

## CHEMOTHERAPY OF MURINE LEPROSY

### V. THE EFFECTS OF VARIOUS COMBINATIONS OF 4,4'-DIAMINODIPHENYL SULFONE (DDS), STREPTOMYCIN AND ISONIAZID ON MOUSE LEPROSY

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In previous communications (3-5) I have reported, among other things, that 4,4'-diaminodiphenyl sulfone (DDS), streptomycin, and isoniazid (isonicotinic acid hydrazide) possess definite therapeutic activity in mouse leprosy, and that the combination of DDS and streptomycin showed an additive effect. The effects of various combinations of DDS, streptomycin and isoniazid on mouse leprosy are reported here.

#### METHODS AND MATERIAL

The same technique of chemotherapeutic assay in intraperitoneally-infected mice was employed as in the previous studies (2). Female albino mice of the National Institutes of Health general-purpose strain weighing  $\pm 20$  gm. were used in groups of 20, each group caged separately. Inoculations were made of 0.5 cc. of a seed suspension, i. e., a 1:30 suspension of the omenta and pelvic fatty pads of mice which had been infected four to five months previously with the Hawaiian strain of *Mycobacterium leprae murium*.

Experiment 1: Eleven groups of mice were used according to the following schedule:

- Group 1. Normal mice control;
- Group 2. Leprosy control, untreated;
- Groups 3 and 4. DDS, 0.05%;
- Groups 5 and 6. Isoniazid, 0.003%;<sup>3</sup>
- Group 7. Streptomycin, 1 mgm.;
- Groups 8 and 9. Streptomycin, 1 mgm., isoniazid, 0.003%;
- Groups 10 and 11. Isoniazid, 0.003%, DDS, 0.05%.

Experiment 2: Repetition of the above experiment, including also groups treated with combinations of streptomycin and DDS, and of streptomycin, DDS and isoniazid, dosage as before. One group of animals was used for each of the single drugs, and two groups for each combination.

Experiment 3: Repetition of Experiment 2, omitting the normal mice control group.

Treatment was started on the day after inoculation. Streptomycin was injected subcutaneously, daily, 5 days a week. All other drugs were mixed in the food, and the dosage was calculated on the basis of an average daily food intake of 4 gm. per 20-gram mouse. Animals were sacrificed 98-105 days after inoculation in Experiment 1, after 88-94 days in Experiment 2, and after 85-93 days in Experiment 3.

Autopsies were performed by me without knowledge of the treatment the animals had received. As usual, the mortality rate, body weight, weights of omenta and of

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pelvic fatty pads, and the bacillus and leprosy indices were recorded. The omenta and pelvic fatty pads of each group of animals were placed in a Petri dish and photographed. The bacillus index represents the total score given to smears (each smear graded as 0 to 5) made from the following sites and organs: site of inoculation (peritoneum), omentum, pelvic fatty pads, portal lymph nodes, paravertebral lymph nodes, thymus, spleen, liver, lung and kidney. The leprosy index is the total score given to gross pathologic lesions in 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; miscellaneous, including lung, kidney, diaphragm, pericardium, retrosternal region, and thymus, each, 0-1.

Histologic preparations of various sites and organs were made from two representative animals of each group of Experiments 2 and 3, including also the livers of four other animals in the same group. The data as finally analyzed, shown in Table 1,<sup>4</sup> represent the averages of all animals of each group except the bacillus indices, which were averages of two representative animals of each group. Kinyoun's (6) modified acid-fast stain, employing cold staining with concentrated fuchsin, was used in the first and second experiments; warm staining with the same stain was adopted in the third one.

#### RESULTS

*Experiment 1.*—All drugs except streptomycin were tested in duplicate groups of animals in this experiment. In total, 220 mice were used. The summarized results are shown in the first part of Table 1.

