

cells. Regeneration occurs from these remaining lymphocytes.

These observations indicate that the histologic effect of nitrogen mustard on the lymphopoietic system is qualitatively but not quantitatively the same as the effect of X-rays at the same level of general toxicity. The effects on the mesenteric lymph node differ only slightly, the difference being a somewhat earlier regeneration. The difference is greater in the lymphatic tissue of the appendix. This lymphatic tissue shows changes after nitrogen mustard at the LD₅₀ comparable to the changes of the X-rays with a dose of half the LD₅₀. The findings of Jacobson *et al.*(4) demonstrating the more rapid regeneration of the rabbit bone marrow after nitrogen mustard as compared to X-rays and the findings reported here, seem to warrant the conclusion that the hematopoietic system regenerates in general more rapidly after nitrogen mustard than after X-rays with dose levels of the

same general toxicity, keeping in mind local differences such as are reported here for the mesenteric lymph node and the lymphatic tissue of the appendix. We have no definite explanation for these local differences. They may be based on differences in sensitivities of the lymphocytes, but it may also be that the instability of the compound *in vivo* causes differences in the effective concentrations in different organs.

Summary. The histologic effects on the lymphatic nodules of the rabbit after X-rays and after nitrogen mustard are identical in nature. However, there is a quantitative difference in the response of the lymphatic nodules to the LD₅₀ dose of the two agents. The difference is minor in the mesenteric lymph node, where there is a slightly earlier regeneration after nitrogen mustard than after X-rays. The difference is greater in the appendix where the histologic changes after nitrogen mustard at the LD₅₀ dose level are comparable to the changes after half the LD₅₀ dose of X-rays.

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Effect of Cortisone on Production of Granulation Tissue in the Rabbit.* (17555)

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Since Hench and his co-workers(1) demonstrated the dramatic therapeutic effect of cortisone and adrenocorticotrophic hormone (ACTH) in rheumatoid arthritis, numerous

investigators have found that these agents exert beneficial effects in a great array of disorders. These include such "mesenchymal diseases" as rheumatic fever, lupus erythematosus disseminatus and periarteritis nodosa. (2-4). The mechanism by which therapeutic

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TABLE I.
Blood Chemical Determinations on 4 Cortisone-treated Rabbits and 4 Control Rabbits Following Production of Ear Wounds.

	5th post-operative day				8th post-operative day			
	Cortisone-treated rabbits		Control rabbits		Cortisone-treated rabbits		Control rabbits	
	No. 55	No. 57	No. 52	No. 56	No. 54	No. 58	No. 53	No. 59
Serum protein (g%)	5.1	5.0	—	—	5.7	5.1	5.5	6.0
Serum albumin (g%)	4.2	4.3	—	—	3.8	4.1	3.8	4.1
Serum sugar (mg%)	235	170	235	122	294	129 (fasting)	174	122 (fasting)
Plasma vit. C (mg%)	—	.42	—	.65	—	0.34 (fasting)	—	.02 (fasting)

effects are achieved has not, up to the present, been clarified, but it has appeared to the writers that the action of these agents is on "host reactivity."⁽⁵⁾ In the course of treatment of several patients with lupus erythematosus disseminatus and rheumatoid arthritis with ACTH, it was noted that open wounds, such as incised abscesses and decubitus ulcers, failed to show normal granulation tissue.⁽⁵⁾ One such wound, an incised abscess, did not form granulation tissue until 4 days after ACTH was discontinued. Healing of aseptic wounds, made for biopsy, was greatly delayed while ACTH was administered, but these healed promptly when the hormone was discontinued. These observations raised the question of whether the action of ACTH and cortisone in the "mesenchymal diseases" might be due to "inhibition of reactivity" of the connective tissue. Patients with hyperadrenalism as manifested by Cushing's syndrome are known to have a disturbance of connective tissues as exemplified by the "moon" facies, formation of striae, osteoporosis, increased fragility of blood vessels and poor healing of wounds. To test this hypothesis it was decided to determine whether the administration of cortisone might inhibit the normal processes of granulation in artificially produced wounds.

