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THE EFFECT OF CORTISONE ALONE AND IN COMBINATION WITH ISONIAZID ON EXPERIMENTAL MURINE LEPROSY IN MICE

BY

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There has been much work on the effects of cortisone on tuberculosis but no publications on its effects on experimental leprosy. Recently, Naguib and Robson (1955) reported that cortisone exacerbates corneal tuberculosis in mice and overcomes the beneficial effects of isoniazid. In rabbits, Greenburgh, Robson, and Willcox (1953) had previously found that cortisone decreases the severity of corneal tuberculosis. The purpose of the present investigation was to determine the effect of the drug on the development of murine leprosy induced by the intracorneal inoculation of the Mycobacterium in mice.

METHODS

Details of the method used for corneal inoculation with *Myco. lepraemurium* have been described previously (Naguib, Rees, and Robson, 1956). The leprosy material used in the present investigation was obtained from a mouse which had been infected intracorneally from a rat leproma (Douglas strain) and had developed generalized leprosy. The mouse was killed and a suspension in 0.05% Tween 80 saline was prepared from the ground liver and spleen. The inoculum, which was about 0.01 ml., contained approximately 2.3×10^{11} lepra bacilli. Albino mice of the "C" strain of 25 g. average weight were used and were divided into four groups: (1) 10 controls, (2) 10 treated with isoniazid, (3) 16 treated with cortisone, and (4) 16 treated with cortisone and isoniazid. Treatment was started on the day of inoculation. The isoniazid therapy was continued until the end of the experiment (40 weeks). All mice on cortisone (groups 3 and 4) received the drug for 55 days. At the end of this period the surviving mice of groups 3 and 4 were divided into equal subgroups: in one the cortisone was discontinued, and in the other the cortisone was administered until all animals had died.

Cortisone acetate suspended in saline was injected subcutaneously in daily doses of 0.5 mg./mouse for 6 days/week; isoniazid was mixed in M.R.C. diet 41 and given in a dose of 0.3 mg./mouse/day in 5 g. of diet. To prevent secondary infection after corneal inoculation aureomycin was given in the drinking-water in a dose of 100 mg./kg. body weight (on the assumption that each mouse drinks 4 ml. daily) and was continued throughout the experiment.

The lesions which developed on the cornea were examined daily and the size estimated in arbitrary units. Values of 0.6 and under do not represent true lesions, which are represented by the higher values given to areas of denser opacity in the cornea.

RESULTS

The intracorneal inoculation of leprosy material produced an initial reaction in all the mice in the form of an opacity which faded gradually until the true lesion became superimposed upon it.

Control Animals.—Corneal lesions developed as small foci in the centre of the opaque areas in all the control mice after an incubation period of some 17 days. They then increased in size and density over the cornea and spread to the sclera, which became thickened and swollen. The progress of these lesions is shown in Fig. 1.

All the mice of this group survived for periods varying between 23 and 31 weeks. At post-mortem examination they all had severe generalized leprosy involving many of the internal organs, particularly the liver and spleen. In one of the animals leprosy bacilli were found in the spinal cord.

Isoniazid.—The mice treated with isoniazid developed lesions after the same latent period as the controls. These lesions progressed very slowly and remained small, except in one animal, in which two-thirds of the cornea was involved at one stage of the infection. Prolonged treatment resulted in a decrease in both the size and density of the lesions, which in two mice almost disappeared, leaving an opacity similar to the initial reaction. One of these mice was killed at 23 weeks for histological examination and another died at 31 weeks. The other eight were alive and well at 41 weeks, when they were killed for post-mortem

