CHEMOTHERAPY OF MURINE LEPROSY

VI. THE EFFECTS OF ISONICOTINYLHYDRAZONE OF 2-CARBOXYMETHOXY-3-METHOXYBENZALDEHYDE (COMPOUND 373) AND ISONICOTINYLHYDRAZONE OF 2-CARBOXYMETHOXYBENZALDEHYDE (COMPOUND 377) ON MOUSE LEPROSY

Y. T. CHANG, M. D.[1, 2]
National Institute of Arthritis and Metabolic Diseases
National Institutes of Health
Bethesda, Maryland

Since the discovery of the antituberculous activity of isonicotinic acid hydrazide (isoniazid), many related chemical compounds have been studied in the hope of finding compounds still more potent, less toxic, and of longer-lasting activity. Isonicotinylhydrazones of substituted benzaldehydes have been studied by several groups of investigators. A total of 25 derivatives have been reported to be effective in experimental tuberculosis of mice, rabbits and guinea-pigs (1, 2, 10, 14, 18, 22, 23, 24, 25, 26, 28). Two were reported effective also in human tuberculosis (11, 16, 20) and one in leprosy (13). The chemical structures of these compounds and the animals in which they have been found effective are shown in Table 1.

Several of these substances have been claimed to be more effective and less toxic than the parent compound, isoniazid, in experimental tuberculosis (10, 23, 24), and one is said to have produced better results in the treatment of leprosy (13). Siebenmann and Zubrys (26) have reported that, in mouse tuberculosis, isonicotinylhydrazone of 2-carboxymethoxybenzaldehyde (Compound 377) was more effective than isoniazid when the treatment was started immediately following the inoculation, but less active when the treatment was given seven days later; its toxicity in mice was less than one twenty-fifth of that of isoniazid. A related derivative, isonicotinylhydrazone of 2-carboxymethoxy-3-methoxybenzaldehyde (Compound 373), approached isoniazid in its *in vivo* antituberculosis activity, while its toxicity was less than one-fortieth of that of isoniazid. Compound 373 also revealed low toxicity in human beings, and a clinical trial in tuberculosis is in progress (27).

The suppressive activity of isoniazid in murine leprosy has been reported by me and other investigators (3, 5, 6, 8, 9, 12, 15, 21). No report on the effects of isonicotinylhydrazones of substituted benzaldehydes has been made. On account of the high activity and low toxicity of Compound 373 and Compound 377 in experimental tuberculosis, studies of their effects on mouse leprosy were undertaken. By employing equimolar doses, the

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[1] Fellow in Pharmacology, Leonard Wood Memorial (American Leprosy Foundation.)

[2] With the technical assistance of Robert W. Scaggs.

activities of these substances and isoniazid were compared in various classes of treatment. The long-lasting protective effect of Compound 377, which is superior to that of isoniazid and of Compound 373, is reported here.

METHODS

The same technique of chemotherapeutic assay in mice as used in previous studies (4) was employed. Female, albino mice of the National Institutes of Health general purpose strain, weighing 20 ± 2 gm., were used in groups of 20, each group being caged separately. Intraperitoneal inoculations were made with 0.5 cc. of 1/30 suspensions of mixed omenta and pelvic fatty pads of mice that had been infected 4 to 5 months before with the Hawaiian strain of *Mycobacterium leprae murium*. Three drugs were used, as said, isoniazid, Compound 373 and Compound 377.[3] Drugs were mixed in the food in equimolar doses, i. e., 0.1 per cent for isoniazid, 0.024 per cent for Compound 373, and 0.022 per cent for Compound 377. The activities of these compounds were compared in the following four different treatment classes.

Class 1: Animals treated immediately after inoculation for a period of three months, and then sacrificed.

Class 2: Animals whose treatment was delayed for one month, and then treated for two months, and sacrificed.

Class 3: Animals treated for three months and allowed to live after the termination of treatment until the end of the experiment, 444 days (nearly 15 months) after inoculation.

Class 4: Animals treated continuously throughout the experiment until the majority of them died of the infection, i. e., about 44 days after inoculation. The survivors of Classes 3 and 4 were sacrificed at the end of the experiment.

Three groups of animals were used as controls. One untreated murine-leprosy group was sacrificed at the end of three months. In another group the disease was allowed to run its natural course, until death of the animals. The third control group, comprising 23 animals, was inoculated with leprosy material that had been autoclaved at 15 pounds for 30 minutes. Of this last group, 6 animals were sacrificed at the end of three months, and 13 were sacrificed at the termination of the experiment. Six of the latter were used for bacteriologic and histologic specimens, and 7 for photographic preparations. Four of this group died from intercurrent diseases during the experiment. In addition, two groups of uninfected animals were observed to ascertain the mortality rate of normal mice.

Autopsies were performed on animals that were sacrificed, and on those that died during the observation period. These autopsies were done without knowledge of the treatment that the animals had received. The mortality rate, body weight, and weights of omenta and of pelvic fatty pads were recorded. The omenta and pelvic fatty pads of each group of animals were placed in a petri dish and photographed. The bacillus and leprosy indices were recorded according to the scores given for various sites and organs in a previous communication (6). Histologic preparations of various sites and organs were made from two representative animals of each group, including also the livers of four other animals in the same group; specimens were also taken from all survivors of Classes 3 and 4 at the end of the experiment. The data presented in Table 2 represent the average of all animals of each group except the bacillus indices, which are averages of two representative animals of each group.

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[3] Compound 373 and Compound 377 were kindly supplied by Drs. C. O. Siebenmann and A. Zubrys of the Connought Medical Research Laboratories, University of Toronto, Toronto, Canada. Compound 373 contains two molecules of water. Therefore, the dose used here was slightly less than the equimolar one.

