Journal of Toxicology and Environmental Health: Current Issues
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/uteh19

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Published online: 20 Oct 2009.

To cite this article: Ø. Fodstad, J. V. Johannessen, L. Schjerven & A. Pihl (1979) Toxicity of abrin and ricin in mice and dogs, Journal of Toxicology and Environmental Health: Current Issues, 5:6, 1073-1084, DOI: 10.1080/15287397909529815

To link to this article: http://dx.doi.org/10.1080/15287397909529815

# TOXICITY OF ABRIN AND RICIN IN MICE AND DOGS

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*Mice and dogs were treated iv with the cytostatic proteins abrin and ricin and observed for clinical, biochemical, and morphological aberrations. In both mice and dogs death occurred within a narrow dose range. Dogs given toxic doses of ricin and abrin showed weakness, anorexia, apathy, and moderate fever. No signs attributable to the central nervous system were observed. Dogs dying from intoxication expired after 15–40 h. After nonlethal doses the animals recovered, apparently completely, in 1–3 wk. No delayed changes were observed in dogs after 4 mo.*

*Abrin and ricin, in contrast to most other cytostatic agents, did not inhibit myelopoiesis. However, after sublethal doses a rapid but transient decrease of peripheral thrombocytes was observed. No evidence for specific liver damage or impairment of kidney function was obtained. Few abnormalities were observed at autopsy or on microscopic and electron microscopic examination of the tissues, in contrast to the findings of some earlier investigators.*

*The results indicate that in mice and dogs given sublethal doses of highly purified toxins the symptoms are reversible. There was no finding militating against a phase I clinical trial.*



## INTRODUCTION

Abrin and ricin are highly toxic proteins that occur in the seeds of *Abrus precatorius* and *Ricinus communis*, respectively. Numerous cases of intoxication resulting from ingestion of the seeds have been described in humans and animals (Morton, 1977). The toxins have been purified and studied extensively and their mechanism of action on the molecular level is now well understood (Olsnes and Pihl, 1976, 1978). They inhibit the growth of various murine cancers (Lin et al., 1970; Fodstad et al., 1977; Fodstad and Pihl, 1978; Gundersen and Fodstad, 1979) as well as several human xenografts in athymic mice (Fodstad et al., 1977; Fodstad, 1979; Pihl et al., 1979). The results are sufficiently promising to warrant clinical studies in humans.

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Journal of Toxicology and Environmental Health, 5:1073–1084, 1979

0098-4108/79/051073-12$2.25

Previously we reported data on the toxicity, distribution, and elimination of abrin and ricin in mice (Fodstad et al., 1976). However, before a phase I clinical trial could be initiated, it was necessary to explore in more detail the toxicity and clinical signs of these lectins in several animal species.

## MATERIALS AND METHODS

### Animals

Male and female $B_6D_2/F_1$ mice weighing about 20 g and young male or female Labrador retriever dogs weighing 14–19 kg were used.

### Toxins

Ricin and abrin were extracted from the seeds of *Ricinus communis* and *Abrus precatorius* and purified as described earlier (Olsnes and Pihl, 1973a, 1973b).

### Administration

The toxins were given iv in a buffered aqueous solution.

### Blood Examinations

Blood examinations were carried out for the dogs. Samples were withdrawn at 1- to 4-d intervals for 2–10 wk after injection of the toxins. Hemoglobin and sedimentation rate were measured and white blood cell counts, differential counts, reticulocyte counts, and platelet counts were performed. Serum was separated and the following constitutents were determined: transaminases aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT), alkaline phosphatase, lactate dehydrogenase, γ-glutamyltransferase, total bilirubin, total protein, albumin, glucose, amylase, haptoglobin, creatinine, urea, $Na^+$, $K^+$, $Cl^-$, $Ca^{2+}$, and inorganic phosphate. Analyses were carried out at the Central Laboratory of the Norwegian Radium Hospital.

### Autopsy

Postmortem examinations were carried out on all animals immediately after death. Samples from the following tissues were taken for microscopic examination: liver, spleen, kidneys, adrenal glands, stomach, small intestine, colon, ovary or testis, bladder, heart, lung, thymus, brain, and striated muscle.

