sensitive one. This enzymes shows 2.28, 4.00 and 2.93 fold increase after 6, 12 and 18 months of aldrin feeding. The AcP, on the other hand, shows 97% increase, but does not show any significant deviation after prolonged feeding for 12 and 18 months.

The SGPT activity is not significantly altered during first 12 months of feeding, but after 18 months of aldrin feeding, the SGPT activity increases 197%. The SGOT activity, on the other hand, shows significant increase of 102 and 47% after 6 and 12 months of aldrin administration, but shows 32% decrease during 18 months of aldrin feeding. LDH, ICDH, CPK and amylase activities are also drastically increased after aldrin feeding. As is obvious from Table XVI and Figure 47, the LDH activity shows 129, 59 and 46.5% increase over the control; ICDH activity shows 15, 57 and 33.5% increase over the control, and amylase activity shows 52, 8.5 and 40% increase over the control after 6, 12 and 18 months of aldrin feeding. The cholinesterase activity is not significantly altered during the first 12 months of feeding, but is raised 27% after 18 months of aldrin feeding.

From amongst the several other biochemical components tested the glucose, urea and protein contents increases, while free amino acids show significant decrease when compared with the control blood

TABLE - XVI

EFFECT OF FEEDING ALDRIN MIXED DIET (2.5 mg/kg body weight/day) FOR A TOTAL PERIOD OF 6-18 MONTHS ON THE VARIOUS ENZYME ACTIVITIES IN ALBINO RAT BLOOD SERUM.

Parameters	6 months aldrin feeding exp.		12 months aldrin feeding exp.		18 months aldrin feeding exp.	
	Control (n=4)	Aldrin fed (n=6	Control (n=4)	Aldrin fed (n=3)	Control (n=6)	Aldrin fed (n=4)
AP (KAU/dl)	9.57[a] ± 0.39	21.85*** ± 0.67	9.46 ± 0.68	37.92*** ± 0.99	10,21 ± 0.92	29.90*** ± 1.04
AcP (KAU/dl)	5.46 ± 0.48	9.77*** ± 0.38	4.48 ± 0.25	4.99 ± 0.30	5.34 ± 0.59	6.09 ± 0.43
Amylase (Somogyi u/dl)	242.53 ±16,74	368.57** ±22,43	210.29 ±10.57	288.11* ±15.82	202.03 ±12,22	283.48* ±23.65
ChE (Rapp-aport u/ml)	31.80 ± 3.76	27.17 ± 0.79	26.00 ± 1.85	29.83 ± 1.74	31.08 ± 1.82	39.35* ± 1.88
CPK (Sigma u/ml)	10.30 ± 0.77	21.92*** ± 2.64	7.57 ± 0.61	26.00*** ± 0.52	8.75 ± 0.97	30,62*** ± 1.86
GPT (IU/l)	17.72 ± 1.40	26.64 ± 4.14	14.82 ± 0.99	16.66 ± 0.66	18.75 ± 1.87	55.68*** ± 2.76
ICDH (Sigma u/ml)	391.86 ±28.33	449.46 ±10.25	395.22 ±10.33	618.77*** ±11.16	462.53 ±24.13	617.22** ±23.03
LDH (IU/l)	401.44 ±10.87	917.40*** ±21.37	442.20 ±49.62	692.73* ±43.55	509.49 ±27.82	746.64*** ±29.68
GOT (IU/l)	21.02 ± 1.46	42.49*** ± 1.35	28.98 ± 2.73	42.66** ± 1.30	35.66 ± 2.55	24.31** ± 0.94

[a] Mean±SEM, Student's 't' test;

*P < 0.05; **P < 0.01; ***P < 0.001

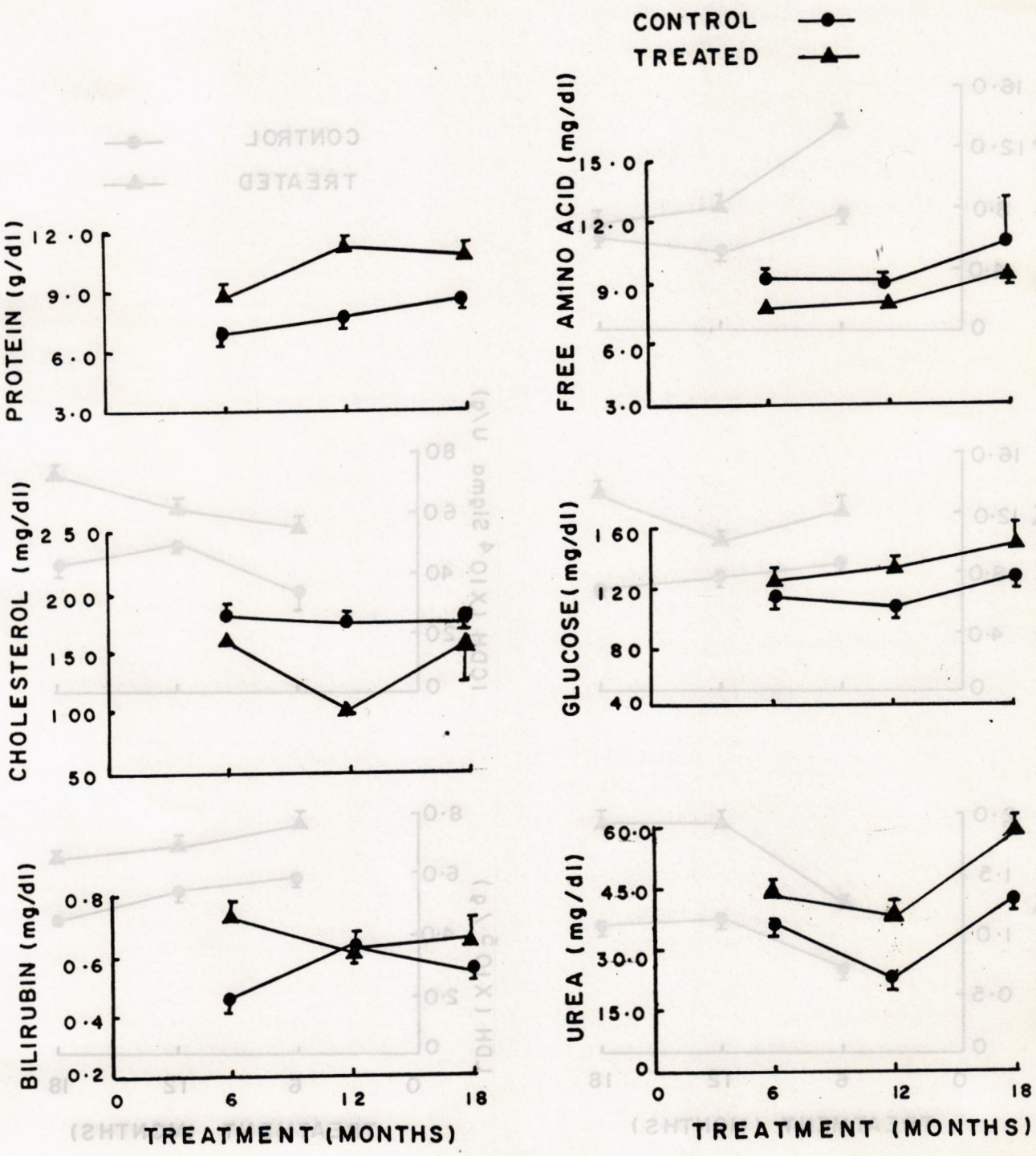


FIG.48: Effect of feeding aldrin mixed diet (2.5 mg/kg body weight/day) for a period of 6-18 months on the various biochemical components of albino rat serum.

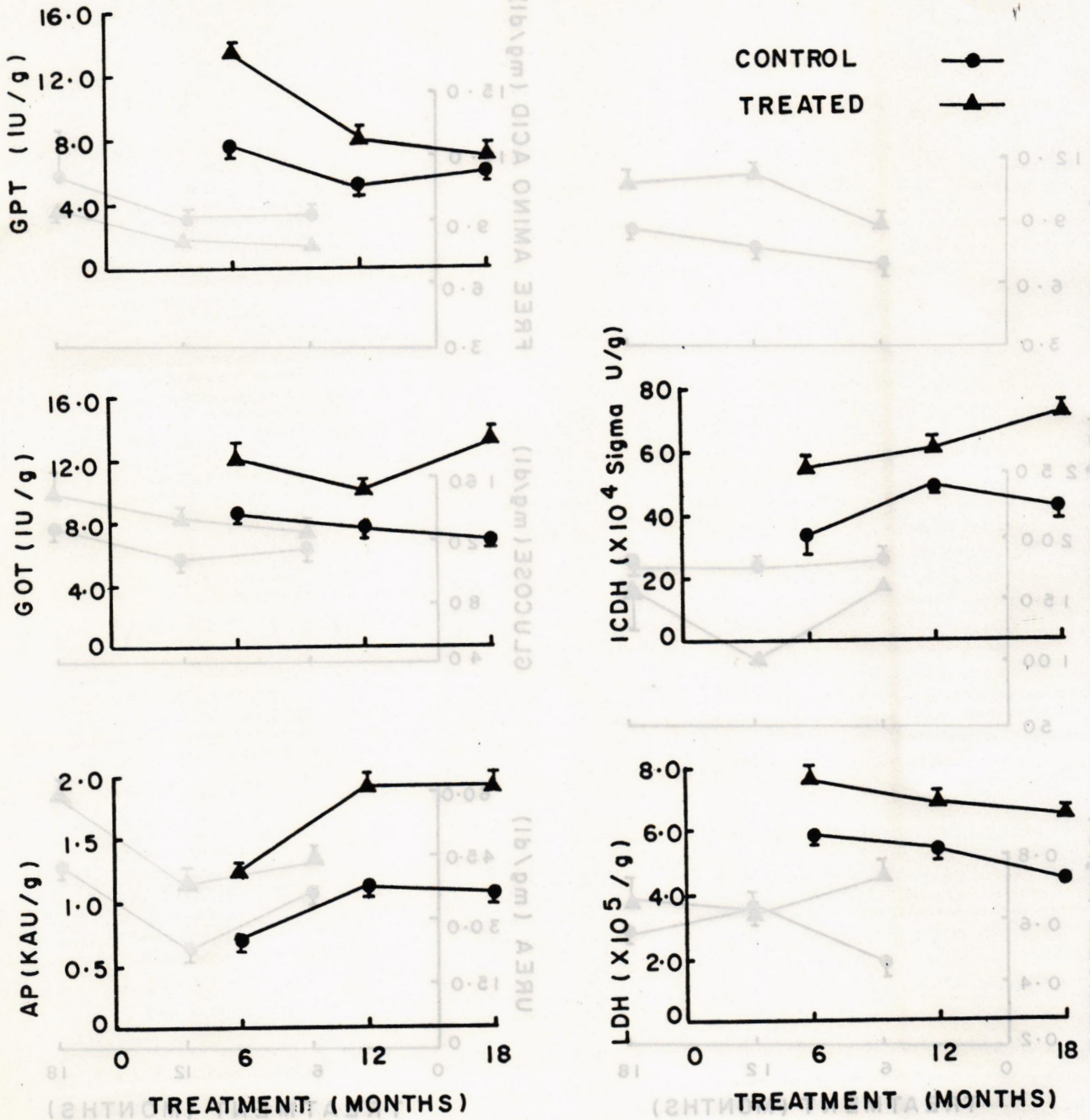


FIG.49. Effect of aldrin mixed diet (2.5 mg/kg body weight/day) for a period of 6-18 months on the activities of various hepatic enzymes in albino rats.

serum (Table XVII, Fig. 48). The bilirubin content decrease by 37% during the first 6 months of feeding, but are then unchanged during subsequent prolonged feeding. The cholesterol content, on the other hand, are significantly lowered after aldrin feeding. (Table XVII, Fig.48).

3.3.4. BIOCHEMICAL ANALYSIS OF LIVER

Figure 49 and Table XVIII shows effect of long term feeding of aldrin on the hepatic enzymes, while Figure 50 and Table XIX shows effect on hepatic biochemical components, other than enzymes. The various hepatic enzymes viz. AP, GOT, GPT, LDH and ICDH are raised after long term administration of insecticide. The increase in AP activity is 68, 71 and 76% after 6, 12 and 18 months of insecticide administration. The GOT activity is increased 44, 38 and 92%, while the GPT activity is increased 72, 62 and 16% when compared with their control activities. The LDH and ICDH activities behave in the like manner. The LDH activity shows 31, 27 and 45% increase, while ICDH activity increases 68, 23 and 71% after 6, 12 and 18 months of aldrin feeding (Table XVIII, Fig.49).

The changes induced by long term feeding of aldrin on the various biochemical components of rat liver, other than enzymes, are shown in Figure 50 and Table XIX. The soluble protein content are increased after insecticide feeding. This increase is 36, 33 and 17% after 6, 12 and 18 months of feeding. The total proteins decrease 27% during the first 6 months, but shows 41 and 6% increase during the rest of the

TABLE - XVII

EFFECT OF FEEDING ALDRIN MIXED DIET (2.5 mg/kg body weight/day) FOR A PERIOD OF 6-18 MONTHS ON THE VARIOUS BIOCHEMICAL COMPONENTS OF ALBINO RAT BLOOD SERUM.

Parameters	6 months aldrin feeding exp.		12 months aldrin feeding exp.		18 months aldrin feeding exp.	
	Control (n=6)	Aldrin fed (n=4)	Control (n=4)	Aldrin fed (n=3)	Control (n=6)	Aldrin fed (n=4)
Bilirubin (mg/dl)	0.74 ± 0.05	0.47** ± 0.05	0.63 ± 0.03	0.63 ± 0.04	0.56 ± 0.04	0.66 ± 0.07
Cholesterol (mg/dl)	182.95 ± 6.25	161.54* ± 6.18	177.19 ± 5.21	104.66*** ± 5.15	180.12 ± 9.80	165.24** ± 5.07
Free amino acids (mg/dl)	9.27 ± 0.34	7.86** ± 0.17	9.00 ± 0.36	7.89* ± 0.07	11.09 ± 2.33	9.30 ± 0.23
Glucose (mg/dl)	114.76 ± 8.52	123.68 ± 5.85	107.45 ± 7.26	132.84 ± 7.04	150.57 ± 3.67	128.63 ±15.35
Protein (g/dl)	6.90 ± 0.37	8.75** ± 0.25	7.68 ± 0.21	11.07*** ± 0.33	8.58 ± 0.45	10.59** ± 0.16
Urea (mg/dl)	36.34 ± 2.20	44.27* ± 1.53	23.55 ± 2.84	39.73* ± 0.91	41.15 ± 1.98	59.58** ± 3.32

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

TABLE - XVIII

EFFECT OF ALDRIN MIXED DIET (2.5 mg/kg body weight/day) FOR A PERIOD OF 6-18 MONTHS ON THE ACTIVITIES OF VARIOUS HEPATIC ENZYMES IN ALBINO RATS.

Parameters	6 months aldrin feeding exp.		12 months aldrin feeding exp.		18 months aldrin feeding exp.	
	Control (n=6)	Aldrin fed (n=3)	Control (t=4)	Aldrin fed (t=3)	Control (t=6)	Aldrin fed (t=4)
AP (KAU/g)	0.74 ± 0.05	1.24*** ± 0.03	1.12 ± 0.07	1.91*** ± 0.09	1.08 ± 0.08	1.90*** ± 0.12
GOT (IU/g)	8.66 ± 0.54	12.48** ± 0.92	7.56 ± 0.46	10.41** ± 0.34	6.79 ± 0.23	13.05*** ± 0.85
GPT (IU/g)	7.71 ± 0.68	13.29*** ± 0.46	5.02 ± 0.54	8.11** ± 0.43	6.05 ± 0.60	7.02 ± 0.49
ICDH ($X10^3$ SU/g)	33.29 ± 6.08	55.99** ± 2.57	49.49 ± 2.52	61.00** ± 0.91	42.50 ± 5.70	72.50** ± 1.77
LDH ($X10^4$ IU/g)	58.29 ± 2.99	76.40* ± 4.53	54.25 ± 3.37	68.60* ± 4.00	44.60 ± 0.30	64.66*** ± 1.79

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

TABLE - XIX

EFFECT OF FEEDING ALDRIN MIXED DIET (2.5mg/kg body weight/day) FOR A PERIOD OF 6-18 MONTHS ON THE VARIOUS BIOCHEMICAL COMPONENTS OF ALBINO RAT LIVER.

Parameters	6 months aldrin feeding exp.		12 months aldrin feeding exp.		18 months aldrin feeding exp.	
	Control (n=6)	Aldrin fed (n=3)	Control (n=4)	Aldrin fed (n=3)	Control (t=6)	Aldrin fed (t=4)
Cholesterol (mg/g)	5.97[a] ± 0.29	5.20 ± 0.80	9.01 ± 0.32	12.86*** ± 1.21	10.50 ± 1.21	9.65** ± 0.21
Free amino acids (ug/g)	217.29 ± 7.70	171.19*** ± 4.01	197.98 ±14.18	170.12 ±11.62	226.70 ±13.24	203.33 ±10.98
Glucose (mg/g)	30.87 ± 2.32	19.11* ± 3.64	27.12 ± 1.37	21.78 ± 3.10	38.32 ± 3.38	18.46 ± 1.93
Soluble protein (mg/g)	124.92 ± 4.67	169.79** ± 7.94	135.22 ± 9.96	180.18** ± 1.73	159.00 ± 2.58	185.23** ± 5.41
Total protein (mg/g)	216.96 ±13.05	159.27** ± 4.10	208.48 ± 6.70	294.20** ±12.88	227.60 ±11.88	241.50 ±12.57
DNA (mg/g)	3.05 ± 0.19	2.90 ± 0.13	2.54 ± 0.14	2.33 ± 0.03	3.06 ± 0.12	2.65 ± 0.40
RNA (mg/g)	9.68 ± 0.62	4.52*** ± 0.37	9.39 ± 0.31	5.74 ± 0.42	11.30 ± 0.49	5.55*** ± 0.44

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

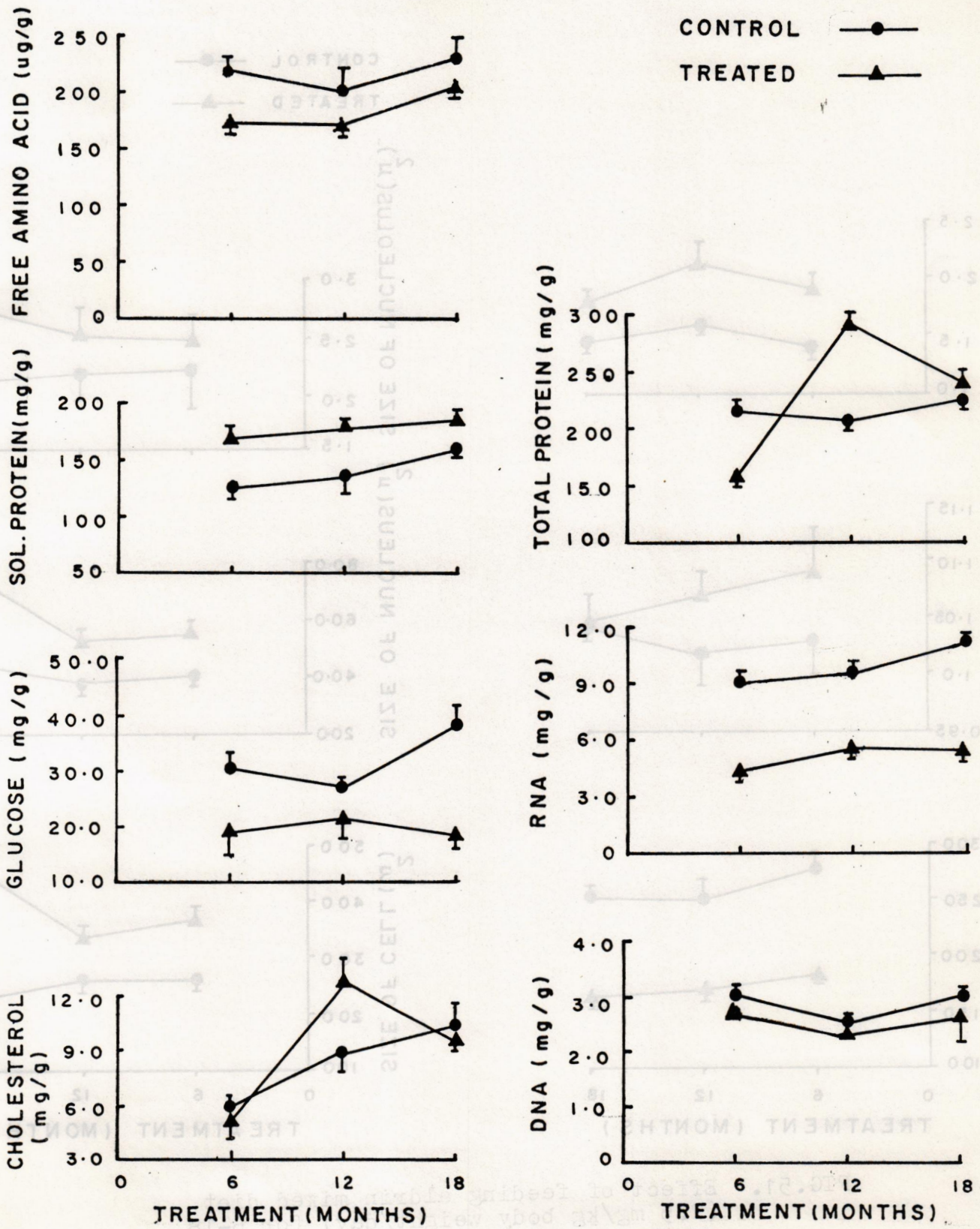


FIG. 50. Effect of feeding aldrin mixed diet (2.5 mg/kg body weight/day) for a period of 6-18 months on the various biochemical components of albino rat liver.

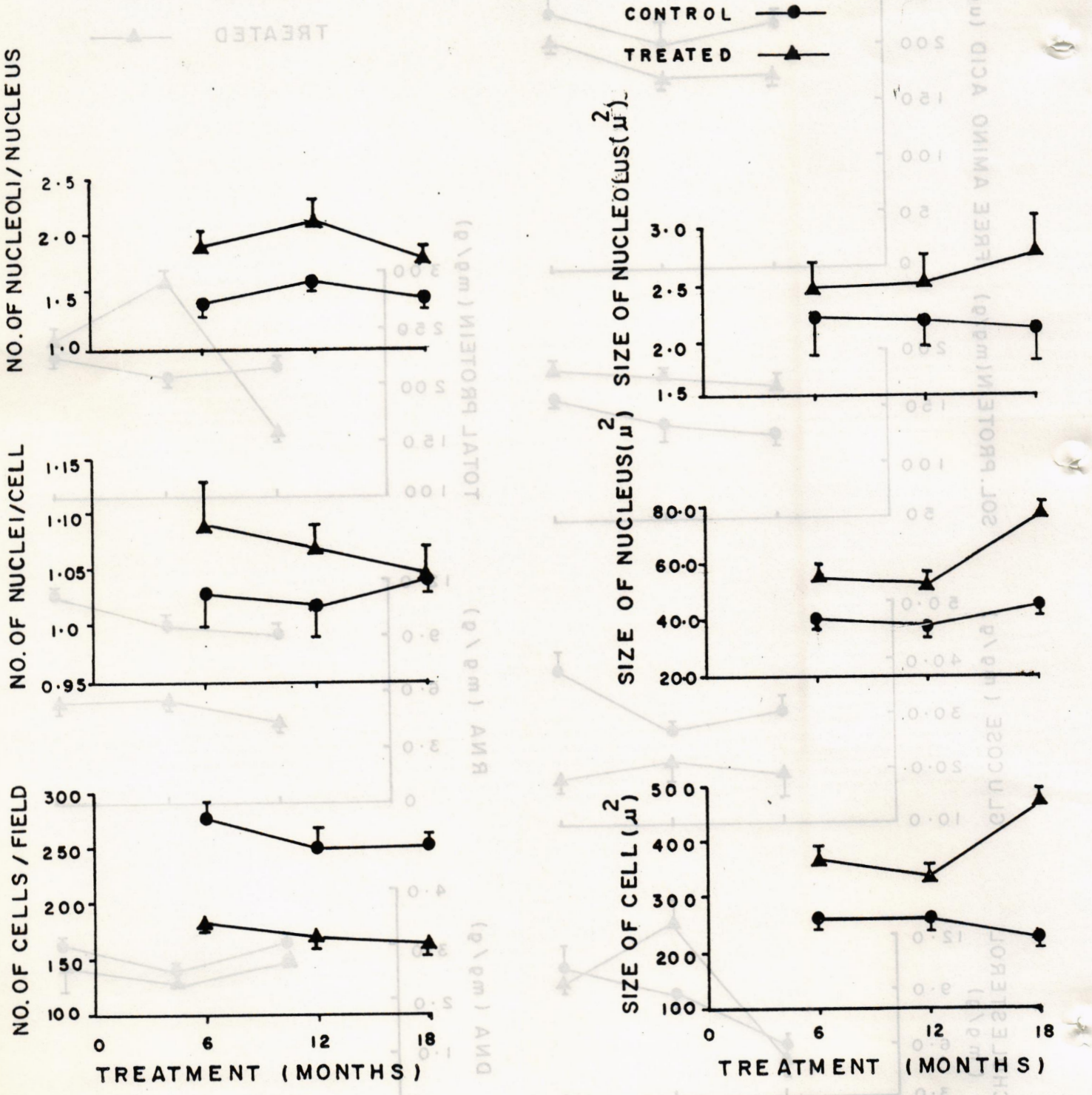


FIG.51. Effect of feeding aldrin mixed diet (2.5 mg/kg body weight/day) for 6-18 months on the various histological parameters of male albino rats.

prolonged administration of 12 and 18 months. The FAA contents conversely show a constant decrease of 21, 14 and 10% during this period. The glucose content likewise show consistant lower values in insecticide treated liver. This decrease is 38, 20 and 52%. The cholesterol content are not significantly affected during the first 6 months but show a prominent increase (43%) after 12 months of aldrin feeding (Table XIX, Fig. 50).

Both the nuclei acids are adversely affected. The DNA, although shows lower values in insecticide treated groups, but most of these values are not statistically significantly different. The RNA content are also decreased after insecticide feeding. This decrease is 53, 39 and 54% after 6, 12 and 18 months of aldrin feeding. (Table XIX, Fig.50).

3.3.5. HISTOLOGICAL STRUCTURE OF LIVER

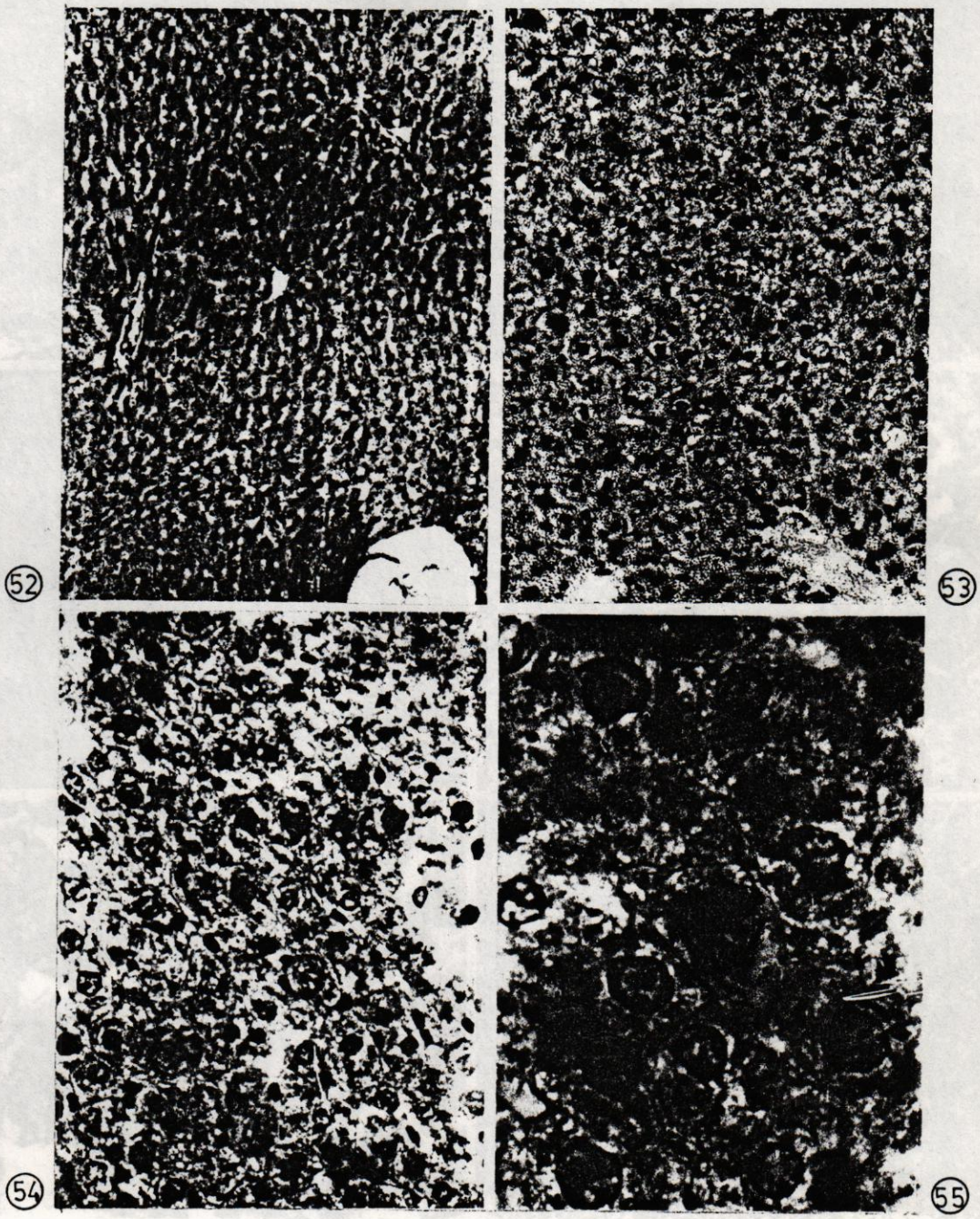
Table XX and Fig. 51 show the effect of long term feeding of aldrin on the various histological parameters of rat liver. The insecticide treatment is marked by hypertrophy of hepatic cells, their nuclei and nucleoli. A typical hepatic cell measures $251.57 \pm 12.84\ \mu^2$ (n =), which increases 48, 29 and 104% after 6, 12 and 18 months of aldrin feeding. The hepatic nuclei likewise show 39, 40 and 70% increase and nucleolar size increases 11, 16 and 34% during the same period. Concomitant with the change in the size of cells, the number of cells/microscopical field decrease in the different insecticide treatment groups (Table XX, Fig. 52).

TABLE - XX

EFFECT OF FEEDING ALDRIN MIXED DIET (2.5 mg/kg body weight/day) FOR 6-18 MONTHS ON THE VARIOUS HISTOLOGICAL PARAMETERS OF MALE ALBINO RATS.

Parameters	6 month aldrin feeding		12 month aldrin feeding		18 month aldrin feeding	
	Control (n= 5)	Aldrin fed (n= 4)	Control (n= 5)	Aldrin fed (n= 4)	Control (n= 5)	Aldrin fed (n= 4)
No.of cells/ microscopical field	277.29[a] ±11.74	184.73*** ± 8.46	249.31 ±13.79	171.22** ± 5.40	251.42 ± 9.68	164.81*** ± 7.24
No. of nuclei/ cell	1.03 ± 0.03	1.09 ± 0.04	1.02 ± 0.03	1.07 ± 0.02	1.04 ± 0.01	1.05 ± 0.02
No. of nucleoli/ nucleus	1.43 ± 0.10	1.91* ± 0.12	1.64 ± 0.11	2.17* ± 0.13	1.46 ± 0.07	1.33 ± 0.09
Size of cell (μ^2)	251.57 ±12.84	371.47*** ± 9.39	262.65 ±14.32	339.35** ±12.81	232.41 ±10.22	474.62*** ±15.81
Size of nucleus(μ^2)	40.23 ± 2.62	55.78** ± 1.77	38.54 ± 2.04	53.96** ± 1.87	46.54 ± 1.92	79.25*** ± 1.76
Size of nucleolus(μ^2)	2.20 ± 0.35	2.44 ± 0.21	2.17 ± 0.26	2.51 ± 0.20	2.09 ± 0.31	2.79 ± 0.29

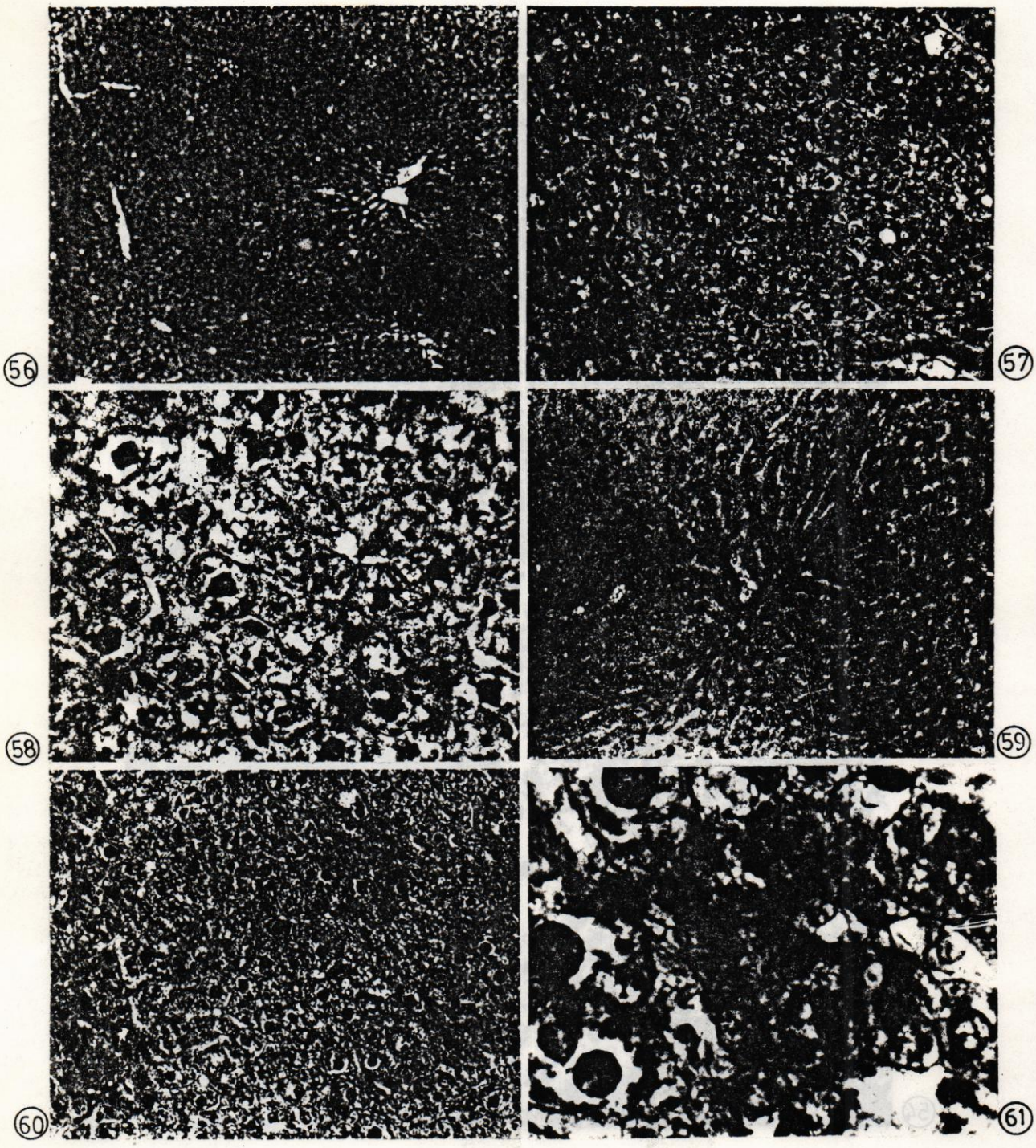
[a]Mean±SEM, student's 't' test; * P 0.05; ** P 0.01; *** P 0.001.



FIGS.52-55:Histological structure of normal rat liver. Note typical hepatic lobule, with many portal areas (52)arrangement of cells and nuclei (53-55).

Stain: Hematoxylin and Eosin.

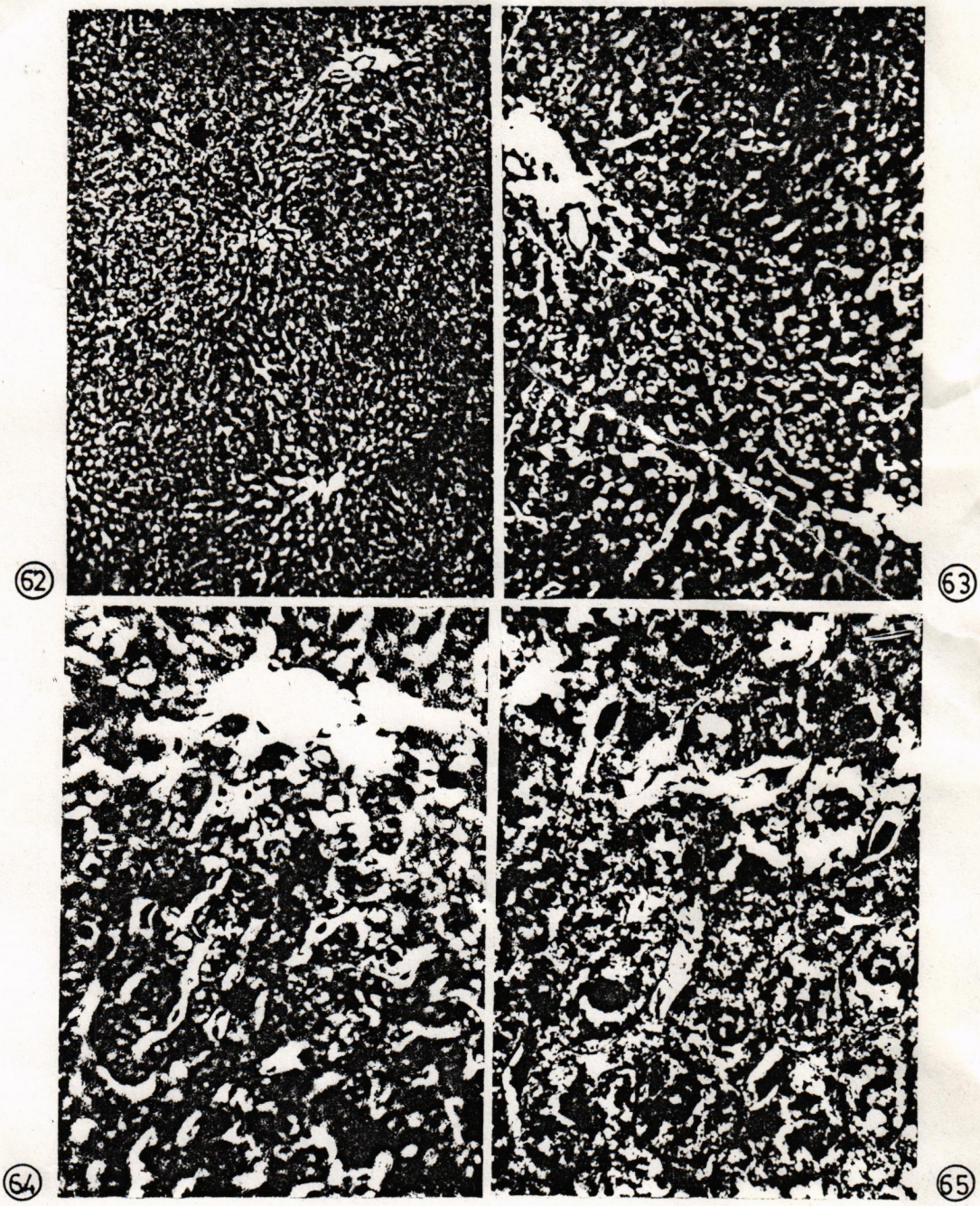
Magnifications: 52,x50; 53,x100; 54,x200; 55,x500.



FIGS.56-61: Histological structure of rat liver fed on aldrin-mixed diet for 6 months (56-58) and 12 months (59-61). Note slightly increased rounded clear areas (56-57,59), cytoplasmic vacuolation arround the nuclei, hypertrophied cells and nuclei (57-58,60-61), and irregular shape nuclei (57-58, 60).

Stain: Hematoxylin and Eosin.

Magnifications: 56,x25; 57,60x100; 59,x50; 58,x250; 61,x500.



FIGS.62-65:Histological structure of rat liver fed on aldrin-mixed diet for 18 months. Note changes in lobular structure (62) greatly enlarged sinusoidal areas with prominent kupffer cells, and hypertrophied cells and nuclei.(63-65).

Stain: Hematoxylin and Eosin.

Magnifications: 62,x25; 63,x50; 64,x100; 65,x250.

Figures 56-58 show hepatic structure after 6 months of aldrin feeding, while Figs. 59-61 show histological structure of 12 month and Figs.62-65 that of 18 month feeding experiments. Figures 52-55 show histological structure of normal, untreated rat liver.

Although the general hepatolobular architecture is maintained throughout the experimental period, the hepatic cells are distinctly enlarged, which are more so prominent in 18 month group (compare Figs. 55 with 65). Majority of the nuclei are converted into condensed bodies, and several darkly stained granules appear in the cytoplasm, most of which is now merginated leaving clear pores (= vacuoles) between the nuclei and marginal cytoplasm.

4. DISCUSSION

Aldrin, just like several other organochlorinated insecticides, are known to contaminate the atmosphere and thus gain entry into non target organisms directly by contact or indirectly through contaminated food (Jager, 1970; Agnihotri et al., 1976; Baldurin et al., 1977). This unintentional insecticidal exposure usually leads to several maladies depending upon the quantity and route of administration (Buck and Van, 1968; Mick et al., 1971; Gertig et al., 1971a,b; Deichmann et al., 1971a,b; Ottolenghii et al., 1973; Reuber, 1976, 1980; Krample and Hladka, 1975; David, 1979; Brandt and Hogman, 1980; and several others). In the presently reported experiment aldrin was mixed in the diet and

then administered to rats as follows: (i) 20 mg/kg body weight for a period of 48 hours (ii) 8 mg aldrin/Kg body weight/day for a total period of 15 days and (iii) 2.5mg/kg body weight/day for period 6-18 months. The first two experiments have been designated as short term experiment, while the third experiment has been designated as long term experiment. All these doses have proved to affect various metabolic processes as evidenced by their effect on various enzymes. Specifically all the liver function tests (LFT) are drastically affected.

Body weight and liver weight

Just as in dieldrin, the body weight is decreased after aldrin feeding. In short term experiment, the rats were exposed to aldrin feeding for only 48 hours, therefore no significant change in the body weight could be recorded. In the second short term experiment, in which the rats were treated with aldrin @ 8 mg/kg body weight/day for 15 day, the body weight decrease significantly after 12 days of continuous feeding. The daily body weight gain decreased 44% after 12-15 days of feeding. The body weight/liver weight ratio was not affected after 3 days of feeding, but then showed 8-12% decrease during subsequent prolonged feeding. In long term experiment the 2.5 mg of aldrin administered per kg body weight/day for a period of 6-18 months did not cause any significant change, while body weight/liver weight ratio decreased 22% after 18 months of feeding. No such change was observed in the 6 month and 12 month group. The small amount of insecticide in the long term experiment probably did not cause any major disturbance in the growth process or if the one had occurred, it got compensated during subsequently prolonged

feeding. The decrease in body weight/liver weight ratio can be attributed to enlarged liver in proportion to body growth rate, which can be attributed to insecticide treatment.

Haematological studies

All the haematological parameters tested behave exactly in the same way irrespective of the dose and duration of aldrin administration. The haemoglobin content and RBC count decrease in all experiments. The haemoglobin content e.g. decrease 11-12% in short term experiment I, 8-12% in short term experiment II and 9-14% in long term experiment. The RBC count decrease 14% in short term experiment I, 10-22% in short term experiment II over a period of 3-15 days and 15-18% in long term experiment over a period of 6-18 months. The insecticide treatment has repeatedly been found to cause a decrease in haemoglobin content and RBC count (Shakoori *et al.*, 1982a,b; Shakoori and Ali, 1982). The decreased synthesis of haemoglobin and slower activity of haematopoietic tissue under the influence of aldrin may explain the low values of haemoglobin and breakdown of RBC.

The PCV on the other hand remains unaffected in short term experiment I, remains unchanged until day 9 in short term experiment II, when it is decreased 3% and further falls down to 6% after 15 days of aldrin feeding. In long term experiment the PCV decreases 5-9% over a period of 6-18 months. This decrease in PCV may be attributed either to decreased cellular content and or increased liquid(plasma) content mainly the water.

WBC count shows typical response to insecticide treatment. This count rises in all experiments. This increase is prominent in both the short term experiments. This increase is 25-28% in 48 hours exposure experiment, and 19-45% in 3-15 day feeding experiment. In long term experiment the WBC count shows 5-11% increase, which is comparatively a little change (through significant). The MCV and MCH values increase under all experimental conditions. The MCV increase is 13% after 20 mg/kg body weight for 48 hours, while it is 10-21% in the second short term experiment, in which the aldrin was administered at a dose of 8 mg/kg body weight/day for 15 days. In long term experiment the increase in MCV ranges between 9-12%. The MCH shows maximum increase (6-13%) in 15 day feeding experiment, while this increase in 48 hour feeding experiment is 3-4% and in long term feeding experiment is 5-8%. The MCHC is not significantly altered in long term experiment, but is decreased 8-9% in the first short term experiment and 6-7% in the second short term experiment.

Blood serum chemistry

Under hepatotoxic conditions, which normally ensue after aldrin treatment, the enzymes of hepatic cells apparently tend to reach out and accumulate in the blood for some time. Until there are cleared from the blood by further metabolic process or degradation (Krample and Hladka, 1975). Almost all the enzymes tested in the present studies were elevated except for ChE and ICDH, which behaved differently under different experimental conditions. In short term experiment, when aldrin was administered @ 20 mg/kg body weight for 48 hours, all enzymes showed elevated activities except

for SGPT, which remained unaltered. The amylase and CPK activities were not significantly deviated after 24 hours of feeding, but showed 60% and 31% increase respectively, after 48 hours of aldrin feeding. From amongst other enzymes, SGOT showed maximum increase i.e. 100% and 370% after 24 and 48 hours of insecticide feeding. This increase in AP activity is 51% and 141%, in AcP activity is 26% and 15%, in LDH activity it is 46 and 78%, in ICDH activity it is 61 and 135% and ChE activity is increased 58% and 88%, respectively in 24 hours and 48 hours feeding experiments.

In the second short term experiment, in which a dose of 8 mg/kg body weight/day was administered for 15 days, all the enzymes generally demonstrated the same trend, as in the first experiment, except for ChE which did not change at this dosage, of all the enzymes tested AP, AcP, SGOT, LDH and amylase activities increased tremendously. The amylase activity is increased 36% after 3 days and 109% after 15 days of continuous feeding. The AP activity increases 94% after 3 days of feeding and is increased 368% after 15 days of feeding. The AcP activity increases 115, 194, 256, 142 and 105% after 3, 6, 9, 12 and 15 days of aldrin feeding. The SGOT activity is not affected until day 9, when it shows 137% increase, and which shoots upto 195% after 15 days of continuous feeding. The SGPT activity, which was not altered in the 48 hour feeding experiment, increases 88%, 117%, 73% and 75% after 3, 6, 9 and 12 days of feeding. The LDH activity increases 39% after 3 days and 115% after 15 days of feeding.

The ICDH activity, on the other hand, was decreased. This decrease was respectively, 41, 40, 11, 23 and 22%, after 3, 6, 9, 12 and 15 days of insecticide feeding. The CPK activity increases 83 and 53% after 9 and 12 days of aldrin feeding. In the long term experiment the extent of elevation of enzyme activities was not of that level as in short term experiments except for CPK activity, which increased 3.099X, 3.43X and 3.499X after aldrin feeding. Like short term experiments, the activities of all the enzymes tested elevated after aldrin feeding. The amylase actitity increased 40-52% after 6-18 months of feeding, while the increase in AP activity was 2.28X, 4.00X and 2.93X, while in AcP this increase was 97%, 11% and 15% after 6, 12 and 18 months of feeding, respectively. The SGOT and SGPT activities likewise increased. This increase was 102, 47 and 32% for SGOT and 50, 12 and 197% for SGPT after 6, 12 and 18 months of aldrin feeding. The LDH activity also increased, while the ICDH activity was increased 57% and 33% after 12 and 18 months of insecticide feeding. The ChE activity was raised (27%) only after 18 months of continuous feeding.

The raised enzymatic activity, especially of liver function is, therefore, direct indicator of disturbed liver function (Gertig et al., 1971a,b; Fitzhugh, et al., 1964). The response of all these doses of aldrin on the activity of blood serum enzymes has been fairly uniform. Although aldrin is metabolized in the liver into dieldrin (Ghiasuddin and Menzer, 1976; Wolff et al.,1980), the sensitivity of different enzymes to aldrin treatment has been clearly manifested in these experiments. The aldrin treatment generally leads to increased CPK activity,

while in dieldrin treated groups, the CPK activity remains unaltered in short term and long term experiment, while a distinct increase has been reported in the case of aldrin-treated blood serum. The CPK activity, therefore, is inhibited or have tendency to be inhibited by dieldrin (Williams and Casterline, 1970; Hendrickson and Bowden, 1976; Meany and Pocker, 1979). The SGPT activity likewise is not drastically affected in short term experiment, and has no effect in the case of long term experiment with dieldrin. In aldrin feeding the SGPT activity is comparatively less affected.

Increase of hepatic enzymes under the influence of aldrin has also been reported with reference to several other enzymes, other than being reported here for LFT. Anastasi and Bannister (1980) have reported that aldrin stimulated mitochondrial enzymes like muscle pyruvate thirace, LDH and malate dehydrogenase, but inhibited mitochondrial enzymes like cytochrome oxidase in Fish.

Besides various enzymatic activities, the other biochemical components of blood serum have also been variously affected by aldrin treatment. The bilirubin content decrease in short term experiment, but show significant increase after 15 day feeding. The bilirubin content decrease 35% after 24 hours of aldrin feeding @ 20 mg/kg body weight/day and decrease 37% after 6 month of feeding @ 2.5 mg/kg body weight/day for 6 months. In short term experiment II (in which the aldrin was administered @ 8 mg/kg body weight/day for 15 days) the bilirubin content increased 148, 121, 77, 71 and 14%, after 3, 6, 9, 12 and 15 days of

insecticide feeding. The blood serum proteins also increase after aldrin treatment. This increase is 13% and 26% after 24 and 48 hours of feeding, while the protein content remain unchanged until day 9 after aldrin treatment @ 8 mg/kg body weight/day, when the protein content increase 22%. During subsequent feeding for 12 and 15 day, the increase is respectively, 21 and 31%. In long term experiment, the blood serum proteins increase 27, 44 and 24% after 6, 12 and 18 months of feeding. The blood urea content decrease 31% after 48 hours of feeding, while this content increase with second short term experiment 28%, 27%, 53%, 102% and 70% after 3, 6, 9, 12 and 15 days of aldrin treatment. This increase in long term experiment is 22, 26 and 45% after 6, 12 and 18 months. The glucose content are not affected in the long term experiment, while in the first short term experiment, the glucose content increase 28% after 48 hours of aldrin feeding. In the second short term experiment, the glucose content decrease 27, 32, 47, 30 and 26% after 3, 6, 9, 12 and 15 days of continuous feeding. The cholesterol content are drastically decreased (31% and 29%) after 24 and 48 hours of feeding, while remain unaltered when 8 mg of aldrin was administered/kg body weight/day for 15 days. In long term experiments the cholesterol content decrease 12% and 41% after 6 and 12 months of aldrin feeding. The FAA content are most drastically affected. These content decrease after short term and long term administration. Aldrin administered for 15 days produced the maximum effect.

The dieldrin, when fed directly to rat result in 67% increase in bilirubin content, while the aldrin feeding does not cause any significant change in this metabolite. The other biochemical parameters e.g. cholesterol, glucose and urea contents decrease in both insecticidal treatments. Although aldrin treatment has caused increase in the total serum protein content, the dieldrin treatment does not cause any appreciable change. Skalsky and Guthrie (1977) have also shown binding of dieldrin with proteins of rat blood. According to Iatropaulos et al. (1975) the dieldrin is quickly absorbed and transported to the liver of sprague Dawley rats. Only a portion is metabolized and excreted. The major portion is redistributed and stored in adipose tissue.

Liver biochemistry

The changes in the parameters of liver function tests are actually reflection of changes in the liver structure and chemistry (Shakoori and Haq, 1982). All the hepatic enzymes tested showed raised activities after aldrin treatment. In short term experiment I, in which aldrin @ 20 mg/kg body weight was administered for 48 hours, the GOT, GPT and LDH activities are not affected until 48 hours, when all their activities increase 28%, 40% and 26% after aldrin treatment. The AP activity appears to be more sensitive and is increased 69% and 17% after 24 and 48 hours of aldrin feeding. The ICDH activity is not affected at all.

In the second short term experiment the ICDH activity is considerably increased 121%, 56%, 132%, 142% and 100% after 3, 6, 9, 12 and 15 days of feeding @ 8 mg/kg body weight/day for 15 days. AP activity is drastically increased. The increase is 80, 73, 133, 144 and 162%, respectively, after 3, 6, 9, 12, and 15 days of aldrin feeding. The GPT activity is not significantly altered, while hepatic GOT activity shows 14, 97, 186, 76 and 171% increase after 3, 6, 9, 12 and 15 days of aldrin feeding. The LDH activity is also significantly increased after 9 and 12 days of insecticide administration.

In long term experiment the activities of all hepatic enzymes are significantly increased.

The raised enzymatic levels in blood are attributable to liver damage under pathological conditions, while their low levels in blood could either be because, of great regenerative power of liver, as a result of which leaking out of the enzymes in blood serum becomes minimal, or due to the biosynthetic activity which implies routing of all the biochemical components towards this activity (Rosen and Nichol, 1963; Knox and Greengard, 1965; Bhatia et al., 1972b, 1973) in liver. The raised enzymatic activity in the liver, on the other hand, may be because of induction of enzyme synthesis (Street, 1969; Kimbrough et al., 1971; Krample and Hladka, 1975), while their low levels could either be because of enzymatic inhibition (Hendrickson and Bowden, 1976; Meany and Pocker, 1979) or due to liver damage without any regeneration.

Besides various enzymatic activities several other biochemical components of the liver were considered to ascertain hepatotoxicity. The cholesterol content decreased under all circumstances. In short term experiment I, the cholesterol content decreased 29%, while in 15 day feeding experiment the cholesterol content decreased 66-72% after 3-15 days of feeding. When feeding of aldrin was extended for 6-18 months, the cholesterol content decreased 8%. The cholesterol content behaved differently under different experimental condition. In short term experiment the glucose content decrease 43%, while in long term experiment a decrease of 38-52% was recorded. In 15 day feeding experiment the glucose content show a gradual increase of 52, 79, 80, 98 and 143% after 3, 6, 9, 12 and 15 days of aldrin feeding. The FAA content likewise behave almost in the same way as in the case of glucose. The FAA content decrease drastically in short term experiment I and long term experiment, while an increase of 53, 28 and 57% was recorded after 9, 12 and 15 days of insecticide feeding. The soluble protein content are not altered in 15 day feeding experiment, while about 29% increase was found in short term experiment I and in long term feeding experiment. A distinct increase in the total hepatic content was observed under all experimental conditions. The decreased cholesterol level, increased glucose content, decreased FAA content are indicative, of hepatotoxicity and corrective measures to acquire more energy by raising level of glucose and increased protein synthesis.

Nucleic acids content

The DNA content remain unaffected after aldrin treatment, while the RNA content increase after 15 days of aldrin mixed diet @ 8 mg/kg body weight/day. This increase is 50, 67, 92, 158 and 113% after 3,6,9,12 and 15 days of feeding. In the long term experiment, however, the RNA content decrease 51-53% after 6-18 months of aldrin feeding. The increase RNA content is related with increased protein content as the two processes are consequent of first into second. This decrease indicates hepatic necrosis, which is also indicated by raised enzymatic levels in blood serum due to cell damage. The raised hepatic enzyme level could be because of enzyme induction. In dieldrin treated rats also the DNA content resist the effect of the insecticides, while the RNA content show increase at the same time.

Hall (1980) has studied biological interaction of aldrin with the nuclear DNA of human fibroblasts. Rocchi et al. (1980) studied action of aldrin on DNA synthesis with a short term in vitro system using rat thymocytes and found that it does not induce damage to human lymphocyte DNA. In swine kidney cells (IBRS 2 cells) the cellular protein, RNA and DNA content decrease, when exposed to 0.1-100 µg/ml aldrin (Rodrigues and Puga, 1979).