Many animals of this experiment suffered from intercurrent diseases. The majority of them recovered from these attacks but 24 died, 5 in the first month and the others in the third month. The losses varied from none in four groups up to 7 in the other seven groups. The average body weight of several groups of animals was lower than that of the control at the end of the experiment.

DDS in a dose-level of 0.05 per cent in the diet, or 100 mgm./kgm. body weight, showed slight suppressive effect on the development of lesions. This was shown by the smaller weights of the omenta and the slightly lower leprosy indices in the mice of the DDS-treated groups than in those of the untreated leprosy control.

Isoniazid in a dose-level of 0.003 per cent in the diet, or 6 mgm./kgm. body weight, caused definite suppression of the infection. Similar activity was also shown by streptomycin in the dose of 50 mgm./kgm. The weights of omenta of the isoniazid- and streptomycin-treated groups were definitely smaller than those of the control. The total leprosy indices of these groups were between two-thirds and one-half of the index of the untreated control group.

In studying the effects of the combinations of streptomycin and isoniazid, and of isoniazid and DDS, the same dosages were used as for

<sup>4</sup> With this manuscript there were three lengthy tables which gave full details of the findings in all of the groups of the three experiments, precisely as in the author's previous reports referred to. As a result of a suggestion by a reviewer of the Editorial Board of the Institute, only the essential findings of the three experiments are given in this condensed table.

each drug alone. Both combinations showed suppressive activity superior to that of the drugs given singly. Marked reduction in the weights of the omenta was noted in all animals, and the weights of the pelvic fatty pads were also lower than those of the control group. The leprosy indices were reduced to between one-third and one-fourth that of the control,

TABLE 1.—Condensed tabulation of the results in the three experiments, the indices of the bacillus content, total leprosy, and chemotherapeutic effectiveness (ICE), of the drugs and combinations used (dosages as in text).<sup>a</sup>

Experiment 1				Experiment 2				Experiment 3				
Drug or combination	Index			Drug or combination	Index			Drug or combination	Index			
	Bacillus	Leprosy	ICE		Bacillus	Leprosy	ICE		Bacillus	Leprosy	ICE	
Leprosy control	24.0	11.99		Leprosy control	22.0	10.99		Leprosy control	33.0	9.60		
DDS	24.5	10.89	1.1	DDS	12.5	5.93	1.9	DDS	28.5	5.50	1.8	
	15.5	10.21	1.2									
Isoniazid	15.5	7.96	1.5	Isoniazid	11.5	2.84	3.9	Isoniazid	22.0	4.78	2.0	
	11.0	6.57	1.8									
Streptomycin	15.5	6.23	1.9	Streptomycin	14.5	4.56	2.4	Streptomycin	27.5	2.96	3.3	
Streptomycin-isoniazid	19.5	4.00	3.0	Streptomycin-isoniazid	8.0	0.72	15.3	Streptomycin-isoniazid	17.0	2.83	3.4	
	17.5	3.69	3.3		3.0	0.77	14.3		15.0	2.63	3.7	
Isoniazid-DDS	16.5	3.12	3.8	Isoniazid-DDS	9.5	1.24	8.9	Isoniazid-DDS	13.0	1.90	5.1	
	14.0	3.37	3.6		9.0	1.24	8.9		14.0	1.74	5.5	
Streptomycin-DDS		10.0	2.49	4.4	Streptomycin-DDS	20.0	3.03	3.2	Streptomycin-DDS	20.0	3.03	3.2
		11.5	2.29	4.8		22.0	3.08	3.1		22.0	3.08	3.1
Streptomycin-DDS-INH		5.5	0.50	22.0	Streptomycin-DDS-INH	10.5	0.90	10.7	Streptomycin-DDS-INH	10.5	0.90	10.7
		5.5	0.70	15.7		8.5	1.35	7.1		8.5	1.35	7.1

<sup>a</sup> When two sets of figures are given for a single drug or a combination, the first set refers to the Group A animals and the second to Group B.

indicating that an additive effect was obtained from both of these combinations.