### Microscopic Examinations

For light microscopy the tissues were fixed in 4% buffered formaldehyde and embedded in paraffin. Sections were stained with hematoxylin and eosin. For electron microscopy tissue cubes 2 mm in diameter were

fixed, immediately after sacrifice of the animals, in 2% phosphate-buffered glutaraldehyde or a formaldehyde-glutaraldehyde mixture (McDowell and Trump, 1976), osmicated, dehydrated, and embedded in Epon-Araldite (Mollenhauer, 1964). For light microscopic evaluation semithin sections were cut with an LKB Pyramitone equipped with glass knives and were stained with toluidine blue. Ultrathin sections were cut with glass or diamond knives, mounted in an LKB Ultratome III, collected on naked Cu grids, stained with uranyl acetate and lead citrate, and examined in a Philips EM 201 electron microscope.

### Bone Marrow Examinations

Bone marrow samples were aspirated from the iliac crest, fixed in Zenker-formol, and stained with hematoxylin-Giemsa. Differential counts were made on representative areas of May-Grünwald-Giemsa stained smears.

### Cells

HeLa cells were maintained in shaker culture at 37°C in Gibco minimum essential medium. The ability of ricin to inhibit cellular protein synthesis was assayed as previously described (Fodstad et al., 1977).

## RESULTS

### Studies in Mice

**Acute Toxicity.** Groups of four mice were injected iv with increasing doses of ricin and abrin and the animals were observed every second hour for clinical signs of toxicity and for death.

**Survival Studies.** The effect of single doses of toxin on survival is shown in Fig. 1. For both toxins the dose-response curves decline steeply after an initial horizontal phase (Fig. 1*a*). Thus all animals died within a narrow dose range, and the minimum lethal dose (MLD), which was about 0.7 and 2.7 μg/kg for abrin and ricin, respectively, is a more meaningful parameter than the LD50.

The relationship between dose and survival time is shown in Fig. 1*b*. Even after supralethal doses no animals died before about 10 h, and the survival time is strictly related to the dose, in agreement with our previous findings (Fodstad et al., 1976). This curve is highly reproducible and is used in our laboratory for biological standardization of our toxin preparations.

**Clinical Observations.** During the first hours after administration of the toxins the animals appeared unaffected. Then, after a time depending on the dose, they showed clear signs of sickness. Dying animals shivered and appeared to have reduced body temperature. The animals showed a loss of body weight of up to 20% with a maximum at about d 5. Animals

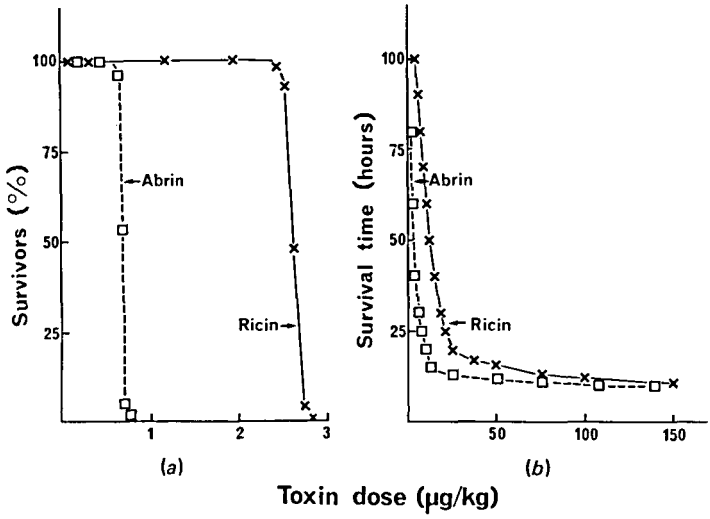


FIGURE 1. Effect of ricin and abrin on survival of mice. Groups of mice were given increasing doses of ricin and abrin iv and (*a*) number of survivors and (*b*) survival time were observed.

surviving for more than 6–7 d seemed to recover completely. Those that died showed no specific or unusual clinical symptoms.

