per kg weight (total dose 1 mg) was given every 4 days for 4 weeks. The antibody titre rose to 1-160 as compared to a control titre of 1-1280.

Comment. The studies of Ehrich[1] have shown that the lymphocytes from lymph of a regional node draining an extremity into which antigen has been injected contain an increased titre of antibody on the fourth to sixth day. Dougherty and White[2,3] have shown that corticosterone will produce a lymphopenia and that the decrease in these cells, with their dissolution is associated with a rise in the antibody titre. Since the nitrogen mustards produce a toxic lymphopenia which is apparent in the peripheral blood[4] and lymph node[5] within 4 days, it might be expected that this toxic dissolution would result in the increase of antibody. With this in view, the nitrogen mustard was given after the normal antibody curve had fallen but no rise in titre similar to the anamnestic reaction was seen. No summation of titre occurred when nitrogen mustard was given at the peak of the antibody response.

These findings do not contest the role of the lymphocyte in antibody formation but suggest that the toxicity of nitrogen mustard interferes with the antibody forming mechanism of the lymphocyte. This hypothesis is supported by the suppression of the antibody formation in animals receiving antigen following pretreatment and concurrent treatment with nitrogen mustard. The dosage used in these experiments are 10-fold or more than that given in human therapy and an investigation of the antibody response in the human is now in progress. It is of interest to mention that in the human given a course of 25 mg nitrogen mustard, the leucopenic phase coincides with a period of active regeneration of the hemopoietic system.[6]

Summary. 1. The lymphocytotoxic effect of the nitrogen mustards will not produce an anamnestic reaction or summation of the antibody titre in the rabbit.

2. Pretreatment and concurrent administration of nitrogen mustard suppress the antibody response to typhoid antigen.

[3] Dougherty, T. F., White, A., and Chase, S. H., PROC. SOC. EXP. BIOL. AND MED., 1944, **56**, 28.

[4] Jacobson, L. O., Spurr, C. L., Barron, E. S. G., Smith, T. R., Lushbaugh, C., and Dick, G. F., *J. A. M. A.*, 1946, **132**, 263.

[5] To be published.

[6] Spurr, C. L., Jacobson, L. O., Smith, T. R., and Barron, E. S. G., A.A.A.S. Gibson Island Conf. on Cancer, *Chemotherapy of Tumors*, in press; *Cancer Research*, 1947, **7**, 51.

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Chemotherapeutic Action of Streptomycin and of Streptomycin with a Sulfone or Sulfadiazine on Tuberculosis.

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Previously published experiments[1] have shown that streptomycin and promin used together in the treatment of experimental tuberculosis in guinea pigs produced a chemotherapeutic effect greater than the sum of effects from the individual components. Equally good results were later reported from the combined use of streptomycin and another sulfone, sodium salt of 4-amino,4'-galacturonylaminodiphenylsulfone.[2] The supply of streptomycin, however, was then so limited that no experiments could be made

[1] Smith, M. I., and McClosky, Wm. T., *Pub. Health Rep.*, 1945, **60**, 1129.

[2] Smith, M. I., McClosky, W. T., and Jackson, E. L., *Am. Rev. Tub.*, in press.

with streptomycin alone to appraise fully the value of the combined treatment.

Data have been accumulated on the relative chemotherapeutic value of several sulfone derivatives with the object of finding a compound less toxic, and if possible more effective, than promin or the parent substance 4,4′-diaminodiphenylsulfone (DDS). Studies on this phase of the problem have indicated that the mono *n*-propyl derivative of DDS was much less toxic than promin and at least as effective as the latter.[2] Another derivative of DDS, the mono-succinimide, synthesized in this laboratory by one of us (H.B.), was also found to have a low degree of toxicity and appeared worthy of therapeutic trial with streptomycin. A trial experiment with streptomycin and a sulfonamide also appeared desirable, especially since sulfadiazine had previously given some indication of chemotherapeutic activity in experimental tuberculosis, even if only of a very low order, compared with the sulfones.[3]

Accordingly a series of experiments were set up to determine (a) the chemotherapeutic effectiveness of streptomycin alone at a dose level of 40 mg per kg per day, a dose 2 to 4 times as great as had been used hitherto, (b) the effect of streptomycin at half the above dose in combination with one of the 2 sulfones mentioned earlier or with sulfadiazine.