Liver histology

All the above biochemical changes are indicated in the typical liver histological changes due to chemical toxicity. The appearance of vacuoles and

hypertrophy of cell and its inclusions are the typical signs of hepatotoxicity. Inspite of the fact that biochemical state predicts mild necrotic region, this is apparently not substantiated by the histological studies.

Although the number of nuclei/cell and number of nucleoli/nucleus are not drastically altered, but the size of hepatic cell, its nucleus and nucleolus hypertrophy considerably. The hypertrophied cell, its nucleolus and nucleus is very typical response of hepatic cells to xenobiotics.

The carcinogenic potential of aldrin in mice, which has so frequently been reported in the previous literature (David, 1979; Davis and Fitzhugh, 1962; Fitzhugh et al., 1964) was not seen in the present studies.

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C H A P T E R III

EFFECTS OF GAMMA BHC (= LINDANE)

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1. INTRODUCTION

Pest control chemicals are poisons, they may present immediate danger to the user if applied improperly or without sufficient knowledge of their toxic effects. Some are highly toxic and may cause serious illness and even death if spilled on the skin, inhaled or otherwise used carelessly. In addition potential future hazards to human health and wildlife can be created by residues from some long lived pesticides that may build up in the food chain and cause widespread contamination of the environment. The contamination of the environment by pesticides has been a subject of concern for the last 30-35 years. The pesticides most frequently involved are the organochlorine insecticide DDT, TDE, endrin, heptachlor, aldrin, dieldrin, chlordane, toxaphene,

Strobane and BHC or its γ-isomer, lindane (Nicholson, 1969). The reports of contamination of human environment with these insecticides are piling up. The contamination of drinking water and food; and accumulation of insecticides in plants and various human and animal organs are being increasingly reported from different parts of the world e.g. (Milby et al., 1968; Milby and Samuels, 1971; Hill et al., 1973; Simonffy et al., 1973; Villeneone, 1975; Bluemthal and Cerny, 1976; Vrochinskii et al., 1976; Traczyk et al., 1977; Joia et al., 1978; Yadar et al., 1978; Wells and Johnstone, 1978; Williams et al., 1979; Battu et al., 1980; Kapoor et al., 1980; Saxena et al., 1980; Crissman, 1980; Harrison et al., 1980; Barquet et al., 1981; Brassow et al., 1981; Siddiqui et al., 1981; Kaphalia and Leth, 1981; Blakley, 1982; Telch and Jarvis, 1982; Wickstrom et al., 1983).

Gamma BHC or lindane is one of the most important and common chlorinated insecticides, which contaminate the environment. Like any other insecticides, lindane has also gained entry into non target organisms frequently with serious consequences (Samuel and Milby, 1971; Milby and Samuel, 1971). Strik (1973) has reported weight loss, tremor, liver injury and eventual mortality as a result of administration of polychlorinated aromatic hexachlorocyclohexane. Toxicity of lindane in different animals has been described from different laboratories (e.g. Thorpe and Walker, 1973; Powell, 1980; Bargman, 1982; Bakthavathsalam and Reddy, 1983; Frank and Braun, 1984; Henschler, 1985).

Convincing evidences are also available to prove carcinogenicity of lindane. Most of the evidences however prove tumorigenesis in mice and rats (e.g. Thorpe and Walker, Herbst et al., 1975; Weisse and Herbst, 1977; Kashyap et al., 1979; Reuber, 1979; Hirayama et al., 1979; Tatematsu et al., 1979; Axelson, 1980; Bhatt et al., 1981a,b,c,d; Thakore et al., 1981; Oasch et al., 1982). Inspite of its elaborate metabolic pathway (Chadwick et al., 1975, 1977), in which it has been reported to ferm- -2,3,4,5,6, pentachlorocyclohexane, pentachlorobenzene and pentachlorobenzene (Engst et al., 197[illegible],1977,1979) and mercapturic acid (Kuribara et al., 1979), lindane has strong tendencies to get accumulated in different organs and tissues of the body e.g. blood, fat body (Crist et al., 1975; Arthur et al., 1975; Hashemy-Tonkabony and Soleimani-Amiri, 1978; Ramachandran et al., 1984), lung (Sadowski, 1980), Brain (Seidler et al., 1975).

Since liver is the main metabolic centre, where most of the xenobiotics are metabolized and detoxified, this is therefore one of the forefront organs of the body, which fall the major conslaught of a unwanted chemical invasion. The liver function is therefore likely to be disturbed. Mehendale (1978) has studied the modification of hepatobiliary function induced by hexanchlorobenzene. Lyubchenko et al. (1974) have reported that lindane causes wild shifts in the activity of cholinesterase and histidase, and also impairs the

excretory function of liver, Gertig and Nowaczyk (1975) have also reported decreased alkaline and acid phosphatases, glutamate oxaleacetate transaminase and LDH activities in blood serum and decreased hepatic glutamate pyruvic transaminase and LDH activities. Khaikina (1972) have also seen appreciable diminution of total LDH activity in the serum as well as liver after prolonged feeding of lindane for 180 days.

The prophyrogenic action of hexachlorobenzene is also well known, though the mechanism is not well understood (Grant et al., 1974; Koszo et al., 1974; Stonard, 1974; Lissner et al., 1975; Goldstein et al., 1978; Graef et al., 1982; DeCalmanovici et al., 1984).

The porphyrogenesis is usually accompanied by increased drug metabolizing enzymes (Grant et al., 1974). Lindane induces O-demethylase, activates benzo (α) pyrene and reduces acid phosphatase (Mikol et al., 1980). A significant increase in cytochroma P450 and activities of mixed function oxygenases was observed in polychlorinated hydrocarbon treated animals (Konat and Clausen, 1973; Brade et al., 1974; Den et al., 1974; Pelissier et al., 1975; Farber et al., 1976; Pelissier and Albrecht, 1976;

Oesch et al., 1982; Albrecht et al., 1981; Srinivasan and Radhakrishna-Murty, 1983).

The chlorinated insecticides, like other toxic chemicals cause liver malfunction, which is evidenced by several liver function tests. The present report deals with the effect of different doses of lindane, administered for different periods of time, on the haematological parameters, biochemical composition of blood and liver and hepatic histological structure.

2. MATERIALS AND METHODS

2.1. ANIMALS

A colony of sprague Dawley albino rats raised as described in the first Chapter was used for the present studies. The rats used were as follows:

a) For short term experiments: two groups of female rats, weighing about 175-200 g and 6-8 months of age were used. One group was used for feeding insecticide for 48 hours, while the second was used for feeding insecticide for 15 days.

b) For long term experiment/ rats of about 90 - 100gm and 3 - 4 months of age were used.

2.2. PREPARATION OF FEED

The rat feed was prepared in the same way as described in Chapter I of this Report.

2.3. INSECTICIDE USED

Gamma BHC (=Lindane, 1α, 2α, 3β, 4α, 5α, 6β-hexachlorocyclohexane), a chlorinated insecticide (26% powder) were obtained from Jafer Brothers, Lahore and administered to the animals orally alongwith feed.

2.4. ADMINISTRATION OF INSECTICIDE

Gamma BHC was administered to rats as strong and weak doses as follow:

a) Strong dose:

For short term experiment, two levels of strong doses were administered. In one group of rats a strong dose of 18 mg/kg body weight/day was administered for a total period of 15 days. In the second group 30 mg BHC/kg body weight/ day was administered for a total period of

48 hours.

b) Weak dose:

A weak dose at a rate of 9 mg/kg body weight/day was administered to another group of rats for 6-18 months.

2.4.1. SHORT TERM EXPERIMENTS

For short term experiments, in which the total duration is 48 hours in one case and 15 days in the other the insecticide was administered as follows:

a) For 48 hour experiment the insecticide mixed diet was prepared by mixing 3.84gm of 26% powdered γBHC in 1 kg of rat feed. Since each experimental rat on the average consumed 30 g of feed daily, it will get 30 mg γBHC/kg body wt./day.

b) For 15 day experiment, the insecticide mixed diet was prepared by mixing 2.31gm of 26% powdered γBHC in 1 Kg of rat feed. The rats in this way got 18 mg γBHC/kg body weight/day.

2.4.2. LONG TERM EXPERIMENT

The insecticide mixed diet was prepared by adding 1.15gm of 26% γBHC powder in small amount of water and then that insecticide mixed water was thoroughly mixed with 1 Kg of ingredient mixed feed. That way the rats consumed 9mh BHC /kg body weight/day.

2.5. PROCEDURE ADOPTED

Experimental procedure adopted for the two short term and one long term experiments is the same as described in Chapter I for dieldrin experiments. The procedures adopted for collection of blood, liver

processing, haematological studies, biochemical analysis of blood, biochemical analysis of liver and histological studies were the same as described in Chapter I.

3. RESULTS

3.1. EFFECT OF γBHC ADMINISTERED AT A DOSE OF 30 mg/kg body weight/day FOR A PERIOD OF 48 HOURS

3.1.1. HAEMATOLOGICAL STUDIES

Figure 1 and Table I show the effect of γ-BHC, administered at a dose of 30 mg/kg body weight/day for a total period of 48 hours on the various haematological parameters of albino rat blood. The haemoglobin content decrease after insecticide treatment. A control rat has 13.27 ± 0.14 g haemoglobin/100 ml of blood, which decreases 7% after 48 hours of γBHC feeding. The RBC count and PCV also decrease under the influence of γBHC feeding. RBC counts decreases 11%, while PCV shows 7% decrease during the same period. Feeding for 24 hours does not cause any significant change. The WBC count, on the other hand shows 39 and 46% increase after 24 and 48 hours of γBHC feeding, respectively. The MCV and MCH increase, while MCHC decrease after insecticide feeding (Table I, Fig.1). The MCV shows 10% increase, while MCH shows 5% increase after 48 hours of feeding. The MCHC in control rats is 31.52 ± 0.09 g/100 ml, which decreases 5% after insecticide feeding.

TABLE - I

EFFECT OF FEEDING γ-BHC MIXED DIET (30 mg/kg body weight/day) FOR 48 HOURS ON THE VARIOUS HAEMATOLOGICAL PARAMETERS OF ALBINO RATS.

Parameters	Control (n = 7)	γ-BHC feeding	
		24 hours (n = 3)	48 hours (n = 3)
Hb (g/dl)	13.27±0.14[a]	12.43±0.37	12.36±0.33*
RBC ($X10^5$ cells/µl)	68.37±2.53	63.63±1.48	61.07±0.91*
WBC ($X10^2$ cells/µl)	63.64±2.60	88.33±4.37**	92.83±8.97*
PCV (%)	42.09±0.34	41.71±0.61	39.18±0.64**
MCV (fl)	61.56±0.17	65.39±2.42	67.45±1.71**
MCH (Pg)	19.38±0.08	19.53±0.14	20.24±0.27*
MCHC (g/dl)	31.52±0.09	29.79±0.49**	29.99±0.37**

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

C = CONTROL
24 = 24 HOURS γ-BHC FEEDING
48 = 48 HOURS γ-BHC FEEDING

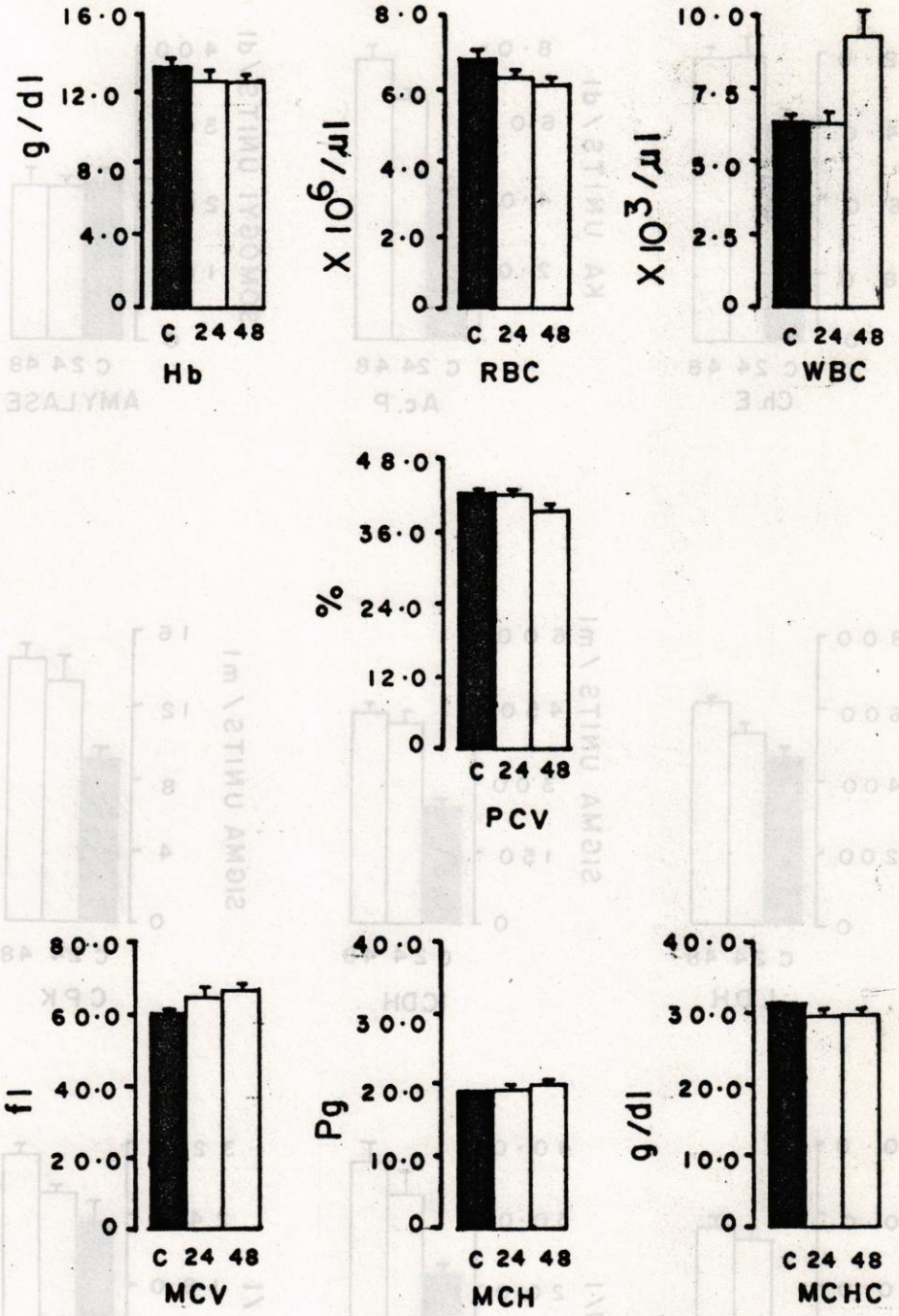


Fig.1. Effect of feeding gamma-BHC mixed diet (30 mg/kg body weight/day) for 48 hours on the various haematological parameters of albino rats.

C = CONTROL
24 = 24 HOURS γ-B H C FEEDING
48 = 48 HOURS γ-B H C FEEDING

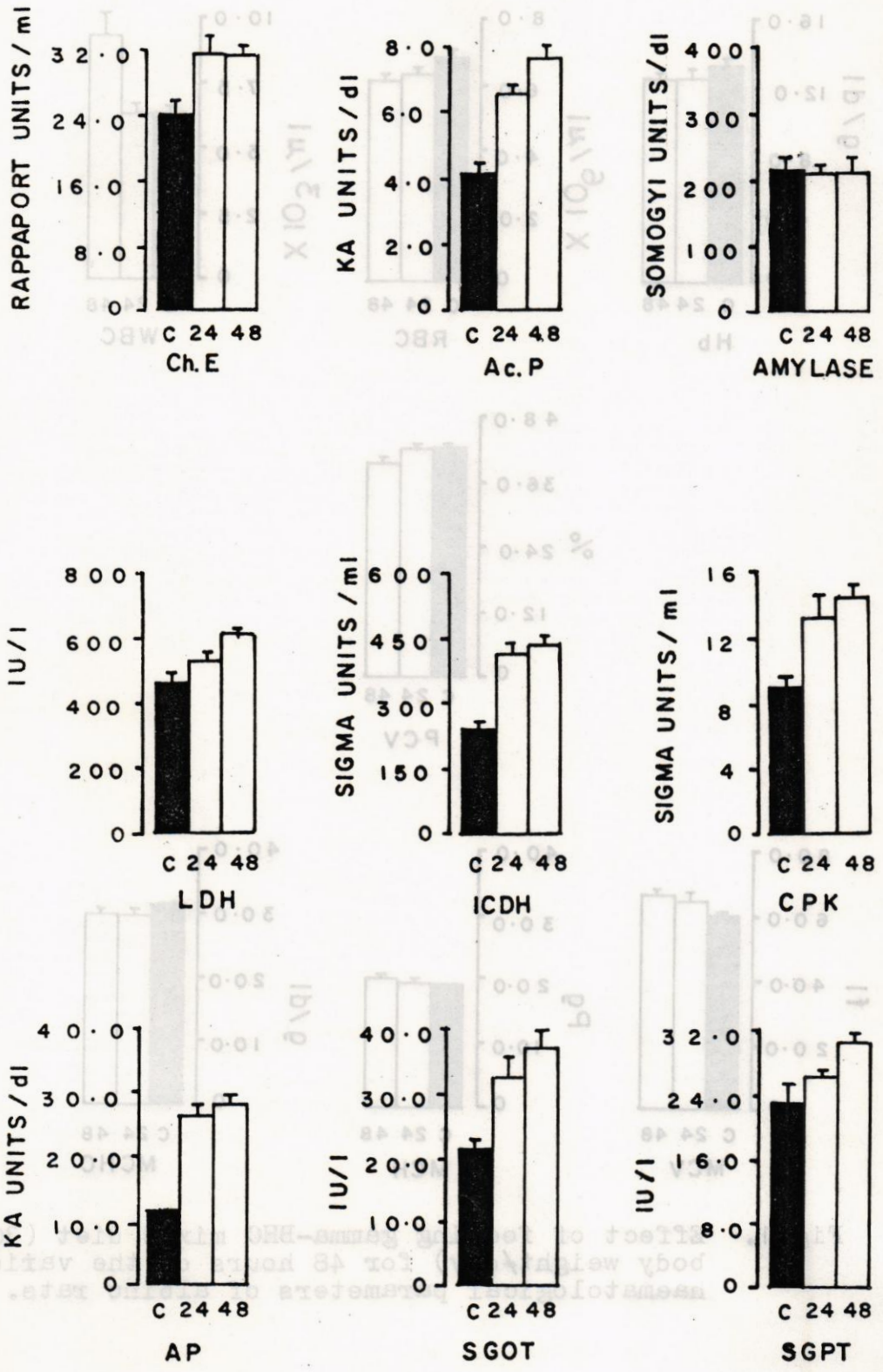


Fig.2. Effect of feeding gamma-BHC mixed diet (30 mg/kg body weight/day) for 48 hours on the activities of various enzymes in albino rat blood serum.

3.1.2. BIOCHEMICAL ANALYSIS OF BLOOD

Several blood serum enzymes, besides other biochemical components were tested to ascertain the effects of rBHC on the blood of albino rats, which was administered for 48 hours (Figs.2,3; Tables II, III).

Figure 2 and Table II shows the effect of insecticides on the blood serum enzyme. Except for the activity of amylase which is not affected at all, all other enzymes/activities were raised after insecticide administration. A control rat shows 11.67 ± 0.16 KAU activity of AP/dl of blood serum, and 4.23 ± 0.2 KAU activity of AcP/dl of blood serum. After rBHC administration the former activity is raised 126 and 144%, while the latter is raised 56 and 81%, respectively after 24 and 48 hours of rBHC administration. The SGOT activity is increased 52 and 72%, while SGPT activity is increased 14 and 33% after 24 and 48 hours of insecticide feeding. From amongst the two dehydrogenases LDH show 1.15 times and 1.31 times increase after 24 and 48 hours of feeding, while ICDH activity increases 1.714 and 1.804 times respectively after 24 and 48 hours of feeding.

Control blood serum shows 24.37 ± 1.42 Rappaport unit of ChE/ml of blood serum. This activity increases 29-30% after insecticide administration. The CPK activity shows 49 and 62% increase after 24 and 48 hours of insecticide feeding.

Several other biochemical components of blood serum are also affected and all of them showed raised level is evident from Table III and Fig.3. The Bilirubin content are not affected after 24 hours of rBHC feeding, but is raised 46% after 48 hours of feeding. The

TABLE - II

EFFECT OF FEEDING γ-BHC MIXED DIET(30 mg/kg body weight/day) FOR 48 HOURS ON THE VARIOUS ENZYMATIC ACTIVITIES OF ALBINO RAT SERUM.

Parameters	Control (n = 8)	γ-BHC feeding	
		24 hours (n = 3)	48 hours (n = 3)
AP (KAU/dl)	11.67±0.16[a]	26.40±1.50***	28.42±1.02***
Ac.P (KAU/dl)	4.23±0.20	6.60±0.23***	7.66±0.35***
Amylase (Somogyi U/dl)	216.77±18.02	214.70±7.24	214.70±22.39
ChE (Rapp-aport U/ml)	24.37±1.42	31.66±2.03*	31.50±1.44***
CPK (Sigma U/ml)	9.01±0.63	13.40±1.30*	14.60±0.61***
SGOT (IU/l)	21.73±1.65	33.10±2.92**	37.40±2.31***
SGPT (IU/l)	23.05±2.40	26.33±0.51	30.66±0.96*
ICDH (Sigma U/ml)	243.18±14.80	416.95±24.40***	438.83±17.02***
LDH (IU/l)	465.66±26.29	534.08±23.87	608.80±25.64**

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

C = CONTROL
24 = 24 HOURS γ- B H C FEEDING
48 = 48 HOURS γ- B H C FEEDING

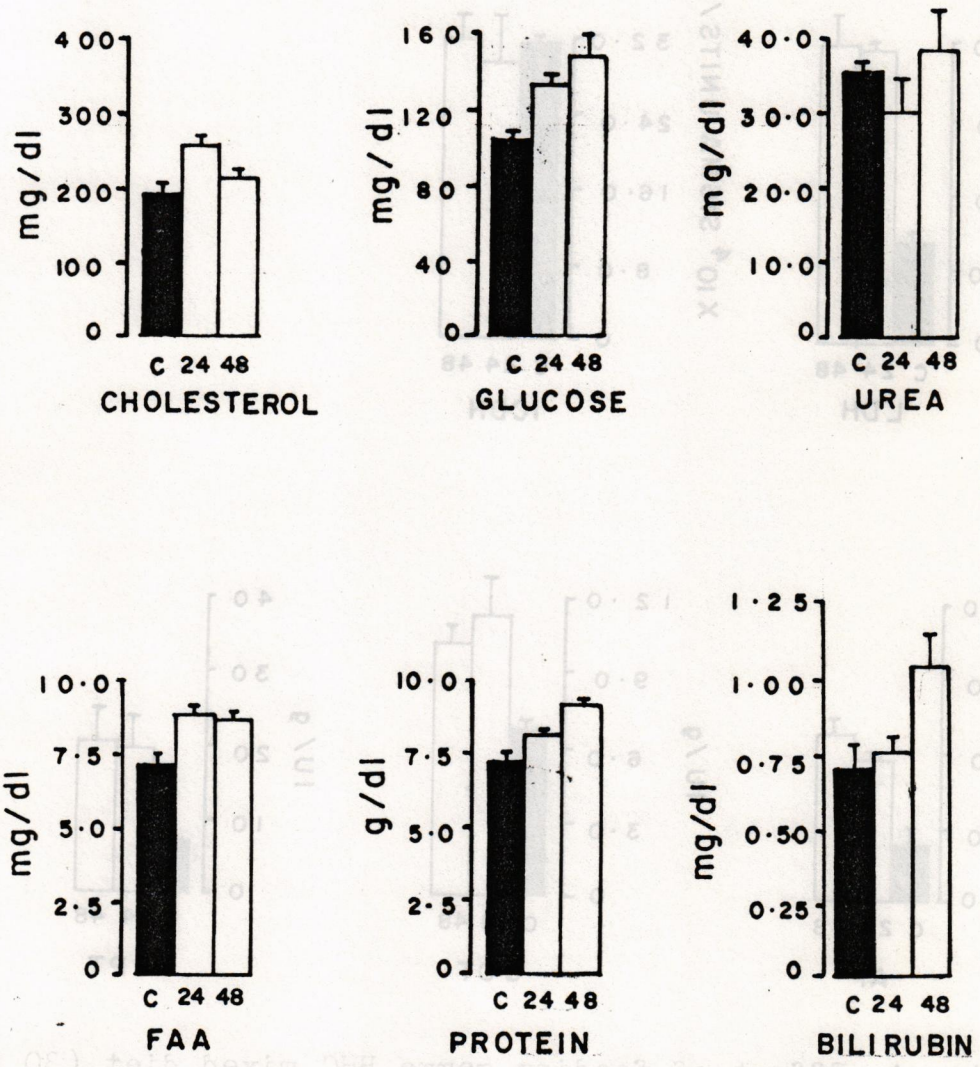


Fig.3. Effect of feeding gamma-BHC mixed diet (30 mg/kg body weight/day) for 48 hours on the various biochemical components of rat blood serum.

C = CONTROL
24 = 24 HOURS γ-B H C FEEDING
48 = 48 HOURS γ-B H C FEEDING

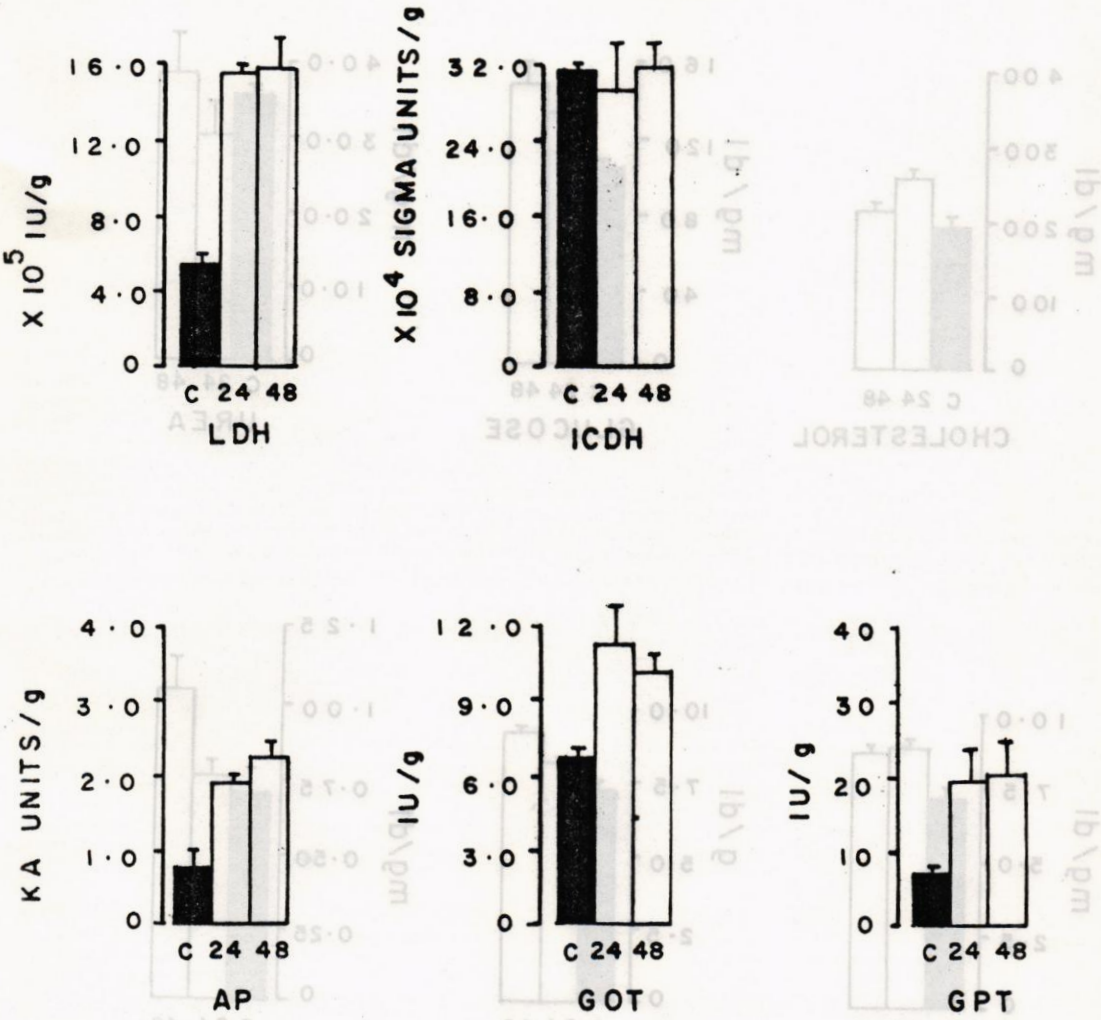


Fig. 4. Effect of feeding gamma BHC mixed diet (30 mg/kg body weight/day) for 48 hours on the hepatic anzyme activities of rat.

TABLE - III

EFFECT OF FEEDING γ-BHC MIXED DIET (30 mg/kg body weight/day) FOR 48 HOURS ON THE VARIOUS BIOCHEMICAL COMPONENTS OF RAT BLOOD SERUM.

Parameters	Control (n = 8)	γ-BHC feeding	
		24 hours (n = 3)	48 hours (n = 3)
Bilirubin (mg/dl)	0.72±0.07[a]	0.77±0.06	1.05±0.11*
Cholesterol (mg/dl)	195.64±6.94	258.92±9.02***	216.28±10.73
FAA (mg/dl)	7.26±0.19	8.92±0.13***	8.77±0.23***
Glucose (mg/dl)	107.37±3.36	136.00±9.45*	150.00±13.11*
Protein (g/dl)	7.30±0.13	8.21±0.06***	9.20±0.14***
Urea (mg/dl)	35.49±0.96	30.02±4.41	38.73±4.82

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

TABLE - IV

EFFECT OF FEEDING -BHC MIXED DIET (30 mg/kg body weight/day) FOR 48 HOURS ON THE ACTIVITIES OF VARIOUS HEPATIC ENZYMES OF RAT.

Parameters	Control (n = 5)	γ-BHC feeding	
		24 hours (n = 3)	48 hours (n = 3)
AP (KAU/g)	0.80±0.16[a]	1.94±0.06*	2.29±0.15**
GOT (IU/g)	7.11±0.38	11.36±1.47*	10.25±0.74**
GPT (IU/g)	7.32±0.68	19.40±4.66*	20.65±4.71*
ICDH ($X10^3$ Sigma U/g)	31.39±0.78	29.25±5.30	31.87±2.58
LDH ($X10^4$ IU/g)	56.57±4.43	157.17±12.93***	159.47±14.82***

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

cholesterol content increase 1.32 times and 1.11 times after 24 and 48 hours of insecticide treatment. The control rat blood serum contains 7.30 ± 0.13 g protein/100 ml of serum and 7.26 ± 0.19 mg FAA/100 ml serum. The protein content are increased 13 and 26%, while FAA content increase 23 and 21%, after 24 and 48 hours of rBHC administration, respectively. The glucose content also increase viz 27% after 24 hours of insecticide treatment, and 40% after 48 hours of insecticide administration. The blood serum Urea is also raised, but the deviation is statistically non-significant (Table III, Fig.3).

3.1.3. BIOCHEMICAL ANALYSIS OF LIVER

Table IV and Figure IV shows the effect of feeding rBHC mixed diet at a dose of 30 mg/kg body weight/day for 48 hours on the activities of various hepatic enzymes. Of all the enzymes tested, ICDH activity remained unaltered, while all other activities viz. AP, GOT, GPT and LDH were considerably increased. The hepatic AP activity increases 141% (24 hour feeding) and 184% (48 hour feeding), while the LDH activity increases 178% and 182%, respectively. Out of the two transaminases tested, GPT was more drastically affected. A control rat liver contains 7.11 ± 0.38 IU of GOT activity/g of liver and 7.32 ± 0.68 IU of GPT activity/g of liver. The former enzymatic activity is raised 60% and 44% after 24 and 48 hours of feeding, while the latter activity is raised 165% and 182%, respectively.

Besides hepatic enzymes, some other biochemical components were also tested to ascertain the effect of rBHC feeding.

TABLE - V

EFFECT OF FEEDING γ-BHC MIXED DIET (30 mg/kg body weight/day) FOR 48 HOURS ON THE VARIOUS BIOCHEMICAL COMPONENTS OF RAT LIVER.

Parameters	Control (n = 5)	γ-BHC feeding	
		24 hours (n = 3)	48 hours (n = 3)
Cholesterol (mg/g)	7.62±0.22[a]	5.07±0.69**	5.11±0.29**
FAA (ug/g)	399.21±18.13	282.20±4.68***	190.04±4.70***
Glucose (mg/g)	20.14±0.52	20.29±1.45	20.83±1.92
Soluble Protein (mg/g)	111.18±5.08	129.47±5.62	144.17±10.32*
Total protein (mg/g)	199.33±6.11	183.16±15.94	197.48±4.38
DNA (mg/g)	3.84±0.44	3.90±0.26	3.23±0.59
RNA (mg/g)	9.53±0.55	5.46±0.61**	4.89±0.09***

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

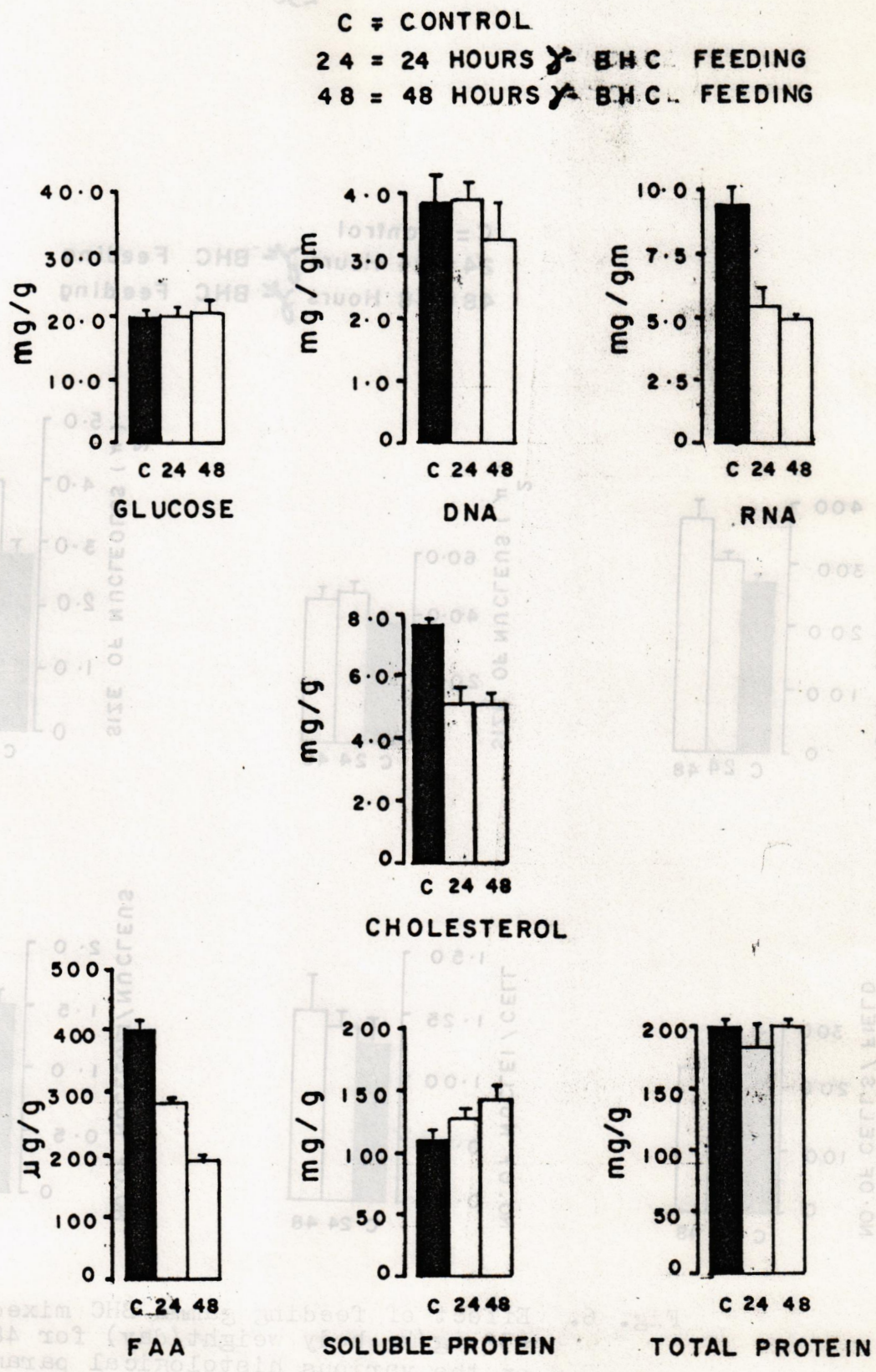


Fig.5. Effect of feeding gamma BHC mixed diet (30 mg/kg body weight/day) for 48 hours on the various biochemical components of albino rat liver.

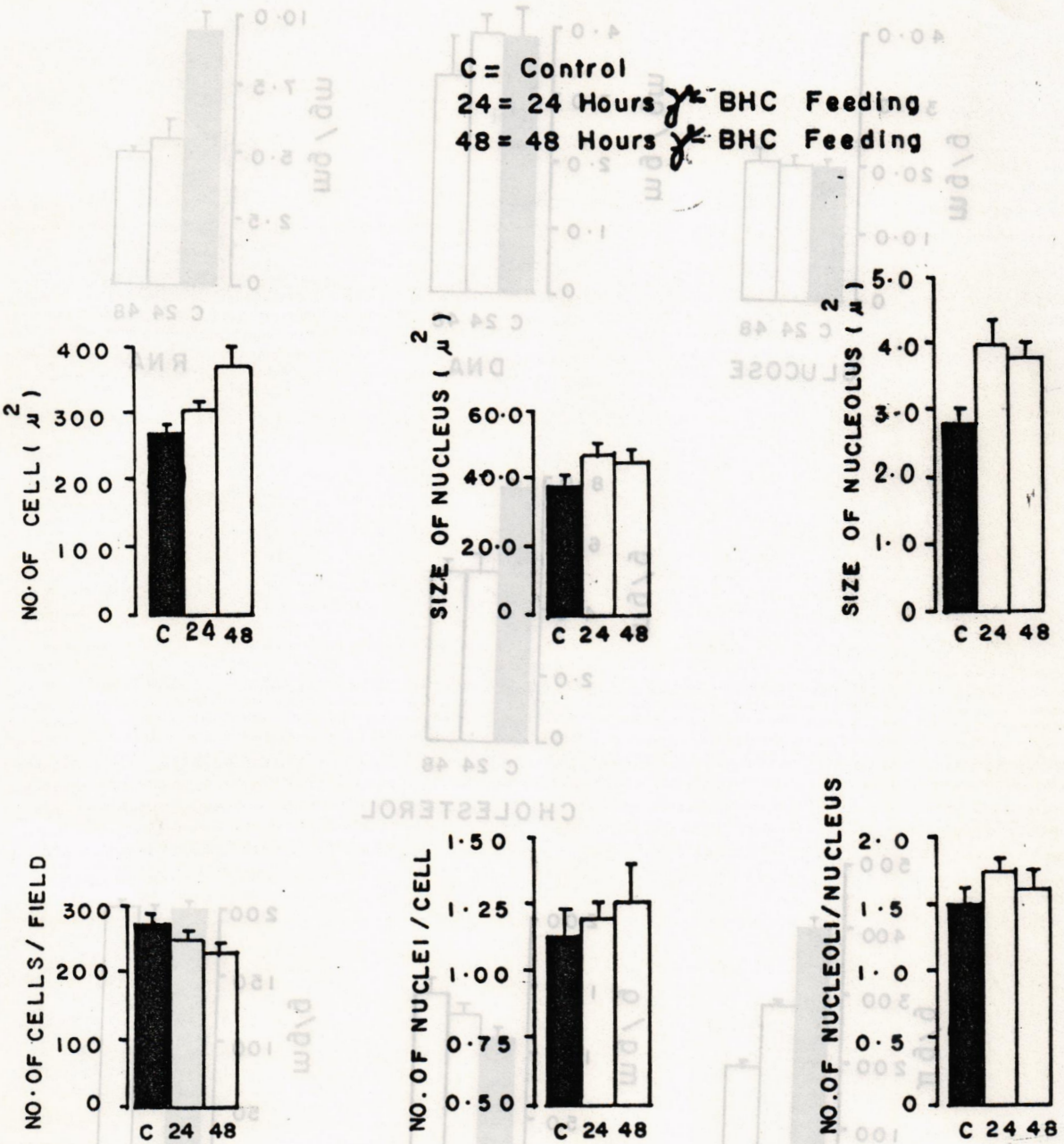


Fig. 6. Effect of feeding gamma BHC mixed diet (30 mg/kg body weight/day) for 48 hours on the various histological parameters of albino rat liver.

TABLE - VI

EFFECT OF FEEDING OF γ BHC MIXED DIET (30 mg/kg body weight/day) FOR 48 HOURS ON THE VARIOUS HISTOLOGICAL PARAMETER OF RAT LIVER.

Parameters	gamma -BHC feeding		
	Control (n = 5)	24 hours (n = 4)	48 hours (n = 4)
No.of cells/ field	276.54±9.62[a]	247.92±11.39	226.13±14.45*
No. of nuclei/ cell	1.12±0.83	1.19 ±0.05	1.26 ±0.15
No.of nucleoli/ nucleus	1.52±0.17	1.77 ±0.09	1.63 ±0.13
Size of cell (μ^2)	269.27±8.49	306.91±4.92**	373.95±9.09 **
Size of nucleus(μ^2)	37.71±0.92	47.39 ±1.18***	44.45±1.33**
Size of nucleolus (μ^2)	2.81±0.18	3.98 ±0.30 *	3.80 ±0.21**

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

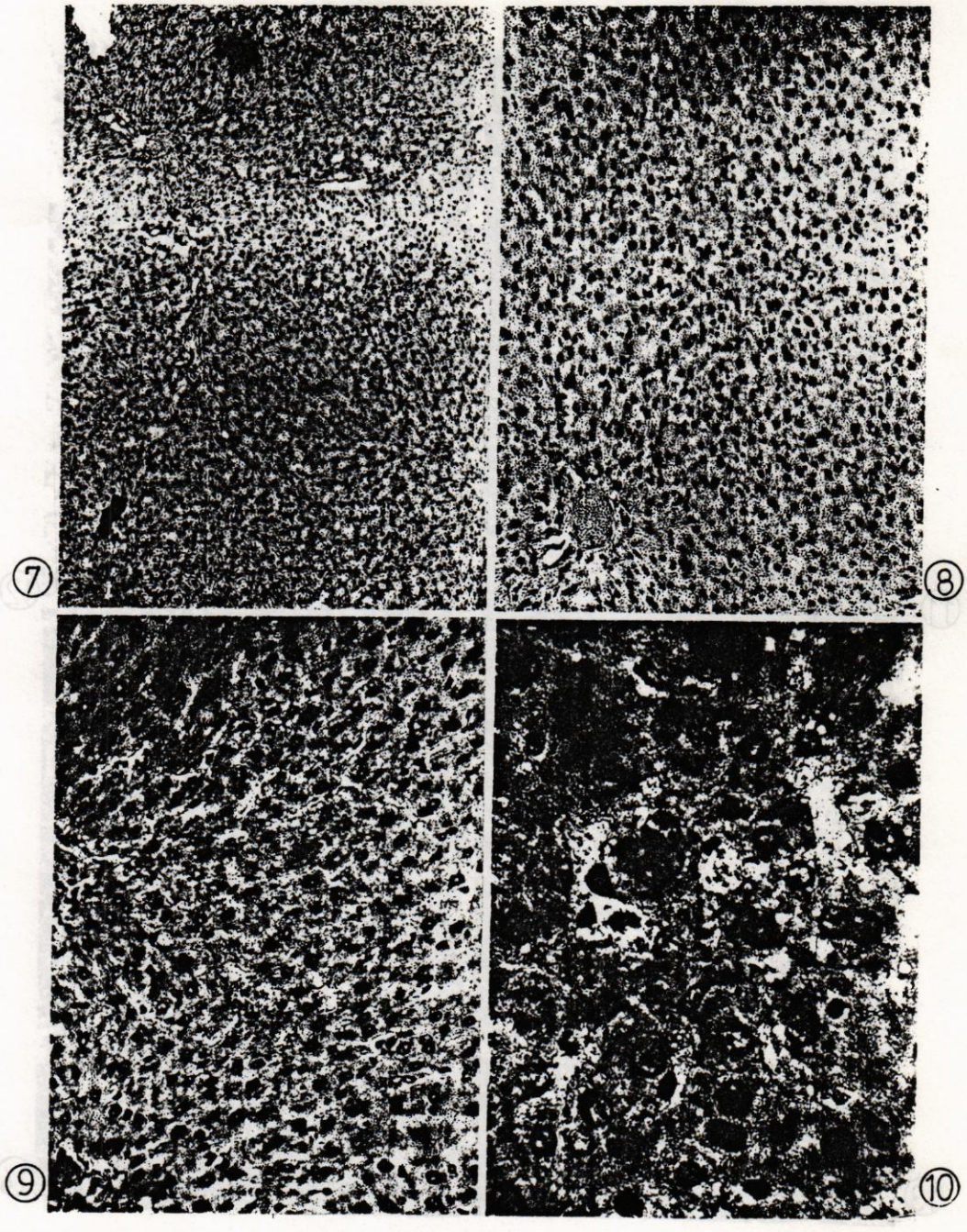
The hepatic cholesterol content decrease 33% after rBHC feeding while glucose and total protein remain unaltered. The soluble protein content are not affected after 24 hours of feeding, but show 30% increase after 48 hours of feeding. The hepatic FAA content are drastically decreased. These content decrease 29% and 52% after 24 and 48 hours after insecticide administration (Fig. 5, Table V).

DNA content do not show any significant deviation, while the RNA content are decreased 43 and 49%, respectively (Fig. 5, Table V).

3.1.4. HISTOLOGICAL STRUCTURE OF LIVER

Figure 6 and Table VI show the effect of feeding aldrin mixed diet at a dose of 30 mg/kg body weight/day for a total period of 48 hours on the various histological parameters of rat liver. As is obvious the hepatic cell, its nucleus and nucleolus are hypertrophied after insecticide treatment. The hepatic cell size increases 14% and 39% after 24 hours and 48 hours of feeding. The nuclear size increases 26% and 18%, while the size of nucleolus increases 42% and 35% during the same exposure period, respectively. The number of nuclei/cell and number of nucleoli/nucleus are not significantly altered after insecticide treatment.

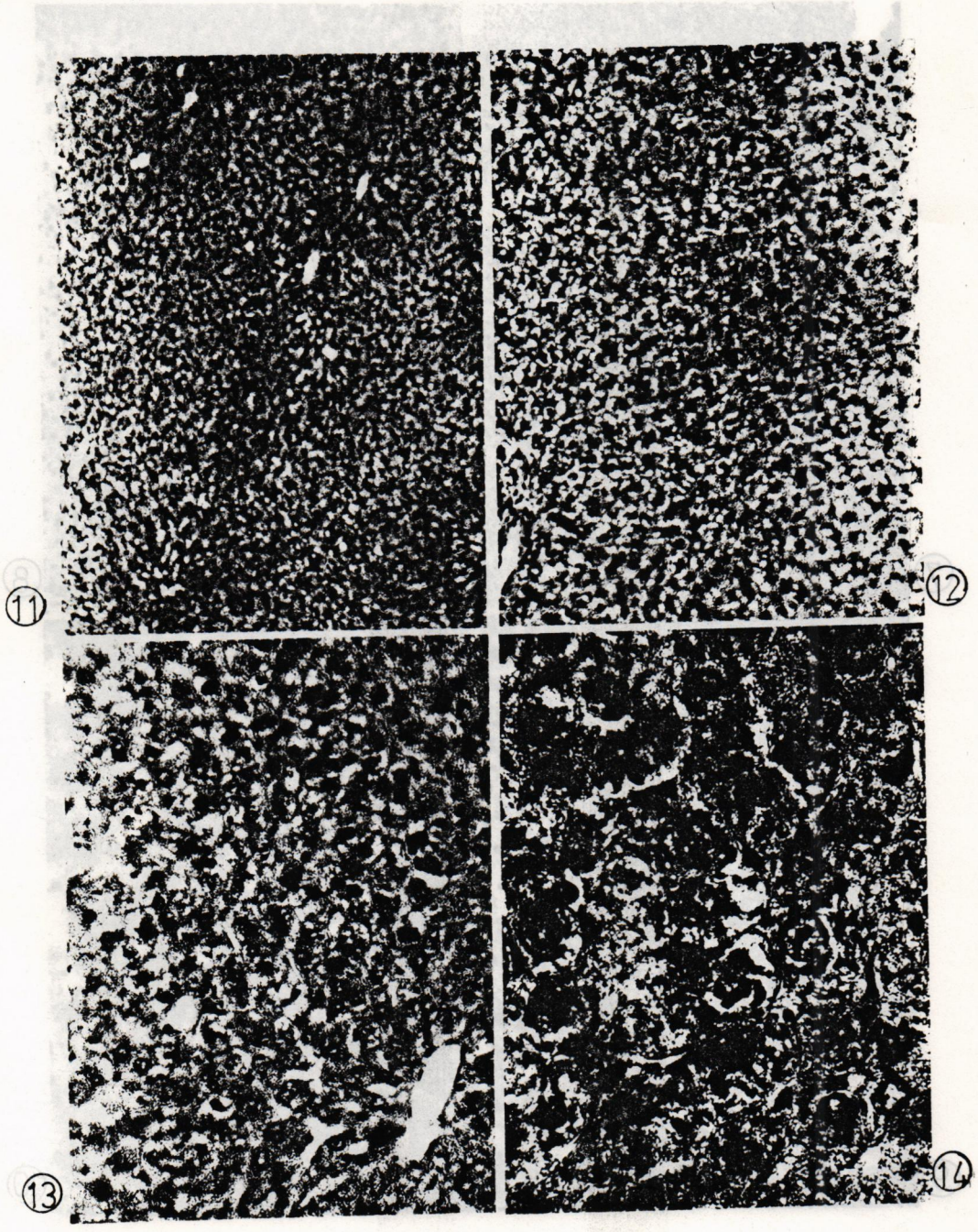
Figures 11-14 shows histological structure after 24 hours of rBHC treatment, while Figures 15-18 show histological structure of liver after 48 hours of insecticide treatment. The hepatolobular architecture is maintained, though very prominently hypertrophied



FIGS.7-10: Histological structure of normal rat liver. Note the normal hepatic lobule, central vein with portal areas (7,8), hepatic cord structure sinusoidal spaces with rod shape kupffer cells,cell and nuclear arrangement (9,10).

Stain: Hematoxylin and Eosin.

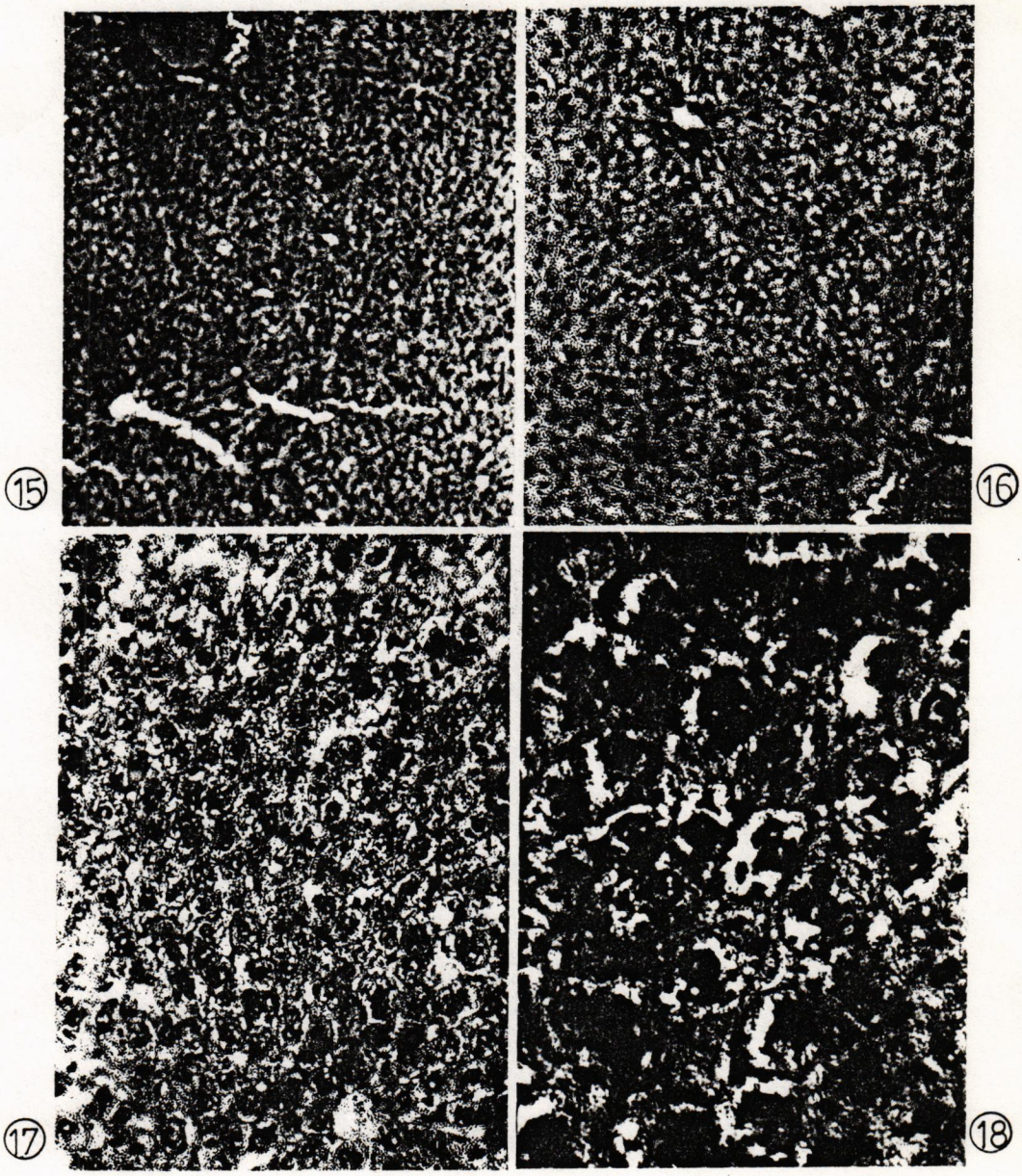
Magnifications: 7,x25; 8,x50; 9,x100; 10,x250.



FIGS.11-14: Histological structure of rat liver fed on gamma-BHC-mixed diet for 24 hours. Note the changes in lobular morphology, disorganised tissue (11,12), slightly increased sinusoidal area and vacuolation, irregular shape nuclei (13), hypertrophied cell and nuclei (14).

Stain: Hematoxylin and Eosin.

Magnifications: 11,x25; 12,x50; 13,x100; 14,x250.



FIGS.15-18: Histological structure of rat liver fed on gamma-BHC-mixed diet for 48 hours. Note disorganised lobular structure (15,16),nuclei with irregular margin (16, 17), cytoplasmic margination and hypertrophied cells (17,18).

Stain: Hematoxylin and Eosin.

Magnifications: 15,x25; 16,x50; 17,x100; 18,x250.

Control (n = 6)		
3 day (n = 3)	203.37±17.89	216.00±17.78
6 day (n = 3)	202.33±14.51	218.50±14.18
9 day (n = 3)	192.00±8.02	212.33±8.14
12 day (n = 3)	174.33±8.74	202.00±11.92
15 day (n = 3)	179.33±10.87	204.17±11.07

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

hepatic cells with vesiculated nuclei, prominent nucleoli and granulated cytoplasm (compare Figures 10,14 and 18).

3.2. EFFECT OF γBHC ADMINISTERED AT A DOSE OF 18 mg/kg body weight/day FOR A TOTAL PERIOD OF 15 DAYS

3.2.1. EFFECT ON BODY WEIGHT AND LIVER WEIGHT

Table VII shows the effect of feeding dieldrin mixed diet at a dose of 18 mg/kg body weight/day for a total period of 15 days on the total body weight and liver weight of albino rats. A control rat shows a growth rate of 1.99 ± 0.15% (n = 6) per day. This growth rate is significantly decreased after γBHC feeding. The percent growth rate in 3, 6, 9, 12 and 15 day feeding group is reduced to 1.78, 1.35, 1.18, 0.94 and 0.93%. Conversely the liver weight, when considered in terms of per cent of the body weight increases after insecticide feeding. The liver weight (% body weight) is 2.76 ± 0.05 (n = 6) in control rats, which is increased to 2.94 ± 0.05 after 15 days of feeding. This weight increase however, statistically non-significant.

