

phonamides, showed the usual α -hemolysis except in those strains of *D. pneumoniae* which showed a conversion of α - to β -hemolysis at low concentration of penicillin.

The colonies of *D. pneumoniae* growing in the clear ring around the optochin disc were not bigger than the ordinary colonies of the same strains; in fact the marginal colonies of most strains were smaller than the ordinary colonies growing far away from the disc. This seems to rule out the possibility that the strongly hemolytic marginal colonies got a better nutrition at the edge of the inhibition zone of optochin; or that, similarly to the stimulation of the bacterial growth by low concentration of penicillin already known^{7,8}, they were simply stimulated in their growth by suitably low concentration of optochin.

However, the possibility still exists that, similarly to the observation of TODD⁹, who found that pneumococci growing in subbacteriostatic concentration of penicillin show a sequence of multiplication, lysis and renewed multiplication, there may, in suitable concentrations of optochin, also be increased multiplication besides increased disruption of pneumococci. This would liberate more hemolytic substance but the colonies would all the same remain small.

The subcultures of colonies of *D. pneumoniae* growing in the clear ring, i.e. showing β -hemolysis, yielded only

α -hemolytic colonies, which seems to rule out the possibility that optochin was merely selecting out β -hemolytic variants of *D. pneumoniae*. These α -hemolytic colonies, however, showed similar stimulation of their hemolysis when subjected to subbactericidal concentration of optochin again.

Zusammenfassung. Bei niedrigen Konzentrationen von Optochin wird die Hämolyse der Pneumokokken verstärkt. Die Umwandlung der α - in β -Hämolyse von Pneumokokken, welche in der subbakteriziden Zone wachsen, wird beschrieben.

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⁷ L. P. GARROD, Br. med. J. 1, 205 (1951).

⁸ Y. AOKI, Y. TANEMORI and T. NODE, Kitasato Archs exp. Med. 22, 103 (1949).

⁹ E. W. TODD, Lancet 1, 74 (1945).

Selection of Streptomycin-Resistant Cells of *Salmonella* in a Minimal Medium

It is generally assumed that repeated contact of bacteria with sub-bacteriostatic concentrations of some antibiotics, such as penicillin and oxytetracyclin, in artificial media, favours the selection of strains which are highly resistant to these antibiotics.

Experiments were undertaken to isolate streptomycin-resistant cells from *Salmonella* cultures, grown without any contact of antibiotics.

Three strains were used: *Salmonella newport*, *S. senftenberg* and *S. minnesota* R 2051. The sensitivity of these strains to streptomycin was examined on MacConkey agar containing different concentrations of this antibiotic. The results indicated that the 3 strains were sensitive to 8, 10 and 7 μ g/ml streptomycin respectively. No resistant colonies could be detected on the plates with these concentrations of streptomycin, although the inoculum contained approximately 10^9 cells/ml.

The strains were grown on nutrient agar. The inoculum was prepared in sterile water and contained 10^9 cells/ml. A quantity of 0.25 ml of this suspension was used to inoculate 25 ml of a minimal medium composed according to MANDELSTAM¹ and containing sodium succinate M/9 as sole source of carbon. These cultures were shaken for 24 h at 37°C. This procedure was repeated several times. After each third transfer the purity of the cultures was checked and the sensitivity to streptomycin was estimated. For this purpose 0.25 ml was inoculated in 25 ml of antibiotic medium No. 3 containing 50 μ g/ml streptomycin. After 30 transfers on the minimal succinate medium growth on the antibiotic-streptomycin medium was observed. Then 0.25 ml of these cultures was transferred to antibiotic medium No. 3 containing 100 μ g/ml streptomycin. After 2 repeated transfers (with 200 and

500 μ g/ml streptomycin in the medium) the cultures could grow in the presence of 1000 μ g/ml streptomycin. The resistant strains thus obtained were grown on MacConkey agar slopes containing 1000 μ g/ml streptomycin. After 6 months, with weekly transfer to the same fresh medium, the strains still possess their resistant qualities to this antibiotic. The biochemical and serological tests have proved that no changes occurred in their original specific entities.

These results are in complete agreement with those obtained by KRČMÉRÝ et al.² using *E. coli* for oxytetracycline-resistance.

Experiments are now undertaken to transfer the streptomycin-resistance to other members of the Enterobacteriaceae.

Résumé. Trois souches, *Salmonella newport*, *S. senftenberg* et *S. minnesota* R 2051, sensibles à la streptomycine ont été cultivées sur un milieu minimal liquide en présence de succinate de sodium. Après une trentaine de repiquages, des colonies résistantes à la streptomycine furent isolées.

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¹ J. MANDELSTAM, Biochem. J. 82, 489 (1962).

² V. KRČMÉRÝ, E. PARRAKOVA and M. PAPPOVA, Z. Mikrobiol. 8, 151 (1968).