(SP) than control (C) fish (SP < C), whereas positive values denote faster learning in SP fish (SP > C) (*14*). A differential effect of scotophobin may be seen on light-avoidance and dark-avoidance learning (*15*). This differential inhibition or facilitation varies over the period of conditioning. When averaged across days (Fig. 2), the effect may also be seen.

It is unlikely that the effect of scotophobin should be equally strong at all dosage levels used; however, we have not considered it appropriate in this report to omit any doses or experiments in which the effect was diminished or absent (*16*). Differential effects were observed with the lowest nominal dose (12.5 ng), and these effects were generally absent with the highest dose (120 ng). The optimum dose for facilitating dark-avoidance learning appears to be lower than the optimum dose for inhibiting light-avoidance learning.

Although we worked with the synthetic substance, we cannot of course say that the entire sequence of 15 amino acid residues is required for the activity we observed.

Caution is particularly indicated in "reading into," interpreting, or extrapolating from these data. We regard the work as evidence that scotophobin does have behavioral activity in fish; that this activity is at least consistent with that reported for mice; and that it interacts with learning in goldfish in a somewhat specific manner, either facilitating or inhibiting learning, depending on the task itself. Although a variety of mechanisms has been proposed whereby such an effect might occur (*17*), we do not at this time propose a mechanism mediating the effect.

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5. We thank G. Ungar for samples of synthetic scotophobin. These samples were synthesized by W. Parr and purified by G. Ungar.
6. The activity of synthetic scotophobin in goldfish was described at the 1971 annual meeting of the Society for Neuroscience [(R. C. Bryant, *Society for Neuroscience Abstracts* 1971)].
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11. In the first two experiments, both experimental and control injections contained approximately 0.1 percent (v/v) methanol, since the scotophobin sample obtained was in an aqueous stock solution containing methanol. In all other experiments, the vehicle was distilled water.
12. In this procedure, level of correct responding for the first day was typically 30 percent for light-avoidance controls and near zero for dark-avoidance controls; on the fourth day, the level for light-avoidance controls was approximately 80 percent and that for dark-avoidance controls was 50 percent.
13. Total scotophobin recipients: light-avoidance (LA) fish, 120; dark-avoidance (DA) fish, 91. Controls: LA, 60; DA, 42.
14. The measure is (Response/Fish)/(Trial). For example, if on a given session (day), a total of 17 avoidance responses was recorded for a single group of seven fish during ten training trials, the measure would be (17 Responses/7 Fish)/(10 Trials) = .243 (R/F)/(T).
15. Probabilities in all cases calculated by the two-tailed t statistic, with 18 degrees of freedom (light-avoidance experiments, 12; dark-avoidance experiments, 8). The values on which Figs. 1 and 2 are based were calculated from means of 20 separate experiments, not 20 individual fish.
16. Nominal doses used were 12.5, 25, 30, 50, 60, and 120 ng. Whether the full dose was administered in each case is not assured, since there is some uncertainty as to the degree of stability or nature of degradation of synthetic scotophobin (G. Ungar, R. Bowman, personal communications).
17. See W. L. Byrne, Ed. *Molecular Approaches to Learning and Memory* (Academic Press, New York, 1970).
18. Partially supported by the Tennessee Department of Mental Health and the U.S. Public Health Service. We thank Dr. L. A. Kepner, Dr. B. M. Kulig, Dr. F. Petty, and Dr. O. L. Wolthuis for criticism and discussion.