TABLE 1.—*Isonicotinyl hydrazones of substituted benzaldehydes; chemical structures, and animals in which they have been found effective.*

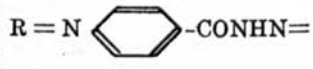
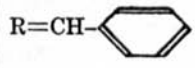
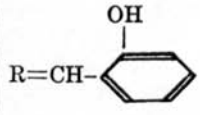
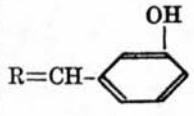
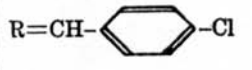
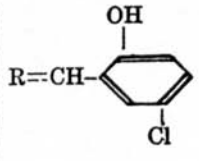
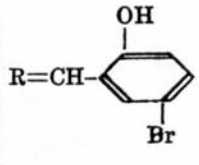
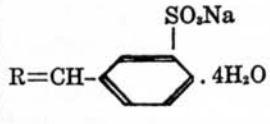
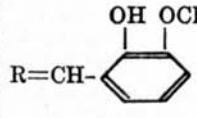
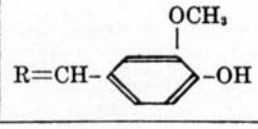
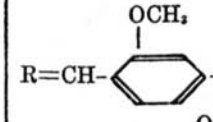
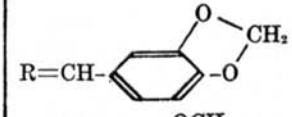
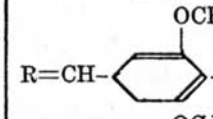
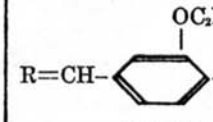
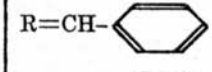
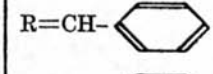
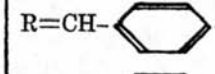
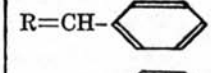
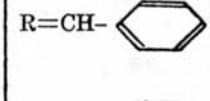
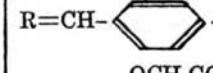
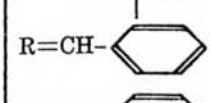
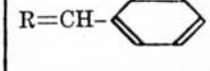
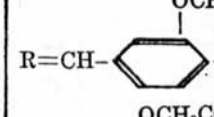
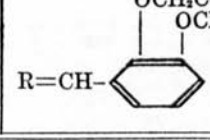
No.	Compound	Structure formula	Effective in tuberculosis of	Reference
				
1.	Isoteben		Guinea-pig, rabbit, mouse	10, 14, 23
2.	Salizid, INHS, Compound 330		Human, mouse, guinea-pig, rabbit	10, 16, 20, 23, 24, 26, 28
3.	Acroteben		Guinea-pig, rabbit, mouse	10, 23
4.	Flavoteben, Compound 327		Guinea-pig, rabbit, mouse	10, 23, 24, 25, 26
5.			Guinea-pig, rabbit, mouse	10, 23
6.	INHCS		Mouse	23
7.	INHBS		Mouse	23
8.	G605		Mouse, human tuberculosis; human leprosy	11, 13
9.	INHA		Mouse	14, 23
10.	Compound 363		Mouse	26
11.	Compound 335		Mouse	23, 24, 26

TABLE 1.—Continued

No.	Compound	Structure formula	Effective in tuberculosis of	Reference
12.			Mouse	14
13.			Mouse	14
14.	Verazide		Mouse, guinea-pig	14, 22
15.			Mouse	23
16.			Mouse	2
17.			Mouse	2
18.			Mouse	1, 2, 23, 24
19.			Mouse	23, 24
20.			Mouse	2
21.			Mouse	2, 23
22.	Compound 377		Mouse	26
23.	G594, Compound 369		Mouse	18, 26
24.	Compound 376		Mouse	26
25.	Compound 373		Mouse	26

Kinyoun's [17] modified acid-fast stain, employing cold staining with concentrated fuchsin, was used in the early part of this experiment; warm staining with the same stain was adopted later.

RESULTS

Class 1.—Three groups of animals were fed equimolar doses of isoniazid, Compound 373 and Compound 377, respectively, immediately after inoculation for a period of three months. One group of untreated leprosy controls and one group of autoclaved-bacteria controls were included. One animal of the untreated leprosy control group died of extensive lesions at the end of three months. One animal of the isoniazid- and two of the Compound 373-treated group died of intercurrent diseases in the early days of the experiment. At the end of three months all remaining animals were sacrificed. The results may be seen in Table 2.

All three drugs showed marked activity in the suppression of the growth of the leprous lesions in the mouse. The weight of omenta of all treated groups was from one-eighth to one-fifth of that of the untreated leprosy control, and was slightly greater than that of animals of the killed-bacteria control. The weight of pelvic fatty pads of all treated animals was smaller than that of the untreated leprosy control. The bacillus index of all treated groups was from one-fourth to one-third of that of the untreated leprosy controls, and was practically the same as that of the killed-bacteria controls. The total leprosy index was 0.53 for isoniazid-, 0.30 for Compound 373- and 0.46 for Compound 377-treated groups, while that of the untreated leprosy control groups was 11.13. The indices of chemotherapeutic effectiveness[4] for isoniazid, Compound 373 and Compound 377 were 21.0, 37.1 and 24.2, respectively. It is noteworthy that the activities of the three drugs were about equal.

Class 2.—The effects of delayed treatment with equimolar doses of isoniazid, Compound 373, and Compound 377 were studied in three groups of animals. Treatment was started in the second month and continued for two months. Three animals of the isoniazid-treated and two each of the Compound 373- and Compound 377-treated groups died of intercurrent diseases. The remaining animals were sacrificed at the end of the two months treatment. The results are shown in Table 2.

The weights of the omenta and the pelvic fatty pads and the bacillus indices of all treated groups were smaller than those of the untreated leprosy control, but larger than those of animals receiving three-months treatment. The leprosy index was 2.49 for the isoniazid-, 2.73 for the Compound 373-, and 2.83 for the Compound 377-treated groups. Accordingly, the ICE was 4.5, 4.1 and 3.9, respectively. Thus, in delayed therapy

[4] The index of chemotherapeutic effectiveness, or ICE, was calculated as follows: $\frac{\text{Total leprosy index of the control group}}{\text{Total leprosy index of the treated group}}$. The larger the figure the higher the activity. Unity means no action.

