THE MECHANISM OF ACTION OF 17-HYDROXY-11-DEHYDROCORTICOSTERONE (COMPOUND E) AND OF THE ADRENOCORTICOTROPIC HORMONE IN EXPERIMENTAL HYPERSENSITIVITY IN RABBITS

By FREDERICK G. GERMUTH, Jr., M.D., JIRO OYAMA, and BARBARA OTTINGER

With the Technical Assistance of Kenneth Q. Grow

(From the Federal Security Agency, Public Health Service, National Institutes of Health, National Microbiological Institute, Laboratory of Infectious Diseases, Bethesda)

(Received for publication, April 23, 1951)

In previous studies reported from this laboratory (1, 2), data were presented which indicated that the adrenal hormone, compound E, and the adrenocorticotropic hormone inhibited the production of experimental hypersensitivity of the Arthus type in rabbits sensitized by daily intracutaneous injections of crystalline egg albumin. This inhibition was shown to result from the hormonal suppression of circulating antibody. In contrast to their marked effect on the active Arthus reaction, compound E and ACTH had no noticeable effect on the *passive* Arthus reaction, suggesting that treatment with the hormones had neither interfered with the antibody-antigen combination in the tissues nor appreciably altered the allergic inflammatory response of the host.

Numerous anatomic changes were observed following the hormone administration. However the most marked alteration common to both the ACTH- and the compound E-treated animals consisted in extensive atrophy of the thymus and spleen and a reduction in circulating lymphocytes. In light of the present theories on the sites of antibody formation, the possibility was presented that the reduced serum antibody levels following treatment with the hormones might have resulted from these marked changes in the lymphoid tissue.

There is now considerable evidence indicating that treatment with compound E and ACTH significantly alters the course of experimental allergy. Rich and his colleagues (3, 4) have demonstrated that the lesions of experimental allergic carditis and arteritis are inhibited by treatment with either ACTH or compound E. Similar observations on the inhibitory effect of cortisone on experimental serum disease have been published by Seifter and his coworkers (5).

The effect which these hormones exert on circulating antibody is not generally agreed upon. Several years ago, it was reported that a single injection of ACTH or adrenal cortical extract resulted in a demonstrable rise in serum antibody (6). However, this observation has not been upheld by subsequent studies in which more

precise methods for the determination of antibody have been employed (7, 8). Other workers (3, 4) have reported that serum antibody levels were unaffected by treatment with compound E and ACTH. In these experiments the relative potencies of the treated and control sera were determined by the antigen dilution method, in which successive dilutions of *antigen* are layered over equal amounts of serum. Although this method is widely used, it is well known that it does not give a measure of the antibody content of a serum since the end-point titer of an antigen does not vary with the amount of antibody present (10).

The results of a recent study by Bjørneboe, Fischel, and Stoerk (9) on the effect of compound E and ACTH on antibody levels are in agreement with those obtained in this laboratory. The administration of ACTH and compound E to rabbits immunized with suspensions of formalin-killed pneumococci resulted in a marked reduction in the concentration of circulating antipneumococcal antibody.

The present studies further demonstrate the reduction in the concentration of circulating antibody by compound E and ACTH and the role that this effect plays in the hormonal inhibition of experimental allergy.

Materials and Methods

The ACTH was obtained from Armour and Company in the form of unsterile powder. Each vial contained an equivalent of 100 mg. of Armour standard ACTH LA-1-A. Fresh saline solutions of ACTH containing 5 mg. per ml. were made approximately every other day and kept cold when not in use.

Compound E was obtained as cortisone acetate from Merck and Company, Inc., in the form of unsterile crystals. After solution in warm anhydrous acetone, the crystals were suspended in saline and most of the acetone removed by evacuation. Finally, the suspension was made up to a volume such that 1 ml. of the suspension contained 10 mg. or 4 mg. of compound E. Suspensions of cholesterol were prepared similarly and used in the control animals of the first experiment to be described. Previous experimentation had indicated that cholesterol had no effect on the development of hypersensitivity or on antibody levels.

Crystalline hen's egg albumin was prepared according to the method of Kekwick and Cannan (11). This antigen is homogeneous from the immunological standpoint so that the quantitative techniques of immunology are readily applicable.

For sensitization, male albino rabbits weighing approximately 2 kg. each were injected intracutaneously daily with 0.2 ml. of crystalline egg albumin solution containing 0.8 mg. of egg albumin nitrogen. Positive skin reactions were usually obtained by the 8th to the 12th injection. The presence of edema, erythema, hemorrhage, and necrosis was recorded and the length, width, and height of the skin reactions were measured with a centimeter rule. The height of a lesion was obtained by measuring the thickness of a doubled layer of skin at the site of greatest swelling, subtracting the thickness of a fold of normal skin, and dividing by 2 to obtain the thickness of a single layer. The sizes of the Arthus reactions are presented in the figures and tables simply as the product of their width, length, and height measured in centimeters.

Quantitative precipitin determinations were performed as follows: The serum which had been preserved in a frozen state was clarified by centrifugation for 45 minutes at 2200 R.P.M. in a refrigerated centrifuge. Some of the samples of sera obtained from the compound E–treated rabbits were considerably lipemic. In this case, the lipoids were removed by centrifugation at

18,000 R.P.M. for 1 hour in the cold. Egg albumin was added to 0.5 ml. or 1 ml. of serum in increments of 1 to 8 μg. of nitrogen until a final addition of antigen produced no further precipitation. The larger increments of antigen were added in the beginning of the test when successive serum samples from the same rabbit showed an increasing amount of circulating antibody. After each addition of antigen, the serum was incubated at 37° C. for 1 hour, placed in an ice box overnight, and then centrifuged. Finally after 3 to 5 days at 0–5° C., the precipitate was collected by centrifugation in the cold and washed twice with cold saline (12). The washed precipitates were dissolved in 4.0 ml. of 0.1 N sodium hydroxide and the absorption of the resulting solution was determined in a 1 cm. quartz cell at a wave length of 280 mμ with a Beckman spectrophotometer. The observed optical density was converted into precipitate nitrogen from a standard reference curve. Since complement is not present in aged serum, the antibody nitrogen content was obtained by subtraction of the antigen nitrogen from the total value. Tests performed on the supernatant sera were all positive for slight excess antigen and negative for antibody. Duplicate determinations were done on the majority of the samples of serum. These agreed within ±5 per cent with values above 100 μg. of nitrogen.

The present method in which the antigen is added in increments differs from the standard technique of Heidelberger and Kendall (12) in which the required amount of antigen is given by a single addition. In this respect it is less precise, since with the serial addition of antigen a portion of the antibody ("univalent" antibody) may fail to precipitate even when there is a slight antigen excess. However, the repeated bleeding of each animal made advisable the use of the present method in which smaller quantities of serum were required. The amount of serum from the final bleedings was sufficient to enable a comparison of the two methods. It was found that although the comparative values given by the two methods were slightly different, the relative potencies of the numerous antisera tested were approximately the same.

OUTLINE OF THE MAIN EXPERIMENT

Forty rabbits were injected intracutaneously every day with crystalline egg albumin. Ten of the 40 rabbits were treated concurrently with 4 mg. of ACTH per day administered in 4 intramuscular doses of 1 mg. each every 6 hours, 15 others with a daily intramuscular injection of 4 mg. of compound E, and the remaining 15 with a daily intramuscular injection of 4 mg. of cholesterol. The latter group of animals served as controls. These daily dosages of compound E and ACTH are approximately equivalent to those generally employed in the treatment of man (100 mg. per 70 kg. body weight).

Treatment with ACTH was discontinued on the 24th day and, at that time, 4 of the animals were killed and autopsied. Two of the 10 ACTH–treated animals died during the experimental period.

By the 14th day of treatment, 3 of the 15 animals receiving compound E had died: one on the 8th day following severe diarrhea and 2 others on the 13th day, one following bloody diarrhea and the other following a massive intraperitoneal hemorrhage of undetermined etiology. At this time the 12 survivors were divided into 2 groups of 6 each: one group continued to receive compound E, while, in the other, compound E was discontinued and injections of cholesterol were begun. All the animals were killed and autopsied on the 28th to the 30th day. One of the animals receiving cholesterol died on the 25th day following diarrhea.

Two weeks after the onset of sensitization, the control animals receiving injections of cholesterol were divided into two groups. Six rabbits continued to receive cholesterol, while in the remaining 8, cholesterol was discontinued and treatment with compound E at a daily dose of 10 mg. was begun. One of the rabbits receiving cholesterol died on the 24th day due to accidental trauma and 2 of the 8 rabbits receiving compound E died on the 18th and 20th days.

The intracutaneous injections of egg albumin were withheld from all of the compound E–

and cholesterol-treated rabbits between the 23rd and 27th days in order to determine the effect of withholding antigen on the Arthus reaction and antibody titers of these animals.

In summary, there were five groups of experimental animals receiving injections of egg albumin:—

1. One control group of 6 rabbits injected with cholesterol.
2. A group of 8 rabbits treated with ACTH.
3. A group of 6 rabbits treated with compound E.
4. A group of 6 rabbits treated with compound E for 2 weeks followed by a 2 weeks course of cholesterol injections for the purpose of showing the duration of the effect of compound E following termination of treatment.
5. A group of 6 rabbits treated with cholesterol for 2 weeks followed by a 2 weeks course of treatment with compound E to show the effect of compound E on animals sensitized prior to treatment.

For the antibody studies to be described, approximately 4 ml. of blood was drawn from the marginal ear vein of each rabbit at the beginning of the experiment and usually on the 7th, 9th, 12th, 14th, 16th, 19th, 21st, 23rd, and 26th days thereafter. Rabbits were bled by cardiac puncture at the termination of the experiment and killed. The weights of the thymus, spleen, liver, adrenals, and kidneys were recorded and the organs sectioned for gross examination. A detailed histopathological study of the effects of compound E and ACTH in the rabbit has been reported elsewhere (2).

The Effects of Compound E and ACTH on the Development of Hypersensitivity and on Antibody Levels When the Hormones Were Administered from the Onset of Sensitization

In a previous study (1), it was shown that the administration of compound E or ACTH in daily dosages of 5 mg. per kg. to rabbits suppressed the development of experimental hypersensitivity and reduced the concentration of circulating antibody. These dosages were approximately equal to 3.5 times those generally employed in the treatment of man. The results of the present experiment indicate that similar effects on hypersensitivity and antibody levels are obtained when the hormones are employed in amounts that are essentially equivalent to those advocated in the treatment of man.

As shown in Table I (group I) by the 14th day of sensitization, all 6 of the control animals receiving injections of cholesterol were responding with large hemorrhagic Arthus reactions to the intracutaneous injections of antigen. If the 6 animals of group V which received cholesterol up until the 14th day, are also included in the control group for the first 2 weeks of the experiment, then actually there were 12 animals which were producing a maximal allergic inflammatory response at this time.

As was noted in a previous study (1), treatment with ACTH did not result in a striking suppression of the Arthus state. Six of 8 rabbits showed hemorrhagic reactions similar to those of the controls while 2 others produced small pink areas of erythema and edema in response to the antigen. However, there was a tendency for the lesions of the ACTH-treated rabbits to be smaller in size than those of the controls.

Treatment with compound E very definitely inhibited the Arthus reaction.

