EFFECT OF CARTILAGE AND OTHER TISSUE SUSPENSIONS ON REPARATIVE PROCESSES OF CORTISONE-TREATED ANIMALS*

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In previous reports from this laboratory,[1,2] experimental evidence has been brought forth which suggests that cortisone in adequate doses probably blocks the release from injured tissues of chemical substances which are necessary for the series of events known as inflammation and repair.

Experiments aimed at identifying this elusive material have continued. Hyaluronic acid, hyaluronidases (from testicular and bacterial sources), lysozyme, ion exchange resins, embryo extract, gelatin, oxidized cellulose, collagenase, suspensions of talc powder, and mechanical trauma have failed in successive series of cortisone-treated and control rats, using the method described in a previous paper,[2] to overcome appreciably the effect of this steroid (unpublished experiments).

In a more recent series of experiments, suspensions and homogenates of different tissues were tested for their possible rôle in modifying the cortisone-induced depression of the reparative process.

METHOD, MATERIALS, AND RESULTS

A. Cartilage

It has been our suspicion that the substances which fail to be released in the injured tissues under the effect of cortisone are probably mucopolysaccharide in nature.[1,2] Consequently, suspensions of powdered cartilage of different origins, which are rich sources of acid mucopolysaccharides and especially of the chondroitin sulfates, were tested.

These studies were made on 29 cortisone-treated and 21 control rats. White male Wistar rats weighing around 200 gm. were given a daily intramuscular injection of 15 mg. of cortisone acetate until sacrificed. On the fourth day, a sterile surgical gauze pledget was

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placed aseptically in the back at the termination of a subcutaneous tunnel, using a technique described previously.[3]

The following preparations were used. 1. Commercial powdered bovine tracheal cartilage prepared by short treatment with acid pepsin was autoclaved for 45 minutes in steam and a suspension was made in the proportion of 1 gm. to 7.5 ml. of an antibiotic solution, each ml. containing 4,000 units of penicillin and 8 mg. of streptomycin. Ten cortisone-treated and 6 control rats were used in this experiment.

2. Finely ground bovine tracheal cartilage, not treated with acid pepsin, was autoclaved and then added to the antibiotic solution, as outlined previously. Ten cortisone-treated and 3 control rats were used in this experiment.

3. Fresh pig's knuckle cartilage was finely minced and put in the antibiotic solution in the same proportions. Four cortisone-treated and 4 control rats were used in this experiment.

4. Finely ground bovine tracheal cartilage was subjected to extraction with water and the insoluble residue was used. Five cortisone-treated and 5 control rats were used in this experiment.

Each day 1 ml. of one of the cartilage preparations and 1 ml. of penicillin-streptomycin mixture were injected through the skin onto the pledget. Control groups received cholesterol suspension in the same vehicle, intramuscularly, in lieu of cortisone acetate. On the fifth postoperative day the animals were sacrificed and bacterial cultures were taken from the gauze pledgets. Infection, or at least positive cultures at necropsy, occurred in 5 cortisone-treated and 4 control animals. These were not included in the analysis of the results since it had previously been demonstrated that pyogenic infection might overcome the inhibitory effect of cortisone on granulation tissue.[2]

Results. In contrast to the picture previously reported[1,2,4] in which the pledgets remained free in cortisone-treated animals, the pledgets were, in general, encapsulated and adherent to the surrounding tissue of the cortisone-treated, cartilage-injected animals and some force had to be employed to free them. The microscopic examination (Fig. 1) showed consistently abundant granulation tissue, including large numbers of fibroblasts and new blood vessels, with minimal acute inflammation. This effect was purely local and dependent upon contact with the cartilage, since the skin wounds of the cartilage-injected, cortisone-treated animals showed little or no connective tissue repair. In these experiments the acid-pepsin-treated, autoclaved, beef tracheal cartilage apparently was more potent in producing this picture than

either autoclaved beef tracheal cartilage or fresh pig's knuckle cartilage. These preparations, obviously, cannot be compared quantitatively because crude suspensions were utilized and consequently there were variations in the amount of cartilage in each milliliter of suspension. The amount of suspension in contact with the pledgets also varied from day to day and from animal to animal and two of the preparations were autoclaved, while the fresh cartilage was not dehydrated.

In the cholesterol-treated control rats, reparative functions were very abundant and the pledgets were encased in a firm capsule of granulation tissue (Fig. 2).

B. Beef Bone

Experiments similar to those of group A were made on 12 cortisone-treated and 3 control rats. The same technique was used, only instead of cartilage a suspension of acetone-dried, powdered, but not decalcified beef bone was used in 6 cortisone-treated animals and in 3 controls; and bone spicules from human ilium were used in 6 cortisone-treated animals. The material was inserted with the pledget at the time of operation but not injected daily, as in the previous experiments. When the animals were killed on the fifth postoperative day, the gauze pledgets in the cortisone-treated rats were either free or loosely adherent. Microscopically, minimal fibro-angioplasia was present in the tissues surrounding the gauze. In the control animals the gauze pledget was encapsulated (Figs. 3 and 4) and there was abundant granulation tissue surrounding it.

