The Influence of Cortisone on Experimental Tuberculosis of Guinea Pigs

ALFRED G. KARLSON, D.V.M., Ph.D.† and
JOSEPH H. GAINER, D.V.M., M.S.‡
Rochester, Minnesota

The marked effects of cortisone on a disease such as rheumatoid arthritis,1 in which bacterial hypersensitivity is said to play a part, suggested to us that perhaps cortisone would alter the pathogenesis of tuberculosis, a disease in which bacterial hypersensitivity occurs as a prominent feature. The following report is an account of an experiment in which it was found that the administration of cortisone to tuberculous guinea pigs was not beneficial and may even have been harmful. Furthermore, the use of this hormone appeared to inhibit the beneficial effects of a low dose of streptomycin. It was also found that tuberculin hypersensitivity was diminished by the administration of cortisone.

Methods

Forty-eight mature male guinea pigs ranging in weight from 750 to 800 gm. were each inoculated subcutaneously over the sternum with 0.1 mg. of virulent human-type tubercle bacilli (H37Rv.). On the 18th day of infection the animals were each injected intradermally on the left side of the belly wall with 0.1 ml. of 1:100 Old Tuberculin which resulted in a positive reaction in each animal when examined 48 hours later. On the 20th day of infection six animals (pretreatment controls) were killed and were found to have grossly visible lesions of tuberculosis in the liver and spleen, the site of inoculation and the axillary lymph nodes as shown in figure 1. Although only one of these animals had grossly apparent lesions in the lungs, subsequent histopathologic examination disclosed that all had small tuberculous foci in the pulmonary tissue. It is presumed that the remaining 42 animals had progressing tuberculosis comparable to that seen in the pretreatment controls.

On the 21st day of infection the remaining 42 animals were divided into five groups as shown in figure 1 consisting respectively of 10 untreated animals to serve as controls; eight animals

†Division of Experimental Medicine, Mayo Foundation, University of Minnesota, Rochester, Minnesota.
‡Research Assistant, Mayo Foundation, University of Minnesota, Rochester, Minnesota.
to be treated once daily with 6 mg. of streptomycin; eight to be treated once daily with 2 mg. of streptomycin; eight to be treated once daily with 2 mg. of cortisone* and lastly, a group of eight animals to be treated once daily with 2 mg. of streptomycin plus 2 mg. of cortisone. In addition to the infected animals, there were also six normal guinea pigs treated once daily with 2 mg. of corti-

*The cortisone was furnished through the courtesy of Dr. J. M. Carlisle, Merck & Co., Inc., Rahway, New Jersey.

FIGURE 1: Schematic presentation of the extent of tuberculous lesions seen in each animal at necropy. The rectangle and oval represent liver and spleen respectively. The dot or circle in the arrow indicates an abscess or an ulcer. Miliary and nodular lesions in the organs are represented by dots. Complete blackening of the spleen indicates diffuse tuberculous involvement. The numerals indicate the number of days of infection, and the black bar means that the animal died before the end of the experiment. The amount of drug shown was given to each animal once daily starting on the 21st day of infection. Treatment was continued for 62 days.
sone and six normal guinea pigs treated once daily with 2 mg. of cortisone plus 2 mg. of streptomycin. These animals were included for weight controls. The materials were injected into the axillary space in 1 ml. of solution. Treatment started on the 21st day of infection and continued for 62 days, at which time the surviving animals were killed.

The doses of streptomycin were based on experience which showed that the administration of 2 mg. of streptomycin daily for approximately eight weeks would result in only a partial but measurable therapeutic effect in tuberculous guinea pigs, whereas the administration of 6 mg. daily causes almost complete regression and healing of the disease. As previously pointed out, when one is studying the effects of other agents used in combination with streptomycin, it is necessary that streptomycin be used in sub-effective doses in order to enable one to detect any additive effect.2 The daily dose of cortisone, 2 mg. per animal, was arbitrarily selected on the basis of the total amount available for the experiment. During the treatment period all the animals, including the controls, were weighed once weekly. Tuberculin tests were done using 0.1 ml. of 1:100 Old Tuberculin on the 15th day of treatment, 0.1 ml. of 1:1,000 Old Tuberculin on the 35th day of treatment and a third test at the end of the treatment period using 0.1 ml. of 1:10,000 Old Tuberculin. The tuberculin was injected intradermally on the left belly wall 48 hours prior to recording the results. The lower concentrations of tuberculin were used in the last two tests because of the work of Sarber3 who found that the inhibitory effects of benadryl on tuberculin reactions in guinea pigs were more pronounced using 1:1,000 Old Tuberculin than when a dilution of 1:100 was used. It was felt that if cortisone had any inhibiting effect on the tuberculin reaction it would possibly be more easily detected using low concentrations of tuberculin. As described later, this eventually proved to be the case.

