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Concanavalin A Toxicity: Histological Studies[1] (39513)

W. NOPANITAYA,[2] J. HANKER,[3] AND M. TYAN[3,4]

[2]*Department of Pathology, School of Medicine, and* [3]*Dental Research Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514*

Concanavalin A (Con A), a carbohydrate-free metalloprotein that specifically binds to α-D-glucosyl and sterically related residues, produces vascular endothelial damage, hepatic necrosis, lymphoid atrophy, and up to 100% mortality within 48 hr when injected intravenously into mice in doses of 200–800 μg (1). Preliminary ultrastructural studies of livers from mice given Con A iv 24 hr previously revealed an accumulation of lipid droplets in hepatocytes, variable degrees of cell organelle degeneration, and, most striking, giant mitochondria that appeared to be formed as the result of coalescence of two or more adjacent swollen mitochondria (2). In this report we will present (a) a description of the gross, ultrastructural, and histochemical changes found 6 hr after the injection of 600 μg of Con A and (b) the histopathological changes noted over the period of 1 year following the iv injection of 200–600 μg.

Materials and methods. The experimental animals were 12-week-old male B6D2F_1 mice. Groups of mice were given 200, 400, or 600 μg of Con A (Miles Laboratories, Kankakee, Ill.) or saline iv; 6 hr and 1, 2, 7, 14, 28, and 362 days later four experimental animals from each group and four saline-injected controls were killed and autopsied. Selected tissues were fixed in 10% neutral formalin and H & E sections were prepared for study.

In addition, the livers of mice killed 6 hr after the injection of 600 μg of Con A were fixed by perfusion *in situ* with chilled (4°) 1.25% glutaraldehyde in 0.1 *M* phosphate-buffered saline (PBS), pH 7.3; the livers were then removed, cut into thin pieces, and placed in 1.25% glutaraldehyde at 4° for 30 min.

The method of Bernhard and Avrameas (3) was used for the ultrastructural visualization of Con A. Briefly, the tissues were frozen and sectioned at 1 μm. They were washed three times in PBS at 4° and incubated for 30 min at room temperature with type IV horseradish peroxidase (Sigma Chemical Co., St. Louis, Mo.) prepared in isotonic saline at a concentration of 50 μg/ml (horseradish peroxidase is 18% carbohydrate by weight and is bound by Con A already specifically attached to tissue sugar). After three washes in cold PBS, the tissues were fixed in 3% glutaraldehyde for 30 min, and the binding of peroxidase was evaluated by the diaminobenzidine (DAB) method (4). The tissues were postfixed with 1% osmium tetroxide, dehydrated in graded alcohols, and embedded in Epon. Ultrathin sections were examined unstained, or lightly poststained with lead citrate, using a transmission electron microscope, JEOLCO JEM-T7, at 60 kV. Tissues from mice injected with saline were processed in the same manner, and they served as controls.

Results. No deaths occurred among the mice given up to 600 μg of Con A iv, and at no time did they appear ill. At autopsy, no gross abnormalities were noted among the mice given 200 μg of Con A. However, histological studies of tissues taken during the 2 days following injection of Con A revealed occasional small areas of periportal necrosis in the livers and mild atrophy of splenic white and red pulp associated with dilated sinusoids and small subcapsular hemorrhages. In addition, the subpleural alveolar capillaries were mildly congested; the renal glomeruli were moderately congested, and the proximal tubules were slightly vacuolated. No significant microscopic abnormalities were found 7, 14, or 28 days after

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[4] Address correspondence to Marvin L. Tyan, Dental Research Center, University of North Carolina at Chapel Hill, Chapel Hill, North Carolina 27514.

the injection of Con A. The remaining animals in this group survived 1 year at which time gross and microscopic findings were within normal limits and indistinguishable from those of the saline-injected controls.

The gross and histological changes produced by 400 and 600 μg of Con A, while somewhat more severe at the higher dose, were similar and, therefore, they will be described together. All experimental mice appeared well 6 hr after the injection of 400–600 μg of Con A. At autopsy, however, the visceral organs showed marked congestion, and there were subcapsular hemorrhages ranging in size from petechial to 5-mm diameter in the spleen, lungs, liver, and kidneys. There was no evidence on gross inspection of hepatic necrosis at this time.

Ultrastructural examination of livers from mice given saline revealed no abnormalities. In sections treated with peroxidase and DAB, electron-dense material was found in the granules of macrophages and polymorphonuclear cells; in hepatocytes, precipitated material was scattered lightly through the endoplasmic reticulum, and moderately heavy deposits were found along the inner membranes and cristae of mitochondria (Fig. 1). These findings are consistent with the distribution of tissue peroxidases (5).

