milliequivalents per day, and this decreased to 19 milliequivalents when the antrum was exteriorized. When subsequently the antrum was transplanted into the duodenum, the average output of hydrochloric acid increased to 65 milliequivalents per day.

Conclusions. 1. Exteriorization of the antrum of the stomach produces a marked decrease in the secretion of Pavlov pouch dogs comparable to that secured by excision of the antrum. When this exteriorized antrum is subsequently transplanted into the duodenum or into the colon, a marked stimulation of gastric secretion is produced.

2. Transplantation of the antrum of dogs with totally isolated stomach pouches into the duodenum or into the colon produces a marked increase in gastric secretion. These findings support the view that the antrum mucosa is a specific internal secreting organ distinct in function from the mucous membrane of the stomach or duodenum.

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Effect of Cortisone* on Experimental Fractures in the Rabbit.† (17785)

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It has been observed that wounds of patients treated with adrenocorticotrophic hormone (ACTH) fail at times to heal normally(1). These observations prompted an experimental evaluation of the action of cortisone on standard skin defects induced in rabbits' ears. It was established that cortisone caused a striking depression of all elements of connective tissue, including formation of new blood vessels, and produced evidence of hyperadrenalism as manifested by depression of circulating eosinophiles and mild hyperglycemia(2). In the light of these studies, it seemed of interest to observe the effect of this adrenal steroid on other mesenchymal tissues. Consequently the effect of cortisone on callus formation was investigated and the results are reported in the following experiments.

Methods. Animals. Healthy adult laboratory rabbits of mixed breeds and sexes weighing between 2.6 and 3.2 kg were used.

Method of Fracture. Under nembutal and ether anesthesia the right femur was fractured by a sharp blow with a mallet in the midshaft without breaking the skin.

Methods of Pathological Study. Animals were sacrificed with nembutal or the intracardiac injection of air. The injured extremities were removed *in toto,* frozen, and then split through the fracture site with a band-saw. Both longitudinal and transverse sections were taken through comparable areas of the fracture site. Sections of liver and adrenals were also made. Tissues were fixed in 80% alcohol, chilled acetone and alcohol-formalin, and were imbedded in paraffin. Sections of the fracture site were stained with the following technics: Hematoxylin and chlorazol fast pink B; Tol-

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uidine Blue 0—1:1000 according to Lillie(3), Hale(4), Masson Trichrome; Laidlaw Silver, Feulgen(5), Feulgen without hydrolysis, periodic acid Schiff reaction(6), periodic acid Schiff reaction after diastase(3), alkaline phosphatase(4), and alkaline phosphatase control. Sections of the liver were stained with hematoxylin and chlorazol fast pink B and the periodic acid Schiff reaction after and without diastase. Sections of the adrenals were stained with hematoxylin and chlorazol fast pink B.

Experimental. Paired rabbits were used. One rabbit of each pair was pretreated with 12.5 mg of cortisone acetate intramuscularly twice daily for 3 days before fracture and thereafter until the time of sacrifice. The other animal of each pair was used as a control and was otherwise treated exactly the same as the rabbit receiving cortisone. Following the pretreatment period in 8 pairs of rabbits, fractures were produced according to the technic described. Treatment following this procedure was identical with the preoperative period. No effort was made to splint or otherwise limit motion of the fractured leg. Despite the injury the rabbits ate and drank well and appeared healthy. One pair of animals was sacrificed daily for 8 days following fracture. Autopsies failed to reveal a significant gross difference between the treated rabbits and controls except for the fracture site and the liver.

On *gross* examination, the reparative process was delayed in the cortisone-treated rabbits. At the second post-fracture day, the control animals showed signs of resolution of muscle hematomata but these remained jelly-like in the animals receiving cortisone. By the fifth post-fracture day, a significant zone of healing was grossly apparent in the control while at the fracture site of the treated animals there was only minimal healing between the ends of fractured bone. In the control animals, the zone of healing tissue became progressively larger over the 8-day period. However, the thin band of healing tissue seen on the fourth day in the cortisone-treated rabbit was not appreciably larger on the eighth day.

