

TABLE IV. Composition of Iguana Ash.

Iguana No.	Sex	Wt, g	Dry wt, g	H ₂ O, %	Ash, g	Cl, mg	Na, mg	K, mg	Ca, g	Mg, g
4 (common)	F	932	252.3	72.9	57.88	1144.0	1054.0	1343.4	18.20	.513
8 (black)	M	867	253.4	70.8	50.23	1008.7	900.4	1340.2	15.75	.427

mg/kilo, and blood pH and plasma chloride, CO₂, and sodium were determined 1, 2, 3, 4, 11, and 23 hours after injection. The values obtained were of the same order of magnitude as those of the controls.

Summary. Two species of large lizards, *Iguana iguana rhinolopha* (common iguana) and *Ctenosaura acanthura* (black iguana) were studied. The following analyses were performed on blood and/or plasma of both species: pH, CO₂, Cl, Na, K, Ca, Mg, protein, urea, uric acid, creatinine, glucose, hema-

tocrit, oxygen capacity, and mean corpuscular volume. There was little difference in the average values for the two species with the exception of pH, CO₂, and chlorides. Although the total ionic concentration of the plasma was about the same for both, the Common Iguana appeared to be more alkaline than the Black Iguana. The urines and total ashes were analyzed for Na, K, Ca, Mg, and chloride. Uric acid, Na, K, and Ca analyses were done on dried excreta.

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Anatomic and Histologic Changes in Rabbits with Experimental Hypersensitivity Treated with Compound E and ACTH. (18430)

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Previous work(1) has indicated that Compound E and ACTH inhibit the production of experimental hypersensitivity of the Arthus type in rabbits receiving daily intracutaneous injections of crystalline egg albumin. This inhibition results from the hormonal suppression of antibody formation. The present report describes the anatomic and histologic changes produced in these animals by Compound E and ACTH with emphasis on the histologic appearance of the Arthus reaction and on the organs possibly related to antibody formation.

Methods and materials. Twenty-four male albino rabbits weighing approximately 2 kg each were injected intracutaneously every day for 18 days with 0.2 ml of crystalline egg

albumin solution(2) containing 0.8 mg of egg albumin nitrogen. Simultaneously, 8 of the 24 animals were treated daily with a single intramuscular dose of 10 mg of Compound E and another 8 with 10 mg of ACTH per day administered in 4 intramuscular doses of 2.5 mg every 6 hours. Two to 3 ml of blood were drawn from the marginal ear vein at the beginning of the experiment and on the 4th, 7th, 9th, 11th, 14th and 16th days for the antibody studies previously reported (1). Total white blood counts and differential counts were performed on the 17th day. Eighteen days after the first injection of egg albumin, the surviving animals were bled from the heart and killed. The weights of the thymus, spleen, liver, adrenals and kidneys

1. Germuth, F. G., Jr., and Ottinger, B., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 815.

2. Kekwick, R. A., and Cannan, R. K., *Biochem. J.*, 1936, v30, 227.

were recorded; in addition, sections for microscopic study were taken from the thyroid, thymus, lungs, heart, liver, spleen, pancreas, mesenteric nodes, adrenals, kidneys, testes, leg muscle, femoral marrow and skin at the site of the final injection of egg albumin, and fixed in 10% formalin. All sections were stained with azure-eosin(3). The liver, kidneys, adrenals and hearts were also stained in frozen section with oil red O to demonstrate fat(3), and liver sections of the Compound E-treated and control animals, which had been previously fixed in absolute alcohol, were stained with Best's carmine stain for glycogen. Quantitative glycogen determinations were carried out on weighed portions of the dried alcohol-fixed liver by hydrolyzing the alcohol precipitated glycogen to glucose and measuring the latter according to the method of Hagedorn and Jensen(4). In addition to the 24 experimental animals 5 normal rabbits were autopsied for comparison with the sensitized, untreated controls.

Six of the 24 experimental rabbits died before the experiment was terminated. Four had been treated with Compound E, one with ACTH, and one was an untreated control. All of these animals were affected with severe diarrhea. Another ACTH-treated animal was killed because of diarrhea. Except for these 7 animals which became extremely dehydrated and emaciated prior to death, the rabbits maintained their weight or showed an increase in weight during the experimental period. It is interesting that the rabbits receiving Compound E became increasingly sluggish and irritable after the first several days of treatment. At the same time, their muscles appeared flabby and their abdomens were protuberant. In connection with the serological studies it was observed that the blood of these animals became considerably lipemic. These alterations were not seen with ACTH.

