THE ACCUMULATION OF ISONIAZID IN TISSUES, AND THE COURSE OF EXPERIMENTAL TUBERCULOSIS

BY

J. VENULET, KRZYSTYNA JAKIMOWSKA, M. JANOWIEC, AND ALICJA URBANŚKA

From the Pharmacological Department, Drug Research Institute and Institute of Tuberculosis, Warsaw, Poland

(RECEIVED MAY 12, 1958)

In an attempt to find agents enhancing the infiltration of isoniazid into tissues, the authors have investigated the effect of the drug on oedema due to injection of egg white into the rat paw, when it was given alone and in conjunction with histamine, antazoline, carbachol, chlorpromazine, and a pyrogen. Isoniazid increased the oedema. All the substances, except antazoline, increased the rate at which isoniazid disappeared from the blood, and they all favoured accumulation of the drug in tissues. Treatment of experimental tuberculosis with subthreshold doses of isoniazid, in conjunction with the above compounds, gave better results than when isoniazid was used alone.

Of the numerous factors affecting the final results of tuberculosis treatment, the accessibility of the drugs to the site of infection is of fundamental importance. The duration of the disease and the attendant protective and toxic reactions, as for example fibrosis and caseation, are serious impediments to the drug reaching the infected areas. The positive results obtained when the disease is treated with a potent antitubercular drug in conjunction with either corticotrophin or cortisone are explained by the paradoxically favourable effects of inhibition of some protective processes.

The experiments described in this paper were designed to determine the degree to which some antitubercular drugs and other substances might modify the inflammatory reaction and the permeability of tissues. Their value in treating experimental tuberculosis was also explored.

METHODS

The volume of extracellular fluid was measured in rabbits by the thiocyanate method (Odier, 1948), and the volume of circulating blood by the method of Griesbach (1921) and Heilmeyer (1929). Oedema of the rat paw was induced by injection into the paw of 0.05 ml. of egg white diluted 1 in 3 (Gross, 1950). Isoniazid concentrations were determined in rabbit blood and in rat lungs, liver, and kidney by the method of Deeb and Vitagliano (1955). In order to eliminate the opalescence in liver samples glycogen was hydrolysed with diastase.

Experimental tuberculosis was produced in albino mice weighing about 20 g. and in guinea-pigs

weighing about 350 g. The mice were injected intravenously with 0.1 mg. of strain H₃₇Rv and the guinea-pigs subcutaneously with 0.3 mg. of strain Sz. The drugs were administered subcutaneously daily from the day after inoculation until all the control animals had died. Then the surviving test animals were killed and the degree of the tubercular lesions was estimated microscopically. Three factors were determined with respect to the control group: (a) the death rate in each group, (b) the mean survival time, and (c) the index of tuberculosis.

The substances used in the experiments comprised: Isoniazid (isonicotinic acid hydrazide), histamine, antazoline (Antistine, Ciba), chlorpromazine (Largactil, Specia), carbachol (Doryl, Merck), and lipopolysaccharide from *Escherichia coli* (pyrogen).

RESULTS

Tissue reactions connected with alterations in permeability were studied in the rat paw, where oedema was produced by injection of egg white. Groups of ten rats were given one of the following preparations 30 min. before injection of the protein: isoniazid (0.05 g./kg., subcutaneously), chlorpromazine (1 mg./kg., subcutaneously) and bacterial lipopolysaccharide (0.1 or 1.0 µg./kg., intravenously). The results are shown in Figs. 1 and 2.

The oedema was increased and prolonged by isoniazid and decreased by chlorpromazine. Bacterial lipopolysaccharide in doses of 0.1 µg./kg. increased the oedema, but with larger doses the effect of the egg white was reduced. When given with isoniazid the effects of chlor-

FIG. 1.—Effect of some agents on the volume of the rat paw. Each preparation was injected subcutaneously 30 min. before injection of egg white, which was used to produce oedema. C, control; I, 0.05 g./kg. isoniazid; P1 and P0.1, 1 μ g./kg. and 0.1 μ g./kg. respectively of bacterial lipopolysaccharide (pyrogen); CP, 1 mg./kg. chlorpromazine. For I, $P=0.005$; for P1, $P=0.8$; for P0.1, $P=0.04$; for CP, $P<0.001$.