TABLE 2.—*The effects of equimolar doses of isoniazid, Compound 373 and Compound 377 on murine leprosy in the mouse. Drugs were administered in two classes. In Class 1 the animals were treated immediately after inoculation, for three months. In Class 2 the treatment was delayed for one month, and subsequently given for two months.*

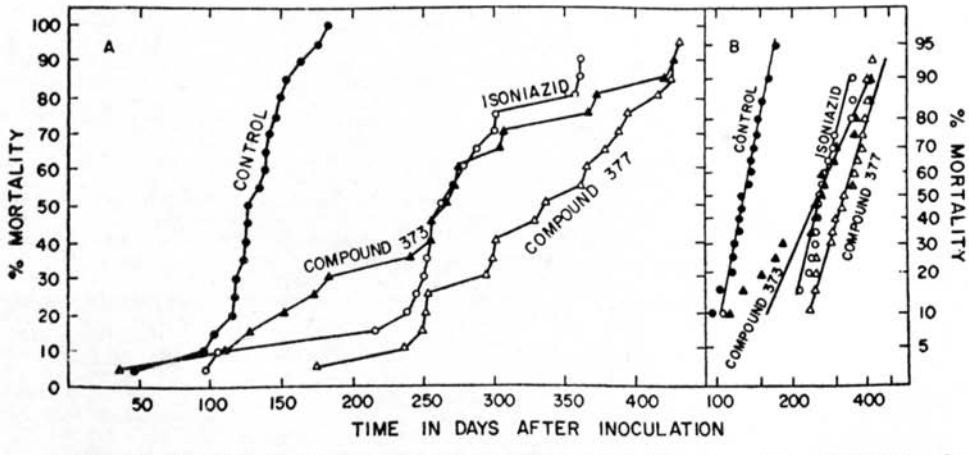
Group	Dose in food, per cent	No. died/ No. used	Body wt., gm.	Weight of omentum, gm.	Weight of pelvic fatty pads, gm.	Bacillus index	Leprosy index								ICE[a]
							Site of inoculation	Omentum and peritoneum	Pelvic fatty pads	Lymph nodes	Spleen	Liver	Misc.	Total index	
Killed-bacteria control		0/6	23.3	0.01	0.79	4.5[b]									
Untreated leprosy control		1/20	21.7	0.16	1.23	21.0	1.11	4.18	3.29	0.11	0.50	0.26	1.68	11.13	
Class 1															
Isoniazid	0.01	1/20	25.6	0.02	0.61	6.5	0.03	0.50	0	0	0	0	0	0.53	21.0
Compound 373	0.024	0/20	26.4	0.03	0.78	6.5	0	0.30	0	0	0	0	0	0.30	37.1
Compound 377	0.022	2/20	23.6	0.02	0.54	5.0	0	0.43	0.03	0	0	0	0	0.46	24.2
Class 2															
Isoniazid	0.01	3/20	24.5	0.06	0.57	9.0	0.27	1.27	0.65	0.03	0.06	0.06	0.15	2.49	4.5
Compound 373	0.024	2/20	23.4	0.05	0.61	15.0	0.58	1.39	0.56	0.03	0.03	0	0.14	2.73	4.1
Compound 377	0.022	2/20	23.9	0.07	0.63	15.0	0.42	1.42	0.72	0	0.08	0.08	0.11	2.83	3.9

a Index of chemotherapeutic effectiveness, see text.
b Average of 6 animals.

all three drugs were effective in the suppression of the infection, and their activities about equal.

The appearance of the pelvic fatty pads and omenta of animals treated with these drugs in Classes 1 and 2, in comparison with that of the normal and of the untreated leprosy controls, may be seen in Figs. 1-8. The lesions in all of the treated groups are markedly smaller than in those of the untreated leprosy control group. The appearance of the fatty pads and omenta of the three treated groups in Fig. 1-4 approaches that of the normal controls.

In summary, Compound 373 and Compound 377 were highly effective in the suppression of the murine leprosy infection of mice. They were most effective when the treatment was started immediately after inoculation and continued for three months, and proportionally less effective when the treatment was delayed for one month. Employing equimolar doses, the three drugs revealed similar activities.



TEXT-FIG. 1.—The mortality curves for the class 3 animals, treated with equimolar doses of isoniazid, Compound 373 and Compound 377 for three months and permitted to live after the termination of treatment. A. The mortality curves for isoniazid and Compound 373 are similar to each other, and far to the right of the curve for the untreated controls, while the curve for the Compound 377-treated group is still further to the right. B. The mortality curves here were plotted on logarithmic-probability graph paper, the ordinate being probability of death, and the abscissa the logarithm of time. The median survival times (ST_{50}) of the various groups of animals were obtained from these curves.

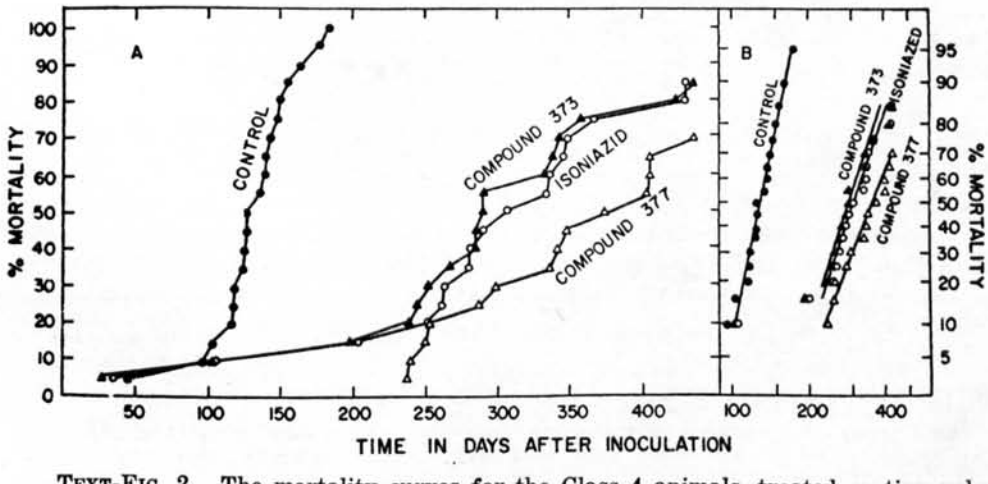
Class 3.—Three groups of animals were treated with equimolar doses of isoniazid, Compound 373 and Compound 377 for three months and permitted to live after termination of the treatment. One group of untreated leprosy animals was used as the control. All animals of the control group died between the 47th and 184th days after inoculation. Deaths of the animals treated with isoniazid and Compound 373 were markedly delayed, and a still greater delay in deaths was observed in those receiving Compound 377. At the end of the experiment, i. e., 444 days after inoculation,

there were two survivors each in the isoniazid- and the Compound 373-treated groups, and one in the Compound 377-treated group. The mortality curves may be seen in Text-fig. 1A.

