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Isolation and Cultivation of Bacterium melaninogenicum.

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We previously pointed out[1] the constant occurrence of *Bacterium melaninogenicum*[2] in the normal mouth and on the external genitalia, and its abundance in connection with various pathologic processes.

Since then, our experience and that of others has amply confirmed the very frequent association of this bacterium with infections of various types, and while there is no satisfactory evidence that the organism is by itself invasive, it is one of the most prominent concomitant organisms in pyorrhea, lung abscess, sub-acute puerperal fever, and similar mixed infections.

Workers have not yet succeeded in maintaining the organism in pure culture, and therefore its systematic description and identification have not been attempted. The organism will develop readily for a time in symbiosis with other organisms (such as *Strep. viridans*), but its ultimate isolation from such mixtures is no easier than from the primary cultures, and it is soon lost.

Recognizing that the formation of pigment by an organism is associated with senescence, Dr. Bronfenbrenner suggested that the almost constant failure to secure pure growth in transfers from pigmented colonies is probably due to loss of viability of the organisms. It was found that this assumption is correct, and that pure cultures can be secured and maintained if transfers are made very frequently from colonies before they develop much pigment. The original material is streaked directly upon plates of freshly made hormone blood agar (pH 7.4) and the plates are incubated for about 4 days in an anaerobic jar. Single colonies which are just beginning to show a brownish color are then fished to warmed plates of the same medium. Thereafter the pure growths must be transplanted at short intervals (48-72 hours) until they become adjusted to artificial conditions. Cultures usually are not viable for longer than 6 or 7 days, except after several months of cultivation. The following are among the principal properties of the several strains which have been studied.

The organism is a Gram negative, non-spore-bearing, non-motile,

[1] Burdon, K. L., *J. Infect. Dis.*, 1928, **42**, 161.

[2] Oliver, W. W., and Wherry, W. B., *J. Infect. Dis.*, 1921, **28**, 341.

anaerobic bacillus, showing considerable variation in morphology from a very small cocco-bacillus to longer rods, and occasional thread forms. The rods are frequently vacuolated and show bipolar staining. There is a marked tendency for the organism to undergo spontaneous autolysis after a few days growth, particularly in liquid media. On blood agar the organisms produce hemolysis which varies in intensity with the amount of the pigment, and is slight when cultures are transplanted frequently on this medium. The organism does not ferment dextrose, nor any other of the 8 common carbohydrates tested. It is strongly proteolytic, causing digestion of coagulated serum, egg, and milk, and to a less extent the proteolysis of meat and brain media, with the slow formation of gas. Gelatin is liquefied. A large amount of indol is produced.

The characteristic pigment is formed only in media containing hemoglobin. The pigment appears late, after active multiplication has ceased. It always develops first in the center of single colonies, in the immediate vicinity of a contaminating colony (where growth is accelerated) and, in pure cultures, in the areas of heaviest growth. The pigment can be extracted from the bacteria in acidified methyl alcohol, and precipitated by neutralization. The amorphous brownish-black powder thus secured is soluble in dilute alkali, but insoluble in water, ether, chloroform, neutral methyl or ethyl alcohol, xylol, dilute acids, carbon disulphide, or carbon tetrachloride.

The organism is not virulent when tested by intraperitoneal and intravenous injections of actively growing pure cultures into guinea pigs and rabbits. One strain, however, has produced an extensive cutaneous gangrene in these animals when inoculated subcutaneously, with death of the animals in about 48 hours. There were no evidences of septicemia.