Tissue Effects of Lysolecithin Injected Subcutaneously in Mice.* (30317)

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Studies with enzyme inhibitors indicate that the reaction of antigen and antibody results in the activation of enzymes, although no particular enzyme has yet been identified positively(1,2). Recent investigations, however, suggest that phospholipase A may be activated and may perhaps be the first in a series of enzymes activated by antigen-antibody union(3,4). Since phospholipase A catalyzes the hydrolysis of lecithin to lysolecithin, one consequence of its activation should be the production of lysolecithin. To determine if any lysolecithin produced could at least in part account for the tissue injury sometimes seen with antigen-antibody reaction, the present study was carried out to investigate the morphological effects of lysolecithin on tissues. To do this, a histological study of the abdominal wall of mice was performed at various intervals following subcutaneous injection of lysolecithin. Since phospholipase A is one of the most potent enzyme activities found in all snake venoms tested(5), the present study, in addition, might contribute to our understanding of the mechanism of snake venom toxicity.

Materials and methods. Two series of injection experiments using white mice of the Swiss-Webster strain were performed. The abdomen of the mouse was shaved, cleansed with zephiran chloride, and the lysolecithin preparation injected with sterile syringe and needle (#26) subcutaneously so that the injected material appeared approximately at the center of the abdomen. At timed intervals, the mice were sacrificed by guillotine and the abdominal wall down through the peritoneum was removed and put immediately into fixative. In the first experiment, 0.1 μmole of lysolecithin in a volume of 0.1 ml was injected and a mouse was sacrificed at 30 minutes and 3, 7, 24, and 78 hours. The fixative used was 10% formalin. Since a volume of 0.1 ml raised the abdominal skin in a large bubble, in the second experiment, 0.1 μmole of lysolecithin was injected in a volume of only 0.025 ml and an animal was sacrificed at 10, 20, and 30 minutes and 1, 2, 3, 4, 24, 48, and 72 hours. A mouse injected with 0.025 ml of isotonic saline and sacrificed at 30 minutes served as a control. The tissues in the second experiment were fixed in Bouin's solution.

The same lysolecithin preparation was used for both experiments. It was prepared from lecithin, isolated from beef brain lipid extract using column chromatography on silicic acid (6), by the action of cobra venom *(naja naja)* as previously described(7). This lysolecithin preparation appeared to be pure by thin-layer silicic acid chromatography using chloroform-methanol-water, 75:25:4 (V/V) as solvent. The solutions of lysolecithin used for injection were made up in sterile isotonic saline. When a 0.1 μmole aliquot was added to 1 ml of a 1% suspension of sheep red blood cells marked hemolysis was observed within a few minutes.

Results. The findings in both series of experiments were essentially the same despite the larger volume injected in the first series and the higher concentration of lysolecithin achieved locally in the second.

Ten minutes after injection of lysolecithin the subcutaneous tissue was edematous, fat cells were disrupted and the small muscle cells of the platysma revealed a hyaline homogeneous, brightly eosinophilic cytoplasm. These changes were confined to the region immediately surrounding the site of injection (Fig. 1 and 2).

At 20 minutes, in addition to the changes noted above, individual muscle fibers of the deep muscle layer had become "hyalinized." Polymorphonuclear leukocytes and a few

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monocytes were also found scattered diffusely throughout the edematous connective tissue. At 30 minutes the muscle fibers of the platysma began to undergo fragmentation and many muscle cells had lost their cytoplasm completely. Capillaries were dilated and contained numerous polymorphonuclear leukocytes.

At one hour the tissue response was essentially the same as at 30 minutes. An important negative finding was the absence of vascular thrombosis or hemorrhage. This was true throughout the 72-hour period of observation.

At 2 hours the myocytolysis was prominent in the cells of the deep muscle layer adjacent to the injection site. The more distant fibers were well preserved indicating damage was due to diffusion of a substance from the injection site, presumably lysolecithin. Polymorphonuclear leukocytes had gathered in focal regions around a few of the degenerating muscle cells and the capillaries in the damaged zone were dilated.

At 3 hours there was little change except for an increasing infiltration by polymorphonuclear leukocytes and capillary dilation in the zone of muscle cell degeneration (Fig. 3).

