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A biochemical, histochemical, and ultrastructural evaluation of the effect of dimethoate intoxication on rat pancreas

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Abstract

Organophosphorus compounds are widely used in industry, agriculture and for public health purposes. They are among the toxic compounds employed for insect control. The purpose of this work was to study biochemical, histochemical, and histological as well as ultrastructural changes that might occur in the pancreas of adult male Wistar rats as a result of chronic dimethoate intoxication. The treated group received dimethoate orally via gavage (21 mg/kg) daily for 2 months while, the control group was given saline orally (0.1 ml/100 g/day) for the same period. Plasma glucose level was significantly increased while, plasma insulin level was decreased in the intoxicated animals compared with the control group. A patchy reduction of histochemically-detected succinic dehydrogenase enzymatic activity was observed in the pancreas of the intoxicated rats. By contrast, acid phosphatase enzymatic activity was markedly increased in the pancreas of the intoxicated group. No changes were observed in alkaline phosphatase or α esterase activities of the intoxicated animals. Light microscopic examination revealed that dimethoate caused patchy degenerative changes of variable severity in many areas of the pancreas affecting both the pancreatic acini and islets of Langerhans. Ultrastructurally, some β cells revealed dense nuclei with wide perinuclear cisternae. Diminution of the number of β granules was evident. One month after discontinuation of the dimethoate, all the above mentioned changes induced by dimethoate intoxication persisted. These findings show that chronic exposure to dimethoate insecticide has clear toxic effect on the rat pancreas, which was not reversible within 1 month. Public health education is necessary to raise people awareness about the hazards accompanying the use of such compounds.

Keywords: Organophosphorus compounds; Dimethoate; Pancreas; Islets of langerhans; Glucose; Insulin; Acid phosphatase; Alkaline phosphatase; Succinic dehydrogenase; Rats

1. Introduction

Nowadays, the overall use pattern of pesticides has changed considerably compared with the past. The hazards of using such chemical compounds have been accentuated by the sharp rise in their

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use in agriculture and industry and by householders and governments.

Exposure to organophosphorus pesticides in agriculture is one of the occupational hazards. Organophosphorus insecticides include derivatives of phosphoric acid (e.g. dichlorovos), phosphorothioic acid (e.g. parathion-methyl, pirimiphos-methyl and profenofos) and phosphonic acid (e.g. trichlorfon), while other organophosphorus insecticides are phosphoroamidothioates, for example acephate (Lancer). Organophosphorus insecticides represent one group of pesticides that is widely used and has been shown to have toxic effects in man (Tsatsakis et al., 1998; Betrosian et al., 1995; De-Bleecker et al., 1993). Some organophosphorus compounds have pesticidal activity other than against insects; an example is pyrazophos, a phosphorothioate, which is a fungicide.

Toxicity of organophosphorus pesticides results in effects on many organs (Betrosian et al., 1995), particularly the nervous system (Desi et al., 1998; Nagymajtenyi et al., 1998). These studies have shown that organophosphate intoxication caused abnormal EEGs and CNS changes characterized by higher excitation state of the CNS and in some cases polyneuropathy.

Other systems that could be affected by organophosphorus intoxication include liver (Gomes et al., 1999), immune system (Aly and El-Gend, 2000), kidney (Kossmann et al., 1997) and reproductive system (Rawlings et al., 1998). In addition, behavioral effects (Bazylewicz-Walczak et al., 1999) and psychological dysfunction (Kedzierski, 1990) have been detected following chronic exposure to organophosphorus pesticides.

Dimethoate (*O*,*O*-dimethyl s-*N*-methyl carbamoyl methyl phosphodithioate) is one of the most important organophosphorus insecticides and it is frequently used in agriculture. Dimethoate poisoning is usually associated with neuromuscular transmission block in both animals and humans (De-Bleecker et al., 1993; Dongren et al., 1999). Immunotoxicological effect due to dimethoate have also been reported (Institoris et al., 1999). Moreover, dimethoate increased the frequency of chromosomal aberrations and sister chromatid exchanges in peripheral lymphocytes from male pesticides applicators (Rupa et al., 1991). Genotoxic effects of dimethoate were also reported in mice (Hoda and Sinha, 1993). No fetotoxic effects were observed at doses up to 30 mg/kg of dimethoate (Srivastava and Raizada, 1996).