Results of pathological studies. A. Rabbits injected with egg albumin and receiving no

treatment (control animals). After a preliminary period of sensitization, 6 of 8 control animals produced large, hemorrhagic and necrotic Arthus reactions. Sections of these lesions showed (a) marked edema, hemorrhage, and leucocytic infiltration of the derma, (b) necrosis and acute inflammation of the subcutaneous muscle, and (c) focal fibrinoid necrosis, acute inflammation, and thrombosis of the blood vessels, particularly of the small arteries lying between the derma and the subcutis. These vascular lesions were similar in appearance to those seen in periarteritis nodosa. Two of the 8 rabbits produced extensive erythema and edema in response to the final injection of the antigen. Microscopic

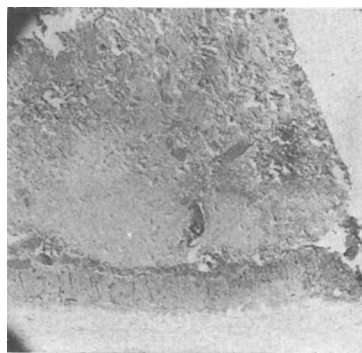


FIG. 1.

Section of derma and subcutis of a control rabbit at site of Arthus reaction showing hemorrhage, edema, leucocytic infiltration, and thrombosis of blood vessels. 10 $\times$.

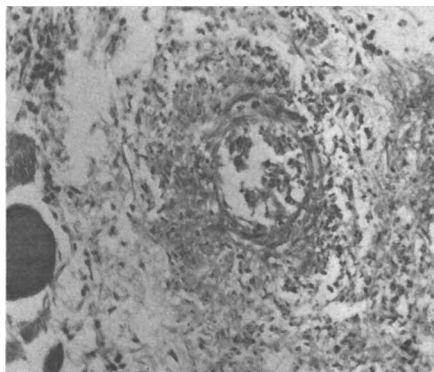


FIG. 2.

Polymorphonuclear infiltration and fibrinoid necrosis of an artery in the skin of a control rabbit at the site of the Arthus reaction. This lesion is similar to that seen in periarteritis nodosa. 200 $\times$.

3. Lillie, R. D., *Histopathologic Technic*, Blakiston Co., Phila., 1948.

4. Hagedorn, H. C., and Jensen, B. N., *Biochem. Z.*, 1923, v137, 92.

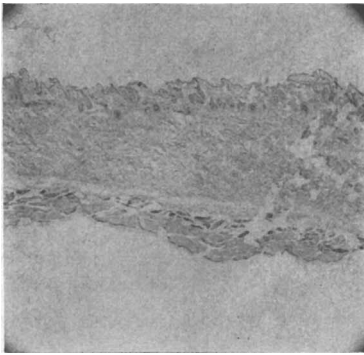


FIG. 3.

Section of skin of a compound E-treated rabbit at site of Arthus reaction. Derma is slightly distended by edema and contains an occasional leucocyte. Note the absence of hemorrhage, necrosis, and vascular lesions. 10 $\times$.

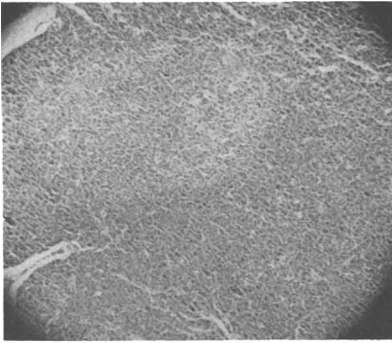


FIG. 4.

Section of thymus of a control rabbit. 33 $\times$.

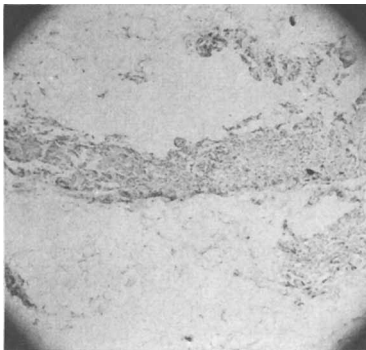


FIG. 5.