C. Beef Tendon

Using the same technique, autoclaved, shredded beef tendon suspension was used daily in 13 cortisone-treated and 8 control rats. At necropsy on the fifth day the gauze pledgets of the cortisone-treated animals appeared to be surrounded by a semitransparent adherent membrane; and, microscopically, focal areas of loosely cellular, young granulation tissue were seen (Fig. 5). In the controls, there was the usual firm encapsulation consisting of abundant granulation tissue (Fig. 6).

D. Chondroitin Sulfate C

Four cortisone-treated and 4 control rats were used in a similar experiment, in which chondroitin sulfate C (dose: 1 cc. of a 2 per cent solution) was injected daily upon the implanted pledget of gauze.

On the fifth day, necropsy showed that the foreign body was either free or loosely attached to the surrounding tissues. Microscopic study showed rare and small foci of minimal fibro-angioblastic proliferation (Fig. 7). The controls showed the usual fibrous granulation tissue response (Fig. 8).

E. Chondroitin Sulfate A and Gelatin

Ten cortisone-treated rats, prepared as before, received daily injections of chondroitin sulfate A and gelatin (6 gm. of chondroitin sulfate and 2 gm. of gelatin in 75 cc. of distilled water). One cc. was injected daily upon the implanted foreign body. No controls were used. On the fifth day, necropsy showed that the gauze pledget was moderately adherent to the surrounding tissues by a thin hyperemic membrane. Histologically, minimal to slight focal repair (Fig. 9) was observed, the foci of granulation tissue being associated with pools of mucoid metachromatic material.

F. Umbilical Cord

In 6 cortisone-treated rats, using the same technique, dried (human) umbilical cord was introduced subcutaneously together with the pledget of gauze. No controls were used in this experiment. On the fifth day, necropsy showed minimal to moderate reparative tissue reaction around the foreign body (Fig. 10).

In all of these experiments, the skin wounds of the cortisone-treated animals, which were not in immediate contact with the substances tested, failed to show evidence of repair.

Discussion

From previous studies[1,2,4] it has been suggested that under the influence of cortisone, some substance, perhaps chemotactic for the cells necessary to complete the reparative process, is not elaborated or is inactivated locally. The observation that a suspension of autoclaved cartilage can overcome the cortisone effect seems to imply that this postulated substance is present in such a suspension and is not heat labile because it withstands steam sterilization. The fact that minimal to moderate reparative responses were obtained using suspensions of bone, tendon, and umbilical cord, respectively, should not be surprising in the light of our working hypothesis. In fact, the composition of the ground substance of these tissues is, in certain respects, similar to that of cartilage, even though it differs in concentration and patterns of mucopolysaccharides.

The practically negative results obtained with daily injections of chondroitin sulfate C solutions might be due simply to the rapid disappearance of a water-soluble substance from the site of injection. The more marked effect of chondroitin sulfate A and gelatin might result from less rapid diffusion due to binding by the gelatin. Of course, there may be other reasons. For instance, these acid mucopolysaccharide salts in the form and composition used might well be different from the active principles necessary in the reparative processes. Since gelatin alone does not exhibit a similar effect (unpublished experiments), the active principle presumably is not in the collagen moiety of cartilage. Whether this substance is released from damaged cells or is elaborated by surviving cells in the area of trauma is still to be established. It is possible that the preparations used may contain a substance which inhibits the steroid "pharmacologically." Experiments are continuing in this laboratory with the aim to characterize the hypothetic active material present in these crude suspensions which appears to overcome the blocking effect of cortisone on the reparative phenomena of the connective tissues. Perhaps such studies may be carried out best by tissue culture methods, testing a series of purified mucopolysaccharides and other substances. In support of this is the fact that workers in the field of tissue culture have been aware for many years that fragments of cartilage added to a culture of fibroblasts will stimulate their growth.[5,6] Healy *et al.*[6] demonstrated that at least part of this effect was caused by glucuronic acid.

Summary

Several different crude preparations of cartilage, when injected as suspensions into cortisone-treated animals, were observed to overcome locally the cortisone-induced depression of reparative processes. Similar but much less marked effects were obtained with suspensions of powdered bone, tendon, umbilical cord, and with a preparation containing chondroitin sulfate with gelatin. These findings suggest that cortisone interferes with the reparative processes by blocking the local elaboration or activation of some chemical substance. This chemical factor (or factors) apparently is present in tissues whose ground substance is rich in acid mucopolysaccharides.

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REFERENCES

1. Lattes, R.; Blunt, J. W., Jr.; Rose, H. M.; Jessar, R. A.; Vaillancourt, deG., and Ragan, C. Lack of cortisone effect in the early stages of inflammation and repair. *Am. J. Path.*, 1953, **29**, 1–19.

