CHEMOTHERAPY OF MURINE LEPROSY III. THE EFFECTS OF NICOTINAMIDE AND PYRAZINAMIDE (ALDINAMIDE) ON MOUSE LEPROSY

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Nicotinamide has been found to possess a marked suppressive activity in experimental tuberculosis and in murine leprosy. The antituberculosis activity of its isomeric derivatives, the isonicotinylhydrazines, has been extensively studied in recent years, both experimentally and in the clinic. Clinical trials of the original compound, nicotinamide, have been limited but favorable results have been reported in the treatment of pulmonary tuberculosis by Huant (35, 36), Tanner (61), Radenback (55) and Lehmann (41). Large amounts may be given orally or parenterally for long periods of time without evidence of toxicity (38, 41).

There have been many laboratory studies of nicotinamide and its allied compounds in the past 15 years. A review of the principal results, especially as they relate to mycobacterial infections, is given here.

Chorine (6) first reported that a large dose of nicotinamide was highly effective in experimental tuberculosis of guinea-pigs and in murine leprosy of rats, while nicotinic acid was ineffective. Varying degrees of activity of nicotinamide against tuberculosis have been demonstrated by various investigators in mice (22, 23, 25, 43, 51,60, 67, rats (23), and guinea-pigs (23). It is at least as effective as streptomycin in mice and guinea-pigs. The oral and subcutaneous routes of administration are equally effective.

Pyrazinamide (Aldinamide), the isoster of nicotinamide, was found by Malone and associates (⁴⁵) to be more effective than nicotinamide in the suppression of experimental tuberculosis of mice. Antituberculosis activity in mice was also reported by



Nicotinamide



Pyrazinamide

Solotorovsky and associates $({}^{60})$, and by Dessau and associates $({}^{9})$ in guineapigs. Clinical trial by Yeager and associates $({}^{66})$ revealed effectiveness of this drug in pulmonary tuberculosis. Their series included patients with streptomycin-resistant bacilli. A serious drawback was rapid emergence of pyrazinamide-resistant bacilli. Its usefulness in short-term chemotherapy, e.g., in connection with the surgical treatment of pulmonary tuberculosis, has been emphasized by some authors $({}^{52}, {}^{58})$.

The effect of pyrazinamide in experimental tuberculosis of guinea-pigs has been found to be somewhat increased by concomitant administration of either streptomycin

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or para-aminosalicylic acid (9), but clinical trials of these combinations have not been encouraging (53, 58).

A combination of pyrazinamide and isoniazid, however, has been shown to be much more effective than other drugs in experimental tuberculosis in mice (4^7) . Recent clinical trials by several groups of investigators (3, 47, 53, 58) have revealed that this combination is highly efficacious, and perhaps superior to other current antituberculosis drugs used either singly or together. The occurrence of toxic hepatitis has been reported.

Both nicotinic acid and nicotinamide have been shown by Koser and Kasai (39 , 40) to possess a weak bacteriostatic action *in vitro* on a number of microorganisms, including pneumococci, stapylococci and various strains of streptococci and of enteric bacilli. Tuberculostatic concentration of nicotinamide (nicotinic acid was less effective) has been reported to vary from 1-2 µgm. per ml against the H37RV strain (25 , 34) to 2 µgm. per ml against another strain of *M. tuberculosis* also probably of human origin (6). It was inactive on an avian strain (37). The tuberculostatic concentration of pyrazinamide also has been found to vary from 100 to 1000 µgm. per ml, with a partial inhibition at 1 to 10 µgm per ml. There was considerable variation in the values from one experiment to another, and from one strain to another (10). Tarshis and Weed (62) found a lack of significant sensitivity to pyrazinamide of a number of strains of the tubercel bacillus on solid media.

The weak and uncertain *in vitro* activity of these compounds is not consistent with their chemotherapeutic activity *in vivo*. Nor does the pellagra-preventing action have any relation to their antituberculosis activity (⁶).

The tubercle bacillus has been shown to synthesize nicotinic acid in vitro (2, 15, 54). Contrary to the early findings of Farber and Miller (1^8) that 25 per cent of tuberculous patients were deficient in niacin, Eissa and Nour El Din (1^5) and El Ride and associates (1^6) have reported that blood nicotinic acid levels in tuberculous guineapigs, rabbits and human beings were much higher than those of normal subjects and increased with the progress of the infection. Excretion of nicotinamide methochloride in the urine of tuberculous patients was higher than normal.