Examination of hepatocytes from mice given 600 μg of Con A revealed an increase in intracellular lipid droplets, dilation of the endoplasmic reticulum, and mild to marked swelling of mitochondria. The cristae of the enlarged mitochondria were greatly dilated and many were poorly defined. Histochemical studies showed electron-dense material in the granules of macrophages and poly-

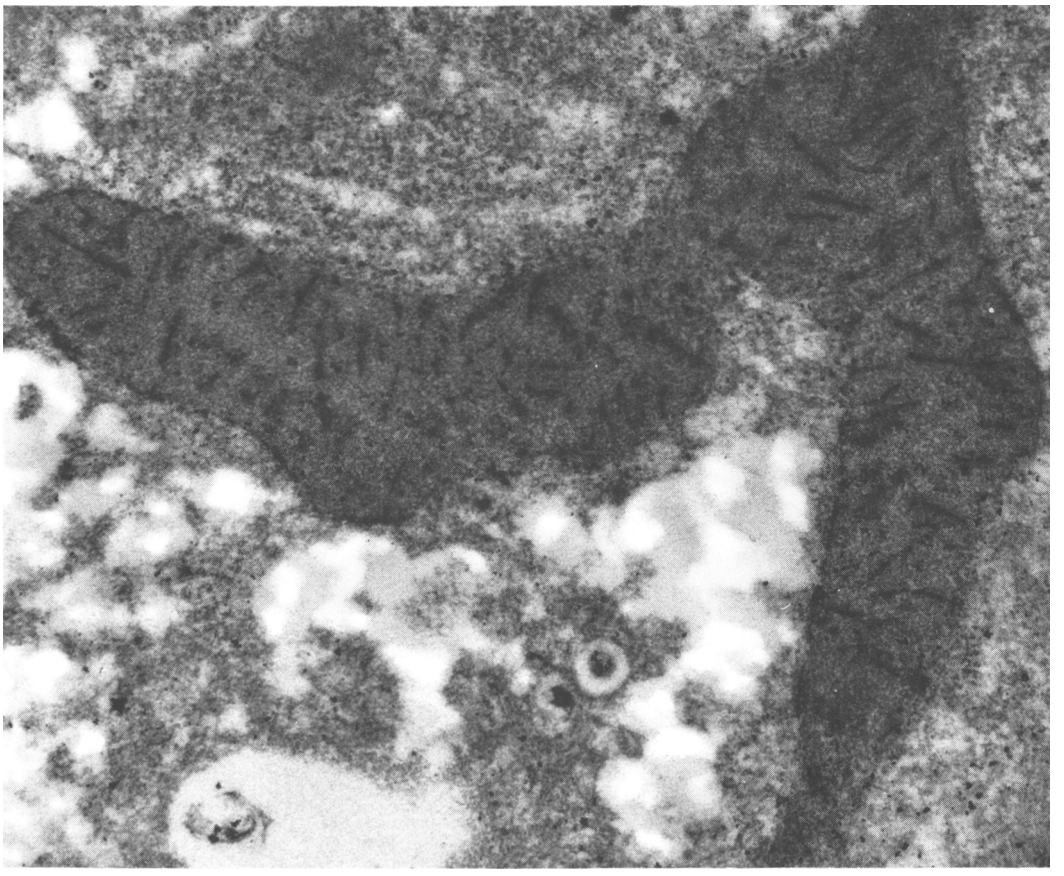


FIG. 1. Liver from a mouse injected with saline. In sections treated with peroxidase and DAB, electron-dense material can be seen in the hepatocytes scattered lightly through the endoplasmic reticulum and in moderately heavy deposits along the inner membranes and cristae of mitochondria. ×49,000.

morphonuclear cells. Precipitated material was found in the dilated endoplasmic reticulum of hepatocytes; there were focal accumulations of electron-dense material within the matrix of swollen mitochondria, but there was an absence of precipitate on the cristae and inner mitochondrial membranes (Fig. 2). Heavy deposits of stain were found on the intraluminal surfaces of capillary and sinusoidal endothelial cells, and degenerated endothelial cells were frequently noted (Fig. 3).