Skin wounds were made on both ears of six pairs of rabbits weighing between 2.4 kg and 3.0 kg.⁽⁶⁾ One rabbit of each pair was

treated with cortisone according to the following schedule: 12.5 mg of cortisone acetate was administered intramuscularly twice daily for 3 days before the wound was made and for 5 to 8 days thereafter until the time of sacrifice. The other rabbit of each pair served as a control and received no cortisone but was otherwise treated exactly the same. The animals were kept in a thermostatically controlled room and identical local treatment to prevent wound infection, as reported elsewhere,⁽⁶⁾ was carried out in each pair. Control and cortisone-treated rabbits ate a similar diet, both groups ate well and weight remained relatively constant.

The development of granulation tissue in the cortisone-treated animals was markedly delayed in all cases. Grossly, at both 5 and 8 days following operation, these rabbits showed little or no granulation tissue and the blood vessels of the wound stood out clearly, while the controls showed good granulation tissue which obscured the blood vessels. Two pairs of rabbits were sacrificed on the fifth post-operative day and two pairs were sacrificed on the eighth post-operative day. The remaining two pairs were not sacrificed but showed similar gross changes and are being used for further long-term experiments. The cortisone-treated rabbits developed such manifestations of hyperadrenalism as the disappearance of circulating eosinophils, a moderate postprandial hyperglycemia and a great increase

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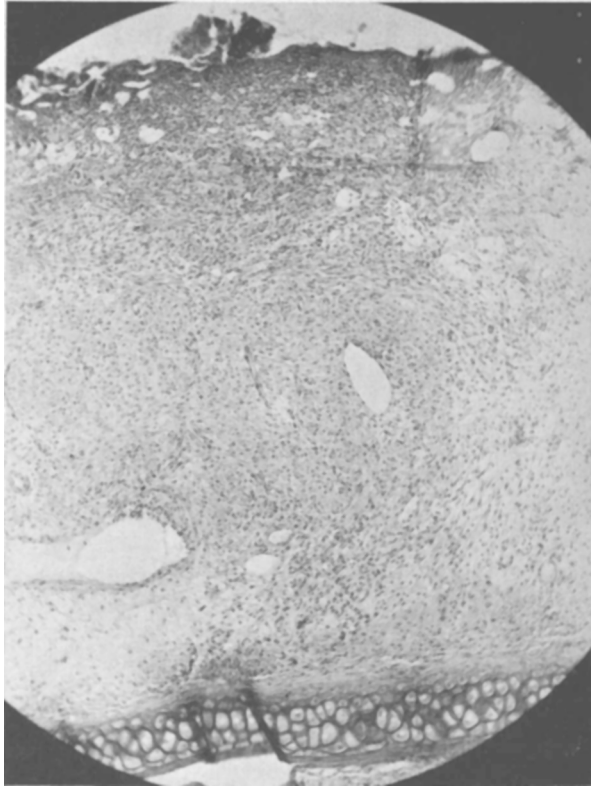


FIG. 1.

Cross-section of rabbit's ear wound, 110 $\times$, eighth post-operative day, control rabbit.

At the base is the cartilage of the rabbit's ear. Note the great thickness of the granulation tissue, the presence of newly-formed blood vessels and the elaboration of reticulin fibrils.

in glycogen deposition in the liver. Serum protein partitions in the animals receiving cortisone were comparable to control animals. (Table I). There was no evidence of wound infection in either the cortisone-treated or control rabbits.

Histologically, the cortisone-treated rabbits at both 5 and 8 days post-operatively showed striking depression of new growth of all elements of the connective tissue. (Fig. 1 and 2). The height of the granulation tissue formed was markedly decreased. Few, if any, new blood vessels could be seen. On the eighth post-operative day, fibroblasts in compact arrangement were present in nests about old blood vessels. In the control animals the new fibroblasts were present throughout the wound and appeared to be much less compact.

