

THE ACTIVITY OF
ISO-NICOTINIC ACID HYDRAZIDE
IN MURINE LEPROSY

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The chemotherapeutic activity of *iso*-nicotinic acid hydrazide (INAH) in an experimental infection of mice with *Mycobacterium lepraemurium* has been compared with that of the two sulphones, 4:4'-diaminodiphenylsulphone (DDS) and its disubstituted derivative, sulphetrone, and that of streptomycin and *p*-acetylaminobenzaldehyde thiosemicarbazone (T.B.1./698).

This organism has been used by other workers for assessing the anti-leprotic activity of the antitubercular drugs, but the conditions of the tests have varied. Chaussinand, Paris and Crouge (1948) found that streptomycin had no effect on the subcutaneous leproma in rats, and Carpenter, Stokinger, Suhrland and Ackerman (1949) found that whilst promin produced slight suppression of the same type of lesion, diasone and streptomycin, were without effect but the streptomycin was only used late in the disease. Levaditi and Chaigneau-Erhard (1951), using mice infected intracerebrally and basing the effect of treatment on the number of organisms in the lesions of the meninges after 77 days, found that DDS was superior

to streptomycin, but the latter was superior to *p*-aminosalicylic acid (PAS). The differences, however, were not large and only single doses of the drugs were given subcutaneously each day. Grunberg and Schnitzer (1951), using mice infected intraperitoneally, found that streptomycin, PAS and DDS had no significant effect on the number of organisms present in the intraperitoneal lesions, but promin and T.B.1/698 had some discernible effect. The experiment lasted only 28 days. We have also used in unpublished experiments the size and degree of infection of the leproma produced subcutaneously in white rats as a measure of the activity of streptomycin and the sulphones. These experiments lasted five months and only streptomycin had a discernible, but small effect.

In the present experiment we used white mice (Schofield) infected intravenously with a mouse-passaged strain of *Mycobacterium lepraemurium* obtained originally from the leproma of an infected rat, kindly given by Dr. Gönnert. In these mice, the lesions became widespread, with the organisms mainly within histiocytes. During the first month of the infection, few if any organisms could be found in the viscera and skin, but after this period they became progressively more numerous. By the fifth month the tissues contained very many organisms and deaths began to occur in the untreated animals. The disease was very chronic and some mice may survive eight months or more in spite of the presence of innumerable organisms.

In this experiment, groups of ten mice were injected into the tail vein with 0.5 ml of a suspension of the spleen and liver from a heavily infected mouse. The organs were cut into small pieces, pulped by manual shaking with glass beads for two or three minutes, suspended in Dubos medium to give a volume of 20 ml, centrifuged for 2 minutes at 2000 r.p.m. to remove the coarser particles, and the supernatant fluid diluted 1:20 with Dubos medium. This suspension contains approximately 100 million organisms per ml. Grunberg and Schnitzer (1951) found the intravenous route unsatisfactory because a large percentage of the animals died after injection of 0.1 ml of a 10^{-1} dilution of rat leproma, but we find that no deaths occur from injection of the infected mouse tissue provided it is diluted 1:200; with less dilute suspensions convulsions and death may occur.

Treatment was started on the day of infection, and in the case of the oral drugs which were given in the diet, it was continued daily till the end of the experiment. The streptomycin was administered subcutaneously at 9 a.m. and 5 p.m., except on Saturdays when the 5 p.m. dose was omitted and on Sundays when no drug was given. The doses selected for this experiment were

the maximum doses that previous experience had shown the mice could tolerate. The oral doses given in Chart 1 are the amounts that the mice received provided they eat 5 g. of food. (Controlled experiments showed however, that in fact the mice eat quantities ranging from 3 to 8 g.). On the 140th, 147th, and 160th day of the experiment one of the control untreated mice died, and on the 180th day the experiment was terminated.

Post-mortem examinations were made on all the animals, but apart from some enlargement of the lungs, liver and spleen, which in some animals contained small visible lepromata, there were no gross macroscopic changes. The effect of treatment was assessed by the degree of enlargement of the spleen, by histological examination of the liver, spleen and skin, and by the number of organisms present in smears of the liver and spleen.

ENLARGEMENT OF THE SPLEEN.

The effect of treatment on the enlargement of the spleen was measured by comparing the mean estimate of area of the spleens of each group, the individual areas being calculated by multiplying the average width by the average length of the organ. The estimate for the control group excluded the spleens of the three animals which died before the end of the experiment.

Statistical analysis of the results, given in Table I showed that the spleens of the animals treated with INAH were significantly smaller than those of any of the other groups. Treatment with streptomycin and T.B.1/698 had a definite effect for the spleens of both groups were smaller than those of the untreated animals. Neither of the sulphones had a significant effect.

HISTOLOGY.

In the livers of the control animals the lesions were of variable size but with a distribution suggesting that they started from around the veins, but there was much individual variation in the extent of the disease. The spleens showed gross involvement of the pulp and follicles. In the skin dense masses of large histiocytes, packed with organisms, were present in the corium and panniculus adiposus.