**Pathology.** In some of the animals that died, small amounts of clear fluid were seen in the serous cavities and the spleens appeared to be congested. Otherwise, no pathological findings were made on gross examination.

Light and electron microscopic examinations revealed no obvious differences between treated and untreated animals. No definite abnormalities were found in the intestine, spleen, or kidneys. The results do not agree with earlier findings on rat spleen and liver (Derenzini et al., 1976) and on the intestinal tract of rabbits (Müller, 1899).

**Delayed Toxicity in Mice.** Animals showed few or no clinical symptoms after repeated doses (0.5 MLD) given weekly for 4 wk. Thus, this regimen did not appear to result in accumulated toxicity. Examination of the tissues after 6 wk, 6 mo, and 12 mo after single or multiple doses did not reveal any macroscopic, microscopic, or electron microscopic abnormalities.

### Studies in Rats, Guinea Pigs, and Rabbits

With abrin, survival studies were also carried out in rats, guinea pigs, and rabbits; the MLDs were 0.35–0.5, 0.40–0.50, and 0.03–0.06 µg/kg, respectively. Thus, on the basis of weight, the rabbit is considerably more sensitive to abrin than the mouse, rat, or guinea pig.

### Studies in Dogs

**Acute Toxicity.** The maximum tolerated dose (MTD)—the dose giving only minimal and reversible toxicity (Schein et al., 1970)—was 0.6 and 0.80 μg/kg for abrin and ricin, respectively. One dog died after 1.75 μg/kg and 1 after 2.0 μg/kg ricin, whereas other dogs survived repeated doses of 1.6 μg/kg. Hence, the MLD of ricin falls in the range 1.6–1.75 μg/kg. For abrin the corresponding dose range was 1.2–1.35 μg/kg.

**Clinical Observations.** After abrin and ricin treatment the dogs lost appetite and body weight (up to 10%) and had a slight fever of 0.5–1°. The clinical symptoms were somewhat less pronounced after ricin than after abrin. Animals given high doses showed general weakness and were unable to stand. Three dogs had edema in the extremities and one dog developed ascites. Two dogs that died after acute intoxication had small amounts of fresh blood in the stools. Death occurred about 15–40 h after lethal doses of abrin and ricin.

Animals that survived abrin or ricin treatment recovered gradually in 1–5 d, depending on dose. They regained their body weights in 1–3 wk and appeared to recover completely.

Quiescent infections were activated by treatment with the toxins. In some cases small sc infections developed into suppurative lesions. In some dogs a local reaction was observed at the site of injection, probably because of paravenous deposition of toxin.

**Hematologic and Biochemical Studies.** A number of hematologic and biochemical parameters were followed at intervals for several weeks. Considerable variations between individual dogs were found.

Hematologic parameters in peripheral blood of one of the dogs given a sublethal dose of ricin are shown in Fig. 2. The hemoglobin value was slightly increased on d 3 and gradually declined to a nadir at d 7. Findings were similar in the other dogs. The initial increase in hemoglobin was probably due to hemoconcentration. Concurrent examinations of the bone marrow showed an initial reduction in the number of erythroblasts and reduced cellularity; these changes disappeared after about 1 wk.

Leukocytes showed an initial strong increase, a rapid decline to subnormal values, and another increase. A new dose of ricin again resulted in a transient increase (data not shown). Variations occurred primarily in the neutrophils. Bone marrow examinations revealed no clear abnormality in myelopoiesis.

Thrombocytes first decreased and then increased after 12 d to higher than normal values. Subsequent repeated sublethal doses did not give clinically significant depressions. Similar results were obtained with abrin.

