Reports

"Bioconvection Patterns" in Cultures of Free-Swimming Organisms

Abstract. The moving polygonal patterns in dense cultures of *Tetrahymena* and other ciliates and flagellates look like "Benard cells," but are not due to thermal convection. They seem to be due to a similar dynamic instability that occurs when the energy input is internal and mechanical. The high concentration in the patterns may be useful in fertilization.

The purpose of this report is to call renewed attention to the physics of the curious streaming patterns observed in dense cultures of free-swimming organisms. Such patterns are well known for several different kinds of ciliates and flagellates—bull sperm (*1*) *Euglena* (*2*), *Tetrahymena* (*3*), and *Arbacia* larvae (*4*)—but there seems to have been no theoretical analysis and little systematic experimental analysis, except in the work of Loefer and Mefferd (*3*), on *Tetrahymena pyriformis*.

In a *T. pyriformis* culture 1 or 2 cm deep and a few centimeters across, in an open or closed vessel, the organisms are concentrated into flowing patterns of polygonal shape, as seen from above. Individual organisms swim upward in the centers of the polygons and move outward a few millimeters to the edges of the polygons where they fall to the bottom in dense, almost vertical sheets or skeins. The size of the polygons and the sharpness of their boundaries depend on the density and age of the culture, but the density of organisms in the falling sheets may reach the order of 100 times the density in the centers of the polygons. Loefer and Mefferd showed that the liquid is in motion in these sheets, carrying the organisms (and inert particles as well) faster than the individuals can swim. They also showed that the patterns are formed only when the depth of the culture is several millimeters or more and when the average density of motile organisms is more than 150,000 per milliliter. Loefer and Mefferd further showed that the patterns are unaffected by *p*H, temperature, osmotic pressure, anaerobic conditions, ultraviolet, surface-active chemicals, viscosity, or age, except as these factors affect motility. The speeds of fluid motion are enough to carry an individual organism to the bottom and back to the top in a time of the order of 15 to 30 sec; the patterns change completely in this time, and after stirring they re-form in this time or less.

A number of additional observations and experiments have now been made on the patterns in *T. pyriformis*. They show that surface-tension explanations can be ruled out, because of (i) the random angles of intersection of the polygon edges with each other and with the walls, (ii) the fact that the pattern shapes are indifferent to underfilling or overfilling of an open glass container, and (iii) the fact that they are indifferent to glass cover slips covering or half-covering the solution. Magnetic fields of 9000 gauss had no effect on the shapes of the patterns or on their rate of formation or change either in an ordinary *T. pyriformis* culture or in a conducting 1-percent NaCl solution of an NaCl-adapted strain. Strong light, heat, or electrodes (a-c) push the patterns "away," but this can be explained as the effect of thermal convection on already formed patterns.

Possible explanations of the patterns might include (i) directed motion of individuals due to exhaustion of oxygen or nutrients in the centers of the polygons; (ii) thermal convection (suggested by N. Spratt); (iii) viscous attachment of individuals (suggested by M. Sussman) or reduction of swimming on collision (suggested by F. Child), with the consequent formation of dense aggregates which fall; (iv) an essential dynamic instability in a group of randomly free-swimming organisms heavier than water (like the instabilities in a group of hovering helicopters if some get in the downdraft of others); or (v) dynamic instability due to density inversion when heavy organisms tend to swim to the top of the liquid (like the instability and downstreaming patterns observed when heavy powder is sprinkled on a water surface) (suggested by R. Donnelly).

The first explanation seems unlikely because of the speed of self-stirring of the solutions and the indifference of the patterns to anaerobic conditions. The second explanation is supported by the close resemblance of the polygonal patterns to the "Benard cells" of classical thermal convection experiments; and in fact the patterns might well be called "bioconvection patterns." But this explanation is ruled out by a crucial experiment of R. Donnelly's, in which he placed a culture dish of *T. pyriformis* on ice and found that the patterns re-formed as rapidly as before, in spite of the negative (stabilizing) thermal gradient (*5*). The third explanation seems unlikely because there is no evidence for it under the microscope (*6*), and because washed cultures in pure water (with any high-viscosity filaments presumably removed) still show the same patterns.