TABLE I

The Effect of Compound E and ACTH on Arthus Reactions in Rabbits Receiving Daily Intracutaneous Injections of Egg Albumin

Group	Treatment	Rabbit No.	Magnitude* and appearance of the 24 hr. skin reactions observed on the following days							
			Days 8	10	12	14	16	18	20	22	28
Group I (control)	*Cholesterol*, 2 mg. per kg. per day	29	0	3.4 r	21 h	11 h	11 h	5.4 h	11 h	4.0 h	4.6 h
		32	0	2.8 h	24 h	17 h	11 h	7.5 h	0.4 r	2.0 h	Dead
		34	0.8 p	0	2.6 r	18 h	13 h	7.5 h	7.5 r	15 h	3.8 h
		36	0	4.8 h	21 h	8.3 h	3.6 h	6.3 h	3.9 p	3.0 h	4.2 h
		38	0	13 p	17 h	17 h	7.4 h	2.6 h	4.0 r	2.9 r	1.5 p
		40	0	18 p	12 h	16 h	7.5 r	4.7 h	3.2 r	4.3 p	2.1 p
		Average..	0.1	7.0	16	14	8.9	5.7	5.0	5.2	3.2
Group II	*ACTH*, 2 mg. per kg. per day = total of 4 mg. per day given in 4 divided doses of 1 mg. every 6 hrs.	16	0	0	1.7 p	1.0 r	0	0	0	0	0‡
		18	0	1.4 p	8.4 p	4.7 p	0	1.4 r	0.7 r	2.7 r	2.5 r
		20	0	7.8 h	15 h	13 h	7.6 h	6.5 h	3.4 h	1.2 h	0.8 r
		21	0	0	21 h	13 h	6.2 h	5.9 h	5.2 h	2.1 h	1.4 r
		22	0	2.0 p	10 h	4.8 h	4.5 h	3.7 r	1.9 r	2.7 r	2.4 r
		23	0	0	3.2 h	0.1 r	5.4 h	4.4 h	9.6 h	3.1 h	2.3 r
		24	0	0	8.9 h	3.1 h	5.2 h	2.4 h	2.0 r	6.3 h	3.7 h
		25	0	0	2.5 h	1.7 r	1.9 r	2.3 r	1.6 h	3.1 r	1.9 h
		Average..	0	1.4	8.8	5.2	3.9	3.3	3.1	2.7	1.9
Group III	*Compound E*, 2 mg. per kg. per day	1	0	0	0	0	0	0	0	0	0
		3	0	0	0	0	0	0	0	0.1 r	0.6 r
		5	0	0	0	0.1 p	0	0	0	0	0
		11	0	0	0.2 p	0.6 p	0	0	0	0	0
		13	0	0	0.2 p	0.5 r	0.2 r	0	0	0.3 p	0
		15	0	0	0	0.7 p	0.5 p	0	0.4 p	0.9 p	0
		Average..	0	0	0.1	0.3	0.1	0	0.1	0.2	0.1
Group IV	*Compound E*, 2 mg. per kg. per day, 0 to 13th day *Cholesterol*, 2 mg. per kg. per day, 14th day to termination	4	0	0	0.3 p	0	1.1 p	4.6 p	3.0 p	2.6 p	2.1 r
		6	0	1.0 h	3.1 h	5.4 h	6.6 h	5.6 h	5.2 h	2.6 p	Dead
		8	0	0	0	0.8 p	1.3 r	2.0 p	3.8 r	5.3 r	5.1 h
		10	0	0	0	0	0	0	0	0	0
		12	0	1.4 r	0.2 p	1.1 r	2.0 r	1.8 r	5.3 r	3.9 r	4.1 h
		14	0	1.3 r	7.9 h	11 h	13 h	13 h	7.0 h	5.9 h	4.2 h
		Average§.	0	0.4	0.1	0.5	1.1	2.1	3.0	3.0	2.8
Group V	*Cholesterol*, 2 mg. per kg. per day, 0 to 13th day *Compound E*, 5 mg. per kg. per day, 14th day to termination	27	0	7.9 p	16 h	12 h	3.4 h	1.8 h	0.9 r	0	1.7 r
		28‖	0	3.1 h	33 h	12 h	4.5 h	3.7 h	8.2 h	2.1 h	2.4 h
		31‖	3.2 p	6.4 h	24 h	12 h	3.9 h	6.0 h	3.6 h	1.1 h	0
		35	1.4 h	6.0 h	13 h	8.1 h	2.9 h	2.5 h	1.2 h	3.4 r	0.2 r
		37	0	6.7 h	26 h	7.4 h	3.2 h	7.9 h	2.6 h	2.3 r	1.8 h
		39	0	4.2 h	17 h	7.2 h	3.7 h	3.7 h	1.8 h	1.6 r	1.4 r
		Average..	0.8	5.7	22	9.8	3.6	4.3	3.1	1.8	1.3

* Magnitude of the skin reactions is represented by the product of their length, width, and height measured in centimeters. For example, a lesion measuring 5 cm. by 4 cm. and 0.5 cm. in height would be designated by the value 10. p = pink, r = red, h = hemorrhagic.

‡ Reactions of the ACTH-treated rabbits to the 24th injection of egg albumin.

§ Rabbits 6 and 14 which developed severe Arthus reactions despite treatment by compound E are not included in the averages.

‖ Received compound E at a dosage of 2 mg. per kg. per day from 14th to 18th day.

By the 14th day of sensitization, only 2 of 12 animals (groups III and IV) showed a response to the antigen similar in severity to that of the controls. These 2 rabbits (Nos. 6 and 14) alone were capable of producing a hemorrhagic skin reaction. Six of the remaining 10 animals showed small areas of erythema and edema while 4 others failed to react to the antigen.

The results of treatment with ACTH and compound E are presented graphically in Fig. 1. This figure includes the animals of groups I, II, and III and not those of groups IV and V in which treatment was changed during the experimental period. The ordinate represents the size of the Arthus reaction in terms of the product of its length, width, and height measured in centimeters. The skin reactions to every other daily injection of antigen are plotted in the figure in which each line represents the skin responses of a single animal.

The serum antibody concentrations of the experimental animals at various intervals after sensitization are shown in Table II. Two weeks after the beginning of sensitization at a time when all the cholesterol-treated (control) rabbits were reacting with maximum intensity, the average antibody content of the sera of the 12 control rabbits of groups I and V was 144 μg. of antibody nitrogen per ml. However, the average serum antibody concentration of the ACTH-treated rabbits was 44 μg. of antibody nitrogen per ml. or approximately one-third of the control. Five of the 8 ACTH-treated animals had a circulating antibody level of less than 30 μg. of antibody nitrogen per ml. while all 12 of the control rabbits had levels higher than this value. After 23 days of treatment with ACTH, the average antibody content of the treated group was still about one-third that of the control, being respectively 105 and 285 μg. of antibody nitrogen per ml.

The serum antibody concentrations of the compound E-treated rabbits were even lower than those of the ACTH-treated group. After 14 days of sensitization and treatment, the average serum antibody concentration was 13 μg. of antibody nitrogen per ml. At the termination of treatment, the average circulating antibody content of these animals was less than 5 per cent of that of the control (10 and 320 μg. of antibody nitrogen per ml. respectively).

It is well known that the active Arthus reaction is dependent on circulating antibody (13). A comparison of the severity of the Arthus reactions with the corresponding serum antibody concentrations of the control animals suggests that, with the procedure used, the production of a maximal Arthus response characterized by the presence of gross hemorrhage requires a circulating antibody level of approximately 40 μg. of antibody nitrogen per ml. of serum and any increase above this level does not alter the Arthus response appreciably. With antibody concentrations between 0 and 40 μg. of antibody nitrogen per ml. of serum, 1 out of 24 Arthus reactions (4 per cent) was grossly hemorrhagic whereas with antibody levels above this amount, 43 of 52 reactions, or 83 per cent, were hemorrhagic. It should be noted that the 2 compound E-treated rabbits (Nos. 6 and 14) which had developed severe skin lesions by the 14th

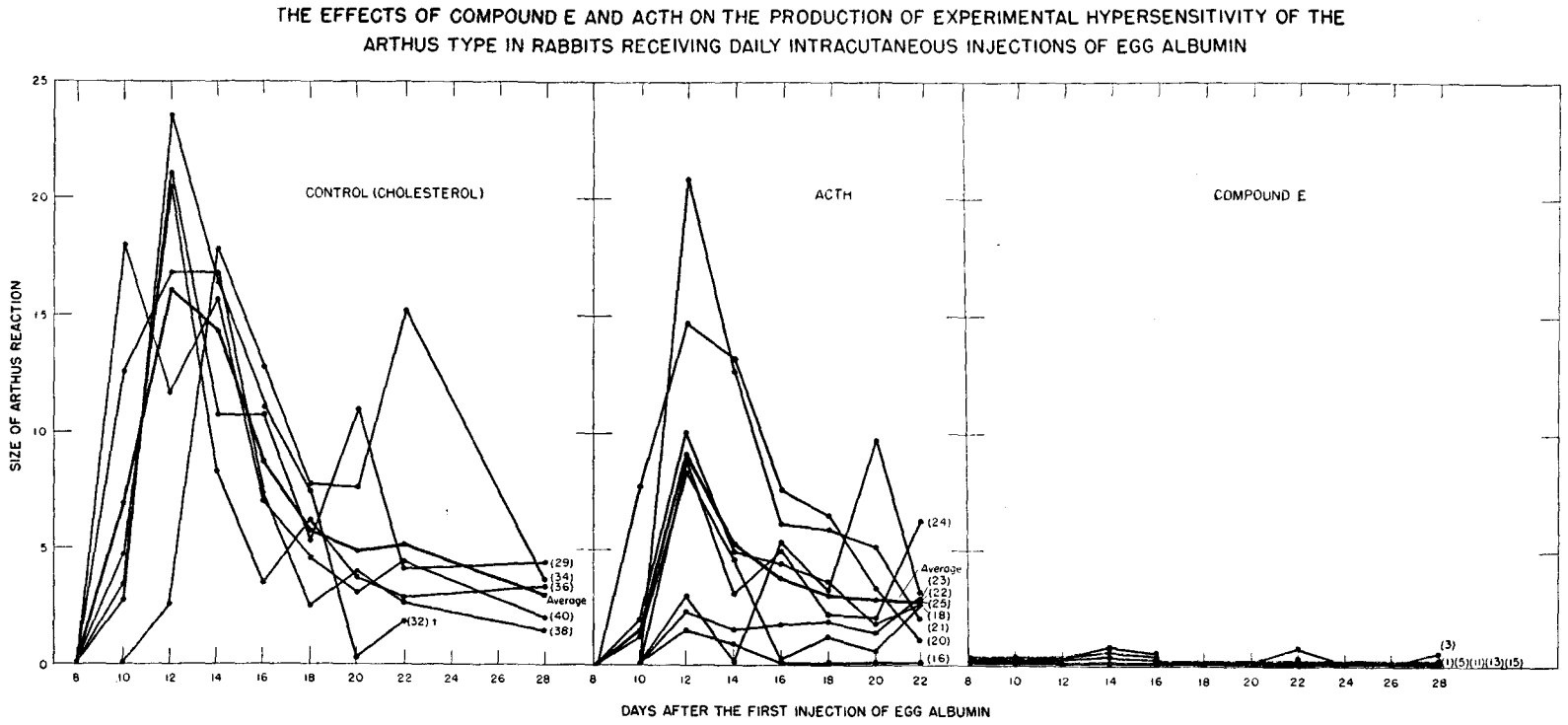


FIG. 1. After data of groups I, II, and III of Table I. The ordinate represents the size of the Arthus reaction in terms of the product of its length, width, and height measured in centimeters. The skin reactions to every other daily injection of antigen are plotted for each animal.

