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STUDIES IN THE CHEMOTHERAPY OF TUBERCULOSIS:

PART VI. THE COMPARISON OF SOME SYNTHETIC SUBSTANCES WITH STREPTOMYCIN USING MICE INFECTED WITH A LIGHT INOCULUM OF *MYCOBACTERIUM TUBERCULOSIS*

BY

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In preceding papers we have described the preliminary examination of a number of types of synthetic substances for activity against a tuberculous infection in mice. In these experiments the animals were infected with a relatively large dose of *Mycobacterium tuberculosis* given intravenously, and the compounds were administered at doses ranging downwards from the maximum tolerated dose, beginning just before infection. Among the compounds found to show activity under these conditions were the following: 4 : 4′-diaminodiphenyl sulphone (Hoggarth and Martin, 1948), 2-*p*-chloroanilino-4-δ-diethylamino-α-methylbutylamino-6-methylpyrimidine dihydrochloride (Hoggarth, Martin, Paige, Scott, and Young, 1948), and a number of thiosemicarbazones derived from *p*-substituted benzaldehydes (Hoggarth, Martin, Storey, and Young, 1949). We have now examined these compounds further under other test conditions and compared their effects with those of *p*-aminosalicylic acid and streptomycin. Under the conditions of the preliminary tests, the lesions produced in the lungs of the mice are predominantly necrotic; by reducing the infecting inoculum from 0.75 mg. of moist organisms to 0.02 mg. or less, the disease is still progressive and unremitting, but in the majority of the animals the lesions are essentially proliferative, with a much smaller degree of necrosis (Stewart, 1950). Using animals infected with these smaller inocula, we have compared the effects produced by the above compounds, when given from the time of infection or in two cases after a delay of 21 days, by which time the disease had become far advanced.

EXPERIMENTAL METHODS

Methods of balancing the groups of mice and infecting them so as to ensure the highest degree of uniformity between the groups will be described in detail by Stewart (1950). The numbers of animals in each group, which varied in the different experiments, are shown in parentheses against the graphs (below) to which they relate. With the

exception of streptomycin (which was given subcutaneously morning and evening daily except on Saturdays and Sundays) and of No. 5961 (see below), the drugs were given every day mixed with the food, under conditions which permitted a check to be made on the amount of food consumed. The concentration of the various drugs in the food was adjusted to provide the desired daily dose on the assumption that the amount of food eaten per mouse per day was 4 g. per 20 g. of body weight. All the animals received powdered food of the same composition as that described by Thomson (1936), and the drugs were passed through a 60-mesh sieve before being mixed with the food. Water was freely available to the mice at all times. One of the drugs (No. 5961) made the food so unpalatable that the mice refused to eat it, and this drug was given by syringe and catheter as an aqueous suspension morning and evening for five days a week.

No attempt was made in these experiments to carry out a full histological study of the animals as they died, but naked-eye examination of the lungs showed the presence of obvious tuberculous lesions in all except a few mice. Those mice which died without evidence of severe involvement of the lungs were not included in the data from which the graphs were constructed. Such non-specific deaths occurred during the first few weeks of the experiment and did not usually exceed 5 per cent of the total.

RESULTS

4 : 4′-Diaminodiphenyl sulphone (No. 371) was included in five experiments (Figs. 1 to 5). In each experiment this substance produced a very marked effect, much greater than had been expected from the results previously obtained under the conditions of the preliminary test. In unpublished experiments we have confirmed the observations of other workers (e.g., Raleigh and Youmans, 1948) that both *p*-aminosalicylic acid (No. 8005) and streptomycin prolong the life of mice

