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National Research Centre, Dokki, Cairo (Egypt)

Acute toxicity of organophosphorus and organochlorine insecticides in laboratory animals

T. H. Mikhail, N. Aggour, R. Awadallah, M. N. Boulos, E. A. El-Dessoukey, and *A. I. Karima*

With 14 figures and 1 table

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The importance of organophosphorus (O.P.I.) and organochlorine insecticides (O.cl.I.) is because of their established value and their widespread use as agricultural insecticides.

Unfortunately the administration of such compounds resulted in dangerous effects; not only on plants but also on animals.

Two of these insecticides were accused for the death of 1200 buffalos in Garbia province (Egypt) after accidental feeding of animals by plants sprayed with cyolane and phosvel (14).

There is, therefore, a need for information on the toxicity, clinical symptoms, and hazards of the use of these compounds in domestic animals.

Since organophosphorous insecticides cause an inhibition of acetyl-cholinesterase, cholinesterase activity has been used widely as a diagnostic tool for insecticidal poisoning (29).

However, *Zemaitis* et al. (32) found that cholinesterase activity was unaltered in males after either acute or chronic exposure to dieldrine (O.cl.I.), but was significantly reduced in plasma from females fed dietary dieldrin for 8 weeks.

Study of serum enzymes is useful in detecting the point of action of toxic substance. Estimation of SGPT and SGOT were found to be very sensitive indices of hepato-cellular injury, and it was found that SGPT is a more specific and sensitive index than SGOT (7).

The purpose of this investigation is to study the effect of two of the commonly used insecticides, namely Dursban (O.P.I.) and DDT (O.cl.I.) on the liver, since it is the main site of detoxication.

The relationship between enzyme activity changes from normal, and certain histochemical and histopathological changes of the liver, kidney and testes were also examined.

Materials and methods

Male rats, weighing 150–200 g, of the Sprague-Dawley strain were used in this study. The animals were maintained on ad-libitum diet and water.

The insecticides used in the tests were Dursban which is a halogenated organophosphorus insecticide (0.0 diethyl 0-3,5,6-trichloro 2-pyridyl phosphorothiate) and DDT which is an organo-chlorine insecticide. (2,2 bis-(p-chlorophenyl)1,1,1-trichloroethane).

The Dursban was diluted in water in the ratio 1:20. Two repeated doses in two successive days of half the L.D. 50 was administered to the animals by intraperitoneal injection.

In case of DDT it was dissolved in vegetable oil and administered to the animals by stomach tube. The dose used was 150 mg/kg body wt. in three repeated doses.

The animals were then sacrificed by light ether anesthesia, and blood samples were taken for enzyme analysis.

Serum glutamic oxaloacetic transaminase (GOT) and serum glutamic pyruvic transminase (GPT) were assayed by the method of *Reitman* and *Frankel* (23). Serum alkaline phosphatase was assayed by the method of *King* and *Armstrong* (12). Serum cholinesterase activity was assayed by the method of *Bigg* (1).

Samples of the liver, kidney and testes were taken for histopathological and histochemical examinations, the material was fixed, sectioned and stained as follows:

For histopathological examination, Ehrlich's Haematoxylin and Eosin (H and E) was used.

For histochemical examination, the following methods were performed on frozen sections.

1. Periodic Acid-Schiff (PAS) to demonstrate carbohydrate substances, particularly glycogen, which is strongly positive (10, 21).
2. For demonstration of fat, the frozen sections were stained with oil blue N (10).

Results and discussion

As breakdown of absorbed foreign substances takes place mainly in the liver, biochemical changes associated with liver function and, to a lesser extent, changes in blood and renal parameters may be expected first after absorption (5).

Kagan et al. (11) found that DDT, which is an organochlorine insecticide, affected liver function. Also, *Kulagin* (13) reported a decrease of albumin and an increase in the globulin contents in the blood serum of rats after oral administration of DDT in a dose corresponding to the LD 50 value.

A similar effect was indicated in the work of *Menrath* (15) who found that oral administration of an O.P.I. caused liver damage in dogs. Indirect evidence of liver damage was provided by an increase in serum enzymes.