TABLE II

The Antibody Content of Sera from Rabbits Sensitized by Daily Intracutaneous Injections of Egg Albumin and Treated with Cholesterol, ACTH, or Compound E

Group	Treatment	Rabbit No.	Antibody nitrogen content of serum on following days after first sensitizing injection of egg albumin									
			Days 7	9	12	14	16	19	21	23	25	28
			μg./ml.	μg./ml.	μg./ml.	μg./ml.	μg./ml.	μg./ml.	μg./ml.	μg./ml.	μg./ml.	μg./ml.
Group I	*Cholesterol*, 2 mg. per kg. per day	29	1	16	66	68	118	196*	211	185	280	208 (216)‡
		32	2	26	111	148	243	321	359	392	Dead	
		34	1	10	26	42	86	162	192	196	271	276 (330)
		36	6	42	—	205	330	438	510	508	625	711 (850)
		38	2	24	60	130	162	209	262	258	330	212 (280)
		40	0	17	41	70	116	185	178	172	222	194 (188)
		Average.	2	23	61	111	176	252	285	285	346	320
Group II	*ACTH*, 2 mg. per kg. per day = total of 4 mg. per day given in 4 divided doses of 1 mg. every 6 hours	16	5	11	—	25	16	—	14	15		
		18	8	10	34	26	20	—	30	38		
		20	2	20	74	108	194	—	294	278		
		21	4	10	10	94	138	—	—	—		
		22	5	9	44	34	56	—	85	118		
		23	2	—	20	17	30	—	151	159		
		24	4	12	24	20	34	—	49	58		
		25	3	10	35	30	42	—	60	71		
		Average.	4	12	34	44	66	—	98	105		
Group III	*Compound E*, 2 mg. per kg. per day	1	4	7	4	2	0	2	3	4	4	1
		3	5	-	28	3	15	15	17	30	65	21
		5	9	6	25	12	9	15	17	24	46	15 (17)
		11	0	6	7	0	0	2	4	28	45	6 (12)
		13	2	2	7	0	1	3*	2	5	33	5 (7)
		15	3	3	1	0	0	0	0	4	6	—
		Average.	4	5	12	3	4	6	7	16	33	10
Group IV	*Compound E*, 2 mg. per kg. per day, 0 to 13th day *Cholesterol*, 2 mg. per kg., per day 14th day to termination	4	6	9	22	5	2	13	12	32	63	38 (40)
		6	10	26	58	53	120	199	204	218	Dead	
		8	5	3	15	0	3	26	34	42	80	70 (60)
		10	7	3	14	0	0	2	1	4	34	3 (5)
		12	7	17	18	7	18	28	34	46	—	109 (128)
		14	2	13	66	71	122	178*	207	270	386	362 (400)
		Average§.	7	8	17	3	6	17	20	31	59	55

TABLE II—*Concluded*

Group	Treatment	Rabbit No.	Antibody nitrogen content of serum on following days after first sensitizing injection of egg albumin									
			Days 7	9	12	14	16	19	21	23	25	28
			μg./ml.	μg./ml.	μg./ml.	μg./ml.	μg./ml.	μg./ml.	μg./ml.	μg./ml.	μg./ml.	μg./ml.
		27	0	11	38	48	83	64*	49	46	62	40(46)
	Cholesterol, 2	28‖	3	27	84	166	225	218*	177	146	162	147(160)
	mg. per kg.	31‖	1	2	142	166	—	196*	226	149	139	75(72)
	per day, 0 to	35	1	—	238	331	442	439	385	343	360	278(324)
	13th day	37	4	—	133	222	275	220	198	172	148	76(74)
Group V	*Compound E*, 5	39	0	24	63	123	162	164	109	114	89	34(40)
	mg. per kg. per day, 14th day to termination											
		Average.	2	16	116	176	237	217	191	162	160	108

* Serum sample was obtained on 20th day.

‡ Values in parentheses were obtained by the quantitative precipitin method of Heidelberger and Kendall.

§ Rabbits 6 and 14 which produced high levels of circulating antibody despite treatment by compound E are not included in the averages.

‖ Received compound E at a dosage of 2 mg. per kg. per day from 14th to 18th day.

day of sensitization despite treatment were the only rabbits of this group which produced a high level of circulating antibody, that is, antibody levels in excess of 40 μg. of antibody nitrogen per ml. On the other hand, the 2 ACTH-treated rabbits (Nos. 16 and 18) which responded only with a mild allergic reaction to injection antigen were the only rabbits of this group whose sera contained antibody in concentrations less than this amount. If the effect of ACTH on the Arthus reaction is due to its suppressive effect on circulating antibody, the disparity between the very slight effect of ACTH on the Arthus reaction and its rather great effect on serum antibody is now understandable, for although ACTH suppressed the antibody levels so that they were approximately one-third those of the controls, the circulating antibody content of the majority of these animals was nevertheless higher than the level required to give a maximum response in the skin test.

From the 23rd to the 26th days of the experiment, the intracutaneous injections of antigen were withheld from the control and compound E-treated rabbits. As shown in Fig. 2, there was a more rapid increase in circulating antibody during this period followed by a sharp decrease (particularly in the compound E-treated group) when the antigen injections were resumed. This change probably reflects the neutralizing capacity of the antigen. Since the daily injection consisted of 0.8 mg. antigen nitrogen which at the equivalence point can combine with approximately 8 mg. of antibody nitrogen, this effect

is considerable. In the case of animals treated with compound E, in which only small amounts of antibody are produced, the injection of this amount of antigen is apparently sufficient to remove almost all of the circulating antibody.

As shown in Table III, treatment with ACTH and compound E produced marked changes in the weights of the thymus, spleen, adrenals, and liver. As a result of treatment with ACTH, there was an 80 per cent reduction in the weight of thymus and, perhaps, a slight reduction in the size of the spleen. The adrenals were approximately 50 per cent larger than those of the controls. In the compound E–treated rabbits, there was a 70 per cent reduction in the weight of the thymus, a 60 per cent reduction in the weight of the spleen, and the adrenals weighed on the average 40 per cent less than those of the controls. In addition, the livers of 4 of 8 animals were larger than normal due to deposition of glycogen.

The Duration of the Inhibitory Effect of Compound E on the Arthus Reaction and on Antibody Production after Termination of Treatment

After 14 days of treatment, cholesterol was substituted for compound E in 6 of 12 animals (group IV, Table I). In this group in which the animals were chosen at random, there were 2 rabbits (Nos. 6 and 14) which had developed Arthus reactions and high circulating antibody levels despite treatment with compound E. Disregarding these 2 rabbits, however, at 2 weeks after termination of treatment with compound E, 2 of the 4 remaining animals had developed hemorrhagic lesions, 1, a red wheal, and a 4th (No. 10) continued to show no reaction. At this time, of the 6 rabbits which continued to receive compound E, 5 showed no reaction and 1 showed a slight erythema. These data are presented graphically in Fig. 3. As shown in Fig. 4, there was an increase in circulating antibody in 5 of 6 rabbits after compound E was discontinued. In rabbit 10, which never showed a reaction to the antigen, antibody continued to remain at a low level. The rise in antibody levels between the 23rd and 26th days is due to temporary cessation of the injections of antigen. Concomitant with the development of hypersensitivity and increased antibody levels following the termination of treatment with compound E, the organ changes previously produced by this hormone tend to revert to normal. As shown in group IV of Table III, 14 days after the termination of treatment, the average weights of the thymus and spleen were 2.0 and 1.2 gm. respectively, approximately 150 per cent and 50 per cent greater than the average weights of the thymus and spleen of the rabbits which received compound E throughout the experiment.

The Effect of Compound E on the Arthus Reaction and Circulating Antibody Levels When the Hormone Was Administered after the Onset of Sensitization

Eight of the 14 cholesterol–treated control rabbits previously sensitized by 14 daily intracutaneous injections of egg albumin were treated with com-

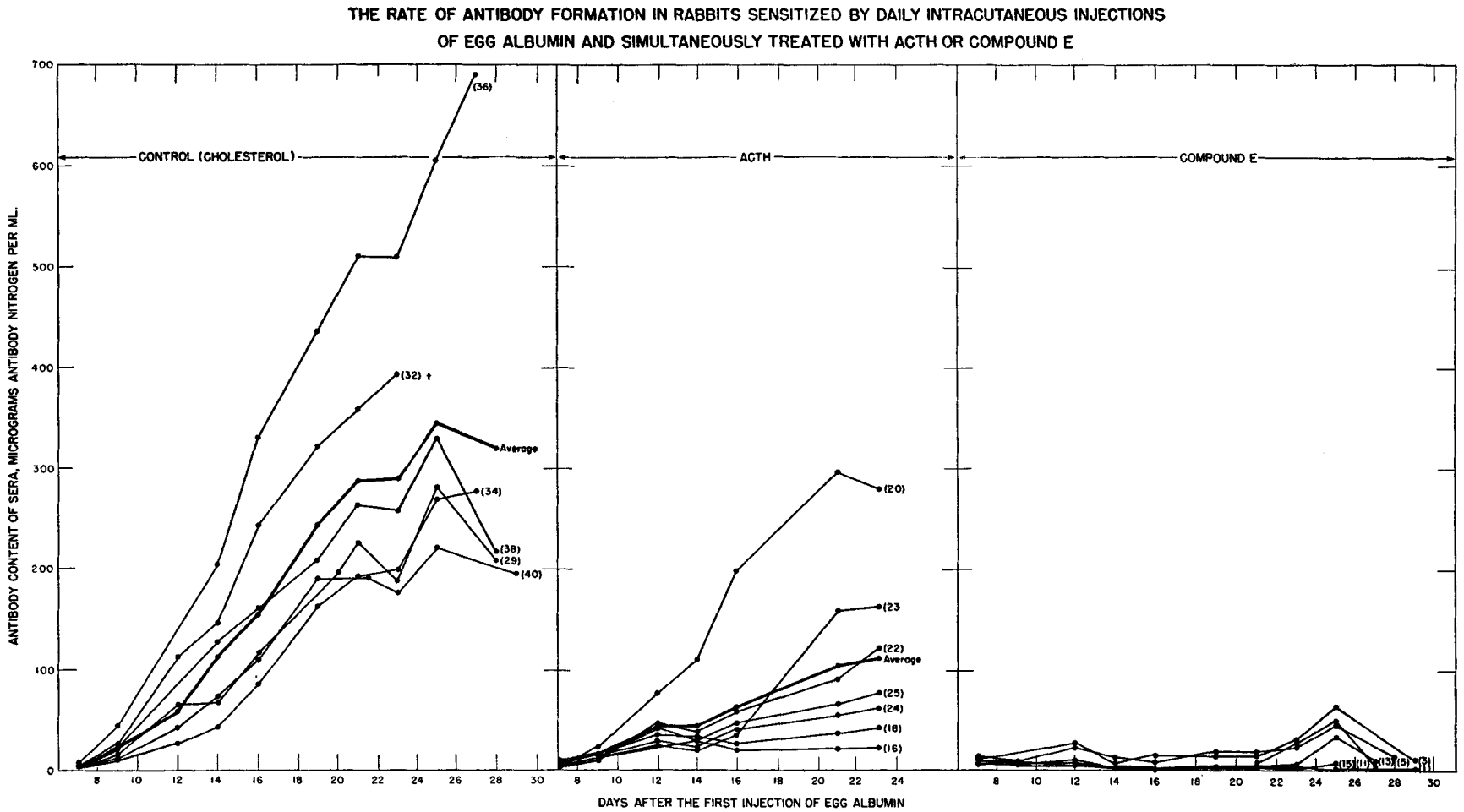


FIG. 2. After data of groups I, II, and III of Table II.

TABLE III

The Anatomical Changes in Rabbits Receiving Compound E or ACTH and Sensitized to Crystalline Egg Albumin

Group	Treatment	Rabbit No.	Weight	Weight of following organs					Day killed or dead
				Thymus	Spleen	Adrenals	Liver	Kidneys	
			kg.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
Group I	*Cholesterol*, 2 mg. per kg. per day	29	2.6	4.7	1.9	0.19	105	16.0	29K
		32	2.4	1.3	2.2	0.23	91	19.2	24D
		34	2.6	3.5	2.9	0.13	80	15.5	28K
		36	2.3	2.3	1.9	0.19	73	14.3	28K
		38	2.5	4.5	1.5	0.18	70	14.0	29K
		40	2.2	3.1	1.6	0.15	67	12.8	30K
		Average...	2.4	3.2	2.0	0.18	81	15.3	
Group II*	*ACTH*, 2 mg. per kg. per day = total of 4 mg. per day given in 4 divided doses of 1 mg. every 6 hours	16	1.8	0.37	1.6	0.30	67	14.7	26K
		17	1.8	1.2	2.7	0.24	70	18.8	13D
		18	2.3	1.4	2.9	0.25	86	16.7	26K
		19	1.2	0	0.48	0.21	49	14.1	19D
		20	1.7	0.46	1.2	0.37	72	15.4	26K
		23	2.0	0.62	2.1	0.27	76	17.3	26K
		Average...	1.8	0.68	1.8	0.27	70	16.2	
Group III	*Compound E*, 2 mg. per kg. per day	1	2.4	0.77	1.4	0.08	130	21.6	30K
		2	2.3	0.54	0.41	0.08	69	16.4	13D
		3	2.3	0.89	1.0	0.14	120	17.7	30K
		5	2.2	1.4	0.89	0.08	130	17.9	29K
		7	2.3	1.1	0.66	0.14	95	18.1	13D
		11	2.2	0.60	1.1	0.12	140	17.0	28K
		13	2.0	0.69	0.60	0.13	65	16.7	28K
		15	1.7	0.86	0.65	0.09	63	17.4	29K
		Average...	2.2	0.85	0.84	0.11	102	17.9	
Group IV	*Compound E*, 2 mg. per kg. per day, 0 to 13th day *Cholesterol*, 2 mg. per kg. per day, 14th day to termination	4	2.2	2.2	1.3	0.13	56	14.1	28K
		6	1.9	0.78	3.0	0.18	76	16.2	26D
		8	2.4	1.3	1.2	0.16	76	15.6	28K
		10	2.3	2.3	0.86	0.16	82	12.8	29K
		12	2.3	2.3	1.4	0.16	65	13.8	29K
		14	2.2	2.2	1.8	0.13	65	12.7	30K
		Average‡..	2.3	2.0	1.2	0.15	70	14.1	

TABLE III—*Concluded*

Group	Treatment	Rabbit No.	Weight	Weight of following organs					Day killed or dead
				Thymus	Spleen	Adrenals	Liver	Kidneys	
			kg.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
Group V	*Cholesterol*, 2	27	2.1	1.3	1.2	0.08	120	20.4	28K
	mg. per kg.	28§	2.3	2.3	1.2	0.12	151	19.4	28K
	per day, 0	31§	2.3	1.2	0.79	0.09	275	19.1	29K
	to 13th day	35	2.4	1.1	0.83	0.12	155	20.3	29K
	Compound E,	37	2.2	1.4	1.4	0.09	220	18.4	30K
	5 mg. per kg. per day, 14th day to termination.	39	1.9	1.1	1.1	0.13	70	17.9	30K
		Average...	2.2	1.4	1.1	0.11	165	19.3	

* Rabbits 21, 22, 24, and 25 which were allowed to survive following termination of the experiment are not included here.

