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Effect of Viscosity on Bacterial Motility

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The behavior of a number of motile flagellated bacteria toward viscosity characteristics of their fluid environments was observed. All showed an increase in velocity (micrometers per second) in more viscous solutions. Velocity reached a maximum at a characteristic value, however, and thereafter decreased with higher viscosities. Peritrichously flagellated bacteria had maximum velocities at higher viscosities than polarly flagellated bacteria. Effects of temperature, and possible utilization of chemical constituents in the viscous solutions, were studied and found to be negligible factors under the experimental conditions used. Different agents produced the same phenomenon, thus indicating that there probably were no chemically induced metabolic effects. Loss of available water and the possibility of a variable energy supply to the flagellar propulsive system were considered but are believed minimal. Theoretically derived thermodynamic equations were utilized and suggest that the conformation of the flagellar helix affects efficiency of propulsion. Such a relationship between helix waveform and velocity was experimentally observed with *Thiospirillum jenese*.

Factors influencing bacterial motility have ecological implications as motile bacteria may migrate from one environment to another, and they have biomedical implications as bacteria often encounter internal body fluids. There are only rare instances where one can obtain specific individual measurements of some bacterial characteristic, but motility is one biological property of an individual organism which may be referred to with a degree of accuracy.

Motile flagellated bacteria move in a fluid environment, and their swimming characteristics are influenced by viscosity. Up to now the effects of viscosity on the motion of bacteria have not been closely examined. Shoesmith (12) reported that a slight increase in viscosity of a suspending medium above that of a buffer solution had the effect of increasing bacterial velocity, whereas a further increase reduced it. Existing theoretical hydrodynamic equations (5, 6, 8, 11), applicable to helical flagellar waveforms typical of bacteria, do not predict this phenomenon.

Other motile cells, such as spermatozoa, which possess a complex flagellum constructed of nine outer and two core filaments and exhibit a planar waveform, do not show this velocity increase with slight increases in viscosity (2). Obviously, bacterial flagella are analogous, not homologous, propulsive organelles, and their responses need not necessarily be those of the eukaryotic type.

This study was undertaken to confirm and illuminate viscosity effects on the behavior of bacteria and to investigate the apparent discrepancy between observed and theoretically predicted behavior.

MATERIALS AND METHODS

Bacteria studied. Bacteria were selected to provide a diversity of flagellated morphological types (Table 1). *Thiospirillum jenense*, obtained from R. L. Gherna, was grown in the medium of Pfennig and Lippert (10). All other bacteria were from the University of Maryland culture collection and were grown in 7-ml amounts of Trypticase soy broth (TSB) from BBL in screw-top vials (12 by 125 mm) for 12 to 18 h

TABLE 1. *Dimensions and types of flagellation represented by bacteria used*

Organism	Dimensions (μm)[a]	Flagellar arrangement
Bacillus megaterium	1.5 × 3.0	Peritrichous
Escherichia coli	0.5 × 1.0	Peritrichous
Pseudomonas aeruginosa	0.5 × 1.5	Polar
Sarcina ureae	2.0 diameter	One flagellum/cell[b]
Serratia marcescens	0.5× 1.0	Peritrichous
Spirillum serpens	1.0 × 3.0	Bipolar
Thiospirillum jenense ...	4.0 × 40	Polar

[a] Average measurements of 10 unstained organisms in phosphate motility buffer, obtained by means of a calibrated ocular micrometer.

[b] Occurs in packets of eight cocci with one flagellum per cell.

at 30 C in a reciprocal shaker (Lab-line Instruments, Melrose Park, Ill.).

Preparation of viscous suspensions. A 10% (wt/vol) polyvinylpyrollidone (PVP Nutritional Biochemicals Corp, K-90, molecular weight 360,000) solution was prepared in TSB or modified Adler motility buffer (1) (in grams per liter): K_2HPO_4, 2.28; KH_2PO_4, 1.36; ethylenediaminetetraacetic acid, 0.028; and $MgSO_4 \cdot H_2O$, 0.12 (pH 7.0). Solutions of lower viscosities were prepared by serial dilutions of PVP in TSB or motility buffer. Methyl cellulose (M-280, 400 cp, Fisher Scientific Co.) was prepared by making a 1% (wt/vol) solution in motility buffer and diluting as required. Viscosities were measured with a modified Ostwald-type viscometer in a water bath at 25 C and were converted to centipoise (cp).

