TABLE IV. Influence of Ionic Strength (μ) on Hemolysis of Guinea Pig Erythrocytes.

Supernates from	μ .05	.1	.15	.2	.4
B. dermatitidis	32	32	16	8	4
H. capsulatum	64	64	64	32	16

NaH_2PO_4 buffer was varied at pH 6.7 from 0.05 to 0.4, and the effect on hemolysis of guinea pig cells examined (Table IV).

An ionic strength of 0.1 was therefore used for future experiments.

The hemolysins of *B. dermatitidis* and *H. capsulatum* lost no activity after exposure to 60°C for 30 minutes, but were destroyed after autoclaving for 15 minutes at 15 lb. pressure.

C. albicans and *C. neoformans* hemolyzed guinea pig erythrocytes in supernate dilutions of 1:4 and 1:2 respectively, when buffered at pH 6.7. This action seemed especially interesting since these two fungus supernates had no detectable nitrogen (Table I), but had the highest carbohydrate concentration. In addition, since *H. capsulatum* supernate had the highest nitrogen content and the highest hemolysin activity, the probability presents itself that the hemolysin is a protein or protein-like substance.

Attempts were made to detect specific antibodies (both human and rabbit) by absorption of the hemolysin with specific immune serum. Thus far, however, the test has not proved of value because of the strong hemolysin-absorption properties of "normal" rabbit and human sera.

Summary. One or more hemolysins were demonstrated in soluble extracts prepared from the yeastlike phases of *Histoplasma capsulatum, Blastomyces dermatitidis, Candida albicans,* and *Cryptococcus neoformans.* The hemolysin was most active against hen and guinea pig erythrocytes. Some properties of the hemolysin were investigated.

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Local Effects of Cortisone on Granulation Tissue and the Role of Denervation and Ischemia.* (18654)

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The effect of cortisone upon connective tissue structures has been well established. All the elements of such tissues seem to be influenced by this steroid(1-10). The mechanisms, by which cortisone acts upon connective tissue structures, are unknown. In the light of our present knowledge, a number of possible mechanisms warranted investigation. It has been well established that C_{11} oxysteroids have a catabolic effect on tissue proteins(11). It therefore seemed possible that the inhibition of granulation

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tissue formation might be simply the result of lack of available protein material. Another possible mechanism was suggested when it became known that C_{11} steroids exert an effect upon vasomotor mechanisms(12). This effect may underlie the changes in granulation tissue observed. A third possible mechanism of action had to be considered, namely, the effect of altered transmission of nervous impulses, as it has been shown that cortisone profoundly influences the metabolism and function of the central nervous system(13) and the acetyl choline metabolism of peripheral nerves(14). Here again there may be some relationship between altered nervous transmission and the morphologic changes in connective tissues. The purpose of the work to be presented was to investigate the three possible inter-relationships.

Methods and results. Granulation tissue forming around turpentine abscesses in white male rats of the Sprague Dawley strain was examined histologically in the same manner as previously described(15). The first series of experiments was designed to determine whether cortisone exerts its action locally. One-half cubic centimeter of turpentine mixed with 1 mg of cortisone was injected subcutaneously into the right suprascapular region of 5 animals as a control procedure, the same amount of turpentine mixed with 1 mg of cholesterol was injected into the left suprascapular region of the same animals. After 4 days the animals were sacrificed, the abscesses removed and fixed in 10% formalin, and hematoxylin-eosin and Van Gieson sections were studied.

The leukocytic barrier did not differ significantly in thickness in the two abscesses but seemed moderately more necrotic on the cortisone side. It also invaded the surrounding tissues to a greater extent, probably because of the decrease in quantity and density of granulation tissue on this side. When, as occurred in certain small areas, the granulation tissue was completely absent, the invasion of the surrounding tissue was quite marked. The granulation tissue layer on the cortisone side differed from that of the control side in the following respects:

(a) It was much thinner—usually less than one-half the control and sometimes practically completely absent.

(b) The fibroblasts were smaller, flatter and less branched. They appeared more compact in some regions while in others they were quite widely separated by edema. They did not tend to align themselves in rows parallel to the young capillary beds, as did the controls.

(c) There was a greater profusion of histiocytes and lymphocytes as well as polymorphonuclear infiltration into the granulation tissue.

(d) Vascularization seemed to be as prominent as on the control side, but very young capillary buds were more sparse.

In order to study the possible effect of vasoconstriction or ischemia upon granulation tissue in comparison with the inhibitory effect of cortisone, the following experiments were performed: The left iliac artery and vein were ligated just below the bifurcation of the aorta in 5 rats. One-half cubic centimeter of turpentine was then injected subcutaneously into the thigh on the ligated side and the normal side as well. The ischemic leg remained viable and after 4 days the animals were sacrificed. Toluidin blue was injected into the abdominal aorta and the distribution of dye in both extremities was observed. In all instances, dye entered the normal side immediately and the entire limb became deeply stained. On the ligated side there was a gradual and only slight evidence of dye penetration. The abscesses were removed, fixed and examined as before. The iliac arteries of three other animals were ligated in the same fashion and daily skin temperatures were taken on each thigh with a Taylor Diatherm. The skin temperatures

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showed a consistent difference between the ligated and the normal sides, in that the operated side was one to two degrees (C) cooler than the normal leg. Despite the undoubted restriction of blood supply on the operated side, no significant difference was found in the granulation tissue as compared to the control side, both presenting typical turpentine abscesses of 4 days' duration and showing intense leukocytic and good granulation response. No necrosis of the structures surrounding the abscess was observed.