3.2.2. HAEMATOLOGICAL STUDIES

Gamma BHC, like a typical insecticide exposure response has detrimental effect on the haemoglobin content of rat blood. A control rat blood contains 13.04 ± 0.16 g haemoglobin/dl of blood (n = 6), which after 15 days of insecticide feeding is reduced by 8% (Table VIII, Fig. 19). The number of RBC count and PCV is likewise significantly reduced. The RBC count decreases 13, 20, 18, 14 and 16% after 3, 6, 9, 12 and 15 days of feeding, while PCV shows 7, 14, 9, 8 and 9%

TABLE - VIII

EFFECT OF FEEDING BHC MIXED DILT (18 mg/kg body weight) FOR 15 DAYS ON THE HAEMATOLOGICAL PARAMETERS OF ALBINO RATS.

Parameters	Control (n = 6)	% BHC feeding				
		3 days (n = 3)	6 days (n = 3)	9 days (n = 3)	12 days (n = 3)	15 days (n = 3)
Hb (g/dl)	13.04±0.16[a]	12.70±0.12	11.14±0.56*	12.26±0.31	12.23±0.33	12.00±0.35*
RBC ($X10^5$ cells/ul)	69.10±1.20	59.86±1.53**	55.43±1.62***	57.03±2.43***	59.66±1.65**	58.13±1.88**
WBC ($X10^2$ cells/µl)	65.50±5.20	89.00±5.50*	82.66±4.34	91.33±5.34*	82.17±5.34	81.50±2.51*
PCV (%)	43.28±0.40	40.18±1.00*	37.18±0.63***	39.37±1.15*	39.66±0.76**	39.36±1.24*
MCV (fl)	62.64±0.46	67.12±0.61***	67.11±0.63*	69.11±0.97***	66.51±0.61*	67.73±0.71
MCH (Pg)	18.88±0.28	20.30±1.07	20.07±0.47	21.53±0.39***	20.50±0.13**	20.65±0.07***
MCHC (g/dl)	30.15±0.36	31.64±0.54	29.91±0.93	31.15±0.15*	30.82±0.83	30.49±0.34

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

C = CONTROL
3 = 3 DAYS γ- B H C FEEDING
6 = 6 DAYS γ- B H C FEEDING
9 = 9 DAYS γ- B H C FEEDING
12 = 12 DAYS γ- B H C FEEDING
15 = 15 DAYS γ- B H C FEEDING

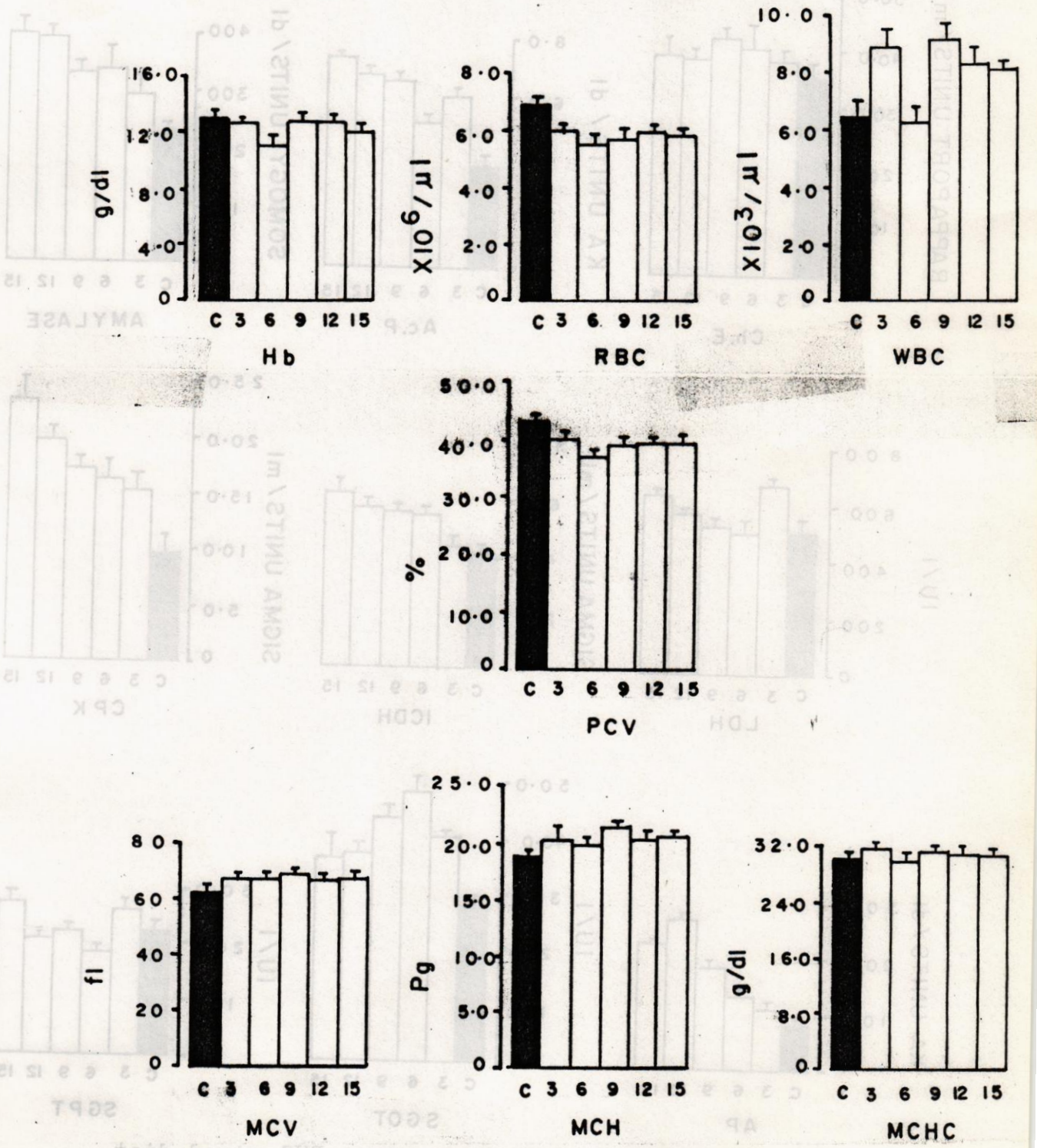


Fig.19. Effect of feeding gamma BHC mixed diet (18 mg/kg body weight/day) for 15 days on the various haematological parameters of rat blood.

C = CONTROL
3 = 3 DAYS γ- B H C FEEDING
6 = 6 DAYS γ- B H C FEEDING
9 = 9 DAYS γ- B H C FEEDING
12 = 12 DAYS γ- B H C FEEDING
15 = 15 DAYS γ- B H C FEEDING

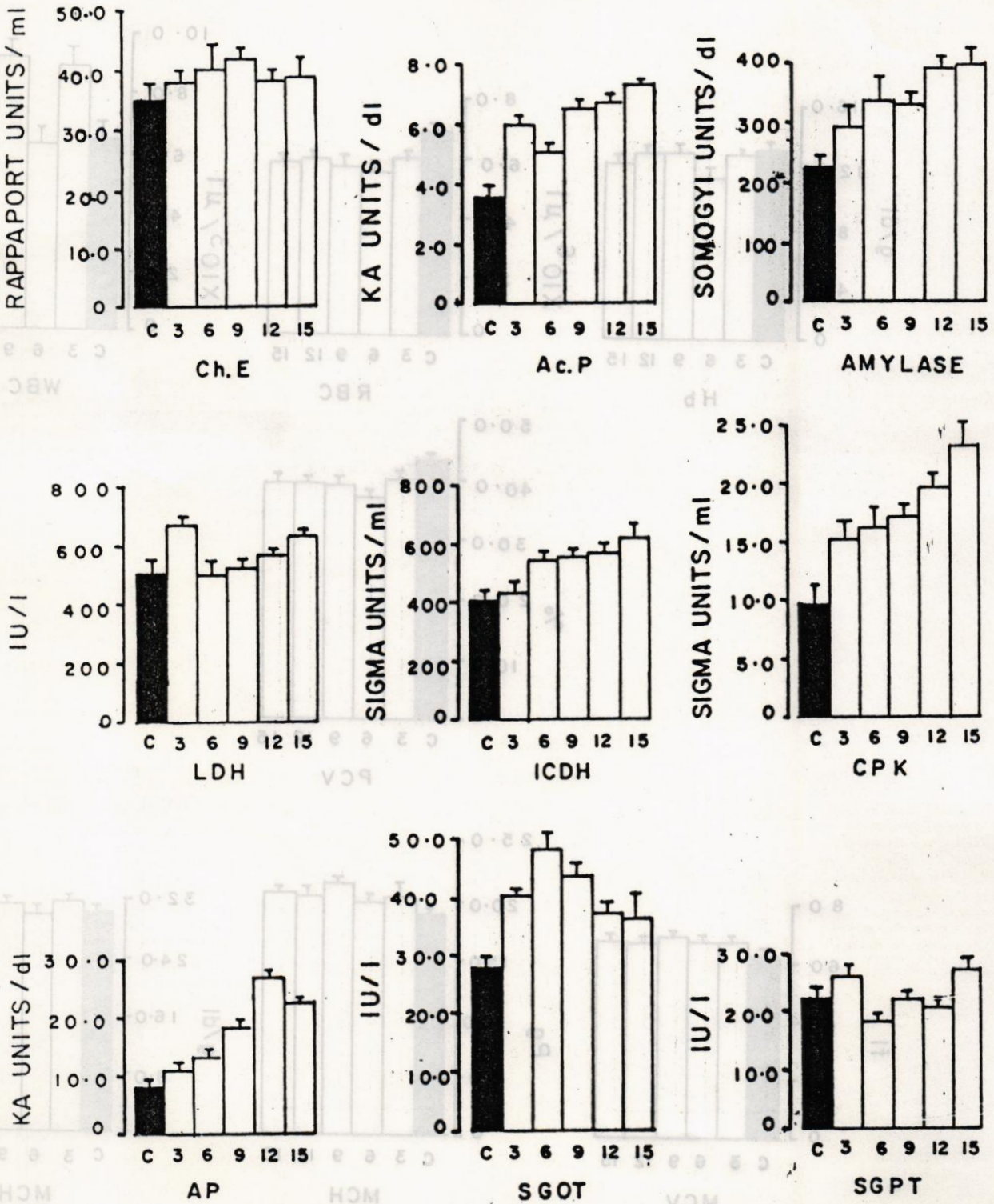


Fig.20. Effect of feeding gamma BHC mixed diet (18 mg/kg body weight/day) for 15 days on the activities of various enzymes of rat blood serum.

TABLE - IX

EFFECT OF FEEDING γ-BHC MIXED DIET (18 mg/kg body weight) FOR 15 DAYS ON THE ACTIVITIES OF VARIOUS ENZYMES IN RAT BLOOD SERUM

Parameters	Control (n=6)	BHC feeding 3 days (n=3)	6 days (n=4)	9 days (n=4)	12 days (n=3)	15 days (n=3)
AP (KAU/dl)	8.57[a] ± 0.39	11.22* ± 0.83	13.23*** ± 0.52	18.23*** ± 0.52	27.05*** ± 0.77	22.58*** ± 0.45
AcP (KAU/dl)	3.63 ± 0.26	6.03*** ± 0.21	5.11** ± 0.27	6.51*** ± 0.28	6.71*** ± 0.22	7.27*** ± 0.19
Amylase (Somogyi U/dl)	226.76 ±14.45	296.13 ±28.87	338.77* ±37.04	332.27** ±17.66	392.98*** ±15.82	398.59*** ±24.47
ChE (Rapp-aport U/ml)	34.77 ± 2.34	37.83 ± 1.74	40.00 ± 4.04	41.75* ± 1.61	38.33 ± 1.28	38.66 ± 2.91
CPK (Sigma U/ml)	9.66 ± 1.60	15.33* ± 1.45	16.20* ± 1.68	16.95** ± 0.76	19.80*** ± 0.92	23.13*** ± 1.69
GOT (IU/l)	27.80 ± 1.48	40.44*** ± 1.06	48.27*** ± 2.32	43.62*** ± 1.46	37.32** ± 1.21	36.66 ± 4.41
GPT (IU/l)	22.39 ± 1.60	26.15 ± 1.40	20 .21 ± 0.59	21.87 ± 1.03	20.63 ± 0.73	27.40 ± 1.91
ICDH (Sigma u/ml)	428.76 ±12.93	435.18 ±35.02	543.98** ±21.58	562.97*** ±12.12	570.72* ±38.58	625.42** ±49.06
LDH (IU/l)	506.56 ±21.91	678.72*** ±19.59	507.52 ±31.53	522.24 ±12.12	574.08* ±15.45	636.64** ±14.56

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

during the same experimental period. The WBC count, just as expected increases after rBHC feeding. The maximum increase of 40% was recorded after 9 days of feeding. The MCH in control rats is 18.8 ± 0.28 Pg (n = 6) which increases 6-14%, while MCHC remains unaltered after insecticide treatment (Fig. 19, Table VIII).

3.2.3. BIOCHEMICAL ANALYSIS OF BLOOD

Table IX and Figure 20 show the effect of feeding gamma BHC on the various enzymatic activities in rat blood serum. A control rat blood serum shows 8.57 ± 0.39 KAU of AP/100 ml and 3.63 ± 0.26 KAU of AcP/100 ml. Both these enzymes show drastic increase. The former enzymatic activity increases 31, 54, 113, 216 and 164%, while the latter enzyme shows 66, 41, 79, 85 and 106% increase after 3, 6, 9, 12 and 15 days of r-BHC feeding. Out of two transaminases tested, SGPT remains unaffected, while SGOT increased 45, 74, 57, 34 and 32% after 3, 6, 9, 12 and 15 days of insecticide feeding. The LDH activity increases34% after 3 days of insecticide feeding and shows 26% increase after 15 days of feeding. The ICDH activity is not significantly affected after 3 days, but shows27%, 31%, 37% and 46% increase after 6, 9, 12 and 15 days of lindane feeding. Besides phosphatases, CPK activity is also considerably affected. A control blood serum shows 9.66 ± 1.60 CPK activity in terms of sigma units/ml, which shoots up 59, 68, 76, 105 and 140% after insecticide treatment.

The Cholinesterase activity, generally remains unaltered except for 9 day treatment, when the ChE activity shows 20% increase over the normal enzymatic

		(n = 3)
Bilirubin (mg/dl)	0.76±0.04[a]	0.71±0.07
Cholesterol (mg/dl)	180.73±7.16	178.65±5.62
FAA (mg/dl)	9.81±0.14	7.13±0.44***
Glucose (mg/dl)	129.60±7.08	118.92±7.28
Protein (g/dl)	8.04±0.21	8.28±0.25
Urea (mg/dl)	33.56±1.19	28.19±0.82**

[a] Mean±SEM, Student's 't' test; * P < 0.0

level. The amylase activity also increases 31, 49, 47, 73 and 76% after 3, 6, 9, 12 and 15 days of γBHC feeding (Table IX, Fig.20).

Besides enzyme activities, several other serum components were also tested for ascertaining effect of γ-BHC on the general metabolic processes of rat. Figure 21 and Table X show the data in that context. The bilirubin content are not affected until day 9, when it increases 31%. After 15 days of feeding the bilirubin content increase 69%. The cholesterol content are not affected at all. The blood serum proteins increase 18 and 21% after 6 and 9 days of feeding, while the FAA content decrease considerably. The decrease is 27, 34, 35, 28 and 19% after γBHC feeding. The glucose and urea content likewise show significant decrease. The glucose content decrease 16% - 19% after 9, 12 and 15 days of insecticide treatment, while the blood urea shows 16-22% decrease during first 9 days of treatment. During the latter part of experimental period, the urea content does not show appreciable deviation.

3.2.4. BIOCHEMICAL ANALYSIS OF LIVER

All hepatic enzymes are elevated after γBHC feeding. AP, GPT, ICDH and LDH activities are raised within three days of feeding, while GPT activity is significantly affected after 6 days of feeding. The hepatic AP activity in control rats is 0.64 ± 0.02 KAU/g (n = 5), which increases 118, 147, 77, 64 and 34% after 3, 6, 9, 12 and 15 days of feeding (Table XI, Fig.22). The GPT activity increases 73, 104, 90, 73 and 65% after 3, 6, 9, 12 and 15 days of feeding. The GOT activity, on the other hand shows 81, 74, 67 and 21%

C = CONTROL
3 = 3 DAYS γ- B H C FEEDING
6 = 6 DAYS γ- B H C FEEDING
9 = 9 DAYS γ- B H C FEEDING
12 = 12 DAYS γ- B H C FEEDING
15 = 15 DAYS γ- B H C FEEDING

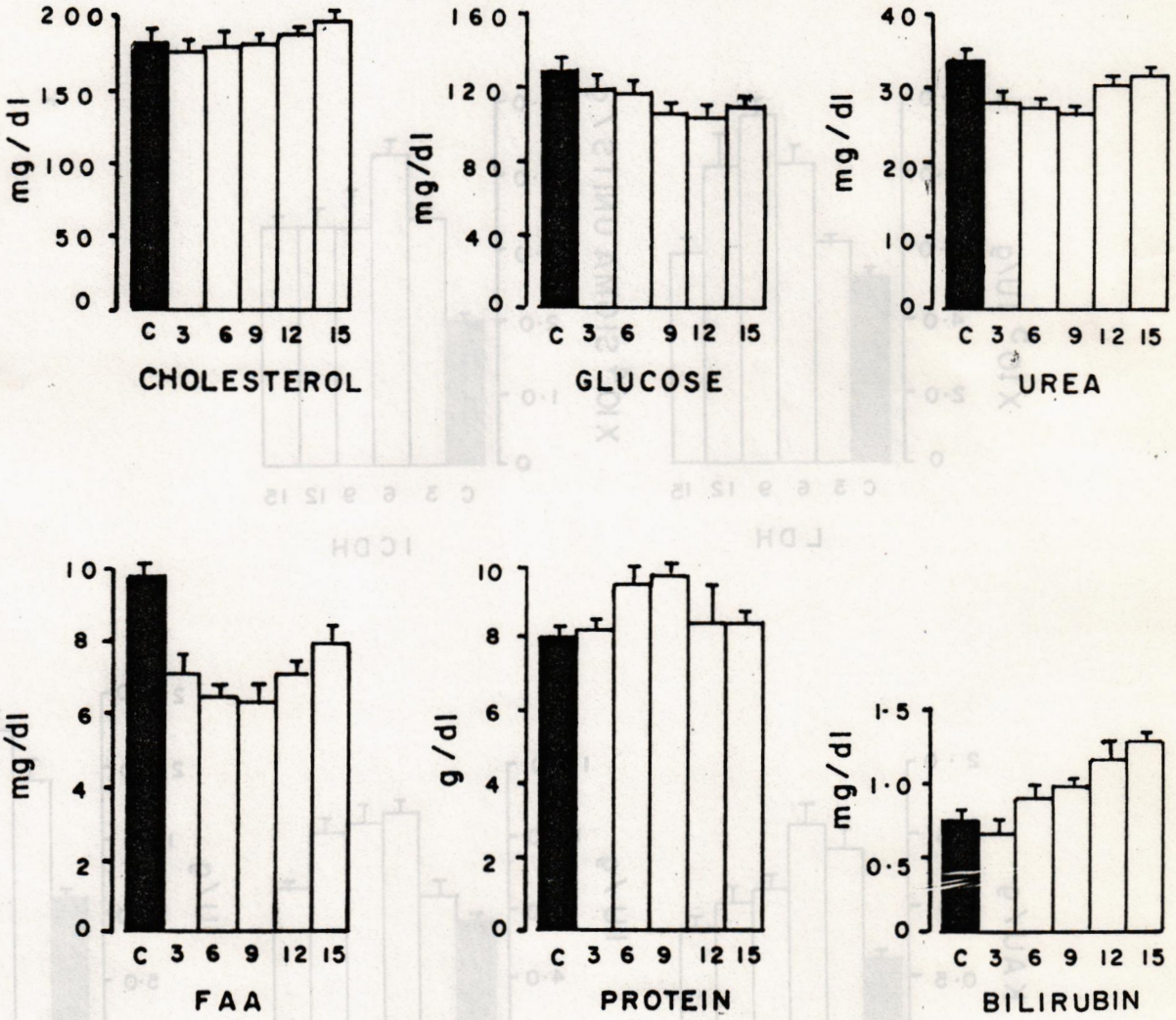


Fig. 21. Effect of feeding gamma BHC mixed diet (18 mg/kg body weight/day) for 15 days on the various biochemical components of rat blood serum.

C = CONTROL
3 = 3 DAYS γ- B H C FEEDING
6 = 6 DAYS γ- B H C FEEDING
9 = 9 DAYS γ- B H C FEEDING
12 = 12 DAYS γ- B H C FEEDING
15 = 15 DAYS γ- B H C FEEDING

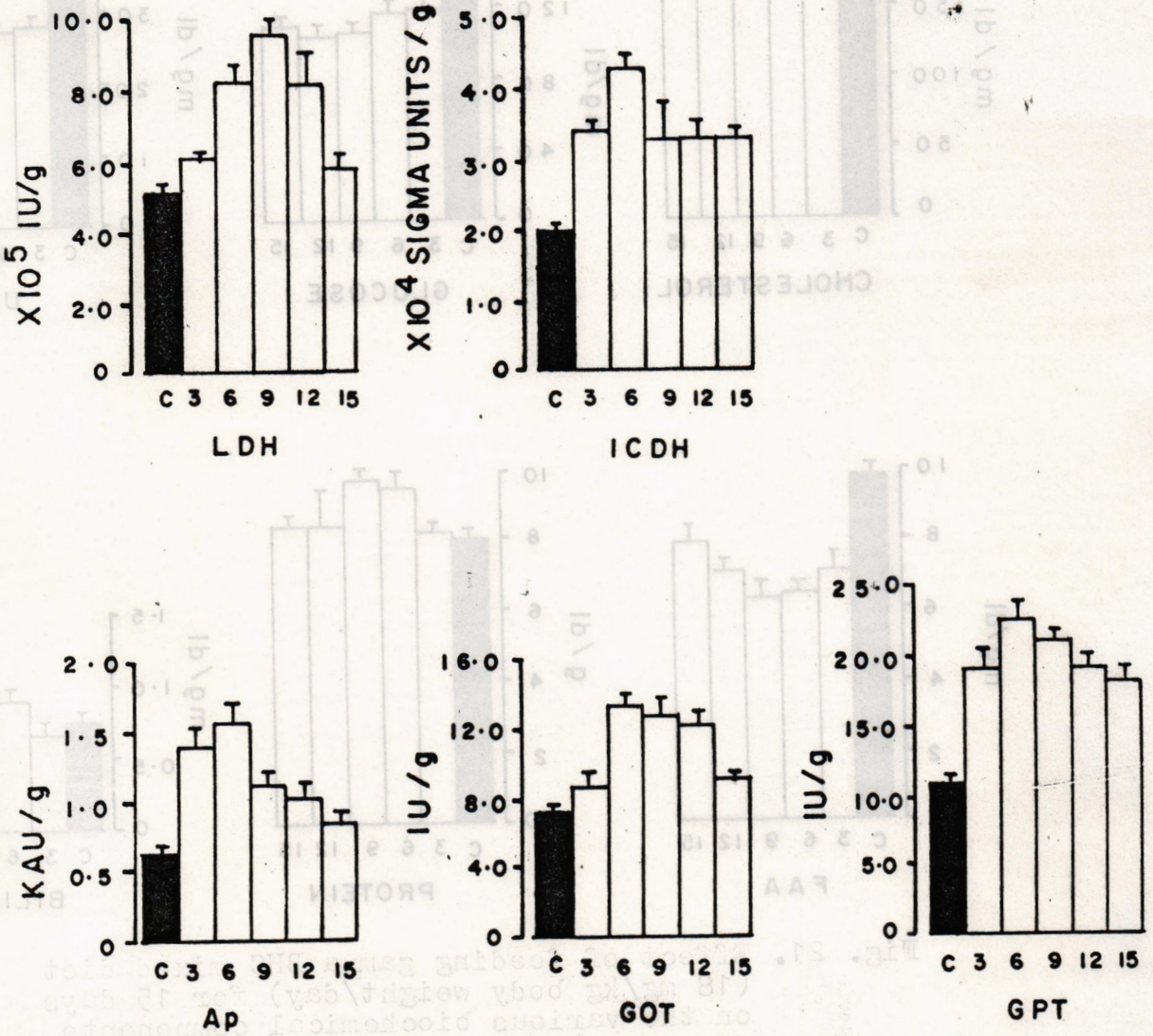


Fig. 22. Effect of feeding gamma BHC mixed diet (18 mg/kg body weight/day) for 15 days on the various hepatic enzymes activities in rat.

meters	3 days (n = 5)	5 days (n = 3)	(
AP (KAU/g)	0.64±0.02[a]	1.40±0.13**	
GOT (IU/g)	7.34±0.17	8.84±0.64	
GPT (IU/g)	11.07±0.25	19.15±1.38**	
ICDH ($X10^3$ sigma u/g)	20.10±0.50	34.59±0.88***	
LDH ($X10^4$ IU/g)	51.90±1.20	60.80±0.77***	

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

TABLE - XII

EFFECT OF FEEDING γ-BHC MIXED DIET (18 mg/kg body weight/day) FOR 15 DAYS ON THE VARIOUS BIOCHEMICAL COMPONENTS OF RAT LIVER.

Parameters	Control (n = 5)	BHC feeding				
		3 days (n = 3)	6 days (n = 3)	9 days (n = 3)	12 days (n = 4)	15 days (n = 4)
Cholesterol (mg/g)	15.72±0.35	7.40±0.40***	7.87±0.57***	6.73±0.48***	5.60±0.28***	5.82±0.34***
FAA (µg/g)	319.95±12.68	269.74±17.99	262.68±24.22	247.77±17.92*	186.58±8.51***	210.52±13.24***
Glucose (mg/g)	12.93±0.61	15.78±1.30	17.87±1.39*	20.16±1.96*	14.57±0.64	14.47±1.04
Soluble Protein (mg/g)	152.99±5.62	123.24±0.83**	126.32±6.69**	139.88±5.44	143.31±5.31	154.92±7.85
Total protein (mg/g)	237.67±7.45	207.14±11.99	218.54±5.21	225.27±6.10	228.56±15.34	241.52±12.53
DNA (mg/g)	2.42±0.17	4.44±0.20***	4.55±0.13***	5.46±0.29***	4.22±0.29**	3.91±0.20***
RNA (mg/g)	8.62±0.38	7.86±0.48	7.14±0.05**	5.94±0.09***	5.22±0.46***	5.80±0.95

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

C = CONTROL
3 = 3 DAYS γ- BHC FEEDING
6 = 6 DAYS γ- BHC FEEDING
9 = 9 DAYS γ- BHC FEEDING
12 = 12 DAYS γ- BHC FEEDING
15 = 15 DAYS γ- BHC FEEDING

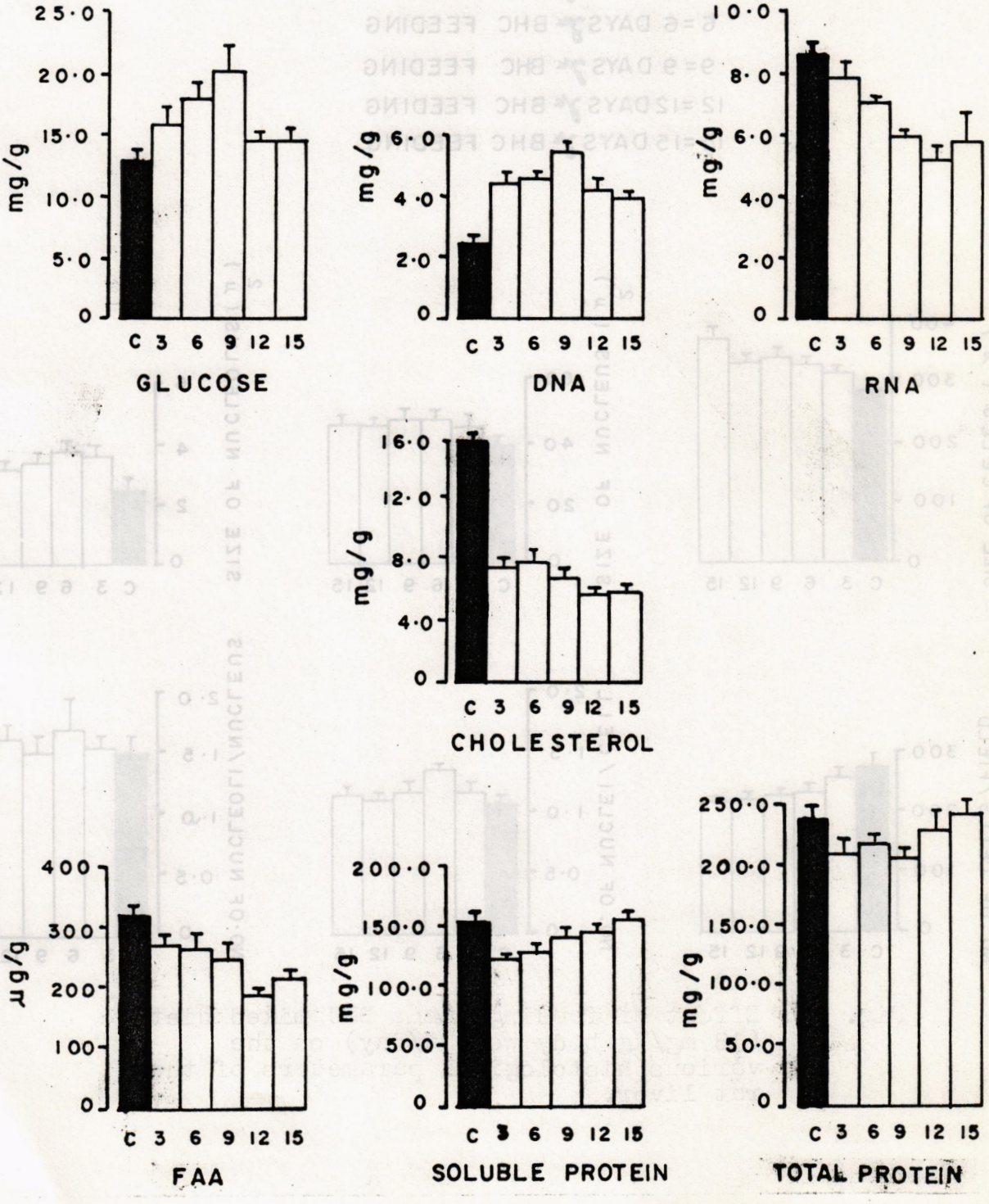


Fig. 23. Effect of feeding gamma BHC mixed diet (18 mg/kg body weight/day) on the various biochemical components of rat liver.

C = CONTROL
3 = 3 DAYS γ-BHC FEEDING
6 = 6 DAYS γ-BHC FEEDING
9 = 9 DAYS γ-BHC FEEDING
12 = 12 DAYS γ-BHC FEEDING
15 = 15 DAYS γ-BHC FEEDING

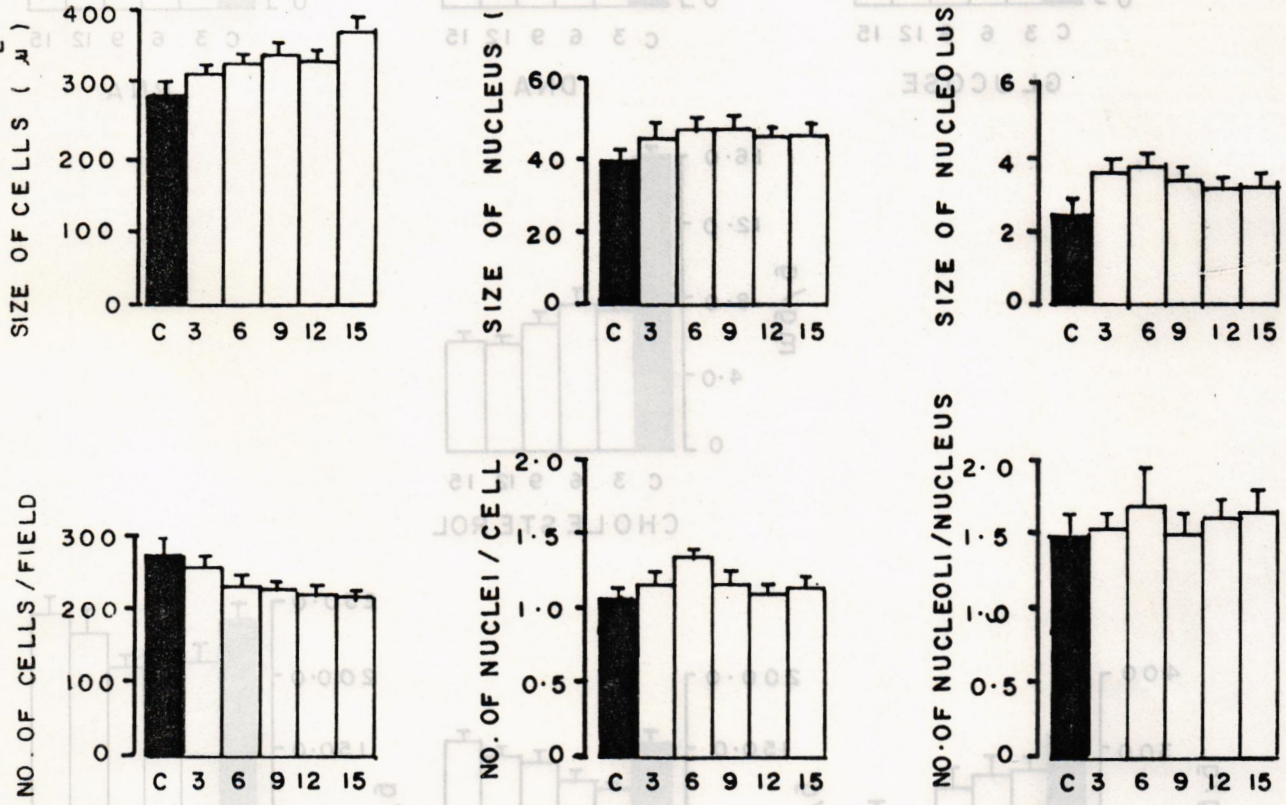


Fig. 24. Effect of feeding gamma BHC mixed diet (18 mg/kg body weight/day) on the various histological parameters of the rat liver.

No.of cells/ field	274.67[a] ±12.47	253.32 ± 8.40
No.of nuclei/ cell	1.08 ± 0.04	1.166 ± 0.04
No.ofnucleoli/ nucleus	1.49 ± 0.11	1.54 ± 0.09
Size of cell(μ^2)	289.23 ± 8.81	317.01* ± 5.61
Size of nucleus(μ^2)	39.45 ± 1.18	45.79* ± 1.49
Size of nucleolus(μ^2)	2.57 ± 0.30	3.68* ± 0.22

[a]Mean±SEM, students 't' test; *P <

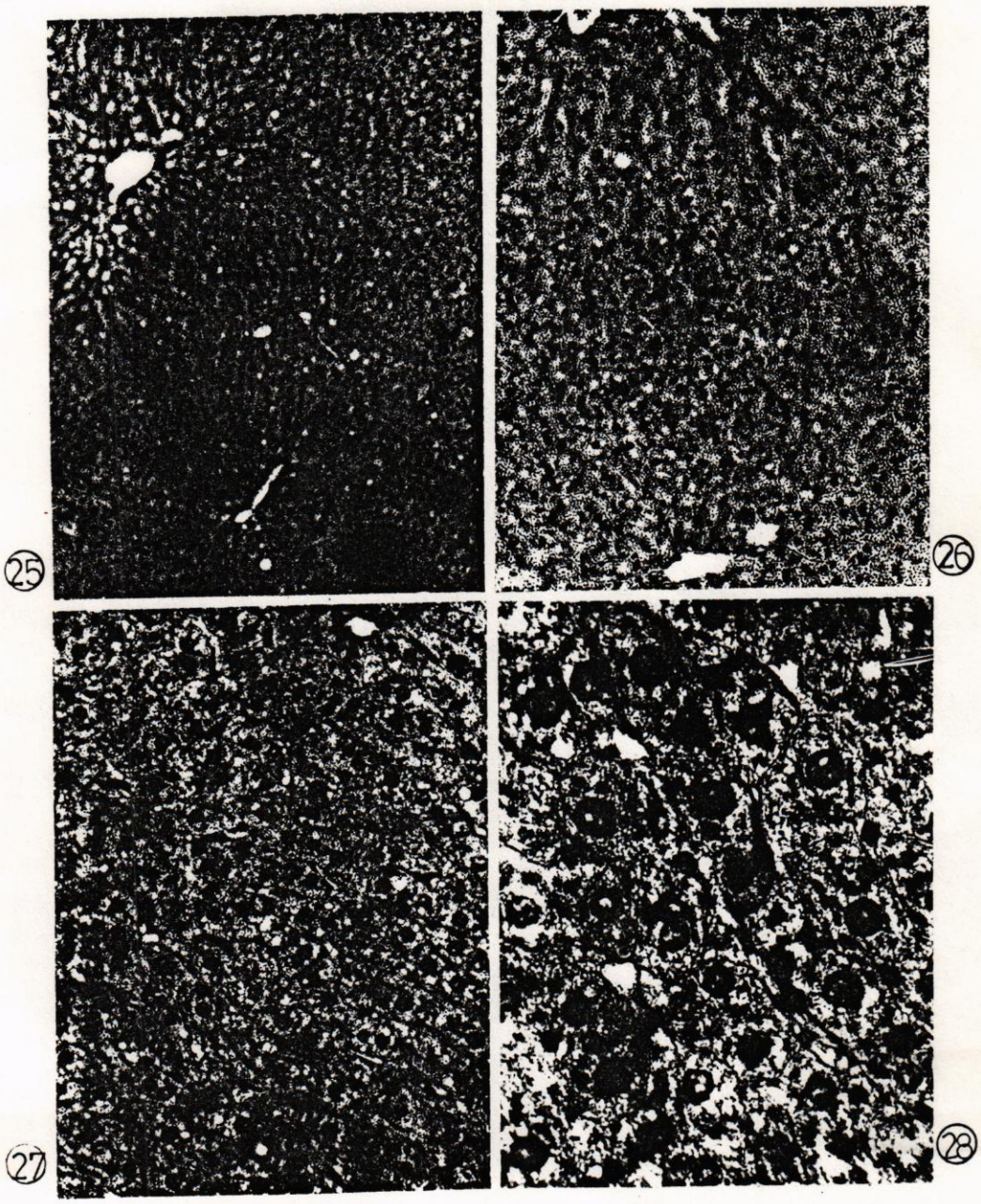
after 6, 9, 12 and 15 days of insecticide administration. The hepatic LDH and ICDH activities increase significantly. This increase is 17, 60, 85, 58 and 53% with case of LDH, and 72, 114, 64, 68, and 66% in the case of ICDH after 3, 6, 9, 12 and 15 days of γBHC feeding.

Table XII and Figure 23 show effect of γBHC feeding on the various hepatic biochemical components other than enzymes. The hepatic cholesterol content are most drastically reduced. The decrease varies from 50 - 64% during 15 days of insecticide feeding. The glucose content show increase after insecticide administration, but it is only during the first 9 days that alteration is significant. The total proteins are unaffected, although the soluble protein component is decreased 17-19% during the first week of insecticide administration. The FAA content also decrease, which is 16, 18, 23, 42 and 34% after 3, 6, 9, 12 and 15 days of feeding.

The hepatic nucleic acid component is considerably affected. The DNA content increase significantly. A control rat liver contains 2.42 ± 0.17 mg DNA/g liver tissue (n = 5). This content increases 84, 88, 123, 75 and 62% after 3, 6, 9, 12 and 15 days of γBHC feeding. The RNA content, conversely is significantly decreases, which is 9, 17, 31, 39, and 33% after 3, 6, 9, 12 and 15 days of lindane feeding (Table XII, Fig. 23).

3.2.5. HISTOLOGICAL STRUCTURE OF LIVER

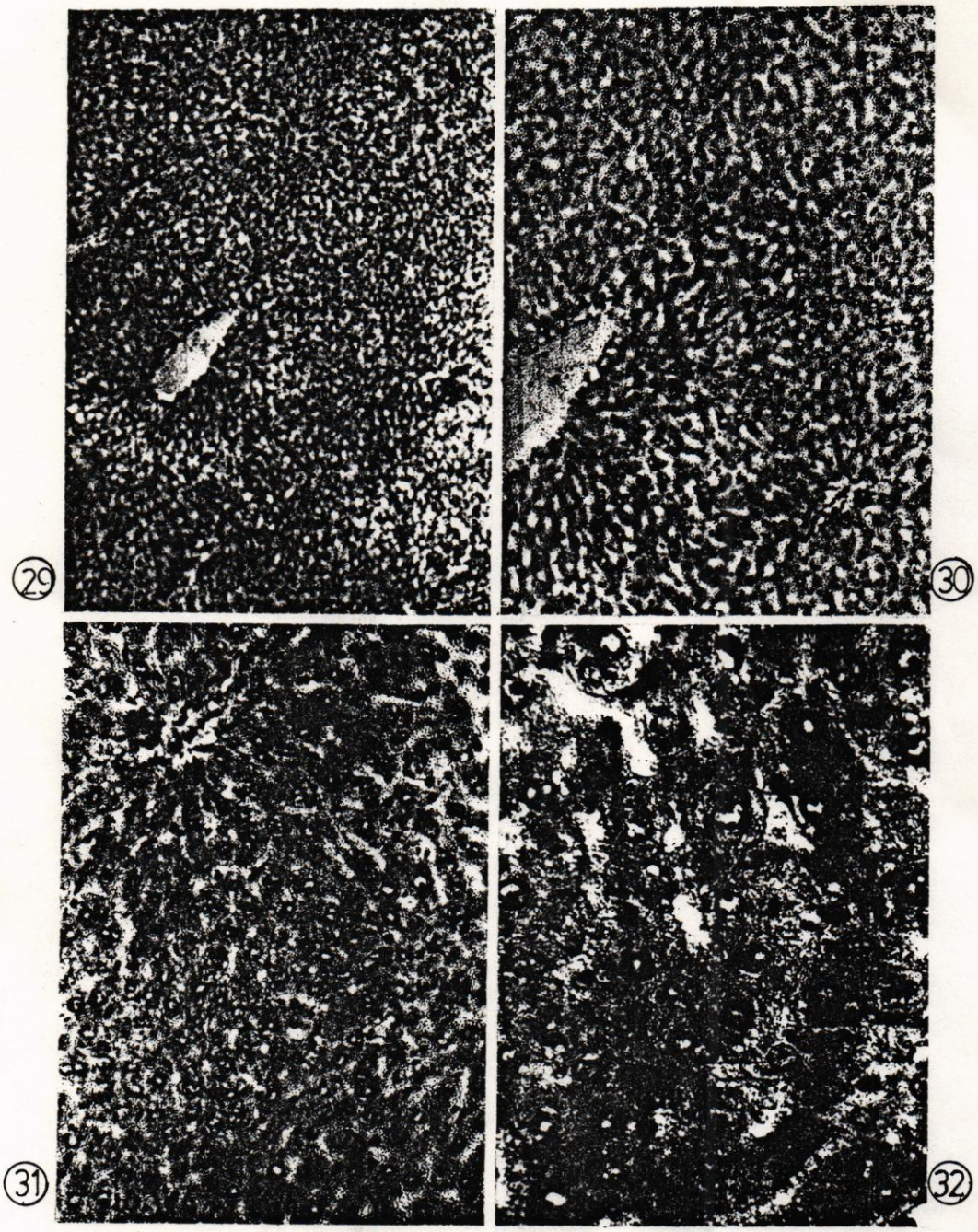
As a typical toxic response the hepatic cells, their nuclei and nucleoli increase in size (Table XIII, Fig. 24). A control hepatic cell measures 289.23 ± 8.81 μ^2, while its nucleus measures 39.45±1.18 μ^2 and



IGS.25-28: Histological structure of normal rat liver. Note a portion of liver lobule, with portal areas, hepatic cords (25-27), sinusoidal spaces, rounded nuclei and almost compact tissue (27-28).

Stain: Hematoxylin and Eosin.

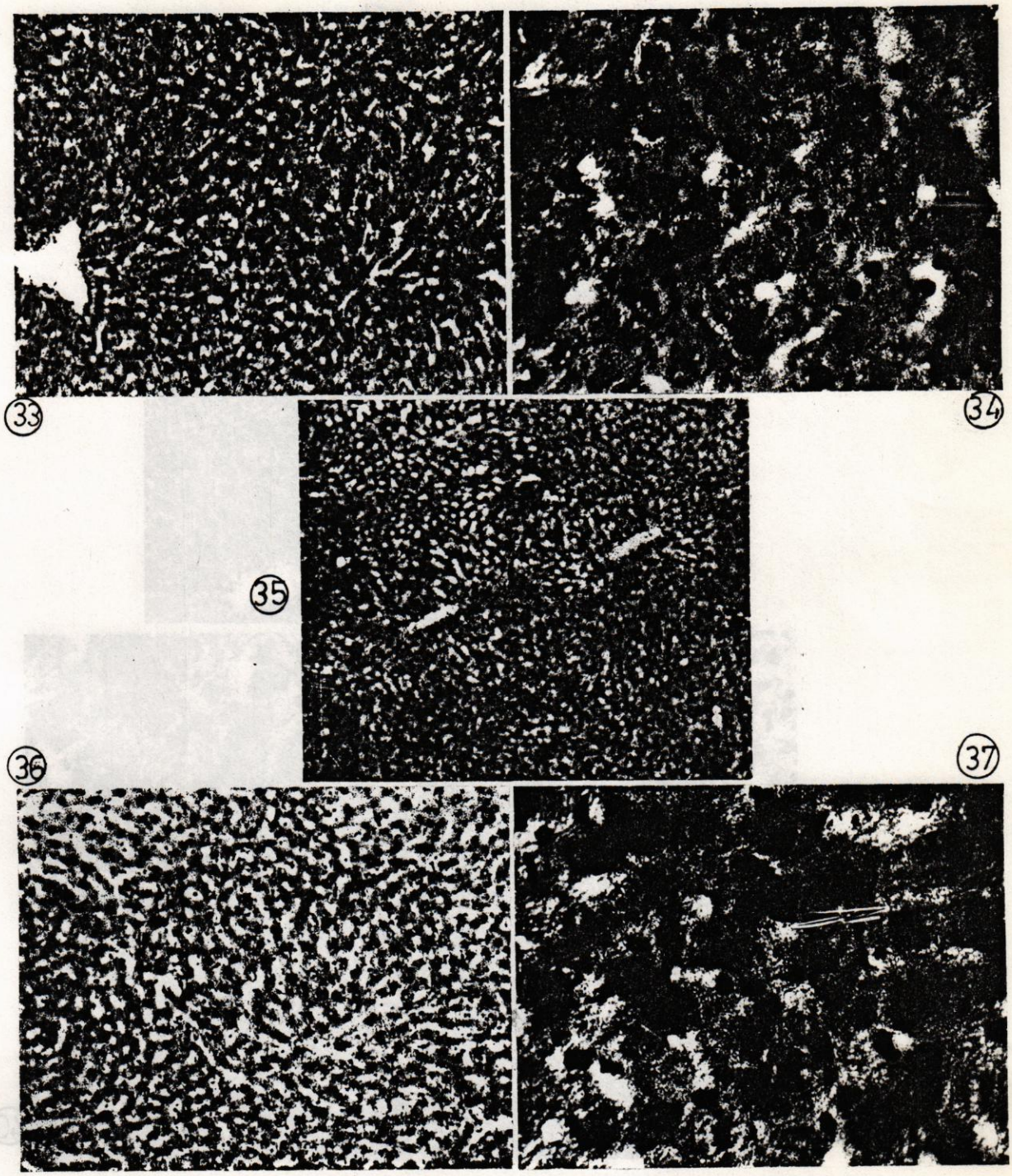
Magnifications: 25,x25; 26,x50; 27,x100; 28,x250.



FIGS.29-32: Histological structure of rat liver fed on gamma-BHC-mixed diet for 3 days. Note hepatic lobule with slightly disturbed cords with few lightly stained irregular zones (29,30) and numerous clear areas in the nucleus (30-32).

Stain: Hematoxylin and Eosin.

Magnifications: 29,x25; 30,x50; 31,x100; 32,x250.

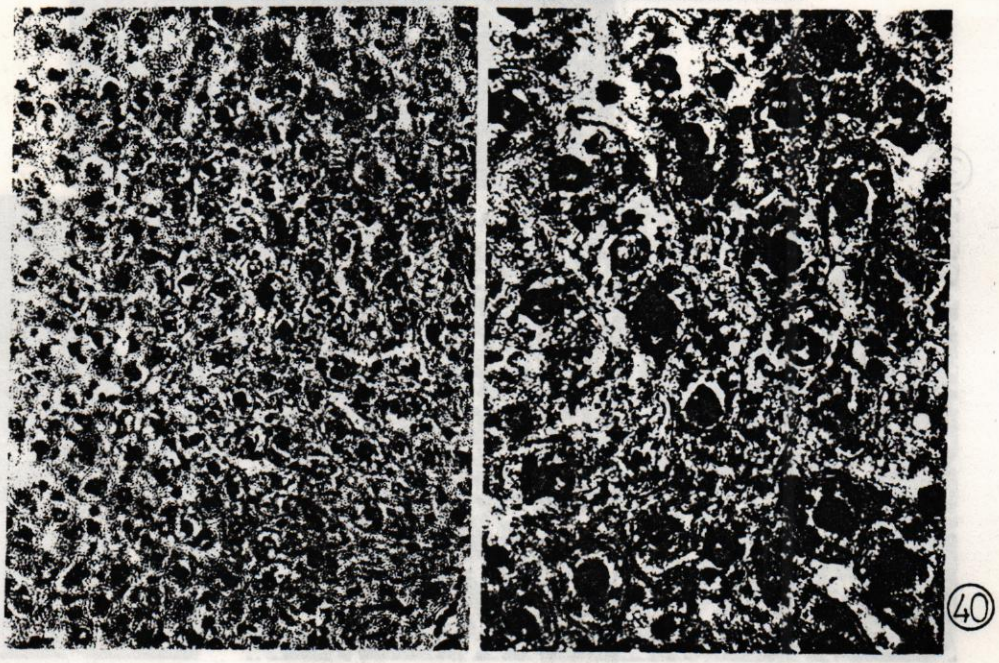


FIGS.33-37: Histological structure of rat liver fed on gamma-BHC-mixed diet for 6 days (33-34) and 9 days (35-37). Note disorganised lobular, and cord structure (33,35), increased sinusoidal spaces with swollen (33,36), almost rounded, kupffer cells,loss of cell structure with irregular nuclear margins (necrotic region) (34,37).

Stain: Hematoxylin and Eosin.

Magnifications: 33,35,x25; 36,x50; 34,37,x250.

(38)

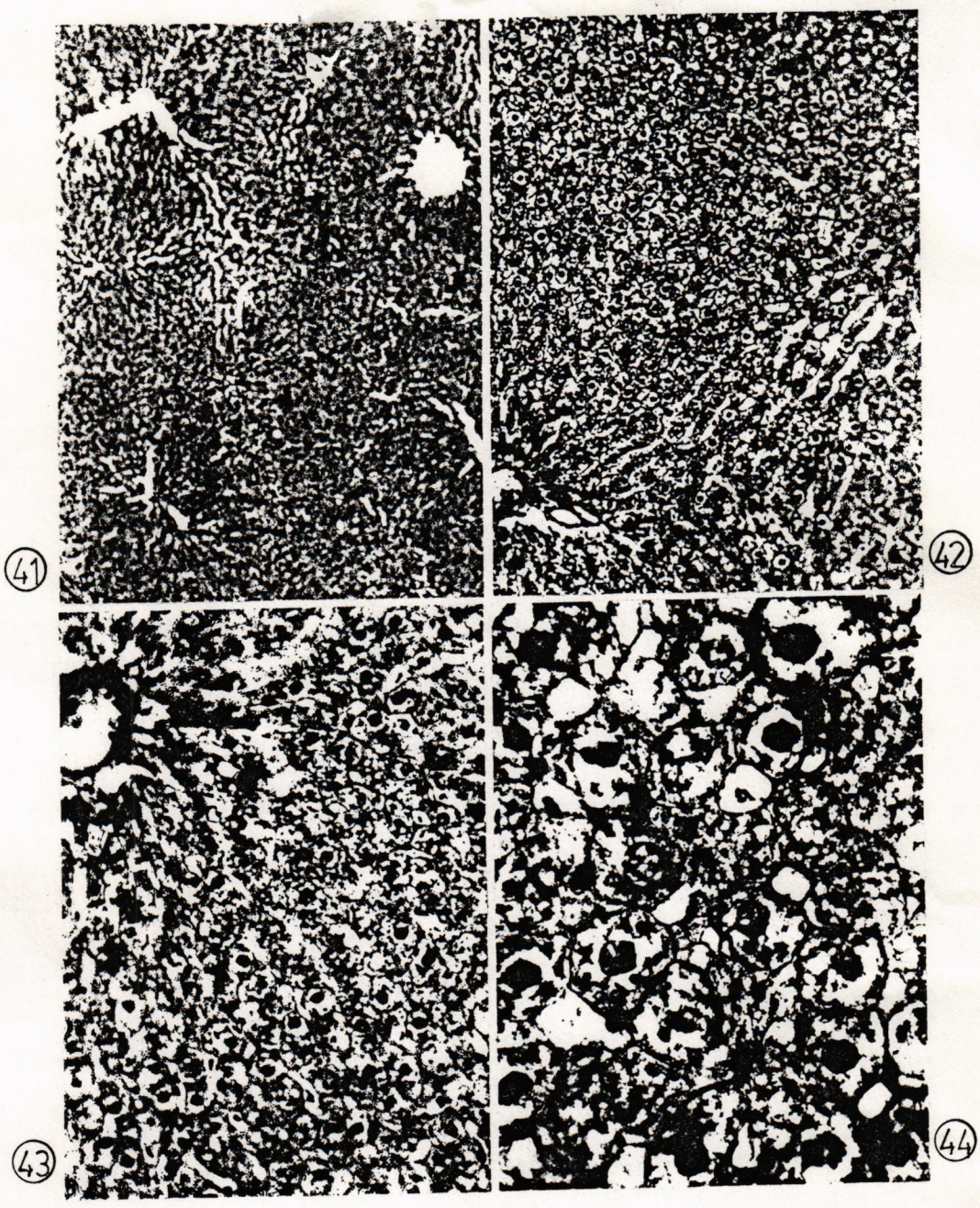


(39) (40)

FIGS. 38-40: Histological structure of rat liver fed on gamma-BHC-mixed diet for 12 days. Note disturbed lobular architecture with disorganised region (38,39), slight cytoplasmic margination and hypertrophied cells (39,40).

Stain: Hematoxylin and Eosin.

Magnifications: 38, x50; 39, x100; 40, x250.



FIGS. 41-44: Histological structure of rat liver fed on gamma-BHC-mixed diet for 15 days. Note disrupted hepatic lobular morphology with several portal areas (41), enlarged sinusoidal spaces (42,43), cytoplasmic margination with dull irregular shape nuclei and hypertrophied cells (42-44).

Stain: Hematoxylin and Eosin.

Magnifications: 41, x25; 42, x50; 43, x100; 44, x250.

nucleolus as 2.57 ± 0.30 μ^2. After γBHC feeding the hepatic cell size increases gradually and show 30% i increase after 15 days of insecticide feeding. The nuclei increase 20% during the same period. The nucleoli show 52% increase during first week of insecticide administration, although during the later part of the experimental period the nucleoli no longer show any significant deviation from the control size.

The number of nuclei/cell and the number of nucleoli/nucleus are not affected, although the number of cells/microscopical field decrease from 274.67 ± 12.47 (n = 5) to 213.15 ± 10.44 (n = 4) after 15 days of γBHC feeding.

Figures 29 - 44 show effect of γBHC on the histological structure of rat liver after feeding for 3 days (Figs. 29-32), 6 days (Figs. 33-34), 9 days (Figs. 35-37), 12 days (Figs. 38-40) and 15 days (Figs. 41-44). The histological structure of control rats, who have been fed on normal diet is given in Figures 25-28.

The hepatolobular architecture is maintained. The nuclei are compact structures, with distinct vacuolar spaces which appear prominently during the first 3 day treatment (Figs. 31-32). The cytoplasm is not granulated until day 15, when vacuoles appear in the cytoplasm and slight granulation is visible (Fig.44). Although the hepatic cell and nuclei appear to be larger in size, but the typical indication of insecticide toxicity i.e. vacuolation is not distinilty visible in these groups of animals.

3.3. EFFECT OF GAMMA BHC ADMINISTERED AT A DOSE OF 9 mg/kg body weight/day FOR A PERIOD OF 6-18 MONTHS

3.3.1. BODY WEIGHT AND LIVER WEIGHT

Table XIV shows the effect of long term feeding of gamma BHC on the Body weight/liver weight ratio and the weight of liver (in terms of % of the body weight). The body weight liver weight ratio decreases 4 and 9% during 6 and 12 months feeding respectively, while a significant decrease of 30% was recorded after 18 months of feeding. The liver weight behaved exactly in the same pattern. It is only after 18 months of feeding that about 24% increase was observed in liver weight considered in terms of percent of body weight.

