

CHEMOTAXIS OF BRACKEN SPERMATIZOIDS
IMPLICATIONS OF ELECTROCHEMICAL ORIENTATION

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(With Plate 7)

In the original experiments on the chemotaxis of fern spermatozoids, Pfeffer (1884) inserted small glass capillaries filled with a sodium malate solution into suspensions of spermatozoids, and observed that chemotactic aggregation resulted from precise orientation of the spermatozoids to the gradients produced by diffusion of malate from the open tip of the capillary. More recently, Rothschild (1952) provided definite confirmation of the precision of orientation in a chemical gradient by cinemicrographic records of chemotaxis, using bracken spermatozoids.

Pfeffer found that to obtain aggregation of spermatozoids at the tip of a capillary, there had to be a ratio of at least 30 between the original concentration of malate in the capillary and the concentration of malate in the sperm suspension. This 'difference threshold' was constant over a 100-fold range of concentrations. This indicated a relationship between the chemotactic response and the gradient of the logarithm of malate concentration, and Pfeffer emphasized the analogy between this relationship and the psychophysical principle known as Weber's Law.

The morphology of bracken spermatozoids corresponds closely to that of other fern spermatozoids described by Dracinschi (1930). The body contains a narrow nuclear strip, about 35μ long, twisted into about three turns of a tapered spiral. Several dozen flagella, about 15μ long, emerge from a strip of cytoplasm lying along the anterior half of the nucleus. A spherical cytoplasmic vesicle, about 10μ in diameter, is attached to the posterior coil of the body.

The flagella propel the spermatozoid forward at speeds which may reach $300\mu/\text{sec}$. As it moves forward, it rolls over three to six times per second, in the sense of a left-handed screw. The result is a helical path resembling those made by sea-urchin spermatozoa (Gray, 1955). Frequent changes in the direction of the axis of the helical path occur more or less at random, but there are no abrupt reversing reactions like those which have been held responsible for the chemotaxis of some bacteria and protozoa (Jennings, 1906).

Bracken spermatozoids orientate themselves with respect to an electric field when in a solution containing sodium L-malate (Brokaw, 1957). Further study of this phenomenon, and the chemical gradients existing in chemotaxis experiments, has suggested a tentative answer to the primary question posed by precise chemotactic orientation. How does the information available in a chemical gradient provide a directional stimulus to the organism, enabling it to turn and swim up the gradient?

CHEMICAL GRADIENTS

To study chemotaxis, known gradients of a chemotactically active substance must be established in a sperm suspension. This is done most conveniently by allowing the substance to diffuse into the sperm suspension from a region of higher concentration. The gradients must be calculated from an appropriate solution of the partial differential equation for diffusion, as no satisfactory method for direct measurement of the gradients within the suspension is available.

Pfeffer's capillary technique, as modified by Rothschild (1956) by using agar in the capillary to prevent hydrodynamic flow, thus ensuring that entrance of the active substance into the solution takes place entirely by diffusion, is experimentally convenient. The capillary can be introduced at a precise time with relatively little disturbance of the sperm suspension. Diffusion from the end of a cylindrical region into a thin layer of sperm suspension between slide and cover-glass must be considered. The solution of the diffusion equation for these boundary conditions has not been available in the past, but Prof. D. R. Hartree kindly examined this problem for me and obtained a solution to a somewhat idealized version. This shows that the concentration of diffusing substance will be nearly uniform throughout the depth of the suspension at distances from the tip of the capillary greater than the depth of the suspension, and has been used in the following form:

$$C = C_0 + (C_1 - C_0) (a^2/8b) (\pi Dt)^{-1/2} \exp(-\frac{1}{2}w^2) K_0(\frac{1}{2}w^2), \quad (1)$$

where C = concentration of diffusing substance at a distance r from the tip of the capillary at a time t after the start of diffusion, C_0 = initial concentration in the suspension, C_1 = initial concentration in the capillary, $w = \frac{1}{2}r(Dt)^{-1/2}$, D = diffusion constant, a = internal radius of the capillary, b = half the thickness of the layer of sperm suspension, and K_0 = the modified Bessel function of the second kind, of zero order.

Before this solution was available, some work was done using capillaries filled with malate solution in agar, which had been rinsed for a known time, T , in distilled water immediately before insertion into the sperm suspension. The rate of diffusion from the capillary in the first few seconds after insertion was assumed to be relatively constant, and determined by the gradients established within the capillary during the period of rinsing. Diffusion in the suspension was then found using the solution for a continuous line source (Carslaw & Jaeger, 1947)

$$C = C_0 - C_1 (a^2/8b) (\pi DT)^{-1/2} Ei(-w^2), \quad (2)$$

where Ei is the exponential integral, defined by $Ei(x) = \int_{-\infty}^x \frac{e^u}{u} du$. This solution cannot be used for $r < 2b$.

Linear diffusion would be preferable to the radial diffusion obtained with Pfeffer's technique, as the mathematics of diffusion and of the analysis of sperm response to the gradients is simpler. Attempts to establish accurate and reproducible linear diffusion on a microscopic scale were unsuccessful, but a crude approximation to linear diffusion was obtained by placing two drops of solution in contact with each

other on a glass slide and attempting to get a straight boundary between the two drops. The solution of the diffusion equation used for linear diffusion is

$$C = C_0 + \frac{1}{2}(C_1 - C_0)(1 - \operatorname{erf} w), \quad (3)$$

where C_0 and C_1 are the initial concentrations in the two drops, r = distance from the boundary in the drop of initial concentration C_0 , and erf is the error function

defined by $\operatorname{erf} x = \frac{2}{\sqrt{\pi}} \int_0^x e^{-u^2} du$.