Beneficial action of nicotinic acid on the unpleasant side effects of sulfanilamide has been reported $(^{8, 11, 14, 48})$. General malaise, cyanosis, gastrointestinal symptoms, headache, and mental apathy are relieved. Floch and Horth $(^{20})$ have reported that nicotinamide increased tolerance to sulfones in leprosy patients. It controlled lepra reaction which occurred frequently at the beginning of sulfone therapy, more effectively than did such drugs as antihistaminics, antimony and vitamin E.

Nicotinamide is a component of pyridine nucleotides, DPN and TPN, or coenzymes I and II, the prosthetic groups of a number of dehydrogenases related to the oxidation of carbohydrates and fatty acids. Pyridine nucleotides have been shown to inhibit the bacteriostatic action of sulfapyridine on staphylococci *in vitro*, whereas nicotinic acid failed to do so (⁶⁴). Sulfapyridine and sulfathiazone inhibited the stimulating effect of nicotinamide on the respiration of dysentery bacilli *in vitro*; sulfanilamide did not. The inhibition could be partially reversed by the addition of an excess of nicotinamide or by washing the cells with buffer (^{12, 13}). 2-Sulfanilamide-5-nitropyridine reversibly inhibited the growth-stimulating action of nicotinamide on *Lactobacillus arabinosus* (⁷). Sulfapyridine greatly inhibited the dehydrogenase activity of pneumococci for glycerol, lactate, and pyruvate (⁴⁴). Prontosil suppressed the activity of catalase on the decomposition of hydrogen peroxide; nicotinic acid and nicotinamide were found to possess an antisulfonamide activity against this suppression (⁵⁷).

All these findings lend support to the opinion that an antagonistic action may exist between sulfonamides and nicotinamide or its allied compounds. Several hypotheses have been proposed to explain this phenomenon but no agreement has been reached $(1^{2}, 5^{9}, 6^{5})$. Large quantities of nicotinamide have been found to inhibit completely the growth of rats, but not of guinea-pigs and rabbits. Methionine was found to alleviate this inhibition. Rats excreted extra N¹-methylnicotinamide after nicotinamide feeding, while guinea-pigs and rabbits did not. It was suggested that the toxic effects of nicotinamide in rats were due to the synthesis of N¹-methylnicotinamide and subsequent depletion of "labile methyl groups" (17, 32).

Nicotinamide is thought to be converted in the body into pyridine nucleotides. The metabolism of the tubercle bacillus may be adversely affected either by an excess of nucleotide or by a resultant depletion of ribose or adenine required for other purposes (56). It has been reported that riboflavin possesses approximately one-third of the tuberculostatic activity of nicotinamide, as measured by treatment of experimental tuberculosis in mice (51).

DPN has been cleaved at the nicotinamide-ribose linkage by diphosphopyridine nucleotidases from various sources. Nicotinamide antagonized this cleavage, thus preventing destruction of the nicotinamide-ribose linkage (^{31, 46, 49, 50, 56, 69}). Isoniazid, structually related to nicotinamide, was approximately ten times as effective as the latter in inhibiting the enzyme (⁶⁸).

These actions of nicotinamide, i.e., the depletion of methyl or ribose and the inhibition of nucleotidases, have been considered in the explanation of the tuberculostatic action of this compound. However, its isoster, pyrazinamide, did not exhibit these activities at a similar level, although the tuberculostatic activity of pyrazinamide was stronger than that of nicotinamide (⁵⁶).

Although the mode of action of nicotinamide and its allied compounds is still obscure, their importance in mycobacterial infections is evident. While many reports of the treatment of tuberculosis with these compounds have appeared, no data on murine leprosy have been published since 1945, when Chorine first demonstrated definite suppressive activity of nicotinamide.

In previous communications I have reported a simple and reliable chemotherapeutic test, employing the intraperitoneally-infected mouse (4, 5). The therapeutic activity of sulfones, streptomycin and isonicotinylhydrazines has been reported. The present communication deals primarily with the activity of nicotinamide and pyrazinamide in mouse leprosy as determined by that technique. Also included are comparative studies on the relative activities of these two drugs and isoniazid, streptomycin and 4, 4'-diaminodiphenylsulfone (DDS) in animals which were treated either immediately after inoculation or after delays of 1 or 2 months.

METHODS

Female albino mice of the National Institutes of Health general-purpose strain, weighing 20 ± 2 gm., were used in groups of twenty, each group being caged separately. Inoculations were made with 0.5 ml of the seed suspension, i.e., a 1:30 suspension made of the omenta and pelvic fatty pads of mice infected 4 to 5 months before with the Hawaiian strain of Mycobacterium leprae murium.

