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THE ACTIVITY OF THIOSEMICARBAZONES ALONE AND IN COMBINATION WITH OTHER DRUGS IN EXPERIMENTAL CORNEAL TUBERCULOSIS

BY

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The discovery of the antituberculosis activity of a number of benzaldehyde thiosemicarbazones was first announced by Domagk and his co-workers in 1946. Their results have since been confirmed, both experimentally and clinically, by a number of investigators. Unfortunately the more recent clinical studies (Mertens and Bunge, 1950; Simmons, Hobson, Resnick, DeNicola, and Bennett, 1950) have shown that the thiosemicarbazones so far tested clinically may produce serious toxic effects which limit their usefulness. Nevertheless these compounds remain of considerable importance especially as it is possible that thiosemicarbazones may eventually be found whose action will not be associated with serious toxic effects. Another possibility is that satisfactory chemotherapy of tuberculosis may result from the combination of thiosemicarbazones with other substances. There are indeed several findings which suggest that the combination of chemotherapeutic substances may prove of value; for example, a preliminary report by the Medical Research Council (1950) demonstrated quite clearly the effectiveness of using *p*-aminosalicylic acid with streptomycin in order to reduce the emergence of streptomycin-resistant tubercle bacilli in man.

Other workers have tried a combination of a thiosemicarbazone with another substance in experimental tuberculosis. Hurni (1949) used a thiosemicarbazone (TB1/698) with streptomycin in mice, but did not find any increased beneficial effect; on the other hand Karlson, Gainer, and Feldman (1950) were able to demonstrate the value of such a combination in experimental tuberculosis in the guinea-pig. Ulstrup (1950) used a combination of the thiosemicarbazone TB1/698 with *p*-aminosalicylic acid in experimental tuberculosis in the guinea-pig, but found only a slight potentiation.

We have used the mouse corneal technique (Rees and Robson, 1950) to test the activity of a number of thiosemicarbazones, either alone or in combination with streptomycin or *p*-aminosalicylic acid.

METHODS AND MATERIALS

The full details of the method used have been described previously by Rees and Robson (1950), who also discuss the use of the method in assessing the activity of a drug. Corneal

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tuberculosis was produced by intracorneal injections of about 1,000 viable organisms of a bovine strain of *Mycobact. tuberculosis*.

In the present investigation all treatments were maintained for a standard period of twenty-eight days. Streptomycin was given subcutaneously, and the total daily dose of 4 mg. was injected in two halves, i.e., 2 mg. in 0.2 ml. sterile distilled water, morning and evening. On Saturdays and Sundays only a single dose of 2 mg. was given in the morning. Treatment was started within a few hours of inoculation. *p*-Aminosalicylic acid (2 per cent) and thiosemicarbazone (0.085 per cent) were given thoroughly mixed into the powdered diet and treatment was started some twenty-four hours before inoculation.

RESULTS

Thiosemicarbazones.—A number of thiosemicarbazones were investigated (Bavin, Rees, Robson, Seiler, Seymour, and Suddaby, 1950), including 4-acetamidobenzaldehyde thiosemicarbazone (TB1/698) and 4-ethylsulphonylbenzaldehyde thiosemicarbazone (TB3/1374). All those which showed activity *in vivo* behaved in a very similar manner, so that only the results obtained with the ethylsulphonylbenzaldehyde compound need be described here. Preliminary toxicity experiments, in which these compounds were mixed in the diet, showed that mice tolerated approximately 5 to 10 mg. per day. With these doses there is an initial loss of weight due to loss of appetite; this confirms the observation of Hoggarth, Martin, Storey, and Young (1949). With the lower dose the mice recover after a week, but with the higher dose a number of mice ultimately die. The 5-mg. dose level was used in all the present experiments, and in fact gave chemotherapeutic results as good as the higher dose.

