Electron Microscopy of Solitary and Aggregated Slime Mould Cells

By E. H. MERCER, Ph.D., and B. M. SHAFFER, Ph.D.


PLATES 182 TO 185

(Received for publication, September 30, 1959)

ABSTRACT

Polysphondylium violaceum and Dictyostelium discoideum myxamoebae have simple double-layered nuclear membranes, a cytoplasmic reticulum of particle-covered membranes, and small mitochondria consisting of convoluted tubules tightly packed in double membranes. In addition to objects still recognisable as bacteria, their food vacuoles contain concentric (or spiral) membranes, apparently formed secondarily from undigested material; these are ultimately ejected.

Where the triple-layered plasma membranes (~70 A wide) of cells in the early aggregates are apposed to one another, they run parallel but separated by a layer of rather constant thickness (~200 A), as in many unspecialised metazoan tissues. Thus studies on slime moulds may well increase our understanding of cell adhesion and tissue formation in metazoa.

INTRODUCTION

The cellular slime moulds alternate between a vegetative phase, in which the myxamoebae are solitary, feed on bacteria, and multiply, and a fruiting phase, in which they flow in streams towards centres of aggregation, and then build stalked, aerial fruiting bodies. In Dictyostelium discoideum, the cell masses elongate immediately after aggregation and migrate as “slugs” over the substratum for some time before starting to make a stalk. Because of the virtual separation of growth and morphogenesis, these organisms have recently been studied to throw light on a variety of developmental processes of the widest biological significance, such as cell migration, cell differentiation, and regulation. Bonner (1959) gives further details and references, and Shaffer (1957 a) a general account of aggregation. Of particular importance is the alteration in cell properties, including an increase in mutual adhesiveness, that brings about the actual changeover from solitary to social, from protozoan to metazoan, a change that is, moreover, spontaneously reversible (Shaffer, 1957 b; 1958).

In the metazoa, the gametes are separate, and intercellular adhesiveness returns at fertilisation. At first it binds all the cells indifferently, but during development it becomes increasingly specific and restricted (Holtfreter, 1948 and earlier; Moscona, 1957). Cells that adhere may, nevertheless, move relatively to one another. When different cell species are mixed together, they may form a common aggregate at first and only subsequently segregate themselves (Townes and Holtfreter, 1955; Weiss and Moscona, in Weiss, 1958). In ascitic tumours, adhesiveness is again lost, and the cells revert to a separate existence. In the cellular slime moulds, cells in an aggregation stream or in a slug can flow past one another; and mixtures of different species and strains may form joint aggregations initially and sort themselves out later (Raper and Thom, 1941; Bonner, 1952; Shaffer, 1957; Bonner and Adams, 1958).

The electron microscope has thus far been relatively little used for studying intercellular adhesion. A single cell, as seen in sections of material fixed in buffered osmium tetroxide (Palade, 1952) or permanganate (Luft, 1956), is bounded by a dense line, less than 100 A thick,
which may be resolved at higher magnification into two dense lines enclosing a lighter area (Robertson, 1959), though the exact interpretation of this image is obscure. Over the greater part of the area in which two tissue cells are in contact, their dense surface membranes are parallel and closely apposed but separated by a poorly scattering region of the order of 100 to 200 A wide, though varying somewhat from tissue to tissue (Text-fig. 1). Any material in it must be of low density or dissolved during processing, and we may regard it either as an integral part of the cell surface, or as an extracellular secretion or exudate. It is convenient, however, to describe only the well-defined dense line as the surface on plasma membrane.

No systematic attempt has been made to correlate changes in cell adhesion in embryonic development with changes in the relationships of the plasma membranes. In some adult tissues that are renewed by cell replacement throughout life, such as skin and its derivatives, the electron microscope has shown that the plasma membranes of the newly divided and undifferentiated cells are somewhat convoluted, and readily separated during processing (Birbeck and Mercer, 1956, 1957). During differentiation they become smoothed out, and the intercellular layer acquires a definite width. Later, during keratinisation, dense specialised intercellular sheets are deposited. In other tissues, localised membrane thickenings (desmosomes) have been described (Sjöstrand and Anderson, 1954; Porter, 1956; Selby, 1956; Fawcett, 1958; Horstmann and Knoop, 1958; Odland, 1958) and interpreted as areas of enhanced adhesion (Weiss, 1958).

