THE EFFECT OF ADRENAL STEROIDS, CORTICOTROPIN, AND GROWTH HORMONE ON RESISTANCE TO EXPERIMENTAL INFECTIONS*

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There is now ample evidence to indicate that large doses of adrenocorticotropin or of cortisone depress resistance to a wide variety of experimental infections in many animal species (1). Although the effects of both of these hormones have been similar in most of the species studied, mice (2) and hamsters (3) have not become more susceptible to certain bacterial or viral infections following the administration of large doses of corticotropin. Indeed, cortisone diminished by tenfold the dose of pneumococci or influenza virus necessary to cause death in mice, and also hastened the time of death in such animals, whereas corticotropin did not have these effects even when given in doses which caused more loss of weight than did those doses of cortisone which depressed resistance to infection (2).

Recent evidence suggests that cortisone is produced in but small amounts, if at all, by the adrenal cortex in those animals that have been studied, whereas hydrocortisone and corticosterone, singly or together, represent the predominant steroid fractions found in adrenal vein blood (4). It is to be recalled that hydrocortisone is the only steroid thus far found that has an action similar to that of cortisone in altering the clinical course of patients with inflammatory lesions (5, 6). Corticosterone appears to be relatively ineffective as an antiarthritic agent in man (5, 6).

Inasmuch as growth hormone (GH, somatotropic hormone) may reverse many of the metabolic changes induced by corticotropin or cortisone (7, 8), it was considered possible that the depression of resistance that is induced by cortisone might also be overcome by GH, and, indeed, such an effect has been reported. Rats given cortisone may develop spontaneous and often fatal exacerbations of otherwise chronic and insignificant pulmonary infections; the administration of GH concurrently with cortisone prevented fatalities (9).

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The need for investigation of the effect of various adrenal steroids on mechanisms of immunity is apparent. In the following experiments, the effects of cortisone on experimental pneumococcal and influenza viral infections were studied in greater detail, and the effects of corticosterone, hydrocortisone, highly purified adrenocorticotropin, and growth hormone were also investigated. A preliminary account of some of these data has been presented elsewhere (10).

Materials and Methods

The methods have been described previously (2). Briefly, two experimental systems were employed. In the first, a mouse-virulent strain of pneumococcus, Type II, was injected in varying dilutions of an overnight blood broth culture into white Swiss mice. The inoculum was mixed with a constant amount of specific antiserum (sufficient to protect about half the mice receiving about 50,000 organisms) and promptly injected intraperitoneally. The animals were observed frequently and many of those that succumbed were autopsied and cultures were made to confirm the nature of the lethal infection. The second experimental system consisted in the use of a mouse-adapted strain of influenza A virus (PR8), which was given intranasally in varying dilutions to lightly anesthetized mice, as has been described (2). When titrations of the rate of multiplication of virus in the murine lung were to be performed, animals were sacrificed in groups of 5 at appropriate intervals after instillation of virus, and the lungs stored at −70°C. until used. The lungs were triturated in 20 per cent serum broth and the viral content estimated in the usual manner by egg-infectivity titrations, using 10 eggs per dilution.

Hormones[1].—Cortisone (compound E of Kendall, 11-dehydro-17-hydroxycorticosterone) was used as a saline suspension containing 25 mg. of cortisone acetate per ml. plus suspending agents, as previously described (2). Suspensions containing 20 mg. per ml. of cortisone acetate (without any suspending agents) were made in saline containing 0.9 per cent butyl alcohol. These suspensions were shaken vigorously before use. Various schedules of injection were employed; when cortisone was given after the infectious agent had been inoculated into the test animals, separate needles were used for each animal, in order to minimize cross-infection.

Hydrocortisone (compound F of Kendall, or 17-hydroxycorticosterone) was used both as the acetate (F Ac) and as the free alcohol (F) in saline suspensions containing 0.9 or 1.5 per cent butyl alcohol. Corticosterone (compound B of Kendall) was prepared in a similar manner.

Adrenocorticotropin (ACTH) was kindly supplied by Drs. E. B. Astwood and M. Raben. Their dry powder, which is of high purity, was suspended in a concentration of 1 mg. per ml. of peanut oil containing 5 per cent beeswax. Preliminary experiments were conducted to determine the biologic activity and toxicity of this preparation, as compared with a less pure, aqueous preparation from a commercial source. The oil-beeswax was injected subcutaneously, and induced no significant local reactions. The methods for studying the hematologic responses of mice to these hormones have been described previously (11). Significant eosinopenia, leukopenia, and weight loss may be induced for as long as 72 hours by the injection of as little as 0.3 mg. of corticotropin in oil and beeswax (Table I), so that the purified material, given in the oil-beeswax menstruum, has approximately 25 to 50 times the activity of the commercial standard aqueous preparation. It is noteworthy that 1 of each group of 15 mice injected with corticotropin in oil and beeswax died but that no deaths occurred in animals that received oil

[1] We are grateful to Armour Laboratories, Chicago, Merck & Co., Inc., Rahway, N. J., G. D. Searle & Co., Chicago, The Upjohn Co., Kalamazoo, Chemical Specialties Co., Inc., New York, and F. W. Horner Co., Montreal, for supplies of hormones.

