THE SCREENING TEST FOR CHEMOTHERAPEUTIC AGENTS FOR LEPROSY

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INTRODUCTION

Most of the chemotherapeutic agents for leprosy in use today were selected on the basis of their efficacy in the treatment of tuberculosis. Unless newly developed agents first pass screening tests with respect to tuberculosis, they are discarded even though they may contain components effective against leprosy. A different medium for discovering new therapeutic agents for leprosy should be employed, and for that murine leprosy is to be considered.

Many investigators have utilized murine leprosy in studying chemotherapeutic substances for leprosy, but the results have been conflicting because of differences in the experimental methods used. The results even with identical agents do not agree. The relationship of an agent to murine and human leprosy has not been clarified, so that it is not certain whether the results obtained in murine leprosy can be applied to human leprosy. In order to attain these objectives the experimental methods must be unified, a simple standard for evaluation set up, and the length of time required for evaluation shortened. For the past several years we have conducted a series of studies in an attempt to find a method which would fulfill these requirements. As a result we have devised one which we believe to be suitable. The experiments and the results are presented here in summary fashion.

EXPERIMENTAL WORK

I. SELECTION OF STANDARD STRAIN OF INOCULUM

A standard strain of *M. leprae murium* for screening chemotherapeutic agents for leprosy therapy, analogous to the H37Rv strain of *M. tuberculosis* used in experimental tuberculosis, should be selected.

Comparative tests were conducted with four strains of murine leprosy, including the three most commonly used in Japan (the Kumamoto strain, the Fukuoka strain, and the Police Department strain), and also the Hawaiian strain,[2] in order to select the most virulent one.

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Experimental method.—The sizes of the lepromas produced after subcutaneous inoculation in mice by the four strains used were compared. The method used in this exploratory experiment was as follows:

Inoculum: To maintain a constant bacterial number, the lepromas of 3-5 mice were collected, weighed, pooled, and ground finely in a mortar. A 1/100 suspension in physiologic saline was filtered through four layers of sterile gauze. The number of bacilli of each preparation was calculated by the Breed method and was found to be about 50,000,000 per cc.

Animal, site and inoculum: For the animal, uniform strains NA1 and NA2 male mice weighing 15 ±1 gm. were selected. The inoculations were given subcutaneously over the abdomen. For the inoculation dose we used 0.2 cc. of, besides the original 10^{-2} (1/100) suspension, also 10^{-4}, 10^{-6}, 10^{-7} and 10^{-8} dilutions.

Evaluation of the results: Two different criteria were used for the evaluation of results. One was the size (spread, area) of the subcutaneous leproma at the site of inoculation. The other was the distribution of the bacilli to the organs as determined on autopsy.

(*a*) The size of the lepromas was measured with a micrometer gauge and the following standard was used for their evaluation.

Size of Lepromas and Standard for Evaluation

Size	*Palpation*	*Designation*	*Index*
1 to 10 mm^2	trace, millet-size	±	1
11 to 30 mm^2	half rice grain-size	1+	2
31 to 70 mm^2	rice grain-size	2+	3
71 mm^2 or more	red bean-size	3+	4

(*b*) The distribution of the bacilli in the superficial structures of the body is for the most part in the inguinal, and they also are found in the deeper bronchial, greater omental, vertebral and hilar lymph nodes. Pathologic changes in the visceral organs are most severe in the liver, spleen, lungs and kidney. Impression smears for bacteriological examination were made from the cut surfaces of those tissues which showed the severest changes. The bacillus aggregates or masses within the cells were counted under low power, and when no such masses were present in the smear the individual bacilli were counted under high power. The results were evaluated by the following standard.

Grading of Concentration of Bacilli

Number of masses	*Designation*	*Index*
None [a]	±	1
1 to 10	1+	2
11 to 50	2+	3
51 to 100	3+	4
>100	00	5

[a] Only solitary bacilli.

Results evaluated by spread (size) of leproma.—This experiment was done twice, in both instances the animals being observed for 50 to 90 days. As shown in the first section of Table 1, no great differences were noted in the first experiment between the Kumamoto, Hawaiian and Police Department strains, but the Fukuoka strain was somewhat less infective. Onset of infection by the Fukuoka strain was also

delayed in the second experiment, clearly showing that this strain was the least virulent.

