THE EFFECT OF CORTICOTROPIN (ACTH) AND STREPTOMYCIN ON EXPERIMENTALLY INDUCED PULMONARY TUBERCULOSIS IN RABBITS

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The clinical evidence for the harmful effect of cortisone on patients infected with tubercle bacilli (American Trudeau Society, 1952; Fred et al., 1951; Traut and Ellman, 1952) has been supported by a series of laboratory studies on mice (Hart and Ress, 1950), rats (Cummings et al., 1952; Michael et al., 1950), guinea pigs (Spain and Molomut, 1950), and rabbits (Lurie et al., 1951). In the preceding study, it was shown that streptomycin could compensate for the deleterious effect of small but not large doses of cortisone.

In this study corticotropin (ACTH) was administered to both primarily infected rabbits and rabbits previously sensitized. In contrast to the results with cortisone, corticotropin produced no deleterious effects on the animals when doses of 4 times the usual human therapeutic doses were employed. However, there was evidence of damage to the animals' resistance when doses 8 times the usual human dose were employed. Streptomycin prevented the harmful effect of the large doses of corticotropin.

Since one of the purposes of this work was to compare the effects of corticotropin and cortisone on the tuberculous process, it was necessary to find some way of comparing the dosages of these two hormones to determine if they were physiologically of the same magnitude. Use was made of the lymphocyte depressing ability of corticotropin and cortisone (Dougherty and White, 1944, 1945) to accomplish this.

MATERIALS AND METHODS

Seventy-five white rabbits of mixed sexes weighing approximately two kg were used in this experiment. All rabbits were etherized, their tracheae were exposed, and 0.25 ml of homogenate containing 5 mg of Mycobacterium tuberculosis var bovis, Ravenel strain, was injected into the tracheae. The rabbits were held in such a manner that the homogenate drained into the right upper lobe of the lung. They were divided into 10 groups and used in the following experiments.

Experiment A. These rabbits were normal, unsensitized rabbits at the beginning of the experiment.

Group I: Four rabbits were used as a control group and were not treated in any way. One died at 13 days and one at 16 days. Another was killed at one month, and the last one was killed at 3 months, the termination of the experiment.

Group II: Five rabbits were treated with 60 mg streptomycin sulfate in sterile saline injected intramuscularly once a day. At 30 days this dose was cut in half and continued until the end of the experiment. One rabbit was killed at one month and the remainder after 3 months.

Group III: Seven rabbits were treated with 2.5 units porcine corticotropin (ACTH, Armour) intramuscularly every 6 hours for a total of 10 units per day. At the end of 10 days this dose was reduced to 1.25 units every 6 hours, at the end of 20 days to 1.25 units every 12 hours, and at the end of 30 days it was discontinued. One rabbit was killed at one month, one died a week before the end of the experiment, and the remainder were killed after 3 months.

Group IV: Seven rabbits were treated with corticotropin and streptomycin using exactly the same procedure for dose reduction of corticotropin as in group III. Streptomycin was administered as in group II. One rabbit was killed at one month and the remainder at 3 months.

Experiment B. These rabbits were sensitized and partially immunized by the subcutaneous injection of 0.5 ml of a homogenate of a living relatively avirulent Saranac R 1 V strain of M. tuberculosis. Sensitivity was allowed to develop for 4 to 6 weeks.

Group I: Eleven rabbits were used as a control.
group and were not treated in any way. One rabbit was killed at 4 weeks, one at 7 weeks, 3 at 11 weeks, and the remainder that did not die spontaneously were killed at 20 weeks.

**Group II:** Fourteen rabbits were treated with 60 mg streptomycin sulfate in sterile saline administered intramuscularly once a day. At 30 days this dose was cut in half and continued until the end of the experiment. One rabbit was killed at 4 weeks, one at 7 weeks, 7 at 11 weeks, and the remainder at 20 weeks.

