responded to folic acid administration inasmuch as they were presumably deficient in this factor; whereas animals fed the same ration *ad lib* had sufficient amounts of folic acid available presumably through intestinal synthesis for an optimal effect.

Summary. A significant reduction in total granulocytes was observed in female rats fed a synthetic ration at reduced caloric intake. Such reduction was not observed in animals fed a similar ration *ad lib* or yeast in place of the synthetic B vitamins. The low granulocyte counts were corrected by treatment with folic acid.

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Influence of Streptomycin and Promin* on Proliferation of Tubercle Bacilli in the Tissues of Albino Rat.

M. I. SMITH, WM. T. MCCLOSKY, AND E. W. EMMART.

From the Division of Physiology, National Institute of Health, Bethesda, Md.

It has been shown[1] that streptomycin and promin when administered together, the one intramuscularly and the other orally, to guinea pigs infected with a human strain of tubercle bacilli, exerted a chemotherapeutic action greater than what might have been anticipated from simple summation of effects. No information is as yet available on the mechanisms that might be involved in this synergistic action. The effect might conceivably be a direct one, on the invading microorganism, or it might be an indirect effect on the defense mechanisms of this normally susceptible host.

In the present work experiments were undertaken to study this phenomenon in the albino rat, normally a highly resistant host to tubercle bacillus infection. It was shown in 1926[2] and amply confirmed since then[3-8] that intraperitoneal or intravenous inoculation of a virulent strain of human or bovine tubercle bacilli in the white rat results within a month in progressive proliferation of monocytes, forming aggregates of epithelioid cells, with some multinucleated, and occasionally a few giant cells. These cellular aggregates are found predominantly in the lungs and to a lesser extent in the liver and spleen. Tubercle bacilli multiply at the same time in great numbers in these cells without producing necrosis, caseation, or other serious tissue damage. The whole process appears to present a simple host-parasite symbiosis having little effect on the general health of the animal until much of the normal lung tissue is replaced by the epithelioid masses which ultimately so impair the normal respiratory functions by reducing the available alveolar space that the animal dies of asphyxia.

In an attempt to learn more about the manner of action of streptomycin and promin it was aimed in the present experiments to ascertain the influence of these drugs, when used individually or in combination, on the proliferation of tubercle bacilli in the tissues of the rat, their viability when planted on culture media, and their pathogenicity when inoculated into guinea pigs.

Experimental. Four groups of rats, 12 each, weighing 100 to 150 g, were inoculated intraperitoneally with 5 mg tubercle bacilli, human

* Sodium p-p'diaminodiphenylsulfone N-N'didextrose sulfonate.

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

2 Smith, M. I., and Hendrick, E. G., *J. Lab. Clin. Med.*, 1926, **11**, 712.

3 Steinbach, M., *Am. Rev. Tub.*, 1932, **26**, 52.

4 Long, E. R., and Vorwald, A. J., *Nat. Tub. Ass. Tr.*, 1930, **26**, 205.

5 Vorwald, A. J., *Am. Rev. Tub.*, 1933, **27**, 270.

6 Hehre, E., and Freund, J., *Arch. Path.*, 1939, **27**, 289.

7 Oswald, N. C., *Arch. Path.*, 1940, **29**, 678.

8 Wessels, C. C., *Am. Rev. Tub.*, 1941, **43**, 449, 637.

TABLE I.

Group A. Effect of Streptomycin and Promin on Rat Tuberculosis. Sept. 17 5 mg Tubercle Bacilli, Human Strain A27, Injected Intraperitoneally. Treatment: Streptomycin 50,000 Units per kg Intramuscularly Once a Day and Promin 0.7% in the Diet, Both 35 Days.

				Subinoculation in guinea pigs		
No.	Days	Tissue smears	Lung subcultures	PPD	Organs affected	T.B. index
1	28	—	—	2+	Glands, spleen, liver	7
2	30	—	+	—	None	0
3	32	—	+	—	"	0
				—	"	0
4	35	—	—	—	"	0
				—	"	0
5	37	—	—	—	"	0
				—	"	0
6	38	—	—	+	Glands, spleen	3
				—	None	0
7	42	—	—	—	"	0
				—	"	0
8	44	—	+	—	"	0
				—	"	0
				—	"	0
				+	Glands	1
9	46	—	—	+	"	1
				—	None	0
				+	Glands, spleen	4
10	49	—	—	—	None	0
				—	"	0
11	52	—	—	—	"	0
12	53	—	—		"	0
				+	None	0

TABLE II.