Although, a large number of studies have been reported on detrimental and deleterious effects of organophosphate insecticides, their damaging effect on the endocrine functions of the pancreas has not attracted the interest of researchers. There has been only very few clinical reports dealing with dimethoate—induced pancreatitis (Marsh et al., 1988). Therefore, this work was conducted to throw light on the possible biochemical, histochemical, histological and ultrastructural changes in islets of Langerhans of the rat pancreas as a result of chronic intoxication with dimethoate.

2. Materials and methods

2.1. Animals and treatment

Fifty adult male wistar rats from the animal house of Pharmacology Department, College of Medicine, King Saud University, weighing (220–270 g) were used in the present study. All animals were housed under standard laboratory conditions and allowed free access to normal laboratory rat chow and water ad libitum. Protocols were approved by the Institutional Animal Care and Use Committee of the University. Animals were divided into three groups. The first group of animals ($n = 20$) received dimethoate dissolved in saline (Bayer Company, West Haven, Connecticut) orally via gavage in a dose of 21 mg/kg (1/10 of the LD50; Hayes, 1975) daily for 2 months. The second group ($n = 20$) received dimethoate as in the previous group for a period of 2 months and was then left for another 1 month after dimethoate discontinuation. Animals in the third group ($n = 10$) were given saline orally in a dose of (0.1 ml/100 g/day) for 2 months and were used as the control group. At the end of the specified treatment (2 h after the last dose of dimethoate), blood samples were taken from the animals by a retrobulbar technique and serum was separated and kept at -20 °C for subsequent biochemical

studies. The animals were then killed by cervical dislocation and specimens were taken from the pancreas for histochemical and histopathological studies.

2.2. Biochemical assays

Serum glucose level was measured according to Trinder (1964) while serum insulin level was assayed using radioimmunoassay as described by Herbert et al. (1965).

2.3. Histochemical studies

Succinic dehydrogenase enzyme was estimated according to the technique of Nachlas et al. (1957). Alkaline and acid phosphatase enzymes were determined using Malaty's modified simultaneous coupling azo dye method (Malaty, 1971). α esterase enzyme was evaluated using coupling azo dye method (Malaty, 1969).

2.4. Histopathological studies

Blocks were prepared from pieces of the pancreas and sections were cut and stained using modified Gomori's aldehyde fuchsin trichrome stain (Gomori, 1950) to demonstrate the various cell types in the islets of Langerhans. Sections were examined under light microscope. For electron microscopy, specimens were treated according to the method of Glauret and Glauret (1958). The specimens were cut into small pieces 1–2 mm^3 in size and were immediately fixed in 3% glutraldehyde pH 7.4 at 4 °C for 2 h. Examination of sections was done by the transmission electron microscope (JEOL 100 CX) at the Electron Microscopic Unit, Faculty of Medicine, King Khalid Hospital, King Saud University.

Table 1
The effect of chronic oral administration of dimethoate (21 mg/kg) daily for 2 months (dimethoate group) and 1 month after dimethoate discontinuation (post-intoxicated group) on serum glucose and insulin levels in adult male Wistar rats

Group	Serum glucose level (mg/dl)	Insulin level (μU/ml)
Control	90.47 ± 6.50	33.38 ± 1.31
Dimethoate	115.1 ± 7.85*	23.63 ± 0.98*
Post-intoxicated	110.31 ± 10.51*	24.21 ± 0.95*

Data are expressed as the mean ± S.E.M. *, $P < 0.05$ compared with the control group.

2.5. Statistical analysis

All results were expressed as mean ± S.E.M. and analyzed using Student's t-test. The significance level was accepted at $P < 0.05$ (Xar, 1984).

3. Results

3.1. Biochemical findings

The administration of dimethoate for 2 months produced a significant increase in serum glucose level while serum insulin level was significantly decreased as compared with the control group (Table 1). One month after dimethoate discontinuation, there was still a significant ($P < 0.05$) elevation in serum glucose level and a decrease in serum insulin level in comparison with the control group (Table 1).

3.2. Histochemical findings

In comparison with the control group (Fig. 1A), pancreatic tissue of the intoxicated group showed a reduction in the succinic dehydrogenase activity while, negative reaction was seen in the severely affected cells (Fig. 1B). By contrast, acid phosphatase enzymatic activity was increased. An intense reaction was observed in the islets of Langerhans while, a moderate one was detected in the pancreatic acini (Fig. 2B) compared with the control group (Fig. 2A). Pancreatic tissue of the intoxicated rats showed more or less the same distribution of the enzymatic activities of alkaline phosphatase (Fig. 3B) and α esterase (Fig. 4B) as that of the control groups (Fig. 3A, Fig. 4A), respectively.