At 24 hours there was a marked inflammatory infiltrate of the edematous connective tissue layer beneath the dermis. The cellular components of this inflammation were histiocytes and polymorphonuclear leukocytes (Fig. 4). In addition, there were swollen connective tissue cells indicating an activation of fibroblasts. Macrophages were concentrated in the zone of degenerating muscle cells. Vascular dilation, predominantly of post capillary venules was present in all the affected tissues.

At 48 hours, the nuclei of the damaged muscle cells were becoming prominent and were frequently seen enveloping fragments of dead muscle cell cytoplasm. By 72 hours the regenerating muscle cells were very prominent and were conspicuous because of their large vesicular nuclei with prominent nucleoli and abundant basophilic cytoplasm (Fig. 5 and 6).

Discussion. The evidence presented indicates that lysolecithin is highly injurious to tissues. The sharp demarcation observed between the normal and damaged tissue was related presumably to the extent of diffusion of the lysolecithin. This finding along with the absence of thrombosis or hemorrhage suggested that the lysolecithin was directly toxic to the cells damaged. One of the most striking early changes observed after injection of lysolecithin was a profound local edema, suggesting an effect on small blood vessels. Such an effect has also been found in human skin, where lysolecithin injection was followed by a typical local wheal and erythema reaction (8). Since this wheal and erythema reaction could be blocked by simultaneous injection of an antihistaminic(8), it appeared to be mediated through histamine, which might have been produced as a result of disruption of mast cells by lysolecithin(9). Lysolecithin has also been shown to release histamine activity from dog liver(10) and guinea pig lung (4). The edema observed in these studies, therefore, could have been mediated at least in part through a histamine effect on the blood vessels. In support of this possibility was the absence of demonstrable blood vessel abnormalities by light microscopy. The fat and muscle cells showed extensive necrosis, although it is possible that with lower concentrations of lysolecithin these cells would have shown more subtle histological changes associated with any degree of functional alteration. The necrosis was followed by an infiltration of predominantly neutrophil leukocytes which was later replaced by histiocytes.

Previous studies on the histological effect of lysolecithin injected into the tissues of animals have been reported by Belfanti(11) and Guerrini(12). These investigators also observed the rapid development of marked edema when lysolecithin was injected subcutaneously into the ear of the rabbit as well as into other tissues of the rabbit and dog. In addition, however, they noted vessel engorgement, seen also in the present study, and extensive capillary hemorrhage leading to gross hemorrhage in the various tissues into which it was injected. The discrepancy between the consistent finding of hemorrhages by these investigators and the lack of hemorrhage noted in the present investigation is un-

