THE MOTION OF *EUGLENA VIRIDIS:* THE ROLE OF FLAGELLA

By M. E. J. HOLWILL

Department of Physics, Queen Elizabeth College, Campden Hill Road, London, W. 8

INTRODUCTION

A description of the movement of *Euglena viridis* has previously been published (Lowndes, 1941, 1944), but the present study has yielded additional information, particularly concerning the flagellar wave parameters.

Although it is generally accepted that the flagella of such organisms as spermatozoa are directly responsible for propelling the organism through its liquid environment, it has been suggested by Lowndes (1944, 1945) that the flagella of certain organisms, e.g. *E. viridis* and *Monas stigmatica*, are only indirectly responsible for the movement of the organism. Lowndes considers that the flagellum causes rotation and gyration of the organism's body which thus acts as a rotating inclined plane and produces the main propulsive thrust. Experiments with model organisms have been carried out by Lowndes (1944) and Brown (1945) to test the feasibility of such a system and they conclude that gyrating organisms could propel themselves by body movement alone although the flagellum might also provide some forward thrust. More recent observations (Bovee, Jahn, Fonseca & Landman, 1963) suggest that the body gyrations of *Mastigamoeba* contribute to the propulsive force.

In the present paper the movement of *E. viridis* is analysed experimentally and also theoretically using equations developed by the method of Gray & Hancock (1955) and the propulsive effects of body gyration and flagellar undulation are compared.

MATERIAL AND METHODS

Cultures of *E. viridis* were obtained from the culture collection at Cambridge and experiments were conducted on them immediately they were received. For the purpose of observation drops of the culture were placed on a cavity slide so that movements of the specimens would not be impaired.

High-speed cinephotomicrographs of *E. viridis* were obtained using a Stalex high-speed camera in conjunction with a Zeiss WL research microscope as described in detail elsewhere (Holwill, 1965 a, b).

OBSERVATIONS

E. viridis is a well-known organism having a somewhat tapered body about 60 μ long and 10 μ in diameter. During translational movement the body rotates about the axis of progression approximately once a second in the manner indicated in Fig. 1 which shows ten consecutive positions of the long axis of the body. If the organism oscillated without moving forward, the envelope of the body movement would be as

shown in Fig. 2. Close similarity exists between this envelope and the photograph of a hyperboloid of one sheet shown in Fig. 3.* These results are not in accord with Lowndes's (1941) description, in which the envelope of the body movement would be a cone under this hypothetical condition. The difference between the two figures is important from the point of view of propulsion by body gyration, as will be seen in the theoretical section.
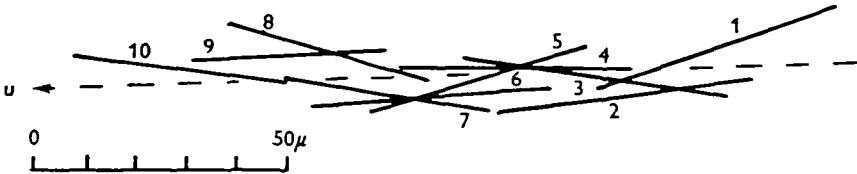

Fig. 1. Diagram showing ten successive positions of the long axis of the body of *E. viridis*. The dashed line is the axis of progression.
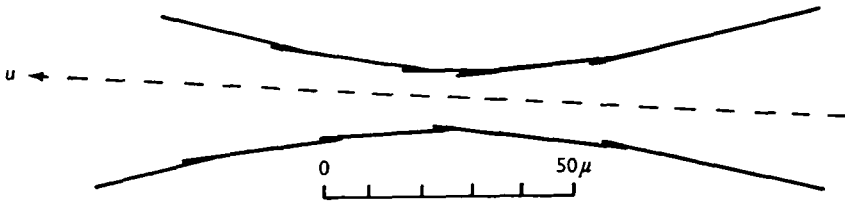

Fig. 2. Envelope of the body movement if the translational velocity were zero.

The average value for the translatory velocity of *E. viridis* was found to be 80 μ/sec., a figure about half that quoted by Lowndes (1941). On no occasion was an organism observed to move more rapidly than 100 μ/sec.