Tables of the mathematical functions appearing in these equations are available (Flügge, 1954).

In these experiments, neutral solutions of sodium L-malate were used to establish malate diffusion gradients. It has been assumed that malate diffuses with a constant diffusion coefficient, D . The value of D used in calculations, 7×10^{-8} cm.²/sec., is the mean of the values available for succinic and tartaric acids (Washburn, 1929). As the exact rate of diffusion of malate ion will depend on the ionic composition of the solutions used and on the concentration of malate, only approximate results can be expected when any of these methods of establishing and calculating malate gradients are used.

METHODS

(a) Preparation of sperm suspensions

Suspensions of bracken spermatozooids (*Pteridium aquilinum* (L.) Kuhn) were obtained as described in the preceding paper.

In experiments in which spermatozooids were placed in a voltage gradient, the suspension was buffered with 0.005 M *tris* (hydroxymethyl)aminomethane—hydrochloric acid buffer, '*tris* buffer', pH 8.1, to obtain maximum buffer capacity with minimum conductivity and, therefore, minimum production of toxic substances at the electrodes. In other experiments, 0.005 or 0.01 M sodium phosphate buffer, pH 7.5, was used, as the spermatozooids survived longer in this medium. Addition of small amounts of sodium and potassium ions to the *tris* buffer solution did not lead to longer survival.

(b) Photographic records

The movements of bracken spermatozooids were photographed with dark-ground illumination and exposures lasting several seconds. This is the 'dark-ground track' method used by Gebauer (1930) to study the galvanotaxis of *Polytoma uvella*, by Rothschild & Swann (1949) to study the movements of sea-urchin spermatozoa, and by Harris (1953) to study the chemotaxis of granulocytes. The sperm suspension was contained in a haemocytometer slide, providing a uniform depth of suspension of 0.10 mm. A 3 in. objective which was able to resolve sperm tracks at any level in the suspension was used. Short gaps were made in the track records by briefly interrupting the light beam, to record the times at which experimental changes were made, the direction of sperm movement, or a time scale.

(c) Electrodes

Silver-silver chloride electrodes were used to establish a voltage gradient in the sperm suspension. Two parallel electrodes, 5 mm. long and usually 10 mm. apart, made from 0.25 mm. silver wire, were used in experiments in an open drop of sperm suspension. In other experiments, four electrodes 4 mm. long, made from 0.05 mm. silver wire, were arranged as sides of a square 5 mm. wide in the haemocytometer slide. A silver-chloride layer was electrolytically deposited on the surfaces of the silver-wire electrodes. A roughly linear relationship between current and voltage was observed with these electrodes: when calculating the voltage gradient to which the spermatozooids were exposed the fall in potential between the electrodes was assumed to be linear. The effects of cell polarization, which might occur at the higher voltages used, and of the presence of the second pair of electrodes, parallel to the voltage gradients, were not determined.

The square array of electrodes was connected to a switching circuit so that a pre-determined voltage gradient could be suddenly applied in any one of four directions. By this means, an individual spermatozoid could be kept in the microscope field, so that several responses to different gradients could be photographed.

