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THE EFFECT OF CORTISONE ALONE AND IN COMBINATION WITH ISONIAZID IN EXPERIMENTAL CORNEAL TUBERCULOSIS IN MICE

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It is well known that cortisone has a striking effect in certain conditions, notably rheumatoid arthritis, and that this effect is due to its action on collagenous tissue. Indeed, it is now generally accepted that cortisone prevents the formation of new connective tissue (Ragan, Howes, Plotz, Meyer, and Blunt, 1949; Spain, Molomut, and Haber, 1950; Shapiro, Taylor, and Taubenhaus, 1951). Since such an effect would hinder the encapsulation of tubercle bacilli, and hence make them more accessible to antibiotics, many workers have studied the action of cortisone on experimental tuberculosis by a variety of methods. The results have been diverse. Thus, some investigators have found that cortisone aggravates tuberculous lesions (Hart and Rees, 1950; Bunn and Drobeck, 1952; Michael, Cummings, and Bloom, 1950) whereas others have stated that it either has no deleterious effect or even some beneficial effect (Le Maistre and Tompsett, 1951; Le Maistre, Tompsett, Muschenheim, Moore, and Dermott, 1951; Weimer, Boak, Bogen, Drusch, Miller, Moshing, and Carpenter, 1953; Greenburgh, Robson, and Willcox, 1953). Most, if not all, such investigations have been carried out on animals with intact adrenals.

In view of the discrepant results on intact animals, it seemed desirable to investigate the effect of the hormone on adrenalectomized as well as on intact animals. Since these experiments revealed striking deleterious actions, it became of interest to find how cortisone modified the effects of isoniazid, and thus a combination of the two drugs was used in both intact and adrenalectomized mice. The effects of the drugs were evaluated quantitatively by the mouse corneal method.

METHODS

All experiments were performed on mice of the albino C strain. The cornea was infected by the technique of, and with the strain of bovine tubercle bacilli used by, Rees and Robson (1950). Adrenalectomy was performed, by the dorsal approach, under ether anaesthesia one week before the corneal inoculation. The operated mice were given normal saline to drink, but this was discontinued when the hormone treatment was started. Cortisone acetate suspended in saline was injected subcutaneously, in daily doses as described in the different experiments. Isoniazid was given in M.R.C. diet 41. The daily dose in 5 g. of diet was 0.3 mg. to intact and 0.2 mg. to adrenalectomized mice, as the latter were unable to tolerate the larger dose. Treatment was started on the same day as corneal inoculation and was continued for 28 days (except in experiment 1, which lasted for 25 days), after which all surviving animals, except those treated with isoniazid alone, were killed and a post-mortem examination carried out. The isoniazid-treated animals were kept for 6–7 weeks and were then killed and examined.

To prevent secondary infection each mouse was injected daily with 2,000 units of penicillin for a few days after the corneal inoculation. However, in one experiment intensive antibiotic therapy was given: this consisted of penicillin in the same dose together with chlortetracycline (aureomycin) dissolved in the drinking-water, or saline in a dose of 100 mg./kg. body weight (on the assumption that each mouse drinks daily some 4 cc.), and was continued for the whole period of the experiment.

RESULTS

Effects of Cortisone

Two experiments were first performed to determine the effect of cortisone on corneal tuberculosis in the absence of endogenous adrenal secretion. In each experiment there were four groups of 14–18 mice: (1) intact control, (2) adrenalectomized control, (3) and (4) adrenalectomized treated with cortisone (0.1 and 1.0 mg./day respectively in Expt. 1, and 0.5 and 1.0 mg./day respectively in Expt. 2). The first experiment was stopped 25 days after inoculation, but the second was continued for a somewhat longer period and revealed important additional effects of the hormone.

Corneal lesions developed in all the inoculated eyes after the usual latent period of 10–12 days. The lesions in the control groups developed gradually, becoming dense with a caseous appearance, and were accompanied by oedema and vascularization from the limbus.