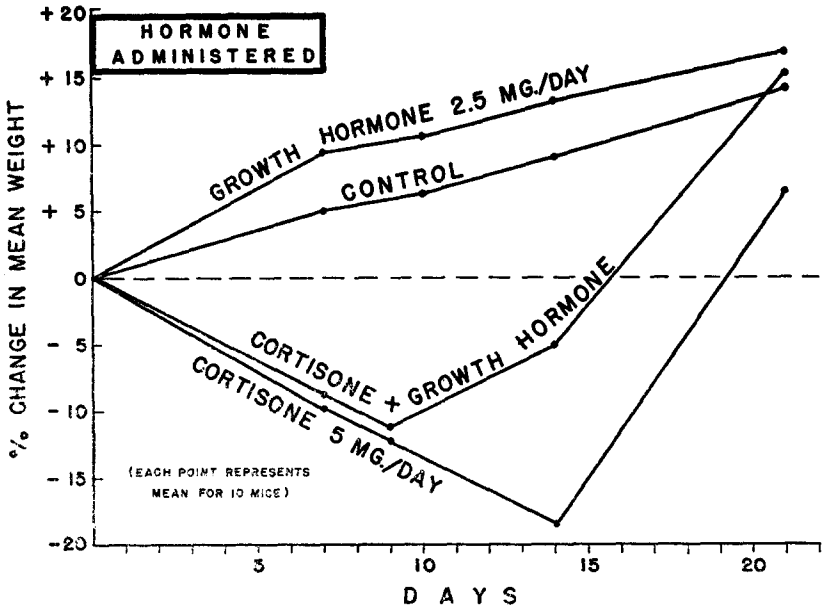

FIG. 1. Effect of cortisone and growth hormone on weights of normal mice.

TABLE I

Leukocyte and Eosinophil Counts and Weight Changes in Mice after Single Injections of Corticotropin in Oil and Beeswax

Corticotropin	Length of time after injection	No. of animals*	Eosinophils‡	Leukocytes‡	Mean change in weight
mg.	*hrs.*				*per cent*
0.1	24	5/5	59 ± 72	6.5 ± 2.0	−3
	48	4/5	147 ± 77	4.6 ± 0.8	−2
	72	5/5	147 ± 32	4.1 ± 1.1	−6
0.2	24	5/5	0	3.1 ± 0.7	−14
	48	4/5	123 ± 101	4.8 ± 0.7	−23
	72	5/5	152 ± 56	4.7 ± 0.7	−7
0.3	24	5/5	0	2.0 ± 0.3	−8
	48	4/5	52 ± 51	3.9 ± 1.1	−24
	72	5/5	19 ± 5.3	6.9 ± 2.7	−13
Oil and beeswax alone	24	5/5	151 ± 43	7.0 ± 1.5	−2
	48	5/5	128 ± 15	7.1 ± 0.4	−2
Controls§	(Not injected)	37	120 ± 9.1	6.9 ± 0.6	

* Survivors/total injected.

‡ Eosinophils expressed in absolute numbers and leukocytes in thousands per cubic centimeter.

§ See reference 11.

and beeswax alone. For these reasons, mice were given 0.3 mg. corticotropin every 3 days, and it was felt that about 7 per cent of deaths should be regarded as due to the drug alone. However, no corrections for the toxicity of the drug were applied to any of the data to be presented.

Growth hormone was originally supplied in a highly purified form by Drs. Raben and Astwood. Solutions of the hormone were adjusted to pH 6.0–6.5 and injected intraperitoneally once daily in a volume of 0.5 ml. The effect of this preparation on weights of growing mice is seen in Fig. 1, in which four groups of male mice, each containing 10 animals, with a mean weight of 15 ± 2 gm. were followed for 3 weeks during and after the administration of growth hormone and cortisone. Growth hormone increased the rate of growth of mice slightly over that of non-treated controls. However, it did not reverse the effect of cortisone in causing loss of weight. Nevertheless, recovery from the effects of administering cortisone was more rapid in animals that received growth hormone and cortisone than in those receiving cortisone alone. After it had been noted that the adverse effects of cortisone on infection were not overcome by growth hormone, purified material was obtained from another source, and this gave substantially the same results as those with the material obtained from Drs. Astwood and Raben.

EXPERIMENTAL

Effect of Cortisone on Resistance to Pneumococcal Infection.—Previous studies had shown that an initial dose of 10 mg. of cortisone followed by 5 mg. daily for 5 days diminished the LD_{50} of pneumococci in partially protected mice, by approximately tenfold (2). Furthermore, the mice succumbing to the fatal infection died sooner than controls. This phenomenon was studied further with respect to dosage schedules, and the results are shown in Fig. 2, in which they are compared with the data previously reported.

The data in the chart represent the accumulation of a large number of experiments and are charted as the ratio of the logarithms of the dilutions of infectious agent necessary to kill 50 per cent of mice in treated mice to the corresponding values in control animals. Repeated experiments indicated that neither butyl aclohol nor oil and beeswax significantly altered the LD_{50}, hence the effects were presumed to be due to the hormones administered. The bars that end with irregular edges and broken lines indicate that an LD_{50} end-point was not attained in that particular group of animals. In every case this was due to a larger difference between the treated and control animals than the dilutions used could discern. As a result, the jagged portion of the bar shows the least possible difference in titer and it is possible that these differences in titer would have been exceeded in some cases had greater dilutions of the organism been used, and a definitive LD_{50} value obtained.