INAH had a very definite effect. Although there were a few enlarged histiocytes around some of the veins of the liver very few contained bacilli, and in the spleen there was no disorganisation of the tissue except for a few enlarged histiocytes. There was no evidence of infection of the skin.

In the tissues of the streptomycin-treated animals the number of bacilli was much reduced as compared with the controls, except in one animal where they were numerous. In the liver the few organ-

isms present were mainly in histiocytes within the parenchyma, but in the skin there were small growing lepromata in the suprapannicular corium and among the hair follicles.

Treatment with thiosemicarbazone had apparently changed the character of the disease for the lesions in the liver consisted mainly of many small foci of phagocytosed bacilli in the parenchyma, with little perivenous infiltration, and the lesions in the skin were much smaller than in those of the control animals. The involvement of the spleens was definitely less than in those of the controls.

The tissues of the animals treated with the sulphones were so similar in appearance to some of the untreated animals that it was difficult to assess the effect of the drug, but in general the involvement of the organs appeared to be less than in the controls.

SMEARS.

Smears were made from the cut surface of the livers and spleens and stained by the Ziehl-Neelsen method counterstaining with methylene blue. The organisms were present either singly or in globular masses and although we are referring to the latter as "globi" the majority were obviously intracellular clumps and therefore not typical of the globi of Neisser. The single organisms were extracellular but were obviously derived from cells broken during the making of the films as there were few extracellular organisms in the sections. The free bacilli and the globi were counted in fields containing approximately 200 spleen cells or 20 liver cells and grouped according to the classification given in chart No. 1. The chart shows the distribution within these groups of the tissues of the mice given the various treatments.

The pronounced inhibitory action of INAH and streptomycin on the multiplication of the organisms is striking. The thiosemicarbazone has apparently had some effect, but the sulphones have had little or none.

DISCUSSION.

In this experiment, INAH has almost completely inhibited the multiplication of *Myc. lepraemurium*, but similar activity, although somewhat less is shown by streptomycin and T.B.1/698, both of which have proved to be almost completely inactive in experiments by other workers. These differences with streptomycin and T.B.1/698 may be due to conditions of the experiments especially in the manner of timing and giving the drugs. In the present experiment the oral drugs were given in the diet, and the streptomycin was injected twice daily, thus ensuring almost constant bacteriostatic blood concentrations.

Although mice heavily infected with tubercle bacilli have

proved satisfactory for screening antitubercular drugs, using *Myc. lepraemurium* for forecasting the chemotherapeutic activity of drugs in human leprosy may be doubted. The antitubercular test uses the organisms responsible for the human disease, but the relationship between *Myc. leprae* and *Myc. lepraemurium* is still undecided. Nevertheless, the two organisms have much in common; both are acid-fast, neither has been grown with certainty on artificial media and both cause an essentially intracellular infection in their natural host, and Wilson and Miles, (1947) go so far as to state that "more recent observations suggest that some cases at least of human leprosy are caused by the rat leprosy bacillus." Mukerji (1951) however noted differences between the two organisms in acid-fastness and morphology after irradiation with ultraviolet light.

In this present experiment the sulphones showed only very doubtful activity, suggesting that either the test does not accurately forecast activity in human leprosy, or only that the sulphones are less active than either INAH, carbazone. Most leprologists consider the sulphones to be the most useful antileprotic drugs at present available, but their choice of drug must necessarily depend a great deal on cheapness, low toxicity, and the ease of administration of the drugs.

The high activity shown by streptomycin is perhaps unexpected for although the antibiotic has been available sufficiently long for its antileprotic value to be assessed in man it has not become widely used. High cost and toxicity when used over long periods have undoubtedly been restricting factors, but there is evidence that streptomycin is more active than the sulphones in the human disease. Faget and Erickson (1948) reported favourable initial responses to streptomycin, to the development of resistance. Erickson (1951) has however, more recently decided that its action is more rapid than that of the sulphones. Saenz (1952) considers it to be as effective as the sulphones, opinion he considers that in practice it may precipitate an acute exacerbation of the disease as well as an erythema nodosum leprosum. Both of these sequelae may be manifestations of the high activity of streptomycin for Cochrane considers them to be related to the Herxheimer reaction. Nevertheless these reports do not suggest that the difference in activity of streptomycin and the sulphones in human leprosy is as marked as is shown in our experimental infection. This may be due to the poor penetration of cells, shown by Mackaness (1952), for Tzanck and Basset (1950) interpreted their experiences with streptomycin in leprosy as indicating

that the antibiotic is effective only against extracellular bacilli. In our experiment treatment not only started at the time of infection when the organisms were extracellular, but very much higher blood concentrations of streptomycin were present in the animals than is possible in man.

There is also evidence that the high experimental antileprotic activity of the thiosemicarbazone applies to the human disease. Ryrie (1950) considered them more rapid in action than the sulphones although Cochrane (1951) was unable to confirm this observation. Keil (1951) however in a review of the use of T.B.1/698, concludes that it has a greater effect in a shorter time than the sulphones, but it is more expensive and the side effects more unpleasant. More recently, Lowe (1952) has decided that T.B.1/698 is at least as active as the sulphones, and Gil (1952) considers that the clinical response is often more impressive than that of the sulphones, although the decrease in the number of organisms in nasal scraping is slower. This latter observation is of interest especially in view of the change produced by T.B.1/698 in the character of the disease in our experiment.