Group B. Effect of Promin on Rat Tuberculosis. Sept. 17 5 mg Tubercle Bacilli Human Strain A27 Injected Intraperitoneally. Promin Fed 0.7% in the Diet Throughout the Experimental Period.

				Subinoculation in guinea pigs		
No.	Days	Tissue smears	Lung subcultures	PPD	Organs affected	T.B. index
1	28	+	+	+	Omentum, spleen, liver	4
2	30	+		+	Liver	1
3	32	+		+	None	0
				+	"	0
4	35	—	+	+	Spleen	1
				+	Omentum, spleen, liver	4
5	37	+		3+	Glands, spleen, lung	6
				2+	" "	4
6	38	+	+	2+	" " lung	7
				—	" "	3
7	42	+	+	2+	Omentum, spleen, liver	7
				+	Glands, lung	2
8	44	—		—	None	0
				+	Omentum, spleen, lung	8
				—	" " "	6
9	46	+		+	Glands, spleen	4
				+	" lung	3
				+	" " omentum	3
10	49	—	+	2+	" liver	3
				+	" spleen	3
				—	" "	2
11	52	+	+	—	"	1
				+	" spleen	3
				+	"	2
12	53	+	+	2+	" liver	3
				—	" spleen	3
				—	"	1

TABLE III.

Group C. Effect of Streptomycin on Rat Tuberculosis. Sept. 17 5 mg Tubercle Bacilli Human Strain A27 Intraperitoneally. Streptomycin Treatment 50,000 Units per kg Intramuscularly Once a Day for 35 Days.

No.	Days	Tissue smears	Lung subcultures	Subinoculation in guinea pigs		
				PPD	Organs affected	T.B. index
1	28	—	—	—	None	0
2	30	—	+			
3	32	—	—	2+	Glands, spleen, lung	5
4	35	—	+	2+	Liver	1
				2+	Omentum, lung	3
5	37	—	—	2+	Glands, spleen, liver, lung	7
				±	Omentum, spleen, lung	3
6	38	—	+	+	Glands, spleen	5
				+	Omentum, glands, spleen, lung	7
7	42	—	+	—	None	0
				—	”	0
8	44	+	+	+	Glands, liver, spleen	5
				+	” ” ”	5
				+	Omentum, liver, spleen, lung	9
				+	Glands, liver, spleen	5
9	46	—	+	3+	” ” ” lung	4
				+	” ” ” ”	8
				+	” ” ” ”	6
				—	” ” ” ”	4
10	49	—	—	+	None	0
				—	”	0
				+	Glands	2
11	52	+	+	2+	” liver lung	5
				+	” ” ”	4
				+	” ”	6
12	53	—	+	+	” liver, spleen	3
				±	” omentum	2
				±	” spleen, lung	4

strain A27. Immediately following inoculation treatment was begun and continued as follows:

Group A. Streptomycin 50,000 units per kg injected intramuscularly once daily and promin fed in the diet at a level of 0.7%. This treatment was continued 35 days.

Group B. Promin fed in the diet at a level of 0.7% to the end of the experiment when the animals were killed.

Group C. Streptomycin 50,000 units per kg as in group A, but no promin. This was continued as in group A for 35 days.

Group D. Untreated controls.

At 28 to 53 days following inoculation the animals were killed by decapitation in sets of 4, one from each group, and under aseptic conditions the left lobes of the lungs were removed, finely minced with iris knives, macerated with 5 cc sterile saline and centrifuged. After removing the coarse particles the centrifugate was resuspended in the saline and 0.1 cc of this was planted on each of several glycerine-egg slants, while 1.0 cc of the same suspension was injected subcutaneously into the right groin to each of several guinea pigs. Smears were made at the same time from the lungs, liver, spleen and kidney for Ziehl-Neelsen stain and microscopic examination.

The slants were examined for growth at regular intervals, and after an incubation period of from 38 to 63 days the colonies if present were counted and an average for each lung suspension obtained. At from 30 to 45 days following the subinoculation of the lung suspensions into the guinea pigs they were tuberculin tested, using 0.01 mg PPD intracutaneously, and 3 days later they were killed with chloroform, autopsied, and the extent of tuberculous involvement (tuberculosis index) rated on the basis of 0 to 4 in the organs of predelection *viz,* glands, omentum, liver, spleen and lungs, as previously described.[9]

Results. These are given in detail in Tables

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

TABLE IV.

Group D. Sept. 17 5 mg Tubercle Bacilli Human Strain A27 Intraperitoneally. No Treatment.

