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T-2 TOXIN-INDUCED PATHOLOGY IN THE HEARTS OF RATS

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Summary.—T-2 toxin is the major lethal component of several *Fusarium* fungi implicated in diseases of man and animals. We report here on the histological and ultrastructural changes in hearts of rats given i.p. single large or repeated small doses of T-2 toxin.

One, 2 and 3 days after a single large dose, there were lesions in most hearts. They consisted of interstitial oedema, focal cellularity and damage to single or groups of myocytes. The small intramural coronaries were constricted, swollen and sometimes disrupted. After 7 days, most of the changes subsided.

In rats killed 1 or 2 months after the last of 10 daily injections of T-2 toxin, cardiomyopathy-like changes were seen with hypertrophy, focal fibrosis and abundant cellularity especially in the subendocardial regions of the left ventricle.

The findings, although non-specific, indicate that T-2 toxin is cardiotoxic.

T-2 toxin (3α-hydroxy-,4β,15-diacetoxy-8α-(3-methyl butyryloxy)-12,13-epoxytrichothec-9-en) is a trichothecene which was shown to be the main toxic component of several strains of *Fusarium* fungi (Lutsky *et al.*, 1978; Ciegler, 1978; Joffe, 1971). These fungi are responsible for severe, acute and chronic disease in men and domestic animals (Joffe, 1971; Hsu *et al.*, 1972; Petrie, Robb and Stewart, 1977).

Experimentally, administration of the crude extracts of the *Fusarium* fungi or T-2 toxin to various animals reproduced the signs and symptoms of the clinical disease (Lutsky *et al.*, 1978; Rukmini, Prasad and Rao, 1980). This consists of a multisystem syndrome which in the acute form is characterized by haemorrhages, sepsis and cardiopulmonary failure. In the more chronic forms of exposure to the drug cardiovascular and immunological systems seem to be predominant targets of toxicity (Lutsky *et al.*, 1978; Ciegler, 1978; Joffe, 1971; Hsu *et al.*, 1972; Petrie *et al.*, 1977; Rukmini *et al.*, 1980).

The subject of fungal toxins and especially that of T-2 toxin has recently aroused interest, as accumulating evidence points towards their use in biological warfare as a component of "yellow rain" of Southeast Asia and elsewhere (Robinson, 1982; Holden, 1982; Mirocha, Watson and Hayes, 1982).

Cardiovascular changes produced by *Fusarium* fungi or T-2 toxin-infected feeds have been observed by several authors (Kurmanov, 1978; Schoental, Joffe and Yagen, 1979; Wilson, Everard and Schoental, 1982). It has even been suggested that toxic fungi may be involved in the aetiology of some forms of human cardiomyopathy (Schoental, 1980). The mechanism of the T-2 toxin cardiotoxicity has not been elucidated, nor has the ultrastructural cardiac pathology been studied *in vivo*.

Our previous work dealt with the effect of T-2 toxin on isolated hearts of rats (Yarom *et al.*, 1983). We found a dose-related decrease of contractility, ECG changes and ultrastructural damage to myocytes, possibly originating in the plasma membrane.

This report deals with histological and

ultrastructural pathology in myocardia of rats injected with single or repeated doses of T-2 toxin.

MATERIALS AND METHODS

Male rats aged 2–3 months of the Hebrew University "Sabra" strain and weighing 200–300 g were injected i.p. with various doses of T-2 toxin. Six rats received a single large dose of 3 mg/kg. Twenty rats received 2 mg/kg. They were killed 6 h, 1, 2, 3 and 7 days later, 4 or 5 at each time period. Four rats received 0·5 mg/kg daily for 5 days. Ten rats received 0·3 mg/kg, daily for 10 days. The animals given repeated doses were killed 1 or 2 months after the last injection. [The LD_{50} for rats is 3 mg/kg (*6*).]

The rats were anaesthesized with ether and perfusion fixations was performed with 0·5% glutaraldehyde in 0·1M cacodylate injected slowly into the left ventricle. After 10 min the heart was removed and further processed. For electron microscopy, samples were taken from posterior papillary muscle, septum and free walls of the right and left ventricles. These samples were further fixed in cacodylate buffered 2% glutaraldehyde, postfixed in osmium tetroxide, dehydrated and embedded in Araldite. Semithin sections were stained with toluidine blue and examined by light microscopy. Ultrathin sections were stained with uranyl and lead; they were viewed with a Philips 400 electron microscope.

