RECURRENT INFECTIONS BY A STABLE DWARF-COLONY VARIANT OF *STAPHYLOCOCCUS AUREUS*

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Small-colony variants of *Staphylococcus aureus* are sometimes seen among the normal colonies obtained in primary cultures from pus. On sub-culture these small colonies usually yield growths of typical large colonies. Many workers, however, have obtained stable small-colony variants *in vitro* by selecting them from cultures grown in media containing inhibitory salts, antiseptics, and antibiotics, as summarized by Chain, Florey, and Jennings (1949; see also Browning and Adamson, 1950). Wise and Spink (1954) also review the literature on G (dwarf-colony) variants and have themselves produced them *in vitro* and isolated them from eight patients, seven of whom were being treated with antibiotics or sulphonamide. Hale (1951) was the first to report a pure growth of a dwarf-colony variant of staphylococcus in primary culture from an abscess; it gave the normal large-colony type of growth in the presence of 10% CO₂. Two similar cases were reported by Sherris (1952). A similar case, but with recurrent infections, is reported in this paper.

Clinical Description

A male second-year medical student aged 21 years had a history of nine pulp infections of the fingers during January to April, 1954. Intramuscular crystalline penicillin was used without effect to treat the first, the lesion progressing to the formation of an abscess, which healed rapidly, however, after surgical incision, drainage, and treatment with oral chloramphenicol. Later lesions also resolved satisfactorily with chloramphenicol (January–April, total probably 30–40 g.), including the ninth, which required surgical drainage early in April. At this time a tenth lesion continued to progress although the patient was still taking chloramphenicol. Eight days after it first appeared this lesion was incised by a practitioner, and a day later the patient came for the first time to the hospital out-patient department with an open sore on the medial side of the middle phalanx of the left index finger. Thick pus was present in the base of the lesion and the whole finger was painful, stiff, and swollen, with an inflammatory swelling spreading on

to the dorsum of the hand. When the patient arrived at hospital the pus was taken for the first time for bacteriological investigation. Treatment was continued with splinting, chloramphenicol, and later by a course of aureomycin (total 8 g. in four days), but it was not until June, eight weeks later, that the lesion had resolved and a crust had formed. On the three occasions on which swabs were taken from this lesion for bacteriological examination, twice in April and once in May, pure cultures of the dwarf-colony variant *Staphylococcus aureus* described below were obtained.

The patient was then free from septic lesions until the last day in August, when another whitlow developed while he was on holiday, working at forestry. He had a few septic spots on his legs, buttocks, and neck in September, but these healed rapidly when treated with chloramphenicol and penicillin for a few days. In mid-October, when he returned to Glasgow for classes, he reported once more to the Western Infirmary; the whitlow, now six weeks old, still had pus in the base of the wound, and a swab from it gave a pure heavy culture of dwarf-colony staphylococci. At this time he had not had any antibacterial drugs for more than a month; a boil which was rapidly developing in his neck ruptured a few days later, and the swab from it also yielded on culture a pure growth of the dwarf-colony staphylococcus. The whitlow, like the previous one, required eight weeks to heal.

Bacteriological Findings

The organism to be described was obtained in pure culture on seven separate examinations: three times from swabs of pus from the first lesion examined, twice from the last whitlow, and twice from the boil in the neck.

Morphology and Staining.—Smears from pus and from cultures showed typical clusters of Gram-positive spherical staphylococci 0.7–0.9 μ in diameter.

Cultural Characters and Physiology.—After 18 hours' growth at 37° C. on 8% horse-blood-agar or nutrient-agar plates, the colonies were almost invisible to the naked eye, but by 24 hours were

just visible as smooth, shiny, colourless, translucent discs, measuring 0.01 to 0.1 mm. in diameter (Figs. 1 and 2). When these dwarf colonies were heaped up on the medium the aggregate was opaque and creamy, and after exposure to daylight at room temperature for a few hours it developed the golden yellow of a typical *Staph. aureus*. After 96 hours' incubation in air at 37° C., some discrete colonies had grown up to 1 mm. in diameter, as did a few in the more heavily grown parts of the plates. During 40 serial subcultures made at one- or two-day intervals with loopfuls of organisms from the butt the colonial appearances did not alter. The larger colonies which were subcultured also produced only the minute dwarf colonies after 24 hours' incubation.