FIG. 2.—The effect of isoniazid combined with some agents on the volume of the rat paw. The combinations, which were 0.05 g./kg. isoniazid with 0.1 μ g./kg. (IP0.1) and 1 μ g./kg. (IP1) of bacterial lipopolysaccharide (pyrogen) and with 1 mg./kg. chlorpromazine (ICP), were injected subcutaneously 30 min. before injection of egg white, which was used to produce oedema. For IP0.1, $P=0.04$; for IP1, $P=0.8$; for ICP, $P>0.3$.

promazine and the pyrogen were similar to those obtained when they were given alone.

The effect of isoniazid on the distribution of extracellular fluid in rabbits was also studied. For this purpose, the volumes of total extracellular fluid and of plasma were measured in 10 animals before and after they had been injected with

isoniazid (10 mg./kg.) twice daily for two days. The results are given in Table I, in which the coefficient I denotes the ratio of extracellular fluid to plasma.

TABLE I
DISTRIBUTION OF WATER IN CONTROL RABBITS AND IN RABBITS TREATED WITH ISONIAZID (10 mg./kg.)

I = extracellular fluid volume/plasma volume.

Control		Isoniazid	
Extracellular Volume (ml./kg.)	Plasma Volume (ml./kg.)	Extracellular Volume (ml./kg.)	Plasma Volume (ml./kg.)
252	66	307	78
I = 3.8		I = 3.9	

Isoniazid raised the overall retention of water in the animals without, however, modifying the rate of its distribution in tissues and blood.

Further experiments were aimed at determining the contents of isoniazid in tissues and in blood as modified by various agents. Groups of 10 rabbits were given histamine (0.5 mg./kg., subcutaneously), antazoline (5 mg./kg., subcutaneously), carbachol (15 μ g./kg., subcutaneously), or chlorpromazine (1 mg./kg., subcutaneously), 60 min. before 20 mg./kg. of isoniazid, while in other groups bacterial lipopolysaccharide (0.01 μ g./kg., intravenously) was given 30 min. before isoniazid and histamine was given simultaneously with isoniazid. The level of isoniazid in the blood 4, 30, 60, and 120 min. after injection is shown in Figs. 3 and 4.

All the preparations, except antazoline, significantly reduced the concentration of isoniazid in the blood. The effect of histamine was obviously dependent upon the timing of its administration. When it was given 1 hr. before isoniazid it increased the tissue permeability to such an extent that isoniazid immediately began to disappear from the blood. Even after 4 min. the concentration of isoniazid was much below that of the controls. When histamine and isoniazid were administered simultaneously the initial values were the same as for the controls. However, subsequently, the concentration of isoniazid fell rapidly and approached the values obtained in the experiments in which histamine was given before isoniazid.

For determining the isoniazid content in rat tissues, the animals were divided into groups of

ten. Group 1 served as a control. Group 2 received histamine, 0.5 mg./kg. subcutaneously 30 min. before, and 0.2 mg./kg. together with isoniazid. Group 3 received antazoline, 5 mg./kg. subcutaneously 30 min. before, and 0.5 mg./kg. intravenously together with isoniazid. Group 4 received carbachol, 15 μ g./kg. subcutaneously 30 min. before, and 10 μ g./kg. together with isoniazid. Group 5 was given chlorpromazine, 1 mg./kg. subcutaneously 30 min. before, and 0.25 mg./kg. intravenously at the same time as isoniazid. Group 6 was given the pyrogen, 0.1 μ g./kg. intravenously 30 min. before isoniazid. The animals were sacrificed 30 min. after the injection of isoniazid. The circulatory system was rapidly perfused through the aorta with warm Ringer solution, and samples of the lung, liver and kidney were taken for isoniazid determinations. The results are shown in Figs. 3 and 4, together with curves illustrating the concentrations of isoniazid in blood.