One day after the injection of 400 or 600 μg of Con A all mice had gross evidence of marked hepatic damage, and the congestion and subcapsular hemorrhages previously noted in the lungs, spleen, and kidneys were more severe. In addition there appeared to be moderate atrophy of the thymus. Microscopically, extensive areas of central and periportal necrosis were observed in the livers; in addition, there was bile stasis and marked congestion of capillaries and sinusoids. The splenic white and red pulp was mildly to markedly atrophic, and there were extensive subcapsular hemorrhages from dilated sinusoids. The thymic cortex was moderately atrophic. The pulmonary alveolar capillaries were congested, and there were large areas of subpleural and alveolar hemorrhage. The renal glomeruli were congested and the proximal convoluted tubules were moderately vacuolated. The following day the gross and microscopic findings were similar although somewhat more advanced (Figs. 4–6).

Seven days after the injection of Con A the livers were finely nodular but much less

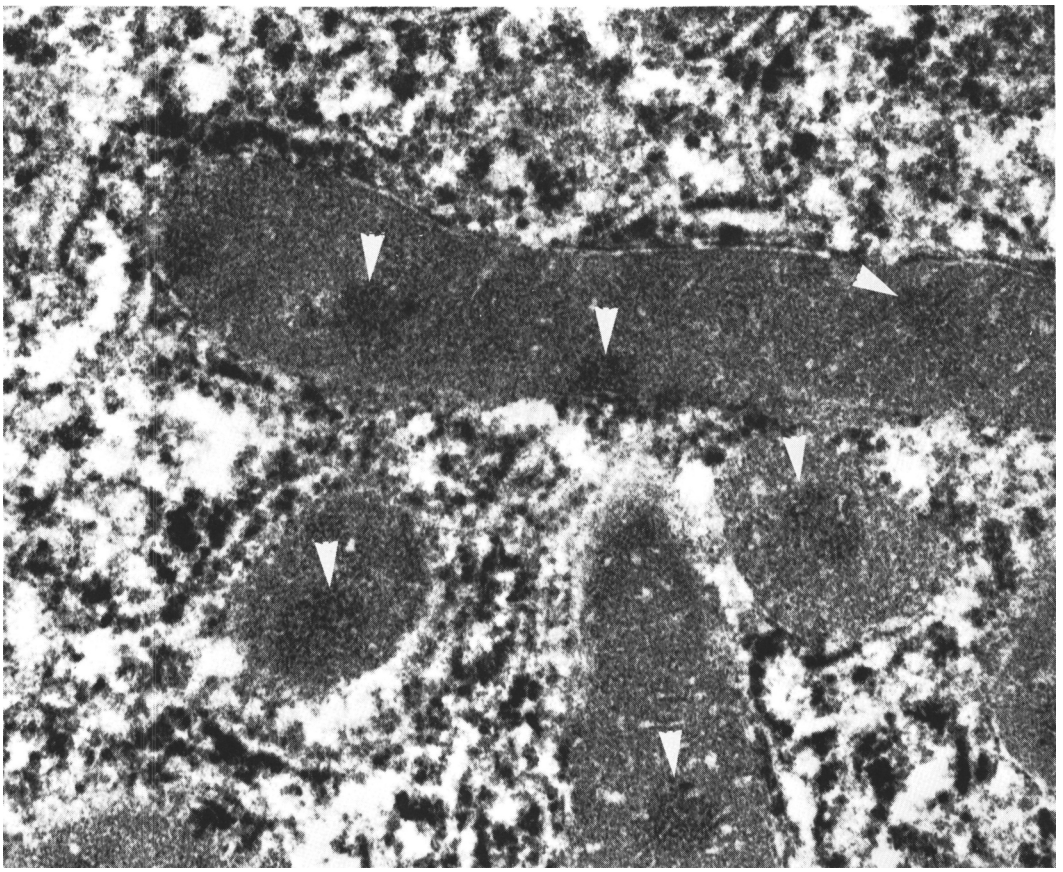


FIG. 2. Liver from a mouse injected 6 hr before with 600 μg of Con A. The sections were treated with peroxidase and DAB. Heavy deposits of electron-dense material are found in the dilated endoplasmic reticulum. The mitochondria are greatly enlarged and the cristae are dilated and poorly defined. Note the absence of stain on the mitochondrial membranes and the focal accumulations of dense precipitate within the matrix. ×45,000.