Experiment 1.—Six groups of mice were used according to the following schedule: Group 1. Murine leprosy control, untreated.

Group 2. DDS 0.1 per cent, as the standard of reference for treatment.

³ Nicotinamide and pyrazinamide (Aldinamide) were kindly supplied by Lederle Laboratories Division, American Cyanamid Company.

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Group 3 and 4. Duplicate experiment, Nicotinamide 0.5 per cent.³ Group 5 and 6. Duplicate experiment, Pyrazinamide 0.2 per cent.³ The treatment was started on the day after inoculation.

Experiment 2.—The following groups were used:Group 1. Normal-mouse control.Group 2. Killed-bacillus control (autoclaved leprous material inoculated into 3)

mice).

Group 3. Murine leprosy control, untreated.

Group 4. Nicotinamide 0.5 per cent, started on the day after inoculation. Group 5. Nicotinamide 0.5 per cent, started 1 month after inoculation. Group 6. Nicotinamide 0.5 per cent, started 2 months after inoculation. Group 7. Pyrazinamide 0.5 per cent, started on the day after inoculation. Group 8. Pyrazinamide 0.5 per cent, started 1 month after inoculation. Group 9. Pyrazinamide 0.5 per cent, started 2 months after inoculation. Group 10. Isoniazid 0.01 per cent, started on the day after inoculation.4 Group 11. Isoniazid 0.01 per cent, started 1 month after inoculation. Group 12 Isoniazid 0.01 per cent, started 2 months after inoculation. Group 13. Streptomycin 2 mgm., started on the day after inoculation. Group 14. Streptomycin 2 mgm., started 1 month after inoculation. Group 15. Streptomycin 2 mgm., started 2 months after inoculation. DDS 0.1 per cent, started on the day after inoculation. Group 16. Group 17. DDS 0.1 per cent, started 1 month after inoculation. Group 18. DDS 0.1 per cent, started 2 months after inoculation.

The streptomycin was injected subcutaneously 5 days a week. All the other drugs were mixed in the food (ground pellets of Purina rat chow). Dosages are expressed in mgm. per kgm. of body weight, and are calculated on the basis of an average daily food intake of 4 gm. per 20-gm. mouse.

All animals were sacrificed at the end of the experiment, approximately three months after inoculation. The autopsies were all performed by the same operator, without knowledge of the treatment the animals had received. The mortality rate, the body weights, the weights and photographs of omenta and of pelvic fatty pads, the bacillus and leprosy indices, and the index of chemotherapeutic effectiveness (ICE) of each group of animals were recorded according to the schedules previously described (⁴). A minor change in the preparation of smears was that the thymus gland was used instead of the tracheobronchial lymph nodes. The data presented in the first two tables represent the average of all animals of each group, except that the bacillus indices are averages of two representative animals of each group.

RESULTS

Experiment 1.—The results are shown in Table 1. Among five of the six groups a total of 15 animals died at various intervals after inoculation, all of intercurrent diseases. The rest of the animals remained in fairly good condition, although the average body weights of those treated with nicotinamide and pyrazinamide were slightly lower than those of the untreated controls and the DDS-treated group.

⁴ Isoniazid (Nydrazid) was obtained through the courtesy of E. R. Squibb & Sons.

	Dose	No.	Dod.	Weight	Weight					Leprosy	indexb				
Drug	diet, per cent	of No.	weight gm.	onen- tum, gm.	pelvic fatty pads, gm.	Bacillus indexa	Site of inocu- lation	Omen- tum mesen- tery	Pelvic fatty pads	Lymph nodes	Spleen	Liver	Misc.	Total index	ICEc
Leprosy control:	0	3/20	24.1	0.14	62.0	15.0	0.53	3.97	1.41	0.50	0.38	0.44	1.32	8.55	:
DDS	0.1	2/20	24.7	0.11	0.67	8.0	0.64	3.33	0.83	0.08	0	0.08	0.53	5.49	1.6
Nicotinamide, Group A	0.5	1/20	22.2	0.05	0.55	11.0	0.13	1.61	0.11	0	0.08	0	0.16	2.09	4.1
Nicotinamide, Group B	0.5	2/20	22.7	0.04	0.40	6.5	0.19	1.75	0.11	0.03	0.08	0.22	0.08	2.46	3.5
Pyrazinamide, Group A	0.2	0/20	23.3	0.07	0.54	5.0	0.18	2.60	0.30	0.25	0.15	0.05	0.43	3.96	2.2
Pyrazinamide, Group B	0.2	7/20	22.9	0.07	0.48	12.0	0.58	2.04	0.42	0.04	0.08	0	0.69	3.85	2.2

