

H. capsulatum seen in the cover-glass cultures, cannot be given at this time. This phenomenon is not dependent upon the presence of living tissues, since the sac-like type of colonies were observed in the controls which lacked tissue fragments. Of possible significance is the fact that this type of colony occurred only when the inoculated yeast cells were fixed *in situ* and surrounded by clotted plasma; in the supernatant of the flask cultures, only free, individual yeast cells were seen. Further work is in progress in an attempt to clarify these problems.

ADDENDUM. Since submitting this paper for publication an article by O. H. Duque has come to our attention. Writing in *Bol. Clin., Univ. Antioquis*, 1937, v9, 312, he reports the results of the cultivation of several pathogenic fungi with fibroblasts grown *in vitro*. The only positive findings concerned a culture inoculated with the mycelial phase of *H. capsulatum* which developed intracellular bodies interpreted as spores.

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Effects of Cortisone and Adrenocorticotrophic Hormone on Wound Healing in Normal and Scorbatic Guinea Pigs. (18709)

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Evidence suggests that ascorbic acid may influence the secretory activity of the adrenal cortex(1,2). However, the rôle of this vitamin in adrenal metabolism is still obscure. A possible mode of action was indicated by Lowenstein and Zwemer(3), who isolated an ascorbic acid-adrenal corticosteroid conjugate. Their work is yet to be confirmed. Symptoms of adrenocortical insufficiency resemble those of scurvy(4). Both conditions are characterized by hypersensitivity to stress(5) and may be favorably modified by adrenocortical hormone or by ACTH(4,6-9). Furthermore, in-

creased utilization or destruction of ascorbic acid has been observed in stress(10). Recent investigations have demonstrated that adrenal cortical hormone may inhibit the growth of connective tissue, thus simulating, in some respects, the pathologic changes seen in scurvy(11-13). Moreover, like ACTH and Cortisone, vit. C in massive doses(14) or in conjunction with desoxycorticosterone(15,16)

1. Giroud, A., *Presse méd.*, 1940, v48, 841.
2. Long, C. N. H., *Recent Progr. Hormone Research*, 1947, v1, 99.
3. Lowenstein, B. E., and Zwemer, R. L., *Endocrinology*, 1946, v39, 63.
4. Lockwood, J. E., and Hartman, F. A., *Endocrinology*, 1933, v17, 501.
5. Parrot, J. L., and Richet, G., *Compt. rend. Soc. de biol.*, 1945, v139, 1072.
6. Hartman, F. A., and Brownell, K. A., *The Adrenal Gland*, Philadelphia, Lea and Febiger, 1949, 286.
7. Schaffenburg, C., Masson, G. M. C., and Corcoran, A. C., *Proc. Soc. Exp. Biol. and Med.*, 1950, v74, 358.
8. Hyman, G. A., Ragan, C., and Turner, J. C., *Proc. Soc. Exp. Biol. and Med.*, 1950, v75, 470.

9. Treager, H. S., Gabuzda, G. J., Zamcheck, N., and Davidson, C. S., *Proc. Soc. Exp. Biol. and Med.*, 1950, v75, 517.
10. Lund, C. C., Levenson, S. M., Green, R. W., Paige, R. W., Robinson, P. E., Adams, M. A., Macdonald, A. H., Taylor, F. H. L., and Johnson, R. E., *Arch. Surg.*, 1947, v55, 557.
11. Ragan, C., Howes, E. L., Plotz, C. M., Meyer, K., and Blunt, J. W., *Proc. Soc. Exp. Biol. and Med.*, 1949, v72, 718.
12. Baker, B. L., in *Symposium on Pituitary-Adrenal Function*, American Association for the Advancement of Science, 1951, 88.
13. Spain, D. M., Molomut, N., and Haber, A., *Science*, 1950, v112, 335.
14. Massell, B. F., Warren, J. E., Patterson, P. R., and Lehmus, H. J., *New England J. Med.*, 1950, v242, 614.
15. Lewin, E., and Wassén, E., *Lancet*, 1949, v2, 993.
16. Le Vay, D., and Loxton, G. E., *Lancet*, 1950, v1, 209.

TABLE I. Comparison of Findings in Hormone-Treated and Untreated, Normal and Scorbatic Guinea Pigs.

Diet	No. of animals	Group	Wounds			Avg adrenal/body wt ratio X 10 ⁻⁵	Avg No. eosinophils per mm ³ of blood	Hyperplasia (+) or atrophy (—) of the zona fasciculata	Avg conc. of adrenal ascorbic acid in γ /100 mg
			Hemorrhage	New formation of collagen	Fibroblastic proliferation				
Scorbutigenic + ascorbic acid	10	Control	+	+	++	20	55	++	126
	10	ACTH	++	++	+++	33	0	3+	114
	5	ACTH vehicle	+	+	++	19	60	++	117
	16	Cortisone	++	++	+++	16	0	—	86
	5	" vehicle	+	+	++	20	70	++	115
Scorbutigenic	10	Control	3+	—	+	31	0	3+	2±
	10	ACTH	3+	—	+	40	0	4+	2±
	10	ACTH vehicle	3+	—	+	28	0	3+	2±
	10	Cortisone	3+	—	+	19	0	±	6±
	8	" vehicle	3+	—	+	30	0	3+	5±

has been reported to benefit patients with rheumatoid arthritis.