Experimental. A series of male guinea pigs weighing 300 to 350 g, usually about 325 g, were inoculated intraperitoneally with 1 cc of a homogeneous bacillary suspension in saline containing 0.4 mg moist weight of tubercle bacilli, human strain, H37Rv.* The animals were divided into 6 groups of 20 each and treated as follows:

Group A. Streptomycin, 20 mg† per kg intramuscularly twice daily, 9 a. m. and 4 p. m.

Group B. Streptomycin, 10 mg per kg intramuscularly twice daily, and *n*-propyl derivative of diaminodiphenylsulfone (*n*-propyl),‡ 500 mg per kg per day, orally.

Group C. Streptomycin as in Group B and 250 mg per kg per day of the succinimide derivative of diaminodiphenylsulfone (succinimido)§ orally.

Group D. Streptomycin as in Group B and 500 mg per kg per day of sulfadiazine orally.

Group E. *n*-Propyl as in Group B but no streptomycin.

Group F. Succinimido as in Group C, but no streptomycin.

Group G. Untreated controls.

The sulfones and sulfadiazine were administered by stomach tube in 10% aqueous suspension with 5% gum acacia.

Treatment was begun the day after inoculation and continued for 11 weeks, 5 days a week, with a double dose on the fifth day. Three weeks after treatment was discontinued the survivors were tuberculin tested, using 0.01 mg PPD in 0.1 cc saline intracutaneously. Readings were made 24 and 48 hours later. Eighteen to 20 weeks after infection all the survivors were killed with chloroform, autopsied, and the extent of tuberculous involvement recorded as previously described.[5]

In addition to the 6 foregoing groups a small group (L) of 7 guinea pigs was set aside for treatment, beginning 23 days after infection with 10 mg per kg streptomycin twice daily. Treatment in this group was continued for 11 weeks as in the preceding groups. These animals were killed and autopsy findings recorded as in the others.

Results. Table I gives in detail the extent of tuberculous involvement (tuberculosis index, T.B.I.) in the individual animals in each group at autopsy and also the average for each group. The ratings are all based on a possible maximum of 20. The sign ±

[3] Smith, M. I., Emmart, E. W., and Westfall, B. B., *J. Pharm. and Exp. Therap.*, 1942, **74**, 163.

* Courtesy Mr. W. Steenken, Jr., acting for the Committee on Standard Cultures of the Medical Research Committee of the National Tuberculosis Association.[4]

† 1 mg = 1000 units.

‡ $NH_2 \cdot C_6H_4 \cdot SO_2 \cdot C_6H_4 \cdot NH \cdot C_3H_7$.

§ $NH_2 \cdot C_6H_4 \cdot SO_2 \cdot C_6H_4 \cdot N$ (CO · CH_2 / CO · CH_2)

[4] A Depot for Standard Cultures of Tubercle Bacilli, *Am. Rev. Tub.*, 1946, **53**, 511.

[5] Smith, M. I., Emmart, E. W., and Stohlman, E. F., *Am. Rev. Tub.*, 1943, **48**, 32.

TABLE I.

The Tuberculin Reaction (PPD) and the Extent of Tuberculous Involvement (Tuberculosis Index, T.B.I.) at Autopsy in the Individual Animals in the Several Groups Treated with Streptomycin Alone or in Combination with Derivatives of Diaminodiphenylsulfone (DDS) or Sulfadiazine. All Animals Inoculated with 0.4 mg H37Rv Intraperitoneally.

	G	A		B		C		D		E	F	L
	Controls	Streptomycin		Streptomycin + *n*-propyl DDS		Streptomycin + succinimido DDS		Streptomycin + sulfadiazine		*n*-Propyl DDS	Succinimido DDS	Streptomycin, 23 days after infection
No.	T.B.I.	PPD	T.B.I.	PPD	T.B.I.	PPD	T.B.I.	PPD	T.B.I.	T.B.I.	T.B.I.	T.B.I.
1	13	+	0	*	*	+	±	+	2	2	9	0
2	6	+	0	+	0	+	1	±	2	2	3	2
3	10	+	±	± +	0	+	2	± +	1	6	4	2
4	20	+	±	+	0	0	±	+	1	3	11	6
5	5	+	2	+	0	±	2	± +	±	1	6	± 2
6	19	0	1	± +	0	+	1	0	0	3	14	5
7	20	0	0	+	0	±	±	0	0	3	2	
8	12	+	2	±	0	±	0	+	2	1	13	
9	20	+	2	0	1	+	1	0	2	3	14	
10	15	0	0	+	±	+	0	+	±	2	1	
11	9	+	1	+	0	0	1	+	2	6	10	
12	18	±	1	0	0	±	0	+	1	4	11	
13	20	+	2	0	±	+	1	0	0	2	15	
14	11	0	1	± *	0	±	0	0	±	9	8	
15	20	+	1	*	*	±	1	+	±	5	2	
16	11	+	0	+	3	±	±	+	0	6	2	
17	16	+	2	± +	2	+	±	±	2	12	8	
18	20	±	±	+	1	+	0	0	±	1	10	
19	10	+	2	+	0		±	±	0	2	13	
20	13	+	1	+	1	+	3	+	2	6	3	
Avg	14.4		1.0		0.5		0.8		1.0	3.9	7.9	2.4

