TECHNICAL PAPERS

Streptomycin-sensitive, -dependent, and -resistant Bacteria [1]

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The occurrence of a few apparently "resistant" variants in a large inoculum of streptomycin-"sensitive" organisms has been noted in strains of *Shigella* (*3*), *Hemophilus influenzae* (*1*), and meningococcus (*5*). Similar resistant variants of many organisms have usually been obtained from smaller inocula by repeated subculture in increasing concentrations of the antibiotic (*2, 4, 6, 7*). Miller (*5*) described another type of variant of the meningococcus, which apparently requires streptomycin for growth and which was obtained after one exposure to a large concentration of streptomycin.

In this laboratory, two types of variants have been derived from the same "sensitive" strains of the following organisms: *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Proteus morgani.* One of the variants, hereafter referred to as "resistant," could grow either in high concentrations of streptomycin or in its absence; the other, which will be referred to as "dependent," grew only in the presence of streptomycin. These variants were obtained by exposure of large numbers of organisms to high concentrations of streptomycin. They usually appeared following the initial exposure of the "sensitive" culture to the antibiotic and represented only a very small proportion of the total number of organisms which had been exposed to the drug.

When these 4 "sensitive" organisms and their derived variants were subcultured in broth containing graded concentrations of streptomycin, using an inoculum of approximately 50,000 organisms/ml, it was noted that growth ceased in the case of both the sensitive and the dependent strains of the same organism at about the same concentration of streptomycin. In other words, the critical concentrations of streptomycin—above which the sensitive strain did not grow and below which the dependent variant failed to grow—were approximately equal for these two strains of the same organism. This is shown in Table 1.

In the course of isolating the dependent variants it was noted that single colonies which had appeared following the first few exposures to streptomycin contained both dependent and sensitive organisms. By repeated subculture of the apparently dependent strains and sensitive strains in both streptomycin-free and streptomycin-containing media, it was possible to obtain pure strains that were either inhibited by streptomycin or dependent on it for growth. The resistant variants, on the other hand, have continued to breed true from the time of their initial isolation.

TABLE 1

GROWTH OF SENSITIVE AND DERIVED VARIANTS IN BROTH CONTAINING GRADED CONCENTRATIONS OF STREPTOMYCIN

Organism	Variant	Streptomycin (μg/ml)												
		50,000	10,000	1,000	500	200	100	50	25	12.5	6.2	3.1	1.5	0
Staph. aureus	Sensitive	0	0	0	0	0	0	0	0	++	+++	+++	+++	+++
	Dependent	+++	+++	+++	+++	+++	+++	++	0	0	0	0	0	0
	Resistant	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
E. coli	Sensitive	0	0	0	0	0	0	0	0	0	0	+++	+++	+++
	Dependent	+++	+++	+++	+++	+++	+++	+++	++	+	±	0	0	0
	Resistant	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Ps. aeruginosa	Sensitive	0	0	0	0	0	0	0	±	++	+++	+++	+++	+++
	Dependent	+++	+++	+++	+++	+++	+++	+++	++	+	±	0	0	0
	Resistant	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
P. morgani	Sensitive	0	0	0	0	0	0	++	+++	+++	+++	+++	+++	+++
	Dependent	+++	+++	+++	+++	+++	+++	+++	±	0	0	0	0	0
	Resistant	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++

These observations are in accord with the widely accepted concept of the action of antibacterial agents as metabolite antagonists. Thus, in the case of the sensitive strain, streptomycin may be considered as interfering with some essential metabolite or metabolic process. The dependent strain, on the other hand, utilizes streptomycin as an essential metabolite or growth factor. Furthermore, the same concentrations of streptomycin which interfere with the growth of the sensitive strain are essential for the growth of the dependent strain.

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Growth of the resistant strain, on the other hand, may take place either in the presence or absence of streptomycin. The latter property appears to be a relatively permanent alteration, while in the case of the dependent strain the relationship between streptomycin and the essential metabolite may be a reversible one.

Details of these and related studies will be presented at a later date.

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The Static Electrification of Dust Clouds

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In studying the passage of finely powdered minerals through a metal tube, we have discovered that the dust coating acquired by the inside of the tube has an important role in the electrostatic charging of the dust clouds formed in that process.

The observations leading to this discovery were made using an apparatus and method similar to that of Wilson, Janes, and Campau (*10*). Attaclay, pyrophyllite, bauxite, and mixtures of these were studied.

Charging tests. The tube was made of brass tubing of $\frac{5}{8}$″ internal diameter, 20″ long, fitted with a jet and venturi orifice at the hopper inlet to increase the suction on the hopper. The tube was insulated by Lucite at its supports and connected to the gas line by a polystyrene adaptor. The blowing gas was dry nitrogen, passed into the tube through a rotameter. The exit end of the tube extended 3″ into a large box used to collect the dust clouds and was paraffined on the outside to prevent discharging of the tube by attraction of the dust clouds onto the metal. A Kelvin voltmeter was used to determine the sign and magnitude of the charge placed on the tube.

A small copper cone was set in the top opening of the hopper. Its bottom opening was easily constricted or widened to regulate the passage of the powder into the tube. Because of the differences in flowability of the powders studied, it was found advantageous to fill the cone rather than the hopper. Furthermore, by separate passage of small units of powder a more detailed picture of the coating and charging was obtained.

The materials used in this study were characterized by the following properties:

	Attaclay	*Pyrophyllite*	*Activated bauxite*
Mineralogical content	Attapulgite: magnesium aluminum silicate	Pyrophyllite: aluminum silicate	γ-Alumina containing kaolinite and anatase
Volatile matter (%)	7.9	3.7	7.5
Packed bulk density, lbs/cu ft	29.7	52.4	60.6
Specific gravity	2.35	2.80	3.19
Screen analysis:			
on 200 mesh	3	0	4
200–325 mesh	11	5	12
through 325 mesh	86	95	84

Before each powder or powder mixture was studied, the tube was cleaned thoroughly. The cone opening was adjusted to suit the powder studied; the cone was filled and put in place in the hopper; and the tube was grounded to remove any precharging, especially that due to contact of the powder with its glass container (*4*, *9*). The cone opening adjustment varied considerably with the powders, since Attaclay flows more readily than pyrophyllite or bauxite. A gas flow of between 1.4 and 1.7 cu ft/min was used throughout. Each coneful was passed through the blower in approximately the same time, 4.5–6 min, and each powder met about the same frictional force in its passage. The tube was discharged after each passage to permit filling the cone with the next sample.

Adhesion tests. These were made with the aid of the blowing system just described. A 3″ square of clean, 0.008″ electrolytic sheet copper was hung vertically by a wire attached to a grounded metal support. It was backed by a piece of cardboard, which prevented motion of the plate and protected its back from deposition of dust. The blower tube was pointed at the center of this plate, the end of the tube being held at a distance of 3 or 6″ from the plate. The dust was blown at the same rate as in the charging experiments and, following the passage of a coneful, the plate was removed, tapped to shake off the looser material, and weighed to determine that which adhered firmly. The tapping procedure was easily made sufficiently uniform because of the sharp demarcation between the adhesion of the loose and the firmly-held material, as attested by the reproducibility of the results. The plate was cleaned carefully between determinations.

Early in the studies, the importance of the coating inside the tube was noticed. Until this had formed, the charge placed on the tube was low and not reproducible, but when the lining had formed, a higher, more constant value was shown. The ease of formation of the lining varied noticeably with the nature of the powder. With Attaclay, this coating was apparently fully formed before the second coneful had passed. With pyrophyllite, 3 conefuls sufficed in general, and with bauxite as many as

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