This study was undertaken in an effort to elucidate the rôle of ascorbic acid in wound repair and in adrenal metabolism. The effects of scurvy upon wound healing in guinea pigs have been compared with those of ACTH and Cortisone, and the influence of these hormones upon the scorbutic process has been evaluated.

Experimental. One hundred guinea pigs, weighing 300 to 400 g. were divided into 10 groups, as outlined in Table I. Males and females were caged separately. All were fed a standard scorbutigenic ration(17), to which were added supplements of ascorbic acid, 20 mg daily *per os*, for those not intended to develop scurvy. Utilizing Wolbach's technic (18), each animal was surgically wounded, in order to secure an area in which formation of connective tissue might be studied. This procedure was carried out on the twelfth day of the scorbutigenic regimen. Beginning at the time of operation, hormones were administered as indicated in the table, ACTH* being injected subcutaneously, 1.0 mg per 100 g

body weight daily, in divided doses at intervals of approximately 6 hours. This amount was doubled at the end of the first 5 days. Cortisone† was given daily in single subcutaneous injections, 4.0 mg per 100 g body weight. Five days after operation one-half of the animals in each group were sacrificed; the remaining half were killed on the tenth postoperative day. Each guinea pig was killed by a blow on the head and subsequent exsanguination from the carotid artery. One adrenal gland from each animal was assayed for total ascorbic acid by a modification of the method of Roe and Kuether(19). Total eosinophil counts by the method of Thorn (20), as well as differential blood counts, were performed at the time of death. Sections of the wounds were stained by hemalum and eosin, toluidin blue, the periodic acid-leukofuchsin reaction, the Mallory-Heidenhain azan procedure, the Wilder silver stain for reticulum, and by Gomori's method for alkaline phosphatase. Sections of adrenal glands were stained by hemalum and eosin, and, in addition, frozen sections of adrenal gland were

17. Sherman, H. C., La Mer, V. K., and Campbell, H. L., *J. Am. Chem. Soc.*, 1922, v44, 165.

18. Wolbach, S. B., *Am. J. Path.*, 1933, v9, 689.

* The ACTH used in these studies was provided by Armour and Co. Dosages are equivalent to Armour Standard LA-1-A.

† The Cortisone employed in this experiment was donated by Merck and Co.

19. Roe, J. H., and Kuether, C. A., *J. Biol. Chem.*, 1943, v147, 399.

20. Thorn, G. W., Forsham, P. H., Prunty, F. T. G., and Hills, A. G., *J.A.M.A.*, 1948, v137, 1005.

stained for neutral fat with oil-red-O.

Results. Our observations are outlined in Table I. (Histologic details are to be described in a separate report.) The wounds of the scorbutic animals showed in each instance a failure to heal and were characterized by a relative absence of collagen; this process was not detectably altered by either ACTH or Cortisone. Likewise, in the non-scorbutic guinea pigs these hormones were apparently without effect upon wound repair. Morphologic alterations were produced by ACTH and by Cortisone in the adrenal glands. Following ACTH therapy there was elevation of the adrenal/body weight ratio in both scorbutic and non-scorbutic animals, accompanied by histologic evidence of hyperplasia and increased sudanophilia in the zona fasciculata of the adrenal cortex. With Cortisone, on the other hand, in both scorbutic and non-scorbutic guinea pigs the adrenal/body weight ratio was reduced and there was atrophy and storage of lipid in the zona fasciculata. Adrenal concentrations of ascorbic acid were of the same magnitude in all non-scorbutic animals, except for the Cortisone-treated group in which vitamin C levels were uniformly depressed. All animals with scurvy, irrespective of treatment, showed almost negligible amounts of ascorbic acid in the adrenal glands and absence of circulating eosinophils. Eosinophil counts were also reduced by ACTH and Cortisone in the nonscorbutic guinea pigs.

Discussion. It has been suggested that scurvy and the "collagen diseases" have in common an insufficiency of Cortisone-like steroids coupled with continued output of desoxycorticoids(7). This notion is based in part on observations suggesting that Cortisone exerts a beneficial effect in scurvy; yet, paradoxically, the action of this substance on connective tissue has appeared to be inhibitory, or "fibrolytic," in most instances(11-13); however, under certain circumstances fibrosis has been produced(21).