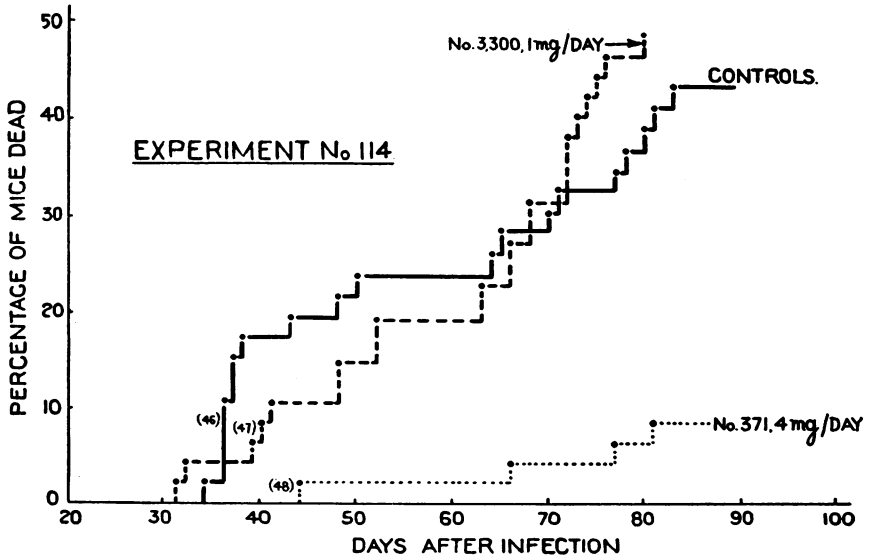

FIG. 1.—Comparison of therapeutic effect of 4 : 4′-diaminodiphenyl sulphone (No. 371) with a pyrimidine compound, 2-*p*-chloroanilino-4-δ-diethylamino-α-methylbutylamino-6-methylpyrimidine hydrochloride (No. 3300).

receiving a large inoculum of tubercle bacilli. The effect of *p*-aminosalicylic acid (at the relatively high dose of 40 mg. per 20 g. per day) on mice infected with 0.02 mg. of bacilli (Fig. 4) we found to be somewhat inferior to that of 4 : 4′-diaminodiphenyl sulphone. When the dose of *p*-aminosalicylic acid was reduced to 8 mg.

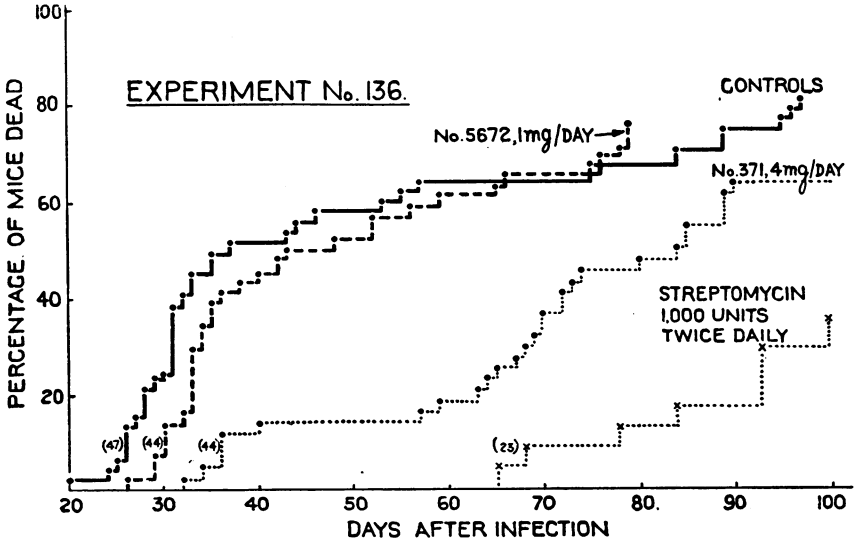

FIG. 2.—Comparison of therapeutic effect of the thiosemicarbazone of *p*-dimethylaminobenzaldehyde (No. 5672) with streptomycin and 4 : 4′-diaminodiphenyl sulphone (No. 371).

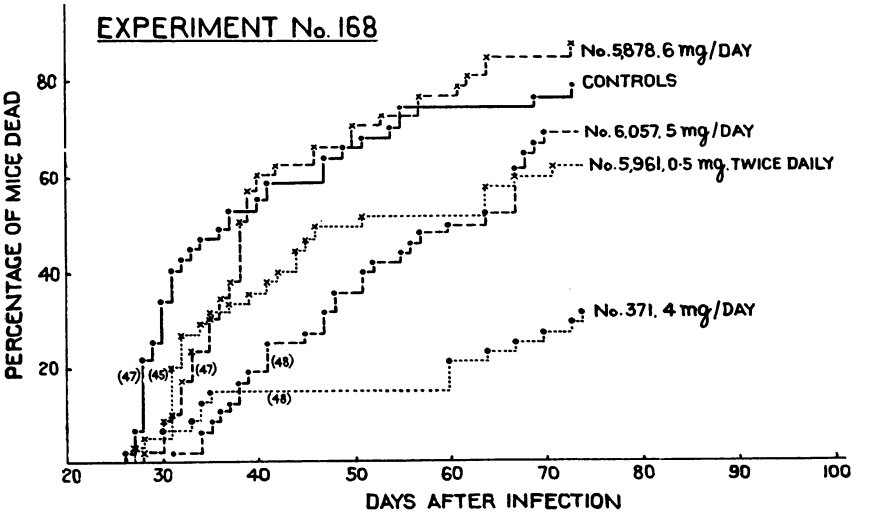

FIG. 3.—Comparison of therapeutic effects of three thiosemicarbazones: *p*-dimethylaminobenzaldehyde 4-*iso*butylthiosemicarbazone (No. 5878), *p*-N-pyrrolidinobenzaldehyde thiosemicarbazone (No. 5691), and *p*-methoxybenzaldehyde thiosemicarbazone (No. 6057) with 4 : 4′-diaminodiphenyl sulphone (No. 371).