The experiments demonstrated that the course of the corneal tuberculosis was affected both by adrenalectomy and by cortisone. In both experiments the lesions developed rather more slowly in the adrenalectomized than in the control animals, though there was no qualitative difference in the course of the infection. This is illustrated in Figs. 2, 3, and 4.

Cortisone (0.5 or 1.0 mg./day) produced initially a slight beneficial effect, since it decreased the size of the corneal lesions by diminishing the amount of oedema and vascularization. Occasionally it also caused a slight increase in the latent period. At about the third week after inoculation mice treated with cortisone developed a typical corneal bleb (Fig. 1). The bleb first appeared in the centre of the caseous lesion as a transparent spot, which grew and became herniated and filled with transparent fluid. Ultimately perforation led to complete destruction of the eye, with the formation of a caseous mass. Hence with the appearance of the corneal bleb the expected course of the corneal lesion was completely changed and progressed instead in a fulminating manner (Fig. 2). This phenomenon was seen in most of the mice which survived more than some three weeks (Table I).

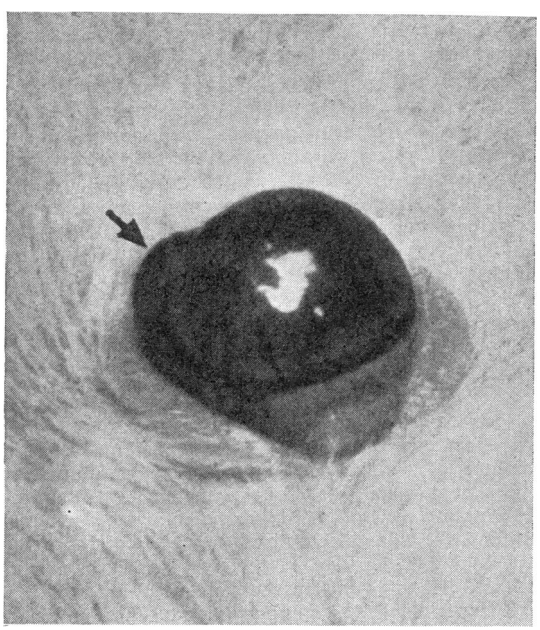

FIG. 1.—Enlarged photograph of mouse eye, showing developing bleb (marked with arrow) in an animal treated with cortisone, 0.5 mg./day for 21 days after inoculation.

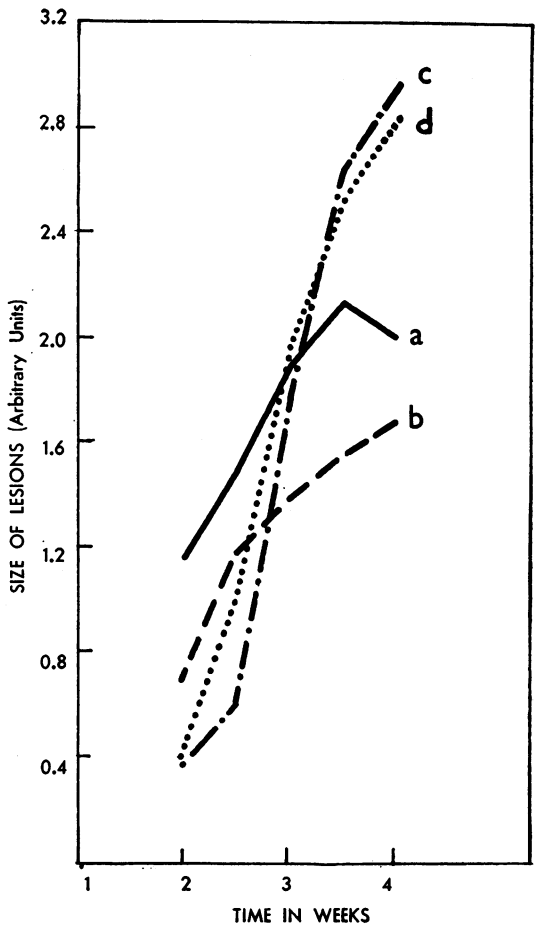

FIG. 2.—Showing the effect of the adrenal gland and of cortisone on the course of corneal tuberculosis. Average size of lesions (arbitrary units) in: (a) intact controls, (b) adrenalectomized controls, (c) adrenalectomized and treated with cortisone (1.0 mg./day), and (d) adrenalectomized and treated with cortisone (0.5 mg./day).