*Experiment 2.*—This experiment, as said, was a repetition of the first one with the addition of the combinations of streptomycin and DDS, and of streptomycin, DDS and isoniazid (INH). In this instance all three individual drugs were tested in single groups of animals while the various combinations were studied in duplicate groups, a total of 260 mice being employed. The principal results are shown in the second part of Table 1.

Three animals died in the early days of this experiment, probably from intercurrent diseases: 1 of the group receiving the combination of streptomycin and DDS, and 1 each of the two groups receiving the combination of isoniazid and DDS.

DDS alone showed definite activity here, the leprosy index being reduced to about one-half of that of the control; but it was the least effective of all, the index of chemotherapeutic effectiveness (ICE)<sup>5</sup> being only 1.9. Streptomycin alone showed a closely similar degree of activity, its ICE 2.4. Isoniazid alone proved to be more active than either DDS or streptomycin, the leprosy index of that group being about one-fourth of that of the control, the ICE 3.9. The average weights of omenta and pelvic fatty pads of all these three groups were definitely smaller than that of the control.

The combination of streptomycin and DDS showed an additive effect. The leprosy indices of the two duplicate groups were 2.29 and 2.49, while that of the control was 10.99. The average ICE of the combination was 4.6, which was materially higher than those of the same drugs given singly. This is in agreement with the previous observation that a combination of these two drugs, in doses higher than those used in the present test, had an additive effect in mouse leprosy (3).

The combination of isoniazid and DDS (2 groups) showed better activity than the streptomycin-DDS combination. The average leprosy index of the former combination was 1.24, the average ICE 8.9.

The combination of streptomycin and isoniazid was found to be the most effective two-drug combination employed in this experiment. The average leprosy index was 0.75, and the average ICE 14.8.

The triple combination, streptomycin, DDS and isoniazid revealed an activity similar, if not superior, to that of the combination of streptomycin and isoniazid. In one group the leprosy index was 0.70 and the ICE was 15.7 not significantly better than with the streptomycin-isoniazid combination, but in the other group these factors were 0.50 and 22.0 respectively.

In the animals treated with both of these combinations—the best of the two-drug ones and the triple one—the lesions were minimal, so that differences between them could hardly be detected. The appearance of the pelvic fatty pads and omenta also showed marked differences among various groups (Plates 1 and 2). They were smaller in the groups treated with various combinations than in those treated with single compounds.

*Experiment 3.*—This experiment was a repetition of the second one, the normal control group omitted. Of the total of 240 mice used, 9 animals died: 8 died from intercurrent diseases, while 1 of the leprosy control group died just before the termination of the experiment from extensive leprosy lesions.

DDS showed essentially the same activity as in Experiment 2. Streptomycin had slightly more effect, and isoniazid slightly less effect, than

<sup>5</sup> The index of chemotherapeutic effectiveness (ICE) is 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 action.

in the second experiment. The combination of isoniazid and DDS, and that of all three drugs, displayed activity superior to that of the individual drugs added together.<sup>6</sup>

## DISCUSSION

Variations of drug activity among these three experiments are to be seen. Final evaluation of the activities of the various drugs and their combinations may be obtained by combining the results of all three experiments, as in Table 2. Each drug or combination was tested in from 60 to 120 animals. Despite the variations between the experiments, the average leprosy indices reveal definite differences of effectiveness among the various drugs and combinations. The averaged results obtained, in terms of the combined ICE, are shown in Text-fig. 1. Each combination shows an activity higher than that of any of the individual drugs.

