

CHEMOTHERAPY OF MURINE LEPROSY
VII. THE EFFECT OF CYCLOSERINE (SEROMYCIN)
ON MOUSE LEPROSY

Y. T. CHANG, M. D.^{1,2}

*National Institute of Arthritis and Metabolic Diseases
Bethesda, Maryland*

Cycloserine (Seromycin) has been reported to have definite antituberculosis activity in human infection (6, 7, 8, 11, 12), but no effect in the experimental tuberculosis of mice (5, 13). In experimental tuberculosis of guinea-pigs, the antibiotic was also found inactive by two groups of investigators (9, 13), although later some histological improvement was reported by one of these groups (10). For a better understanding of the variations in the activity of cycloserine in different hosts, studies on experimental mycobacterial infections other than tuberculosis seem desirable. Probably, also, an infection can be found that is more closely correlated with human tuberculosis in respect to the action of this antibiotic than is tuberculosis in the mouse or guinea-pig.

Since murine leprosy has been found to be a useful tool in studies of the antimycobacterial activity of various compounds (2, 3, 4), the effect of cycloserine was studied in this disease.³

METHODS

The author's technique of chemotherapeutic assay in mice (1) was employed. Female albino mice of 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 from mice that had been infected 4 to 5 months before with the Hawaiian strain of *Mycobacterium leprae murium*.

The effects of various doses of cycloserine were tested in two successive experiments. Isoniazid was used as the standard of reference for drug activity in the first experiment, streptomycin and DDS (4,4'-diaminodiphenyl sulfone) in the second. Cycloserine and DDS were mixed in the diet of ground purina rat chow. Streptomycin was injected subcutaneously once daily, five days a week. Treatment was started on the day after inoculation and terminated about three months later. An untreated leprosy control group was observed in each experiment.

Autopsies were performed by the author at the end of each experiment, without knowledge of the treatment the animals had received. The mortality rates, body weights, 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. As reported in a previous communication (3), bacillus and leprosy indices were computed according to scores given for various sites and organs. The data pre-

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

² With technical assistance of Robert W. Scaggs.

³ The cycloserine, Seromycin, was kindly supplied by Dr. R. S. Griffith of Lilly Laboratory for Clinical Research.

sented in the tables represent the averages of all animals of each group except the bacillus indices, which are averages of two representative animals of each group. The Ziehl-Neelsen method was used for staining the bacilli; decolorization was with 3 per cent hydrochloric acid in 95 per cent ethyl alcohol.

RESULTS

Experiment 1.—One group of mice was treated with 0.2 per cent and another with 0.5 per cent of cycloserine in the food. The dosage of isoniazid was 0.01 per cent in the food. Thirteen animals of the three treated groups (total 60 mice), and 1 of the untreated leprosy control group, died in the early days of intercurrent diseases. The results are shown in Table 1.

Isoniazid revealed marked suppressive activity as shown by the very low average weights for omenta and pelvic fatty pads, and by the bacillus and leprosy indices, in comparison with those for untreated leprosy controls. The smaller dose of cycloserine displayed little activity. The leprosy index of that group was similar to that of the untreated control group, although the bacillus index and the weights of omenta and pelvic fatty pads were slightly lower. The larger dose of cycloserine showed a definite suppressive effect. The average weights of omenta and pelvic fatty pads, and the bacillus index were definitely lower than those of the untreated controls, and the leprosy index, was only about one-half of that of the controls. The index of chemotherapeutic effectiveness⁴ for the larger dose was, however, only 1.9, as compared to 21.1 for isoniazid.

Experiment 2.—Four dosages of cycloserine, i.e., 0.2, 0.5, 0.75 and 1.0 per cent in the food, were each tested in one group of mice. The dosage of DDS was 0.1 per cent, and that of streptomycin was 2 mgm., injected subcutaneously. Five mice in the streptomycin-treated and DDS-treated groups, and two in the groups receiving the highest doses of cycloserine, died from intercurrent diseases. The antibiotic was well-tolerated. The results are shown in Table 2.

Streptomycin and DDS revealed their regular suppressive activity as reported previously (2, 3). The smallest dosage of cycloserine, i.e., 0.2 per cent, showed only slight suppressive activity. Although the leprosy index was definitely smaller than that of the untreated leprosy controls, the average weights of omenta and the bacillus indices of these two groups were similar to each other. The three higher doses of cycloserine displayed marked suppressive activity, and their activities were similar. The average weights of omenta and pelvic fatty pads and the bacillus indices of these three groups were markedly lower than those of the control group. Their leprosy indices ranged from 1.26 to 1.93, in comparison with 8.28 for the control group. The ICE of the these groups of animals receiving 0.5,

⁴ Index of chemotherapeutic effectiveness, or ICE, was calculated as follows:

$$\frac{\text{Total leprosy index of control group}}{\text{Total leprosy index of treated group}}$$
 The larger the figure the higher the activity.
 Unity means no activity.

TABLE 1.—Experiment 1. The effects of cycloserine on mouse leprosy.