Section of thymus of a compound E rabbit. The lymphocytes have almost completely disappeared and only collapsed stroma, blood vessels, and Hassall's bodies remain. 65 $\times$.

examination of one of these lesions showed marked leucocytic infiltration of the derma and subcutis. Heart sections from 4 of 6

animals contained minute foci of round cells and occasional polymorphonuclear leucocytes situated in the myocardium. These slight but definite areas of interstitial myocarditis were not seen in any of the sections of the normal, Compound E or ACTH-treated animals.

B. Rabbits injected with egg albumen and treated with Compound E. In contrast to the histological appearance of the skin lesions of the control animals, sections of the small skin lesions of the Compound-E-treated animals showed, at most, slight edema of the derma with few polymorphonuclear leucocytes and round cells scattered here and there. Noteworthy was the absence of the necrotizing arteritis, the hemorrhage, and the necrosis of

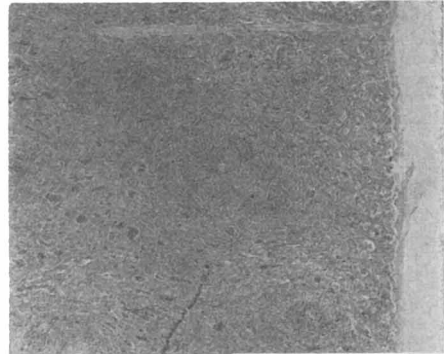


FIG. 6.

Section of spleen of a control rabbit. 18 $\times$.

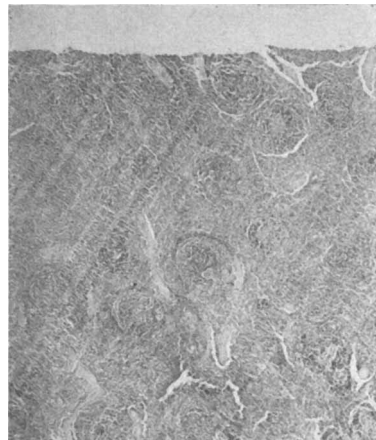


FIG. 7.

Section of atrophic spleen of a compound E rabbit. Note the prominence of the trabeculae and the great number of small malpighian bodies. 18 $\times$.

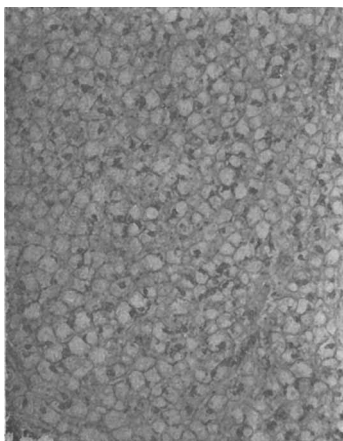


FIG. 8.

Section of liver of a compound E rabbit stained with azure-eosin. The cells are extensively vacuolated and contain little cytoplasm. An occasional hepatic cell appears necrotic. 70X.

subcutaneous muscle, all of which were so prominent in the sections of the skin lesions of 6 of the 8 controls. In all of the Compound E-treated animals the thymus was atrophic. In many instances the lymphocytes had completely disappeared and only the collapsed stroma, blood vessels, and Hassall's bodies remained. As a result of this massive loss of lymphoid elements, the thymuses of the Compound E-treated animals weighed only one-eighth as much as those of the untreated controls (Table I). Like the thymus, the spleens were reduced in size as a result of treatment. They weighed one-third as much as the spleens of the controls. Microscopically, the trabeculae appeared prominent and there was a massive loss of lymphocytes which was reflected by a great increase in the number of small Malpighian bodies occupying a single low-power field.