Cortisone in a dose of 0.1 mg./day to adrenalectomized mice had no noteworthy effect, as the lesions developed and progressed in the same manner as in the control and were accompanied by the usual oedema and vascularization.

Cortisone not only had a harmful effect in aggravating the corneal lesions after the initial stage but also led to the development of generalized tuberculosis. This was found in all the mice

TABLE I

EFFECT OF CORTISONE ON DEVELOPMENT OF BLEBS AND ON SUBSEQUENT DESTRUCTION OF THE EYES, IN MICE INOCULATED INTRACORNEALLY WITH *MYCO. TUBERCULOSIS*

Note that 0·1 mg./day had no effect, whereas 0·5 or 1 mg./day produced a striking effect

Expt.	No. of Treated Mice	Dose of Cortisone (mg.)	Mice Developing Blebs	Mice with Eyes Destroyed	Remarks
1	14	0·1	0	0	—
	16	1·0	10	1	Experiment terminated at 25 days
2	14	0·5	14	11	Three mice died before their eyes were destroyed*
	14	1·0	10	6	3 mice died before 21 days—one did not develop a bleb

* One mouse died on 20th day, a second died on 24th day, and the third was killed at the end of the experiment.

which were autopsied—five mice of the group receiving 1.0 mg. cortisone in the first experiment, and 21 mice of the two groups treated with the hormone in the second experiment. Obvious macroscopic lesions were seen in many organs, including the liver, spleen, kidneys, heart, and serous membranes, in 15 out of the 26 animals; in the remaining 11 animals there was no obvious macroscopic tuberculosis, yet microscopic examination of smears from the liver and spleen revealed the presence of enormous numbers of tubercle bacilli.

The animals lost weight, their skin lost its sheen, they became debilitated and emaciated and looked generally ill. Moreover, the dorsal adrenalectomy wound did not show signs of healing during the period of the experiment.

Effects of Isoniazid together with Cortisone

Having obtained these results with cortisone, investigations were performed to determine how cortisone modified the response to isoniazid both in intact and in adrenalectomized animals. It has been shown (Goulding and Robson, 1952) that isoniazid produces a strikingly beneficial effect on the course of corneal tuberculosis in non-adrenalectomized mice.

Two experiments were performed. In each there were 8 groups of 13–18 mice: (1) intact control, (2) adrenalectomized control, (3) intact treated with cortisone (0.5 mg./day), (4) adrenalectomized treated with cortisone (0.5 mg./day), (5) intact treated with isoniazid (0.3 mg./day), (6) adrenalectomized treated with isoniazid (0.2 mg./day), (7) intact treated with cortisone (0.5 mg./day) and isoniazid (0.3 mg./day), and (8) adrenalectomized treated with cortisone (0.5 mg./day) and isoniazid (0.2 mg./day). The isoniazid and cortisone were given for the usual period of 28 days.

In the first experiment the mice received the usual antibiotic treatment of daily penicillin. The effects of combining cortisone with isoniazid were, however, so striking—in that the infection had become more severe—that it was considered desirable to take all possible precautions to exclude secondary infection. The second experiment was therefore a repetition of the first, except that the mice received intensive antibiotic therapy, consisting of penicillin and chlortetracycline, as described under Methods.

