the adrenals substantially modifies the response to ACTH in the dog, but is without effect on pyrogen induced leukocyte responses. It is concluded that pyrogen produces its characteristic changes in the W.B.C. count by mechanisms other than pituitary-adrenal discharge.

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Anti-Tubercular Activity *in vivo* of Nicotinaldehyde Thiosemicarbazone and Its Isomers. (18673)

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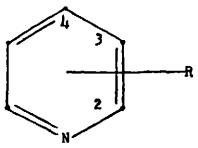
The anti-tubercular activity of 4-acetylaminobenzaldehyde thiosemicarbazone (Tibione) has been well established in experimental infections(1-5). The extensive chemical variations in the series of thiosemicarbazones has not yet resulted in chemically related compounds of appreciably higher activity(6-8), although the ethyl sulfonate of Tibione appeared somewhat superior to the parent substance in the studies by Hoggarth and Martin (4,8). These authors(4) also mention one heterocyclic compound of marked activity: quinoline-4-aldehyde thiosemicarbazone. The recent observations of Levaditi and his coworkers(9) according to which the thiosemicarbazone of nicotinaldehyde exerted a marked effect in experimental tuberculosis of mice seemed to offer a new approach to chemotherapeutic agents with anti-tubercular activity. Levaditi's findings are in agreement with the results of studies with compounds of this type carried out in our laboratories during the last year. This work was undertaken in connection with the studies of Chorine(10) and of McKenzie *et al.*(11) on the effect of niacinamide on experimental tuberculosis. While we were able to confirm the activity of this substance, we failed, as had McKenzie and his group(12) to find derivatives of niacinamide which were superior to the parent substance, until the thiosemicarbazone derivatives were synthesized in the Roche Chemical Laboratories. Of the numerous compounds synthesized by Drs. H. H. Fox, T. S. Gardner, F. A. Smith and E. Wenis and investigated during recent months, the biological properties of the following three substances will be described: 1. the thiosemicarbazone of picolinaldehyde (I); 2. the thiosemicarbazone of nicotinaldehyde (II), and 3. the thiosemicarbazone of isonicotinaldehyde (III). Tibione was used as the reference substance.



$R = CH{=}N{-}NH{-}\overset{\overset{S}{\|}}{C}{-}NH_2$

I in 2—position
II in 3— ''
III in 4— ''

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Materials and methods. Strain: The H37Rv strain of *M. tuberculosis* obtained through the courtesy of Dr. W. Steenken, Jr., of Trudeau Sanatorium on December 20, 1947 was used in all the experiments. *Culture*: The cultures were grown in 5.0 ml Dubos' medium containing Tween 80, but without albumin.