Compound E had no apparent effect on the size of the mesenteric lymph nodes. However, histologically, germinal centers present in the sections of the nodes from the control animals were absent in the nodes of the animals treated with Compound E. The adrenals of the Compound E animals weighed on the average only one-half as much as the adrenals of the controls but approximately the same as those from 5 normal rabbits. Histologically, the fascicular zones of the cortices appeared to be more narrow than those of the controls. How-

ever, lipoid was present in all 3 zones of the cortex. The livers of the Compound E-treated animals presented an extraordinary appearance. In contrast to the purple livers of the control animals, these were gray-yellow in color and approximately three times larger. With azure-eosin stain the hepatic cells appeared greatly enlarged and extensively vacuolated. Small amounts of cytoplasm were present about the nucleus of each cell and, to a lesser extent, just inside the cell membrane. Glycogen and fat stains demonstrated that these otherwise empty cells contained large quantities of glycogen and smaller amounts of fat. Quantitative glycogen determinations demonstrated that, per gram of dried tissue, the livers of the Compound E-treated rabbits contained approximately three times as much glycogen as those of the controls. The livers from 4 control animals contained 34-35 mg of glycogen per g, averaging 63 mg, while those from 4 Compound E-treated rabbits contained as much as 138-233 mg per g averaging 188 mg.

Numerous areas of necrotic cells were encountered in the livers of 5 of the 8 rabbits. The cause of these foci of coagulative necrosis was not apparent. The question, however, arises whether the presence of large quantities of glycogen and fat in the cells might have interfered with their metabolic activities in such a way that they were no longer able to survive. For example, it has been demonstrated that the regenerative capacity of hepatic cells that are overburdened with fat is far less than that of fat-free cells(6). It should be noted that the livers of the animals which had died during the experimental period were only slightly larger than those of the normal and did not contain glycogen. The kidneys were definitely larger than normal. Whether this enlargement was due to the presence of glycogen was not determined. The renal epithelium of the treated animals was no more vacuolated than that of the controls.

Sections of skeletal muscle were removed

5. Rich, A. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1935, v32, 1349.

6. Rich, A. R., Berthrong, M., and Germuth, F. G., Jr., *Trans. Assn. Am. Phys.*, 1948, v61, 263.

TABLE I. Anatomic Changes in Rabbits Sensitized by Repeated Intracutaneous Injections of Crystalline Egg Albumin and Treated with Compound E or ACTH.

No. of rabbits	Treatment	Avg wt of following organs in g				
		Thymus	Spleen	Adrenals	Liver	Kidneys
8	Sensitized (control)	3.1	2.4	.28	79	15
8	Sensitized and treated with compound E	0.56	0.86	.13	212 (94)*	20
7	Sensitized and treated with ACTH	0.71	1.6	.37	80	16
5	Normal	5.3	1.5	.13	69	17

* The figure in parentheses is the average weight of the livers of the animals which died during the experimental period.

TABLE II. Effect of Compound E and ACTH on Circulating Leucocytes.

Group	Total W.B.C. per cu. mm blood	No. of W.B.C./cu. mm/blood			Normoblasts per 100 leucocytes
		Lymphocytes	Monocytes	Neutrophiles	
Control (avg of 7 animals)	10340	7130	320	2790	120
Compound E (avg of 4 animals)	10190	2230	102	7850	292
ACTH (avg of 6 animals)	9800	5510	153	4140	35

from the right hind leg of 5 animals at a site distant from the local injections of Compound E and egg albumin. These showed numerous small areas of necrotic fibers surrounded by mononuclear cells and an occasional giant cell. The number of circulating lymphocytes was greatly reduced (Table II) by treatment with Compound E. However, there was an increase in the total number of polymorphonuclear leucocytes so that the total white blood counts were unaltered. Numerous nucleated red cells were encountered in the blood, probably as the result of the repeated bleedings. These were somewhat more numerous in the blood of the Compound E-treated animals than in that of the controls. Because of the presence of pseudoeosinophils in the blood of rabbits, accurate eosinophil counts could not be obtained.

C. Rabbits injected with egg albumin and treated with ACTH. The skin lesions of these rabbits varied considerably in severity. Four showed only slight edema and, at least in one case, slight leukocytic infiltration of the derma. Three others showed (a) marked edema, hemorrhage, and neurotropic infiltration of the derma, (b) necrosis of the subcutaneous muscle, and (c) periarteritic lesions of the small arteries. The thymuses of 5 of

the 7 animals examined were atrophic. However, lymphocytes were present in greater numbers than in those of the Compound E-treated animals. Two were entirely normal. Although the average weight of the spleens was somewhat less than that of the controls, only two out of seven were definitely atrophic. In contrast to the small adrenals of the Compound E animals, the adrenals of the ACTH animals were large. Grossly, the cortices were unusually thick, but lacking in lipid. Microscopically the cortex was normal except for the cells of the zona fasciculata which were large, contained abundant granular cytoplasm, and showed almost complete absence of lipid. The livers from 2 animals contained moderate amounts of glycogen; those from 4 showed slight to moderate fat, and 2 appeared normal. There was also a lymphocytopenia which was not as marked as with compound E. (Table II).