TABLE 3.—*The median survival time (ST_{50}) of animals treated with equimolar doses of isoniazid, Compound 373 and Compound 377.*

Drug	Dose in food, per cent	ST_{50} in days		
		Untreated control	Treated for three months and allowed to live	Treated continuously for 444 days
(None)		130		
Isoniazid	0.01		265	312
Compound 373	0.024		260	290
Compound 377	0.022		330	362

The median survival time (ST_{50}) of animals may be determined by the rapid graphic method of Litchfield (19). In Text-fig. 1B the cumulative percentage mortality has been plotted against time in days after inoculation on logarithmic-probability paper. The ST_{50} of the untreated control group and of the groups treated with the various drugs are shown



TEXT-FIG. 2.—The mortality curves for the Class 4 animals, treated continuously with equimolar doses of isoniazid, Compound 373 and Compound 377 for 444 days. A. The mortality curves for isoniazid and Compound 373 are similar. They are still further to the right of the curve for untreated controls than those for the same drugs in Text-fig. 1. The curve for Compound 377 is again the farthest to the right. B. Same explanation as in Text-fig. 1.

in Table 3. The ST_{50} of the untreated leprosy control group was 130 days, and of the isoniazid- and Compound 373-treated groups 265 and 260 days, respectively, indicating that the death was delayed for approximately 130 days in animals receiving the latter two substances. The ST_{50} of the Compound 377-treated animals was 330 days, i. e., approximately 70 days longer than those receiving the other two drugs.

All five survivors of the treated groups were autopsied at the end of the experiment, as were all animals found dead during the period of observation. Animals which were found with profound postmortem changes or which had been destroyed by others were discarded. The leprosy indices of all treated groups as computed from the remaining animals and those of the two control groups are shown in Table 4. Data were obtained from 14 animals in the untreated leprosy control group, 6 in the killed-bacteria control group, 10 in the isoniazid-, 8 in the Compound 373- and 12 in the Compound 377-treated groups. Extensive leprous lesions were present in all animals that died during observation, excepting two of the Compound 377-treated group, one of them revealed slight and the other minimal lesions. All survivors showed extensive lesions, while animals receiving killed bacteria had only minimal lesions.

The average leprosy indices of the untreated control animals and of those treated with isoniazid, Compound 373 and Compound 377 were 18.07, 18.75, 18.63 and 15.25, respectively; that of the killed-bacteria controls was 0.60. The bacillus indices of the five survivors ranged from 31 to 42, and of those receiving killed bacteria, from 1 to 8. Apparently, these drugs were effective only temporarily.

In summary, isoniazid, Compound 373 and Compound 377, administered in equimolar doses for three months, were able to suppress the growth of mouse leprosy for a short time; the infection then progressed, killing the animals.

Class 4.—Three groups of animals were treated continuously with equimolar doses of isoniazid, Compound 373 and Compound 377, respectively, for 444 days. At the end of the experiment there were three survivors each in the isoniazid- and Compound 373-treated groups, and 6 in the Compound 377-treated group. All other animals died during the course of the experiment. Their mortality curves are shown in Text-fig. 2, and their median survival time in Table 3. The ST_{50} of the isoniazid- and Compound 373-treated animals was about 170 days longer than that of the untreated controls, and that of Compound 377-treated animals 232 days longer. Thus, the suppressive activity was definitely higher than in Class 3.

The available leprosy indices of these animals may be seen in Table 4. The data were obtained from 12, 14, and 13 animals in the isoniazid-, Compound 373-, and Compound 377-treated groups, respectively. All animals in the isoniazid-treated group showed extensive lesions, with an average leprosy index of 18.08.

The lesions of the Compound 373-treated group were less than those of the isoniazid-treated group. Four animals of this group revealed slight or minimal lesions; in the others, including the three survivors, the lesions were extensive. The average leprosy index of this group was 12.61. The

TABLE 4.—*Leprosy index of animals treated with equimolar doses of isoniazid, Compound 373 and Compound 377 in Class 3 animals (treated for 3 months and allowed animals to live) and Class 4 (treated continuously for 444 days).*

Group	No. of available animals	Leprosy index	
		Range	Average
Untreated control	14	14.0–23.5	18.07
Class 3			
Isoniazid	10	8.0–24.0	18.75
Compound 373	8	14.0–25.5	18.63
Compound 377	12	0.5–22.5	15.25
Class 4			
Isoniazid	12	8.5–24.0	18.08
Compound 373	14	1.5–22.0	12.61
Compound 377	13	0.5–20.5	5.85
Killed-bacteria control	6	0.5– 1.0	0.60