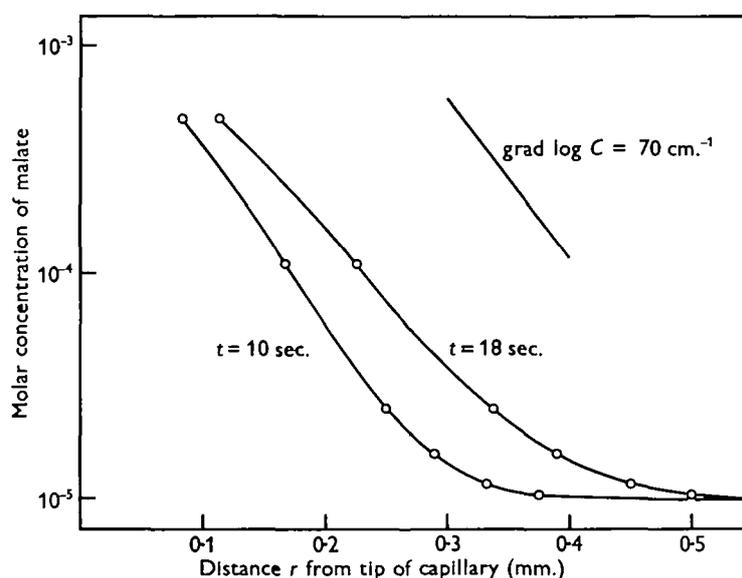
RESULTS

(a) Chemotaxis

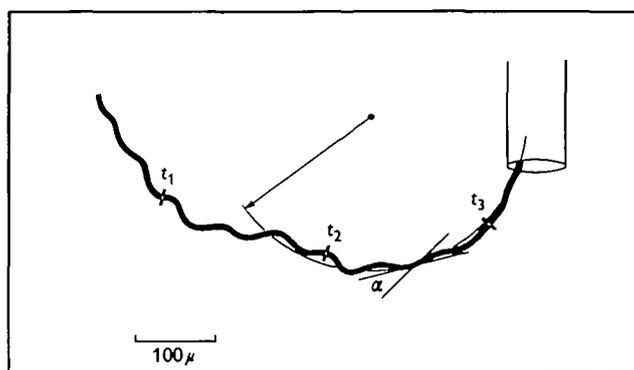
Many tracks of bracken spermatozooids swimming in a diffusion gradient near the tip of a capillary containing sodium L-malate in agar were recorded. Some of these are shown in Pl. 7, figs. 1-3. Pl. 7, figs. 1, 2, are mainly of qualitative interest, as no information about the malate gradients in these experiments was available. These tracks show that a gradual turning towards the tip of the capillary is superimposed on an apparently unaltered normal forward movement. In the experiment depicted in Pl. 7, fig. 3, the capillary was rinsed in water before insertion into the sperm suspension and the diffusion of malate was calculated from equation (2). The exposure started 10 sec. after the beginning of diffusion and lasted 8 sec. The malate concentration curves at 10 and 18 sec. are shown in Text-fig. 1. These curves are drawn on a logarithmic scale, following Pfeffer's demonstration of the importance of the relative concentration gradient, $\text{grad log } C$. Although, as discussed in the preceding paper, the spermatozooids are sensitive to the bimalate ion, this distinction is experimentally unimportant at constant pH, where the gradient of the logarithm of bimalate concentration will also equal $\text{grad log } C$.

Text-fig. 1 shows that near the capillary tip there is a region where $\text{grad log } C$ is nearly constant. Calculation of $\text{grad log } C$ is most accurate in this region. To determine the relationship between turning and the gradient, tracks such as that in Pl. 7, fig. 2, where all the turning takes place in the region of nearly constant $\text{grad log } C$, are of special interest. These are seldom found, as the weaker, less accurately known peripheral gradients generally influence the motion of the spermatozooids, before they enter the region of more constant $\text{grad log } C$. This can be seen in

Pl. 7, fig. 3, where two of the three spermatozooids which reached the tip were travelling in approximately the right direction before they entered the region where their motion was obviously influenced by the gradient. Most records were of this type. However, in Pl. 7, fig. 3, the third spermatozoid reaching the tip, whose track has a smoother curvature, allows a very rough calculation of the relationship between turning rate and grad log C , Text-fig. 2. Assuming that the turning rate



Text-fig. 1. Concentration of malate (logarithmic scale) for times at the start and finish of the exposure in Pl. 7, fig. 3, calculated by equation (2), with $T = 571$ sec., $a = 19 \mu$, $C_1 = 0.5$ M, $C_0 = 10^{-8}$ M.



Text-fig. 2. Calculation of turning rate for a sperm track from Pl. 7, fig. 3. t_1 , t_2 , t_3 , indicate 0.86 sec. intervals; the average swimming speed is therefore about 250μ /sec. The curved track is approximately an arc of radius 200μ , corresponding to a turning rate of 1.25 rad./sec. From Text-fig. 1, grad log C is about 70 /cm., and the angle α between the gradient and the direction of motion of the spermatozoid, is relatively constant and about 30° . The average turning rate per unit gradient is therefore 0.035 rad. sec. $^{-1}$ /cm. $^{-1}$.

is proportional to the component of grad log C perpendicular to the direction of motion, the factor of proportionality is

$$\frac{\text{turning rate}}{\text{component of grad log } C} = 0.035 \text{ rad. sec.}^{-1}/\text{cm.}^{-1}. \quad (4)$$

No other tracks which could be treated in this manner were obtained, so that it has not been possible to test the assumption of proportionality for chemotactic orientation. This isolated result, when the spermatozooids show so much variation in behaviour, is, of course, of limited value.

The photographic records of chemotaxis and the malate diffusion curves calculated from equations (1) and (2) show that unmistakable chemotaxis is observed when grad log C is greater than 25/cm., and that no chemotaxis is detectable when grad log C is less than 10/cm. In other experiments, the number of spermatozooids aggregating at the tip of the capillary was counted. When C_1/C_0 is high, a rapid aggregation of spermatozooids occurs in the first 2–3 min.; this is followed by a sharp levelling off, and after 5–8 min., by a slow decline in the number of spermatozooids near the tip. At 6 min., grad log C calculated from equation (1) for the region near the tip is 10/cm. A value of grad log C greater than about 10/cm. is therefore required to observe chemotactic aggregation with Pfeffer's technique.

Observation of a 'difference threshold' for chemotaxis with the capillary technique is not inconsistent with the assumption of a linear relationship between turning rate and grad log C . To observe chemotactic aggregation at the tip of a capillary, spermatozooids must be 'trapped' by the diffusion gradient. The turning rate of a spermatozoid entering the region around the capillary tip must be great enough for the spermatozoid to complete its turn towards the tip before it swims out of this region (Pl. 7, fig. 2). The path of a spermatozoid near a capillary will depend on its swimming speed, its random approach to the region around the tip, its turning rate in a gradient, and the magnitude of the gradient. As information about all these factors is available, it should be possible to calculate under what conditions chemotactic aggregation will be observed; but the mathematics of this problem are formidable. However, inspection of the diffusion curves calculated from equation (1) suggests that Pfeffer's threshold value for C_1/C_0 , 30, is consistent with my measurements of the relationship between turning rate and grad log C .