The hematologic and biochemical results are briefly summarized in Table 1. ASAT and ALAT showed transient increases in some animals and small or no increases in others. Somewhat larger increases of alkaline phosphatase and lactic dehydrogenase were observed. However, because

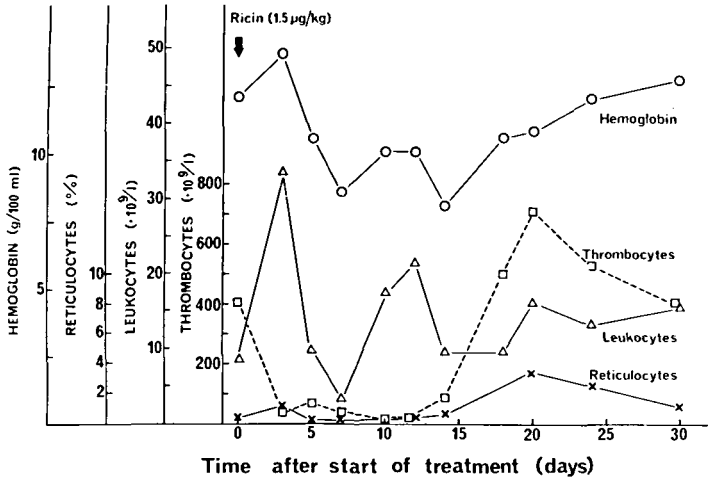


FIGURE 2. Hematologic parameters in a dog treated with ricin. A Labrador retriever weighing 19 kg was given a sublethal dose of ricin iv and blood samples were examined at intervals as indicated.

TABLE 1. Effect of Abrin and Ricin on Hematologic and Biochemical Parameters in Dogs

| Constituent | Aberration[a] | |
|---|---|---|
| | Abrin | Ricin |
| Hemoglobin | (↓) | (↓) |
| Hematocrit | ↓ | ↓ |
| Reticulocytes | (↑) | (↑) |
| Leukocytes | ↑ | ↑ |
| Thrombocytes | ↓ | ↓ |
| Sedimentation rate | None | None |
| Transaminase | | |
| ALAT | (↑) | (↑) |
| ASAT | (↑) | (↑) |
| Alkaline phosphatase | ↑ | ↑ |
| Lactate dehydrogenase | ↑ | ↑ |
| γ-Glutamyltransferase | None | None |
| Bilirubin | None | None |
| Total protein | ↓ | ↓ |
| Albumin | ↓ | ↓ |
| Glucose | (↓) | (↓) |
| Amylase | (↑) | (↑) |
| Creatinine | None | None |
| Urea | None | None |
| Haptoglobin | None | None |
| $Na^+$ | None | None |
| $K^+$ | (↑) | (↑) |
| $Cl^-$ | None | None |
| $Ca^{2+}$ | None | None |
| Inorganic phosphate | None | None |

[a] (↑) or (↓): increased or decreased values in some animals, small or none in others. ↑ or ↓: considerably increased or decreased values in some or all animals, depending on dose.

glutamyl transferase and bilirubin values did not show significant deviations, the results do not provide evidence for liver damage.

Total protein and albumin decreased in the treated animals; the values became normal in 1–2 wk. Creatinine, urea, $Na^+$, $Cl^-$ values remained normal. A transient increase in $K^+$ levels was observed, probably caused by leakage from damaged cells. No abnormalities were observed in $Ca^{2+}$ and inorganic phosphate levels. Amylase values moderately increased whereas glucose levels decreased after treatment.

**Formation of Antibodies.** Because abrin and ricin are proteins, treatment with them might be expected to induce antibody formation. The presence of antibodies in the serum of toxin-treated animals was investigated by a specific and highly sensitive method: measuring the ability of serum to counteract the toxin inhibition of cellular protein synthesis. Data for a ricin-treated dog are shown in Fig. 3. Serum taken 8 wk after toxin treatment counteracted ricin doses that gave almost complete inhibition in the control cells, demonstrating the presence in the serum of specific antibodies to ricin. Serum known to contain antibodies against abrin had no similar effect on the sensitivity of the cells to ricin.