Viscosity experiments. The organisms in the culture to be observed were centrifuged at 3,000 × *g* and resuspended in 7 ml of motility buffer. Immediately prior to observation, 0.01 ml of this suspension was mixed with 0.5 ml of viscous solution, and 1 drop from a Pasteur pipette (approximately 0.05 ml) was placed on a microscope slide and covered with a no. 2 cover slip. The edges were sealed with Dow fluid (DC200, Dow Corning Corp.) to prevent drifting due to evaporation and also to permit a minimal specimen depth. Motility was generally measured at room temperature which was monitored and maintained within 1 C over a range of 19 to 25 C. Experimental temperatures other than room temperature were attained by use of a warm stage (Chicago Surgical and Electric Co.). The microscope slide and preparation were placed on it and allowed to equilibrate for 5 min before observations were made.

Motility measurements. Unless noted in the text, velocity data were obtained by using a 1-inch (2.54-cm) video tape recorder (Panasonic NV-504) coupled to a television camera (Concord MTC-21) attached to a Zeiss II photomicroscope, incorporating a "phase 2" system (×40 Neofluar objective lens and a ×10 eyepiece), and illuminated by a 12-V 60-W incandescent lamp. The tape was played back on a monitor (Electrohome EMV-23AG), and paths of individual bacteria traced on a transparent plastic sheet were measured with a calibrated planimeter and replayed and timed with a 1/100-s stopwatch. The 10 greatest velocities were used to calculate the average velocity.

Several measurements were made by using "motility tracks" as developed by Vaituzis and Doetsch (13). A Zeiss I photomicroscope (×10 achromatic objective lens, "phase 3" annular ring in the condenser, and ×3.2 or ×6.3 film projection lens) was set up with the exposure controlled by an electronic timer. Exposures of 2 to 10 s were made on Tri X film which was overdeveloped (12 min in Kodak D-76 developer) to increase the ASA to 1,200. The films were projected on a screen, and "tracks" were measured with a calibrated planimeter.

RESULTS

Effect of viscosity on polarly flagellated bacteria. The average velocity of the bacteria was plotted against fluidity, the reciprocal of the viscosity (Table 2), instead of viscosity, since the reciprocal function better separated the experimental viscosities. *Pseudomonas aeruginosa* (Fig. 1) was the fastest organism observed, being recorded at a maximum speed of 81.4 μm/s. Exact measurement of linear velocity at this rate was difficult; however, accuracy

TABLE 2. *Viscosity of methyl cellulose and PVP solutions*

Solution	Percent (wt/vol)	Viscosity (cp)	Fluidity (cp^{-1})
PVP	0.25	1.17	0.855
	0.50	1.66	0.602
	0.75	2.06	0.485
	1.00	2.50	0.400
	1.25	2.82	0.355
	1.50	3.38	0.295
	1.75	4.09	0.245
	2.00	4.65	0.215
	2.50	6.46	0.155
	3.0	8.36	0.125
	4.0	13.2	0.090
	5.0	20.2	0.055
	7.5	53.7	0.019
	10.0	249.0	0.004
Methyl cellulose	0.025	1.69	0.592
	0.050	2.08	0.481
	0.075	2.30	0.435
	0.100	2.62	0.377
	0.125	3.06	0.327
	0.150	3.25	0.208
	0.175	3.92	0.255
	0.200	4.51	0.221
	0.225	4.71	0.212
	0.250	4.85	0.206
	0.500	13.17	0.075
	0.750	34.64	0.029
	1.00	74.73	0.013

