Delta J. Sci. 12 (3) 1988 1119-1140

EFFECT OF DIMETHOATE ON THE ENZYMATIC ACTIVITIES IN THE LIVER OF TOAD (Bufo regularis).

BY

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Received : 7 . 7. 1988

ABSTRACT

Adult toads were fed the organophosphorous insecticide dimethoate at a dose level of (40 mg/kg body weight), for two and seven days. Histochemical investigations of the activity of acid phosphatase, alkaline phosphatase and succinic dehydrogenase showed that after 2 days acid phosphatase containing particles (Lysosomes) were increased in both number and size, then after a week liver tissue exhibited more intensive reaction. Succinic dehyrogenase activity was also increased according to the period of treatment. On the other hand, a considerable depletion of alkaline phosphatase activity was seen in the liver of 48 hours fed toads whereas this depletion showed a more or less increased in liver of animals fed for a week.

INTRODUCTION

Dimethoate is one of the organophosphorous insecticides which are useful in controlling many agricultural insects (Martin and Worthing , 1976). The wide spread

use of dimethoate for eradication of insects makes it possible to reach the food of animals or even human beings (Sannderson and Edson 1964).

Several investigations have pointed out that dimethoate toxicity caused a degenerative effects, chronic leasions and destruction of the cells (Gobliks and Friedman 1965; Yasnova and Abdasov 1972; El-Ganzuri, (1975) and Moussa and Hafez 1982).

Murphy, 1966; Tandon et al., 1978; Mikhail et al, 1979; Nigam et al., 1981 and Verma et al., 1983 pointed out that acid and alkaline phosphatase showed significant alterations under the effect of insecticides. Khattab et al, 1986 studied the effect of curacron and dimethoate on acid phosphatase activity in the neurones of rat. Effect of cyolane intoxication on the protein content of the ileal epithelial cells of Clarias lazera were declared by Banhawy et al, (1986).

Activity of the respiratory enzyme (succinic dehydrogenase) affected by insecticides was also investigated by Bahig, 1975; Sitkiewcz et al, 1976; Gil et al, 1980 and Al- Dahaby, 1986.

The present study declares the effect of dimethoate toxicity on the activity of acid phosphatase, alkaline phosphatase and succinic dehydrogenase enzymes in the liver

of toad as well as the pattern of distribution and localization of these enzymes in liver tissue.

MATERIAL AND METHODS

Thirty adult healthy toads (Bufo regularis) weighing 40-50 g. were divided into three groups. The first group was orally fed a dose of dimethoate (40 mg/Kg body weight) daily for two days. The second group was given the same dose of dimethoate every day for one week, while the third group was used as a control.

After the different experimental periods, the toads were killed and dissected, suitable blocks of liver were quickly excised and then freshly sectioned by cryostat.

Sites of acid phosphatase activity were visulaized in 10% neutral formalin-fixed frozen sections of 10 microns thick. These were incubated for 2 hours at 37°c - in Gomori medium (1952).

Alkaline phosphatase was examined in the formalin postfixed cryostate sections following incubation for 1½ hours at 37°C according to Gomori (1952). Succinic dehydrogenase activity was detected by the method of Nachlas et al. (1957).

RESULTS

- Acid phosphatase :-

In normal toads, enzyme activity was seen in the cytoplasm of hepatocytes as fine particles. Endothelial cells of sinusoids were also positively stained (Fig., 1). Liver cells of animals fed dimethoate for 48 hours exihibited marked increase in enzyme activity than those observed in controls; the cytoplasm of hepatocytes exhibited a high intensity of enzyme reaction. Sites of reaction were seen as intensely stained plaques giving cytosol appearances. Endothelium of bile ducts were positively stained while the blood vessels endothelia were negatively stained . (Figs., 2 and 3) .

High enzymatic activity was seen in hepatocytes of animals treated for seven days. Also marked damage in liver tissue was seen. The ground cytoplasm of hepatocytes in portal areas showed a pale colour in staining . Endothelia of blood vessels were positively stained . (Figs., 4 and 5) .

