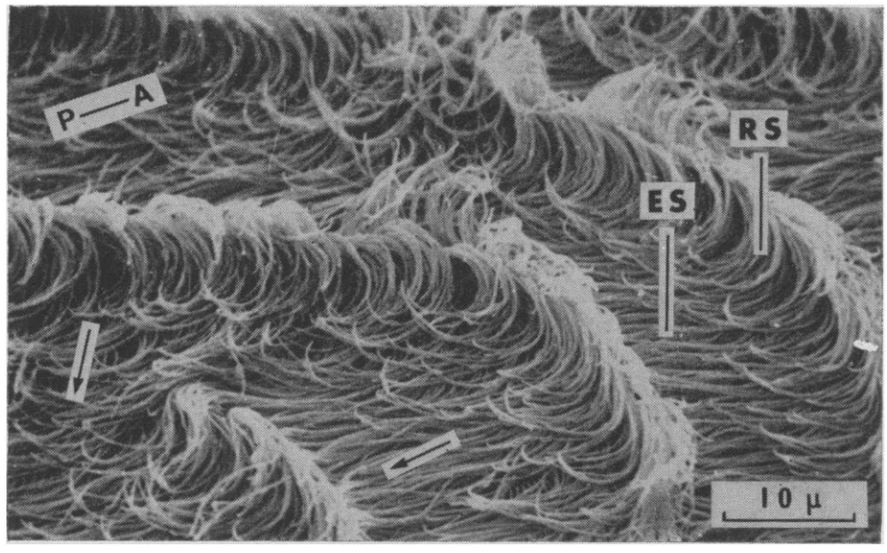

Fig. 2. Higher magnification of metachronal waves on a different animal, showing posterior side of waves. Arrows have the same meaning as in Fig. 1. *RS*, cilia in recovery stroke; *ES*, cilia in effective stroke; *P–A*, posterior-anterior axis.

stub allowed the organism to be viewed at 45° from different sides.

Opalina can change its direction of swimming by changing the direction of the effective stroke of ciliary beating, with corresponding changes in the direction of transmission of metachronal waves (*7*). This situation is illustrated in Fig. 1 in which the organism is fixed with waves traveling simultaneously in different directions. The wave pattern is similar to that in a living animal photographed while turning to the left by Sleigh (*8*). We find that the form of the waves is the same for waves moving in different directions.

The form of the ciliary beat and pattern of coordination in *Opalina* has recently been described with the use of photography of living animals (*8*) and also by the instantaneous fixation technique coupled with hematoxylin staining and light microscopy (*2*). We confirm Párducz's statement that ". . . contrary to Sleigh's description, the whole of the beating cycle involves more than a single plane . . . the cilium bends out of the plane of the effective beat after performing it, and during the greater part of the recovery phase it rotates counterclockwise, parallel to the body surface, gradually emerging into the preparatory position for the next stroke" (*2*, p. 107). The counterclockwise bending of cilia in the recovery stroke can be clearly seen in Fig. 2 (*RS* cilia). Apart from this large divergence from one plane, the general form of the beat is as figured by Sleigh (*8*).

In conclusion, the combination of instantaneous fixation, critical point drying, and scanning electron microscopy offers a powerful new method for the study of ciliary motion, not only in Protozoa, but in metazoan tissues as well. The introduction of the critical point drying technique greatly increases the usefulness of the scanning electron microscope for studying any material that suffers distortion by air drying.

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Microspikes on the Lymphocyte Uropod