TABLE 2.—Combined results concerning the activities of all drugs and combinations employed in the three experiments.<sup>a</sup>

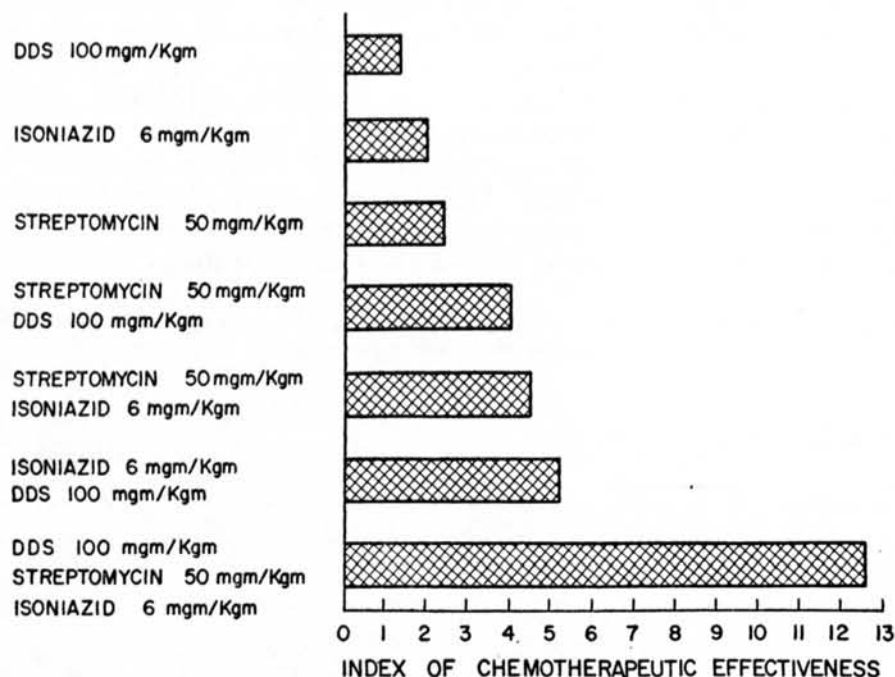
Drug or combination	Dose mgm./ kgm.	Total mice used	Leprosy index				ICE
			Exp. 1	Exp. 2	Exp. 3	Average	
Leprosy controls		60	11.99	10.99	9.60	10.86	
DDS	100	80	10.89 10.21	5.93	5.50	8.13	1.3
Isoniazid	6	80	7.96 6.57	2.84	4.78	5.54	2.0
Streptomycin	50	60	6.23	4.56	2.96	4.58	2.4
Streptomycin and DDS	50 100	80	—	2.49 2.29	3.03 3.08	2.72	4.0
Streptomycin and isoniazid	50 6	120	4.00 3.69	0.72 0.77	2.83 2.63	2.44	4.5
Isoniazid and DDS	6 100	120	3.12 3.37	1.24 1.24	1.90 1.74	2.10	5.2
Streptomycin, isoniazid, DDS	50 6/100	80	—	0.50 0.70	0.90 1.35	0.86	12.6

<sup>a</sup> Arranged in the order of increasing chemotherapeutic effectiveness (ICE).

The bacillus indices in all three experiments also revealed increased activity of the combination treatments. The lowest of these indices, ranging from 3 to 8, were seen in the second experiment in the groups treated with the combination of all three drugs, and the streptomycin-isoniazid

<sup>6</sup> The ICE of DDS, 1.8, and that of INH, 2.0, added together totals 3.8; whereas that of the combination (average of the two groups) is 5.3. The total ICE of the three individual drugs (that of streptomycin being 3.3) is 7.1; the average ICE of the triple combination is 8.9.

combination. These figures are similar to the average bacillus index of the autoclaved bacterial control, i. e., 7.3, reported in a previous communication (4). The bacillus indices of Experiment 3 are generally higher than those in the other two experiments. This is chiefly due to the stated modification of the staining technique.



TEXT-FIG. 1. Comparison of the activities of DDS, streptomycin, isoniazid and their various combinations in mouse leprosy, in terms of the indices of chemotherapeutic effectiveness from the combined results of three experiments. The longer the bar, the higher is the activity. Note that the various combinations are superior to the individual drugs.