- Alkaline phosphatase :-

Control liver cells exhibited a normal level of enzyme activity, sites of alkaline phosphatase activity were seen as fine black deposits lying in cytoplasm. A regular appearance was seen through different hepatic areas. In dimethoate fed toad for 48 hours, alkaline phosphatase activity was

considerably deceased. The intensity of enzyme reaction was highly deleted in hepatocytes arround hepatic artery. (Figs., 6 and 7)

The pigments were increased in number and coalesed in liver tissue. Alkaline phosphatase activity gradally increased in liver of toad treated for seven days, compared with that of two days treated liver. Sinusoidal boundaries of hepatocytes exhibited a moderate activity while ground cytoplasm appeared less stainable. Hepatocytes surrounding large blood vessels showed more enzyme reaction.(Fig. 8)

- Succinic dehydrogenase:-

The activity of enzyme was seen as deep purple to blue fine granules in the cytoplasm of hepatocytes. Nuclei as well as endothelium of blood vessels were unstained. Endothelium of bile ducts was positively stained . (Fig. 9).

Forty eight hours after fed dimethoate , activity of enzyme was increased in hepatocytes. Liver cells arround the blood vessels exhibit more activity . Sites of enzyme activity were seen as fine to large granules scattered in the cytoplasm arround the nuclei. (Figs., 10 and 11).

A marked increase in enzyme activity was seen in the liver cells of toads treated for seven days. Cytoplasm of hepatocytes exhibited more intensity of enzymatic reaction.

Sites of enzyme activity were variable along the sinusoidal margins. The final reaction products were sit on the sinusoidal borders of hepatocytes as fine, small granules and dense masses. Activity of enzyme was strong in the centerolobular areas. Cells of bile ducts were strongly stained while endothelium of blood vessels appeared negatively stained. (Figs., 12 and 13).

The activity of the enzymes under study and its distribution patterns and localization can be summarized in the following Table (1) .

Table (1):- The activity of the enzymes under study, its distribution patterns and localization

Enzymes	Activity / Localization	Control toads	2 days treated toads	7 days treated toads
Acid phosphatase	1	++	+++	+++
	2	-	-	-
	3	+	-	++
Alkaline phosphatase	1	++	+	+ + +
	2	-	-	+
	3	+	-	+
Succinic dehydrogenase	1	++	+++	+++
	2	+	++	+++
	3	-	+	+

1. Hepatocytes
2. Endothelium of bile duct
3. Endothelium of blood vessels

+++ Strong activity.
++ Moderate activity.
+ Weak activity.
- No activity.

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DISCUSSION

Acid phosphatase is localized mainly in the lysosomes. There is also extralysosomal acid phosphatase, which is found in endoplasmic reticulum and possibly in the hydroplasm (Lojda et al, 1979) . Lysosomes have proved to be implicated in many plysiological and pathological conditions in animal cells. Banhawy, (1974) reported the higher diffused enzymatic activity of acid phosphatase-containing particles (Lysosomes),and the consequent release of the enclosed enzymes into the ground cytoplasm. He added that the release of acid phosphatase together with the other hydrolases and their increase in the cytoplasm is believed to play an impotant role in the initiation of the autolytic processes which ultimately lead to cellular autolysis and degeneration. This assumption was also declared by Sastry and Malik (1979).

The present investigation revealed that acid phosphatase activity was increased in the liver of dimethoate treated toads, and this elevation was more clear as the time of treatment increased.

The present results are in agreement with those of Bryc (1972) and Niepolomski, et al. (1972), who noticed that, liver cells of rats displayed a higher activity of acid phosphatase under thioridazine intoxication and emphetamine treatment. Similar observations were also reported by Cavallero, et al.