3.2.2. HAEMATOLOGICAL STUDIES

Gamma BHC (=Lindane) administered for a period of 6-18 months does not appear to have brought about any drastic change in haematological parameters as was seen in other chlorinated insecticides (Fig. 45, Table XV). The haemoglobin content show slight decrease from the control level, but except for 6 month observation, which show 10% significant decrease, all other deviations are non significant. The RBC count is significantly decreased, which is 19%, 14% and 10% after 6, 12 and 18 months of feeding. The WBC count, on the other hand, does not change significantly. The PCV behaves just like Haemoglobin content. The six month feeding causes about 12% decrease, while prolonged feeding does not have any significant effect. The MCV and MCH is increased after γBHC feeding. The MCH shows 11, 14 and 8% increase, while MCV shows 10, 15 and 10%

Parameters	Control (n = 6)	ABHC fed (n = 4)
Body wt/ liver wt.ratio	39.27±0.73[a]	37.84±2.80
Liver wt. (% body wt.)	2.55±0.05	2.69±0.19

[a]Mean±SEM, Student's 't' test; *P< 0.0

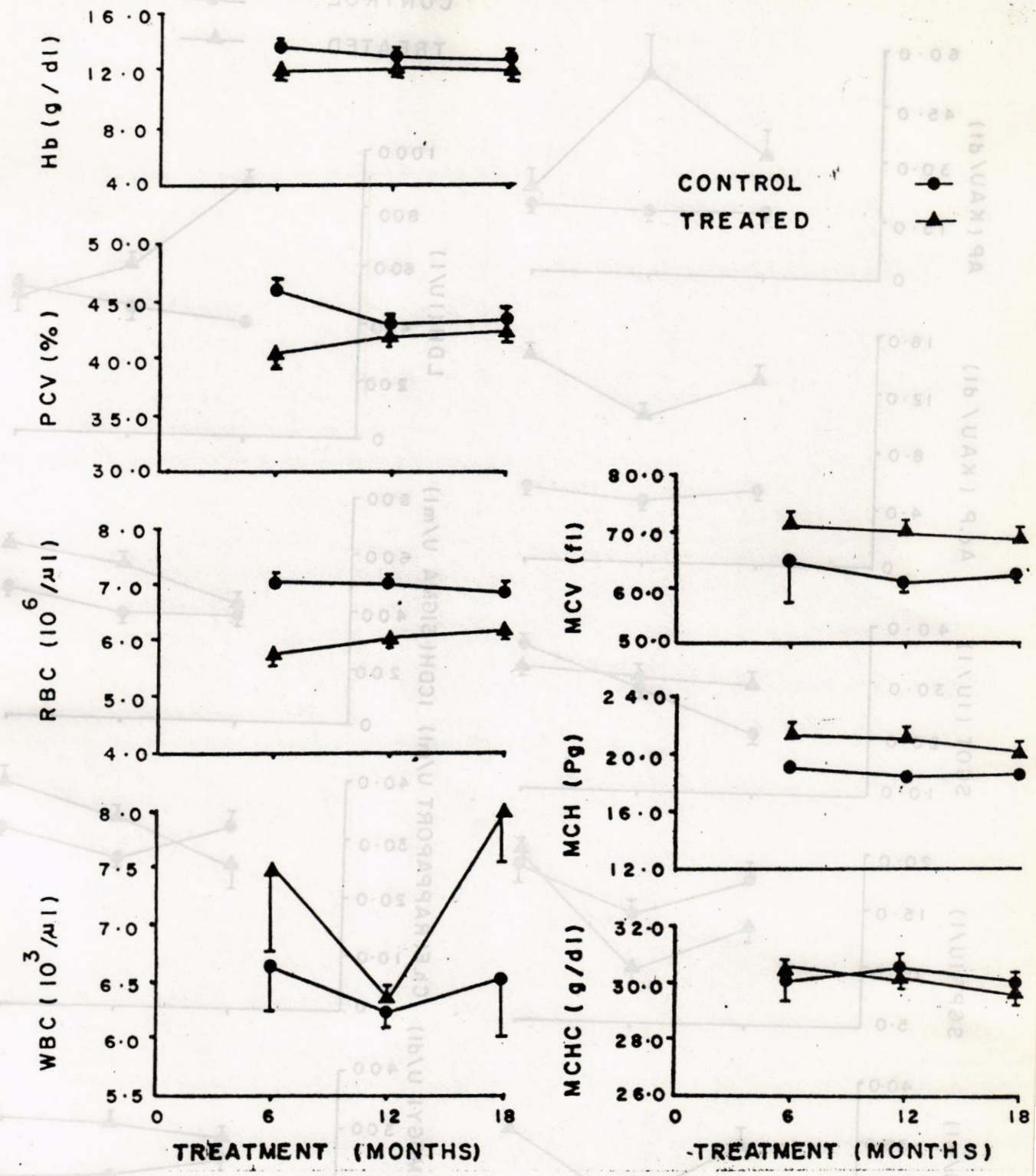


Fig.45. Effect of feeding gamma BHC mixed diet (9 mg/kg body weight/day) on 6-18 months on the various haematological parameters of albino rats.

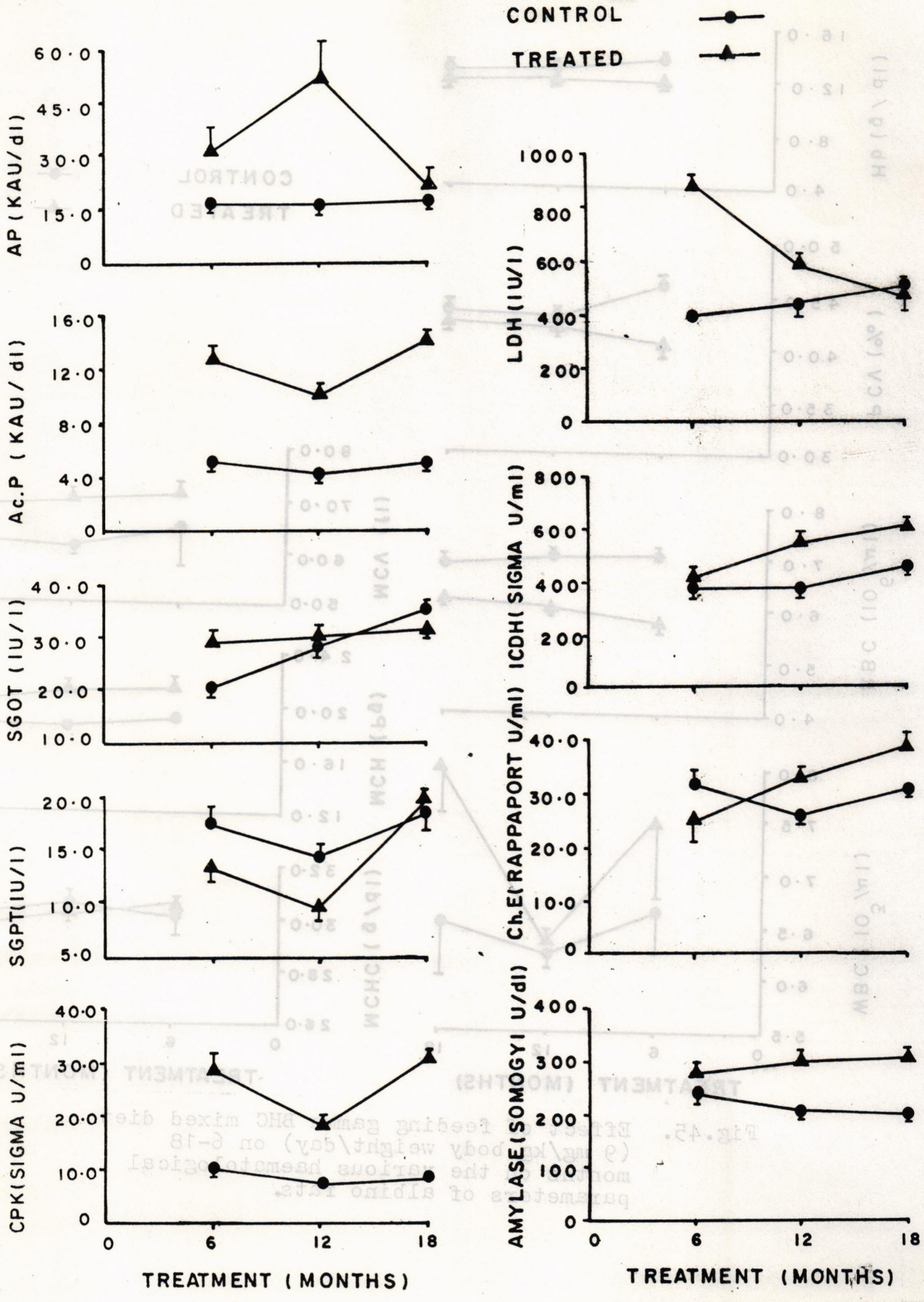


Fig.46. Effect of feeding gamma BHC mixed diet (9 mg/kg body weight/day) for 6-18 months on the activities of various enzymes in rat blood serum.

increase, in 6, 12 and 18 month groups, respectively. The MCHC remains unaltered (Table XV, Fig. 45).

3.3.3. BIOCHEMICAL ANALYSIS OF BLOOD

All blood serum enzymes, except for SGOT and SGPT increase after long term feeding of γ-BHC and generally follow the same pattern (Fig. 46; Table XVI). The SGPT decreases 23 and 34% after 6 and 12 months of γ-BHC administration, but the enzyme activity is recovered inspite of continuous feeding for another 6 months. The SGOT activity on the other hand is significantly increased (41%) after 6 months of feeding, but then later on does not shown any deviation from the normal after prolonged feeding upto 12 or 18 months.

From amongst the most drastically affected enzymes, phosphatases (both acidic and alkaline) are at the top. AP activity increases 236, 460 and 113% while AcP activity increases 140, 131 and 171% after 6, 12 and 18 months of feeding.

The two dehydrogenases follow opposite routes after γ-BHC feeding. The LDH activity shows 123% increase in 6 months and 33% increase in 12 month group, while after 18 months of feeding the LDH activity shows no deviation at all. The ICDH activity, on the other hand, shows significant change (33% increase) after 12 months of γ-BHC feeding, while the same increase after 18 months of feeding is 32%.

The CPK activity is significantly increased (102%) within 6 months of feeding, while this increase after 12 and 18 months of feeding is respectively, 147

TABLE - XV

EFFECT OF FEEDING γ-BHC MIXED DIET (9 mg/kg body weight/day) FOR 6-18 MONTHS ON THE VARIOUS HAEMATOLOGICAL PARAMETERS OF ALBINO RATS.

Parameters	6 months γBHC feeding experiment		12 months γBHC feeding experiment		18 months γBHC feeding experiment	
	Control (n=6)	γBHC fed (n=4)	Control (n=4)	γBHC fed (n=3)	Control (n=6)	γBHC fed (n=4)
Hb (g/dl)	13.79[a] ± 0.32	12.36** ± 0.26	13.14 ± 0.23	12.83 ± 0.14	13.04 ± 0.16	12.66 ± 0.17
RBC ($X10^5$ cells/µl)	70.87 ± 1.26	57.50** ± 2.48	70.48 ± 1.28	60.40*** ± 1.25	69.10 ± 1.20	62.28** ± 1.45
WBC ($X10^2$ cells/µl)	66.50 ± 3.99	75.00 ± 7.60	62.38 ± 1.34	63.33 ± 1.09	65.50 ± 5.20	80.88 ± 4.60
PCV (%)	45.92 ± 0.44	40.55*** ± 0.94	42.89 ± 0.19	42.18 ± 0.55	43.28 ± 0.40	42.64 ± 0.22
MCV (fl)	64.38 ± 0.65	70.71** ± 1.60	60.91 ± 0.86	69.86*** ± 0.56	62.64 ± 0.46	68.56** ± 1.29
MCH (Pg)	19.45 ± 0.32	21.56** ± 0.47	18.64 ± 0.26	21.24*** ± 0.10	18.88 ± 0.28	20.35** ± 0.21
MCHC (g/dl)	30.24 ± 0.65	30.49 ± 0.21	30.62 ± 0.41	30.40 ± 0.36	30.15 ± 0.36	29.69 ± 0.29

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

TABLE - XVI

EFFECT OF FEEDING γ-BHC MIXED DIET (9 mg/kg body weight/day) FOR 6-18 MONTHS FOR ACTIVITIES OF VARIOUS ENZYMES OF RAT SERUM.

Parameters	6 months γBHC feeding experiment		12 months γBHC feeding experiment		18 months γBHC feeding experiment	
	Control (n=6)	γBHC fed (n=4)	Control (n=4)	γBHC fed (n=3)	Control (n=6)	γBHC fed (n=4)
AP (KAU/dl)	9.57[a] ± 0.39	32.19*** ± 2.41	9.46 ± 0.68	52.46*** ± 3.24	10.21 ± 0.97	21.78*** ± 0.45
AcP (KAU/dl)	5.46 ± 0.48	13.11*** ± 1.11	4.48 ± 0.25	10.35*** ± 0.49	5.34 ± 0.59	14.46*** ± 0.52
Amylase (Somogyi U/dl)	242.53 ±16.74	279.24 ±15.97	210.29 ±10.57	299.03*** ±10.42	202.03 ±12.22	306.25*** ±14.77
ChE (Rappaport u/ml)	31.80 ± 3.76	25.33 ± 3.38	26.00 ± 1.85	33.00* ± 1.80	31.08 ± 1.82	38.75* ± 2.31
CPK (Sigma U/ml)	10.30 ± 0.77	29.03*** ± 3.20	7.57 ± 0.61	18.66*** ± 0.58	8.75 ± 0.97	31.25*** ± 0.75
SGOT (IU/l)	21.02 ± 1.46	29.58* ± 2.54	28.98 ± 2.73	30.22 ± 0.48	35.66 ± 2.55	32.00 ± 1.69
SGPT (IU/l)	17.72 ± 1.40	13.62* ± 1.26	14.82 ± 0.99	9.77* ± 1.42	18.75 ± 1.87	19.20 ± 0.99
ICDH (Sigma U/ml)	391.86 ±28.33	401.14 ±22.35	395.23 ±10.33	547.02*** ±11.14	462.53 ±24.13	609.01** ±16.77
LDH (IU/l)	401.44 ±10.87	895.80*** ±18.93	442.20 ±49.62	586.24 ±47.94	509.49 ±27.82	501.12 ±58.43

[a] Mean±SEM, Student's 't' test;

*P < 0.05; **P < 0.01; ***P < 0.001

and 257%.

The ChE and amylase activities are affected after 12 months of feeding. The six month group does not show any appreciable change. The ChE activity is increased 27 and 25%, while amylase activity is increased 42 and 52% after 12 and 18 months of feeding (Table XVI, Fig. 46).

A few other biochemical components of rat blood serum were also tested to find out the effect of long term feeding of γ-BHC to albino rats for 6-18 months, (Table XVII, Fig. 47). The bilirubin content decrease 18 and 36% during the first 6 and 12 months of feeding, but after 18 months of prolonged feeding the bilirubin content increase 30%. The Cholesterol content remain unchanged except for 12 month feeding group, when an increase of 23% was recorded. The FAA content likewise showed no appreciable throughout except for 3 month feeding group when a 24% increase was observed. The blood serum protein content are totally unaffected, while urea content show a significant decrease of 18% after 18 months of feeding. The only component, which is consistantly deviated is blood sugar level. The glucose content increase 47 and 31% after 6 and 12 months feeding, while a significant decrease of 20% was observed after 18 months of γ-BHC feeding (Table XVII, Fig. 47).

3.3.4. BIOCHEMICAL ANALYSIS OF LIVER

Figure 48 and Table XVIII show the effect of feeding γ-BHC to male albino rats on the activities of hepatic enzymes, while Figure 49 and Table XIX show

TABLE - XVII

EFFECT OF FEEDING γ-BHC MIXED DIET (9 mg/kg body weight/day) FOR 6-18 MONTHS ON THE VARIOUS BIOCHEMICAL COMPONENTS OF ALBINO RAT BLOOD SERUM

Parameters	6 months γBHC feeding experiment		12 months γBHC feeding experiment		18 months γBHC feeding experiment	
	Control (n=6)	γBHC fed (n=4)	Control (n=4)	γBHC fed (n=3)	Control (n=6)	γBHC fed (n=4)
Bilirubin (mg/dl)	0.73[a] ± 0.60	0.52* ± 0.06	0.63 ± 0.03	0.41** ± 0.04	0.56 ± 0.04	0.73** ± 0.03
Cholesterol (mg/dl)	182.95 ± 6.25	196.09 ± 6.91	177.19 ± 5.21	218.22*** ± 2.36	180.12 ± 9.80	199.11 ± 9.62
FAA (mg/dl)	9.27 ± 0.34	11.45** ± 0.48	9.00 ± 0.36	9.92 ± 0.19	11.09 ± 2.33	10.31 ± 0.23
Glucose (mg/dl)	114.76 ± 8.52	168.25* ± 14.75	107.45 ± 7.26	140.47* ± 3.92	150.57 ± 3.63	120.34*** ± 3.40
Protein (g/dl)	6.90 ± 0.04	7.18 ± 0.29	7.68 ± 0.21	8.15 ± 0.32	8.58 ± 0.45	7.69 ± 0.27
Urea (mg/dl)	36.34 ± 2.20	32.65 ± 4.43	23.55 ± 6.84	28.26 ± 2.71	41.15 ± 1.98	33.75* ± 2.11

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

TABLE - XVIII

EFFECT OF FEEDING γ-BHC MIXED DIET(9 mg/kg body weight/day) FOR 6-18 MONTHS ON THE ACTIVITIES OF VARIOUS HEPATIC ENZYMES OF ALBINO RATS.

Parameters	6 months γ-BHC feeding experiment		12 months γ-BHC feeding experiment		18 months γ-BHC feeding experiment	
	Control (n=6)	γ-BHC fed (n=4)	Control (n=4)	γ-BHC fed (n=3)	Control (n=6)	γ-BHC fed (n=4)
AP (KAU/g)	0.74[a] ± 0.05	1.68** ± 0.19	1.12 ± 0.07	0.82* ± 0.08	1.08 ± 0.08	1.06 ± 0.09
GOT (IU/g)	8.66 ± 0.54	8.14 ± 0.61	7.56 ± 0.46	7.53 ± 0.45	6.79 ± 0.23	9.62 ± 0.83
GPT (IU/g)	7.71 ± 0.68	14.83*** ± 0.34	5.02 ± 0.54	8.59*** ± 0.06	6.05 ± 0.60	17.19** ± 2.33
ICDH ($X10^3$ Sigma U/g)	33.29 ± 6.08	38.85 ± 8.65	49.49 ± 2.52	40.45* ± 1.89	42.50 ± 5.70	31.04 ± 2.18
LDH ($X10^4$ IU/g)	58.29 ± 2.99	63.02 ±10.42	54.25 ± 5.37	99.48* ± 9.98	44.60 ± 0.31	56.63* ± 5.69

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

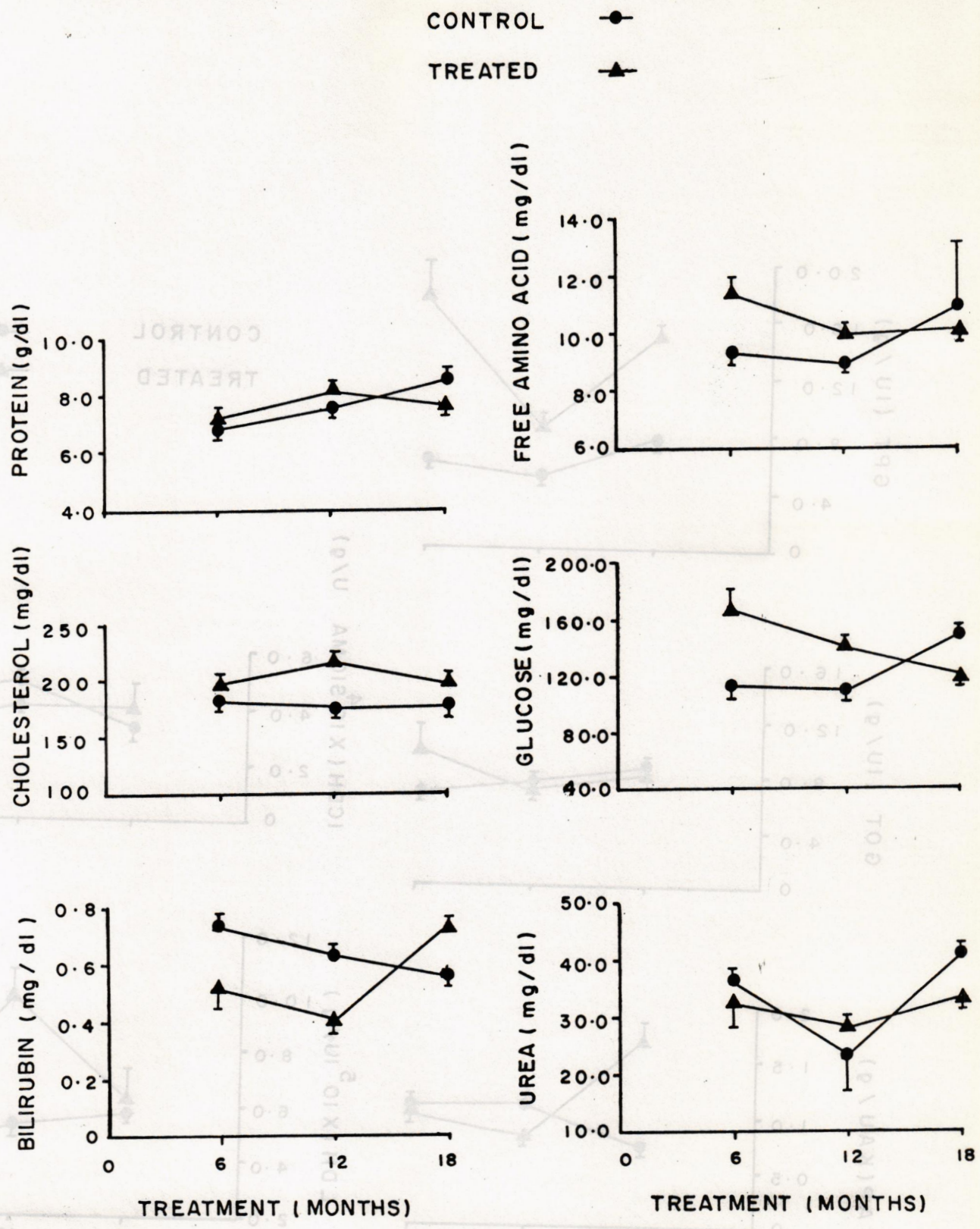


Fig.47. Effect of feeding gamma BHC mixed diet (9 mg/kg body weight/day) for 6-18 months on the various biochemical components of rat blood serum.

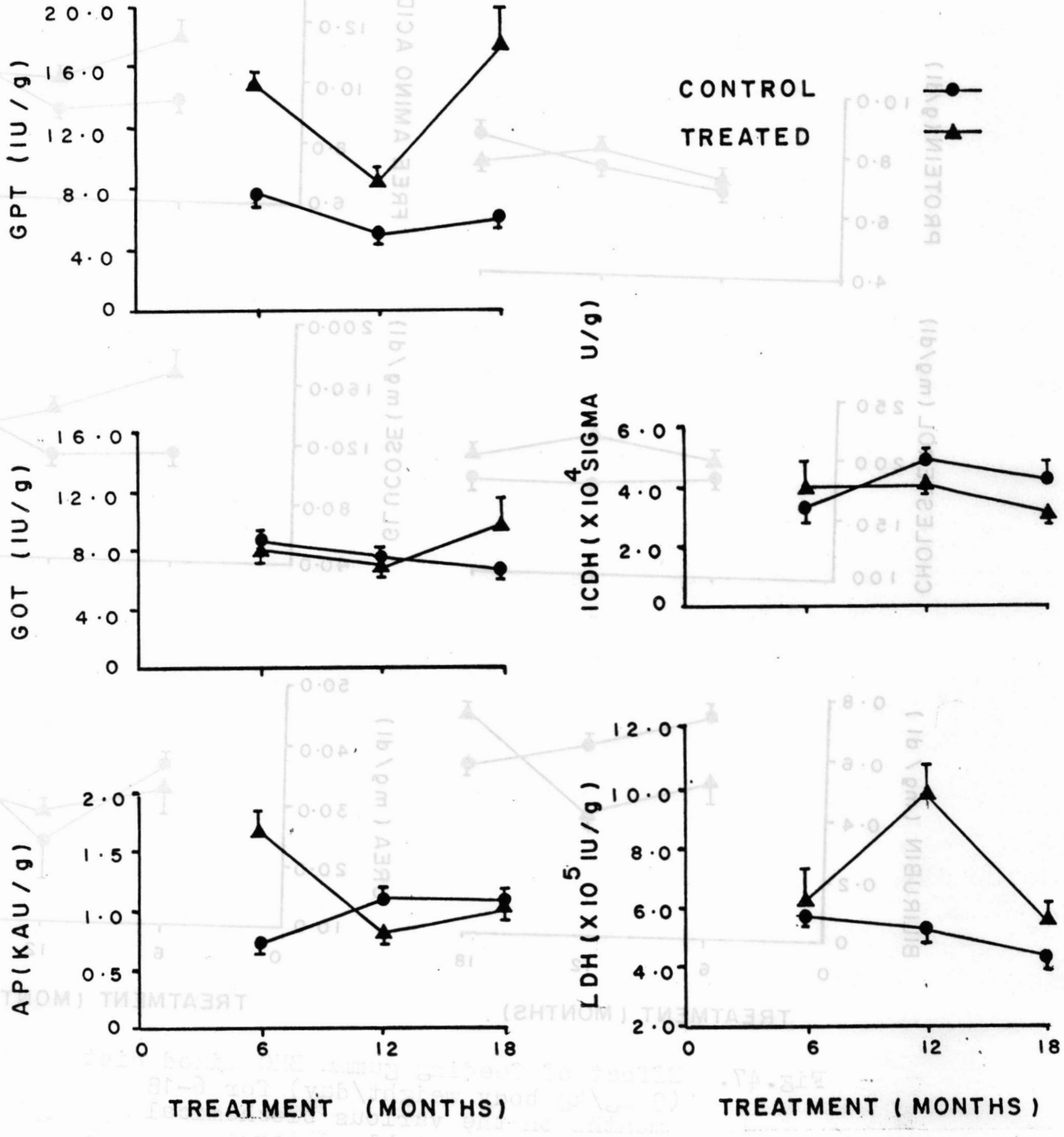


Fig.48. Effect of feeding gamma BHC mixed diet (9 mg/kg body weight/day) for 6-18 months on the activities of various hepatic enzymes in rat.

changes in the various other biochemical components of rat liver.

Two enzymes i.e. GPT and LDH activities are prominently affected. The GPT activity is increased 92, 71 and 184% after 6, 12 and 18 months, while LDH activity is increased 8, 83 and 27% during the same period, respectively. AP activity is increased 126% after 6 months of feeding, while it shows 27% decrease after 12 months of feeding and no change was observed after 18 months. The hepatic GOT activity is significantly affected and is raised 42% after 18 months of γ-BHC feeding. The ICDH activity is increased 17, 18 and 27% after 6, 12 and 18 months of feeding (Table XVIII, Fig. 48).

Figure 49 and Table XIX show the effect of feeding γ-BHC (= Lindane) at a dose of 9 mg/kg body weight/day for 6-18 months on the various biochemical components of liver other than enzymatic activities. The cholesterol content increase 55% after 6 months of feeding, but do not show any significant change after prolonged feeding. The glucose content are drastically decreased. The decrease is 53, 60 and 49% after 6, 12 and 18 months of feeding. The total protein content remain unchanged, while the soluble proteins decrease 18, 20 and 30% after 6, 12 and 18 months of γ-BHC feeding. The FAA content, on the other hand, show very significant increase of 14 and 28, and 15% during 6 and 12 and 18 months of insecticide feeding. The increase in 18 months feeding group is, however, not significant.

TABLE - XIX

EFFECT OF FEEDING γ-BHC MIXED DIET (9 mg/kg body weight/day) FOR 6-18 MONTHS ON THE VARIOUS BIOCHEMICAL COMPONENTS OF ALBINO RAT LIVERS.

Parameters	6 months γ-BHC feeding experiment		12 months γ-BHC feeding experiment		18 months γ-BHC feeding experiment	
	Control (N=6)	γ-BHC fed (n=4)	Control (n=4)	γ-BHC fed (n=3)	Control (n=6)	γ-BHC fed (n=4)
Cholesterol (mg/g)	5.97[a] ± 0.29	9.23* ± 0.99	9.01 ± 0.32	8.97 ± 0.41	10.50 ± 1.21	8.48 ± 0.28
FAA (µg/g)	217.29 ± 7.70	247.49* ± 7.69	197.98 ±14.18	252.53* ±15.62	170.12 ±11.59	197.19 ±21.32
Glucose (mg/g)	30.87 ± 2.32	14.61*** ± 1.78	27.12 ± 1.37	10.89*** ± 1.38	38.32 ± 3.38	19.71** ± 1.91
Soluble Protein (mg/g)	124.92 ± 4.67	102.99* ± 4.63	135.22 ± 9.96	108.25 ± 6.00	159.00 ± 2.58	111.62*** ± 5.62
Total Protein (mg/g)	216.96 ±13.05	267.51 ± 7.22	208.48 ± 6.70	240.10 ±11.57	227.60 ±11.88	239.49 ±11.46
DNA (mg/g)	3.05 ± 0.11	2.81 ± 0.25	2.54 ± 0.14	2.60 ± 0.37	3.06 ± 0.12	4.89*** ± 0.36
RNA (mg/g)	9.68 ± 0.62	12.78* ± 0.89	6.39 ± 0.31	6.92 ± 0.91	11.30 ± 0.49	9.93 ± 1.13

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

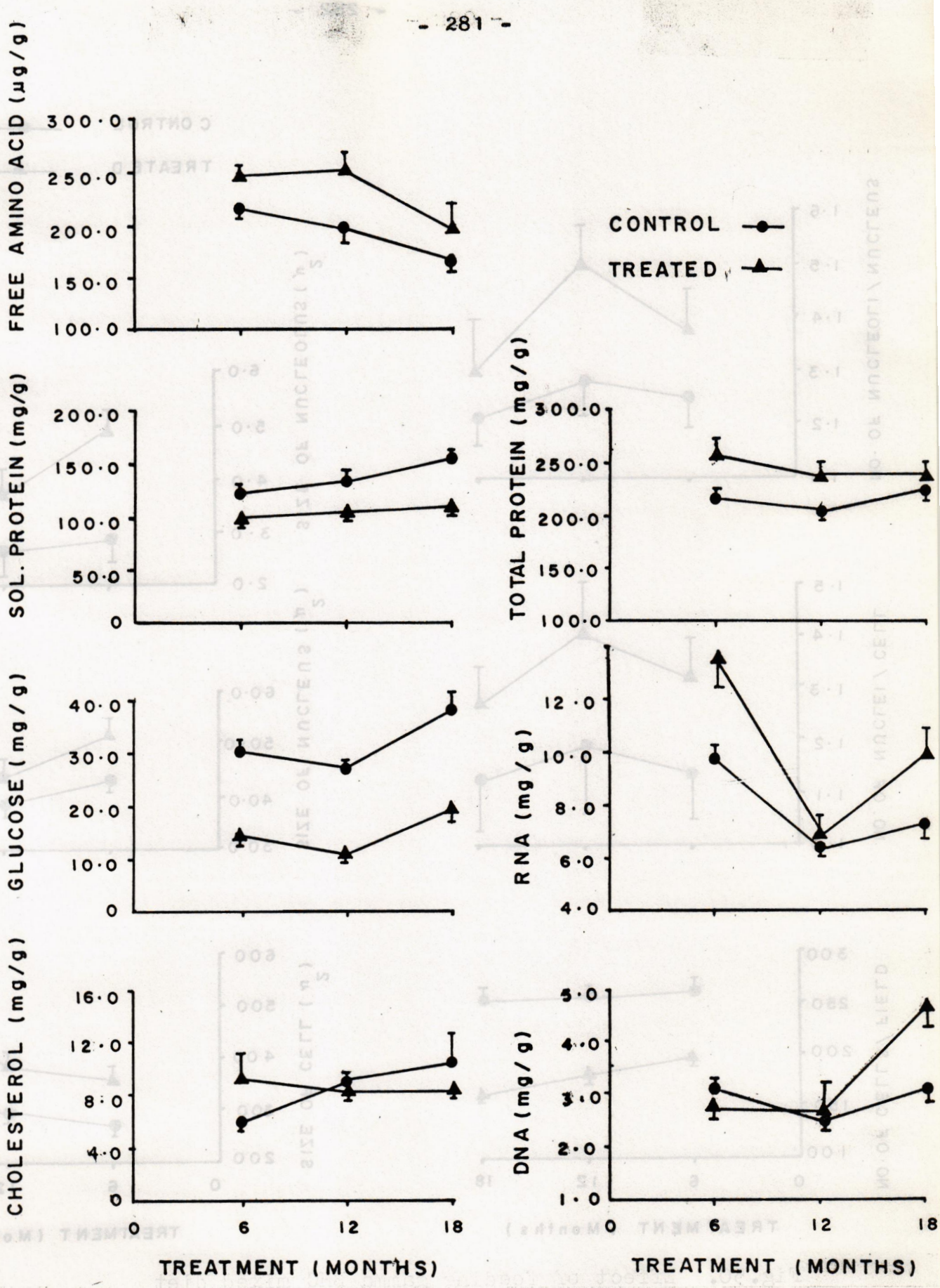


Fig.49. Effect of feeding gamma BHC mixed diet (9 mg/kg body weight/day) for 6-18 months on the various biochemical components of rat liver.

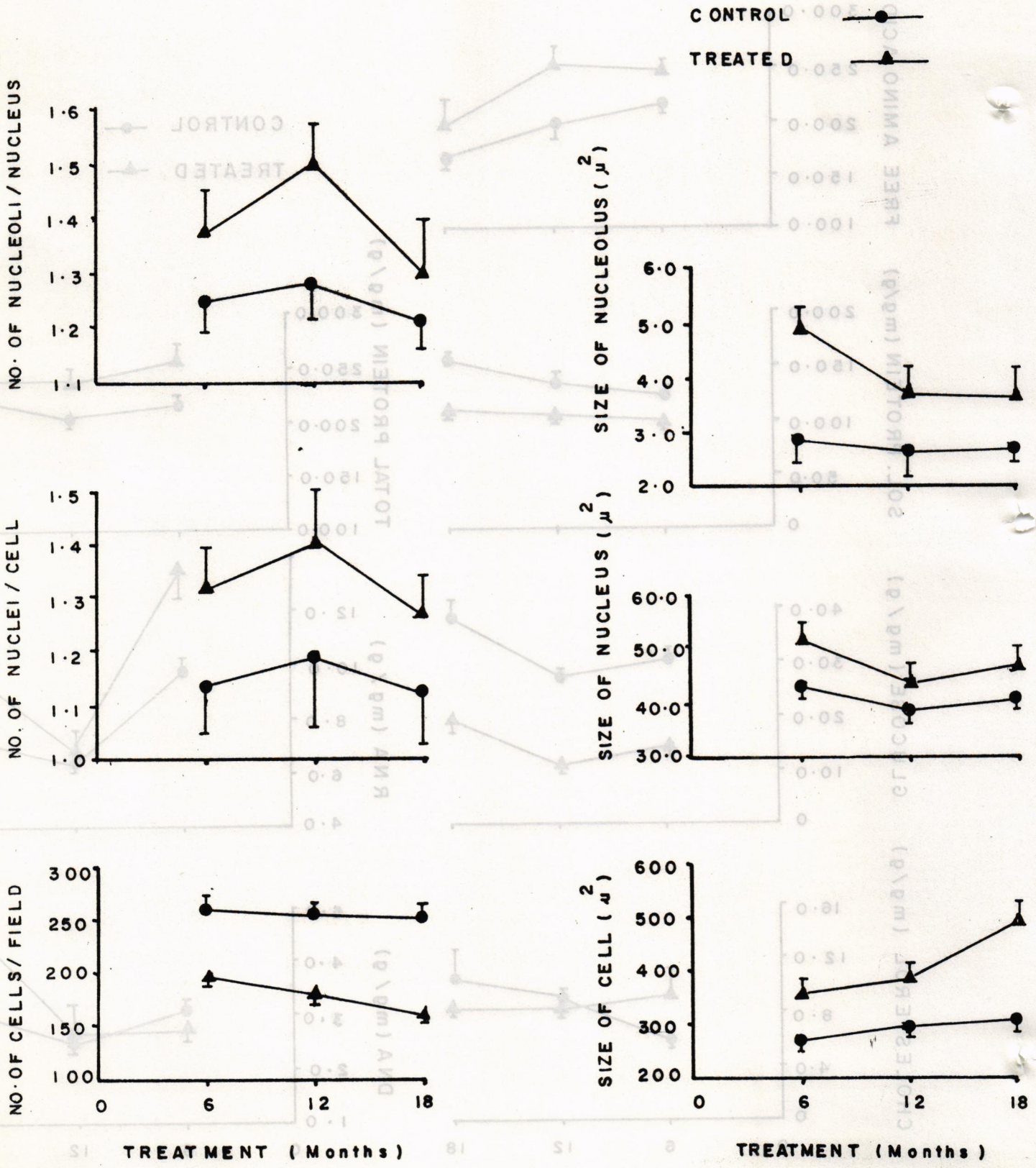


Fig.50. Effect of feeding gamma BHC mixed diet (9 mg/kg body weight/day) for 6-18 months on the various histological parameters of rat liver.

The DNA content are not changed until month 18 of feeding, when 55% increase in the DNA content was recorded. The RNA content generally was unchanged, except for six month feeding group, when the RNA content increased 32%.

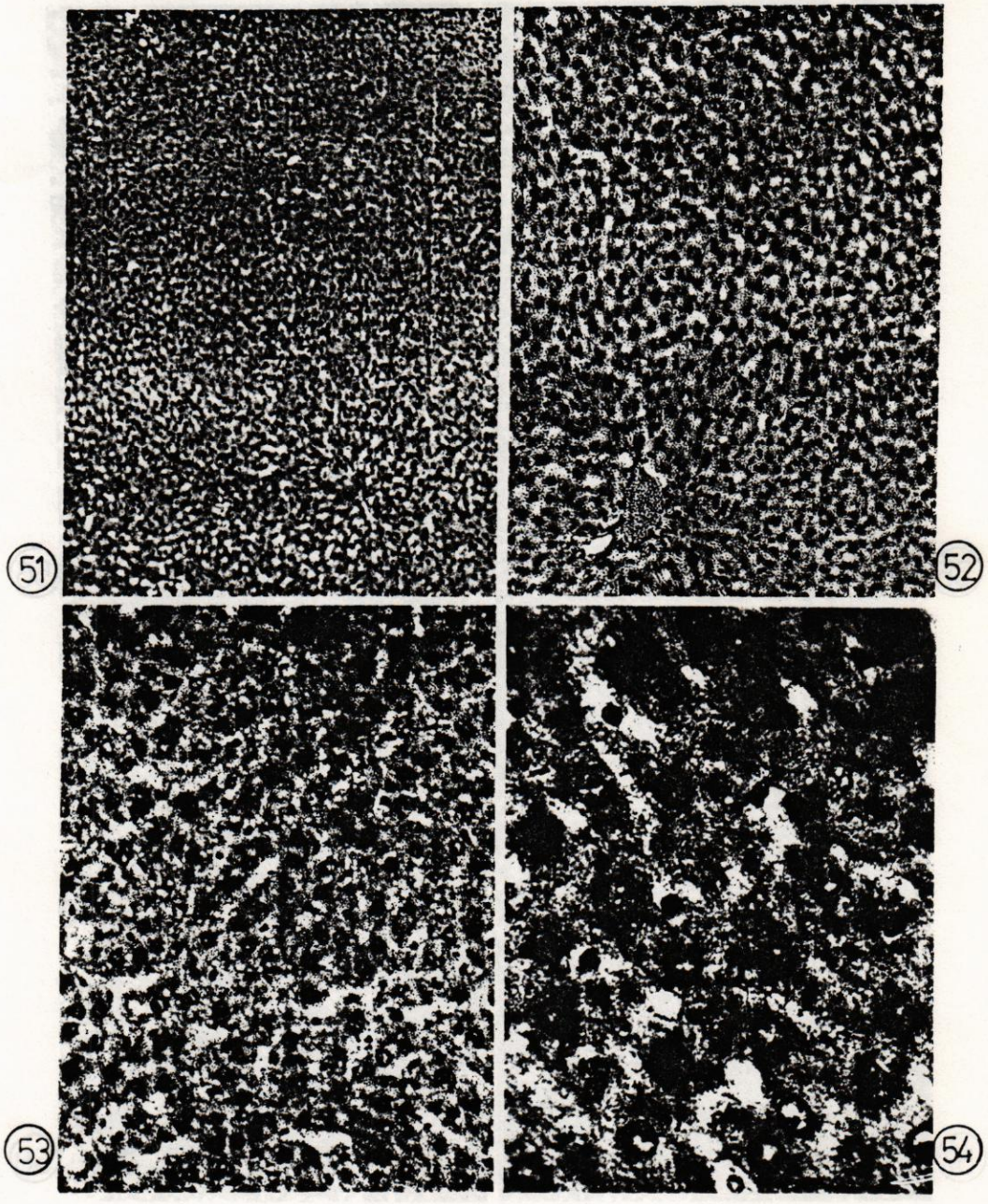
3.3.5. HISTOLOGICAL STRUCTURE OF LIVER

Typical effects of insecticidal toxicity are visible in rat liver after γ BHC feeding. Table XX and Fig. 50 shows the effect of long term feeding of γ BHC on the various histological parameters of rat liver. The hepatic cells, their nuclei and nucleoli register a significant increase. The hepatic cells increase 34, 28 and 59% respectively after 6, 12 and 18 months, while the nucleus shows 20, 13 and 14% and nucleolus shows 73, 42 and 37% increase during the same period. The number of nuclei/cell and number of nucleoli/nucleus remain unaltered and do not show any significant change. The number of cell/microscopical field are decreased because of hypertrophy of cells.

Figures 55-58 show effect of γ BHC fed for 6 month on the hepatic histological structure, while Figures 59-63 and Figures 64-67 show effect of insecticides fed for 12 and 18 months respectively on the hepatic structure. Although a prominent well defined hypertrophied hepatic cells and hypertrophied and condensed hepatic nuclei can be seen (Compare Figures 58 and 63 with Figure 54). The most prominent and significant change is observed in 18 month group, where cytoplasm is mostly vacuolated and marginated, while hepatic nuclei are considerably condensed (Figures 64-67; compare Figs. 54 with 67). Although the general

No.of cells/ Field	263.31 ±13.42	197.24 ±15.63
No.ofnuclei/ cell	1.14 ± 0.09	1.32 ± 0.26
No.of nucleoli/ nucleus	1.25 ± 0.05	1.38 ± 0.07
Size of cell (μ^2)	272.41 ± 9.13	365.34*** ±10.75
Size of nucleus(u^2)	42.78 ± 1.29	51.35** ± 1.57
Size of nucleolus (μ^2)	2.88 ± 0.31	4.97** ± 0.25

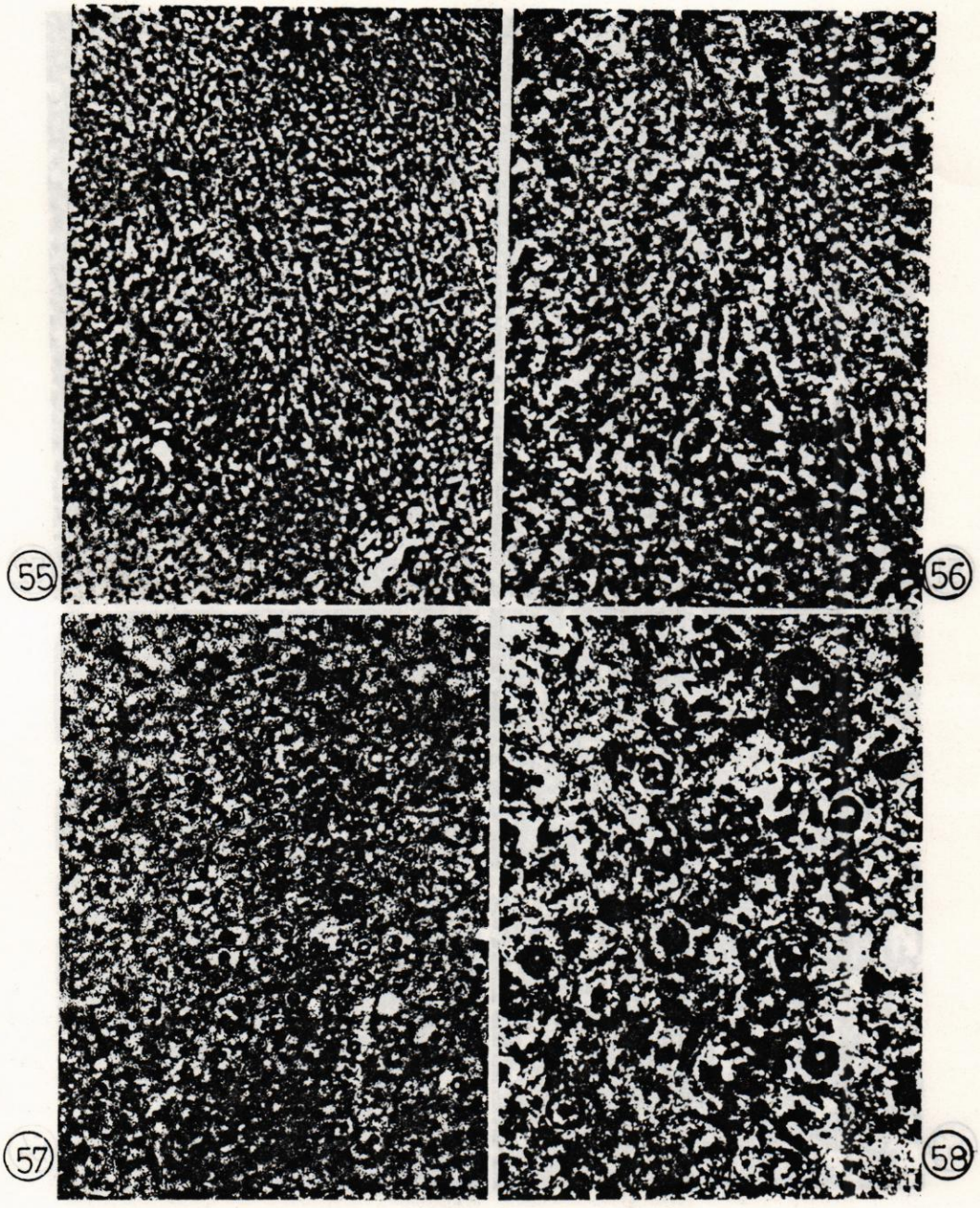
[a]Mean±SEM, students 't' test; *P



FIGS.51-54: Histological structure of normal rat liver. Note typical hepatic lobule with portal areas (51), central vein, normal hepatic cord structure (52,53), cellular and nuclear arrangement (54).

Stain: Hematoxylin and Eosin.

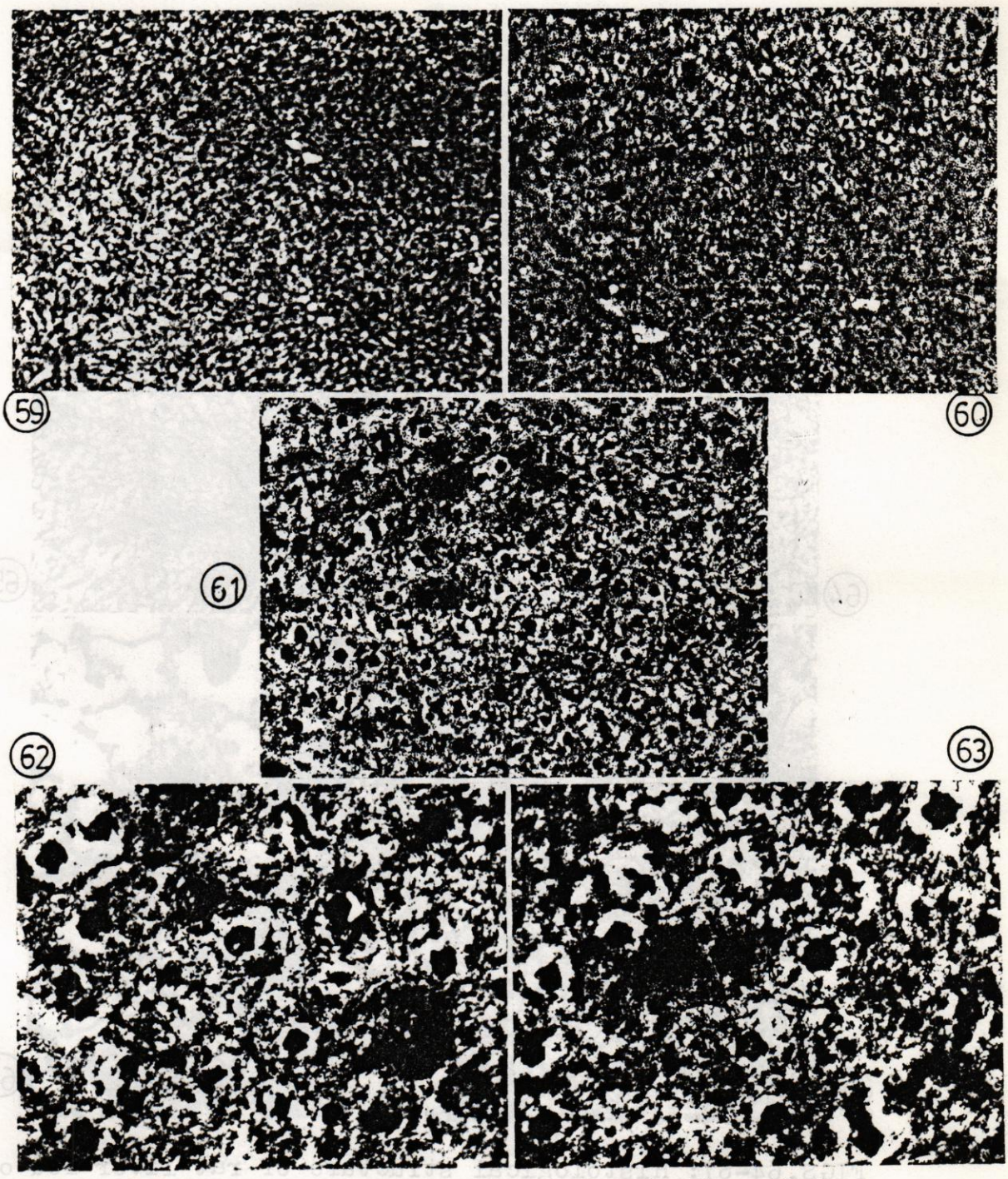
Magnifications: 51,x25; 52,x50; 53,x100; 54,x250.



FIGS.55-58: Histological structure of rat liver fed on gamma-BHC-mixed diet for 6 months. Note totally disorganised regions of hepatic lobule with disturbed cord structure(55,57),enlarged sinusoidal spaces(57), hypertrophied cells and nuclei (57,58).

Stain: Hematoxylin and Eosin.

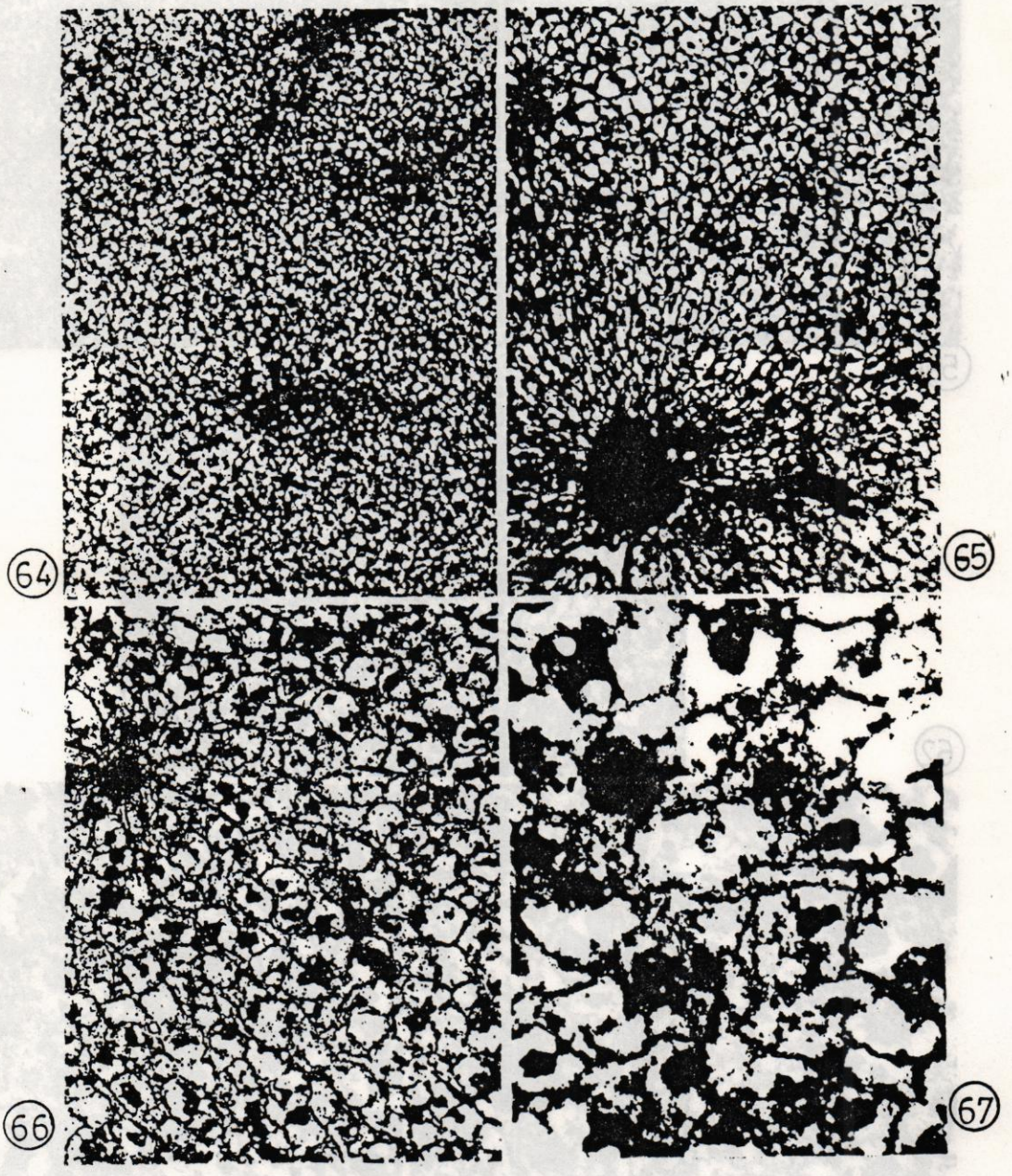
Magnifications: 55,x25; 56,x50; 57,x100; 58,x250.



FIGS.59-63: Histological structure of rat liver fed on gamma-BHC-mixed diet for 12 months. Note disturbed lobular morphology with slight vacuolation (59,60), cytoplasmic margination (60-63),hypertrophied cells and hypertrophied nuclei with irregular margins (62,63).

Stain: Hematoxylin and Eosin.

Magnifications: 59,x25; 60,x50; 61,x100; 62-63,x250.



FIGS. 64-67: Histological structure of rat liver fed on gamma-BHC-mixed diet for 18 months. Note totally disorganised lobular structure with heavy vacuolation (64), extensive cytoplasmic margination, prominent ducts of bilecanaliculi, deshaped nuclei and hypertrophied cells (65-67).

Stain: Hematoxylin and Eosin.

Magnifications: 64, x25; 65, x50; 66, x100; 67, x250.

hepatolobular architecture is not disturbed but the liver of insecticide fed rat is marked by distended central vein and prominent blood vessels and bile canaliculi on the sides of cords of cells (Figs.65-67).

4. DISCUSSION

Lindane, 1,2,3,4,5,6,- hexa chlorocyclohexane (gamma-isomer) has been used as effective controlling chemical against various mites infesting man and livestock. It is also remarkably toxic to locusts and grasshopper that have developed resistance against DDT. Because of its high vapour pressure it also possess fumigant properties and in warmer climates it is very effective against insects that hide in corners and crevices. Lindane therefore is a very effective insecticide against cotton and livestock. Because of its special properties and specificity, this insecticide is likely to gain entry into the non target organisms more easily (Wells and Johnstone, 1978; Crissman, 1980; Angerer et al., 1983).

