

stable and retained their properties on passages. Of the 16 biochemical mutants, 11 required amino-acids. Examination of 504 colonies from the control plates of the same strain, that is, from the plates without phage, revealed no biochemical mutants.

This confirms our assumption that actinophage is a mutagenic factor; but whether this mutagenic action is based on transduction, and, if so, how the emergence of the auxotroph under the action of actinophage grown on a prototrophic strain can be explained, are as yet unknown.

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¹ Alikhanian, S. I., and Iljina, T. S., *Nature*, 179, 784 (1957).

Chaining and Unchaining *Streptococcus faecalis*—a Hypothesis of the Mechanism of Bacterial Cell Separation

It was observed that in young broth cultures, *Streptococcus faecalis* (enterococcus) grows in marked chains which later break up rather abruptly; the chains can be maintained by spinning young cultures and resuspending the cells in fresh medium. It was further found that many unrelated compounds are capable to a greater or lesser degree of inducing chain formation in enterococci, the most active being detergents, suramin and crystal and gentian violet; bile¹ was moderately active. In the presence of these compounds enterococci grow rough, with a clear supernatant and a heavy granular deposit consisting of long, tangled chains. On transfer to ordinary media the organisms revert to the typical, smooth, diffuse type of growth with little sediment—singles, pairs and short chains predominating.

It was assumed, as working hypothesis, that the final stage of cell division, that is, cell separation, is due to an enzyme system, its deficiency or inactivity leading to the formation of larger or smaller aggregates. Indeed, Webb² has already shown that chains of *Clostridium welchii* can be broken up by an enzyme obtained by disintegrating cells of this species. In the light of the present hypothesis compounds inducing chain formation would do so by inhibiting either the synthesis or the activity (or both) of the cell-separating system.

Direct proof of the idea came from experiments in which chains were broken up by supernatants of short-form cultures. Horse heat-infusion broth with 1 per cent 'Oxoid' peptone was used throughout. Supernatants of 18–24 hr.-old cultures of five strains of enterococci served as source of the cell-separating system, autoclaved supernatants as controls. Chains were obtained by growing the same strains in the presence of detergents or, more commonly, suramin (0.5–1 per cent) afterwards removed by washing with saline; alternatively, an atypical strain of enterococcus growing naturally in long chains (N.C.T.C. 2400) was used. The tubes containing mixtures of chains and supernatants were placed in the water-bath at 37° C. and the length of chains in experimental and control tubes compared at intervals by examining wet and Gram-stained preparations. In the experimental tubes 'induced chains' became visibly shorter after a few minutes and broke up within 15 min. to 3 hr.; in the control tubes the chains persisted for 6–24 hr., but eventually

broke up. Chains of strain N.C.T.C. 2400 were slower to break up and, as expected, persisted in the controls. The cell-separating system was found to be considerably impaired by heating for 30 min. at 70° C. and completely destroyed by boiling for 5 min.; it is largely retained by Seitz filters, partly by sintered glass filters; its activity appears greatest around pH 7. It acts on cells killed by boiling; here the chains become very rapidly Gram-negative prior to unchaining.

During the unchaining of live cells, lysis invariably occurred, though its degree varied with the strain used as source of the unchaining system. Lysis showed first as single Gram-negative cells within Gram-positive chains, their numbers increasing with time. At later stages the outline of such cells becomes indistinct and it is assumed that they eventually disintegrate. The possibility that Gram-positive cells remained connected by Gram non-staining material was considered: it was ruled out by the results of examination of wet preparations in which short chains, pairs and singles showed independent drifting and Brownian movement. That the cell-separating system is identical with or part of a lytic system is a distinct possibility; on the other hand, lysis may be incidental or even a consequence of unchaining. Preliminary results seem to indicate the existence of a similar cell-separating system in Gram-positive and Gram-negative rods; a full account of the results will be published elsewhere.

We are indebted to the Rankin Research Fund for a grant towards the expenses of this work.

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¹ Weissenbach, R. J., *C.R. Soc. Biol.*, 81, 559 (1918).

² Webb, M., *J. Gen. Microbiol.*, 5, 497 (1951).

Spontaneous Occurrence of Intranuclear Inclusion Bodies in Continuous Cultures of Renal Cells

THIS communication describes the spontaneous occurrence of intranuclear inclusion bodies of A type¹ in renal cells of the dog which have been propagated in continuous tissue cultures. Spontaneous occurrence of the intranuclear inclusion bodies in normal tissue cultures has, to the best of my knowledge, never been reported. The primary tissue cultures were prepared by a modification of a trypsin digestion technique². The cells were grown on histological coverslips (2 in. × ½ in.) inside 1-oz. screw-capped bottles, which were incubated in a horizontal position at 37° C. Each bottle contained 3 ml. of a nutrient medium (pH 7.0–7.2) composed of 1.0 per cent lactalbumin hydrolysate and 20 per cent chicken serum in Hanks's balanced salt solution. Phenol red (0.002 per cent), penicillin (100 u./ml.) and streptomycin (100 µgm./ml.) were added to the medium. When cell sheets were established (5–6 days), the cells were dispersed with 0.5 per cent versene and re-suspended in a fresh portion of the medium, using the method of Jordan³, to give the desired count.