Fig. 2 shows that 5 mg. of cortisone acetate daily for 3 days was as effective as the original dosage of 10 mg. initially, followed by 5 mg. daily for 5 days. The persistence of loss of weight for at least a week after the administration of cortisone had ceased (Fig. 1), suggested that some of the hormone was held locally and continued to exert an effect over a prolonged time. Additional experiments were, therefore, carried out with cortisone given as two injections 4 hours apart, the second being given just before the introduction of the organisms, and no further cortisone was then administered. Both 5 mg. and 10 mg. dosages given in this manner were effective in depressing resistance,

with the latter somewhat more so. Even 5 mg. administered once, at the time that the bacteria were injected, depressed resistance and survival time significantly.

That cortisone shortens the survival time of those animals that are destined to die has been reported previously. The magnitude of this effect is charted in Fig. 3, in which the mean survival time of all the animals injected with the test substance is compared with the mean survival time of the control animals in

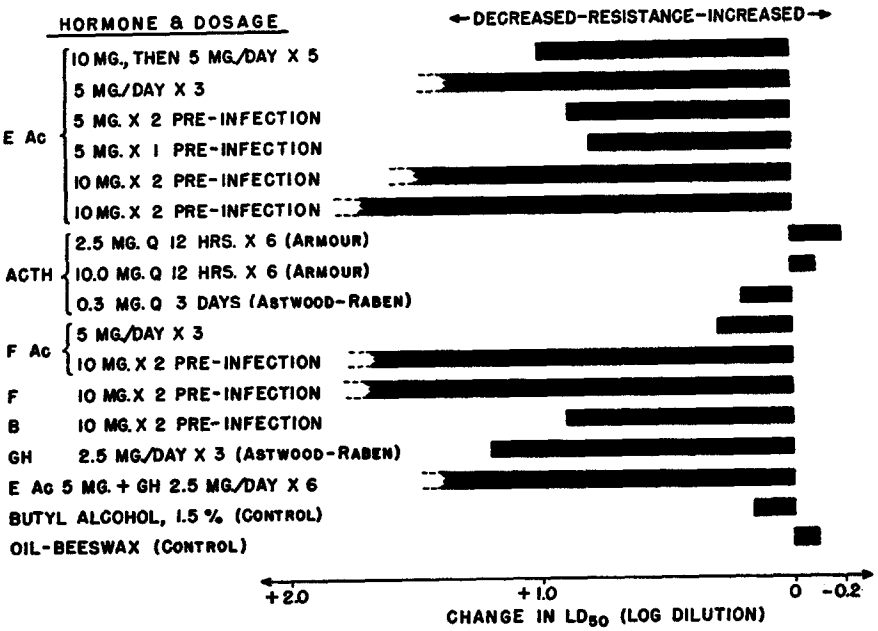

FIG. 2. Effect of certain steroids, ACTH, and growth hormone on LD_{50} in passively immunized mice infected with Type II pneumococcus.

each experiment. Representative control experiments with butyl alcohol and oil and beeswax are included.

It should be noted that for the purposes of calculation, all the surviving animals were assumed to have succumbed on the 12th day, when the observations in each experiment were terminated. This manifestly extreme assumption weighted the results heavily against an effect of cortisone on survival time, particularly because the method fails to distinguish sharply between those animals dying of infection during the 8 to 12 day period after the injection of the bacteria (as occurred occasionally in the cortisone-treated group) and those surviving. The mean survival time of animals was therefore markedly shortened by administration of cortisone in any of the dosage schedules used. The effect of cortisone was approximately as great when given in crude saline suspension, as when given in saline suspension with added suspending agents, although accurate measurement of the dose in the former instance was difficult.

As shown in Fig. 1, the loss of weight which accompanied the administration of cortisone persisted after all injections had ceased. It was, therefore, of interest to determine whether a similar effect on resistance to infection would persist. One hundred and fifty mice were each given 5 mg. of cortisone subcutaneously.

