TABLE I. Rats Dying or Showing CNS Involvement Following Injection with Bat-Brain Suspension. One rat in each experiment.

Bat group	Inoculum: Days after inj. tissues were harvested	Inoculum: Pool	Incubation, days	Symptoms or death
Oral	5	Feces and intestines	11	Paralysis hind legs
	12	" " "	12	Slight tremors; partial paralysis
	12	Hearts	6	Paralysis
	30	Brains and cords	8	Dead*
	30	Kidneys and bladders	4	Paralysis hind legs
Intranasal	12	Feces and intestines	6	Dead*
	30	Brains and cords	7	" *
Intracerebral	5	" " "	8	" *
	12	Feces and intestines	6	" *

* These rats were dead when examined in the morning although they had shown no symptoms the previous afternoon.

gitis were noted on histopathological examinations nor could the agent be passed serially to more rats. Furthermore, the pathological findings in all 4 cases were almost identical with those in the other 5 rats which succumbed following injection with bacteria-free material.

Summary. Different groups of bats (*Eptesicus fuscus*) were exposed to the Lansing strain of poliomyelitis virus by the oral, intranasal, and intracerebral routes. Attempts were made to isolate the virus in cotton rats from suspensions of various tissues and feces of bats sacrificed on the 5th, 12th, 20th, and 30th days after exposure. Nine of the rats died or were paralyzed on the first passage. Four of these rats had been injected with bat feces and intestines, 3 with bat brains and cords, one with bat hearts, and one with bat kidneys and bladders. However, histopathological examinations showed no typical lesions of poliomyelitis, and attempts to produce symptoms in rats of the 2nd passage were unsuccessful.

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Received May 9, 1952. P.S.E.B.M., 1952, v80.

Effects of Isonicotinic Acid Derivatives on Tubercle Bacilli.* (19588)

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The mechanism underlying the action of synthetic chemicals and antibiotics on bacteria remains a debated question. The possibility that the tuberculocidal or tuberculostatic effect of some chemicals and antibiotics may be due to their action on the respiratory enzymes of tubercle bacilli has long been under investigation in this laboratory.

The effect of isonicotinic acid hydrazide and 1-isonicotinyl-2-isopropylhydrazine on oxygen consumption, succinic dehydrogenase and catalase activity of virulent, streptomycin-sensitive and streptomycin-resistant strains of human type tubercle bacilli, as well as on the attenuated, streptomycin-sensitive, bovine type tubercle bacilli BCG, was studied.

Material and methods. Cultures of tubercle bacilli were grown on Sautons synthetic medium for 7 to 19 days, during which time they produced a heavy wrinkled film on the surface of the medium while the underlying culture medium remained clear. The bacilli

* This study was supported by a grant from the National Institutes of Health, U. S. Public Service.

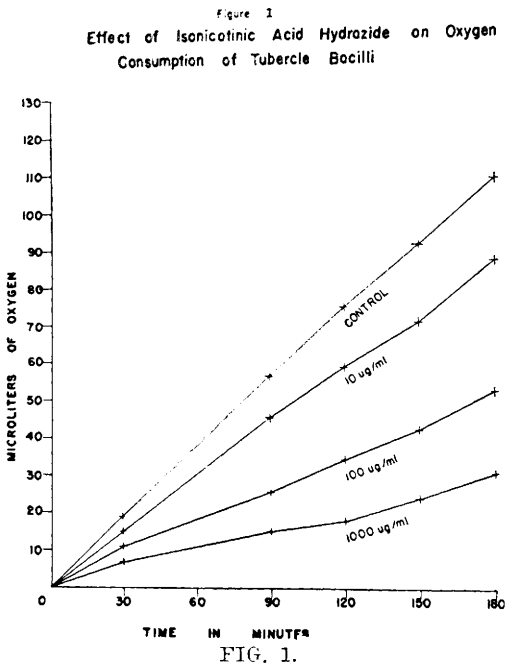

FIG. 1.

were collected on a sintered glass filter of coarse porosity, where they were washed several times with isotonic saline solution buffered to pH 7.0. The washed bacillary mass was weighed, ground to a uniform paste with glass beads and suspended in buffered saline to yield a concentration of 50 mg moist weight per ml. Oxygen consumption was determined by the usual Warburg technic. One ml of the bacillary suspension was transferred to Warburg flasks and one ml of a solution of the isonicotinic acid hydrazide or 1-isonicotinyl-2-isopropylhydrazine, made up to the desired concentration with buffered saline, was added. The mixture was permitted to equilibrate for 30 minutes at 37°C, after which time readings were recorded at 30 minute intervals for a period from 2 to 3 hours.