In many organisms, notably the Trypanosomatidae (e.g. Holwill, 1964, 1965a; Jahn & Fonseca, 1963), the Chrysomonadida (e.g. Jahn, Landman & Fonseca, 1963, 1964) and many spermatozoa (e.g. Gray, 1955, 1958; Brokaw, 1965; Rikmenspoel, 1962; Zorgniotti, Hotchkiss & Wall, 1958) it is a relatively easy matter to photograph the flagellum since it is directed either anteriorly or posteriorly while being attached to the corresponding extremity of the organism. *E. viridis*, on the other hand, has its free flagellum attached to the anterior end and directed posteriorly so as to lie alongside the body. Because of the body rotation and the three-dimensional character of the flagellar movement, photography of the flagellum is very difficult and on no occasion was a record obtained of the whole length of a moving flagellum. From film sequences showing the movement of different parts of the flagellum it is possible to reconstruct the movement of the entire flagellum. A wave originates at the base of the flagellum and is almost planar in this region, becoming helical as the wave progresses towards the flagellar tip. Average values for the wavelength and amplitude of the helical wave are 35 and 6μ respectively, while the average beat frequency is 12 cyc./sec.

The thin appendages which are known to be attached to the flagellum along its length (e.g. Pitelka, 1963) were not observed during flagellar movement.

* The hyperboloid of one sheet is a quadric surface having a finite centre and some of its plane sections hyperbolae (e.g. James & James, 1949). The surface can be generated by a moving straight line. A set of lines lying on a hyperboloid is shown in Fig. 3.

THEORETICAL ANALYSIS

It is intended to derive an expression for the thrust developed by a cylindrical body moving in the surface of a hyperboloid in a fluid under conditions such that viscous forces predominate over inertial ones. The last stipulation is necessary to enable valid comparisons to be made between the theoretical predictions and the experimental
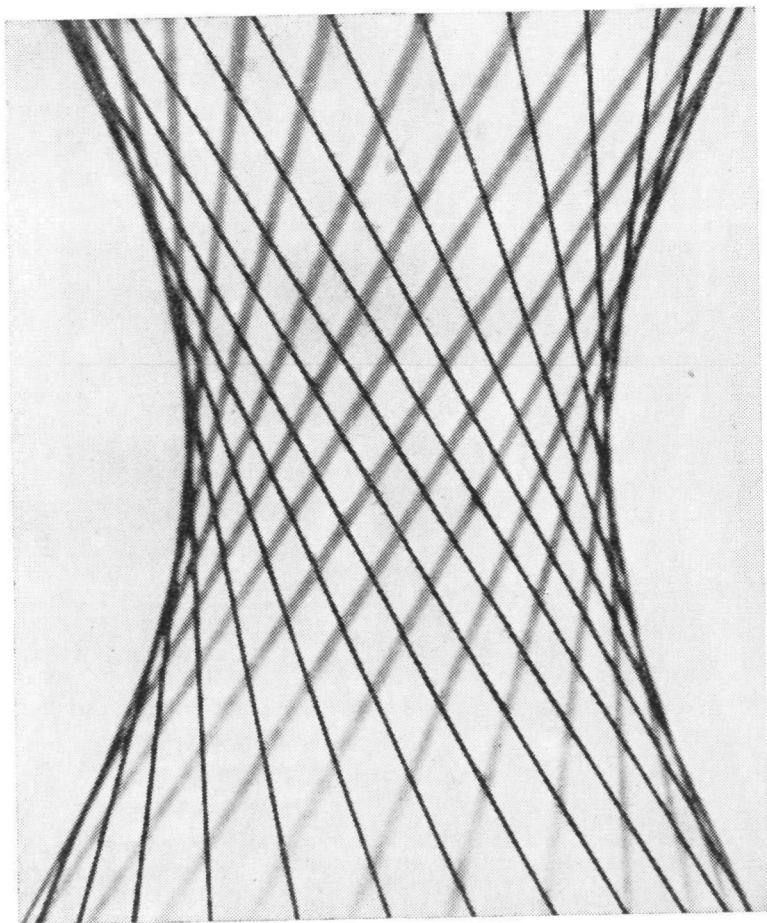

Fig. 3. A set of straight lines lying on a hyperboloid.

observations on *Euglena*. The ratio of inertial to viscous forces acting on micro-organisms is very small (e.g. Bidder, 1923; Taylor, 1951; Hancock, 1953; Burge & Holwill, 1965).