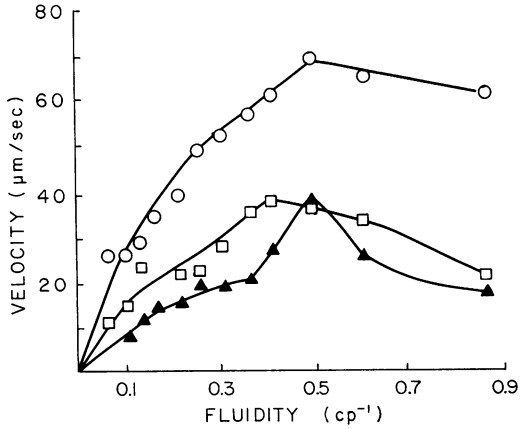

FIG. 1. *Effect of viscosity on the average velocity of the polarly flagellated bacteria, P. aeruginosa* (○), *S. serpens* (□), *and T. jenense* (▲).

increased as the organism slowed down in PVP-buffer solutions above viscosity values of 2.06 cp. Velocity decreased rapidly as viscosity increased above that at which maximum average velocity was observed.

Spirillum serpens, when observed by the motility track method, showed that as viscosity increased velocity increased to a maximum of 38.5 μm/s at a viscosity of 2.5 cp of PVP, but beyond this value velocity decreased rapidly. *T. jenense*, a "giant" bacterium, showed a similarly shaped velocity/viscosity curve despite its relatively great size.

Effect of viscosity on peritrichously flagellated bacteria (Fig. 2). *Bacillus megaterium* is a very difficult organism with which to work. It is extremely aerobic and, in buffer alone on a slide under a cover slip, becomes nonmotile within seconds as the available oxygen is consumed. It did, however, maintain a constant motility in PVP solutions under a cover slip, and was evidently obtaining energy for motility by anaerobically metabolizing the PVP or some contaminating compound associated with the PVP. To provide a constant energy source in excess of requirements, independent of the PVP concentration, PVP was made up in TSB instead of the motility buffer. The resulting motility characteristics, as observed under a cover slip, remained unchanged over a period of 5 min. In contrast to the polarly flagellated bacteria, *B. megaterium* has a maximum velocity at a much greater viscosity (4.7 cp) and could swim at its initial velocity even in the solutions of higher viscosity.

Since *Serratia marcescens* did not require an externally added energy source to maintain its motility, the buffer-PVP viscosity series was used. The maximum velocity of *S. marcescens* occurred at the same viscosity as did *B. megaterium*. *Escherichia coli* was also tested in the buffer-PVP system, and, although its maximum velocity was observed at 1.66 cp, a plateau at this velocity was observed up to 4.7 cp. The velocity of *Sarcina ureae* was measured by means of the motility track method. Although its speed is relatively constant up to 6.5 cp, its maximum velocity (at 1.66 cp) is at a relatively low viscosity compared to the peritrichous organisms.

Viscous agent as an energy source. The observed increases in velocity might be explained by postulating an increase in energy input to the flagellum propulsive mechanism. If a bacterium obtained no energy from the buffer solution but could metabolize the viscous agent or some contaminating component, it is possible that, as the amount of viscous agent increased, more of the material would be supplied and thus increase the velocity of the organism.

To investigate the possibility, a series of dilutions of 10% (wt/vol) PVP was prepared in both buffer and TSB. The average velocities of *P. aeruginosa* in both series were nearly identical (Fig. 3). Substitution of TSB for buffer had no effect on the behavior of this organism. In addition, *B. megaterium* also was examined in solutions of PVP made up in TSB, and again the "increase in velocity phenomenon" was noted. TSB ought to supply most of the requirements for optimum motility, and an adventitious energy source in the viscous agent would no longer be a limiting factor. It appears that the increase in velocity observed was not due to

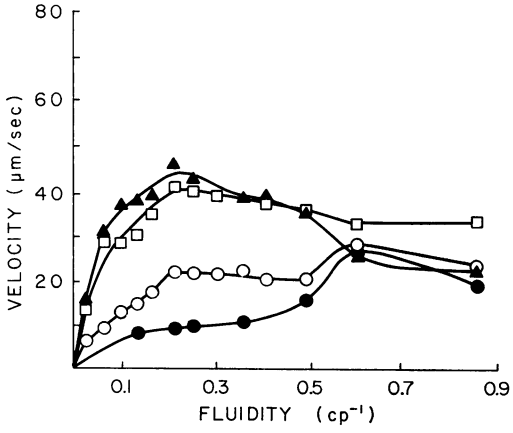

FIG. 2. *Effect of viscosity on the average velocity of the peritrichously flagellated bacteria B. megaterium* (▲), *S. marcescens* (□), *E. coli* (○), *and S. ureae* (●).