**Group III:** Six rabbits were treated with 2.5 units porcine corticotropin (ACTH, Armour) intramuscularly every 6 hours for a total of 10 units per day. At the end of 10 days this dose was reduced to 1.25 units every 6 hours, at the end of 20 days to 1.25 units every 12 hours, and at the end of 30 days it was discontinued. One rabbit was killed at 31 days, and the one remaining alive at the end of the experiment was killed at 20 weeks.

**Group IV:** Seven rabbits were treated with corticotropin and streptomycin using the same procedure for dose reduction of corticotropin as in group III which began with 2.5 units every 6 hours. Streptomycin was administered as in group II. One rabbit was killed at 4 weeks, and those remaining alive were killed at 20 weeks.

**Group V:** Six rabbits were treated with 5 units of corticotropin every 6 hours administered as in group III. At the end of 10 days the dose was reduced to 2.5 units every 6 hours, at the end of 20 days to 2.5 units every 12 hours, and at the end of 30 days it was discontinued. One rabbit was killed at 7 weeks and the remainder at 11 weeks.

**Group VI:** Seven rabbits were treated with corticotropin and streptomycin using the same procedure for dose reduction of corticotropin as in group V which began with 5 units. Streptomycin was administered as in group II. One rabbit was killed at 7 weeks, and the remainder were killed at 11 weeks.

The methods of killing the animals and studying the disease produced in them were exactly the same as in the preceding study with the cortisone treated animals. All the lungs were studied grossly and microscopically and were evaluated by the same numerical grading system as described before.

**Experiment C.** To determine the smallest amounts of corticotropin that have any depressive effect on the circulating lymphocytes, normal rabbits were given single injections of corticotropin in dosages ranging from 5.0 to 0.15 units, and the smallest amount showing physiological activity according to this criterion was found. The white counts and differentials were done as described in the preceding study.

**RESULTS**

**Experiment A—Group I:** There were 4 animals in the control, unsensitized group, two of which died spontaneously. All 4 had extensive disease involving the hilar and tracheal lymph nodes. Cavitation and extension to almost all lobes of the lung were the rule. Microscopically, the disease was of a spreading type with areas of pneumonia surrounding the active process. There were moderate fibrosis and poor fibroblastic proliferation. A few tubercle bacilli were seen in every rabbit.

**Group II:** There were 5 rabbits in the streptomycin group, none of which died spontaneously. Two of them had cavities with pus and adhesions; the rest had moderate disease. In general the disease was much less extensive than in the control group. Microscopically, the disease was of a spreading type with poor delimitation. A few tubercle bacilli were seen in every rabbit.

**Group III:** There were 7 rabbits in the corticotropin group, one of which died spontaneously. Pleural adhesions, large cavities, extension to all lobes, involvement of hilar and tracheal nodes, and enlargement of the right lobes to 2 to 3 times normal size characterized the disease in these animals. Microscopically, there was extensive cavitation, which was defined rather well by epithelioid and fibroblastic proliferation, that was better than that seen in any other group. Many tubercle bacilli were seen.

**Group IV:** There were 7 rabbits in the corticotropin-streptomycin group, none of which died spontaneously. The disease was much less than that of the controls and was about equal to that in the rabbits receiving streptomycin alone. There were few adhesions, little extension throughout the lung, few cavities, and no enlargement of lobes. The epithelioid and fibroblastic proliferation was good with discrete, fairly well defined nodules being present. Few tubercle bacilli were seen.

**Experiment B—Group I:** There were 11 control animals of which 3 died spontaneously before termination of the experiment at 140 days. Two of the latter had secondary infection. There was no gross enlargement of the lungs except in one
rabbit. All rabbits except one had extensive disease in the right upper lobe (figure 1A). In only two rabbits was there marked spread to the left lung. Epithelioid proliferation was rather marked. Tubercle bacilli were difficult to find, and when they occurred, they were chiefly in the centers of necrotic areas.

**Group II:** There were 14 animals in the streptomycin group of which one died spontaneously. There was no secondary infection, extension to the left lung, or enlargement. The severity of disease in this group was much less than in the control group, 6 of them having only very few scattered, discrete tubercles 1 mm or less in diameter. Tubercle bacilli were seen in the tissue sections of only one rabbit, but from another one, streptomycin resistant tubercle bacilli were cultured.