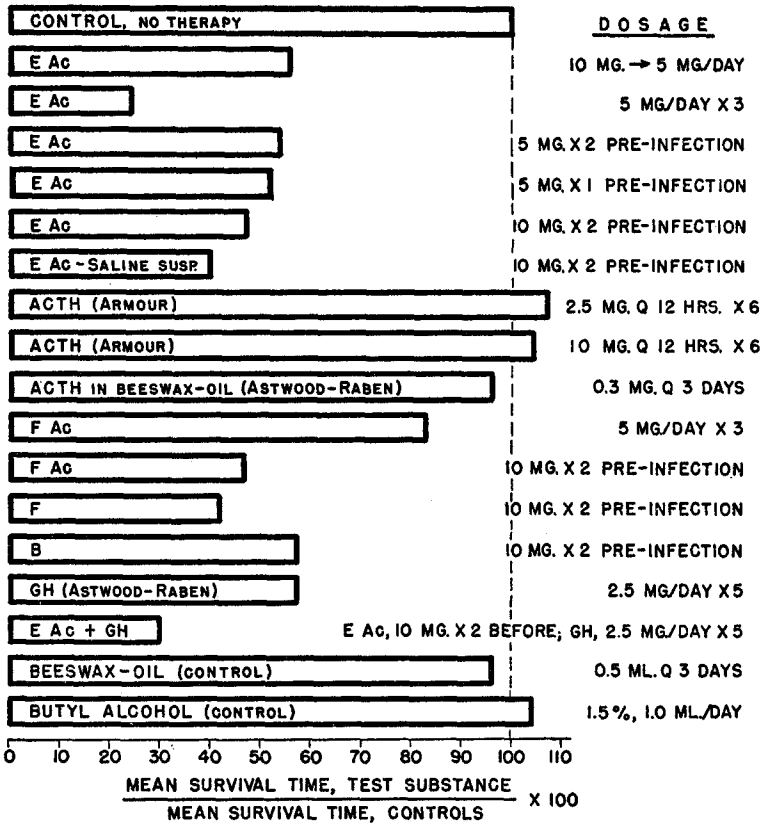

FIG. 3. Effect of certain steroids, ACTH, and growth hormone on pneumococcal infections in partially immunized mice.

Three groups of 10 were immediately given 3 standard dilutions of pneumococci and the constant amount of antiserum, and a like number of control animals were also injected. This procedure was repeated in the remaining mice at 1, 3, 6, and 9 days after the injection of the hormone.

The data in Fig. 4 show that the effect of cortisone on resistance and survival time persisted for between 3 and 6 days after the last injection. This suggests that the cortisone is absorbed slowly from the site of injection.

When corticotropin was tested, it was found to have no significant effect on resistance to infection, or on survival time (Figs. 2 and 3); this was demonstrated using 0.3 mg. of the purified material or as much as 20 mg. of the less purified material daily.

Hydrocortisone acetate gave different results in two experiments. In the first, 5 mg. daily was given, whereas in the second, 10 mg. was given twice, 4 hours apart, before infection. The latter schedule gave results that were

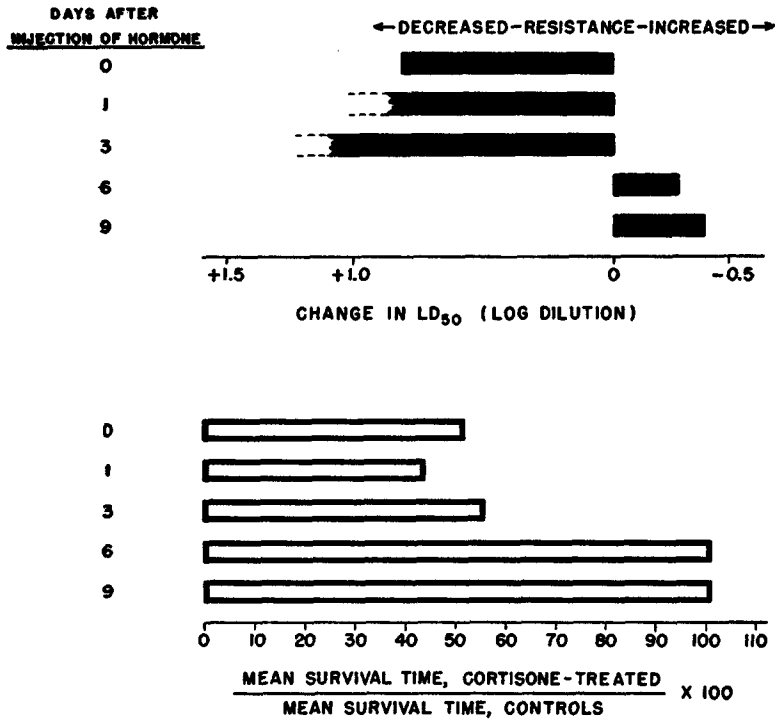

FIG. 4. Persistence of depression of resistance to pneumococcal infection in passively immunized mice after a single injection of 5 mg. of cortisone.

roughly similar to those obtained using cortisone, whereas hydrocortisone acetate by the former schedule was less effective. Inasmuch as hydrocortisone acetate in man is less effective by parenteral routes than by mouth, it was assumed that the present variability in results was due to varied rates of hydrolysis of the acetate in tissues. Hydrocortisone, as the free alcohol, depressed both resistance and survival time. Corticosterone appeared to be somewhat less effective than hydrocortisone or cortisone; nevertheless it significantly depressed resistance to infection. In these experiments growth hormone, given in large doses, acted like cortisone, and when given along

with the latter did not overcome the effect. This amount of growth hormone, as was seen in the preliminary data, was apparently not toxic to the mice.

Experiments with Influenza Virus.—Similar studies were conducted in relation to influenza viral infection in mice.

The schedules of injection of hormones were similar to those used in the pneumococcal infections. Inasmuch as deaths rarely occur sooner than 4 days after inoculation of lethal amounts of the virus in the dilutions used, the schedules of injection of hormones were modified to include an additional dose of hormone on the 4th day in those experiments in which two pre-infection doses of hormone were administered. In the case of corticosterone, the lot of hormone used was different from that used in the pneumococcal experiments, and was better tolerated by the mice when given as 2.5 mg. twice daily on the day preceding infection, and again on the day of infection. This dose was repeated once on the 4th day after infection. Larger doses than this, with the lot used, led to intercurrent deaths—none were encountered when this smaller dose of hormone was used. The cause of these intercurrent deaths was not determined but inasmuch as the animals died within the 1st day after injections of 5 to 10 mg. of this lot of corticosterone, and heart's blood cultures were sterile, it was assumed that these were acute toxic deaths due to an unknown agent in the preparation of hormone.