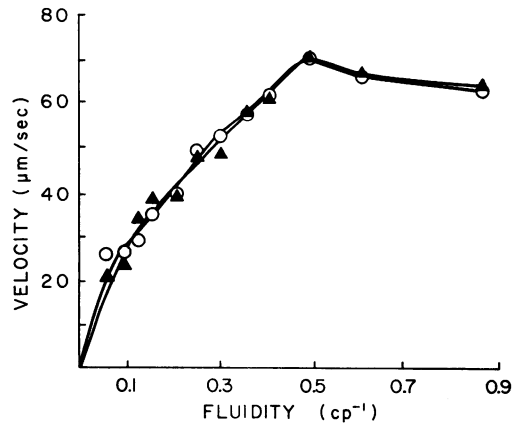

FIG. 3. *Effect of viscosity on the average velocity of P. aeruginosa in dilutions of PVP with phosphate buffer* (▲) *and PVP with TSB* (○).

the viscous agent acting as an energy source.

Effect of temperature. Temperature may affect the motile behavior of bacteria. A rise of 7.8 C, from 17.2 to 25 C, increased the average velocity of *S. marcescens* in 0.05% (wt/vol) methyl cellulose and buffer 6% (Table 3). A rise of 2.8 C, from 22.2 to 25 C, increased the average velocity 0.5%. This increase in velocity is substantially smaller than the potential error of measurement inherent in the television recording system (3%). In the course of an individual experiment, the temperature might vary about 1 C at most. If the slide was kept on the microscope stage, it could absorb heat from the illumination source; however, since each slide was recorded (10 to 15 s) immediately after being placed on the stage, heating was minimal and constant throughout a given series. Temperature variation is not considered, therefore, to have been a significant variable in these experiments.

TABLE 3. *Effect of temperature on motility of Serratia marcescens in methyl cellulose*[a]

Temp (C)	Distance[b] (cm)	Time[c] (s)	Velocity[d] (μm/s)
17.2	29.5	3.05	28.92
	28.5	3.65	23.35
	43.5	4.25	30.60
	36.0	4.60	23.40
	34.0	4.40	23.11
	18.5	2.20	25.14
	29.0	3.80	22.82
	31.0	2.80	33.10
	15.0	1.75	25.63
	24.0	2.80	25.63
22.2	29.5	3.10	28.45
	26.0	2.50	31.10
	13.0	1.40	27.76
	16.0	2.10	22.78
	33.0	3.45	28.60
	21.0	2.05	30.63
	29.0	3.00	28.90
	21.5	2.20	29.22
	29.0	3.20	27.00
	13.0	1.70	22.86
25.0	26.0	2.30	33.80
	17.0	2.00	25.42
	33.0	3.10	31.83
	22.5	2.30	29.25
	15.0	1.80	24.92
	38.5	3.90	29.52
	35.0	4.20	24.92
	25.0	2.60	28.75
	19.5	2.45	23.80
	23.0	2.60	26.45

[a] *S. marcescens* in 0.05% (wt/vol) methyl cellulose and phosphate motility buffer.

[b] Path length of organism, measured from monitor screen with a calibrated planimeter.

[c] Time for organism to traverse path length, measured with 1/100-s stopwatch.

[d] Average velocities: 26.17, 27.73, and 27.86, at 17.2, 22.2, and 25.0 C, respectively.