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Capillary Suction Test of the Pressure Gradient Theory of Amoeboid Motion

A contraction-hydraulic (rear contraction) theory has been proposed to explain the amoeboid movement of *Amoeba proteus* (*1, 2*), *Pelomyxa palustris* (*3*), and *Endamoeba invadens* (*4*), as well as certain other amoebae. In the contraction-hydraulic theory a positive pressure gradient is responsible for protoplasmic flow. Recently, Allen *et al.* (*5*) sucked the posterior ends of specimens of *Chaos carolinensis* and *Amoeba proteus* into capillary micropipettes subjected to a pressure reduction of 30 to 35 cm of water. When this pressure was applied, protoplasm flowed backward into the pipettes, but the pseudopodal flow continued in the opposite direction. Allen *et al.* assumed that only endoplasm was flowing into the pipette and that lack of reversal of pseudopodal flow ruled out the presence of a contraction-hydraulic mechanism in these pseudopods. The frontal contraction model (*6*) was proposed as an alternative mechanism. In support of their theory Allen *et al.* also cite the observation of Kanno (*7*) that sucking more than half the cytoplasm from the tail region of a proteus-like amoeba that is moving in a forward direction in a capillary still allows forward movement to continue.

A contraction-hydraulic mechanism explains the data of Allen *et al.*, since their pipettes must have withdrawn mostly or only ectoplasm (gel). They made the assumption that the pipette withdrew mostly or only endoplasm (sol) from the amoeba. The diameter of their pipettes prohibited insertion into the amoeba, and also prohibited the withdrawal of only endoplasm. As the ectoplasm was sucked into the pipette (arrow 1 in Fig. 1), gel was drawn in from the sides (arrows 2 and 3). Because of the structural viscosity (*8*) of the gel and its glutinosity, ropiness, and elasticity (*9*), gel should be drawn in from the sides. This will squeeze the sol and force it forward (arrows 4, 5, and 6). In this way a positive and not

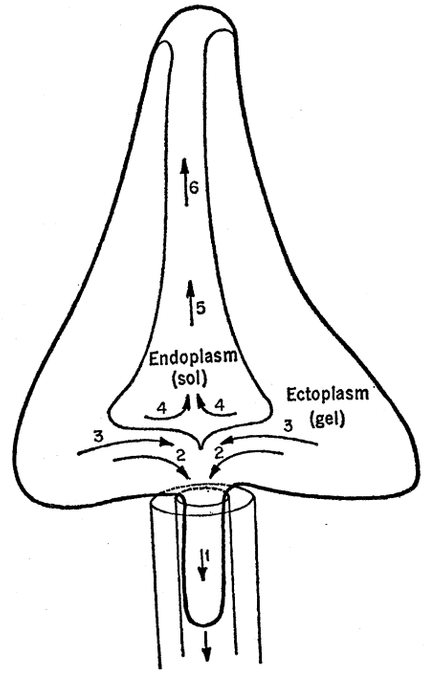

Fig. 1. Flow of ectoplasm and endoplasm according to the contraction-hydraulic theory.

a negative pressure is created in the area of arrow 4. Photographs of the flow in the region of arrows 2 and 3 were not shown by Allen *et al.* Therefore, flow in this area could equally well be assumed to be as we suggest (Fig. 1) than as suggested by Allen *et al.* The mechanism by which the sol is forced forward is essentially the same as that which involves the active contraction of the posterior gel, as in normal movement. Furthermore, Goldacre (*10*) tore a hole in the rear of an amoeba and protoplasm flowed outward; this contradicts the observation of Kanno. Therefore, *Chaos carolinensis* and *Amoeba proteus* should be included in the list of amoebae that locomote by means of a posterior contraction-hydraulic mechanism (*11*). The advantage of the contraction-hydraulic system over the frontal contraction system was pointed out by Jahn (*2*).

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15 March 1972

Observations of amoebae being sucked into a capillary by vacuum pressure were reported by Allen *et al.* (*1*) as inconsistent with Mast's hydraulic theory of amoeboid motion (*2*). Allen *et al.* found that sucking one pseudopod into a capillary does not prevent the extension of other pseudopods. They interpret this to mean that the streaming in the extending pseudopods "cannot be a result of a positive pressure gradient generated along the length of the stream"