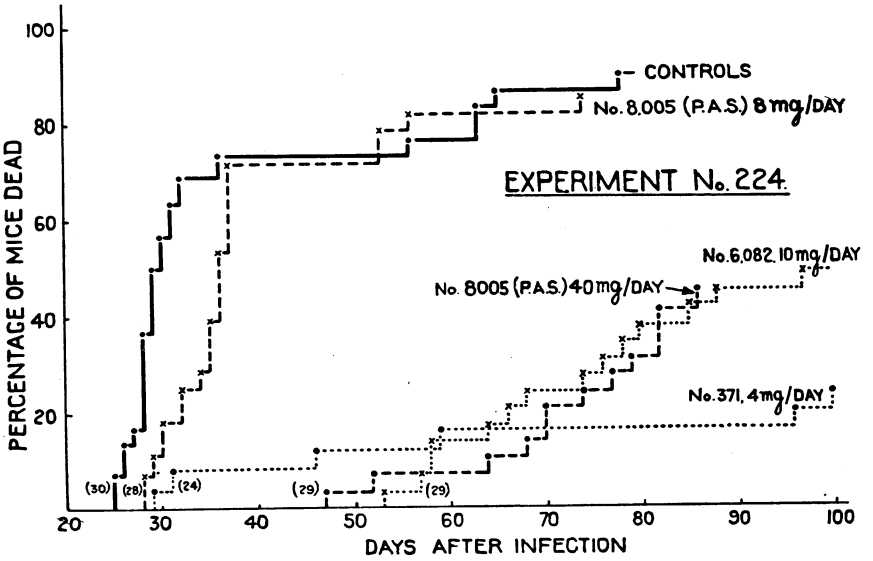

FIG. 4.—Comparison of therapeutic effects of *p*-hydroxybenzaldehyde thiosemicarbazone (No. 6082) and *p*-aminosalicylic acid (No. 8005) with 4:4′-diaminodiphenyl sulphone (No. 371).

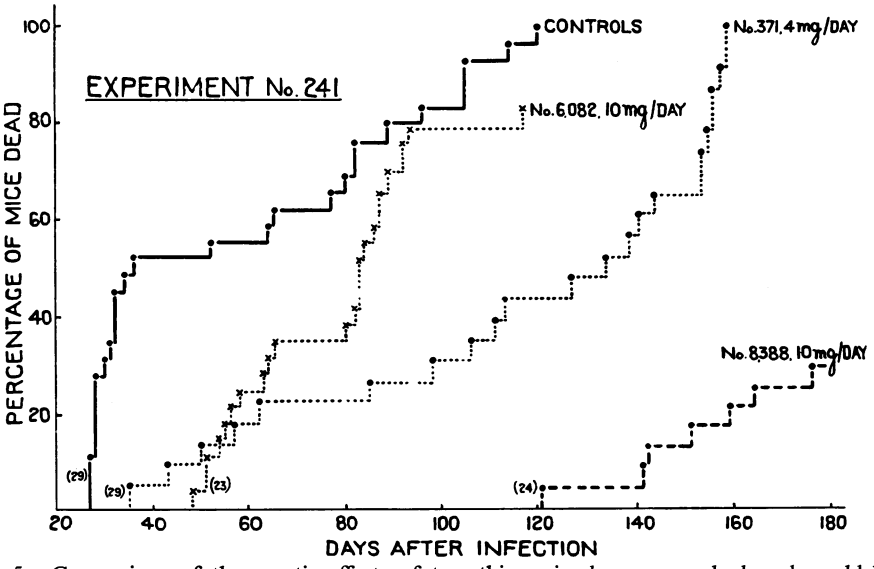

FIG. 5.—Comparison of therapeutic effects of two thiosemicarbazones: *p*-hydroxybenzaldehyde thiosemicarbazone (No. 6082) and *p*-ethylsulphonylbenzaldehyde thiosemicarbazone (No. 8388) with 4:4′-diaminodiphenyl sulphone (No. 371).

per 20 g. per day, no effect was detected. A very marked effect was produced by streptomycin (1,000 units per mouse twice daily) in another experiment of this type (Fig. 2).