2. Lattes, R.; Martin, J. R., and Ragan, C. Suppression of cortisone effect on repair in the presence of local bacterial infection. *Am. J. Path.*, 1954, 30, 901–911.

3. Lattes, R., and Frantz, V. K. Absorbable sponge tests. *Ann. Surg.*, 1945, 121, 894–896.

4. Ragan, C.; Lattes, R.; Blunt, J. W., Jr.; Vaillancourt, deG.; Jessar, R. A., and Epstein, W. The effect of cortisone upon repair processes in dense and loose connective tissues. In: The Effect of ACTH and Cortisone upon Infection and Resistance, Shwartzman, G. (ed.). Symposia of the Section on Microbiology, New York Academy of Medicine, Columbia University Press, 1953, No. 6, p. 46–55.

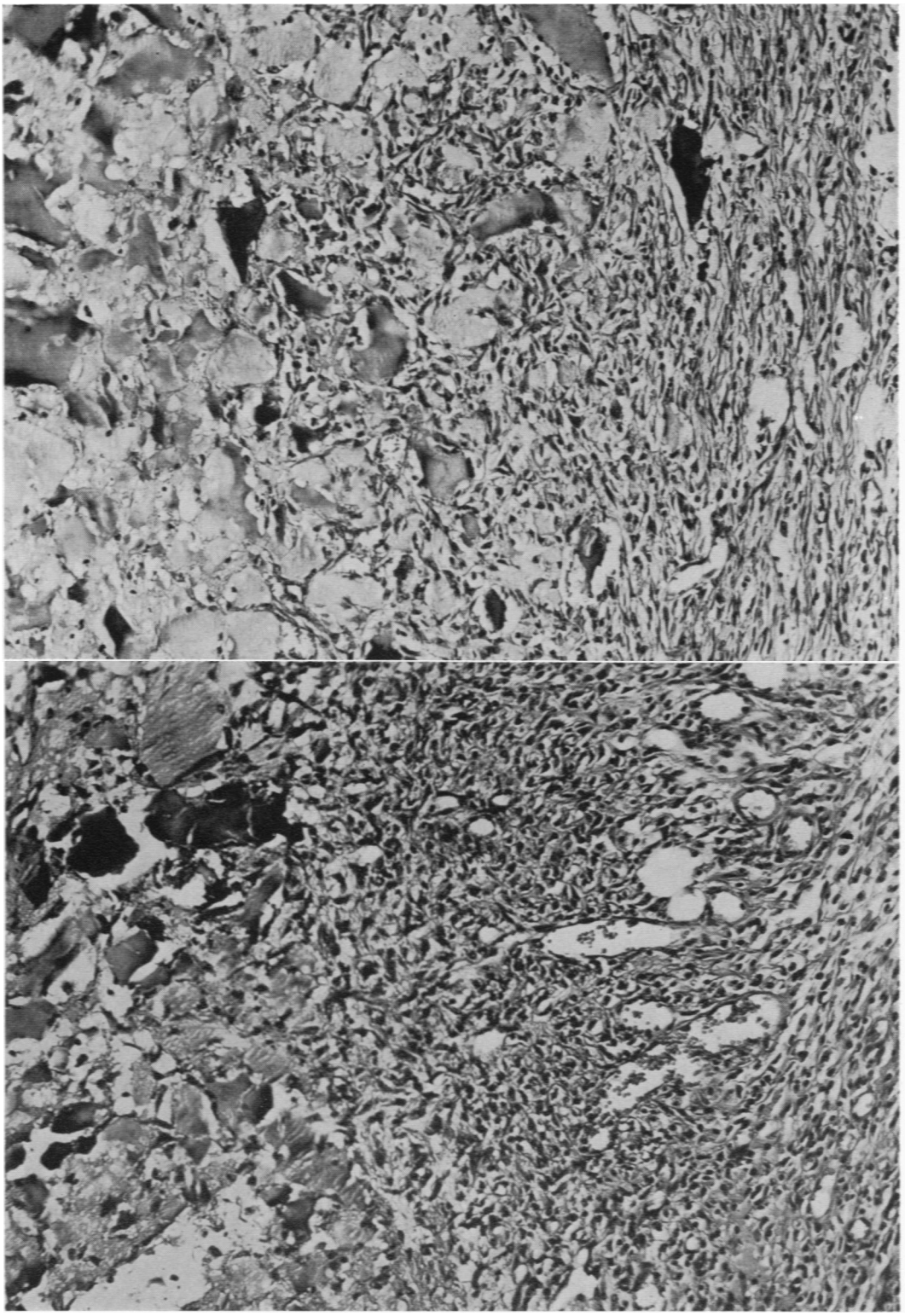
5. Davidson, J. N., and Waymouth, C. Factors influencing the nucleoprotein content of fibroblasts growing in vitro. *Biochem. J.*, 1943, 37, 271–277.

6. Healy, G. M.; Fisher, D. C., and Parker, R. C. Nutrition of animal cells in tissue culture. X. Synthetic medium No. 858. *Proc. Soc. Exper. Biol. & Med.*, 1955, 89, 71–77.