The results of these experiments, in so far as they concern the cornea, are recorded in Figs. 3 and 4. They confirm previous results: that adrenalectomy leads to a somewhat lesser development of the tuberculous infection of the cornea and

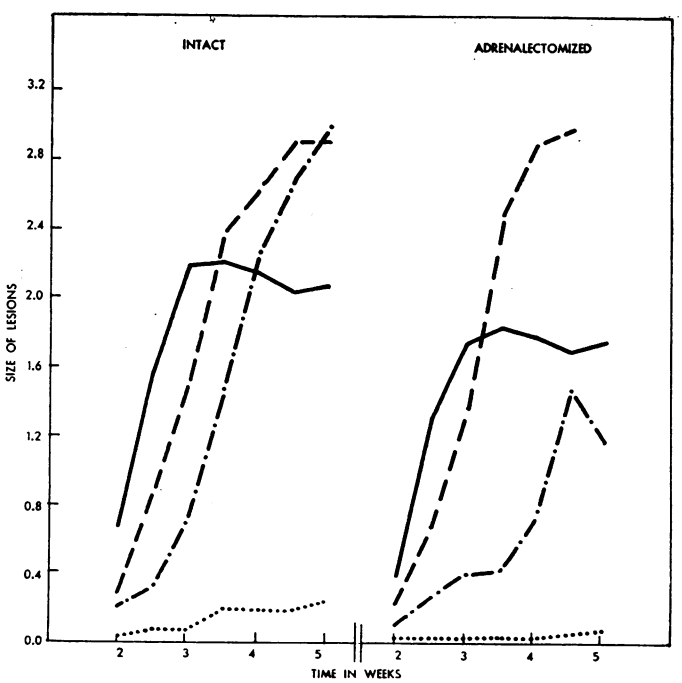

FIG. 3.—Showing the effect on the development of corneal tuberculosis of cortisone and isoniazid, in intact and adrenalectomized animals (Expt. 3). Average size of lesions in: controls (——), animals treated with isoniazid (. . . .), animals treated with cortisone (– – –) and animals treated with cortisone and isoniazid (_ . _ . _).

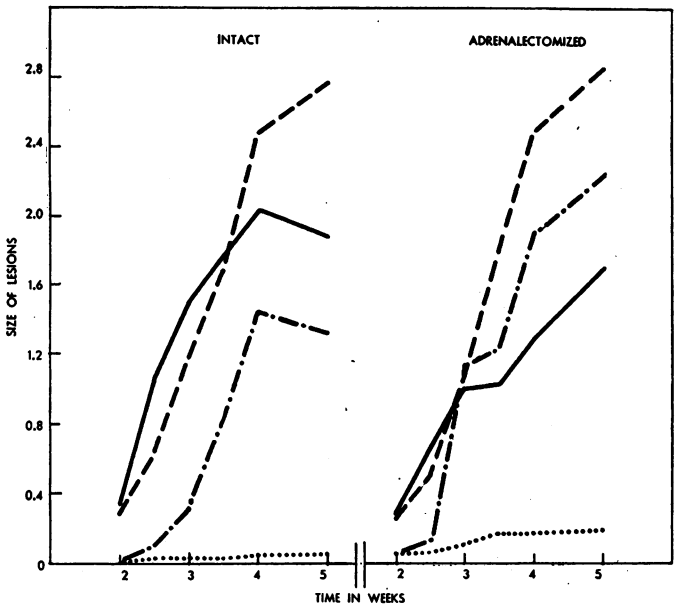

FIG. 4.—Experiment similar to that of Fig. 3, except that all animals received intense antibiotic treatment with penicillin and chlortetracycline (Expt. 4). Control (——), isoniazid (....), cortisone (– – –), isoniazid plus cortisone (_ . _ .).

that the administration of cortisone to adrenalectomized mice markedly aggravates the infection after the first three weeks during which there is a slight beneficial effect. They also show that cortisone has a similar biphasic effect in animals with intact adrenal glands. These findings are remarkably similar in both experiments showing that the intensive antibiotic therapy did not appreciably diminish the deleterious action of cortisone (Table II).