(b) *Electrochemical orientation*

When a voltage gradient of more than 0.5 V./cm. is established in a sperm suspension containing *tris* buffer, pH 8.1, and 10^{-4} M sodium L-malate, the spermatozooids orientate and swim towards the anode. The voltage required is not noticeably altered by lowering the pH to 6.4 (sodium phosphate buffer, 0.01M), by a tenfold increase in the ionic strength of the suspension, nor by increasing the concentration of sodium L-malate to 10^{-3} M. When the concentration of sodium L-malate in the suspension is decreased, a higher voltage must be applied to cause orientation of the spermatozooids. With 10^{-6} M malate at pH 8.1, no orientation is

observed at 7 V./cm.; the effect of higher voltage gradients was not investigated because of the toxic effects of the electrolytic products arising at the electrodes.

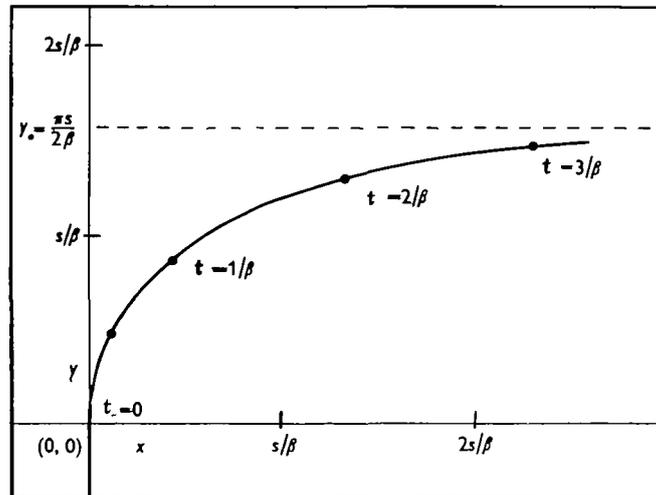
Sperm orientation occurs at 7 V./cm. with 10^{-4} M sodium L-malate or 10^{-4} M sodium maleate in the suspension, but not with 10^{-4} M sodium fumarate or 10^{-4} M sodium succinate (Brokaw, 1957). The chemical specificity of this phenomenon is, therefore, identical with that observed in classical chemotaxis experiments (Rothschild, 1956).

Voltage gradients in a sperm suspension can be manipulated more easily than chemical gradients, and therefore provide a more favourable situation for quantitative study of the response of spermatozooids to a gradient. Pl. 7, fig. 4, shows the response of several spermatozooids to a high voltage gradient applied at right angles to the direction of swimming. The spermatozooids were initially swimming towards the top of the plate under the influence of a voltage gradient in that direction. At the time indicated by the first gap in the tracks, this gradient was removed, which produced no response. At the time indicated by the second gap in the tracks, a horizontal gradient was applied. This experiment shows that the spermatozooids can turn very rapidly and also that there is much individual variability in their movements. The endosmosis of particles in the suspending medium, also seen in this photograph, indicates the exact direction of the voltage gradients. The turns do not show the consistent dependence on orientation within the path-helix indicative of a single-unit klinotactic mechanism, as in the photo-orientation of *Euglena* (Mast, 1938).

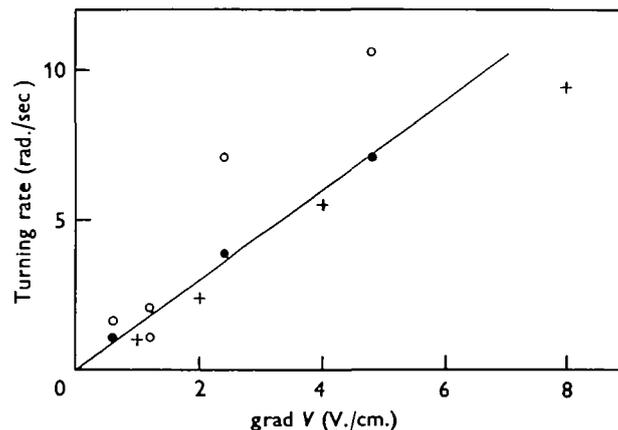
Responses to lower voltage gradients are shown in Pl. 7, figs. 5, 6. In Pl. 7, fig. 5, a spermatozoid was initially swimming towards the top of the plate from the lower left-hand corner. A horizontal gradient of 1 V./cm. was then applied, causing a slow turn to the right. A gradient of 2 V./cm. was then applied in the opposite direction, causing a turn of 180° . The turning rate is higher at the higher voltage and highest when the spermatozoid is swimming at right angles to the gradient. This suggests a direct proportionality between turning rate and the component of the voltage gradient perpendicular to the axis of the spermatozoid's helical path. If this is so, the expected shape of the curved track can be calculated (see Appendix 1) and is shown in Text-fig. 3. This is similar to the curves in the track in Pl. 7, fig. 5. Most tracks do not have exactly the form shown in Text-fig. 3, as the normal helical motion of the spermatozoid obscures the form of rapid turns at high voltage gradients; while at lower voltage gradients, the probability is high of a spermatozoid making random turns before completing orientation to the gradient. However, turns resembling that drawn in Text-fig. 3 are observed often enough to suggest that this is an accurate representation of the turning of the spermatozooids.

The relationship between turning rate and voltage gradient was also investigated by photographing a series of responses of an individual spermatozoid to various voltage gradients. Part of one series of results is shown in Pl. 7, fig. 6. This spermatozoid was initially swimming towards the top of the plate, on the left-hand side. A horizontal voltage gradient of 4.8 V./cm. was applied, followed by a vertical gradient of 0.6 V./cm., and then a horizontal gradient of 2.4 V./cm. The

higher voltage gradients caused sharper turns. Assuming that these turns have the shape shown in Text-fig. 3, the distance y_0 can be measured, and one can compute β , the turning rate when the spermatozoid is swimming at right angles to the gradient. Data for eight turns made by the spermatozoid in Pl. 7, fig. 6, and



Text-fig. 3. Predicted path of a spermatozoid orientating to a gradient applied in the x direction at $t=0$. At $t=0$, the spermatozoid is at $(0, 0)$, travelling in the y direction with speed s (see Appendix 1).



