EFFECTS OF CORTICOSTERONE, HYDROCORTISONE, AND CORTICOTROPIN ON PRODUCTION OF ANTIBODIES IN RABBITS*

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That cortisone (compound E of Kendall, 11-dehydro-17-OH corticosterone) and pituitary adrenocorticotropin (ACTH) decrease the production of antibodies is well documented (1–4). However, there are no comparable data with respect to the effects of hydrocortisone (compound F of Kendall, 17-OH corticosterone) and corticosterone (compound B of Kendall). Several considerations make it desirable to obtain such information about the latter steroids. First, hydrocortisone and corticosterone are the predominant steroids secreted by the adrenal cortex in all the animal species thus far studied and these two hormones may occur singly or together; cortisone, on the other hand, is secreted only in trace amounts if at all (5). Second, hydrocortisone and corticosterone, in the same dosages, exert different effects on clinical inflammatory disorders (4, 6), on the size and nucleic acid concentrations of lymph nodes (7, 8), and on resistance to infections (4, 9). The effect of corticosterone is variable but usually negligible, whereas the activity of hydrocortisone is similar to that of cortisone. Third, adrenal secretion is not constant in rabbits at least, and the administration of ACTH, or of certain environmental stimuli, may cause marked alterations in the nature of the steroids secreted (8, 10).

Thus, the adrenal cortices of rabbits ordinarily secrete corticosterone as the predominant steroid, but after prolonged stimulation to steroidogenesis hydrocortisone may become the predominant steroid that is secreted (8, 10). It would be anticipated that any stimuli that alter the relative amounts of these two steroids in the adrenal secretion would also alter the response of the host in accordance with the differences in the biologic activity of these steroids.

The considerations indicate the need for detailed studies of the differences in the effects of corticosterone and hydrocortisone on various mechanisms of resistance to infection. The experiments to be presented show that these two steroids affect antibody production differently. In addition, the influence of

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TABLE I

Effects of Hydrocortisone, Corticosterone, and Corticotropin on Antibody Production in Rabbits

Rabbit No.	Treatment	Antibody content, mg./ml. serum			
		Bleeding			
		1	2	3	4
1	None	0.220	0.559	0.370	0.307
2		0.385	0.775	0.588	0.465
3		0.805	1.640	1.165	1.134
4		0.186	0.446	0.377	0.345
5		0.225	0.830	0.866	0.855
6		0.166	0.530	0.400	0.230
7		0.275	1.219	0.790	0.690
8		0.160	0.443	0.373	0.280
9		0.230	1.063	0.620	0.324
10		0.269	1.167	0.946	0.900
Mean ±		0.292±	0.867±	0.631±	0.553±
S.E.		0.061	0.70	0.101	0.101
11	ACTH 15 units daily	0.405	1.725	3.065	2.683
12		0.317	1.449	0.821	0.617
13		0.163	0.307	0.241	0.320
14		0.281	0.700	0.546	0.480
15		0.105	0.317	0.202	0.193
16		0.096	0.232	0.187	0.219
17		1.094	D*		
18		0.297	1.203	0.900	0.845
19		0.159	0.293	0.358	0.401
20		0.017	0.381	0.291	0.300
Mean ±		0.293±	0.734±	0.735±	0.673±
S.E.		0.097	0.193	0.304	0.261
21	ACTH 20 units daily	0.298	0.400	0.526	0.622
22		0.198	0.671	0.504	0.394
23		0.137	0.333	0.411	0.374
24		0.348	1.055	2.280	2.000
25		0.155	0.298	0.304	0.251
Mean ±		0.227±	0.551±	0.805±	0.728±
S.E.		0.041	0.110	0.370	0.321
26	ACTH 30 units daily	D			
27		0.170	0.435	0.503	0.666
28		0.044	0.256	0.323	0.330
29		0.221	D		
30		0.116	0.353	0.266	0.304
Mean ±		0.138±	0.348±	0.364±	0.433±
S.E.		0.120	0.066	0.148	0.117

*D, died.