TABLE II

EFFECT OF CORTISONE ALONE OR IN COMBINATION WITH ISONIAZID ON THE DEVELOPMENT OF BLEBS AND SUBSEQUENT DESTRUCTION OF THE EYES, IN MICE INOCULATED INTRACORNEALLY WITH *MYCO. TUBERCULOSIS*

Isoniazid did not prevent the deleterious effect of cortisone

Expt.	No. of Treated Mice*	Treatment	Mice Developing Blebs	Mice with Eyes Destroyed	Remarks
3	36	Cortisone	29	26	7 died before 21 days
	35	Cortisone and isoniazid	16	8	10 died before 21 days and 11 did not develop blebs
4	27	Cortisone	23	18	2 died before 21 days and 2 did not develop blebs
	28	Cortisone and isoniazid	16	3	5 died before 21 days and 7 did not develop blebs

* Both intact and adrenalectomized.

This effect was also striking in mice on isoniazid therapy (Fig. 4 and Table II). Both in intact and in adrenalectomized mice the combination of cortisone with isoniazid greatly diminished the efficacy of the tuberculostatic drug. This is seen in Expts. 3 and 4, though it is slightly less marked in the latter with its intensive antibiotic therapy. Even so, out of 28 normal or adrenalectomized mice on isoniazid and cortisone, 16 developed blebs, which led to complete destruction of the eye in 3, whereas in Expt. 3, out of 35 animals with no intensive antibiotic therapy, there were 16 blebs and 8 destructions of the eye.

The effect of cortisone in overcoming the beneficial action of isoniazid was also seen when the systemic spread of the disease was investigated (Table III). In many of the animals on combined treatment, both in Expts. 3 and 4, multiple tuberculous lesions, mainly involving the liver and spleen, were found; microscopic examination of such lesions showed many tubercle bacilli. In other animals, though no macroscopic lesions were found, smears from the liver and spleen revealed large numbers of acid-fast bacilli. It should be emphasized that in the absence of cortisone treatment we have consistently failed to find systemic spread, either macroscopically or microscopically, in many animals examined for this purpose.

Histological examination of the corneas of the various groups was performed in the hope of finding why the course of the disease was altered by the administration of the hormone. There was, however, no difference in the fibrous tissue formation or in the cellular invasion in the various groups. The corneal stroma was thickened in all the animals at the site of the lesion. In the cortisone-treated mice there was diffusion of fluid from the invading capillary blood vessels (Fig. 5). This fluid started forming in a part of the cornea adjacent to the posterior surface, gradually accumulated in an anterior direction, and led to the development of the corneal bleb with ultimate perforation. One clear result, for which there is at present no explanation in histological terms, is that the lesions in animals treated with cortisone, and some treated with cortisone and isoniazid, were teeming with

TABLE III

EFFECT OF CORTISONE ALONE OR IN COMBINATION WITH ISONIAZID ON THE DEVELOPMENT OF SYSTEMIC TUBERCULOSIS IN MICE INOCULATED INTRACORNEALLY WITH *MYCO. TUBERCULOSIS*

Note the striking development of systemic tuberculosis in the cortisone-treated mice, which was not prevented by the concomitant administration of isoniazid

Treatment	Experiment 3				Experiment 4			
	No. of Mice Treated (i)	Mice with Generalized T.B. (ii)	Mice with No Systemic Spread	No Autopsy	No. of Mice Treated (i)	Mice with Generalized T.B. (ii)	Mice with No Systemic Spread	No Autopsy
Cortisone	36	29 (3)	4	3	27	20 (3)	2	5
Cortisone and isoniazid ..	35	20 (3)	6	9	28	14 (4)	11	3

(i) Both intact and adrenalectomized. (ii) No. with microscopic lesions only in parentheses.

tubercle bacilli (Fig. 6) in contrast to the fewer organisms seen in lesions from the other groups. Nevertheless, no secondary infection was detected in Gram-stained preparations from the corneal lesions of the cortisone-treated mice.

DISCUSSION

These experiments have shown consistently that adrenalectomy produces a small, but definite, favourable effect on the course of corneal tuberculosis in the mouse. The mechanism of this action is at present unknown. It cannot be attributed to the cortisone naturally secreted by the animal's adrenals since, at this stage of the disease, cortisone produces a beneficial effect.