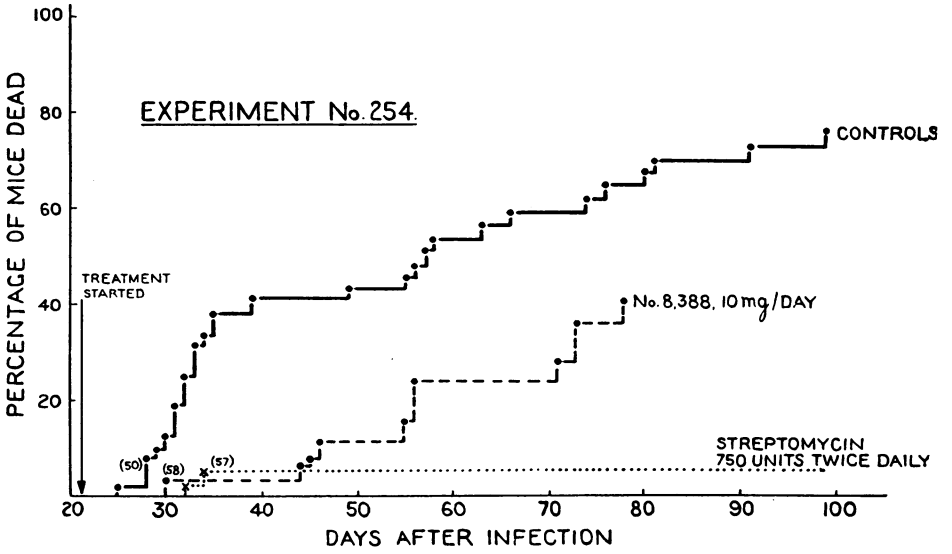

FIG. 6.—Comparison of therapeutic effect of *p*-ethylsulphonylbenzaldehyde thiosemicarbazone (No. 8388) with streptomycin.

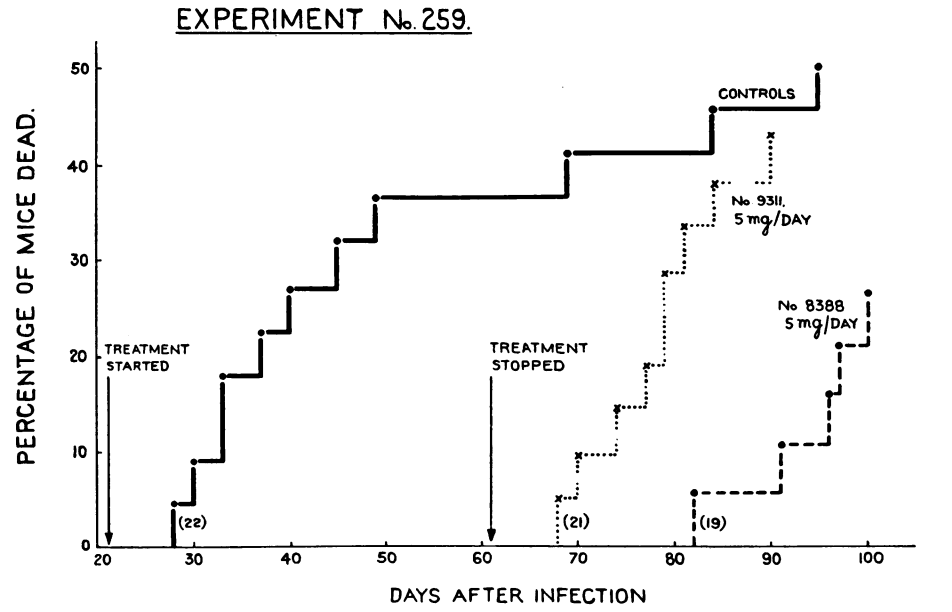

FIG. 7.—Comparison of the therapeutic effects of two thiosemicarbazones: *p*-ethylsulphonylbenzaldehyde thiosemicarbazone (No. 8388) and *p*-acetylaminobenzaldehyde thiosemicarbazone (No. 9311) with streptomycin. (No animals treated with streptomycin died before 100 days.)

Of the synthetic compounds which we have examined, the following had no effect upon animals dying from the proliferative lesions produced by the lighter infection: 2-*p*-chloroanilino-4-δ-diethylamino-α-methylbutylamino-6-methylpyrimidine dihydrochloride (No. 3300, Fig. 1), the thiosemicarbazones of *p*-dimethylamino- and *p*-N-pyrrolidino-benzaldehyde (Nos. 5672 and 5961, Figs. 2 and 3) and *p*-dimethylaminobenzaldehyde 4-*iso*butylthiosemicarbazone (No. 5878, Fig. 3). The thiosemicarbazones of *p*-nitrobenzaldehyde (No. 6056), *p*-methoxybenzaldehyde (No. 6057), and *p*-hydroxybenzaldehyde (No. 6082) all exerted some effect upon the disease produced by the lighter infection (Figs. 3, 4, and 5). The graph for No. 6056, included in Experiment 168 (Fig. 3), is not drawn, as it was almost coincident with that for No. 6057.