It is readily apparent (Fig. 5) that the results of the studies with the influenza virus are similar to those obtained using pneumococci. Cortisone in several dosage schedules depressed resistance to the viral infection, and even the highly purified corticotropin failed to alter resistance significantly. Compound F also depressed resistance, and, although the effect of growth hormone was variable and not consistent, growth hormone did not overcome the effects of cortisone. Corticosterone, in one experiment, had no significant effect on resistance, in contrast to the effects observed in the pneumococcal infections. Inasmuch as the dose of corticosterone used in the viral studies was lower than that used in the bacterial infection, it is difficult to draw definitive conclusions. The availability of but small amounts of corticosterone precluded extensive study of the effects of this hormone or repetition of these experiments.

A study of survival times in this influenzal infection is less fruitful than in the pneumococcal infections because with the amounts of virus used, deaths usually do not occur sooner than 4 to 5 days after infection, and may be deferred for another 4 to 6 days. Inasmuch as surviving animals have been arbitrarily assigned a death time of 12 days, when all the experiments were terminated, the differences in survival time in the most extreme instances of depression of resistance to influenzal infection were necessarily small. For this reason the data are not presented in detail; suffice it to say that even under these relatively weighted circumstances there was good correlation between the capacities of a hormone to reduce resistance to infection, and to lower the survival time of the animals dying of infection.

Effect of Adrenocortical Hormones on Viral Multiplication.—The rate of multiplication of viral particles in infected murine lungs was studied.

For this purpose, groups of 20 mice were given hormone, and a standard dose of influenza virus, as has been described. Groups of 5 mice were then sacrificed at appropriate time intervals thereafter, and the lungs prepared for titration of the viral content in embryonated eggs. In all these experiments the same source of virus was used, and no detectable deterioration of the source material was observed in repeated control titrations. The results are presented in Fig. 6; 6 control titrations, performed on different days, are compared with a single control titration in animals receiving oil and beeswax alone.

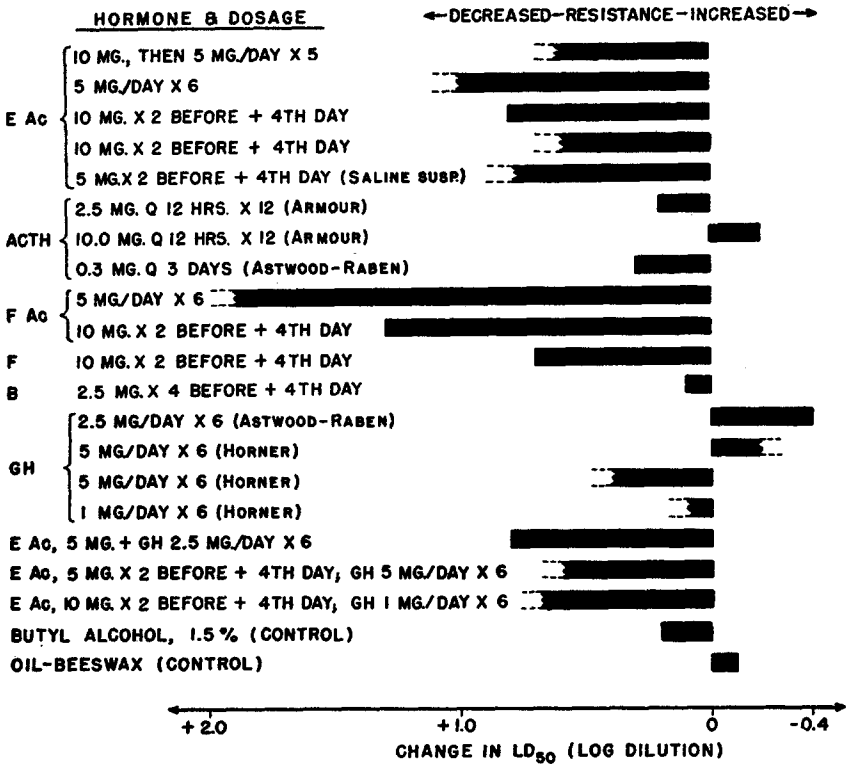

FIG. 5. Effect of certain steroids, ACTH, and growth hormone on LD_{50} in mice infected with influenza A virus.

It is seen that the latter treatment did not affect substantially the titer of virus, as compared with the range encountered in the 6 control titrations. The characteristic pattern of multiplication of the virus in control animals is apparent. During the 1st day after inoculation of virus, there is rapid multiplication in the lung to a broad range of values varying at least 200-fold. By the 2nd day, the range of values is still about 100-fold, but the viral titers have increased to their peak values. By the 4th day there is a small and inconstant decrease in viral concentration in the lungs, but the variation is but fivefold,