As the results of this experiment are consistent with clinical experiences so far, it is highly probable that drugs which are active against *Myc. lepraemurium* are also active against *Myc. leprae*. INAH therefore should possess very high antileprotic activity, and the fact that it readily penetrates cells as shown by Mackaness and Smith (1952) is very much in its favour. So far there are only preliminary reports from clinical trials in human leprosy and in these the opinions of the effects varies; Gil (1952) states that no other drug produces such rapid changes in the morphology of the organisms, and Latapi and Rubio (1952) found favourable effects on skin nodules and nasal lesions in 13 of 14 cases, but Lowe (1952) concludes from the results of treatment of 27 patients for periods of 14 to 23 weeks that INAH is possibly of slight benefit but its action is much less than that of the sulphones or thiosemicarbazone. The antileprotic value of INAH in the human disease is therefore still in doubt, but it is only a question of time before the answer will be known for, unlike streptomycin or T.B.1/698, INAH is inexpensive, relatively non-toxic and easily administered.

In this experiment INAH has shown greater activity than streptomycin and if the precipitation of acute exacerbation of the disease and of erythema nodosum leprosum by streptomycin are due to high antileprotic activity, then such sequelae may be expected to be even more frequent with INAH. Even more serious, however, are the suggestions that streptomycin fails in human leprosy because of the development of resistance, for experience

with INAH in tuberculosis shows that this phenomenon occurs equally as rapidly with INAH as with streptomycin. Except for the one mouse containing large numbers of organisms in the streptomycin-treated group, there was, however, no suggestion of resistance developing in either the streptomycin or INAH-treated animals, but an endeavour is being made to assess the ability of *Mycobacterium lepraemurium* to develop resistance by examining the surviving organisms present in the treated mice of this experiment.

The antibacterial activity of INAH has so far proved to be remarkably specific for *Mycobacterium tuberculosis*, but the result of this experiment shows that it undoubtedly extends to other pathogenic species of the genus, *Mycobacterium*.

SUMMARY

In mice infected intravenously with *Mycobacterium lepraemurium*, INAH has almost completely suppressed the infection. Streptomycin was almost as effective, and T.B.1/698 somewhat less effective but the two sulphones had only a doubtful effect on the development of the disease.

Evidence is presented for assuming that the activity shown against this organism also applies to *Mycobacterium leprae*.

We are indebted to Dr. David Trevan for the histological examinations, and to Mr. P. A. Young for the statistical analysis.

TABLE I.

Treatment group	Mean Surface areas + Std. error (cm) ²	Significance of comparison with group:					
		1	2	3	4	5	6
1. INAH	1.12 \pm 0.079		+	+	+	+	+
2. Streptomycin	1.50 \pm 0.161	—		=	+	+	+
3. T.B.1/698	1.50 \pm 0.114	—	=		+	+	+
4. Sulphetrone	2.43 \pm 0.256	—	—	—		=	=
5. Untreated	2.73 \pm 0.213	—	—	—	=		=
6. DDS	2.75 \pm 0.238	—	—	—	=	=	

+ = superior effect ($P = <0.05$)

— = inferior effect ($P = <0.05$)

= = similar effect ($P = >0.35$)

Table I. Statistical analysis of the surface areas of the spleens from each group of mice. The body of the table indicates the significance of the difference between pairs of treatment ("t" test).

DRUG	ROUTE	DAILY DOSE mg./ mouse	GROUP						
			7	6	5	4	3	2	1
I.N.A.H.	Diet	2						○○○○ ○○○○ ●●●● ●	○○ ●●●
Streptomycin	S.C	2x1			●	○	○	○○○ ○○○ ●●●	○○
T.B.1/698	Diet	10		○		●●● ●●●	○○○ ○○○ ●●●	○○○	
Sulphetrone	Diet	100	●	●●	○○ ●	○○○ ○○○ ●●● ●	○○ ●●		
D.D.S.	Diet	2.5	○ ●●	○○○ ●●	●●● ●	○ ○○○	○○ ●●		
Controls	—	—	○	○○ ○○ ●● ●●	○ ●●	○○○ ●●			

Liver ○ Spleen ●

CHART I.

Distribution of the liver and spleen smears within groups, based on the number of *Myco. lepraemurium* and globi per field.

Group 1. — <0.1 organisms.

Group 2. — 0.1 to 2 organisms.

Group 3. — 2 to 30 organisms and <0.1 globus.

Group 4. — 30 to 300 organisms and 0.1 to 1.0 globus.

Group 5. — 30 to 300 organisms and 1 to 2 globi.

Group 6. — >300 organisms and 2-5 globi.

Group 7. — >300 organisms and >5 globi.

Each open circle represents the liver and each black circle represents the spleen of one animal.

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