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.

of 1% glucose nutrient broth is fitted with a rubber stopper which supports 2 platinum electrodes, a glass electrode, a salt bridge to a calomel cell, and inlet and outlet tubes for the circulation of a gas mixture. After inoculation with 2 cc of a 36-48 hour brain-broth culture of *Bacteroides vulgatus,* strain *marino,* a gas mixture of purified nitrogen containing about 2% CO_2 is circulated through the medium, and when, after several hours, the oxidation-reduction potential observed with the shiny platinum electrodes has fallen to about +0.100 volt, the oxygen content of the gas is gradually increased so that the potential remains at an E_h of about +0.100 volt, never rising above 0.150. After about 12 hours evidence of growth appears in terms of increasing turbidity, and a gradual fall in pH, of about 0.1 pH per hour.

At this time, by means of a closed-system, gas-circulating pump, a mixture of about 6% O_2, 2% CO_2, and the rest nitrogen is circulated through the culture at such a rate that the E_h remains below 0.150 volt and growth continues. The gas-circulating device consists of a 300-cc tonometer with both inlet and outlet tubes leading from and to the culture vessel, with a pair of glass valves, so that the gas can pass only in one direction. A mercury leveling-bulb attached to the bottom of the tonometer which is alternately raised and lowered about 10 cm once every 3 seconds by a windshield-wiper motor provides the pressure changes necessary for the motion of the gas. At suitable times 50 cc samples of gas are withdrawn through a side-arm stopcock for analysis for O_2 and CO_2 by the method of Van Slyke and Sendroy.[3]

Controls were studied in which the potential was maintained above 0.150 volt throughout, by starting early the circulation of an oxygen-containing gas mixture. These controls, which differed in their preparation from the test vessels only in the time at which the circulation of the oxygen-containing gas was started, showed no evidence of growth, that is, no turbidity and no significant pH change. Other observations were made in which the potential was kept below 0.150 volt until growth was well started, and then elevated above 0.150 volt during the circulation of the analyzed gas mixture.

Results. Table I gives the result of one typical control and test experiment. Eight observations in 4 experiments where growth occurred while circulating gas mixtures containing 3 to 8% O_2, showed an average oxygen consumption of 0.17 cc pure O_2 per hour. The control O_2 consumption was 0.01 cc per hour which is just

[3] Van Slyke, D. D., and Sendroy, J., Jr., *J. Biol. Chem.,* 1932, **95**, 509.

TABLE I.
Oxidation-reduction Potential, pH, and Oxygen Consumption in Cultures of *Bacteroides vulgatus.*

Time, hr	Avg potential, millivolts	pH	Δ pH per hr	% O_2 in gas	Volume of gas in cc	cc pure O_2 consumed by culture: in time interval	cc pure O_2 consumed by culture: per hour
0		6.5					
	210		.01				
10.5		6.4		9.41			
	170		.00				
14.0		6.4		9.45	150	(+.06)	(+.02)
	240		.01				
25		6.3		9.35	100	.10	.01
0		6.5					
	130		0				
4.0		6.5					
	75		.10				
10.0		5.9		6.79			
	75		.13				
11.5		5.7		6.72	225	.16	.10
	80		.25				
13.5		5.2		6.36	150	.54	.27
	130		.04				
23		4.8		5.63	100	.73	.06

With potential above 150 millivolts, there is no growth, no turbidity, no pH change, and no oxygen consumption.

With potential below 150 millivolts, there is extensive growth, marked turbidity (15 mg dry weight of organisms at end), definite pH change, and oxygen consumption.

within the limit of experimental error. In the experiments where growth was allowed to begin but then was diminished by elevating the potential above 0.150 volt, the O_2 consumption was 0.06 cc per hour.

The dry weight of organisms in cultures showing maximum growth averaged 15 mg and the Qo_2 on this basis is 11. This 15 mg represents the final weight at the end of the experiment, and the average weight during the circulation of the gas was undoubtedly less; so the Qo_2 of 11 is a minimal value. This value is close to the Qo_2 of 6-12 calculated by Stickland[4] from observations on methylene blue reduction in a Thunberg tube by *Clostridium sporogenes.*

Conclusion. Although *Bacteroides vulgatus* grows in the absence of oxygen and is killed by air, its growth at low oxidation-reduction potential in the presence of 3 to 8% oxygen is characterized by oxygen consumption.

[4] Stickland, L. H., *Biochem. J.*, 1934, **28**, 1746.