EFFECTS OF CORTISONE ON EXPERIMENTAL MURINE TYPHUS

III. Effect of Cortisone on the Immune Response in Mice, Rabbits, and Guinea Pigs

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Many attempts have been made to determine the effect of adrenal cortical hormones on immunization and immunity to various infectious and noninfectious agents. There are at least three questions to be considered in connection with the study of the immune response: first, the effect of cortical hormones on the development of antibodies, second, the effect these hormones may have on already formed antibodies, and, third, the effect that the hormones have on anaphylactic reactions. The effect of cortical hormones on antibodies has been a very controversial question with the results falling into three possible categories: first, no effects being noted, and second, an inhibitory effect, either on the production of antibodies, or reduction in the antibody titer after immunization by either adrenalectomy or administration of excess hormone. The third possible group of results in these hormonal studies is an enhancement of antibody production or its release into the blood serum.

The role of the adrenals as set forth in early literature is confusing and contradictory. Much of the evidence is indirect due in part to the introduction of many unknown factors without critical analysis of the mechanisms involved. Some thirty years ago considerable work was done on the effects of adrenalectomy on immunization. The results of these workers show that some found a stimulatory effect resulting from adrenalectomy, while others reported no effect or a depression in antibody production. Many early workers failed to confirm the completeness of adrenalectomy by autopsy and thus failed to rule out regrowth or the presence of small cortical remnants.

The work of Fox and Whitehead (1935, 1936) marked the beginning of the use of adrenal cortical extracts in the study of immunity. These early studies were hampered by the lack of potent materials and standardization of materials used. Although extracts prepared by different investigators were adequate to maintain life of adrenalectomized animals, they differed widely in the extent of purification and in various types of biological activity. Six of the 28 crystalline steroids that have been isolated from the adrenal cortex have been shown to have physiologic activity. All are capable of replacing one or more functions of the adrenal cortex.

Dougherty et al. (1944, 1945) have compiled considerable evidence for the presence of antibody within the lymphocyte. They have also extended the concept of pituitary-adrenal cortical control of lymphoid tissue structure and function to include control of antibody release from tissue cells. Evidence has been presented by Dougherty et al. to suggest that alterations in the functions of the adrenal cortex are of importance in the immunological response. They have reported an increase of serum antibody titer in mice by injection of adrenal cortical hormone or by exposure of the animals to various noxious stimuli designed to promote increased hormonal secretion by the adrenal cortex (Dougherty and White, 1946) (White and Dougherty, 1945).

Fischel et al. (1949), Fischel (1950), and De Vries (1950) were unable to demonstrate an anamnestic rise in circulating antibody following ACTH treatment despite a concomitant decrease in circulating lymphocytes. These results are in marked contrast to the work of Chase et al. (1946) and Hammon and Novak (1950) who found that an anamnestic reaction was produced.

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by mass administration of adrenal cortical steroid to rabbits, mice, and rats.

Germuth and Ottinger (1950) found that cortisone and ACTH suppressed antibody formation to crystalline egg albumin in the rabbit. Slanetz (1954) demonstrated that agglutination titer for Salmonella enteritidis in cortisone treated rats were approximately one half that of the controls. In contrast to these findings Mirick (1950) found that patients being treated with ACTH or cortisone produced as prompt and as high a titer to pneumococcal polysaccharides as did patients not receiving cortisone or ACTH.

Not only is there controversy as to the effects of cortisone on the production of antibodies, but there are also discrepancies in the observations on the effect of the hormone on antibodies already formed. Fischel et al. (1951) have shown that cortisone and ACTH fail to effect the disappearance of pneumococcal antibody passively administered to rabbits. Van der Slikke and Keuning (1953) have reported a decrease in antibody titers to para B vaccine in rabbits after immunization. Stoner and Goodwin (1954) reported that cortisone treatment of mice immunized with active infection with Trichinella spiralis and challenged with trichinella larva produced a breakdown in their immunity sufficient to permit reinfection.

