THE EFFECT OF CORTISONE AND STREPTOMYCIN ON EXPERIMENTALLY INDUCED PULMONARY TUBERCULOSIS IN RABBITS

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Clinical experience suggests that the administration of cortisone may reactivate an apparently healed tuberculous infection in man (King et al., 1951; American Trudeau Society, 1952a). Laboratory studies with mice (Hart and Ress, 1950), rats (Michael et al., 1950; Cummings et al., 1952), guinea pigs (Spain and Molomut, 1950), and rabbits (Lurie et al., 1951) infected with tubercle bacilli have shown that the administration of cortisone causes a more extensive disease than that exhibited by the controls (American Trudeau Society, 1952b).

The clinical evidence for the reactivation of tuberculosis by the administration of corticotropin (ACTH) is suggestive (Tompsett et al., 1950; LeMaistre et al., 1950; Muschenheim, 1951) but not as conclusive as for cortisone. A few laboratory studies with corticotropin (ACTH) therapy in guinea pigs (LeMaistre and Tompsett, 1952a) and rats (LeMaistre and Tompsett, 1952b) experimentally infected with tubercle bacilli have failed to show any evidence that the hormone influenced in an unfavorable manner the progress of the disease.

With the exception of one study from this laboratory (Bacon and Smith, 1953), all of the studies with cortisone or corticotropin (ACTH) have been carried out in primarily infected, and therefore unsensitized, animals; hence, the results may not apply to patients who are already infected and sensitized. The doses of these hormones employed by the various investigators have varied considerably and were in many instances proportionately much larger than those used in clinical therapy. For these reasons it seemed advisable to restudy the problem using both previously normal and previously sensitized rabbits and to administer doses of the hormones comparable to those used for man. For comparative purposes larger doses were employed in control experiments. Therapeutic doses of streptomycin were administered to a part of each group of animals to evaluate its effect with the small and large dose of each hormone (Spain and Molomut, 1953). The same size inoculum of a virulent bovine tubercle bacillus was employed in all the animals, and the experiments with cortisone and corticotropin (ACTH) were carried out simultaneously.

In order to determine the physiological activity of the dose levels of cortisone employed in this experiment and to compare these doses with similar therapy using corticotropin (ACTH), the depression of circulating lymphocytes has been used as an index of the activity of cortisone (Harris and Harris, 1950; Dougherty and White, 1944).

For convenience in presentation the results of the studies with cortisone will be presented in this report and those with corticotropin (ACTH) in the following report.

MATERIALS AND METHODS

Seventy-four white rabbits of mixed sexes weighing approximately two kg were employed in this study. 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 trachea. 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 animals were normal rabbits unsensitized at the beginning of the experiment.

Group I: Four rabbits were used as 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 at the end of the experiment.

Group III: Six rabbits were treated with two mg "cortone" acetate Merck (cortisone) administered intramuscularly once a day. At the end of 10 days this dose was reduced to one mg, at the end of 20 days to 0.5 mg, and at the end of 30 days it was discontinued. One rabbit was killed at one month and one at 3 months; the remainder died spontaneously.

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

Experiment B. These rabbits were sensitized and partially immunized by the subcutaneous injection of 0.5 ml of a homogenate of living avirulent Saranac R. 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. One rabbit was killed at 4 weeks, one at 7 weeks, 7 at 11 weeks, and the remainder at 20 weeks.

Group III: Seven rabbits were treated with two mg "cortone" acetate Merck (cortisone) following the plan for therapy and dose reduction given above (A, group III). All rabbits remaining alive at 20 weeks were killed.

Group IV: Seven rabbits were treated with cortisone and streptomycin using exactly the same procedure for dose reduction of cortisone as in group III. Streptomycin was administered as in group II. One rabbit was killed at 4 weeks and the remainder at 20 weeks.

Group V: Six rabbits were treated with 20 mg cortisone administered as above. At the end of 10 and 20 days the dose was reduced to 10 mg and 5 mg, respectively. At the end of 30 days it was discontinued. One rabbit was killed at 7 weeks; all others died spontaneously.

Group VI: Seven rabbits were treated with cortisone and streptomycin using the same procedure for dose reduction of cortisone as in group V. Streptomycin was administered as in group II. One rabbit was killed at 7 weeks, and those rabbits remaining alive were killed at 11 weeks.

