

Short Communications and Preliminary Notes

A colicin produced by cells that are sensitive to it

When cultures of *Escherichia coli*, strain 15 h-, are irradiated with ultraviolet light their optical density rises for several hours and then drops precipitously. Unirradiated control cultures continue their growth during this time and eventually reach a stable level. No bacteriophage have been detected in the ultraviolet-induced lysates; rather they can be shown to contain colicins (see JACOB, SIMINOVITCH AND WOLLMAN¹).

The antibacterial activity of the lysate is demonstrated after vigorous centrifugation, by placing drops of the supernatant on the surface of a plate containing a top layer of soft agar in which the test bacteria are dispersed. After incubation these background bacteria grow and cloud the surface layer of the plate except where the lysates have been placed. Here clear areas, containing some small colonies, can be seen sharply delimited from the surrounding growth. The only strain which has been found sensitive to this lysate is the same h-strain that produces it; other strains of *A. coli* such as ML, B, 122, Bordet, K12 and W1485 have repeatedly shown themselves to be resistant.

This specificity of action demonstrates that the lysate activity is not due to some nondiscriminating toxic product. Furthermore, two sorts of resistant mutants of strain h- have been secured, one of which cannot, in addition, produce this activity. When active lysates are diluted and tested, the clarity of the spots fades out with decreasing concentration; in no case have particulate plaques been observed within the spot, nor could any activity be transferred from a clear spot. Consequently, the active agent is not reproducible. Lysates can be stored at 5–10° for weeks, exposed to 8700 ergs of ultraviolet light per mm², treated with chloroform or sterile-filtered through sintered glass without losing activity; they will not withstand, however, 70° for one-half hour. These are properties compatible with colicins. Further, the fact that antibacterial activity cannot be demonstrated in unirradiated h-cultures, whether they are grown in nutrient broth or in defined medium, indicates that h-cells contain a procolicin which is stimulated to develop by ultraviolet light.

The surprising feature of this situation is that cells carrying the procolicin are sensitive to the colicin they produce (see FREDERICQ²). Usually colicinogenic strains do not produce activity against themselves; for example, the colicin from strain ML bacteria will not inhibit their growth but acts against bacteria of the Bordet strain. In order to test the hypothesis that strain h- was heterogeneous, containing some colicinogenic bacteria and some that were non-colicinogenic but sensitive, single colony isolations were made. Each of these isolates showed the same behavior as its parent, producing colicins in response to ultraviolet irradiation that were active against itself and had a specificity like that of colicins from the parent.

The small colonies within the clear areas produced on test plates consist of bacteria that, after some hours, do not grow when transferred to nutrient agar slants. The fresh lysates contain, however, small numbers of viable cells (usually removed by centrifugation). Some of these (2 out of 5 tested) were like the original h-strain, producing the same colicins as h- and being sensitive to them. Their survival in the active lysates must be explained as a result of a transitory resistance. The remainder of the cells isolated from lysates possessed a heritable resistance while still containing their procolicin. They produced colicins with the same specificity as those from the original h-strain but were *not* sensitive to them.

Presumably these resistant strains were mutants which had arisen in the original sensitive population. Their existence in small numbers demonstrates that unirradiated cultures, in which no colicins can be found, actually contain no colicins or at least so few as to confer no selective advantage on resistant mutants. Otherwise they would be expected to form the preponderant part of a stock culture. On the other hand, the existence of colicinogenic bacteria sensitive to the colicins they produce indicates that the presence of the procolicin is not sufficient to cause resistance.

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¹ F. JACOB, L. SIMINOVITCH AND E. WOLLMAN, *Ann. Inst. Pasteur*, 83 (1952) 295.

² P. FREDERICQ, *Ann. Inst. Pasteur*, 84 (1953) 294.

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