In the present work two repeated i.p. injections of Dursban in a dose of half the LD 50 resulted in a significant increase in serum GOT, GPT and alkaline phosphatase activity and a decrease of cholinesterase (Table 1).

In case of DDT, two doses of 150 mg/kg orally resulted in a significant increase in the activity of serum GPT only, while three doses increased serum GOT and GPT. No significant change was observed in serum alkaline phosphatase and cholinesterase.

The early change of serum GPT in case of DDT is in agreement with our previous finding that serum GPT is a more specific and sensitive index of hepato-cellular injury than serum GOT (7).

The results in case of Dursban are in accord with the results of *Wright* et al. (31) who found that the cattle poisoned by oral administration of O.P.I. had a serum of highly GOT and GPT activities; this increase correlated well with the depression of cholinesterase activity.

It is clear that cholinesterase was inhibited by Dursban, which is an O.P.I., and not by DDT, which is an O.cl.I.

Weak esterase inhibitory action of several chlorinated hydrocarbon insecticides has been reported by *Geike* (9) and *Bogusz* et al. (2).

Table 1. Effect of Dursban and DDT on serum enzymes.

		Control	Dursban two doses	DDT two doses	DDT three doses
GOT U/ml	Mean	79.00	126.00	70.89	114.00
	SD ±	6.14	36.30	10.75	24.90
	n	(8)	(5)	(9)	(5)
	p		< .05	> .05	< .05
GPT U/ml	Mean	32.38	110.40	44.67	76.40
	SD ±	2.26	61.01	7.07	10.14
	n	(8)	(5)	(9)	(5)
	p		< .05	< .05	< .05
Alk. phosphatase	Mean	28.38	65.40	39.22	37.20
	SD ±	6.67	15.69	14.86	22.22
	n	(8)	(5)	(9)	(5)
	p		< .05	> .05	> .05
Cholinesterase I U/ml	Mean	1.75	0.55	1.81	
	SD ±	0.71	0.39	0.68	
	n	(10)	(9)	(14)	
	p		< .05	> .05	

Also the activity of plasma cholinesterase is regulated in part by estrogen (24).

Zemaitis et al. (32) suggested that depression of cholinesterase activity may result from an increased rate of steroid metabolism in dieldrin-treated female rats, while in male rats plasma-cholinesterase activity was unaffected by either acute or chronic dieldrin administration.

Also alkaline-phosphatase was increased by Dursban and was not affected by DDT under the present experimental conditions.

It was found that alkaline-phosphatase increases 24–48 hrs after administration of high doses of hepatotoxic substances when hepatobiliary injury is produced (28).

Pathological examination of the liver showed that in Dursban-intoxicated rats there is liver necrosis of midzonal type. The necrotic part, which appears pale, occupies the intermediate zone. There is fatty change at the periphery (Figs. 1, 2). There is moderate mononuclear cell infiltrate and early formation of new bile ducts (Fig. 3). There is marked congestion of the central vein with dilated sinusoids (Fig. 4).

However, in case of DDT the liver cells lost their radial arrangements and showed fatty change. There is cellular infiltration, mostly mononucleolar cells, and early formation of new bile ducts (Figs. 5, 6).

Domschke et al. (6) observed that liver cell damage by some O.P. insecticides in rats was due to an impaired phospholipid biosynthesis, especially that of lecithin. Liver ATP was decreased in comparison with the control.

In both insecticides there is a sign of gonadal and kidney damage. There is necrosis of some of the seminiferous tubules of the testes (Figs. 7, 8, 9) and clowdy swelling of the convoluted tubules of the kidney (Fig. 10).