The rabbits which were sacrificed were anesthetized with sodium pentothal and exsanguinated. The lungs of all rabbits were dissected free, fixed with Helly's solution, sectioned to show representative areas, and stained with hematoxylin and eosin and by Ziehl-Neelsen's method for microscopic study. Representative lungs of each group were photographed in color and in black and white before processing. Cultures were made from all lungs for secondary infection and tubercle bacilli. From those rabbits receiving streptomycin, cultures for streptomycin-resistant tubercle bacilli were made on media containing varying concentrations of streptomycin (Steenken, 1949).

Comparisons as to the severity of disease among the various animals in the experiment were made on the basis of the following criteria: mode of death, presence or absence of secondary infection, gross appearance, microscopic appearance, and bacteriological studies. An attempt was made to rate the extension and severity of the disease in each lobe of the lungs by assigning a numerical scale (figure 2). By rating each lobe according to the scale it was possible to arrive at a numerical classification of each rabbit's disease and in turn to compare one group with another on a numerical basis. This rating system was applied to all animals studied in the present series of experiments including those receiving corticotropin (ACTH).

Experiment C. To determine the smallest amount of cortisone that would have any effect on the circulating lymphocytes, normal rabbits were given single injections of cortisone in dosages ranging from 1.0 to 0.125 mg. The rabbits were bled from an ear vein, and total white counts and differentials were done just before each injection and at 1 and 3 hours following injections.

The ability of corticotropin to prevent the tuberculin reaction in the rabbit lung was seen earlier in this laboratory (Reimnuth and Smith, 1951). As a comparison this experiment was repeated with cortisone using 21 sensitized rabbits. These rabbits were injected intra-
Results

Experiment A—Group I: There were 4 animals in the control unsensitized group, two of which died spontaneously. All 4 of them 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 sections from 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 6 animals in the cortisone group, 3 of which died spontaneously. In general the disease was about equal to that found in the unsensitized control group. Cavitation, many adhesions, and enlargement of the right lobes to 2 to 3 times normal size were characteristic. A pneumonitis was present in 4 of the 5 rabbits with cocci being seen inside macrophages in 3 of the 5. Epithelioid and fibroblastic proliferation was poor. Many tubercle bacilli were seen.

Group IV: There were 7 rabbits in the cortisone-streptomycin group, one of which died spontaneously. The disease was about equivalent to that in the group of nonsensitive animals receiving streptomycin alone. There were no adhesions, cavitation, or marked enlargement of lobes. The lesions were of a nodular type with spreading edges that were poorly delimited. There was fair fibroblastic and epithelioid proliferation. Moderate numbers of tubercle bacilli were seen.

Experiment B—Group I: There were 11 sensitized control animals of which 3 died spontaneously before the termination of the experiment at 140 days. Two of the latter had secondary infection. There was a gross enlargement of the lungs in one rabbit only. The margins of the individual tubercles were well delimited. In only two rabbits was there extensive spread to the left lung. The reaction was typically one of epithelioid proliferation, moderate numbers of lymphocytes and giant cells, and few polymorphonuclear leucocytes. Tubercle bacilli were difficult to find, and when they occurred, they were chiefly in the centers of necrotic areas.

Group II: The 14 animals in this group were treated with streptomycin, and only one died spontaneously. There was no secondary infection, extension to the left lung, or enlargement of the lungs. The severity of disease in this group was much less than in the sensitized control group, 6 of them having only very few scattered discrete tubercles one mm or less in diameter. In the remaining ones, the disease was still much less severe than the average control. 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 7 animals in this group receiving two mg of cortisone, of which two died spontaneously with secondary infection. The remainder were killed at the termination of the experiment. All except one had disease equal to or worse than the sensitized animals of the control group. There was moderate to marked extension to the left lung in all except two. In 4 of the 7 the right lung was almost entirely filled with disease; and cavitation, marked caseation, and enlargement were present. Three of the right lungs were covered partially or completely with a fibrous capsule. Giant cells were few and epithelioid cells moderate in number; many tubercle bacilli were seen.

Group IV: The 7 animals in this group received both cortisone and streptomycin; none died spontaneously or contracted secondary infection. This group resembles closely the streptomycin group of sensitized animals in severity of disease. The typical histological picture was one of a moderate number of small, discrete tubercles with little or no necrosis.

