MEASUREMENT OF PROTEUS CELL MOTILITY DURING SWARMING

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PLATE IX

During the progress of a proteus swarm over the surface of a solid medium, concentric zones of thickened growth are almost always found within the colony (Russ-Münzer, 1935). These zones have been assumed to be caused by a periodic negative chemotaxis of long swarming forms away from a toxic metabolite thought to accumulate in the medium (Lominski and Lendrum, 1947; Hoeniger, 1964; Grabow, 1972; Smith, 1972). When the long forms reach an area of the medium that is free from the toxic metabolite they stop and divide into short non-swarming organisms. Recently, however, neither cessation of motion nor a morphological transition was found to be necessary for concentric zone formation (Bisset, 1973; Bisset and Douglas, 1976; Douglas and Bisset, 1976). Hence, the negative chemotactic explanation of the phenomenon is questionable.

Chemotactic behaviour has been much studied recently (for reviews see Berg, 1975 and Adler, 1976) and is now widely accepted as being controlled by the frequency of reversal of flagellar rotation, which produces a random change of direction of the bacteria without change of velocity (Macnab and Koshland, 1972; Larsen et al., 1974). Evidence presented in this paper, however, shows that the formation of concentric zones is associated with a reduction in velocity of long forms at the edge of the swarm and so may not be due to chemotaxis.

MATERIALS AND METHODS

Nutrient-agar plates (BBL-Trypticase Soy) were inoculated centrally with a loopful of growth from overnight cultures of three strains of Proteus mirabilis (PM, PMS, and 20) and four P. vulgaris (PVM, PVS, PVC, 16) isolated in this department. Another P. mirabilis strain, designated P11, was kindly supplied by Dr J. Armitage, University College, London. The plates were incubated at 37°C until swarming growth was visible to the naked eye. Then, with a scalpel blade, a small block of agar supporting the outer 1–2 mm of growth was removed from the extreme edge of the colony and the organisms were washed from its surface by inverting and gently agitating it in a drop of 0.1 M phosphate buffer, pH 7.0. After the preparation had been held for 5 min. at room temperature a portion of the resultant suspension was examined microscopically. Samples were taken in this way at 30-min. intervals and immediately afterwards the distance between the edge of the inoculum and the edge of the swarm was measured.

Received 24 July 1978; accepted 16 Oct. 1978.

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J. MED. MICROBIOL.—VOL. 12 (1979) 195
Photographs of the preparations were taken on Kodak Tri-X film and an 8-s exposure by the darkground method of Vaituzis and Doetsch (1969). Best results were obtained with scrupulously dust-free slides and with a very thin film of suspension under the coverslip. Motility tracks produced by long forms only were measured and by averaging the five maximum track lengths on each photograph the mean maximum velocity of the organisms was determined (see Vaituzis and Doetsch, 1969).

RESULTS

Preliminary observations showed that at periods when swarming movement at the edge of the colony was slow or had ceased altogether, cellular motility in liquid suspension was also slow. An attempt was made, therefore, to measure these apparent changes in velocity by the motility-track method of Vaituzis and Doetsch (1969) and to relate them to spreading of the colony and concentric zone formation.

A typical example of bright-line motility tracks is shown in fig. 1 in which two types of track are visible: some that are relatively thick, bright and corkscrew like and others that are thin and fainter. Long swarming forms produced the thick tracks (mean width 3-4 μm) because during motion these cells were invariably curved to some extent and so, while rotating about their long axis, scribed a relatively wide arc (fig. 2, lower). In contrast, the short non-swarming cells were not curved and so rotated much more closely about their axes, producing a considerably thinner track (mean width 1-0 μm) (fig 2, upper); a short-form cell, 1-3 μm long and 0-1-0-6 μm wide, could produce a thick motility track only by moving with its long axis perpendicular to the direction of motion. This was not observed and therefore by measurement of only the thick motility tracks on a photograph the mean maximum velocity of the long swarming cells

![Diagram](https://via.placeholder.com/150)