The following results were obtained in a typical experiment: seven mice in the untreated control group all developed tuberculous corneal lesions between the 11th and 14th days after inoculation, and these rapidly increased in size (Fig. 1A);

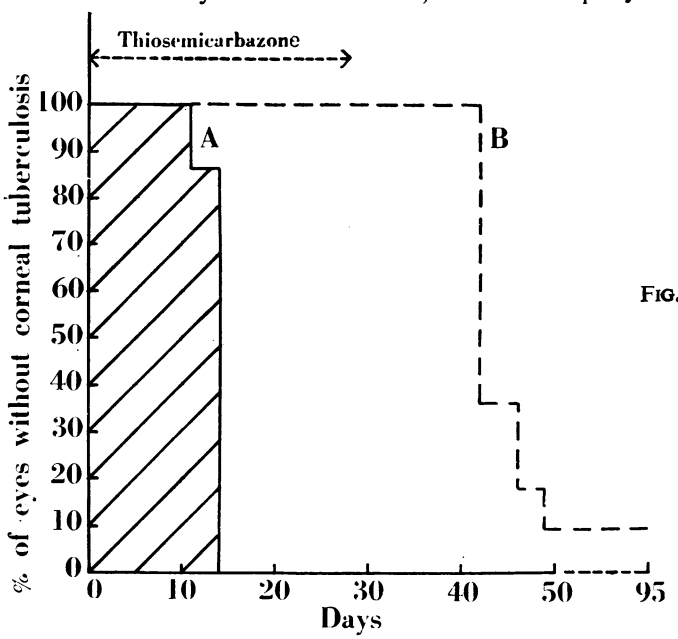

FIG. 1.—To show the effect of thiosemicarbazone on the development of corneal tuberculosis. (A) Control group. (B) Mice treated with 4-ethylsulphonylbenzaldehyde thiosemicarbazone.

eleven mice in the group treated with thiosemicarbazone remained free from corneal tuberculosis throughout the period of treatment; seven of these developed lesions on the 42nd day (i.e., 14 days after cessation of treatment), two on the 46th day, and one on the 49th day after inoculation. The one remaining mouse had a clear cornea when the experiment was terminated on the 95th day (Fig. 1B). The incubation period was markedly prolonged in all the treated mice, but the lesions, once they had appeared, rapidly increased in size and in no way differed from those seen in the untreated control animals.

Thiosemicarbazone and p-*aminosalicylic acid.*—In this experiment seven untreated control mice all developed corneal tuberculosis between the 11th and 14th days after inoculation. Of the six mice on *p*-aminosalicylic acid, five developed lesions between the 18th and 24th days, while still under treatment, and the remaining mouse on the 32nd day (Fig. 2A). All these lesions were active in type. The six

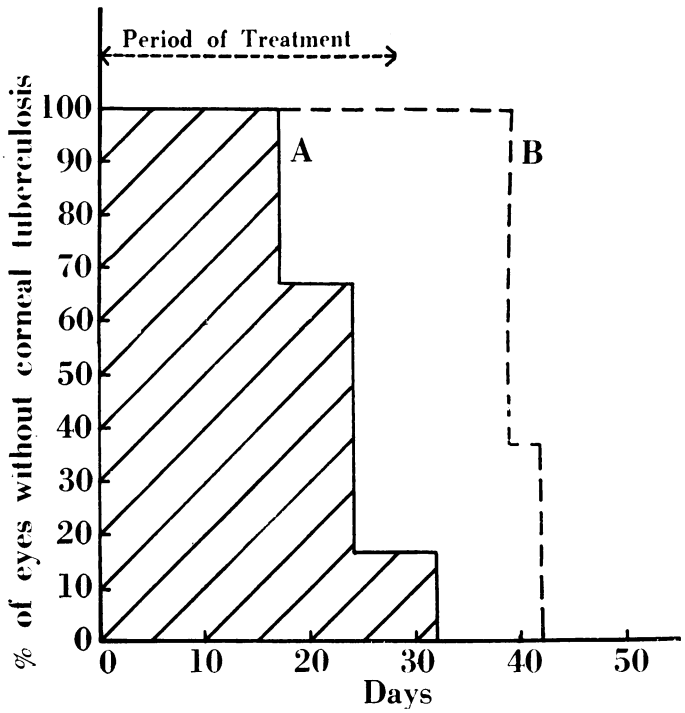

FIG. 2.—To show the effect of *p*-aminosalicylic acid alone (A) and in combination with thiosemicarbazone (B) on the development of corneal tuberculosis.

mice on thiosemicarbazone remained free from corneal tuberculosis throughout the 28-day period of treatment, but all developed active lesions between the 39th and 50th days. Eight mice were given both *p*-aminosalicylic acid and thiosemicarbazone; they remained free from corneal tuberculosis during the period of treatment, but all developed active corneal lesions between the 39th and 42nd days (Fig. 2B.)