Group V: The 6 rabbits comprising this group received 20 mg of cortisone, and 5 of them died spontaneously. Three of the deaths were accompanied by secondary infection. All of these animals had much worse disease than the
controls, and 4 of the 6 lungs were moderately to greatly enlarged. Necrosis was marked and poorly delimited; in 4 of the 6 it almost completely filled the right lung. Few giant cells, many epithelioid cells, and many tubercle bacilli were present. An intraalveolar fibrinous exudate was seen in many parts of some of the lungs.

Group VI: There were 7 animals in this group that received streptomycin and 20 mg of cortisol; one died spontaneously without secondary infection. Two of the group had very slight extension to the left lung. The disease was about equal in extent to that in the controls and less than the group receiving two mg cortisol dose without streptomycin. Tubercle bacilli ranged from none to very numerous, and from two of the animals streptomycin resistant tubercle bacilli were cultured.

Experiment C. The effect of cortisol on the numbers of circulating lymphocytes was pronounced. The small lymphocytes were taken as a criterion rather than eosinophilic leucocytes because the blood of rabbits contains "pseudo-eosinophils" which behave as to neutrophils under cortisol therapy and which are often difficult to distinguish from true eosinophils. These small lymphocytes were depressed 1/4 or more from control values by cortisol doses greater than 0.25 mg. The experimental results are tabulated in Table 1.

An inflammatory lesion was produced in the lungs of all of the 21 rabbits receiving intratracheal injections of Old Tuberculin. These lesions were not reduced significantly by any dose of cortisol up to and including doses of 5 mg per kg.

**DISCUSSION**

It should be kept in mind that this experimental work is different from most previous research in the field of experimental tuberculosis.

**TABLE 1**

*Depression of circulating lymphocytes of rabbits by cortisol*

<table>
<thead>
<tr>
<th>Cortisone Doses</th>
<th>No. Animals Tested</th>
<th>No. Animals Showing Depression</th>
<th>% Showing Depression</th>
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<tr>
<td>mg</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>1.0</td>
<td>2</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>0.5</td>
<td>6</td>
<td>4</td>
<td>67</td>
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<td>6</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>0.12</td>
<td>4</td>
<td>0</td>
<td>0</td>
</tr>
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</table>

In that most of the animals used here were sensitized and partially immunized, whereas other investigators in the past have used nonsensitized animals. We have employed also nonsensitized tuberculous animals with which we may compare our animals directly. The sensitized experimental animal certainly simulates the human with tuberculosis more closely than the nonsensitized, and we have been able to demonstrate differences in the progression of disease in the sensitized and nonsensitized animals.

The animals of both sensitivity states which were not treated in any way (controls) showed evidence of severe, progressive disease (figure 1A). However, the unsensitized animals had a higher mortality rate with increased disease extension to other lobes and to hilar and paratracheal lymph nodes, and microscopically they showed markedly increased numbers of tubercle bacilli and a paucity of the epithelioid and fibroplastic proliferation characteristically seen surrounding areas of necrosis in lungs of animals previously sensitized to the tubercle bacillus. These differences were seen with some modifications in all treatment groups.

The streptomycin animals (A, B; group II) bear out what has been shown previously by many investigators. Nearly half of the 14 in the sensitized group had almost completely resolved their disease and possessed lungs that were normal throughout most portions. The other sensitized animals had evident disease, but the lesions in the main were in a stage of healing with evidence of scarring and little necrosis. Among the unsensitized animals there was much better arrest of the disease process compared to the control unsensitized rabbits although streptomycin failed to alter the basic process or achieve the same degree of healing seen in the sensitized streptomycin animals. The results in the unsensitized streptomycin treated animals were approximately equal to those in sensitized animals which received no treatment.

Those animals that received two mg of cortisol without other therapy (A, B; group III) showed extensive disease that was much worse than in the control group (figure 1B). In both sensitized and unsensitized animals the disease was about equal in severity and extent. In both, stained sections of the lungs showed decreased epithelioid and fibroplastic proliferation; and, particularly in the unsensitized animals, there
Figure 1. A—Lungs of untreated control rabbit, killed at 75 days; B—lungs of rabbit treated with 2 mg cortisone, killed at 140 days; C—lungs of rabbit treated with streptomycin and 2 mg cortisone, killed at 140 days; D—lungs of rabbit treated with streptomycin and 20 mg cortisone. All 4 rabbits were sensitized before infection.

was a pneumonia-like lobar disease with numerous mononuclear cells containing gram positive cocci, indicating reduced resistance to secondary infection. Thus, in the sensitized rabbit, as in the unsensitized, cortisone is deleterious to the tuberculous animal, resulting in extension and spread of the disease with poor fibroblastic proliferation, delimitation, and healing by the host.