After 62 days of treatment all the surviving animals, including the controls, were killed. A record was made at necropsy of the extent of disease in each animal as presented schematically in figure 1. Portions of lung, liver, spleen, site of inoculation, axillary lymph nodes and tracheobronchial lymph nodes were selected for histopathologic study.

Results

Weight Change: During the course of the experiment it became evident that the administration of cortisone or cortisone plus streptomycin to the tuberculous guinea pigs was harmful or at least not beneficial as indicated by their failure to gain weight in comparison to each other group of animals including the in-
fected control animals. Referring to figure 2 it is seen that the normal animals given cortisone alone or in combination with streptomycin gained weight rapidly, which was in marked contrast to the tuberculous animals similarly treated. Beginning with the fourth week of treatment the infected animals given cortisone alone or with streptomycin had an average loss of weight, whereas each of the other groups, including the infected controls, showed some gain of weight by the end of the experiment.

Tuberculin Tests: The tuberculin tests done with 0.1 ml. of 1:100 Old Tuberculin and read 48 hours later on the 15th day of treatment revealed no significant differences between the various groups of animals regarding the size of the area of erythema and the number of animals with necrosis of the reaction. However, a second test which was done on the 35th day of treatment using 0.1 ml. of 1:1,000 Old Tuberculin did indicate that the administration of cortisone reduced the size of the reaction and the number of reactions with necrosis. A third tuberculin test was applied on the 60th day of treatment using 0.1 ml. of 1:10,000 Old Tuberculin. When examined 48 hours later there was a strik-
ing difference between the group that received cortisone and those that did not, as shown in table I.

**Deaths of Animals:** Only a few animals died before the end of the experiment, as indicated in figure 1. The control animal that died on the 60th day of infection had sufficient tuberculosis disease to account for death, but for the animal that died on the 77th day the cause of death is not known. The gross and especially the microscopic appearance of the lesions was not severe enough to be incompatible with life. This was also true of the two animals treated with 2 mg. of streptomycin daily that died on the 63rd and 66th day of infection respectively. However, each of the three animals in the group treated with cortisone that did not survive did have sufficiently extensive tuberculous involvement of the parenchymal organs to account for their death. Microscopic examination disclosed disseminated involvement especially of the pulmonary tissue. In the animal in this group that died on the 69th day of infection there were innumerable microscopically evident tuberculous foci in the liver which were not observed at necropsy. In the group treated with cortisone plus streptomycin the first animal to die succumbed on the 64th day of infection. It was found to have very little gross or microscopic evidence of active tuberculous disease, suggesting that perhaps the administration of cortisone had augmented the beneficial effects of the low dose of streptomycin. This impression, however, was neutralized by the finding of extensive involvement especially of the spleen and lungs of the animal that died on the 82nd day, which was one day preceding termination of the experiment. Microscopic examination disclosed unrestricted progression of the lesions in all the parenchymal organs with no evidence that the animal had been given streptomycin in addition to cortisone.

**Necropsy Observations:** Figure 1 presents schematically the extent of the grossly visible tuberculous lesions seen when the

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<td><strong>REATIONS TO 0.1 ML. OF 1:10,000 OLD TUBERCULIN INJECTED ON THE SIXTIETH DAY OF TREATMENT AND READ FORTY-EIGHT HOURS LATER</strong></td>
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animals were killed at the end of the experiment. In comparison to the amount of disease seen in the controls it is apparent that the administration of 6 mg. of streptomycin once daily for 62 days caused a marked reversal of the progressive disease that is presumed to have been present when treatment started. There were, however, residual foci at the site of inoculation in a few animals and also in the axillary lymph nodes. The administration of 2 mg. of streptomycin resulted in only partial inhibition of the disease, although two animals had no grossly apparent lesions in the lungs, liver or spleen. The extent of the lesions seen at necropsy in the animals given cortisone is comparable to that seen in the untreated control animals; and, with two exceptions, this is true also of those treated with streptomycin plus cortisone. It appeared that in six of the eight animals in this group the beneficial effect of treatment with 2 mg. of streptomycin daily was not seen when 2 mg. of cortisone was given concomitantly.

