TWO SMALL COLONY VARIANTS OF *STAPH. AUREUS* ISOLATED IN PURE CULTURE FROM CLOSED INFECTED LESIONS AND THEIR CARBON DIOXIDE REQUIREMENTS

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An occasional small colony variant or "G" form of *Staph. aureus* may often be seen among the normal colonies on first isolating the organism from infected lesions. A pure growth of small colony variants in primary culture is, however, a rarity, and this note concerns two such strains isolated within a week, both of which closely resembled a strain investigated by Hale (1951).

Case Histories

Case 21269/52.—H.N., a machinist, suffered a penetrating wound of the right hand five days before attending the casualty department of the Radcliffe Infirmary. It had become painful and was treated with hot poultices and one intramuscular injection of 500,000 units of penicillin. There was no history of other recent sepsis.

On examination there was an area of cellulitis surrounding the small scar of a puncture wound and the axillary lymph nodes were tender. Radiology revealed no foreign bodies. Twice-daily treatment with 500,000 units of penicillin intramuscularly was instituted and the hand immobilized.

On the following day the lesion was found to be pointing and was incised. Pus and necrotic tissue were removed and a swab of pus sent to the laboratory. Some oedema and discharge continued for three days, but the condition of the hand steadily improved and healing was complete 12 days after operation.

Case 21362/52.—P.H., a student, attended the casualty department suffering from a tender swelling in front of the left ear which was beginning to point. It had been present for 36 hours and was diagnosed as an infected sebaceous cyst. The cervical lymph nodes were enlarged and tender. Penicillin treatment of 500,000 units intramuscularly twice daily was begun and magnesium sulphate dressings applied. On the following day the lesion was incised and a deep pouch of pus was found, but there was no evidence of a cyst wall. A swab of pus was sent to the laboratory. Healing was almost complete five days after incision.

This patient had never before been treated with antibiotics.

Laboratory Findings

The findings on primary culture of the swabs from both these patients were identical and are described together.

Two horse blood agar plates were inoculated from each swab, one of which was incubated aerobically and the other anaerobically. A direct film of pus showed, in each case, pus cells and Gram-positive cocci.

After approximately 16 hours' incubation no growth was apparent on the aerobic plates, but numerous small colonies were visible on the anaerobic plates, which, on staining, showed organisms morphologically typical of staphylococci. After 40 hours' incubation a profuse growth of minute colonies was visible on the aerobic plates and the colonies on the anaerobic plate had enlarged and become pigmented to resemble those of a normal *Staph. aureus* grown under similar conditions. Both strains were coagulase positive and as sensitive to penicillin as the Oxford *H. staphylococcus* when tested by the ditch plate method.

Subcultures of the small colonies from the aerobic plates were made on horse blood agar and the plates were incubated aerobically, anaerobically, and in 10% CO₂. One anaerobic culture was made in the presence of soda lime to absorb any CO₂ resulting from the organism's metabolism. These cultures were incubated for 18 hours at 37° C., and the average diameter in millimetres of the isolated colonies is shown in Table I. The colonies which developed in an atmosphere of CO₂ were typical, normal-sized staphylococcal colonies and were both haemolytic and pigmented.

To exclude the possibility that the stimulation of growth was due to the increased humidity in sealed containers, cultures were incubated in an atmosphere of 10% CO₂ in a desiccator. Normal large staphylococcal colonies developed from both strains under these conditions.

TABLE I

COLONY SIZE (MM.) OF THE STAPHYLOCOCCAL VARIANTS AND OF A NORMAL STAPHYLOCOCCUS GROWN UNDER DIFFERENT ATMOSPHERIC CONDITIONS

Condition of Incubation	Strain		
	H.N.	P.H.	Control
Aerobic	<0.1	<0.1	1.5
10% CO ₂	1.0	1.2	1.5
Anaerobic	0.5	0.3	0.5
Anaerobic + soda lime ..	<0.1	<0.1	0.6

The small colony variants were subcultured on to nutrient agar plates and on to blood agar plates at pH 6.5, 7.0, and 7.5, and incubated for 18 hours at 37° C. Minute colonies only developed under these conditions, indicating that the inhibition of the staphylococci was not due to the presence of blood nor was their stimulation due to alteration of the surface pH of the medium in the presence of 10% CO₂.

Aerobic subcultures of the normal-sized colonies which had developed in CO₂ resulted in a reversion to the small colony type.

On continued incubation of the primary cultures from the original swabs, and also of subcultures of the small colonies, an occasional larger colony developed which bred true on subculture.

The two strains were reported by Dr. R. E. O. Williams to be of different phage-types. Strain H.N. was of phage-type 3C+ and strain P.H. was of type 52A+.

Discussion

These two small colony variants of *Staph. aureus* appear to be similar to the strain recorded by Hale (1951) in all the properties for which they were tested.

In metabolic studies he showed that the small variant brought about the dismutation of pyruvic

acid and the decarboxylation of oxaloacetate less readily than a normal staphylococcus, but that this function was restored after growth in 10% CO₂.

The origin of these strains is of interest. It is possible in both cases reported here that the penicillin injections given before operation may either have selectively inhibited the large colony staphylococci and allowed the development of the small variant, or, alternatively, have altered the metabolism of the parent strain. As the small colony variant was fully sensitive to penicillin, the former alternative seems less probable. In the case reported by Hale (1951) the small colony variant derived from an abscess was also present in the patient's nose, and it is possible that in a small proportion of the population such variants may colonize the nose under natural conditions.

In both the cases recorded here no growth was apparent on the primary aerobic plates after overnight incubation, and it is important to bear in mind the existence of these strains, as their presence might readily be overlooked.

Summary

Two small colony variants of *Staph. aureus* were isolated in pure culture from closed septic lesions.

Subcultures in the presence of 10% CO₂ gave rise to normal-sized staphylococcal colonies.

On primary aerobic culture no growth was visible after overnight incubation, and attention is drawn to the risk of overlooking this variant in routine cultures.

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REFERENCE

Hale, J. H. (1951). *Brit. J. exp. Path.*, **32**, 307.