TABLE I—*Continued*

Rabbit No.	Treatment	Antibody content, mg./ml. serum			
		Bleeding			
		1	2	3	4
31	Corticosterone 10 mg./day 6 times weekly	0.310	0.420	0.424	0.305
32		0.120	0.035	0.035	0.025
33		0.120	0.218	0.222	0.174
34		0.640	2.317	2.714	3.886
35		0.168	0.608	0.450	0.609
36		0.442	2.374	D	
37		0.095	0.524	D	
Mean ±		0.271±	0.928±	0.769±	0.999±
S.E.		0.078	0.372	0.491	0.727
38	Hydrocortisone 10 mg./day 6 times weekly	0.160	0.155	0.180	0.140
39		0.087	0.035	D	
40		0.065	0.107	0.081	0.037
41		0.047	0.010	D	
42		0.114	0.025	0.002	0.000
Mean ±		0.095±	0.066±	0.088±	0.059±
S.E.		0.020	0.028	0.055	0.042
43	Hydrocortisone 2.5 mg./day 6 times weekly	0.340	0.379	0.350	0.484
44		0.115	0.075	0.068	0.168
45		0.085	0.140	0.106	0.249
46		0.069	0.014	0.044	0.000
47		0.094	0.114	0.099	0.073
Mean ±		0.141±	0.144±	0.133±	0.206±
S.E.		0.051	0.062	0.055	0.083
48	Hydrocortisone 10 mg./day and corticosterone 10 mg./day 6 times weekly	D			
49		0.001	0.001	0.010	0.034
50		0.000	D		
51		0.002	0.022	0.010	0.025
52		0.005	D		
53		0.000	0.001	0.022	0.073
54		0.016	0.040	0.028	0.094
55		0.017	D		
56		0.005	0.106	0.022	0.136
57		0.000	0.001	0.046	0.072
Mean ±		0.005±	0.029±	0.023±	0.072±
S.E.		0.002	0.017	0.006	0.017

TABLE I—*Concluded*

Rabbit No,	Treatment	Antibody content, mg./ml. serum			
		Bleeding			
		1	2	3	4
58	Hydrocortisone 2.5 mg./ day and corticosterone 10 mg./ day 6 times weekly	0.000	0.001	D	
59		0.047	0.018	0.058	0.065
60		0.001	0.000	0.012	0.000
61		0.000	0.000	D	
62		0.047	0.058	0.211	0.207
Mean ±		0.019±	0.015±	0.094±	0.091±
S.E.		0.011	0.011	0.060	0.061

various doses of corticotropin on antibody production was studied, since it seemed likely that because the relative amounts of hydrocortisone and corticosterone that are secreted are altered by the administration of ACTH, different effects on antibody production might be obtained with different doses of ACTH.

Materials and Methods

Male albino rabbits weighing 2.5 to 3.5 kg. were used. They were fed the stock laboratory diet *ad libitum*, and were weighed every 2 or 3 days. The antigen consisted of highly purified human serum gamma globulin.[1] This was dissolved in saline in a concentration of 50 mg. per ml. and the solution was mixed with an equal volume of aluminum hydroxide gel (amphojel, Wyeth). Two ml. of the final suspension were given intramuscularly three times weekly for 2 weeks and the rabbits were bled 10 days after the last injection. A second course of three injections of antigen in 1 week began immediately after this bleeding; and the rabbits were again bled 5 days after the completion of this course. No more antigen was administered, but two subsequent bleedings were taken at 10 day intervals. At each bleeding 10 to 15 ml. of whole blood were taken from the marginal ear veins. The serum was stored at −25°C. until used. Control sera, obtained before immunization, proved to be free of detectable antibody to the antigen used. The antibody content of the antisera was determined quantitatively (11), the protein content of the washed antigen-antibody precipitates being measured spectrophotometrically (12, 13).

Corticosterone and hydrocortisone alcohols were suspended in 20 per cent ethanol with 0.9 per cent benzl alcohol added. The final concentration of steroid was adjusted so that the injected volume did not exceed 1 ml. Single daily injections of the steroid hormones were given intramuscularly six times weekly. The corticotropin[2] consisted of relatively crude material containing 2.0 units per mg. The dry powder was suspended in peanut oil containing 5 per cent beeswax, using a sterile Waring blendor. The corticotropin was administered daily in a single injection intramuscularly, in a volume not exceeding 1 ml. Administration of the hormones was begun on the 1st day of immunization and was continued for a total of 36 days; *i.e.*, until the day of the bleeding that followed the completion of the injections of antigen.