In vitro experiments. Serial dilutions of the compounds to be tested were made in 5.0 ml Dubos' medium containing Tween 80. Each test tube was inoculated with 0.05 ml of a 10^{-1} dilution of a 10- to 14-day-old culture of *M. tuberculosis* and incubated at 37°C. A final reading for the presence or absence of growth was taken at the end of 21 days. *In vivo experiments.* The technic of these experiments was similar to that recommended by Youmans(13). Adult albino mice of 18-20 g from one colony were used throughout the investigation. The mice were infected intravenously by route of the tail vein with 0.5 ml of a 10^{-1} dilution of a 7-10-day-old culture of *M. tuberculosis* in Dubos' medium which corresponds approximately to 100 minimal infective doses. Cultures of this age had a density of 65-70% light transmission determined by the lumetron photometer. The compounds to be tested were administered to the animals in the form of a medicated diet. This was accomplished by incorporating the desired concentration of the drug to be tested in powdered Rockland mouse food and allowing the animals to feed *ad libitum* from waste proof mouse feeders. At the end of 21 days, at which time all control animals were usually still alive, all mice were sacrificed and the lungs examined for the presence or absence of tuberculous lesions. The number of lesions present in lungs were usually graded in the following manner: 4+ = widespread foci in lungs; 3+ = numerous foci in lungs; 2+ = few foci in lungs; 1+ = 1-2 foci in lungs, and 0 = no observable foci. In the present note only the percentage of animals which were completely protected, *i.e.* absence of gross lesions, will be given. In order to characterize the response of this infection to the known anti-tubercular agents, the following data from numerous experiments are given: streptomycin, 50 mg/kg/day subcutaneously: 362 out of 453 mice protected = 80%; para-aminosalicylic acid, 2500 mg/kg/day by diet: 154 out of 159 mice protected = 96%; niacinamide, 2500 mg/kg/day by diet: 48 out of 50 mice protected = 96%. In addition to the intravenous infection the intranasal route leading to bronchogenic spreading has been used. The same culture dilution (10^{-1}) was selected as in the experiments with intravenous infection, since virulence tests had shown that the mice were equally susceptible to both routes of infection. The technic of the intranasal infection was the same as that described by Buck *et al.*(14) for the intranasal pneumococcus infection. Mice were infected, under light ether anesthesia, with four drops of a 10^{-1} dilution of a 7-10-day-old culture of *M. tuberculosis* H37Rv in Dubos' medium instilled by means of a 25 gauge needle from a 2.0 ml syringe. The mice were kept on medicated diet for 21 days after which time they were sacrificed and the lungs examined for lesions. These lesions were found to be not of the miliary type but of the confluent type characteristic for bronchogenic spreading. The lesions were graded as 4+ and 3+ where the entire lungs were affected in the sense of a tuberculous pneumonia while in the case of a lower degree of infection multiple smaller lesions of bronchogenic character were observed. In this note only the percentages of fully protected animals (absence of gross lesions) are given.

Experimental results. In vitro experiments. The soluble hydrochloride of nicotinaldehyde thiosemicarbazone was found to be bacteriostatic against *M. tuberculosis* H37Rv at a concentration of 1.6-3.2 mg % which agrees fairly well with the value of 6.0 mg % published by Levaditi *et al.*(9). The other 2 compounds were not tested because of their low solubility. Tibione was active at a concentration of 0.2 mg %.

Toxicity. Acute toxicity tests carried out by administering a single dose of the com-

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

Thiosemicarbazone	No. of mice	% medicated diet	Approx. dose: mg/kg	% protection
I	14	.02	50	0
II	48	.2	500	100
	59	.1	250	96.8
	10	.05	125	100
	18	.02	50	83.3
	20	.01	25	0
III	18	.2	500	100
	28	.1	250	90
	10	.05	125	80
	18	.02	50	80
	20	.01	25	0
Tibione	10	.2	500	100
	39	.1	250	97.5
	40	.05	125	95
	30	.02	50	50
	56	.01	25	1.8
Controls	141	—	—	0

pound by gavage gave an LD_{50} of 51.1 mg/kg for picolinaldehyde thiosemicarbazone, of 340.0 mg/kg for nicotinaldehyde thiosemicarbazone and of 931.0 mg/kg for isonicotinaldehyde thiosemicarbazone.

Since in the protection experiments with infected mice the drugs were given in the form of medicated diet over a 21-day period, the doses of the compounds are given in the tables as percent of drug in diet as well as in approximate mg drug/kg/day. This figure was calculated on the basis of the observation that mice normally consume 5 g of the medicated food per day so that 1% diet corresponds to 2500 mg/kg/day. The values thus calculated are generally but not always in reasonable agreement with the tolerated dose as evaluated by oral administration of a single dose by gavage. It happens sometimes that the animals tolerate more drug by medicated diet when the amount is consumed over a 24-hour period. In the experiments to be described this was the case with picolinaldehyde thiosemicarbazone.

In vivo experiments. The results of the experiments using the intravenous route of infection are given in Table I. It is evident from the data in this table that only the derivatives of nicotinaldehyde and isonicotinaldehyde thiosemicarbazone exerted an appreciable protective effect equal or even slightly better than that of Tibione. The thiosemicarbazone of picolinaldehyde was inactive.

