

## LETTERS TO THE EDITORS

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## 'Electro-Chemical' Orientation of Bracken Spermatozooids

THE chemotaxis of bracken spermatozooids, first demonstrated by Pfeffer<sup>1</sup>, has so far been investigated by introducing a concentrated source of a stimulating substance into a suspension of spermatozooids and

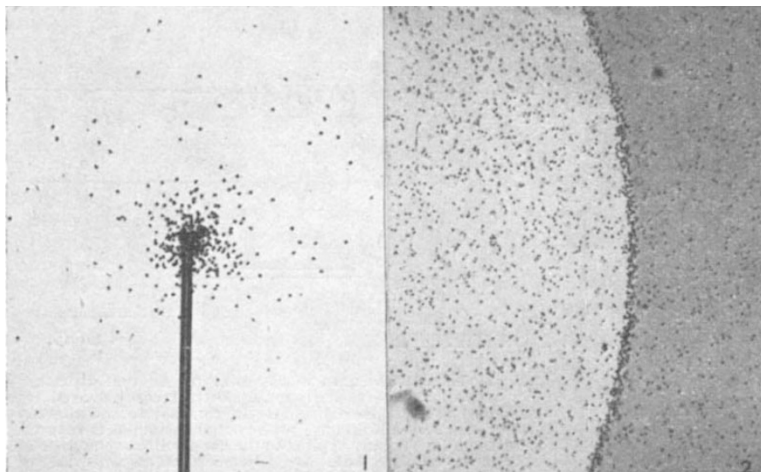


Fig. 1. Aggregation of spermatozooids at the tip of a capillary containing sodium L-malate. ( $\times 84$ )

Fig. 2. Aggregation of spermatozooids at border between buffered region (darkened by indicator) and acidified region. ( $\times 22$ )

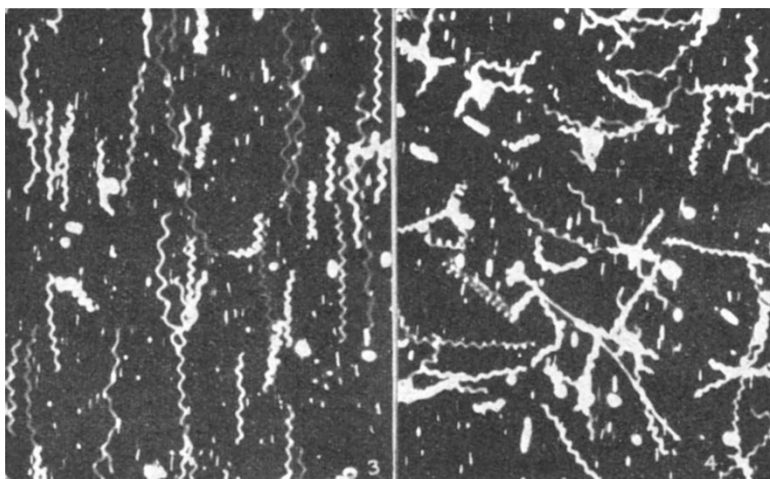


Fig. 3. Spermatozooids swimming to anode (at top) in electric field, in presence of  $10^{-4}$  M sodium maleate. Projection of helical paths recorded during 3-sec. exposure. ( $\times 50$ )

Fig. 4. With  $10^{-4}$  M sodium fumarate and same electric field, no orientation. ( $\times 50$ )

observing their behaviour in the resultant chemical gradient. In Pfeffer's original method, a fine glass capillary containing a solution of sodium L-malate provided the source; in these conditions spermatozooids aggregate around the tip (Fig. 1). Such experiments have revealed the highly directional character of the response<sup>2</sup>, its chemical specificity (salts of L-malic acid and a few closely related dicarboxylic acids are active)<sup>3</sup>, and that the response depends on the ratio between the applied and background con-

centrations in the suspension<sup>1</sup>. To permit more quantitative study of bracken sperm chemotaxis, a search has been made for conditions experimentally more favourable than those obtained with the classical methods of establishing chemical gradients. Two new phenomena, closely related to 'classical' chemotaxis, have been observed.

(1) When a small drop of 0.1 M hydrochloric acid is placed in contact with a sperm suspension containing  $10^{-4}$  M sodium L-malate in 0.005 M tris(hydroxymethyl) amino methane-hydrochloric acid buffer, pH 8.1, actively swimming spermatozooids aggregate in a band in the region of sharp pH change. The result of such an experiment, with an indicator added to locate the region of sharp pH change, is shown in Fig. 2. No such aggregation is observed unless sodium-L-malate is present. Similar results have been obtained with the sperm suspension buffered at various pH's, down to 0.01 M sodium citrate buffer at pH 5.7.

(2) If an electric field of 7 volts/cm. is established within a suspension of bracken spermatozooids when the suspending medium contains  $10^{-4}$  M sodium maleate or  $10^{-4}$  M sodium maleate in tris buffer (0.005 M, pH 8.1), the spermatozooids turn and swim directly towards the anode (Fig. 3). No reaction is observed if the concentration of sodium L-malate is less than  $10^{-6}$  M; nor if  $10^{-4}$  M sodium succinate is substituted for maleate; nor if  $10^{-4}$  M sodium fumarate is substituted for its isomer, sodium maleate (Fig. 4). The chemical specificity of the 'electro-chemical' reaction is, therefore, identical with that observed when classical methods of establishing chemical gradients are used.

As might be predicted from this experiment, the chemotaxis of bracken spermatozooids in a chemical gradient can be inhibited by application of the appropriate electric field. No differences in the morphology of the rapid and precise orienting responses occurring in these various situations have been detected, either by photographic path records of the type shown in Figs. 3 and 4, or by direct observations at higher magnification.

Further details of these experiments will be published in due course.

I am indebted to Sir James Gray for allowing me to work in his Department, and to Lord Rothschild for suggesting an investigation of this problem.

C. J. BROKAW\*

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<sup>1</sup> Pfeffer, W., *Unter. Bot. Inst. Tübingen*, 1, 363 (1884).

<sup>2</sup> Rothschild, Lord, *Internat. Rev. Cytology*, 1, 257 (1952).

<sup>3</sup> Rothschild, Lord, "Fertilization" (Methuen and Co., 1956).