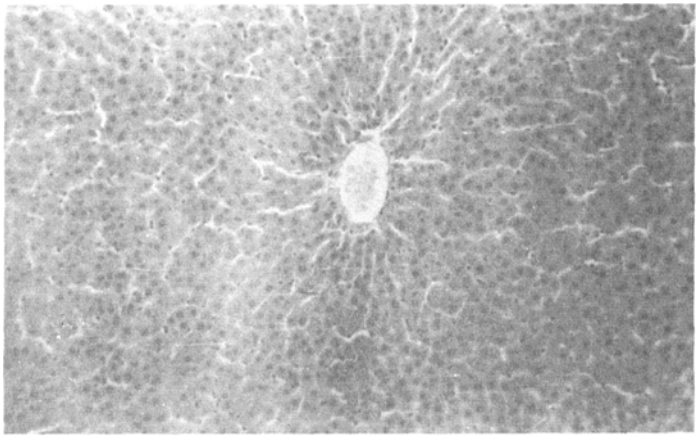

Fig. 1. Microphotograph showing liver cells of control rat.

Testicular damage may be due to the direct toxic effect of the insecticides and/or through hormonal effect.

The stress of hepatotoxic agents administration causes ACTH release (21).

The present results show that Dursban and DDT are hepatotoxic.

Competitive inhibition of the pituitary gonadotrophin release by an excessive secretion of corticotrophin or adrenal cortical growth factor has been suggested after a number of experimental observations (3, 19, 27).

Thus blockade of the pituitary gonadotrophin release as a result of insecticide administration may explain the gonadal damage found in the present work.

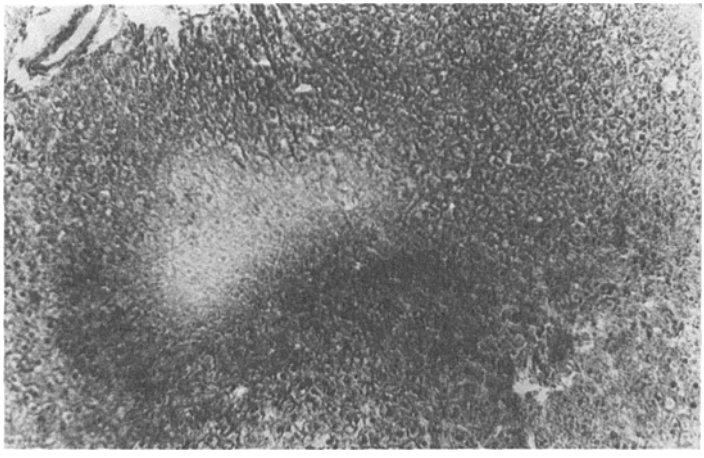

Fig. 2. Microphotograph showing necrosis of mid-zonal type and fatty change at the periphery of the liver of rat treated with Dursban.

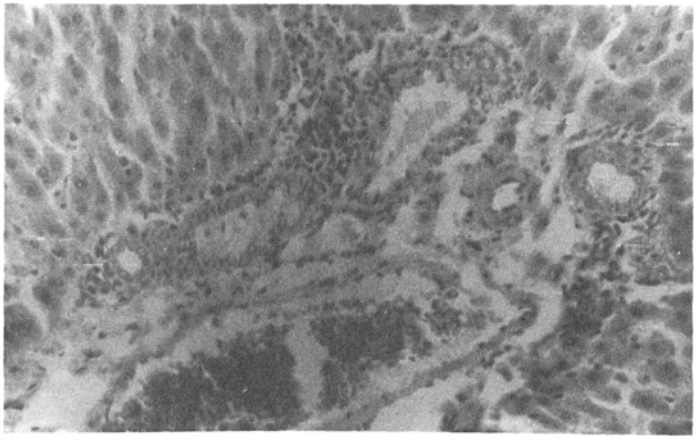

Fig. 3. Microphotograph showing moderately intense mononuclear cell infiltrate and early formation of new bile ducts in the liver of rat treated with Dursban.

Histochemical study of the liver in animals treated with Dursban showed that there is depletion of glycogen especially around the central vein (Figs. 11, 12). The PAS positive material was deposited at one side of the cell.

Hyperglycemic effect was previously reported after organophosphate poisoning (17, 30).

Fatty infiltration of the liver was also obtained in animals after liver damage by Dursban and DDT.