[1] Kindly supplied by Charles Pfizer & Co., and Sharp & Dohme, Inc.

[2] Kindly supplied by Armour Laboratories.

RESULTS

The antibody titers for each rabbit and the treatment given to each group of rabbits are listed in Table I. The mean values for each treatment group are charted in Fig. 1.

Hydrocortisone markedly depressed the antibody response, an effect comparable to that observed by others (1–4) using cortisone; even doses as low as 2.5 mg. per day significantly depressed antibody production. Corticosterone, on the other hand, had no significant effect on the antibody response, even in

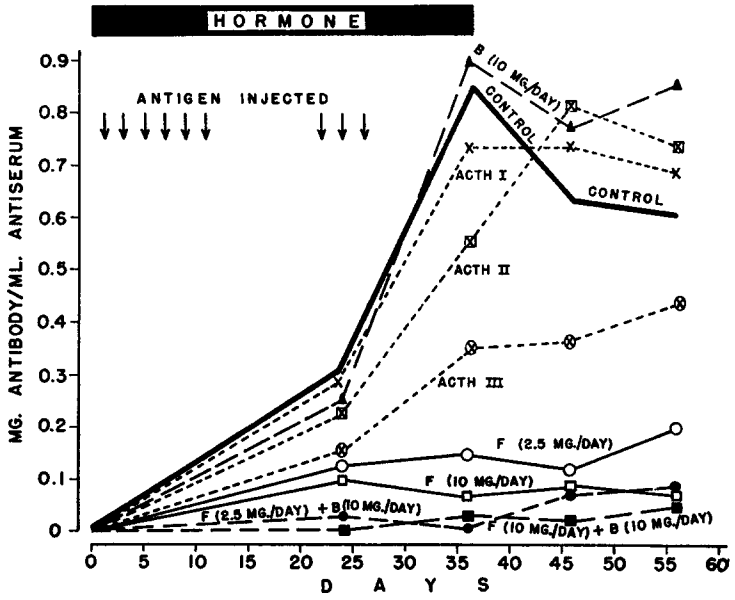

FIG. 1. Effect of adrenal steroids on antibody production.

doses of 10 mg. per day. Furthermore, corticosterone did not overcome the adverse effect of hydrocortisone, and, indeed, seemed to act additively.

The effects of corticotropin varied with the dosage used. Fifteen units daily exerted little effect on the antibody response, but as the dose of corticotropin was increased, there was a progressive fall in the antibody titers.

The effects of adrenal steroids on the weights of the test animals are shown in Fig. 2. Corticosterone in doses of 10 mg. per day induced weight changes comparable to those that occurred following the administration of 15 units of corticotropin daily. Hydrocortisone, in the same dosage as corticosterone, produced a striking loss of weight—greater than that which occurred when 30 units of corticotropin were given. The effects on antibody production were not

due solely to the effect on nitrogen balance; 2.5 mg. of hydrocortisone and 20 units of ACTH produced comparable changes in weight, but the former was considerably more active than the latter in reducing the antibody response.