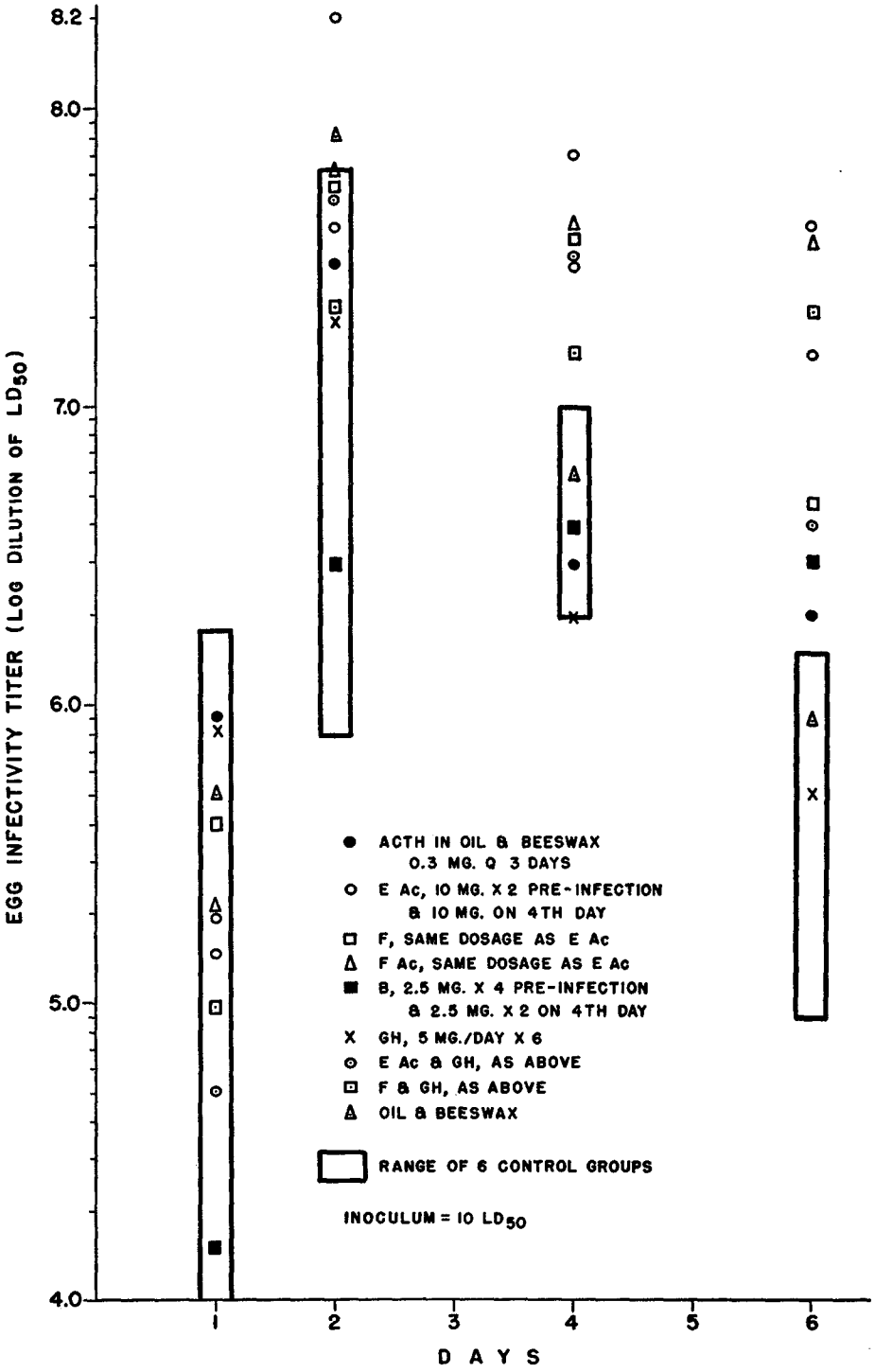

FIG. 6. Effect of certain steroids, corticotropin, and growth hormone on titers of influenza virus in murine lungs.

and by the 6th day there is a distinct fall in titers to levels about equal to those observed on the 1st day, and the range of values is about 20-fold. Because 10 lethal doses were instilled as the inoculum, the animals began to die of the infection after the 6th day, so that titrations were not extended beyond this time in these experiments.

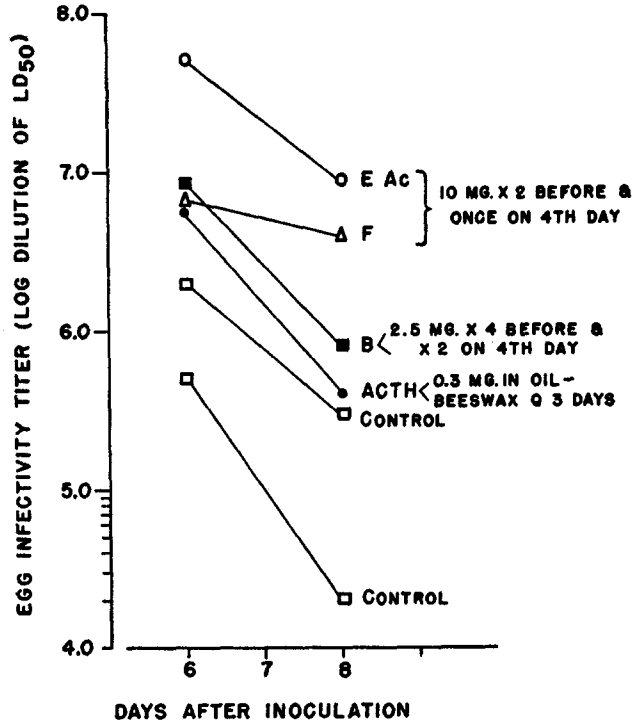

FIG. 7. Effect of ACTH and adrenocortical steroids on titers of virus in lungs of mice infected with sublethal amounts of influenza virus.