(1972), and Banhawy and El-Ganzuri (1980 b). The decrease in the activity of alkaline phosphatase, probably indicates a disturbance in the metabolic processes of the liver. This is in accordance with the findings of Batzinger and Bueding, (1977) and Bideand Boward (1970). Mukhopadhyay and Dehadrai (1980) reported that intestinal alkaline phosphatase activity was markedly altered by nutritional status.

Sastry and Malik, (1979) observed that dinecron increased the acid phosphatase but decreased the alkaline phosphatase activities in the intestinal mucosa of the fresh water Channa punctatus. Also Sharma and Sastry (1980) stated that the activity of the two phosphatases was inhibited in the liver cells but some what elevated in the kidney cell post-endrin exposed.

The above findings confirm the study which showed a marked depletion in the activity of alkaline phosphatase in liver of two days treated toads, then enzyme activity displayed a considerable recovery in the liver of seven days treated animals. Boseila et al. (1987) also noticed variable decreases in alkaline phosphatase activity in the kidney of rat affected by niridazole (ambihar).

Succinic dehyrogenase enzyme, plays an important role in the respiratory processes of most living cells (Novikoff and Essner 1960).

Succinic dehyrogenase activity was noticed to be

definitely increased in both liver of toads treated for two and seven days respectively . The enzymatic increased activity extended in all hepatic tissue components. This indicates that dimethoate has a definite lesion action on the cellular mitochondrial membranes which are responsible for the increased succinic dehyrogenase activity. This result is in agreement with Johnson and Daniels (1979). Similar observations were repoted by Boseila et al (1987) on the colonic mucosa of human infected with carcinoma.

Organophosphorous insecticides, apart from inhibiting acetyl cholinesterase activity , cause disturbances of other metabolic processes (Heidker and Pardini 1972). It seems that these insecticides are capable of binding the lipid components of mitochindrial membranes and consequently may alter mitochondrial functions (Sitiewicz and Zalewska, 1975).

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Fig. 1. Normal liver section .Acid phosphatase activity in hepatic cells (granular staining). X 100.

Fig.2. Two days treated liver. High activity of enzyme in different hepatic areas. Densely stained cytoplasm (cytosol).X 160.

Fig. 3. High magnification of the same above figure. X 250.

Fig. 4. High activity of acid phosphatase surrounding blood vessels X 160.

Fig. 5. 7 days treated liver. High activity of enzyme. Endothelial of blood vessels are positivly stained. X 60.

Fig. 6 . Normal activity of alkaline phosphatase. Endothelial of blood vessel positively stained . X 100.