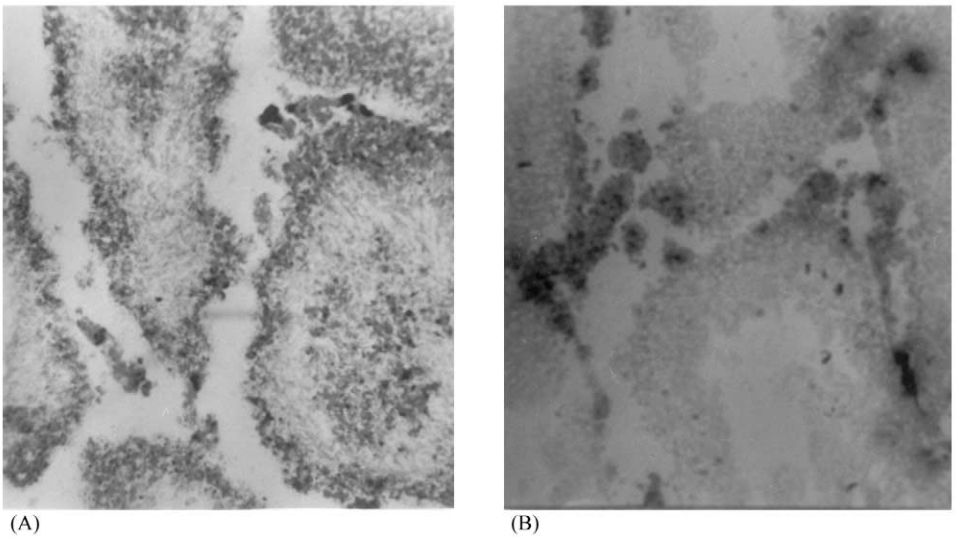

Fig. 1. (A) Normal control rat pancreas, showing the distribution of succinic dehydrogenase enzyme. The acini show intense reaction while islets of Langerhans show moderate granular reaction (× 100). (B) Intoxicated rat pancreas, showing a patchy reduction in the succinic dehydrogenase enzymatic activity in the degenerated acini and islets of Langerhans (× 100).

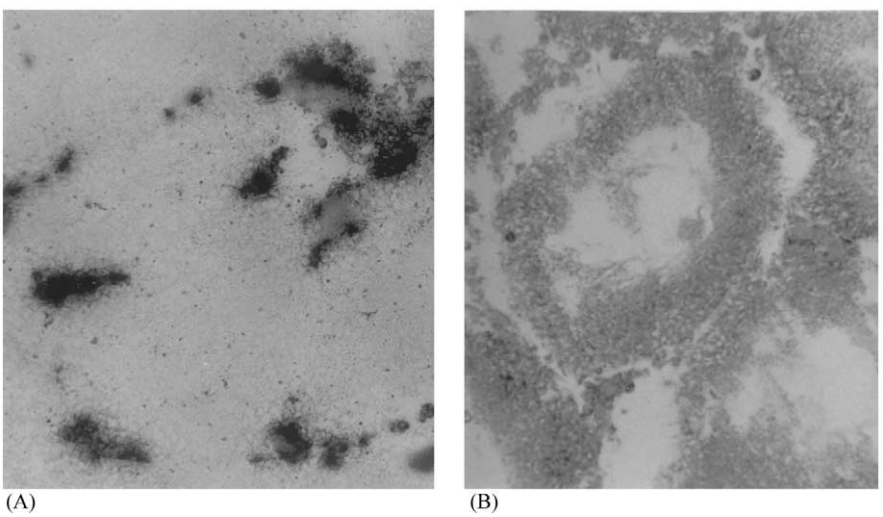

Fig. 2. (A) Normal control rat pancreas, showing the normal distribution of acid phosphatase enzyme. The islet cells show strong reaction while, a moderate reaction is seen in the acini (× 100). (B) Intoxicated rat pancreas, showing a definite increase in the acid phosphatase activity in both the cells of islets and acini (× 100).