The work of Hanan and Overman (1953) and Hanan and Oyama (1954) has presented a new idea as to why cortical hormones may vary in their effect on antibody production. The results of their studies provide evidence that the antibody response to antigen of large particle size, as sheep red cell stroma, is less influenced by cortisone treatment than is the case with smaller soluble antigen as bovine serum albumin. Antibody production to mumps virus is affected only irregularly. They suggest that cortisone exerts its effect at some point before the antigen reaches the site of antibody synthesis. It is possible that due to the differing physical state of the antigens, they are captured by two different elements of the reticulo-endothelial system, and that one of these elements is affected by cortisone whereas the other is not. It is believed by these workers that cortisone interference with γ-globulin synthesis is not responsible for the hormonal effects on antibody production. If this work can be verified by others with other antigenic agents this may possibly explain the varied effects of cortical hormones on the immunological response to different antigens.

The role of cortisone and ACTH in the production and life of antibody globulin is not clear at this time. Undoubtedly there are many factors involved in the part played by the hormone in antibody formation. The dosage of hormone, the antigen, and the general design of the experiment must be considered in comparing the varied results found in the literature.

The effects of cortisone on susceptibility to murine typhus infection in the mouse, cotton rat, hamster, and guinea pig have previously been presented. In this paper the effect of cortisone was studied in the immune processes of these animals and also the rabbit.

**EXPERIMENTAL METHODS**

Experiment I. The effect of cortisone on the production of complement fixing antibody in the guinea pig, cotton rat, hamster, and white mouse. In a previous study (Whitmire and Downs, 1957) the susceptibility of these animals to murine typhus and the effect of cortisone was described. The animals which served for the susceptibility study also served as the basis for the results presented here in the study of complement fixing antibody production. The animals were divided into two groups, one receiving cortisone, the other receiving salt solution. All were infected with murine typhus in fourfold dilutions (62.5 to 64,000) of infected yolk sac, 4 animals per dilution, the yolk sac material having a mouse toxicity of LD₉₀ of 1:640.

The schedule for cortisone treatment was as follows. Mice received 2.5 mg cortisone 4 hr prior to infection. Cotton rats, group A, 2.5 mg cortisone 24 and 48 hr prior to infection and 2.5 mg daily for 10 days, total of 30 mg per animal; group B received 2.5 mg cortisone 24 and 48 hr prior to infection and 1.25 mg daily for 10 days, total 17.5 mg per animal. Hamsters received 5 mg cortisone 24 hr prior to infection and 1.25 mg daily for 10 days, total 17.5 mg per animal. Guinea pigs received 10 mg 24 hr prior to infection and 20 mg daily for 10 days, total 210 mg per animal. All sera were negative for complement fixing antibodies on the
4th day. The titers for the later periods are presented in Table 1. It would appear from the average complement fixing titer of the 4 animals of each group that cortisone caused a slower development of antibodies. On the 21st day, however, there was a tendency in most instances for a higher level of complement fixing antibody to be present in the cortisone treated animals. No conclusion can be drawn concerning the average titers of the cortisone treated animals at 40 days due to the lack of complete results. The cortisone dosage was such that after withdrawal a number of animals died which had been treated with cortisone.

All mice, cotton rats, and hamsters surviving on the 20th day were bled and their sera were titrated for complement fixing antibodies. The results presented in Table 2 represent the complement fixing antibody titers for mice, cotton rats and hamsters surviving on the 20th day. Mice gave relatively unsatisfactory results, since only one group gave an appreciable titer. The titers in the cortisone treated group suggested that less antibody was produced than in the untreated infected group.

On the whole the complement fixing titers were higher in the cortisone treated cotton rats than in the untreated rats. With the hamsters, unsatisfactory data was obtained since many deaths in the treated group prevented accurate sampling.