* Died accidentally at an early date.

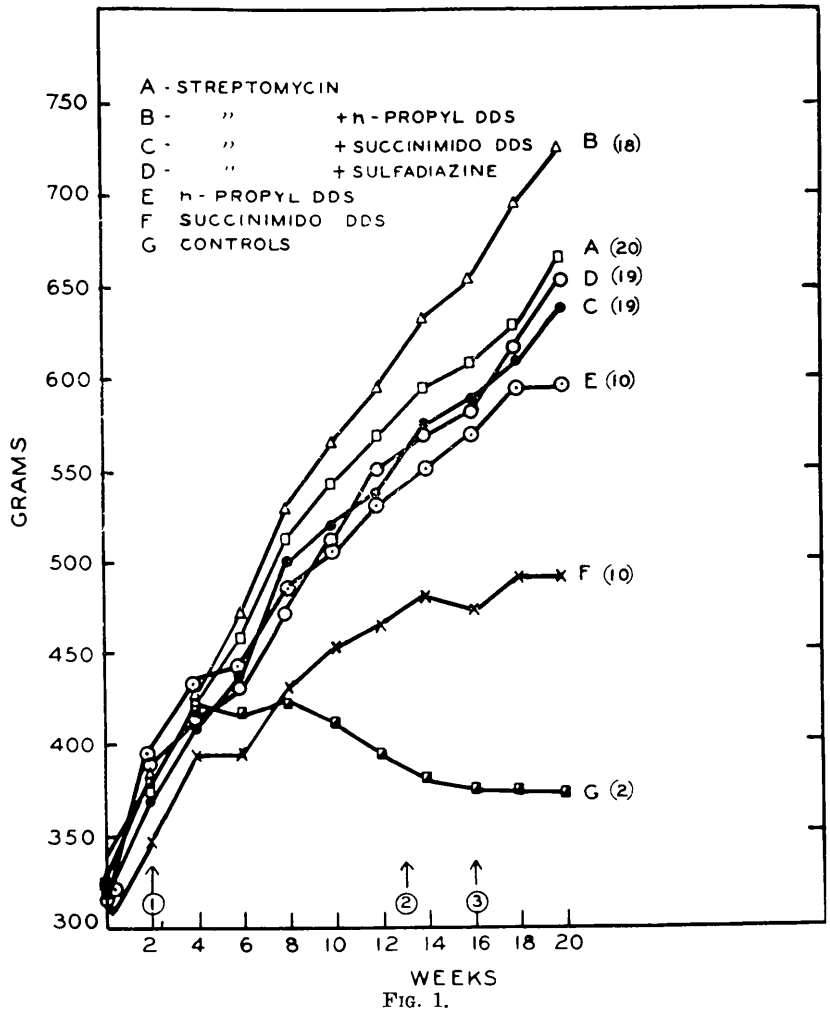

FIG. 1.

Average weight curves of groups of tuberculous guinea pigs treated with streptomycin (A), streptomycin and a derivative of diaminodiphenylsulfone (B and C), streptomycin and sulfadiazine (D), sulfone derivatives alone (E and F). G represents a group of untreated controls. Figures in parentheses indicate number of animals surviving at termination of experiment. First arrow shows time of infection and when treatment was begun; second arrow indicates end of treatment; at third arrow the survivors were tuberculin tested.

For details as to dosage and other pertinent data see Table II.

designates doubtful lesions and has been arbitrarily assigned a numerical value of 0.5. The results of the tuberculin tests for Groups A, B, C, and D are given in the PPD columns of the respective groups.