TABLE 1.-Experiment 1: Evaluation of nicotinamide and pyrazinamide, with DDS as the standard of reference.

and organs: site of inoculation, omentum, pelvic fatty pads, portal lymph nodes, paravertebral lymph nodes, thymus gland, spleen, liver, lung and kidney.

b The leprosy index in this and other tables represents the amount of gross pathological lesions in the various sites and organs graded as follows: Site of inoculation and lymph nodes, each, 0-2; omentum and mesentery, 0-6; pelvic fatty pads, spleen, and liver, each, 0-4; miscellanous, including lungs, kidneys, diaphragm, pericardium, retrostermal region, and thymus gland, each, 0-1. 0 represents no visible lesions.

c Index of chemotherapeutic effectiveness (see text).

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Marked suppression of the leprous growth in the omenta and pelvic fatty pads was shown in the animals treated with nicotinamide and pyrazinamide. The average weight of the omenta in each of the duplicate groups (A and B) treated with these drugs was markedly less than that in the untreated control group. The weights of pelvic fatty pads also indicated suppression although less marked.

The differences in size and appearance of the pelvic fatty pads and the omenta between the untreated control and the three groups treated with DDS, nicotinamide and pyrazinamide are shown in Plate 24. The activity of these drugs is evident.

The leprosy index, i.e., the amount of gross pathological lesions in the various organs, also revealed a marked difference between the treated and untreated groups. This index of the control group was 8.55. The indices of the two nicotinamide-treated groups were 2.09 and 2.46, and those of the two pyrazinamide-treated groups were 3.96 and 3.85. Therefore, the average index of chemotherapeutic effectiveness ⁵ was 3.8 for nicotinamide and 2.2 for pyrazinamide.

DDS revealed a definite activity in this experiment (ICE 1.6) but less than previously reported.

Comparing the figures of the duplicate groups, A and B, of the nicotinamide and pyrazinamide-treated groups, respectively, close similarity is seen in the values for each component of the leprosy index and also in the weights of the omenta and pelvic fatty pads. This indicates that the activity of these drugs was fairly uniform in this experiment.

All of the bacillus indices of the five treated groups are smaller than that of the untreated control, but it will be noted that the variations between the duplicated groups are great.

From these findings it may be concluded that nicotinamide and pyrazinamide, in the dosages used, exercised definite activity in intraperitoneally-infected mouse leprosy. Their activity was slightly superior to that of DDS. The early finding of Chorine regarding the effectiveness of nicotinamide was confirmed.

Experiment 2.—A repetition of the experiment with nicotinamide and pyrazinamide was performed, using a higher dose of the latter. In addition, a comparison of the effects of these drugs was made with those of three compounds known to be active, isoniazid, streptomycin and DDS. To compare these drugs with respect to both suppressive and therapeutic effectiveness, observations were made on groups of mice in which administration of the drugs was (a) started immediately after inoculation, continued 3 months; (b) delayed 1 month, continued 2 months; and, (c) delayed 2 months, continued 1 month.

⁵ The index of chemotherapeutic effectiveness, or ICE, was calculated as follows: Total leprosy index of the control group.

Total leprosy index of the treated group.

The larger the figure the higher the activity. Unity means no action.