LEGENDS FOR FIGURES

FIG. 1. Zone of granulation tissue associated with implanted pledget of gauze and injections of cartilage suspensions in a cortisone-treated rat, 5 days postoperatively. The cartilage preparation used was autoclaved, pepsin-treated, bovine tracheal cartilage. Hematoxylin and eosin stain. × 165.

FIG. 2. Zone of granulation tissue associated with implanted pledget of gauze and injections of cartilage suspensions in a control rat, 5 days postoperatively. The cartilage preparation used was autoclaved, pepsin-treated, bovine tracheal cartilage. Hematoxylin and eosin stain. × 165.



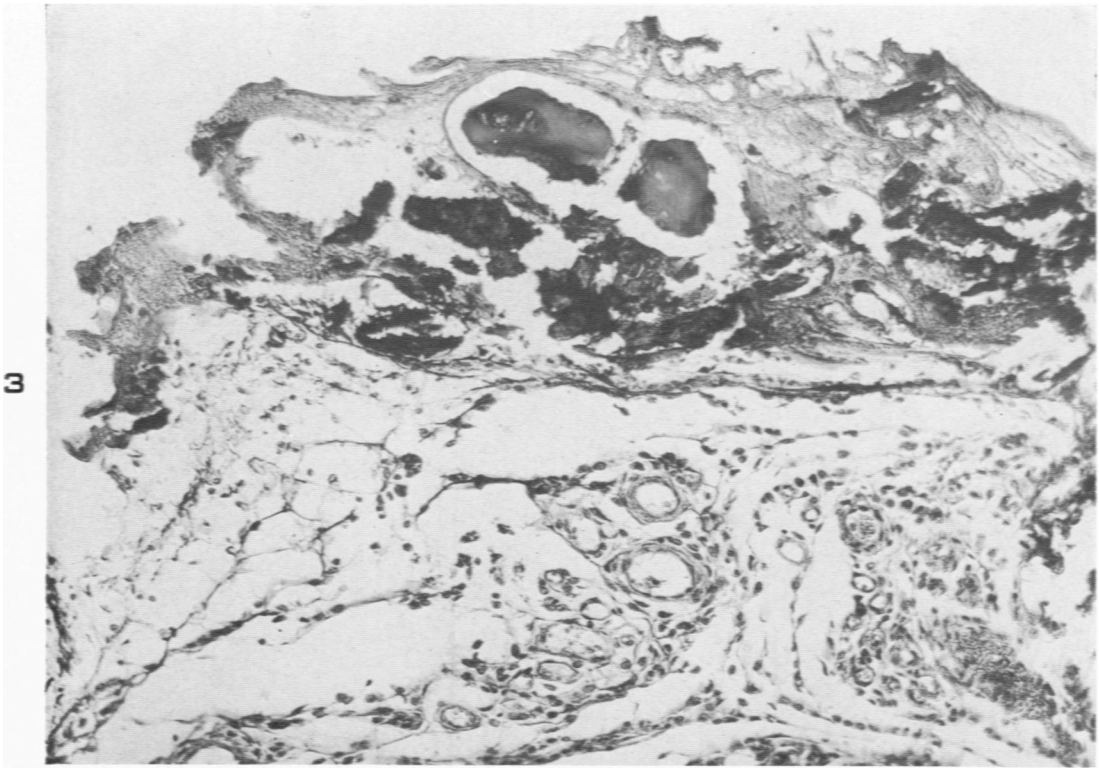
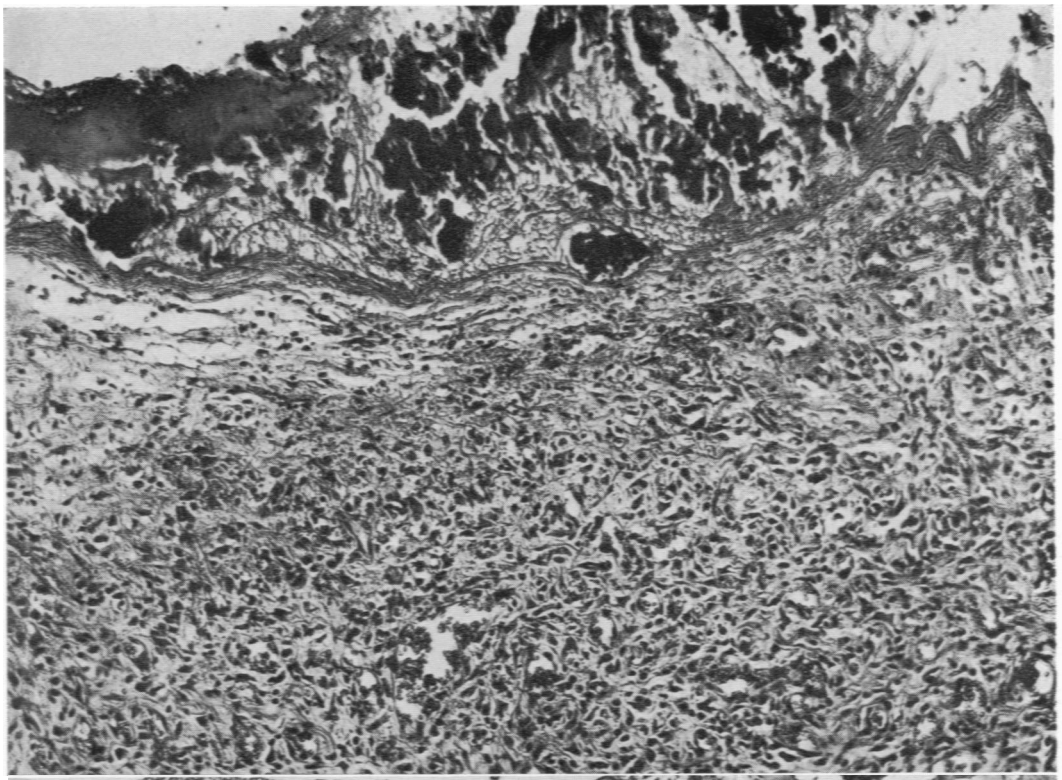