No.	Days	Tissue smears	Lung subcultures	Subinoculation in guinea pigs		
				PPD	Organs affected	T.B. index
1	28	+	—	+	Omentum	1
2	30	—	+			
3	32	+	+	+	Glands, liver	2
4	35	+	+		"	2
5	37	+	+	2+	" liver, spleen	7
					" omentum, spleen, lung	7
6	38	+	+	±	" spleen	5
				+	" "	4
7	42	—	+	+	" liver	3
				2+	" spleen	6
8	44	+	+	+	" omentum, liver, lung	6
				2+	Omentum, liver, spleen	7
				+	" " " lung	10
				2+	" " " "	5
9	46	+	+	2+	Glands, spleen	5
				+	" "	3
				—	" " liver	6
				+	" "	3
10	49	—	+	+	" "	3
				±	"	2
11	52	+	+	2+	Omentum, spleen, liver, lung	6
				2+	" liver, lung	5
				3+	" " spleen	9
12	53	—	+	—	Glands	2
				—	"	1

I to IV. The rats in group A treated with streptomycin and promin, shown in Table I, had no acid fast organisms in the tissues as determined by microscopic examination of the smears. The lung suspensions of only 3 of the animals yielded positive cultures in the form of a few colonies, and these did not appear until 45 to 50 days following planting as shown in Table V. Subinoculation of the lung suspensions in guinea pigs indicated that only 5 of the animals of this group harbored pathogenic bacilli, while 5 of the 12 rats (41.6%) appear to have been free from tubercle bacilli by all the tests used. The average tuberculosis index of all the subinoculated guinea pigs in this group was only 0.7.

The animals of group B treated with promin alone to the termination of the experiment harbored viable and pathogenic bacilli in the tissues since all the culture tubes, except those that were contaminated, showed good growth, and all but one of the subinoculated guinea pigs developed macroscopic tuberculosis with an average tuberculosis index of 3.1. The results in this promin-treated group were not much better than in the control group D, detailed in Table IV, in which all but one of the cultures were positive and all the subinoculated guinea pigs were positive with an average tuberculosis index of 4.6.

Blood level determinations for promin in the animals of groups A and B done at necropsy revealed a mean concentration of 4.4 mg % with a variation of 2.9 to 7.6 mg % in the animals of the A group, and a mean concentration of 4.1 mg % with a variation of 1.8 to 13.0 mg % in the animals of the B group.

The results of treatment with streptomycin alone in group C as shown in Table III, were somewhat better than in the controls or in the animals treated with promin, but not nearly as good as in the animals receiving the combined treatment. The subcultures of the lung suspensions in group C were positive in 8 of the 12 animals, and the subinoculation tests in guinea pigs indicated 9 of the 11 animals to harbor pathogenic organisms. The guinea pigs subinoculated with the lung suspension of one of the rats of this group (No. 2) died too soon after inoculation. The

TABLE V.
Data of Subculture Tests of Lung Suspensions on Glycerine-Egg Slants. Maximum Incubation period 63 days.

Rat No.	Interval from inoculation of slants to appearance of colonies, days				Avg No. of colonies at end of incubation period			
	Group A promin and streptomycin	Group B promin	Group C streptomycin	Group D control	Group A promin and streptomycin	Group B promin	Group C streptomycin	Group D control
1	*	49	*	*	0	4	0	0
2	47	†	47	47	0.5	†	6	3
3	45	†	*	38	0.5	†	0	10
4	*	12	35	35	0	12	0.2	40
5	*	†	*	10	0	†	0	200
6	*	19	32	19	0	200	12	33
7	*	15	35	28	0	187	0.5	200
8	50	†	26	26	0.3	†	150	175
9	*	†	24	24	0	†	150	150
10	*	28	*	21	0	7	0	7
11	*	25	25	25	0	12	17	17
12	*	24	24	17	0	75	13	4

* No visible colonies after 63 days incubation.
† Contaminated.

TABLE VI.
Summary of All Tests.

Group	Positive smears	Positive subcultures	Guinea pig subinoculation tests		
			Positive PPD	Macroscopic tuberculosis	Avg T.B. index
Streptomycin and promin	0/12	3/12	5/12	5/12	0.7
Promin	9/12	7/7*	12/12	11/12	3.1
Streptomycin	2/12	8/12	9/11	9/11	3.8
Controls	8/12	11/12	10/11	11/11	4.6

* Five contaminated.

average tuberculosis index for the whole group of subinoculated guinea pigs in this series was 3.8.