‡ Rabbits 6 and 14 which developed severe Arthus reactions despite treatment with compound E are not included in the averages.

§ Received compound E at a dosage of 2 mg. per kg. per day from 14th to 28th day.

pound E at a daily dosage of 10 mg. until the termination of the experiment approximately 2 weeks later. As indicated previously, 2 of these animals died. The size of the Arthus reactions, in terms of the product of their length, width, and height measured in centimeters, to alternate daily injections of egg albumin is recorded in Table I (groups I and V) and Fig. 5. As shown there, even in the control animals after the 12th daily injection of egg albumin, succeeding injections of the antigen produced progressively smaller skin reactions. This occurred even at a time when the circulating antibody was sharply increasing. This diminution in size of the Arthus reaction may have been due to at least two circumstances: (1) Previous injections were made in the lower sides of the animal where the skin is loose and consequently the inflammatory edema has a greater tendency to spread. (2) The character of the Arthus reaction changed to one showing less edema but more induration and hemorrhage.[1] Nevertheless, it is obvious that treatment with compound E did not produce a profound effect on the Arthus reaction. Even after 2 weeks of treatment, the skin lesions of the compound E–treated animals were only slightly smaller and less severe than those of the controls.

[1] The decrease in the area of skin involved in the Arthus reaction as the serum antibody concentrations increase may be the result of a more rapid fixation and localization of antigen. In terms of tissue damage, the large edematous reactions are less severe than the localized hemorrhagic and necrotic lesions. In this respect, the figures which only give an indication of the size of the Arthus reaction and not its severity may be misleading.

Although treatment with compound E failed to alter the Arthus reaction appreciably, its suppressive action on circulating antibody was striking. When treatment with compound E was instituted, the 6 experimental animals contained circulating antibody levels ranging from 48 to 331 μg. of antibody nitrogen per ml. Following treatment, the antibody levels increased for 2 days, then reached a plateau, and gradually decreased as shown in Fig. 6.

THE DURATION OF THE INHIBITORY EFFECT OF COMPOUND E ON THE ARTHUS REACTION IN RABBITS FOLLOWING TERMINATION OF TREATMENT

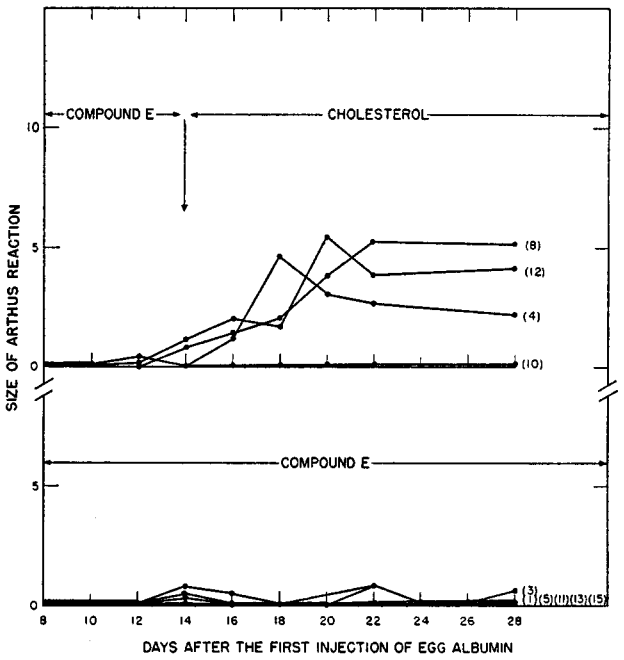


FIG. 3. After data of group IV of Table I. Rabbits 6 and 14 which developed severe Arthsu reactions before treatment with compound E was discontinued are not included in the figure.

After 2 weeks of treatment, the serum antibody concentrations of the treated and control animals were 108 and 320 μg. of antibody nitrogen per ml. respectively (groups I and V, Table II). The rate of change in the antibody content of the serum of each rabbit following treatment is presented in Fig. 7. As shown there, the antibody levels of the controls continued to rise and then began to level off at the end of the experiment. At this time, the average antibody concentration of the control rabbits was 349 per cent of the average at the end of 2 weeks of sensitization. On the other hand, after 2 days treatment, the antibody levels of the compound E–treated animals began to fall so that by the end of the experiment the average antibody concentration was

60 per cent of the pretreatment value. The inflections in the antibody levels between the 8th and 12th day of treatment are again the result of a temporary cessation of the injections of antigen. As shown in Table III, treatment with compound E produced the characteristic atrophy of the thymus, spleen, and

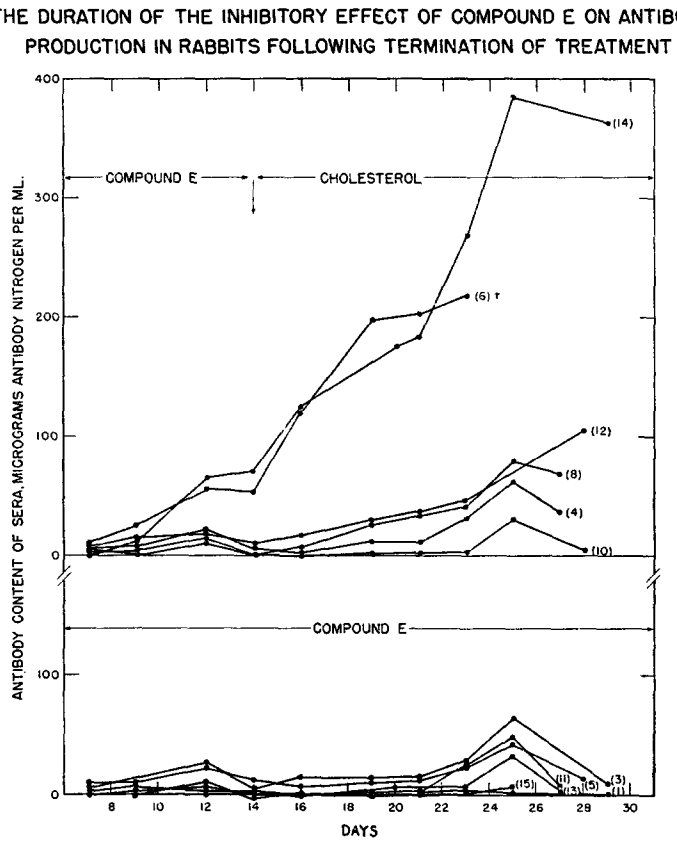


FIG. 4. After data of group IV of Table II.

adrenals and enlargement of the liver. As indicated in a previous report (2), with a daily dosage of 10 mg. of compound E per day, as employed in the present experiment, the deposition of glycogen in the liver is considerable.

Subsidiary Experiment on the Effect of Compound E on the Arthus Reaction and Antibody Levels When the Hormone Was Administered after the Onset of Hypersensitivity

In the previous experiment it was found that although treatment with compound E following sensitization produced a striking reduction of the anti-

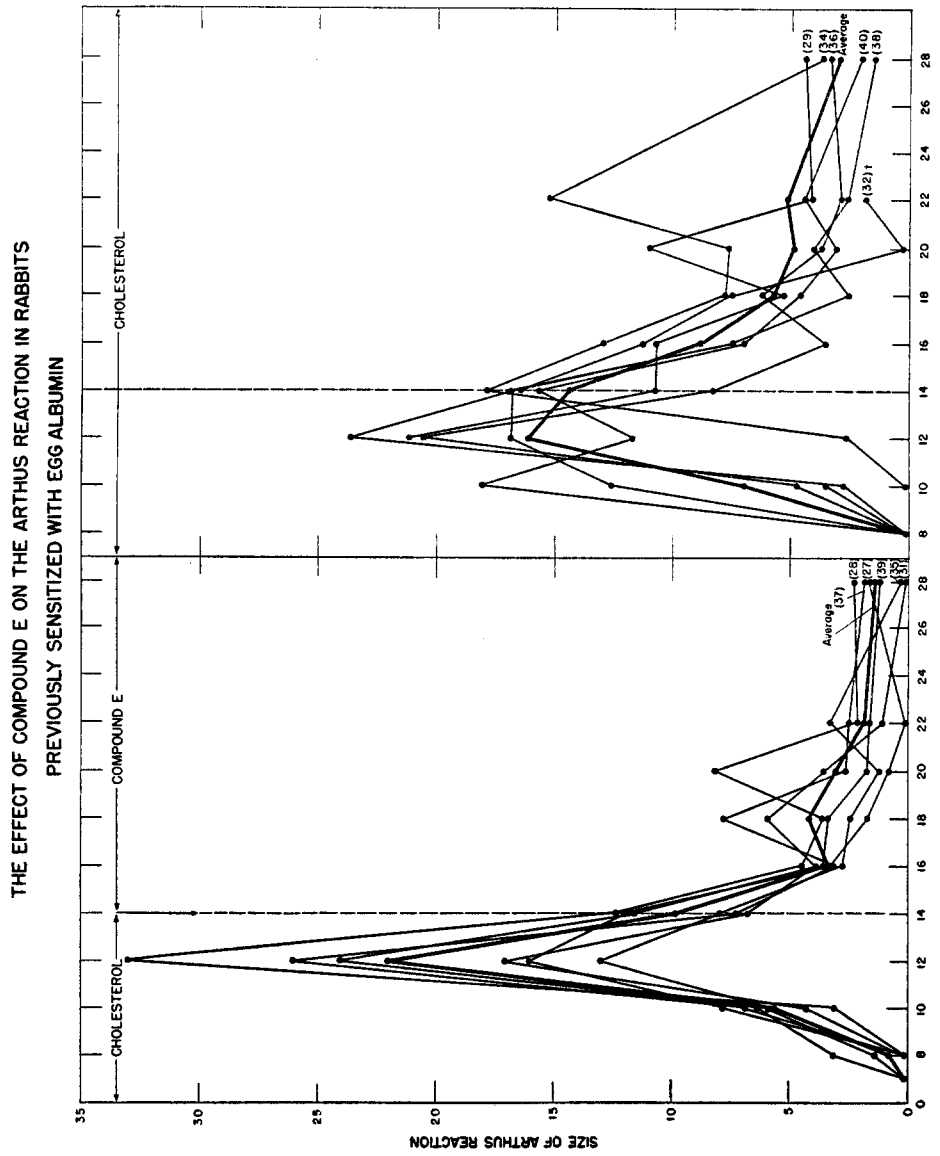


FIG. 5. After data of groups I and V of Table I.

body levels, there was only a slight diminution in the skin responses of the rabbits to the injected antigen. It has already been noted that a circulating antibody level of approximately 40 μg. of antibody nitrogen per ml. of serum is sufficient to produce the most intense Arthus response in terms of maximum