Group G Controls. Eighteen animals in this group (90%) died with extensive generalized tuberculosis in from 44 to 97 days. The remaining 2 animals were killed at the termination of the experiment, 126 days after infection, and these, also, had advanced tuberculosis of the viscera. The tuberculosis index in the individual animals varied from 5 to 20 (out of a possible maximum of 20) with an average of 14.4.

Group A. All the animals treated with 20 mg per kg streptomycin twice daily survived the experimental period, and made good gains in weight, as shown in curve A, Fig. 1. Though 14 animals in this group gave positive tuberculin reactions, 8 (40%) showed none or only doubtful lesions at necropsy. The remainder had a tuberculosis index of 1 to 2, with an average of 1 for the entire group.

Group B. Two of the animals treated with

streptomycin and the *n*-propyl compound died in 27 and 32 days respectively. At autopsy there were emphysema and congestion of the lungs in one and pulmonary edema in the other. The animals had shown no signs of illness up to the last drug feeding by stomach tube, and death in these 2 animals was regarded as accidental. The remaining 18 animals in this group gained weight normally, survived the full experimental period, and while 10 of them gave positive tuberculin reactions, 13 (72%) showed no gross evidence of tuberculosis at autopsy. The animals in this group showed the highest average gain in weight (curve B, Fig. 1). The average tuberculosis index for the entire group was 0.5.

Groups C and D. The animals treated with streptomycin in combination with the succinimido compound and sulfadiazine respectively compared favorably with the animals in Group A receiving streptomycin alone at twice the dose level. One animal in each of these groups died, at 71 and 104 days respectively, and while pulmonary emphysema was present in both neither showed much evidence of tuberculosis. The average tuberculosis index for Group C was 0.8, and 11 animals (55%) showed no gross evidence of tuberculosis. Ten animals in this group gave positive tuberculin reactions. The average tuberculosis index for Group D was 1.0, with 10 animals (50%) showing no macroscopic lesions, and 9 reacting to tuberculin. The weight curves of the animals of these 2 groups were nearly identical with that of Group A.

Groups E and F. Treatment with the *n*-propyl and succinimido derivatives respectively resulted in the mortality of 50% in each group. However, all of the survivors in Group E were in very good condition, while 4 of the survivors in Group F at the termination of the experiment were losing weight. The average weight curve for Group E was decidedly better than that of Group F. At autopsy all the animals in both groups had definite macroscopic tuberculosis. However, the average tuberculosis index for Group E was 3.9 with a variation of 1 to 12, while

TABLE II.
Summary of All Tests in the Several Groups.

Group and drug, mg/kg/day	G Controls	A Streptomycin 2 × 20	B Streptomycin 2 × 10 + *n*-Propyl DDS 500	C Streptomycin 2 × 10 + succinimido DDS 250	D Streptomycin 2 × 10 + sulfadiazine 500	E *n*-Propyl DDS 500	F Succinimido DDS 250
Mortality % at termination of exp. 130-140 days after infection	90	0	0	5	5	50	50
Avg T.B. index computed on basis of 100 for controls	100	6.9	3.5	5.5	6.9	26.9	54.5
Percent with no or doubtful lesions (T.B. rating of 0 or ±)	0	40	72	55	50	0	0
Chemotherapeutic effectiveness (ratio of T.B. index of controls and treated groups)		14.4	28.6	18.2	14.5	3.7	1.8
Avg wt gains, g	53	342	402	332	351	278	207
Wt of spleen, g							
Range	1.0-12.0	0.7-1.8	0.7-1.6	0.7-1.4	0.6-1.2	0.9-7.0	0.7-9.9
Avg	5.4	1.0	1.2	0.9	0.9	2.8	2.5

TABLE III.
Blood Levels. Average of 3 to 4 Animals.