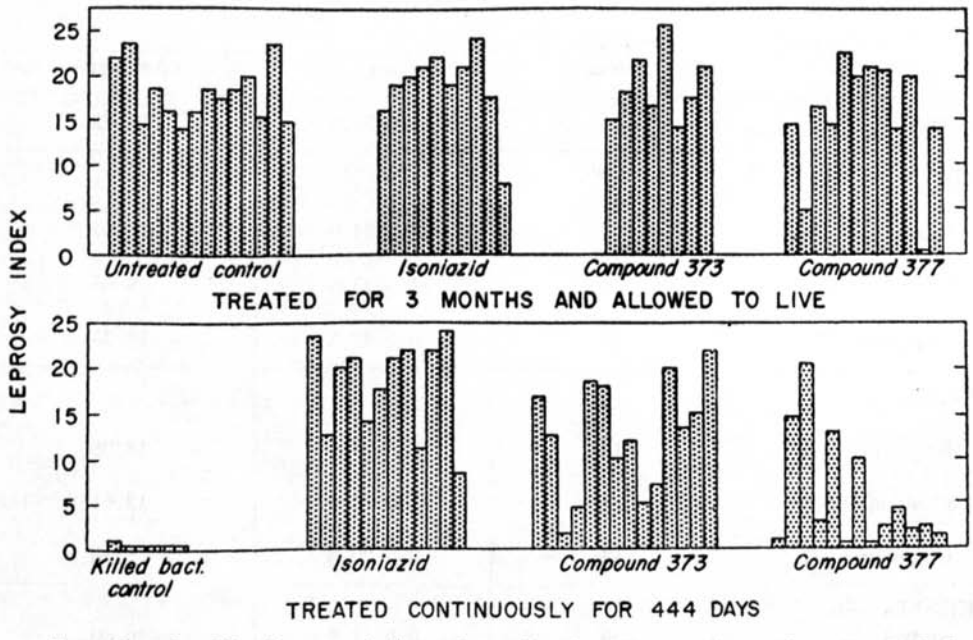
lesions of the Compound 377-treated group were markedly less than those of the other two groups. Nine out of 14 indices of this group were minimal. The six survivors showed only slight lesions in the site of inoculation, omentum and mesentery. The average leprosy index was 5.85.

A comparison of the leprosy indices of all available animals receiving treatment in the various groups of Classes 3 and 4 may be seen in Text-fig. 3. The activity of Compound 377 was definitely superior to that of the other two drugs in the animals treated for 444 days.

The appearance of the pelvic fatty pads and omenta of all survivors in Classes 3 and 4 is shown in Figs. 9-12. Tremendous leprous lesions were seen in all the specimens except those treated with Compound 377 for 444 days. The lesions of the last-mentioned group were very similar to those of the killed-bacteria control.

The bacillus indices of the three survivors each in the isoniazid- and

the Compound 373-treated animals were very high, ranging from 33 to 45; while those of the six survivors of the Compound 377-treated animals were low, ranging from 8 to 28, with an average of 14.8. In these six survivors, acid-fast bacilli were invariably found in the site of inoculation, omentum and lymph nodes; a few organisms were present in the pelvic fat, thymus gland, spleen, liver and lung. In animals which were inoculated with killed bacteria and sacrificed 15 months after inoculation, bacilli were present in site of inoculation, omentum and lymph nodes. Their average bacillus index was 3.1.



TEXT-FIG. 3.—The leprosy indices for mice in Class 3, i. e., those treated for three months and allowed to live; for Class 4, i. e., those treated continuously for 444 days; for untreated controls; and for killed-bacteria controls. Compound 377 gave the most favorable result.

Histologic studies were made of the lesions of animals receiving the various classes of treatment. The untreated leprosy control animals which were sacrificed three months after inoculation were heavily infected. Typical lesions were present in the site of inoculation, omentum, pelvic fat, lymph node, spleen, liver and uterus. Animals of Class 1 showed only a few lesions—in the site of inoculation, omentum and pelvic fat. In the animals of Class 2 more lesions were present; they were found in site of inoculation, omentum, pelvic fat, lymph node, spleen, liver and uterus. The survivors of Class 3 revealed the heaviest infections. Swarms of lepra cells were found in all the above-mentioned sites and organs. In additon, a few lesions were found in the lung, kidney, heart, and intestine. The survivors of Class 4 showed distinct differences between animals treated

with isoniazid or Compound 373 and those given Compound 377. In the former, the animals were very heavily infected, resembling the survivors of Class 3, while in those treated with Compound 377 there were only a few lesions in the site of inoculation and omentum, with occasional ones in spleen, liver and uterus. The killed-bacteria animals revealed only small, atypical lesions in the omentum.

In all animals of Class 1 and in the survivors of Class 4 receiving Compound 377, the lesions consisted mostly of atypical lepra cells. The bacteria in these atypical cells were fewer in comparison with those in typical lesions. Groups of apparently extracellular bacteria appeared in some lesions. These findings were somewhat similar to those found in animals inoculated with killed bacteria. These atypical lepra cells may be residual leprous lesions from the early stage of the infection, the mycobacteria having been held in check by the drug for as long as 15 months. Whether the bacteria were dead or still alive at the end of the experiment was not determined.

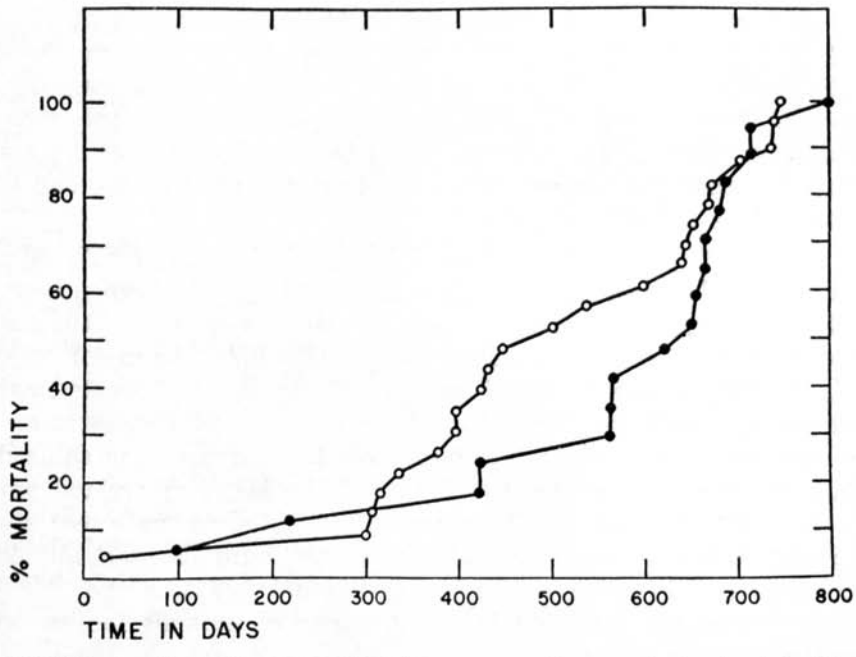
In summary, isoniazid, Compound 373 and Compound 377, administered in equimolar doses for 444 days, revealed activity higher than that exhibited when these drugs were given for three months and the animals allowed to live (Class 3). In the animals in which treatment was continued, the ST_{50} of the isoniazid- and the Compound 373-treated animals was about 40 days longer, and that of the Compound 377-treated animals 107 days longer, than the ST_{50} of animals treated with the same drugs in Class 3. The average leprosy index of the isoniazid-treated animals was very high; that of the Compound 373-treated animals was slightly lower; and that of the Compound 377-treated animals markedly lower, 9 out of 14 indices being minimal. Compound 377 was the only drug able to suppress the infection in the majority of animals for as long as 15 months.