**Pathology.** Only moderate pathological manifestations were observed in animals that died. After ricin and abrin treatment, one of three animals showed a large and congested spleen. One animal given a sublethal dose of

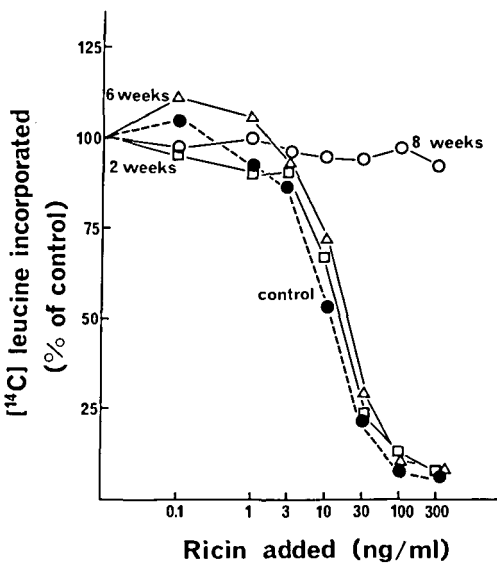


FIGURE 3. Demonstration of the presence of antibodies to ricin in a ricin-treated dog. Serum samples were taken at intervals from a dog treated with 1.4 μg/kg ricin (on d 1, 5, and 29), and their ability to counteract the inhibiting effect of ricin on protein synthesis in HeLa cells was measured. The assay system was as previously described (Fodstad et al., 1977), with 25 μl dog serum added.

abrin followed by a lethal dose 3 mo later developed petechial bleeding shortly before death. In one dog given a lethal dose of ricin serosanguinous fluid was found in the pleural cavity. Otherwise, no gross abnormalities were found at autopsy.

Light and electron microscopic examination revealed no evidence of necrosis in the tissues examined, as shown for pancreas in Fig. 4. However, in some animals the reticuloendothelial cells of liver and spleen were swollen and showed evidence of increased phagocytic activity (Fig. 5).

**Delayed Toxicity.** Repeated doses of 0.6 μg/kg abrin or 0.8 μg/kg ricin every second week produced almost no clinical symptoms. Light and electron microscopic examination of the tissues of these animals showed the same changes as described above for acute intoxication. The data indicate that doses given at these intervals do not result in accumulated toxicity.

## DISCUSSION

Ricin and abrin inhibit protein synthesis in eukaryotic cells by inactivating the large ribosomal subunit. The high toxicity results because



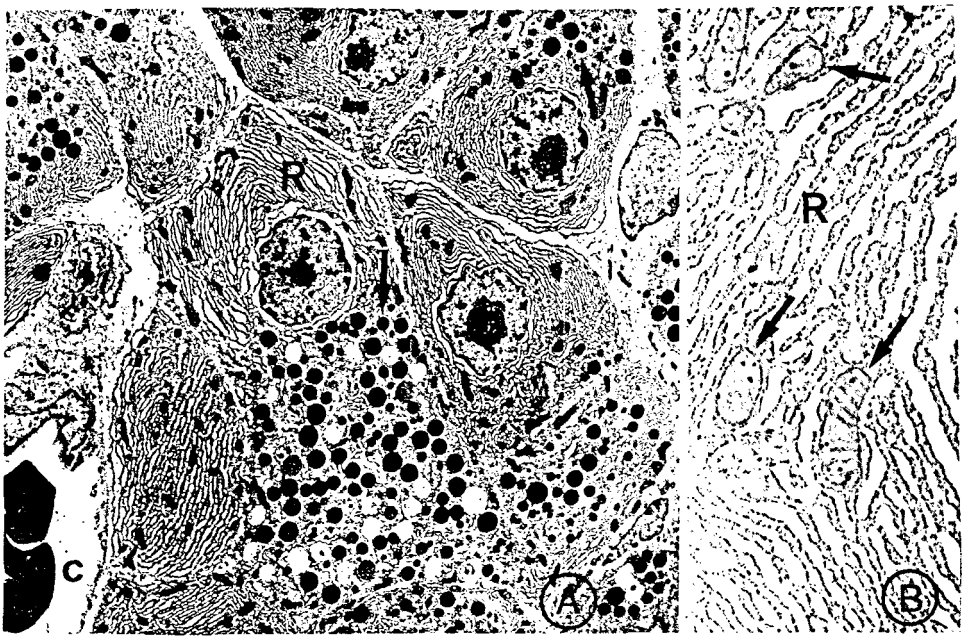


FIGURE 4. (A) Electron micrograph of exocrine pancreas with normal-appearing cisternae of rough endoplasmic reticulum (R) and zymogen granules (arrows). A blood capillary (c) is seen in the lower left corner (X2392). (B) Higher magnification. Note the orderly arrangement of ribosomes along the cisternae of the rough endoplasmic reticulum (R) as well as the normal-appearing mitochondria (arrows). Uranyl acetate and lead citrate stain (X16,500).