At any given instant the position of the axis of the cylinder will be as shown in Fig. 4; one end of the cylinder is assumed to lie at the waist of the hyperboloid. The cylinder lies in a plane parallel to the axis of progression. The angle θ is measured in this plane between the cylinder and the orthogonal projection of the axis of progression on the plane. Each element of the cylinder moves with angular velocity ω in a circle,

but it is important to notice that the radii of the circles at their points of contact with the axis of the cylinder do not lie in a plane; rather, they lie in the surface of a hyperbolic paraboloid. The vectors representing the instantaneous rotational velocities, $r\omega$, of the elements of the cylinder are therefore not coplanar. To calculate the forces
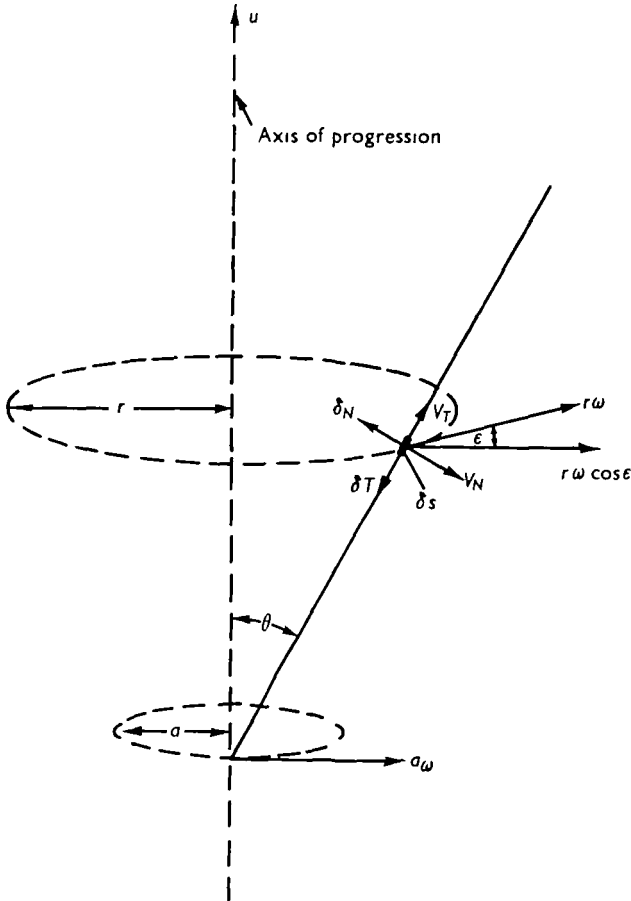

Fig. 4. An instantaneous position of a thin cylinder traversing a hyperboloid, showing the forces acting on an element δs. The cylinder lies in a plane parallel to the axis of progression. The angle θ is measured in this plane from the cylinder to the orthogonal projection of the axis of progression on the plane. The velocity component $r\omega \cos \epsilon$ lies in the plane containing the cylinder and the velocity $a\omega$.

acting on the cylinder it is sufficient to consider only those velocities or component velocities lying in the plane containing the cylinder and parallel to the axis of progression. Velocities normal to this plane will not contribute to the propulsive thrust. Let V_N, V_T (Fig. 4.) be the velocities in the defined plane normal to and tangential to an element of the cylinder δs which moves in a circle of radius r. The element δs will elicit from the fluid normal and tangential reactions of magnitudes:

$$\delta N = V_N C_N \, \delta s, \tag{i}$$

and

$$\delta T = V_T C_T \, \delta s, \tag{ii}$$

respectively. Here C_N and C_T are the surface coefficients of resistance as defined by Gray and Hancock (1955); for a smooth, thin cylinder:

$$C_N = 2C_T. \tag{iii}$$

The thrust developed in the direction of motion (the x-direction) is thus

$$\delta F = \delta N \sin \theta - \delta T \cos \theta. \tag{iv}$$

Now
$$V_N = r\omega \cos \epsilon \cos \theta - u \sin \theta, \tag{v}$$

and
$$V_T = r\omega \cos \epsilon \sin \theta + u \cos \theta, \tag{vi}$$

where ϵ is the angle between the planes parallel to the x-axis containing the vectors $a\omega$ and $r\omega$. From equations (i) to (vi) we have

$$\delta F = C_T[r\omega \cos \epsilon \cos \theta \sin \theta - u(\cos^2 \theta + 2 \sin^2 \theta)] \, \delta s, \tag{vii}$$