In the present experiments, three different doses were administered to albino rats for three different periods of time, i.e. 30mg lindane/kg body weight for 48 hours, 18mg lindane/kg body weight for 3-15 days and 9mg lindane/kg body weight for 6-18 months.

Haematological parameters

The haemoglobin content, RBC count and PCV decrease under all experimental conditions. The haemoglobin content decrease 7% after 48 hours of lindane treatment

at 30mg/kg body weight and 8% after 15 days of lindane treatment at 18mg/kg body weight. In long term feeding experiment, the haemoglobin content decrease 10% after 6 months of feeding. The 12 and 18 months of feeding does not produce any significant change. Severe cases of hypoplastic anemia and aplastic anemia have been reported previously (Reeves et al., 1981; Schimmel et al., 1980; Morgan et al., 1980). The RBC count decreases significantly. A strong dose of 30mg/kg body weight did not cause much change in the various haematological parameters except for WBC count, which rises 39% within 24 hours of insecticide treatment. When insecticide treatment was extended for another 24 hours, the RBC, PCV and MCHC decrease 11, 7 and 5%, respectively. The WBC, MCV and MCH, however, increase 46, 10 and 4%, respectively. Lindane administered at a dose of 18mg/kg body weight for 15 days causes decrease in the haemoglobin content RBC count and PCV, which is respectively 8, 16 and 9%, while WBC, MCV and MCH shows 24%, 8% and 9% increase, respectively, during the same period. In the long term feeding experiment of 18 months, the haemoglobin content decrease 10% after 6 months of feeding at a dose of 9mg/kg body weight/day. No significant change was observed after prolonged feeding. The PCV behaved the same way, while RBC count decreases 10% after 18 months of feeding. The WBC, PCV and MCH increase respectively, 24%, 10% and 8% during the same period.

The decrease in RBC count is much more drastic than in the haemoglobin content. This is perhaps due to special property of lindane to bind with membrane of erythrocyte (Yang et al., 1975; Antunes-Madeira et al., 1981). The hepatic heme biosynthesis has also been

reported to be affected, which contributes to decreased haemoglobin content (Taljaard et al., 1972). Lindane has also been reported to produce abnormalities in different haematological parameters from other laboratories (Mengle et al., 1967; Christophers, 1969; Traczyk et al., 1977; Buteiko, 1980). Long term treatment of lindane has also been reported to cause pronounced morphological changes in haematopoeitic organs like hyperplasia of reticular cells, increased number of lymphocytes and plasma cells (Komarova and Gorbachevskava, 1979).

Blood serum biochemistry

The blood serum was tested with a view to ascertain effects of lindane administration on the various liver function tests (LFT). The typical LFT viz. activities of AP, AcP, SGOT, SGPT, LDH and contents of bilirubin, protein, cholesterol and FAA were evaluated. A high dose of 30mg/kg body weight/day, administered twice resulted in significant increase in all LFT parameters. The most drastic changes were recorded in AP (2.44 fold), AcP (81%), SGOT (72%), SGPT (33%), LDH (1.31 fold), bilirubin (46%), protein (26%), cholesterol (1.11 fold) and FAA (21%) increase after 48 hours of insecticide treatment. Almost similar trend was observed, when weaker doses of lindane were administered for longer periods of time. A dose of 18mg/kg body weight/day administered for 15 days resulted in significant increase in AP (164%), AcP (100%), SGOT (32%), LDH (26%), and bilirubin content (69%). The SGPT, protein and cholesterol content were not affected, while FAA content showed 19% decrease.

A dose of 9mg lindane/kg body weight/day administered for a total period of 6 months caused drastic increase in AP (236%), AcP (140%), SGOT (41%), LDH (123%) and FAA content (24%). The SGPT, protein and cholesterol did not show any appreciable change while bilirubin content decrease 18%. When this insecticide treatment was extended for 18 months, the rats apparently seem to have adjusted themselves to this long term treatment. The AP and AcP activities and bilirubin content show 113, 171 and 30%, increase; while SGOT, SGPT, LDH, protein, cholesterol and FAA content do not show any significant deviation.

Rivett et al. (1978) has reported increased serum alkaline phosphatase activity in beagle dogs, which were fed on lindane (100 and 200 ppm) for two years, without any detectable histopathological change.

Significant changes in the GOT, GPT and AP activities in the blood serum and liver have been reported by Dikshith et al. (1978) in guinea pigs after dermal application of gamma-BHC in daily dose of 100, 200 and 500mg/kg for 30 days.

The increased serum acid phosphatase activity, which is indicative of lysosomal enzyme activation/synthesis in lymphocytes of peripheral blood has also been reported by Komarova and Gorbachevskava (1979).

Brassow et al. (1981) did not observe any significant difference in neurological status and ECG of normal and lindane treated animal. Significant differences were, however, ascertained viz. higher polymorphonuclear leukocytic count, low lymphocyte count, higher reticulocyte

count, lower prothrombin test and lower blood concentration of creatinine and uric acid. No significant differences were observed in total red and white blood cell or platelets count, haemoglobin content, gamma-glutamyltransferase, GOT, GPT, LDH, ChE, triglyceride, cholesterol and urea. No signs of severe health impairment were observed in workers of lindane factory. Lyub-Chenko et al. (1974) have likewise not reported any major change in the LFT in workers having direct contact with lindane. Gertig et al. (1971) have reported increase in AP and AcP activities after lindane treatment in rats, while similar trend in GOT and GPT activities has been reported by Bakthavathsalam and Srinivasan Reddy (1982) in a fish. Gertig and Nowaczyk (1975) have reported decrease serum AP, AcP and LDH activities after a dose of 0.1 LD_{50} given to rats. In the presence of protein rich diet this trend is further intensified.

Prolonged treatment of rats with lindane (1.7mg/kg) for 180 days produced an appreciable diminution of total LDH activity in blood and converse relationship in liver (Alekhina and Khaikina, 1972). Thakore et al. (1981a,b) have also reported decreased LDH activity in blood serum after lindane treatment. Meany and Pocker (1979) have shown behaviour of LDH in in vitro system.

Increased cholesterol level after feeding lindane at a dose of 40mg/kg body weight for 27-33 weeks has been reported by Davey and Johnson (1974). Carlson and Kolmadin-Hedman (1977) have however shown that when 80 men were exposed to lindane, α-lipoprotein (HDL) cholesterol decreased significantly within two years after exposure.

Besides LFT, several other biochemical components of the blood serum were tested to evaluate certain other aspects of metabolism. The amylase, ICDH, CPK and ChE activities increase under all experimental conditions. In 48 hour treatment experiment, amylase activity is not significantly affected, while the ICDH, CPK and ChE increase respectively 1.8 fold, 62% and 29%. The glucose content also increase 40% after 48 hours of treatment with 30mg lindane/kg body weight. In 15 day treatment experiment, in which lindane has been administered at a dose of 18mg/kg body weight/day for 15 days, the amylase, ICDH and CPK activities increase 76%, 46% and 139%, respectively. The ChE activities, urea content and glucose content are not significantly changed.

A same trend was recorded, when lindane was administered for a period of 18 months. The amylase, ICDH, CPK and ChE activities show 52%, 32%, 257% and 25% increase, respectively. The urea and the glucose content show a decrease of 18% and 20%, respectively after 18 months of feeding.

The raised LFT enzymes and bilirubin content is an indication of liver malfunction due to lindane treatment. Although the FAA and protein content increase in the 48 hour treatment experiment, but remain non-significantly modified in long term experiments. The amylase activity increases after lindane treatment, which points towards increased flow of glucose from glycogen into the main metabolic pathway. This is accompanied by accelerated kreb's cycle, as indicated by enhanced ICDH activity. The CPK activity reflects muscle damage, which is significantly affected after lindane treatment. The ChE activity which is

usually the target of organophosphorous insecticides is enhanced about 20% in 48 hour experiment and shows about 20-27% increase in 18 month feeding experiment. The increased ChE activity probably sensitizes the organisms and allows the nerve impulses to travel much more efficiently and quickly than they would normally do.

Liver biochemistry

All hepatic enzymes tested were elevated after lindane treatment. In 48 hours treatment experiment, in which lindane was administered at a dose of 30mg/kg body weight/day, the AP, GOT, GPT and LDH activities increase 2.84 fold, 44%, 2.82 fold and 2.82 fold respectively, the ICDH activity remains unaltered. In 15 day treatment experiment in which lindane was administered at a dose of 18mg/kg body weight/day, the maximum increase was recorded after 6 days of lindane treatment. After 15 days of continuous feeding the various enzyme activities are elevated, but not to the same extent on 6th day. For example hepatic AP, GOT, GPT, LDH and ICDH activities show, respectively, 34%, 21%, 65%, 53% and 66% increase after 15 days, as against increase of 147%, 81%, 104%, 60% and 114%, respectively, on day 6. In long term experiment, in which lindane was administered at a dose of 9mg/kg body weight/day for 6-18 months, only AP and GPT activities showed 126% and 92% increase after 6 months of feeding. All other enzyme activities did not deviate significantly. After 18 months of lindane feeding, however, GPT and LDH activities increased 184% and 27%, respectively, while the changes in hepatic AP, GOT were non significant, and ICDH activity showed 27% decrease.

Alekhina and Khaikina (1972) have reported reduced LDH activity in liver after administration of lindane at a dose of 17mg/kg for 180 days in rats. Almost similar type of observation has been made by Gertig and Nowaczyk (1975) and Boulekbache and Spiess (1974). This is in contrast to the results being reported here, which show high LDH activity. This is because of 6 fold more lindane content of each dose.

The increased hepatic enzyme content are probably because of enzyme induction. Lindane has been shown to induce several other enzymes viz drug metabolizing enzymes or microsomal monoxygenase systems (Taljaard et al., 1972; Konat and Clavsen, 1973; Dent et al., 1974; Herbst et al., 1974; Mikol et al., 1980; Oesch et al., 1982; Sharmanov et al., 1985), lysosomal enzymes (Roux et al., 1974; 1976; Carevic, 1977), Carbohydrate metabolizing enzymes (Escoubet and Vicente, 1976; Barros and Saliba, 1978; Strebocan et al., 1978), enzyme of steroid metabolism (Graef et al., 1979). Besides that several other reports exist in literature in which changes in various enzymatic activities have been related with the development of hepatic tumors (Srinivasan and Radha-Krishnamurtay, 1977; Schulte-Hermann and Schmitz, 1980; Bhatt et al., 1981a,b,c; Thakore et al., 1981; Pereira et al., 1982).

The hepatic cholesterol content decrease in 48 hour and 15 day feeding experiment. The decrease in the former dose is about 33%, while in the 15 day feeding experiment it varies between 50-60%. In the long term experiment, the cholesterol increase 55% after 6 months of feeding. No significant effect was observed after 12 and 18 months of feeding. The

glucose content remain unchange after strong dose administration, but increase 56% after 6-9 days of lindane feeding at a dose of 18mg/kg body weight/day. These content however decrease 53%, 60% and 49% after 6, 12 and 18 months of feeding. The total protein content remain unchanged under all experimental conditions, while the soluble protein content increase 30% after 48 hours of lindane feeding at a dose of 30mg/kg body weight/day. In 15 day feeding experiment, the soluble protein decrease 17-19% during 3-6 days of lindane feeding at a dose of 18mg/kg body weight/day, while this decrease was 18-30% after 6-18 months of lindane feeding. The FAA decrease after lindane feeding.

The DNA content is not affected in 18 month feeding experiment, but shows significant increase of 84%, 88%, 123%, 75% and 62% after 3,6,9,12 and 15 days of lindane feeding at a dose of 18mg/kg body weight/day. In long term feeding experiment the DNA content are not affected after 6 and 12 months of feeding, but shows 55% increase after 18 months of feeding at a dose of 9mg/kg body wt./ day. The RNA content decreased after lindane feeding. This decrease was 43-49% in 48 hour feeding experiment, while it ranged between 17-39% after 6-15 days of feeding. The RNA content however increase 32% after 6 months of feeding.

Liver histology

The size of hepatic cell, its nucleus and nucleolus increase considerably after lindane treatment. The extent of hypertrophy of hepatic cells, nuclei and nucleoli is greatest in long term experiments. The

various histochemical and histological and ultra-structural changes have been reported from different labs.(Medline et al., 1973; Obuchowska and Pawlowska-Tochman, 1973; Grzycki and Zarebska, 1973; Kimbrough and Linder, 1974; Zufarov et al., 1975; Dikshith et al., 1978a,b; Jeanne, 1979; Shiranandappa and Krishnakumari, 1981; Gupta and Singh, 1982; Nigam et al., 1984).

Rivett et al. (1978) however could not find any detectable histopathological changes after treatment of dogs with 100 and 200 ppm of lindane for 104 week.

Lindane stimulates liver cell proliferation (Brade et al., 1974) and in several cases development of liver cancers have been reported (Ito et al., 1975; Herbst et al., 1975; Weisse and Herbst, 1977; Tatematsu et al., 1979; Reuber, 1979; Kashyap et al., 1979; Bhatt et al.,1981d; IARC, 1982; Nigam et al., 1984).

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C H A P T E R-IV

EFFECT OF DDT

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I. INTRODUCTION

DDT is one of the first, quite extensively used chlorinated hydrocarbon insecticide throughout the world, including Pakistan, primarily for the control of insect pests of agriculture and medical importance.

Like other organochlorinated compounds, DDT is a very stable substance, which is only slowly biodegraded into different metabolites, which are also not less toxic than DDT itself. It's persistent nature (Adamczyk, 1971; Deichmann et al., 1971; Halacka and Vymetal, 1973; Waldron and Naber, 1974; Harri et al., 1979; Krauthacker et al., 1980) and mobility in food chains is an important factor in chronic toxicity.

The deleterious effects of DDT on nontarget organisms are largely due to its indiscriminate and unplanned use in domestic places, on fruits, vegetables, crops, and in forests. Consequently the whole environment became polluted with DDT and its metabolites which are present in air, rainwater, in large water bodies and in aquatic life (Hoffmann, 1953; Bulter and Drooz, 1969; Johns et al., 1971; Davies et al., 1975; Spencer, 1975; Young et al., 1976; Milillo, 1978; Skaftason and Johannesson, 1979; Mukherjee et al., 1980).

After absorption into the system by inhalation, ingestion or contact with the skin, it is transported to the blood. So blood is the first target of insecticide in the body (Morgan et al., 1972; Hesselberg and Scherr,

1974; Liedtke et al., 1976; Lone and Javaid, 1976) where it becomes chemically linked with the lipid fraction especially with the lipoprotein complexes of the blood, (Tinsley et al., 1971; Pocock and Vost,1974; Skalsky, 1978; Plack et al., 1979; Ciupe et al., 1979; Leighty et al., 1980; Nageswara et al., 1980) and enters into the liver.

There are many reports of storage of large amounts of DDT residues in animal tissues, such as liver,muscles, brain, adipose tissue and lactating organs (Laug et al., 1950; Dale et al., 1962; Brown et al.,1966; Zimak and Zero, 1970; Morgan and Roan, 1971;1972; Simonffy et al.,1973; Whiting et al., 1973; Avavindakshan et al.,1974; Fang et al.,1977).

Quite extensive work has been done on metabolism, distribution(Hunnego, 1971; Feil et al., 1973; Kuzinskaya and Girenko, 1973; Morgan and Roan, 1974; Vaskovskaya, 1974; Watson et al.,1975; Yadav et al., 1976; Ahmed and Walker,1979) and residue analysis of DDT in different tissues of invertebrates and vertebrates including man (Keil et al.,1972; Pordab and Maik,1972; Bronise and Ochynski,1973; Ramachandran,1974; Zobel, 1975; Agarwal et al.,1976; Latimer and Seigel,1977; Dhaliwal and Kalra, 1977; Dikshith,1978; Delos Reyes and Mora, 1979).

Due to their persistence in the animal tissues, (Adamczyk,1971; Halacka and Vymetal,1973) the DDT and its metabolites cause abnormal alterations in the physiology which are obviously a consequence of their interference with different metabolic pathways (Sanchez,1967;Clifford and Weil,1972; Haynes,1972; Kacew et al.,1972; Konat and Clausen,1973; Darsie et al.,1976; Sanyal et al.,1979; Agarwal et al.,1980; Dudeja et al.,1980; Sampson et al., 1980). As a result DDT has a quite large range of animal toxicity which is evident from the work of

different laboratories. Considerable information is available in the literature on the toxicity (Adams et al., 1949; El-Samra, 1971; Kashyp and Gupta, 1971; Deichmann, 1972; Terracini et al., 1973; Okey and Page, 1974; Cordes, 1976), mortality, absorption and excretion of DDT and its metabolites in nontarget animals especially in fish, birds and mammals (Dale et al., 1962; Brown et al., 1966; Zimak and Zero, 1970; Morgan and Roan, 1971, 1972; Whiting et al., 1973; Paschal et al., 1974; Ohmiya and Nakai, 1977).

Effect on reproduction and hormonal system is reported by many authors (Clement and Okey, 1974; Copeland and Cranmer, 1974; Orberg and Lundberg, 1974; Ware, 1975). Allison (1963, 1964) reported that DDT has no effect on ova production and ombryonic development in Cutthoart trout. However, Jonsson (1975-76) concluded that prolonged ingestion of DDT affects the progesterone level and in turn reproduction in female rat.

Histopathology is another aspect of great importance (Gabr and Al-Hussaini, 1967-68; Delos Reyes and Mora, 1979). Kimbrough et al. (1971) has shown increased liver size after DDT administration. He also noticed the moderate vacuolation of liver cells and decrease in glycogen contents. There are other conflicting reports about the carcinogenic potential of DDT (Grey et al., 1970; Svendson, 1973; Thrope, 1973; Rossi et al., 1977; Renber, 1978, 1979). Tomatis et al. (1974) and Kashyap et al. (1977) reported the initiation of tumours in mice after chronic administration of DDT. Life time feeding experiments on an especially tumour prone strain of laboratory mice showed that DDT increases the incidence of liver tumour (Turusov et al., 1973). The carcinogenic activity of DDT has been challenged by Laws (1971) and Cabral et al., (1982a, b). Agthe et al. (1970) also reported that no increase in tumaurs were induced in golden hamsters after DDT feeding.

Hayes et al. (1971) showed that feeding DDT to man for two years did not result in tumours.

Quite a few reports are available on the effects of DDT on different biochemical aspects of vertebrates especially mammals (Kagan et al., 1970; Dvorchik et al., 1971; Clifford and Weil, 1972; Cranmer et al., 1972; Haynes, 1972; Byczkowski, 1973; Davison, 1973; Konat and Claussen, 1973; Dinu et al., 1974; Kohli et al., 1975; Traczyk et al., 1977; Sanyal et al.,1979; Agarwal et al., 1980; Down and Casseaud, 1981) but none of these has shown any comprehensive evaluation of liver function due to DDT feeding. Since, like all xenobiotics, DDT will be metabolized in the liver,it is therefore likely to cause liver dysfunctioning. Gertig et al., (1971, 1975) has reported the effect of DDT on alkaline phosphatase, acid phosphatase, alanine transaminase and pyruvate transaminase activities. There are reports of increased protein synthesis in the rat liver after DDT administration. (Coppon and Nicholls, 1973, 1975). Dudeja et al., (1980) reported the effects of DDT on carbohydrate metabolism in the rhesus monkey. Story and Freedland (1978) reported that DDT feeding inhibitis the gluconeogenesis in the isolated hepatocytes of rat liver. Increased glucose 6-phosphatase activity was reported by Kacew and Singhal (1973). Darsie (1976) and Sampson et al. (1980) have concluded that dietary

DDT causes defficiency of essential fatty acids.

These incomplete informations on biochemical and haematological aspects reveal the need of further more elaborate studies on these lines to understand the mode of action of this compound. The objective of the present study was mainly to investigate the response of liver to continuous feeding of DDT for short and long periods. This response was evaluated at biochemical, histopathological and haematological level. These results are meant for later on extrapolation to human beings and other useful vertebrates of the ecosystem who are exposed to insecticidal hazards due to improper safety measures in Pakistan and most of the third world.

2. MATERIALS AND METHODS

2.1. ANIMALS

A colony of sprague Dawley albino rats raised as described in the first Chapter was used for the present studies. The rats were used as follows:

a) For short term experiments: Two groups of female rats, weighing about 120-180gm and 5-6 months of age were used. One group was used for feeding insecticide for 48 hours, while the second was used for feeding insecticide for 15 days.

b) For long term experiment about 70-90gms and three months of age were used.

2.2. PREPARATION OF FEED

The rat feed was prepared in the same way as described in Cahpter I of this Report.

2.3. INSECTICIDE USED

A Chlorinated insecticide DDT [1,1,1-trichloro-2,2-bis (p-Chlorophenyl) ethane] was obtained from the plant Protection Division of Punjab Agriculture Department, in the form of 75% powder which is administered to animals alongwith feed.

2.4. ADMINISTRATION OF INSECTICIDE

DDT was administered to rats as strong and weak doses as follows:

a) Strong dose:

For short term experiment, two levels of

strong doses were administered. In one group of rats a strong dose of 20 mg/kg body weight/day was administered for a total period of 15 days. In the second group 100 mg/kg body weight/day was administered for a total period of 48 hours.

b) Weak Dose:
A weak dose at a rate of 10 mg/kg body weight/day was administered to another group of rats for 18 months.

2.4.1. SHORT TERM EXPERIMENTS

For short term experiments, in which the total duration is 48 hours in one case and 15 days in the other, the insecticide was administered as follows:

a) For 48 hour experiment, the DDT mixed diet was prepared by mixing 800 mg of 75% DDT in 1 kg of dry food. Since each experimental rat on the average consumed 30 g of feed daily, it will get 100 mg/kg body weight/day.

b) For 15 day experiment, the insecticide mixed diet was prepared by mixing 525 mg of 75% DDT powder in about one litre molasses mixed water. Each rat of about 200 g weight consumed about 35 g of this feed daily. So in this way rats got a DDT dose of 20 mg/kg body weight/day.

2.4.2. LONG TERM EXPERIMENT

The insecticide mixed diet was prepared by adding mg of 75% DDT powder in 1 kg of ingradient

mixed feed. That way the rats consumed 10 mg DDT/kg body weight/day.

2.5. PROCEDURE ADOPTED

Experimental procedure adopted for the two short term and one long term experiments is the same as described in Chapter I for DDT experiments. The procedure adopted for collection of blood, liver processing, haematological studies, biochemical analysis of blood, biochemical analysis of liver and histological studies were the same as described in Chapter I.

3. RESULTS

3.1. EFFECT OF DDT ADMINISTERED AT A DOSE OF 100 mg/kg body weight/day FOR A PERIOD OF 48 HOURS

3.1.1. HAEMATOLOGICAL STUDIES

Figure 1 and Table I shows effect of feeding DDT mixed diet at a dose of 100 mg/kg body weight/day for a total period of 48 hours on the various haematological parameters. The haemoglobin content decrease 6 and 11% respectively, after 24 and 48 hours of DDT feeding. The RBC count decreases 16%, while PCV remains unaffected.

The MCH and MCV increase after insecticide treatment. The MCV shows 19% increase, while MCH shows 7.72% increase after 48 hours of DDT feeding. The MCHC, on the other hand, shows 6 and 10% decrease after 24 and 48 hours of insecticide feeding.

TABLE - I

EFFECT OF FEEDING DDT MIXED DIET (100mg/kg body weight/ day) FOR 48 HOURS ON THE VARIOUS HAEMATOLOGICAL PARAMETERS OF ALBINO RATS.

Parameters	Control (n = 7)	DDT fed	
		24 hours (n = 4)	48 hours (n = 4)
Hb (g/dl)	13.27±0.14[a]	12.47±0.25*	11.87±0.22***
RBC ($X10^5$/ul)	68.37±2.53	57.60±1.70**	57.40±1.30**
WBC ($X10^2$/µl)	63.60±2.0	90.00±4.40***	94.40±2.00***
PCV (%)	42.09±0.34	41.94±0.29	41.81±0.20
MCV (fl)	61.56±0.17	72.95±1.66***	72.93±1.34***
MCH (Pg)	19.38±0.08	21.67±0.53**	20.69±0.42*
MCHC (g/dl)	31.52±0.09	29.72±0.39**	28.39±0.39***

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

C = CONTROL
24 = 24 HOURS DDT FEEDING
48 = 48 HOURS DDT FEEDING

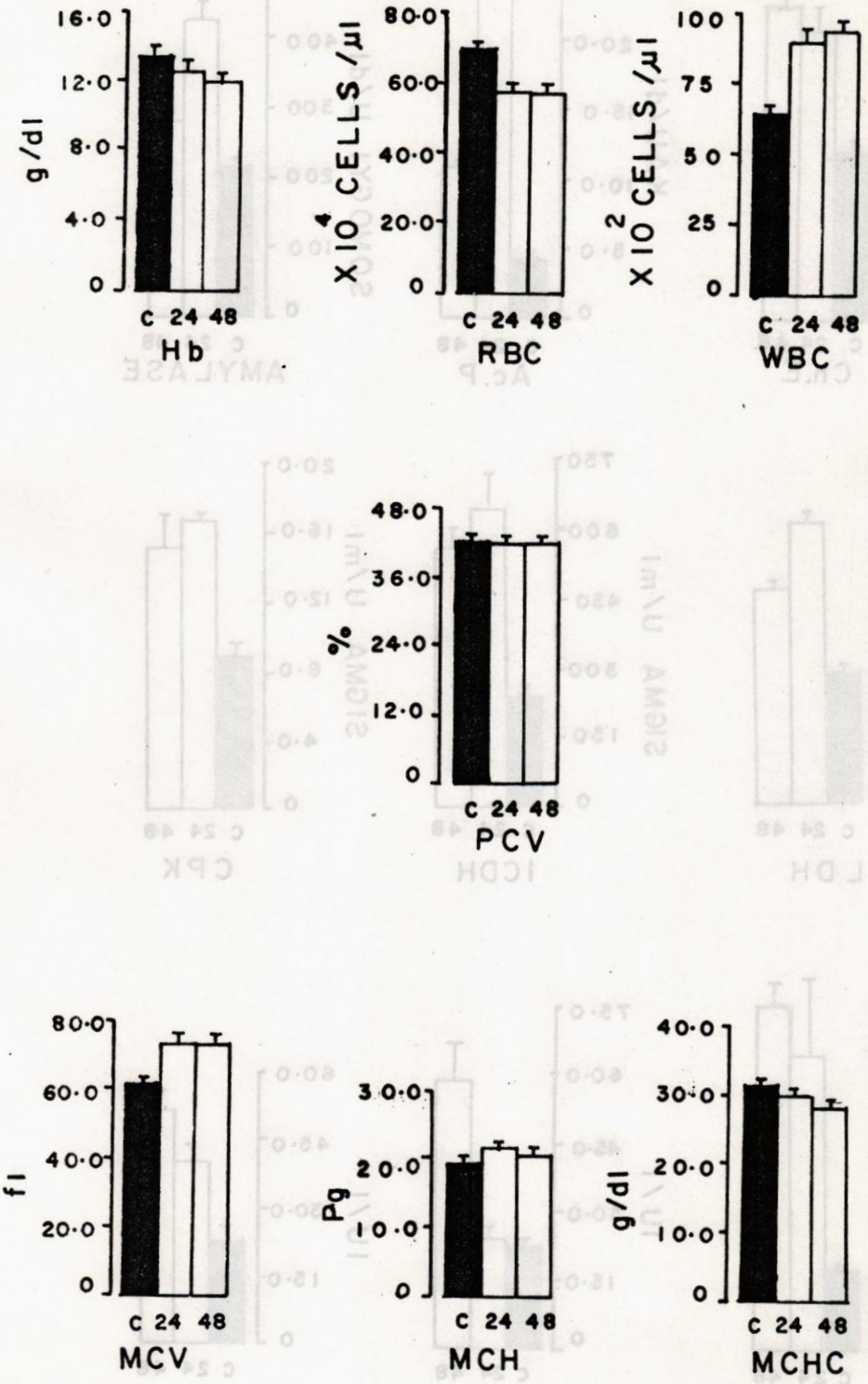


Fig.1: Effect of feeding DDT-mixed diet (100mg/kg body weight/day) for 48 hours on the various haematological parameters of albino rats.

C = CONTROL
24 = 24 HOURS DDT FEEDING
48 = 48 HOURS DDT FEEDING

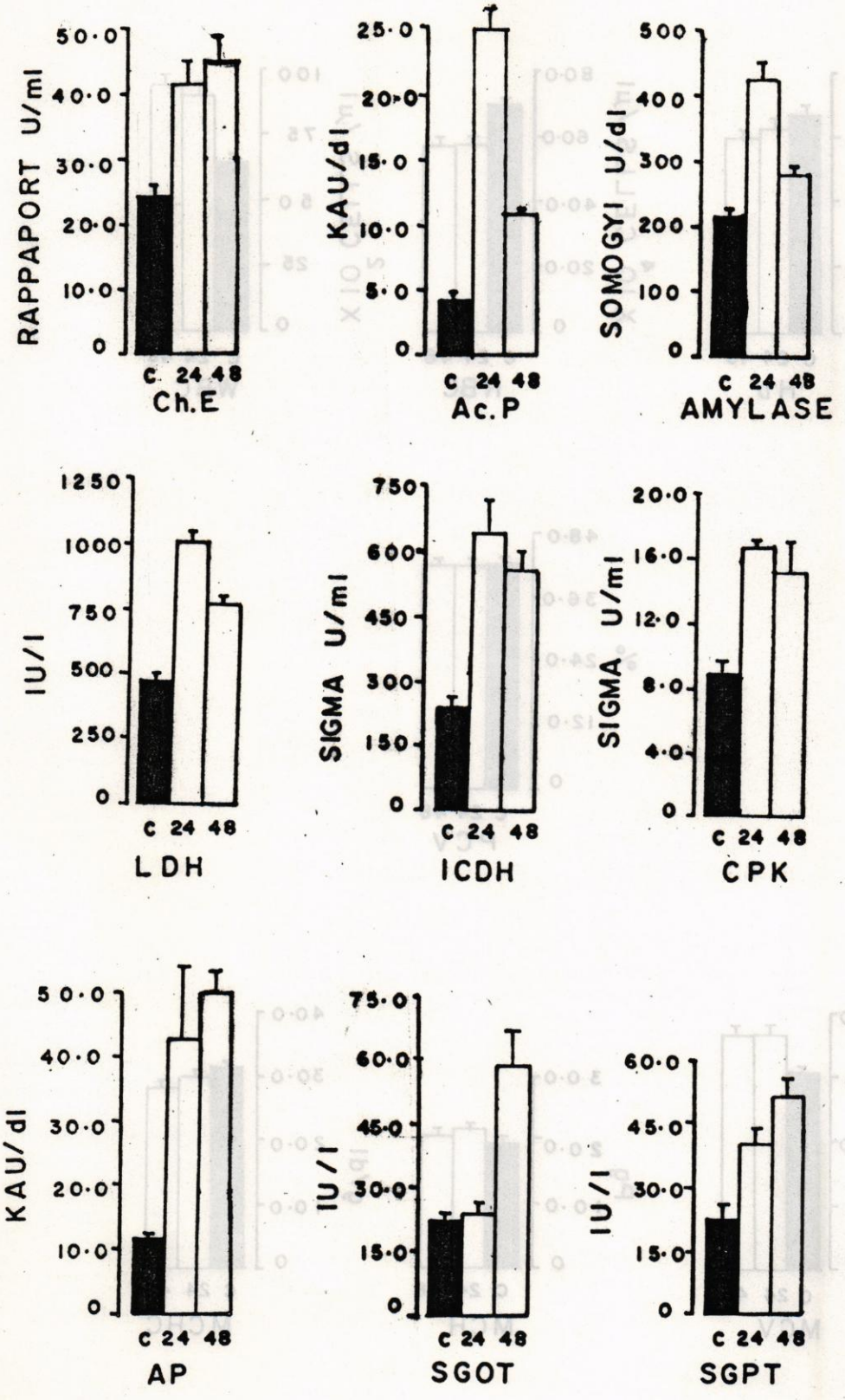


Fig.2: Effect of feeding DDT-mixed diet (100mg/kg body weight/day) for 48 hours on the activities of various enzymes of rat blood serum.

3.1.2. BIOCHEMICAL ANALYSIS OF BLOOD

The rat blood serum was tested for several enzymatic activities, to ascertain the toxic effect of DDT. Figure 2 and Table II show such changes. Almost all the enzymes tested are significantly increased. The AP activity increases 268 and 323% , while AcP activity show 489 and 158 % increase, respectively after 24 and 48 hours of DDT feeding Cholinesterase, SGOT and SGPT activities are increased after both feedings, though 24 hour feeding has more drastic effect that 48 hour feeding. The cholinesterase activity increase 70 and 77% after 24 and 48 hours of feeding. The SGPT activity likewise show 76 and 124% increase, respectively, while SGOT activity is affected (% increase) only after 48 hours of feeding. The LDH and ICDH activities are also elevated. LDH activity show 117 and 66% increase, while ICDH activity show 166 and 131% increase after 24 and 48 hours of DDT feeding. The CPK activity in control blood serum is 9.01 $\pm$ 0.63 sigma units/ml, while increases 85% after 24 hours and 69% after 48 hours of insecticide feeding. The amylase activity is also increased 95 and 29% after 24 and 48 hours of DDT feeding.

Figure 3 and Table III show the effect of DDT feeding on the various biochemical components of rat blood serum. The bilirubin content remain unaffected, while glucose and urea are not affected until 48 hours of DDT feeding. The glucose content show 36% increase, while urea content are decreased 32% after 48 hours of DDT feeding. The Cholesterol content decrease 57% and 40% after 24 and 48 hours of insecticide feeding, while FAA content show 17% decrease within 24 hours of DDT

TABLE - II

EFFECT OF FEEDING DDT MIXED DIET (100mg/kg body weight/ day) FOR 48 HOURS ON THE ACTIVITIES OF VARIOUS ENZYMES OF RAT BLOOD SERUM.

Parameters	Control (n = 8)	DDT fed	
		24 hours (n = 4)	48 hours (n = 4)
AP (KAU/dl)	11.67±0.16[a]	42.94±13.84*	49.08±1.28
AcP (KAU/dl)	4.23±0.20	24.93±1.41***	10.90±0.38***
Amylase (Somogyi units/dl)	216.77±8.02	423.53±23.76***	280.42±8.18***
ChE (Rappaport units/ml)	24.37±1.42	41.51±3.29***	43.15±4.59***
CPK (Sigma Units/ml)	9.01±0.63	16.65±0.53***	15.22±1.80**
ICDH (Sigma Units/ml)	243.18±14.80	646.85±74.91***	562.05±50.20***
LDH (IU/l)	465.66±26.29	1012.44±27.84***	774.84±26.98***
SGOT (IU/l)	21.73±1.65	24.14±1.77	58.66±8.03**
SGPT (IU/l)	23.05±2.40	40.60±3.31**	51.66±3.74***

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

C = CONTROL
24 = 24 HOURS DDT FEEDING
48 = 48 HOURS DDT FEEDING

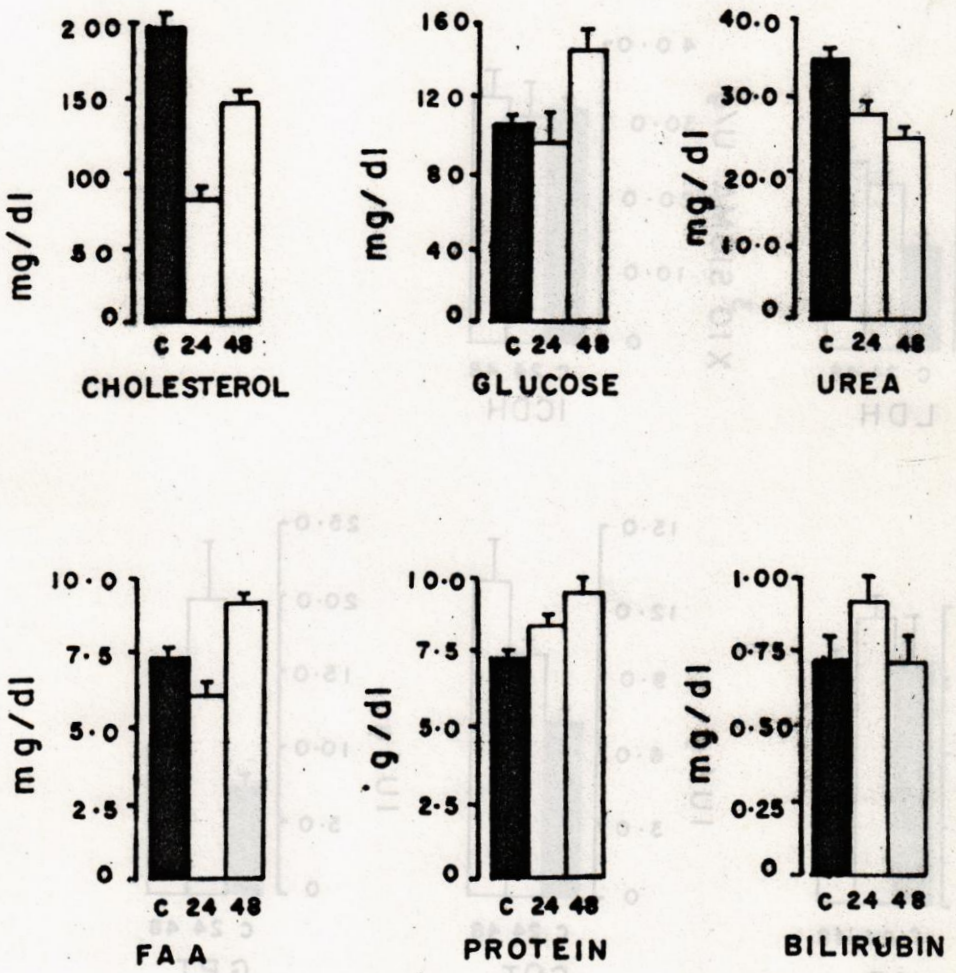


Fig.3: Effect of feeding DDT-mixed diet (100mg/kg body weight/day) for 48 hours on the various biochemical components of albino rat blood serum.

C = CONTROL
24 = 24 HOURS DDT FEEDING
48 = 48 HOURS DDT FEEDING

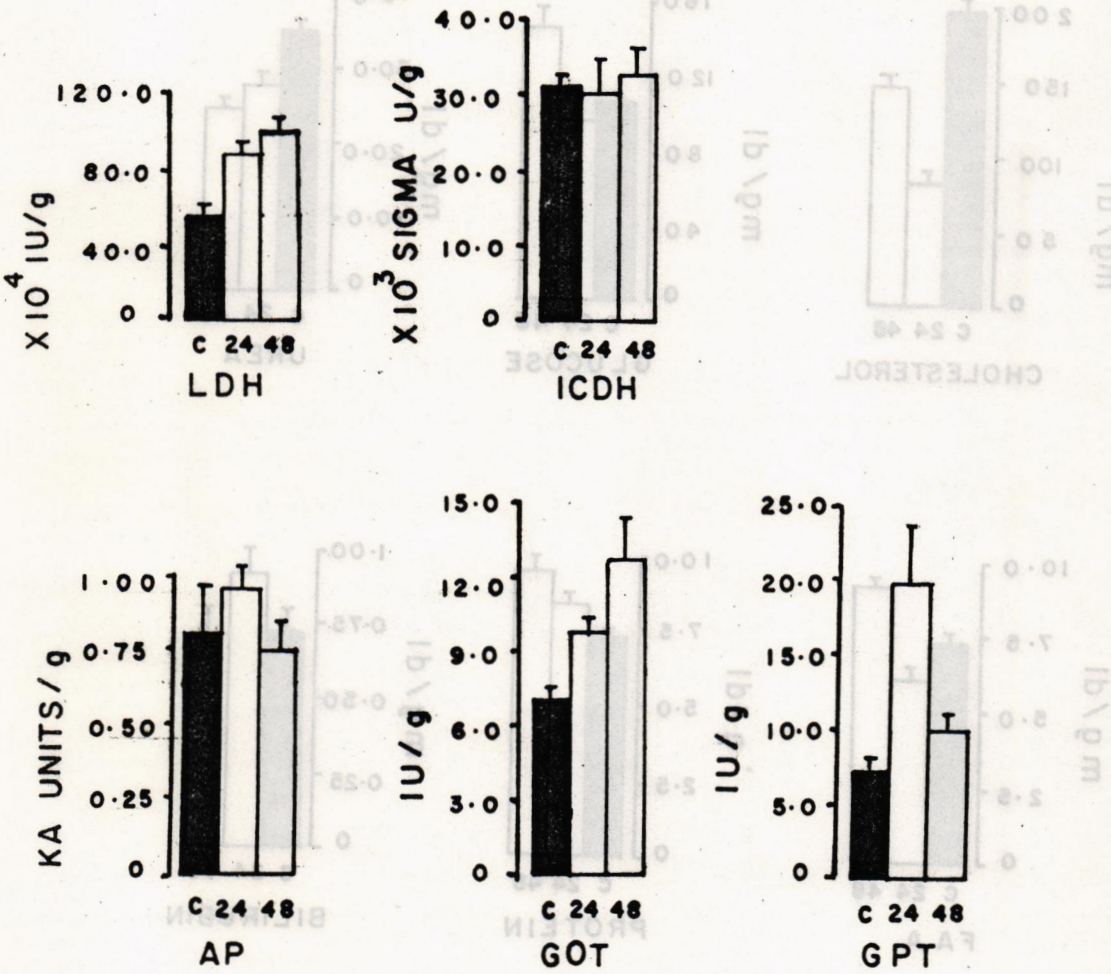


Fig. 4: Effect of feeding DDT-mixed diet (100mg/kg body weight/day) for 48 hours on some of the hepatic enzymes of albino rats.

TABLE - III

EFFECT OF FEEDING DDT MIXED DIET (100 mg/kg body weight/ day) FOR 48 HOURS ON THE VARIOUS BIOCHEMICAL COMPONENTS OF ALBINO RAT BLOOD SERUM

Parameters	Control (n = 8)	DDT fed	
		24 hours (n = 4)	48 hours (n = 4)
Bilirubin (mg/dl)	0.72±0.07[a]	0.91±0.09	0.71±0.08
Cholesterol (mg/dl)	195.64±6.94	84.20±5.50***	147.01±3.31***
FAA (mg/dl)	7.26±0.19	6.03±0.42*	7.90±0.24
Glucose (mg/dl)	107.37±3.36	97.50±16.90	146.32±10.56**
Protein (g/dl)	7.30±0.13	8.45±0.26*	9.49±0.51**
Urea (mg/dl)	35.49±0.96	32.68±1.57	24.09±0.70***

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

TABLE - IV

EFFECT OF FEEDING DDT MIXED DIET (100 mg/kg body weight/ day) FOR 48 HOURS ON THE ACTIVITIES OF SOME HEPATIC ENZYMES OF ALBINO RAT.

Parameters	Control (n = 5)	DDT fed	
		24 hours (n = 4)	48 hours (n = 4)
AP (KAU/g)	0.80±0.16[a]	0.95±0.06	0.74±0.09
GOT (IU/g)	7.11±0.38	9.76±0.33**	12.61±1.79*
GPT (IU/g)	7.32±0.68	19.87±3.52**	9.91±0.86*
ICDH ($X10^3$ Sigma Units/g)	31.39±0.78	30.59±4.24	32.60±3.06
LDH ($X10^4$ IU/g)	56.56±4.43	90.96±4.94**	96.94±2.30**

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

feeding. The blood serum protein increase 16 and 30% after 24 and 48 hours of DDT feeding.

3.1.3. BIOCHEMICAL ANALYSIS OF LIVER

Just like serum enzyme activities, the hepatic enzyme activities are also raised after DDT feeding (Table IV; Fig.4). The GOT activity shows 37 and 77% increase, while GPT activity shows 171% and 35% increase after 24 and 48 hours of DDT feeding. The LDH activity shows 61% and 71% increase during the same period. The AP and ICDH activities are not affected.

Several other hepatic biochemical components were tested (Table V, Fig.5). The nucleic acids and total protein content were not altered, while the soluble protein content increased 33% after 48 hours of DDT feeding. The FAA content, on the other hand, show drastic decrease. After 24 and 48 hours of DDT feeding @ 100 mg/kg body weight/day, the FAA content decrease 51% and 43%, respectively. The glucose content also decrease (21%) after 48 hours of feeding. The cholesterol content are not affected after 24 hours of feeding, but when this feeding is prolonged for 48 hours, these content decrease 44%.

3.1.4 HISTOLOGICAL STRUCTURE OF LIVER

Table VI and Figure 6 show the effect of 100 mg DDT/kg body weight/day for 48 hours on the histological structure of liver.

The hepatic cells increase in size. This increase is 29% in 24 hour feeding group and 23% in 48 hour feeding. The nuclear size is slightly increased,

TABLE - V

EFFECT OF FEEDING DDT MIXED DIET (100 mg/kg body weight/ day) FOR 48 HOURS ON THE VARIOUS BIOCHEMICAL COMPONENTS OF ALBINO RAT LIVER.

Parameters	Control (n = 5)	DDT fed	
		24 hours (n = 4)	48 hours (n = 4)
Cholesterol (mg/g)	7.62±0.22[a]	7.64±0.78	4.23±0.68**
FAA (µg/g)	399.21±18.13	194.85±17.51***	226.11±16.43***
Glucose (mg/g)	20.14±0.53	16.06±1.41*	16.00±1.88*
Soluble Protein (mg/g)	111.18±5.08	122.06±8.06	158.45±8.45**
Total Protein (mg/g)	199.33±6.11	211.58±16.29	218.44±11.58
DNA (mg/g)	3.84±0.44	2.64±0.50	3.68±0.58
RNA (mg/g)	9.53±0.55	7.86±0.90	9.61±0.29

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

C = CONTROL
24 = 24 HOURS DDT FEEDING
48 = 48 HOURS DDT FEEDING

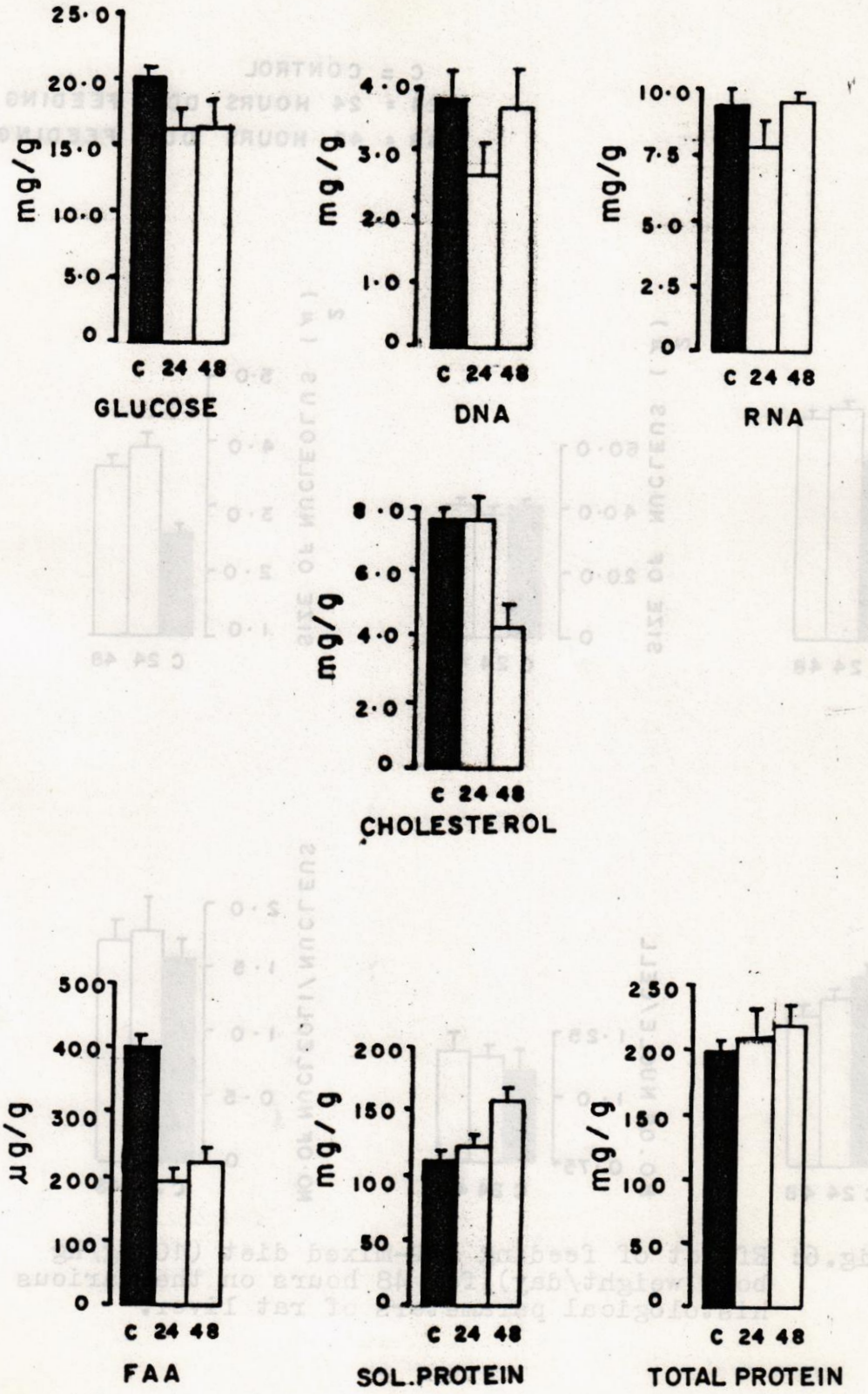


Fig.5: Effect of feeding DDT mixed diet (100mg/kg body weight/day) for 48 hours on the various biochemical components of albino rat livers.

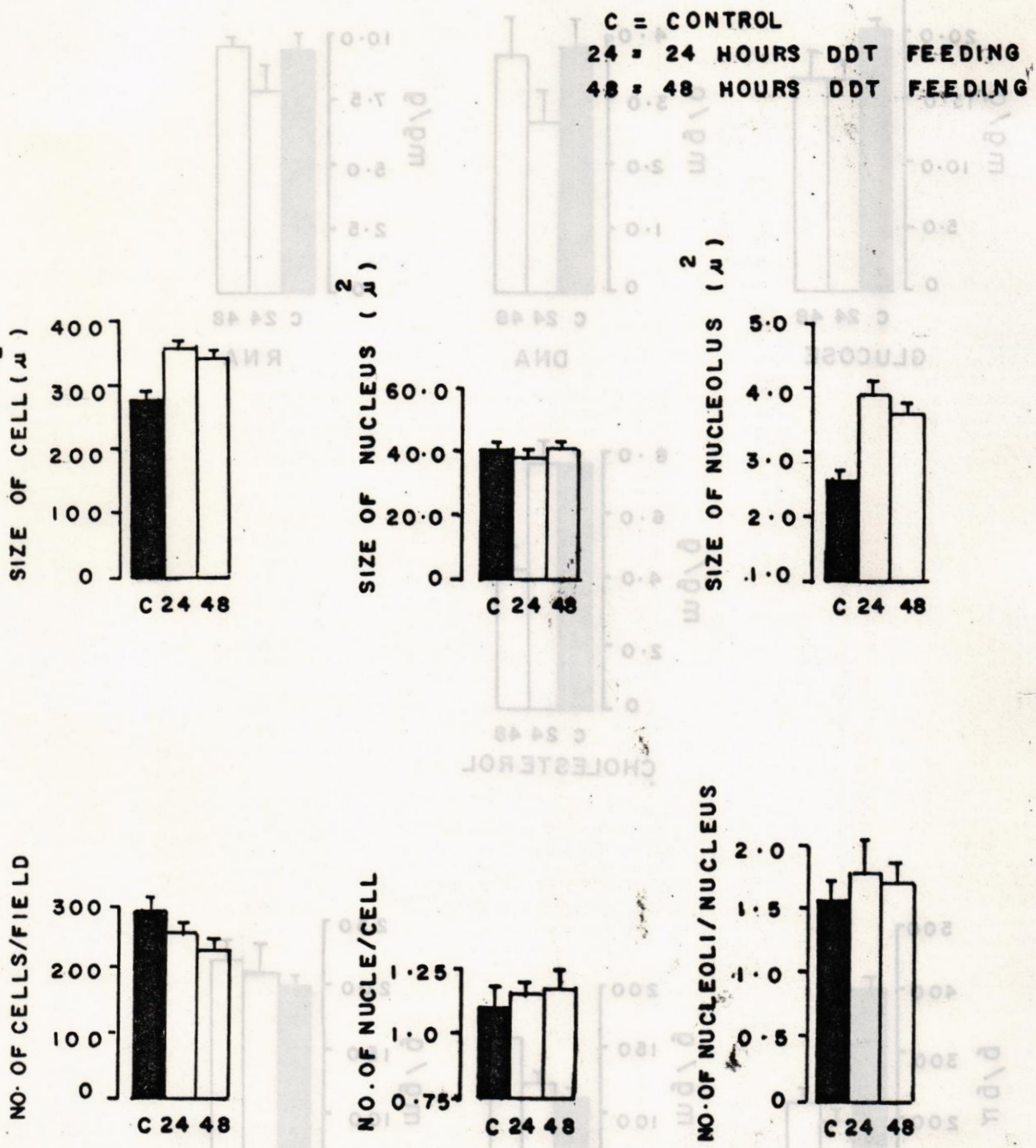


Fig.6: Effect of feeding DDT-mixed diet (100mg/kg body weight/day) for 48 hours on the various histological parameters of rat liver.

TABLE - VI

EFFECT OF FEEDING DDT-MIXED DIET (100mg/kg body weight/ day) ON THE VARIOUS HISTOLOGICAL PARAMETERS OF RAT LIVER.

Parameters	Control (n = 90)	DDT- fed	
		24 hours (n=90)	48 hours (n=90)
No. of cells/ field	291.42±16.73[a]	244.55±9.78*	228.49±13.52*
No. of nuclei/ cell	1.10±0.08	1.15±0.05	1.18±0.07
No. of nucleoli/ nucleus	1.58±0.16	1.78±0.25	1.72±0.16
Size of cell (μ^2)	281.77±11.46	363.72±9.02***	345.57±6.30**
Size of nucleus (μ^2)	39.57±2.04	37.54±1.18	40.98±1.34
Size of nucleolus (μ^2)	2.52±0.34	3.93±0.38	3.62±0.26

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

but this increase is not statistically significant. The nucleolar size, on the other hand, is significantly increased. This increase is 56% in 24 hours and 44% in 48 hour feeding group. The number of nuclei/cell and number of nucleoli/nucleus are not changed and remain unaffected after DDT feeding.

Figures 11-14 show histological structure of liver of rat fed on DDT mixed diet for 24 hours, while Figures 15-18 show histological changes after 48 hours of feeding.

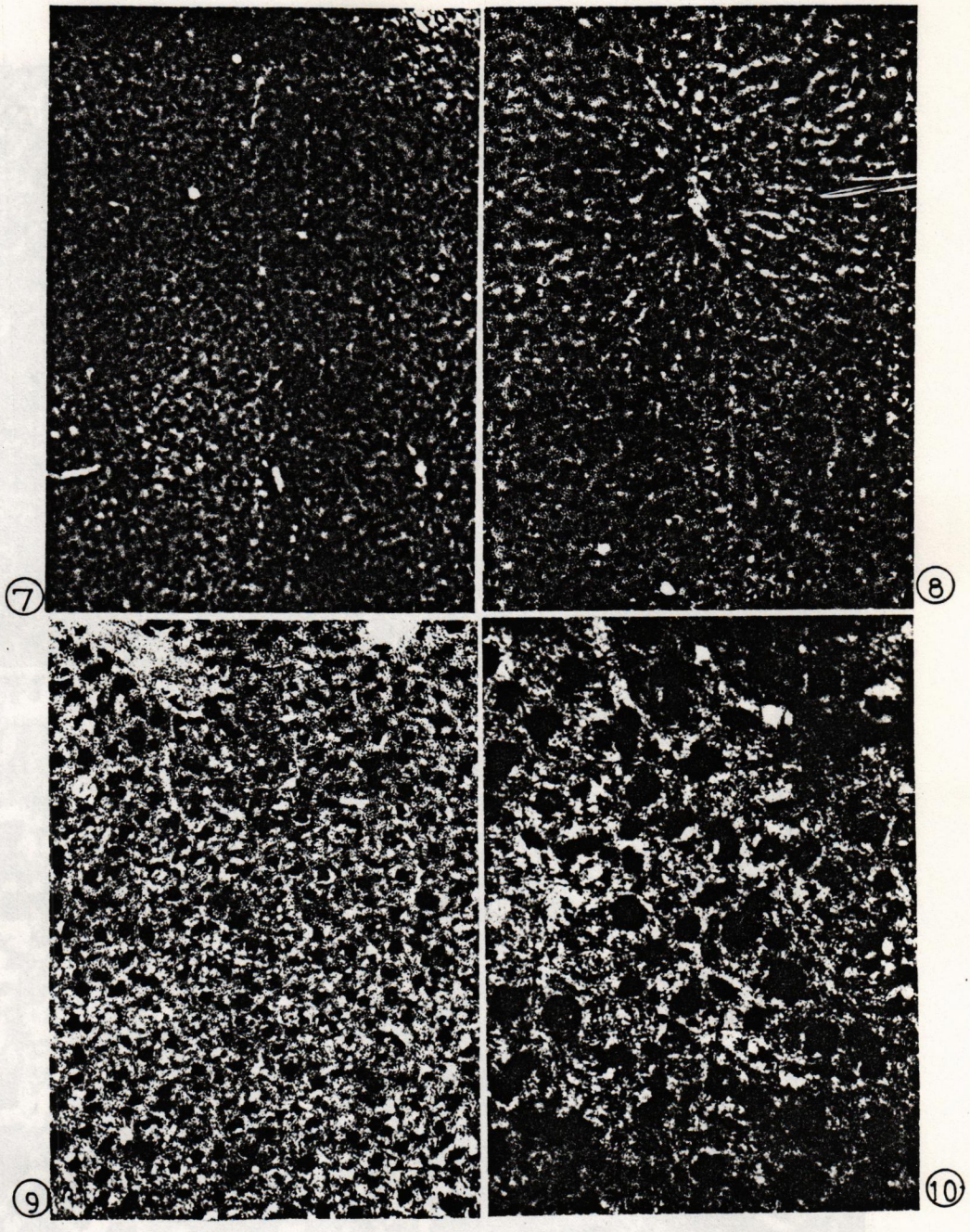
The hepatic cells, nuclei and nucleoli hypertrophy. The nuclei in 24 hour group are condensed with a clear space around nuclei. The blood vessels and bile canaliculi are prominently enlarged (Figures 17 and 18; compare with Figures 10 and 14). The nuclei are distinctly enlarged and vesicular.

