mine solutions, but on the basis of the dialysis-equilibrium experiments this effect cannot be attributed to binding of histamine by the protein. Schayer(5) has pointed out the non-equilibration of guinea pig organs with exogenous histamine *in vivo*. The present results show that *in vitro* guinea pig lung is incapable of binding added histamine or of equilibrating added with bound histamine. Apparently, binding of histamine requires formation at the binding site.

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Anti-mycobacterial Properties of a New Derivative of Isoniazid. (20595)

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Since the first description of the marked anti-tuberculous properties of isoniazid(1,2,3, 4) and iproniazid, it has been shown that derivatives of isoniazid in which one or both of the terminal hydrogens have been replaced by substitutions might possess similar high activity both *in vitro* and *in vivo*(5,6,7,8). Of the numerous compounds of this type synthesized by Dr. H. H. Fox in the Roche Chemical Research Laboratories, which were studied for their activity against *Mycobacterium tuberculosis* and were found to exert the expected effect, one substance appeared to have favorable properties of both toxicity and chemotherapeutic activity. This substance, 1,1'-methylenebis(2-isonicotinylhydrazine) from now on designated as Ro 2-4969, which contains 2 isoniazid molecules linked by the methylene group is characterized by the lack of solubility in water, alcohol and most other organic solvents. Despite this physical property which distinguishes it sharply from other active members of the hydrazine series, it exhibited an effect comparable to the parent substance. The results of the experimental studies carried out with Ro 2-4969 are reported in the present note.

Materials and methods. The H37Rv strain of *M. tuberculosis* obtained through the courtesy of Mr. W. Steenken, Jr., of Trudeau Sanatorium on December 20, 1947 was used in all experiments. Adult albino mice of 18-20 g from our own colony were used throughout the investigation. The technics of the experiments in mice used in the evaluation of Ro 2-4969 are identical with those described in earlier publications(2,9) and are based on the effect of a 21 day treatment with medicated diet and examination of the lungs both immediately after discontinuation of treatment as well as 21 days after termination of therapy. The lack of solubility of Ro 2-4969 prompted also experiments in which small pellets were inserted in the subcutaneous tissue. This was followed by intravenous infection with *M. tuberculosis* H37Rv at different intervals after the implantation. Further details of the procedure are given in the text.

Toxicity for mice. Acute toxicity tests carried out by administering a single dose of the compound by gavage gave an LD_{50} of 3900.0 mg/kg.*

In vivo experiments with M. tuberculosis. 1. *Effect of oral drug administration.* If medicated diet with Ro 2-4969 was started immediately after the intravenous infection with strain H37Rv and continued for 21 days at which time the mice were sacrificed and examined, the majority of animals were found to be protected at a dose of 25.0 mg/kg/day

* Experiments in different species of animals including chronic toxicity tests have been carried out by Dr. Benson and associates in the Roche Pharmacology Laboratory and will be reported elsewhere.

TABLE I. Antitubercular Activity of Ro 2-4969 in Intravenous Infection of Mice with Strain H37Rv. Infection: 0.5 ml of a 10^{-1} dilution of a 7- to 10-day-old culture in Dubos' medium. Treatment: Medicated diet for a 21-day period.

Daily dose, mg/kg	No. of mice	Mice protected No.	Mice protected %	No. neg. cultures / No. cultures taken
25.0	40	39	97.5	21/27
12.5	19	6	31.6	1/19
6.25	20	0	0.0	4/20
Controls	39	0	0.0	3/39

TABLE II. Antitubercular Activity of Ro 2-4969 in Intravenous Infection of Mice with Strain H37Rv. Infection: 0.5 ml of a 10^{-1} dilution of a 7- to 10-day-old culture in Dubos' medium. Treatment: Medicated diet for 21 days. Normal diet for 21 days.

Daily dose, mg/kg	No. of mice	Mice protected No.	Mice protected %	No. neg. cultures / No. cultures taken
500	17	16	94.1	10/14
250	20	8	40.0	11/20
125	17	10	58.8	5/14
50	10	3	30.0	3/10
Controls	17	0	0.0	1/9

(Table I). These mice also showed the phenomenon of lack of growth of cultures taken directly from lung tissue. This observation which seems to be characteristic for isoniazid and related compounds(1) has meanwhile been observed by other investigators (10,11,12) with isoniazid. The 50% protective dose (PD_{50}) as calculated according to Reed and Muench was found to be 15.3 mg/kg/day for Ro2-4969. If the observation period was prolonged for an additional 3 weeks without therapy, the low doses used in the first group of experiments could be expected to be inactive since isoniazid required doses of more than 50.0 mg/kg/day for lasting protection(2). This indeed was the case and a dose of 125.0 mg/kg/day or more of Ro 2-4969 was necessary to produce lasting protection (Table II). The PD_{50} for this type of experiment was calculated to be 171 mg/kg/day. The higher dose of 250.0 and 500.0 mg/kg/day also showed absence of viable bacilli in at least 70% of the animals cultured. That this observation does not constitute a claim of "sterilization" of the lung tissue has been mentioned in earlier papers(1,2).