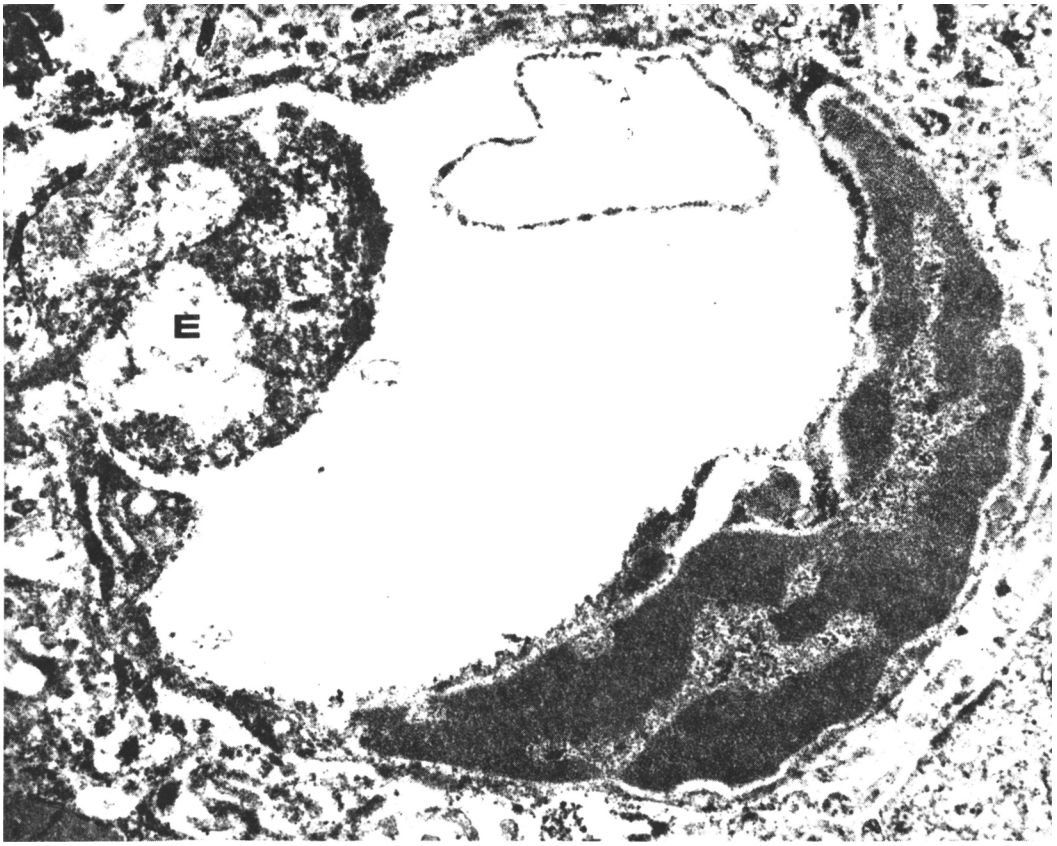
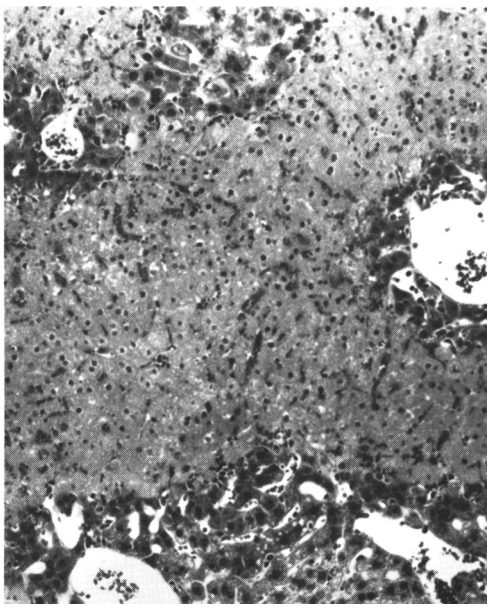


FIG. 3. Hepatic sinusoid of a mouse given 600 μg of Con A iv 6 hr before. The section was stained with peroxidase and DAB. Electron-dense material lines the entire luminal surface of the endothelial cells, and a degenerating endothelial cell (E) is seen on the left. ×23,000.



congested. The lungs, spleen, and kidneys remained congested but the majority of the hemorrhages had been resorbed. Microscopically, few necrotic areas were seen in the liver and there was evidence of early regeneration of the hepatocytes. The capillaries and sinusoids were much less congested and focal accumulations of lymphocytes were seen among the hepatocytes. The lungs and spleen remained moderately congested, and there were areas of hemorrhage noted in both organs. In addition, the pulmonary septa were edematous, and there were many small areas of atelectasis. The kidneys were essentially unchanged; the cortical areas of the thymus were hypertrophic.