The fourth or fifth explanations seem the most probable. They are supported by the resemblance to "Benard cells" (thermal instability), especially in the requirement of a minimum critical depth and a minimum critical density for formation of the patterns, the density of motile organisms being presumably the biological analogue of the energy input in the thermal case. The patterns can also be stabilized, like Benard cells, by mechanical obstacles in the solution, such as glass rods and plates and bubbles, or strings hanging from the top surface of the culture. Studies of *T. pyriformis* in a sucrose solution as dense as the organisms, or denser, might permit a decision between the last two alternatives.

Dynamic instabilities leading to channeled streaming patterns are well known in the thunderstorm instabilities of meteorology and in the "rip tide" streams where ocean breakers push onto a straight shoreline (*7*). It is interesting to see that they may be of major importance in determining the aggregate and concentration behavior of living organisms where the energy input is from an internal metabolic source in each individual rather than from an external source. Possibly the Chandrasekhar theory of thermal instabilities in hydrodynamic systems can be modified to explain these bioconvection patterns and their sizes, veloc-

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Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to contributors" [*Science* **125**, 16 (1957)].

ities, and critical parameters. The increased concentrations in bioconvective streams might be important in fertilization and in initial streaming and schooling behavior in many small marine organisms (*8*).

JOHN R. PLATT*
Marine Biological Laboratories, Woods Hole, Massachusetts

References and Notes

1. L. Rothschild, *Nature* **163**, 358 (1949).
2. W. J. Robbins, *Bull. Torrey Botan. Club* **79**, 107 (1952).
3. J. B. Loefer and R. B. Mefferd, Jr., *Am. Naturalist* **86**, 325 (1952).
4. L. Rebhun, unpublished data.
5. R. J. Donnelly, unpublished data.
6. P. Dunham, unpublished data.
7. W. Bascom, *Sci. American* **203**, 81 (1960).
8. This problem was called to my attention by Burr Steinbach and Nelson Spratt. I am indebted to the colleagues mentioned, and to a number of others, for helpful discussions; I wish to thank Philip Dunham for the preparation of several *T. pyriformis* cultures, Leonard Rebhun for cultures of *Arbacia* larvae, and Irvin Isenberg for assistance with the magnetic field experiments. The work was assisted by a grant from the U.S. Public Health Service.
* Present address: Physics Department, University of Chicago, Chicago, Ill.

12 January 1961

Recording of Single Unit Activity in Isolated Central Nervous Tissue

Abstract. The retina and attached segment of optic nerve isolated from the rabbit were maintained in a functioning state in vitro. Microelectrodes, introduced into the nerve, recorded unit discharges in response to light stimuli. The characteristics of these evoked discharges are described.

Nervous tissue that has been isolated from the host and is being maintained in a suitable incubation medium offers three advantages for experimentation not shared by the same tissue studied in the intact animal: (i) Its chemical and physical milieu can be precisely defined and easily altered. (ii) Certain metabolic processes can be monitored continuously during an experiment. (iii) The tissue can be removed without trauma at any instant for anatomical or chemical analysis. To be most useful, such an in vitro preparation must have been isolated without irreversible damage, must be maintained in a nearly physiological state, and must be amenable to measurement of function.

Rabbit retina has been used as an example of central nervous gray matter that is suited to in vitro study. It can be isolated and maintained in an incubating fluid without significant shifts in intracellular electrolytes (*1*) and with maintenance of function as recorded by gross electrodes (*2*). The present report describes measurement of single unit activity in this isolated preparation.

Details of the methods used in isolating and incubating the tissue have been presented (*2*). In brief, an eyeball with 1 cm of optic nerve is removed from an anesthetized, dark-adapted rabbit; and, as rapidly as possible, the retina and the attached segment of nerve are separated, under cooled medium, from the other tissues of the eye. The nerve is mounted on two 24-gauge platinum wires, one tied to the cut end of the nerve and the other encircling it near the disk. Besides their use as gross electrodes, these wires serve to suspend the preparation in the incubation medium and to fix the nerve for the introduction of the microelectrode. The medium resembles cerebrospinal fluid in electrolyte composition, is equilibrated with 5 percent CO_2 and 95 percent O_2, and is maintained at 30°C.

