in the infrared spectrum despite little or no removal of fatty acid esters from the substance. These results together with those reported by Noll and Braude make it clear that no evidence has been established for correlation between fatty acid ester content and toxic properties of the endotoxin. It is conceivable that, in the case of *E. coli,* the endotoxin in the Boivin extract is accompanied by nontoxic protective substances. The treatment with lithium aluminum hydride then would not selectively modify a portion of the endotoxin and render it nontoxic, but would separate the nontoxic antigen from the endotoxin by subsequent precipitation with aluminum hydroxide. Further discussion is best deferred until the nontoxic antigenic fraction from *E. coli* has been chemically characterized.

Summary. Treatment of conventional aqueous ether extracted endotoxins from *Salmonella enteritidis* with aqueous phenol, followed by high speed centrifugation, yielded material having high biological potency with a nitrogen content of 0.4-0.5% and a fatty acid content of 3-4%. Upon reacting this purified endotoxin with lithium aluminum hydride and citric acid solution, an aluminum citrate-endotoxin complex was recovered which had the desirable features of high biological potency and a rapid rate of solution in water. Non-phenolized endotoxins extracted from *S. enteritidis* with aqueous ether or trichloroacetic acid also formed aluminum citrate complexes under the same conditions. These were biologically potent but somewhat more difficult to dissolve. After prolonged treatment with lithium aluminum hydride, the aluminum citrate complexes still contained 1-2% esterified fatty acids. Lipid fractions isolated from acid hydrolysates of the complexes were biologically inactive.

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Effect of Hydrocortisone on Experimental Silicotic Nodule.* (27967)

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There have been conflicting reports on the effects of corticosteroids on the progression and maturation of the silicotic nodule, one report even denying that steroids reduce total fibrosis(1). While a number of other studies have confirmed a diminished quantity of fibrosis in steroid-treated animals(2-9), some have suggested that this is due to an arrest in the transformation of reticulin to collagen(7), while others indicate that the maturity of fibrosis is unaffected by corticosteroid administration(4,5). Both decreased cellularity of treated lesions(2,3) and increased cellularity(7) have been reported. In the opinion of some investigators, the principal effect of cortisone is on the migration of dust cells and dispersion of silica dust(1,4,6).

The present study concerns the effect of hydrocortisone on the progression and maturation of the silicotic nodule. In an attempt to minimize the effects of steroids on the mo-

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bility of phagocytic cells, the method of Zaidi, *et al.*(10) was used to produce silicotic nodules in mouse livers.

Materials and methods. Swiss strain, white, virgin, female mice were selected as the experimental animal. Silica nodules were induced by a suspension of 7.5 mg of 1-2 μ particles of St. Peters sandstone† injected into the tail vein. Hydrocortisone‡ was administered subcutaneously in a daily dose of 2.5 mg.

Mice were divided randomly into 6 groups: 1) Given neither silica nor hydrocortisone, 2) Given no silica but treated with hydrocortisone, 3) Given silica but not hydrocortisone, 4) Given silica and treated with hydrocortisone until sacrifice of the animal, 5) Given silica and treated with hydrocortisone for 150 days, 6) Given silica, with hydrocortisone therapy begun 95 days after silica injection and continued for 105 days.

Mice were sacrificed at 30, 95, 150, 200 and 250 days after silica injection. A total of 110 mice were sacrificed; in each, 4 sections of the left lobe of the liver were made after fixation in Bouin's solution. One section was stained with hematoxylin and eosin, one with silver, one with trichrome, and one unstained but mounted. Slides were then randomized and graded on an arbitrary scale to evaluate the extent of fibrosis, reticulin, and collagen formation. This grading was done "blindly," observers being unaware which animal the slide represented. The silver and trichrome stains were graded separately, each slide being graded twice by 2 observers. Final grades represent an average of these values.

Histological interpretations were made by both authors with the guidance of Dr. Frank W. Fitch, Dept. of Pathology, University of Chicago.

† St. Peters sandstone from Ottawa, Ill. was kindly supplied by Mr. Pitchard of Western Material Co., Chicago, Ill. Its composition is: SiO_2 99.820%, Fe_2O 0.019%, Al_2O_3 0.049%, TiO_2 0.012%, CaO 0.006%, and MgO 0.031%. One to 2 μ particles were prepared by the method of Cummings(11).

‡ Hydrocortisone phosphate was kindly supplied by Merck, Sharp & Dohme.