FIG. 3. Two small foci of fibro-angioplasia associated with a pledget of gauze and powdered bone suspension, in a cortisone-treated rat, 5 days postoperatively. Hematoxylin and eosin stain. × 165.

FIG. 4. Abundant granulation tissue associated with a pledget of gauze and powdered bone suspension in a control rat, 5 days postoperatively. Hematoxylin and eosin stain. × 165.

FIG. 5. Small focus of fibro-angioplasia associated with a pledget of gauze and shredded beef tendon suspension in a cortisone-treated rat, 5 days postoperatively. Hematoxylin and eosin stain. × 165.



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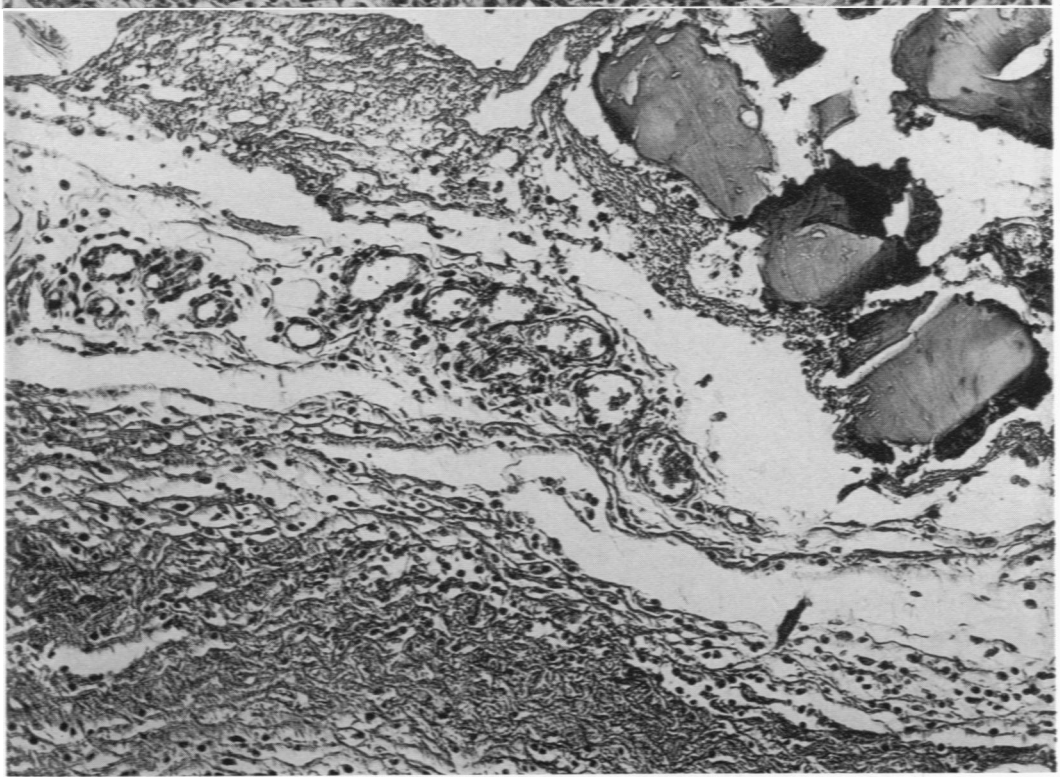