In the experiments reported here, the microelectrode was of tungsten, sharpened electrolytically to a tip diameter of less than ½ μ and insulated with vinyl lacquer according to the method of Hubel (*3*). It was advanced by means of a micrometer drive into the nerve about 4 mm from the disk at an angle of 45° from normal. One of the gross electrodes served as electrical reference. The signal was led through a high-input-impedance cathode follower to a preamplifier, having a band

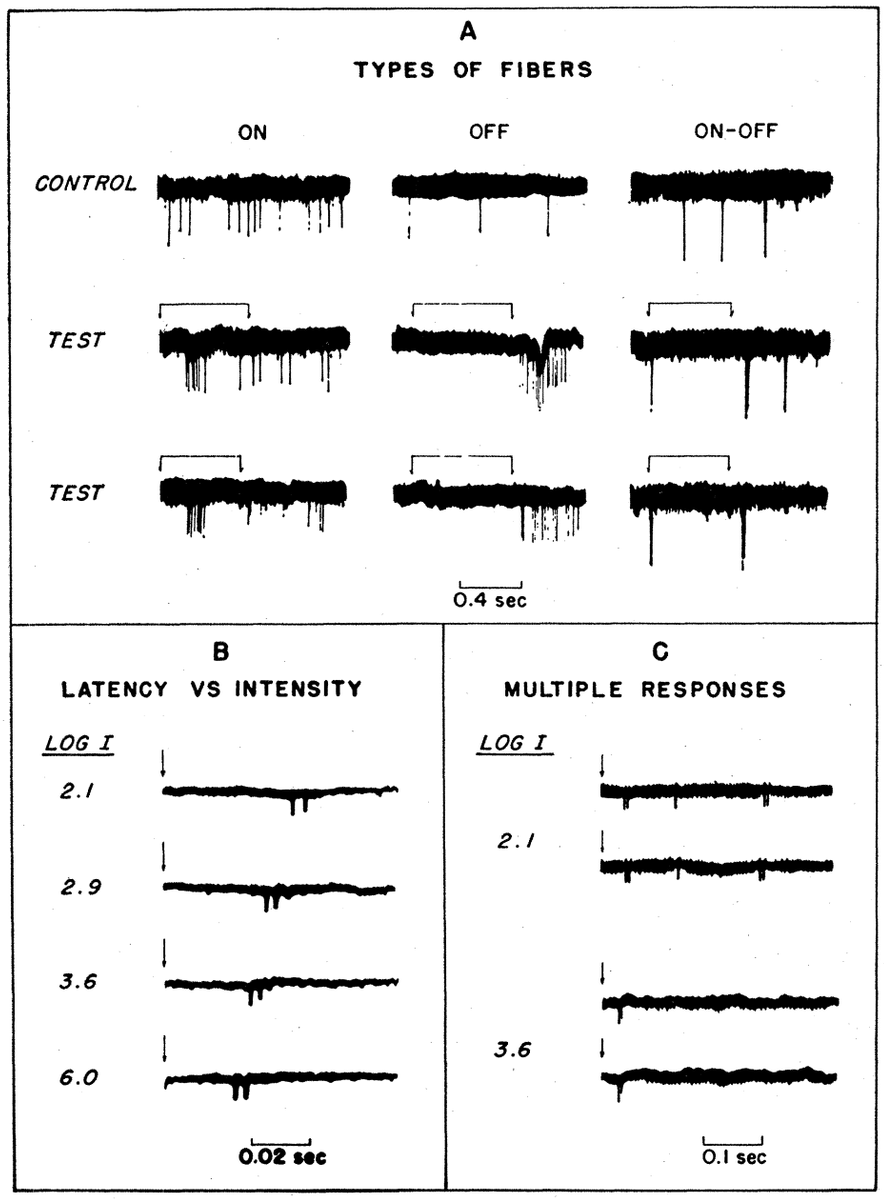

Fig. 1. (*A*) Spontaneous and evoked activity recorded from three fibers of different response types. (*B*) Relation of response to stimulus intensity in an "on" fiber. (*C*) Multiple responses following stimuli of low but not of high intensity. Durations of the longer light stimuli are indicated by horizontal lines above traces in (*A*). The times of presentation of the short (about 10 μsec) light stimuli are indicated by arrows in (*B*) and (*C*).