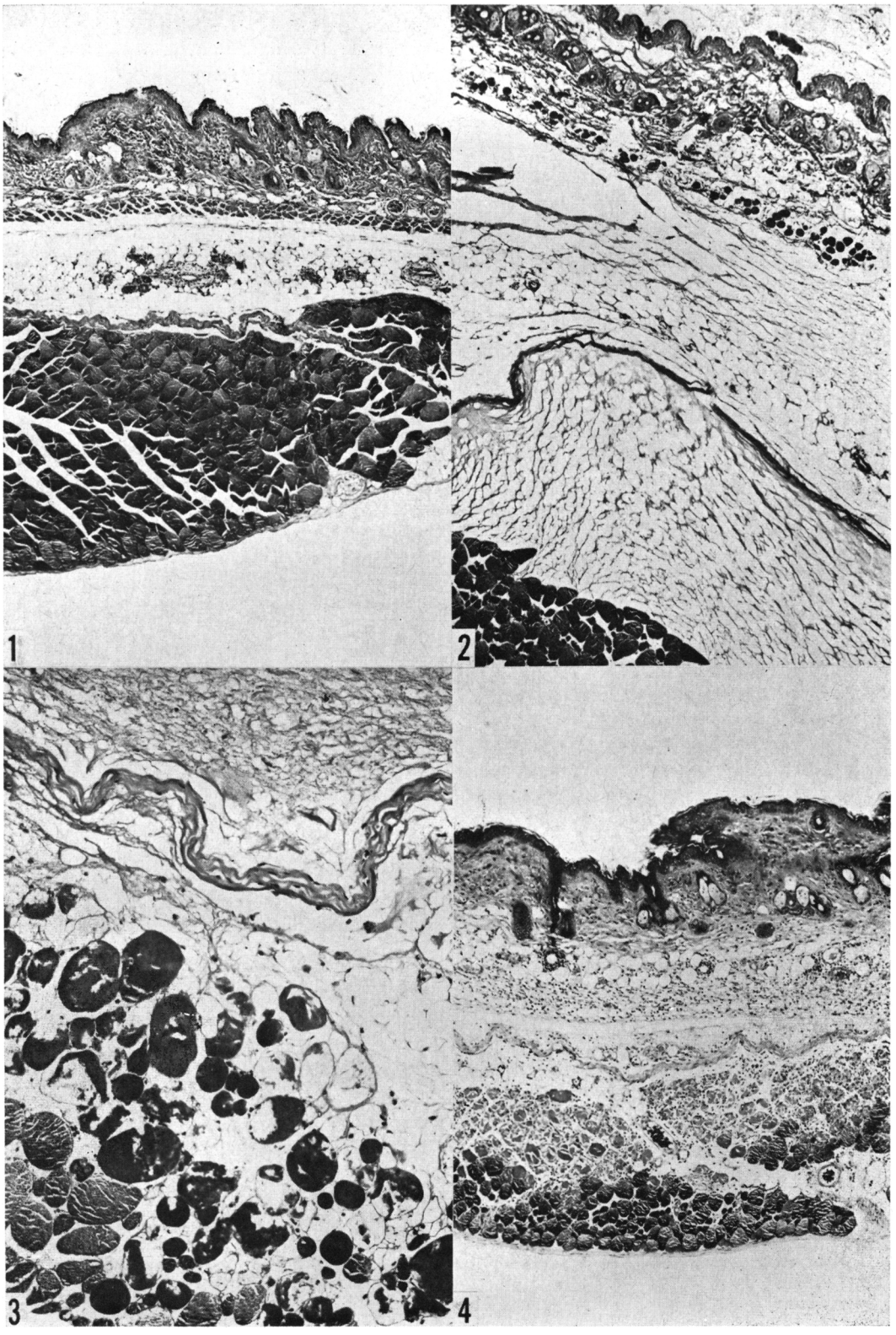
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FIG. 1. Normal skin and muscle of abdominal wall of mouse 30 min after saline injection (control). Hematoxylin and Eosin × 32.

FIG. 2. Same magnification as Fig. 1 ten min after lysolecithin injection. Massive edema in fat tissue and hyalinization of platysma muscle immediately beneath epidermis is seen. Hematoxylin and Eosin × 32.

FIG. 3. Degenerative muscle changes are prominent at 3 hr. Note hyalinization and fragmentation of fibers. Hematoxylin and Eosin × 75.

FIG. 4. At 24 hours extensive necrosis of platysma muscle and the superficial portion of the muscle of abdominal wall is evident, associated with a cellular reaction as well as edema and fat necrosis. Dilated but intact vessels can be seen. Hematoxylin and Eosin × 32.

explained. One possible explanation is that the hemorrhage might have resulted from the larger amount of lysolecithin injected by these investigators, although the amounts they used may have been smaller than stated due to impurities in their preparation. Another possibility is that the lysolecithin preparation used by these investigators contained some of the snake or bee venom that was used to prepare it. Guerrini(12), in fact, observed that the histological changes produced by injection of lysolecithin were essentially the same as those following injection of the venom of *Lachesis neuwiedii* when injected subdurally in the rabbit. However, he noted that similar effects were produced by the subdural injection of lysolecithin isolated from tissues which were not exposed to venom. Finally, impurities other than venom in their preparations might have been responsible.

Whether lysolecithin is produced during the immune reaction cannot be stated. Fischer and Haupt(13) have reported formation of lysolecithin in serum during antigen-antibody-complement reactions *in vitro*, but evi-