FIG. 3.—Effects of histamine and antazoline on the concentration of isoniazid in blood of rabbits and tissues of rats. The curves represent the concentration of isoniazid in the blood after injection of 20 mg./kg. isoniazid alone (I) and followed 60 min. later by 5 mg./kg. antazoline (IA), and by 0.5 mg./kg. histamine (IH). 20 mg./kg. isoniazid administered simultaneously with 0.5 mg./kg. histamine (IHS). For IA, $P=0.434$; for IHS, $P=0.015$; for IH, $P=0.001$. The columns represent the concentration of isoniazid in μ g./g. in the lungs (Lu), liver (Li) and kidneys (K). For antazoline, $P=0.694$; for histamine, $P<0.001$.

FIG. 4.—Effects of carbachol, chlorpromazine and bacterial lipopolysaccharide (pyrogen) on the concentration of isoniazid in blood of rabbits and tissues of rats. The curves represent the concentration of isoniazid in the blood after injection of 20 mg./kg. isoniazid alone (I) and followed 60 min. later by 15 μ g./kg. carbachol (IC), 1 mg./kg. chlorpromazine (ICP) and by 0.01 μ g./kg. bacterial lipopolysaccharide (pyrogen) (IP). For IC, $P=0.018$; for ICP, $P=0.018$; for IP, $P=0.010$. The columns represent the concentration of isoniazid in μ g./g. in the lungs (Lu), liver (Li) and kidneys (K). For chlorpromazine, $P<0.001$; for bacterial lipopolysaccharide, $P<0.001$; for carbachol, $P<0.001$.

Since carbachol and the pyrogen proved very active in aiding infiltration of isoniazid into tissues, additional determinations with these two substances were carried out 1 hr. after the isoniazid injections. The results are shown in Figs. 5 and 6.

The isoniazid levels in various tissues differed according to the drugs used. Histamine, carbachol, bacterial lipopolysaccharide, and chlorpromazine significantly increased the content of isoniazid in all tissues. Consequently the reduction of isoniazid in the blood was attended by an increased infiltration into the tissues. This accounts, at least partly, for its disappearance from the blood. In spite of considerable dosage, only traces of isoniazid were found in the lungs after 30 min. when it was given alone. Even after 1 hr. the concentration was still lower than after the pyrogen. The highest concentration of isoniazid was found in the kidneys, and this is likely to be related to their excretory function.

FIG. 5.—The effect of 15 µg./kg. carbachol on the concentration of isoniazid in lungs (cross-hatched), liver (horizontal hatched), and kidneys (double cross-hatched) of rats 30 and 60 min. after injection of 20 mg./kg. isoniazid. Black columns represent the concentrations after isoniazid treatment alone.

FIG. 6.—The effect of 0.01 µg./kg. bacterial lipopolysaccharide (pyrogen) on the concentration of isoniazid in lungs (cross-hatched), liver (horizontal hatched) and kidneys (double cross-hatched) of rats 30 and 60 min. after injection of 20 mg./kg. isoniazid. Black columns represent the concentrations after isoniazid treatment alone.

In the kidney the effects of the pyrogen and carbachol were most pronounced. In the control animals the concentration of isoniazid in the liver was only one-third of that in the kidneys.

When examined 1 hr. after the administration of isoniazid, the effects of carbachol and bacterial lipopolysaccharide were considerably smaller.

The overall pattern of these observations prompted us to test their possible usefulness in treating experimental tuberculosis in mice and guinea-pigs. In carrying out these experiments isoniazid was given in a dose below that currently used. It was considered that this dose was subthreshold, that is, inadequate for controlling the disease. The course of the experiments and the results obtained are shown in Tables II and III.

TABLE II
THE TREATMENT OF EXPERIMENTAL TUBERCULOSIS IN EIGHT GROUPS, EACH OF TEN MICE, FOR 35 DAYS, WITH ISONIAZID ALONE AND IN CONJUNCTION WITH BACTERIAL LIPOPOLYSACCHARIDE, HISTAMINE OR CARBACHOL

Injections were subcutaneous. INH=isoniazid.