DISCUSSION

Although in the Compound 377-treated animals of Class 4 the lesions were minimal, 70 per cent of these animals died before termination of the experiment. The mortality rate was not in agreement with the extent of leprous involvement. As shown in Text-fig. 3, quite a few of the mice died with minimal or moderate leprous lesions, indicating that death was attributable to other causes. As stated above, 4 out of the 23 animals in the killed-bacteria control group died within 444 days. In the two groups of normal animals which were observed for 800 days in the same room as the leprosy animals, the mortality for the first 15 months was 23.5 per cent in one group, and 47.8 per cent in the other (Text-fig. 4). Therefore, in long-term experiments designed to ascertain the activity of drugs in murine leprosy, the mortality rate is not as precise a measure as the leprosy index.

Isoniazid, Compound 373 and Compound 377 showed quite similar

activities in the three-months experiments, but definitely different results when treatment was continued for 15 months. In the long-term experiments Compound 377 displayed long-lasting activity, while isoniazid and Compound 373 did not. While the similarity of the drugs observed in the short-term experiments may be simply explained by the liberation of the parent compound, isoniazid, from the two hydrazones, this hypothesis does not account for the superior activity of the hydrazones and particularly of Compound 377 in the long-term experiments. If only a fraction of



TEXT-FIG. 4.—Mortality curves for two groups of normal mice kept in the same room as the murine-leprosy animals. Note that 4 out of 17 animals in one group (solid-circle curve), and 11 out of 23 in the other group (open-circle curve) died within 450 days.

the available isoniazid is liberated from these derivatives a lowering of the activity would be expected, as it has been found that isoniazid in a dose of about one-third of that used in the present study caused only slight suppression of the infection (7). Apparently the whole molecule of Compound 377, or its degradation product(s) other than isoniazid, is responsible for its superior activity.

The lack of lasting protective effect of isoniazid in murine leprosy has been reported by several groups of investigators. Bushby and Barnett (3) reported that mice died in one year under continued treatment with isoniazid, while untreated controls died in 150 days. When tissues from treated animals were subinoculated into fresh animals, isoniazid failed to give any protection. Grunberg and his associates (15) found that the

majority of rats treated with isoniazid for 22 weeks survived for 56 weeks, while untreated controls died in about 30 weeks. Cruickshank (9) reported that the average survival time of isoniazid-treated rats was 70.7 weeks against only 34.6 weeks for untreated controls. Fegeler (12) reported that the effect of isoniazid in mice was only bacteriostatic, relapse occurring after cessation of treatment. After relapse, treatment was even less effective than before. Chaussinand and his associates (8) and Nishmura and Masuda (21) reported that in some of their experiments the lesions were as severe in treated rats as in controls. These findings are in agreement with the results of the present studies in that isoniazid only prolonged the survival time. Eventually the treated animals died of extensive leprous infection.

All these results indicate that *M. leprae murium* develops resistance to isoniazid in animals treated for a long period of time. Compound 377 appears to be the first compound to hold this infection in check in the majority of animals for as long as 15 months. Based on these findings, the clinical trial of this compound in human leprosy may be justified.

SUMMARY

The activity of isoniazid, isonicotinylhydrazone of 2-carboxymethoxy-3-methoxybenzaldehyde (Compound 373), and isonicotinylhydrazone of 2-carboxymethoxybenzaldehyde (Compound 377) was compared, in equimolar doses, in mice infected intraperitoneally with *M. leprae murium*.

All three compounds were found to be highly effective in the suppression of the murine leprosy infection in the three-months experiment. They were most effective when treatment was started immediately after inoculation and continued for three months, and proportionally less effective when treatment was delayed for one month and then continued for two months. The degree of activity of the three compounds was more or less equal.

When mice were treated for three months and allowed to live, all three compounds suppressed the infection for a period; then the disease remanifested itself, killing the animals. The suppressive activity of isoniazid and Compound 373 was similar, while that of Compound 377 was the highest.

When mice were treated with either isoniazid or Compound 373 continuously for 15 months from the time of inoculation, the survival time was definitely longer than when they were treated for only three months. Eventually, however, the infection led to death regardless of the duration of treatment. Isoniazid revealed activity similar to that of Compound 373.

On the other hand, the continuous administration of Compound 377 for 15 months suppressed the infection in the majority of the mice. This appears to be the first drug to hold this infection in check for as long as 15 months.

RESUMEN

Comparóse la actividad de la isoniacida, la isonicotinilhidrazona del 2-carboximetoxi-3-metoxibenzaldehido (Compuesto 373) y la isonicotinilhidrazona del 2-carboximetoxibenzaldehido (Compuesto 377), a dosis equimolares, en ratones infectados intraperitonealmente con *M. leprae murium*.

Los tres compuestos se mostraron altamente eficaces en la supresión de la infección por lepra murina en el experimento de tres meses. Mostraron su eficacia máxima cuando se iniciaba el tratamiento inmediatamente después de la inoculación y se continuaba por tres meses, y proporcionalmente menos eficacia cuando se demoraba el tratamiento por un mes y se continuaba luego por dos meses. El grado de actividad de los tres compuestos fué más o menos igual.