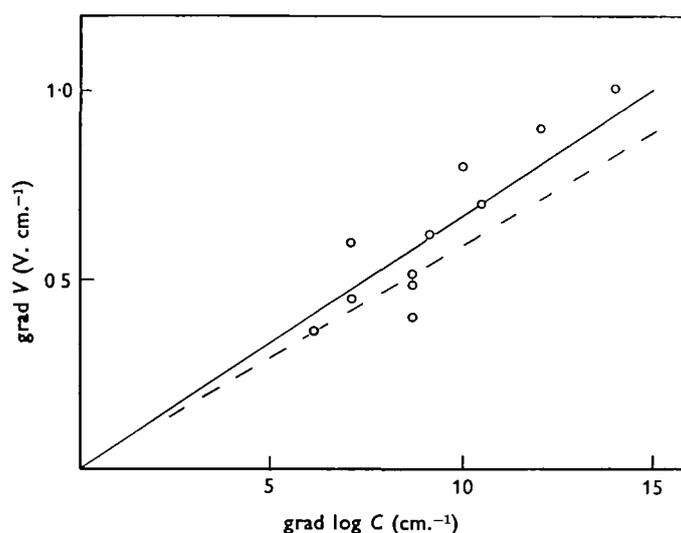
Text-fig. 4. Turning rates of spermatozooids reacting to voltage gradients. \bullet = turns shown in Pl. 7, fig. 4. \circ = other turns of this spermatozoid. $+$ = turns of another spermatozoid.

four turns made by another spermatozoid are shown in Text-fig. 4, where β is plotted against the applied voltage gradient, $\text{grad } V$. These results suggest that the assumption of a linear relationship between turning rate and the magnitude of the gradient is reasonable, and that, at least for these two spermatozooids,

$$\beta/\text{grad } V = 1.5 \text{ rad. sec.}^{-1}/V. \text{ cm.}^{-1}. \quad (5)$$

(c) *Combination of chemical and electric gradients*

A direct determination of the quantitative relationship between the responses to chemical and electric gradients was effected as follows: linear diffusion of malate was established between two drops of solution placed in contact on a glass slide, and two electrodes were arranged parallel to the boundary between the drops. The voltage across the electrodes was varied and the voltage which just neutralized the effect of the concentration gradient, so that the spermatozooids swam in random directions, was noted. The precision of the results is limited by the difficulty of establishing exact linear diffusion and of determining when the gradients are



Text-fig. 5. Voltage gradient required to neutralize chemical gradient. Solid line represents mean of observed points, dashed line represents the ratio predicted by equation (9).

balanced. Errors may also be introduced if the voltage gradient is not linear, due to variation in the depth of the drops. The diffusion junction potential and the effect of the applied voltage gradient on the diffusion of malate have been neglected, which may introduce second-order errors.

The drop of sperm suspension contained 2×10^{-4} M sodium L-malate and 0.005 M *tris* buffer, pH 8.1, and the other drop contained 2×10^{-3} M sodium L-malate and 0.005 M *tris* buffer. Debris in the sperm suspension indicated the boundary between the two drops and only those experiments in which a fairly straight boundary was obtained were continued.

In this linear diffusion situation there will be a fairly broad region on the low-concentration side of the boundary between the drops, where grad log C is roughly constant near its maximum value. In these experiments, the applied voltage was raised until the spermatozooids began to swim across the region of maximum grad log C into the drop of lower malate concentration; the voltage was then lowered

until the spermatozooids began to swim in the opposite direction; the mean of these two voltages was recorded together with the time elapsed after joining the two drops. The results from four successful experiments are shown in Text-fig. 5, where the applied voltage gradients ($\text{grad } V$) are plotted against $\text{grad log } C$ calculated by equation (3) for the region of maximum $\text{grad log } C$ at the time of the determination. These results provide some support for the assumption of a linear relationship between responses to chemical and electric gradients; the factor of proportionality between the two gradients for equal effects, $\text{grad log } C/\text{grad } V$, is about 15.

The same result was obtained when the experiment was repeated with malate concentrations of 2×10^{-5} and 2×10^{-4} M in the two drops.

This result is compared with two other estimates of the factor of proportionality between the gradients in Table 1.

Table 1. *Summary of data on the equivalence between the effects of chemical and electric gradients*

Experiment	Result in electric gradient	Result in chemical gradient	$\frac{\text{grad log } C}{\text{grad } V}$
Measurement of minimum gradient required for observation of tactic response	0.5 V. cm. ⁻¹	10 cm. ⁻¹	20
Measurement of rate of turning of individual spermatozooids in a gradient	1.5 rad. sec. ⁻¹ /V. cm. ⁻¹	0.035 rad. sec. ⁻¹ /cm. ⁻¹	42
Direct balancing of voltage gradient and diffusion gradient	—	—	15

DISCUSSION

These results indicate that there is a close relationship between chemotaxis and electrochemical orientation. When a spermatozoid is in a solution containing bimalate ions, a voltage gradient produces an effect which the sensory elements of the spermatozoid cannot distinguish from a concentration gradient of bimalate ions.