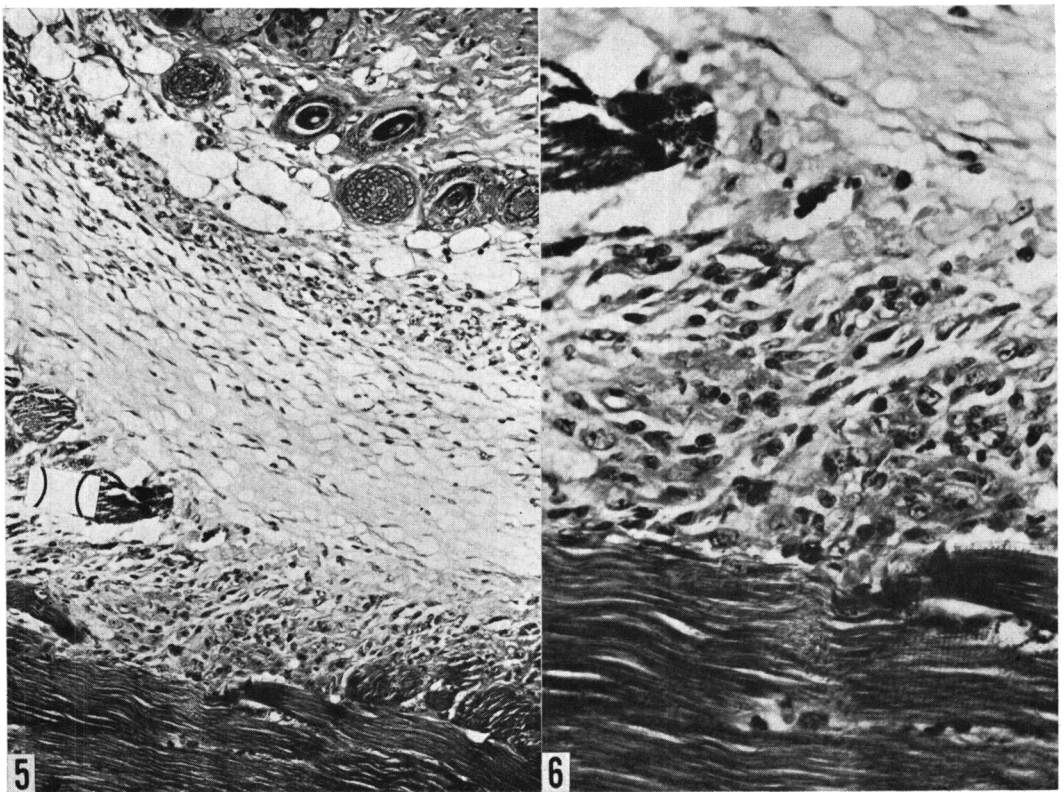

FIG. 5. At 78 hours there is a broad band of necrosis in superficial portion of abdominal wall muscle. Platysma muscle is represented only by a rim of histiocytes and regenerating muscle nuclei in superficial corium. Hematoxylin and Eosin × 75.

FIG. 6. A portion of Fig. 5 at a greater magnification. Note histiocytic character of cellular reaction. Hematoxylin and Eosin × 188.

dence has been presented to the contrary(7). Immune hemolysis and tissue immune reactions, however, differ in several respects(2). Indeed, Keller(14) has reported formation of lysolecithin in rat mast cells during antigen-antibody reaction *in vitro*. If lysolecithin were formed during tissue immune reactions, the evidence presented here indicates that it could contribute significantly to tissue damage. It could also account for edema production, as seen with urticaria and angioneurotic edema. The potent cytotoxic effect of lysolecithin, in addition, could account in large part for the deep necrosis often seen at the site of a snakebite.

Summary. A series of mice was injected subcutaneously with lysolecithin and the tissues examined at measured intervals. Within 10 minutes after injection, striking edema with necrosis of fat tissue was evident at the injection site. Subsequent changes included infiltration with neutrophil leukocytes and histiocytes and hyalinization and necrosis of muscle cells. Despite the marked edema and contrary to previous reports, vascular damage was not demonstrated by light microscopy. The production of lysolecithin could account for much of the local necrosis resulting from snakebite.

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Effect of Defatted Brain Extract and Soy Sterols on Plasma Cholesterol Levels and Atherogenesis in Cholesterol-Oil Fed Cockerels.* (30318)

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It has long been inferred that elevated serum cholesterol levels are positively correlated with degree of atherosclerosis, both in animals and man. Consequently, intensive efforts have been made to devise means to reduce serum cholesterol levels in the hope of preventing atherosclerosis. The search goes on for an ideal hypocholesterolemic agent which would be practical to administer and harmless even on extended use.

Brain extract (cerebroside), and sitosterols from plants have been reported by various workers to have a favorable hypocholesterolemic effect both in clinical trials and in animal studies(1-6). The present study further evaluates the status of these products in re-

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