ϵ and r may be expressed in terms of s and θ:

$$\cos \epsilon = \left(1 - \frac{s^2 \sin^4 \theta}{a^2 + s^2 \sin^2 \theta}\right)^{\frac{1}{2}} \tag{viii}$$

and
$$r^2 = a^2 + s^2 \sin^2 \theta, \tag{ix}$$

where a is the radius of the smaller circle described by an end of the cylinder. Substituting (viii) and (ix) in equation (vii) and expanding we have

$$\delta F = C_T \left\{ a\omega \sin \theta \cos \theta \left[1 + \frac{s^2 \sin^2 \theta}{a^2} - \frac{s^2 \sin^4 \theta}{a^2 + s^2 \sin^2 \theta} - \frac{s^4 \sin^6 \theta}{a^2(a^2 + s^2 \sin^2 \theta)} \right]^{\frac{1}{2}} - u(\cos^2 \theta + 2 \sin^2 \theta) \right\} \delta s. \tag{x}$$

To obtain the thrust developed by the whole cylinder it is necessary to integrate equation (x) over the length of the cylinder. As it stands the expression is not directly integrable, but in the practical case to be discussed the contribution to the integral of the terms in $\sin^4 \theta$ and $\sin^6 \theta$ in the square bracket is less than 0·7 % and hence may be neglected. The thrust developed by the cylinder is thus

$$\int_{s=0}^{s=l} \delta F = F = \tfrac{1}{2} C_T \, \omega \sin^2 \theta \cos \theta \left\{ l \left(\frac{a^2}{\sin^2 \theta} + l^2 \right)^{\frac{1}{2}} + \frac{a^2}{\sin^2 \theta} \ln \left[\frac{l \sin \theta}{a} \right. \right.$$
$$\left. \left. + \left(1 + \frac{l^2 \sin^2 \theta}{a^2} \right)^{\frac{1}{2}} \right] \right\} - ulC_T \left(\cos^2 \theta + 2 \sin^2 \theta \right), \tag{xi}$$

where l is the length of the cylinder.

APPLICATION OF THE HYDRODYNAMIC EQUATIONS TO THE MOTION OF *EUGLENA VIRIDIS*

Propulsion by rotation of the body

When an organism is moving through a fluid with uniform velocity, the propulsive thrust developed by the body rotation must be exactly balanced by the retarding forces acting on the body; in the case of *E. viridis* the retarding forces would be accentuated by the flagellum. The maximum forward velocity would evidently result if the retard-

ing forces produced by the flagellum were zero, and from equation (xi) this velocity is given by

$$u_{max.} = \frac{\omega \sin^2 \theta \cos \theta}{2} \cdot \frac{l\left(\dfrac{a^2}{\sin^2 \theta}+l^2\right)^{\frac{1}{2}}+\dfrac{a^2}{\sin^2 \theta}\ln\left[\dfrac{l\sin\theta}{a}+\left(1+\dfrac{l^2\sin^2\theta}{a^2}\right)^{\frac{1}{2}}\right]}{l(\cos^2\theta+2\sin^2\theta)}. \quad \text{(xii)}$$

Inserting the parameters of the hyperboloidal path of *E. viridis* into equation (xii) we obtain $u_{max.} = 12\cdot3 \,\mu/\text{sec}$.

If the posterior end of the organism traversed a circular path at some region of the hyperboloid other than the waist, then an inspection of equation (xii) shows that in general the translatory velocity will be somewhat less than the maximum value obtained above.

If the organism moved in the surface of a cone then, to represent the motion in terms of the parameters already used, a and θ are zero. It follows from equation (xii) that the translatory velocity is also zero. If the organism followed a cylindrical path, $\theta = 0$ while a is finite, and again the translatory velocity is zero.