Fibrils appeared between the fibroblasts and there was more ground substance as determined roughly by metachromasia with toluidine blue stains and by the Hale(7) stain.

This suppression of all new elements of the connective tissue is similar to that seen in scurvy. Plasma ascorbic acid levels in the cortisone-treated rabbits tended to be somewhat lower than the control rabbits but the levels did not reach zero in the plasma. In this connection it is of interest that low ascorbic acid levels have been observed in our clinic in three patients with Cushing's syndrome. In the production of scurvy by deficiency of vitamin C, complete disappearance of this vitamin from the plasma is found

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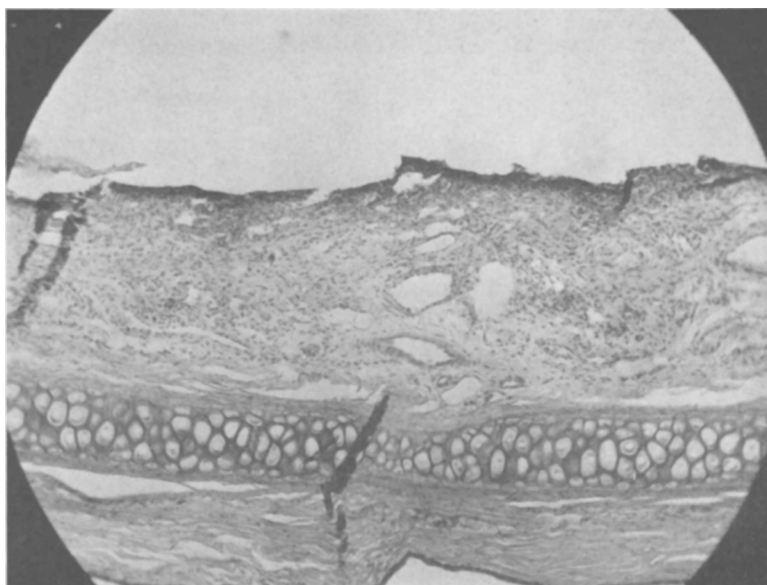


FIG. 2.

Cross-section of rabbit's ear wound, 124 $\times$, eighth post-operative day, cortisone-treated rabbit.

Pre-formed blood vessels left in the connective tissue at the time the skin was removed may be seen in the center of the field. Note the narrow area of granulation tissue with densely-packed fibroblasts.

months before any interference with wound healing is noted. Thus if this effect be related to vitamin C deficiency the result might be due to impairment of utilization of available ascorbic acid. No manifestations of the hemorrhagic diathesis were observed in the animals receiving cortisone. This correlates well with clinical findings in humans since no manifestations of scurvy have developed in patients we have treated with ACTH for as long as 4 months, and 600-900 mg of ascorbic acid administered daily to patients being treated for rheumatoid arthritis with ACTH or cortisone have failed to change the beneficial effects. However, it is of interest that preliminary experiments in the rat, an animal which is resistant to the development of vitamin C deficiency, show that wound healing is not modified by the administration of cortisone in a dose of 1 mg per day per 100 g of rat. It is noteworthy also that the rat is remarkably resistant to histamine while the rabbit is a histamine-sensitive animal. Rats

in general are refractory to the pharmacological action of most drugs and it is possible that the dose of cortisone was too low to affect wound healing. Further studies are in progress to clarify this species difference as well as to attempt to "titrate" the minimal dosage of cortisone necessary to inhibit the growth of granulation tissue in the rabbit.

Summary. 11 dehydro 17 hydroxycorticosterone (Compound E of Kendall, cortisone) administered to rabbits in large doses caused a delay in the development of all elements of connective tissue. It is suggested that the action of cortisone and ACTH on the "mesenchymal diseases" is due, at least in part, to "inhibition of reactivity" of the connective tissue. It is furthermore suggested that the inhibition of the growth of granulation tissue in the rabbit may prove of value in assay of compounds active in the treatment of "mesenchymal diseases."

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