'Viscotaxis', a New Behavioural Response of *Leptospira interrogans* (*biflexa*) Strain b16

By MARIANNE G. PETRINO AND R. N. DOETSCH

Department of Microbiology, University of Maryland, College Park, Maryland 20742, U.S.A.

(Received 25 April 1978)

When in a non-viscous environment and confronted with a viscous one, *Leptospira interrogans* (*biflexa*) strain b16 preferentially selected the latter. We have designated this positive response to a viscosity gradient as 'viscotaxis'. Using an originally designed experimental chamber, a pool of leptospires were faced with capillary tubes containing either polyvinylpyrrolidone (PVP) or N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES). Leptospires suspended in HEPES responded positively to 2% (w/v) PVP in capillaries, and migrated into them in large numbers in 1 h. No response was observed when the chamber and capillary tubes contained solutions of the same viscosity. As the viscosity of PVP was increased, a proportionally larger number of leptospires migrated into it. This newly observed aspect of leptospiral behaviour may have ecological significance.

INTRODUCTION

From the earliest observations on isolation and cultivation of spirochaetes to the present day, experimental data on leptospires has been difficult to obtain and is essentially qualitative in nature. Recently, Greenberg & Canale-Parola (1977) demonstrated a positive chemotactic response to several carbohydrates by *Spirochaeta aurantia* M1. However, no other studies have been published on chemoreception or other physical tactic behaviour in leptospires.

This paper presents observations on the positive response of leptospires to viscosity gradients, a phenomenon we describe as 'viscotaxis'. This hitherto unknown behaviour supports the previous work of Kaiser & Doetsch (1975) which indicated that leptospires swam more efficiently in increasingly viscous materials.

The transport of pathogenic leptospires into host tissues through mucous membranes could be considered as a viscotactic phenomenon. A mechanism for 'viscotaxis' is proposed and its possible ecological importance is discussed.

METHODS

*Organism and growth conditions.* *Leptospira interrogans* (*biflexa*) strain b16 was studied. It was isolated from fresh water (Baseman *et al.*, 1966) and is easily cultivated and maintained in synthetic medium SM-4 (Henneberry *et al.*, 1970). This culture was kindly provided by Dr C. D. Cox, Department of Microbiology, University of Massachusetts, Amherst, Mass., U.S.A. Fresh cultures were prepared every 3 to 4 d by transferring 1 ml of stock culture to 5 ml SM-4; these were kept at 28 to 30°C.

Portions (5 ml) of sterile SM-4 in screw-capped tubes were each inoculated with 1 ml of a 2 to 4 d-old stock culture. These cultures were incubated for 48 to 72 h at 30°C prior to use, to ensure good leptospiral motility and provide a culture in the stationary growth phase.

*Preparation of chemicals.* The buffer (pH 7.3) used in all the experiments was 7.5 mM-N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid (HEPES; Sigma). The viscous agent used was polyvinylpyrrolidone.
Fig. 1. Experimental chamber for studying viscosity responses: 1, plastic cap; 2, outer tube; 3, fluid level in the capillary; 4, small inner tube; 5, pool of bacteria; 6, capillary tube.

(PVP-360; Sigma). Stock solutions of 4, 6 and 8% (w/v) PVP were prepared by dissolving, with mild steam heating, the appropriate quantity of PVP in stock HEPES buffer. After cooling, the pH was adjusted to 7.3 ± 0.1 with 1 M NaOH, and the solutions were sterilized by autoclaving for 15 min at 121 °C.

Preparation of SM-4 plates. SM-4 medium (100 ml) was supplemented with 0.1 ml tryptose phosphate broth (Difco) and 1 g Bacto-agar (Difco). After the pH was adjusted to 7.6 ± 0.1 with 1 M NaOH, the medium was sterilized for 15 min at 121 °C. Upon cooling, 1 ml of filter-sterilized 4% (v/v) Tween 80 (Fischer Scientific Co., Fairlawn, N.J., U.S.A.) was added.

Description and preparation of the experimental chamber. The chamber employed was different from that used by Adler (1973) and others. It consists of three parts: (i) a screw-capped outer glass tube, 13 x 100 mm, fitted with a plastic cap, (ii) an inner glass culture tube, 6 x 50 mm (Kimble, Owens, Ill., U.S.A.), and (iii) a capillary tube 1.6 to 1.8 mm diam. x 100 mm (Kimble) sealed at one end (Fig. 1). This apparatus has several advantages over the generally used slide chamber. All materials are contained in small sterile culture tubes rigidly held by outer tubes, and leakage, 'overlap' or other mishaps cannot readily occur. In addition, contamination is minimal, since the chambers are capped, and sample removal is easy. Furthermore, the incubation temperature can be closely controlled using a water bath, rather than relying on the uneven heat distribution supplied by a so-called slide warmer. It is possible that this chamber will be applicable for other studies of bacterial taxis. The chambers were sterilized (15 min at 121 °C) in advance without capillaries. Capillaries were prepared separately. Using forceps, one end was sealed by flaming and drawn into a small hook in order to hang the capillary on the lip of the outer tube of the chamber.