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ICE 6.0 2.7 13.8 2.6 27.3 3.0 1.8 40.0 1.9 4.9 1.4 2.6 1.9 1.1 2.1 Total 12.00 0.44 6.48 2.47 5.71 8.92 6.50 4.01 6.61 0.30 2.00 4.49 0.87 4.67 4.67 10.54 Misc. 0.42 0.79 0.12 0.46 0.21 0.73 0.14 0.68 1.23 0.45 0.65 1.58 1.64 0 0 0 Liver 0.72 0 0.03 0.08 0.04 0.32 0.58 0.58 0.21 0.70 0 0 0 0 0 0 Spleen 0.05 0.29 0.26 29.0 0.45 0.21 0.50 Leprosy index 0.63 0.21 0 0 0 0 0 0 0 Lymph nodes 0.29 0.15 0.11 0.19 0.08 0.83 0.24 0.30 0.02 0.26 0.23 0.53 0 0 0 0 Pelvic fatty pads 0.74 2.28 0.32 0.08 0.54 0.05 0.68 1.18 1.45 0.08 0.87 0.40 1.21 0 0 Omen-tum & mesen-tery 0.39 2.45 3.42 4.72 0.30 1.38 2.33 0.82 2.29 2.83 3.00 3.55 1.92 2.90 3.32 4.25 Site of inocu-lation 0.63 1.00 0.23 1.04 0.76 1.18 1.14 0.05 0.48 0.33 0.50 0.90 0.85 1.08 0 0 Bacillus index 7.3b 20.0 4.0 12.0 16.0 4.0 11.0 17.0 10.5 11.0 11.5 5.0 17.5 12.0 16.5 16.0 14.5 Weight of pelvic fatty pads, gm. 0.43 0.52 1.00 0.60 0.73 0.73 0.75 1.04 0.55 0.57 0.41 0.52 0.32 0.50 0.61 0.57 0.81 1.00 Weight of tum, gm. 0.10 0.01 0.13 0.01 0.08 0.12 0.02 0.03 0.08 0.01 20.0 0.09 0.04 0.10 0.10 0.11 0.17 0.01 Body weight, gm. 25.0 23.6 24.2 25.7 23.7 24.9 26.0 27.3 24.9 26.2 24.3 24.3 24.5 24.9 24.3 25.5 23.3 24.1 a Delay in treatment; see text. 1/200/12 2/200/200/20 0/13 1/203/20 0/20 2/20 1/200/20 0/20 3/20 No. of No. No. 0/20 1/20 0/20 0/3 mgm.c 2 mgm.c 2 mgm.c Dose in diet, per cent 0.01 0.01 0.01 0.5 0.5 0.5 0.5 0.1 0.5 0.5 0.1 Delay in treat-ment (mos.)a 0 0 01 01 0 3 0 0 0 Autoclaved inocu-lum control Leprosy control, untreated Drug Nicotinamide Pyrazinamide Pyrazinamide Nicotinamide Nicotinamide Pyrazinamide Streptomycin Streptomycin Streptomycin Normal mice Isoniazid Isoniazid Isoniazid DDS DDS DDS

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b Average of 3 animals. c Injected subcutaneously.

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The results are shown in Table 2. Two of the untreated control animals died of the disease on the 71st and 95th days of the experiment. A total of 12 deaths in the nicotinamide, isoniazid, streptomycin, and DDS groups were probably due to intercurrent diseases, since no evidence of toxicity of these drugs was noticed in these experiments or the previous ones. Seven animals in one group and 8 in another group of the pyrazinamide-treated mice unfortunately escaped shortly before the end of the experiment. The rest of the animals were in fairly good condition at the end. The average body weight was within the normal range, indicating that the drugs tested were well tolerated.

All five drugs were found to be effective in suppression of the infection. The medication was most effective in the groups whose treatment was started at once after inoculation. Nicotinamide, pyrazinamide and isoniazid were the most effective. No gross lesions were found in the animals receiving full therapy with these three compounds, except a few tiny nodules on the omentum and mesentery. Lesions at the site of inoculation were rare. The omenta and pelvic fatty pads were normal The bacillus indices were only one-fourth to one-fifth of that in size. of the control group. The most marked differences were observed in the leprosy indices, that of the nicotinamide group being 0.44; of the pyrazinamide, 0.30, and of the isoniazid, 0.87; while that of the control The ICE were, therefore, 27.3 for nicotinamide, 40.0 for was 12.00. pyrazinamide, and 13.8 for isoniazid. The larger dose level (0.5%) of pyrazinamide was much more effective than the smaller one (0.2%) used in Experiment 1.

Streptomycin and DDS were less active. The total leprosy indices of the animals receiving full therapy with these drugs were 2.47 and 4.67, respectively. Thus, the ICE for the former was 4.9, and for the latter 2.6. Streptomycin was more active than DDS in this experiment, although previously the two had been much alike (5).

Marked activity of the three most effective drugs was also observed when the treatment was delayed for 1 month. Between the control and treated groups, the differences in the weights of omenta and pelvic fatty pads and the differences in the bacillus and leprosy indices were obvious. The ICE were 3.0 for nicotinamide, 6.0 for pyrazinamide, and 2.6 for isoniazid. Streptomycin and DDS were again less active, with ICE of 2.1 and 1.9, respectively.

In the animals whose treatment was delayed for 2 months and continued only 1 month, the effectiveness of the three active drugs was slight but definite; streptomycin and DDS were inactive.