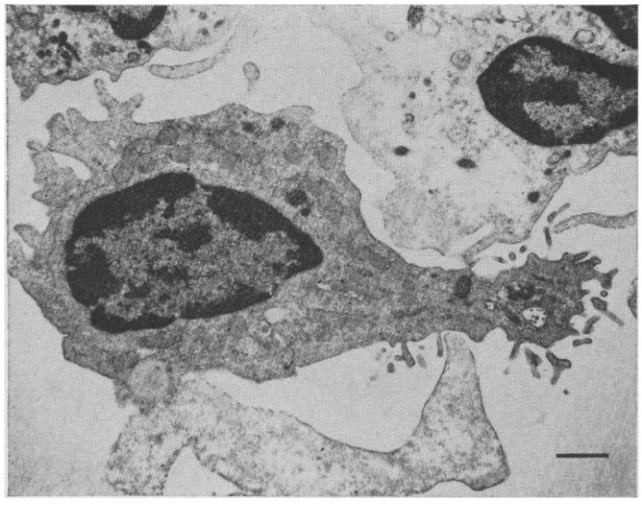
Abstract. *Lymphocytes have anatomic and functional characteristics reminiscent of the amoeba. The capacity to form microspikes on the uropod suggests a high degree of specialization essential to the lymphocyte's function in immunologic reactions.*

The motile lymphocyte in vitro has a characteristic configuration (*1*). Locomotion is essentially amoeboid, but the tail section of the cell is so prominent (*2*) that the cell can readily be distinguished from other motile leukocytes which may, irregularly and briefly, display a "tail."

Cinephotomicrographic studies of human lymphocytes in immunological reactions in vitro indicate that the cytoplasmic process forming the tail is used by the lymphocyte to contact and attach to debris, the surface of the culture vessel, and other cells in the environment (*3*). In many instances, as during interaction with macrophages, this attachment lasts for prolonged periods, even hours, with the tail process forming the connecting stalk. Thus the term "uropod" was applied to describe a process which was both a tail and a connecting stalk (*3*). By means of phase contrast microscopy, threadlike projections could often be seen extending from the uropod to contact neighboring objects. I have studied the uropod with electron microscopy to determine its structure in more detail.

Leukocytes (0.5×10^6) from two unrelated human donors were cultured in medium 199 containing 20 percent autologous plasma derived equally from the cell donors (*4*). On day 3, while the culture was maintained at 37°C, the supernate was decanted quickly, and 2.5 percent gluteraldehyde at 4°C was added gently and rapidly. The cell button was loosened with a rubber policeman, fixed in osmium tetroxide, and embedded in Epon for examination in an RCA EMU-4 electron microscope.

Most lymphocytes appeared round in cross section, suggesting a spherical shape with occasional regularly spaced microvilli projecting from the cell's surface. In some instances, the microvilli were so numerous that the lymphocyte appeared shaggy or burrlike. Occasional lymphocytes were elongated and had the configuration typical of the



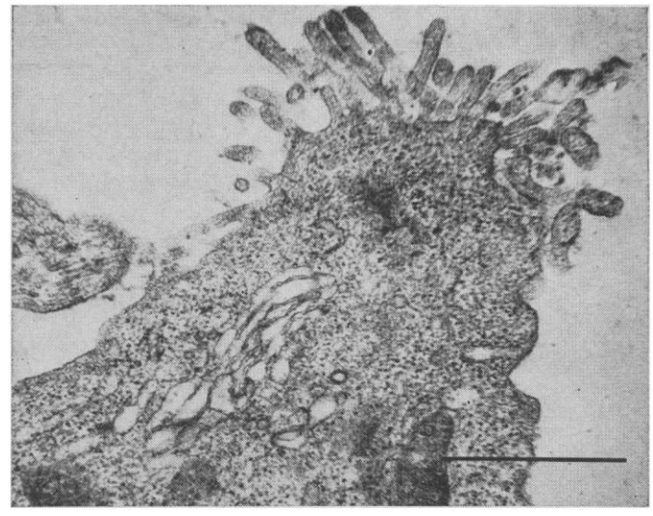


Fig. 1 (left). The motile lymphocyte in vitro has a characteristic hand mirror shape. Pseudopods and the nucleus are at the anterior end of the cell; the uropod with projecting microspikes forms the tail. (solid bar, 1.0 μ). Fig. 2 (right). Luxuriant microspikes on another uropod. Each microspike contains linear striations that are arranged radially in cross section (solid bar, 1.0 μ).

motile lymphocyte (Fig. 1). The pseudopods and nucleus are found at one end, usually the anterior end of a motile cell; the uropod comprises the posterior end. The greater portion of the cell membrane is devoid of microvilli, whereas the terminal portion of the uropod is studded with microvilli.