Measurement of rate of migration into capillaries. Three systems were used: control, motility and 'viscotaxis'. In the control, both the capillary tube and the pool contained HEPES (7.5 mM; pH 7.3); for motility, both contained 2% (w/v) PVP in HEPES; for 'viscotaxis', the capillary tube contained 2% PVP in HEPES and the pool was HEPES. All pools contained 0.1 ml of standard leptospire culture. Migration was assessed at 15 min intervals up to 1 h.

A 48 to 72 h culture was centrifuged for 5 min at 551 g (Safety-Head Centrifuge, Clay Adams, N.Y., U.S.A.) and the pellet was resuspended in 5 ml of sterile HEPES with mixing for 10 s on a vortex mixer (Scientific Industries, Springfield, Mass., U.S.A.). A sample (0.1 ml) of this washed culture was added to HEPES (0.4 ml) to obtain a total leptospire count with a Petroff-Hauser counting chamber. A standard suspension of 1 x 10^7 leptospires ml^-1 was then made in HEPES buffer. Samples (0.1 ml) of standard suspension were transferred to the inner tube of each chamber with 1.0 ml sterile pipettes. All chambers were prepared in duplicate. After adding the leptospires, 0.1 ml of either 2% (w/v) PVP or HEPES was also added to the inner tube. Each chamber was mixed gently and incubated for 20 min at 30 ± 1 °C in a water bath to equilibrate.

Each capillary was filled by lightly flaming the open end for 4 s and then dropping it into 8 ml of reagent in a 17 x 150 mm test tube. By capillary attraction, fluid rose in each tube so treated. After 20 min, each capillary was lowered into the bacterial pool of the inner tube. It could be suspended either by hooking the closed end over the outer tube lip or by resting the capillary against one side of the inner tube. In either case, the capillary opening did not touch the bottom of the inner tube, but was submerged below the surface of the bacterial pool. The chamber with capillary was incubated at 30 ± 1 °C in the water bath for the appropriate time. After incubation, the capillary was removed from the chamber, the outer surface was
wiped with 70% (v/v) ethanol and the closed end was broken. The contents of each capillary were diluted into known volumes of HEPES. From each dilution, duplicate samples were removed with a 25 µl micropipette (Kimble) and spread on solid SM-4 medium. Plates were incubated at 33°C for 72 h before counting.

At the end of the experiment, four samples of the standard suspension, diluted 10⁻⁴, were plated on solid SM-4 medium to obtain a viable count of leptospires. Plates were incubated at 33°C for 72 h and colonies were counted daily for the next 4 d.

Effect of PVP concentration on migration. The method was the same as before but all tubes were incubated for 45 min. The concentrations of PVP (in HEPES) used were 0, 1, 2, 3 and 4% (w/v), corresponding to viscosities of 0.90, 3.3, 7.36, 14.43 and 25.09 cP, respectively. HEPES was considered as 0% (w/v) PVP and represented the control.

Viscosity determinations. The viscosity (cP) of test solutions of different concentrations of PVP and HEPES at 30°C was determined with a falling ball viscosimeter (V-2000, Roger Gilmont Instruments, Great Neck, N.Y., U.S.A.). Six readings for each solution were averaged.

RESULTS

When the leptospires were suspended in HEPES buffer and faced with the same buffer in the capillary, no significant movement was observed (Fig. 2). A peak influx was reached in 30 min for the average (log₁₀) of total leptospires in the capillary (TLIC). From a maximum of 3.3, at equilibrium, the average log₁₀TLIC decreased to 3.1 at 1 h. Similarly, when leptospires suspended in 2% (w/v) PVP were faced with 2% (w/v) PVP in the capillary, no influx of any significance occurred in the capillary (Fig. 2). When there was a viscosity gradient formed by the HEPES buffer in the pool to 2% (w/v) PVP in the capillary, the leptospires swam up it. Over 1 h, the average log₁₀TLIC increased from 2.9 to 4.2 (Fig. 2). Thus, leptospires suspended in buffer leave a region of low viscosity and accumulate in a region of higher viscosity.

With increased, but still identical, concentrations of PVP in the pool and capillary, migration into and out of the capillaries was again random (Fig. 3). However, when leptospires suspended in HEPES were faced with increasing concentrations of PVP in the capillary, the average log₁₀TLIC accumulated in 45 min increased from 3.1 for 0% (w/v) PVP (=HEPES) to 4.1 for 4% (w/v) PVP (Fig. 3). As the viscosity of the PVP solution in the capillary was increased up to 8 cP, leptospires swam into the capillary in greater numbers, but further increases in viscosity did not significantly alter the number of leptospires in the capillary.