Drug	Dose in food per cent	No. died/No. used	Body wt., gm.	Weight of omentum, gm.	Weight of pelvic pads, gm.	Bacillus index	Leprosy index						ICE ^a		
							Site of inoculation	Omentum and mesentery	Pelvic fatty pads	Lymph nodes	Spleen	Liver		Misc.	Total index
Leprosy control, untreated		1/20	24.6	0.17	1.15	34.0	0.47	4.18	2.73	0.42	0.71	0.47	1.58	10.56	—
Isoniazid	0.01	3/20	23.8	0.03	0.50	11.5	0	0.50	0	0	0	0	0	0.50	21.1
Cycloserine	0.2	6/20	21.4	0.15	1.00	30.0	1.07	3.96	2.21	0.32	0.25	0.50	1.71	10.02	1.1
Cycloserine	0.5	4/20	21.8	0.10	0.60	23.5	0.41	2.28	1.16	0.09	0.56	0.63	0.44	5.57	1.9

^a Index of chemotherapeutic effectiveness (see text).

TABLE 2.—Experiment 2. Repetition of Experiment 1, including higher doses of cycloserine.

Drug	Dose in food per cent	No. died/No. used	Body wt., gm.	Weight of omentum, gm.	Weight of pelvic pads, gm.	Bacillus index	Leprosy index						ICE		
							Site of inoculation	Omentum and mesentery	Pelvic fatty pads	Lymph nodes	Spleen	Liver		Misc.	Total index
Normal mice		0/20	25.2	0.01	0.85	—	—	—	—	—	—	—	—	—	—
Leprosy control, untreated		0/20	25.8	0.12	1.25	31.0	0.73	2.93	1.93	0.13	1.28	0.25	1.03	8.28	—
Streptomycins ^a		4/20	24.6	0.05	0.75	15.5	0.78	0.81	0.31	0	0.03	0	0.09	2.02	4.1
DDS	0.1	1/20	23.5	0.08	0.74	20.0	0.71	1.55	0.68	0	0.03	0.79	0.26	4.02	2.1
Cycloserine	0.2	0/20	23.5	0.13	0.93	30.5	0.58	2.23	1.23	0	0.83	0.10	0.58	5.55	1.5
Cycloserine	0.5	0/20	24.1	0.05	0.53	22.0	0.35	1.05	0.28	0	0.25	0	0	1.93	4.3
Cycloserine	0.75	1/20	22.5	0.04	0.60	11.0	0.18	0.55	0.24	0	0.29	0	0	1.26	6.6
Cycloserine	1.0	1/20	24.0	0.03	0.66	17.0	0.11	0.68	0.21	0	0.47	0	0	1.47	5.6

^a 2 mgm. per mouse, injected subcutaneously.

0.75 and 1.0 per cent of the antibiotic were 4.3, 6.6 and 5.6 respectively; those of streptomycin and of DDS were 4.1 and 2.1, respectively. Thus the activity of the higher doses of cycloserine in this experiment was superior to that of DDS and approached that of streptomycin.

The appearance of the pelvic fatty pads and omenta of various groups receiving cycloserine in Experiment 2 may be seen in Figs. 1-6. The lesions in the mice receiving the three larger doses of cycloserine are obviously smaller than those of the untreated controls. Only slight activity by the smallest dose of the antibiotic is evident.

DISCUSSION

Cycloserine has been shown to be effective in tuberculosis of man, but inactive in the experimental disease in mice and guinea-pigs. This disagreement has puzzled investigators a great deal, and gives added interest and value to the study of the effectiveness of this antibiotic in other mycobacterial diseases. In the search for an experimental infection that might respond to treatment with cycloserine, murine leprosy was selected. In this infection the antibiotic was found to have effectiveness greater than DDS and about the same as that of streptomycin, but much lower than that of isoniazid.

In murine leprosy the bacilli are exclusively intracellular, while in murine tuberculosis they multiply both intracellularly and extracellularly. Histological sections of the tuberculous lung of the mouse show swarms of extracellular tubercle bacilli, having an appearance of bacterial growth *in vitro*. In the treatment of murine leprosy, a drug must reach intracellular parasites; in murine tuberculosis, it may act on either extracellular or intracellular organisms or on both. This may be one reason why cycloserine acts differently in these two infections.

SUMMARY

Cycloserine (Seromycin) was found to be moderately effective in the suppression of intraperitoneally infected mouse leprosy. The smallest dosages used, 0.2 per cent in the food, showed minimal effects. Higher doses showed greater activity, of about the same order as that exhibited by streptomycin, but much less than that of isoniazid.

Cycloserine has been reported to be beneficial in human tuberculosis but to have no effect in experimental tuberculosis of mice and guinea-pigs. The discovery of its suppressive action in murine leprosy suggests the desirability of the extension of screening tests to other mycobacterial infections in the search for more active antituberculosis agents.

RESUMEN

La cicloserina (Seromicina) resultó ser moderadamente eficaz para la supresión de la lepra en ratones infectados intraperitonealmente. La dosis más pequeña utilizada, 0.2 por ciento en el alimento, produjo efectos mínimos. Las dosis más altas mostraron

mayor actividad, más o menos del mismo orden que la manifestada por la estreptomina, per mucho menor que la de la isoniacida.