Histopathologic Examination: Microscopic study of tissues from the pretreatment controls provided evidence that an actively progressive disease had been established by the 20th day of infection. As noted previously, there were tuberculous foci in the pulmonary tissue of those animals whose lungs appeared grossly normal at necropsy. The parenchymal organs, the site of inoculation and the axillary nodes of the untreated controls presented the picture of conglomerate destructive lesions typical of inoculation tuberculosis in guinea pigs. Figure 3a is that of a section of lung from a control animal showing the unrestricted morbid process replacing the pulmonary tissue. The microscopic appearance of the lesions in the animals given 2 mg. of cortisone daily (fig. 3b) was similar to that seen in the untreated animals. While the histologic preparations were being studied the impression was obtained that the disease in the animals given cortisone alone was progressing more rapidly and was more extensive than in the control animals. This was difficult to evaluate, however.

Treatment with 6 mg. of streptomycin once daily appeared to have effected regression and healing of lesions which may be presumed to have been active when treatment started. There were no microscopically evident lesions of active tuberculosis in the lungs or spleens of these animals. Only one animal had residual lesions in the liver, most of which were becoming fibrotic. However, the axillary lymph nodes in all these animals retained areas of necrosis.

The administration of 2 mg. of streptomycin daily resulted in a definite retardation of the disease, but in contrast to the animals treated with 6 mg. of streptomycin daily there were still some recognizable tubercles in the parenchymal organs in all but two
FIGURE 3: (a) Lung of control animal killed 83 days after infection.—(b) Lung of animal treated with 2 mg. of cortisone daily for 62 days starting on the 21st day of infection.—(c) Lung of animal treated daily with 2 mg. of streptomycin for 62 days starting on the 21st day of infection.—(d) Lung of animal treated daily with 2 mg. of cortisone plus 2 mg. of streptomycin for 62 days starting 21 days after infection. The disseminating morbid process shown in (d) is similar to that shown in (a) and (b) and is in marked contrast to the restriction of the disease shown in (c).
of the eight animals. Figure 3c presents a typical pulmonary lesion in an animal treated with only 2 mg. of streptomycin.

Of particular interest were the histologic findings in the animals treated daily with 2 mg. of streptomycin plus 2 mg. of cortisone. With the exception of two animals, the lesions were comparable in extent and appearance to those seen in the controls and in those given cortisone alone. There were none of the microscopic features of healing such as the fibrosis seen in those given 2 mg. of streptomycin alone. Figure 3d is that of a lung from an animal given cortisone plus streptomycin. The two exceptions noted earlier in this paragraph were the animals that at necropsy were found to have little macroscopic evidence of tuberculosis. Histopathologic study of tissues from these two animals revealed that unlike the others in the group, they did have a beneficial response to the administration of streptomycin. Sections of the lungs and liver revealed no active lesions. One had a few active tuberculous foci in the spleen.

An index of infection based on the character of the lesions and the extent of involvement was determined for each group of animals in the experiment. By this method, values of 10 to 35 are given to the parenchymal organs with actively progressing tuberculous lesions, depending on the degree of involvement. For inac-

![Index of infection diagram](https://example.com/diagram.png)

**FIGURE 4:** Average index of infection for each group based on histopathologic characteristics. The procedure is described in the text.
tive lesions with evidences of healing such as fibrosis and calcification values of one to three are given. A comparison of the average index for each group may be made by referring to figure 4. In comparison to the untreated controls and those given cortisone alone, the lower index obtained for the group treated with 2 mg. of streptomycin plus 2 mg. of cortisone is due to the two animals in this group that, unlike the others, did have histologic evidence of healing and regression of the disease presumably due to the administration of 2 mg. of streptomycin. If these two were excluded, the index of infection for the group given cortisone plus streptomycin would be the same as that obtained for the animals given cortisone alone.