To determine whether leptospires would swim down a gradient, they were suspended in 2% (w/v) PVP and were faced with HEPES buffer in the capillary. After 45 min, the average log₁₀TLIC was 3.4, a value as low as in the control assays, indicating that the
leptospires remained in the region of higher viscosity. There was no significant change in the leptospiral population during any of the experiments.

DISCUSSION

The experimental results showed that *L. interrogans* s16 responded positively to viscosity gradients. This is the first time that bacterial behaviour of this kind has been reported and no other description of this phenomenon has been found in the literature for either bacteria or higher organisms. We propose to designate it 'viscotaxis'.

Taxes may be defined as responses to stimuli in which affected organisms are directed towards (positive) or away from (negative) the stimuli (Fraenkel & Gunn, 1940). The presence of a stimulus and a response to it implies the existence of receptors and effectors. If this is true, must receptors specific for 'viscotaxis' be postulated?

With respect to the question of receptors, one must consider the nature of the stimulus, namely viscosity. According to Fraenkel & Gunn (1940), mechanical stimuli are forces which deform or tend to deform an organism or its parts. Subjecting a leptospire to a gradient of increasing viscosity may induce physical changes of this sort which, in turn, could serve as a stimulus not requiring a special receptor. It may be argued that increased osmolarity might serve as a signal to the organism to swim faster by inducing enzymic processes similar to those postulated to occur in chemotaxis (Taylor & Koshland, 1975). However, it seems reasonable to assume that a mechanical deformation altering the leptospiral helix configuration may allow it to swim faster because of increased hydrodynamic efficiency. A deformation of this sort is not easy to detect microscopically. Changes of helicity with viscosity increments may lead the organism up a viscosity gradient by continuously improving its translational swimming ability. Although such movement is directed (they clearly proceed up the gradient), the use of the term 'taxis' for this response could be questioned. In this sense, our use of the term 'viscotaxis' describes a unique behavioural phenomenon, but does not prove it to be a classical stimulus–response event; therefore, we have placed this word in quotation marks.

While minute differences in osmolarity, osmotic pressure and density between the HEPES/PVP interface exist, at time 0, it is believed that they exert negligible effects, a conclusion that follows similar assumptions made in numerous studies on chemotactic responses which involve similar differences in these parameters.

Preliminary experimental data on other viscous agents, such as Tween 80 and solutions of gelatin and methyl cellulose, reveal responses similar to those detailed here for PVP. Finally, different lots of PVP from several suppliers (Sigma; Nutritional Biochemicals Co.,
Cleveland, Ohio, U.S.A.) gave similar behavioural responses, thus ruling out possible responses to unknown chemical contaminants.

It is known from the work of Cox & Twigg (1974) and Kaiser & Doetsch (1975) that increasing the viscosity of a medium containing leptospires results in more efficient translational movement and higher velocity. Random non-translatory movement is replaced by directed translational motion. Furthermore, within the leptospiral population, the number of organisms displaying efficient translatory movement increases significantly with increased viscosity.

With the above in mind, the following mechanism is proposed for ‘viscotaxis’. The moment the PVP-filled capillary comes into contact with the leptospiral pool (time 0), a sharp boundary exists at the viscous–non-viscous interface. A random number of leptospires will swim into the capillary by immediate contact with the viscous layer, because of their proximity to it. As time passes, the sharp boundary dissipates and a viscosity gradient is established. Randomly moving leptospires will either be engulfed or blunder into this region. Once inside, however, the organisms swim with greater velocity in response to the increased viscosity. Velocity is increased by increments as the leptospires are propelled directly up the gradient, until they reach the region of maximum viscosity. Only 15 min is required for a significant response to occur. In the control and motility assay, an isophagic condition exists and there is no significant net migration of the leptospires into the capillary.

Does ‘viscotaxis’ have ecological significance? Or to put it another way, of what value is swimming more efficiently to the organism? If saprophytic, free-living leptospires move more efficiently in a more viscous milieu, the survival value when dwelling in moist soil and mud is apparent. Indeed, it has been demonstrated (Johnson, 1977) that free-living leptospires, although associated with water, are found in larger numbers in the surrounding soils and muds. They are believed to be present in the water from run-off and leaching from the soil.

Finally, vicosotactic behaviour may facilitate entry of spirochaetes into host tissues in certain diseases, since penetration may be achieved by successfully moving across mucous membrane boundaries. It is known that the movement of exoflagellated bacteria is impeded in viscous materials (Schneider & Doetsch, 1974), so mucous membranes may present an effective barrier to invasion by such organisms. However, the ability of leptospires to swim faster, and move more efficiently, in viscous media (Cox & Twigg, 1974; Kaiser & Doetsch, 1975) suggests that they might cross such barriers easily. Viscotactic responses would enable leptospires to move efficiently through natural viscosity gradients in the host, for example, from mucous membranes into body fluids, such as blood or lymph. Although the role of ‘viscotaxis’ as a virulence factor has yet to be elucidated, it poses an interesting problem in pathogenesis.

REFERENCES