Figure 2 shows in higher magnification the terminal end of another uropod which contained approximately 78 microvilli, according to serial sections. In cross section these microvilli are 125 to 2500 Å in diameter, and lengths are variable up to approximately 0.8 μ. Cross section of one microvillus suggests a group of five or six radially spaced densities or cores which appear on longitudinal sections as linear striations. The internal structure and the general appearance of the processes imply a degree of rigidity. Thus they are consistent with microspikes described by Weiss (*5*) and further elaborated by Taylor and Robbins (*6*). They are active processes thrust from the cell membrane and are constantly subject to retraction or change in form. Taylor and Robbins felt that microspikes represented "real organelles which are admirably adapted to provide for tactile exploration of and selective attachment to solid structures within the immediate fluid environment of a cell process" (*6*).

Microspikes on the lymphocyte may be unique among cell processes. Microspikes are reportedly more common in the advancing cytoplasmic border of other mammalian cells in vitro, whereas in the motile lymphocyte they appear to be concentrated in the terminal uropod. As seen in time-lapse cine studies, as the motile lymphocyte passes debris or other cells, such as macrophages, the uropod is swung laterally on occasion to establish contact. On other occasions the lymphocyte backs up in order to contact a cell or debris with the microspike-laden uropod.

Uropods also contain microtubules, mitochondria, pinocytosis vacuoles, and rough endoplasmic reticulum. Microtubules are presumed to function in pumping interstitial matrix from the posterior to the anterior portions of the cell in the formation of pseudopods during locomotion (*7*). They are also involved in the extension of microspikes from the surface of the cell.

In addition to its manner of locomotion, the lymphocyte possesses other features which may be analogous to those of the amoeba. In amoebae, pinocytosis begins in the tail area and the lining of the pinocytosis vacuole consists of cell membrane which previously covered a portion of the tail section (*8*). If the same mechanism prevails in the lymphocyte, and the presence of pinocytosis vacuoles and multivesicular bodies in the uropod favors it, any materials, including antigens presumably, that may attach to receptor sites on the membrane of the uropod can be pinocytized early. The effect of the momentary but marked increase in the surface area of the uropod by a profuse outburst of microspikes may have to be considered in any estimate of receptor sites on lymphocytes. Interaction between the lymphocyte and macrophages, other lymphocytes, or lymphoblasts in vitro is by means of the uropod, suggesting that in the interest of efficiency the organ of attachment is also the main receptor organ.

Certain characteristics of lymphocyte behavior in vitro also apply to the situation in vivo. In experimental allergic neuritis, lymphocytes attach to the endothelial lining of blood vessels by means of the uropod before migrating through the vessel wall (*9*).

Thus, combined light- and electron-microscopic studies indicate that the lymphocyte possesses anatomic and functional features of unicellular organisms. Moreover, in some lymphocytes, certain features appear to have become highly specialized. It remains to be determined whether this high degree of specialization is the property of all cells recognized morphologically as lymphocytes.

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Hemicholinium-3: Noncholinergic Effects on Squid Axons

Abstract. *Hemicholinium-3, when applied to the inside of a squid axon, is effective in blocking the action potential. This action is not antagonized by the addition of choline or acetylcholine to the perfusate. Voltage-clamp experiments show that hemicholinium-3 depresses both the early transient and late steady-state components of membrane ionic conductances, with a greater effect on the peak transient component.*

Hemicholinium-3 (HC-3) inhibits effectively the synthesis of acetylcholine (*1*). This action of HC-3 has been attributed to a competitive inhibition of active choline transport across biological membranes (*2*). Hemicholinium-3 blocks impulse propagation in adrenergic (*3*) and frog sciatic (*4*) fibers. To decide whether HC-3 might have an effect on the sodium or potassium conductance change of nerve membranes which could explain its action on peripheral nerves without the necessity of invoking a cholinergic mechanism, we conducted experiments with single squid axons. We used standard microelectrode techniques in observations of membrane potentials and the sucrose-gap voltage clamp to measure ionic conductance changes.