2. *Protective effect of implanted pellets.* Small pellets containing 25.0 mg of Ro 2-4969 which were prepared for us by Dr. L. Magid of the Roche Pharmaceutical Laboratories, were implanted in the dorsal subcutaneous tissue of mice. While in the majority of animals the pellet caused no untoward effects, some animals showed signs of necrotization or at least loss of hair at the site of implantation. The pellets were, however, palpable for periods of 2-3 weeks. At intervals of 0, 3, 7, 14, 21 and 42 days following implantation of the pellet, the animals were infected with *M. tuberculosis* H37Rv intravenously. Each group of animals originally consisting of 10 was sacrificed on the 21-25th day after infection and the lungs examined for the presence or absence of gross lesions.

The results of these experiments (Table III) indicate that marked protection was conferred on the majority of animals infected within the first 3 days after the implantation of the pellet. Even after 7 days 50% of the animals were free of lesions. If 2 weeks or longer had elapsed, no appreciable protective effect was observed. The slow release of Ro 2-4969 from the site of implantation was seemingly sufficient to produce an effect similar to the continuous drug absorption from the intestinal tract even to the extent that also about 60% of the animals which were infected within 3 days after pellet implantation showed negative cultures from their lungs. One can, therefore, characterize the effect of the implantation of the pellet by stating that a sufficient amount of the compound seems to be released for a period of about 25 days so as

TABLE III. Protective Effect of Pellets of Ro 2-4969 in Intravenous Infection of Mice with Strain H37Rv. Infection: 0.5 ml of a 10^{-1} dilution of a 7- to 10-day-old culture in Dubos' medium. Treatment: 25 mg pellet implanted subcutaneously.

Insertion of pellets .... days before infection	Total observation (days)	Protection rate*	No. neg. cultures / No. cultures taken
0	25	9/9	5/9
3	24	8/9	5/8
7	28	5/10	1/10
14	35	3/10	1/10
21	42	0/10	1/9
42	63	0/9	0/8
Controls	24–25	0/20	1/20

* No. of mice free of lesions/No. of treated mice.

to inhibit the lesions in a majority of animals and even to interfere with the recovery of the causative organism.

Discussion. The experiments presented seem to indicate that the insoluble isoniazid derivative, 1,1'-methylenebis(2-isonicotinyl hydrazine), is apparently absorbed both from the gastro-intestinal tract and the subcutaneous tissue in sufficient amounts to exert a marked protective effect in the intravenous *M. tuberculosis* infection of mice. Since a method of isoniazid determination can be used successfully for the determination of blood concentrations of this compound(13), one might assume that a breakdown to isoniazid takes place in the body.

It is, therefore, also not surprising that the activity of this substance is fundamentally the same as that of isoniazid. Due probably to its slower and perhaps less complete distribution, the activity appears to be about 3-4 times less than that of isoniazid. The low toxicity results in a more favorable chemotherapeutic ratio of an LD_{50}/PD_{50} of 255 as compared to 44.1 for isoniazid(1). The assumption that a compound like Ro 2-4969 with its still quite appreciable anti-tuberculous activity might be suitable for producing a therapeutically favorable slow absorption appropriate to build up a consistently high blood level seems to be born out by the investigations of Larson and Dickie(13).

It might also be mentioned at this point that preliminary experiments indicate that rats infected with *M. lepraemurium* and kept on a diet of 0.4% Ro 2-4969 for a period of 4 months failed to develop any lesions even 5 months after discontinuance of the medicated diet.

Summary. (1) Chemotherapeutic experiments with a new insoluble derivative of isoniazid, 1,1'-methylenebis(2-isonicotinyl hydrazine), are described which show that this compound which was of very low toxicity possessed marked anti-tuberculous properties in mice infected with *M. tuberculosis* H37Rv. (2) This substance was evidently absorbed from the intestinal tract as well as from the subcutaneous tissue where it was implanted in the form of a pellet. (3) Although the new compound was found to be 3-4 times less active than isoniazid on the basis of the absolute value obtained for the 50% protective dose, the ratio LD_{50}/PD_{50} was very favorable (255) due to the low toxicity.

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