The rest of the hearts were fixed in formaline and embedded in paraffin. Multiple sections were stained with haematoxylin and eosin and Van Gieson stains. They were viewed by light microscopy.

RESULTS

Of the 6 rats that received a 3 mg/kg dose of T-2 toxin, 4 died 8—20 h later. Post-mortem examinations revealed haemorrhages in their lungs and other organs. The hearts, in addition to focal haemorrhages, showed marked interstitial oedema and infiltration with mononuclear cells (Fig. 1). Small intramural coronary vessels were often swollen and hypercellular. Rats receiving 2 mg/kg survived. Those killed 6 h after the toxin showed no definite cardiac pathology. Those killed after 1, 2 and 3 days showed swelling of single or groups of myocytes associated with interstitial oedema and extravasation of erythrocytes (Fig. 2). There were foci of interstitial cells, often related to small hypercellular blood vessels (Fig. 3), and sometimes an excessive number of mast cells in the subendocardial and subepicardial regions (Fig. 4).

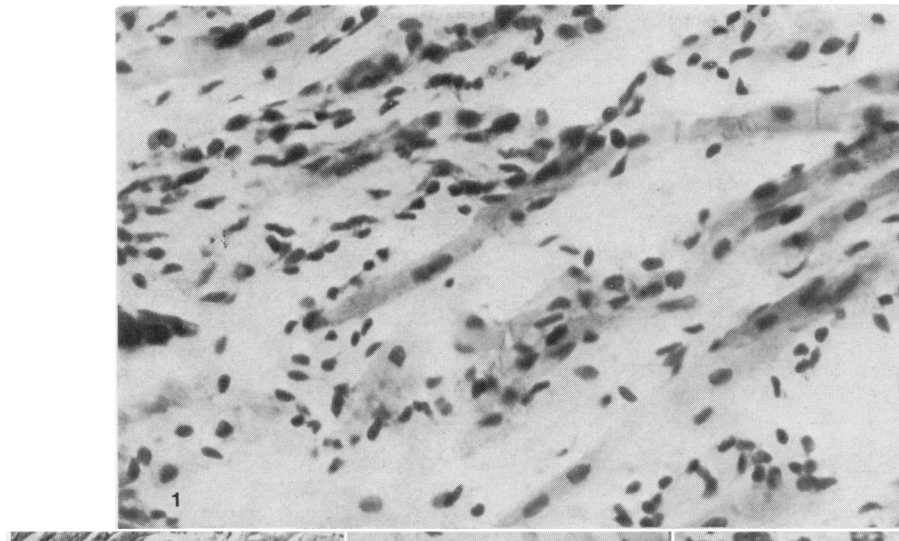
Electronmicroscopically groups of oedematous, disorganized myocytes could be seen (Fig. 5). The small vessels had swollen, pinocytic endothelium (Fig. 6) and sometimes a vacuolated, necrotic appearance (Fig. 7). In the interstitium, the cells were mainly histiocytic but lymphoid and vessel related cells were also frequent.

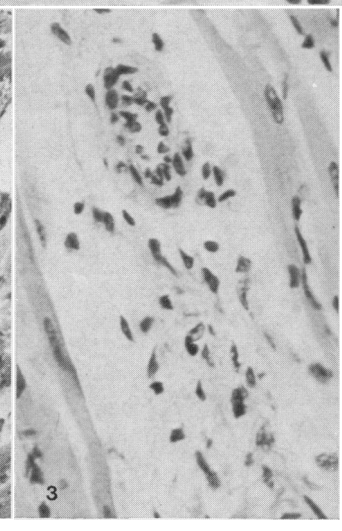
In the rats killed 1 week after a single dose of 2 mg/kg of T-2 toxin occasional foci of interstitial cellularity could still be found but there seemed to be little other histological or ultrastructural pathology.

In the hearts of rats killed 1 or 2 months after a series of small doses of T-2 toxin, the changes varied. In 2 of the rats who received 5 injections and in 3 of the 10 given 10 doses there were large areas of cellular infiltration and patchy fibrosis (Figs 8 and 9). The changes were mainly in the subendocardial region. In addition to lymphocytes and histiocytes the infiltrates consisted of eosinophiles, connective tissue and capillary related cells. In most of the other hearts small foci of cellularity were seen.

Electronmicroscopically there were nonspecific signs of hypertrophy with large or double nuclei, accumulation of mitochondria and hypercontracted myofibrils (Fig. 10). In some myocytes there were degenerative changes with decrease of organelles and focal oedema (Fig. 11). In others, excess of disorganised, intercalated disc material was seen (Fig. 12). No definite sarcolemmal changes were observed.