Initially, we wanted to establish whether or not HC-3 had any effect on the resting and action potentials when applied either inside or outside the axon. Internal perfusion was carried out as follows (*5*). A giant axon was isolated and partially cleaned, its axoplasm was squeezed out by means of a small roller, and the crushed axon was inflated with an internal perfusate. All experiments with external applications were performed using isolated intact axon preparations. In all experiments the perfusion was continuous. The internal perfusate contained 400 mmole of K^+, 370 mmole of glutamate$^-$, 15 mmole of $H_2PO_4^-$, and 333 mmole of sucrose; the *p*H was adjusted to 7.3. Artificial seawater was used as the external bathing medium; it contained 449 mmole of Na^+, 10 mmole of K^+, 50 mmole of Ca^{2+}, 30 mmole of tris(hydroxymethyl)aminomethane, and 559 mmole of Cl^-, and the *p*H was adjusted to 8.0. A micropipette electrode was used to sense the resting and action potentials of the membrane. The maximum rate of rise of the action potential (*dv/dt*) was used as a measure of excitability because it is proportional to the inward current at that moment and is a more sensitive index than the height of the action potential. In those cases in which the resting potential slowly drifted, the measurements of the action potential and its rate of rise were made after the resting potential was brought back to its original level by application of an appropriate current through a separate microelectrode.

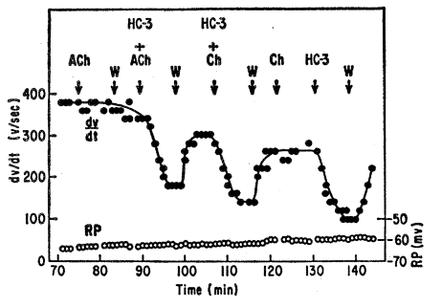

Fig. 1. Effect of internal applications of 1 mmole/liter of acetylcholine (ACh), choline (Ch), and hemicholinium-3 (HC-3) on the maximum rate of rise of the action potential (*dv/dt*) and the resting potential (RP); W refers to washing with normal internal perfusate.

Hemicholinium-3 had little or no effect on either the resting or action potential when applied externally in concentrations up to 10 m*M*. However, when 10 m*M* HC-3 was applied internally at this concentration the action potential was completely abolished with no obvious effect on the resting potential. Reducing the concentration to 1 m*M* still caused a significant depression of the rate of rise of the action potential. The effect was partially reversible with washing (*6*).

We repeated similar experiments with acetylcholine and choline chloride added to the perfusate to see whether these compounds exhibit any protective action against the blockage by internally applied HC-3 (Fig. 1). Choline (1 m*M*) or acetylcholine (1 m*M*) had no effect on the action or resting potential when given alone. When added to a 1 m*M* solution of HC-3 they did not inhibit the HC-3 action. These results indicate that the nerve blockage with HC-3 is not due to the lack of either choline or acetylcholine. However, the possibility that HC-3 is forming complexes with cholinergic structures in the membrane essential for

Table 1. Peak transient conductance (g_p) and late steady-state conductance (g_{ss}) before and after application of HC-3.

Preparation	Conc. (mmole/liter)	g_p (mmho/cm²)			g_{ss} (mmho/cm²)		
		Before	After	A/B	Before	After	A/B
			External application				
1	10	93	102	1.09	127	118	0.92
2	10	93	110	1.18	127	118	.92
3	10	91	83	0.91	105	92	.87
4	10	91	84	.92	105	86	.81
5	10	120	100	.83	108	83	.76
6	10	56	60	1.07	53	53	1.00
7	10	56	54	0.96	53	50	0.94
Mean				.99			.88
			Internal application				
1	10	96	27	0.28	76	62	0.81
2	10	96	23	.23	76	61	.80
3	10	100	30	.30	97	49	.50
4	10	114	33	.28	125	78	.62
5	10	129	43	.33	140	85	.60
Mean				.28			.66
6	1	113	79	0.69	125	89	0.71
7	1	101	71	.70	119	97	.81
8	1	101	74	.73	98	80	.81
Mean				.70			.77