The appearance of the pelvic pads and omenta of normal mice, of the untreated leprosy controls and of the 15 groups treated with the five drugs for different lengths of time are shown in Plates 25 to 28. For each drug, the differences in the degree of activity among the three groups treated for different lengths of time can be seen. The differences among

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groups treated with the five drugs for the same length of time are obvious. The greater effectiveness of nicotinamide, pyrazinamide and isoniazid is illustrated by the close resemblance between normal tissues and the tissues of animals receiving full therapy with these compounds. With the two months' delay, the gross appearance of the DDS animals was similar to that of the control group.

The leprosy index revealed a quantitative relationship among the various compounds. For each compound, the lowest index was obtained from the full therapy; while the delayed treatments gave progressively higher indices. The relative activity of the five compounds is shown in Text-fig. 1.



TEXT-FIG. 1. The relative activity of nicotinamide, pyrazinamide, isoniazid, streptomycin and DDS in 3 groups of animals inoculated with *M. leprae murium*.

I. Treated immediately after inoculation for 3 months.

II. Treatment delayed 1 month, subsequently treated for 2 months.

III. Treatment delayed 2 months, subsequently treated for 1 month.

The ordinate represents the total leprosy index; the lower the column the higher the activity of the drug.

The bacillus indices of all the treated groups were lower than the index of the untreated control. The lowest figures, ranging from 4 to 5, were found in groups receiving full therapy of nicotinamide, pyrazinamide and isoniazid. It is known that killed acid-fast bacilli may remain in tissues for a long time. Three animals inoculated with autoclaved leprous material were included in this experiment as a control. When sacrificed at the end of the three months the average bacillus index was found to be 7.3, similar to the lowest figures found in the groups receiving the most effective therapy. Whether the residual organisms in these treated animals were dead or alive was not determined.

Histological examinations revealed minimal lesions in the animals which received full treatment with the three most active drugs in the second experiment. Only a few scattered acid-fast bacilli were found at the sites of inoculation, or in the omenta or pelvic fatty pads. Some organisms were occasionally found in the uterus and the liver. The typical lepra cell was rarely seen.⁶ However, the lepra cells in the rest of the animals did not differ in appearance from those in the controls.

Drug	Dose (mgm./kgm.)	Delay in treatment (mos.)	Total leprosy index	Histological lesions in liver
Leprosy control, untreated			12.00	Extensive
Nicotinamide	1,000	0	0.44	Minimal
Nicotinamide	1,000	1	4.01	Slight
Nicotinamide	1,000	2	6 61	Moderate
Pyrazinamide	1,000	0	0.30	Minimal
Pyrazinamide	1,000	1	2.00	Slight
Pyrazinamide	1,000	2	4.49	Moderate
Isoniazid	20	0	0.87	None
Isoniazid	20	1	4.67	Minimal
Isoniazid	20	2	6.48	Moderate
Streptomycin	100	0	2.47	Slight
Streptomycin	100	1	5.71	Moderate
Streptomycin	100	2	8.92	Moderate
DDS	200	0	4.67	Moderate
DDS	200	1	6.50	Moderate
DDS	200	2	10.54	(Not recorded

 TABLE 3.—Comparison of the leprosy index with the amount of histological lesions in the liver of mice. Experiment 2.

⁶ Dr. G. L. Fite of the Laboratory of Pathology of this institute, considered that the scattered bacilli were probably located intracellularly, although they seemed to be extracellular. He has also observed scattered bacilli in rats in which the leprous lesions were not well developed. No changes in the character of the lesions were found as a result of chemotherapy.

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Various degrees of leprous involvement were found in all other groups, the extent of lesions being inversely proportional to the duration of the treatment. There was a correlation between effectiveness and the lesions of certain organs. Table 3 shows the relationship between the total leprosy index and the extent of histological lesions in the liver in the animals of the second experiment. The leprosy index was roughly equivalent to the degree of liver involvement. This supports the opinion of Fite (19) that the histological findings in the liver may be used as a criterion in evaluating chemotherapeutic activity in experimental murine leprosy.

These observations lead to the conclusion that, under the conditions of these experiments, nicotinamide, pyrazinamide, and isoniazid showed the highest activity in the suppression of murine leprosy, streptomycin the next highest and DDS the lowest. The effectiveness of pyrazinamide was similar, if not superior, to that of nicotinamide and of isoniazid. All the five drugs possessed some activity when the treatment was delayed for one month. Only the three most active compounds showed definite effect of treatment for one month after the delay of two months, while streptomycin and DDS were ineffective under these circumstances.