Thiosemicarbazone and streptomycin.—In one such experiment there were eight untreated control mice, of which seven developed corneal lesions on the 11th day,

and the remaining one on the 18th day after inoculation; all were typical acute lesions. There were eleven mice on streptomycin, four of which developed corneal lesions between the 18th and 25th days while still under treatment; six of the remaining seven developed lesions between the 32nd and 46th days, and the remaining mouse was clear when the experiment was terminated 168 days after inoculation (Fig. 3A). However, the lesions in these animals on streptomycin were not all typical and acute. In six of the mice they remained minimal in size and in only four did the lesion become acute and similar to those seen in the untreated control series. There were eight mice on thiosemicarbazone, and they all remained free from corneal

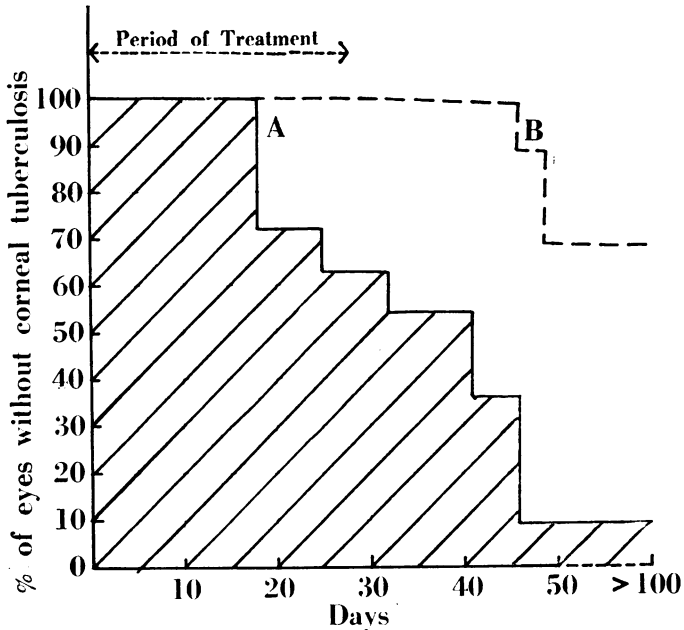

FIG. 3.—To show the effect of streptomycin alone (A) and in combination with thiosemicarbazone (B) on the development of corneal tuberculosis.

tuberculosis throughout the period of treatment; six of them developed lesions between the 42nd and 46th days and the remaining two on the 63rd and 100th day after inoculation respectively. All were typical acute lesions. On the other hand, in the group of ten mice on combined treatment only three developed corneal tuberculosis; one lesion appeared on the 46th day and remained minimal in size, and the other two appeared on the 49th day and developed into acute lesions. The remaining seven mice were still free from corneal tuberculosis when the experiment was terminated on the 168th day (Fig. 3B).

DISCUSSION

The results show that the mouse corneal method has again proved satisfactory for screening compounds for antituberculous activity, demonstrating that thiosemicarbazones possess marked chemotherapeutic activity. Indeed, additional information has been obtained which may help to throw some light on the mode of action of various drugs active in tuberculosis.