Comparison of viscous agents. Velocities of *S. marcescens* were measured in dilutions of methyl cellulose and compared with velocities in comparable serial dilutions of PVP (Fig. 4). Although not identical, the resulting curves are quite similar. Maximum average velocity in PVP was 42.6 μm/s, whereas in methyl cellulose it was 44.3 μm/s. Both curves show an initial increase in velocity as viscosity increases, followed by a leveling off or decline.

Flagellar helix diameter as related to viscosity. *T. jenense* has a flagellar fascicle made up of at least 60 individual flagella, thereby giving it an overall diameter of about 0.2 μm; it therefore can be resolved with phase-contrast optics. This organism was examined with the view of observing the relationship between flagellar helix radius and velocity. It is, however, technically difficult to express this quantitatively. Since the fascicle is not sufficiently long to exhibit a complete helical wave, its position in a photograph may not be at the point in its cycle of revolution that corresponds to the radius. Thus, a photographic method is not accurate. Television methods would not have this problem but lack sufficient resolution to visualize the fascicle. A relationship between flagellar fascicle waveform and velocity was observed, however, even though quantitation was not possible. The flagellar fascicles of slowly moving thiospirilla had a helix waveform

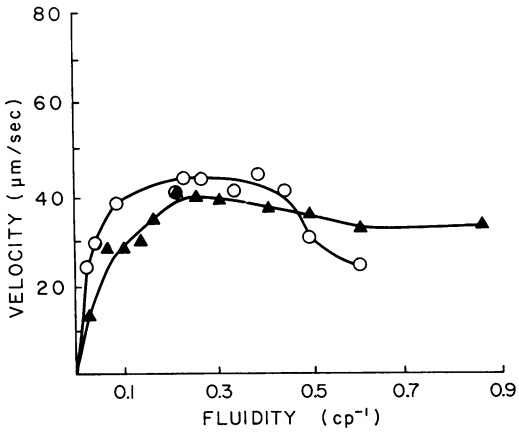

FIG. 4. *Effect of viscosity on the average velocity of S. marcescens in dilutions of PVP with phosphate buffer (▲) and methyl cellulose with phosphate buffer (O).*

characterized by a large radius (amplitude) and a relatively short wavelength. Thiospirilla moving at higher velocities had a characteristic waveform of small radius and large wavelength. The waveform varied in an individual organism if its velocity changed, and, in general, helix radius was found to be inversely proportional to velocity.

DISCUSSION

Shoesmith's observation (12), that there is an initial increase in the velocity of bacteria with an increase in viscosity, has been confirmed and extended. This was seen in all the bacteria examined here; gram positive and gram negative, aerobic, facultative, and anaerobic, monotrichous and peritrichous, large and small, sporeformers and nonsporeformers, rods and cocci. This indicates that it is a general behavioral phenomenon of motile bacteria; however, it has not been reported in larger organisms, and the existing theoretical treatments of flagellar action do not predict it.

Lubliner and Blum (7) derived theoretical equations relating wave speed in flagella to viscosity. Their equations predict only small decreases in velocity as the viscosity increases from 1 cp. At viscosities above 5 cp, the observed velocity should decline more rapidly. Similarly, Schreiner's (11) equations predict a velocity inversely proportional to the square root of the coefficient of viscosity. Neither of these equations account for the velocity increases observed experimentally. There must, therefore, be a factor not accounted for in existing equations, and this missing component must increase in value rapidly as the viscosity initially increases.

An inherent problem in comparing a more viscous with a less viscous solution is that more of the viscosity-conferring compound must be used. This in turn will displace other molecules in the solution, in this case water molecules. Loss of available water will affect bacterial multiplication and also might affect motility; however, a solution of as much as 10% (wt/vol) PVP will become turbid with contaminating bacteria after as little as 8 h at room temperature. Many of the bacteria, upon dilution of the 10% PVP, have been observed to be motile, possessing swimming patterns characteristic of *Pseudomonas*. Since the highest concentration of PVP used did not displace enough water molecules to affect multiplication, it would seem likely that motility systems would not be affected by the more dilute solutions in which the increase in velocity was noted (0.5 to 2.0% [wt/vol] PVP). Moreover, this effect upon motility has not been noted in plasmolysis experiments using sucrose solutions, which also reduce available water molecules (9). It would seem, therefore, that the deprivation of water molecules is not sufficient to cause the observed responses.