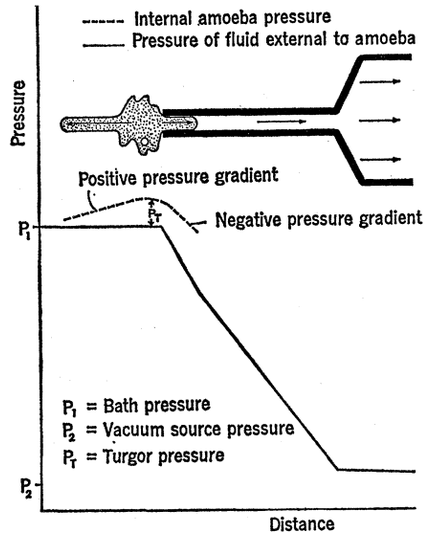

Fig. 1. Model of internal and external pressure distributions. The model is consistent with both experimental observations and the positive pressure gradient theory of amoeboid motion (not drawn to scale).

because the applied suction establishes a pressure gradient in the endoplasm of the opposite sign required. Although we have verified the laboratory observations, we find that alternative interpretations are tenable.

First, the vacuum pressure actually exerted on the advancing front of the amoeba within the capillary is considerably less than that indicated by the manometer in these experiments. The manometer is located adjacent to the vacuum source and responds to the pressure there. The pressure being exerted on the amoeba front is dependent upon the location of the front within the capillary (Fig. 1). The measured pressure difference will be distributed along the entire length of the capillary because the viscous shear stress at the tube wall resists the fluid flow (*3*). The resulting external pressure will be virtually equal to the bath pressure near the capillary orifice and will fall to virtually the manometer pressure only at the downstream end of the capillary. Thus a pseudopod being sucked into the capillary will certainly not be "subjected to a pressure reduction of 30 to 35 cm of water."

The positive pressure gradient theory of amoeboid motion, as proposed by Mast, suggests that the internal turgor pressure, being higher than the external atmospheric pressure, drives the endoplasm toward a local pressure reduction caused by local swelling of the plasmagel at the tip of an advancing pseudopod. If the turgor pressure of the amoeba is maintained throughout the sucking experiment, flow could be generated in any direction toward an area of reduced pressure at an advancing pseudopod tip, in accord with the pressure gradient theory. If the cited experiments are to provide "a direct test of the positive pressure gradient theory," it must be shown that turgidity is lost as a result of the suction. Because no instrumentation is available to measure the internal pressure, one can only guess at its magnitude and distribution. One might guess that the experimentally applied pressure difference, even though much less than the apparent difference of 30 to 35 cm of water, is sufficient to substantially reduce the pressure inside the cell. That this is not necessarily the case is suggested by the following analogy. A balloon full of water can be made to flow into a tube by the application of suction pressure, but if one punctures the surface, creating a local pressure reduction, the water will be caused by the turgidity of the balloon to flow out through the puncture. Even though the turgor pressure of an amoeba is generated by a different mechanism than that of the balloon, the analogy does describe how the pressure could be distributed within the amoeba to cause streaming away from the capillary by a positive pressure gradient while the amoeba is simultaneously being sucked into the capillary.

Figure 1 depicts an interpretation of the pressure distributions, both internal and external to the amoeba, that will account for the experimental observations and is consistent with the positive pressure gradient flow theory. The turgor pressure is defined as the difference between the local internal pressure and the bath pressure. The external pressure distribution indicated is for laminar flow of a Newtonian fluid in a capillary; this type of flow is known from hydrodynamics to be linear and of the form shown (*3*). The internal pressure distribution is that predicted by Mast's theory. The amoeba is made to flow into the capillary under the influence of the negative external pressure gradient along the capillary. It is being pushed into the capillary by the pressure difference between the bath and the capillary interior. Because the turgor pressure is maintained, the pseudopod extends under the influence of the internal positive pressure gradient and may or may not make net

progress away from the capillary tip depending on the speed with which the amoeba flows into the capillary.

