THE CONCEPTS OF MEMBRANE FLOW AND MEMBRANE VESICULATION AS MECHANISMS FOR ACTIVE TRANSPORT AND ION PUMPING

By H. STANLEY BENNETT, M.D.

(From the Department of Anatomy, School of Medicine, University of Washington, Seattle)

Dr. Palade has shown that in many cells the cell membrane is infolded extensively and that such folds may carry the cell membrane to positions deep in the cell. Moreover, he has shown that such deep folds often appear to lie close to isolated vesicles in the cytoplasm. Dr. Palade has pointed out that such vesicles are often arranged so as to suggest that they might have formed from the pinching off of a recessed tip of such a fold, or that they might represent vesicles destined to coalesce with such a recessed fold.

Dr. Palade referred to the classical paper on pinocytosis by Lewis (8). Lewis showed cells in which the cell surface and adjacent cytoplasm were in a state of vigorous activity, with an orderly flow of granules and vesicles from one portion of a cell to another. Similar activity and orderly movements within cells have been demonstrated by Gey, Shapras, and Borysko (6), by Frederic and Chèvremont (5), and by Blandau, De Marsh, and Ralph (1).

Dr. Palade's pictures suggest that in such cells as macrophages, in which pinocytosis and orderly flow of cytoplasmic particles are evident, the cytoplasmic membranes of the endoplasmic reticulum, particularly those components representing membranes folded inwards from the outer cell border, might participate in or even mediate these activities.

I would like to introduce the hypothesis that membrane flow may be an important part of a type of active transport mechanism carrying particles, including ions, along, within, into, and out of cells. If membrane is being formed or synthesized in one region, and is being broken down or enzymatically destroyed at another, it would be expected to flow from the membrane source to the membrane sink or site of breakdown. If the source is at the exposed cell surface, as at A and A' in Fig. 1, and the sink is deep in the cell as at B (Fig. 1), the membrane would flow from the surface to a position deep in the recess as indicated by the arrows. A membrane flow in the reverse direction would result from a source at the tip of the recess at B and a sink on the surface at A or A'. Membranes entirely within the cell or entirely on the exposed cell surface or extending through the cell as tunnels or slits could be envisioned as similarly motivated to flow from sources to appropriately placed sinks. The energy required for such kinesis would be provided by oxidative mechanisms in the cell.

As an alternative or additional mechanism to be invoked in membrane movement or flow, one may hypothesize that some membranes may have contractile properties, such as have been demonstrated in monolayers of actomyosin by Hayashi (7).

Dr. Palade has shown that cytoplasmic membranes are often closely associated with mitochondria or other particles or granules of a size resolvable with the light microscope. A moving or flowing membrane might transmit some of

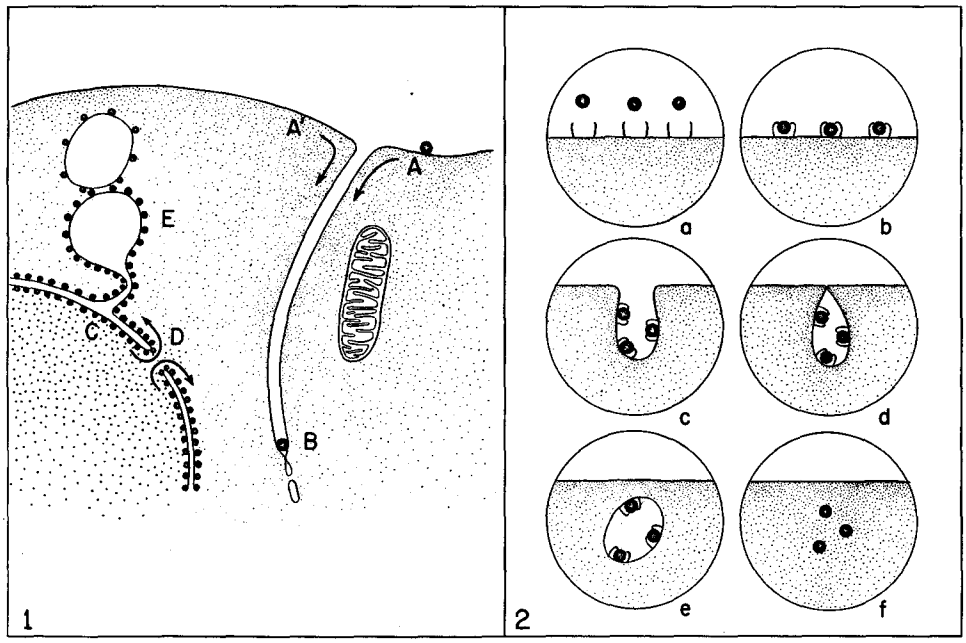

FIG. 1. Diagram representing the concept of transport by membrane flow. For explanation see text.

FIG. 2. Diagram representing the concept of transport by membrane vesiculation. For explanation see text.

its energy of motion to a nearby particle and carry it along by viscous drag or by some other type of linkage. This may account for the orderly parade of mitochondria and other particles seen passing in definite pathways to and from centers of cell activity such as the cell center. Such orderly progression has been observed by Blandau, De Marsh, and Ralph (1) in motion pictures of blood leucocytes. Vesicles, droplets, or globules of fluid or viscous material imprisoned in bulbous cisternae between pairs of such membranes could also be translated across the cell if paired membranes flowed in the fashion postulated. This would provide a mechanism for the movement of fluid droplets within cells seen by Lewis (8) and by Gey, Shapras, and Borysko (6).

