jugular vein without the subsequent shocking dose of egg albumin. The latter 5 animals showed no ill effect of the intravenous injection. The results are given in Table I. Autopsy of the fatalities disclosed typical pulmonary edema and emphysema in 17 of the controls, while in 2 animals the emphysema was patchy, and in one animal gross lesions were absent. In the fatalities of the histaminase group, findings typical of anaphylaxis were present in 18 of the animals, while in the remaining one, emphysematous blebs were present.

Discussion. The first experiment involving a small group in which the administration of histamine apparently effected a survival rate of 100%, was very promising and appeared to confirm the findings of Karady and Browne. However, subsequent experiments involving a total number of animals more than 3½ times as great as that used by the above workers failed to show any effect of the histaminase preparations on anaphylactic shock of guinea pigs. The results were negative when the histaminase preparations were injected intravenously or intraäbdominally.

Conclusion. The protective action of histaminase on anaphylactic shock of guinea pigs as reported by Karady and Browne could not be confirmed.

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Bacteriostatic and Bactericidal Substances Produced by a Soil Actinomyces.*

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The ability to antagonize other microörganisms is widely distributed among actinomycetes, a large group of microörganisms, present in soils, composts, water basins, dust, and in other natural substrates.[1] The nature of the antagonistic action exerted by these

* Journal Series Paper, New Jersey Agricultural Experiment Station, Department of Soil Chemistry and Microbiology.

[1] Greig-Smith, R., *Proc. Linn. Soc. N. S. Wales*, 1917, **42**, 162; Lieske, R., *Morphologie und Biologie der Strahlenpilze*, Leipzig, 1921, p. 138; Gratia, A., and Dath, S., *Compt. rend. Soc. Biol.*, 1925, **93**, 451; 1926, **94**, 1267; Welsch, M., *Compt. rend. Soc. Biol.*, 1937, **124**, 575; Millard, W. A., and Taylor, C. B., *Ann. Appl. Biol.*, 1927, **14**, 202; Borodulina, J. A., *Microbiologia*, 1935, **4**, 561; Waksman, S. A., and Foster, J. W., *Soil Sci.*, 1937, **43**, 69; Nakhimovskaia, M. I., *Microbiologia*, 1937, **6**, 131; Krassilnikov, N. A., and Koreniako, A. I., *Ibid.*, 1939, **8**, 673.

organisms is not always the same: either fungi or bacteria were reported to be affected, dead or living cells of bacteria, as well as various types of bacteria. These varying observations point to the fact that one is dealing not with a single type of reaction in all cases, but with different mechanisms, possibly characteristic of the different antagonistic organisms.

By a method previously described,[2] the authors isolated from the soil a species of *Actinomyces* which has marked bacteriostatic and bactericidal effects upon all bacteria, belonging both to the Gram-positive and Gram-negative types. This organism was found to belong to the chromogenic type of actinomycetes, characterized by the production of black pigments on protein- and peptone-containing media. No relation was found between the formation of this pigment and the active substance as will be shown in detail elsewhere,[3] since the substance is also formed on protein-free synthetic media, though in lower concentrations. Certain amino acids, such as phenyl-alanine favor its formation; however, it is produced in media containing sodium nitrate and starch as the only sources of nitrogen and carbon.

The organism was grown on agar media, for 6-8 days, and the active substance extracted with ether; it was redissolved in 95% alcohol, to give a stock solution containing 5 mg per 1 cc; for making the tests, the alcoholic solution was diluted with water. The aqueous solution was turbid; however, when the ether-soluble material was treated with petrol ether, a readily crystallizable preparation was obtained which gave a clear aqueous solution. The active substance could thus be separated into 2 fractions: (A) soluble in ether and in alcohol, but not in petrol ether, and giving a clear solution in water; (B) soluble in ether and in petrol ether, soluble with difficulty in alcohol, and giving a turbid suspension with water. Fraction A was bright red in color and gave a yellow solution even in concentrations of 0.001 mg per 1 cc; it possessed extremely high bacteriostatic properties, but was rather slowly bactericidal. Fraction B was colorless, had comparatively little bacteriostatic action and was markedly bactericidal. It may be added here that the younger cultures of the organism had a higher concentration of the B fraction, as compared with the A fraction; as the culture grew older, the relative yield of the former diminished and of the latter increased. The active substance was tentatively designated as "actinomycin". It is proposed here to designate the above two preparations as "actinomycin A" and "actinomycin B".

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2 Waksman, S. A., and Woodruff, H. B., *J. Bact.*, 1940, **40**, 581.