One effect common to these two situations is a net flux of bimalate ions. The velocity of a univalent anion exposed to a concentration gradient and a voltage gradient will be (Höber, 1950)

$$v = -2.3D \text{ grad log } C + u \text{ grad } V, \quad (6)$$

where v = net velocity of the ion, D = its diffusion coefficient, and u = its mobility. Since, at 25° C.,

$$u/2.3D = f/2.3RT = 17, \quad (7)$$

where f = faraday, R = the gas constant, and T = absolute temperature, the movement of bimalate ions will be neutralized by an applied voltage gradient when

$$\text{grad log } C/\text{grad } V = 17. \quad (8)$$

However, to do this, the direction of the voltage gradient must be the reverse of that applied to prevent chemotaxis in the experiment described above. The spermatozooids cannot, therefore, be sensitive to the flux of bimalate ions. However, the numerical agreement between the experimentally determined ratios (Table 1) and that in equation (8), and the appearance of $\text{grad log } C$ in these equations, suggests that some physicochemically similar sensory effect is involved.

A possible explanation is that the sensory elements of the spermatozoid adsorb bimalate ions. A body carrying reversibly adsorbed bimalate ions will, in a voltage gradient, experience a force tending to move it towards the anode. In a bimalate concentration gradient it will experience a force tending to move it up the gradient (see Appendix 2). Such forces could most simply cause an orientating response if bimalate ions were adsorbed on the anterior end of the spermatozoid, so that the forces would directly pull the anterior end around until the spermatozoid was swimming up the gradient.

This suggestion can be treated more exactly by considering a model consisting of a sphere of radius $\rho = 5 \mu$. A small region on the surface of the sphere contains 'bimalate combining sites' which reversibly adsorb bimalate ions. The sphere moves at constant speed through the solution with the adsorbing region in front. Its tactic responses are the sum of this constant movement and the independent orientation of the sphere caused by forces acting on the adsorbing region.

If the effect of viscous drag is not considered, the force acting on the model when it is moving at right angles to a gradient will be (see Appendix 2)

$$\text{turning force} = N(2.3RT \text{ grad log } C + f \text{ grad } V), \quad (9)$$

where N = moles of adsorbed bimalate. The factor $f/2.3RT$ also appears here, agreeing in both direction and magnitude with the observed relationship between the tactic effects of chemical and electric gradients (Table 1). Using equation (9) and Stoke's law for the frictional resistance of a rotating sphere, it can be estimated that about 10^5 bimalate ions would have to be adsorbed to give the model the turning rate given by equation (5).

However, when viscous effects are considered, the force predicted by equation (9) will not be attained, because when the sphere turns it will drag along with it the layer of solution near its surface. In a voltage gradient, the movement of the excess cations in this layer will produce a retarding force, and the resultant net movement must be calculated by the quite different approach used for electrophoresis (Abramson, Moyer & Gorin, 1942). When this is done, the estimate of the number of bimalate ions needed to turn the model is increased to 10^7 – 10^8 . 10^8 ions is probably close to an upper limit for the number of bimalate ions which could be adsorbed on the anterior surface of a spermatozoid.

In a concentration gradient, the movement of the surface layer of the solution along with the sphere means that the adsorbing surface is not really moving relative to the gradient, as implied in the derivation of equation (9), and the force will therefore be less. Since both these effects are due to the movement of the surface layer of solution, they may, perhaps, be of similar magnitude, so that equation (9) will still

explain the experimental results. Further information about the physical chemistry of an adsorbing body in a gradient is needed before it can be concluded that the behaviour of this model will duplicate the tactic behaviour of bracken spermatozoids.

Previous students of the chemotaxis of fern spermatozoids have held that turning is a result of alterations in the activity of the flagella (Hoyt, 1910; Metzner, 1926). However, no mechanism describing the relationship between flagellar activity and malate concentration has been offered which is consistent with all the following observations.

(1) No observable change in swimming speed or any other parameter of general flagellar activity is found when a spermatozoid is turning and moving up a gradient (see Pl. 7, figs. 1, 2).

(2) There is a linear relationship between turning rate and $\text{grad log } C$ or $\text{grad } V$, without a measurable threshold.

(3) There is no delay, at least in responding to a voltage gradient (see Pl. 7, fig. 4).

(4) The sensitivity to a voltage gradient requires bimalate or other chemotactically active ions.

The hypothesis suggested here, in which turning is independent of flagellar activity, satisfies all the above conditions, and is also consistent with the following observations.

There is no evidence that malate is metabolized by bracken spermatozoids.

The ratio between the gradients required for equal tactic effects is in agreement with equation (9): subject to further information about the validity of equation (9).

SUMMARY

1. The chemotaxis of bracken spermatozoids involves their precise orientation in a gradient of bimalate or a few other similar ions. When a voltage gradient is established in a sperm suspension containing bimalate or other chemotactically active ions, a similar orientation is observed, causing the spermatozoids to swim towards the anode.

2. Photographic records of sperm responses reveal a linear relationship between turning rate and the component of the gradient perpendicular to the direction of swimming.

3. Estimates have been obtained of the ratio of the magnitudes of the two types of gradient required to produce an equal tactic response.

4. The results suggest that the sensory elements of the spermatozoids adsorb bimalate ions. The reversible adsorption of bimalate ions on 'bimalate-combining sites' on the anterior end of a spermatozoid might fully explain its tactic behaviour, without requiring any modification of flagellar activity by bimalate.