Let us assume that points on the exposed cell surface might display ion exchange groups, or hydrogen bonding or other binding groups. Such points would then be able to tie suitable particles to the external surface of the membrane. The particle might be a calcium ion bound by means of side chains capable of appropriate chelation; or a water molecule tied to hydroxyl groups on the cell surface by hydrogen bonding; or a protein molecule or other particle, large or small, tied to the membrane by some sort of a specific or non-specific linkage which would depend in part on the molecular configuration at that point. Such a particle would be carried along by the moving membrane. An example of such a particle is represented by the small circle on the outside of the cell membrane of Fig. 1 at *A*. A flow of the membrane as indicated by the arrow would carry the particle deep into the cytoplasm to a position *B* at the bottom of the fold. Here the particle might be included in a vesicle pinched off from the tip of the recess, as postulated by Palade, and thence be moved off to some other portion of the cell.

A possible corollary of this membrane flow hypothesis may be derived from Watson's (13) recent demonstration that the inner and outer nuclear membrane are continuous at the edge of the "nuclear membrane pores," and that the outer nuclear membrane is often reflected into the cytoplasm of the cell, becoming continuous with the endoplasmic reticulum. These continuous membranes are often studded with the particles of Palade (11), which appear to contain ribonucleoprotein. Some of the ribose nucleic acid newly formed in the nucleus might become attached to the inner nuclear membrane in a position indicated by *C* (Fig. 1). A flow of this membrane might then take place, carrying the particle along to a pore, out of the pore at *D* to the outer nuclear membrane, and thence out onto the ergastoplasmic portions of the endoplasmic reticulum represented by *E*. This hypothetical flow might provide one of several mechanisms for transmission of nucleoprotein from nucleus to cytoplasm.

Dr. Palade's concept of vesiculation of cell membranes can be invoked in conceiving of a second mechanism whereby particles could be transported. The extracellular fluid may contain particles (such as ions or protein molecules) capable of engaging binding sites on the cell surface. Such particles and binding sites are represented in Fig. 2 *a*. As a result of appropriate collisions and engagement of specific complementary groups, the particles would become bound to the external surface of the membrane (Fig. 2 *b*). If the cell responded by causing the membrane to invaginate in the region of the bound particles, the portion of the membrane bearing the particles would soon be located in a recess or pocket or caveola intracellularis of Yamada (14), as shown in Fig. 2 *c*. Further development of the process would cause the invaginated stretch of membrane bearing the particles to be pinched off (Fig. 2 *d*), and then isolated from the free cell surface membrane, as represented in Fig. 2 *e*, and as postulated by Palade (9). If the isolated membrane of the vesicle were then

broken down by cytoplasmic enzymes, the particles would be free within the cytoplasm, as represented by Fig. 2 *f*.

This process could just as well proceed in reverse order. Particles or ions in the cytoplasm as at *f* could be selected by the cell. The cell could then synthesize or form a membrane around them, as at *e*. The particles might first be bound to proteins by means of groups capable of specific binding, and the proteins carrying the particles be incorporated in the membrane of the vesicle represented at *e*. The vesicle could then be moved by the cell towards a portion of its surface membrane, made to fuse with the surface membrane as at *d*, and then caused to open out to the surface as at *c* and *b*. The particles could then be liberated to the surrounding extracellular fluid, perhaps as a consequence of a destruction or change in the binding side chains resulting from appropriate enzymatic activity.

This membrane vesiculation mechanism for transfer across cell membranes represented in Fig. 2 has a number of interesting features. First, it provides a mechanism whereby particles can move from one side of a membrane to the other without going through the membrane. Second, it makes it unnecessary to postulate any holes or pores in a membrane in order to explain the passage of particles from one side of a membrane to the other. Third, specificity of binding groups on the membrane would permit a great selectivity in transfer operations. It is presented for consideration as one type of mechanism which might play a role in active transport.

Many features of this vesiculation mechanism have been conceived previously and were postulated by Palade (9) in 1953 in connection with his discovery of vesicles ranged along the inner and outer cytoplasmic membranes of capillary endothelial cells, and by De Robertis and Bennett (3) in relation to vesicles seen in Schwann cells. Evidence that this mechanism operates in mesothelial cells will be presented to this Conference by Dr. Odor (p. 105). Mr. James Hampton, a graduate student in our Department, has found that vesiculation figures in active transfer of colloidal particles by endothelial and parenchymal cells in the liver.

In conclusion, one is tempted to guess that membrane vesiculation might be a feature of certain ion pump mechanisms. The synaptic vesicles discovered by De Robertis and Bennett (2, 4) and by Palay (12) and Palade (10) might be manifestations of such a process. Here one can imagine acetylcholine or some other specific humoral agent active at the synapse to be synthesized in or transported to the synaptic terminal and there segregated in packets enclosed by the membranes of the synaptic vesicles. The corresponding ions of opposite charge might be excluded from the vesicle, and thus potential energy would be stored in electrostatic form, with the membrane serving as the dielectric medium of a condenser. The vesicle could then be moved to a position adjacent to the synaptic membrane, where a propagated disturbance could bring to

pass an opening of the vesicle to the outside with a liberation of its contents into the synaptic interspace. This could produce a surge of ions reflected by an electrical impulse.

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