It was noted that the radius of the flagellar fascicle helix in *T. jenense* decreased as the velocity of the organism increased. This fact assumes more importance when the theoretical equations are analyzed. Schreiner (11) calculated that:

$$U = \sqrt{(E/D)\ (dw/dt)}$$

where U is the velocity of the organism, E is the efficiency of the propulsive system, D is the translational drag on the head, and dw/dt is the amount of work done by the organism on the surrounding liquid. An increase in viscosity will increase the translational drag on the head; that is:

$$D = 6\pi\mu A$$

where μ is the coefficient of viscosity and A is the radius of the head. The increase in drag on the head will in turn decrease the velocity of the organism.

Any increase in velocity associated with an increase in viscosity can be related to only two terms: dw/dt or E. The amount of work (dw/dt) might vary. This could be caused by an increase of energy input to the flagellum. An energy-regulating mechanism thus would be postulated in bacterial motility systems. It would seem unlikely that bacteria would evolve an elaborate and largely unneeded system such as this. A simpler explanation for the increased velocity phenomenon would be desirable.

The efficiency (E) is the remaining term in the velocity relationship derived by Schreiner. He calculated efficiency to be inversely proportional to the radius and pitch angle of the helix. He had assumed, however, a constant helix radius and pitch angle for the flagellum in any given microorganism, but it has been shown here that these configurations do, in fact, vary with the velocity of the organism.

Now, considering the shape of the individual flagellum, Holwill and Burge (4) calculated the maximum efficiency to be at an amplitude/wavelength relationship of: $nk = 1$, where n is the amplitude of the flagellar waveform and k is $2\ \pi$/wavelength for planar waves. Their efficiency ratio is the square of the propulsive velocity divided by the power expended by the flagellum. The efficiency was halved as nk

decreased to 0.25 or increased to 2.0.

Holwill and Coakley (5) have extended these calculations for helical and non-uniform waves and found the relationship of n to k to be generally unchanged. Thus, the velocity of a bacterium maintaining a constant power input to its flagellum may be varied by altering the conformation of its flagellum.

Machin (8) derived an expression for the displacement (y) of an elastic flagellum by means of a two-dimensional bending movement, $M(x, t)$:

$$\frac{d^2M}{dx^2} + a \frac{d^4y}{dx^4} + b \frac{dy}{dt} = 0$$

The constant a describes the magnitude of the internal elastic forces of the flagellum, resisting the bending movement, and is characteristic of any given flagellum. The constant b, however, may vary since it has the form (6):

$$b = (4\pi\mu)/(2 - \ln R)$$

where R is the Reynolds number for the system and μ is the viscosity external to the flagellum. This equation shows that the external viscosity can alter flagellar conformation.

The theoretical equations and experimental work presented here both suggest that bacteria have a constant power input to the flagellum. The viscosity of the medium will then control flagellar helix conformation, and, as the helix radius decreases (and the wavelength increases) with increasing viscosity, the efficiency of the flagellum increases to a peak (at $nk = 1$) and then decreases. The initial gain in propulsive efficiency of the flagellum is responsible for the increase in velocity when viscosity is raised.

Another effect observed in our experiments was the maintenance of velocity by peritrichous bacteria in more viscous solutions as compared with polarly flagellated bacteria, whose velocity dropped more rapidly as viscosity increased. It has been debated whether all peritrichous flagella are active or if only a few at polar regions are active (3). Our results are most easily explained by assuming that only flagella at the polar regions are active and that the other flagella stream passively by hydrodynamic drag to the rear. These extra, inactive flagella, bunched together at the pole of the bacterium, would contribute to the effective stiffness of the active flagella and thereby increase the value of constant "a" in Machin's equation. This increased internal elasticity would result in maximum efficiency being reached at a much higher external viscosity than for a normal single flagellum, and, indeed, this is what was observed.

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