In liver treated with DDT there were large globules of fat inside the liver cells compared to tiny minute droplets of fat in the control liver (Figs 13, 14). This is in accord with the results of *Singlevich* et al. (26) who

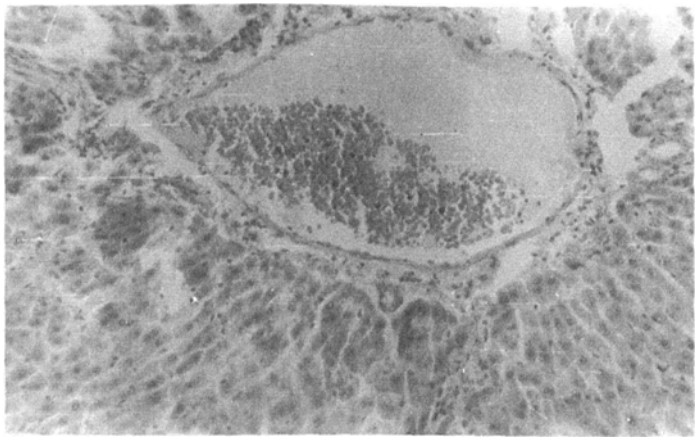

Fig. 4. Microphotograph showing marked congestion of the central vein with dilated sinusoids of the liver of rat treated with Dursban.

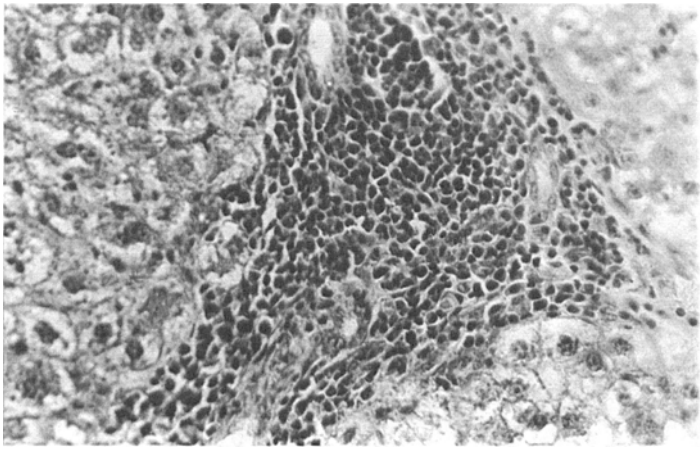

Fig. 5. Microphotograph showing cellular infiltration; the liver cells lost their radial arrangement and showed fatty change in rat treated with DDT.

found that DDT produced an increase in hepatic triglyceride level and histological examination showed fatty infiltration.

Damage of endoplasmic reticulum, increased glycolysis, and mitochondrial respiration are primary signs of liver damage after treatment with hepatotoxins (8).

Frunder (8) found that liver damage by hepatotoxins is followed by an increased concentration of triglyceride in liver cells and a decreased serum lipoprotein synthesis. Metabolism of phospholipids in the endoplasmic reticulum is decreased, but remains unaltered in mitochondria. ATP is transported from the cytoplasm into mitochondria where also the concentration of ADP and inorganic P increases.

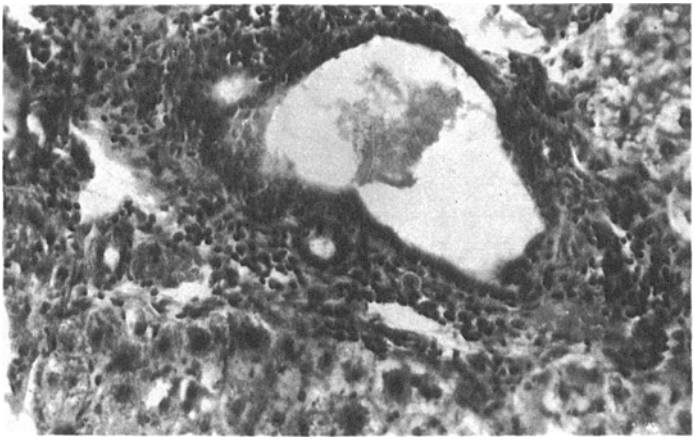

Fig. 6. Microphotograph showing dilatation, cellular infiltration and early formation of new bile ducts in the liver of rat treated with DDT.

