THE EFFECT OF ADRENOCORTICOTROPIC HORMONE ON INFLAMMATION DUE TO TUBERCULIN HYPERSENSITIVITY AND TURPENTINE AND ON CIRCULATING ANTIBODY LEVELS*

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For a long time the adrenal gland has been known to play a role in the course of many infectious and toxic states. Earlier work using adrenalectomized animals and animals treated with excess amounts of various adrenal extracts was limited by many technical difficulties.

The first reports suggesting adrenal cortical involvement in defense against infection came from pathological studies of animals and human beings dying from bacterial infection or intoxication. Roux and Yersin (1) noted hemorrhagic adrenal cortices in guinea pigs killed with diphtheria toxin. Observing changes in lipid content of the adrenal cortex during infections, some German pathologists felt these variations in the store of lipids represented alteration in fat metabolism. In careful studies on the cortical lipid content in various fatal human diseases, Elliot (2) reported lack of correlation between adrenal fat content and that found elsewhere. In acute infections, he found marked loss of lipids in the adrenals in contrast to their accumulation in the heart and kidneys. In contrast, the adrenals maintained their lipids until death in debilitating diseases such as diabetes, cancer, and anorexia nervosa. Dietrich (3) compared normal adrenals with those of infected individuals by detailed autopsies of soldiers killed suddenly and those dying of wound infections. Consistent pathological changes were found in the adrenal cortices of infected individuals. First, there were splitting and loss of lipid droplets, followed by cellular vacuolization and necrosis. Leucocytes infiltrated the areas of cell necrosis. In the more severely damaged glands, marked blood vessel reaction with inflammatory exudate, hyperemia, hemorrhage, and thrombosis occurred. Adrenal damage sustained in severe septicemia, especially meningococcemia, has provided a better known example of these pathological changes (4, 5). Despite adequate documentation, pathological changes have not proved that adrenal insufficiency plays a role in most human infections (6 *a*, 7).

Experimental work with adrenalectomized animals has provided a more direct

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approach to this problem. As early as 1896, unilaterally adrenalectomized guinea pigs were tested for resistance to bacterial infection (8). It was not until 1920, however, that techniques were developed which allowed survival of bilaterally adrenalectomized animals. In such studies difficulties were encountered in prolonged maintenance of

TABLE I

Effect of Adrenalectomy on Antibody Formation

Author	Animal	Antigen	Average antibody titers				Cortical remnants	Remarks
			Adrenalectomized		Controls			
			No.	Titer	No.	Titer		
Také (19)	Rabbit	Sheep red blood cells	14	2700	30	1100	13	
Jaffe (20)	Rat	Typhoid vaccine	14	821	34	512	2*	
Marmorston-Gottesman (21)	Rat	1 cc. 10 per cent sheep red blood cells	24	2300	43	5656	‡	Composite titers 5 days after antigenic stimulus
Marmorston-Gottesman (21)	Rat	1 cc. undiluted sheep red blood cells	6	7333	18	2022	‡	
Marmorston-Gottesman (22)	Rat	1 cc. 10 per cent sheep red blood cells	33	3418	32	5257	‡	Animal given isotonic salt solution
Khorazo (23)	Rat	Typhoid vaccine	20	1030	27	1120	0	
Eisen (24)	Rat	Pneumococcus vaccine	11	67	6	56	0	
Eisen (24)	Rat	Sheep red blood cells	11	160	6	85	0	
Eisen (24)	Rat	Pneumococcus vaccine	11	35.6 mg.	6	45.3 mg.	0	Quantitative precipitin test

* 8 animal autopsies reported.

‡ No report of autopsy findings.

animals and in determining whether lack of epinephrine or one or more cortical hormones was responsible for experimental findings. It was also found that data are of doubtful significance unless autopsies following experimentation show no regrowth of small cortical remnants (6 *b*, 9). Despite these pitfalls, the experimentation of Scott (10–12) and Jaffe (13) corroborated and extended by incompletely controlled studies of others (14–17), has shown that adrenalectomized rats and rabbits are more susceptible to the effects of a wide variety of toxic substances including drugs, histamine, bacterial vaccines and toxins, and active bacterial infections. Moreover, clinical ex-

perience has shown that patients with Addison's disease are hypersusceptible to stress conditions, including bacterial infections (6 *c*, 18).

Another type of investigation has involved the study of antibody production in the adrenalectomized rabbit and rat. Table I summarizes many of these studies (19–24).

If variations in the data in individual experiments, errors in the dilution technique, and the doubtful value of studies done without careful autopsies are considered, these reports reveal small differences between adrenalectomized and control animals. The rat and rabbit form antibodies without the adrenal, implying a lack of direct participation of cortical steroids in acquired immunity.