3.3. Histological and electron microscopic studies

3.3.1. Light microscopic examination

The administration of dimethoate for 2 months resulted in patchy degenerative changes of variable degrees among many areas of the pancreas affecting both the pancreatic acini and the islets of Langerhans. These degenerative changes ranged from cloudy swelling with granulation of the cytoplasm to vacuolation or even hydropic degenera-

tion. The degenerated cells of pancreatic acini appeared ballooned with vacuolated cytoplasm and eccentric pyknotic nuclei. Large foci of cellular infiltration especially perivasuclar sometimes were observed in relation to the severely affected acini (Fig. 5B) as compared with the normal

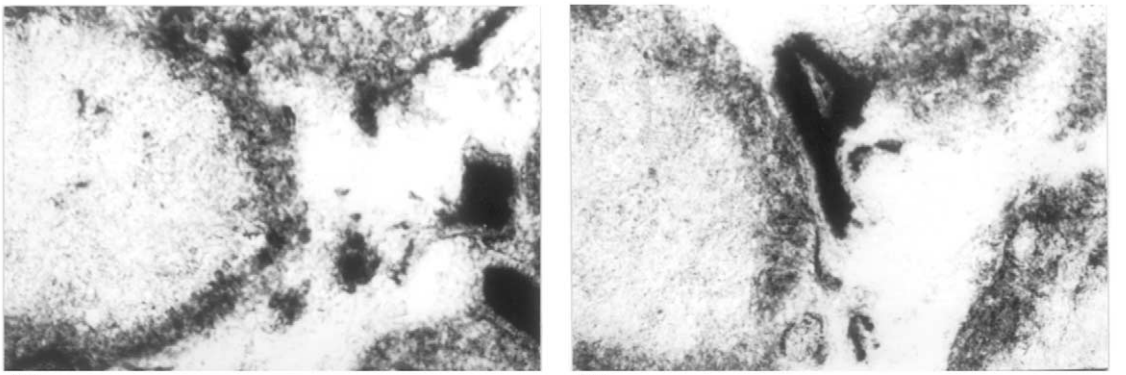

Fig. 3. (A) Normal control rat pancreas, showing the distribution of alkaline phosphatase enzyme. An intense reaction is seen in blood vessels between the lobules. A moderate reactions is seen in the blood capillaries between acini and in islets. Acinar and endocrine cells show negative reaction (× 100). (B) Intoxicated rat pancreas, showing more or less the same alkaline phosphatase enzymatic activity as that of the control group (× 100).

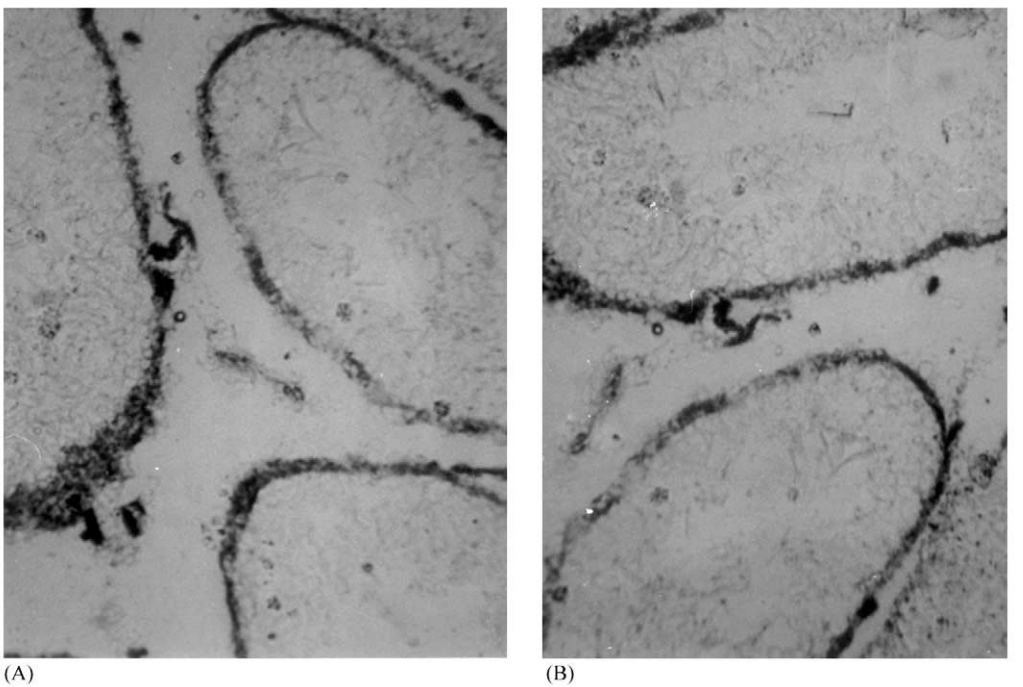

Fig. 4. (A) Normal control rat pancreas, showing the distribution of α esterase. A moderate reaction is seen in the acini, while, the islets of Langerhans show negative reaction (× 100). (B) Intoxicated rat pancreas, showing more or less the same α esterase activity as that of the control group (× 100).