Experiment II. Immunization of rabbits with whole rickettsial antigen and sonic treated rickettsial antigen and the effect of cortisone on antibody production. Recent work of Hanan and co-workers (1953, 1954) presented evidence that the effects cortisone may have on antibody production are related to the particle size of the antigen. With this in mind, the following experiment was undertaken to determine if cortisone would interfere with the immunization of rabbits when sonically disintegrated rickettsiae were used. The vaccination schedule consisted of 1 ml antigen given intravenously every second day for a series of 7 injections over 14 days. The rabbits were divided into 4 groups of 2 animals each and injected as follows: group A (rabbits 1 and 2) saline treated,

**TABLE 1**

Complement fixation titers of guinea pig sera at intervals during infection and the effect of cortisone on antibody production

<table>
<thead>
<tr>
<th>Infectious Dose:</th>
<th>Average Complement Fixation Titer for 4 Animals on Various Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilution of Infected Yolk Sac*</td>
<td>No cortisone treatment</td>
</tr>
<tr>
<td></td>
<td>13</td>
</tr>
<tr>
<td>1:250</td>
<td>640</td>
</tr>
<tr>
<td>1:1,000</td>
<td>832</td>
</tr>
<tr>
<td>1:4,000</td>
<td>672</td>
</tr>
<tr>
<td>1:16,000</td>
<td>320</td>
</tr>
<tr>
<td>1:64,000</td>
<td>200</td>
</tr>
</tbody>
</table>


* Mouse toxic LD₅₀ 1:640/ml.
† Cortisone treatment: 10 mg. 24 hr prior to infection and 20 mg daily for 10 days, total 210 mg per animal.

**TABLE 2**

Complement fixation titers of mouse, cotton rat, and hamster sera and the effects of cortisone on antibody production

<table>
<thead>
<tr>
<th>Species</th>
<th>Complement Fixation Titors: Dilutions of Infected Yolk Sac* Used in Inoculation of Animals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No cortisone</td>
</tr>
<tr>
<td></td>
<td>250</td>
</tr>
<tr>
<td>Mice</td>
<td>512</td>
</tr>
<tr>
<td>Cotton rats</td>
<td>512</td>
</tr>
<tr>
<td>Hamsters</td>
<td>256</td>
</tr>
</tbody>
</table>

* Toxic LD₅₀ for mice 1:640/ml.
† 2.5 mg 24 and 48 hours prior to infection and 2.5 mg daily for 10 days.
‡ 2.5 mg 24 and 48 hours prior to infection and 1.25 mg daily for 10 days.
immunized with whole rickettsial antigen; group B (3 and 4) cortisone treated, whole antigen; group C (5 and 6) saline treated, sonic antigen; group D (7 and 8) cortisone treated, sonic antigen. The treatment was started 3 days prior to immunization and continued until 6 days after immunization, a total of 23 days of treatment. Saline was given intramuscularly in 0.2 ml amounts, cortisone in 5 mg amounts. The total cortisone treatment over the 23 days was 115 mg per animal.

The rabbits were bled on the 10th day after immunization was begun, the animals having received 5 injections of antigen. One of the rabbits (5) died as a result of trauma. On the 21st day, 8 days after the last injection of vaccine and 2 days after the last cortisone treatment, the remaining animals were bled for the second time.

To test the effect of cortisone on already formed antibody, the animals were bled on the 31st day after immunization was begun and 25 mg cortisone was given to animals numbers 6, 7, and 8. These 3 animals were bled again after 4 hr. To test the effect of cortisone on an anamnestic response these same rabbits were continued on a daily dose of 5 mg cortisone and rabbits number 1 and 2 received saline for 5 days. The animals were given a desensitizing injection intraperitoneally of 1 ml of antigen and 2 hr later received 2 ml intravenously. The final bleeding was 1 week after the final injection of antigen, the 40th day after the initiation of immunization.

The sera from these 5 bleedings were titrated for complement fixing antibody and the titers are presented in table 3. No consistent effect of cortisone is evident with either the sonic or whole rickettsial antigen. With the whole rickettsial antigen and cortisone treatment the animals showed a tendency to develop lower titers than the animals receiving whole rickettsiae and saline. These cortisone treated rabbits died prior to the 31st day. Therefore, it is not known whether the titers would have increased and were merely delayed in developing. No significant effect was noted on the complement fixation titer of those animals bled 4 hr after cortisone treatment on the 31st day. No anamnestic response occurred after the injection of antigen on the 33rd day and the second course of cortisone treatment. The same titers were obtained on the 40th day in all animals regardless of the antigen or treatment used.