The small blood vessels were hypercellular, they had focal hyperplasia of smooth muscle cells and increased numbers of pericytes and perivascular cells (Figs 13 and 14).





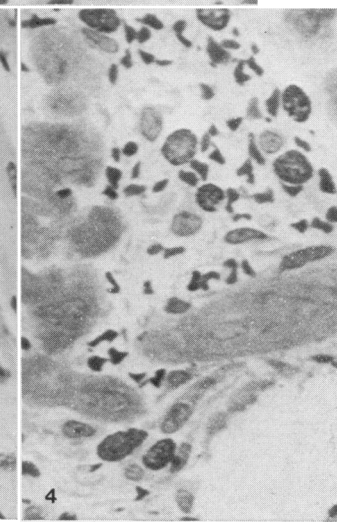


FIG. 1.—Myocardium of rat who died 20 h after 3 mg/kg of T-2 toxin. There is interstitial oedema and mononuclear cell infiltration. Some myofibres are damaged (H. & E. × 150).

FIG. 2.—Myocardium of rat killed 3 days after 2 mg/kg of T-2 toxin. There is a subendocardial focus of oedema and myofibre necrosis (H. & E. × 90).

FIG. 3.—Myocardium of rat killed 1 day after 2 mg/kg of T-2 toxin. The small vessel is contracted and hypercellular. There is perivascular oedema and mononuclear cellularity (H. & E. × 150).

FIG. 4.—Subendocardial region 1 day after 2 mg/kg T-2 toxin with extravasated erythrocytes and numerous mast cells (toluidine blue × 240).

DISCUSSION

It has been reported previously that cardiac pathology is found in most animals given lethal doses of feeds containing *Fusarial* fungi (Lutsky *et al.*, 1978; Rukmini *et al.*, 1980; Schoental *et al.*, 1979; Buja, Ferrans and Maron, 1974). Haemorrhages, myofibre necrosis, phagocytosis and fibrosis have been described, mainly in the posterior papillary muscle and the subendocardial region of the left ventricle. The lesions were more prominent with smaller doses and longer survival of the animals.

In long term experiments with rats given T-2 toxin, chronic inflammatory

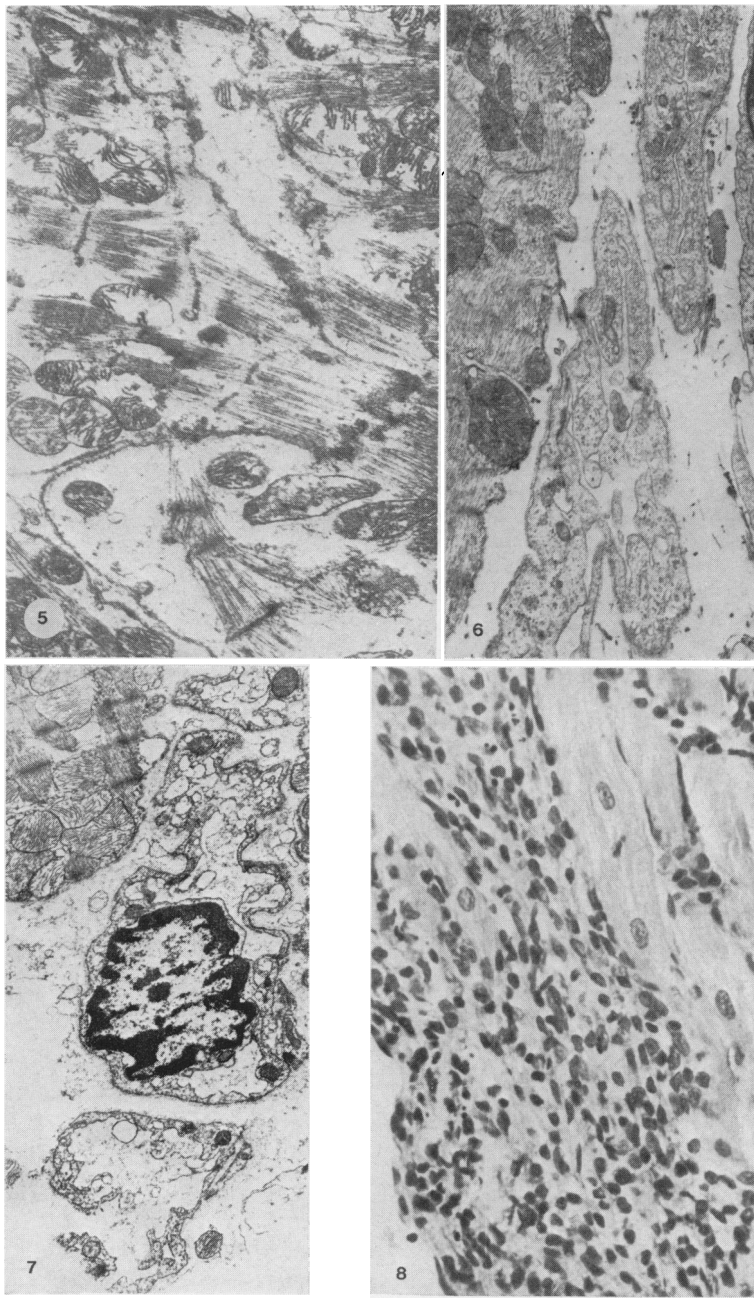
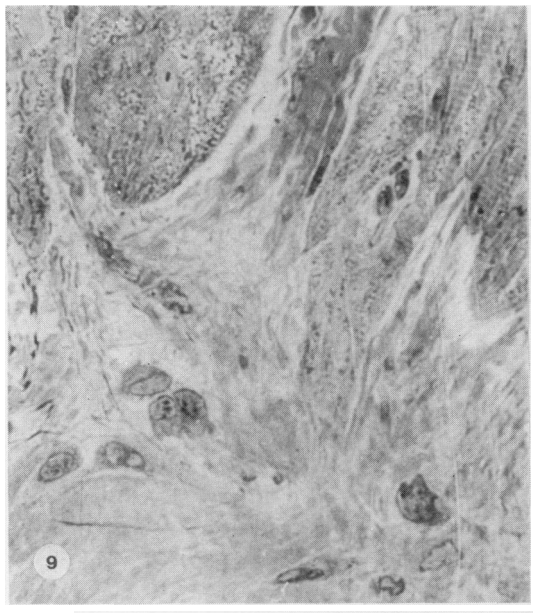