On *microscopic* section, the repair process was seen to pursue the following pattern: By the second post-fracture day, early fibroplasia could be seen and for the first 3 or 4 days there was a local reaction of edema, inflammatory infiltration and tissue autolysis. During this period of time there was little difference between the control and the cortisone-treated rabbits. Beginning with the fourth or fifth day, however, the difference between the 2 groups was striking. In the *control animals,* well-defined areas of new cartilage were seen near the old bone surrounded by a wide margin of granulation tissue which encompassed some muscle fibers. By the eighth day (Fig. 1a), well-defined soft bone trabeculae were present in association with the cartilage and the granulation tissue. The calcium of the engulfed muscle bundles decreased as the calcified trabeculae appeared. In the *cortisone-treated rabbits* (Fig. 1b), there was a retardation of all phases of healing after the fourth day. Metachromasia was decreased and the intercellular matrix appeared quite irregular in structure. Fibroblasts showed rounded forms and bizarre arrangements of nuclear chromatin. The cartilage which was seen on the eighth day in the treated animal had small lacunae, sparse perilacunar reticulum fibers, only irregular metachromasia of the matrix, minimal and irregular alkaline phosphatase content and only sparse small glycogen granules in the cytoplasm. No bone trabeculation was seen in the treated rabbits within the experimental period. In essence, up to the fourth post-fracture day, the control and cortisone-treated rabbits exhibited little difference in the reparative process. After the fourth day at about which time new blood vessels began to appear, there was a marked retardation of repair in the cortisone-treated group.

The effect on certain other organs was studied. The gross appearance of the liver of the cortisone-treated rabbits differed from

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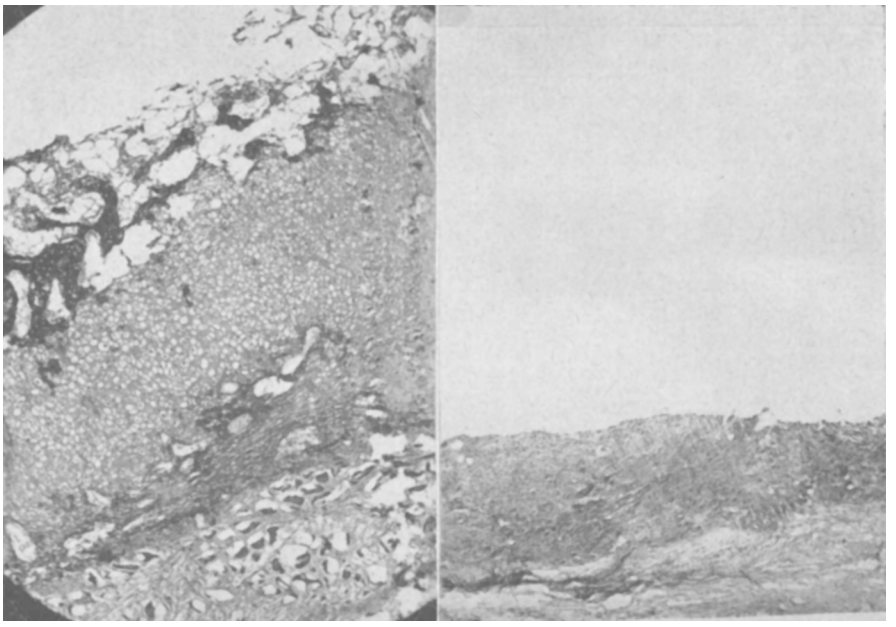

Fig. 1a. Section through fracture site, 8th post-fracture day, control rabbit, 38 ×, hematoxylin-chlorazol fast pink B. At the top, below the margin of injury, is a dense area of fibroplasia. There is a wide area of well-differentiated cartilage below that and at the bottom osteoid with bone trabeculation is noted.

Fig. 1b. Section through comparable fracture site, 8th post-fracture day, cortisone-treated rabbit, 38 ×, hematoxylin-chlorazol fast pink B. The entire healing zone is represented by the relatively narrow area on the right. No clearly defined cartilage and no osteoid can be seen.

the controls. From the first day of sacrifice (after 4 days of cortisone), the liver of the treated animal was larger, firmer and paler than the control, bled less and on cut section showed disturbance of the architecture by infiltration of a pale yellow substance. On the eighth day following fracture, the liver of the control animal weighed 85 g while that of the treated rabbit weighed 175 g. Histologically, the increase in weight and change in gross appearance were shown to be due, at least in part, to a marked deposition of glycogen (Fig. 2). There was no other significant histological difference noted between the livers of the two groups. The adrenals of the control and treated animals appeared grossly the same and histologically no distinct differences could be seen.