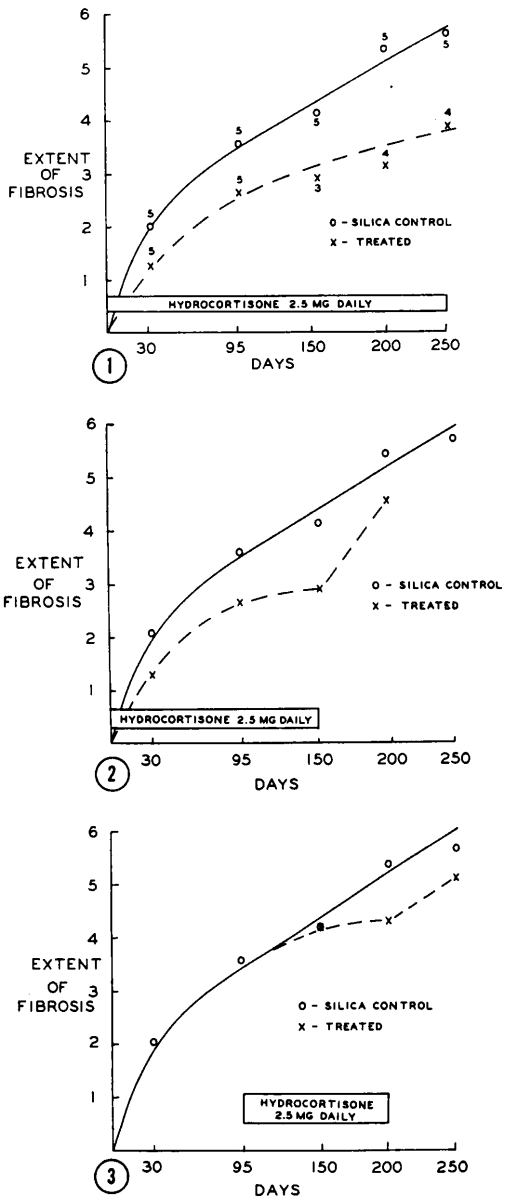

FIG. 1. Effect of treatment with 2.5 mg of hydrocortisone phosphate daily from day 1-day 250. The number at each point indicates number of animals. The difference between the 2 curves is significant by Student's Test beyond 150 days. (P <.01 of chance occurrence.)

FIG. 2. Effect of treatment with 2.5 mg of hydrocortisone phosphate daily from day 1 to day 150. Each point represents avg value for 5 animals.

FIG. 3. Effect of treatment with 2.5 mg of hydrocortisone phosphate daily from day 95 to day 200. Each point represents avg value for 5 animals.

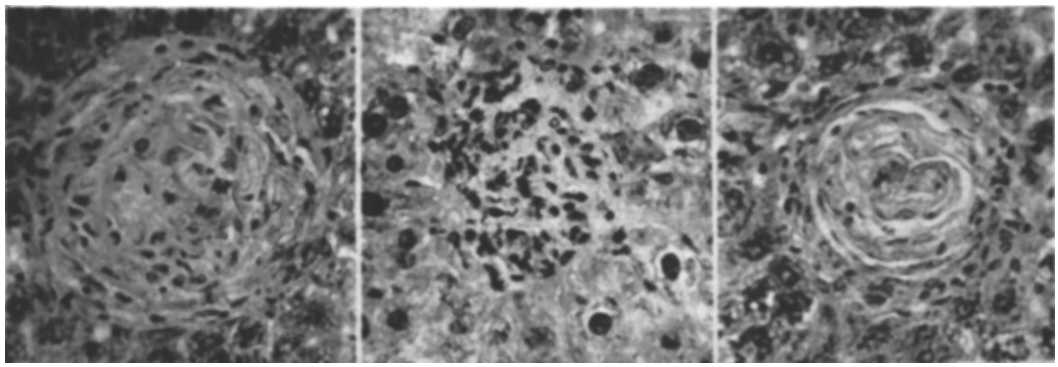

FIG. 4. Photomicrographs of silicotic nodules (×330). On left, a control nodule at 250 days. In the center, a nodule in a hydrocortisone treated animal at 250 days, and on right, a control lesion at 150 days. (Hematoxylin and eosin stain.)

Results. Extent of lesions. Control Groups 1 and 2 show no pathologic change in the liver throughout the 250-day observation period. Thymus size is markedly reduced in Group 2 (hydrocortisone treated), but these animals otherwise appear as healthy as Group 1 (untreated).