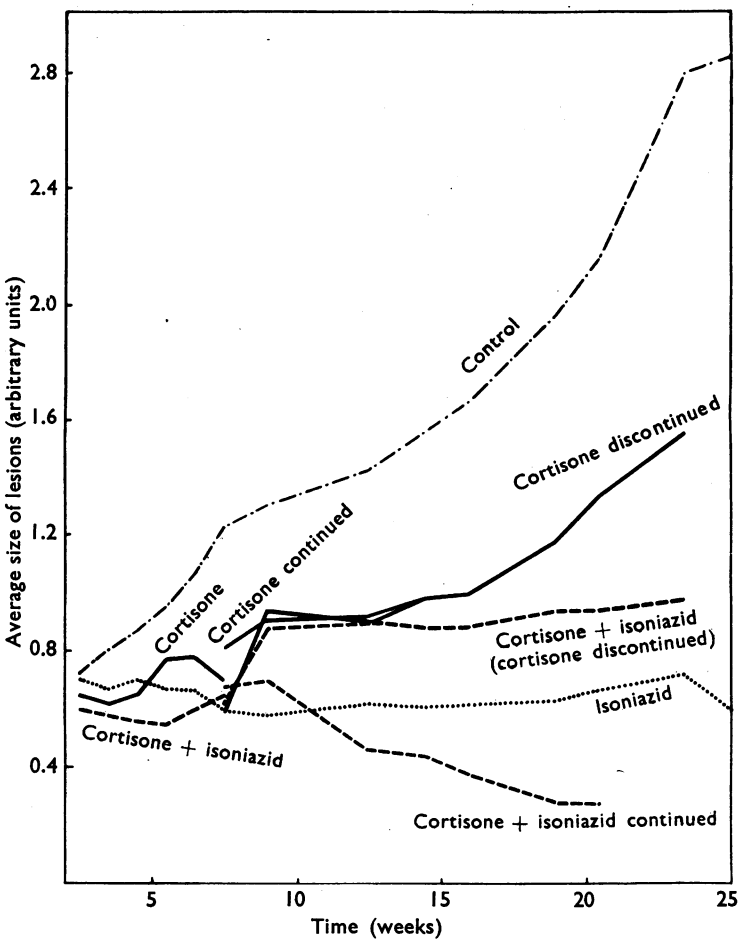

FIG. 1.—Showing the development of corneal lesions in the various groups of animals. — · — · — Control group. . . . . Isoniazid group. ———— Animals on cortisone; 55 days after inoculation the surviving animals were divided into two subgroups, in one of which the cortisone was discontinued and in the other continued. - - - - - - - Animals on cortisone and isoniazid. The isoniazid was given until the end of the experiment. At 55 days the surviving animals were divided into two subgroups, in one of which the cortisone was discontinued and in the other continued.

examination. In six of them no evidence of infection, either macroscopic or microscopic, was found in the various internal organs. In the other two a few bacilli were found on microscopic examination of the liver and spleen, but there were no macroscopic lesions. The isoniazid treatment had thus produced a striking effect on the course of the infection, both in the cornea and in the internal organs.

Cortisone.—The latent period preceding the appearance of lesions was considerably increased, and for 7 weeks no typical lesions were seen. One of the mice died 17 days after inoculation and showed no changes in the cornea. In all the others, blebs appeared in the cornea in the period preceding the development of typical lesions. The bleb usually appeared as a transparent spot in the centre of an opaque area (the site of inoculation), but sometimes elsewhere in the cornea, and in a number of mice more than one bleb developed. The bleb grew and became filled with fluid, resembling a blister. Some of the blebs disappeared and reappeared after various periods. They never became herniated, and never spread to destroy the cornea and the eye as has been seen in corneal tuberculosis treated with cortisone (Naguib and Robson, 1955).

Fifty-five days after inoculation 14 animals of this group were still alive and in seven the cortisone was continued. Two of these died 9 weeks after inoculation without developing corneal lesions, and the other 5 developed corneal lesions at periods after inoculation varying from 87 to 144 days. In the animals in which the cortisone was discontinued, true lesions appeared about 10 days after cessation of treatment, except in one animal where the latent period was much longer (110 days). The average size of the lesions in the various groups is shown in Fig. 1.

Five of the animals on continued cortisone survived for periods varying between 15 and 23 weeks. At post-mortem examination comparatively few macroscopic lesions were found and these were small and scattered ; sections or smears of the liver and spleen of 3 of the animals which died 16–21 weeks after inoculation showed a large number of lepra cells filled with leprosy bacilli. The effect in these animals, therefore, is similar to that described by Lurie and Zappasodi (1955) in rabbits infected by inhalation of *M. tuberculosis* and treated with cortisone.

The mice in which cortisone was discontinued survived for periods varying between 23 and 36 weeks, and in all but one of these the macroscopic

lesions were either small and not numerous, or totally absent. In 5 out of 7 animals, however, section or smears showed many lepra cells with very large numbers of bacilli. In the other 2 mice, the number of bacilli found in the liver and spleen was appreciably smaller.

Cortisone and Isoniazid.—No true corneal lesions appeared during the first 7 weeks following inoculation, and bleb formation was seen as in the mice on cortisone alone. Six of these animals died before 55 days. In 4 of the animals in which cortisone was continued, no corneal lesions developed and in one a lesion appeared 87 days after inoculation. On the other hand, in 4 of the animals in which cortisone was discontinued, true corneal lesions appeared at periods varying from 62 to 101 days after inoculation; in the fifth the cornea remained clear until the end of the experiment.