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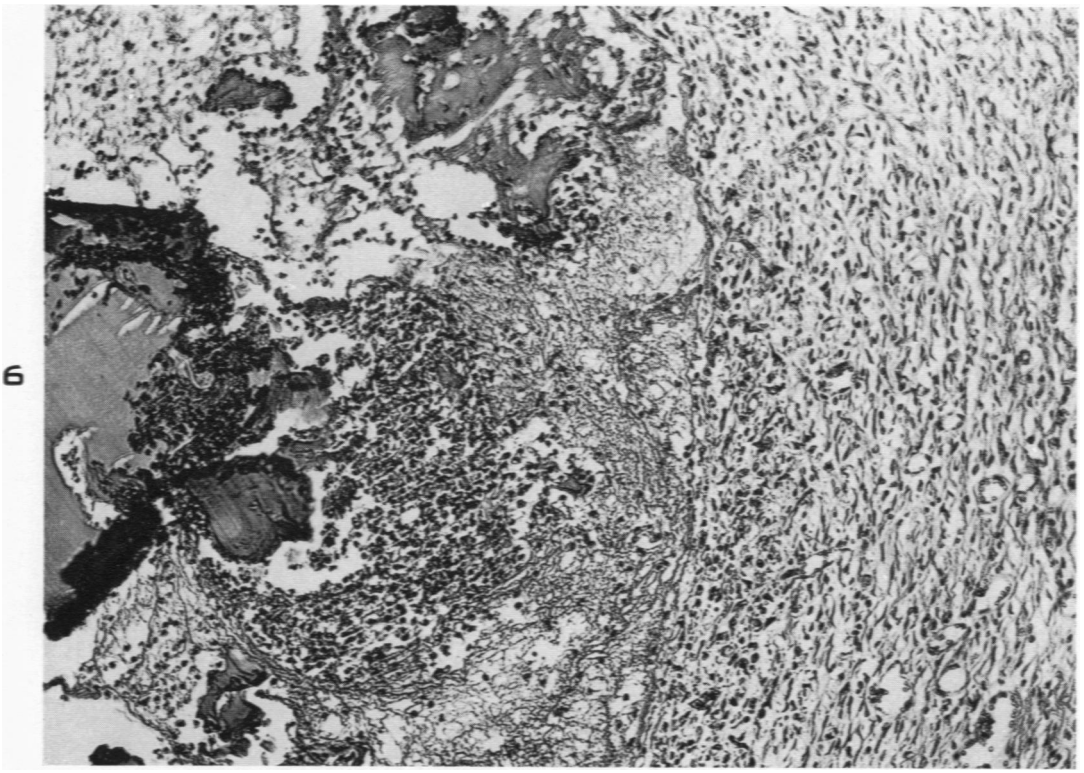
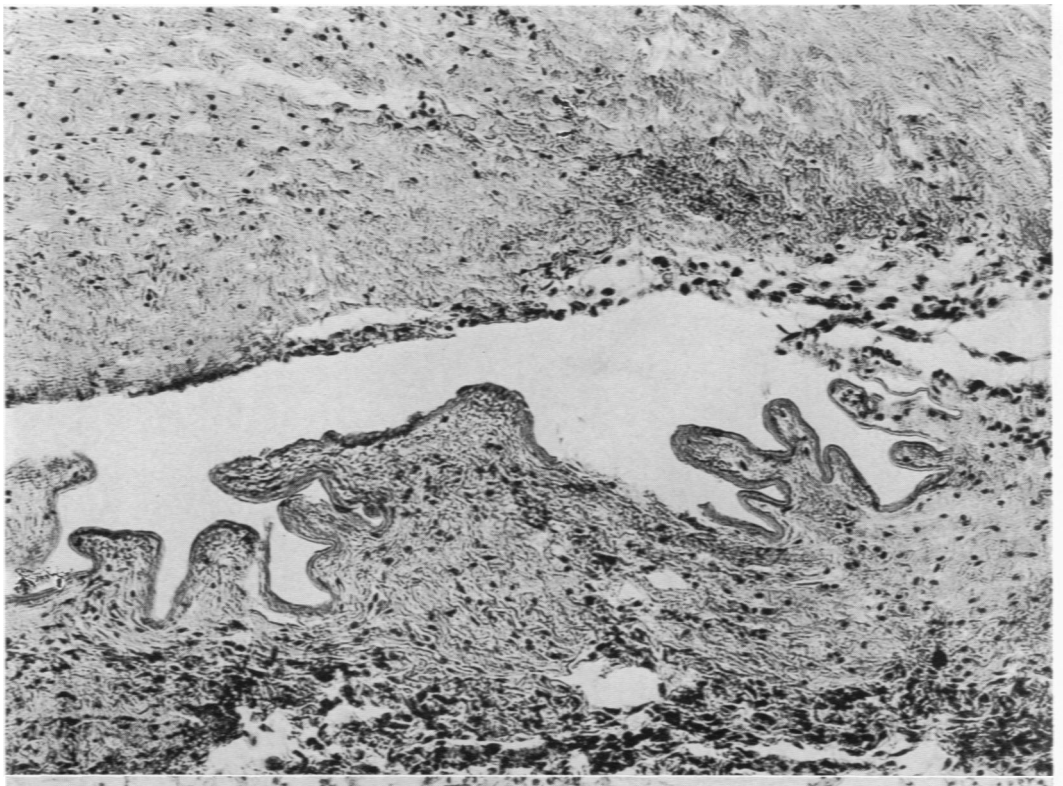


FIG. 6. Abundant granulation tissue associated with a pledget of gauze and shredded beef tendon suspension in a control rat, 5 days postoperatively. Hematoxylin and eosin stain. × 165.