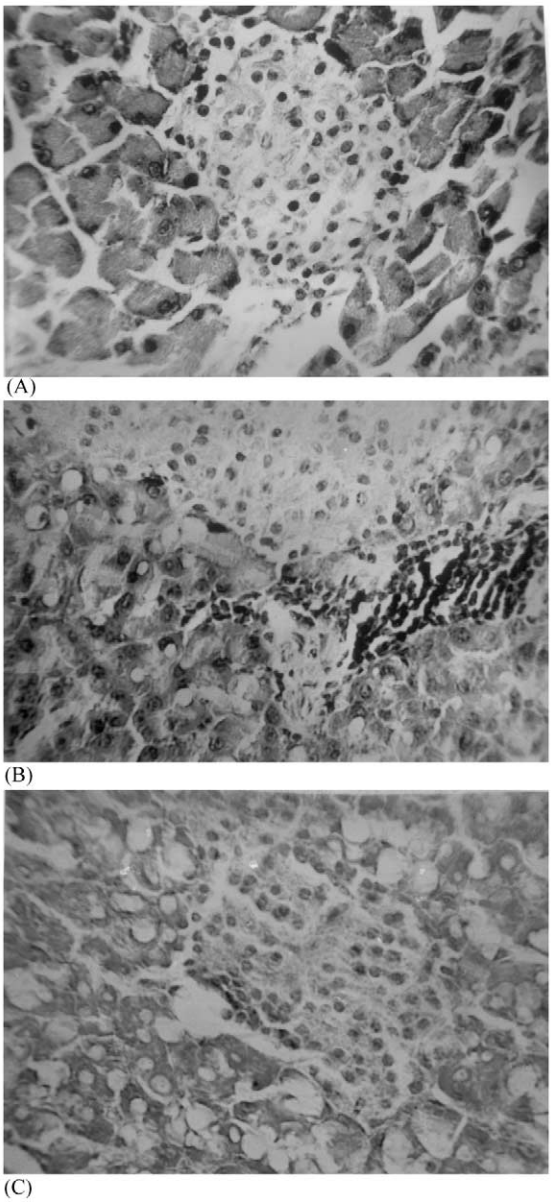

Fig. 5. (A) Normal control rat pancreas, H and E stain (× 400). (B) Intoxicated rat pancreas, showing details of degenerated acini with ballooning of the cells and eccentration of the nuclei. Islet cells show cloudy swelling with granulated cytoplasm. H and E (× 400). (C) Intoxicated rat pancreas, 1 month after dimethoate withdrawal, showing islets of Langerhans with scattered degenerated cells varied from cloudy swelling with granular cytoplasm to hydropic degeneration with vacuolated cytoplasm. Perivascular cellular infiltration is present H and E (× 400).

pancreas (Fig. 5A). These changes persisted even after dimethoate discontinuation (Fig. 5C).

3.3.2. Electron microscopic examination

In normal control rat pancreas (Fig. 6A), the β cells appeared with their secretory granules (sg), and a large amount of mitochondria (m) with lamellar cristae and electron dense matrix. Fig. 6B shows the ultrastructural changes after dimethoate administration for 2 months. The β-cells were the most affected cells among the injured islets as compared with the control group (Fig. 6A). Most of the β-cells showed a marked diminution in the number of their granules with the appearance of multiple empty vacuoles and dilatation of the rough endoplasmic reticulum. The α-cells were generally less affected than the β-cells.

4. Discussion

During the last decades, the extensive use of different pesticides in agriculture and for public health purposes, has led to drastic effects especially in animals and human (Pesticides residues in foods, 1996). Most of these chemicals are not highly selective but generally they proved to be toxic to many non-target species including man and other desirable forms of life that coinhabit the environment, therefore, their improper application may result in serious illness and even death.

Dimethoate, the insecticide used in this study, is a widely used organophosphate compound which has a significant contact and systemic action against a wide variety of insects and pests of both plants and animals (Westcott et al., 1987).