The animals in which cortisone was continued survived for periods varying between 19 and 25 weeks. On post-mortem examination no macroscopic lesions were present in the internal organs in 4 out of 5 of the mice, and in the fifth a single minute macroscopic lesion was found in the liver. Smears or sections of the liver and spleen of these animals showed either a few bacilli (in 3) cr none at all (in the other 2).

The animals in which cortisone was discontinued survived for periods varying between 23 and 40 weeks. In 2 of these, post-mortem examination revealed extensive macroscopic involvement of the liver and spleen, but in the other 3 no macroscopic lesions were found. Smears from the organs of the animals with gross macroscopic lesions showed huge numbers of lepra cells with many bacilli, but in the other 3 animals few or no bacilli were recovered.

Discussion

Inoculation of *M. lepraemurium* into the cornea of mice leads to progressive involvement of the eye and systemic spread of the disease, which ultimately kills the animals. Administration of isoniazid has a striking inhibitory effect on the course of both the ocular and systemic infections.

Cortisone modifies the spread of murine leprosy; but the effects are rather complex and the results are summarized in Table I. Cortisone delayed the appearance of macroscopic lesions, both locally and systemically. When its administration was stopped at 55 days, lesions quickly made their appearance in the cornea, as was evident from the sudden increase in the density of the corneal opacities; this increase was not seen in animals on continued cortisone therapy. The inhibitory effect on the spread of the corneal lesions is similar to that obtained with corneal tuberculosis in the rabbit (Greenburgh *et al.*, 1953).

The systemic lesions in the liver and spleen were much smaller than in the untreated animals, but sections or smears from these were swarming with bacilli.

The combination of cortisone with isoniazid produced effects similar to those produced by isoniazid alone; the adrenal steroid did not overcome the beneficial effect of isoniazid. On the other hand, and rather unexpectedly, when the cortisone administration was stopped at 55 days, two of the animals on continued isoniazid (which survived respectively for 23 and 41 weeks) did develop gross systemic leprosy, which was never seen in animals on isoniazid alone. This suggests that occasionally cortisone can reverse the effect of isoniazid, an action similar to that consistently observed in tuberculous infection arising in the cornea (Naguib and Robson, 1955).

TABLE I

SHOWING THE COURSE OF THE INFECTION IN THE VARIOUS GROUPS OF ANIMALS

(For further information see text)

Group	No. of Mice	Mean Survival Time (Weeks)	Cornea	Systemic Disease
Controls	9*	27	Progressive disease	Gross macroscopic lesions
Isoniazid	8†	40	Small lesions	Nil or very slight
Cortisone for whole period	7‡	16	Small delayed lesions ‖	Small macroscopic lesions. Masses of bacilli
Cortisone discontinued at 55 days	7	26	Lesions showing after‖ discontinuation of cortisone	Idem
Cortisone + isoniazid for whole period	5§	22	Nil or slight‖	Slight or nil
Cortisone discontinued at 55 days	5	36	Lesions at 7–46 days‖ after discontinuation of cortisone in 4 animals	Gross macroscopic lesions in 2 mice. Slight or nil in 3

* 1 killed for histology. † 1 killed for histology; 1 died at 3 weeks. ‡ 2 excluded which died at 2–3 weeks. § 6 excluded which died at 3–7 weeks.
‖ Apart from blebs in early stage.

The development of blebs in the cornea in the early stages of cortisone therapy, both with and without isoniazid, is puzzling. The similar phenomenon observed in tuberculosis of the cornea in animals receiving cortisone was accompanied by multiplication of the bacilli (Naguib and Robson, 1955; Robson and Didcock, 1956) so that the area of bleb formation contained a mass of bacilli. It seemed possible that this led to the production of some toxic substance responsible for the bleb formation and subsequent perforation of the cornea. In the leprosy infection, however, bleb formation occurred in the absence of any true macroscopic lesion and never was the prelude to perforation and destruction of the eye. The mechanism of bleb formation in the cornea under these circumstances requires further investigation.

One last point deserves mention. It has been found that in some cases of human leprosy, particularly in leprous eye inflammation, cortisone may be of value to supplement other drug treatment (Lowe, 1952). This is in keeping with the results of the present experiments.

SUMMARY

1. Mice were inoculated intracorneally with *M. lepraemurium* and treated with cortisone, or with a combination of cortisone and isoniazid.

2. Cortisone decreased the development of both macroscopic corneal and systemic lesions; nevertheless, the liver and spleen in most animals contained very large numbers of lepra cells filled with bacilli. Cortisone did not appreciably modify the beneficial action of isoniazid, except in two mice in which gross systemic lesions occurred; this was not seen in mice on isoniazid alone.

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