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APPENDIX 1

Calculation of the path of a particle moving in a plane at constant speed and turning at a rate determined by the angle between its direction of motion and a fixed direction

The basic assumptions are contained in the following differential equation:

$$d\theta/dt = -\beta \sin \theta, \tag{i}$$

where θ = angle between the direction of motion of the particle and a fixed direction, t = time, and β = a constant, the turning rate when $\theta = \frac{1}{2}\pi$. If the fixed direction (the direction of the gradient) is along the positive x axis, and the particle is at (0, 0) at $t=0$, moving along the y axis with speed s , the equations

$$\left. \begin{aligned} \tan \frac{1}{2}\theta &= \exp -\beta t, \\ y &= s \int_0^t \sin \theta \, dt, \\ x &= s \int_0^t \cos \theta \, dt, \end{aligned} \right\} \tag{ii}$$

determine the curve. Integrating and eliminating θ ,

$$\left. \begin{aligned} y &= s/\beta (\frac{1}{2}\pi - 2 \tan^{-1} (\exp -\beta t)), \\ x &= st - s/\beta \ln \frac{1}{2}(1 + \exp -2\beta t). \end{aligned} \right\} \tag{iii}$$

The curve in Fig. 3 can be drawn from (iii). For large t ,

$$y_0 \equiv \lim_{t \rightarrow \infty} y = \pi s/2\beta. \tag{iv}$$

y_0 can conveniently be measured to determine β .

APPENDIX 2

Prediction of the behaviour of an adsorbing surface in a concentration gradient

Consider first the case of a cylindrical surface of circumference c , extending through the interface between two immiscible liquids, A and B (Text-fig. 6a). If γ_A is the interfacial tension between liquid A and the surface of the cylinder and γ_B is that between liquid B and the surface, a force $-F$ will be required to hold the system in equilibrium. When the cylinder is allowed to move a distance δx through the interface against this force at equilibrium, the work done will be

$$\delta W = -F \delta x. \tag{v}$$

The interfacial tension of the surface element moved through the interface will change from γ_B to γ_A , corresponding to a change in surface energy

$$\delta E = c \delta x (\gamma_A - \gamma_B). \tag{vi}$$

Since, for the process at equilibrium,

$$\delta E + \delta W = 0, \quad (\text{vii})$$

$$-F = c(\gamma_B - \gamma_A). \quad (\text{viii})$$

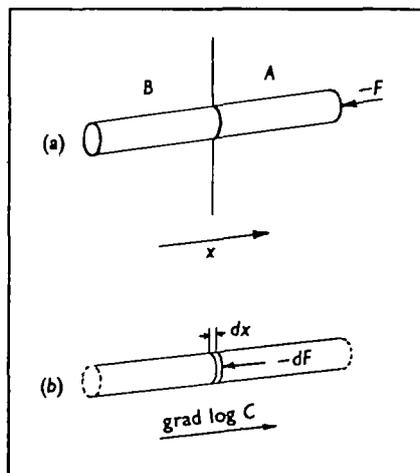
$-F$ will be positive if $\gamma_B > \gamma_A$, so that the cylinder will tend to move through the interface into the liquid of lower interfacial tension. This has been qualitatively demonstrated (Freundlich, 1926) and the quantitative relationships are straightforward.

Consider now that A and B are two solutions containing a solute which is adsorbed on the surface. The interface will be replaced by a stiff membrane which fits closely around the cylinder. The interfacial tension between the surface and the solutions will depend on the concentration of solute, according to the Gibb's adsorption equation

$$\Gamma_A = -\partial\gamma_A/\partial\mu_A, \quad (\text{ix})$$

where μ_A = chemical potential of the solute in A , and Γ_A = excess number of moles of solute per unit area adsorbed on the surface in A . If the solute concentrations in A and B are not too different, the force required to hold the cylinder in equilibrium will be, in the same way,

$$-F = c(-\Gamma_B\mu_B + \Gamma_A\mu_A). \quad (\text{x})$$



Text-fig. 6. Forces on adsorbing cylinder.

If the surface of the cylinder is nearly saturated with an adsorbed solute, Γ will not vary much with concentration, and

$$-F = c\Gamma(\mu_A - \mu_B). \quad (\text{xi})$$

Since

$$\mu_A = \mu_A^* + 2.3RT \log C_A, \quad (\text{xii})$$

where μ_A^* is a constant,

$$-F = c\Gamma 2.3RT (\log C_A - \log C_B). \quad (\text{xiii})$$

The cylinder will, therefore, tend to move through the membrane into the solution of higher concentration, since the higher concentration lowers the interfacial tension.

If the membrane is removed, a gradient of solute concentration will be established but the same force should be available. If a small element of surface of the cylinder $c dx$, is moved a distance δx ($dx \ll \delta x \ll 1$) along a gradient at equilibrium (Text-fig. 6b), the change in surface energy will be

$$\delta E = (c dx) \delta \gamma = -(c dx) \Gamma 2 \cdot 3 RT \text{ grad log } C \delta x, \quad (\text{xiv})$$

and the force, $-dF$, to hold this surface element at equilibrium will be

$$-dF = c \Gamma 2 \cdot 3 RT \text{ grad log } C dx. \quad (\text{xv})$$

If the adsorbing surface has total area A , with $\text{grad log } C$ constant over the surface, and

$$N \equiv \Gamma A, \quad (\text{xvi})$$

the total force tangential to the adsorbing surface required to prevent the surface from moving up the gradient will be

$$-F = N 2 \cdot 3 RT \text{ grad log } C. \quad (\text{xvii})$$

This equation will not hold for a surface of arbitrary shape, but will be satisfactory for a small element of the surface of a sphere, as needed for equation (9).