The general hepatolobular architecture remains undisturbed.

3.2. EFFECT OF DDT ADMINISTERED AT A DOSE OF 20 mg/kg body weight/day FOR A PERIOD OF 15 DAYS

3.2.1. BODY WEIGHT AND LIVER WEIGHT

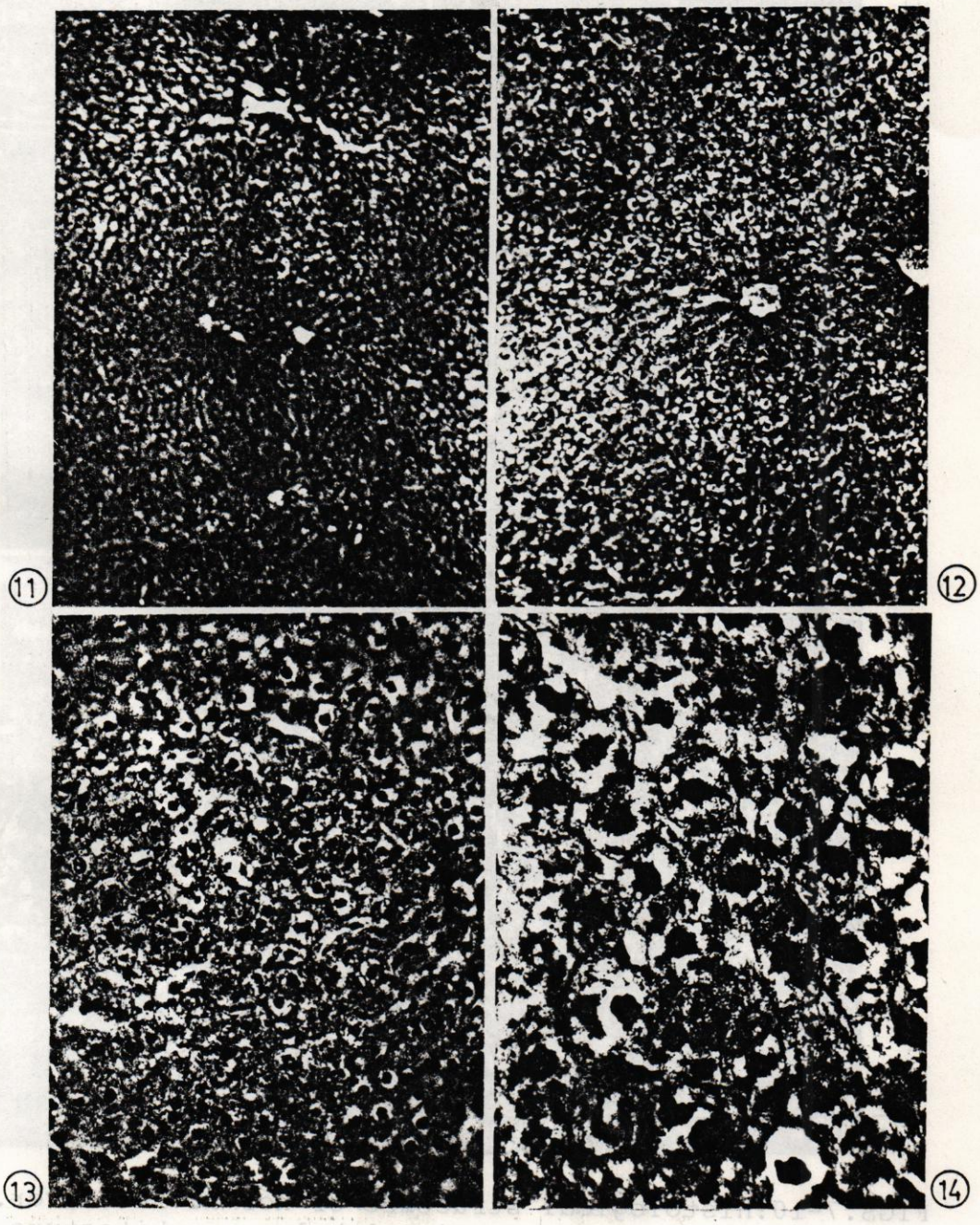
The body weight is decreased after DDT administration for 15 days. In control rats the body weight gains at a rate of 1.99 $\pm$ 0.15% per day (n = 6). The percent body weight gain is decreased to 1.82, 1.32, 1.11 and 1.07 after 6, 9, 12 and 15 days of DDT feeding (Table VII).



FIGS.7-10:Histological structure of normal rat liver. Note the normal hepatic lobular architecture (7) sinusoidal spacing, hepatic cords, hepatocytes and nuclear arrangement (8-10).

Stain: Hematoxylin and Eosin.

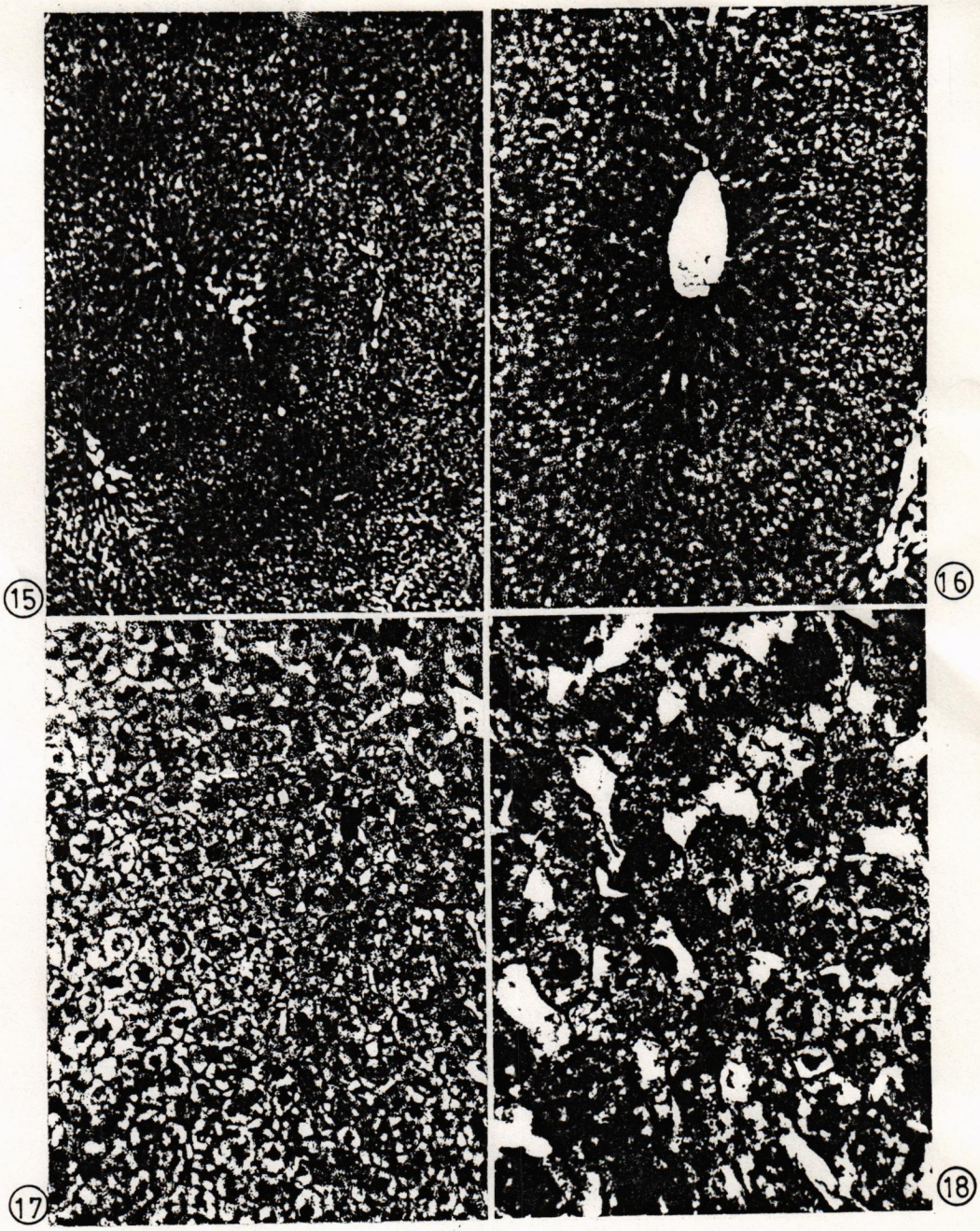
Magnifications: 7,x25; 8,x50; 9,x100; 10,x250.



FIGS.11-14:Histological structure of rat liver fed on DDT-mixed diet for 24 hours. Note the disturbed arrangement of hepatic cords (11) mildly enlarged sinusoidal areas (11-13) clear area in the cytoplasm arround the nuclei (12-14) and hypertrophy of the hepatocytes (13,14).

Stain: Hematoxylin and Eosin.

Magnifications: 11,x25; 12,x50; 13,x100; 14,x250.



FIGS.15-18: Histological structure of rat liver fed on DDT-mixed diet for 48 hours. Note the large number of vacuoles in the tissues (15-17), high degree of cytoplasmic margination arround the nuclei (16-17), large sinusoidal spaces (16-18) and hypertrophied cells and nuclei (17-18). Also note the darkly stained oblong area in the center of the hepatic lobule (18).

Stain: Hematoxylin and Eosin.

Magnifications: 15,x25; 16,x50; 17,x100; 18,x250.

	3 day	slaughtering
Control (n = 6)	185.00±11.86[a]	214.42±12.10
3 day (n = 3)	182.62±6.82	193.50±6.85
6 day (n = 3)	163.25±4.82	181.12±6.15
9 day (n = 3)	159.25±5.45	178.12±5.40
12 day (n = 3)	167.13±4.09	189.37±3.21
15 day (n = 3)	166.37±6.53	192.87±7.12

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

The liver weight is, on the other hand, increased after DDT feeding. The liver weight in control animals is 5.89 ± 0.41 (n = 6). After 15 days of insecticide feeding the liver weighs 7.09 ± 0.27 gm (n = 3). The liver weight, when considered in terms of per cent body weight, increases after DDT feeding. In control rats the liver weight is 2.76 ± 0.05(n = 5) per cent of body weight, which increases to 3.68 ± 0.09% (n = 5) after 15 days of DDT administration at a dose of 20 mg/kg body weight/day (Table VII).

3.2.2. HAEMATOLOGICAL STUDIES

Table VIII and Figure 19 show changes in the various haematological parameters after DDT feeding. The haemoglobin content do not change after insecticide feeding. The RBC count, however, decreases considerably. A control rat contains 69.10 ± 1.20 X 10^5 RBCs/µl (n=6), which after DDT treatment decrease significantly and are reduced by 17% after 15 days of insecticide treatment. The WBCs, on the other hand, increase significantly after 6 days of DDT feeding. After 15 days of insecticide feeding the WBC count increases 46%. The PCV is not changed until day 9, when it is reduced by 6%. On day 15 PCV is reduced by 7%.

The MCV in control rats is 62.64 ± 0.46 fl(n=6), which is increased considerably after DDT administration. This increase is 6% after 3 days of insecticide treatment and 12.40% after 15 days of insecticide treatment. The MCH is increased 7% after 9 days and 9% after 15 days. The MCHC is decreased after 9 days of DDT feeding (Table VIII; Fig. 19).

Hb (g/dl)	13.04±0.16[a]	12.37±0.39
RBC ($X10^5$ cells/µl)	69.10±1.20	63.63±1.17**
WBC ($X10^2$ cells/µl)	65.50±5.20	77.00±5.63
PCV (%)	43.28±0.40	42.17±0.93
MCV (fl)	62.64±0.46	66.26±0.36***
MCH (Pg)	18.88±0.28	19.42±0.27
MCHC (g/dl)	30.15±0.36	29.31±0.32

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

C = CONTROL
3 = 3 DAY DDT FEEDING
6 = 6 DAY DDT FEEDING
9 = 9 DAY DDT FEEDING
12 = 12 DAY DDT FEEDING
15 = 15 DAY DDT FEEDING

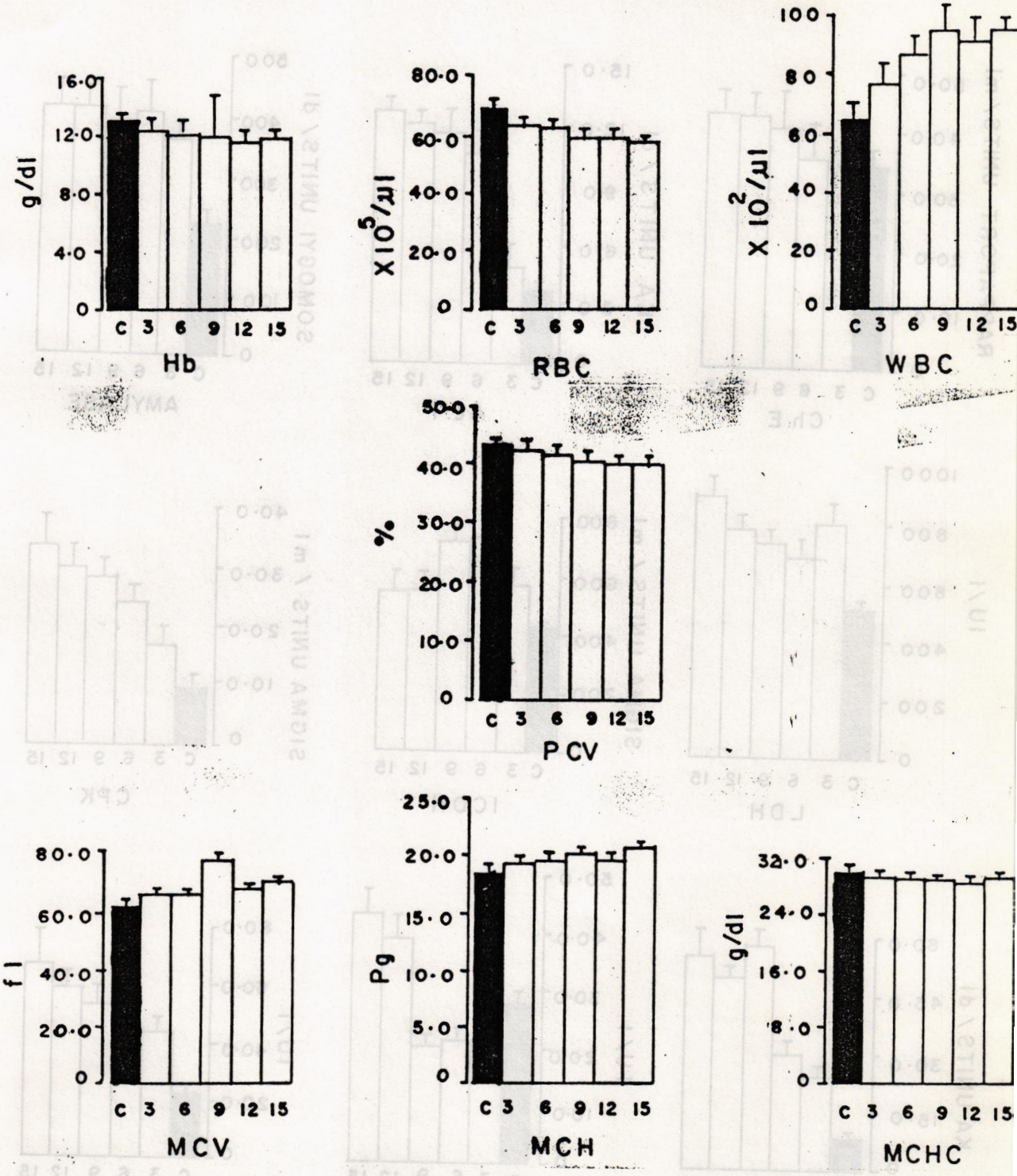


Fig.19: Effect of feeding DDT-mixed diet (20mg/kg body weight/day) for 15 days on the haematological parameters of albino rats.

C = CONTROL
3 = 3 DAY DDT FEEDING
6 = 6 DAY DDT FEEDING
9 = 9 DAY DDT FEEDING
12 = 12 DAY DDT FEEDING
15 = 15 DAY DDT FEEDING

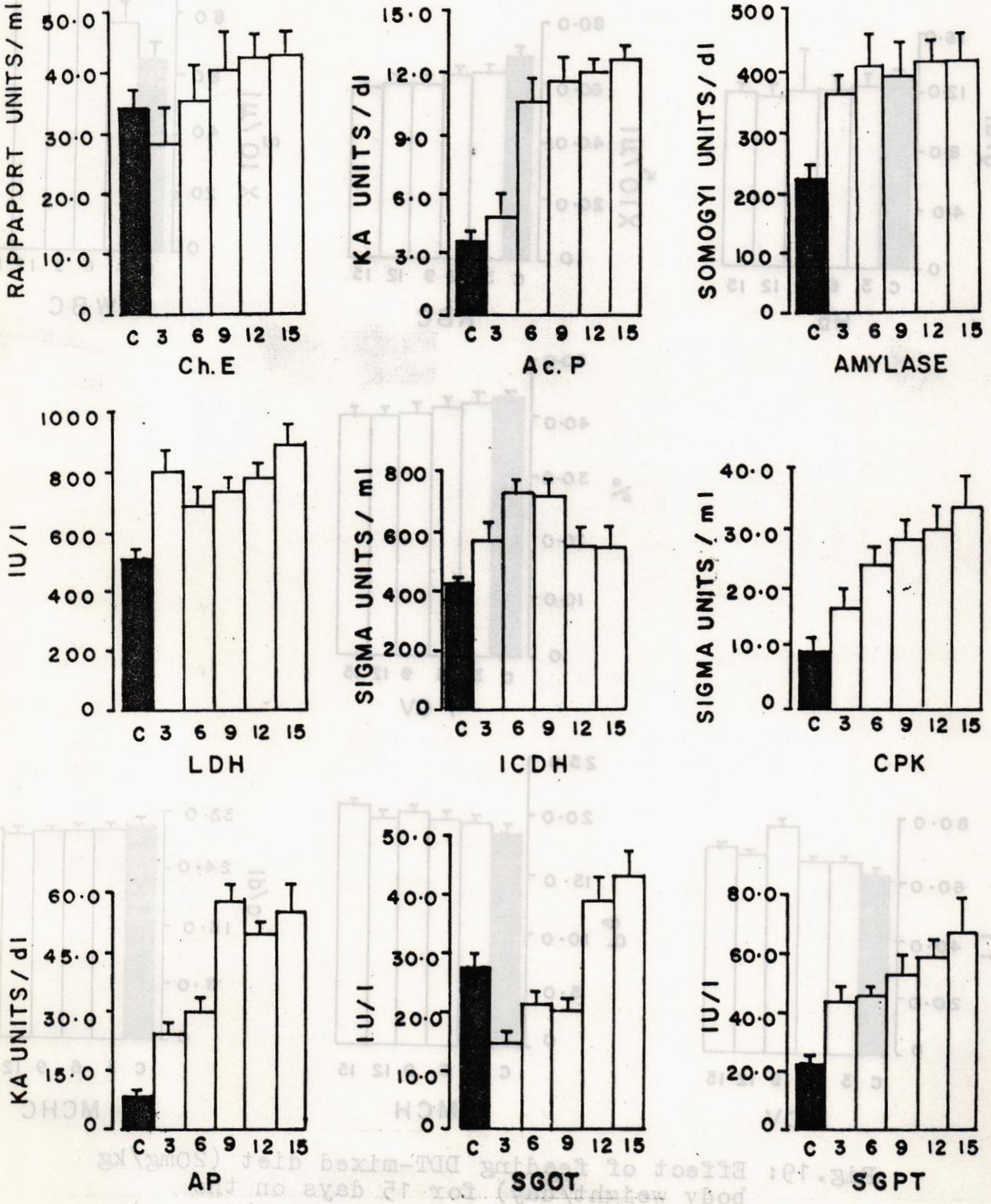


Fig.20: Effect of feeding DDT-mixed diet (20mg/kg body weight/day) for 15 days on the various enzymatic activities in rat blood serum.

TABLE - IX

EFFECT OF FEEDING DDT MIXED DIET (20 mg/kg body weight/day) FOR 15 DAYS ON THE ACTIVITIES OF VARIOUS ENZYMES IN RAT BLOOD SERUM.

para-meters	Control (n = 6)	DDT feeding 3 days (n=4)	6 days (n=4)	9 days (n=4)	12days (n=4)	15days (n=4)
AP (KAU/dl)	8.57[a] ± 0.39	24.90*** ± 2.28	29.67*** ± 3.27	58.67*** ± 3.25	49.60*** ± 2.94	55.27*** ± 6.00
AcP (KAU/dl)	3.63 ± 0.26	4.92 ± 0.96	10.68*** ± 0.96	11.62*** ± 0.81	12.09*** ± 0.57	12.64*** ± 0.49
Amylase (Somogyi U/dl)	226.76 ±14.45	366.66*** ±23.57	410.98** ±44.88	392.85* ±53.92	414.81*** ±24.93	416.66** ±39.09
ChE (Rappaport U/ml)	34.77 ± 2.34	28.87 ± 5.55	35.76 ± 5.25	40.34 ± 5.85	42.53 ± 3.02	42.72 ± 3.17
CPK (Sigma U/ml)	9.66 ± 1.60	16.47* ± 2.33	24.05*** ± 2.03	28.32*** ±2.66	29.85*** ± 3.16	33.60*** ± 4.38
SGOT (IU/l)	27.80 ± 1.48	14.99*** ± 1.08	21.45* ± 1.67	20.29** ± 1.35	38.99* ± 3.37	43.15** ± 3.44
SGPT (IU/l)	22.39 ± 1.60	43.58** ± 4.34	45.87*** ± 2.66	52.93*** ± 5.62	59.24*** ± 4.36	67.16** ±10.07
ICDH (Sigma U/ml)	428.76 ±12.93	591.66* ±53.48	732.09** ±37.14	722.98*** ±45.14	756.59*** ±61.94	751.12*** ±69.42
LDH (IU/l)	506.56 ±21.91	801.00** ±65.18	689.88* ±56.98	739.80*** ±37.51	780.00*** ±39.95	897.12*** ±46.84

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

3.2.3. BIOCHEMICAL ANALYSIS OF BLOOD

Table IX and Figure 20 show the effect of DDT on the activities of various enzymes in Rat blood serum. The AP and AcP activities are considerably elevated. The AP activity shows 2.90 X 3.46 X, 6.85X, 5.79X and 6.45X increase over the control level after 3,6,9,12 and 15 days of DDT feeding. The AcP activity likewise is increased 1.36X, 2.94X, 3.20X, 3.33X, and 3.48X after 3,6,9,12 and 15 days of DDT feeding.

The amylase activity is raised 62%, 81%, 73%, 83% and 84% after 3, 6, 9, 12 and 15 days of insecticide feeding. The ChE activity is not affected after DDT feeding. The CPK activity in control rat blood serum is 9.66 ± 1.60 sigma units/ml (n=6). This activity increases 73%, 149%, 193%, 209% and 248% after 3, 6, 9, 12 and 15 days of DDT feeding.

The SGOT activity is decreased until day 9 and is then increased on day 12 and 15. The decrease is 46%, 23% and 27% after 3, 6 and 9 days of feeding. After 12 days and 15 days of DDT feeding the GOT activity is increased 40% and 55%. The SGPT activity is continuously increased. The increase is 95%, 105%, 136%, 165% and 200%, 3, 6, 9, 12 and 15 days of insecticide feeding.

The activities of two dehydrogenases i.e. ICDH and LDH are considerably increased. The ICDH activity in blood serum of control rats is 428.76 ± 12.93 sigma units/ml (n = 6), which increases 38%, 71%, 69%, 75% and 75% after 3, 6, 9, 12 and 15 days of DDT feeding. The LDH activity is increased 58%, 36%, 46%, 54% and 77% after 3, 6, 9, 12 and 15 days of DDT administration (Table IX, Fig. 20).

C = CONTROL
3 = 3 DAY DDT FEEDING
6 = 6 DAY DDT FEEDING
9 = 9 DAY DDT FEEDING
12 = 12 DAY DDT FEEDING
15 = 15 DAY DDT FEEDING

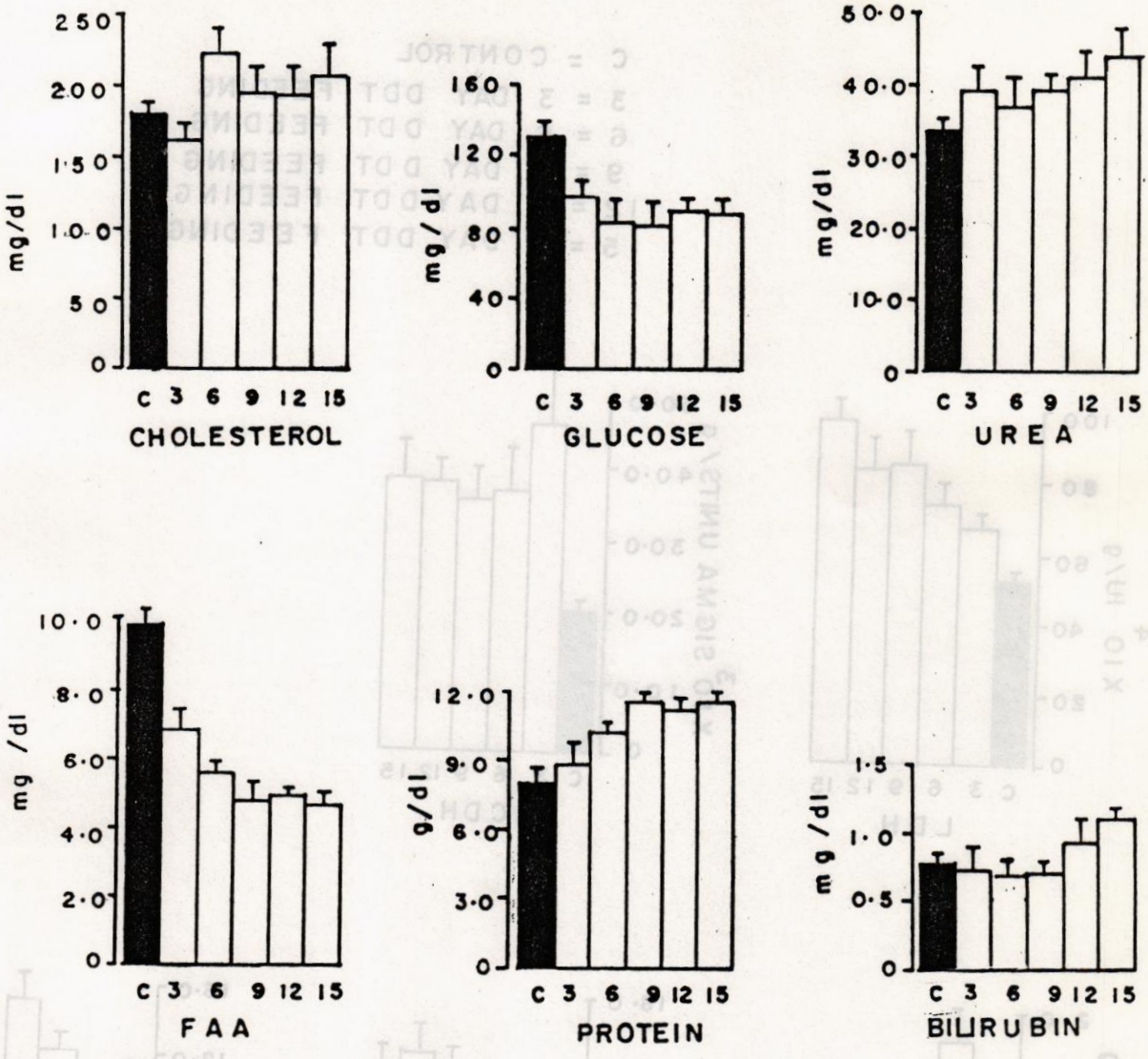


Fig.21: Effect of DDT-mixed diet (20mg/kg body weigh day) for 15 days on the various biochemical components of rat blood serum.

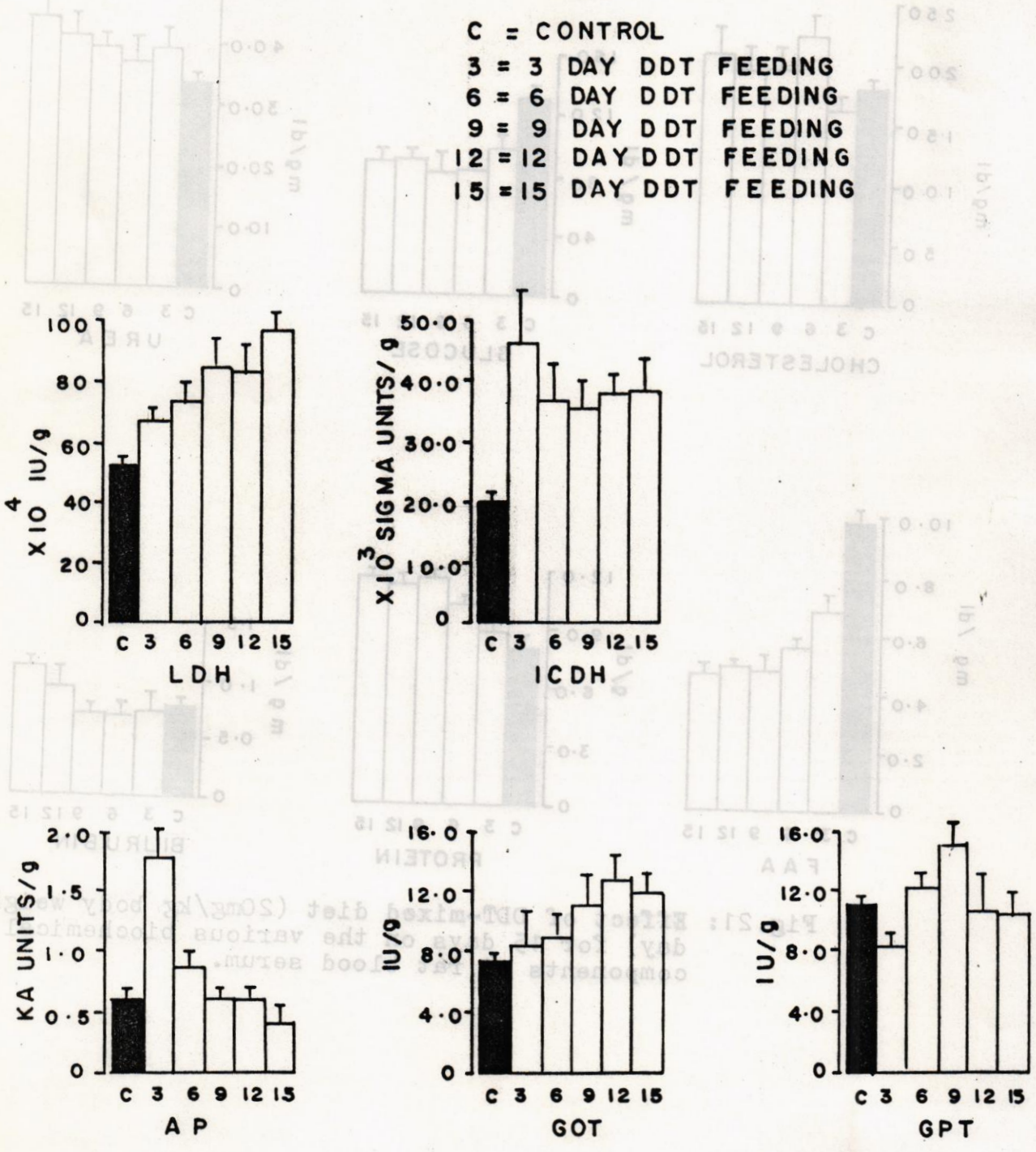


Fig.22: Effect of DDT-mixed diet (20mg/kg body weight/day) for 15 days on activities of some hepatic enzymes of albino rats.

Cholesterol (mg/dl)	180.73±7.16	161.92±9.64
FAA (mg/dl)	9.81±0.14	6.83±0.55***
Glucose (mg/dl)	129.60±7.08	97.72±7.56*
Protein (g/dl)	8.04±0.21	8.89±0.73
Urea (mg/dl)	33.56±1.19	39.16±2.66

[a]Mean±SEM, Student's 't' test; *P < 0.

TABLE - XI

EFFECT OF FEEDING DDT MIXED DIET (20 mg/kg body weight/day) FOR 15 DAYS ON THE ACTIVITIES OF VARIOUS HEPATIC ENZYMES.

Parameters	Control (n = 5)	DDT feeding				
		3 days (n = 4)	6 days (n = 4)	9 days (n = 4)	12 days (n = 4)	15 days (n = 4)
AP (KAU/g)	0.64±0.02[a]	1.79±0.21***	0.87±0.11	0.61±0.05	0.62±0.06	0.43±0.12
GOT (IU/g)	7.34±0.17	8.79±2.26	8.89±1.28	11.03±1.77	12.80±1.51**	11.82±1.15**
GPT (IU/g)	11.07±0.25	8.29±0.83*	12.10±0.59	14.86±1.38*	10.45±2.25	10.31±1.29
ICDH ($X10^3$ Sigma (U/g)	20.10±0.50	46.32±8.60*	36.71±5.88*	36.54±4.44*	36.9 ±3.09**	37.25±5.10*
LDH ($X10^4$IU/g)	51.90±1.20	67.14±3.60**	73.12±5.48**	84.27±9.11**	83.13±8.84**	94.61±5.15***

[a]Mean±SEM, Student's 't' test; *P< 0.05; **P< 0.01; ***P< 0:01

Besides enzyme activities, a few other biochemical components were tested (Table X, Figure 21). The cholesterol and urea content remain unchanged. The bilirubin content are also not significantly affected until day 15, when these content increase 45%. The FAA content decrease significantly, while protein content show significant increase. The decrease in FAA content is 30%, 43%, 51%, 50% and 52%, after 3, 6, 9, 12 and 15 days of DDT feeding. The protein content conversely increase 27%, 44%, 39% and 44% after 6, 9, 12 and 15 days of insecticide feeding. The glucose content decrease. This decrease is 25% after 3 days of feeding and 32% after 15 days of feeding (Table X, Fig. 21).

3.2.4. BIOCHEMICAL ANALYSIS OF LIVER

Table XI and Figure 22 show effect of DDT feeding on the activities of various hepatic enzymes in rat liver. The AP activity is raised 180% after 3 days of DDT feeding, but is then normalized with increasing period of administration. In fact with continuous feeding the AP activity is indicated to be inhibited. The GOT and GPT activities are also mildly affected. A control rat liver shows 11.07 $\pm$ 0.25 IU GPT activity/g tissue, which is reduced 25% after 3 days of feeding, while GOT activity is raised 74% after 12 days of feeding. The ICDH and LDH activities appear to be highly sensitive to DDT feeding. The ICDH activity is raised 130% after 3 days of feeding, and to 85% after 15 days of insecticide administration. The LDH activity in control rat liver is 51.90 $\pm$ 1.20 X 10^4IU/g. This activity increases 29%, 41%, 62%, 60% and 82% after 3, 6, 9, 12 and 15 days of feeding (Fig.22;Table XI).

TABLE - XII

EFFECT OF FEEDING DDT MIXED DIET (20 mg/kg body weight/ day) FOR 15 DAYS ON THE VARIOUS BIOCHEMICAL COMPONENTS OF RAT LIVER.

Parameters	Control (n= 5)	DDT feeding 3 days (n=4)	6 days (n=4)	9 days (n=4)	12days (n=4)	15days (n=4)
Cholesterol (mg/g)	15.72[a] ± 0.35	5.06*** ± 0.95	8.91** ± 1.42	7.26*** ± 0.71	9.47*** ± 0.37	9.77** ± 1.18
FAA (ug/g)	319.95 ±12.68	147.09*** ± 5.15	163.46*** ± 7.89	192.73*** ±12.36	222.23** ±23.41	220.75** ±18.97
Glucose (mg/g)	12.93 ± 0.61	10.56 ± 1.78	18.11* ± 1.38	22.61*** ± 1.65	25.75** ± 2.31	24.28** ± 2.66
Soluble Proteins (mg/g)	152.99 ± 5.62	116.28** ± 7.17	99.44** ± 4.24	106.45* ±11.45	104.25** ± 9.58	100.88** ± 7.99
Total Protein (mg/g)	237.67 ± 7.45	224.83 ±12.15	211.02 ±12.73	204.59 ±11.80	198.49* ± 9.37	188.35** ±10.81
DNA (mg/g)	2.42 ± 0.17	2.70 ± 0.21	3.19* ± 0.24	3.40* ± 0.25	3.43* ± 0.33	3.38* ± 0.25
RNA (mg/g)	8.62 ± 0.38	8.92 ± 0.73	9.91 ± 0.94	9.26 ± 0.77	8.83 ± 0.78	7.82 ± 0.62

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

Several other hepatic biochemical components, besides enzyme activities were also analyzed (Table XII; Fig.23). Almost all hepatic components were significantly affected. The cholesterol content is drastically decreased i.e. 68% after 3 days of DDT feeding. The decrease on day 6, 9, 12 and 15 is respectively, 43%, 54%, 40% and 38%, respectively. Total protein content are not altered until day 12, when 16% decrease was recorded. After 15 days of feeding, the total protein was found to decrease 21%. The soluble proteins, and FAA content likewise decrease after DDT administration for 15 days. A control rat liver contains 152.99 ± 5.62 mg soluble proteins/g liver. After 3 days of feeding the soluble protein content decrease 24%. It continues to decrease till day 15 of DDT feeding, when soluble protein content decrease 34%. The FAA are also drastically decreased. These content decrease 54%, 49%, 40%, 31% and 31% respectively after 3, 6, 9, 12 and 15 days of DDT feeding. The glucose content remain unaffected after 3 days of feeding. The first significant change (40% increase) is noted after 6 days of feeding. The glucose content increase 88% after 15 days of feeding DDT mixed diet.

Out of the two nucleic acids the RNA does not show any significant change, while DNA exhibits significant increase after prolonged feeding period. The DNA content in control rat liver is 2.42 ± 0.17 mg/g liver weight. This content increases 32%, 40%, 42% and 40% after, 6, 9, 12 and 15 days of DDT feeding. (Table XII, Fig. 23).

TABLE - XIII

EFFECT OF FEEDING DDT MIXED DIET (20 mg/kg body weight/day) ON THE VARIOUS HISTOLOGICAL PARAMETERS OF RAT LIVER.

Parameter	Control (n =)	DDT - fed 3 days (n =)	6 days (n =)	9 days (n =)	12 days (n =)	15 days (n =)
No. of cells/ field	267.44[a] ±15.13	242.39 ±11.19	221.29* ± 9.48	232.61 ±12.77	205.61* ±10.37	193.48** ± 9.87
No. of nuclei/ cell	1.12 ± 0.04	1.25 ± 0.06	1.15 ± 0.07	1.12 ± 0.04	1.19 ± 0.04	1.23 ± 0.09
No. of nucleoli/ nucleus	1.56 ± 0.15	1.60 ± 0.14	1.82 ± 0.13	1.80 ± 0.24	1.70 ± 0.11	1.80 ± 0.25
Size of cell (μ^2)	270.63 ±11.21	298.36 ± 8.05	308.11* ± 7.86	309.12* ± 6.25	334.82** ± 8.05	341.05** ±15.13
Size of nucleus (μ^2)	43.34 ± 1.20	45.40 ± 1.75	44.36 ± 1.47	46.98 ± 1.56	51.09** ± 1.55	48.87* ±1.35
Size of Nucleolus(μ^2)	2.87 ± 0.21	3.70* ± 0.25	3.35 ± 0.23	3.26 ± 0.36	3.80 ± 0.45	3.09 ± 0.60

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

C= CONTROL
3=3 DAY DDT FEEDING
6=6 DAY DDT FEEDING
9=9 DAY DDT FEEDING
12=12 DAY DDT FEEDING
15=15 DAY DDT FEEDING

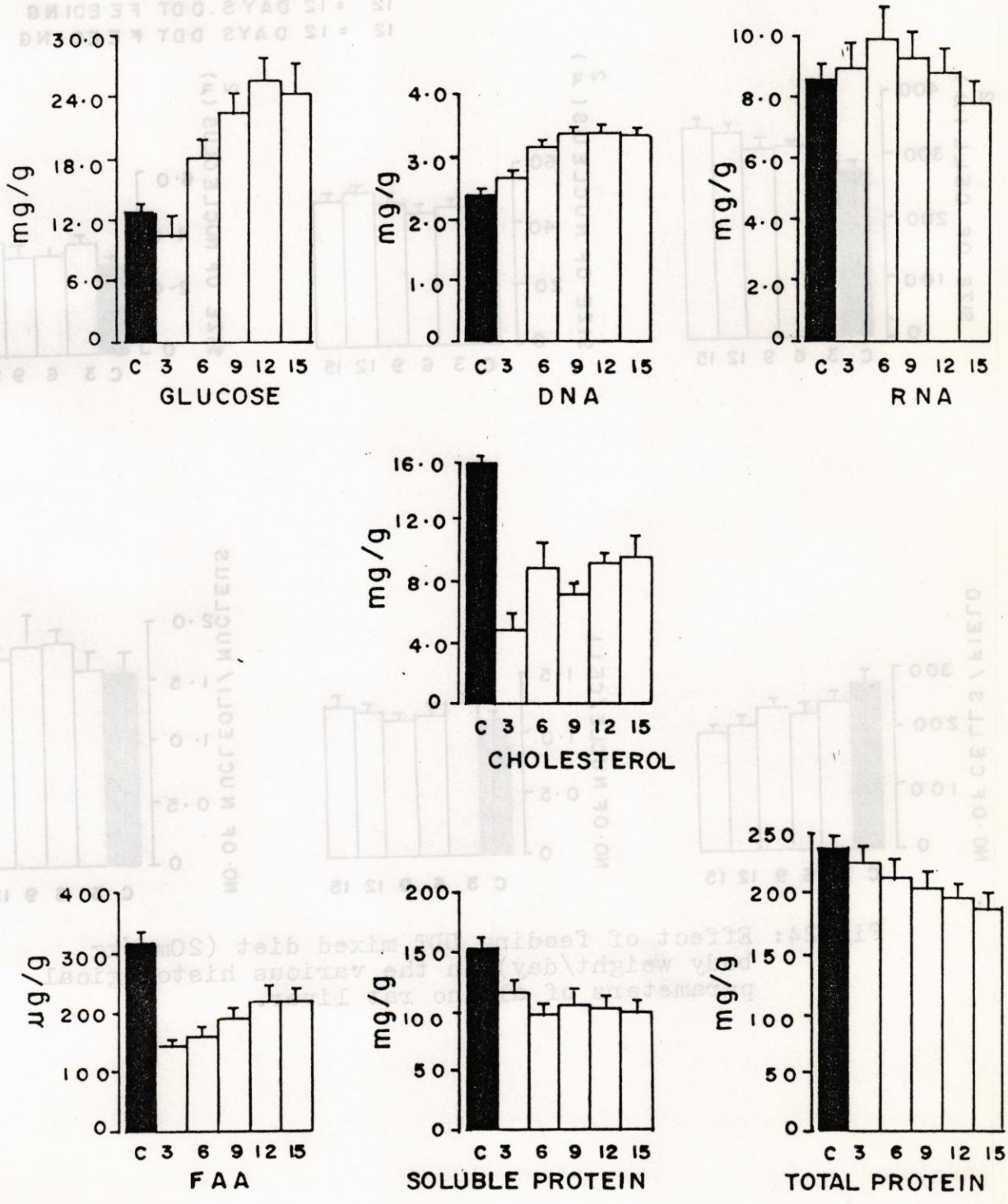


Fig.23: Effect of feeding DDT mixed diet (20mg/kg body weight/day) on the various biochemical components of rat liver.

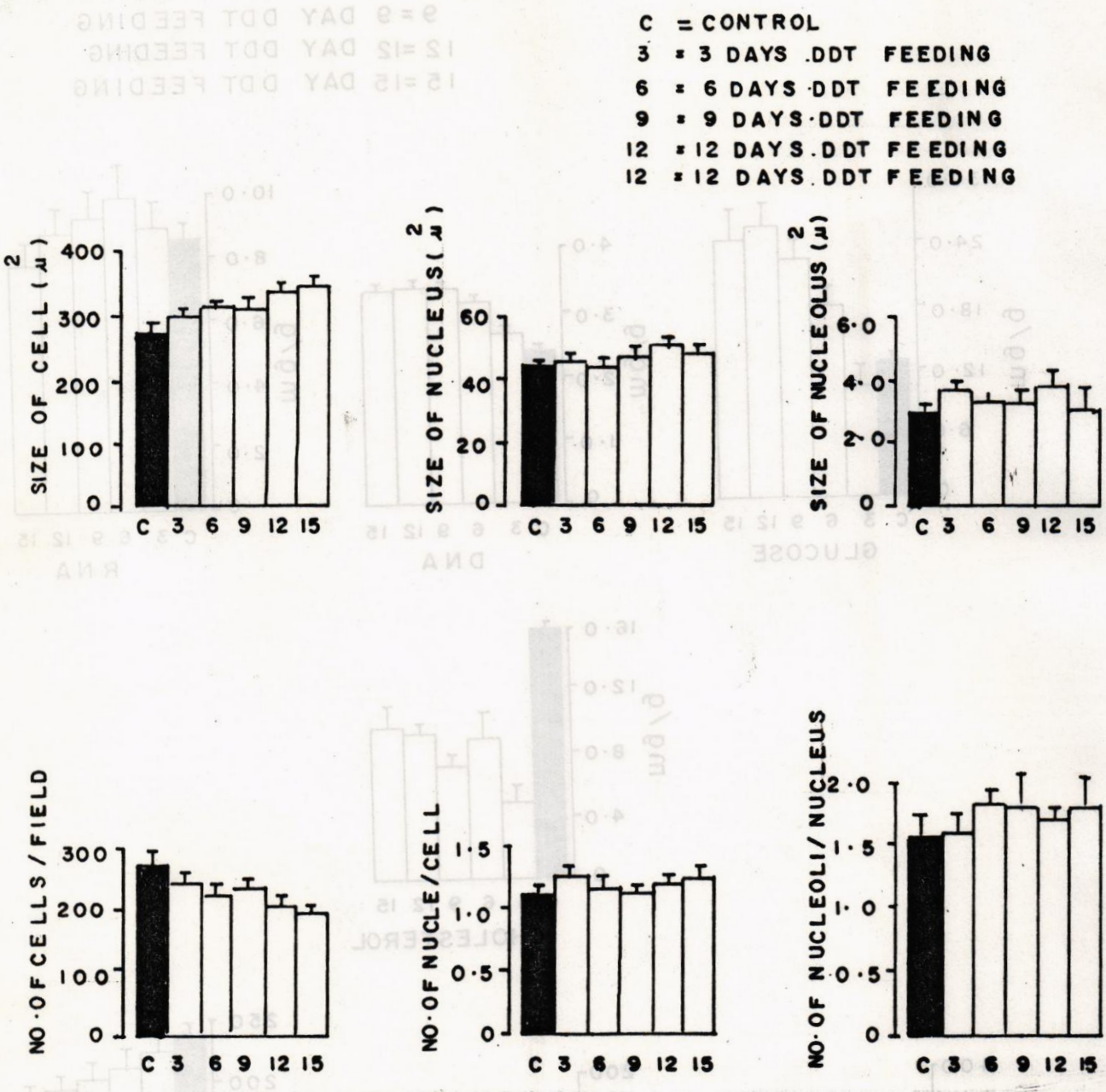


Fig.24: Effect of feeding DDT mixed diet (20mg/kg body weight/day) on the various histological parameters of albino rat liver.

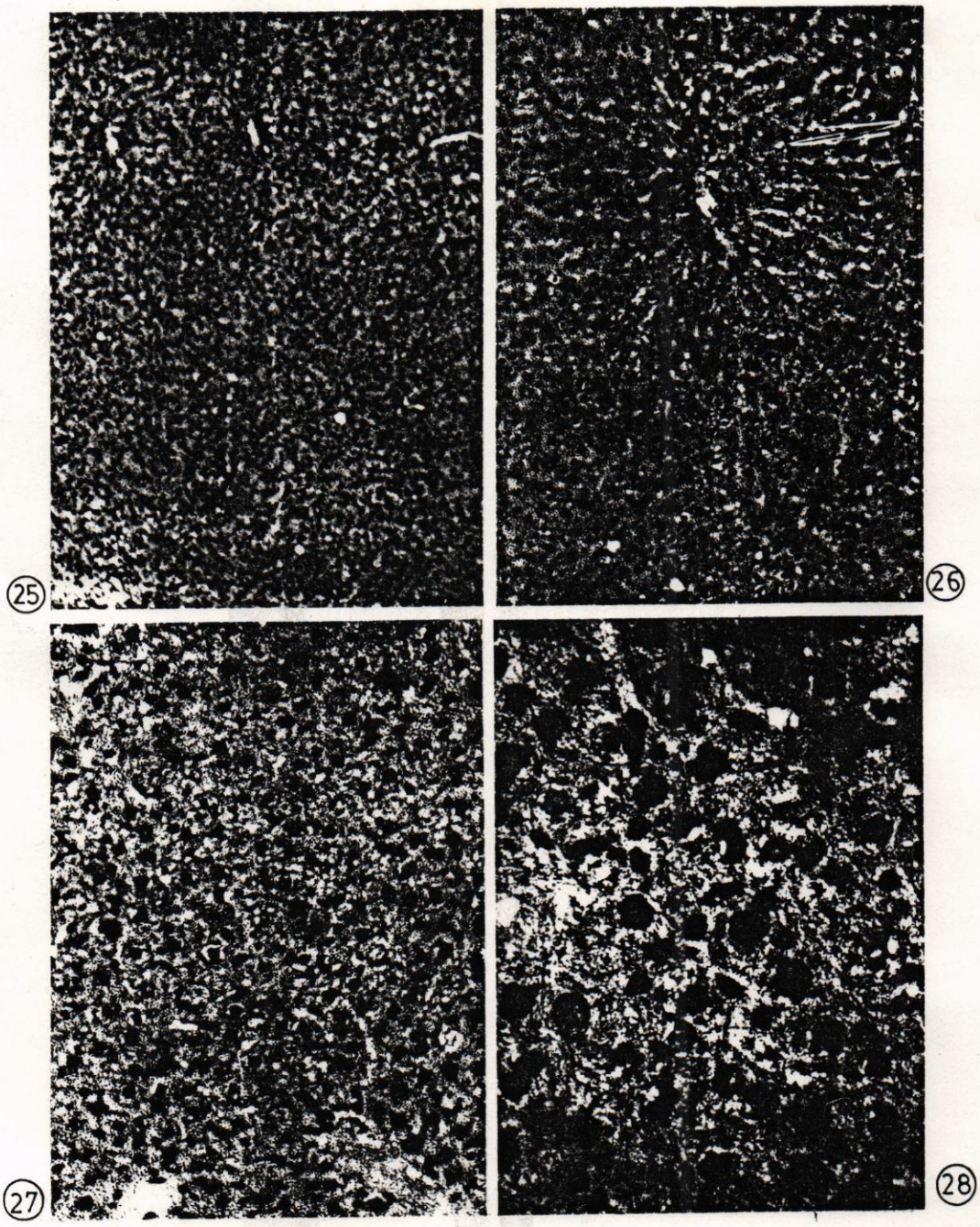
3.2.5. HISTOLOGICAL STRUCTURE OF LIVER

The histological changes induced by feeding of DDT mixed diet in rat liver are quantified in Table XIII and Figure 24. The hepatic cell size increases after DDT feeding. This increase is 14%, 14%, 24% and 26% after 6, 9, 12 and 15 days of feeding. The size of the nucleus is not changed until day 12, when it increases 18%. Although the size of nucleolus is also increased after DDT feeding, but this increase is not statistically significant.

The number of nuclei/cell and number of nucleoli/nucleus remain unaltered. Due to increase in the size of hepatic cells, the number of hepatic cells per microscopical field are decreased. For example this number decreases 28% after 15 days of feeding (Table XIII, Fig. 24).

Figures 29-33 show histological structure of liver of rat fed on DDT mixed diet for 3 days. Figs.34-37 show hepatic structure of liver of 6 day feeding group. Figures 38-41 show structure of liver of 9 day feeding group. Figures 42-45 represent hepatic structure of 12 days and Figures 46-50 show structure of 15 day feed group.

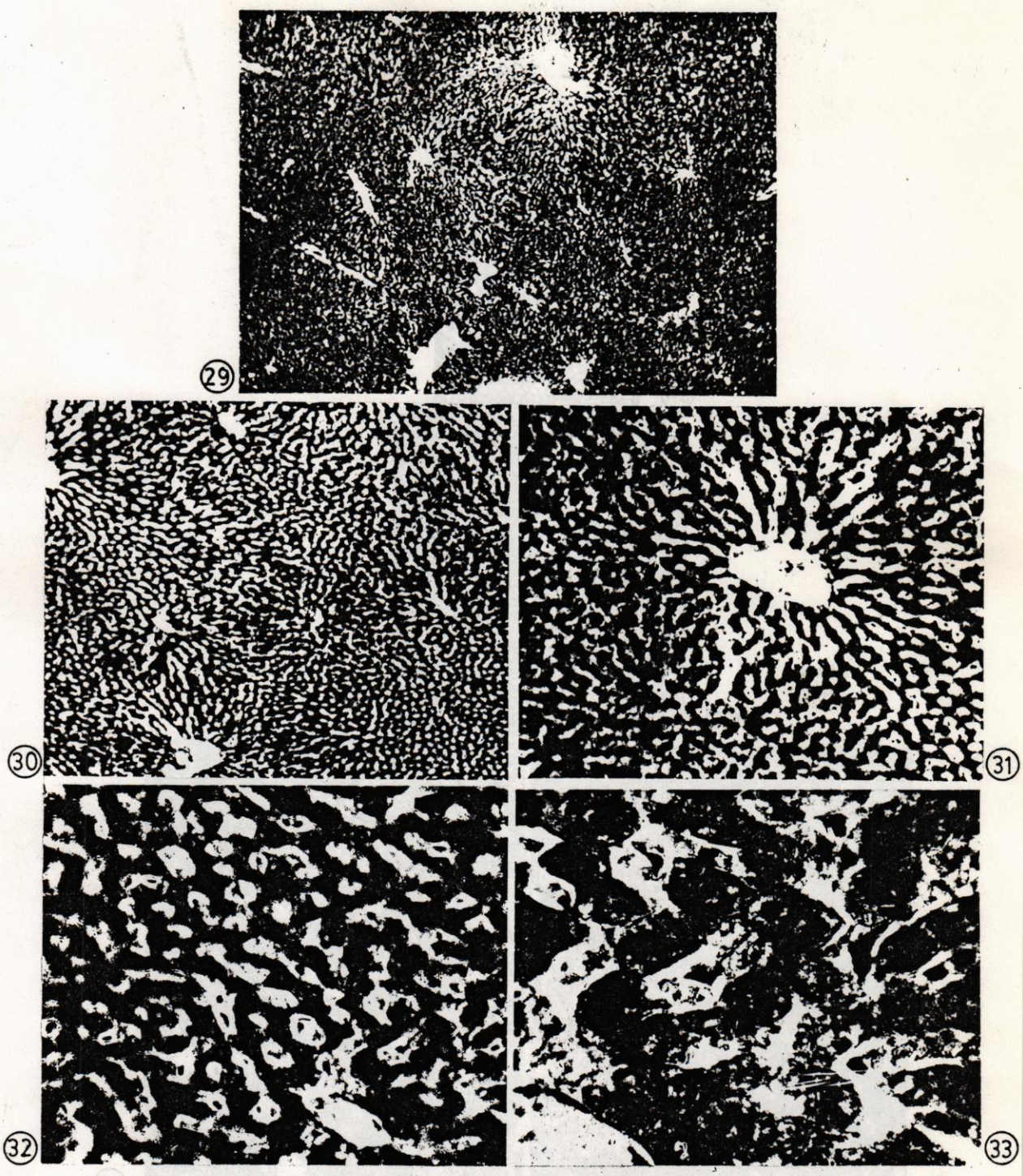
The hypertrophied cells, well defined vesicular nuclei, swollen bile canaliculi and other blood vessels (Figures 5, 13; compare with Fig.28) are the prominent structural changes in liver, with increasing duration of administration. The cellular hypertrophy and formation of vesicular nucleus becomes more prominent.