Experimentation in animal and man using excess amounts of cortical hormone has been too limited for accurate evaluation. Early studies using cortical extracts were hampered by use of insufficient amounts of potent material. Furthermore, even the inadequate methods presently available for judging when a pharmacologically active excess of hormone has been administered were unknown to early investigators. Recent studies have indicated that adrenal steroids either decrease (25, 26), or leave unaltered (27, 28) survival time or recovery rate from infectious processes, depending on the species, type of organism, and dose of hormone. In man, signs of toxicity in pneumonia (29), typhoid fever (30, 31), and tuberculosis (32, 33) have been suppressed with ACTH or cortisone therapy.

The effects of excess cortical hormone on antibody production have varied not only with the species and the amount of hormone administered, but also with the immunological method employed. Using relatively small amounts of cortical extracts and ACTH, earlier investigators showed increased antibody titers in rats, mice, and rabbits (34, 35). More recent reports have indicated that ACTH and cortisone fail to affect, or even suppress, circulating antibody levels (36, 37). Using quantitative immunochemical techniques, two recent observers have demonstrated marked suppression of antibody production and lowering of circulating antibody levels in rabbits immunized with egg albumin and pneumococcal polysaccharide (38, 39). Studies in human beings to date, though scanty in nature, have failed to demonstrate changes in antibody levels with doses of hormone which affect allergic and toxic processes (29, 31, 40). In the series reported by Hahn and others (41), in which no real changes in the clinical course of streptococcal infections were noted, the dose of cortisone was relatively small.

In summary, experimental work has shown that although adrenal cortical hormones are necessary to protect the body against many forms of stress, including those associated with bacterial infections, they are not essential for antibody formation. Data concerning the effects of excess amounts of these steroids on acquired and natural resistance are conflicting and too limited for satisfactory evaluation.

The recent availability of adrenocorticotropic hormone (ACTH) and 17-hydroxy-11-dehydrocorticosterone (cortisone) has made possible a more careful analysis of adrenal cortical effects upon immunological processes. The suppression of signs of toxicity in certain bacterial infections by treatment with ACTH and cortisone has suggested altered body response to noxious stimuli. Several observers have noted that adrenal steroids will suppress inflammatory

reactions (42–46). The series of experiments reported here describes the effect of ACTH on inflammation produced by a specific immunological system, tuberculin hypersensitivity, and a non-specific irritant, turpentine. Guinea pigs sensitized with heat-killed tubercle bacilli were used, because high degrees of sensitivity could be developed, and it was felt that these animals would withstand multiple procedures better than animals actively infected. Tuberculin complement-fixing antibodies were measured on sera from most of the tuberculin-sensitive animals. Although these antibodies have been shown to be unrelated to hypersensitivity (47, 48), a study of them is germane to the problem of the adrenal and acquired immunity. In addition, hematological and weight data were recorded to show the animal response to ACTH.

TABLE II

Plan of Experimentation

Group	No. of animals	Sensitized or unsensitized	Treatment
A	23	Tuberculin-sensitive	ACTH
B	23	Tuberculin-sensitive	Saline
C	24	Tuberculin-sensitive	None
D	10	Normal	ACTH
E	9	Normal	Saline

EXPERIMENTAL

1. The Effect of ACTH on the Tuberculin Skin Reaction

Animals.—Guinea pigs weighing 400 to 800 gm., which showed steady weight gain and appeared in good health, were used for experimentation in September, November, February, and October. Animals used in November showed considerable weight loss, but otherwise the animals remained in good condition during experimentation.

Sensitization.—Seventy guinea pigs received 2.5 to 5.0 mg. of heat-killed tubercle bacilli (H37Rv) subcutaneously in each groin on one or more occasions. Animals chosen for experimentation responded to 5 gamma of PPD intradermally with at least 1 cm. of induration and erythema. All animals had been immunized at least 6 weeks, but not more than 5 months, prior to experimentation.

ACTH.—Dosages for the four lots of ACTH[1] (64A, 60–61, H8412, 128-105R) used in this study were adjusted to Armour standard La-1-A. Animals received ACTH every 8 hours in a saline suspension, daily doses ranging from 2.0 to 3.0 mg. per 0.1 kg. The drug was administered subcutaneously in the abdominal wall.

Skin Testing.—Tuberculin testing was done with 5 gamma of PPD in 0.1 cc. of buffered saline placed intradermally on the back. The average diameter of the area of erythema was measured at 24 and 48 hours. Since higher values were usually obtained at 24 hours, the figures listed in the tables are the averages of the 24 hour diameters.

The animals were grouped as indicated in Table II.

All the animals, with the exception of those in group C, were bled from the heart (2.0 cc.)