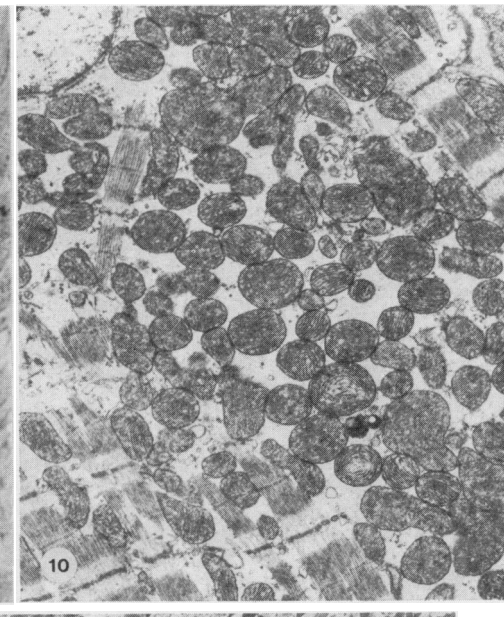
FIG. 5.—Electronmicrograph showing group of oedematous, disorganized myocytes 1 day after T-2 toxin (× 9,300).

FIG. 6.—Electronmicrograph showing contracted small vessels with swollen endotheline cells, 1 day after T-2 toxin (× 5,300).

FIG. 7.—Electronmicrograph 2 days after the toxin showing damaged small blood vessels (× 6,600).

FIG. 8.—Myocardium of rat killed one month after the last of 10 small doses of T-2 toxin. There are extensive subendocardial areas of cellularity (H. & E. × 133).