Cuando se trataba a los ratones por tres meses y se les dejaba vivir, los tres compuestos suprimieron la infección por un período de tiempo; luego volvió a manifestarse la enfermedad, matando a los animales. La actividad supresora de la isoniacida y del Compuesto 373 fué semejante, mientras que la del Compuesto 377 fué la mayor.

Cuando se trató a los ratones ya con isoniacida o con Compuesto 373 continuamente por 15 meses desde la fecha de inoculación, el tiempo de sobrevivencia fué decididamente más largo que cuando se les trataba solamente por tres meses. Sin embargo, a la larga, la infección condujo a la muerte independientemente de la duración del tratamiento. La isoniacida reveló actividad semejante a la del Compuesto 373.

Por otro lado, la administración continua del Compuesto 377 por 15 meses suprimió la infección en la mayoría de los ratones. Esta droga pareçe ser la primera que haya mantenido esta infección cohibida hasta 15 meses.

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Description of Plates

Plate 6

Comparison of the pelvic fatty pads and the omenta of untreated and treated murine-leprosy mice in Class 1, i. e., animals treated from the time of inoculation for three months. In each picture the larger objects are the pelvic fatty pads and the smaller ones the omenta.

Fig. 1. Leprosy control group, untreated.

Fig. 2. Isoniazid, 0.01 per cent.

Fig. 3. Compound 373, 0.024 per cent.

Fig. 4. Compound 377, 0.022 per cent.

The pelvic fatty pads of all untreated control animals are much rougher and, in most cases, larger, than those of the three treatment groups. The omenta are much larger in the untreated mice. The fatty pads and omenta of the treatment groups are of similar appearance.

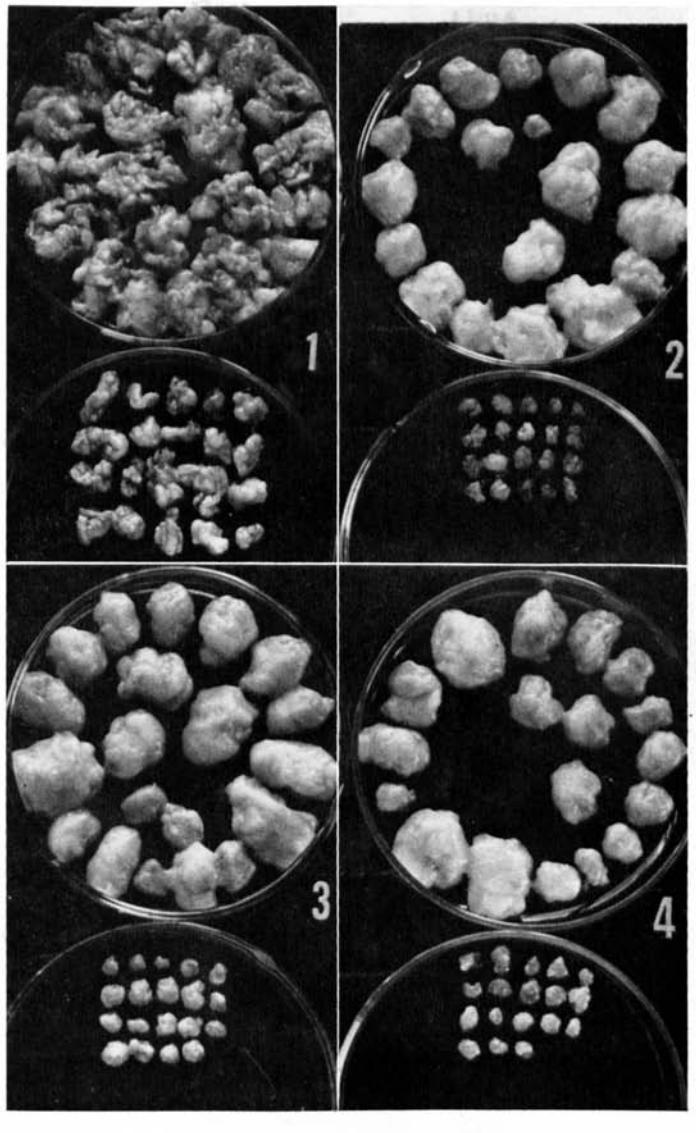

PLATE 6

PLATE 7

Comparison of pelvic fatty pads and omenta of mice in Class 2, i. e., those in which treatment was delayed for one month after inoculation and subsequently carried on for two months.

FIG. 5. Isoniazid, 0.01 per cent.

FIG. 6. Compound 373, 0.024 per cent.

FIG. 7. Compound 377, 0.022 per cent.

FIG. 8. Normal mice group.

The lesions of all the three treated groups are smaller than those of untreated leprosy group (Fig. 1), but somewhat larger than those of the animals receiving three months treatment (Figs. 2 to 4).