[1] We are indebted to Dr. John R. Mote of the Armour Laboratories for providing the ACTH used in these studies.

TABLE III

Group A. Tuberculin-Sensitized Guinea Pigs

Effect of ACTH on Tuberculin Skin Reactions, Complement-Fixing Antibody Titers, Leucocyte Counts, and Weights

Animal	Month	Color	Tuberculin skin reaction			Complement-fixing antibody titer		Leucocyte count						Weig	
								White blood cells		Lymphocytes		Eosinophils			
			Before ACTH	During ACTH	2 wks. after ACTH	Before ACTH	During ACTH	Before ACTH	During ACTH	Before ACTH	During ACTH	Before ACTH	During ACTH	Before ACTH	During ACTH
			mm.	*mm.*	*mm.*			*per c.mm.*	*per c.mm.*	*per c.mm.*	*per c.mm.*	*per c.mm.*	*per c.mm.*	*gm.*	*gm.*
8-1	Sept.	Black	11	0	13			5390	4780	1842	660	108	0		
8-3	Sept.	Brown	10	0	4			5890	6060	1800	968	18	0		
8-5	Sept.	Brown	10	0	0			8820	6180	5400	1273	35	0		
8-7	Sept.	White	17	2	15			6770	3340	2965	1543	636	7		
2-5	Nov.	White	16	7	*	240	240	16,650	10,375	4998	2758	666	21	535	405
3-4	Nov.	White	16	9	*	240	480	13,650	17,150	4830	2265	600	34	815	680
5-0	Nov.	Brown	14	5	12	480	240	10,475	12,555	3520	1230	126	0	825	720
4-6	Nov.	White	15	8	14	960	960	13,475	9675	5760	2225	81	0	550	445
4-7	Nov.	Brown	17	12	*	120	120	17,225	17,900	6500	1720	576	0	630	555
5-6	Nov.	White	15	8	14	120	120	12,500	6925	5520	1122	700	0	650	520
4-0	Nov.	Black	12	0	0	60	0	9050	7150	3150	1945	36	14	625	490
1-18	Feb.	White	25	11	26	0	30	16,680	9740	3535	2980	466	156	555	625
1-21	Feb.	White	18	9	‡	0	0	15,980	16,900	2680	3140	863	135	720	670
2-2	Feb.	White	22	12	22	0	0	22,580	13,700	4850	3425	6080	27	695	760
2-4	Feb.	White	17	10	17	30	0	18,500	10,380	4660	1390	925	104	720	695
2-6	Feb.	White	21	14	32	240	240	19,120	6640	3480	1128	4825	27	655	560
1-37	Feb.	White	16	11	20	60	120	9030	7880	2455	1813	505	32	640	560
5-8	Feb.	Brown	15	0	19	120	60	13,430	8400	2930	1562	2930	17	590	530
3-2	Oct.	White	15	12	16	30	30	3700	3400	1518	1665	96	27	770	790
1-40	Oct.	White	21	17	22	30	30	7450	3730	3100	1038	372	0	660	720
4-5	Oct.	White	24	15	19	60	120	11,800	5800	4360	2005	590	12	565	580
1-71	Oct.	White	18	9	‡	0	60	4800	6600	2010	951	38	26	550	580
1-77	Oct.	White	17	4	16	0	0	3420	3700	1676	985	137	15	620	620

* Died.

‡ Killed by faulty cardiac puncture.

The difference in millimeters between the mean diameter of erythema before and during treatment has been analyzed by the t test.

$$t = \left(\sqrt{\frac{Z(x_t - \bar{x}_t)^2 + Z(x_c - \bar{x}_c)^2}{n_t + n_c - 2}} \right) \frac{\bar{x}_t - \bar{x}_c}{\frac{n_t n_c}{n_t + n_c}}$$

x_t, n_t refer to treated animals

x_c, n_c refer to control animals

$t = 7.89$

$p < 1 \times 10^{-9}$

prior to performing the PPD skin tests. 48 hours later, when the reading of the skin tests was complete, ACTH or saline administration was begun. The animals receiving saline followed the same treatment schedule as those receiving ACTH. Rebleeding and retesting were done on the 7th and 14th days. Treatment was continued for 16 days. Animals were again bled and tested 2 weeks after cessation of hormone or saline treatment. The control animals, group C, which received no injections, were bled and skin-tested on two occasions 2 weeks apart.