To investigate the possible nervous mechanism we studied abscess formation in denervated areas. In 5 animals the left sciatic nerve was transsected and turpentine was injected subcutaneously into both thighs posteriorly, on the day of operation. Daily injections of 5 mg cortisone were given into the scapular regions for five days, the animals were then sacrificed, the abscesses removed and prepared as above. Three other animals were similarly operated on, and one week was permitted to elapse, to allow degeneration of nerve endings beyond the transsection. Abscesses were then induced and cortisone given as before. The abscesses on both the denervated and control sides exhibited typical cortisone effects, no appreciable difference being noted in either leukocytic barrier or qualitative and quantitative aspects of the granulation tissue layer.

To study the effect of a more radical and extensive denervation, spinal cord transsection at the level of about T_8 was performed and the distal portion of the cord removed as much as possible. Twenty-four hours later the lumbar sympathetic fibers were removed. Turpentine was then placed subcutaneously in the suprascapular region, which was well supplied by nerves, and the lumbar region which was included in the paralyzed portions of the animal. Two animals received cortisone and 3 were kept as controls. Four-day-old abscesses were then studied, the abscess of the denervated part compared with the abscess of the intact site. The granulation tissue of these animals in general was poorer than normal, due to the general debilitated state of these rats following the operative procedure. The degree of inhibition however was far greater in the cortisone treated animals. No difference was noted in the intact and denervated parts of the animals. There was no interference with cortisone action in the denervated area.

Discussion. The local action of cortisone was demonstrated by mixing and injecting it with turpentine and showing typical inhibition of the granulation tissue in the developing abscess. This inhibition did not follow absorption of the cortisone from the site of injection with a general cortisone effect in the whole animal, for granulation tissue in a simultaneously developing abscess following turpentine injection (without cortisone) in another site in the same animal showed no inhibition. It is therefore clear that the changes seen in granulation tissue developing under cortisone influence do not depend on an altered metabolic state induced by cortisone in the whole animal, *e.g.* the catabolic action of cortisone on proteins in the whole animal is not responsible for its inhibitory effect on granulation tissue. In a previous paper(1) inhibition of granulation tissue proliferation following sex steroid administration was reported and evidence was presented that this might be due to inhibition of growth hormone output from the anterior pituitary by the steroid injected in these animals. Our experiments show that cortisone inhibition of granulation tissue could not occur by this mechanism, if so, both the cortisone and the control sides would have been inhibited in the same manner. The local action of cortisone has also been demonstrated by other methods in experimental animals(9) and by clinical observations on inflammatory conditions of the eye(16).

Although it has been shown that animals lacking C_{11} oxysteroids cannot make adequate adjustments in the dynamics of circulation (17), and that adrenal extracts potentiate the vasoconstrictive action of nor-adrenaline in splanchnic vessels(12), our experiments demonstrated that granulation tissue de-

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veloped well in spite of inadequate blood supply. The inadequacy of the blood supply was proven by delayed and decreased entrance of dye into the extremity and by the persistent lowering of the skin temperature. The ischemic extremity, however, received some blood as evidenced histologically by the lack of necrosis. The limb may have been kept viable by collaterals from the pelvic arteries and skin vessels. In these experiments it was shown that granulation tissue developed well in spite of inadequate blood supply. This seems to rule out ischemia as a cause for inhibition of fibroblastic proliferation by cortisone.

In our experiments, in which the sciatic nerve was transsected, the action of cortisone was evident in the denervated as well as in the intact extremity. The damage induced to motor and sensory fibers by transsection of the sciatic nerve of the rat has not been adequately studied and the amount of sympathetic fibers destroyed by this procedure is unknown. It was thought advisable therefore to perform a more radical denervation to corroborate these results. In animals with cord transsection and lumbar sympathectomy granulation tissue in denervated areas was affected by cortisone to the same degree as in intact areas.

The mechanism of the inhibitory effect of cortisone upon granulation tissue remains unknown. As each of the possibilities discussed above is ruled out, it becomes more likely that the effect is direct and locally exerted; presumably upon the growing fibroblasts and/or the matrix in which these cells develop.

Summary. Granulation tissue developing around turpentine abscesses in rats is inhibited if cortisone is suspended in the turpentine. This effect is a local one, because in the same animal a simultaneously developing abscess caused by turpentine in which cholesterol is suspended showed no such inhibition. Moderate ischemia does not inhibit granulation tissue formation and presumably does not underlie the inhibitory action of cortisone upon granulation tissue. Denervation does not interfere with this effect of cortisone. The implication of these findings on the possibility of a direct cortisone action upon granulation tissues is discussed.

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A Study of the Fibrous Components of the Vitreous Body of the Electron Microscope.* (18655)

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(Introduced by Chester N. Frazier)

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The vitreous humor of the mammalian eye has been described by Robertson and Duke-Elder(1) as "a gel composed of a meshwork of elastic fibrillae suspended in a viscous fluid." Young(2) and Mörner(3) considered the fibrous residue of the vitreous humor to be collagenous since it dissolved in boiling water and gelled on cooling. Pirie, Schmidt and Waters(4) extracted ox vitreous body with saline, solubilized the fibrous residue

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