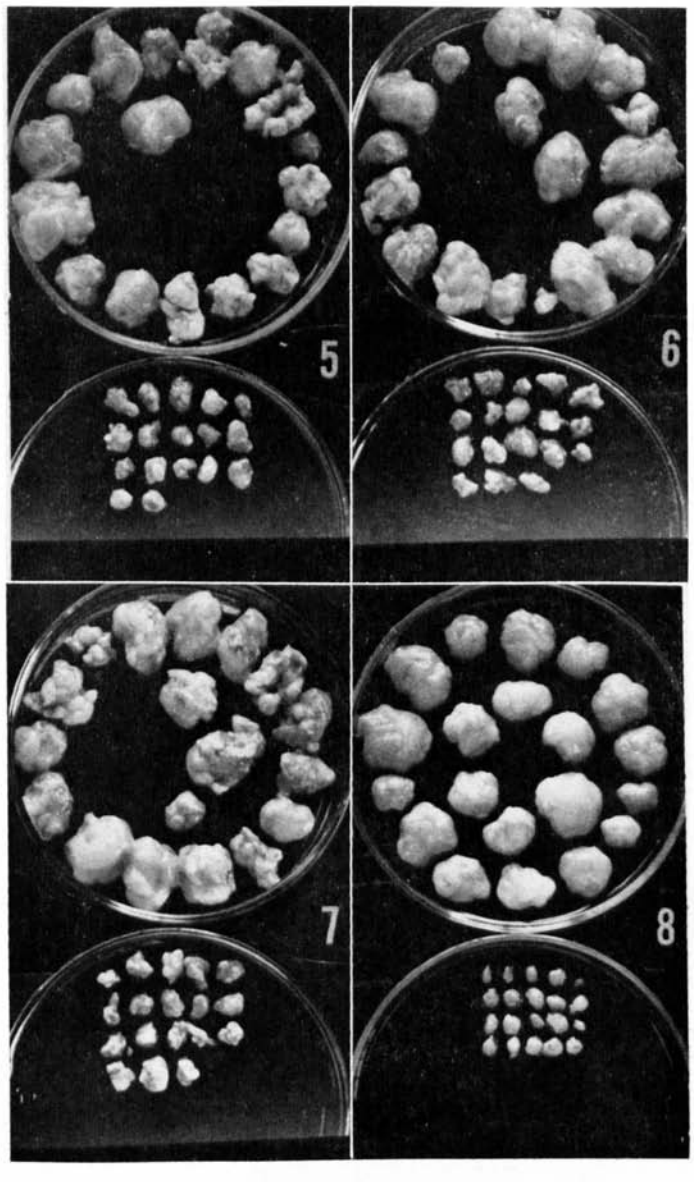

PLATE 7

PLATE 8

Comparison of pelvic fatty pads and omenta of survivors in Class 3, i. e., animals treated for three months and allowed to live after termination of treatment; and in Class 4, i. e., animals treated continuously for 444 days. In Figs. 9, 10 and 11, the upper halves are of Class 3 and the lower halves, of Class 4. In each picture the outer, larger objects are pelvic fatty pads, and the inner, smaller ones are omenta.

FIG. 9. Isoniazid, 0.01 per cent.

FIG. 10. Compound 373, 0.024 per cent.

FIG. 11. Compound 377, 0.022 per cent.

FIG. 12. Killed-bacteria control.

The lesions in Figs. 9 and 10, and in the upper part of Fig. 11, are large and rough. Those in the lower half of Fig. 11 are small and smooth. The growth of the lesions in the survivors receiving continued treatment with Compound 377 was well checked. Note the smooth appearance of pelvic fatty pads and omenta (with the exception of one omentum) in the killed-bacteria group.

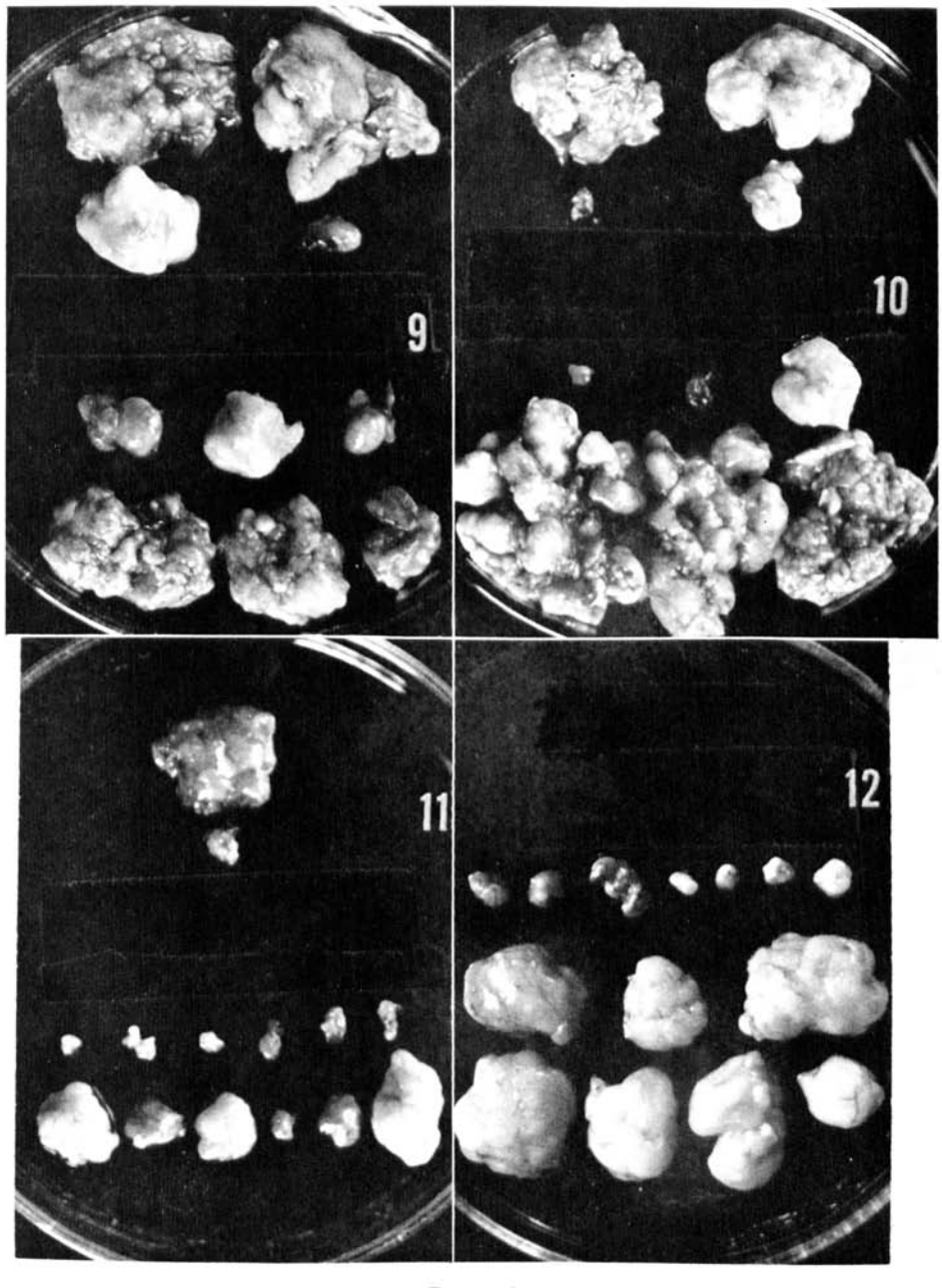

PLATE 8