TABLE 1.—*Leproma development at site of subcutaneous inoculation, in two experiments with various dosages of four strains of murine leprosy.*

Strain of inoculum	Dilution [a]	Experiment 1				Experiment 2			
		No. mice	Results, days			No. mice	Results, days		
			50	70	90		50	70	90
Kumamoto	10^{-2}	7	2.1	3.3	3.6	—	—	—	—
	10^{-4}	7	0.3	1.2	2.6	20	1.5	2.9	3.7
	10^{-6}	6	0	0.5	2.3	20	0.1	1.1	2.4
	10^{-7}	4	0	0	0.5	26	0	0.8	2.0
	10^{-8}	6	0	0	0	21	0	0.1	1.2
Hawaiian	10^{-2}	7	2.5	3.1	3.4	—	—	—	—
	10^{-4}	6	0	1.2	2.8	19	1.4	2.8	3.1
	10^{-6}	5	0	1.3	2.5	21	0.1	1.0	2.5
	10^{-7}	4	0	0.5	0.8	26	0	0.6	1.3
	10^{-8}	6	0	0	0	21	0	0.3	1.0
Fukuoka	10^{-2}	8	0.9	1.2	1.8	—	—	—	—
	10^{-4}	6	0	1.2	1.3	18	0.3	1.9	2.9
	10^{-6}	6	0	0.8	1.0	18	0	0.2	1.5
	10^{-7}	5	0	0	0.4	24	0.1	0.5	1.4
	10^{-8}	5	0	0	0	20	0	0.1	0.5
Police	10^{-2}	8	1.9	2.5	3.0	—	—	—	—
	10^{-4}	7	0	1.7	2.4	18	0.8	2.8	3.4
	10^{-6}	5	0	0	1.3	18	0.3	1.1	2.3
	10^{-7}	3	0	0	1.0	24	0	0.4	1.6
	10^{-8}	5	0	0	0	20	0	0.3	0.9

[a] Dose, 0.2 cc. throughout.

Results evaluated by the distribution of bacilli.—For investigation of the distribution of bacilli in the organs, the animals were killed 150 days after subcutaneous inoculation and examined in detail microscopically. Tissues were taken from six sites: inguinal lymph node, axillary lymph node, submaxillary lymph node, liver, spleen and lung. The distribution of bacilli is closely related to the quantity of inoculum, so three dilutions (10^{-4}, 10^{-6}, 10^{-7}) were compared. As can be seen in

Table 2, examination of 324 samples of tissues of 54 animals inoculated with the 10^{-4} suspension revealed acid-fast bacilli in 160. Conversion to the indices in the table (see above) gives an average of 7.4 per animal. Values higher than this average were given by the Kumamoto, Hawaiian and Police Department strains, while the Fukuoka strain gave a value lower than the average. Similar results were obtained with the 10^{-6} and 10^{-7} dilutions.

TABLE 2.—*Distribution of bacilli in organs of mice 150 days after subcutaneous inoculations of various dilutions, comparing the four strains of murine leprosy.*

Dilution [a]	Strain of inoculum	Number of mice	Number of sections examined	Number of organs positive for bacilli	Average number of positive sections	Index of average number of positive sections	Index of the averages
10^{-4}	Kumamoto	17	102	51	50.0	131	7.7
	Hawaiian	12	72	35	48.5	90	7.5
	Fukuoka	15	90	37	41.1	90	6.6
	Police	10	60	37	61.1	82	8.2
	Total	54	324	160	49.4	402	7.4
10^{-6}	Kumamoto	15	90	29	32.2	58	3.8
	Hawaiian	17	102	42	41.1	88	5.2
	Fukuoka	15	90	18	20.0	38	2.6
	Police	16	96	33	34.4	75	4.7
	Total	63	378	122	32.3	259	4.1
10^{-7}	Kumamoto	21	126	30	23.8	54	2.6
	Hawaiian	20	120	25	20.8	54	2.7
	Fukuoka	20	120	19	15.8	32	1.6
	Police	24	144	34	23.6	72	3.0
	Total	85	510	108	21.1	212	2.5

[a] Dose, 0.2 cc.

On the basis of the results of these comparisons, it can be seen that there are no great differences between the Kumamoto, Hawaiian and Police Department strains as regards either the size of the leproma or the distribution of the bacilli, whereas the Fukuoka strain is less virulent. Any one of the first three strains could therefore be used, but

it was decided to employ the internationally known Hawaiian strain in further experiments.

