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Enhancement of Clear Spot Formation on *Pseudomonas aeruginosa* Lawns by Chloramphenicol, Tetracycline, and Sulfisoxazole

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The spontaneous appearance of clear spots on *Pseudomonas aeruginosa* agar cultures has long plagued those working with the bacteriophage of this organism. Upon close examination, these spots appear to be due to a material that is often iridescent by reflected light. Efforts are being made to discourage the formation of this material (V. L. Sutter, V. Hurst, and J. Fennell, J. Bacteriol. **86**:1354, 1963). We wish to report on the enhancement of clear spot production on *P. aeruginosa* lawns by certain chemotherapeutic agents.

In the course of antimicrobial disc sensitivity testing of clinical isolates on Mueller Hinton Medium (Difco), it was observed that chloramphenicol, tetracycline, and sulfisoxazole caused the production of plaque-like clearings on the lawn of many strains of *P. aeruginosa*. The clear spots appeared in the form of a ring of apparent lysis around the discs. This is illustrated in Fig. 1; no clear spots appear on the bacterial lawn except in the vicinity of the discs. Figure 2 depicts the lawn of a strain of *P. aeruginosa* on which numerous small clear spots appeared spontaneously. In the immediate vicinity of the sulfisoxazole disc, spot formation on the sulfa-resistant organism appears to be inhibited. At some distance from the disc, in the vicinity of apparently lower concentrations of the drug, spot formation is greatly enhanced. In this area, the spots seem more numerous as well as larger.

These clearings were almost typical of the

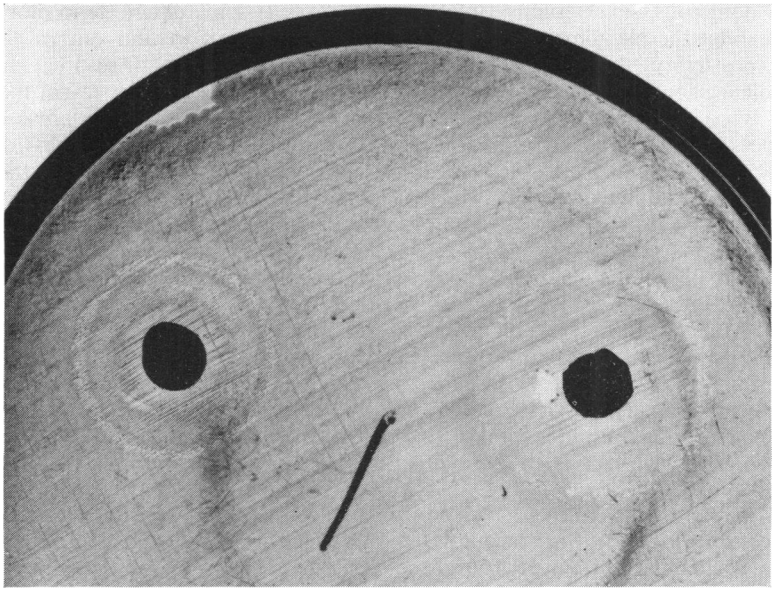

FIG. 1. *Formation of a ring of clearings around tetracycline (left) and chloramphenicol sensitivity discs (30 μg) on a strain of Pseudomonas aeruginosa. No spontaneous clearings appear in the lawn. Mueller Hinton Medium plate incubated at 37 C for 18 hr.*