The results of the tuberculin skin reactions are reported in Tables III to V. All ACTH-treated animals showed marked reduction of the area of erythema. When analyzed, these changes are found to be very significant. The changes

TABLE IV

Group B. Tuberculin-Sensitized Guinea Pigs

Effect of Saline on Tuberculin Skin Reactions, Complement-Fixing Antibody Titers, Leucocyte Counts, and Weights

Animal	Month	Color	Tuberculin skin reaction			Complement-fixing antibody titer		Leucocyte count						Weight	
								White blood cells		Lymphocytes		Eosinophils			
			Before saline	During saline	2 wks. after saline	Before saline	During saline	Before saline	During saline	Before saline	During saline	Before saline	During saline	Before saline	During saline
			mm.	*mm.*	*mm.*			*per c.mm.*	*per c.mm.*	*per c.mm.*	*per c.mm.*	*per c.mm.*	*per c.mm.*	*gm.*	*gm.*
8-2	Sept.	Brown	12	15	10			7100	5900	2960	2515	811	838		
8-4	Sept.	Black	8	11	6			13,300	7200	7360	3400	1063	504		
8-6	Sept.	White	14	13	*			7860	7280	3215	2590	1510	44		
8-8	Sept.	White	16	0	*			9180	5410	3580	2065	1745	0		
3-3	Nov.	Brown	16	12	13	960	960	14,550	9750	4600	2600	232	0	740	625
3-5	Nov.	White	16	14	16	60	480	11,200	8275	4700	3390	605	66	620	480
3-7	Nov.	White	13	16	16	120	60	11,950	8775	4060	2140	48	35	545	475
4-2	Nov.	Tan	12	12	*	120	120	8725	7825	3440	1283	698	0	595	490
4-4	Nov.	White	16	9	15	120	120	16,175	12,000	6630	6120	65	0	670	535
1-19	Feb.	White	17	20	21	0	0	19,420	17,520	6060	4070	1515	3120	525	535
1-44	Feb.	White	18	16	17	30	0	14,820	18,300	3350	4100	504	183	765	695
5-4	Feb.	White	21	14	19	30	0	7640	7450	2400	2625	107	93	595	645
8-0	Feb.	White	21	25	19	30	30	15,000	8000	4500	2765	180	144	680	660
1-86	Feb.	White	20	23	25	30	120	13,850	31,100	2230	5200	3435	11,200	715	725
1-90	Feb.	White	24	25	22	0	0	22,610	17,250	5200	5100	2760	2240	715	700
1-91	Feb.	White	34	43	34	240	120	10,520	7990	2105	2490	147	96	650	650
5-2	Oct.	White	16	18	16	120	120	5800	3730	2005	1350	12	22	620	560
1-95	Oct.	White	17	18	21	120	120	5560	7600	2270	3085	78	76	610	570
1-00	Oct.	White	15	16	17	60	60	5020	9500	2310	4120	80	133	640	650
1-48	Oct.	White	16	20	15	0	0	6500	6750	2625	2270	65	68	600	620
1-63	Oct.	White	17	19	20	60	240	6100	7550	2135	1570	61	76	520	510
4-92	Oct.	White	16	18	18	480	240	6750	5850	2340	2200	68	70	610	540
1-78	Oct.	White	18	22	‡	30	480	7750	10,300	4030	5090	124	473	445	440

* Died.

‡ Killed by faulty cardiac puncture.

The difference between the mean diameter of erythema before and during treatment is 0.1 mm.,

in induration were even more striking. Because of the difficulty in accurate measurement, these values were not tabulated. However, all animals demonstrated marked induration before experimentation, while 18 of the 23 receiving ACTH showed none during treatment. Only 5 dark skinned animals, in whom erythema was difficult to determine, completely lost their skin reactions. There were 7 dark animals out of a total of 23 in group A. The average diameter of the skin reactions did not change in the saline or untreated groups.

Although the mean value for the skin tests was unaltered during saline treatment, animal 8-8 in group B completely lost its skin reaction to tuberculin.

TABLE V

Group C. Tuberculin-Sensitized Guinea Pigs

Tuberculin Skin Reactions, Complement-Fixing Antibody Titers, Leucocyte Counts, and Weights of Untreated Animals