Our examination of the slime moulds differs from earlier ones made with the electron microscope (Mühlthaler, 1956; Gezelius and Ränby, 1957) in that our attention has been directed primarily to the plasma membrane in the solitary and the aggregated phase. But we shall also briefly describe the general cytology of the cells.

Species and Methods

Two species were examined: Dictyostelium discoideum and Polysphondylium violaceum. They were inoculated in limited areas of non-nutrient-agar plates spread uniformly with Aerobacter aerogenes or Escherichia coli, and kept at room temperature. Plates bearing the required stages of development were flooded with cooled fixative (1 per cent OsO₄ buffered at pH 7–8). After 2 hours at 0–5°C., the osmium solution was poured off, and the plates washed with water several times and then covered with water for 15 minutes. Small blocks of agar from selected areas of the cultures were cut out, dehydrated, and finally embedded in an araldite resin (Glauert, Rogers, and Glauert, 1956). One difficulty was that frequently all the older aggregation centres and streams, as well as some of the younger ones and the separate myxamoebae, floated off the surface of the solution when the plate was flooded. For the vegetative phase, the cells were grown on Bonner's nutrient agar. When staining with phosphotungstic acid (PTA) or uranyl acetate (UA) was desired, the agar blocks in 70 per cent or absolute ethanol were transferred for 30 minutes to 1 per cent solutions in ethanol of the same strength. Sections of the blocks were cut usually at right angles to the agar surface by means of a modified Cambridge or a Porter-Blum microtome and were examined in a Siemen's microscope.

Description and Discussion of Results

The following observations apply to both slime mould species.

The Vegetative Myxamoeba.—The cytoplasm is bounded by a dense plasma membrane 60 A thick, strongly stained by PTA or UA, and readily resolved into two dense lines enclosing a light area (Text-fig. 1 and Fig. 6). This membrane lacks the external fringe of fine threads found in Amoeba proteus (Pappas, 1959; Mercer, 1959).

The round nucleus (N in Figs. 1 and 4) is bounded by a simple double membrane, as in most metazoan cells and also (Mercer, unpublished) in the small solitary Acanthamoeba. There is no elaborate, thick, latticed inner membrane, as there is in Amoeba proteus (Pappas, 1959; Mercer, 1959), suggesting that perhaps this is a specialisation necessitated by the size of its nucleus—a biconcave disc 40 μ in diameter and 5 to 10 μ thick. Nucleolar material (nu in Figs. 1 and 4) is found within domed evaginations of the nuclear membrane in the myxamoebae and in Acanthamoeba, occasionally with deposits resembling ferritin (D in Fig. 1). A mitochondrion (m in Figs. 1, 4, and 5) is about 0.5 μ in diameter and consists of a bunch of convoluted tubules enclosed in a double membrane. A similar arrangement has been described in other protozoa (Sedar and Porter, 1955; Powers et al., 1956; Mercer, 1959; Pappas, 1959).

Bacteria may adhere closely to the plasma membrane and are sometimes seen partly engulfed as this folds round them. Within the food vacuoles, they are found in varying stages of digestion (Fig. 3), being gradually replaced by clusters of con-
centric or spiral membranes, which appear quite dense in living myxamoebae observed under phase contrast. Scrutiny of the morphology of these membranes makes it obvious that they are secondary formations not structurally continuous with the bacteria, though perhaps their material is derived from the bacterial wall. They resemble the phospholipid bodies noted in a wide variety of cells and also prepared artificially (Stoeckenius, 1959), but the intermembrane spacing, even in compact examples (such as seen in Fig. 2), is greater than in phospholipid preparations, although this may be influenced by the entrance of water. Ultimately, the membrane whorls are ejected from the cell (W in Fig. 1). However, in parts of some vacuoles, the limiting membrane breaks down (F in Fig. 1) and digested material presumably passes directly into the cytoplasm.

Contractile vacuoles (Figs. 4 and 5) range from several microns across down to 0.05 μ. The numerous small ones may coalesce with larger ones or grow independently. Mitochondria are not concentrated in their vicinity, as in A. proteus (Pappas and Brandt, 1958; Mercer, 1959), perhaps simply because the cell is too small.