Histologic studies of the animals of Experiments 2 and 3 revealed fewer and smaller leprosy lesions in all treated groups than in the controls, especially in those which received combinations of drugs. The extent of the lesions in the liver was observed in 5 or 6 of the mice receiving each drug alone, and 10 to 12 of those receiving the combinations. The findings are given in Table 3. The extent of these lesions in the various groups was in fair agreement with their leprosy indices.

On the basis of these findings, it may be concluded that various combinations of small doses of DDS, streptomycin and isoniazid produce suppressive effects in mouse leprosy that are definitely superior to those obtained from any individual drugs alone. Clinical trial of combinations of these drugs in human leprosy may be justified.

The small doses of DDS, of streptomycin, and of isoniazid used alone caused definite suppressive effect. The most active drug was isoniazid;

even the dose of 6 mgm. per kgm. showed marked activity. This agrees with the well-known marked antituberculosis activity of this drug in experimental tuberculosis of mice (1), and indicates that mouse leprosy

TABLE 3.—Comparison of the average leprosy indices with the amount of histological lesions in the liver, mice of Experiments 2 and 3.<sup>a</sup>

Drug or combination	Experiment 2		Experiment 3	
	Average leprosy index	Histological lesions in liver	Average leprosy index	Histological lesions in liver
Leprosy controls	10.99	Extensive	9.60	Extensive
DDS	5.93	Moderate	5.50	Moderate
Isoniazid	2.84	Moderate	4.78	Slight
Streptomycin	4.56	Moderate	2.96	Slight
Streptomycin and DDS	2.39	Slight	3.06	Minimal
Streptomycin and isoniazid	0.75	Minimal	2.73	Slight
Isoniazid and DDS	1.24	Minimal	1.82	Minimal
Streptomycin, isoniazid, DDS	0.60	Minimal	1.13	Minimal

<sup>a</sup> Arranged in the order of increasing total chemotherapeutic effectiveness (Table 2).

is essentially as sensitive as mouse tuberculosis in the study of the activity of chemotherapeutic agents.

#### SUMMARY

The effects of various combinations of small doses of 4,4'-diaminodiphenyl sulfone (DDS), streptomycin and isoniazid (isonicotinic acid hydrazide) have been studied in intraperitoneally-infected mouse leprosy.

The suppressive effects of various combinations of these drugs were definitely superior to those obtained with the individual drugs alone. The order of activity of the drugs and combinations used, based on the decreasing total indices of chemotherapeutic effectiveness, may be expressed as follows: (1) streptomycin, isoniazid, and DDS combined; (2) isoniazid and DDS; (3) streptomycin and isoniazid; (4) streptomycin and DDS; (5) streptomycin alone; (6) isoniazid; (7) DDS.

#### SUMARIO

Se han estudiado los efectos de varias combinaciones de pequeñas dosis de 4,4'-diaminodifenil-sulfona (DDS), estreptomycin e isoniazida (hidracida del ácido isonicotínico) en la lepra murina inoculada intraperitonealmente.

Los efectos supresivos de varias combinaciones de esas drogas fueron decididamente superiores a los obtenidos con las distintas drogas por sí solas. Cabe expresar en la forma siguiente el orden de actividad de las drogas y de las combinaciones, a base de

los decrecientes índices totales de eficacia terapéutica: (1) estreptomycin, isoniácida y DDS combinadas; (2) isoniácida y DDS; (3) estreptomycin e isoniácida; (4) estreptomycin y DDS; (5) estreptomycin sola; (6) isoniácida; (7) DDD.

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#### DESCRIPTION OF PLATES

Comparison of the pelvic fatty pads and the omenta of untreated and treated leprosy mice of Experiment 2. In each pair of pictures the larger one is of the pelvic fatty pads, the smaller one of the omenta.

#### PLATE 9

FIG. 1. Leprosy control group, untreated.

FIG. 2. DDS group. The lesions of the pelvic fatty pads are smaller than those of the control.