FIG. 4. H & E section of liver from a mouse given 600 μg of Con A iv 2 days before. A typical area of massive hepatocellular necrosis is shown. ×170.

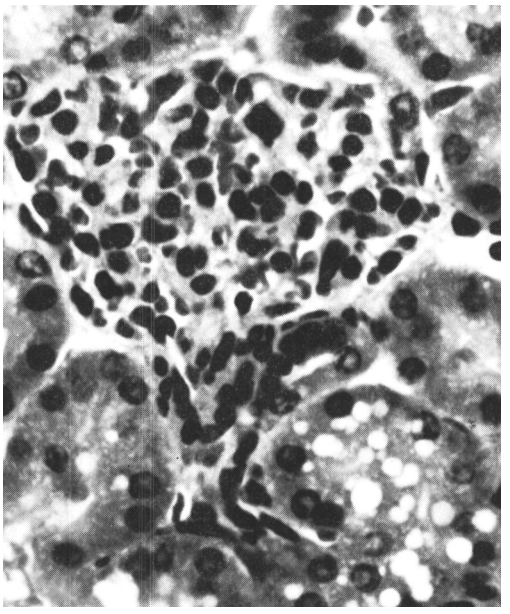


FIG. 5. H & E section of kidney from a mouse given 600 μg Con A iv 2 days before. The glomerulus is congested and the proximal convoluted tubules are vacuolated. ×850.

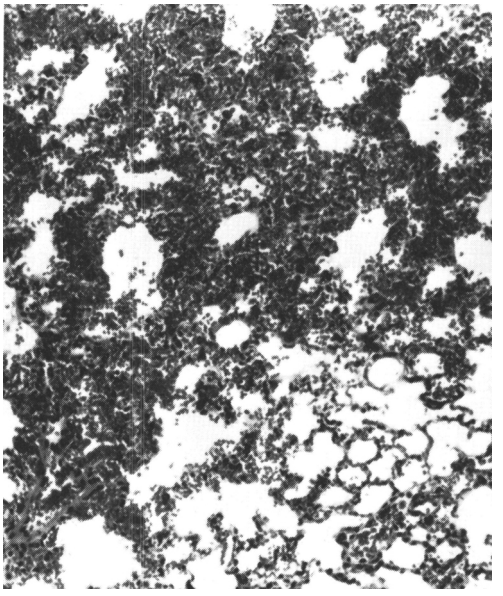


FIG. 6. H & E section of lung from a mouse given 600 μg of Con A iv 2 days before. There is marked capillary congestion and many areas of hemorrhage are present. ×170.

Fourteen days after the injection of Con A the findings on gross examination were essentially those noted 7 days earlier. Microscopically, there was evidence of continuing hepatic regeneration. The vasculature of the lungs, spleen, and kidneys remained congested, and many fibrinous thrombi were noted in pulmonary vessels. The proximal convoluted tubules were vacuolated in areas; the thymus was normal.

Twenty-eight days after injection the liver appeared normal on gross examination but the lungs and spleen were still somewhat hyperemic. Microscopically, the liver was minimally congested, there were no areas of necrosis, and regeneration was almost complete (Fig. 7). On the other hand, the lungs and kidneys revealed the same vascular changes noted 2 weeks earlier (Figs. 8 and 9). One year after the injection of Con A no significant gross or microscopic changes were noted.

Discussion. These and previous studies suggest that the mechanisms of Con A toxicity in the intact organism and in the isolated cell are complex. It has been shown *in vitro* that within minutes concentrations of Con A as low as 20 μg/ml produce marked changes in the net fluxes of sodium and potassium in mouse tumor cells (6) and that within 2 hours DNA and protein synthesis by mouse thymocytes are decreased and significant

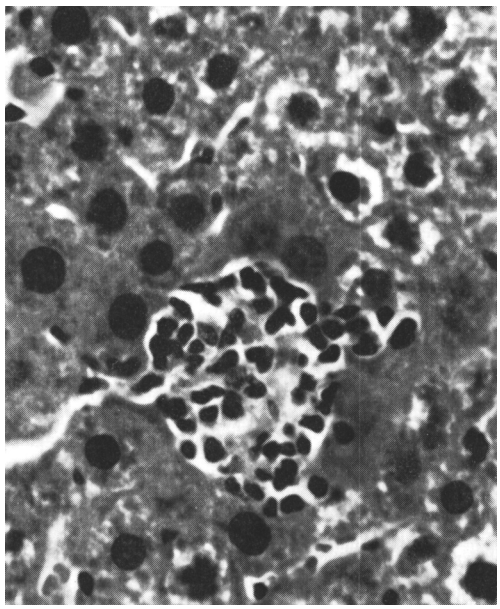


FIG. 7. H & E section of liver from a mouse given 600 μg of Con A iv 28 days before. A focal accumulation of lymphocytes is present among the regenerating hepatocytes. ×850.