This investigation was undertaken to compare scurvy with hyperadrenalcorticism and

to evaluate similarities or differences in their effects upon connective tissue. It was anticipated that scurvy would interfere principally with the formation of collagen. On the other hand, it was thought that the adrenal corticoids would inhibit primarily proliferation of fibroblasts and angioblasts and that the production of ground substance and fibers would be affected only secondarily.

The disturbance of healing manifested by the wounds of our scorbutic animals conforms to that previously described(17,22) and needs no further comment. Surprising, however, was the observation that Cortisone and ACTH, in both scorbutic and non-scorbutic guinea pigs, were without apparent influence upon wound repair. This lack of effect may have been due to inadequate dosage, yet hormone-induced changes were noted in adrenal glands and in eosinophil levels. It remains to be determined whether wound healing might have been affected by larger doses of the hormones or by induction of hyperadrenalcorticism prior to operation. The fact remains that the methods used in this study are essentially like those employed in investigations with other species. It appears, therefore, that in the guinea pig the connective tissue may respond to adrenal hormone, if at all, less readily than in the rat(12,23), the rabbit(11), the mouse(13), and in man(24). These findings also cast doubt on the specificity of the "antiscorbutic" effect of Cortisone reported elsewhere(7-9); such action may represent protection against the non-specific stress of vit. C depletion. This problem clearly demands further study.

The virtual absence of vit. C in the adrenals of all scorbutic animals, despite treatment with ACTH or Cortisone, appears significant. Thus, it may be assumed that the guinea pigs sacrificed on the tenth postoperative day survived at least 5 days with but traces of adrenal ascorbic acid. Furthermore, evidence indicates that adrenal cortical hyperplasia and

22. Bunting, H., and White, R. F., *Arch. Path.*, 1950, v49, 590.

23. Baker, B. L., and Whitaker, W. L., *Endocrinology*, 1950, v46, 544.

24. Ragan, C., Grokoest, A. W., and Boots, R. H., *Am. J. Med.*, 1949, v7, 741.

21. Baggenstoss, A. H., Shick, R. M., and Polley, H. F., *Am. J. Path.*, 1950, v26, 709.

sudanophilia increased during this interval, particularly in the ACTH-treated group, in which the response surpassed that obtained in the corresponding non-scorbutic series. These observations are in accord with those of Hyman, Ragan, and Turner(8), strongly suggesting that vit. C is not essential for the secretion of adrenal hormones. The depletion of adrenal ascorbic acid seen in the non-scorbutic animals receiving Cortisone may be correlated with cortical atrophy and lessened secretory activity; this change was maximal at the end of 10 days. That adrenal concentrations of vit. C were within the normal range following ACTH therapy in the non-scorbutic guinea pigs is consistent with the observed histologic changes and with the duration of the treatment(25).

Our data give no conclusive evidence that either ACTH or Cortisone influenced the rate of growth, nutrition, or hemorrhagic tendency of the scorbutic animals in this study. Definite

effects might have been observed had the treatment been prolonged, possibly until spontaneous death. This, however, was deliberately avoided in an effort to secure optimum conditions for appraisal of wound repair.

Summary. 1. ACTH and Cortisone, in doses sufficient to alter the adrenal glands and depress the level of circulating eosinophils, failed to influence the repair of wounds in normal and in scorbutic guinea pigs. 2. It is suggested, therefore, that the connective tissue of the guinea pig may respond to adrenal hormone, if at all, less readily than that of other species studied. 3. The specificity of the "anti-scorbutic" action of Cortisone and of ACTH is questioned. 4. The adrenal response of scorbutic guinea pigs to ACTH, in the virtual absence of adrenal vit. C, suggests that ascorbic acid is not essential for the secretion of adrenal hormone. 5. The relationship of ascorbic acid to the metabolism of the adrenal cortex remains obscure.

25. Sayers, G., *Physiol. Rev.*, 1950, v30, 241.

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Iodinated Organic Compounds as Contrast Media for Radiographic Diagnoses. X. Interrelationship of Gallbladder and Pancreas.* (18710)

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During a study of the factors influencing the accumulation of cholecystographic media in the gallbladder of dogs, it was found that complete ligation and division of the pancreatic ducts inhibited the visualization of the viscus. This observation indicates that the pancreas plays an intimate role in gallbladder function, and, experimentally, suggests a simple method to test the completeness of ligation and division of pancreatic ducts.

The mechanisms involved in the inhibition of gallbladder visualization after pancreatic

duct ligation and division have not been determined as yet. It appears that the effect requires several days for development since dogs with pancreatic duct ligation, or with complete pancreatectomy, visualize satisfactorily if intravenous cholecystography is carried out immediately after the completion of the operative procedure. Provisionally, the inhibition of visualization seems to be due to some alteration in the concentrating mechanism of the gallbladder.

Experimental. Studies were carried out on 14 healthy, mongrel dogs ranging in weight from 13.5 to 25 kg. Preoperative control cholecystograms were made, usually by intravenous administration of the sodium salt of

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