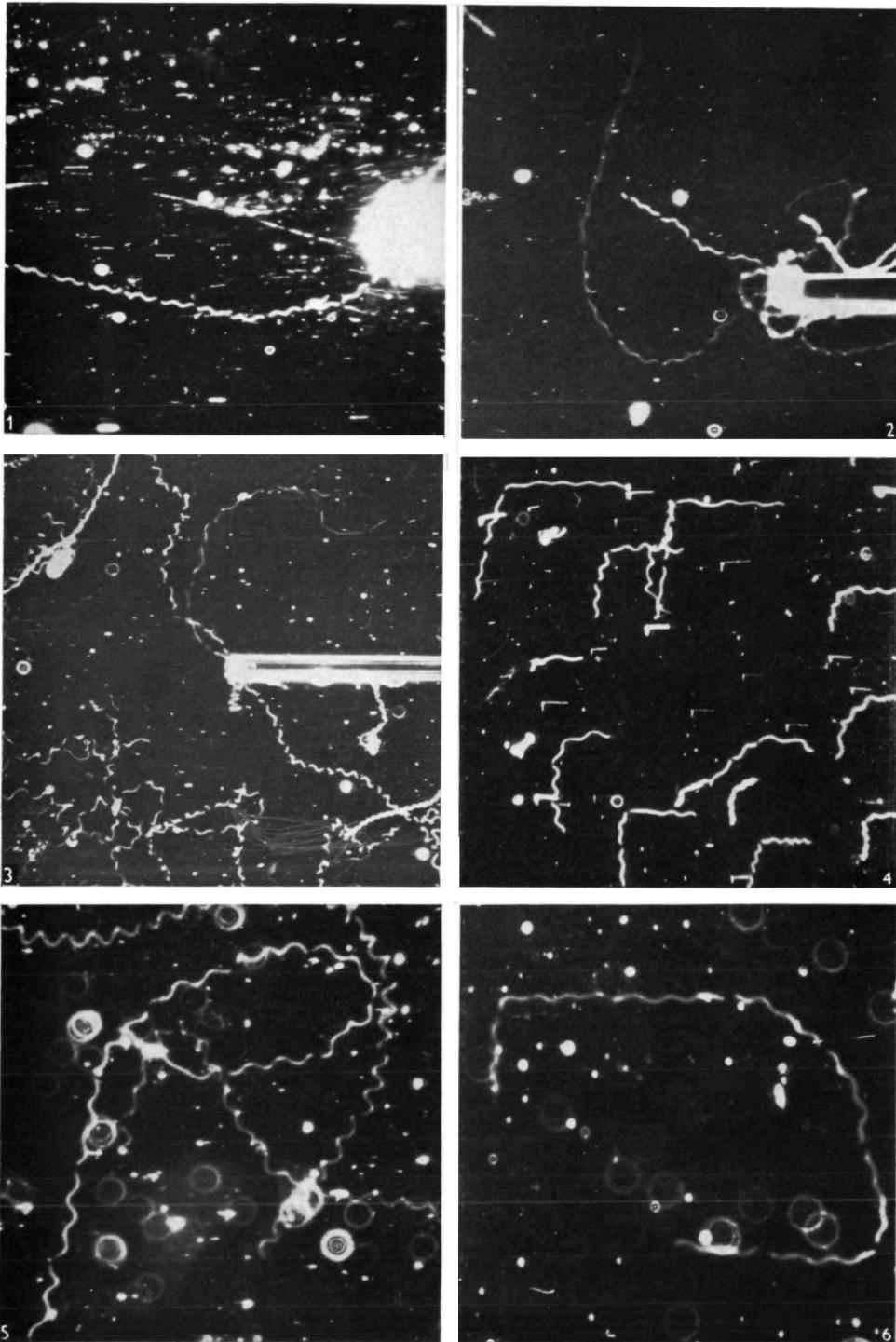
When the membrane is removed, the surface layers of the solution will no longer be prevented from moving along with the surface, and the surface will not actually move relative to the gradient, as stated above. No calculation of the magnitude of this effect is available and no experimental work on this problem is known.

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EXPLANATION OF PLATE 7

- Figs. 1, 2. Examples of sperm tracks approaching tip of a capillary source of sodium L-malate. Time intervals of $\frac{1}{4}$ sec. are indicated by gaps in the tracks. Direction of swimming, towards tip, was determined by observation during exposure. $\times 60$.
- Fig. 3. Tracks of three spermatozooids approaching the tip of a rinsed capillary source of sodium L-malate, 10–18 sec. after the start of diffusion. Gaps in the tracks producing an asymmetric sequence of increasing lengths indicate the direction of swimming and the time scale. Time for one cycle of the sequence = 0.86 sec. $\times 45$.
- Fig. 4. Tracks of spermatozooids reacting to a strong voltage gradient. Gaps in the tracks indicate changes in the applied gradient (see text). $\times 45$.
- Figs. 5, 6. Tracks showing the responses of individual spermatozooids to several changes in the voltage gradient (see text). Gaps in the tracks indicate time intervals of 1 sec. $\times 75$.



BROKAW—CHEMOTAXIS OF BRACKEN SPERMATOZOIDS.

(Facing p. 212)