Table V gives the detailed data on the subculture tests of the lung suspensions on glycerine-egg slants. Comparison of the average number of colonies on the slants at the end of the incubation period in the several groups indicates marked reduction on the slants planted with lung suspensions from the combined treatment group A, slight reduction in the case of the streptomycin group C, and little difference as between the promin-treated group B and the controls of group D. The relatively low counts on the slants from the first 3 animals of the series, sacrificed at from 28 to 32 days after incubation, may be accounted for by the relatively shorter infection period, while the low counts in the last 3 animals of the series is probably due to the relatively shorter incubation period between the inoculation of the slants and the counting of the colonies.

A summary tabulation of the results of all the tests employed in each of the four groups is given in Table VI.

Discussion. The data shown in Table VI, summarizing the results of all the tests, indicate that the combined treatment with streptomycin and promin was moderately effective in eradicating the tubercle bacilli from the tissues or in preventing their multiplication. Neither streptomycin nor promin alone had any such effects. The former may have exerted a slight bacteriostatic action as evidenced by the reduced colony counts when compared with the controls. Promin alone had no deterrent effect. These findings harmonize well with the previously observed synergistic action of streptomycin and promin in the chemotherapeutic treatment of experimental tuberculosis in guinea pigs and the

relative ineffectiveness of promin alone.[1] It was previously suggested that the slight retardation of the tuberculous process in guinea pigs by promin is probably due to an attenuating action on the tubercle bacillus.[10] The present experiments appear to indicate a slight bacteriostatic action by streptomycin against the tubercle bacillus *in vivo*, and a tuberculocidal action by the simultaneous application of streptomycin and promin. The mechanism of the combined action of the 2 drugs still remains to be determined.

It may be of interest to point out the relative value of the 3 tests employed to determine the presence of tubercle bacilli in the tissues. By direct smear examinations 19 or 39.7% of the 48 infected rats in the 4 groups gave positive results, by the subculture technic 29 or 60.4% were positive, and by the guinea pig subinoculation tests 36 or 75% were positive.

Summary and Conclusions. Rats inoculated with a human strain of tubercle bacilli were treated with streptomycin and promin, individually and in combination, and the effect of treatment determined by (a) direct tissue smears, (b) subculturing of lung suspensions, (c) subinoculation of lung suspensions in guinea pigs. Treatment with promin alone showed no beneficial effects, treatment with streptomycin alone resulted in an average lower colony count than in the controls, while treatment with both drugs appeared to indicate sterilization of 41.6% of the animals and marked decrease and attenuation of persisting viable tubercle bacilli in the remainder.

[10] Emmart, E. W., and Smith, M. L., Proc. Soc. Exp. Biol. and Med., 1942, **50**, 320.

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Streptomycin and Penicillin Resistant Staphylococci; Influence of pH, Body Fluids on Streptomycin Action.

Emanuel Wolinsky and William Steenken, Jr. (Introduced by L. U. Gardiner).

From the Research and Clinical Laboratory, Trudeau Sanatorium, Trudeau, N. Y.

Interest in streptomycin has grown rapidly since its discovery by Schatz, Bugie, and Waksman.[1] The purpose of this report is to present experimental observations upon the development of a streptomycin- and penicillin-resistant strain of *Staphylococcus aureus*.

Materials and Methods. The *Staphylococcus aureus* used was the Smith strain.* Its viability was maintained by daily transfer in F.D.A.† broth, and it was found to be sensitive to 1.0 to 2.0 units of streptomycin per cc of medium. The same broth of pH 7.5 was employed for all sensitivity and culture studies. Streptomycin hydrochloride‡ of one lot number was used throughout the study. Streptomycin sensitivity tests were done by the serial broth dilution method, and assays by the agar cup plate method of Stebbins and Robinson.[2]

Organisms Naturally Resistant to Streptomycin. In making routine streptomycin assays by the cup plate method, it was noted that a few isolated colonies of the test organism, the Smith *Staphylococcus aureus*, grew within the clear zone of inhibition produced by the drug. These colonies, when picked and subcultured, produced organisms that were about 10 times more resistant to

[1] Schatz, A., Bugie, E., and Waksman, S. A., Proc. Soc. Exp. Biol. and Med., 1944, **55**, 66.

* Received from Merck and Co., Rahway, N. J.

† Formula: Peptone (Siccum, Armour) 10.0; Bacto Beef Extract (Difco) 5.0; NaCl 2.5; Distilled H_2O ad 1000 cc

‡ Streptomycin hydrochloride, lot No. 165, kindly supplied by Merck and Co., through the courtesy of Drs. Carlisle and Robertson.

[2] Stebbins, R. B., and Robinson, H. J., Proc. Soc. Exp. Biol. and Med., 1945, **59**, 255.