DISCUSSION

The index of chemotherapeutic effectiveness (ICE) used in this and previous reports (4, 5) is the ratio of the total leprosy index in a control group to that in a treated group. The total leprosy index is the sum of the grades assigned to gross lesions in certain sites and organs. It does not take into account the number of bacilli and other data. The ICE represents the activity of a drug at a specified dosage and should not be confused with a chemotherapeutic index based upon the minimal effective and the maximal tolerated doses.

Short-term chemotherapeutic tests with intraperitoneally-infected mouse leprosy have been reported by two groups of investigators. Grunberg and associates (26, 27) sacrificed their animals four weeks after inoculation. Comparison of the bacillus counts was made on localized lesions removed from the peritoneum of treated and untreated animals. A modification of this procedure was used by Hobby and her associates (33), who employed spleen suspensions as the material for the bacillus count. Quantitative differences in the action of antimicrobial agents were most apparent after treatment for at least 42 days after inoculation. In some of their experiments the observation period was more than 60 days.

Isoniazid and iproniazid were found highly effective by both groups of investigators. This agrees with my findings (5). However, both streptomycin and DDS were found ineffective by Grunberg and associates, while streptomycin was found active and DDS ineffective by Hobby and associates. By using a longer observation period, i.e., three months, and an ICE based upon gross lesions, I have found that both streptomycin and DDS are active. The longer the observation period the more accurate seems to be the test. An observation period shorter than three months is probably inadequate for the evaluation of a less-active compound such as DDS in intraperitoneally-infected mouse leprosy.

Barnett and Bushby (1) reported a chemotherapeutic test using intravenously-infected mice. Goulding and associates (24) employed mice infected by the intracorneal route. Excellent response to isoniazid was obtained by both methods, but no effect from sulfones. Their observation periods were 180 days and four months, respectively. Levaditi and Chaigneau-Erhard (42) reported some activity of streptomycin and of DDS in mice infected intracerebrally; the observation period was 77 days. Their method, involving chiefly a histological technique, is tedious for routine assay.

Comparing the various methods of chemotherapeutic assay in mouse leprosy, my technique seems to be simple, and sufficiently sensitive for quantitative evaluation of chemotherapeutic activity.

From the highly effective activity of nicotinamide and pyrazinamide in murine leprosy, clinical trial of these compounds in leprosy seems to be indicated.

SUMMARY

A brief review of the pharmacological actions of nicotinamide and allied compounds in relation to the mycobacterial infections has been presented.

The activity of nicotinamide and pyrazinamide (Aldinamide) in mouse leprosy has been studied, employing the intraperitoneal route for infection. Comparative studies of the activity of these compounds with that of three known effective drugs—isoniazid, streptomycin and DDS—were made in animals treated immediately after inoculation or after delays of one or two months. The duration of the experiments was three months.

Both nicotinamide and pyrazinamide were found to be highly effective in the suppression of the leprous infection. Nicotinamide was found to have a degree of activity similar to that of pyrazinamide and of isoniazid, and superior to that of streptomycin; DDS was the least active.

All these five compounds were most effective when the administration was started immediately after the inoculation and continued for three months, proportionately less effective when the treatment was delayed for one or two months. Only nicotinamide, pyrazinamide and isoniazid possessed any significant activity when the treatment was delayed for two months and then carried out for only one month.

RESÚMEN

Se presenta un breve resúmen de la acción farmacalógica de la nicotinamida y compuestos aliados en relación a las infecciones por micobacterias. Se estudió la actividad de la nicotinamida y la pirazinamida en la lepra murina, producida por inyeccion intraperitoneal. Estudios comparativos de la actividad de éstos compuestos y la de 3 otras drogas efectivas (isoniazid, estreptomicina y D.D.S.) se hicieron en animales tratados inmediatamente después de la inoculacion o despues de 1 ó 2 meses. La duración de las experimentos fue 3 meses.

Tanto la nicotinamide como la pirazinamide fueron altamente efectivas en la supresión de la infección leprosa. La nicotinamide tuvo una acividad similar a la de pirazinamida y a la de isoniazid, y superior a la estreptomicina; D.D.S. fué la menos activa.

Todos éstos 5 compuestos fueron mas efectivos cuando la administración se empezó inmediatamente despues de la inoculación y se continuó por 3 meses, proparcionalmente menos efectivo cuando el tratamiento se pospuso por 1 ó 2 meses. Sólo la nicotinamide, la pirazinamida y la isoniazid demostraron actividad significante cuando el tratamiento se pospuso por 2 meses y entonces se llevó a cabo por un mes.