Organophosphorus pesticides were claimed to have a harmful effect on the endocrinal and biochemical functions of the pancreas (Hsiao et al., 1996; Sikk et al., 1985). In the present study, chronic administration of dimethoate for 2 months resulted in a significant decrease in plasma insulin level and a pronounced increase in blood glucose level. These effects persisted even after discontinuation of the insecticide. These biochemical changes were accompanied by histologi-

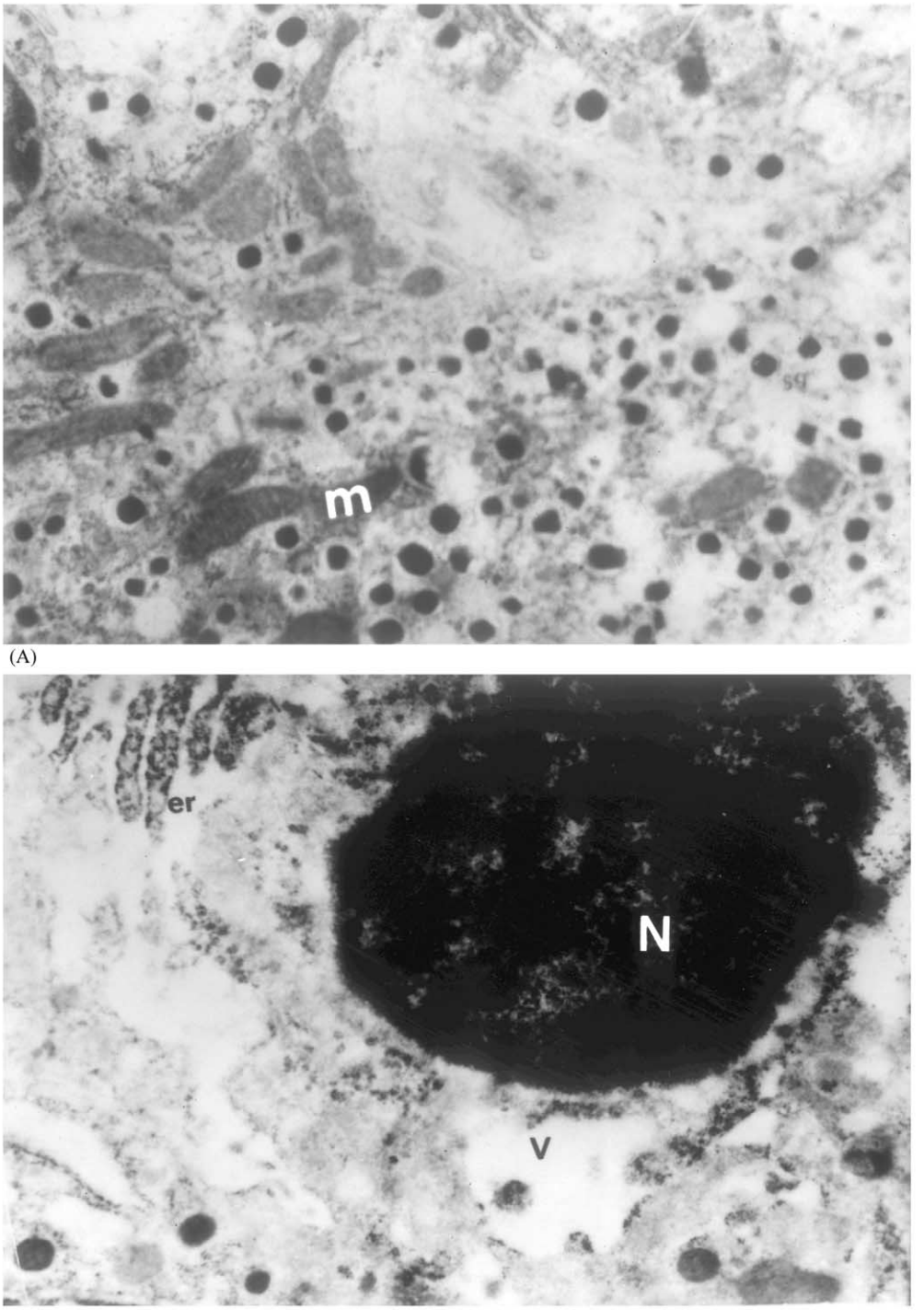

Fig. 6. (A) Electron photomicrograph of (A). Normal control rat pancreas, showing β cells with their sg, large amount of mitochondria (m) with lamellar cristae and electron dense matrix (× 10 000). (B) Intoxicated rat pancreas, showing β cells with dense pyknotic nucleus (N). The cytoplasm shows degranulation dilated endoplasmic reticulum (er) and multiple vacuoles (V) (× 10 000).

cal and histochemical changes with destruction of the β-cells that could account for the observed elevation in blood glucose level after dimethoate intoxication. Similar results have been obtained for other organophosphorus compounds (Fletcher et al., 1988).