FIGS.25-28: Histological structure of normal rat liver. Note the hepatic lobular arrangement (25,26), normal sinusoidal areas, hepatocytes and nuclear pattern (26-28).

Stain: Hematoxylin and Eosin.

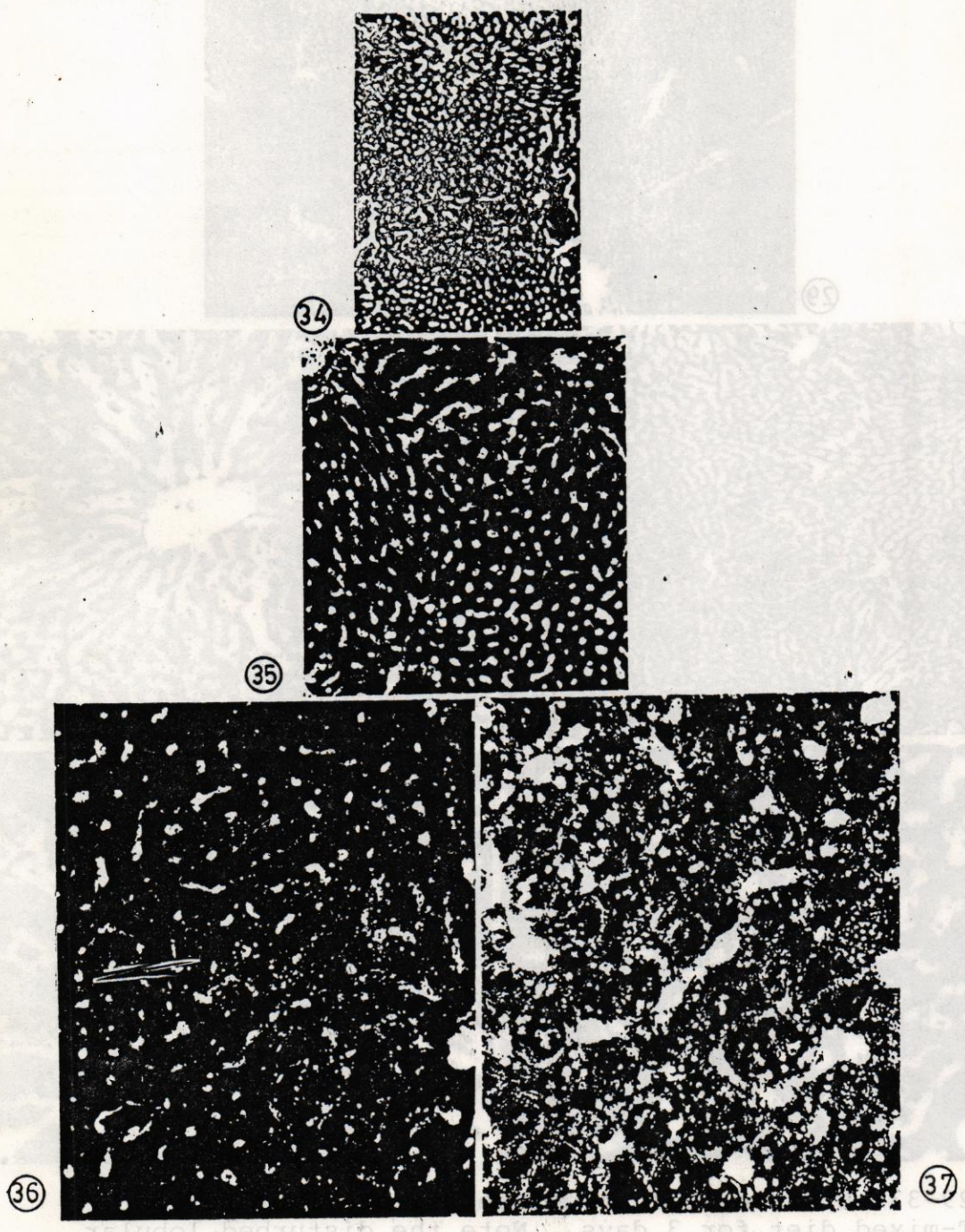
Magnifications: 25,x25; 26,x50; 27,x100; 28,x250.



FIGS.29-33: Histological structure of rat liver fed on DDT-mixed diet for 3 days. Note the disturbed lobular structure (29), disruption of hepatic cords (30-32), increase in the number od kupffer cells (30,31), greatly enlarged sinusoids (29-33) and cellular and nuclear hypertrophy (32-33).

Stain: Hematoxylin and Eosin.

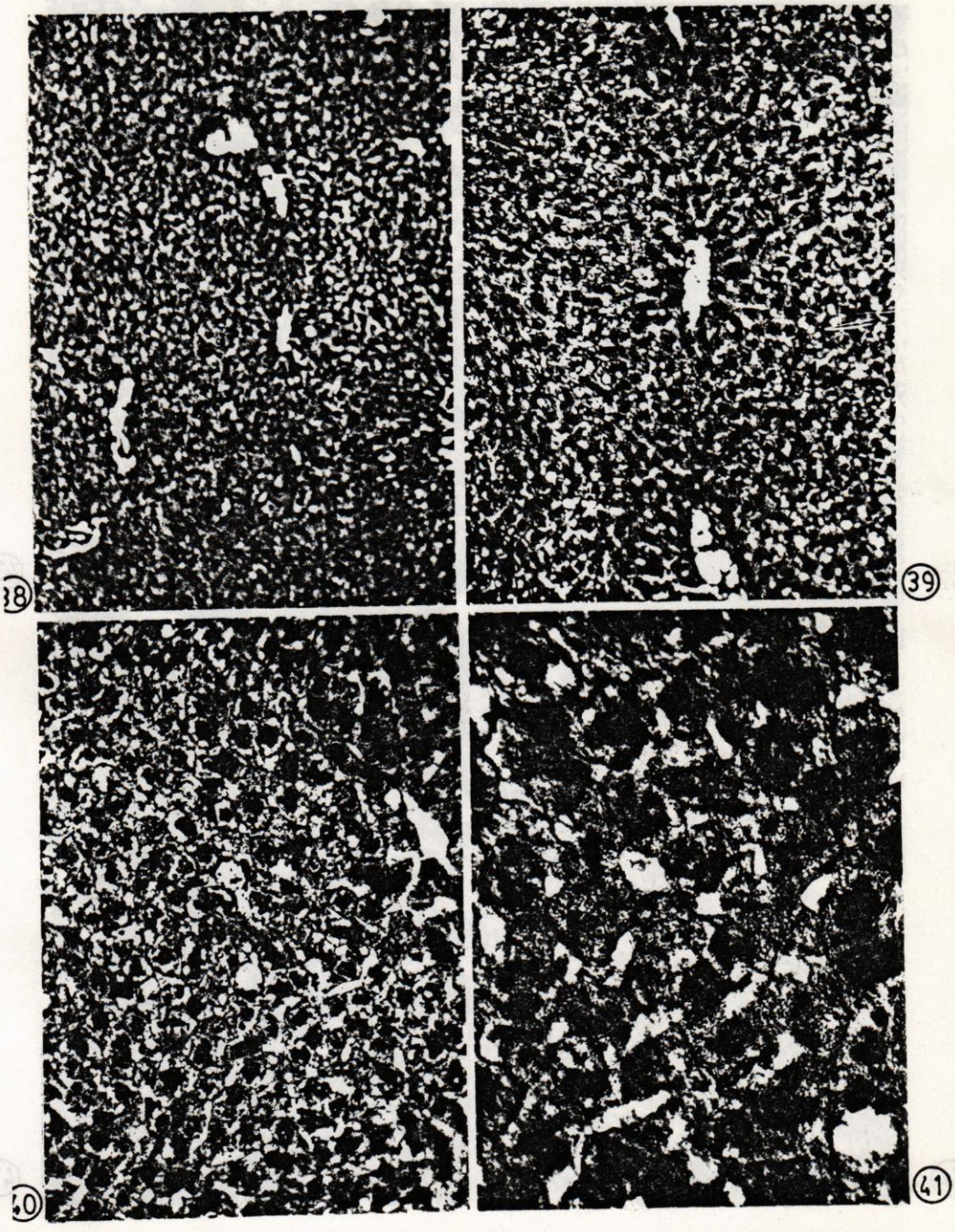
Magnifications: 29,x10; 30,x25; 31,x50; 32,x100; 33,x250.



FIGS.34-37: Histological structure of rat liver fed on DDT-mixed diet for 6 days. Note the altered lobular morphology (34,35), rounded vacuoles inside cytoplasm (35), enlarged sinusoidal spaces(35,36), and hypertrophy of the hepatocytes and nuclei (36,37).

Stain: Hematoxylin and Eosin.

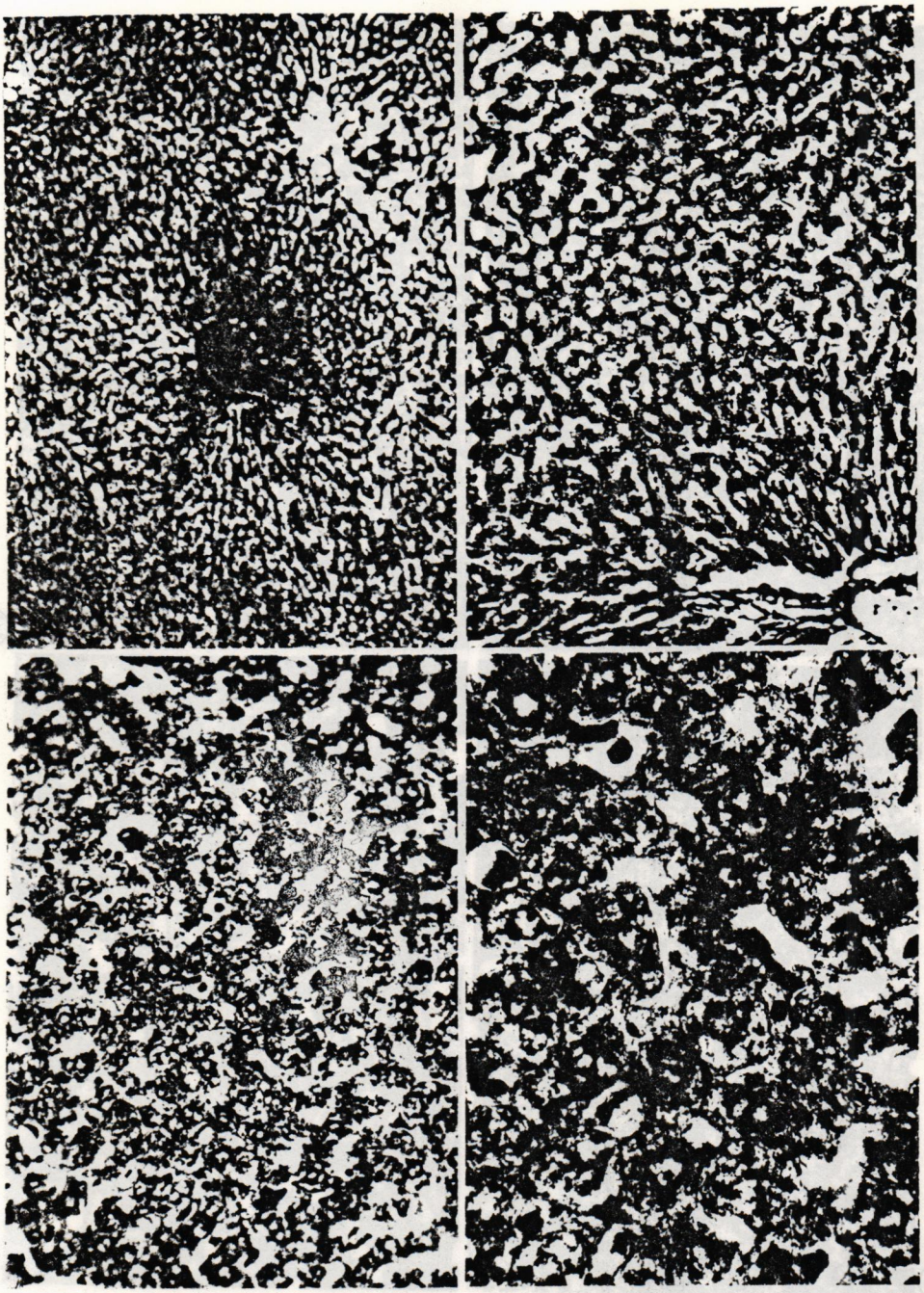
Magnifications: 34,x25; 35,x50; 36,x100; 37,x250.



FIGS. 38-41: Histological structure of rat liver fed on DDT-mixed diet for 9 days. Note enlarged blood vessels (38, 39), prominent vacuoles (39-41), cytoplasmic margination, increased sinusoidal areas and hypertrophied cells and nuclei (40,41).

Stain: Hematoxylin and Eosin.

Magnifications: 38,x25; 39,x50; 40,x100; 41,x250.

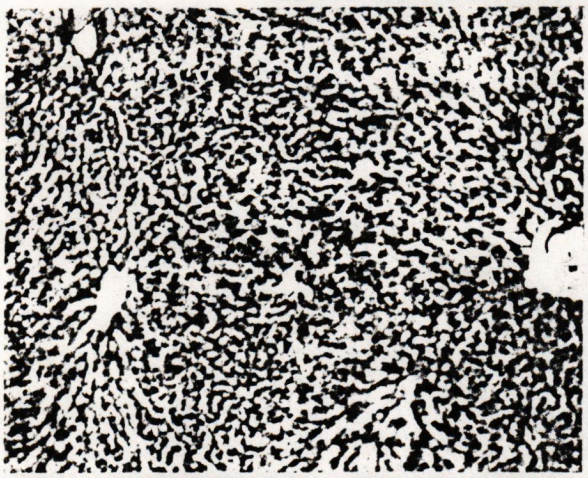


FIGS. 42-45: Histological structure of rat liver fed on DDT-mixed diet for 12 days. Note altered hepatic lobular structure (42), highly disorganised cord morphology (43-44), fairly abnormal sinusoids with great number of rounded and swollen kupffer cells (43-45). Also note the hypertrophy of hepatocytes and nuclei (44,45).

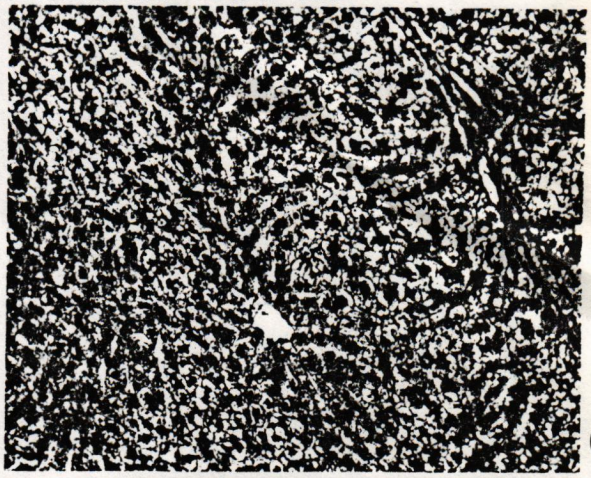
Stain: Hematoxylin and Eosin.

Magnifications: 42, x25; 43, x50; 44, x100; 45, x250.

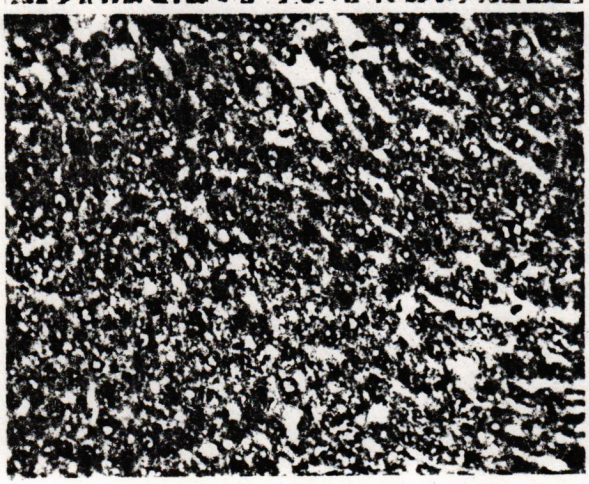
(46)



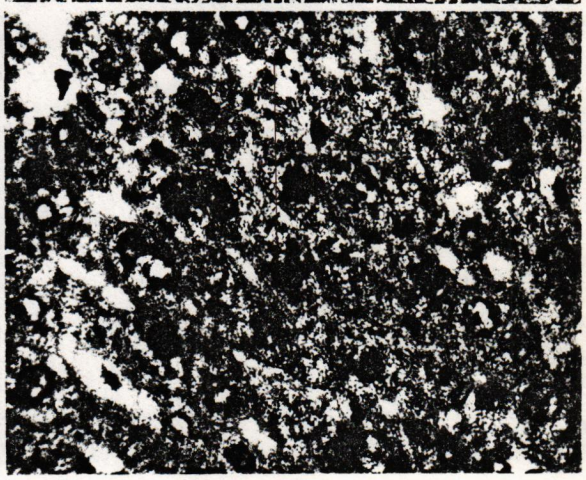
(47)



(48)



(49)



(50)

FIGS.46-50: Histological structure of rat liver fed on DDT-mixed diet for 15 days. Note the irregular margins of blood vessels (46), enlarged sinusoids (47), cytoplasmic margination **in** the cells (48), many clear areas, and hypertrophied cells and nuclei (49,50).

Stain: Hematoxylin and Eosin.

Magnifications: 46,x10; 47,x25; 48,x50; 49,x100; 50,x250.

	(n = 6)	(n = 3)
Body wt./ liver wt. ratio	39.27±0.73[a]	33.74±1.22*
Liver wt. (% body wt.)	2.55±0.05	2.97±0.11*

[a]Mean±SEM, Student's 't' test; *P < 0.0

3.3. EFFECT OF DDT ADMINISTERED AT A DOSE OF 10 mg/kg body weight/day FOR A PERIOD OF 18 MONTHS

3.3.1. BODY WEIGHT AND LIVER WEIGHT

Table XIV shows effect of long term feeding of DDT on Body wt./liver weight ratio and on the liver weight in terms of per cent body weight. The body wt./ liver weight ratio decreases 14%, 16% and 33% after 6, 12 and 18 months of DDT feeding at a rate of 10 mg/kg body weight/day. The liver weight (% body weight) is increased, which in 6, 12 and 18 months feeding experiments was recorded as 17%, 19% and 29%(Table XIV).

3.3.2. HAEMATOLOGICAL STUDIES

Table XV and Figure 51 show the effect of long term feeding of DDT on the various haematological parameters of rat. The RBC count and PCV are significantly decreased after DDT feeding. The haemoglobin count are affected significantly only after 18 months of feeding. The RBC count is decreased 13-14%, while PCV show 6-8% significant decrease. The WBC count is increased 8%,19% and 29% after 6, 12 and 18 months of feeding.

The MCV and MCH are significantly increased after insecticidal administration. After 6, 12 and 18 months of feeding the MCV is increased 8, 13 and 10%, while the increase in MCH during the same period is, respectively, 8, 11 and 6%. The MCHC is not significantly deviated (Table XV, Fig. 51).

3.3.3. BIOCHEMICAL ANALYSIS OF BLOOD

Blood serum enzymes representing different

TABLE - XV

EFFECT OF FEEDING DDT MIXED DIET (10 mg/kg body weight/day) FOR 6-18 MONTHS ON THE VARIOUS HAEMATOLOGICAL PARAMETERS OF ALBINO RATS

Parameters	6 months DDT feeding experiment		12 months DDT feeding experiment		18 months DDT feeding experiment	
	Control (n=6)	DDT fed (n=3)	Control (n=4)	DDT fed (n=3)	Control (n=6)	DDT fed (n=3)
Hb (g/dl)	13.79[a] ± 0.32	12.79 ± 0.51	13.14 ± 0.23	12.64 ± 0.26	13.04 ± 0.16	12.02** ± 0.19
RBC ($X10^5$ cells/µl)	70.87 ± 1.26	60.83*** ± 1.07	70.48 ± 1.28	61.40** ± 1.47	69.10 ± 1.20	59.93** ± 1.33
WBC ($X10^2$ cells/µl)	66.50 ± 3.99	72.00 ± 1.15	62.38 ± 1.34	74.33* ± 3.21	65.50 ± 5.20	84.33* ± 2.85
PCV (%)	45.92 ± 0.44	42.45*** ± 0.22	42.90 ± 0.20	42.37 ± 0.39	43.28 ± 0.40	40.75* ± 0.63
MCV (fl)	64.38 ± 0.65	69.81** ± 0.81	60.91 ± 0.86	69.06*** ± 1.10	62.64 ± 0.46	68.01** ± 0.92
MCH (Pg)	19.45 ± 0.32	21.01* ± 0.55	18.64 ± 0.06	20.60*** ± 0.22	18.88 ± 0.28	20.05* ± 0.21
MCHC (g/dl)	30.24 ± 0.65	30.12 ± 1.05	30.62 ± 0.41	29.84 ± 0.33	30.15 ± 0.36	29.48 ± 0.09

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

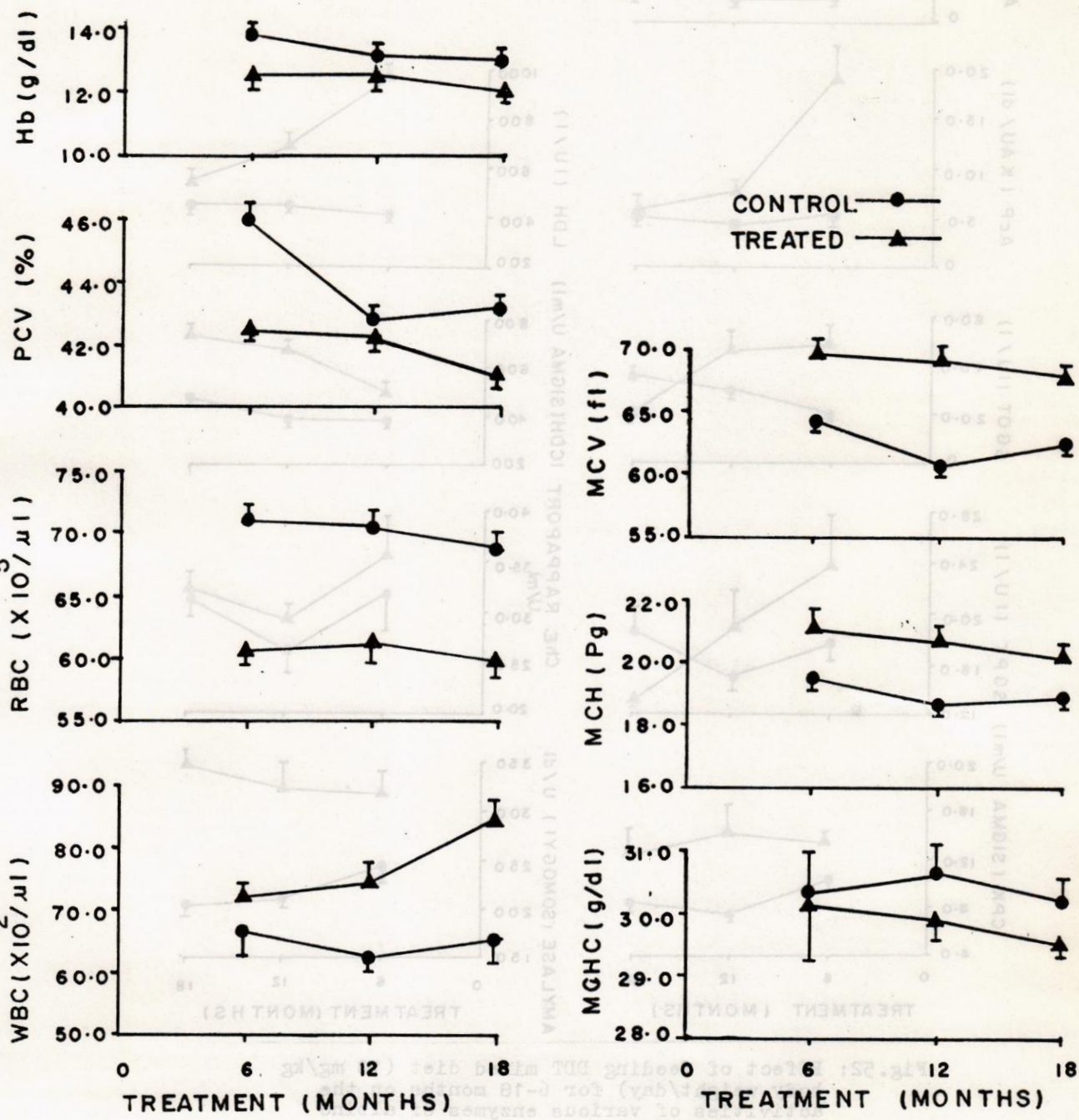


Fig.51: Effect of feeding DDT mixed diet (10mg/kg body weight/day) for 6-18 months on the various haematological parameters of albino rats.

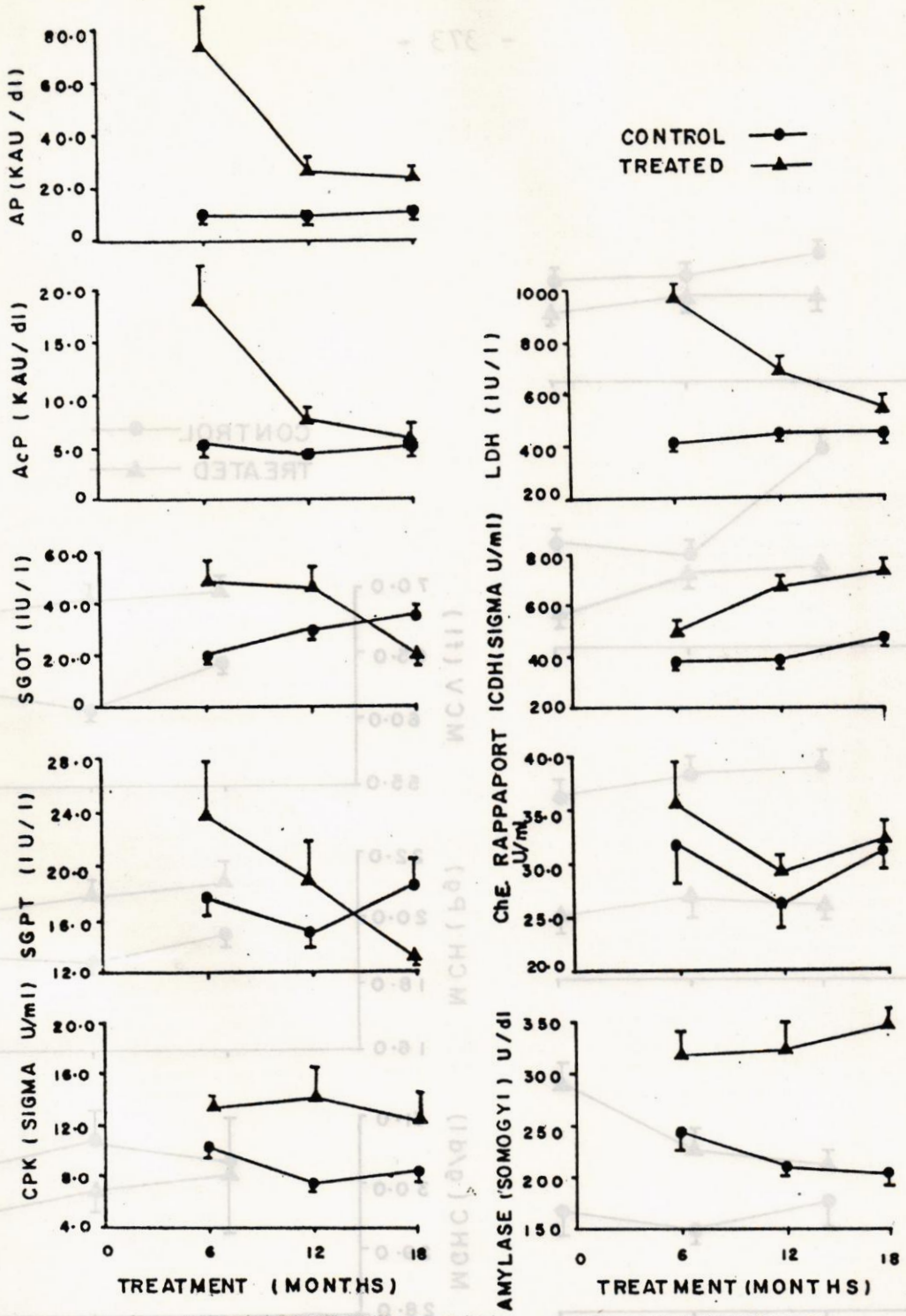


Fig.52: Effect of feeding DDT mixed diet (10 mg/kg body weight/day) for 6-18 months on the activities of various enzymes of albino rat blood serum.

TABLE - XVI

EFFECT OF FEEDING DDT MIXED DIET (10 mg/kg body weight/ day) FOR 6-18 MONTHS ON THE ACTIVITIES OF VARIOUS ENZYMES OF ALBINO RAT BLOOD SERUM

Parameters	6 months DDT feeding experiment		12 months DDT feeding experiment		18 months DDT feeding experiment	
	Control (n=6)	DDT fed (n=3)	Control (n=4)	DDT fed (n=3)	Control (n=6)	DDT fed (n=3)
AP (KAU/dl)	9.57[a] ± 0.39	75.65*** ±11.73	9.46 ± 0.68	26.89*** ± 2.45	10.21 ± 0.92	23.85*** ± 1.27
AcP (KAU/dl)	5.46 ± 0.48	19.11** ± 3.36	4.48 ± 0.25	7.92*** ± 0.38	5.34 ± 0.59	5.76** ± 0.64
Amylase (Somogyi U/dl)	242.53 ±15.41	317.24 ±21.07	210.29 ±10.57	323.81** ±21.96	202.03 ±12.22	345.00*** ±14.43
ChE (Rappaport U/ml)	31.80 ± 3.76	35.33 ± 4.33	26.00 ± 1.85	29.02 ± 1.55	31.08 ± 1.82	32.00 ± 1.60
CPK (Sigma U/ml)	10.30 ± 0.77	13.23* ± 0.67	7.57 ± 0.61	14.27* ± 2.15	8.75 ± 0.97	12.56* ± 0.99
GOT (IU/l)	21.02 ± 1.46	50.78** ± 5.69	28.98 ± 2.73	47.20 ± 6.66	35.66 ± 2.55	21.55* ± 4.24
GPT (IU/l)	17.72 ± 1.40	23.91 ± 4.46	14.82 ± 0.99	18.99 ± 3.05	18.75 ± 1.87	13.15* ± 0.98
ICDH (Sigma U/ml)	391.86 ±18.33	494.75* ±23.33	395.22 ±10.33	674.65*** ±23.91	462.53 ±24.13	731.18*** ±13.19
LDH (IU/l)	401.44 ±10.87	966.40*** ±24.05	442.20 ±19.62	608.64** ±22.25	409.49** ±27.82	539.08 ±18.22

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

aspects of metabolism were tested after DDT feeding in order to correlate the insecticidal treatment with macromolecular abnormalities. In blood serum the activities of almost all the enzymes tested were elevated after DDT feeding. The AP activity is increased 7.90, 2.84 and 2.34 fold over the control after 6, 12 and 18 months of feeding, respectively. The AcP activity is also increased. This increase is only 3.5 fold, 1.77 fold and 1.08 fold after 6, 12 and 18 months of DDT feeding.

The two transaminases (i.e. SGOT and SGPT) show significant increase during 6 and 12 months feeding, but then show drastic decrease, when DDT mixed diet is fed for 18 months. In SGOT, the activity increases 142% and 63% after 6 and 12 months of insecticide feeding, but after 18 months this activity decreases 40%. The SGPT follows almost the same pattern. The SGPT activity is raised 35% and 28% after 6 and 12 months, and is lowered by 30% after 18 months.

Two dehydrogenase i.e. ICDH and LDH were also tested. The LDH activity is increased 141% and 38% after 6 and 12 months feeding, but then shows non-significant increase after 18 months of feeding. The ICDH activity generally follows the same pattern. The ICDH enzyme activity is increased 26%, 71% and 58% after 6, 12 and 18 months of DDT feeding. The CPK activity is likewise raised after insecticide administration (Fig. 52; Table XVI). The CPK activity is raised 29%, 89% and 44% after 6, 12 and 18 months of DDT feeding.

The ChE activity is not affected. Slight deviation from the control group is just non significant.

TABLE - XVII

EFFECT OF FEEDING DDT MIXED DIET (10 mg/kg body weight/ day) FOR 6-18 MONTHS ON THE VARIOUS BIOCHEMICAL COMPONENTS OF ALBINO RAT BLOOD SERUM.

Parameters	6 months DDT feeding experiment		12 months DDT feeding experiment		18 months DDT feeding experiment	
	Control (n=6)	DDT fed (n=3)	Control (n4)	DDT fed (n=3)	Control (n=6)	DDT fed (n=3)
Bilirubin (mg/dl)	0.74[a] ± 0.05	0.55 ± 0.07	0.63 ± 0.03	0.60 ± 0.08	0.56 ± 0.04	0.62 ± 0.07
Cholesterol (mg/dl)	182.95 ± 6.25	224.02 ±17.39	177.19 ± 5.21	178.97 ± 6.24	180.12 ± 9.80	167.99 ± 5.29
FAA (mg/dl)	9.27 ± 0.34	8.57 ± 0.25	9.00 ± 0.36	9.44 ± 0.27	11.09 ± 2.33	11.50 ± 0.39
Glucose (mg/dl)	111.76 ± 8.52	92.95 ±21.86	107.45 ± 7.26	109.47 ±11.14	150.57 ± 3.67	172.45* ± 7.14
Protein (g/dl)	6.90 ± 0.04	8.12* ± 0.42	7.68 ± 0.21	6.88 ± 0.62	8.58 ± 0.45	11.83*** ± 0.13
Urea (mg/dl)	36.34 ± 2.20	37.94 ± 2.67	23.55 ± 6.84	47.94* ± 1.16	41.15 ± 1.98	35.05 ± 1.83

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

The amylase activity is distinctly affected. This enzyme shows 31, 54 and 71% increase after 6, 12 and 18 months of DDT feeding.

Although several enzymes tested in this study were significantly altered after DDT feeding, but some other biochemical components did not evince any notable response (Table XVII, Fig. 53). The bilirubin, cholesterol and FAA content remain unaltered. The glucose content are not altered until 18 months of feeding, when 15% significant increase was recorded. The protein content likewise increased 38% after 18 months of feeding.

The Urea content generally remain unaltered after insecticide feeding except for 12 month feeding when 2.04 fold increase was observed.

3.3.4. BIOCHEMICAL ANALYSIS OF LIVER

Figure 54 and Table XVIII show the effect of long term feeding of DDT on the activities of various hepatic enzymes. The AP and LDH activities show very prominent effect. The AP activity is increased 64, 24, and 39% after 6, 12 and 18 months of feeding, respectively. The LDH activity, during the same period is increased 42, 39 and 39%, respectively. The hepatic GOT and ICDH activities remain unaltered except for 12 month group in which the former enzyme activity is raised 2.12 fold while the latter enzyme shows 22% increase. The GPT activity is raised 1.24 fold, 5.27 fold and 1.86 fold after 6, 12 and 18 months of feeding (Table XVIII, Fig. 54).

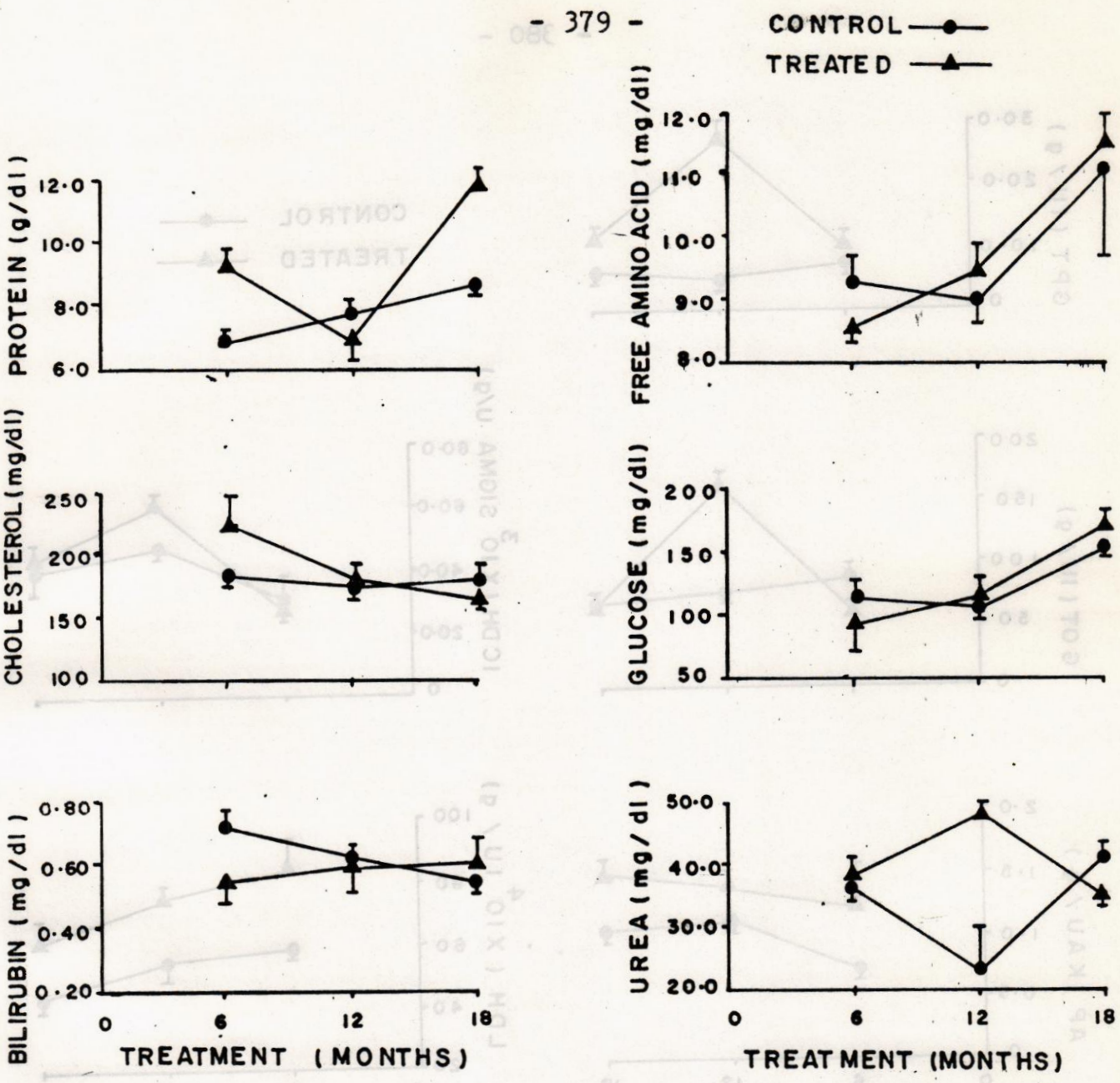


Fig.53: Effect of feeding DDT mixed diet (10mg/kg body weight/day) for 6-18 months on the various biochemical components of albino rat blood serum.

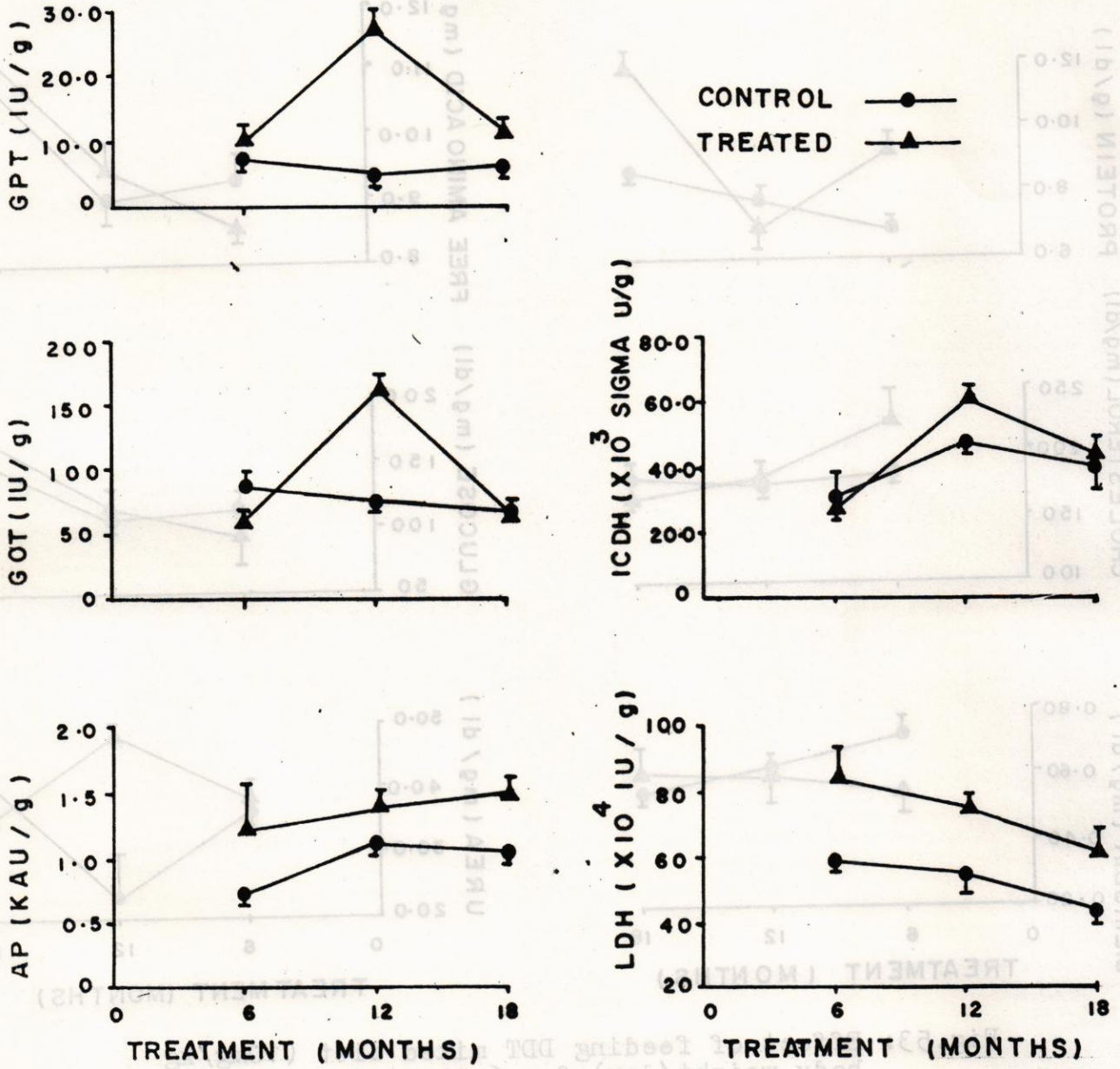


Fig.54: Effect of feeding DDT mixed diet (10mg/kg body weight/day) for 6-18 months on the activities of various hepatic enzymes of albino rats.

TABLE - XVIII

EFFECT OF FEEDING DDT MIXED DIET (10 mg/kg body weight/ day) FOR 6-18 MONTHS ON THE ACTIVITIES OF VARIOUS HEPATIC ENZYMES OF ALBINO RATS.

Parameters	6 months DDT feeding experiment		12 months DDT feeding experiment		18 months DDT feeding experiment	
	Control (n=6)	DDT fed (n=3)	Control (n=4)	DDT fed (n=3)	Control (n=6)	DDT fed (n=3)
AP (KAU/g)	0.74[a] ± 0.05	1.22* ± 0.08	1.12 ± 0.07	1.39* ± 0.04	1.08 ± 0.08	1.49*** ± 0.11
GOT (IU/g)	8.66 ± 0.54	6.09 ± 0.18	7.56 ± 0.46	16.05*** ± 0.49	6.79 ± 0.23	6.60 ± 1.13
GPT (IU/g)	7.71 ± 0.68	9.55 ± 0.53	5.02 ± 0.54	26.47*** ± 3.03	6.05 ± 0.60	11.26** ± 0.62
ICDH$_3$ ($\times 10^3$ Sigma U/g)	33.29 ± 6.08	29.12 ± 1.44	49.49 ± 2.52	60.33* ± 1.66	42.50 ± 5.70	45.11 ± 3.56
LDH$_4$ ($\times 10^4$ IU/g)	58.29 ± 2.99	82.64* ± 8.57	54.25 ± 5.37	75.17* ± 0.89	44.60 ± 0.30	61.81* ± 5.33

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

TABLE - XIX

EFFECT OF FEEDING DDT MIXED DIET @10 mg/kg body weight/ day) FOR A PERIOD OF 6-18 MONTHS ON THE VARIOUS BIOCHEMICAL COMPONENTS OF ALBINO RAT LIVER.

Parameters	6 months DDT feeding experiment		12 months DDT feeding experiment		18 months DDT feeding experiment	
	Control (n=6)	DDT fed (n=3)	Control (n=4)	DDT fed (n=3)	Control (n=6)	DDT fed (n=3)
Cholesterol (mg/g)	5.97[a] ± 0.29	6.89 ± 0.76	9.01 ± 0.32	6.32* ± 0.70	10.50 ± 1.21	7.11* ± 0.39
FAA (µg/g)	217.29 ± 7.70	221.83 ±21.81	197.98 ±14.18	216.00 ±13.41	170.12 ±11.59	187.47 ±13.04
Glucose (mg/g)	30.87 ± 2.32	12.71*** ± 1.22	37.12* ± 1.37	22.75 ± 3.21	38.32 ± 3.38	26.11* ± 3.23
Soluble Protein (mg/g)	124.92 ± 4.67	106.73 ±10.96	135.22 ± 9.96	111.48 ±17.23	159.00 ± 2.58	128.08* ±10.93
Total Protein (mg/g)	216.96 ±13.05	145.88* ±15.76	208.48 ± 6.70	228.54 ±12.38	227.60 ±11.88	274.41* ± 8.65
DNA (mg/g)	3.05 ± 0.11	3.15 ± 0.16	2.54 ± 0.14	2.78 ± 0.30	3.06 ± 0.12	2.66* ± 0.09
RNA (mg/g)	9.68 ± 0.62	5.35** ± 0.86	10.39 ± 0.31	7.68*** ± 1.09	11.30 ± 0.49	6.86*** ± 0.26

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

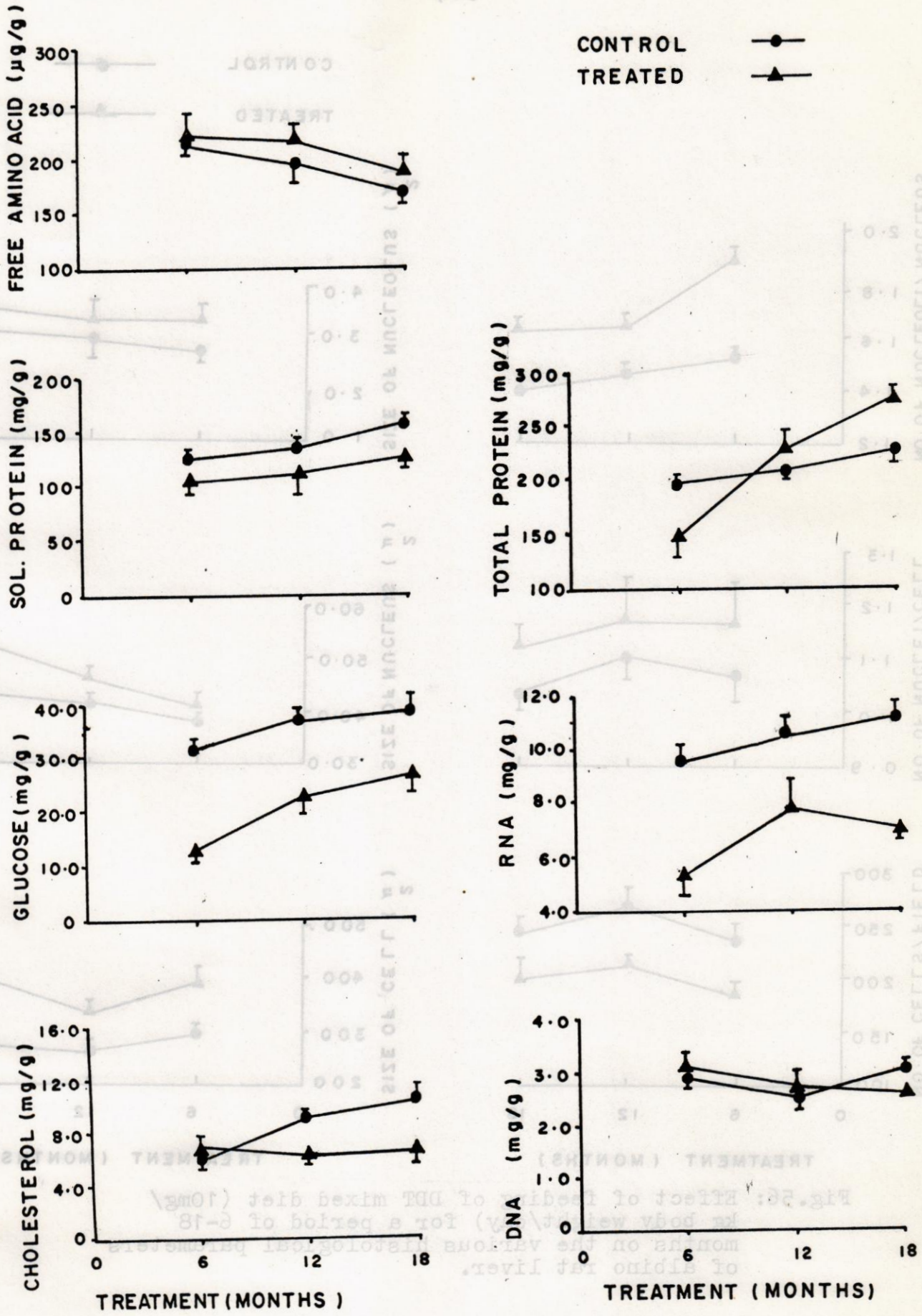


Fig.55: Effect of feeding DDT mixed diet (10mg/kg body weight/day) for a period of 6-18 months on the various biochemical components of albino rat liver.

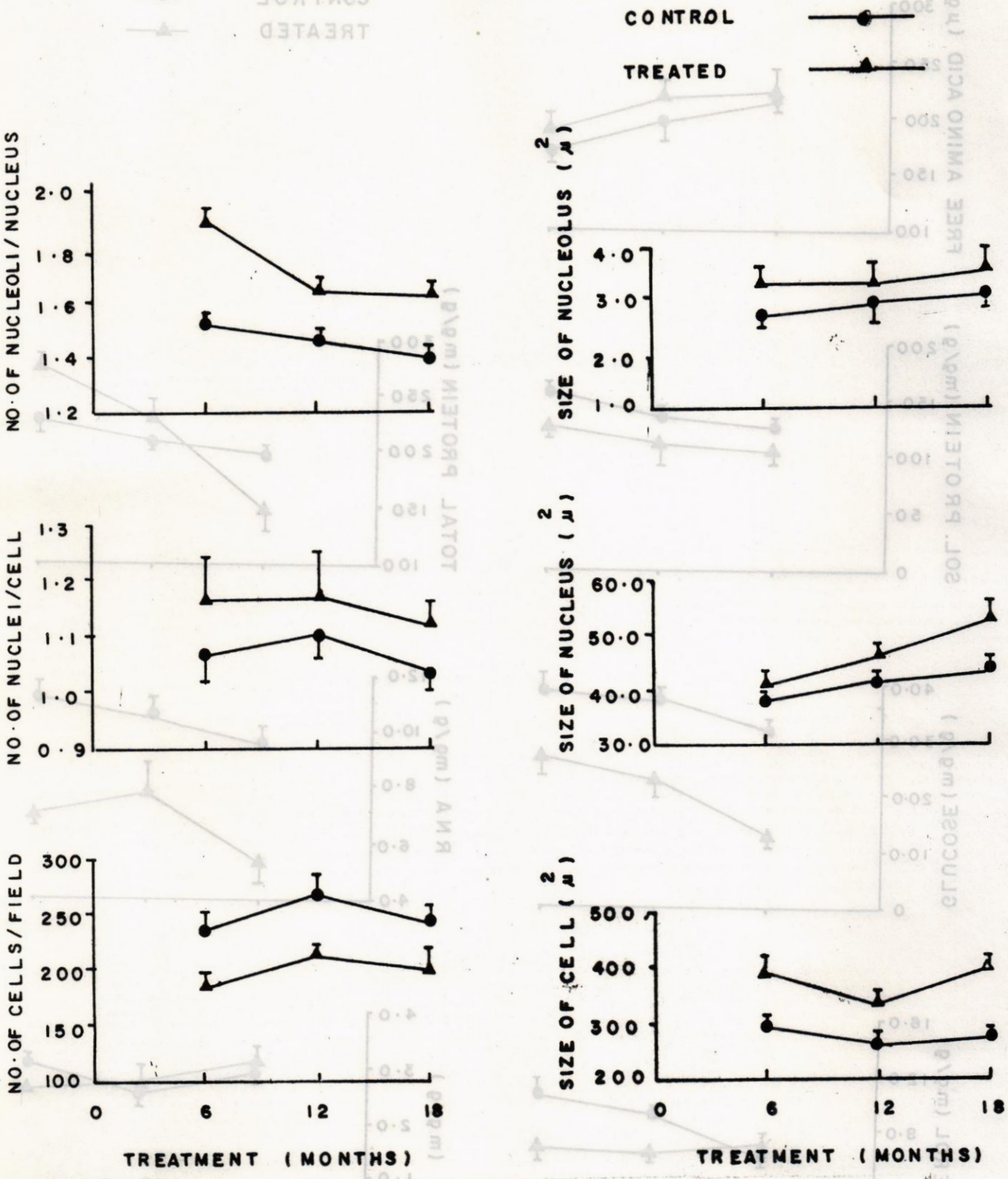


Fig.56: Effect of feeding of DDT mixed diet (10mg/kg body weight/day) for a period of 6-18 months on the various histological parameters of albino rat liver.

The effect of DDT feeding on the various biochemical components of rat liver other than enzymes is shown in Figure 55 and Table XIX.

The FAA content are not affected at all, while soluble proteins are reduced 20% after 18 months of DDT feeding. The total protein content of liver decrease 33% after 6 months of DDT feeding, while these content increase 21% when DDT feeding was extended till 18 months. The glucose content also decrease. This decrease is 59%, 39% and 31% after 6, 12 and 18 months of DDT feeding.

The DNA content are not affected until 18 months when these content show 13% decrease. The RNA content decrease 45%, 26% and 39% after 6, 12 and 18 months of DDT feeding (Table XIX, Fig. 55).

