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Toxicity of Actinomycin.

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An *Actinomyces* possessing strong antagonistic properties to all the bacteria so far tested and to a number of fungi has been isolated from the soil by two of us and described as *Act. antibioticus.*[1] An active substance was obtained in crystalline form from cultures of this organism grown on organic and inorganic media and designated as actinomycin. This substance has strong bacteriostatic and bactericidal properties.

The following observations are presented here to illustrate the extreme toxicity of actinomycin. In most experiments, the purified crystalline actinomycin A was employed. It was dissolved in alcohol to give 5-10 mg per 1 cc, and diluted further with sterile water or saline solution. The acute toxicity of actinomycin was determined in 320 mice, 60 rats, 19 guinea pigs, 32 rabbits, 3 hens and 50 15-day-old chick embryos. Doses ranging from 0.10 to 30 mg per kg body weight were given intravenously, intraperitoneally, subcutaneously and orally. Observations were made frequently during the first 12 hours and thereafter twice daily for varying additional periods. The results obtained in one group of

TABLE I.
Acute Toxicity of Actinomycin in Mice.
10 mice used for each treatment.

Dose, mg/kg	No. dead, after days							
	Intrav.		Intrap.		Subcut.		Oral	
	1	7	1	7	1	7	1	7
0.15	0	0	0	0	0	0	—	—
0.25	0	2	0	2	0	1	—	—
0.50	0	8	0	10	0	10	—	—
1.0	9	10	0	10	0	10	—	—
2.0	10	10	8	10	10	10	0	0
5.0	10	10	10	10	10	10	0	0
10.0	—	—	—	—	—	—	0	3
15.0	—	—	—	—	—	—	0	7
20.0	—	—	—	—	—	—	10	10

[1] Waksman, S. A., and Woodruff, H. B., *J. Bact.*, 1940, **40**, 581; 1941, **42**, in press; PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 609.

experiments on mice are presented in Table I. Similar results were obtained in rats, rabbits and other animals.

It is evident that actinomycin is extremely toxic to experimental animals, being considerably more toxic than gramicidin, tyrocidin, or penicillin. Doses of 1 mg or more per kg were lethal, the toxicity becoming more apparent when observations were extended over a 7-day period. Toxic signs consisting of weakness, languor, anorexia and diarrhea, developed 6-12 hours following actinomycin administration. Gross hematuria occurred frequently in rabbits from 4 to 6 hours after intravenous administration of 1 mg per kg. As intoxication progressed, the nervous system became involved as evidenced by ataxia and tonic convulsions. The immediate cause of death appeared to be respiratory failure, since the heart continued to beat for some time after respiration ceased. Congestion in the lungs, liver, spleen and intestinal viscera was found, the most striking phenomeon being the diminution in the size and weight of the spleen.

The 15-day-old chick embryos tolerated a dose of 1 mg per kg weight. However, this dose was sufficient to prevent their hatching.

Working with strains of *Staph. aureus, Cl. welchii, Strep. hemolyticus* and Pneumococcus Type I, actinomycin was found to have a powerful bacteriostatic effect. Over a 5-day period, with or without the presence of 10% serum, this bacteriostatic action slowly became bactericidal. Actinomycin appears to be somewhat more effective against pneumococci and streptococci than against staphylococci. Anaerobic bacteria are less sensitive than the above aerobic forms.

The antibacterial action of actinomycin *in vivo* was studied in mice using a mouse virulent strain of Pneumococcus Type I and a hemolytic strain of streptococci Lancefield group A. The infected mice were treated immediately after the inoculation with single doses of 0.25, 0.5 and 1.0 μg of actinomycin intraperitoneally, subcutaneously or orally. A second series of experiments consisted of infecting mice intraperitoneally and treating them orally, subcutaneously or intraperitoneally, every 4 hours, until death, with doses of 0.1 and 0.2 μg. Under these conditions actinomycin afforded little or no protection. In a control group of mice 3 μg of gramicidin afforded complete protection. However, some effect was obtained with actinomycin against infection with *Trypanosoma equiperdum.* It was also found that a dose 50 μg was ineffective in protecting guinea pigs and incubated eggs against a virulent strain of *Brucella abortus.*

A dose of 2.5 μg actinomycin per 20 g of animal weight, using mice, rats and chickens, was found to disappear from the circulating blood within a period of 60 minutes. Analysis of the urine indicated that within a period of 6 hours from 10-20% of actinomycin was excreted in the urine of rabbits.

Summary. Actinomycin is found to be a powerful bacteriostatic and bactericidal agent *in vitro*. The presence of serum does not diminish the efficacy of this substance. However, no protection is afforded to mice inoculated with cultures of *Streptococcus hemolyticus* or *Pneumococcus* Type I, or to guinea pigs inoculated with *Brucella abortus*. The lack of *in vivo* activity may be due, among other things, to the rapid disappearance of actinomycin from the blood. Actinomycin is extremely toxic to all animal species, death apparently resulting from respiratory failure. Most deaths do not occur until 15-20 hours after actinomycin inoculation.

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Oxygen Consumption and Growth in Cultures of an Obligate Anaerobe, *Bacteroides vulgatus*.

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Following the observations of Knight and Fildes[1] on *Clostridium tetani*, and of Vennesland and Hanke[2] on *Bacteroides vulgatus*, that these two kinds of anaerobes grow in the presence of controlled and limited tensions of oxgen, provided the E_h is kept below a certain level, it was of interest to determine whether this growth is characterized by oxygen consumption. The latter authors in fact observed that when cultures of *Bacteroides vulgatus* are exposed to pure O_2, this gas is consumed at a slow rate, but here the organisms were dying rapidly and this oxygen consumption could hardly be related to growth of the anaerobe. In this study oxygen consumption is measured during growth.

The apparatus and procedure were essentially those of Vennesland and Hanke.[2] A 4-ounce wide-mouth bottle containing 75 cc

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1 Knight, B. C. J. G., and Fildes, P., *Biochem. J.*, 1930, **24**, 1496.

2 Vennesland, B., and Hanke, M. E., *J. Bact.*, 1940, **39**, 139.