FIG. 3. Streptomycin group. The lesions of the pelvic fatty pads and the omenta are distinctly smaller than those of the control, and slightly smaller than those of DDS-treated group (Fig. 2).

FIG. 4. Isoniazid group. The lesions are markedly smaller than those of the control, and slightly smaller than those of the streptomycin-treated group (Fig. 3).



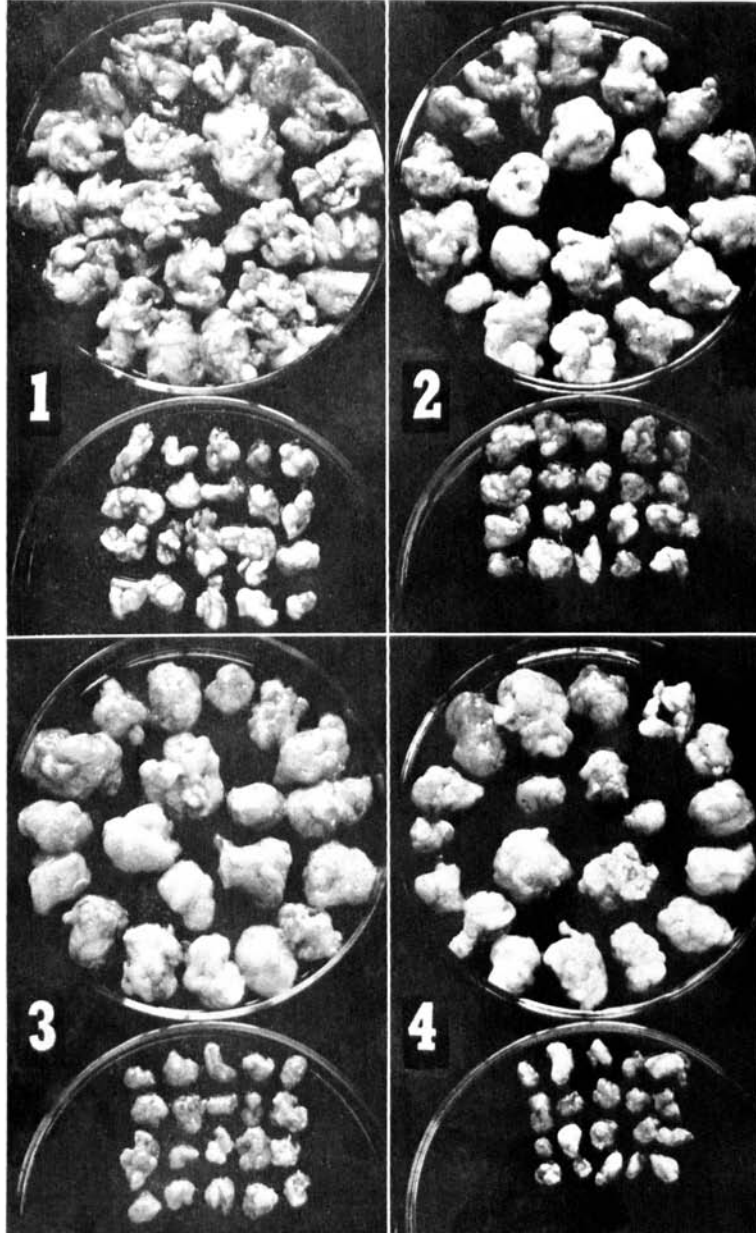


PLATE 9

PLATE 10

FIG. 5. Combination of streptomycin and DDS. The lesions are distinctly smaller than those of the groups receiving either of those drugs alone (Figs. 2 and 3).

FIG. 6. Combination of isoniazid and DDS. The lesions are markedly smaller than those of the DDS group (Fig. 2), and slightly smaller than those of the isoniazid group (Fig. 4).

FIG. 7. Combination of streptomycin and isoniazid. The lesions are distinctly smaller than those of the groups receiving either drug alone (Figs. 3 and 4).