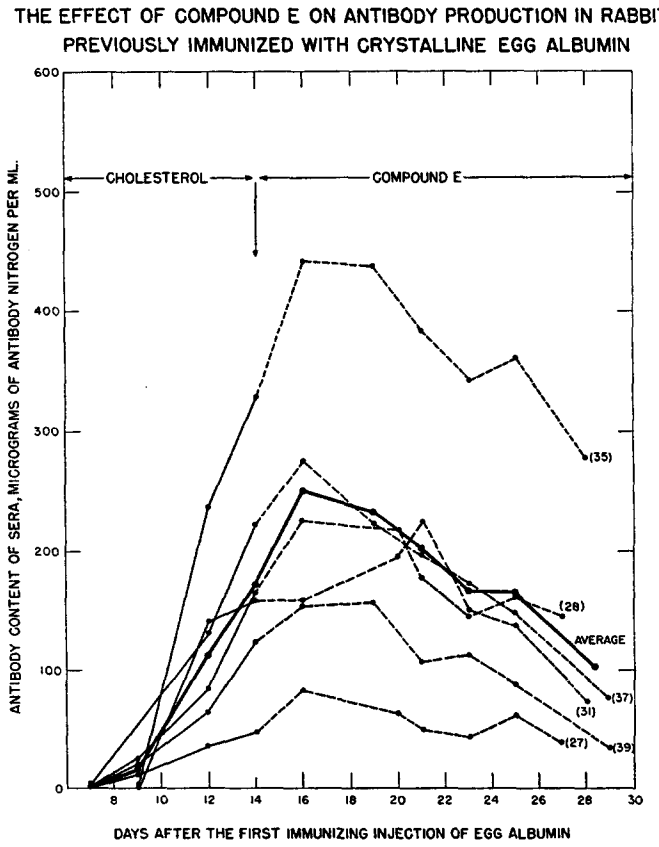


FIG. 6. After data of group V of Table II. Refer to the first section of FIG. 2 for the immune response of the control animals.

tissue damage. In the light of this observation, if the inhibitory action of compound E on the Arthus reaction is due to suppression of circulating antibody, the reason for the failure of compound E to reduce the Arthus response in this experiment was apparent. Even though compound E reduced circulating antibody levels considerably, these were at no time reduced below the value of 40 μg. of antibody nitrogen per ml. during the experimental period, and, therefore, the treated animals could continue to react maximally to the antigen.

In order to learn whether treatment with compound E would inhibit the

Arthus reactions in rabbits previously sensitized with egg albumin if the serum antibody concentrations were sufficiently reduced, a second experiment was undertaken. Instead of treating animals after they had attained high levels of circulating antibody and had been reacting maximally to the intracutaneously injected antigen, animals were treated as soon as they developed a definite response.

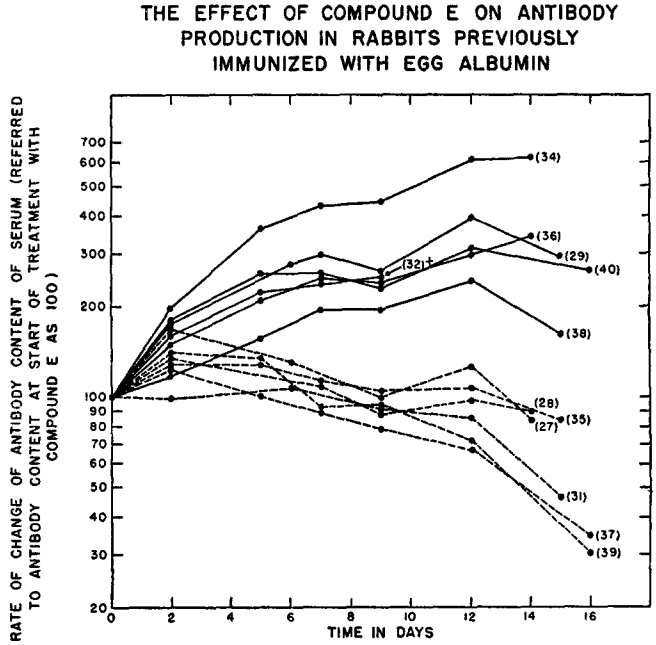


FIG. 7. After data of groups I and V of Table II. The serum antibody concentrations on the 14th day of sensitization when treatment with compound E was instituted are referred to as 100 per cent. The control animals are represented by solid lines and the rabbits treated with compound E by interrupted lines.

A series of rabbits were sensitized as previously described and following the onset of hypersensitivity, every other rabbit was treated with compound E. Five were treated at a daily dosage of 4 mg. of compound E for 5 days and then with 10 mg. of the hormone for the next 5 days, while 4 others received 10 mg. per day throughout the experiment. The animals continued to receive the injections of the antigen in order to determine the effect of treatment on the Arthus reaction. The animals were bled for 10 ml. of blood on the 1st day of treatment and at varying intervals thereafter. Quantitative precipitin determinations were performed according to the method of Heidelberger and Kendall and the precipitate nitrogen was determined by the Markham modification of the micro Kjeldahl technique (14).

The results of this experiment are recorded in Tables IV and V and are shown graphically in Figs. 8 and 9. Six of 9 control animals showed increasing sensitivity and produced maximal responses to the injected antigen in the

form of indurated and hemorrhagic skin reactions. In marked contrast, 7 of 9 treated animals showed a progressively milder reaction to the antigen and failed to react at all by the 6th day of treatment. Two animals (Nos. 7 and 17) represented by the broken lines in Fig. 8, which had been given a daily dosage of 4 mg. of compound E during the first 5 days were seemingly unaffected by

TABLE IV

The Effect of Compound E on the Arthus Reaction in Rabbits Previously Sensitized with Egg Albumin

Treatment	Rabbit No.	Day of sensitization or when treatment was begun	Magnitude and appearance of Arthus reaction on following days after treatment was instituted										
			Days.. 0	1	2	3	4	5	6	7	8	9	10
None, control	12	8	1.0 p	5.3 p	16 p	4.5 p	7.6 r	14 p	4.8 r	3.4 p	3.6 p	8.8 r	8.2 h
	4	10	7.7 p	34 p	34 p	37 p	6.8 p	7.9 p	5.4 p	9.8 p	3.4 p	1.2 p	0.4 p
	5	11	2.0 p	1.8 p	2.4 p	1.1 p	4.1 p	3.4 p	6.6 p	5.1 p	3.0 p	3.1 p	3.0 p
	19	11	14 p	12 r	7.0 h	8.7 h	2.8 r	4.9 h	8.6 h	1.9 r	4.7 h	10 h	7.7 r
	22	11	15 p	66 r	26 p	13 p	—	4.0 p	7.6 r	8.0 h	2.8 h	2.5 r	4.5 h
	18	13	7.2 p	5.7 r	4.1 r	3.9 h	2.3 h	5.3 h	9.7 h	5.0 r	7.6 r	12 r	6.0 r
	24	13	23 p	5.7 p	4.9 p	3.9 p	9.0 p	2.5 p	6.2 r	2.4 r	1.8 p	4.6 r	1.3 r
	23	14	4.3 p	8.9 h	6.7 h	9.9 h	10 h	5.4 r	6.6 r	4.7 r	8.8 r	4.5 h	11 h
	20	14	4.2 h	4.8 h	9.2 h	7.2 h	4.5 r	7.9 r	7.0 r	18 r	4.2 r	6.0 p	
	Median.	11	7.2	5.7	7.0	7.2	6.8	5.3	6.6	5.0	3.6	4.6	6.0
Compound E*	17	8	7.9 p	6.4 p	15 p	3.6 p	32 h	20 p	22 p	8.8 p	5.1 p	13 p	2.9 p
	7	10	4.6 p	1.8 p	11 p	11 p	6.5 p	2.6 p	6.1 p	11 p	13 p	5.1 p	8.2 p
	8	11	4.4 p	4.9 r	4.1 p	3.5 h	0.8 p	0.5 p	0	0	0	0	0D‡
	21	11	0.3 p	2.2 r	0.7 p	0.8 p	0.4 p	0	0	0	0	0	0
	16	11	7.9 p	4.3 r	4.5 r	4.0 p	3.0 p	0.6 p	0.2 p	0	0	0.5 p	0
	11	13	3.8 h	0.6 h	1.4 r	2.6 h	0.7 p	0	0	0	0	0	0
	15	13	10 p	2.1 p	1.3 p	0	0	0D	0	0	0	0	0
	9	14	2.9 p	0.7 p	1.5 r	1.3 r	0.5 p	0	0	0	0	0	0
	6	15	5.0 p	1.0 p	0.9 p	0.4 p	0	0	0	0	0	0	0
	Median.	11	4.6	2.1	1.5	2.6	0.7	0	0	0	0	0	0

* Rabbits 17, 7, 8, 21, and 16 received compound E at a dosage of 2 mg. per kg. per day for the first 5 days and 5 mg. per kg. per day thereafter.

Rabbits 11, 15, 9, and 6 were treated throughout with a dosage of 5 mg. per kg. per day.

‡ Diarrhea.

treatment and continued to produce an erythematous and edematous lesion to the injected antigen.

While in the control rabbits the serum antibody concentrations were increasing, reaching by the termination of the experiment a median level of 68 μg. of antibody nitrogen per ml., in 7 of 9 animals receiving compound E the antibody decreased and had all but disappeared by the 10th day of treatment. It is noteworthy that the 2 animals (Nos. 7 and 17) whose skin reactivity was unaltered by treatment, maintained a constant level of circulating antibody despite the administration of compound E.