The effect of cortisone administration, both in intact and in adrenalectomized mice, can be divided into two stages. In the first, lasting for about three weeks, there is a beneficial effect which is probably due to the anti-inflammatory action of cortisone which is known to inhibit markedly inflammation and vascularization of the cornea as well as of other parts of the body (Jones and Meyer, 1950; Michaelson, 1952; Harvey, Howard, and Kattus, 1950; Moon and Tershakovec, 1952; Ebert, 1952).

This transient beneficial effect of cortisone is followed by a deleterious action which starts with the formation of a corneal bleb at the centre of the lesion and may ultimately result in perforation of the eye and its destruction by caseation; there is also widespread dissemination of tubercle bacilli throughout the body. These findings are in general agreement with those of Hart and Rees (1950) and other investigators, who state that cortisone exacerbates tuberculosis and produces widespread infection. It is obvious that at this stage cortisone markedly reduces resistance to the infection. Since

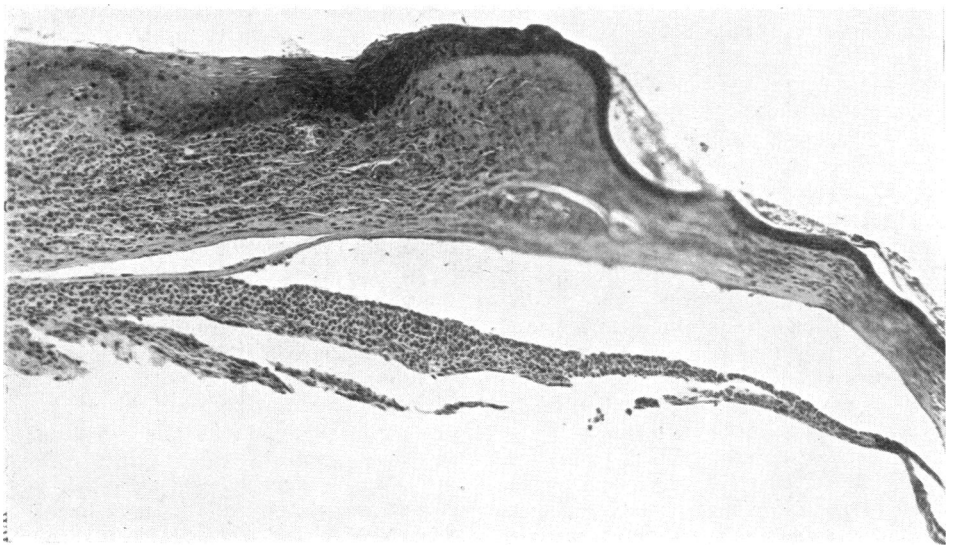

FIG. 5.—Section of cornea, showing the development of bleb. Note space in posterior part of cornea and, anterior to this, exudation of fluid from capillaries, ×100.