The best results which we have observed with a thiosemicarbazone in mice infected with 0.02 mg. of bacilli were obtained with the thiosemicarbazone of *p*-ethylsulphonylbenzaldehyde (No. 8388, Figs. 5 and 6). The graph representing the results of Experiment No. 241 (Fig. 5) shows the effect produced by this drug on a group of animals to which it was administered, as was usual, over the whole period of the experiment; a similar group was included in which administration of drug was stopped at 85 days. The animals in this latter group began to die more quickly after withdrawal of the drug than did the animals under continued treatment. For example, at 180 days, 60 per cent of the animals dosed for only 85 days were dead, as compared with 29 per cent of the animals under continued treatment. The closely related compound No. 8574, the thiosemicarbazone of *p*-methylsulphonylbenzaldehyde, was much less effective. The graph for this compound is not drawn as it was almost coincident with that for 4 : 4′-diaminodiphenyl sulphone (No. 371). In Experiment No. 254 (Fig. 6), treatment was delayed until 21 days after infection, by which time the disease was well advanced, since the control animals left untreated began to die on the 25th day. A marked effect was observed with No. 8388 at a dose of 10 mg. per 20 g. per day, and even at a dose of 2.5 mg. per 20 g. per day the effect was only slightly smaller. Streptomycin (750 units per mouse twice daily) produced a much better effect.

Recent publications (e.g., Heilmeyer, 1949; *Lancet*, 1950) show that the thiosemicarbazone of *p*-acetylaminobenzaldehyde (under the code number Tb.I/698) has been used clinically in the treatment of tuberculosis. We have compared this compound (No. 9311) with the thiosemicarbazone of *p*-ethylsulphonylbenzaldehyde (No. 8388) and with streptomycin in an experiment (Fig. 7) in which dosing was delayed until 21 days after infection and then continued for 40 days. Both thiosemicarbazones markedly prolonged the lives of the treated animals, the effect of No. 8388 being somewhat the greater. Streptomycin was more effective than either, since mice receiving 1,000 units twice daily were all alive at the conclusion of the experiment—hence these animals are not represented in Fig. 7. Although the mice receiving streptomycin appeared at the end of the experiment to be in good condition and their average weight was considerably greater than that of the other treated mice, their lungs, when examined *post mortem*, were found to be grossly enlarged and abnormal, being scarcely distinguishable from those of the mice treated with Nos. 8388 and 9311.

Discussion

The results of the first five experiments described above lead to the conclusion that the synthetic compounds examined are in general placed in the same order when tested in mice infected with a small inoculum (as described here) as they are when tested in mice infected with a large dose of organisms (as described in previous reports). 4 : 4′-Diaminodiphenyl sulphone is exceptional in producing an unexpectedly good response under the new conditions. It is possible that the predominantly necrotic lesions found in the lungs of the more heavily infected animals contain substances antagonistic to the action of 4 : 4′-diaminodiphenyl sulphone, and that these substances are present to a smaller extent in the essentially proliferative lesions produced by the smaller inoculum. In experiments in which dosing was delayed the superiority of streptomycin was apparent in the improved condition and the gain in weight of the animals soon after starting treatment. On the other hand, animals in the groups treated with No. 8388 showed a temporary loss in weight and condition immediately after administration of this drug. The inferiority of No. 8388 may in part have been due to the temporary reduction of the intake of food and loss in weight which we have observed to take place when mice are offered food containing this drug. Animals offered such food are found to eat only 60–70 per cent of the normal amount during the first seven days. A temporary loss in weight has been observed also, when this drug is first given by mouth.

Summary

A number of synthetic substances, shown in earlier papers to extend the survival time of mice infected intravenously with a heavy inoculum of *Mycobacterium tuberculosis*, have been examined under the different conditions which prevail when the infecting dose is reduced. The less active compounds have no significant effect upon the course of the disease under the new conditions, but a definite response has been observed with the more active members of a group of thiosemicarbazones. The best of these compounds show "activity" greater than that observed with *p*-aminosalicylic acid or with 4 : 4′-diaminodiphenyl sulphone, but this activity is much less than that exerted by streptomycin under the same conditions.

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