II. SITE OF INOCULATION AND QUANTITY OF INOCULUM

The favorite sites of lesion formation in murine leprosy are the loose subcutaneous connective tissue and the lymph nodes, followed by the liver and spleen which are rich in reticuloendothelial elements. The most suitable method for enhancing infection would be to fix accurately the inoculation bacilli in these tissues. However, if too large a quantity of inoculum is injected, evaluation of the effect of any drug administered afterward is difficult, so it would be ideal to employ a quantity of inoculum sufficient to induce infection and to afford simple, accurate judgment of the pathologic changes macroscopically.

In experimental methods used heretofore, highly concentrated suspensions such as 1/5 to 1/50 have been used. They have been inoculated by different investigators subcutaneously, intraperitoneally, intratesticularly, intravenously, intramuscularly, intranasally, intrathecally, intraocularly, and intracorneally. In our studies, with the object of determining the relation between speed of infection and quantity of inoculum, and also the ease of judgment, we compared the subcutaneous, intramuscular and intravenous routes.

1. *Subcutaneous inoculation.*—Quantity of inoculum and leproma development: The number of days required for development of the leproma to 1+, or an index of about 2, was investigated in the 372 animals inoculated subcutaneously with various dilutions from 10^{-2} to 10^{-8}, as shown in Table 1. It was found that the 10^{-2} dilution required 30 to 60 days, the 10^{-4} dilution 60 to 90 days, the 10^{-6} dilution 90 to 150 days, the 10^{-7} dilution 120 to 180 days, and the 10^{-8} dilution more than 150 days. With dilutions higher than 10^{-6} there were great individual variations. From these results it can be seen that the minimal quantity for development of a leproma within 90 days is 0.2 cc. of the 10^{-4} suspension, and that for development within 60 days the same dose of the 10^{-2} dilution is required. The latter suspension, however, is quite concentrated, and the evaluation of the effects of weakly-acting drugs appears somewhat more difficult.

Ease of evaluation: It is very easy to measure the leproma macroscopically, and by use of a micrometer gauge the measurement is made accurate. Pathologic changes occur in the organs and are found most frequently in the lymph nodes with swelling, tubercle formation and necrosis; and, with progression of the disease, such changes also occur in the spleen, liver and lungs. In the majority of animals, however, such changes in the organs are found only after a lapse of more than five months, and so evaluation by pathologic changes of that kind is not practicable.

Study of the distribution of the bacilli shows that a large number are present chiefly in the lymph nodes, and after inoculation with the 10^{-2} suspension, bacilli are found in the inguinal and axillary nodes of the majority at 60 days. With the 10^{-4} suspension, however, bacilli are present in only a few places in 90 days, and even after 150 days they are found in only about one-half. Evaluation on the basis of distribution of the bacilli is therefore difficult when a dilute inoculum is used.

These results show that the inoculation of 0.2 cc. of the 10^{-4} or the 10^{-2} suspension is best suited for short-term observation of leproma development after subcutaneous inoculation, and that evaluation of the results by size of the leproma is relatively easy.

2. *Intraperitoneal inoculation.*—A total of 146 NA1 mice were used in two series of experiments by intraperitoneal inoculation. It is known that with this route a large quantity of bacilli must be inoculated to attain development of pathologic changes within a short time, so 1/50 and 1/100 (10^{-2}) suspensions were used.

In the first series the animals were sacrificed and the organs examined for macroscopic changes and the distribution of bacilli every 30 days, from the 30th to the 150th days after inoculation. In the second series the animals were sacrificed and examined on the 60th and 90th days.

Quantity of inoculum and speed of development of pathologic changes: As shown in the first part of Table 3, pathologic changes became apparent in 60 per cent of the animals only after 90 days following the inoculation of the 1/100 dilution. With the 1/50 suspension changes were found in about one-third in 30 days, 70 per cent in 60 days, and about 85 per cent after 90 days.

No greater increase in rate of pathologic changes was found thereafter. On the contrary, the percentages were lower in the animals examined after 150 days. It is believed, however, that this finding was due to the previous death of severely infected animals, and the survival of those only slightly infected.

Ease of evaluation: The index of macroscopic evaluation, i.e., the formation of nodules, appears to be most marked in the greater omentum, followed in order by the bronchial nodes, spleen, liver, and hilar nodes of the lungs. The occurrence of lesions, however, is not constant even in the greater omentum, and lesions were observed in no more than 61 per cent of the animals 90 days after inoculation with the 1/50 suspension.