Two main criteria can be used in assessing the activity of a drug given for the standard period of 28 days: (1) the incubation period, which may fall within the 12–14 days range usually found in untreated animals or be prolonged; and (2) the type and development of lesions appearing after the incubation period. By recording these effects it has been found that each drug, or group of drugs, produces a characteristic and reproducible pattern of response. For example, with the thiosemicarbazones, and this applies not only to the 4-ethylsulphonylbenzaldehyde compound tested here but to all the active derivatives we have so far tested (Bavin *et al.*, 1950), no tuberculous lesions appear in the cornea during the 28-day period of treatment; this in itself is a characteristic which distinguishes this group of drugs from any other so far studied, but even more interesting is the observation that, despite this prolongation of the incubation period beyond the period of treatment, the eyes eventually develop tuberculous lesions at a characteristic period. The resistance of almost every eye breaks down between the 40th and 45th day, and a progressive lesion then appears which is in every way as severe as those seen in the untreated control group. Thus fifty-two mice have so far been treated for the standard 28-day period with an active thiosemicarbazone, and forty-eight of these have developed lesions with a mean incubation period of 43.8 days. The mean incubation period for the controls to these experiments was 13.3 days. Hence the mice treated with thiosemicarbazone developed lesions 15.8 days after the cessation of treatment, a period which, allowing for the elimination of the drug present in the mice on the last day of treatment, corresponds almost exactly with the mean incubation period of their controls. These results suggest that, under the conditions of these experiments, thiosemicarbazones exert essentially a bacteriostatic action, and that once the treatment has come to an end tubercle bacilli are still present in the cornea of most of the mice in sufficient number and with a sufficient degree of virulence to produce a corneal lesion with an incubation period and a subsequent rate of progress identical with that seen in untreated control mice.

The two experiments in which ethylsulphonylbenzaldehyde thiosemicarbazone was used in combination with another drug gave clear-cut results; this cannot always be achieved with other methods. Indeed, in experiments with potent chemotherapeutic substances it is often difficult to detect any advantage from combined therapy, and it may be necessary to reduce the doses considerably before it can be said with confidence that the result is not attributable to either drug used; this does not apply to the mouse corneal test, at least not with the drugs tested so far. Although streptomycin, thiosemicarbazones, and *p*-aminosalicylic acid all prolong the incubation period, there is subsequently a high incidence of lesions; even with streptomycin 50–90 per cent of the eyes develop lesions (though about half of them remain minimal), and with *p*-aminosalicylic acid and thiosemicarbazones more than 90 per cent succumb to the infection and develop progressive lesions. There remains therefore considerable scope for demonstrating any therapeutic advantage which a combined therapy may possess.

In the experiment in which a combination of *p*-aminosalicylic acid and thiosemicarbazone was used there was no evidence of an additive effect: the group of mice which received the combined treatment was no better than that receiving thiosemicarbazone only. This suggests, incidentally, that the mechanism of action

of *p*-aminosalicylic acid and thiosemicarbazones is rather similar, the latter group of drugs being more effective than *p*-aminosalicylic acid.

On the other hand the results in the mice treated with a combination of thiosemicarbazone and streptomycin were unequivocally better than those in the animals treated with either drug alone. In the experiment described here seven out of ten mice on combined therapy were free from corneal tuberculosis when the experiment was terminated 140 days after the end of treatment. This represents an additive effect even more striking than that obtained by the same method with a combination of streptomycin and *p*-aminosalicylic acid (Rees and Robson, 1950).

SUMMARY

1. Corneal tuberculosis, produced by an intracorneal injection of about 1,000 viable organisms of a bovine strain of *Mycobact. tuberculosis*, was used to assess the chemotherapeutic activity of 4-ethylsulphonylbenzaldehyde thiosemicarbazone (TB3/1374) given alone and in combination with *p*-aminosalicylic acid or streptomycin.

2. TB3 and other active thiosemicarbazones tested showed marked antituberculous activity, producing a prolongation of the incubation period, but over 90 per cent of eyes eventually developed active tuberculosis. The results suggest that, under the conditions of this test, the action of thiosemicarbazones is essentially bacteriostatic.

3. A combination of *p*-aminosalicylic acid and thiosemicarbazone showed no advantage over thiosemicarbazone alone.

4. A combination of streptomycin and thiosemicarbazone showed a definite additive effect, greater than that produced under the same experimental conditions by a combination of streptomycin and *p*-aminosalicylic acid.

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