Animal	Month	Color	Tuberculin skin reaction		Complement-fixing antibody titer		Leucocyte count						Weight	
							White blood cells		Lymphocytes		Eosinophils			
			First	Second	First	Second	First	Second	First	Second	First	Second	First	Second
			mm.	*mm.*			*per c.mm.*	*per c.mm.*	*per c.mm.*	*per c.mm.*	*per c.mm.*	*per c.mm.*	*gm.*	*gm.*
2-44	Oct.	White	12	16	120	30	7150	5700	2585	1720	429	274	880	860
2-19	Oct.	White	11	13	30	30	5780	7700	2095	2400	984	1540	915	850
2-33	Oct.	Brown	6	13	960	480	4740	7900	2425	2720	218	190	790	750
2-68	Oct.	White	19	18	60	60	11,800	10,900	7320	4990	1675	1440	860	830
2-18	Oct.	White	20	23	60	30	6100	11,400	2520	4990	561	775	765	720
3-19	Oct.	White	20	17	30	30	12,600	12,700	3905	5305	3150	2260	875	890
2-26	Oct.	White	18	17	60	60	9400	9600	3380	2500	1615	1613	800	780
4-89	Oct.	Brown	17	17	30	30	5300	3500	2280	1310	584	343	945	1010
2-55	Oct.	White	20	17	0	0	8500	9200	2840	2890	1412	828	850	850
2-70	Oct.	White	15	17	60	60	6700	7200	1610	2045	1610	2045	680	700
2-73	Oct.	White	20	15	0	0	7800	7100	3120	2970	733	426	785	700
2-75	Oct.	White	18	20	480	480	9200	8200	3290	3540	810	262	730	770
2-66	Oct.	White	21	13	60	120	9700	14,100	4360	5850	136	338	520	570
2-32	Feb.	White	15	18	120	120	8650	8000	4180	4760	329	208	870	910
2-77	Feb.	White	16	19	960	240	14,750	15,600	6740	6500	797	950	760	750
2-10	Feb.	White	15	18	0	120	10,550	10,350	4220	4740	127	186	770	760
2-35	Feb.	White	14	15	0	30	9650	16,650	3900	7390	154	200	695	700
3-66	Feb.	Black	15	20	480	480	10,600	13,900	4875	5500	720	1000	820	870
2-71	Feb.	Black	15	17	60	60	9400	19,100	3440	7370	715	1720	750	760
2-40	Feb.	White	16	21	60	480	35,800	15,000	8100	5340	788	450	645	620
2-76	Feb.	Brown	19	14	30	0	16,650	19,150	7710	7280	1700	2070	710	720
2-79	Feb.	Brown	21	16	480	240	14,500	10,200	5650	3630	493	403	770	760
1-82	Feb.	Tan	24	19	240	240	17,750	18,000	7880	6590	923	1440	850	860
2-14	Feb.	Brown	10	15	0	0	17,550	18,000	6740	5250	1040	1260	875	840

This animal died on the 16th day, 48 hours after skin testing. In this same group, animals 3-3, 3-5, 3-7, 4-2, and 4-4, all of which were in the November series, lost a considerable amount of weight and vigor. Some of these animals had decreased skin reactivity while receiving saline. Although this is not apparent in the measurements of erythema, animals 3-5, 4-2, and 4-4 of this group demonstrated no induration after 2 weeks of saline injections. Though

the numbers are too small for analysis, these findings demonstrate the influence of over-all health of the host on skin reactivity to tuberculin.

The findings in the control group demonstrated the normal variations which occur in reactions to tuberculin in the guinea pig. These were not entirely due to the mode of sensitization or the species since we have observed the differences in human beings (50), and similar observations have been reviewed by Rich (49). The difficulty in analysis which these variations cause can be obviated by using adequate numbers of animals.

TABLE VI

Group D. Normal Guinea Pigs

Effect of ACTH on Leucocyte Counts and Weights

Animals	Month	Color	Leucocyte count						Weight	
			White blood cells		Lymphocytes		Eosinophils		Before ACTH	During ACTH
			Before ACTH	During ACTH	Before ACTH	During ACTH	Before ACTH	During ACTH		
			per c.mm.	*per c.mm.*	*per c.mm.*	*per c.mm.*	*per c.mm.*	*per c.mm.*	*gm.*	*gm.*
8-9	Sept.	Tan	3866	4460	1865	858	38	9		
9-0	Sept.	White	4810	4070	2585	1197	212	8		
9-1	Sept.	White	3310	2880	1886	375	199	17		
9-2	Sept.	White	6020	2580	4130	660	193	5		
2-1	Nov.	White	11,500	11,500	4280	2530	1230	23	645	420
2-9	Nov.	Brown	8800	8275	4680	1820	370	0	665	535
2-8	Nov.	White	4975	8925	1970	1965	139	0	760	610
1-9	Nov.	White	4825	10,140	2890	1848	58	0	570	500
5-3	Feb.	Black	11,380	5780	4500	1550	1500	35	590	555
6-3	Feb.	Brown	7950	6680	3055	1483	40	40	610	565

Another interesting detail which does not appear in the tables was the period of time following testing for the development of a maximum skin response. Minimal reactions were at their peak at 24 to 32 hours, while necrotic lesions were maximal between 40 and 48 hours. If early readings had not been made, especially in the ACTH-treated animals, many weak positive tests would have been called negative.

The tuberculin-negative animals in groups D and E did not react to PPD at any time. Observations on these animals are listed in Tables VI and VII.