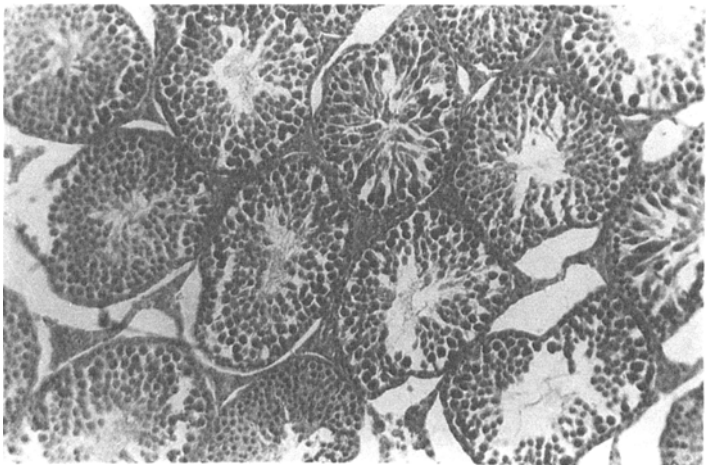

Fig. 7. Microphotograph showing normal pattern of the seminiferous tubules of the testis of a control rat.

This causes the cellular nuclei to begin the proliferative processes. On the other hand, lipolecithin is produced by damaged cellular membranes which induced degenerative processes. These lead to liver cell necrosis within 48–200 hrs after intoxication.

The present results revealed that serum enzyme changes associated with insecticides intoxication indicates liver-cell alteration.

However, no direct correlation was found by *Rees* et al. (22) between degree of liver injury and serum enzyme levels. Only recent injury may be measured by the enzyme levels in serum since the rise in most cases being transitory.

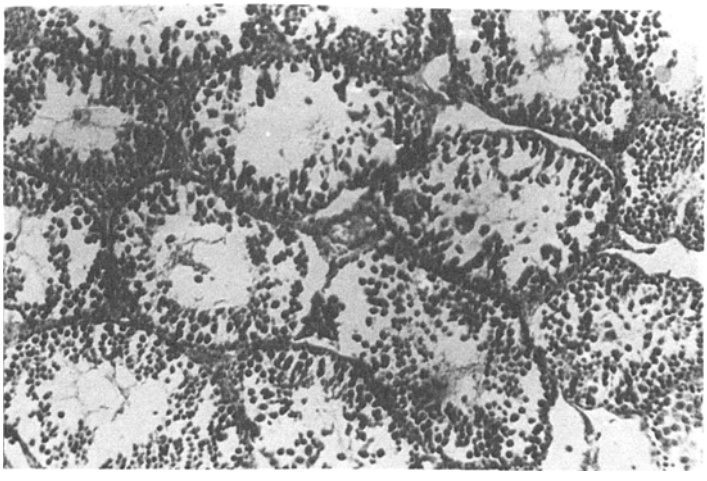

Fig. 8. Microphotograph showing necrosis of some of the seminiferous tubules of the testis of rat treated with Dursban.

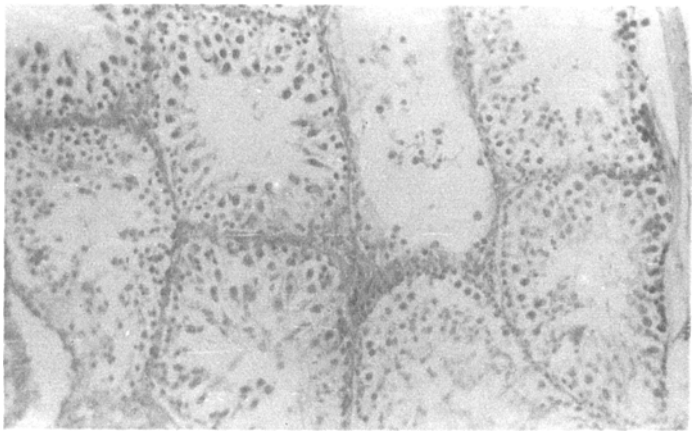

Fig. 9. Microphotograph showing degenerative changes and necrosis of some of the seminiferous tubules of the testis of rat treated with DDT.