The administration of the various hormones did not alter the initial rate of accumulation of virus in the murine lungs; and even by the 2nd day only one group of animals, namely, one of the two groups treated with cortisone acetate, had titers exceeding the range of control values. There was, therefore, no striking effect of the hormones on the rate of accumulation of virus in the lungs. On the other hand, by the 4th and 6th days significant and reproducible differences appeared. Growth hormone alone exerted no significant effect on the rate at which the viral titers declined. Corticotropin and corticosterone exerted a minimal effect in that there was no deviation from control values on the 4th day, and but small increases in titer over control values on the 6th

day. However, the viral titers in the lungs of the animals treated with cortisone or hydrocortisone, the latter given either as the alcohol or the acetate, declined more slowly than in controls and remained as much as 200 times greater than in the controls.

In order to study this effect further, the experiment was repeated using an inoculum of 0.1 LD_{50}, so that there was no mortality among the animals during the experiment and the lungs were examined on the 8th day. Under these circumstances, the decline in titer was most rapid in the control animals, less rapid in the animals receiving corticotropin or corticosterone, and least in the animals receiving cortisone or hydrocortisone (Fig. 7).

Inasmuch as cortisone, and presumably also hydrocortisone, may depress antibody formation, it was possible that the delayed reduction in viral titers was due simply to diminished production of antibodies. Furthermore, since the larger antigenic stimulation occurred in the experiment in which 10 LD_{50} were inoculated, and the most striking effect of the adrenal steroids was noted on the 6th day after instillation of the virus, all the mice that were sacrificed on the 6th day were bled, before the lungs were removed, and the serum titrated for its antibody content by a micro-modification of the Hirst method, as well as by neutralization of egg infectivity of about 100 infective doses of virus. The technique is such that the minimal dilution of serum that can be tested is 1:4. Twenty normal mice were sacrificed and their sera failed to show any detectable antibody. Similarly, no detectable antibody was found in any of the mice that had received virus 6 days previously, whether or not the animals were receiving hormonal treatment. Although these studies do not eliminate the possibility that local antibody formation, or fixation of antibody at the site of the local lesions, could be affected by the adrenocortical steroids, they do not lend substance to the possibility that alterations in antibody response are the primary reason for the delayed decrease in viral titers in the lungs.

DISCUSSION

The mechanisms by which adrenal steroids may alter resistance to infection have been discussed in detail elsewhere (1, 12). The effect of such steroids is consistent and reproducible, and a variety of animal species are affected in a similar manner, when infected with diverse infectious agents.

The experiments herein reported add some information concerning the mechanisms of action. Cortisone and its congeners may exert a significant effect within the first 24 hours after they have been injected into infected animals, suggesting that the effect of these steroids on antibody formation does not appear to be a primary factor in the decreased resistance to infection. Similarly, the loss of weight which occurs when the steroids are given is probably not a significant feature of the effect on infection both because weight loss during the first 24 hours after giving the hormone is usually not

over 2 to 3 per cent, and because in mice adrenocorticotropin may induce greater loss of weight than does cortisone without any noticeable change in resistance to infection. Indeed, in most viral diseases, loss of weight and inanition more commonly increase resistance to infection, in contrast to the situation in bacterial infections (13).

The similarity of effects of hydrocortisone and cortisone is consistent with clinical experience in the treatment of various inflammatory disorders; hydrocortisone acetate is clinically relatively less effective than the free alcohol when given parenterally and the difference is presumed to be due to slow hydrolysis of the ester (14). Similar observations were made in the studies herein reported.

The failure of adrenocorticotropin to influence significantly resistance to infection, or survival, in either pneumococcal or influenza viral infections is in striking contrast to the effect of this hormone in man, rabbits, monkeys, and probably also other mammals (1). That mice and hamsters do not manifest significant depression of resistance to several viral infections after injection of ACTH has been observed repeatedly (15–18), and in each of these infections in which it was studied, cortisone depressed resistance when ACTH did not (17, 18). In one series of experiments, however, large doses of ACTH given in a gelatin menstruum seemed to depress resistance of mice to infection with the West Nile virus (17). Unfortunately, in those experiments, only 4 uninfected mice were given the hormone and it is not clear whether the hormonal preparation was itself toxic.

Several possible explanations may be offered to account for the divergent actions of corticotropin and cortisone. It is possible that ACTH does not stimulate the murine adrenal gland; there is evidence that stress and corticotropin produce little effect on the lipid components of the adrenal in mice (19). Yet the mice receiving corticotropin exhibit loss of weight, and marked eosinopenia and lymphopenia. Thus, it appears likely that stimulation of the adrenal cortex occurs after the administration of corticotropin to mice, although such an effect is not demonstrable by the study of the lipids of the adrenal gland. The possibility that the effects of corticotropin on the hematologic patterns, as well as the weight loss, in mice are due to an extra-adrenal effect of the hormone does not appear to be an adequate explanation. Finally, it may be that corticotropin stimulates the murine adrenal to release hormones that do not suppress resistance to infection.