Discussion. The present studies confirm and extend previous observations on the inhibitory effect of cortisone on the response of the connective tissues to trauma. By means of serial daily observations, a histological feature of these experiments became evident which may help to clarify the mechanism involved. Up to the fourth post-fracture day, the inflammatory and reparative responses of the connective tissue were practically the same in both control and cortisone-treated animals. However, at about that time, new vessels began to penetrate the area of injury in both groups, but in the control animals the number of new vessels appeared to be greater. Following the ingrowth of new vessels in the control animals, reconstruction and differentiation of tissue continued rapidly, but in the cortisone-treated animals the reparative process remained sluggish with relatively little tissue differentiation. This difference may be ascribed to one of two possible situations: first, that in the cortisone-treated animals, the blood supply was inadequate due to a dearth of new blood vessels with inadequate nutrition of tissue; or secondly, that through the few new blood vessels which did penetrate the area, a material, perhaps cortisone itself, was

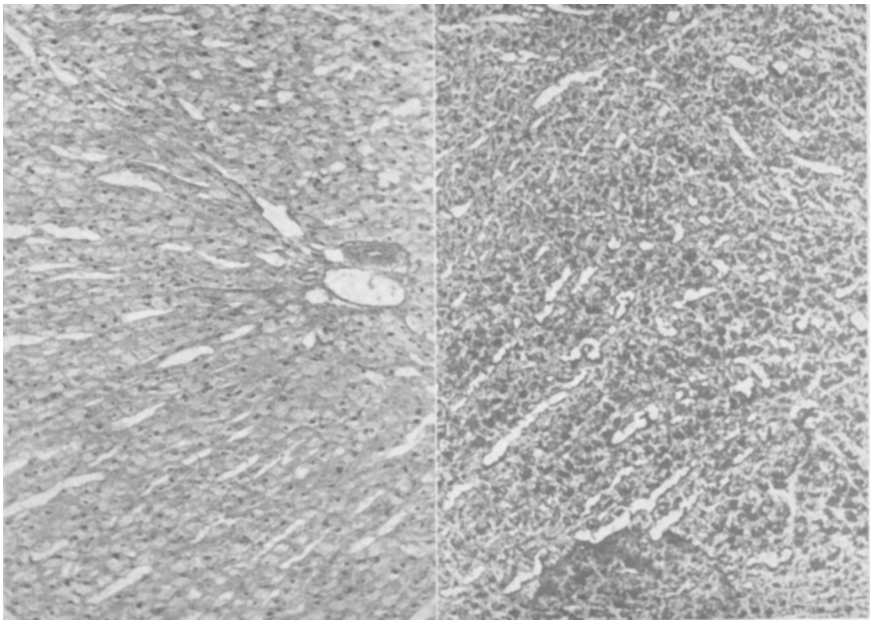

Fig. 2a. Liver, 8-day cortisone-treated rabbit, 78 ×, hematoxylin-chlorazol fast pink B. Note the enlargement of the hepatic cells and vacuolization of the cytoplasm.

Fig. 2b. Liver, same cortisone-treated rabbit, 78 ×, periodic acid Schiff stain. The cytoplasmic enlargement is seen here to be due largely to glycogen infiltration.

transported which inhibited growth and development of connective tissue. Although our data do not permit a definite conclusion as to the mechanism involved, the latter view receives some support from the observations of Baker(7), who demonstrated an inhibitory effect of cortisone applied locally to open wounds.

Summary. 1. Sixteen rabbits were subjected to fractures of the femur. Eight of these were under treatment with cortisone.

2. One control and one treated animal were sacrificed serially for eight days following fracture.

3. Gross healing of the fracture and absorption of the hematoma were greatly delayed in the animals receiving cortisone.

4. Histologically, the chief differences were seen following the fourth post-fracture day when retardation of all phases of healing were noted in the cortisone-treated animals. At the fourth day, new blood vessels began to appear in both control and cortisone-treated rabbits, but were more numerous in the control animals.

5. Failure of connective tissue regeneration following the fourth post-fracture day in the cortisone-treated rabbits may have been the result of poor tissue nutrition due to an inadequate blood supply or may have been due to the action of a circulating material possibly cortisone itself, brought to the area through new vessels.

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