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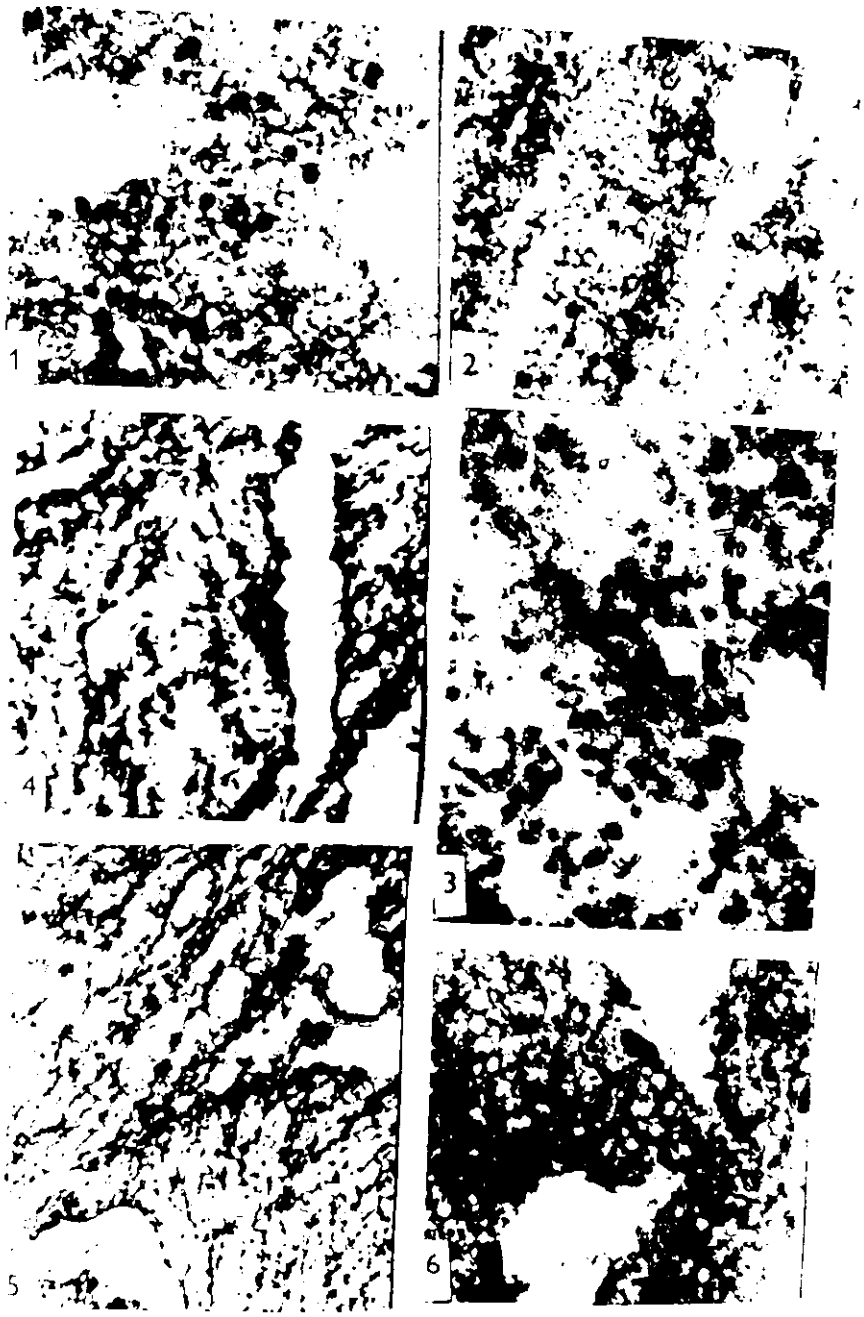