**Fig. 2.—Diagrams indicating how different motility tracks are formed: (lower) rotation of a long form about its longitudinal axis, scribing a relatively wide arc due to the curvature of these cells in motion; (upper) three possible ways in which movement of a short non-swarming cell might produce a motility track. Note that a thick track could be produced only by a short cell moving with its longitudinal axis perpendicular to the direction of motion. This was not observed.**
FIG. 1.—Darkground photograph of bright-line motility tracks in a suspension of *Proteus* from the edge of a swarming colony. Note the thick, bright, corkscrew-like tracks caused by movement of long swarming forms and the thin, fainter tracks produced by short non-swarming forms. ×400.
FIG. 3.—Graphs of change in velocity of cellular motility during swarming growth of *Proteus* species on nutrient agar in relation to time of incubation: (left) *P. vulgaris* strain 16; (right) *P. mirabilis* strain P11 incubated at 37°C. ○ = Velocity of motility; ● = radius of swarm colony.

could be determined, even when these were present among a variety of morphological types.

Two types of result were obtained and are represented by strains 16 and P11 (fig. 3 left and right respectively). The graphs show the progress of the colony over the solid medium in relation to the velocities of the long forms at the colonial edge. One type of organism (strain 16) produced diffuse rings on solid medium and showed only small changes in velocity during incubation. Strains PVM and PVS behaved similarly. In contrast, the other type, exemplified by strain P11, gave sharply defined step-like rings and exhibited striking changes of velocity. Strains PM, PMS, PVC and 20 belonged to this latter category. The periods of slowed colonial advancement produced by the strain that gave rise to diffuse rings (strain 16) were relatively short and coincided with a decrease in cell motility. With strain P11, swarming stopped completely 2-5 h after inoculation, leaving a sharply defined zone of thickening which coincided with a considerable decrease in the motility of the long forms.

**DISCUSSION**

The periods of reduced spreading of the proteus swarm on solid medium are the result of a reduction in the velocity of motility of the cells at the colony edge. These velocities are not mean motilities for the whole population but are average values for long forms moving in the plane of focus of the microscope.
(for further discussion see Segel, Chet and Henis, 1977). The motilities were lower and considerably less variable for strain 16 than for strain P11 and reflected the way in which the concentric rings were formed (Douglas and Bisset, 1976). As the colony of strain 16 spread, the edge slowed only briefly, with much less piling up of growth and hence diffuse rings. With strain P11, advancement of the swarm ceased entirely, producing sharply defined rings.

The periodic alteration in the velocity of the swarming cells might be considered to be the result of a changing chemotactic stimulus. However, Koshland and his co-workers showed that the chemotactic response was due to the frequency with which the cells changed their direction of motion rather than to changes of velocity (Macnab and Koshland, 1972; Tsang, Macnab and Koshland, 1973). Thus, the reduction in velocity of motility and hence the slowing of advancement of the swarm may not be the result of removal of a chemotactic stimulus unless Proteus species behave differently from the other motile bacteria studied for mechanism of chemotaxis. These observations support those of Williams et al. (1976) on non-chemotactic and non-swarming mutants and of Sturdza (1973) who showed that the long swarming forms continued to move when transplanted to fresh regions of agar away from any possible chemotactic influence.

The cause of the periodic variation of motility of Proteus species has yet to be elucidated but it is clear that the zonation phenomenon can no longer adequately be explained on chemotactic grounds alone.

**SUMMARY**

The motilities of Proteus long forms during swarming on agar were measured on cells transferred to liquid suspension. During concentric-ring formation on solid medium, when the edge of the swarm was advancing slowly or had stopped, the velocity of long-form motility was low. When the colony was spreading rapidly, long-form velocity was relatively high. This periodic variation in cell velocity, which determines the zones formed during swarming, cannot adequately be explained by negative chemotaxis.

I wish to acknowledge receipt of a studentship from the Medical Research Council during this work.

**REFERENCES**


MOTILITY DURING PROTEUS SWARMING