The distribution of bacilli is shown in the second part of Table 3. Here too, the highest percentage was found in the greater omentum, but this again was not constant, and bacilli could not be regularly detected.

These results suggest that the 1/50 suspension is more suited for intraperitoneal inoculation than the 1/100 dilution. Even with the more concentrated inoculum, however, macroscopically detectable lesions did

TABLE 3.—*Results of intraperitoneal inoculation of 0.2 cc. doses of concentrated suspensions of the Hawaiian strain into NA1 mice, two experiments, as evaluated* (a) *by macroscopic changes in the organs and* (b) *microscopically by distribution of bacilli.*

Dilution of inoculum	Days after inoculation	No. of mice	Mice with organic lesions								Distribution of bacilli						
			Positive, No. & p.c.	Bronchial N.	Portal N.	Paravert. N.	Omentum	Spleen	Liver	Lung	Bronchial N.	Portal N.	Paravert. N.	Omentum	Spleen	Liver	Lung
First Experiment																	
1/50	30	6	2 (33)	—	—	—	2	—	—	—	2	1	1	2	2	1	0
	60	7	6 (85)	2	—	1	6	2	1	1	6	4	5	7	4	3	1
	90	6	5 (83)	2	—	—	4	4	1	—	3	3	2	5	4	1	0
	120	9	8 (89)	5	2	1	8	6	5	—	7	6	4	7	6	6	4
	150	7	3 (43)	2	2	1	3	3	2	3	4	4	2	5	3	3	1
1/100	30	7	1 (14)	—	—	—	1	—	—	—	7	0	2	2	0	0	0
	60	7	4 (57)	2	—	—	4	2	—	—	4	2	3	7	3	2	1
	90	6	5 (83)	3	1	1	5	2	2	—	5	4	2	6	3	1	0
	120	6	4 (67)	1	1	—	4	1	1	—	3	3	1	5	3	1	1
	150	6	3 (50)	2	—	—	3	2	—	—	3	3	0	6	2	1	0
Second Experiment																	
1/50	60	19	12 (63)	7	2	1	10	3	1	—	9	4	3	13	6	5	1
	90	20	17 (85)	6	4	2	12	3	4	1	11	10	4	13	12	8	4
1/100	60	20	8 (40)	5	—	1	5	1	—	—	11	2	1	7	2	3	0
	90	20	12 (60)	7	1	1	10	6	6	—	9	7	2	12	9	9	1
	(Total)	146									84	53	30	97	59	44	14
											57.8	36.3	20.5	66.4	40.4	30.1	9.6
	(Order)			2	5	6	1	3	4	7							

not always develop, even in the greater omentum, where they are most frequently found. Neither was microscopic detection of bacilli in the greater omentum constant. Consequently, there is no means for accurate evaluation of results after inoculation by the intraperitoneal route.

3. *Intravenous inoculation.*—Twenty mice of the NA1 strain were inoculated intravenously with 0.2 cc. of 10^{-2} suspension, and 10 animals were sacrificed and examined after 60 days and the other 10 after 90 days.

The findings may be presented without resort to a tabulation. The most interesting point is that there was almost no macroscopic pathology in the organs. The spleen and liver showed some nodule formation in only 3 or 4 of the animals of either group—not significantly more after 90 days than after 60 days. Microscopically, bacilli were most frequently detected in the liver and spleen, but not in all cases.

These findings indicate that early leproma development cannot be expected by the method of intravenous inoculation.

4. *Comparison of the three routes of inoculation.*—The factors that have been found operative with respect to the three routes of inoculation studied are compared in the following tabulation with a view to the selection of the most suitable method for the screening test.

Factor	*Subcutaneous*	*Intraperitoneal*	*Intravenous*
Inoculation technique	Easy	Easy	Difficult
Most suitable concentration (dose: 0.2 cc.)	10^{-2} or 10^{-4}	1/50 or 1/100	10^{-2}
Minimal time for evaluation	10^{-2}: 1-2 mos. 10^{-4}: 3 mos.	1/50: 3 mos. 1/100: 4 mos.	3 to 4 mos.
Most frequent site of leproma formation	Inoculation site; 100%	Greater omentum; 61%	Liver, spleen, 30 to 35%
Greatest bacillus concentration	Subcutaneous lymph nodes	Greater omentum, bronchial nodes, spleen and liver	Liver and spleen
Method of evaluation	Observation of leproma at inoculation site	Autopsy: gross changes and bacillus distribution in greater omentum, spleen and liver	Autopsy: gross changes and bacillus distribution in liver and spleen

It can be clearly seen that the *intravenous* route is inferior in all respects in comparison with the other two routes of inoculation. To secure early leproma development after *intraperitoneal* inoculation requires a concentration of inoculum (1/50) which interferes with accurate judgment, and it also requires a period of at least three months —and, furthermore, there is no organ in which lesions are constantly

found. Macroscopic evaluation of the lesion also lacks objectivity, so their route is inferior to subcutaneous inoculation.