Propulsion by flagellar activity

If it is assumed that the flagellum executes a circular helical wave along its entire length and propels the organism through the fluid with velocity u, then the relation between u and the wave velocity V_w is given by

$$\frac{u}{V_w} = \frac{\eta^2 k^2}{1+2\eta^2 k^2+(3r_B/n\lambda)(1+\eta^2 k^2)^{\frac{1}{2}}[\ln(2\lambda/d)-\frac{1}{2}]}, \quad \text{(xiii)}$$

where η, λ are the amplitude and wavelength respectively of the undulation, $k = 2\pi/\lambda$, d is the radius of the flagellum, n is the number of wavelengths sustained by the flagellum and r_B is the radius of the sphere hydrodynamically equivalent to the body (Holwill & Burge, 1963).

An approximate value for the radius of the sphere hydrodynamically equivalent to the body can be obtained by assuming the body to be an ellipsoid since Lamb (1952) has given expressions for the equivalent radii of ellipsoids moving in a viscous fluid parallel to and perpendicular to their major axes. Taking note that the body of *E. viridis* is inclined at an angle of 10° to the axis of progression, the radius of the sphere hydrodynamically equivalent to the body is found to be $9\cdot7\mu$.

Since no record was obtained on film of the whole flagellum, it is impossible to determine directly the number of wavelengths contained by the flagellum. An indirect estimation may be made as follows. The length, L, of flagellum necessary to form a single wavelength is given by

$$L = \lambda(1+\eta^2 k^2)^{\frac{1}{2}}. \quad \text{(xiv)}$$

For $\lambda = 30\,\mu$ and $\eta = 6\,\mu$, $L = 51\cdot5\,\mu$. The total length of the flagellum is about $100\,\mu$ and will thus contain approximately two wavelengths. Substituting the relevant parameters in equation (xiii) we obtain $u = 94\cdot8\,\mu/\text{sec}$.

DISCUSSION

The foregoing theoretical analysis shows that the thrust developed by the body movement of *E. viridis* is insufficient to propel the organism through the fluid at the velocities observed. The propulsive force generated by the flagellum, on the other hand, provides a theoretical forward velocity very close to that which is observed experimentally.

In view of the approximations made in the analysis it is pertinent to discuss the extent of the errors these approximations will introduce. The representation of the body *E. viridis* by a thin cylinder for the purpose of calculating the thrust produced by its movement may appear to be an oversimplification, particularly in view of the fact that the cylinder radius is effectively zero, since it is only in this case that C_N is exactly twice C_T for a cylinder of finite length. Hancock (1953) has shown that when the cylinder has zero radius each small element makes an independent contribution to the thrust, since the effect of one particle on a neighbouring one is negligible. If the cylinder diameter is finite, each element produces a certain amount of backward fluid movement at other regions along the cylinder, so that the elements in these regions will not be so effective in producing forward thrust and the propulsive velocity will fall. The propulsive velocity calculated for the cylinder is in all probability greater than that which would be produced by the body of *E. viridis*.

Since the whole of the flagellum does not form itself into a helix, the number of helical waves sustained by the flagellum will be less than two. The proximal region of the flagellum, which executes a planar wave, will contribute less to the propulsive force than it would if it executed a helical wave (Taylor, 1952; Hancock, 1953; Holwill & Burge, 1963). The wave remains planar for less than half a wavelength and has a small amplitude in this region, so that its effect on the propulsive velocity is slight. The degree to which the velocity would be affected if the flagellum remained quiescent over a distance of half a wavelength may be found by putting $n = 1 \cdot 5$ in equation (xiii). The value for u is then $84 \cdot 8 \, \mu$/sec. The planar nature of the wave at the proximal end of the flagellum will therefore reduce the propulsive velocity by less than $10 \, \mu$/sec.

The presence of mastigonemes on the flagella of certain organisms is known to induce reversal of the direction of fluid motion expected when a smooth flagellum is undulating (Jahn *et al.* 1963, 1964; Sleigh 1964) and, provided the mastigonemes remain rigid and perpendicular to the flagellar surface, the phenomenon can be explained satisfactorily for both planar and helical waves (Jahn *et al.* 1964; Sleigh & Holwill, in preparation). Although *E. viridis* possesses a large number of mastigonemes on its flagellum, it moves in the direction to be expected if the flagellum were smooth. The absence of reversed mobility can be explained in several ways:

(*a*) The mastigonemes may be flexible and may lie almost parallel to the flagellar membrane.