FIG. 8. Combination of DDS, streptomycin and isoniazid. The lesions are distinctly smaller than those of the groups treated with each drug alone (Figs. 2-4). Note the close resemblance between this group and the group treated with the combination of streptomycin and isoniazid (Fig. 7).

FIG. 9. The same tissues from the normal-mice group.

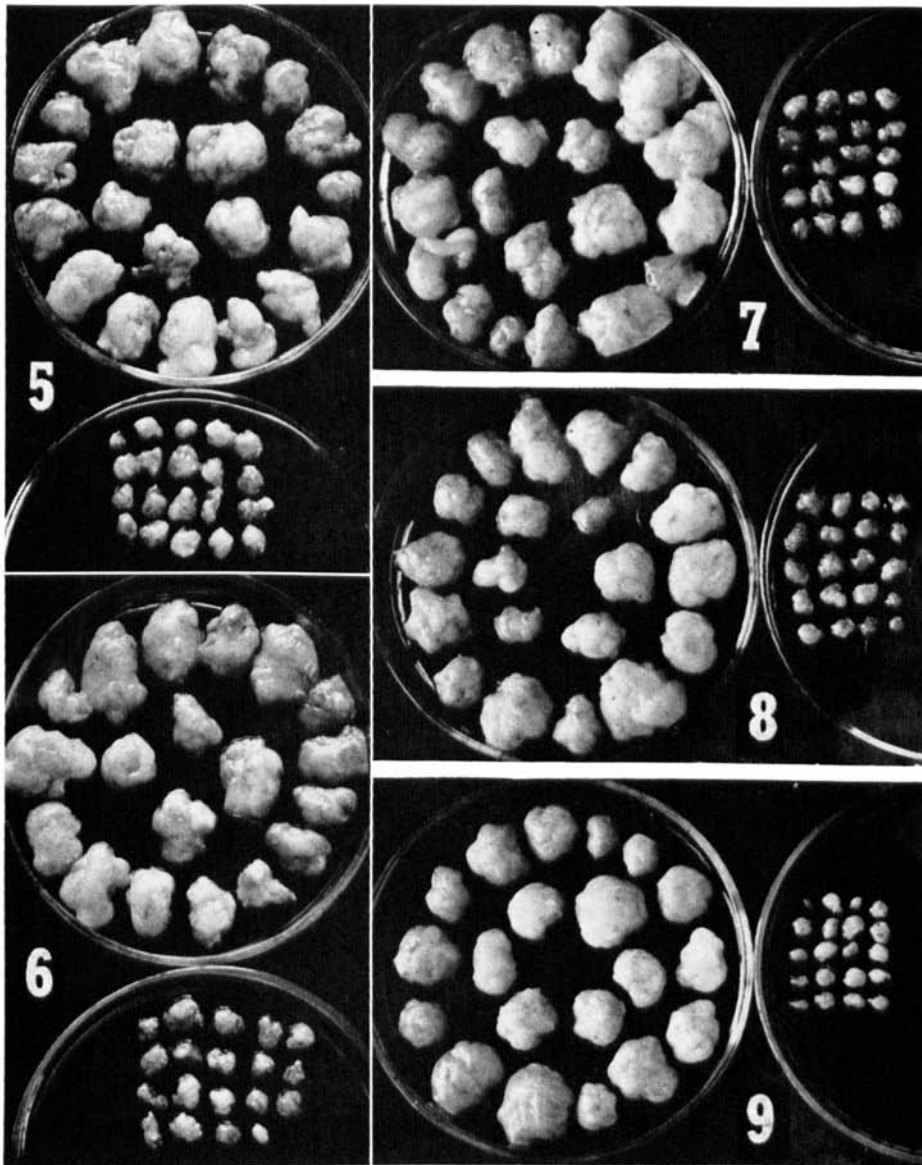


PLATE 10