Group of Animals	Preparation	Dose/kg.	Mortality in Group	Average Time of Survival (Days)	Index of Tubercular Changes
I	Control	—	10/10	28.1	118
II	INH	1.0 mg.	9/10	26.9	52
III	INH+ pyrogen	1.0 mg.+ 1.0 µg.	6/10	28.4	41
IV	INH+ histamine	1.0 mg.+ 0.5 µg.	2/10	35.0	43
V	INH+ carbachol	1.0 mg.+ 20.0 µg.	4/10	27.6	31
VI	Pyrogen	1.0 µg.	10/10	18.3	72
VII	Histamine	0.5 µg.	10/10	20.5	111
VIII	Carbachol	20.0 µg.	9/10	16.4	42

TABLE III
THE TREATMENT OF EXPERIMENTAL TUBERCULOSIS IN EIGHT GROUPS, EACH OF TEN GUINEA-PIGS, FOR 62 DAYS, WITH ISONIAZID ALONE AND IN CONJUNCTION WITH BACTERIAL LIPOPOLYSACCHARIDE, HISTAMINE OR CARBACHOL

Injections were subcutaneous. INH=isoniazid.

Group of Animals	Preparation	Dose per kg.	Mortality in Group	Average Time of Survival (Days)	Index of Tubercular Changes
I	Control	—	10/10	51.1	117
II	INH	1.0 mg.	6/10	52.9	61
III	INH+ pyrogen	1.0 mg.+ 0.5 µg.	1/10	56.9	44
IV	INH+ histamine	1.0 mg.+ 0.2 µg.	2/10	56.9	41
V	INH+ carbachol	1.0 mg.+ 10.0 µg.	1/10	59.8	41
VI	Pyrogen	0.5 µg.	10/10	46.4	89
VII	Histamine	0.2 µg.	10/10	40.2	91
VIII	Carbachol	10.0 µg.	9/10	45.2	96

In evaluating the results two criteria were used: the time of survival and the index of tubercular changes. The second criterion was used only in those instances where the survival time suggested that the results were not worse than those of controls. The first criterion has only a negative value in that it is helpful to show in which groups the results were worse than the control groups. It cannot be a definitive criterion since the proper estimation of the survival time is hampered by the killing of all the animals at the moment of death of the last animal in the control group. In the first experiment, results statistically superior to those of the controls were obtained from animals treated with isoniazid alone, and with isoniazid given in conjunction with bacterial lipopolysaccharide, histamine or carbachol. Isoniazid given with carbachol proved superior to isoniazid given alone; when given in conjunction with the pyrogen or histamine it was also slightly more effective. This is of interest because the pyrogen, histamine and carbachol significantly shortened survival time when used alone.

From Table III it can be seen that in guinea-pigs the treatment with isoniazid combined with the pyrogen or histamine or carbachol gave a reduced mortality rate and a lengthening of survival time. The Tubercular Index was significantly smaller than in the control group. Bacterial lipopolysaccharide and histamine given without isoniazid did not aggravate the course of the disease as they did in the experiment with mice recorded in Table II. All groups treated by these three combinations gave better results than the group treated with isoniazid alone, though the difference was not statistically significant at the 95% level.

DISCUSSION

Oedema of the rat's paw due to injection of egg white provided a suitable criterion for assessing the alteration in tissue permeability due to antitubercular drugs. Isoniazid increased and prolonged the oedema and in other experiments streptomycin and *p*-aminosalicylic acid had a similar effect (Jakimowska and Venulet, 1957). To some extent the results reported here are inconsistent with those of Theobald (1954), who found isoniazid to inhibit oedema production in analogous experiments with dextran. This discrepancy may be explained by a difference in the mechanism of formation of the two kinds of oedema.

Histamine, carbachol, bacterial lipopolysaccharide and chlorpromazine considerably facilitated the accumulation of isoniazid in all the

tissues examined, though the results with carbachol and pyrogen in which tissue concentrations were estimated after both 60 and 30 min. indicate that these effects are of short duration. Nevertheless the concentration of antitubercular drugs in the tissues might be of therapeutic value, and this was further examined by treating infected mice and guinea-pigs with isoniazid alone and in conjunction with those drugs which increased tissue permeability. From these experiments and from others (in which a total of 360 mice and 240 guinea-pigs were used), increasing tissue permeability appeared to be a valuable complement to isoniazid treatment. The relative effectiveness of the drugs used is not yet quite certain, but carbachol appeared to give somewhat superior results to the pyrogen and histamine. However, the side-effects of carbachol and histamine are sufficiently profound to limit their value while bacterial lipopolysaccharide with its milder effects is worthy of further investigation.