2. The Effect of ACTH on Skin Reactivity to Turpentine

Twelve normal guinea pigs, similar to those used previously, were divided into groups F and G of 6 animals each. Group F received ACTH in the same doses and on the same schedule as group A. Group G was treated with saline on the same schedule as group B. Skin reactions were produced by injecting 0.1 cc. of oil of turpentine U.S.P. intradermally. The maximum values which were read at 2, 24, and 48 hours were recorded. The animals were bled from the heart (2.0 cc.) and skin testing was done. After 48 hours, treatment with ACTH or saline

was begun and continued for 16 days. Animals were retested and rebled at 2, 7, and 14 days, and again 2 weeks after discontinuing treatment. The original and final skin reactions were used to obtain values for analysis of untreated animals.

The responses to turpentine are listed in Tables VIII and IX. The reactions consisted of a central area of necrosis with a peripheral zone of erythema and induration. The large amounts of ACTH employed suppressed erythema and induration, but failed to affect the necrotic reactions. Even though the series was small, this change during ACTH administration was highly significant. The wide variations in the reactions which occurred were due to the difficulty in placing exact amounts of irritant intracutaneously. The diminution in the

TABLE VII

Group E. Normal Guinea Pigs

Effect of Saline on Leucocyte Counts and Weights

Animals	Month	Color	Leucocyte count						Weight	
			White blood cells		Lymphocytes		Eosinophils		Before saline	During saline
			Before saline	During saline	Before saline	During saline	Before saline	During saline		
			per c.mm.	*per c.mm.*	*per c.mm.*	*per c.mm.*	*per c.mm.*	*per c.mm.*	*gm.*	*gm.*
9-3	Sept.	White	9500	4840	6100	2290	19	58		
9-4	Sept.	White	3650	2900	2035	1190	263	348		
9-5	Sept.	White	9040	8430	4050	2765	488	711		
9-6	Sept.	White	6640	5960	2975	2240	530	370		
1-8	Nov.	White	5750	12,100	1910	1645	0	0	555	435
2-0	Nov.	White	16,125	7700	5350	3330	0	0	555	435
1-29	Feb.	Tan	13,670	18,300	7620	6180	1500	3330	735	740
6-6	Feb.	White	16,100	17,500	4860	10,120	64	140	610	635
1-93	Feb.	White	19,860	21,300	6070	4690	2620	5450	640	655

average amount of erythema and induration during saline treatment was probably not significant.

3. Effect of ACTH on Tuberculin Complement-Fixing Antibody Titers

Complement fixation tests were performed on the sera of all animals except those in the September group. Sera were obtained from blood drawn by cardiac puncture and stored at −20°C. Values for antibody titers were thus obtained before, during, and after ACTH or saline treatment. Untreated animals were tested after a 2 week interval. Sera for a single animal were checked on at least two occasions, and each series was accompanied by a titered positive serum control.

The method outlined by Kabat and Mayer (51) was employed with the following modifications. (1) The system was incubated at 37°C. for 90 rather than 40 minutes. (2) Sheep red cells were sensitized for 30 rather than 10 minutes. (3) The system was incubated with sensitized red cells for 40 instead of 30 minutes. A 1:50 dilution of old tuberculin (Massachusetts State 200 mg. per 0.1 cc.) which had been titered for maximum activity against a known

TABLE VIII

Group F. Normal Guinea Pigs

Effect of ACTH on Turpentine Skin Reactions, Leucocyte Counts, and Weights

Animal	Month	Color	Turpentine skin reaction						Leucocyte count						Weight	
			Erythema			Necrosis			White blood cells		Lymphocytes		Eosinophils			
			Before ACTH	During ACTH	2 wks. after ACTH	Before ACTH	During ACTH	2 wks. after ACTH	Before ACTH	During ACTH	Before ACTH	During ACTH	Before ACTH	During ACTH	Before ACTH	After ACTH
			mm.	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*per c.mm.*	*per c.mm.*	*per c.mm.*	*per c.mm.*	*per c.mm.*	*per c.mm.*	*gm.*	*gm.*
9	Oct.	White	20	13	22	7	8	12	4700	5030	2415	1690	212	20	750	650
1-0	Oct.	Tan	26	14	31	10	12	12	5450	5120	1935	1945	300	41	740	730
5-0	Oct.	Tan	31	14	33	13	10	12	5070	4560	2020	1598	355	55	740	770
1-47	Oct.	White	29	17	22	12	13	10	4100	4370	1910	1380	33	26	690	640
1-70	Oct.	White	23	12	18	11	10	9	2550	3720	1158	1430	61	22	560	610
1-84	Oct.	White	21	15	25	9	10	12	4300	4700	2100	820	9	18	590	620

The difference between the mean diameter of erythema before and during treatment is — 10.8 mm.: $t = 4.392$; $p < 0.00001$.