Se ha comunicado que la cicloserina es beneficiosa en la tuberculosis humana, pero ineficaz en la tuberculosis experimental del ratón y del cobayo. El descubrimiento de su acción supresiva en la lepra murina sugiere la conveniencia de extender las pruebas de triaje a otras infecciones micobacterianas en busca de medicamentos antituberculosos más activos.

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DESCRIPTION OF PLATE

PLATE 18

Comparison of the pelvic fatty pads and the omenta of untreated and treated murine leprosy in Experiment 2. In each picture the larger objects are pelvic fatty pads and the smaller ones the omenta.

FIG. 1. Leprosy control group, untreated.

FIG. 2. Group treated with cycloserine 0.2 per cent in the food.

FIG. 3. Group treated with cycloserine 0.5 per cent in the food.

FIG. 4. Group treated with cycloserine 0.75 per cent in the food.

FIG. 5. Group treated with cycloserine 1.0 per cent in the food.

FIG. 6. Normal mice group.

The lesions in the untreated leprosy group (Fig. 1) are large and rough. Those in Figs. 2 and 3 are smoother and smaller than those of the untreated control. Those in Figs. 4 and 5 are very slight, and the appearance is very similar to that of the same tissues of normal mice (Fig. 6).

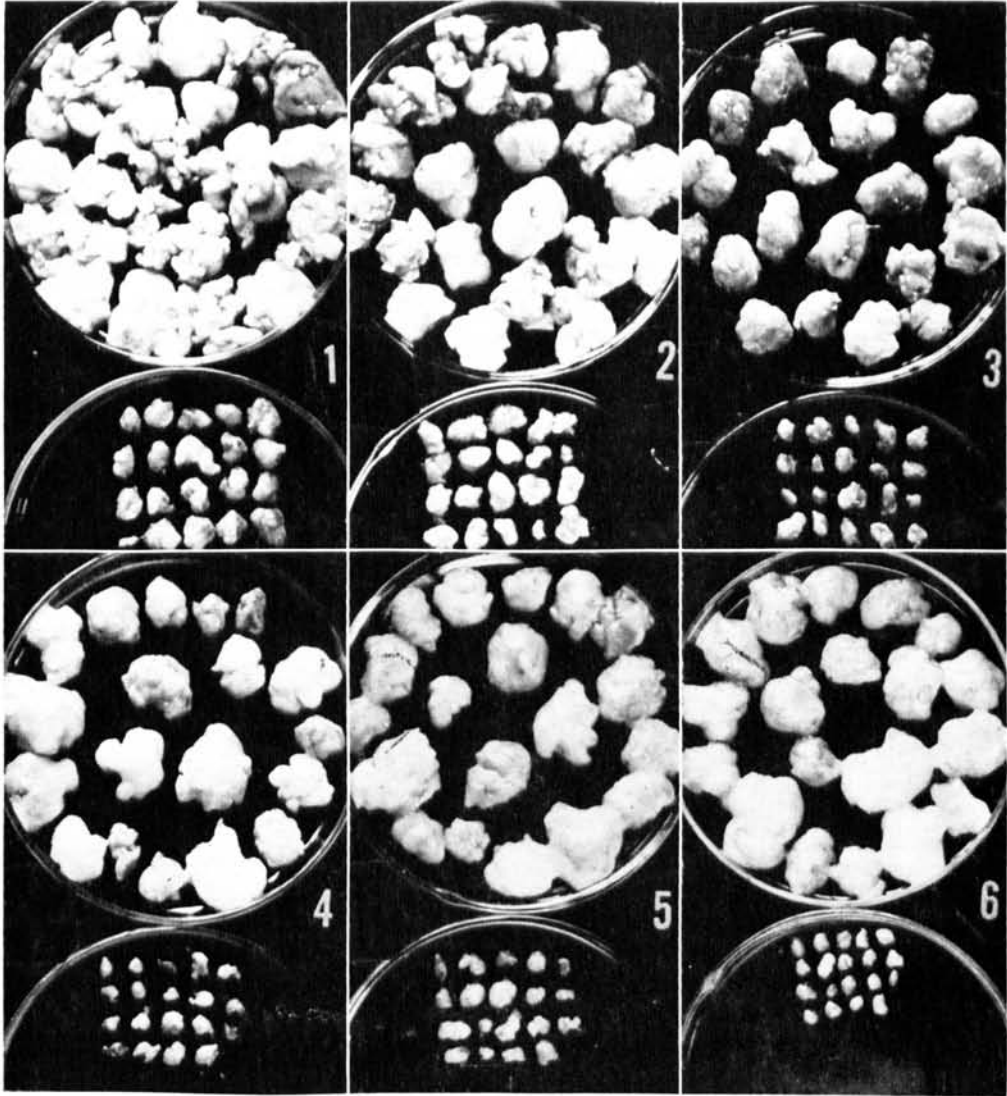


PLATE 18