Previous work on the effect of carbachol, histamine, and bacterial lipopolysaccharide on the course of tuberculosis sheds little light on the problems raised by the present work because they have been used alone and not in conjunction with an antitubercular drug. The effects of histamine are usually believed to be unfavourable and those of antihistamine drugs favourable (Garbiński, 1953; Judd and Henderson, 1949; Duca and Scudi, 1949). Mitchell (1956) points out that there is some evidence that histamine stimulates the hypophyseal-adrenal system and this alone might lead to unfavourable results, but numerous authors have found combined treatment with isoniazid and corticotrophin or cortisone to be effective (Fortier and Favez, 1952; Quiring, Perlia, and Nicole, 1953; Favez and Fortier, 1953). Our results suggest that, in addition, histamine has an important direct action on tissues and vessels. In the light of our experiments antazoline is of no value and chlorpromazine has an intermediate activity, perhaps aiding permeability by its ganglionic blocking and sympatholytic actions. Kellentei and Földers (1954) showed that acetylcholine enhances the permeability of the blood-brain barrier and carbachol favours the accumulation of phenytoin in the brain. This may well be due to a similar mechanism (Porszasz, Venulet, and Gibiszer-Porszasz, 1954).

The role of bacterial lipopolysaccharide is most difficult to evaluate, since little work has been performed on substances of this type. Recently, Dubos and Schaedler (1956) showed the favourable effects exerted by bacterial lipopolysaccharides on the course of tuberculosis in mice.

This preparation distinctly raises the level of plasma β - and γ -globulins (Piechocki and Venulet, 1957), but the effect of pyrogens on permeability was hitherto unknown. In this connexion, it is interesting that Rowley (1956) calls attention to the positive role of pyrogens in immunity.

REFERENCES

- Deeb, E. N., and Vitagliano, G. R. (1955). *J. Amer. pharmac. Ass., Sci. Ed.*, **44**, 182.
- Dubos, R. J., and Schaedler, R. W. (1956). *J. exp. Med.*, **104**, 53.
- Duca, C. J., and Scudi, J. V. (1949). *Ann. Allergy*, **7**, 318.
- Favez, G., and Fortier, C. (1953). *Schweiz. Z. Tuberk.*, **10**, 256.
- Fortier, C., and Favez, G. (1952). *Schweiz. med. Wschr.*, **82**, 953.
- Garbiński, T. (1953). *Tuberculosis, Warsaw*, **21**, 845.
- Griesbach, W. (1921). *Dtsch. med. Wschr.*, **47**, 1289.
- Gross, F. (1950). *Arch. exp. Path. Pharmacol.*, **211**, 421.
- Heilmeyer, L. (1929). *Biochem. Z.*, **212**, 430.
- Jakimowska, K., and Venulet, J. (1957). *Tuberculosis, Warsaw*, **25**, 959.
- Judd, A., and Henderson, A. (1949). *Ann. Allergy*, **7**, 306.
- Kellentei, S., and Földers, J. (1954). *Acta physiol. Acad. Sci., hung.*, **6**, 433.
- Mitchell, R. G. (1956). *Brit. J. Pharmacol.*, **11**, 462.
- Odier, J. (1948). *Helv. med. Acta*, **15**, Suppl. XXI, 1.
- Piechocki, T., and Venulet, J. (1957). *Acta physiol. pol.*, **8**, 507.
- Porszasz, J., Venulet, J., and Gibiszer-Porszasz, K. (1954). *Acta physiol. Acad. Sci. hung.*, **5**, 509.
- Quiring, E., Perlia, C., and Nicole, G. (1953). *Helv. med. Acta*, **20**, 346.
- Rowley, D. (1956). *Brit. J. exp. Path.*, **37**, 223.
- Theobald, W. (1954). Dissertation, Saar.