3 Waksman, S. A., and Woodruff, H. B., *Soil Sci.*, 1940, **50**.

A comparative study of the inhibitory effect of the active substance against a large number of bacteria, using solid or liquid culture methods, brought out the fact that many of the Gram-positive bacteria were largely or completely inhibited by a concentration of 0.1 mg per liter of medium; some were inhibited even by a concentration of 0.01 mg of purified actinomycin A added per liter of nutrient agar or broth; other Gram-positive bacteria required 10 mg per liter for complete inhibition. The 2 species of *Actinomyces* tested were found to be partly or largely inhibited by 10 mg per liter. The Gram-negative bacteria also varied considerably in this respect; some were inhibited even by 100 mg per liter of medium, whereas the growth of others was markedly reduced by 10 mg per liter; the colon-aerogenes and *Serratia* groups were the most resistant (Table I).

In testing the bactericidal properties of the 2 preparations, various

TABLE I.
Inhibitory Effect of Actinomycin upon the Growth of a Number of Bacteria.

Organism	Gram stain	Actinomycin added per liter of medium, mg			
		0.1	1.0	10	100
Serratia marcescens	—	3[1]	3[1]	3[1]	3[1]
Aerobacter aerogenes	—	3	3	3	3[2]
Escherichia coli—intermediate	—	3	3	3	3[2]
Escherichia coli	—	3	3	3	1[2]
Pseudomonas aeruginosa	—	3	3	3	0
Pseudomonas fluorescens	—	3	3	3	0
Brucella abortus	—	3	3	3	0
Neisseria catarrhalis	—	3	3	2	0
Erwinia carotovora	—	3	3	2	0
Shigella gallinarum	—	3	2	2	0
Achromobacter stutzeri	—	3	2	1	0
Hemophilus pertussis	—	3	3	0	0
Azotobacter vinelandii	—	3	0	0	0
Actinomyces cellulosæ	+	3	2	1	0
Actinomyces californicus	+	3	3	2	0
Mycobacterium tuberculosis	+	3	3	0	0
Clostridium welchii	+	3	0	0	0
Bac. macerans	+	3	3	0	0
Bac. megatherium	+	3	0	0	0
Bac. polymyxa	+	3	0	0	0
Bac. mycoides	+	1	0	0	0
Bac. mesentericus	+	1	0	0	0
Bac. cereus	+	1	0	0	0
Bac. subtilis I	+	0	0	0	0
Bac. subtilis II	+	0	0	0	0
Gaffkya tetragena	+	0	0	0	0
Sarcina lutea	+	0	0	0	0
Streptococci and Staphylococci	+	0	0	0	0

10—No growth; 1—Trace; 2—Fair growth; 3—Good growth.

2200 mg per liter usually gave for *A. aerogenes* — 2, *E. coli* int. — 1, and *E. coli* — 0.

methods were employed for different organisms. *Brucella abortus* and *Sarcina lutea* were used as washed suspensions; *Escherichia coli* was grown in broth cultures, for 5 hours at 37°, and the 2 preparations added. *Bacillus subtilis* was grown in broth cultures and 0.2 cc portions added to 100 cc of a phosphate buffer solution and incubated at 28°C.

Actinomycin A exerted, in small amounts, a definite bacteriostatic effect upon the growth of *Bac. subtilis* (Table II), but no bactericidal action; this is clearly brought out by the lack of cell multiplication with 0.01 and 0.1 mg of the substance. In larger concentrations, especially after longer periods of incubation, the A fraction exerted a definite bactericidal effect. On the other hand, actinomycin B had no growth-inhibiting effect in small doses, as shown by the multiplication of the organism with the lower concentrations of the substance; in larger concentrations, it had a marked bactericidal action. The same effects were obtained (Table III) for the 2 preparations acting upon *B. abortus*.

A study of the mechanism of the bactericidal action of actino-

TABLE II.
Bactericidal Action of Actinomycin Fractions upon *Bacillus subtilis*.
Cells added to 100 cc buffer solution—580,000; spores—217,000.

Preparation used	Mg added to 100 cc buffer	No. of viable cells left		
		After 24 hr	After 72 hr	
		Total cells	Total cells	Spores
0	0	2,490,000	15,000,000	61,000
Actinomycin A	0.01	470,000	200,000	32,000
" A	0.10	510,000	150,000	5,000
" A	1.00	180,000	130,000	<1,000
" B	0.01	2,760,000	46,800,000	18,000
" B	0.10	1,040,000	43,600,000	<1,000
" B	1.00	130,000	180,000	17,000

TABLE III.
Bactericidal Action of Actinomycin Fractions upon *Brucella abortus*.
2,530,000,000 per 100 cc of buffer solution.