Drug	Daily dose, g/kg	Mg %, hr after administration		
		3	5	19-24
n-Propyl DDS	0.25	1.5	1.5	0.8
	0.50	2.0	2.1	1.2
	2.00	3.4	3.6	3.8
Succinimido DDS	0.25	1.8	0.7	0.5
	2.00	1.6	1.5	1.6

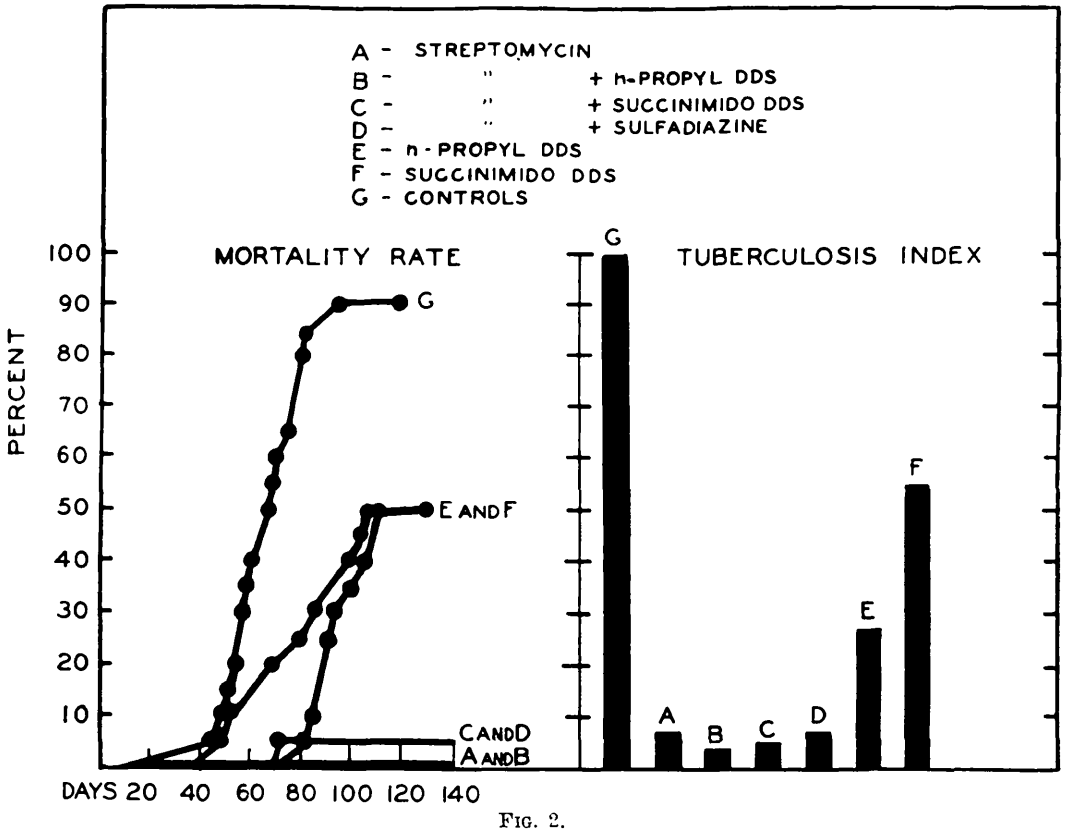

FIG. 2.
Mortality rate and average extent of tuberculous involvement (tuberculosis index) in groups of tuberculous guinea pigs treated as indicated in legend of Fig. 1.

in Group F the average index was 7.9 with a variation of 1 to 15. This evidence appears to indicate that while neither drug eradicated the disease the *n*-propyl derivative had the much greater suppressive effect of the 2.

Group L. The animals in this group in which treatment was begun 23 days after infection all survived the experimental period, with marked improvement in their weight curve after treatment was instituted, and necropsy at the termination of the experiment showed an average tuberculosis index of 2.4 with a variation of 0 to 6. Two of the 7 animals showed no gross evidence of the disease.

Tissues from each of the 7 animals in this group, together with tissues from 5 animals of the control group, were submitted to Dr. R. D. Lillie of the Laboratory of Pathology for microscopic examination. These included liver, spleen, lungs, kidneys, testes, omentum, and diaphragm. His re-

port is summarized as follows:

Of the 5 controls one showed chronic progressive tuberculosis, 2 had subacute progressive tuberculosis, and 2 showed active uninhibited tuberculosis. Two of the treated animals (L 1 and L 5) had no tuberculosis, the latter showing subacute bronchopneumonia. The others presented the following:

L 2. Solitary tubercle of spleen, relatively inactive.

L 3. Old lymphadenoid tuberculosis, relatively inactive.

L 4. Inactive old tuberculosis with calcification and fibrosis in the lungs and lymph nodes. Chronic bronchopneumonia and bronchiectasis.

L 6. Old lymphadenoid and peritesticular tuberculosis with some calcification and fibrosis and chronic interstitial pneumonia.