**Group III:** There were 6 animals in the group that received 10 units of corticotropin and 4 died spontaneously. Two of the latter had secondary infection. With the exception of one, the animals in this group had no worse disease than those in the control group. Rather, in our opinion, they appeared to have slightly less disease than the controls (figure 1B). Tissue reaction was approximately the same as in the controls except that the lesions were slightly more circumscribed and were limited to the peribronchial regions. Tubercle bacilli were difficult to find.

**Group IV:** There were 7 animals in this group that received streptomycin and 10 units of corticotropin; only one died spontaneously with secondary infection. In all except one animal there was much less disease than in the control group and in those receiving corticotropin alone. In no case was there enlargement or extension of disease to the left lung. Most of the lung tissue was normal, but in the areas of disease there was well outlined epithelioid reaction with little or no necrosis. No tubercle bacilli were seen in the tissue sections.

**Group V:** There were 6 rabbits in this group that received corticotropin and none died spontaneously. With the exception of one animal, the disease was in every case slightly to considerably worse than in the control animals (figure 1C). In 3 of the animals, there was marked extension to the left lung. In one of them the right lung was enlarged moderately, and in another it was enlarged to twice the normal size. There was extensive cavitation in 3 of the lungs. In all of the animals, the necrosis was more extensive than in the controls, yet it was sharply demarcated. Many epithelioid cells were present, but few giant cells. Tubercle bacilli were found in all.

**Group VI:** Seven animals comprised this group that received streptomycin and 20 units of corticotropin. There were no secondary infections. The disease in this group was equal to those animals receiving streptomycin and corticotropin in smaller doses and much less than in the control group or where corticotropin alone was employed (figure 1D). It was only slightly worse than the group receiving streptomycin alone. In only one animal was there any extension of disease to the left lung. The histological picture was one of a shotgun-like distribution of a moderate number of tubercles with some necrosis not well delimited. In only one section were tubercle bacilli seen.

**Experiment C.** The small lymphocytes in the circulating blood of normal rabbits were depressed 1/4 or more from control values by doses of corticotropin greater than 0.31 units. The smallest dose used that had any appreciable effect on the lymphocytes was 0.31 units (table 1).

**DISCUSSION**

It has been recognized that animals that are sensitive to tuberculin react to new infection in a
different manner from those that are not sensitive (Rich, 1951). In previous studies on the effect of cortisone and corticotropin on experimental tuberculosis, however, this fact has been largely ignored, and the full importance of differences in reaction of sensitive and nonsensitive animals to new infection has not been appreciated. This and the preceding study indicate that these differences are marked and that they should not be forgotten when results are examined with human disease in mind—a disease that seldom occurs except in sensitized bodies.

It was found generally that all groups of nonsensitive animals had a more rapid and extensive progression of the disease than corresponding groups of sensitive animals. According to the numerical index devised to estimate severity of disease, the severity in the various groups of nonsensitive animals was at least twice that found in the sensitive groups (figure 2). This generality was observed throughout all the groups in the experiment with no difference being made whether the animals were controls or whether they were receiving streptomycin, corticotropin, or a combination of both. This was found to be true also in the preceding study with cortisone.

The nonsensitized rabbits showed a much greater tendency than sensitized ones to cavity formation and extension to many lobes of the lung. Spread to the paratracheal nodes was much more common. Microscopic examination of the lesions of the nonsensitive animals revealed distinctly less fibroblastic proliferation surrounding areas of active disease, and the disease process, especially in the untreated controls, appeared to be more spreading in type than in the sensitized animals. Further, all of the unsensitized animals showed markedly increased numbers of tubercle bacilli in comparison with the sensitized rabbits. From these facts, it is evident that the sensitized state previous to tuberculous infection is an advantageous one, and with its presence the animal possesses a markedly increased resistance to tuberculosis.

In the following discussion of how streptomycin and corticotropin affect the tuberculous process, the same remarks in general will apply both to sensitized and nonsensitized animals, the only important difference being that the nonsensitized animals have an all-round increased severity. The degrees of severity of disease in the control, streptomycin, corticotropin, and streptomycin-corticotropin groups hold the same relative position to one another in both sensitivity states. This can be seen from figure 2.