Experiment III. Studies in the mouse of the effects of cortisone on the development of immunity and on the resistance to challenge with murine typhus. The complement fixation test on the mice in experiment I was not satisfactory as seen in table 1. To clarify the effects that cortisone may have on immunization and the immune state in the mouse the following experiment was undertaken.

Female mice were immunized with modified Plotz rickettsial antigen, each mouse receiving 2 injections of 0.1 ml intraperitoneally 2 days apart. To test the effect of cortisone on immunization, 30 mice were treated with 1.25 mg cortisone subcutaneously 1 day prior to the first dose of antigen and for 3 successive days during immunization, making a total of 5.0 mg cortisone. This amount did not exceed the optimum dosage established for increasing the susceptibility of

### TABLE 3

**Complement fixation titers of rabbit sera at intervals during immunization with whole and sonic treated rickettsial antigens and the effect of cortisone**

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Antigen</th>
<th>Treatment</th>
<th>Days* Animals Bled</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Complement Fixation Titers</td>
</tr>
<tr>
<td>1</td>
<td>Whole</td>
<td>Saline</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Whole</td>
<td>Saline</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Whole</td>
<td>Cortisone</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>Whole</td>
<td>Cortisone</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Sonic</td>
<td>Saline</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>Sonic</td>
<td>Saline</td>
<td>2</td>
</tr>
<tr>
<td>7</td>
<td>Sonic</td>
<td>Cortisone</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>Sonic</td>
<td>Cortisone</td>
<td>2</td>
</tr>
</tbody>
</table>

* The days were numbered from the initial immunizing injection.
mice to typhus infection (Whitmire and Downs, 1954). Thirty control mice were treated with saline for the same period during immunization.

To test the effect of cortisone on the immune state, 72 immunized mice were divided into 6 groups on the 17th day after the last injection of antigen and treated as follows: group A received no treatment; group B, 5 mg cortisone 4 hr before challenge; group C, 2.5 mg cortisone 24 hr before challenge; group D, 2 injections of 2.5 mg cortisone 24 and 48 hr prior to challenge. The control groups E and F received 0.2 ml saline; group E, 4 hr before challenge and group F, 24 hr before challenge.

To eliminate ill effects of handling and treatment, normal mice were treated with cortisone and saline in the same manner as the test animals and challenged with toxic material.

The mice were challenged on the 19th day after the last antigen injection by intravenous injection of three different dilutions of toxic material (1:30, 1:60, and 1:90) which equaled 4.5, 2.25, and 1.5 mouse toxic LD$_{50}$. To determine any drop in toxic strength of the challenge material, a toxicity test was run before and after the test challenge. At the beginning, the infected yolk sac material gave a toxic LD$_{50}$ of 1:100, and after 4 hr storage in an ice water bath during the test it had dropped to 1:103. To arrive at the challenge doses given above an average of these 2 toxic challenge titrations was found to be 1:135. A part of the variation was controlled in the test by inoculation of the challenge dilution in the control animals just prior to the test animals.

All nonimmune mice died from the challenge dose with no difference in effect noted between the untreated, saline, and cortisone treated animals, regardless of the time of treatment or the challenge dosage. No immunized mice died when challenged with the 3 challenge doses in the untreated, saline, or cortisone treated groups. It would seem that the effects of cortisone on susceptibility of the mouse to typhus infection is not related to the immune reaction but must be due to other factors.