These results made it desirable to determine the effect of cortisone on streptomycin in vitro. Two series of twofold dilutions of streptomycin ranging from 2.0 to 0.03 microgram per milliliter were made in Proskauer and Beck liquid medium and distributed in 2 ml. amounts into test tubes. One series was made to contain 0.2 mg. of cortisone per milliliter of medium in addition to the streptomycin. Each tube of medium was inoculated with 0.1 mg. of tubercle bacilli from the same strain used in the animal experiment. After 10 days of incubation and again after 18 days of incubation it was found that in both series growth was inhibited by a concentration of 0.25 microgram of streptomycin per milliliter. There was no evidence that the presence of cortisone interfered with or enhanced the bacteriostatic effect of streptomycin. The tests were repeated using the liquid medium with 10 per cent horse serum, which gave the same results.

Comment

The mechanisms of resistance to tuberculosis and the factors which permit healing of a tuberculous lesion are not known. It is possible that they are not the same in all species. In the experiment described here the histologic evidence suggests that the administration of cortisone prevented restriction of the disease by inhibiting fibrosis. Spain and Molomut conducted a similar experiment in guinea pigs and found that in animals given cortisone plus streptomycin the lesions were more extensive and had much less granulation tissue than did those animals given streptomycin. For mice, however, Hart and Rees concluded that the enhancement of the tuberculous process associated with the administration of cortisone could not be explained on the basis of depression of collagen formation. Solotorovsky and associates reported that in mice the protective effect of vaccination with heat-killed tubercle bacilli was abolished by cortisone owing to interference with the immune response. Michael, Cummings and Bloom found that the
natural resistance of rats to tuberculosis was markedly reduced by the administration of cortisone owing possibly to depression of the inflammatory response. Lurie and associates\(^9\) found that in highly susceptible rabbits the administration of cortisone resulted in a greater number of tubercles being formed in the lungs but that the lesions were smaller than in the untreated animals. Furthermore, the spread of the disease to the tracheobronchial lymph nodes and to other organs from the lung was markedly reduced in infected rabbits given cortisone. Lurie and his associates\(^9\) concluded that cortisone was able to transform a rabbit of high susceptibility to one that had some of the pathologic responses characteristic of the natively resistant rabbit.

The clinical significance of the experimental work is not clear. In a preliminary note\(^10\) regarding the experiment described in this report it was stated that in cases of tuberculosis cortisone should be investigated with caution, and on the basis of experiments on animals others have sounded the same warning.\(^5\,6\,8\,11\) Reports on the effects of ACTH or cortisone on clinical tuberculosis\(^12\,13\) are few and inconclusive concerning the danger of administering these hormones to patients with tuberculosis. It is the general experience, however, that cortisone or ACTH rapidly produces amelioration of symptoms and marked subjective improvement. Withdrawal of these agents is soon followed by a return of the signs of illness, which in some cases have been worse than before treatment was started. However, of particular interest are the reports of Browne\(^14\) and Thorn\(^15\) who described patients with Addison's disease and tuberculosis whose tuberculous disease responded favorably to treatment with streptomycin while the patients were being given cortisone also. Cummings\(^16\) has found that the administration of cortisone to infected rats being treated with streptomycin does not interfere with the protective effect of the antibiotic agent. Although this finding is at variance with results obtained in guinea pigs reported in this paper and by Spain and Molomut\(^5\) and Bogen and associates,\(^13\) it does indicate that there are important species differences which must be considered when relating experiments on animals to clinical practice.

**SUMMARY**

Forty-eight guinea pigs were inoculated with virulent tubercle bacilli and 20 days later six were killed and found to have visible lesions of tuberculosis. The remaining animals were divided into groups as follows: 10 controls, eight treated once daily with 6 mg. of streptomycin, eight treated once daily with 2 mg. of streptomycin, eight treated once daily with 2 mg. of cortisone, and eight treated once daily with 2 mg. of streptomycin plus 2 mg. of cor-
CORTISONE IN EXPERIMENTAL TUBERCULOSIS

Vol. XX

Cortisone. Treatment started on the 21st day of infection and continued for 62 days. During the course of treatment the animals given cortisone alone or in combination with streptomycin showed an average loss of weight and also a decrease of sensitivity to tuberculin in comparison to the other groups. After 62 days of treatment all animals were killed. The untreated controls and those given cortisone alone were found to have extensive tuberculous disease in the lungs, liver and spleen. There was almost complete absence of visible lesions in those given 6 mg. of streptomycin. Those given 2 mg. of streptomycin had less disease than seen in the controls. Only two of eight animals given streptomycin plus cortisone appeared to have responded beneficially. The other six had visible lesions of tuberculosis comparable to those seen in the controls. Histopathologic studies confirmed the necropsy findings. In six of the eight animals treated concomitantly with streptomycin and cortisone, the administration of cortisone appeared to have prevented the restriction and healing of the tuberculous process. Cortisone had no inhibitory effect on streptomycin in vitro.