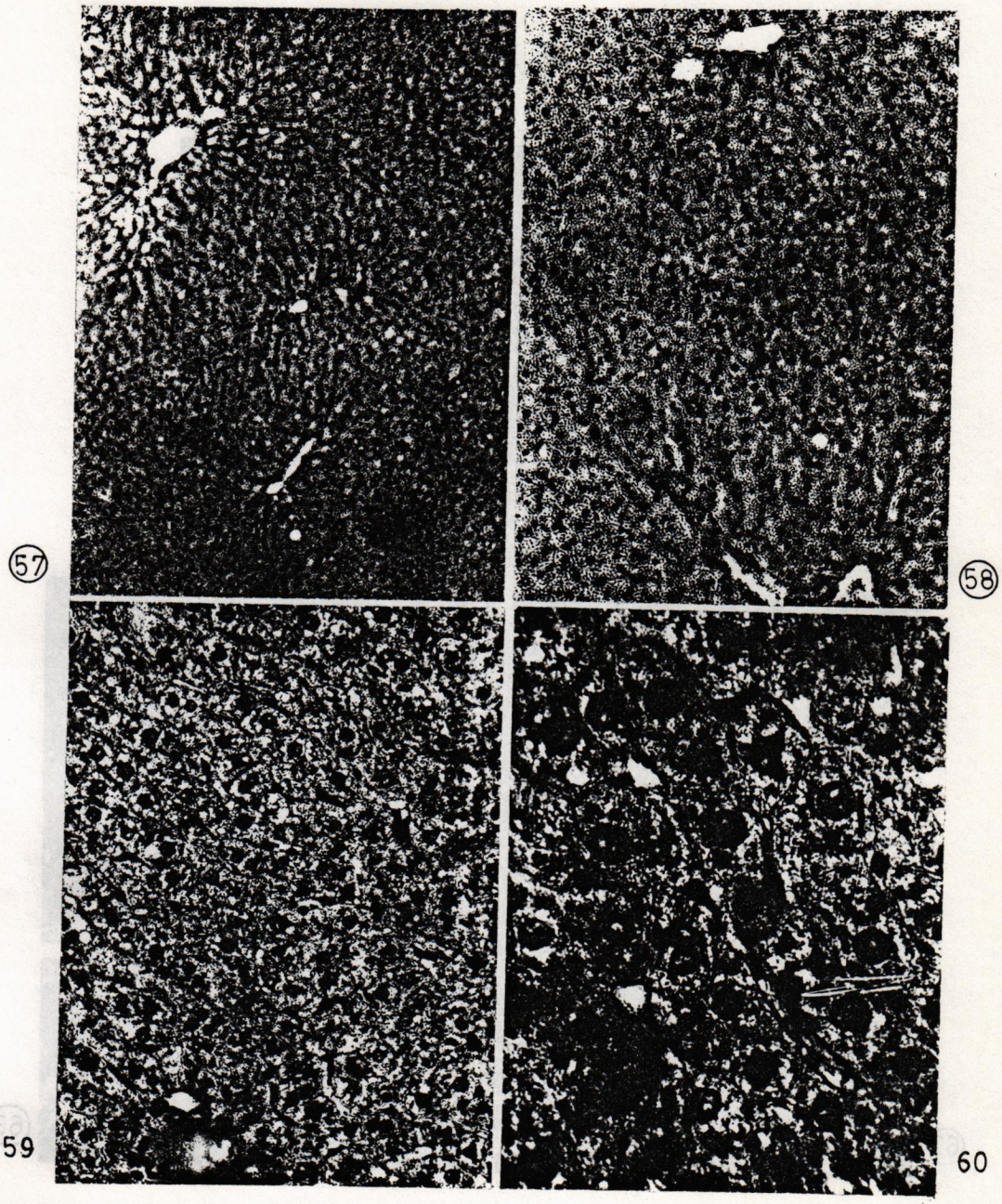
3.3.5. HISTOLOGICAL STRUCTURE OF LIVER

Table XX and Figure 56 show the effect of long term feeding of DDT on the various histological parameters of rat liver. As is typical of all the chlorinated insecticide toxicity in non target organism, the hepatic cells hypertrophy. The hepatic cells increase 33%, 27% and 45% after 6, 12 and 18 months of feeding. The nuclei of hepatic cells increase significantly (20%) after 18 months of insecticide feeding. The nucleolus remains unaltered. The number of nuclei/cell and number of nucleoli/nucleus also remain unaltered.

On comparison of Figures 61-63 (6 month feeding), Figures 64-67 (12 months feeding) and Figures 68-71 (18 months feeding) with Figures 57-60 (Control group) several histological changes can be observed.

No.of cells/ field	238.37[a] ±11.68	184.78* ±10.49	
No.of nuclei/ cell	1.07 ± 0.06	1.17 ± 0.08	
No.of nucleoli/ nucleus	1.52 ± 0.16	1.88 ± 0.31	
Size of cell (u^2)	290.31 ± 8.78	387.12** ±15.76	
Size of nucleus (u^2)	37.91 ± 1.23	40.96 ± 1.50	
Size of nucleolus (u^2)	2.79 ± 0.21	3.29 ± 0.22	

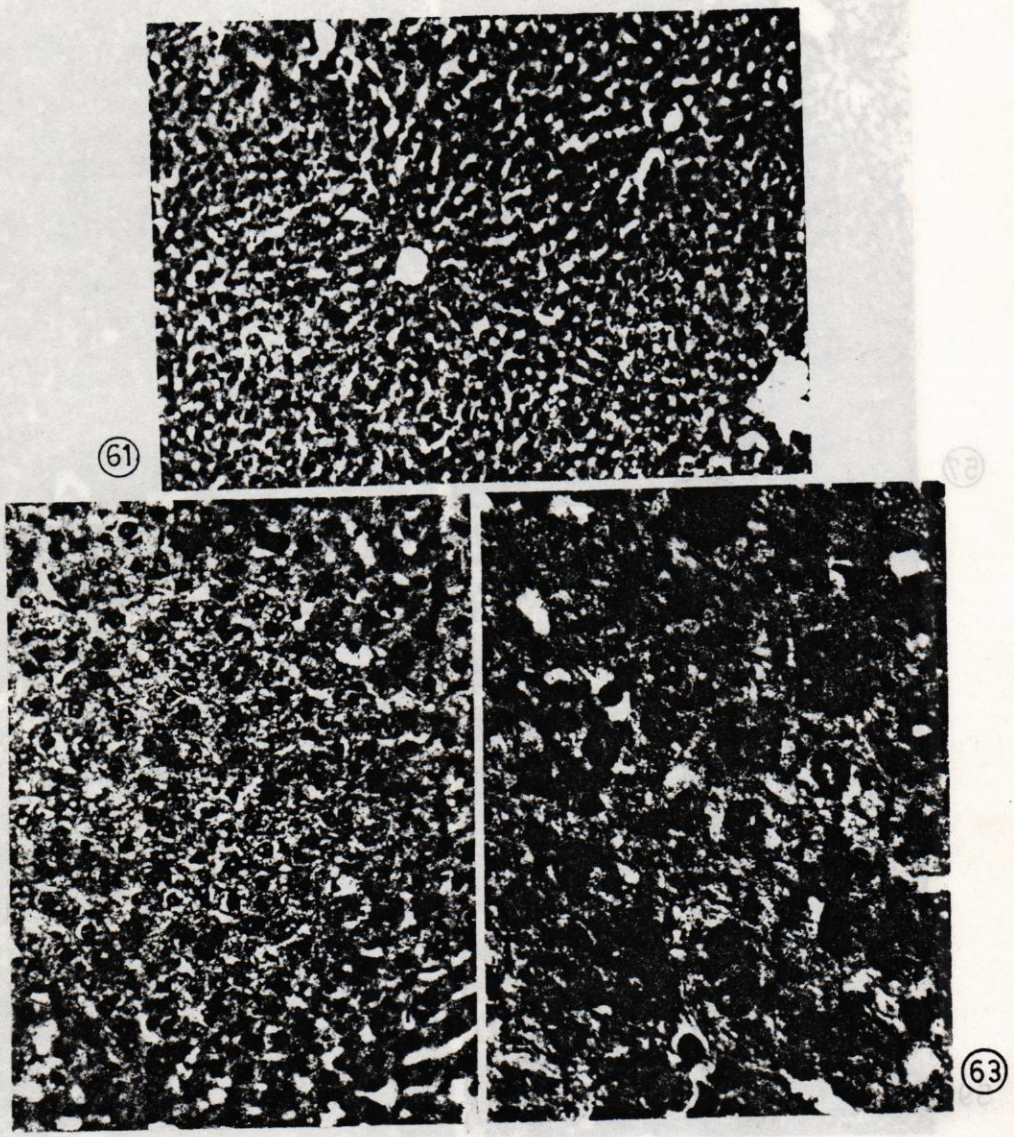
[a]Mean±SEM; Student's 't' test; *P < 0.



FIGS.57-60: Histological structure of normal liver. Note a portion of hepatic lobule, with normal cord structure, portal areas (57-59), sinusoidal spaces, regular shape of nuclei and almost compact arrangement of cells (59-60).

Stain: Hematoxylin and Eosin.

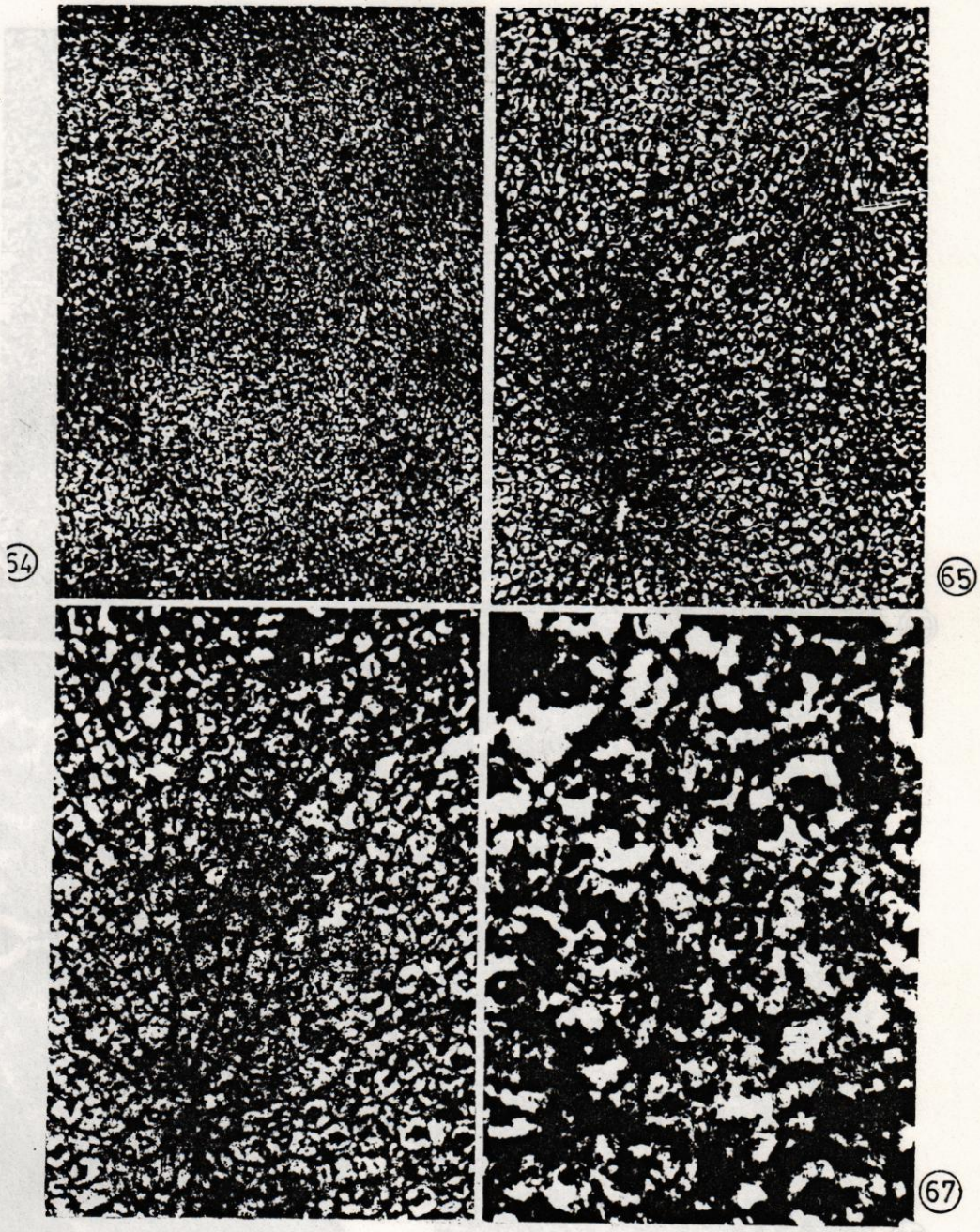
Magnifications: 57,x25; 58,x50; 59,x100; 60,x250.



FIGS.61-63: Histological structure of rat liver fed on DDT-mixed diet for 6 months. Note hepatic lobule with disturbed cord structure, slightly increased sinusoidal areas (61), spacesial, almost rounded, area in the center, slight margination (62), hypertrophied and irregularly arranged cells(61-62).

Stain: Hematoxylin and Eosin.

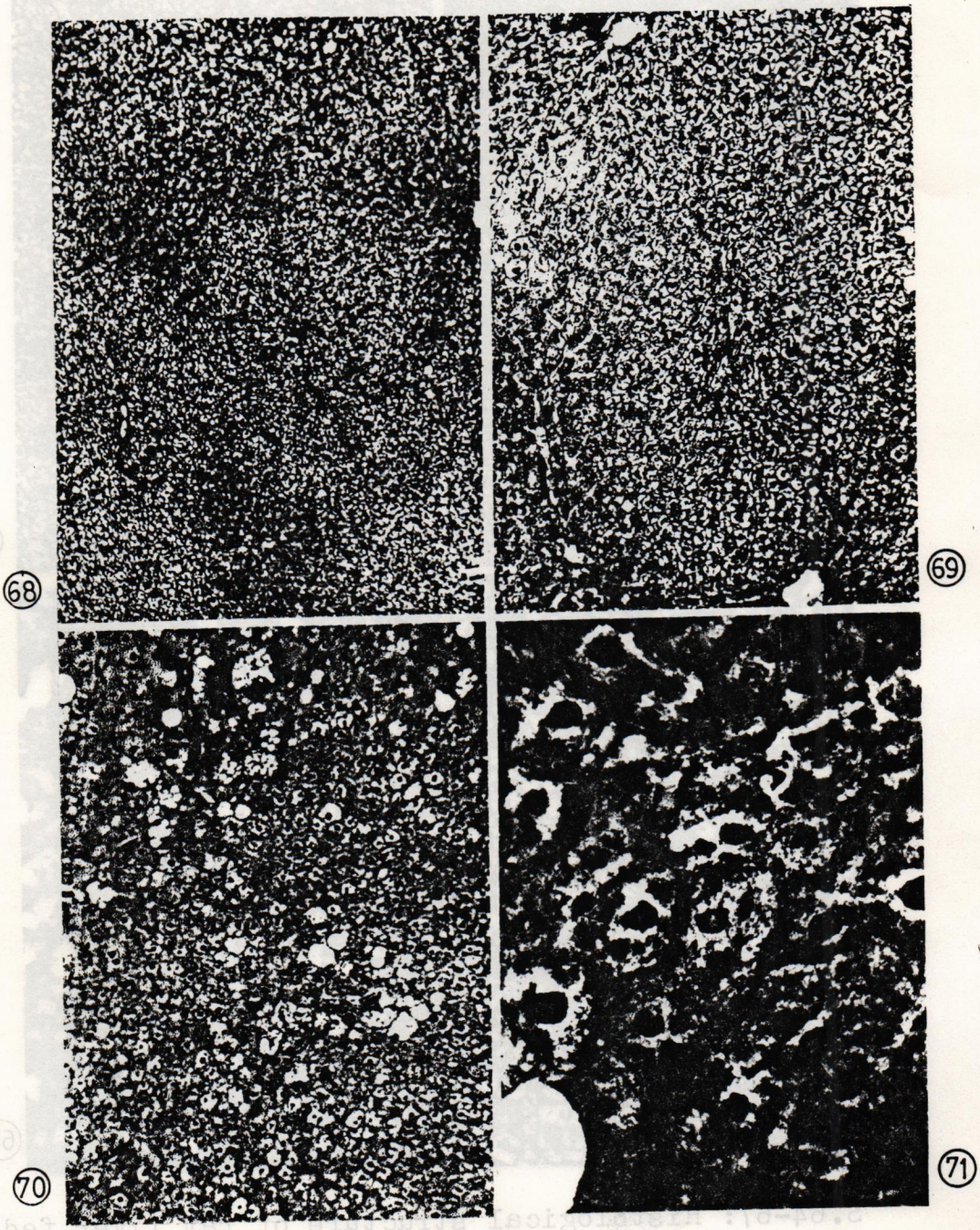
Magnifications: 61,x50; 62,x100; 63,x250.



S.64-67: Histological structure of rat liver fed on DDT-mixed diet for 6 months. Note the vacuolation (64), distinctly prominent membrane network(65,66), cytoplasmic margination, hypertrophied and irregular shape of cell and nuclei (66, 67).

Stain: Hematoxylin and Eosin.

Magnifications: 64,x25; 65,x50; 66,x100; 67,x250.



FIGS.68-71: Histological structure of rat liver fed on DDT-mixed diet for 18 months. Note disturbed hepatic lobular structure (68), tissue vacuolation (68, 70), cytoplasmic margination (69-71) and hypertrophied cells (71).

Stain: Hematoxylin and Eosin.

Magnifications: 68,x25; 69,x50; 70,x100; 71,x250.

Although the general hepatolobular architecture is maintained, but hepatic cells gradually increase in size. After 12 months of feeding of DDT the hepatic cells are hypertrophy (Fig.66), nuclei become well demarcated (Fig.67). The plasma membrane of hepatic cell becomes very prominent (Fig. 67). The bile canaliculi become very prominent (Figs. 65, 66). Eighteen months of feeding results in toxicity, which is evidenced by numerous vacuoles, which can be seen in Figure 70. The hepatic nuclei also become very much condensed and are surrounded by clear zone (Fig. 71).

4. DISCUSSION

DDT administered as three different doses viz. 100mg/kg body weight for 48 hours, 20mg/kg body weight for 15 days and 10mg/kg body weight for 18 months produced almost similar type of effects in the various haematological, biochemical and histological changes in rat blood and liver. The extent of damage, however, depended upon the dose and duration of administration.

Haematological parameters

The haemoglobin, RBC count and PCV decreased after DDT treatment while WBC, MCV and MCH increased at the same time. The haemoglobin content decrease 11% after 48 hours of DDT at a dose of 100mg/kg body weight, while the other two doses did not have any significant effect. The RBC content decrease 16%, 17% and 14% after DDT treatment for 48 hours, 15 days and 18 months administered at their respective doses. The decrease in RBC count

could be attributed to breakdown of erythrocyte due to DDT treatment (O'Brien and Hamilton, 1979). The PCV decreased significantly at the end of experimental period in the 15 day and 18 months feeding experiments. The 48 hour experiment did not produce any change in PCV.

The WBC are drastically increased as a defence mechanism. In 48 hour feeding experiment, the WBC increase 48% while in 15 day experiment it is 64% increase at the end of experimental period. In 18 months long feeding experiment the WBC showed an increase of 8, 19 and 29% after 6, 12 and 18 months of DDT feeding at a dose of 10mg/kg body weight. The MCV and MCH also shows significant increase after DDT treatment.

Blood Biochemistry

All blood serum enzymes under all experimental conditions show raised levels of activities after DDT feeding except for ChE, which is elevated 77% after 48 hours of DDT feeding. This enzymatic activity remains unaltered under all other experimental conditions.

All other blood serum enzymes viz. Amylase, AP, AcP, SGOT, SGPT, LDH, ICDH and CPK are significantly increased. The most prominent changes have been observed in activities of AP, AcP, LDH and GOT. Agarwal, et al. (1978) have reported, marked increase in blood serum LDH, transaminases, amylase and AP after single oral dose of 150mg/kg body weight in rhesus monkey.

The intensity of the insecticide effects depends upon the amount of DDT, which the animal picked up during feeding (Kagan et al., 1970; Gertig et al., 1971a, b). The lower doses caused liver dysfunction as evidenced by raised levels of AP, AcP, LDH, GOT, GPT and low protein content in the blood serum. The intensity of this dysfunction increases with the increased duration of DDT feeding. It also depends upon the amount of DDT, which the rats may consume to the critical level, even in shorter period of time, say 24 hours. The stronger doses have greater damaging effects than the weaker dose administered for a longer period of time. Gertig et al., (1971a, b) have reported similar type of increase in the liver function test enzymes after DDT feeding.

Liver is the main metabolic centre and is also the centre for drug metabolism. The DDT is, therefore, likely to hit the liver directly thus leading to significant deviations in the biochemical profile of blood serum (Kohli et al., 1975). The liver damage may cause leaching out of enzymes and eventual raising their level in the blood. The blood serum analysis, therefore, could be a toxicity indicator for various poisons.

The other biochemical components of blood, other than several enzymes mentioned above were not very drastically or uniformly affected.

In 48 hour feeding experiment, the bilirubin and FAA content are unchanged, protein, urea and glucose increase respectively 30, 32 and 36%, while cholesterol shows 40% decrease after 48 hours of feeding.

In 15 day feeding experiment, in which DDT was administered @ 20mg/kg body weight, the protein and FAA content are the most sensitive indicators. The protein content increase 44%, while FAA content decrease 52% after 15 days of DDT feeding. The urea and cholesterol content remain unaltered, while glucose shows 32% decrease after 15 days. In long term feeding experiments for 18 months, most of the blood serum biochemical components remain unaffected, except for minor changes in protein and glucose contents.

Liver Biochemistry

The various hepatic enzymes behave uniformly after different treatments of rat with DDT. In 48 hour feeding experiments, in which DDT was administered at a dose of 100mg/kg body weight, the hepatic AP and ICDH activities remain unchanged, while hepatic GOT, GPT and LDH is considerably increased. In 15 day DDT feeding experiment, only hepatic LDH and ICDH activities show consistent increase throughout the experimental period, while AP, GOT and GPT activities show occasional increase during 15 day DDT feeding. Some consistent and significant changes were observed however, in the various hepatic enzymes tested after 6-18 months of DDT feeding @ 10mg/kg body weight. The GOT and GPT activities are most significantly affected and show prominent deviation from the control.

DDT has already been reported to induce enzyme systems (Chadwick et al., 1975). The increase in activity of various hepatic enzyme may be due to induced synthesis of these enzymes after DDT treatment. Suhasini et al.(1979)

have also reported increased synthesis of GOT and GPT activities in liver of frog after DDT administeration. Agarwal et al. (1978) have shown increased SDH and transaminases in brain, liver, kidney, adrends and spleen of rhesus monkey after single dose of 150mg/kg body wt. LDH, Mg^{++} ATPase, AcP and amylase increase in some of these tissues, while AP decreased in all organs except kidney (Agarwal et al., 1978).

The liver tissue reacts to DDT feeding by an increase in larger number of biochemical components. The activities of AP, LDH, GOT and GPT enzyme and sugar content of hepatic tissue increase significantly after milder doses of DDT. If the weak doses are prolonged for 15 days the cholesterol contents decreases while the protein contents increase slightly. Stronger doses of DDT do not behave differently. The raised level of different enzymatic activities of liver could be because of (i) liver damage followed by liver proliferation or (ii) because of increase in the synthesis of these particular proteins, which has been stimulated by DDT treatment (Cappon and Nicholls, 1973, 1975). The sugar metabolism is enhanced i.e. glycogenolysis is need of enzyme, which is required for various biochemical processes inside the cell (Haynes, 1972; Kacew et al., 1972). The cholesterol metabolism, however, appears to be hindered or inhibited, partly or wholly.

Besides enzymes, several other biochemical components of liver were tested after DDT feeding. In 48 hour feeding experiment, significant changes were observed only after 48 hours of feeding. FAA content seem to be the most prominent and sensitive parameter.

The hepatic FAA, glucose and cholesterol content decrease 43, 21 and 44%, while soluble protein shows 33% increase during this period. In 15 day feeding experiment however all hepatic biochemical components, except for glucose, decrease significantly. After 15 days of feeding, the cholesterol, FAA, total protein and soluble protein show decrease of 38%, 31%, 21% and 34%, respectively. The glucose content on the other hand show 88% increase during this period. No consistent pattern was visible in 18 month long term feeding experiment. After 18 months of feeding @ 10mg/kg body weight, the glucose and soluble protein showed respectively 31% and 20% decrease, while total protein showed 21% increase.

The nucleic acids contents of liver showed some variable effects of DDT feeding. In 48 hour feeding experiment, both DNA and RNA remain unchanged, while in 15 day feeding experiment, the RNA content remain unchanged and DNA content increase 40% after 15 days of DDT feeding. In long term feeding experiments the DNA content decrease 13% after 18 months of feeding, while RNA content decrease 39% during the same period. Ireland et al. (1980) have reported in-creased DNA synthesis by O,P' - DDT with uterine tissue.

Histological Changes

Just like other insecticide treatments the DDT feeding results in hypertrophy of hepatic cell and its nuclear and nucleolar size. In 48 hour feeding experiment the cell size shows about 23% increase, while nucleolus shows 44%, increase after 48 hours of DDT feeding. In 15 day feeding experiment the cell size

increases 26%, while nucleus shows 18% increase. In 18 month feeding experiment, this increase is respectively, 45% and 20%. The number of cells, nucleic or nucleoli remain unaltered. Besides these changes in sizes of different hepatic cell components, typical hepatic damage as manifested in the form of sinusoided congestion, cellular vacuolation and foamy appearance is evident in the present study. Similar type of morphological changes have been reported from other labs. (Datta and Dikshith, 1973).

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C H A P T E R - V

HAZARDS OF CHLORINATED INSECTICIDES: CONCLUSIONS

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I. INTRODUCTION

The chlorinated insecticides, inspite of being placed on the list of banned chemicals for common and open use, are still being manufactured and extensively used in several countries. Whatever the rationale the users and manufacturers may have, but it has been proved on several occasions that these insecticides are hazardous to human health, as they are likely to gain entry into the food chain directly or indirectly. Frequently residue analysis has shown the deposition of these insecticides in different human tissue with carcinogenic consequences. As liver is the main metabolic centre of the body the chances that it may be affected the most are not remote. The insecticide treatment is likely to affect most of the metabolic processes. Liver function tests are the

most appropriate parameters to look for any such deleterious effects. In the present studies the various enzymes and a few other biochemical components of blood serum, which are known for clinical diagnosis, have been studied with specific objective to record changes due to insecticidal exposure. The corresponding enzymes and biochemical components have also been studied in the liver. This chapter highlights the effects of different doses of insecticides administered under different conditions of experimentation on the various biochemical components and enzymes. After a brief information on their chemical nature and general mode of action, an account of different changes consequent to administration of insecticides have been described under different subheadings.

II. CHLORINATED INSECTICIDES

Man has been concerned with his capacity to metabolize toxicants in his environment, although this is a sadly neglected field of insecticides as far as pesticides are concerned; and most of the available data are derived from studies of drug metabolism. A major question is that of extrapolation of metabolic studies from animals to man (Hollingworth, 1976). It is established that no species is an adequate surrogate for man, but that, as phylogeny would suggest, the old world monkeys are among our closest metabolic consins, with the New world monkeys next, but less closely related. Rodents, particularly the rat, may be used as a predictor for man only with an acute awareness of their many differences from him.

Man may not only sometimes differ from laboratory animals in the mechanisms by which he elimimates xenobiotics, but often in the rate, also. In some important reactions (e.g. with mixed function oxidases and glutathione transferase) there is evidence that man, at least in *in vitro*, has a rather low capacity to degrade foreign compounds compared to laboratory animals (Hollingworth, 1971; Chasseaud, 1973; Nelson *et al.*, 1971).

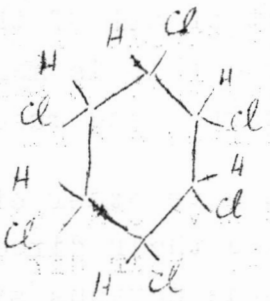
While performing the present studies, the above considerations were kept in mind. A few chlorinated insecticides were fed to rats for different periods of time and their effects noted on different aspects of metabolic processes of the animal.

Types of Chlorinated insecticides:

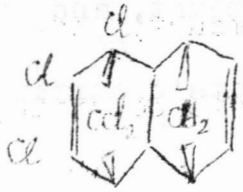
OC insecticides are classified in the three main

families comprising respectively compounds related to DDT, BHC and compounds related to aldrin.

(1) DDT family
eg. DDT



(2) BHC family
(only γ-BHC)



(3) Chlorinated Cyclodiene family
eg. Aldrin

They have common physicochemical characteristics.

(1) Chemical stability because of presence of bonds like C-C, C-H and C-Cl (which are chemically inactive under normal environmental conditions). Accumulation in environment is common.

(2) Low solubility in water coupled with their strongly lipophilic character unless organism's defence mechanism can degrade them to excretable products at a rate that is sufficiently rapid to the rate of entry, accumulation in body lipids will take place.

General mode of action

All are neurotoxic substances. Initial effect of DDT is upon peripheral nervous system, whereas γ- BHC and aldrin appear to attack the central Nervous system. The general effect of all of them is to destabilize neural activity and this is manifested by a hyper excitability of nerves and muscles.

Insecticides have other effects besides acute and chronic toxicity to their direct targets (e.g. ChE of various tissues, the nervous system as a whole).

(1) Side-and after-effects, which generally occur at doses close to the fatal amount, and

(2) Subtle, often unexpected effects occurring at much lower doses.

Effects of insecticides

The chlorinated insecticides have been administered orally alongwith food according to the following schedule:

	Insecticide	Dose (mg/kg/day for 48 hour exp.	15 day exp.	18 months exp.
1.	DDT	100	20	10
2.	γ- BHC	30	18	9
3.	Aldrin	20	8	2.5
4.	Dieldrin	40	12	6

At the end of stipulated period the blood and liver samples were analyzed for various liver function tests. Mainly the changes were recorded in haematological

parameter, biochemical composition of blood and liver and pathological changes in the hepatic structure. The major features of these changes as supported by experimental data given in the previous chapters are being highlighted below:

Haematological parameters

The OC insecticides generally decrease haemoglobin content, RBC count and PCV in both the short term and long term experiments, while the WBC count is very prominantly increased under all these experimental conditions. The MCV and MCH are accordingly increased (Tables I-III).

The increase in WBC is a typical response and is very prominent in the 48 hour and 15 day feeding experiments for all insecticides. In long term experiments, however, the aldrin and dieldrin do not affect the WBC count, while γ BHC and DDT do result in increased count. The decreased haemoglobin content could be attributed either to (i) decreased production of erythronytes or (ii) increased breakdown of RBC and hence haemoglobin. Both these possibilities will result in decreased RBC count and PCV.

Chemical composition of blood

The blood samples were tested for the activities of various blood serum enzymes e.g. amylase, AP, AcP, SGOT, SGPT, LDH, ICDH, CPK and ChE. All these enzymes were elevated after OC insecticide treatment under all experimental conditions. Tables IV-VI show the effect of different insecticides on the various blood serum

TABLE I: Effect of different insecticides administered for 48 hours on the various haematological parameters. The effects have been shown in terms of percent increase(↑) or decrease(↓), with reference to control values.

Parameter		DDT (100mg/kg)	BHC (30mg/kg)	Aldrin (20mg/kg)	Dieldrin (40mg/kg)
Hb	↓	11	7	12	9
RBC	↓	16	11	14	22
PCV	↓	-	7	-	11
WBC	↑	48	46	25	55
MCV	↑	19	10	13	14
MCH	↑	7	4	3	17
MCHC	↓	10	5	9	-

TABLE II: Effect of different chlorinated insecticide administered for 15 days on the various heamatological parameters. The % increase(↑) or decrease (↓)has been calculated with reference to control values.

Parameter		DDT (20mg/kg)	BHC (18mg/kg)	Aldrin (8mg/kg)	Dieldrin (12mg/kg)
Hb	↓	-	8	12	11
RBC	↓	17	16	22	16
PCV	↓	7	9	6	13
WBC	↑	64	24	45	36
MCV	↑	12	8	21	5
MCH	↑	9	9	13	-
MCHC	↓	-	-	6	-

TABLE III: Effect of different chlorinated insecticides administered for 18 months on the various haematological parameter. The % increase(↑) or decrease (↓) has been calculated with reference to control values.

Parameters		DDT (10mg/kg)	BHC (9mg/kg)	Aldrin (2.5mg/kg)	Dieldrin (6mg/kg)
Hb	↓	-	-	10	9
RBC	↓	13	10	15	17
PCV	↓	8	-	7	7
WBC	↑	29	24	-	-
MCV	↑	10	10	9	12
MCH	↑	6	8	5	9
MCHC	↓	-	-	-	-

TABLE IV: Effect of different insecticides administered for 48 hours on the various blood serum enzymes activities. The effects are shown as % increase (↑) or decrease (↓) with reference to control values.

Parameter		DDT (100mg/kg)	BHC (30mg/kg)	Aldrin (20mg/kg)	Dieldrin (40mg/kg)
Amylase	↑	29	-	60	-
AP	↑	323	144	141	56
AcP	↑	158	81	15	49
SGOT	↑	170	72	370	36
SGPT	↑	124	33	-	35
LDH	↑	66	31	78	120
ICDH	↑	131	80	135	105
CPK	↑	69	62	31	63
ChE	↑	77	29	88	57

TABLE V: Effect of different insecticides administered for 15 days on the various blood serum enzyme activities. The effects are shown as % increase (↑) with reference to control.

Parameter		DDT (20/mg/kg)	BHC (18mg/kg)	Aldrin (8mg/kg)	Dieldrin (12mg/kg)
Amylase	↑	84	76	109	63
AP	↑	550	164	368	290
AcP	↑	250	100	105	-
SGOT	↑	55	32	195	71
SGPT	↑	200	-	-	55.4
LDH	↑	77	26	115	120
ICDH	↑	75	46		11
CPK	↑	248	139	-	-
ChE	↑	-	-	-	-

TABLE VI: Effect of different insecticides administered for 18 months on the various blood serum enzyme activities. The effects are shown as % increase (↑) with reference to control.

Parameter		DDT (20mg/kg)	BHC (18mg/kg)	Aldrin (8mg/kg)	Dieldrin (12mg/kg)
Amylase	↑	71	32	40	18
AP	↑	134	113	193	48
AcP	↑	8	171	15	36
SGOT	↑	40	-	32	63
SGPT	↑	30	-	197	-
LDH	↑	-	-	47	17
ICDH	↑	58	32	33	7
CPK	↑	44	257	250	-
ChE	↑	-	25	27	-

enzymes administered at different doses for different time periods. The effect on all enzymes in the 48 hour feeding experiment is very obvious, while in the 15 day feeding experiment the ChE activity remains unchanged. Besides that γ-BHC does not affect SGPT, aldrin has no effect on SGPT and CPK, while CPK and AcP remain unaffected by dieldrin. In 18 months long feeding experiment the DDT does not alter LDH and ChE. The SGOT, SGPT and LDH are unaffected by γ-BHC treatment, while SGPT, CPK and ChE are not affected by dieldrin.

The raised level of these enzymes in serum is generally indicative of cellular damage in the liver muscle, nerve endings etc. The cells thus damaged contribute towards increased enzymatic activities in serum where they have leaked into. In 48 hour treatment all the insecticides have very drastic effect, while in 15 day feeding experiment γ-BHC does not effect two enzymes (SGPT and ChE), while aldrin and dieldrin do not change three enzymes each e.g e.g. SGPT, CPK and ChE in the first case, while AcP, CPK and ChE in the second case. In 18 months long feeding no effect on SGOT, SGPT and LDH. Aldrin produces very drastic effects, while dieldrin does not change SGPT, CPK and ChE.

From amongst several other biochemical components tested bilirubin and blood serum protein contents are raised after insecticide treatments under almost all experimental conditions, while FAA contents are decreased during this period (Tables VII-IX). The increased bilirubin content is in conformation with the typical malfunction of hepatic cells. The blood serum proteins

TABLE VII: Effect of different insecticides administered for 48 hours on the various blood serum component. The effects are shown as % increase (↑) or decrease (↓) with reference to control.

Parameter	DDT (100mg/kg)	BHC (30mg/kg)	Aldrin (20mg/kg)	Dieldrin (40mg/kg)
Bilirubin	↑ -	↑ 46	-	↑ 32
Protein	↑ 30	↑ 26	↑ 26	↑ 17
Urea	↑ 32	-	↓ 31	↑ 42
Glucose	↑ 36	↑ 40	↑ 28	↑ 23
Cholesterol	↓ 40	↑ 11	↓ 29	↓ 28
FAA	-	↑ 21	↓ 18	↓ 29

TABLE VIII: Effect of different insecticides administered for 15 days on the various blood serum components. The effects are % increase (↑) or decrease (↓) with reference to control.

Parameters	DDT (20mg/kg)	BHC (18mg/kg)	Adlrin (8mg/kg)	Dieldrin (12mg/kg)
Bilirubin	↑ 45	↑ 69	↑ 64	-
Protein	↑ 44	-	↑ 31	-
Urea	-	-	↑ 70	-
Glucose	↓ 32	↓ 16	↓ 26	-
Cholesterol	-	-	-	↓ 27
FAA	↓ 52	↓ 19	↓ 56	↓ 53

TABLE IX: Effect of different insecticides administered for 18 months on the various blood serum components. The effects are shown as % increase (↑) or decrease (↓) with reference to control.

Parameters	DDT (10mg/kg)	BHC (9mg/kg)	Aldrin (2.5mg/kg)	Dieldrin (6mg/kg)
Bilirubin	-	↑ 30	↑ 18	↑ 67
Protein	↑ 38	-	↑ 24	-
Urea	-	↓ 18	↑ 45	-
Glucose	↑ 15	↓ 20	-	↓ 19
Cholesterol	-	-	-	↓ 18
FAA	-	-	↓ 16	-

TABLE X: Effect of different insecticides administered for 48 hours on the activities of hepatic enzymes. The effects have been shown as percent increase (↑) or decrease (↓) with reference to control.

Parameters	DDT (100mg/kg)	BHC (30mg/kg)	Aldrin (20mg/kg)	Dieldrin (40mg/kg)
GOT	↑ 77	↑ 44	↑ 28	-
GPT	↑ 35	↑ 181	↑ 40	↑ 100
AP	-	↑ 184	↑ 171	↑ 213
LDH	↑ 71	↑ 181	↑ 26	↑ 36
ICDH	-	-	-	↓ 17

have increased probably because some new protein has been synthesized in response to insecticide treatment. The FAA content of blood serum have decreased, because these are being used up in the synthesis of new protein. The other biochemical components generally have fluctuating response to different insecticides administered at different doses for different periods of times. In 48 hour experiment γ- BHC shows 1.11 fold increase, in cholesterol content; which decrease in all other insecticide treatments. In 15 day experiment DDT, γ- BHC and aldrin has no effect on cholesterol, while dieldrin treatment results in 27% decrease. The same pattern is maintained in 18 months long feeding experiment.

Liver biochemistry

Most of the hepatic enzymes tested were elevated after insecticide feeding (Tables X-XII). In 48 hour feeding experiment γ- BHC shows maximum increase in the activities of GPT, AP and LDH. The aldrin treatment also shows similar trend. Although DDT does not appear to have caused any significant change in AP activity after 48 hour feeding, but this is the most sensitive enzyme so far as other insecticides are concerned. ICDH activity remains unaffected under the influence of DDT, γ- BHC and aldrin, but is decreased 17% after dieldrin feeding for 48 hours. When dieldrin is fed for 15 days or for 18 months the ICDH activity is significantly raised. Besides that other enzymes are also increased (Tables XI-XII). These enzymes are apparently, induced as a result of which their activity is raised in the liver. This increase is then reflected in the blood

TABLE XI: Effect of different insecticides administered for 15 days on the activities of hepatic enzymes. The effects have been shown as % increase (↑) or decrease (↓) with reference to control.

Parameters	DDT (20mg/kg)	BHC (18mg/kg)	Aldrin (8mg/kg)	Dieldrin (12mg/kg)
GOT	↑ 174	↑ 21	↑ 171	-
GPT	↑ 25	↑ 65	-	↑ 55
AP	-	↑ 34	↑ 172	↑ 89
LDH	↑ 82	↑ 53	-	↑ 26
ICDH	↑ 85	↑ 66	↑ 100	-

TABLE XII: Effect of different insecticides administered for 18 months on the activities of hepatic enzymes. The effects have been shown as % increase (↑) or decrease (↓) with reference to control.

Parameters	DDT (10mg/kg)	BHC (9mg/kg)	Aldrin (2.5mg/kg)	Dieldrin (6mg/kg)
GOT	-	-	↑ 92	↑ 26
GPT	↑ 1086	↑ 184	↑ 16	↑ 56
AP	↑ 39	-	↑ 76	↑ 45
LDH	↑ 39	↑ 27	↑ 45	↑ 12
ICDH	-	↑↓ 27	↑ 71	↑ 65

serum, into which the enzymes leak and hence lead to raised enzymatic activities in the blood serum.

Tables XIII-XV show the effect of different doses of different insecticides on the various biochemical components of liver. The hepatic cholesterol show consistent decrease after insecticide treatment under all experimental conditions. Although FAA content are not much affected after 18 months of feeding, but these are otherwise drastically decreased in the other two feeding experiments i.e. 48 hours and 15 days. Total protein remain unaffected in the 15 day experiment, show a tendency towards increase in the long term feeding experiment, but has no definite pattern in 15 day feeding experiment. The soluble protein increase in the 48 hour feeding, but has fluctuating values in other treatments.

The hepatic nucleic acid content behave differently under different experimental conditions. Dieldrin causes a decrease in the RNA and DNA content, while γ-BHC results in RNA decrease only. All other treatments remain non effective. In 15 day feeding experiment the DDT and γ-BHC treatment results in increased DNA, although aldrin and dieldrin do not make any effect. The RNA content, on the other hand, are decreased by γ-BHC and increased by aldrin and are not affected by DDT and dieldrin. In 18 month long feeding experiment the DDT decrease both RNA and DNA content, while γ-BHC results in increase of DNA. The other insecticide do not show any significant change in DNA content. The RNA content are however significantly decreased after dieldrin treatment for 18 months.

TABLE XIII: Effect of different insecticides administered for 48 hours on the various biochemical components of rat liver. The effects have been shown as % increase (↑) or decrease (↓) with reference to control.

Parameters	DDT (100mg/kg)	BHC (30mg/kg)	Aldrin (20mg/kg)	Dieldrin (40mg/kg)
Cholesterol	↓ 44	↓ 34	↓ 29	↓ 24
Glucose	↓ 21	-	↓ 43	↓ 35
FAA	↓ 43	↓ 52	↓ 37	↓ 40
Total Protein	-	-	-	-
Sol.Protein	↑ 33	↑ 30	↑ 29	↑ 40
DNA	-	-	-	↓ 63
RNA	-	↓ 49	-	↓ 47

TABLE XIV: Effect of different insecticides administered for 15 days on the various biochemical components of rat liver. The effects have been shown as % increase (↑) or decrease (↓) with reference to control.

Parameters	DDT (20mg/kg)	BHC (18mg/kg)	Aldrin (8mg/kg)	Dieldrin (12mg/kg)
Cholesterol	↓ 38	↓ 63	↓ 72	-
Glucose	↑ 88	-	↑ 143	↓ 54
FAA	↓ 31	↓ 34	↑ 57	↓ 26
Total Protein	↓ 21	-	↑ 33	-
Soluble Protein	↓ 34	-	-	↑ 30
DNA	↑ 40	↑ 62	-	-
RNA	-	↓ 33	↑ 113	-

Histological changes

All insecticides under all experimental conditions generally lead to hypertrophy of hepatic cells, its nuclei and nucleoli (Tables XVI-XVIII). This is indicative of toxicological symptoms. The number of nuclei/cell and number of nucleoli/nucleus generally remain unaltered. In 15 day feeding experiment however, the dieldrin feeding results in 25% increase in number of nuclei/cell although number of nucleoli/nucleus remain unchanged.

General considerations

It is now clear that chlorinated hydrocarbon insecticides cause liver damage at high concentrations (e.g. at about 1000 ppm). The damage ranges from increase in weight and fat content to cell necrosis. There is great variation in the doses which induce such effects, even among individual animals belonging to the same species, for general health and nutritional conditions are known to greatly affect the susceptibility of the liver. In rat at least, sex also plays an important role in the manifestation of these histopathological changes. Cytological effects, representing induction phenomen can take place at very low doses. Jager (1970) has reported that LFT or aldrin/dieldrin and endrin factor of workers and states that there is a slight increase in SGOT, GPT. The increases suggest liver enzyme induction. West (1967) believes that lindane causes haematological reactions. Tocci et al. (1969) have reported that heavy exposure to pesticide causes changes in kidney and liver function and in the concentration of some circulating amino acids in about 30% of the people studied. The exposed group tended to have higher SGOT, AP, serum osmolality and

TABLE XV: Effect of different insecticides administered for 18 months on the various hepatic biochemical components. The effects have been shown as % increase (↑) or decrease (↓) with reference to control.

Parameters	DDT (10mg/kg)	BHC (9mg/kg)	Aldrin (2.5mg/kg)	Dieldrin (6mg/kg)
Cholesterol	-	-	↓ 18	↓ 61
Glucose	↓ 31	↓ 49	↓ 52	↑ 61
FAA	-	-	↓ 10	
Total Protein	↑ 21	-	↑ 6	↑ 20
Soluble Protein	↓ 20	↓ 30	↑ 17	
DNA	↓ 13	↑ 55	-	-
RNA	↓ 39	- -	↑ 51	↓ 12

TABLE XVI: Effect of different insecticides administered for 48 hours on the various histological parameters of rat liver. The effects have been shown as % increase (↑) or decrease (↓) with reference to control.

Parameters	DDT (100mg/kg)	BHC (30mg/kg)	Aldrin (20mg/kg)	Dieldrin (40mg/kg)
Cell size	↑ 23	39	40	46
Nuclear size	↑ -	18	31	-
Nucleolar size	↑ 44	35	79	-
No.of nuclei/ cell	-	-	-	-
No.of nucleoli/ nucleus	-	-	-	

TABLE XVII: Effect of different insecticides administered for 15 days on the various histological parameters of rat liver. The effects have been shown as % increase(↑)or decrease(↓) with reference to control.

Parameters		DDT (20mg/kg)	BHC (18mg/kg)	Aldrin (8mg/kg)
Cell size	↑	26	30	53
Nuclear size	↑	-	20	61
Nucleolar size	↑	-	-	84
No.of Nuclei/ cell	↑	-	-	25
No.of nucleoli/ nucleus		-	-	-

TABLE XVIII: Effect of different insecticides administered for 18 months on the various histological parameters of rat liver. The effects have been shown as % increase(↑)or decrease(↓) with reference to control.

Parameters		DDT (10mg/kg)	BHC (9mg/kg)	Aldrin (2.5mg/kg)	Dieldrin (6 mg/kg)
Cell size	↑	45	59	104	87
Nuclear size	↑	20	14	70	39
Nucleolar size	↑		37	34	14
No.of nucleic/ cell		-	-	-	-
No.of nucleoli/ nucleus.			-	-	

creatinine values than were found in a control population and 16% of the group were thought to have specific renal tubular transport defect.

Gertig et al. (1971a,b) made efforts to measure biochemical changes induced by various chlorinated hydrocarbons in the serum enzymes by using human blood in vitro. The effects of aldrin, lindane, DDT, DDE and TDE (DDD) on both AcP and AP were slight. Their effects on human aminotransferases in vitro were varied, but on the whole they activated SGOT and SGPT activities. Kacew et al. (1972) injected 100 mg/kg of O,P'-DDT (intramuscularly) in the rat and observed increase in various renal gluconeogenic enzymes-pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose 1,6, difosphatase and glucose-6-phosphatase to the levels of 298, 273, 300 273, 300 and 298% of the control values, respectively.

In studying biochemical lesions caused by pesticidal compounds, it appears necessary to distinguish the lesions resulting from general stress and insult to the body systems from those which represent specific biochemical reactions. The specific reactions include inhibition of cholinesterases and other esterases, organophosphates and carbamates. ATPase inhibition by chlorinated hydrocarbon insecticide , action of DDD analogus on the adrend cortex and aconitase inhibition by fluoroacetate generating agents. Some of the non specific responses are definitely related to the hepatic changes which can be induced by many pesticides, including induction of serum aminotransferases (e.g. SGOT and SGPT) LDH and AP changes in levels of hormones and various biogenic

amino are indirectly related to these hepatic changes. Since this hepatic changes take place at relatively low pesticide concentrations, and since the hormones and biogenic amines do act on vital biological sites at very low doses, it is likely that subtle biochemical changes can be produced by relatively low doses of pesticides.

As for the detection of these subtle effects of pesticides in man, there is no convenient monitoring method sensitive enough to be useful at this level (exclusive of blood ChE test). Naturally it is not feasible to collect liver samples from patients and biochemical changes in the blood, faeces , urine etc., cannot be detected at low pesticide levels. Perhaps it is necessary to develop a reliable assay method for changes in bioenzyme pattern or immunological testing methods. Other alternative would be to conduct a complete diagnostic investigation such as a complete blood count, serum bilirubin, thymol turbidity, goldsol reaction, leucine aminopeptidase, plasma Na^+, K^+, serum carotene and serum protein haemoglobin in addition to other routine tests already discussed.

Are OC insecticides carcinogenic?

The question of the carcinogenicity of chlorinated insecticides is not quite settled. Kemeny and Tarjan (1966) and Tarjan and Kemeny (1969) conducted a long-term multigeneration feeding study on the effects of DDT in mice maintained on a diet containing 2.8-3.0 ppm of p.p'-DDT, which corresponds to 0.4-0.7 mg/kg/day. They observed that the increased incidence of leukemia and tumors was statistically significant with respect to controls in the second and third generations. By the

fifth generation, the incidence of pulmonary carcinoma had increased 25-fold. However, no effects on reproduction were found.

Even though the carcinogenicity of DDT has not been satisfactorily proven, it is clear that tumorigenic - whether malignant or benign - effects intensify during continuous exposure of generations of mice to DDT. Tomatis (1970) found that tumors occurred in the second generation of mice (BALB/c strain) administered 2.8-3.0 ppm of DDT. Tomatis et al. (1972) found that in a two-generation experiment with the CF-1 minimum-inbred strain of mice the incidence of liver tumors, but not of lymphomas, osteomas, or lung tumors, increased at all levels (i.e., 2,10,50, and 250 ppm in the diet) of DDT and also appeared at an earlier age than in controls. According to Tomatis et al, the tumors are "well-differentiated nodular growths, compressing, but not infiltrating, the surrounding parenchyma, or nodular growths in which the architecture of the liver is obliterated, often showing trabecular or glandular patterns".

That DDT causes more profound tumorigenic effects on subsequent generations than on the parental generation has also been shown by Shabad et al. (1972).

It would be unfair not to mention the studies indicating that DDT and its analogues are not carcinogenic and those showing that these compounds have antitumorigenic properties in certain cases. Ortega et al. (1956) and Ottobomi (1969) were not able to show that DDT has carcinogenic effects in the rat. Unfortunately, because

these studies were conducted for a relatively short period, they do not resolve the extremely important question of whether this difference is species specific. Studies comparable to the mice experiments just discussed (including multigeneration studies) would have answered the question. Although there is some evidence that DDT might increase the incidence of hepatic tumors in the rat (Fitzhugh and Nelson, 1947) at very high doses, the issue of the effect of DDT in the rat is not settled.

Laws (1971), on the other hand, found that DDT had an antitumorigenic effect on the rate of success ("take") in transplanting an experimental ependymoma in the mouse. These animals were exposed to a 5.5 mg/kg/day dose of technical DDT (given in the diet at 33.3 mg/kg), and an ependymoma (the Zimerman ependymoma) known to have a 100% "take" rate in mice was transplanted 1 week after the initial feeding of DDT. The incidence of subcutaneous tumors was 92.1% in the DDT treated mice and 100% in controls. Of 89 animals receiving DDT and a transplant, seven never developed any tumors throughout the experiments. Moreover, the DDT-fed mice lived longer. There are a few other studies indicating that DDT-treated animals are less likely to develop cancer in response to experimentally introduced chemical carcinogenic agents, probably because DDT has the ability to induce hepatic microsomal detoxification mechanisms. Okey (1972), for instance, studied this effect by using dimethybenzanthracene, known to cause mammary tumor, against rats. p.p'-DDT concentrations in the diet as low as 10 ppm caused a significant reduction in tumor incidence. It must be remembered, however, that the effects of induction can

be multifold. Induction clearly decreases the danger of carcinogenic chemicals that act directly, because metabolic transformation detoxifies them, but there are other compounds whose metabolic products are the carcinogenic agents (e.g. tryptophan metabolites) so that induction could increase the danger. Various induction aspects of chlorinated hydrocarbons in relation to carcinogenicity have been discussed by Falk (1971).

Studies on other chlorinated hydrocarbon insecticides are less extensive. BHC isomers have been examined by Japanese scientists. Nagasaki et al. (1971) fed mice 6,66, and 660 ppm of technical BHC and found that hepatomas developed in 24 weeks in all 20 of those fed 660 ppm. Later, Nagasaki et al. (1972) compared the effects of four isomers of BHC and found that only α-BHC induced hepatoma in mice at 250-500 ppm levels in the diet after 24 weeks. Yellowish nodules up to 0.3-2.0 cm in diameter appeared at these doses of α-BHC, no carcinogenic effects were observed with the β-, γ- (lindane), and δ-isomers. Independently, Goto et al. (1972) studied the effects of α-, β-, and γ-BHC and their possible metabolites, 1,2,4-trichlorobenzene, 2,3,5-trichlorophenol, and 2,4,5-trichlorophenol, on male mice. Their finding was essentially the same, that α-BHC is the most active analogue in causing hepatoma in mice. They observed tumors of 0.5-1.5 cm diameter.

As for dieldrin and aldrin, Fitzhugh et al. (1964) studied their effects on rats for 2 years. They found 18 tumors (in 41 rats) at 0.5 ppm, 15 tumors (in 41 rats) at 2 ppm, and 16 tumors (in 40 rats) at 10 ppm of aldrin

or dieldrin in the diet. In contrast, Deichmann et al., (1967) and Walker et al. (1968) could not observe any increase in tumor incidence in rats fed aldrin (5ppm) or dieldrin (0.1, 1.0, and 10 ppm) for 2 years. Aldrin and dieldrin are known to increase the occurrence of hepatic tumor in mice. Davis and Fitzhugh (1962) found increased hepatic tumors in mice fed 10 ppm of aldrin/dieldrin for 2 years. The experiments in the Tunstall Laboratory (Shell Chemicals in Britain) confirmed this observation. At 10 ppm, hepatic tumors appeared in about 9 months; however, at 1.0 ppm tumors were rare (Jager, 1970). Assuming that 1.0 ppm in the diet is the minimum effect level, aldrin/dieldrin concentrations in food commodities (0.01-0.1 ppm) are in excess of that allowed by the 100-fold safety factor.

In the rat, there is evidence that aldrin, dieldrin, and endrin have mild antitumorigenic activities. Deichmann et al. (1970) fed diets containing 20, 30, or 50 ppm of aldrin or dieldrin or 2, 6, or 12 ppm of endrin to 900 albino rats for their lifetime. The mean life spans of females fed 50 ppm aldrin and dieldrin were 13.0 and 16.6 months, respectively, compared to the control life span of 19.5 months. Altogether 257 tumors of all types were observed in 793 treated rats and 79 tumors in 163 controls. This reduction in tumor incidence was attributed to increased hepatic activity in the treated rats. The most frequent tumors were in the lungs, mammary tissues, lymph modes, liver, and kidneys. Treon (1956) could not observe any sign of carcinogenicity in rats fed 1,5, 25, 50 and 100 ppm of endrin in their diet for 2 years. The frequency of tumor incidence in the

treated animals was identical to that in controls. Cabral et al. (1972) gave five doses of 10 mg/kg heptachlor in 2 days to 95 suckling rats and compared tumor incidence after 106, 110 weeks with that in control rats (which received only corn oil). They concluded that heptachlor is not carcinogenic, as their studies revealed no statistically significant differences.

In conclusion, it is clear that certain insecticides can induce tumors in some experimental animals (mostly rodents) even at relatively low concentrations; the lowest figure for such effects is approximately 1 ppm. While such effects have not been carefully assessed in terms of human hazard, they certainly represent potential danger, close enough to warrant further investigation.

III. CONCLUDING REMARKS

Synthetic pesticides when used properly have been of tremendous benefit to man and his environment, but when misused or used carelessly they have caused considerable harm. Fortunately the adverse effects have been relatively minor in comparison to the great benefits from pest control. There is little doubt that pesticides have played, and most likely will continue to play, an important role in the production of food as the world's supply of raw agricultural products continues to decline in proportion to the increase in population. The risks or hazards of using chemical pesticides have increased in recent years with the sharp rise in their consumption by agriculture, industry and householders.

Pesticides are of great benefit to man. OC insecticides just like other insecticides have saved millions of lives through control of disease carrying insects. They have minimized catastrophic crop damage by insects, weeds, plant diseases, rodents and other pests, preserved valuable forests and porklands from insect destruction, and protected households against damaging beetles, moths and other bugs. We cannot afford to lose the advantages gained through pesticides, but neither can we ignore the potential dangers. We must therefore find ways to minimize or eliminate the hazards that (may) accompany the application of these chemicals.

One of the problems of OC insecticides is that these chemicals get deposited inside the animal tissue, as well as in various ecological nickes. The recent shift in pesticide usage from the chlorinated hydrocarbons to the so called substitutes or non persistent pesticides is an encouraging trend. However, these less persistent carbomates and organophosphate are more toxic.

In order to minimize a liminate the harmful effects of pesticides, the users may be directed/taught to follow the instructions and precautions prescribed.

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