As noted in Fig. 1-3, treatment at any time causes a slowing of the progression of lesions but does not cause regression or complete cessation of growth of nodules. It also appears that the effect of hydrocortisone lasts only as long as the steroid is administered, treated lesions returning to the levels of the controls when treatment is discontinued. Reticulin and collagen formation as judged histologically from silver and trichrome stains are parallel in all groups studied.

Histologic appearance. By 30 days, lesions are seen in all parts of the liver lobules, and small quantities of collagen are noted. Treated and control nodules are qualitatively similar, both revealing considerable cellularity, primarily with mononucleated cells. Outline of cell "ghosts" may be seen and there are fragments of darkly staining material throughout the nodule. The silica, though scattered throughout the nodule, is clumped, and occasionally a cell outline can be seen surrounding silica particles.

Between 90 and 250 days, control lesions become more patterned, with development of a typical "swirl" appearance of collagen fibers. The cells of early lesions are replaced by spindle shaped cells resembling fibroblasts. By 250 days, control nodules are classical silicotic nodules; some show central necrosis.

Differences between control and treated lesions are noted as early as 90 days but become more obvious in later nodules. Treated lesions show a disrupted pattern of loosely packed collagen and continue to contain many cells similar to those of early lesions as well as dark staining debris. The treated lesions are less well defined than controls; at times, their collagen surrounds parenchymal liver cells.

The difference in nodule size and histologic appearance of control *vs* treated lesions at 250 days may be noted in Fig. 4. A control lesion at 150 days is also shown. The size of treated nodules at 250 days is similar to that of control lesions at 150 days. However, there are qualitative differences in the appearances of these lesions.

Discussion. It seems clear that adrenocortical steroids do affect the nodules of silicosis, slowing the progression of lesions. This effect is probably completely erased when corticosteroids are withdrawn. Histologic sections also reveal an effect of steroids on maturation of the nodules. Differences between control and treated nodules might be interpreted as indicating that treated nodules remain in a state of constant inflammatory reaction, but the mechanism of this effect is not clear. Hydrocortisone does not seem to have any specific effect on formation of collagen from reticulum (if this occurs at all) as suggested by Marenghi(7); in the present study, there was a complete parallelism between the

development of reticulum and collagen in treated as well as in control animals, at least as judged by histologic technics. There is some evidence that fibroblastic proliferation is inhibited by corticosteroids(12,13), and a failure of development of the fibroblast might be responsible for the lack of organization and continued inflammatory response noted in the treated nodules.

Regardless of the mechanism of action of hydrocortisone, in view of the rapid increase in nodule size on cessation of therapy, it would appear that the mouse is incapable of effectively "detoxifying" silica particles while on steroids. This failure to inactivate silica may account for the continued cellular exudate noted in treated lesions. Whether this continued toxicity is directly related to inhibition of fibrosis or is due to another effect of hydrocortisone cannot be determined from these experiments.

Summary. The effect of treatment with hydrocortisone upon growth and maturation of the silicotic nodule has been studied in mouse liver. Treatment slowed growth of lesions and development of collagen and reticulin regardless of the time at which therapy was instituted. When hydrocortisone was discontinued, however, the size of nodules returned close to control level. Histological differences were noted between control and treated nodules after 150 days. These differences (disorganization and cellularity of treated nodules) suggest a continuation of the early inflammatory response in hydrocortisone treated lesions.

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Mammary Gland Enzyme Systems Concerned with Synthesis of Monoiodotyrosine.* (27968)

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Research on the secretion of administered I^{131} into milk has been reviewed by Brown-Grant(1). It is now well established that mammary tissue of man and the common laboratory and domestic animals has an active iodide concentrating mechanism capable of maintaining an elevated milk:plasma I^{131} ratio. Results in goats(2,3) and in rats(4,5) suggest that under conditions of limited iodine intake mammary tissue may actively compete with the thyroid for available iodine, resulting in reduced thyroxine formation. Iodine secreted into milk *in vivo* occurs in part as iodide and in part as monoiodotyrosine in peptide linkage in the milk protein(6).

Recently we reported(7) that lactating rat mammary tissue has the ability to concentrate I^{131} *in vitro* and defined some of the conditions influencing this process, but did not identify the iodinated compounds formed. It was of interest, therefore, to determine the

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