Bush has reported in detail on species differences in the nature and content of the adrenal secretion (4). Although no data are available concerning the nature of the adrenal secretion in mice, the predominant adrenal steroid in rats and rabbits is corticosterone, and small amounts of other steroids may be found in the adrenal secretions of these species. Inasmuch as either hydrocortisone or corticosterone or both have been the predominating steroids re-

leased in all the animal species thus far studied (4), and cortisone or hydrocortisone exerts effects in the mice which are quite different from the effects of adrenocorticotropin, it might be suggested that corticosterone is produced by the murine adrenal gland. However, corticosterone lowered the resistance of mice to pneumococcal infection, whereas corticotropin did not, although both hormones exerted similar effects on influenza viral infections in mice. This difference may be explained by assuming that corticosterone is quantitatively less effective than cortisone or hydrocortisone in reducing resistance to infection, but that the relatively large doses used in these experiments led to effects which could not always be duplicated by the administration of large doses of ACTH. It is also possible that the differences in the effects of corticosterone and corticotropin might represent the result of the action of small amounts of other hormones secreted by the adrenal which could overcome any mild depression of resistance induced by pure corticosterone. Indeed, a substance in adrenal cortical extracts can overcome the capacity of cortisone to reduce resistance to experimental tuberculosis (20). Finally, it is conceivable that the murine adrenal gland produces a hormone which is similar to, but not identical with, corticosterone, hydrocortisone, or cortisone.

The variation in species responsiveness to these hormones is further illustrated by the observation that corticotropin, cortisone, and hydrocortisone induce atrophy of lymphoid tissue in rabbits, and cause certain characteristic alterations in the concentration of pentose nucleic acids, whereas corticosterone does not induce these changes (21); yet rabbits' adrenal glands secrete corticosterone primarily (4). These and many similar problems concerning the species responses of experimental animals to these hormones clearly require further study.

The effect of growth hormone on infections may also indicate species differences. The experiments herein reported show no effect of growth hormone in overcoming the effects of cortisone and similar observations have been made in induced infections in rabbits (22). However, the original observations on the effectiveness of growth hormone were made in rats in which fatal spontaneous infections were induced by the administration of cortisone (9). Growth hormone alone clearly offered no advantage to infected mice in the studies herein reported, and Gordon *et al.* have shown that the hormone does not add to the therapeutic activity of streptomycin in irradiated mice (23).

The effect of hydrocortisone and cortisone on the pattern of multiplication of influenza virus in the murine lung requires further study. These hormones caused the titers of virus to be maintained above control titers, and this effect was not explained on the basis of suppression of antibody response. The titration of serum antibody does not eliminate the possibility of suppression of local antibody formation, or of suppression of antibody formation with sufficiently rapid local fixation of antibody so that differences in serum concentration in

hormone-treated as opposed to control animals would not be apparent. However, the experiments also suggest the possibility that a cellular mechanism by which virus may be removed from the lung is adversely affected by the cortical steroids. It has been found that injected erythrocytes are removed more slowly from the regional lymph nodes of cortisone-treated animals than from those of controls, and that the effect appears to be largely referable to inhibition of the capacity of macrophages to dispose of ingested erythrocytes (21). Such a mechanism may also account for the persistence of virus in the lungs of cortisone-treated mice.

SUMMARY AND CONCLUSIONS

Cortisone acetate, hydrocortisone, and hydrocortisone acetate depress the resistance of mice to pneumococcal and influenza viral infections, although hydrocortisone acetate is somewhat less effective than the free alcohol, when given subcutaneously.

Pituitary adrenocorticotropin, even in highly purified form and in oil and beeswax, does not significantly alter the resistance of mice to these experimental infections, even when given in doses which may cause profound eosinopenia, lymphopenia, and weight loss, and which are at the limit of tolerance of the animals.

Corticosterone depresses resistance to pneumococcal infections significantly, but fails to alter resistance to influenza viral infections. The findings suggest that murine adrenals may produce one of the known adrenal steroids such as corticosterone along with another steroid, or may produce a steroid other than cortisone, hydrocortisone, or corticosterone.

When resistance is decreased by adrenal steroids, survival time is invariably shortened, and the effect of the steroid hormones is frequently demonstrable within the 1st day after infection with pneumococci, making it unlikely that the depression of resistance that is seen is primarily due to depression of antibody formation.

A single dose of 5 mg. of cortisone may cause depression of resistance and may decrease the survival time for 3 to 6 days afterward.

Growth hormone (somatotropic hormone) in highly purified form, and in the doses used, did not overcome the weight loss induced by cortisone, but the animals treated with growth hormone and cortisone regained their lost weight more rapidly than those receiving cortisone alone. Growth hormone alone caused a slight increase in the rate of gain in weight over controls.

Growth hormone alone did not increase resistance to infection, and did not increase the survival time, in mice infected with either pneumococci or influenza virus. Growth hormone in various dosages failed to overcome the effect of cortisone in depressing resistance to these infections.

Cortisone, hydrocortisone, corticosterone, and corticotropin did not alter

significantly the titers of influenza virus attained in the murine lungs during the first 2 days after infection, but cortisone and hydrocortisone markedly delayed the rate at which virus titers declined during the subsequent 6 days. Corticosterone and corticotropin delayed the rate at which the titers declined but slightly, and growth hormone had no apparent effect, as compared with controls. Growth hormone did not overcome the effect of cortisone and hydrocortisone on viral titers. No detectable antibody was found as late as 6 days after infection, in controls or in hormone-treated animals.

We are indebted to Mildred W. Barnes for many of the influenza viral titrations.

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