At room temperature, on horse-blood-agar or nutrient-agar plates, growth became visible only after 72 hours. In anaerobic conditions (McIntosh and Fildes jar) at 37° C., colonies on plates were just visible after 24 hours. When subcultures on blood agar were incubated for 18 hours at 37° C. in an atmosphere of air with 10% CO₂, only large

FIG. 2.—Dwarf staphylococcal colonies on a blood-agar plate; 18-hour culture at 37° C. in air. $\times 8$ (approx.).

FIG. 1.—Normal staphylococcal colonies on a blood-agar plate; 18-hour culture at 37° C. in air for comparison with Figs. 2 and 3. $\times 8$ (approx.).

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colonies, 1.5 to 2 mm. in diameter, typical of *Staph. aureus* were obtained. Under these conditions the colonies were surrounded by zones of complete haemolysis on 8% rabbit blood agar and on horse blood agar. When these large colonies grown in CO₂ were subcultured in air, pure cultures of dwarf colonies were once again grown. On plates streaked both with normal and dwarf staphylococci, satellitism of the dwarf-colony variant around the normal staphylococci was observed (Fig. 3). When grown on slopes in bottles with the caps tightly screwed down, growth became heavy like that of a normal *Staph. aureus*—no doubt because the CO₂ concentration rose from the organism's own metabolic processes (Hale, 1951; Sherris, 1952). In meat-extract broth a uniform turbidity was produced after 18 hours at 37° C. Only slight growth was obtained in peptone water. Subcultures on nutrient agar of five-day-old fluid cultures gave pure growths of minute colonies.

In 24 hours at 37° C., the organism fermented glucose, lactose, sucrose, and maltose, with the production of acid but not of gas; mannitol was

FIG. 3.—Dwarf-colony variant of *Staph. aureus* on a blood-agar plate yields larger colonies near an inoculum (white band near top of figure) of normal staphylococci; 18-hour culture at 37° C. in air. $\times 8$ (approx.).

attacked in the same way only after 72 hours' incubation. The organism did not ferment xylose, glycerol, dulcitol, or salicin; it acidified litmus milk after 72 hours. It produced saccate liquefaction in a gelatin stab culture after two weeks at room temperature, but did not liquefy coagulated serum. The organism produced catalase and urease. Methyl-red and Voges-Proskauer reactions, citrate utilization, nitrate reduction, and indole production were negative. The organism was repeatedly tested and always found to produce coagulase. The organism (grown in CO_2) did not react with known phages.

Sensitivity to Antibiotics.—With sensitivity test tablets ("sentests," Evans) on heavily inoculated plates grown in air, the organism was completely resistant to penicillin (0.5 I.U.), and almost completely resistant to chloramphenicol (40 $\mu\text{g.}$), but sensitive to streptomycin (20 $\mu\text{g.}$) and aureomycin (10 $\mu\text{g.}$).

Further Mutation in Old Cultures.—When plate and slope cultures were left in a cupboard at room temperature for eight weeks and were then subcultured, only small colonies appeared, but these colonies all remained small even after prolonged incubation in air, or in air with 10% CO_2 ; they were no longer *aureus* when heaped up; but they were still strongly coagulase positive.

Isolation from Patient's Nose.—The stable dwarf staphylococcus was also grown from swabs taken on various occasions from the patient's nose, no normal staphylococcal colonies being found at any time. During the mucopurulent stage of a common cold the staphylococci were isolated only from cultures incubated in 10% CO_2 ; at this time on plates incubated in air staphylococcal colonies could not be recognized in the heavy mixed flora of *Strep. viridans* and Gram-negative diplococci. The staphylococcal colonies isolated in an atmosphere of CO_2 gave rise only to minute colonies when subcultured in air.

Discussion

The dwarf-colony variant *Staphylococcus aureus* described was grown pure in primary cultures of pus from two whitlows and a boil. It is of interest that the whitlows from which it was isolated persisted for eight weeks in spite of adequate drainage and, in the first lesion, the use of antibiotics. In contrast, the patient's other lesions all healed rapidly with antibiotic and other appropriate treatment. This was true also of the ninth lesion, which developed along with that which first yielded the dwarf staphylococci and also required incision. Only one lesion remained when the patient first attended hospital, and it is unfortunate that this alone of the 10 lesions which developed between January and April was available to us for bacteriological examination.