Results. Unlike the chemicals and antibiotics studied previously, under the same conditions, isonicotinic acid hydrazide significantly reduced the oxygen consumption of tubercle bacilli. The per cent reduction of oxygen consumption paralleled the concentration of isonicotinic acid hydrazide and bore no relationship to the sensitivity or resistance of the organisms to streptomycin. The results of a typical experiment with a virulent human type streptomycin-sensitive tubercle bacillus are presented in Fig. 1.

The oxygen consumption of the avirulent BCG culture was inhibited to a less degree by isonicotinic acid hydrazide than was that of the virulent strains of tubercle bacilli used in the experiments here recorded. The percent inhibition of oxygen consumption for both virulent and attenuated strains of tubercle bacilli is presented in Table I. The inhibitory action of isonicotinic acid hydrazide on oxygen consumption was most marked on 6- to 12-day-old cultures of tubercle bacilli and significantly less marked on 12- to 19-day-old cultures. The cultures of tubercle bacilli used in this study were inhibited by a concentration of 0.15 µg/ml of isonicotinic acid hydrazide in Youmans medium containing 0.5% fraction V bovine albumen.

Catalase. The effect of isonicotinic acid hydrazide on the enzymatic activity of catalase of virulent and attenuated strains of tubercle bacilli was determined according to the method of Finlayson and Edson(1). One ml of a bacillary suspension containing 0.75 mg moist weight of the virulent organisms was transferred to Warburg flasks while 2.5 mg in 1 ml of 0.015 M phosphate buffer, pH 7.4 of the avirulent BCG culture was transferred to the flasks. The larger amount of the attenuated BCG culture was used, because unpublished studies indicate that the catalase activity of attenuated strains of tubercle bacilli is significantly less than that of virulent strains. To the bacillary suspension there was then added isonicotinic acid hydrazide to give a final concentration of either 10, 100 or 1000 µg/ml. After 30 minutes equilibration at 37°C, 0.3 ml of a 0.05 M solution of hydrogen peroxide in 0.005 M phosphate buffer, pH 7.4, was tipped into the flask from the side arm. Manometric readings of the oxygen evolved were taken at 10-minute intervals for 30 minutes. As a control, distilled water was substituted for the isonicotinic acid hydrazide. The results obtained are presented in Table I.

It will be noted from Table I that while isonicotinic acid hydrazide inhibited the oxygen consumption of the virulent strain of tubercle bacilli to a greater degree than the attenuated strain, no such striking reduction

TABLE I. Effect of Isonicotinic Acid Hydrazide on Oxygen Consumption and Catalase Activity of Virulent and Attenuated Cultures of Tubercle Bacilli.

μg/ml isonicotinic acid hydrazide	% reduction of oxygen consumption		% reduction of catalase activity	
	Strains of tubercle bacilli			
	Virulent	Attenuated	Virulent	Attenuated
10	9	4	4	14
100	53	12	28	36
1000	73	27	87	80

TABLE II. Percentage Inhibition of Oxygen Consumption and Catalase Activity of Virulent Tubercle Bacilli by Isonicotinic Acid Derivatives.

Isonicotinic acid derivatives	% reduction of oxygen consumption			% reduction of catalase activity		
	10 μg/ml	100 μg/ml	1000 μg/ml	10 μg/ml	100 μg/ml	1000 μg/ml
Isonicotinic acid hydrazide	9	53	73	4	28	87
1-isonicotinyl-2-isopropylhydrazine	0	0	12	0	0	0

was evident in the relationship of catalase activity to virulence of the bacilli.

Succinic dehydrogenase. The effect of isonicotinic acid hydrazide on this enzyme was investigated by a modification of the procedure described by Kun and Abood(2). To 1 ml containing 75 mg of a bacillary suspension prepared as described above there was added 1 ml isonicotinic acid hydrazide in M/50 sodium succinate to give a final concentration of 1000 μg/ml. This mixture was incubated for one hour, after which time there was added 1.5 mg of neotetrazolium chloride dissolved in M/15 phosphate buffer pH 7.6. After 3 hours incubation at 37°C the neotetrazolium chloride was reduced to a deep red water-insoluble formazan. At the end of this time the reaction was stopped by the addition of 0.5 ml of a 40% solution of trichloracetic acid. The formazan was extracted by vigorous shaking for 30 seconds with 10 ml of ethyl acetate, after which time the mixture was centrifuged. Five ml of the supernatant fluid was transferred to a Klett tube and read in a Klett-Summerson colorimeter. The results were compared with a control tube to which no isonicotinic acid hydrazide had been added. It was found that under these conditions the succinic dehydrogenase activity was not affected by the amount of isonicotinic acid hydrazide used.