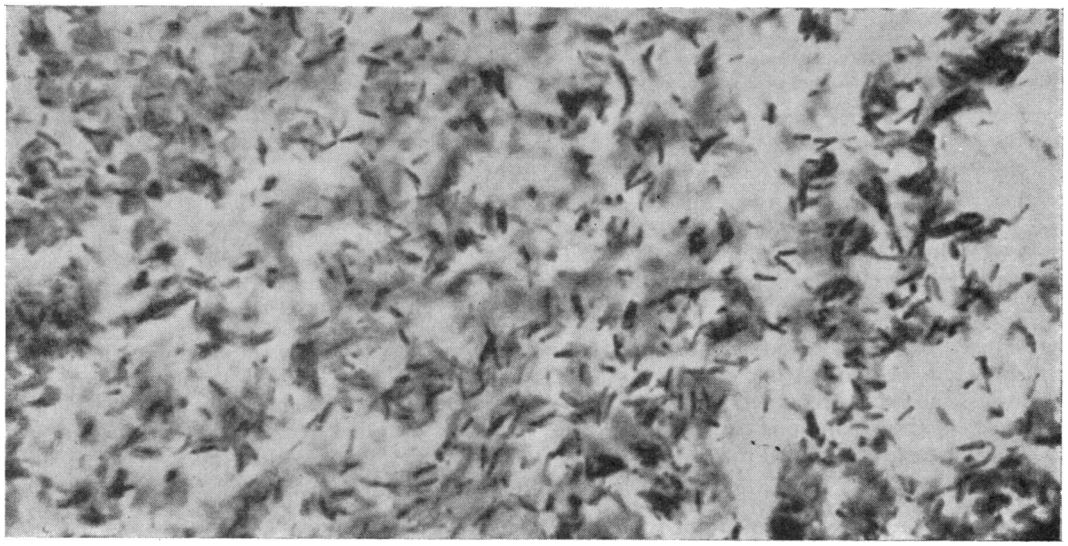

FIG. 6.—Section of the same cornea as in Fig. 5 stained with Ziehl-Neelsen to show the large number of tubercle bacilli. Animals treated with cortisone for 26 days, × 1,100.

cortisone renders animals more susceptible to spontaneous and experimentally induced pyogenic infection (Selye, 1951 ; Thomas, 1953), special precautions were taken to determine whether this effect was not, in part at least, due to secondary infection rather than to a true exacerbation of the tuberculosis. But the action of cortisone remained even when intensive antibiotic therapy was given, and, moreover, histological examination of the material failed to reveal secondary infecting organisms. It must, therefore, be concluded that cortisone truly does lead to a worsening of the tuberculous infection of the eye, as well as to the dissemination of the disease to other parts of the body. It is of interest, in this connexion, that Bunn and Drobeck (1952) found that corticotrophin, and to a lesser extent cortisone, produced a deleterious effect on ocular tuberculosis in the rabbit when the treatment was started at the time of, or shortly after, inoculation of the anterior chamber: the course of the disease was speeded, more rapid destruction of the eye occurred, and metastatic tuberculosis very often developed. In the present experiment this effect of cortisone was accompanied by a very rapid multiplication of the mycobacteria which were present in the cornea in enormous numbers at the time of bleb formation. It is possible, indeed, that the presence of these large numbers of organisms may have contributed, by their toxic effect, to the formation of the bleb and the subsequent perforation of the cornea. At present the course of these lesions is being followed in the unstained cornea by means of the phase-contrast microscope, and the presence of enormous numbers of tubercle bacilli, in cords and in palisades, has been confirmed at the stage preceding the development of the bleb. It is hoped to discuss these findings in detail in a later paper.

The results of combined treatment with cortisone and isoniazid are particularly interesting since they show that cortisone can antagonize the beneficial effect of the tuberculostatic substance, both in intact and in adrenalectomized mice, and lead to caseous destruction of the eye and the development of generalized tuberculosis. This is contrary to the findings of Ilavsky and Foley (1954), who investigated the course of tuberculosis in mice injected intraperitoneally, and found that the effect of isoniazid was not impaired by the concomitant administration of cortisone. It must be noted, however, that though the dose of cortisone used was similar, these workers used appreciably larger amounts of isoniazid than we did (about 0.8 mg./mouse/day as compared with 0.2–0.3 mg./mouse/day), and this may account for the discrepancy. Ilavsky and Foley did find that the effectiveness of streptomycin was impaired by cortisone. The mechanism by which the adrenal steroid antagonizes the strikingly beneficial effect of isoniazid in corneal tuberculosis requires further investigation.

Summary

1. The effect of adrenalectomy and of cortisone on tuberculosis was studied by the corneal method in mice.

2. Adrenalectomy produces a small, but definite, favourable effect on the course of the disease.

3. Administration of cortisone (0.5 or 1 mg./day) to intact or adrenalectomized mice produces a slight beneficial effect, which lasts for about three weeks. This is followed by a deleterious action involving the development of a corneal bleb and leading to perforation of the eye with extensive caseous involvement. Systemic spread of the disease to many organs also occurs.

4. The hormone also antagonizes the beneficial effect of isoniazid both in intact and in adrenalectomized mice; there is exacerbation of the localized tuberculosis and also metastatic infection.

5. Histological investigation of the material has not explained the mechanism of action of cortisone, though it has shown that it involves intense multiplication of the organisms.

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