

The unsuccessful attempts to produce bronchogenic lung abscess in dogs<sup>2, 3, 4</sup> have been attributed to the efficiency of the cough reflex in the removal of foreign substances from the lungs. This factor was eliminated by maintenance of localization of the infective organisms with a heavy viscid media. Thus abscess formation was the usual result. It is not unlikely that maintenance of localization of pathogenic organisms occurs more readily in man than in dogs.

### 7363 P

#### Studies on Small Colony Variants of *Staphylococcus Aureus*.

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Small colony variants of bacteria are regarded by some investigators as a filterable stage in a life cycle of the organisms. Other workers do not feel that there is sufficient evidence in support of this theory. Hadley<sup>1</sup> has been one of the chief exponents of the idea that bacterial variation represents a cyclical development of the organisms and in this connection he described a small colony filterable form of the Shiga dysentery bacillus and other bacteria.

In the present investigation small colony variants of *Staphylococcus aureus* were studied with respect to their occurrence, morphology, physiological and antigenic properties and filterability. The variants were obtained by plating ageing broth or agar cultures and from cultures in lithium chloride broth. Their occurrence was irregular and rare and no means were found by which this type of variation could be induced. Tiny colonies were not observed at any time in very young cultures except occasionally in broth containing lithium chloride. They appeared only after the cultures had aged for a week or two and were more numerous after 3 or 4 weeks.

Well-isolated colonies of *Staph. aureus* are usually from 1 to 2 mm. in diameter when grown on nutrient agar. Those of the small colony variants could scarcely be discerned without the aid of a microscope. In the various strains they ranged from .04 mm., the smallest ever observed, to 0.5 mm., but most frequently they were

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<sup>2</sup> Cutler, E. C., and Schlueter, S. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1926, **23**, 266.

<sup>3</sup> Schlueter, S. A., and Weidlein, I. N., *Arch. Surg.*, 1927, **14**, 457.

<sup>4</sup> Allen, D. S., *Arch. Surg.*, 1928, **16**, 179.

<sup>1</sup> Hadley, P., Delves, E., and Klimek, J., *J. Infect. Dis.*, 1931, **48**, 1.

about 0.1 mm. in diameter. The small colony variants had lost the power of pigment production, the growth on agar slants being white and translucent.

Morphologically some of the variants were not strikingly different from the original strains. In other cultures, however, the cells were very irregular in size. Some were in tetrads, others in pairs or single. Within each cluster of cells there were marked contrasts in size, some being much larger and others smaller than those of normal cultures.

Reversion to the normal\* form occurred occasionally on agar. Sometimes in transferring from a stock culture of a variant strain, one or two large colonies with deep golden pigment appeared on the new slant and stood out sharply against the background of very small colonies which were scarcely visible. If the cultures were inoculated into nutrient broth, reversion to the normal took place more completely. Three strains never gave rise to normal colonies although many attempts were made to induce such a reversion.

These variants were very susceptible to changes in the composition of the media upon which they were grown. Growth could be stimulated in some strains by the use of veal infusion and dextrose and in others by the presence of blood in the medium.

The small colony forms were characterized by a change of physiological properties such as loss of fermentative powers. They all attacked dextrose, producing a definitely acid reaction in 48 hours. Lactose was not fermented and actually seemed to be inhibitory to their growth. The reactions in sucrose were similar to those in lactose. The original staphylococcus strains all attacked dextrose, lactose and sucrose, and readily produced a definite acid reaction in 24 hours.

One of the most striking characteristics of the small colony strains was their relatively slow rate of reproduction. This was shown by a comparison of the growth curves of a small colony variant and its parent strain in nutrient broth. At the end of 12 hours the normal bacteria had increased in numbers 40,000 times while the variants had multiplied to only 16 times the original number.

The variant organisms were avirulent for rabbits in the doses used although the normal strains from which they were derived showed a low degree of virulence.

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\* The word "normal" is used in this paper as the most convenient term to designate the type of colony of *Staph. aureus* usually observed, which is round and smooth, having a diameter of 1 to 2 mm. on nutrient agar. It is recognized that this may not necessarily be the normal form.

Agglutination tests showed most of the variants to be antigenically similar to the normal strains from which they had been obtained.

All attempts to demonstrate a filterable stage in association either with the normal or the variant strains were unsuccessful. The organisms were grown in various media including Kendall's K medium and filtrations were carried out both with very young and old cultures. Growth could not be detected in any of the media, either liquid or solid, inoculated with filtrates from variant or from normal cultures.

It is suggested that these small colony forms occur as the result of injury to an occasional cell of the normal culture which results in a lowered rate of reproduction and the loss of several physiological properties.

### 7364 C

#### Ordinary and Mucoid Encapsulated Hemolytic Streptococci from Scarlet Fever.

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A series of strains of hemolytic streptococci and mucoid encapsulated streptococci was obtained from the throats of scarlet fever patients at Cook County Contagious Hospital.

Laboratory studies were made on a group of these strains to determine. (1) the results of their growth on chocolate agar, (2) their effect on the sugars sorbitol and trehalose and (3) the influence of mouse passage on capsule formation and colony characteristics.

Using chocolate agar Tunnicliff<sup>1</sup> found that hemolytic streptococci of scarlet fever produced no color change in this medium except occasionally a slight greening after several days' growth. The colonies almost always were conical in shape and slightly granular. Occasionally, however, a strain dissociated and produced atypical colonies that were large, moist, smooth and convex and turned chocolate agar green. These atypical colonies were not scarlatinal by the opsonic, agglutination or toxin neutralization tests.<sup>2, 3</sup>

<sup>1</sup> Tunnicliff, R., *J. Am. Med. Assn.*, 1930, **94**, 1213.

<sup>2</sup> Tunnicliff, R., *J. Inf. Dis.*, 1931, **48**, 511.

<sup>3</sup> Tunnicliff, R., *J. Inf. Dis.*, 1931, **49**, 357.