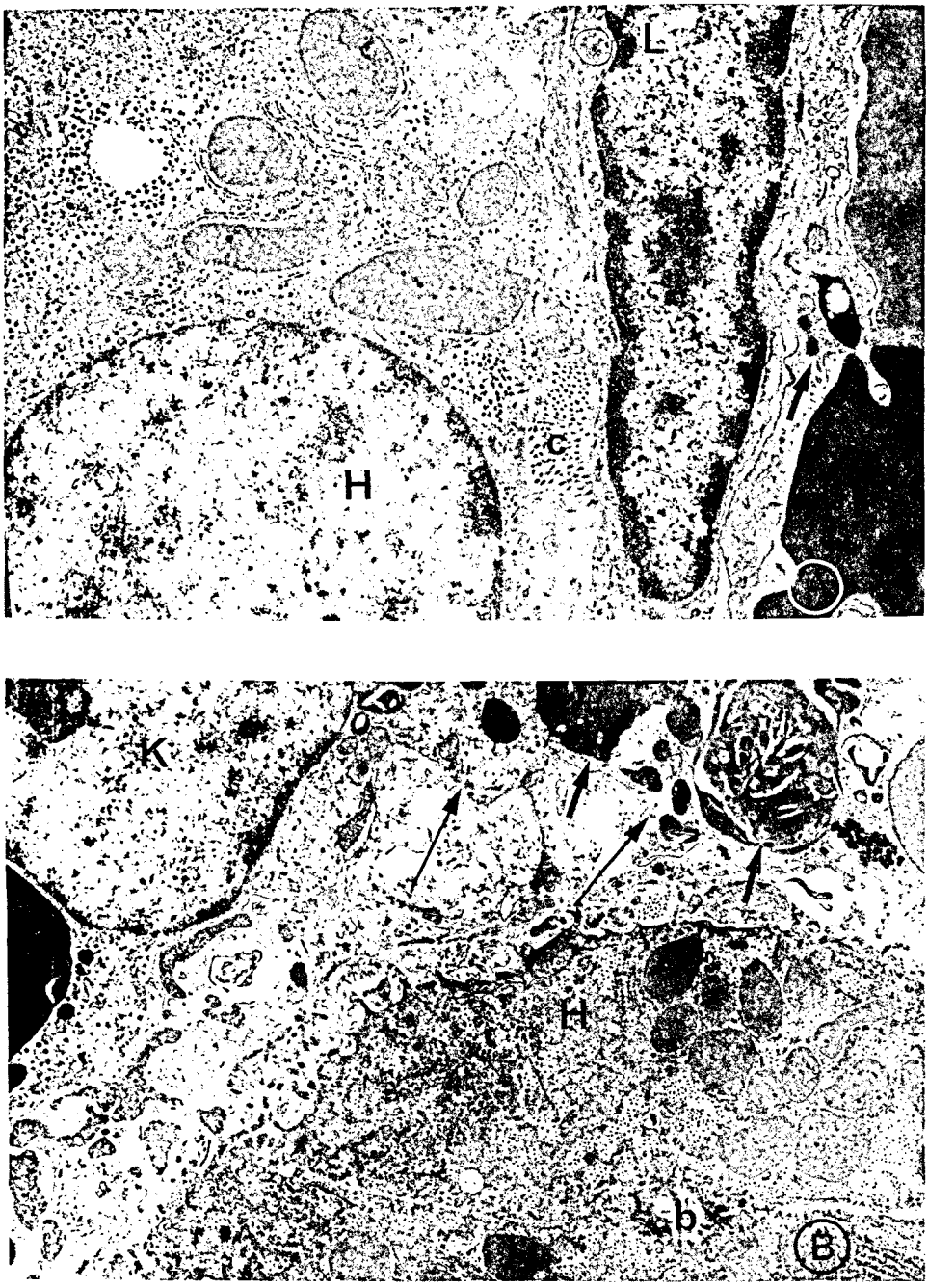


FIGURE 5. (A) Hepatocyte (H) with adjacent littoral cells (L) containing scattered lysosomes (arrow) bordering a sinusoid. A couple of normal erythrocytes (e) are seen in the sinusoid, and collagen fibrils (c) occupy the Disse space (X12,144). (B) Sinusoid with enlarged Kupffer cell (K) with large phagolysosomes (short arrows) as well as recently engulfed erythrocytes (e). Note the ruptured cell membrane (long arrows). Hepatocyte (H) and bile canaliculus (b). Staining as in Fig. 4 (X8280).

the toxin A chain is an enzyme capable of inactivating numerous ribosomes (Olsnes and Pihl, 1978). The lag time characteristic of the intoxication may reflect the time required to decrease various enzymes and essential proteins to certain critical levels.

The main clinical symptoms we observed were weakness, anorexia, weight loss, and moderate fever. Animals given lethal doses died without any unique or unusual symptoms. In particular, no symptoms attributable to the central nervous system were observed. The steep dose-lethality relationship seen in mice also seems to obtain in dogs and may obtain in humans as well.

Most cytotoxic agents in common use have a strong bone marrow depressing action. Present, and previous (Fodstad et al., 1977), findings that abrin and ricin do not depress the number of peripheral leukocytes and that the bone marrow of toxin-treated dogs showed little effect on myelopoiesis are therefore of particular interest.

Using pure preparations of ricin and abrin, we were unable to confirm several findings reported by earlier investigators who used preparations from castor beans and *Abrus* seeds. Stillmark (1888), Ehrlich (1891, 1892), and Flexner (1897) described lesions of the intestines and diffuse nephritis as well as necrosis of the liver, kidneys, and mesenteric lymph nodes in various animal species. In our mice and dogs these tissues appeared almost normal after sublethal or lethal doses, even by electron microscopy. The fresh blood found in the feces of two dying dogs may be related to the initial rapid fall in the thrombocytes.