As many investigators have shown already, the effect of streptomycin is a beneficial one. In the sensitized streptomycin animals, nearly half of them had healed their disease and exhibited essentially normal lungs while the others were in a stage of healing with scar formation and little necrosis. In the nonsensitized rabbits, streptomycin did not produce as good results as were seen in the sensitive rabbits, and the disease approximately equalled in severity that seen in

**Table 1**

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<th>Corticotropin Doses</th>
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<th>% Showing Depression</th>
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**Figure 2.** Degree of tuberculous disease in sensitized and nonsensitized rabbits treated with corticotropin (ACTH).

0—No disease; 1—few tubercles less than 1 mm; 2—moderate number tubercles less than 1 mm; 3—necrosis, few tubercles less than 2 mm; 4—necrosis, many tubercles less than 2 mm; 5—areas of necrosis, confluent disease; 6—much of lobe occupied with necrotic disease; 7—lobe filled with disease. Ratings for the individual 5 lobes were added together for the total rating of each rabbit.
the sensitized untreated controls—that is, moderately severe disease was present.

The animals of both sensitivity states that received an initial dose of 2.5 units of corticotropin every 6 hours developed disease that was approximately equal to that of the control animals. This indicates that corticotropin in this dose is not detrimental to the resistance of rabbits to tuberculosis.

In the group of sensitized animals treated with 5 units of corticotropin every 6 hours, the disease was slightly to considerably worse than that in the control group, there being marked extension, cavitation, and necrosis with tubercle bacilli present in moderately heavy numbers. We believe that this dose of corticotropin produces disease approximately equivalent to that seen in tuberculous rabbits that are treated with two mg of cortisone a day and that this large dose of corticotropin is harmful to the tuberculous host in that it invokes a cortisone effect.

In the sensitive and nonsensitive animals treated with streptomycin and corticotropin in an initial dose of 2.5 units every 6 hours, the disease progressed in a fashion almost identical to those animals treated with streptomycin alone. This shows that corticotropin at this dose level does not interfere with the therapeutic effects of streptomycin (Bacon and Smith, 1953).

The higher dose level of corticotropin (5.0 units every 6 hours) in sensitized animals also did not prevent the therapeutic action of streptomycin, even though this amount of corticotropin when used alone produced disease worse than in the controls. In these animals the disease was approximately equal and similar in type to that seen in the animals receiving streptomycin alone and streptomycin-corticotropin in the smaller dose.

On the basis of the evidence which we have set forth in this and the preceding study, it seems that the effects of corticotropin and cortisone used to treat tuberculous rabbits are essentially different. Corticotropin in small doses (10 units) does not inhibit the host’s ability to combat the disease while with large doses (20 units) the disease is definitely more extensive. Neither the small nor large doses of corticotropin have any effect on the ability of streptomycin to ameliorate the disease process.

On the other hand, cortisone in both large (20 mg) and small (2 mg) doses seems to affect the host’s reaction so that the disease becomes greater, and while streptomycin is able to neutralize the effect of small amounts of cortisone to produce almost complete healing, the larger dose of cortisone allows extensive disease equal to that in the controls to develop even when streptomycin is given at the same time.

We feel that these observations warrant classification of the therapeutic plans into groups as follows. To avoid confusion, only the sensitized animals are presented in this listing.

1) Disease equal to the control group in severity: a. cortisone in large dose (20 mg) with streptomycin (60 mg); b. corticotropin in small dose (10 units).

2) Disease markedly worse than the control group: a. cortisone in large dose (20 mg); b. cortisone in small dose (2 mg); c. corticotropin in large dose (20 units).

3) Disease at or approaching healed state: a. Streptomycin; b. corticotropin in large dose (20 units) with streptomycin; c. corticotropin in small dose (10 units) with streptomycin; d. cortisone in small dose (2 mg) with streptomycin.

