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Peroxide Production by Type 3 Strains of Group A *Beta* Hemolytic Streptococci.

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Penfold[1] reported that peroxide-producing bacteria form black colonies on blood agar containing benzidine. He concluded that all pneumococci, and probably all streptococci, exhibit this reaction, but observed that some streptococcal strains gave both black and white colonies. Tunnicliff,[2] using Penfold's method, noted that streptococci from scarlet fever, giving specific opsonic tests, produced only white colonies while their dissociants, as well as cultures from erysipelas, yielded black colonies. Recently, Fuller and Maxted[3] stated that 10 Type 3 strains of *beta* hemolytic streptococci of Group A failed to reveal peroxide whereas 63 of 65 strains of other types did form it. Since 2 of their peroxide-negative Type 3 strains ("Richards" and "Robb") had been shown, in many earlier studies on sulfanilamide, highly susceptible to this drug, Fuller and Maxted concluded that the theory of Mellon and coworkers[4, 5] regarding the part played by peroxide in bacteriostasis caused by sulfanilamide was invalidated. The present report deals with the development of peroxide-forming variants from apparently peroxide-negative strains of Type 3 streptococci growing on benzidine blood agar.

Methods: Benzidine solution was prepared by grinding 0.5 g of Pfanstiehl C.P. benzidine base with 0.7 cc concentrated HCl and adding gradually 99.3 cc distilled water. This 0.5% solution was sterilized by filtration. At the time of pouring plates 0.5 cc of this solution, together with 1.0 cc of defibrinated rabbit blood, was added to 20 cc of beef-muscle or veal-heart infusion agar, pH 7.6, containing 1% Bacto-peptone, 0.5% salt and 1% sodium glycerophosphate. If the culture fails to grow on this concentration of benzidine the amount may be reduced to 0.1 cc. After a few passages on the lower concentration the amount may be increased to 0.25 cc, which is sufficient to give a positive reaction. Broth cultures of strains to be

1 Penfold, W. J., *Med. J. Australia*, 1922, **9**, 120.

2 Tunnicliff, R., *J. Inf. Dis.*, 1935, **57**, 147.

3 Fuller, A. T., and Maxted, W. R., *Brit. J. Exp. Path.*, 1939, **20**, 177.

4 Main, E. R., Shinn, L. E., and Mellon, R. R., PROC. SOC. EXP. BIOL. AND MED., 1938, **39**, 272.

5 Locke, A. P., Main, E. R., and Mellon, R. R., *J. Immunol.*, 1939, **36**, 183.

tested are streaked on the benzidine medium which is incubated at 37°C. Observations are made daily and plates at first showing only white colonies are incubated for 2 to 4 days and then left at room temperature.

Strains Tested: These included 6 Type 3 strains from Dr. Lancefield and designated by her: D58/36/3 (Richards); P279/7/1; D 121; S84/46/0; F 85 and H 89. Two other strains from Dr. Griffith were designated: US41 and Lewis. When received, all cultures were in mucoid phase except H89, which was smooth, and D58 and P279, which were predominantly mucoid but contained a few smooth-phase organisms.

Results: Streptococcal cultures that readily produce peroxide manifest black colonies within 24 to 48 hours at 37°C. Of the strains mentioned above all gave white colonies when observed after 48 hours. After the plates had stood at room temperature for 3 to 10 days, however, some of the white colonies had developed black papillae (secondary colonies). At first these were present in only a few of the white colonies but, as the plates aged, additional white colonies were observed to contain one or more of them. If growth was sufficiently prolonged they might overgrow the white portions of the original colony. When the entire colony was scraped from the agar the underlying medium was blackened only beneath the papillae.

When black papillae were subcultured to fresh benzidine blood agar pure black and pure white colonies developed, the former showing color in 2 to 4 days. Subculture from the papillae of some strains gave black-ringed colonies or colonies showing black arcs at the periphery. Continued selection of black colonies yielded strains that produced 100% black colonies after 24 hours' growth. While some strains required only 2 or 3 selections, others gave a pure black culture only after 6 or 7 selections. Pure black lines have remained stable for at least 2 months. The virulence for mice is less than that of the parent white strains.

Of 8 Type 3 strains tested we have obtained peroxide-positive variants from 7. Only D121 has thus far given negative results. Of these 7 strains 3 (Lewis, Richards and P279) were reported peroxide-negative by Fuller and Maxted.

Conclusions. These results demonstrate that Type 3 hemolytic streptococci produce peroxide under conditions determined by certain variability factors. This is also true of some other type strains. In manner of origin and in subsequent cultural behavior these peroxide-positive variants resemble the lactose-positive variants of *B. coli mutabile.* Peroxide formation by Type 3 strains was predicted by

Mellon,[6] who believed its apparent absence was due to temporary suppression of a special "growth phase" possessing this function. Whether suppression of peroxide formation in the white colonies is complete, or reduced to a degree not detectable by the present methods, remains a question.

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Microdetermination of Homocystine in Pure Solution with the Dropping Mercury Cathode.*

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In a previous paper the polarographic microdetermination of cystine in globin hydrolysates was reported.[1] Under the conditions outlined for the experiment it was shown that methionine did not give a polarographic effect and was not detected by the mercury cathode. The fact that methionine does not interfere in the polarographic determination of cystine, provided its concentration is not greatly in excess of that of cystine, has been verified by Smith and Rodden.[2] A special calibration method was devised, however, to eliminate any errors in the cystine determination should interference arise from methionine or other amino acids present in the hydrolysates.[1]

Homocystine, which is derived from methionine by demethylation and oxidation, gives the sulfhydryl compound, homocysteine, upon reduction. Inasmuch as homocystine is a homologue of cystine, the behavior of these compounds should be analogous. While homocystine may be determined by the method of Okuda[3, 4] and photometrically,[5] the physiological importance of homocystine makes it desirable to have still another method for determining this substance

[6] Mellon, R. R., 1940, in press.

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[1] Stern, A., Beach, E. F., and Macy, Icie G., *J. Biol. Chem.*, 1939, **130**, 733.

[2] Smith, E. R., and Rodden, C. J., *J. Research Nat. Bur. Standards*, 1939, **22**, 669.

[3] Okuda, Y., *J. Biol. Chem.* (Japan), 1925, **5**, 17.

[4] Brand, E., Cahill, G. F., and Block, R. J., *J. Biol. Chem.*, 1935, **110**, 399.

[5] Kassell, Beatrice, *J. Biol. Chem.*, 1935, **109**, xlix.