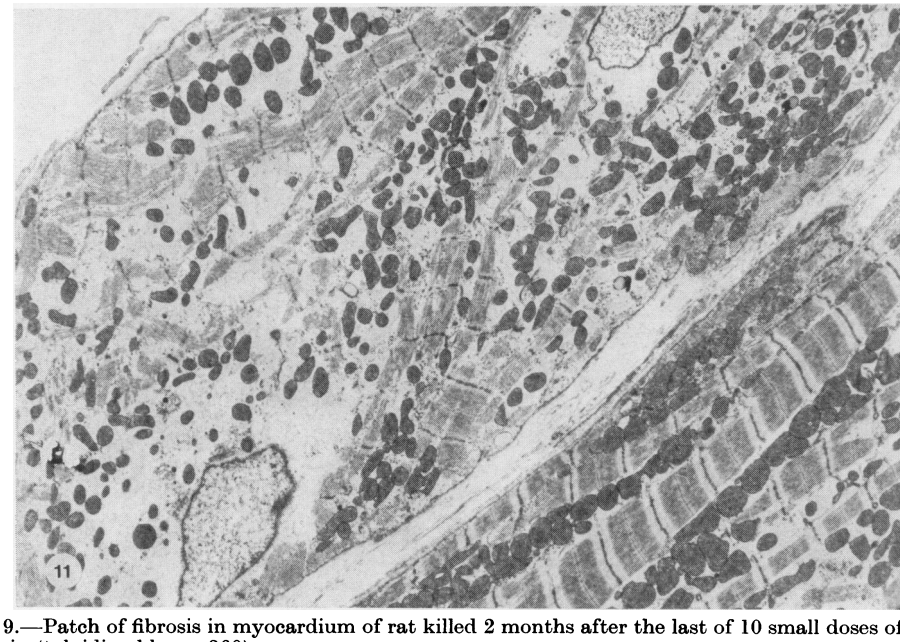
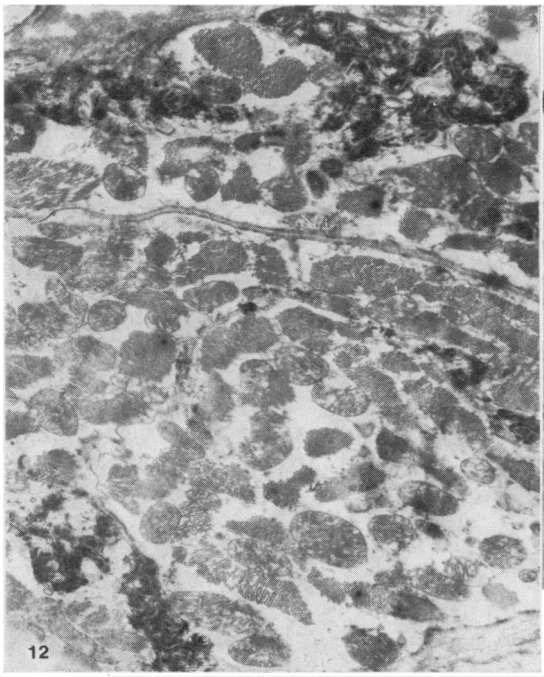
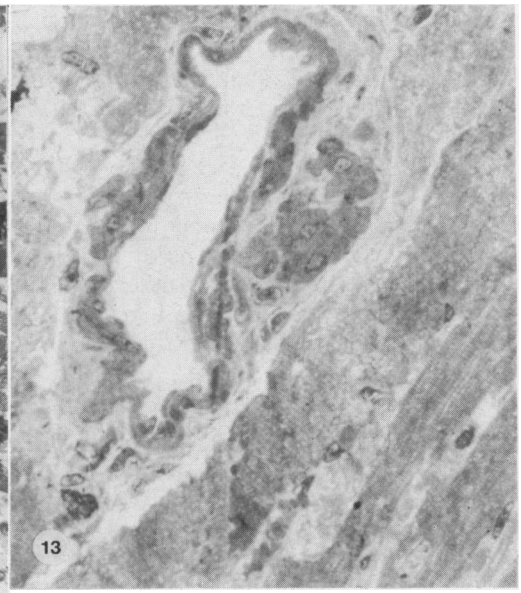


FIG. 9.—Patch of fibrosis in myocardium of rat killed 2 months after the last of 10 small doses of T-2 toxin (toluidine blue × 360).

FIG. 10.—Electron micrograph showing accumulation of mitochondria in myocytes 1 month after repeated doses of T-2 (× 6,800).

FIG. 11.—Electromicrograph showing degenerative changes in a hypertrophied myocyte, 2 months after repeated doses of T-2 (× 4,250).