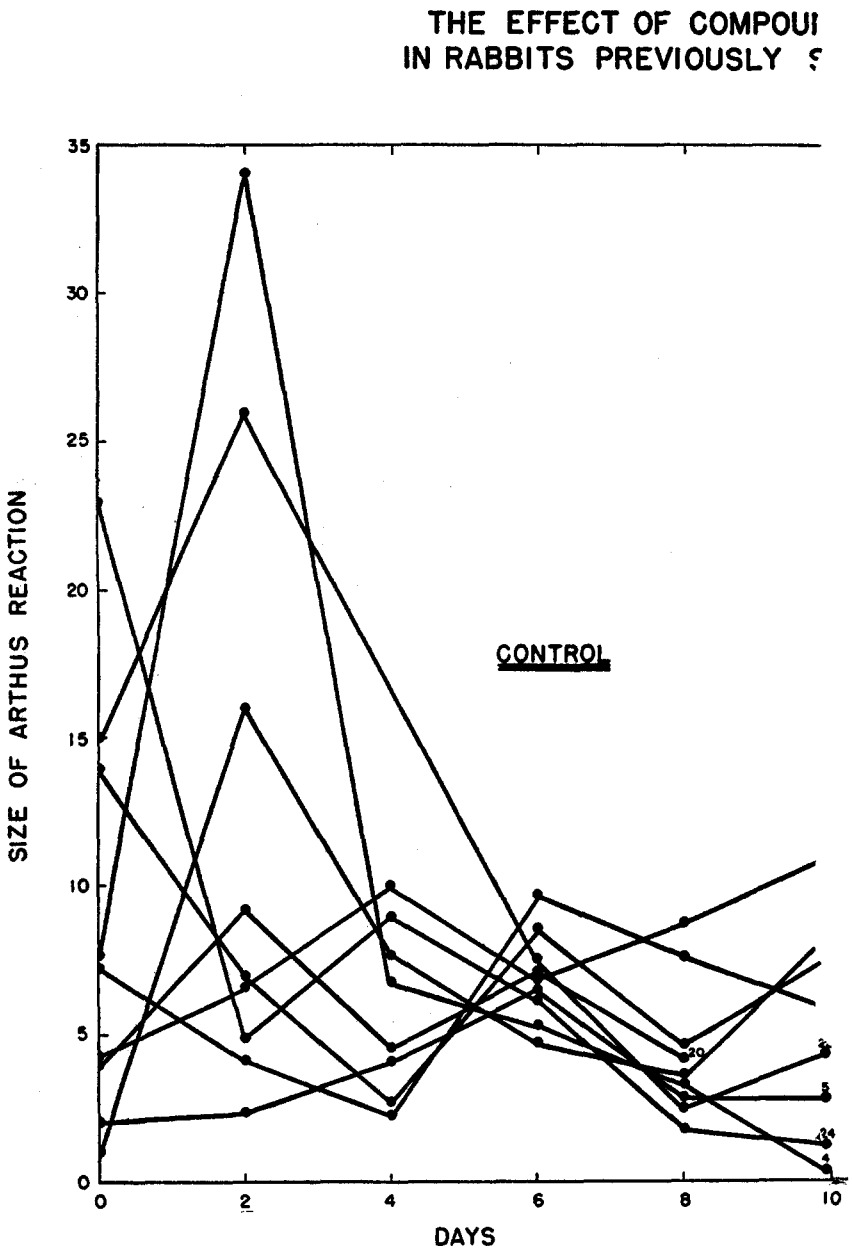
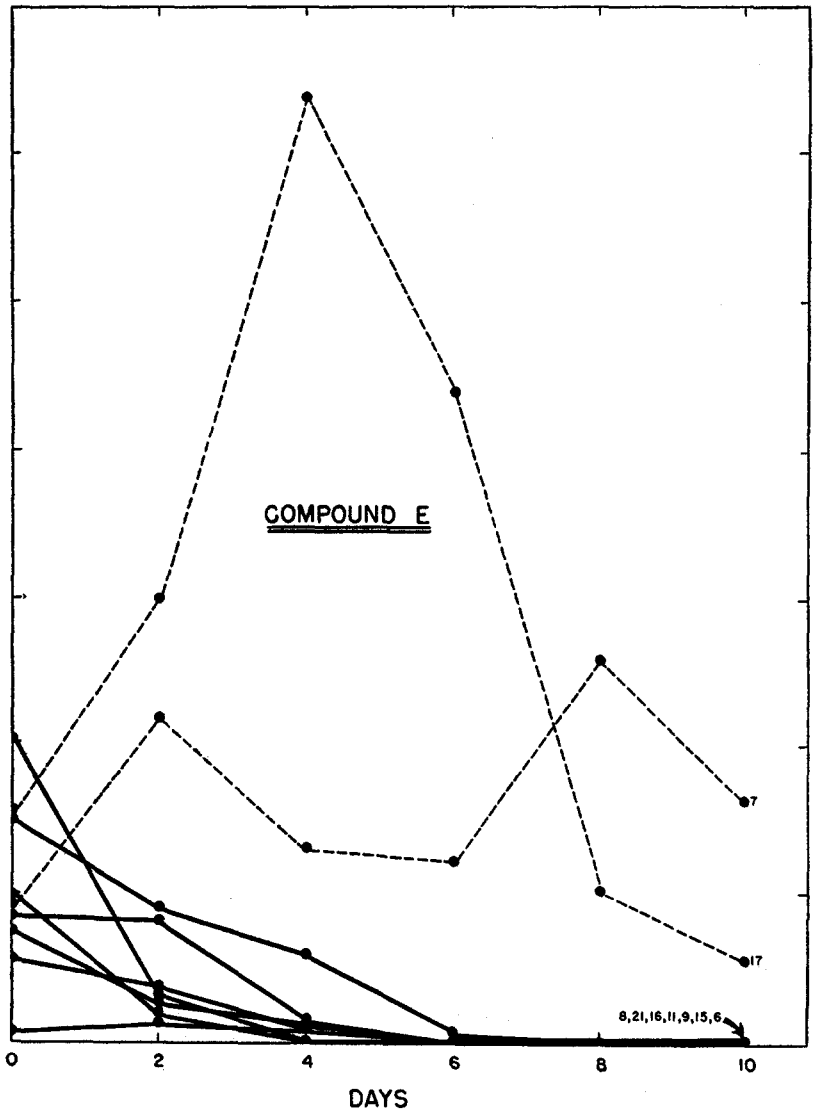


FIG. 8. After data of Table IV. Two rabbits (Nos. 7 and 17) which continued to re- lines.

ON THE ARTHUS REACTION
TIZED WITH EGG ALBUMIN



with an Arthus reaction despite treatment with compound E are represented by interrupted

The Effect of Compound E on Antibody Levels in Passively Immunized Rabbits

In the actively immunized or sensitized animal, antibody is constantly synthesized and destroyed and the level of circulating antibody at any given time

TABLE V

The Effect of Compound E on Serum Antibody Concentrations of Rabbits Previously Immunized with Crystalline Egg Albumin

Treatment	Rabbit No.	Day of sensitization when treatment was begun	Antibody nitrogen content of serum on following days after treatment				
			Days....0	2	4	7	10
			μg./ml.	*μg./ml.*	*μg./ml.*	*μg./ml.*	*μg./ml.*
None, control	12	8	0	9	18	31*	39
	4	10	3	9	14	26*	—
	5	11	1	6	7	14	6
	19	11	12	30	43	67	77
	22	11	9	15	28	71	61
	18	13	17	25	79‡	63*	68
	24	13	21	29	40‡	35*	45
	23	14	35	77	105	104	127
	20	14	37	86§	—	148‖	181¶
	Median....	11	12	25	40	63	68
Compound E	17	8	0	19	25	26*	—
	7	10	0	18	17	16*	—
	8	11	10	12	7	11	7
	21	11	0	2	0	0	0
	16	11	13	12	15	—	0
	11	13	33	32	20‡	8*	3
	15	13	4	1	0*	0*	0
	9	14	15	19	19	5	3
	6	15	6	12§	—	4‖	1
	Median....	11	6	12	15	5	1

* 8 days after treatment.
‡ 5 days after treatment.
§ 3 days after treatment.
‖ 6 days after treatment.
¶ 9 days after treatment.

is dependent in a large measure on the relative rates at which these two opposing processes are operating. In addition, the rate of release of antibody into the blood and its rate of removal from the circulation into the body tissues may also be factors which influence antibody levels. In the passively immunized animal, in which there is an absence of antibody formation, antibody rapidly disappears from circulation both as a result of destruction and as a result of diffusion into the surrounding tissues. In order to clarify the mechanism by

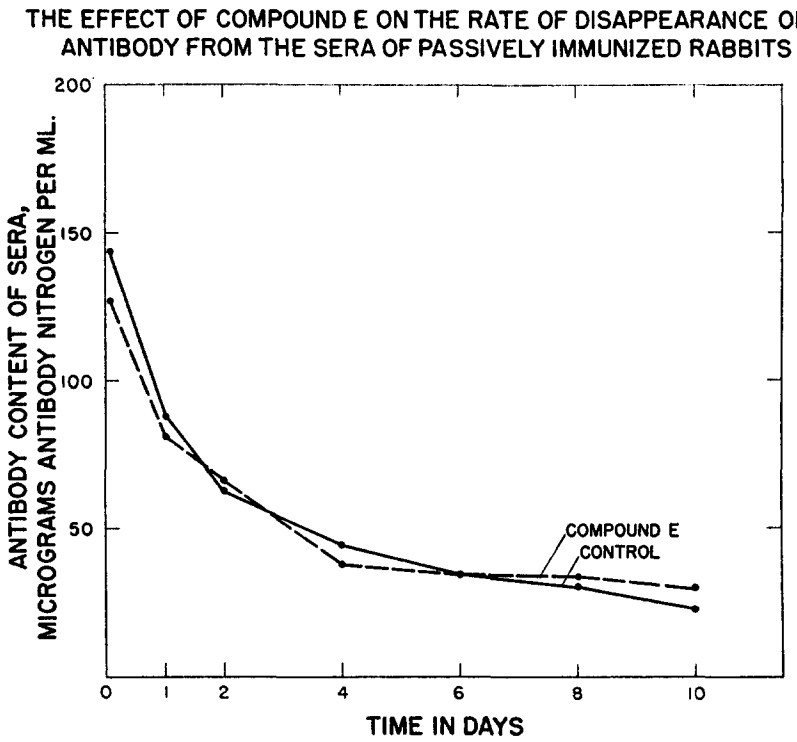


FIG. 10. After data of Table VI. Averages of 6 control and 6 compound E–treated rabbits.

TABLE VI

The Effect of Compound E on the Rate of Disappearance of Antibody from the Sera of Passively Immunized Rabbits

Rabbit No.	Treatment	Antibody nitrogen content of serum on following days after passive immunization						
		Days 0.02	1	2	4	6	8	10
		μg./ml.	μg./ml.	μg./ml.	μg./ml.	μg./ml.	μg./ml.	μg./ml.
1	*Compound E*, 5 mg. per kg. per day for 5 days prior to immunization and for the next 10 days	124	82	60	36	30	26	26
2		128	98	76	36	46	46	30
3		122	74	86	40	26	30	Dead
4		112	52	44	32	32	Dead	
5		142	86	60	40	30	26	24
6		142	94	72	46	46	50	42
Average		128	81	66	38	35	35	31
7	Control	142	78	60	40	24	26	22
8		134	86	66	42	32	28	20
9		160	98	68	52	40	36	28
10		140	90	60	48	40	32	22
11		158	94	68	48	40	32	28
12		130	88	64	42	36	40	20
Average		144	89	64	45	35	32	23