Bhatia et al. (1972), Weiss et al. (1984) reported a pronounced increase in blood sugar level which was going parallel to the inhibition of the cholinesterase and the appearance of manifestation of cholinergic stimulation as a result of parathion intoxication. Berberian (1988)reported hyperglycemia after chronic intoxication by another organophosphate compound, malathion.

Chronic dimethoate administration for 2 months led to degenerative changes of variable degrees of the pancreatic islets as well as the exocrine acini. The degeneration of the acini might be considered as a sort of toxic inflammatory process simulating pancreatitis. Our results are in agreement with Marsh et al. (1988) who reported acute pancreatitis after accidental cutaneous exposure to dimethoate. Other reports suggested that acute pancreatitis may follow the oral ingestion of several organophosphates including mevinphos, malathion, parathion and diazinon (Hsiao et al., 1996; Moore and James, 1989; Dressel et al., 1989)

In the present study, ultrastructural study confirmed the degranulation of β-cells and α-cells with marked dilatation of endoplasmic reticulum. These changes may be due to immunological activation that resulted from dimethoate intoxication. This is confirmed in our study by the presence of inflammatory cells in relation to the affected islets as well as the appearance of large foci of cellular infiltration in the pancreatic tissue. Another explanation for the observed ultrastructural changes is provided by Wilson and Gaines (1989) who found that toxicity of organophosphorus insecticides produced damage to DNA of the cultured rat β-cells which in turn might increase the activity of a poly (ADP-ribose) polymerase involved in DNA excision repair. This enzyme uses nicotinamide adenine dinucleotide (NAD) as its substrate. The over activation of this enzyme may lead to reduction in cellular NAD and and subsequent destruction of the cell (Carson et al., 1986).

In this study, some enzymatic activities of the pancreas were evaluated after intoxication of dimethoate. The succinic dehydrogenase enzyme activity of the acini and islet of Langerhans was decreased in the degenerated cells. The induced decrease of the succinic dehydrogenase activity can be attributed to the ability of dimethoate to inhibit mitochondrial enzymatic activities (Moussa and Hafez, 1995). Curiously, our results on succinate dehydrogenase are similar to those observed by Anastasi and Bannister (1990) who demonstrated inhibition of this enzyme in fish muscles after chronic intoxication with permethrin, which is a synthetic pyrethroid and thus an insecticide with an entirely different action from organophosphorus insecticides.

Dimethoate intoxication led to a definite increase in the acid phosphatase activity in both the cells of islets and acini. Orci et al. (1995) showed that most of the acid phosphatase activity of the islets of Langerhans was concentrated in the β-cells which showed a very strong reaction. Gohlke and Grigorowa (1992) recorded an increase in the acid phosphatase activity in several cell types of rats under dimethoate and parathion-methyl intoxication. In addition, an increased acid phosphatase activity in fish tissues occurred also under the influence of other insecticide, monocrotophos (Joshi and Desal, 1994). The increase in acid phosphatase enzyme was most probably due to disintegration of cells resulting from intoxication with dimethoate (Barger et al., 1993).

In the present work, no change in the α esterase enzyme activity was noted in the cells of islets of Langerhans or in the acini of animals receiving dimethoate. Non-specific esterase enzyme is concerned with metabolism and is probably not involved in the processes of insulin synthesis or release or that of other hormones (Malaty, 1969). Moreover, dimethoate administration had no effect on the activity of alkaline phosphatase in the rat pancreas. Similar results have been obtained by prolonged exposure to a mixture of organophosphorus pesticides (Gomes et al., 1999).

The persistent alteration in the biochemical parameters and histopathological changes in the pancreas after discontinuation of dimethoate indicates a long lasting damaging effect. As the dam-

aging effect of dimethoate is irreversible, great precautions must be taken in order to minimize the harmful side effects of those organophosphorus compounds on all the surroundings especially man to decrease the incidence of environmental pollution.

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