chains was resuspended in sterile sucrose solution and centrifuged. The whole procedure was repeated several times. The supernatants from different centrifugations giving the same percentage of conidia on counting (90–95%), were pooled together and centrifuged at 3000 *g* for 20 min. The conidial pellet so obtained, was washed 3 times with sterile distilled water.

The mainly mycelial pellet of the 0.45 *M* sucrose centrifugation, on the other hand, was resuspended in 0.6 *M* sterile sucrose solution for liberation of conidia still retained in the mycelial mass and centrifuged as described above. The increase in the concentration of sucrose is necessary for the variation in density of mycelial fragments and conidia, after a stay in a first sucrose solution; this finally helps to obtain good separation between the diverse elements. This method when repeated many times produces a pellet with high percentage of free conidia (over 90%). This conidial preparation was mixed with the one obtained with 0.45 *M* sucrose solution ('Sucrose method').

The conidia so obtained constituted a starting material for the inoculation of fresh medium and for the analysis of enzymatic and proteinic activity. The following Table presents an average value of many counts and results from 6 experiments of conidial isolation.

The procedure is relatively simple and rapid, and permits the collection of free conidia in an uniform physiological and pregermination stage. This method is similar to the isopycnic gradient centrifugation, method used for obtaining yeast cells in their synchronous developmental state (MITCHISON[4], and Dr. J. DESHUSSES, personal communication). By dilution plate technique, a classical bacteriological method, we verified the synchronism of growth of isolated conidia.

With this method, we obtained a homogeneous inoculum, as good from the point of view of morphology and physiology for the experiments on the dynamics of *Neurospora* growth. On the other hand, we have now the possibility to analyze a population of conidia which does not present more than 10% of contamination from which half consists of conidia in pairs or chains. The fragments of mycelia represent only 5% of the entire population. Thus we can consider the results acquired as significant from the analytical point of view[5].

Results from preparation of isolated macroconidia

| Type of cells | 'Sucrose method' Number/ml | % | 'Filtration method' % |
|---|---|---|---|
| Total of 'cells' | $7.80 \times 10^7$ | 100 | 100 |
| Free conidia | $7.05 \times 10^7$ | 90.3 | 55–60 |
| Conidial chains and mycelial fragments | $0.75 \times 10^7$ | 9.7 | 45–40 |

*Résumé.* Une méthode simple d'obtention de conidies libres et isolées de *Neurospora crassa* est décrite ici. Après une culture de 4 jours du champignon sur milieu minimal solide additionné de glycine, les conidies sont récoltées; elles sont suspendues dans de l'eau stérile contenant un détergent non-ionique, puis filtrées et soumises ensuite à des centrifugations à basse vitesse dans une solution saccharosée. Les surnageants donnent alors une suspension à fort pourcentage de conidies libres et isolées.

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[4] J. M. MITCHISON, Expl Cell Res. *13*, 244 (1957).

[5] We thank Dr. M. N. OJHA for the English translation of this article.

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## Reversion of α- into β-Hemolysis of *Diplococcus pneumoniae* by Low Concentration of Optochin

The stimulation of bacterial hemolysis by low concentration of some antibiotics is already known. STORY[1] reported his observations on the stimulation of hemolytic activity of *Diplococcus pneumoniae* by subbactericidal concentration of penicillin as early as 1953. Recently, LÖFSTRÖM, HALLANDER and LAURELL[2–4] as well as KIENITZ, RITZERFELD and GRÜN[5], and RITZERFELD, WINTERHOFF and KIENITZ[6], described the enhancement of the production of staphylococcal α-hemolysis by penicillin. The potentiation of hemolysis of some gramnegative bacteria by low concentration of penicillin and other antibiotics was noticed also. Recently, it was observed to happen also at low concentration of kanamycin and gentamicin (unpublished).

Here, the reversion of α into β-hemolysis in *Diplococcus pneumoniae* under the influence of low concentration of optochin is reported. The phenomenon was observed in 2 strains of *D. pneumoniae* isolated from sputum and in several strains isolated from liquor cerebrospinalis and pus. All strains appeared to be typical *D. pneumoniae*. The optochin test was made on 8% horse blood agar, the thickness of which was approximately 1 cm. 'Oxoid' optochin discs were used. The zone of inhibited growth around the disc varied from 2–3 cm in most cases. Following the usual overnight incubation at 37 °C, a narrow (ca. 2 mm in diameter) clear ring of β-hemolysis was visible at the border of the inhibition zone of optochin. Outside this ring of β-hemolysis, all colonies of *D. pneumoniae*, including those growing at the edge of the inhibition zone of antibiotics and sul-

[1] P. STORY, J. Path. Bact. *65*, 61 (1953).

[2] H. O. HALLANDER, G. LAURELL and G. LÖFSTRÖM, Acta path. microbiol. scand. *68*, 142 (1966).

[3] G. LÖFSTRÖM, H. O. HALLANDER and G. LAURELL, Acta path. microbiol. scand. *70*, 633 (1967).

[4] G. LÖFSTRÖM, H. O. HALLANDER and G. LAURELL, Acta path. microbiol. scand. *72*, 453 (1968).

[5] M. KIENITZ, W. RITZERFELD and L. GRÜN, *Staphylokokken in Klinik und Praxis* (Wissenschaftliche Verlagsgesellschaft, Stuttgart 1964).

[6] W. RITZERFELD, D. WINTERHOFF and M. KIENITZ, Zentbl. Bakt. Parasitkde *199*, 478 (1966).

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 TODD[9], 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 μg/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 MANDELSTAM[1] 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 μg/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 μg/ml streptomycin. After 2 repeated transfers (with 200 and 500 μg/ml streptomycin in the medium) the cultures could grow in the presence of 1000 μg/ml streptomycin. The resistant strains thus obtained were grown on MacConkey agar slopes containing 1000 μg/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 KRČMÉRY 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. KRČMÉRY, E. PARRAKOVA and M. PAPPOVA, Z. Mikrobiol. *8*, 151 (1968).