On the other hand, a leproma develops relatively early after subcutaneous inoculation of a small quantity of bacilli, and leproma formation occurs in 100 per cent of the animals. Attention therefore can be centered on this one site, and the development followed macroscopically according to time; the spread can be measured accurately with a micrometer so that the margin of error can be kept at a minimum.

From all this, it was decided that the method of subcutaneous inoculation was the most suitable for the purpose.

III. SIMPLIFICATION OF EVALUATION BY THE SUBCUTANEOUS METHOD

It having been shown that the subcutaneous method is the most logical one, a study was then made to determine the most simple and accurate method of evaluation of the results when this route is used.

Evaluation by spread of leproma alone.—Because leproma development at the site of inoculation occurs in 100 per cent of the animals inoculated subcutaneously, it would be advantageous if this development could be used, by accurately measuring the changes in size of the leproma as an index for evaluation without microscopic examination, or macroscopic observation of other pathologic changes. The relationship of the spread of the leproma to its weight and the index of bacillus distribution was studied statistically in the 274 animals listed in the second half of Table 1, which were sacrificed 5 months after inoculation. Table 4 shows the results.

TABLE 4.—*Relation of size (spread, or area) of leproma to weight of leproma and bacillus distribution.*

Grade of leproma size (mm^2)	Number of mice	Average size of grade (mm^2)	Average weight leproma (mgm.)	Index of bacillus distribution
1-10	103	3.0	2.4	1.6
11-20	35	15.0	6.2	3.1
21-30	29	27.2	7.3	3.4
31-40	27	37.5	20.0	4.4
41-50	20	47.0	35.2	4.2
51-60	13	56.8	29.6	6.1
61-70	9	67.7	40.5	8.3
71-80	15	73.7	43.6	7.0
81-90	5	84.5	38.0	7.0
91 plus	18	129.0	81.1	7.2

The size (area) of the leproma was divided into 10 categories ranging from 1 mm^2 to over 90 mm^2 (actually 196), and for each stage or grade the average size of the lepromas is compared with the average leproma weight and index of bacillus distribution. It was found that increases in spread of the leproma was followed by a corresponding increase in its weight and in the bacillus distribution index, although the rate of increase of the latter factor appeared to be somewhat lower than the rate of increase in weight. This is believed to be due to the slow spread of bacteria to the organs with inocula as dilute as 10^{-4} or greater, as used in this experiment.

A comparison of the size of the leproma and the pathologic changes in the lymph nodes and organs after five months showed that, with these dilute suspensions, there were few such distant lesions even though a leproma of considerable size had developed locally.

It is therefore believed that if the period of observation is limited to three months, macroscopic changes—aside from the local changes—can be disregarded in the screening test, and that evaluation may be carried out, without great error, solely on the basis of the size of the leproma formed locally by subcutaneous inoculation of a 0.2 cc. dose of a 10^{-2} or 10^{-4} bacillus suspension.

Evaluation on the basis of weight of the spleen.—Of the internal organs, the spleen is the favorite site of pathologic change in murine leprosy, so if the weight of the spleen could be taken as an index for evaluation, as in experimental tuberculosis, this would aid in the simplification of the test procedure.

The weight of the spleen was ascertained in 58 mice of the NA1 strain six months after subcutaneous inoculation of 0.2 cc. of a 10^{-4} suspension of the Kumamoto strain, and the relation of the spleen weight to size of the leproma and the bacillus-distribution index was determined. As shown in Table 5, the size of the leproma and the bacillus distribution do not parallel the weight of the spleen in cases where the spleen weighed between 60 and 120 mgm. With an increase in weight to 121 mgm. or more, there is a corresponding increase in size of the leproma and rise in the index of bacillus distribution.