FIG. 7. Cortisone-treated rat, 5 days postoperatively. Practically complete lack of reparative response in association with a pledget of gauze in which daily injections of a 2 per cent solution of chondroitin sulfate C were made. Only scattered wandering fibroblasts are seen but no capillary proliferation. The hyalinized fibrinous membrane was a frequent finding. Hematoxylin and eosin stain. × 165.

FIG. 8. Control rat, 5 days postoperatively. Highly cellular, abundant granulation tissue in association with a pledget of gauze into which daily injections of a 2 per cent solution of chondroitin sulfate C were made. The cellularity is due in part to mononuclear and polymorphonuclear leukocytic infiltration. This was seen in most of the animals of this group treated with chondroitin sulfate C. Bacteriologic cultures were negative. Hematoxylin and eosin stain. × 165.



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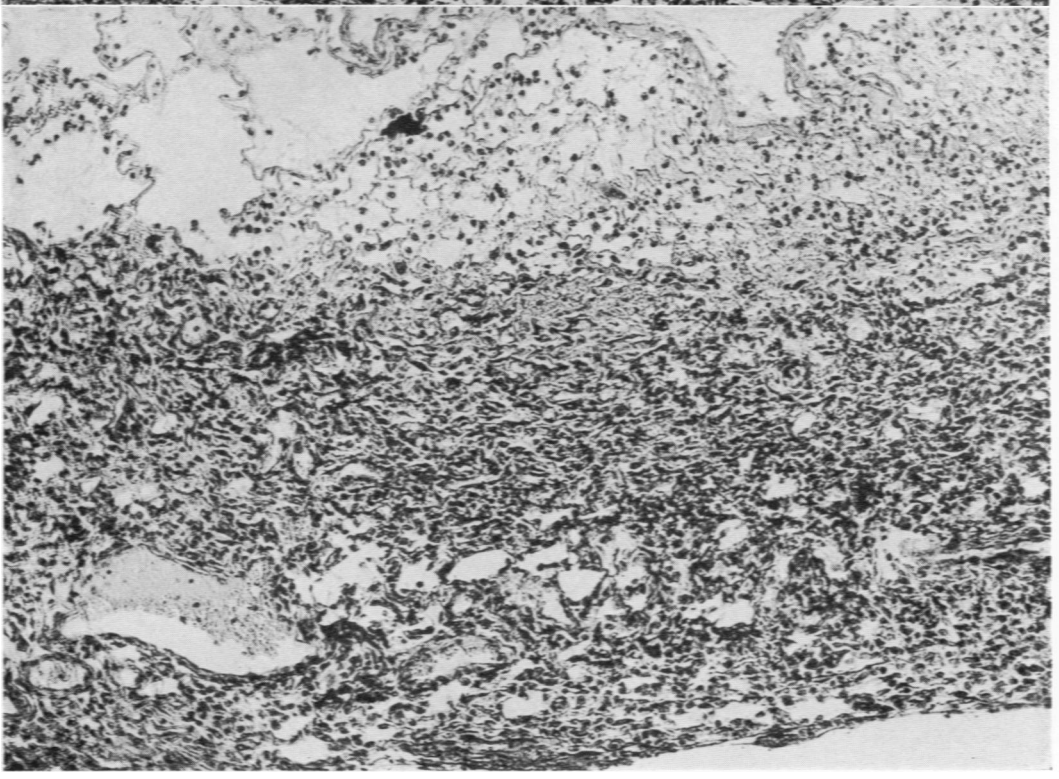