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DESCRIPTION OF PLATES

PLATE (23)

Comparison of the pelvic fatty pads and the omenta of untreated and treated mice three months after infection with murine leprosy. Experiment 1. The upper part of each picture is of the pelvic fatty pads, the lower of the omenta. Note that the tissues of the treated groups are smaller and smoother than those of the control group.

FIG. 1. Untreated control group.

FIG. 2. DDS group. The lesions on the whole are perceptibly smaller than those of the control.

FIG. 3. Nicotinamide-treated mice, Group B. The lesions are distinctly smaller than those of the DDS group (Fig. 2), and slightly smaller than those of the pyrazinamide group (Fig. 4).

FIG. 4. Pyrazinamide-treated mice, Group A. The lesions are smaller than those of the DDS group (Fig. 2), but slightly larger than those of the nicotinamide group (Fig. 3).



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PLATE 23.

PLATE (24)

Comparison of the pelvic fatty pads and the omenta of untreated and treated mice three months after infection with murine leprosy. Experiment 2.

FIG. 5. Normal mouse control group.

FIG. 6. Leprosy control group, untreated.

FIG. 7. Nicotinamide group; treated from the beginning, for 3 months. No apparent lesions are seen; the appearance is similar to that of normal mouse group (Fig. 5).

FIG. 8. Nicotinamide group; treatment delayed for 1 month, subsequently treated for 2 months. The omenta are larger than those of the nicotinamide group treated for 3 months (Fig. 7). Some lesions are seen in the fatty pads.



PLATE 24.

PLATE (25)

Experiment 2, continued.

FIG. 9. Nicotinamide group; treatment delayed for 2 months, subsequently treated for 1 month. The lesions are larger than those of Fig. 8, but definitely smaller than those of the controls (Fig. 6).

FIG. 10. Pyrazinamide group; treated from the beginning, for 3 months. No apparent lesions are seen. Note the close resemblance to the tissues of the normal mice (Fig. 5).

FIG. 11. Pyrazinamide group; treatment delayed for 1 month, subsequently treated for 2 months. There were only 13 animals in this group at the end. Few lesions are seen in the fatty pads. The omenta are slightly enlarged.

FIG. 12. Pyrazinamide group; treatment delayed for 2 months, subsequently treated for 1 month. Only 12 animals in this group at the end. The lesions are larger than those of Fig. 11, but much smaller than those of the controls (Fig. 6).

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PLATE 25.

PLATE (26)

Experiment 2, continued.

FIG. 13. Isoniazid group treated from the beginning for 3 months. Some lesions are seen on omenta, but not in the fatty pads. Note the similarity of appearance to Fig. 5, 7 and 10.

FIG. 14. Isoniazid group; treatment delayed for 1 month, subsequently treated for 2 months. The lesions are larger than those of the isoniazid group treated for 3 months (Fig. 13).

FIG. 15. Isoniazid group; treatment delayed for 2 months, subsequently treated for 1 month. The lesions are larger than these of Fig. 14, but smaller than those of the controls (Fig. 6).

FIG. 16. Streptomycin group; treated from the beginning, for 3 months. The lesions are much smaller than those of the controls (Fig. 6), but distinctly larger than those of the groups receiving full therapy of nicotinamide, pyrazinamide or isoniazid (Fig. 7, 10 and 13).



PLATE 26.

PLATE (27)

Experiment 2, concluded.

FIG. 17. Streptomycin group treatment delayed for 1 month, subsequently treated for 2 months. Lesions are larger than those of the streptomycin group treated for 3 months (Fig. 16), but smaller than those of the controls (Fig. 6).

FIG. 18. Streptomycin group; treatment delayed for 2 months, subsequently treated for 1 month. Lesions are slightly smaller than those of the controls (Fig. 6), but the difference is not obvious.

FIG. 19. DDS group; treated from the beginning, for 3 months. Lesions are smaller than those of the controls (Fig. 6), but larger than those of streptomycin group treated for 3 months (Fig. 16). These tissues are quite similar to those of the second DDS-treated group (not shown here) whose treatment was delayed for 1 month, subsequently treated for 2 months.

FIG. 20. DDS group; treatment delayed for 2 months, subsequently treated for 1 month. Lesions are about the same as in the controls (Fig. 6).



PLATE 27.