In this way, a relationship between the spleen and the other factors becomes apparent only after the weight of the spleen exceeds a certain limit, otherwise there is no relationship. Examination of the bacillus distribution revealed cases in which there was widespread distribution even though the weight of the spleen was light. Furthermore, investigation of the relation between the index of bacillus concentration in the spleen itself and the weight of the spleen showed that here, too, a relationship became apparent only when the spleen weight was 121 mgm. or greater. No bacilli were detected, however, in the three cases in which the weight of the spleen was 141-150 mgm., but it is believed that the splenomegaly in those cases was due to some other cause.

TABLE 5.—*Relation of weight of spleen to weight of leproma and bacillus distribution.*

Weight of spleen (mgm.)	Number of mice	Body weight (average) (gm.)	Leproma weight (average) (mgm.)	Bacillus distribution, body (average)	Bacillus distribution, spleen
60-69	4	19.0	33	6.5	1.0
70-79	4	22.3	48	6.5	0.8
80-89	7	22.0	30	5.9	0.7
90-99	2	20.5	65	5.0	0.5
100-109	19	24.7	32	5.5	0.4
110-119	3	25.3	50	6.0	0.7
120-129	6	24.7	75	8.8	0.8
130-139	4	26.0	88	8.8	1.5
140-149	3	24.3	63	4.0	0
>150	6	25.0	115	12.2	1.7

In consideration of the factors and findings related, it is concluded that the weight of the spleen alone is inadequate as an index for evaluation. This conclusion is indicated in spite of the fact that, in these animals, six months had elapsed since inoculation, and the pathologic changes in the organs were quite marked. If the period of observation should be shortened to three months, it is believed, there would probably be even less relationship between the weight of the spleen and the other factors.

Other considerations.—From the results here recorded it can be seen that the spread (size) of the leproma which results from subcutaneous inoculation of mice is related to its weight and to the index of bacillus distribution. The leproma softens rapidly in the mouse, so that evaluation on the basis of leproma weight is difficult in actual practice. Evaluation by bacillus distribution would be even more difficult when a dilute inoculum is used and the period of observation is short; and evaluation on the basis of gross pathologic change in the lymph nodes or organs, or the weight of the spleen, is not worth considering.

The conclusion was thus reached that measurement of the size of the leproma at the site of inoculation according to time is the most logical standard for evaluation.

IV. SELECTION OF MOUSE STRAIN FOR THE SCREENING EXPERIMENT

Strains of mice best suited for screening anticancer and antituberculosis agents have been selected and are in use today. It would be advan-

tageous if a superior strain of mouse could be found in the case of murine leprosy.

Samples of various pure, uniform strains were obtained from several institutions and tested for the following conditions: (1) high susceptibility to infection by murine leprosy, and uniform production of lesions; (2) resistance to other infections, and fitness for prolonged experiments; (3) high reproducibility, with constant supply of large numbers. The strains examined and their sources were the NA_1, NA_2, S_{417}, from the Osaka Pure Strain Animal Farm; DD, from the Research Laboratory, Takeda Pharmaceutical Ind. Ltd.; dbr, DBA, S, from the National Institute of Genetics; B, from the School of Agriculture, Nagoya University; and SM, from the Tokyo Central Experimental Animal Farm.

Experimental method.—The greatest difficulty encountered in this study was in obtaining animals 5 to 6 weeks old and weighing 15 ± 1 gm. Because of this difficulty it was necessary to conduct the tests in two series. The bacilli were injected subcutaneously, and the Kumamoto, Hawaiian, Fukuoka and Police Department strains were used equally.

In the first series (September 1954-February 1955), 154 mice of the NA1 strain, 105 of the NA2, 48 of the S_{417}, and 100 animals of a mixed strain were tested. In the second series (January-June 1955), 29 mice of the NA1 strain, 99 of the S, 30 of the DD, 21 of the DBA, 22 of the dbr, 11 of the B, and 33 of the SM strain were employed.

Experimental results.—No single especially susceptible strain was found. However, the B strain showed relatively early and constant infection, followed by the dbr and S strains. From the standpoint of low mortality, tolerance to prolonged experiments and availability, the NA_1, NA_2, DD and SM strains were found to be the most suitable. The mixed strains lacked uniformity in the results, and mortality was high.

In the range of this experiment a strain which would satisfy all three conditions was not found, although the B strain appeared to be the best. This strain, however, is not available in large numbers as yet, so for the present the NA_1, NA_2, DD and SM strains must be used in large-scale experiments.