RESUMEN

Se inocularon cuarenta y ocho cuyes con bacilos tuberculosos virulentos, y 20 días más tarde, se sacrificaron 8 de ellos, habiéndolos encontrado lesiones visibles de tuberculosis. Los animales restantes quedaron distribuidos en grupos, en la forma siguiente: 10 testigos; ocho recibieron tratamiento consistente en 6 mg. de estreptomicina una vez al día; ocho recibieron 2 mg. de estreptomicina una vez al día; ocho recibieron tratamiento de 2 mg. de cortisona, una vez al día, y ocho recibieron tratamiento, una vez al día, consistente en 2 mg. de estreptomicina y 2 mg. de cortisona. Se inició el tratamiento el día vigésimo-primer de la infección y se continuó durante 62 días. En el curso del tratamiento, aquéllos animales a quienes les fué administrada la cortisona sola o combinada con estreptomicina, acusaron una pérdida de peso en promedio, así como una disminución de sensibilidad a la tuberculina, en relación a los demás grupos. Pasados los 62 días, todos los animales fueron muertos. Los testigos, que no recibieron tratamiento alguno, y los cuyes a los que se les administró la cortisona sola, indicaron una tuberculosis extensa en los pulmones, el hígado y el bazo. En aquéllos que fueron tratados con 6 mg. de estreptomicina, se pudo apreciar una carencia casi absoluta de lesiones visibles. Los que recibieron una dosis de 2 mg. de estreptomicina acusaban la enfermedad en grado menor que el observado en los testigos. De los animales a quienes se les administró la estreptomicina en combinación con la cortisona, sólo dos respondieron
benéficalement. Los seis restantes tenían lesiones visibles de tuberculosis, comparable a las vistas en los testigos. Los estudios histopatológicos confirmaron los resultados de las autopsias. En seis de los ocho cuyes tratados simultáneamente con estreptomicina y cortisona, la suministración de la cortisona parece haber evitado la restricción y curación del proceso tuberculoso. La cortisona no tuvo ningún efecto inhibitorio sobre la estreptomicina in vitro.

**RESUME**

Les auteurs ont inoculé 48 cobayes avec un bacille de KOCH virulent. Vingt jours après, six d'entre eux furent sacrifiés et se montrèrent porteurs de lésions tuberculeuses apparentes. Le restant des animaux fut divisé en plusieurs lots: 10 comme témoins, 8 traités une fois par jour par 2 mmg. de streptomycine, 8 traités une fois par jour par 2 mmg. de cortison, et 8 traités une fois par jour par l'association de 2 mmg. de streptomycine et de 2 mmg. de cortison. Le traitement débuta le 21ème jour après l'inoculation et fut continué pendant 62 jours. Au cours de ce traitement les animaux du groupe recevant de la cortison isolément ou combinée à la streptomycine accusèrent une chute notable de poids et une diminution de leur sensibilité à la tuberculine par rapport aux animaux des autres lots. Tous les animaux furent sacrifiés après 62 jours de traitement. Les animaux témoins qui n'avaient reçu aucun traitement et ceux qui n'avaient reçu que de la cortison présentaient une tuberculose étendue des poumons, du foie et de la rate. Il n'y avait à peu près aucune lésion visible chez ceux qui avaient reçu 6 mmg. de streptomycine. Ceux qui avaient été traités par 2 mmg. de streptomycine avaient moins de lésions que les témoins. Il n'y eut que deux des huit animaux traités par l'association de streptomycine et de cortison que parurent en tirer avantage. Les six autres cobayes étaient atteints de lésions comparables à celle des témoins. Les examens histopathologiques confirmèrent les constatations faites à l'autopsie. Chez six des huit animaux traités simultanément par la streptomycine et la cortison, il semble que la cortison a empêché l'arrêt du processus tuberculoso et sa guérison. La cortison n'a pas d'action inhibitrice sur la streptomycine in vitro.

**REFERENCES**