This interpretation of the pressure events is certainly not the only plausible one, but because it does satisfy the observations and is consistent with the positive pressure gradient theory of pseudopod extension and retraction, it points out that the cited suction experiments do not constitute a direct test of the hydraulic flow theory but are inconclusive in that regard. Even though the observation that the applied suction "rarely showed any detectable effect on the streaming pattern except in the immediate vicinity of the capillary orifice" can be interpreted as evidence that the negative pressure gradient is established only in the vicinity of the orifice, since no effect is noticed elsewhere; it would seem that directly testing the theory on this basis awaits the deveopment of a method to measure the internal pressure distribution.

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22 March 1972

In their critique of our capillary suction test of the pressure gradient theory of amoeboid movement, Jahn and Votta (*1*) have attempted to substitute a diagrammatic, hypothetical interpretation from a "thought experiment" for well-documented, detailed observations of living amoebae under actual experimental conditions. We stated that our major conclusion was based on many experiments in which the endoplasm was observed (not assumed) to flow into the capillary.

In our report we did point out that a result somewhat similar to Jahn and Votta's hypothetical result [figure 1 in (*1*)] can be achieved by sucking a pseudopod into a tube of slightly smaller diameter so that it is squeezed; this forces the endoplasm to flow forward. As we stated in our report, however, this situation results in the formation of spherical rather than cylindrical pseudopod tips. The outflow of cytoplasm from a tear in the tail of an amoeba, a result less reproducible in our hands than in those of Goldacre (*2*), does not tell us anything about the possible existence of a pressure gradient inside the cell.

The critique of Kirby *et al.* (*3*) is gratifying in that our experiment was repeated and the observations verified. On the other hand they suggest [figure 1 in (*3*)] that virtually the entire pressure drop in our experiments may have taken place along the capillary into which the endoplasm of the amoebae was sucked. This suggestion is demonstrably incorrect.

The pressure drop, Δp, along a capillary of radius r and length L containing a fluid of viscosity μ flowing at the volume rate of flow Q can be estimated quite precisely from the Hagen-Poiseuille equation (*4*), shown below with the appropriate values inserted:

$$\Delta p = \frac{8\mu LQ}{\pi r^4} =$$

$$\frac{(8)(0.015)(2)(25 \times 10^{-8})}{(3.14)(6.25 \times 10^{-10})} = 30.6 \text{ dyne/cm}^2$$

Taking the pressure difference applied as 30 cm of water (2.94×10^4 dyne/cm^2), the pressure drop along the capillary tubing applied to the amoeba amounted to 0.1 percent of the reading of our pressure transducer, well within the accuracy claimed in our report.

The important fact to know is how the pressure drop occurs in the amoeba between the capillary orifice and the bath. The apparent rigidity of the amoeba's ectoplasmic tube and the freedom of the endoplasm to move out of it into the capillary suggest that the pressure drop should occur along the length of the amoeba unless our current views about amoeba structure are incorrect.

It is difficult to see how turgor pressure could be present in an amoeba unrestrained by a cell wall and containing a contractile vacuole to maintain water equilibrium. Even if turgor pressure were present, it is difficult to understand how it might be controlled so as to generate the complicated pattern of pressure gradients required to explain the complexities of amoeboid movement.

The authors of both critiques have failed to recognize the significance of the fact that a "pressure sink" (regardless of its absolute magnitude) down which cytoplasm flows at a rate several times the largest volume rate of flow ever measured in the intact cell does not prevent pseudopods from extending away from the direction of suction. The evidence bearing on theories of amoeboid movement has been reviewed recently (*5*).

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26 June 1972

Memory Transfer: Correction

In our report "Interanimal memory transfer: results from brain and liver homogenates" (*1*) we noted a statistical and typographical error. The chi-square analyses as presented were calculated incorrectly and therefore should be ignored. Reanalysis of all statistical tests used in this experiment indicated no other error. Although these changes do not require reinterpretation of the results, we deeply regret any inconvenience to the readers.

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Reference

1. B. Frank, D. G. Stein, J. Rosen, *Science* **169**, 399 (1970).

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Because of clerical error this correction was not published when received.—Ed.