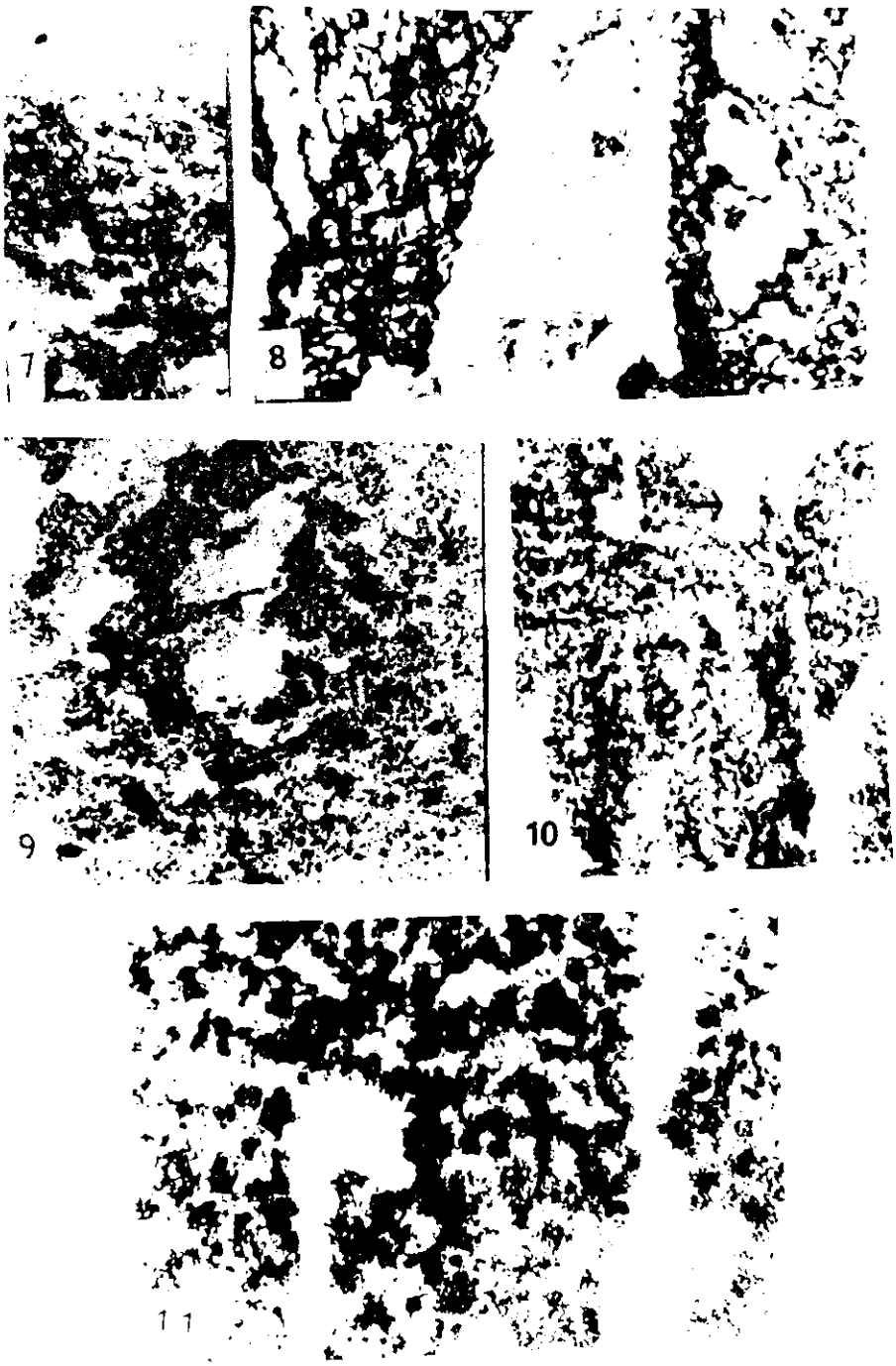
Fig. 7. Two days treated liver. Activity of alkaline phosphatase are highly decreased. X 60.

Fig. 8. Liver section of 7 days treated toad. high activity of enzyme in the sinusoidal borders of hepatocytes. X 60.

Fig. 9. Liver section of normal toad, moderate activity of succinic dehydrogenase which appears fine granules in cytoplasm of hepatocytes. X 250.

Fig.10. Two days treated liver showing increas in activity throughout whole cytoplasm. Bile duct is weakly stained (arrow). X 60.

Fig. 11.High magnification of the above figure.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Figs 12, 13. Liver section of 7 days treated toads. High activity of enzyme. Cells of bile duct are strongly stained. Sites of enzyme activity appeared as fine granules and densely masses. X 250,100 respectivily