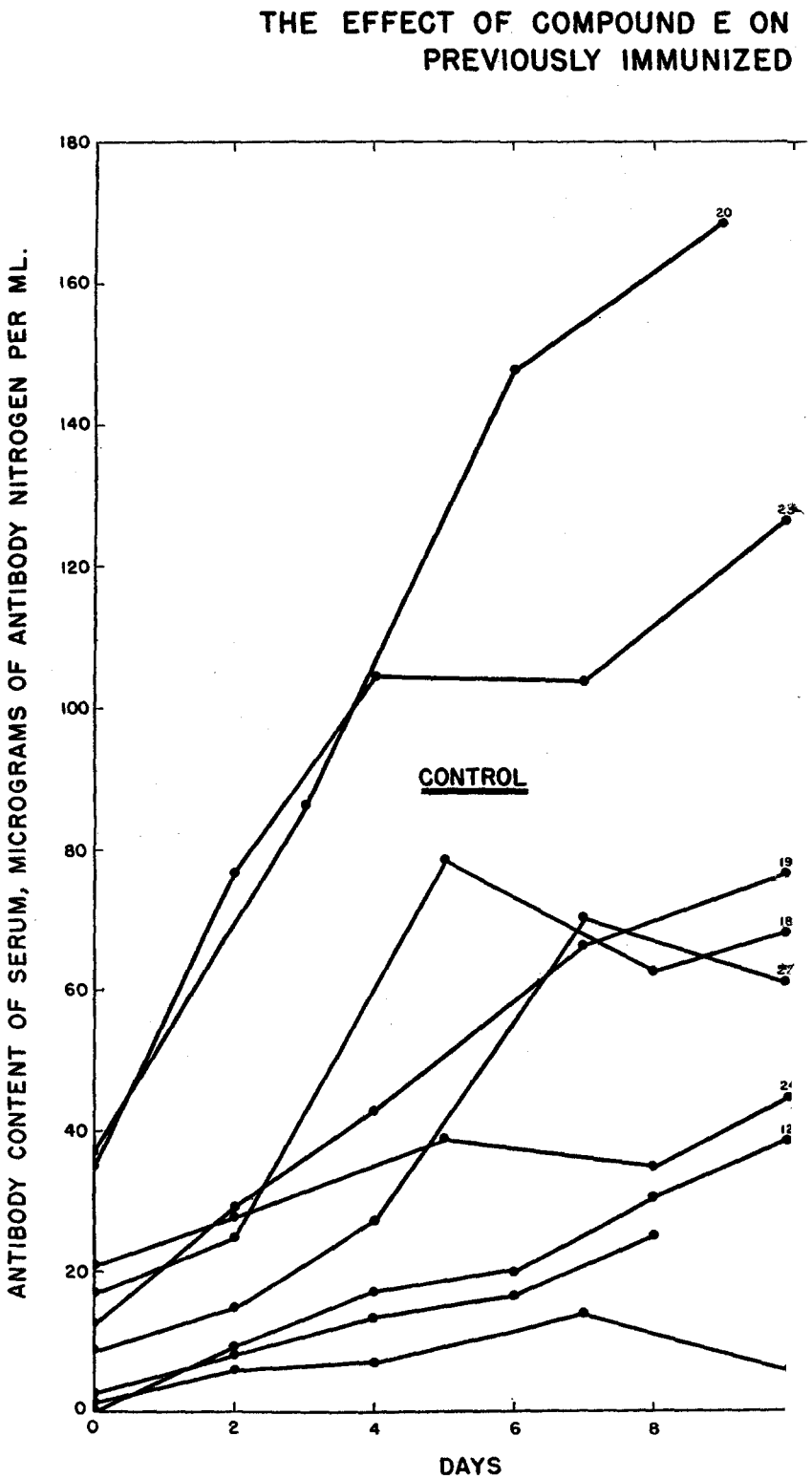
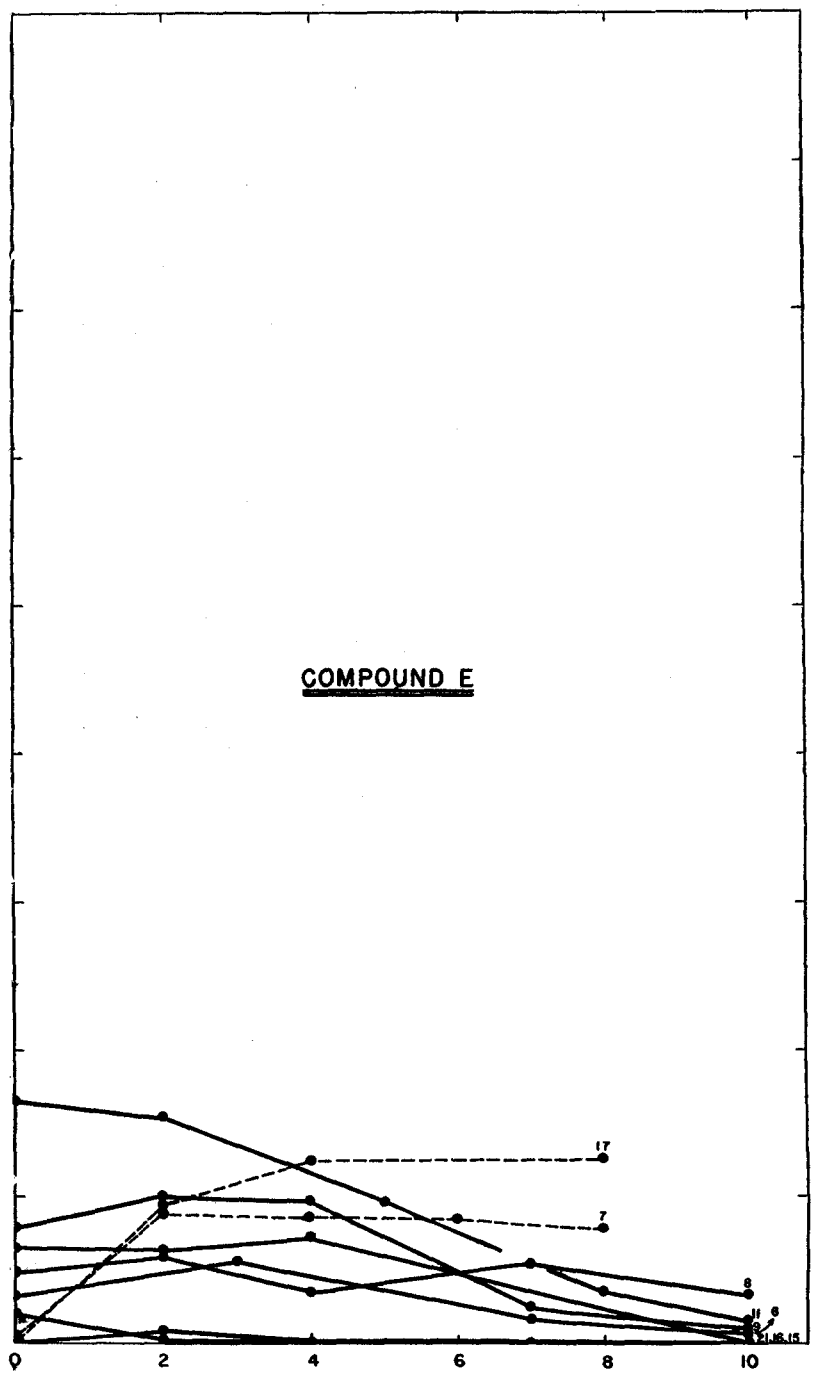


FIG. 9. After data of Table V. Two rabbits (Nos. 7 and 17) which produced and E are represented by interrupted lines.

JLATING ANTIBODY LEVELS IN RABBITS
CRYSTALLINE EGG ALBUMIN



a reasonable concentration of circulating antibody during treatment with compound

which compound E alters antibody levels, the effect of compound E on circulating antibody in passively immunized animals was investigated.

Twelve animals were passively immunized by an intravenous injection of 21 mg. of anti-egg albumin rabbit antibody nitrogen contained in 20 ml. of serum. Six of the 12 rabbits were

TABLE VII

The Effect of Compound E on the Passive Arthus Reaction Following Systemic Sensitization

Rabbit No.	Treatment	Size and appearance of Arthus reaction produced by										
		0.01 mg. bovine albumin			0.1 mg. bovine albumin				1 mg. bovine albumin			
		2 hrs.	4 hrs.	6 hrs.	2 hrs.	4 hrs.	6 hrs.	24 hrs.	2 hrs.	4 hrs.	6 hrs.	24 hrs.
1	*Compound E,*	0.1p	0.4r	Dead	0.2h	0.9h	Dead	—	0.6h	1.5h	Dead	—
2	5 mg. per	0.1h	0.1r	0.2r	0.3h	0.8h	1.4h	1.8r	0.7h	3.0h	3.9h	8.9h
3	kg. per day	0.1p	0.2p	0.4p	0.4p	1.0h	1.4h	0.9r	0.8h	1.9h	3.5h	4.4h
4	for 3 days	0.1p	0.0p	0.1p	0.5p	0.4h	0.1r	0.8r	0.2p	1.5h	3.6h	6.1h
5	prior to sen-	0.2h	0.8p	0.7p	0.5h	1.8h	2.1h	0.9r	0.1p	2.5h	2.4h	3.2h
6	sitization	0.1h	0.5r	0.7r	0.2h	1.2h	2.4h	1.8r	0.1h	1.4h	3.0h	6.1h
7	and on the	0.1p	0.6p	0.4p	0.4p	1.3r	1.7r	0.2p	0.4p	1.5h	3.3h	2.4h
8	day of sen-	0.1p	0.3p	0.1p	0.3p	0.7r	1.0r	0.4r	0.5h	2.1h	3.0h	1.5h
9	sitization	0.0p	0.0p	0.2p	0.1p	1.3r	1.1r	0.8p	0.2p	2.0h	2.5h	3.7h
10		0.2h	0.7r	0.8r	0.2h	1.7h	3.2r	1.1r	0.3h	3.2h	6.5h	8.6r
Average		0.1	0.4	0.4	0.3	1.1	1.7	0.1	0.4	2.1	3.5	5.0
11		0.2p	0.2p	0.3p	0.6h	1.8h	1.6h	0.9r	0.8h	2.4h	3.6h	5.0h
12		0.4p	0.2p	0.5p	0.9h	1.2h	1.8h	0.9r	0.8h	1.8h	3.5h	4.3h
13		0.4p	0.4p	0.5r	0.9h	1.8h	2.6h	1.0p	1.1h	2.7h	3.2h	5.4h
14		0.2p	0.2p	0.2p	1.1h	1.6h	2.2h	1.3r	1.1h	1.9h	3.3h	4.6h
15	None, control	0.5p	0.2p	0.5p	1.5h	1.8h	2.6h	0.9r	0.9h	2.2h	5.4h	5.5h
16		0.2p	0	0.2p	1.1h	1.2h	1.2r	0.7r	1.1h	1.8h	2.8h	3.0h
17		0.1p	0.1p	0.2p	0.2h	0.7h	1.4h	0.7r	0.3h	1.3h	1.4h	3.9h
18		0.1p	0.2r	0.1r	0.2h	1.0h	1.0h	0.7r	0.6h	1.3h	2.6h	5.0h
19		0.1p	0.1p	0	0.6h	1.3r	1.6h	0.1r	0.8h	1.9h	4.3h	7.1h
20		0.1p	0.1p	0.1p	0.2h	0.3h	0.8h	0.5p	0.2p	0.2h	1.1h	3.7h
Average		0.2	0.2	0.3	0.7	1.3	1.7	0.9	0.8	1.7	3.3	4.8

treated for 5 days before immunization and for the next 10 days after immunization with 10 mg. of compound E per day. The antibody nitrogen content of the serum of each rabbit at ½ hour and 1, 2, 4, 6, 8, and 10 days after immunization is recorded in Table VI.

The average antibody nitrogen concentrations of the sera of the 6 treated and 6 control animals for each time interval are plotted in Fig. 10. It is apparent that treatment with compound E had no effect on antibody levels in the passively immunized rabbits, indicating that there is no increased destruction of antibody or increased diffusion of antibody into tissues due to

treatment and suggesting that the effect of compound E on antibody levels in the actively immunized rabbits is due to the suppression of new antibody formation.

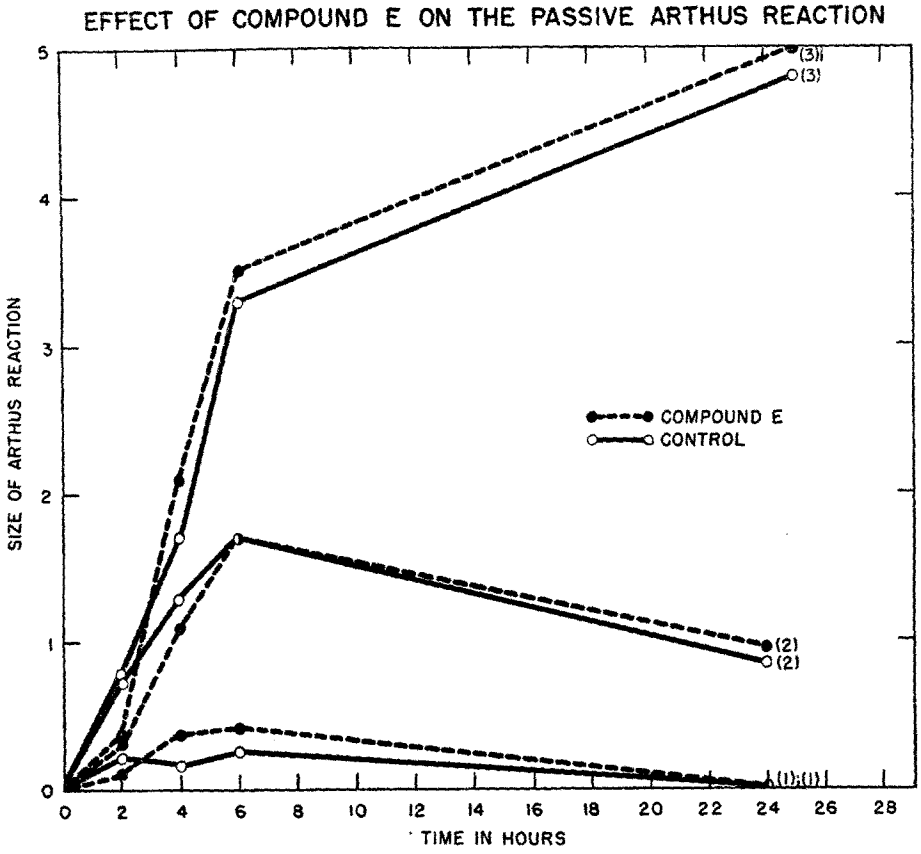


FIG. 11. After data of Table VII. Average skin responses of 10 control and 10 compound E-treated rabbits to (1) 0.01 mg., (2) 0.1 mg., and (3) 1 mg. of bovine albumin.

The Effect of Compound E on the Passive Arthus Reaction Following Systemic Sensitization

In a previous communication, it was reported that treatment with compound E had no effect on the *local* passive Arthus reaction produced by an injection of antigen into a skin site previously sensitized by antibody. In the light of recent observations indicating that treatment with compound E alters capillary and tissue permeability (15), the effect of compound E on the *systemic* passive Arthus reaction was determined. This reaction which follows an intracutaneous injection of antigen into the skin of animals previously sensitized by the intra-

venous administration of antibody is produced by the same sequence of events as the active Arthus reaction.