Molander (16) found that discontinuance of hepatotoxic agents results in rapid decrease of serum transaminase towards normal.

Also there is evidence that in tissue damage cellular enzyme activity actually increases (4, 25) simultaneously with serum-enzyme increase.

The data obtained by *Murphy* (18) demonstrated that acute poisoning of rats by several organophosphorus insecticides or toxic irritants increased the activity of hepatic alkaline-phosphatase and tyrosine-transaminase activity in rat.

These enzymatic effects of O.P.I. or toxic irritants on liver enzymes were prevented or considerably reduced by adrenalectomy or hypophys-

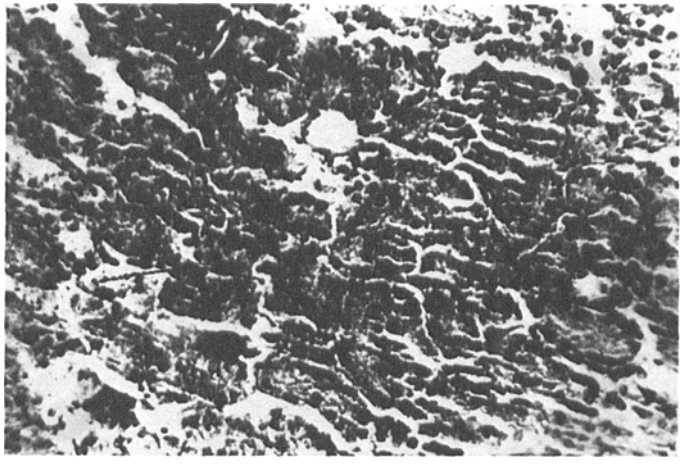

Fig. 10. Microphotograph showing clowdy swelling of the convoluted tubules of the kidney of rat treated with Dursban.

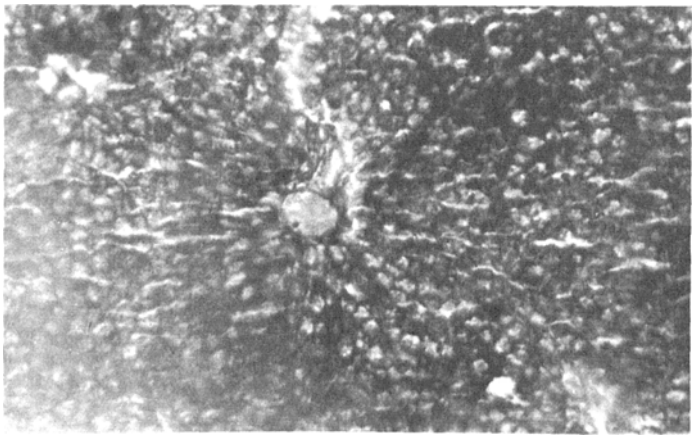

Fig. 11. Microphotograph showing liver cells of control rat loaded with PAS positive material, particularly glycogen.

ectomy. Treatment of adrenalectomized rats with hydrocortisone produced enzymatic effects in the livers which were similar to those produced by toxic substance in intact rats.

From these findings it is concluded that the response of liver or serum enzyme to O.P.I. or toxic irritants is mediated through the pituitary adrenal cortex system.

Summary

The influence of acute poisoning with Dursban (O.P.I.) and D.D.T. (O.cl.I.) on serum enzymes and histopathological examination of the liver, kidney and testes was investigated in albino rats.

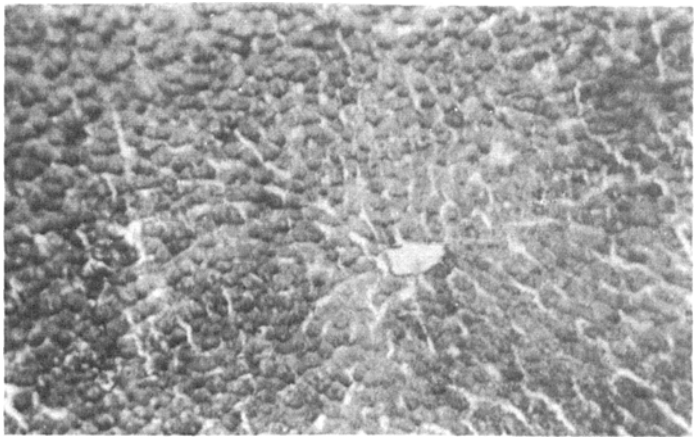

Fig. 12. Microphotograph showing depletion of glycogen especially around the central vein in the liver of rat treated with Dursban.