Preparation used	Mg added to 100 cc buffer	No. of viable cells left	
		After 20 hr total cells	After 72 hr total cells
0	0	4,260,000,000	427,000,000
Actinomycin A	0.1	2,690,000,000	8,000,000
" A	2.0	1,290,000,000	<100,000
" A	5.0	221,000,000	<100,000
" B	0.1	3,990,000,000	210,000,000
" B	2.0	<100,000	<100,000
" B	5.0	<100,000	<100,000

TABLE IV.
Destruction of *E. coli* by Varying Concentrations of Actinomycin.

Actinomycin mg	No. of surviving *E. coli* cells, as determined by plate method, after			
	3 hr	7 hr	24 hr	48 hr
0	215,600,000	231,200,000		238,000,000
0.01	224,000,000	264,200,000	232,800,000	154,400,000
0.10	250,800,000	196,200,000	91,200,000	21,600,000
1.00	126,800,000	44,100,000	0	0

mycin, as measured by the viability of the bacterial cells on culture media and by the methylene blue reduction test brought out the fact that in a 10 cc suspension of *E. coli*, 0.5 mg actinomycin reduced the numbers of living cells from 6,400,000 to 493,000, the M.B. test remaining +; 1 mg of the substance reduced the number of cells to 4,800 with M.B. —; 2 mg brought about complete destruction of the total number of cells.

The destruction of the bacterial cells by actinomycin seems to be due to a chemical interaction, similar to that of other antiseptics. This can be shown by analyzing the results obtained with 0.1 mg actinomycin added to a suspension of *E. coli* cells in a 10 cc buffer solution (Table IV).

$$K = \frac{1}{\text{time}} \log \frac{\text{No. of cells at beginning}}{\text{No. of cells at time—t}}$$

7 hr $K = 1/4 \log \frac{250}{196}$ $K = 0.026$

24 hr $K = 1/21 \log \frac{250}{91}$ $K = 0.021$

48 hr $K = 1/45 \log \frac{250}{22}$ $K = 0.023$

Summary. The bacteriostatic and bactericidal substance produced by a soil *Actinomyces* was shown to consist of 2 compounds, designated as "actinomycin A" and "actinomycin B". The first is highly pigmented (red) and is soluble in ether, ethyl alcohol and water, but not in petrol ether; it gives a clear solution in water. The second is soluble in ether and in petrol ether, soluble with difficulty in alcohol, but not in water. The first (A) is highly bacteriostatic, many Gram-positive bacteria being inhibited by dilutions of 1:100,000,000; the Gram-negative bacteria are inhibited only by higher concentrations, namely, 1:5,000 to 1:100,000; this substance is only weakly bactericidal. The second substance (B) is weakly

bacteriostatic, but more actively bactericidal. Although Gram-negative bacteria are as a rule more resistant against the actinomycin than the Gram-positive forms, there is no sharp line of demarcation between the two groups. Marked differences in sensitivity exist between the bacteria within each group.

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Germicidal Efficiency of Some Medicinal Dyes Compared to a Group of Non-Dye Disinfectants.

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The phenol coefficient test is employed universally for rating disinfectants. The test was designed originally for the evaluation of germicides which were closely related to phenol in structure. However, the test is of little value for determining the efficiency of compounds which are unlike phenol. Also, the test gives no information concerning the degree of inactivation of a germicide in the presence of organic matter, nor its toxicity towards living tissue.

In a series of papers published over a number of years[1] a new method was proposed and employed for the evaluation of germicidal substances intended for clinical application. The germicides were tested for their effect on living embryonic chick heart tissue cultivated *in vitro* as well as for their ability to kill bacteria. All tests were performed at 37°C and in the presence of a standard amount of organic matter. A number known as the toxicity index was determined which was defined as the ratio of the highest dilution of germicide required to kill the tissue in 10 minutes to the highest dilution required to kill the test organism in the same period of time under identical conditions. The smaller the toxicity index the more nearly perfect the chemotherapeutic agent. Theoretically, an index less than one means that the germicide is more toxic to bacteria than to the embryonic tissue while an index greater than one means that the germicide is more toxic to the embryonic tissue than to the bacteria.

Some of the dyes, especially those belonging to the triphenylmethane group, are used clinically and strong claims have been made

[1] Salle, A. J., Shechmeister, I. L., and McOmie, W. A., *J. Bact.*, 1939, **37**, 639.