Twenty rabbits were passively sensitized by an intravenous injection of 12 mg. of anti-bovine albumin rabbit antibody nitrogen contained in 4 ml. of serum. Thirty minutes later, each rabbit was injected intracutaneously at 3 different skin sites with 1 mg., 0.1 mg., and .01 mg. of bovine albumin. Ten of the 20 rabbits were treated daily with 10 mg. of compound E for 3 days prior to and on the day of sensitization. The skin sites were observed at 2, 4, 6, and 24 hours after challenge and all reactions were measured and recorded. The size and appearance of the skin reactions to 1 mg., 0.1 mg., and 0.01 mg. of bovine albumin at each time interval are recorded in Table VII. The average size of the skin reactions of the treated and control animals to each of the three quantities of bovine albumin and at each time interval is plotted in Fig. 11.

All the rabbits, both treated and control, responded to the largest quantity of bovine albumin with a hemorrhagic Arthus reaction. This reaction increased in size and severity for 24 hours after injection. With 0.1 mg. of bovine albumin, a hemorrhagic skin reaction was produced which was greater in size at 6 hours than at 24 hours and with the smallest amount of antigen, 0.01 mg. bovine albumin, only a small pink, slightly hemorrhagic or red evanescent wheal was produced. Two hours after challenging the reactions of the compound E–treated rabbits to all 3 injections of antigen appeared on the average smaller than those of the controls. However, at 4, 6, and 24 hours, there were no differences between the reactions of compound E–treated rabbits and those of the control group even in the case of the very mild reaction to the smallest quantity of antigen.

DISCUSSION

These studies confirm the observations previously reported from this laboratory which indicated that treatment with compound E and, to a lesser degree, ACTH results in a reduction in circulating antibody and a suppression of experimental hypersensitivity of the Arthus type. The present data show that similar effects are produced by compound E when this hormone is administered at a dosage which is approximately equivalent to that generally advocated in the treatment of man (100 mg. per day). In agreement with the findings of the previous study, ACTH produced only a slight inhibition of the Arthus reaction, although the reduction in circulating antibody following treatment with this hormone was considerable. However, the serum antibody concentrations were never maintained at as low a level as those of the compound E–treated animals and, furthermore, in most instances, they reached levels higher than the concentration sufficient for eliciting a maximal Arthus response with the amount of antigen employed (approximately 40 μg. of antibody nitrogen per ml. of serum).

The inhibition of the Arthus state by compound E was only temporary.

When treatment was discontinued, there was a gradual increase in the severity of the skin response and a concomitant rise in circulating antibody.

When treatment with compound E was started after the onset of hypersensitivity, there was a more or less rapid disappearance in circulating antibody. However, the effect on the Arthus reaction was variable and dependent on the magnitude of the pretreatment serum antibody concentration. In one group of animals in which the circulating antibody concentrations were greatly in excess of that required to produce a maximal Arthus response, there was little change in the severity of the Arthus reactions even though treatment was continued for as long as 2 weeks. However, at the end of treatment, the serum antibody concentrations were still in excess of 40 μg. of antibody nitrogen per ml. It would be expected that further treatment, bringing about an additional decline in circulating antibody, would have resulted in a definite inhibition of the Arthus response. In a second group of animals in which treatment was started before the circulating antibody had reached so high a level, there was a rapid loss of skin reactivity and after 6 days of treatment the majority of the animals failed to respond at all to the injected antigen. In these animals, the circulating antibody had all but disappeared owing to treatment.

The data presented here indicate that the inhibitory action of compound E and presumably ACTH on experimental hypersensitivity results from specific desensitization due to the hormonal suppression of the production of circulating antibody. In every instance in which treatment with compound E inhibited the Arthus reaction, the serum antibody concentrations were maintained or reduced to levels far below those required for the maximal Arthus response. Of even greater significance is the fact that in those animals in which the serum antibody concentrations remained high despite treatment, compound E also failed to alter the Arthus reaction.

It is obvious that the rate at which compound E abolishes the hypersensitive state is dependent on at least 2 factors: (1) the level of the pretreatment serum antibody concentration, and (2) the rate of disappearance of circulating antibody following treatment. The importance of the level of pretreatment circulating antibody arises from the fact that the severity of the Arthus reaction varies with circulating antibody only through a limited range of the latter, that is from 0 to 40 μg. of antibody nitrogen per ml. The intensity of the vascular and tissue damage is maximal at approximately 40 μg. of antibody nitrogen per ml. of serum and any antibody in excess of this amount does not increase the severity of the reaction at least with amount of antigen herein employed. Therefore, if treatment is instituted when the serum antibody concentration is very high, no effect on the Arthus response will be observed until the antibody concentration is reduced below the level giving rise to the maximal Arthus response. This concept is presented diagrammatically in Fig. 12. In the case of rabbit A in which treatment is started when the serum antibody

concentration had obtained a level of 200 μg. of antibody nitrogen, there is a latent period of 15 days before antibody is reduced sufficiently for an effect on the Arthus reaction to become apparent. On the other hand, with rabbit B, there is an immediate effect on the Arthus reaction since a slight reduction in circulating antibody will decrease the serum antibody concentration to a level below that required for maximal tissue reactivity.

The mechanism by which serum antibody is reduced by treatment is now under study. The failure of compound E to increase the rate of disappearance of antibody in passively immunized animals suggests that the reduced antibody levels are the result of a suppression of antibody formation rather than a more

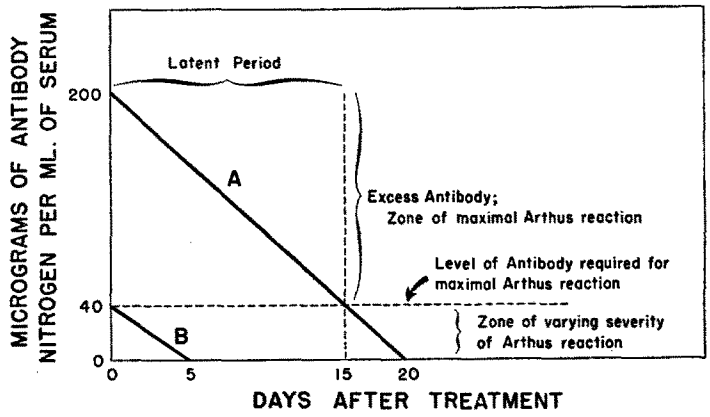


FIG. 12. Diagram showing the relationship between the pretreatment serum antibody concentration and the length of treatment required for inhibition of the Arthus reaction.

rapid breakdown of antibody protein. The relationship between the antibody changes and the marked lymphoid atrophy following treatment is probably more than coincidental. Other procedures such as the administration of x-ray (16) and nitrogen mustards (17) which result in the destruction of lymphoid tissue also inhibit antibody production. Following treatment with ACTH, the lymphoid atrophy was not as marked as with compound E. This is of interest since treatment with this hormone did not result in so great a reduction or suppression of circulating antibody as did the administration of the adrenal hormone.

There is increasing evidence that compound E alters the inflammatory reaction of tissues to injury (18). Studies in this laboratory (19) have shown that treatment with this hormone results in a marked suppression of the local inflammatory response following an intracutaneous injection of viable pneumococci. The present experiments indicate that this is not the case with allergic inflammation. Treatment with compound E did not alter the Arthus response when large concentrations of serum antibody were maintained. Furthermore,

compound E had only a very slight and temporary effect, if any, on the systemic passive Arthus reaction. Previous observations from this laboratory (1) and elsewhere (20) have demonstrated that treatment with either ACTH or compound E had no effect on the *local* passive Arthus response. However, Dougherty (21) has reported that the cellular infiltration in mild allergic inflammatory reaction in sensitized mice was diminished by pretreatment with cortisone and has interpreted this effect as indicating that cortisone inhibits allergic inflammation through an antiphlogistic action. Although the results of the present experiments do not permit such an interpretation, they are not necessarily in disagreement with this observation. The edema and hemorrhage and necrosis of tissue of the Arthus reaction are the direct result of vascular damage following the union of antigen with antibody. To these primary changes are then added the edema and cellular infiltration of the inflammatory reaction which ordinarily occurs following tissue damage. It is probable that only the latter non-specific process is influenced by treatment with cortisone. Therefore, the size of the Arthus reaction would be little affected by compound E although the secondary cellular infiltration might be diminished. Whether the cellular infiltration in the passive Arthus reactions was inhibited by compound E was not determined in the present study.

SUMMARY

The concurrent administration of compound E at a daily dosage of 2 mg. per kg. to rabbits receiving daily intracutaneous injections of crystalline egg albumin markedly inhibited the development of anaphylactic hypersensitivity of the Arthus type. ACTH, when given at a similar dosage, produced a much less marked effect. Both hormones suppressed circulating antibody and as with the Arthus reaction, the suppression produced by compound E was much greater than that obtained with ACTH.

When treatment with compound E was started following sensitization, there was a rapid decline in circulating antibody and, if the pretreatment serum antibody was low, there was also a progressive decrease in skin reactivity, becoming negative after 5 days of treatment. When the pretreatment serum antibody concentration was great, so that by the termination of treatment the antibody concentration was still above the level ordinarily sufficient for a maximal skin response, the Arthus reaction was unaffected by treatment.

These considerations as well as the failure of compound E to inhibit the systemic passive Arthus reaction suggest that the inhibitory effect of compound E and ACTH on the development of experimental hypersensitivity results from the hormonal reduction of circulating antibody.

Treatment with compound E had no effect on the rate of disappearance of circulating antibody in the passively immunized rabbit. This finding suggests that ACTH and compound E reduce circulating antibody by inhibiting antibody formation rather than by promoting antibody destruction.

The question is raised as to whether the marked lymphoid atrophy produced by these hormones may be related to the interference with antibody production.

BIBLIOGRAPHY

1. Germuth F. G., Jr., and Ottinger, B., *Proc. Soc. Exp. Biol. and Med.*, 1950, **74,** 815.
2. Germuth, F. G., Jr., Nedzel, G. A., Ottinger, B., and Oyama, J., *Proc. Soc. Exp. Biol. and Med.*, 1951, **76,** 177.
3. Berthrong, M., Rich, A. R., and Griffith, P. C., *Bull. Johns Hopkins Hosp.*, 1950, **86,** 131.
4. Rich, A. R., Berthrong, M., and Bennett, I. L., Jr., *Bull. Johns Hopkins Hosp.*, 1950, **87,** 549.
5. Seifter, J., Ehrich, W. E., Begany, A. J., and Warren, G. H., *Proc. Soc. Exp. Biol. and Med.*, 1950, **75,** 337.
6. Dougherty, T. F., Chase, J. H., and White, A., *Proc. Soc. Exp. Biol. and Med.*, 1945, **58,** 135.
7. Eisen, H. N., Mayer, M. M., Moore, D. H., Tarr, R., and Stoerk, H. C., *Proc. Soc. Exp. Biol. and Med.*, 1947, **65,** 301.
8. Fishel, E. E., LeMay, M., and Kabat, E. A., *J. Immunol.*, 1949, **61,** 89.
9. Bjørneboe, M., Fischel, E. E., and Stoerk, H. C., *J. Exp. Med.*, 1951, **93,** 37.
10. Kabat, E. A., and Mayer, M. M., Experimental Immunochemistry, Charles C. Thomas, Springfield, Illinois, 1948, 57.
11. Kekwick, R. A., and Cannan, R. K., *Biochem. J.*, 1936, **30,** 227.
12. Heidelberger, M., and Kendall, F. E., *J. Exp. Med.*, 1935, **62,** 697.
13. Culbertson, J. T., *J. Immunol.*, 1935, **29,** 29.
14. Markham, R., *Biochem. J.*, 1942, **36,** 790.
15. Benditt, E. P., Schiller, S., Wong, H., and Dorfman, A., *Proc. Soc. Exp. Biol. and Med.*, 1950, **75,** 782.
16. Hektoen, L., *J. Infect. Dis.*, 1915, **17,** 415.
17. Philips, F. S., Hopkins, F. H., and Freeman, M. L. H., *J. Immuno*., 1947, **55,** 289.
18. Ragan, C., Haves, E. L., Plotz, C. M., Meyer, K., Blunt, W. J., and Lattes, R., *Bull. New York Acad. Med.*, 1950, **26,** 251.
19. Data to be published.
20. Fischel, E. E., *Bull. New York Acad. Med.*, 1950, **26,** 255.
21. Dougherty, T. F., and Schneebeli, G. L., *Proc. Soc. Exp. Biol. and Med.*, 1950, **75,** 854.