p-Anisaldehyde-Thiosemicarbazone in Treatment of Experimental Murine Tuberculosis.* (17985)

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Since Domagk and his coworkers(1) described the antimycobacterial properties of various thiosemicarbazones, these compounds have undergone laboratory and clinical investigation(2-9). This is a preliminary study of the antimycobacterial activity of one of the more promising thiosemicarbazones described by Domagk(1).

Materials and method. In vitro testing. p-anisaldehyde-thiosemicarbazone was prepared for *in vitro* testing as follows: A 0.50% solution of the compound was made up in hot 50% alcohol. This was diluted in Dubos' medium(10) to suitable concentrations, inoculated with *Mycobacterium tuberculosis* $H_{37}RV$ and with *Mycobacterium tuberculosis* B1 and incubated for 14 days. The results indicated that the compound had far greater *in vitro* activity than streptomycin or para-aminosalicylic acid(11). It prevented the growth of both strains of the tubercle bacillus at a concentration of 1.0 μg%.

In vivo testing. Fifty dba mice, weighing approximately 25 g, were infected intravenously with 0.5 cc of a 7-day culture of $H_{37}RV$ grown at 37°C in Dubos' Tween-albumin medium. Twenty-four hours after the infection the mice were divided into 2 groups of 25 each. The first control group was untreated. The second group received the thiosemicarbazone mixed in ground mouse food in 1% concentration *ad libitum*.

Thirty days after the infection all the untreated controls were dead. At autopsy these animals all showed extensive ++++ gross pulmonary tuberculosis. No other gross lesions were observed. Two of the treated animals died within 4 days of infection from causes other than tuberculosis. Fourteen of the remaining treated animals were sacrificed at the end of 30 days. The autopsies revealed no gross pulmonary tuberculosis nor other lesions, except for the spleens, which were markedly enlarged. The remaining 9 treated mice, which appeared to be in good health, were sacrificed 75 days after infection. The autopsies revealed an average of only one plus or very slight pulmonary tuberculosis. The liver and kidneys appeared normal. The spleens, again, were enlarged, as in the treated animals autopsied after 30 days.

Summary. 1. One mg % of p-anisaldehyde-thiosemicarbazone inhibits the growth of the $H_{37}RV$ and Bl strains of *Mycobacterium tuberculosis* in Dubos' medium.

2. Mice infected with the $H_{37}RV$ strain and fed 1% p-anisaldehyde-thiosemicarbazone in their diet had no gross tuberculosis when sacrificed at the end of 30 days, when all controls were dead of the disease. Infected

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Erratum

Article on p-Anisaldehyde, Vol. 74, p. 595, Summary, No. 1, should read

mice, sacrificed after 75 days of treatment with p-anisaldehyde-thiosemicarbazone, showed only one plus gross pulmonary tuberculosis.

3. It may be concluded that p-anisaldehyde-thiosemicarbazone is an effective antimycobacterial agent in the mouse as well as *in vitro*.

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A Comparative Study of Susceptibility of Tubercle Bacillus ($H_{37}RV$) to Aureomycin, Streptomycin, and Para-aminosalicylic Acid.* (17986)

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Aureomycin, an antibiotic derived from *Streptomyces aureofaciens*, has been effective in the treatment of a wide variety of bacterial, rickettsial and virus diseases(1). For the purpose of exploring its possible usefulness as an antibiotic agent in tuberculosis, experiments with this agent were carried out to compare it with streptomycin and para-aminosalicylic acid. The latter 2 antibiotics are known to be active against the tubercle bacillus(2,3), while only brief mention of the activity of aureomycin against this organism has been made(4,5,6).

In vitro experiment. Sterile solutions of streptomycin and aureomycin were prepared in Dubos' Tween-albumin medium. The para-aminosalicylic acid was dissolved in a saturated solution of $NaHCO_3$ and then brought to pH 7.0 with M/15 phosphate buffer. The solution was Seitz-filtered and then added to Dubos' medium. The concentrations of streptomycin, aureomycin, and para-aminosalicylic acid tested, ranged from 0.1 to 30.0 μg/cc of medium. The tubes containing 5 cc of each dilution were inoculated with 0.1 cc of a 10-day $H_{37}RV$ culture from Dubos' medium. The concentrations of aureomycin were maintained by adding suitable amounts of aureomycin every 24 hours for the duration of the experiment, since its rate of deterioration is fairly constant(1). The amount of growth was graded from ++++, representing a milky growth, to 0, representing no growth. Whenever present, growth was checked by stained smears. Aureomycin compares favorably with streptomycin and para-aminosalicylic acid in inhibiting the growth of the tubercle bacillus *in vitro* (Table I).

In vivo experiments. Seventy-five dba mice, averaging 25 g in weight, were infected intravenously with 0.1 mg of $H_{37}RV$ suspended in 0.2 cc of saline. The mice were then divided into 5 groups of 15 each. Group I was untreated, Group II received 1000 μg/cc of streptomycin, injected subcutaneously, in 0.25 cc of saline every 12 hours. Group III received 1.5% para-aminosalicylic acid incorporated in the diet. Group IV and Group V received 1000 and 5000 μg/cc, respectively, of aureomycin, injected subcutaneously in

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