TABLE IX

Group G. Normal Guinea Pigs

Effect of Saline on Turpentine Skin Reactions, Leucocyte Counts, and Weights

Animal	Month	Color	Turpentine skin reaction						Leucocyte count						Weight	
			Erythema			Necrosis			White blood cells		Lymphocytes		Eosinophils			
			Before saline	During saline	2 wks. after saline	Before saline	During saline	2 wks. after saline	Before saline	During saline	Before saline	During saline	Before saline	During saline	Before saline	During saline
			mm.	*mm.*	*mm.*	*mm.*	*mm.*	*mm.*	*per c.mm.*	*per c.mm.*	*per c.mm.*	*per c.mm.*	*per c.mm.*	*per c.mm.*	*gm.*	*gm.*
5-9	Oct.	White	27	26	28	10	14	12	6350	4650	2720	2335	38	49	810	810
1-17	Oct.	White	40	22	20	17	12	10	3600	5500	2010	2290	176	264	630	610
1-30	Oct.	Tan	22	20	25	12	10	14	1650	3350	676	1580	66	20	560	500
1-59	Oct.	White	32	24	30	12	12	9	3010	3840	1475	1705	113	138	650	650
4-99	Oct.	White	14	23	26	8	11	13	3820	3840	2760	2260	49	69	730	750
4-91	Oct.	Tan	26	17	24	10	8	9	8450	8630	4380	3245	676	431	770	780

The difference between the mean diameter of erythema before and during treatment is — 4.2 mm.: $t = 1.229$; $p < 0.3$.

positive serum was used as the antigen. Sera were prepared in doubled dilutions starting at 1:30. Anticomplementary controls were run with each dilution of serum.

The results of the complement fixation tests are found in Tables III to V. The titer listed is the highest dilution showing fixation. A comparison of titers

before and after 2 weeks of treatment shows no change in the average titer of any group. For analysis of this data differences in titers were considered significant by two criteria: the first, a difference of two tubes; the second, the difference between a negative and any positive titer. Table X summarizes the findings for the three tuberculin-positive groups using these criteria. None of the tuberculin-negative animals demonstrated antibody titers.

The data must be further evaluated in terms of the interval between sensitization and experimentation. Baker has shown a rapid rise in complement-fixing antibody titers during the first weeks following the inoculation of tubercle bacilli and a slow fall in titers thereafter (47). All but 14 of the 53 animals demonstrating antibodies were sensitized or resensitized 6 to 8 weeks before experimentation. The remaining animals (No. 1-37 of group A, No. 1-44 of group B, and Nos. 2-44 to 2-75 in group C) were sensitized 3 to 5 months

TABLE X

Significant Changes in Complement-Fixing Antibody Titers ACTH-Treated, Saline-Treated, and Untreated Animals

Group	No. tested	Unchanged	Increased	Decreased
A	16	12	2	2
B	16	10	4	2
C	21	15	3	3

previously. The only difference noted between these groups was a greater degree of stability of titers in animals with longer periods between sensitization and experimentation.

Although the dilution technique will not detect minor changes in antibody titers, these results indicate that guinea pigs receiving large doses of ACTH for 2 weeks and having hematological response to the hormone show no gross changes in circulating complement-fixing antibody levels.

4. Effect of ACTH on Hematological Values and Weight

White cell and differential counts were done before, during, and after treatment on blood samples drawn by cardiac puncture. Blood films were prepared directly from the needle using the cover-slip technique and staining with Wright's stain. Differential white blood cell counts were made by counting 500 cells. Animals were weighed before treatment, then weekly for 4 weeks thereafter.

The hematological data (Tables III–IX) have been grouped for analysis on the basis of treatment. Thus values for all animals of groups A, D, and F (Tables III, VI, and VIII) were combined to show the effects of ACTH, and

of groups B, E, and G (Tables IV, VII, and IX) to show the effects of saline. Group C (Table V) provided the control data.[2]

The most striking hematological change occurred in eosinophil levels. All animals with an appreciable number before treatment showed a very marked fall in eosinophils while receiving ACTH. Because these changes were obvious, and wide variations in the counts make statistical treatment difficult, these data were not analyzed. There was no change in the mean for the saline-treated group. Those saline-treated animals in group B which lost considerable weight, however, had depression of circulating eosinophils. These were the same animals which showed some diminution of skin reactivity to tuberculin. Again, the numbers were too small and the variations too great for statistical analysis. The group of untreated animals demonstrated the wide variations occurring in the eosinophil level of the same animal.

ACTH treatment caused a very significant decrease in the level of circulating lymphocytes. Five ACTH-treated animals failed to show a fall in lymphocyte count. There was not complete correlation between circulating lymphocyte levels and suppression of the tuberculin skin reaction. Saline treatment caused no significant variations in lymphocyte counts.