In the livers evidence of increased phagocytic activity was found; changes were similar to although less pronounced than those described in rats (Derenzini et al., 1976). In the castor beans and *Abrus* seeds several toxic fractions as well as powerful agglutinins are present. Some of the earlier findings may be caused by use of impure preparations.

The transient depression of hemoglobin observed in some dogs after a few days can hardly be explained by the moderate and transient effect on the bone marrow, as dog erythrocytes have a long lifetime (about 50 d). Measurements of serum hemoglobin and of haptoglobin gave no evidence of hemolysis. Whatever the mechanism, the transient decrease in hemoglobin appeared to be of little clinical significance.

The biochemical analyses reported here fail to explain adequately the clinical symptoms. Thus, even though the action of abrin and ricin at the subcellular level is rather well known, the mechanism underlying the lethal and clinical effects of these toxins in animals is as yet inadequately understood. Death may be caused by a decrease in the level of some specific vital proteins. The possibility must also be considered that these toxins may exert effects other than that on protein synthesis.

The clinical, biochemical, and pathological examinations carried out on dogs many months after acute, subacute, and chronic treatment indicate

that the symptoms in animals given sublethal doses are reversible. Observations on dogs are good indicators of all serious organ system toxicities in humans, with the possible exception of central nervous system and dermal toxicity (Schein et al., 1970), and no observations reported here should preclude testing of abrin and ricin in humans. Such a study is now in progress. Our animal data indicate that dose escalations in patients should be based primarily on the subjective and objective clinical symptoms, as none of the test parameters we studied proved to be indicators with high predictive value.

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*Received June 14, 1979*
*Accepted July 16, 1979*