L 7. Minimal chronic tuberculosis of the lungs, spleen and mesenteric lymphnodes with bronchopneumonia and bronchiectasis.

These findings agree well with the data given in Table I, and recorded independently on the basis of gross observation.

In Table II are summarized the results of the entire experiment. Comparison of the relative values of the different forms of treatment, based on the most important criteria, namely mortality per cent, average tuberculosis index, and incidence of freedom from disease as judged by macroscopic examination, all point to the superiority of combined treatment with streptomycin and the *n*-propyl derivative of diaminodiphenylsulfone. The average gains in weight of the animals in the several groups as shown in Table II and Fig. 1 also point to the same conclusion.

Of the 2 sulfones presented in this study the data in Tables I and II and in Fig. 1 and 2 indicate that the *n*-propyl derivative has the greater suppressing effect on the disease. Both compounds have a low degree of toxicity and are characterized by poor absorbability, as shown in Table III. It is to be noted that the succinimido compound was used in the therapeutic tests at half the dose level of the *n*-propyl derivative, but it is doubtful whether it would have been more effective at the higher dose level, since in normal animals little more of this drug was absorbed when administered in daily doses of 2.0 g per kg than when given in doses of 0.25 g per kg.

Discussion. Viewing the results of the present study against the background of those previously reported it is becoming increasingly evident that streptomycin is by far the most effective chemotherapeutic agent in experimental tuberculosis. Working under fairly well standardized conditions, with a rather heavy infection giving a mortality of from 65 to 95% of the controls in 90 to 100 days, treatment with streptomycin at a dose level of 10 to 15 mg per kg per day gave a chemotherapeutic effectiveness of 5.2, no mortality within the experimental period, and absence of macroscopic tuberculosis in 15% of the animals.[1] When the daily dose of streptomycin was increased to 40 mg per day in the present series under very similar conditions a chemotherapeutic effectiveness of 14.4 was obtained, no mortality, and 40% of the animals appeared to be free from lesions. This latter dose of streptomycin is approximately 1/10 of the LD_{50}.[1] It is evident therefore that the response is roughly proportional to dosage, and it is not impossible that further improvement may be had with increasing dosage. It is to be remembered however, that in all cases treatment was begun the day after infection and continued for 60 to 80 days. The picture is not as bright however, when treatment is delayed, as it was done in the present study, Group L, where treatment with streptomycin was not begun until 23 days after infection in which case 5 of the 7 animals had slight to moderate degrees of tuberculosis and a chemotherapeutic effectiveness for the group of only 6.0 (14.4/2.4) was obtained.

Summarizing the present and previous results on the action of streptomycin and the sulfones used individually or in combination it appears that there is good evidence of potentiation every time combined therapy has been tried. Obviously the better the sulfone the more impressive the effect. For convenience the essential data of the present study and of the 2 previous publications[1,2] have been put together in Table IV. Expressing the value of a given treatment

TABLE IV.

Summary of Essential Data on the Effects of Streptomycin Used Alone or in Combination with a Derivative of Diaminodiphenylsulfone in Experimental Tuberculosis.

Streptomycin mg/kg/day	Sulfone derivative	Chemotherapeutic effectiveness	Mortality %	Free from lesions, %	Mortality, % of controls	Duration of exper., days	Reference
10-15	0	5.2	0	15	65	110	1
0	Promin	2.4	15	5	65	110	1
10-15	"	20.0	0	65	65	110	1
0	Galacturonyl	2.7	70	5	95	103	2
20	"	27.0	5	75	95	103	2
40	0	14.4	0	40	90	140	present
0	*n*-Propyl	3.7	50	0	90	"	"
20	"	28.6	0	72	90	"	"
0	Succinimido	1.8	50	0	90	"	"
20	"	18.2	5	55	90	"	"
20	Sulfadiazine	14.5	5	50	90	"	"
20*	0	6.0	0	28	90	"	"

* Treatment delayed for 3 weeks after infection.

in terms of "chemotherapeutic effectiveness" (the ratio of the average extent of tuberculous involvement in the control and treated groups) it is evident that the efficacy of the combination is greater in every instance than the sum of effects from the individual components. Also the highest percentage incidence of animals free from gross lesions has been realized in the experimental groups treated with the combination, though a very substantial percentage appeared to be free from the disease in the group treated with streptomycin alone at the highest dosage level it has been employed.