In direct contrast were the results obtained in rabbits receiving two mg of cortisone with 60 mg of streptomycin (A, B; group IV). All sensitized animals in this group showed marked healing of the tuberculous process with residual scarring and only a few scattered, well delimited tubercles remaining as evidence of disease (figure 1C). The lungs of these sensitized animals were as nearly normal as any in the entire experiment. In the unsensitized animals results were similar to those with streptomycin alone, and any deleterious effect of this small dose of cortisone appeared to have been offset by streptomycin. Healing was not as advanced as in the sensitized animals.

The infectious process in experiment B, group V, comprised of those sensitive animals that received 20 mg of cortisone without other therapy, was much enhanced and even more pronounced. Secondary infection was prevalent as in group III, and the worsening of the disease was plainly evident from the greatly enlarged and pus filled lungs. Microscopically the lesion was spreading and poorly delimited in type, resembling closely the control unsensitized animals.

In the group of animals receiving 20 mg of cortisone and 60 mg of streptomycin (B; group VI), the disease was approximately equal to that found in the control group. The character of the lesions in this sensitized group is distinctly different from that of the other groups receiving streptomycin. The large 20 mg dose of cortisone apparently decreases the epithelioid and fibroblastic proliferation which tends to contain the disease, and the lesion becomes a more spreading and fulminating type with increased numbers of tubercle bacilli as in the unsensitized animals and those that received cortisone alone. Therefore, in the rabbits that received 20 mg of cortisone and 60 mg of streptomycin, the dose of cortisone was too large to allow the streptomycin to offset the disadvantage imposed upon the rabbit by the decreased containment of the lesion (figure 1D). However, in the group of rabbits receiving two mg of cortisone in conjunction with streptomycin,
the action of the antibiotic is sufficient to overcome the disadvantages placed upon the rabbit by the two mg dose of cortisone, which alone has been shown to reduce materially the resistance of rabbits to the disease.

Study of the lymphocyte depression by cortisone in varying dose levels revealed that levels above 0.25 mg per kilo of body weight actively depressed circulating lymphocytes. Thus, all dose levels of cortisone employed here were physiologically active although we have demonstrated that doses of cortisone up to levels above the usual human dose (up to doses equal to 260 mg per day in humans) fail to depress the tuberculin reaction in the rabbit lung (Weimer et al., 1953). Cortisone doses in those rabbits receiving 1 mg per kilo were comparable to human levels (60 to 200 mg per day), but in rabbits receiving 10 mg per kilo these doses were 2 to 6 times the usual 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.

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SUMMARY

Seventy-four white rabbits were used in this study. Fifty-two of these were sensitized and partially immunized by the subcutaneous injection of the relatively avirulent R_Y strain of Mycobacterium tuberculosis, and the others were not sensitized in any way. All of the rabbits were injected intratracheally with 5 mg of the Ravenel strain of bovine tubercle bacilli.

The disease was much more extensive and destructive in the unsensitized rabbits. The unsensitized animals which received streptomycin had less disease than the other unsensitized rabbits. Marked progressive disease of a more severe nature than in the controls developed in both sensitive and nonsensitive rabbits which received two mg of cortisone; and those receiving cortisone in 20 mg doses died spontaneously with extremely extensive, fulminating disease. Both sensitized and nonsensitized animals which received streptomycin and two mg of cortisone showed healing comparable to that seen in those animals receiving streptomycin alone. Animals receiving streptomycin and 20 mg of cortisone showed a diffuse disease similar in extent and severity to that in the control animals.

The experiments support the conclusions of previous work on the effect of cortisone on experimental tuberculosis in that cortisone alone exerts an enhancing effect on the disease process. However, if a small dose of cortisone up to twice the human dose is used together with streptomycin, the deleterious effect may be so reduced that the healing process may proceed equally as well as in rabbits receiving streptomycin alone. With large doses of cortisone which exceed twice the human levels, the disease is enhanced in spite of the action of streptomycin.

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