The decrease in white blood cell counts occurring in the ACTH-treated group was probably significant. This was due to the marked change in lymphocyte levels. There was no significant alteration in polymorphonuclear leucocytes (data not included in the tables). White blood counts were not significantly altered by saline administration.

Treatment with ACTH, but not with saline, resulted in a significant loss of weight. However, if the weight data are analyzed excluding the values for the 19 animals treated in November, there was no significant change in the ACTH-treated group. The guinea pigs in the November experiment, all of whom showed marked weight loss, became lethargic and anorectic during experimentation. All but 4 animals (Nos. 3-3, 3-7, 4-4, and 2-9) died within 2 months following treatment. These animals showed diminution in circulating eosinophils, and even some of the saline-treated animals had alteration in skin reactivity to tuberculin. Although we were unable to determine the cause of this epidemic illness, it emphasized the need for close attention to all phases of animal care

[2] Given below are the data for leucocyte values analyzed by the t test in terms of logarithms of the counts.

	White blood cells			Lymphocytes		
	ACTH-treated	Saline-treated	Untreated controls	ACTH-treated	Saline-treated	Untreated controls
Mean	−0.0776	−0.0177	+0.027	−0.3065	−0.0652	+0.0101
t	2.598	1.631		6.332	0.512	
p	<0.02	<0.2		$<1 \times 10^{-9}$	<0.7	

during such a project. More important, it demonstrated the influence of extraneous factors on skin reactivity and hematological values.

DISCUSSION

It has been suggested that the dramatic remissions in rheumatoid arthritis, rheumatic fever, asthma, and certain infectious diseases under the influence of ACTH or cortisone may be attributed to (1) suppression of antibody production, (2) blocking of antigen-antibody reactions *in vivo*, or (3) suppression of the tissue responses (52).

Antibody production in rabbits has been lowered by cortisone and to a lesser degree by ACTH treatment (38, 39). These reports provide isolated examples in favor of the first explanation. In the studies reported here, the treatment of guinea pigs with large amounts of ACTH failed to alter complement-fixing antibody titers to tuberculin. The results of this and of certain earlier observations (29–31, 40, 41, 45) would indicate that the administration of these hormones to different species using varying types of antigenic stimuli cannot be relied upon to suppress antibody formation. To date there is no evidence in man or animals that antibody production in bacterial infections is lowered by the relatively small amounts of adrenal steroids necessary to suppress evidence of toxicity.

The failure to prevent the passive Arthus phenomenon (38, 52), anaphylactic shock (52, 53), nephrotoxic nephritis (54, 55), or the Prausnitz-Küstner response (56) appears to rule out the second postulated effect of adrenal cortical hormones.

The results of the present study indicate that in the guinea pig inflammation due to a specific immunological system, tuberculin hypersensitivity, and a non-specific irritant, turpentine, has been suppressed by large amounts of ACTH. Similar alteration of inflammation resulting from a variety of specific immunological systems and non-specific irritants has been observed by others (42–46, 50, 57, 58). These findings provide strong evidence for the third hypothesis by indicating that adrenal hormones limit the local tissue reaction secondary to primary cell injury. This is further suggested by the observation that tuberculin hypersensitivity cannot be passively transferred by means of any known circulating antibody, but may be by sensitized leucocytes (59).

Since adrenal cortical hormones do not consistently alter antibody formation or antigen-antibody reactions, it would appear that the major role of these agents in providing relief from a variety of diseases has been the suppression of tissue response to injury. If alleviation of inflammatory or toxic conditions is mediated by altered cell responses, the favorable effects of adrenal steroids on such diverse processes as tuberculin hypersensitivity and chemical insults can be explained. Since the disposal of foreign particles and bacteria usually depends upon their ingestion or destruction by phagocytic cells, the use of

adrenal cortical hormones may prove disadvantageous in those infectious conditions in which a constantly renewed supply of mobile cells is necessary for maximal efficiency of the immunological mechanism.

SUMMARY

The treatment with adrenocorticotropic hormone of guinea pigs sensitized with heat-killed tubercle bacilli caused suppression of their skin reactivity to tuberculin. Similar animals treated with saline did not show this change.

Normal guinea pigs treated with adrenocorticotropic hormone showed suppression of inflammation, but not necrosis, produced by intracutaneous oil of turpentine. There was slight, but probably not significant, diminution of inflammation during saline administration.

Tuberculin complement-fixing antibody titers were not altered by either adrenocorticotropic hormone or saline administration.

Adrenocorticotropic hormone produced marked eosinopenia and lymphopenia in guinea pigs.

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