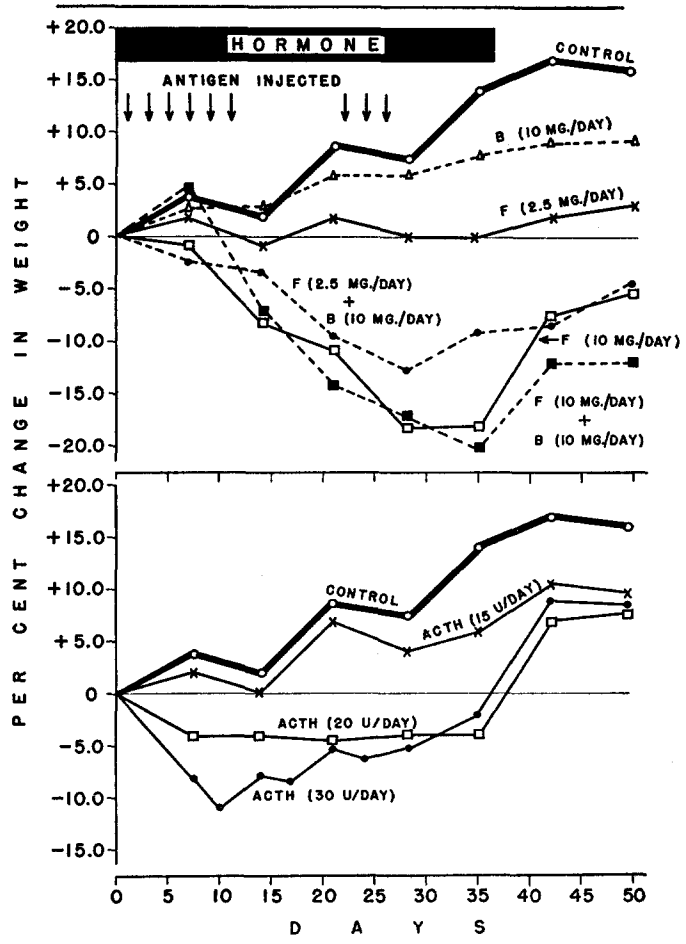

FIG. 2. Effect of corticosterone (B), hydrocortisone (F), and corticotropin (ACTH) on weights of rabbits.

DISCUSSION

The results show that hydrocortisone, which is known to alter the course of clinical inflammatory disorders, also depresses antibody production. In contrast corticosterone, which does not alter inflammatory processes when used in the same doses, and exerts only a variable and often negligible effect on re-

sistance to infection (4, 6, 9), does not alter the antibody response significantly.

The finding that relatively large amounts of corticotropin depress antibody production is consistent with the observations that highly purified corticotropin decreased the ribonucleic acid concentrations of lymph nodes in a manner similar to that of hydrocortisone or cortisone—an effect not produced by corticosterone (7, 8). Such observations are best explained by the finding that persistent administration of corticotropin leads to increased production of hydrocortisone by the adrenal gland of the rabbit (10). The present findings indicate that differences in the pattern of steroidogenesis so produced may induce different host responses, at least with respect to immunologic mechanisms. They further suggest that not only length of time of administration, but also size of the administered dose of corticotropin, are important in determining the ratio of hydrocortisone to corticosterone in the adrenal secretory product of the rabbit, if the effect on the antibody response be used as an indication of the relative amounts of these hormones secreted under stimulation from exogenous ACTH.

Whether and to what extent prolonged application of stimuli other than exogenous corticotropin will lead to increased secretion of hydrocortisone sufficient to impair host defensive mechanisms remains to be investigated. The solution of this problem may be complicated. When infections or sterile abscesses were produced in rabbits and the adrenal secretion studied at the end of 1 week, the response, in terms of total steroid secreted and relative amounts of hydrocortisone and corticosterone secreted, was variable and unpredictable (8). In addition, suggestive evidence was obtained indicating that in certain strains of rabbits, tuberculosis may alter the capacity of the adrenal cortex to respond to infection (8).

The additional possibility must be considered that substances produced in trace amounts by the adrenal cortex may depress or augment resistance to infection. Therefore, not all the effects of corticotropin are necessarily explained by the differing ratios of secretion of hydrocortisone to corticosterone. However, the data thus far presented can be satisfactorily explained on this basis and there are as yet no clear indications that trace steroids significantly alter known mechanisms of resistance to infection.

SUMMARY AND CONCLUSIONS

The effects of hydrocortisone, corticosterone, and pituitary adrenocorticotropin on antibody production were studied immunochemically in rabbits.

Hydrocortisone in doses as low as 2.5 mg. per day (approximately 1.0 mg. per kg. per day) markedly depressed antibody production whereas corticosterone in doses four times as large exerted no significant effect.

Corticotropin in doses of 15 units daily exerted an effect similar to that of corticosterone, but its effect was more like that of hydrocortisone when given

in doses twice as great. This finding is in agreement with observations that the prolonged administration of corticotropin in sufficient dosage leads to increased output of hydrocortisone by the adrenal cortex of the rabbit.

Corticosterone did not antagonize the effect of hydrocortisone on antibody production, but seemed to act additively with it.

Although each of the hormones tested induced some adverse effect on nitrogen balance as measured by the weights of the animals, and hydrocortisone induced the more striking decreases in weight, there was no direct correlation between the effects of these hormones on antibody production and their effects on weight.

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