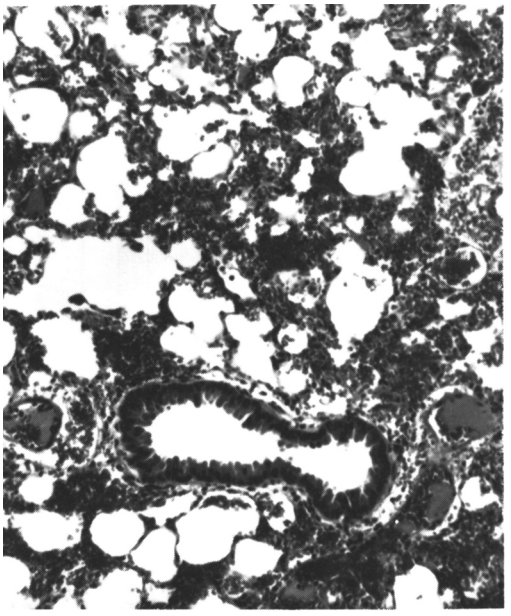


FIG. 8. H & E section of lung from a mouse given 600 μg of Con A iv 28 days before. There is marked capillary congestion and several fibrinous microthrombi can be seen in vessels adjacent to the bronchus. ×170.

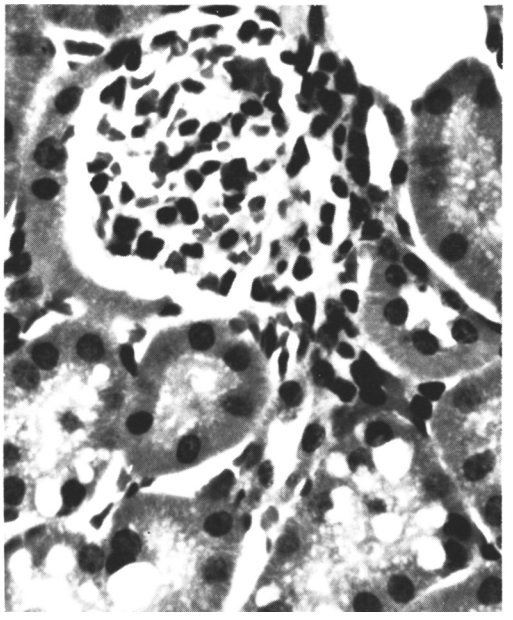


FIG. 9. H & E section of kidney from a mouse given 600 μg of Con A iv 28 days before. The glomerulus is congested and the proximal tubules are vacuolated. ×850.

cell lysis has occurred (7–9). Taken together these results suggest that Con A impairs cell function by changes produced in the plasma membrane and in various intracellular organelles. The present studies indicate that Con A can interact directly with the endoplasmic reticulum and mitochondria, and that at least a portion of the toxic properties of Con A may result from damage to these organelles.

When Con A was injected intravenously in these experiments, damage to vascular endothelial cells and to hepatic parenchymal cells was found to be well advanced 6 h later. The changes found in the lungs, spleen, kidneys, and liver suggest that damage to the vascular endothelial cells which resulted in thrombosis and hemorrhage may have contributed as much to total tissue damage as did the direct effects of Con A on parenchymal cells. Further, repair of the vascular damage, although apparently complete by 1 year, proceeded at a much slower pace than did hepatic or lymphoid regeneration.

Summary. Con A injected iv in doses of 200–600 μg produced vascular endothelial damage, hepatic necrosis, and lymphoid atrophy. Six hours after mice were given 600 μg of Con A iv, subcapsular hemorrhages and other evidence of vascular damage were found in the visceral organs. Ultrastructural studies of livers from these mice revealed damage to the vascular endothelium and to hepatocytes as manifested by the accumulation of lipid droplets, dilation of the endoplasmic reticulum, and the formation of giant degenerating mitochondria. Histochemical studies demonstrated Con A on the cell walls of vascular endothelial cells and in the endoplasmic reticulum and mitochondria of hepatocytes. Con A appeared to destroy the peroxidase activity normally found on the inner membranes and cristae of mitochondria.

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