**DISCUSSION**

The results presented here show no significant effects of cortisone on the production of immunity or on the immune state in the rabbit, guinea pig or mouse. Whitney and Anigstein (1953) reported a limited suppression of antibody formation by cortisone treatment in the rabbit immunized with epidemic typhus. They reported the average titers for sera drawn from rabbits on the 14th day after the first vaccine dose were 1:93 for the nontreated animals and 1:50 for the cortisone treated animals. This apparent suppressive action of cortisone on the antibody formation became negligible during the third week when the average titers for the nontreated and treated rabbits were 1:73 and 1:70 respectively. In the results presented in table 1, the average titers in all instances show lower titers in the cortisone treated animals at 13 days. However, on the 21st day 3 of the groups (1:250, 1:1000, and 1:64,000) show higher complement fixing titers than the nontreated animals. The 1:4000 group showed approximately the same titer in both groups while the 1:16,000 group showed a lower titer in the cortisone treated group. The cortisone dosage was such that after withdrawal, a number of animals which had been treated with cortisone died, thus the result at 40 days is not complete. These deaths were not believed to be due to an enhanced typhus infection since the scrotal reaction and fever had subsided early in infection.

In an attempt to repeat the work of Hanan and co-workers (1953, 1954) which showed that cortisone interfered with the antibody production against small antigens more than that with large antigens, sonic treated rickettsiae were used. As seen in table 3 no differences were noted in the complement fixation titers in relation to either the sonic or whole antigen in either the saline or cortisone treated rabbits. The factor responsible for the different effects noted by Hanan with the sheep red cell stroma and bovine serum albumin does not carry over for the use of two antigens from the same source but of different particle size as the rickettsial antigens used in this experiment.

The inability of cortisone to interfere with the development of immunity to challenge or to the immune state once it has been established was shown in experiment III. Cortisone has been shown to have no effect on the toxic reaction produced by large doses of typhus rickettsiae in the mouse (Kass et al., 1951; Jackson and Smadel, 1950; and Whitmire and Downs, 1954); thus the action of cortisone on immunity in the mouse can be tested by the use of toxic challenge doses of rickettsiae without alteration of sus-
ceptibility of the animal as would be the case in an infective type challenge. It was hoped that a difference in the number of challenge mice dying from the three dilutions of toxic material would show either an increase in immunity or a break-
down in immunity by cortisone treatment. Un-
fortunately, the immunity of the mice was at too high a level to show deaths with any of the chal-
lenge doses used. Chase et al. (1946) found that
adrenal cortical extracts produced a marked
increase in antibody titers within 6 to 12 hr after
injection in the rabbit, with a return to approxi-
mately the initial value in 24 hr after the single
injection of hormone. No reaction of this type
could be demonstrated in mice.

It would appear from the results presented for
the mouse, cotton rat, and guinea pig that the
action of cortisone in altering the course of typhus
infection is not due to the interference with the
immune response. Vaughan et al. (1950) demon-
strated that ACTH treatment of rheumatoid
patients caused a fall in complement titer during
treatment with a rebound to pretreatment levels
upon conclusion of therapy. Stavitsky (1952)
investigated the effects of adrenal cortical
extracts on the union of antigen and antibody in
the circulating blood of the rabbit and concluded
that the effects of these hormones on allergic
reactions are not due to interference with the
in vivo union of antigen and antibody. The effects
on the serum complement level were erratic. It
would appear that the effects of the cortical hor-
mones in increasing susceptibility are not related
to the immune response in typhus and the effects
of cortisone on the antigen-antibody combination
in vivo probably are not of primary importance
in typhus infection.

SUMMARY

A study of the effects of cortisone on immunity
and the immune response in the mouse, cotton
rat, hamster, guinea pig, and rabbit has been
undertaken. Cortisone was found to have no effect
on the development of immunity or on the resist-
ance to challenge with murine typhus in the
mouse. Cortisone had no effects on the immuniza-
tion of rabbits with sonic treated or whole
rickettsial antigens. It would appear that corti-
sone caused a slower development of complement
fixing antibodies in the rabbit, although, by the
21st day in most instances a higher level of com-
plement fixing antibody was present in the corti-
sone treated animals. Insufficient data was pre-
sented for the cotton rat and hamster to draw any
conclusions concerning the effects of immunity
on the development of the complement fixing
titers.

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