Action of 1-isonicotinyl-2-isopropylhydrazine on tubercle bacilli. The action of 1-isonicotinyl-2-isopropylhydrazine on the oxygen consumption, catalase and succinic dehydrogenase activity of tubercle bacilli was determined in the same manner as described above. The growth of the cultures used in these studies was inhibited by 15.0 μg/ml of 1-isonicotinyl-2-isopropylhydrazine in Youmans medium containing 0.5% fraction V albumen. It was found that 100 or 1000 μg/ml of the 1-isonicotinyl-2-isopropylhydrazine slightly inhibited the oxygen consumption of virulent strains of tubercle bacilli as well as the attenuated BCG culture. The catalase and succinic dehydrogenase activity of the virulent strains was not affected by the 1-isonicotinyl-2-isopropylhydrazine. On the other hand, the catalase activity of the attenuated BCG strain was reduced 10, 19 and 25% by 10, 100 and 1000 μg/ml of the compound respectively while the action of succinic dehydrogenase was not affected. The comparative action of isonicotinic acid hydrazide and 1-isonicotinyl-2-isopropylhydrazine on the oxygen consumption and catalase activity of strains of virulent tubercle bacilli is presented in Table II.

It will be observed from this table that comparable amounts of isonicotinic acid hydrazide and 1-isonicotinyl-2-isopropylhydrazine show significant differences in their inhibitory action on oxygen uptake and catalase activity. However, when the inhibitory action of these 2 compounds on the growth of tubercle bacilli is considered as being in the ratio of 100 to 1, it

then becomes evident that 10 μg/ml of isonicotinic acid hydrazide has the same effect on oxygen uptake as has 1000 μg/ml of 1-isonicotinyl-2-isopropylhydrazine.

Conclusions. 1. The oxygen consumption and catalase activity of both virulent and attenuated strains of tubercle bacilli was reduced significantly by isonicotinic acid hydrazide from 2 different laboratories while the activity of succinic dehydrogenase was unaffected. 2. 1-isonicotinyl-2-isopropylhydrazine slightly inhibited the oxygen consumption of both virulent and attenuated strains of tubercle bacilli, but did not modify the action of succinic dehydrogenase of these cultures. However, the catalase activity of the attenuated BCG strain was significantly reduced, while the catalase activity of the virulent strains was unaffected.

We are indebted to Hoffmann-La Roche, Nutley, N. J., for samples of Isonicotinic Acid Hydrazide and 1-isonicotinyl-2-isopropylhydrazine and to E. R. Squibb & Sons, New Brunswick, N. J. for Isonicotinic Acid Hydrazide.

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Received May 19, 1952. P.S.E.B.M., 1952, v80.

Effect of Age on Wallerian Degeneration in the Rat.* (19589)

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If a peripheral nerve is cut that portion of the nerve distal to the point of section undergoes a series of changes characteristic of Wallerian degeneration. Previously it has been shown that in cat sciatic nerve degenerating after nerve section there is an increase in the concentration of nucleic acid (1) related to cellular proliferation, and a decrease in the concentration of phospholipid (2) related to the destruction of the myelin sheath. Similar changes occur in the sciatic nerve of the rat, although the degeneration process is speeded up considerably(3). In experiments preliminary to those described in the above paper it became apparent that in the sciatic nerve of the rat the concentration of both nucleic acid and phospholipid varied with the age of the animal. Since it has been frequently stated that Wallerian degeneration proceeds more rapidly in younger animals(4), it was decided to study the effect of age on the concentration of nucleic acid and phospholipid both in normal nerves and in the peripheral stump of nerves degenerating after nerve section.

Methods. The sciatic nerve on one side of each of 2 groups of male Sprague-Dawley rats was sectioned high in the thigh. One group of rats was 60 days old (body weight = 205 $\pm$ 5 g) and the other was 160 days old (body weight = 419 $\pm$ 8 g). Both groups were maintained on an adequate synthetic diet. After either 8 or 16 days the animals were killed and the distal degenerating segment of nerve removed. In none of the animals was there postmortem evidence of regeneration. A similar length of sciatic nerve was taken from the opposite side to serve as a control. Phospholipid was determined in a lipid extract of the nerve(2). Nucleic acid was determined by ultraviolet absorption using a hot trichloroacetic acid extract of the residue remaining after the extraction of lipids and acid-soluble compounds(1). Details of the operation, analytical procedures, and the method of recording the results have already been published(3).

Results. Nucleic Acid. Table I shows that the concentration of nucleic acid in the sciatic nerves of the 60-day rats was significantly ($P < 0.001$) greater than that in the nerves

* This work was supported by grants from the National Research Council of Canada and the National Mental Health Grants.