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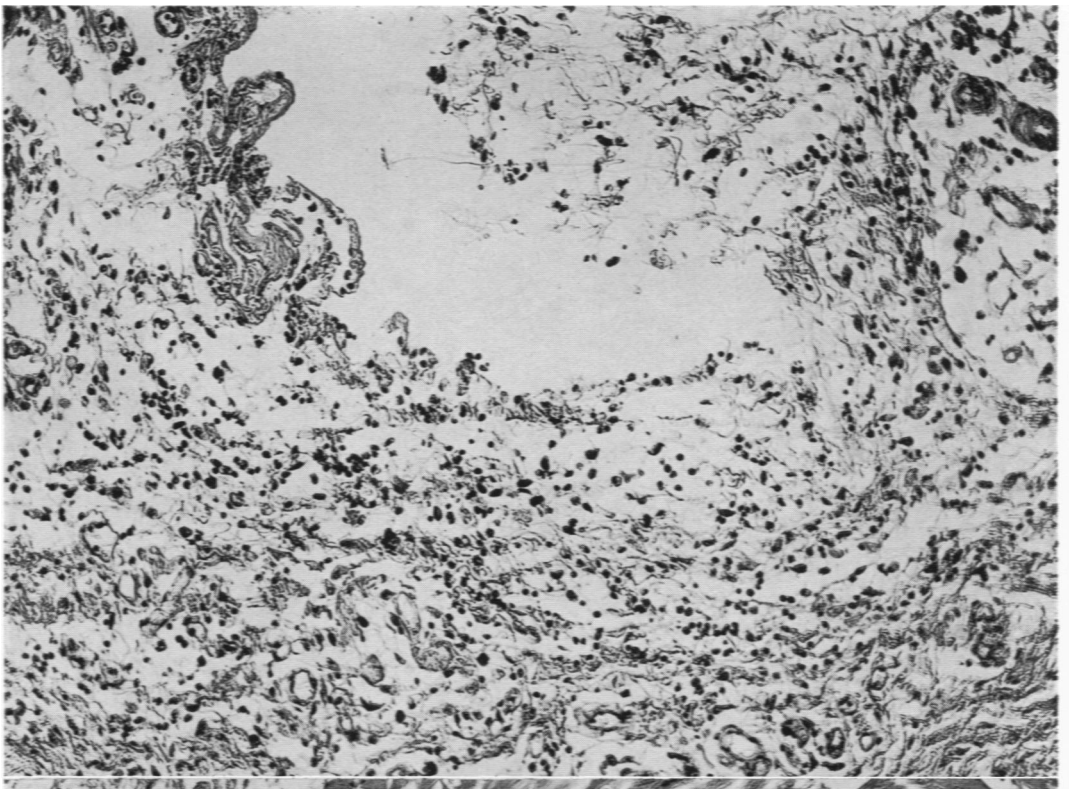
FIG. 9. Cortisone-treated rat. 5 days postoperatively. Tissue surrounding a pledget of gauze into which daily injections of chondroitin sulfate A and gelatin were made. Minimal, sparse, capillary and fibroblastic proliferation is associated with a predominantly mononuclear exudate. Hematoxylin and eosin stain. × 165.

FIG. 10. Cortisone-treated rat. 5 days postoperatively. Tissue surrounding a pledget of gauze associated with a suspension of dried (human) umbilical cord. There is moderate to minimal diffuse fibro-angioplasia. Hematoxylin and eosin stain. × 165.



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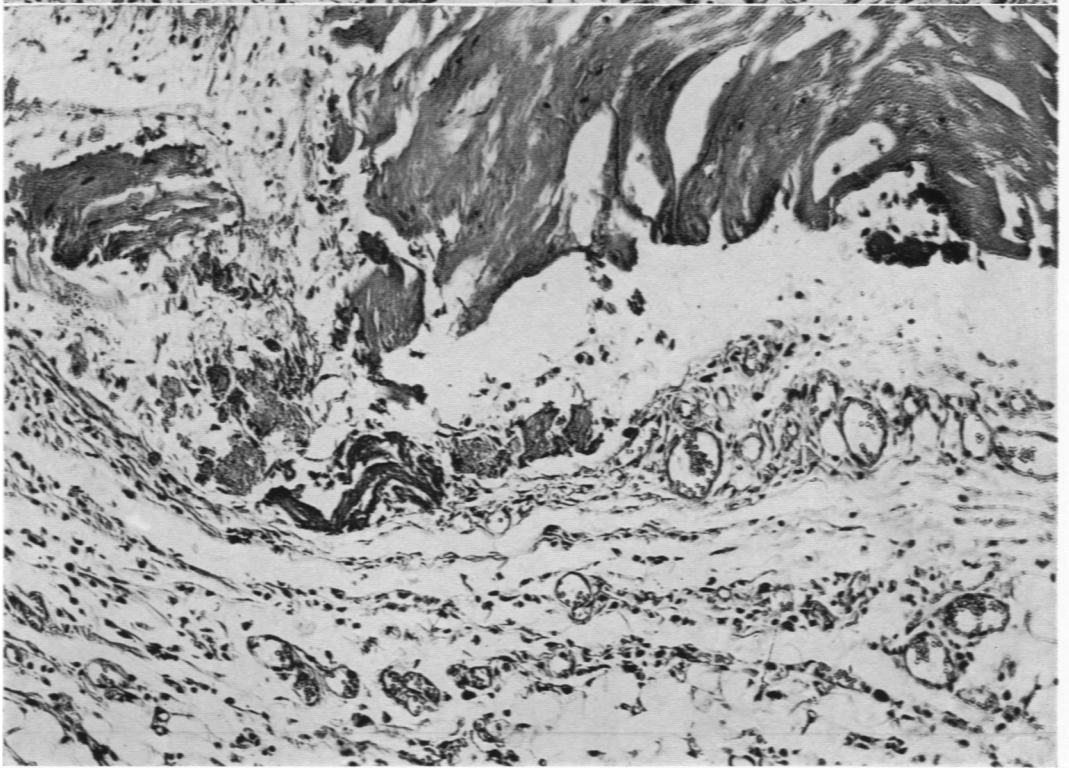

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