Discussion. The microscopic appearance of the Arthus reactions in the Compound E-treated rabbits differed decidedly from that of the controls. While the skin lesions in the untreated animals showed extensive edema, hemorrhage, and acute inflammation, those from the treated rabbits exhibited only slight edema and leukocytic infiltration. Further-

more, in the skin of 5 of 6 control animals examined, there were extensive vascular changes (consisting of focal fibrinoid degeneration, inflammation and thrombosis of the smaller arteries) similar in all respects to those of periarteritic nodosa. These vascular alterations which are probably the immediate result of antigen-antibody combination were completely absent in the skin of the Compound E-treated animals. From these observations, one might anticipate that Compound E would exert a similar inhibitory effect on allergic periarteritis nodosa. As was obvious from the gross appearance, the skin lesions of the ACTH-treated animals varied considerably in severity although on the average, they were less severe than those of the controls.

In the hearts of 4 of 6 control animals, there were small areas of interstitial myocarditis similar to those which have been described as resulting from hypersensitivity (8). These lesions were not observed in rabbits receiving either Compound E or ACTH. Rich and his colleagues(9) have recently shown that ACTH exerts an inhibitory influence on the development of cardiovascular lesions produced by anaphylactic hypersensitivity to normal horse serum.

Both Compound E and ACTH produced a profound alteration in the lymphoid tissues of rabbits. In the ACTH-treated rabbits there were atrophy of the thymus, occasional atrophy of the spleen and a lymphocytopenia. With Compound E these changes were even more extensive. In a previous study it was shown that Compound E and ACTH inhibit the production of antibody(1). This inhibition of antibody formation was found to be greater with Compound E than with ACTH. Similarly, as shown by the present study, the atrophy of the lymphoid tissues is more marked with Compound E. In view of recent work on the lymphoid origin of antibody(10) one might wonder whether the suppressed

antibody formation in rabbits treated with Compound E and ACTH could have resulted from the lymphoid atrophy produced by these two hormones.

The presence of the focal necrosis of skeletal muscle in animals receiving Compound E might well explain the muscular weakness which appeared during treatment. It is interesting to note that excessive doses of this hormone can produce muscular weakness and paralysis in man(11). The Compound E-treated rabbits also showed enlarged livers due to extensive deposition of glycogen and fat. Possibly because of this alteration in fat metabolism the sera became noticeably lipemic after 7 days of treatment. While Compound E produces its effects directly, the effects of ACTH are mediated through the adrenal cortex. This difference in the mode of action of these two hormones was reflected in the size and lipid content of the cortex. With treatment with Compound E the cortex was thin; with ACTH, the cortex was hypertrophic and its fascicular zone was lacking in lipid.

Summary. 1. The experimental data indicate that Compound E markedly suppresses the pathological alterations of the Arthus reaction ordinarily produced in the rabbit by repeated injections of crystalline egg albumin. The adrenocorticotrophic hormone exerted a decidedly less marked effect. 2. Treatment with Compound E and ACTH produced atrophy of the lymphoid tissues, including the thymus and spleen, and a lymphocytopenia. These alterations were greater with Compound E and in addition, in the animals treated with this hormone, there were extensive deposition of glycogen and fat in the liver, a lipemia and focal necrosis of skeletal muscle. In the animals treated with Compound E the adrenals were small while in those treated with ACTH, the adrenals were enlarged but lacking in lipid.

7. Rich, A. R., Charles C. Thomas, Springfield, Ill., 1944.

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9. Berthrong, M., Rich, A. R., and Griffith, P. C., *Bull. Johns Hopkins Hosp.*, 1950, v86, 131.

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11. Thorn, G. W., Forsham, P. H., Frawley, T. F., Hill, S. R., Roche, M., Staebelin, D., and Wilson, D. L., *New England J. Med.*, 1950, v242, 783.