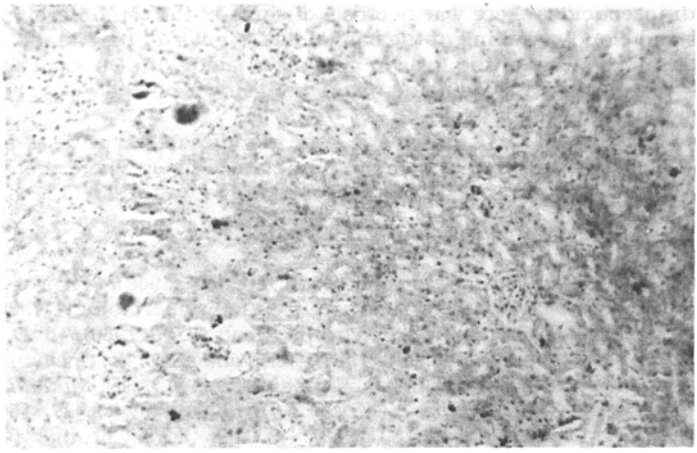

Fig. 13. Microphotograph showing tiny minute droplets of fat stained with oil blue in control liver.

Two repeated i.p. injections of Dursban in a dose of half the LD 50 resulted in a significant increase in serum GOT, GPT and alkaline phosphatase activity and a decrease of cholinesterase.

In case of DDT, two doses of 150 mg/kg orally resulted in a significant increase in the activity of serum GPT only, while three doses increased serum GOT and GPT. No significant change was observed in serum alkaline phosphatase and cholinesterase activity.

Regarding the pathological examination it was found that in animals treated with Dursban there was liver necrosis of mid-zonal type and fatty change at the periphery.

In case of DDT the liver cells lost their radial arrangements and showed fatty change. There was cellular infiltration in the centre, mostly mononucleolar cells.

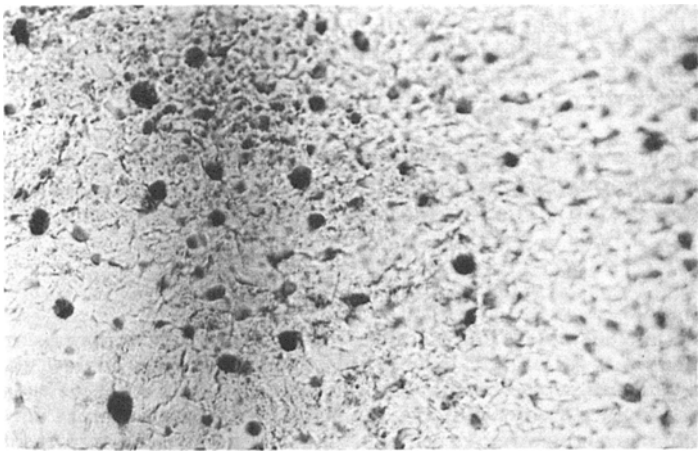

Fig. 14. Microphotograph showing large globules of fat in the liver cells of rat treated with DDT.

In both insecticides there was necrosis of some of the seminiferous tubules of the testes and cloudy swelling of the convoluted tubules of the kidney.

Histochemical study of the liver in animals treated with Dursban showed that glycogen was deposited at one side of the cell. However, there was depletion of glycogen around the central vein.

In liver treated with DDT there were large globules of fat inside the liver cells, indicating increased fat content compared to control liver, where there were tiny minute droplets of fat.

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Authors' address:

Dr. *Tahani H. Mikhail,* National Research Centre, Dokki, Cairo (Egypt)