The bronchogenic tuberculosis responded differently to anti-tubercular agents. The infection by the intranasal route was, as a rule, more resistant to anti-tubercular drugs than was the hematogenous infection. Para-aminosalicylic acid and niacinamide were of low activity even if high doses (2500 mg/kg/day by diet) were given. Our standard dose of streptomycin (50 mg/kg/day subcutaneously) protected not more than 10-20% of the animals, but full protection was observed with a daily dose of 150 mg/kg.

Tibione, on the other hand, and the thiosemicarbazones of nicotinaldehyde and isonicotinaldehyde were markedly effective in this type of infection. This is shown in Table II. From this table it may also be noted that nicotinaldehyde thiosemicarbazone and isonicotinaldehyde thiosemicarbazone were equally effective but 2 to 3 times less active than in the hematogenous infection. Tibione was less active than the heterocyclic thiosemicarbazones.

TABLE II. Anti-tubercular Activity of Thiosemicarbazones in Intranasal Infection of Mice.
Infection: 4 drops of a 10^{-1} dilution of a 7-10-day-old culture in Dubos' medium.
Treatment: Medicated diet for a 21-day period.

Thiosemicarbazone	No. of mice	% medicated diet	Approx. dose: mg/kg	% protection
I	8	.02	50	0
II	12	.2	500	100
	10	.1	250	90
	9	.04	100	55.5
	10	.02	50	0
III	10	.2	500	100
	20	.1	250	75
	10	.04	100	60
	10	.02	50	0
Tibione	10	1	2500	80
	16	.5	1250	62.5
	20	.2	500	30
Controls	38	—	—	2.7

Discussion. From the experiments described in the earlier part of this paper, it is evident that the thiosemicarbazones of nicotinaldehyde and of isonicotinaldehyde exerted a protective effect in the experimental hematogenous tuberculosis of mice. Levaditi's findings with the thiosemicarbazone of nicotinaldehyde were thus confirmed at least in the dosage of 50-100 mg/kg/day used by him. It seems, therefore, evident that the thiosemicarbazones of compounds which do not contain primary or secondary amino groups might exert an activity comparable or superior to Tibione.

Superior activity was particularly evident in the intranasal infection which was more resistant to such agents as para-amino-salicylic acid, niacinamide and also seemed to require considerably higher doses of Tibione than did the thiosemicarbazones of nicotinaldehyde and isonicotinaldehyde. No satisfactory explanation has as yet been found for this difference in activity.

Summary. (1) The anti-tubercular activity of the thiosemicarbazone of nicotinaldehyde in the intravenous infection of mice with *M. tuberculosis* as described by Levaditi was confirmed. (2) The thiosemicarbazone of isonicotinaldehyde was found as active as the thiosemicarbazone of nicotinaldehyde and the effect of both compounds was comparable to that of Tibione. Picolinaldehyde thiosemicarbazone was ineffective. (3) The thiosemicarbazones of nicotinaldehyde and isonicotinaldehyde also were found appreciably active in the therapeutically more resistant bronchogenic type of tuberculosis of mice produced by intranasal infection. Under these experimental conditions the compounds appeared superior to Tibione.

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Effect of Barbiturates on Oxidative Phosphorylation.* (18674)

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There is still much doubt as to the exact mechanism by which barbiturates produce their pharmacologic effects. Quastel and his associates(1) demonstrated that narcotics depress the respiration of brain tissue *in vitro* and postulated that the locus of the blocking action was at a flavoprotein step in the cytochrome chain. This supposition was further supported by the work of Grieg(2). However, both groups of workers used indirect means to demonstrate the postulated locus of action and did not specifically measure the step in question. Persky, *et al.*(3) have recently produced evidence which they interpret as showing that the site of action for the barbiturates is the dehydrogenase moiety of the pyruvic oxidase complex. Work in our laboratory has substantiated the overall inhibition of respiration in brain homogenates, but spectrophotometric measurements of DPN-cytochrome c reductase activity failed to show any unique sensitivity of this step to central nervous system depressants(4). Partial purification of the members of the cytochrome chain: DPN-cytochrome c reductase, cytochrome oxidase, and various dehydrogenases from brain, liver, and heart muscle did not disclose any enzyme which could be specifically inhibited by a reasonable

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