(*b*) The mastigonemes may be of insufficient length to provide a force great enough to overcome that of the flagellum itself.

(*c*) The mastigonemes may lie in planes containing the axis of the helix formed by the flagellum.

(*d*) The mastigonemes may be wrapped around the flagellum during movement as suggested by Pitelka & Schooley (1955).

Although the mathematical analysis would seem to support the last explanation, it is difficult to correlate it with a report by Leedale (personal communication) who has observed flimmer activity on the euglenoid flagellum, though he has found it impossible record the movement of individual mastigonemes on film. The movement of flexible mastigonemes could be such as to enhance the propulsive force, but until more is known about the movement of these flagellar appendages, reliable theoretical calculations cannot be made.

As a result of the theoretical investigation it is concluded that the flagellum of *E. viridis* is the main propulsive element and that body movement contributes little to the forward thrust. This is in direct contradiction to the results of Lowndes (1944) and Brown (1945) who used models to show that propulsion at the experimentally observed speeds is possible by body gyration alone. The following discussion shows that their interpretation of the model experiments is not valid.

The use of models to simulate on a laboratory scale conditions of fluid flow in systems too large to be examined under controlled conditions is standard practice in many branches of engineering, e.g. aeronautical, nautical and civil engineering. In order that predictions made from the model experiments will be valid when applied to the actual structure many precautions must be taken in the operation of the model experiment. One of these is that the Reynolds numbers applying to the model and the structure must be comparable. The Reynolds number is a measure of the ratio of inertial to viscous forces acting on a system, and is given by $R = lv\rho/\mu$, where l is a linear dimension of the system, v is its velocity and ρ, μ are the density and viscosity of the fluid surrounding the structure.

When models of micro-organisms are examined to predict features of the behaviour of the actual micro-organism, it is thus essential that the Reynolds numbers applicable to the organism and the model are of the same order. For most micro-organisms R lies between 10^{-6} and 10^{-3} so that viscous forces predominate over inertial ones. In particular, for the body movement of *E. viridis* R is about 10^{-3}.

The model used by Lowndes (1944) to simulate the movement of *E. viridis* consisted of a cylindrical stick mounted on the shaft of a motor in such a way as to rotate in the surface of a cone. Rotation of the cylinder caused air to be drawn towards the apex of the cone and Lowndes concluded, by analogy, that a free organism executing a conical gyration would draw itself forward. Although no details are given, it is possible to estimate the order of magnitude of the Reynolds number obtaining in the model. The viscosity of air is 18×10^{-5} poise while its density is about $1\cdot2 \times 10^{-3}$ g. cm.$^{-3}$ so that, assuming a cylinder diameter of $0\cdot5$ cm. and a motor speed of 1 cyc./sec. the Reynolds number is about 30.

The speed of rotation required to make the Reynolds number of the model equal to 10^{-3} would be less than 10^{-4} cyc./sec. At this slow speed no air current would be drawn into the cone, a result which may be correctly applied to the organism. Lowndes (1944) also carried out experiments with his apparatus immersed in water. The Reynolds number in this case would be about 10^3. Brown (1945) and Metzner (1920) carried out similar experiments with cylinders and model bodies gyrating in air and in water to simulate the movements of a variety of micro-organisms—and concluded that propulsion by conical gyration of flagella or of bodies is feasible for the organisms considered. The Reynolds numbers obtaining in these experiments were of the same order as those

calculated for Lowdnes's models, indicating that the application of the results of the model experiments to the movement of micro-organisms is completely invalid. The mathematical arguments presented earlier show that propulsion in a viscous fluid is not possible by conical gyration alone; if the body moves in a hyperboloid slow forward movement is possible, but the speeds attained by certain organisms are too great to be achieved by body movement alone.

SUMMARY

1. Analysis of high-speed cine-films of *Euglena viridis* reveal that the organism traverses a complex three-dimensional path while helical waves are propagated from base to tip along the flagellum.

2. Theoretical analysis shows that the rapid forward velocity of the organism cannot be produced by the body movement alone. The propulsive force generated by the flagellum is sufficient to maintain the observed velocities.

3. Although the euglenoid flagellum bears mastigonemes the thrust produced by it is in the direction to be expected if the flagellum were smooth. Possible explanations of this observation are given.

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