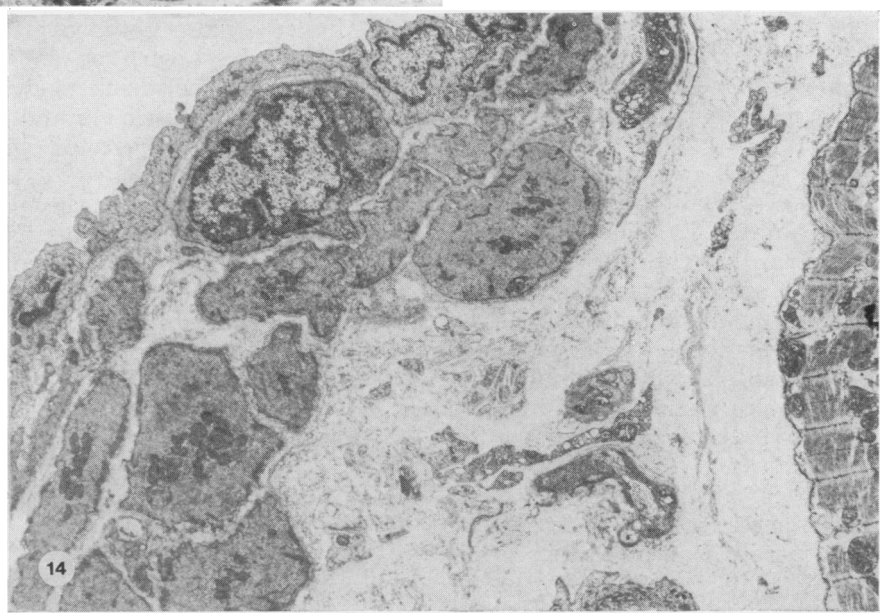


FIG. 12.—Electron micrograph showing accumulation of intercalated disc material in many myocytes 2 months after repeated doses of T-2 (× 6,000).

FIG. 13.—Hypercellular blood vessel with focal hyperplasia of smooth muscle cells (toluidine blue × 270).

FIG. 14.—Electronmicrograph of thickened arteriole with hyperplasia of smooth muscle and perivascular cells (× 4,250).

changes in the cardiovascular system with cellular myocardial fibrosis, thickened blood vessels and frequent mural thrombi were observed (Schoental *et al.*, 1979; Wilson *et al.*, 1982).

Our findings agree with the above reports. In addition, electronmicroscopy shows that intramural blood vessels may be rapidly damaged by the drug. This would explain the early multiple haemorrhages and cellular foci and may contribute towards the focal myofibre damage.

The large number of mast cells, which in rats also contain serotonin, may augment the blood vessel and myocyte damage, be instrumental in attracting eosinophiles and even contribute toward the patchy fibrosis (Ferrans and Roberts, 1976; Yarom, Havivi and Heilig, 1979).

The absence of neutrophilic polymorphs in the early cellular lesions could be due to the selective suppression of these cells known to occur with T-2 intoxication (Stolerman, 1979). Alternatively, the drug may interfere with a chemotactic mechanism for polymorphonuclears.

The acute focal myocardial changes caused by the T-2 toxin are by no means specific. They resemble lesions caused by a number of cardiotoxic drugs especially those used in antidepressant and anticancer therapy (Billingham, 1979). The pathogenic mechanisms of these drugs are varied and often multifactorial. Morphological changes alone cannot indicate the metabolic pathways leading to the damage.

T-2 toxin is an immensely potent substance. It enters most cells freely and affect their metabolism at various levels (Ciegler, 1978). The suppressive effect on cardiac contractility as well as on the conducting system has been demonstrated in isolated heart experiments (Yarom *et al.*, 1983).

In the whole animal, the effects of T-2 toxin on the peripheral vascular system as well as on the brain, lungs and other organs must add to the drugs direct cardiotoxicity and may contribute to fatal acute heart failure.

The changes observed in the chronic experiments are also non-specific (Buja *et al.* 1974). The myofibre and coronary blood vessel changes may be due to myocardial hypertrophy caused by hypertension which is known to occur after prolonged T-2 toxin administration in rats (Wilson *et al.*, 1982).

The pathogenesis of the intense cellularity of the subendocardial regions which bears a resemblance to some forms of cardiomyopathy, especially endomyocardial fibrosis, is speculative at the moment. It is possible that the known suppression of the immune system by the drug (Rukmini *et al.*, 1980; Kriek *et al.*, 1977) opens the animals to viral attack on the heart. It is also possible that the primary injury provokes autoimmune, cell bound responses. Both these theories could be tested in inbred animals.

A fungal toxin aetiology for some cardiomyopathies, such as endomyocardial fibrosis or beer-drinkers cardiomyopathy and even the pellagra and Beri-beri hearts, have been suggested previously (Schoental, 1980). The *in vivo* experiments with T-2 toxin support the possibility of acute and chronic cardiovascular disease occurring in geographical or other circumstances favourable to *Fusarium* growth and point towards the danger of exposure to T-2 toxins.

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