Hale does not mention previous antibiotic treatment in his case; Sherris's two cases were both treated by intramuscular penicillin for 24 hours before the swabs were taken; in both only dwarf colonies grew and both were sensitive to penicillin, the lesions healing in five and 12 days. But in view of other workers' findings that stable dwarf-colony variants can be produced in the presence of antibiotics, previous therapy may have been responsible for the production of our dwarf strain; if so it is possible that this type of infection may be more common than is realized. Unless the bacteriologist is aware of the possibility that dwarf variants may grow in pure culture—without a single typical staphylococcal colony—he may easily fail to recognize the organism as a pathogenic *Staphylococcus aureus*. This is particularly

worth remembering in examining such specimens as urine, or throat-swabs and sputa, where the presence of the minute colonies may be missed altogether, particularly in a mixed flora. Stokes (1955) recommends great extension of the use of cultures in CO₂ in routine bacteriology.

Our strain of dwarf-colony variant seems to have a metabolic defect similar to that of the strains described by Hale and by Sherris. As originally isolated, our strain required CO₂ to develop normally and produced minute colonies when grown only in air. Hale showed that his strain of dwarf staphylococcus when grown in air was deficient in the dismutation of pyruvate, and postulated that this was due to failure to develop an enzyme produced in an atmosphere containing CO₂. The satellitism which we observed is perhaps due to diffusion of the enzyme postulated by Hale from the typical actively growing staphylococcal colonies, or merely to diffusion of CO₂ in the medium. Experiments showed that the small size of the colonies was not due to lack of any of the known vitamins. Even the smallest dwarf colonies on the plate in Fig. 3, which was inoculated with a normal as well as with the dwarf staphylococcus, were very much larger than those on a similar plate without normal staphylococci (Fig. 2), presumably due to the accumulation of CO₂ in the atmosphere and agar from the heavy growth. The larger satellite colonies did not grow as large as normal colonies even on prolonged incubation.

In recent articles, Spink (1954) and Wise and Spink (1954) state that in the absence of antibiotic small staphylococcal colonies revert to large colonies, but this was not so in our case. Pure dwarf colonies were isolated from various lesions and also from the nose of our patient between early April and late October, showing that a nasal carrier may retain even an abnormal staphylococcus for a substantial period (almost seven months). Moreover, unlike the occasional small colonies that grow among large colonies on plates of normal staphylococci, these dwarf colonies were stable *in vitro* for at least 40 subcultures on solid media. Wise and Spink state that G variants lose virulence and remain viable in animal tissues without producing signs of infection. From the descriptions in previous papers and the first "dwarf-staphylococcal lesion" in our case, the dwarf staphylococci may have been surviving mutants from normal staphylococcal populations, but there is very strong evidence that the dwarf-colony staphylococcus was the primary pathogen in the last two lesions in our case, certainly in the boil on the neck.

Summary

In a patient who had recently had multiple whitlows treated with antibiotics, pure growths of a dwarf-colony variant of *Staphylococcus aureus* were obtained repeatedly in primary cultures of pus from two whitlows and a boil, and also from the patient's nose, between early April and late October. This is confirmation that a carrier keeps the same staphylococcus for many months, even if it is an "abnormal" staphylococcus. There is strong evidence that these dwarf-staphylococci were the primary pathogens at least in the last two lesions; the chronicity of the two whitlows (each required eight weeks to crust over) is striking.

Unlike the occasional small colonies seen in plates of normal staphylococci, these dwarf-colony staphylococci were stable both *in vitro* for 40 subcultures, and *in vivo* in the patient for almost seven months. The metabolic defect was overcome while the organism was grown in an atmosphere of air with 10% CO₂. Primary cultures in 10% CO₂ made it possible to isolate the dwarf staphylococcus from mixed cultures from the nose during the mucopurulent stage of a common cold. After cultures had been left at room temperature for eight weeks, the organism had further changed in that, although it still grew only as dwarf colonies and produced coagulase, the growth was no longer *aureus* when heaped up, nor would the colonies become typically staphylococcal when incubated in an atmosphere of air with 10% CO₂.

Bacteriologists engaged in routine work will realize that dwarf staphylococci in pure culture may readily be overlooked. This may be of some importance in view of the evidence cited that pure growths of dwarf-colony staphylococci can be produced *in vitro* by cultivation of normal staphylococci in antiseptics or antibiotics.

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