It is interesting that sulfadiazine appears to have contributed, if anything, less than any of the sulfones in the combination with streptomycin. The effectiveness of sulfadiazine in the treatment of experimental tuberculosis is also slight compared with the sulfones.[3,6]

Little can be said at this time concerning the possible mechanisms that may be involved in the mutual potentiation of streptomycin and the sulfones. Our knowledge of the mechanism of antibacterial action of either streptomycin or of the sulfones is fragmentary and inadequate. However, evidence has been accumulating to indicate that bacteria can acquire a high degree of resistance against the antibiotics including streptomycin.[7-9] Youmans and Williston[10] have also shown recently that the tubercle bacillus may acquire a resistance to streptomycin, that streptomycin resistant strains are equally virulent in mice, and the animals so infected are refractory to treatment with streptomycin. We had previously shown in this laboratory that prolonged cultivation of

[6] Smith, C. R., and Oechsli, F. W., *Am. Rev. Tub.*, 1945, **52**, 86.

[7] Miller, C. P., and Bohnhoff, M., *J. Am. Med. Assn.*, 1946, **130**, 485.

[8] Graessle, O. E., and Frost, B. M., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 171.

[9] Finland, M., Murray, R., Harris, H. W., Kilham, L., and Meads, M., *J. Am. Med. Assn.*, 1946, **132**, 16.

[10] Youmans, G. P., and Williston, E. H., PROC. SOC. EXP. BIOL. AND MED., 1946, **63**, 131.

the tubercle bacillus in a medium containing low concentrations of promin resulted in attenuation of virulence.[11] It is possible therefore that the beneficial effect of the combined action of the 2 chemotherapeutic agents consists in eliminating or attenuating strains which might acquire a resistance to the antibiotic.

Summary and Conclusions. The chemotherapeutic action of streptomycin used alone or in combination with one of two sulfones or sulfadiazine was studied in experimental tuberculosis in guinea pigs.

Evidence has been obtained to indicate that the therapeutic effect from combined treatment is greater than the sum of effects from the individual components.

The chemotherapeutic effectiveness (ratio of extent of tuberculous involvement in a group of untreated controls and treated groups) in a group of guinea pigs treated with 40 mg (40,000 units) streptomycin per kg of body weight daily was 14.4. Previously treatment with 10 to 15 mg per kg per day under similar conditions gave a chemotherapeutic effectiveness of 5.2.

The chemotherapeutic effectiveness of combined therapy with 20 mg streptomycin per kg per day plus 500 mg 4-amino-4′-propylaminodiphenylsulfone per kg per day was 28.6. The chemotherapeutic effectiveness of the sulfone alone was 3.7.

Similar though less marked potentiation was obtained in combined therapy with streptomycin and 4-amino-4′-succinimidodiphenylsulfone. By itself this latter sulfone was less effective than the *n*-propyl derivative.

Combined therapy with streptomycin and sulfadiazine, a substance of doubtful efficacy in experimental tuberculosis, gave inconclusive evidence of potentiation.

11 Emmart, E. W., and Smith, M. I., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 320.

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Further Studies on the Testing of Sterility of Concentrated Streptomycin Solutions.

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The inactivation of streptomycin with semicarbazide, as an aid in testing the sterility of concentrated solutions of streptomycin, was proposed in an earlier paper.[1] A similar method of sterility testing involving the use of hydroxylamine has been described in tentative minimum specifications for streptomycin issued by the Food and Drug Administration.[2] As is shown in Table I, semicarbazide, as compared to hydroxylamine mol for mol, has the advantage for such sterility test procedures in that the bacteriostatic action of the former is less than that of the latter for most organisms tested.* If instead of thioglycolate broth, which was used in the present tests, tryptone broth is used, the differences in the bacteriostatic action of the two compounds are even more marked in favor of semicarbazide.[1] The same is true of effects on bacteria in aqueous streptomycin solutions in which the antibacterial power of the carbonyl reagent would be exerted in the suggested sterility tests.

However, recent experiences lead to the

1 Rake, G., and Donovick, R., PROC. SOC. EXP. BIOL. AND MED., 1946, **62**, 31.

2 *Tentative Minimum Specifications for Streptomycin,* Food and Drug Admin., June 28, 1946.

* Amongst the organisms tested were a number of strains kindly made available to us by Dr. W. A. Randall of the Food and Drug Administration and said to be less susceptible to hydroxylamine than to semicarbazide.