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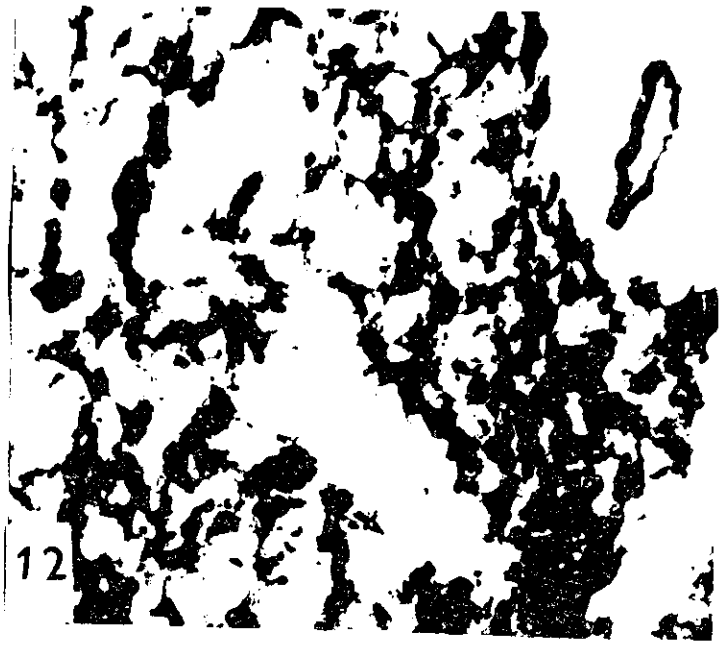

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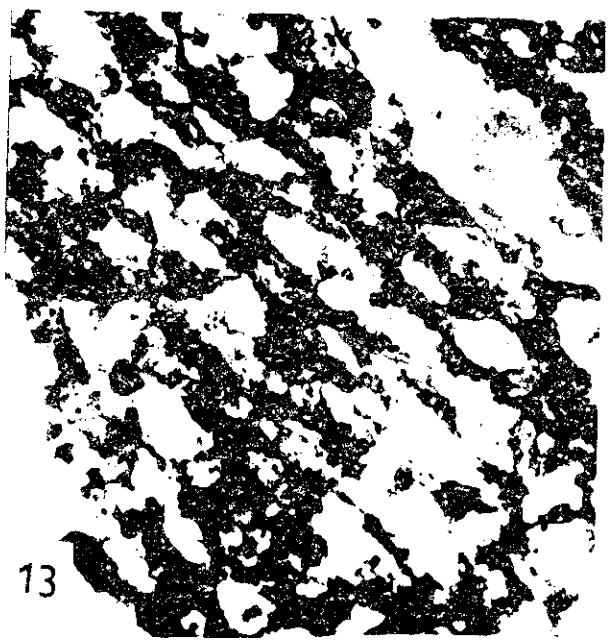

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تأثير المبيد الحشرى (د يموثويت) على الانشطة الانزيمية فى كبد الضفدعة (بوفورجيولارس)

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يتضمن هذا البحث دراسه تأثير المبيد الحشرى الفسفورى د يموثويت على نشاط كل من أنزيم الفوسفاتيز الحمضى والقاعدى وكذلك أنزيم التنفس نازع الهيدروجين السكسينى فى كبد الضفادع بوفورجيولارس وذلك بعد ٤٨ ساعة وأسبوع من المعاملة ٠

وقد تبين من هذا البحث أن كل من أنزيم الفوسفاتيز الحمضى وأنزيم التنفس نازع الهيدروجين السكسينى قد زاد معدل نشاطهم بشكل واضح فى كبد الضفادع المعامله لمده ٤٨ ساعه بالمبيد وكذلك فى تلك المعامله لمده أسبوع ٠ بينما نقص معدل نشاط أنزيم الفوسفاتيز القاعدى بشكل ملحوظ بعد ٤٨ ساعه عن معدله الطبيعى أما فى الحيوانات المعامله لمده أسبوع فقد بدأ يزداد معدل نشاط هذا الانزيم ٠

وعلى هذا فقد أتضح من هذه الدراسه أن المبيد المستعمل قد أثر بصورة ملحوظه على الاغشيه السيتوبلازميه فى خلايا الكبد ٠ فقد تسبب سميه هذا المبيد فى انتفاخ اليسوسومات مما أدى الى تمزق أغشيتها وخروج محتوياتها الهاضمه ٠ وبزياده فترة المعامله يحدث تحلل واضح فى النسيج الكبدى وكذلك أثر المبيد على أنزيمات التنفس الموجوده فى الميتوكوندريا فى خلايا الكبد مما أدى الى زيادتها بصررة ملحوظة ٠