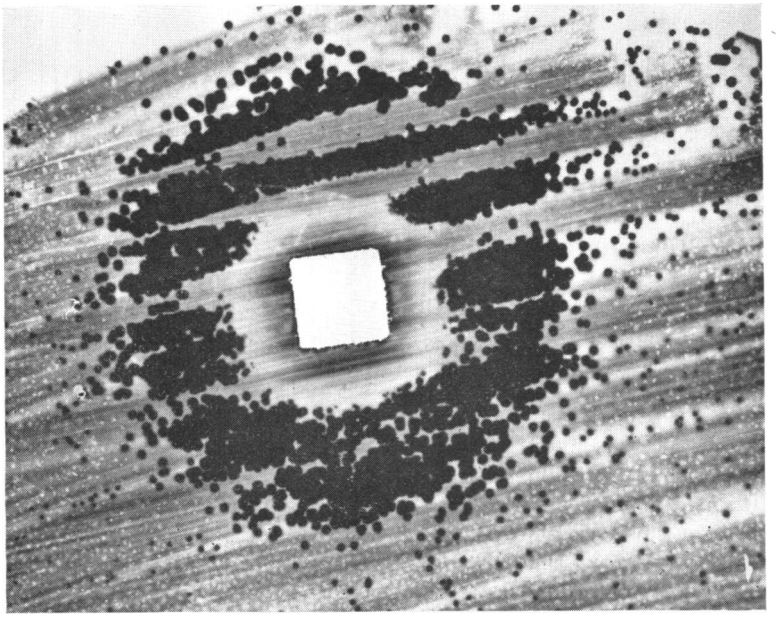

FIG. 2. *Increased clear spot activity due to sulfisoxazole (300 μg disc) on the lawn of a Pseudomonas aeruginosa strain which produces clearings spontaneously. Medium and incubation as in Fig. 1.*

bacteriophage plaques described by P. T. J. C. P. Warner (Brit. J. Exptl. Pathol. **31**:245, 1950). Except for the fact that some were iridescent, they were symmetric and sunk into the surface of the lawn. Consequently, an attempt was made to determine what relationship, if any, existed between this phenomenon and phage activity. Subcultures from numerous single-colony isolations continued to exhibit the phenomenon. This then excluded the possibility that the strains were carrying a virulent phage or that they were mixed cultures of a lysogenic strain and an indicator strain. The spots often resembled the latter system with the appearance of a small colony within the clearings. The only other possibility was that the agents were reversing the normal immunity a lysogenic organism has for its own phage. However, no increase in phage activity in broth due to various concentrations of the agents could be demonstrated. Mueller Hinton Medium broth culture filtrates plated with indicator strains on the NBYE medium of T. W. Feary, E. Fisher, Jr., and T. N. Fisher (J. Bacteriol. **87**:196, 1964) or the sodium lactate medium of Sutter et al. (J. Bacteriol. **86**:1354, 1963) gave plaque counts similar to those of the controls.

Agar plugs of equal size were then taken from the area of concentrated clearings around the discs as well as from the normal lawn and the surfaces washed off in nutrient broth. There was no significant difference in the plaque counts from either area. Of equal importance is that there was also no difference in the viable cell count. One would expect a lower colony count from the area of concentrated clearings if the clearings were due to lyses. Had lower counts been present, the possibility of clear spots being composed of osmotically sensitive protoplasts would have to be investigated, since some sets of dilutions were made in water.

There is some difference in morphology of the cell population in and out of the clear spots. Smears made from these areas and stained for 1 min with crystal violet reveal that the majority of the cells from the clear areas do not readily accept the stain. Those cells that do appear dark and sharp seem to be longer, on the average, than cells from an area without clear spots. Phase-contrast microscopy did not reveal any spheroplasts. Cells from clear areas were rod-shaped and were indistinguishable from those of the controls.

Finally, the pyocine activity of these organisms was not affected by the antimicrobials mentioned. The presence of the agents did not induce any of the strains to be lysed by their own pyocines. Pyocine typing was done by the method of R. R. Gillies and J. R. W. Govan (J. Pathol. Bacteriol. **91**:339, 1966).

It is, therefore, concluded that the phenomenon depicted in Fig. 1 and 2 is an indication of an enhancement of the normal process of clear spot formation and is not related to bacteriophage or bacteriocine activity, nor was there any indication that the clearings seen were due to lysis of the cells.