CONCLUSION

A standard screening test based on the findings reported may be summarized as follows:

Strain of murine leprosy: Hawaiian strain. Inoculum to be a suspension of fresh lepromas removed three months after subcutaneous inoculation of the mouse.

Strain of mouse: Uniform NA_1 strain, female mice, 15 ± 1 gm. in weight, 10 animals per group.

Site of inoculation: Subcutaneous, in the lower abdomen.

Quantity of inoculum: 0.2 cc. of 10^{-2} or 10^{-4} suspension; at times only the 10^{-4} dilution.

Administration of drug: 1/30 to 1/100 LD_{50} administered orally or parenterally 6 times a week, beginning the day following inoculation.

Observation period: Three months, measuring the degree of infection every 15 days after the first month.

Method of judgment: Measure the area of the leproma developing at the site of inoculation, determine the index from the predetermined table, calculate the average for each group (10 animals), record on a histogram, and compare with the untreated control.

Evaluation of efficacy: The lower the index compared to the untreated control, the greater can be considered the effect of the treatment. Numerous experiments show that isoniazid incompletely suppresses infection by 0.2 cc. of a 10^{-2} suspension, and completely suppresses 0.2 cc. of 10^{-4} dilution, so this can be taken as a standard for effect.

SUMMARY

In view of the fact that there is no generally-accepted, uniform method for screening therapeutic agents for leprosy using murine leprosy, the methods used differing with each investigator so that the results are inconsistent, the need for a standard method was felt. Consequently, detailed basic studies were designed for the selection of a standard strain of murine leprosy for the purpose, the most suitable site of inoculation and quantity of inoculum to be used, the simplification and constancy of evaluation of results, and the selection of the best mouse strain. On the basis of the results of these studies a screening method, using the subcutaneous inoculation method, which might serve as a standard was devised.

CONCLUSIONES

Cabe sumarizer en la forma siguiente una prueba normalizada de triaje, basada en los hallazgos expuestos:

Cepa de la lepra murina: Cepa hauaya. El inóculo será una suspensión de lepromas recientes extirpados a los tres meses de la inoculación subcutánea del ratón.

Cepa del ratón: Cepa NA1 uniforme, hembras, 15 ± 1 gm. de peso, 10 animales en cada grupo.

Sitio de la inoculación: Subcutáneo, en la porción inferior del abdomen.

Cantidad de inóculo: 0.2 cc. de supensión al 10^{-2} o 10^{-4}; a veces solamente la dilución al 10^{-4}.

Administración de la droga: 1/30 a 1/100 DL_{50}, administrada oral o parentéricamente 6 veces semanales, comenzando el día después de la inoculación.

Período de observación: Tres meses, midiendo la intensidad de la infección cada 15 días después del primer mes.

Método de apreciación: Mídase el área del leproma que aparece en el sitio de inoculación, *determínese el índice por la table predeterminada,* calcúlese el promedio para cada grupo (10 animales), regístrese en un histograma y compárese con el testigo no tratado.

Justipreciación de la eficacia: Mientras más bajo sea el índice comparado con el testigo no tratado, mayor puede considerarse el efecto del tratamiento. Numerosos experimentos demuestran que la isoniacida *suprime incompletamente la producida por 0.2 cc. de una suspensión al* 10^{-2} *y suprime totalmente la producida por 0.2 cc. de una dilución al* 10^{-4}*, de modo que puede tomarse esto como pauta en cuanto a efecto.*

Nishimura ([2]) ha publicado un estudio en que se compara este método con el de la inoculación intraperitoneal descrito recientemente por Chang ([1])

RESUMEN

En vista de que no hay ningún método uniforme y aceptado generalmente que utilice la lepra murina para el triaje de los agentes terapéuticos para la lepra y de que los métodos usados discrepan con cada investigador de modo que los resultados son inconstantes, se sentía la necesidad de contar con un método normalizado. Por consiguiente, se proyectaron estudios fundamentales pormenorizados para la selección de una cepa normalizada de lepra murina destinada a dicho propósito, del sitio más adecuado de inoculación y de la cantidad de inóculo que se usaría, unido esto a la simplificación y constancia de la justipreciación de los resultados y la selección de la mejor cepa de ratón. A base de los resultados de dichos estudios, se ha elaborado un método de triaje, que utiliza la técnica de la inoculación subcutánea, que podría servir como pauta.