Therefore, we believe that cortisone in tuberculous animals definitely has a more deleterious effect on the course of the disease than does corticotropin. This difference in effect may be due to a more physiological action of corticotropin in stimulating secretion from the adrenal cortex of hormones other than cortisone which have an incompletely understood action on the response of the body to infection.

Two questions now remain to be settled. How do these doses of the two hormones compare with human doses, and how do they compare in units of physiological activity with one another? The latter question must certainly be settled in order to rule out the possibility that relatively larger physiological doses of cortisone were given and that this is the cause of the differences in results of the two hormones.

In those rabbits receiving 1 mg per kilo of cortisone, the dose was somewhat smaller than the usual human doses of 100 to 200 mg. In those rabbits receiving 10 mg per kilo of cortisone, the dose was 3 to 6 times in excess of human doses. Corticotropin doses, on the other hand, were at all times far in excess of human therapeutic levels being 4 to 20 times the 40 to 100 units human dose. It is significant in view of these facts to note that cortisone exerted its deleterious effect both at and above the human levels, whereas corticotropin could be administered in
excess of human dose levels without harmful effect and was deleterious only at levels 20 times the human dose. It is also interesting to note that doses of cortisone at human levels were not sufficient to depress the reaction to tuberculin in the lungs of rabbits although the corticotropin dose of 5 units per kilo was effective in reducing the tuberculin reaction in the lungs by 50 to 90 per cent (Reimnuth and Smith, 1951).

To answer the second question, use was made of the depressive effect of corticotropin and cortisone on the numbers of circulating lymphocytes. If the smallest amounts of these hormones that have any measurable effect on lymphocytes are assumed to represent approximately equal physiological units, it is seen by simple mathematical calculations that corticotropin in the smaller dose (10 units) is approximately 4 times more active physiologically according to the lymphocyte criterion than the small dose of cortisone (2 mg). In the larger doses of 20 units of corticotropin and 20 mg of cortisone, the two hormones are approximately equal in physiological activity. These are by no means complete accurate determinations, but they are accurate enough to show definitely that relatively more corticotropin was given the animals than cortisone according to the above criterion. In spite of this, the results obtained in the rabbits treated with cortisone were consistently much worse than those in rabbits treated with corticotropin. This indicates that the differences observed cannot be due to a relatively greater dose of cortisone but must be due to an actual difference in the actions of the hormones.

It is worth noting that all doses of corticotropin and cortisone that were used in the experiments were markedly higher than the smallest amounts that had a measurable effect on the lymphocytes, the corticotropin doses being around 33 to 66 times this smallest amount and the cortisone doses 8 to 80 times.

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SUMMARY

Seventy-five white rabbits were used in this experiment. Fifty-two were sensitized and partially immunized by the subcutaneous injection of the relatively avirulent R

\textit{v} strain of \textit{Mycobacterium tuberculosis}, and the others were not sensitized in any way. All of the rabbits were injected later intratracheally with 5 mg of the Ravenel strain of bovine tubercle bacillus. They were treated with streptomycin, corticotropin, and a combination of the two.

In general all groups of nonsensitized rabbits including controls showed much more extensive disease than the corresponding groups of sensitized animals.

Corticotropin in a 10 unit per day dose did not exert any damaging influence on the course of untreated tuberculosis, and it did not interfere with the beneficial healing effect of streptomycin. In a 20 unit per day dose, it caused disease slightly to moderately worse than that found in controls. However, this dose did not interfere with the healing effect of streptomycin.

These results are contrasted with those in the preceding study in which it was seen that cortisone in both large (20 mg per day) and small (2 mg per day) doses affected the host's reaction so that the disease became worse than that of controls.

In order to see that the dosage level of the two hormones was not causing this difference, use of the lymphocyte depressing ability of the hormones was made to determine the relative physiological amounts of each that were being administered to the rabbits. It was found that relatively more corticotropin than cortisone was being given, so dosage cannot account for the poor results obtained with cortisone.

Cortisone exerted its deleterious effect both at and above human dosage levels, whereas corticotropin could be administered in excess of human dose levels without harmful effect and was deleterious only at levels 20 times the human dose.

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