The cytoplasmic ground substance of the myxamoebae, as also of Acanthamoeba, contains fine, dense, closely packed granules similar to those carrying RNA in mammalian cells (Palade, 1955). In contrast, the cytoplasm of Amoeba proteus lacks recognisable granules of this kind (Mercer, 1959) and so seems more open.

The myxamoebae also contain flattened sacs bounded by particle-covered membranes (Figs. 4 and 5). These structures, which are occasionally quite elaborately developed (Fig. 7) are similar to the basophilic endoplasmic reticulum (or ergastoplasm) commonly found in the cells of higher organisms; and there, at least, it appears to be involved in the synthesis and secretion of protein. No basophilic reticulum has yet been found in A. proteus.

**Late Aggregation Centres.**—The internal organelles of aggregated cells shown no obvious modifications. Partly digested bacteria (Fig. 5) are still not uncommon, and a cell deep within an aggregate has been found engulfing a bacterium.

Intercellular relationships, as may be appreciated from Figs. 4 and 5, strikingly resemble those in many epithelial tissues of multicellular animals. The individual myxamoebae fit tightly together, their plasma membranes being separated by only a narrow gap (~200 A). Gezelius and Råmy have given a contrary opinion, but their micrographs hardly allow of so definite a decision. The triple-layered structure of each individual membrane is still apparent in the aggregated cells (Fig. 6). Continuous adhesion as the cells move is emphasized by the manner in which the apposed membranes cling together in spite of the often very irregular convolutions of the surfaces of contact. Appearances very like these are to be found in the zone of differentiation in the mammalian hair follicle and in the epidermis. In contrast, in the growing tissues of higher plants, the intercellular gap widens rapidly and marked intercellular deposits are to be seen. However, we have not examined the later stages of slime mould development, in which the cells become encased in cellulose. The apposed plasma membranes of the myxamoebae also show many small areas of higher density (Figs. 4 and 5), which resemble developing desmosomes. However, in these early aggregates we have not seen any additional material either within the cells or between the membranes, such as is present in fully developed desmosomes.

This investigation was in part supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research, Royal Cancer Hospital) from the Medical Research Council, the British Empire Cancer Campaign, the Jane Coffin Childs Memorial Fund for Medical Research, the Anna Fuller Fund, and the National Cancer Institute, National Institutes of Health, United States Public Health Service.

The authors are particularly grateful to Mr. M. J. Docherty for making the photographic enlargements.
BIBLIOGRAPHY

Shaffer, B. M., 1957 *b, Quart. J. Micr. Sc.*, 98, 377 and 393.

EXPLANATION OF PLATES

All micrographs are of *Dictyostelium discoideum.*

PLATE 182

**Fig. 1.** A vegetative myxamoeba. *N,* nucleus; *NN,* nucleolus (here with dense deposits *D* which may be ferritin); *F,* food vacuoles. *FW* are vacuoles in which the undigested bacterial residues have formed concentric membranes; these whorl-like formations are also found outside the cell (*W*) after being ejected. *m,* mitochondrion; *M,* the plasma membrane. × 14,000.

**Fig. 2.** A well-developed mass of concentric membranes external to the cell, as at *W* in Fig. 1. The small vesicles (*v*) also seem to have been ejected by the myxamoebae, as they occur in the agar only in their vicinity. They too may be bacterial residues, as they resemble the vesicles resulting from the lysis of bacteria by bacteriophage.
Stain: uranyl acetate. × 30,000.

**Fig. 3.** Two food vacuoles in a vegetative myxamoeba to show stages in the digestion of bacteria. In one vacuole the bacterium (*B*) is recognisable and is seen to be surrounded by a few membranes; in the second the bacterial cytoplasm and nuclear structures have gone and the system of membranes is more elaborate (*W*). × 30,000.
(Mercer and Shaffer: Slime mould cells)
PLATE 183

Fig. 4. Portions of several cells in a premigratory aggregate. Note the tissue-like appearance and the close contacts of the cell membranes (M) (enlarged in Fig. 6). N, nucleus; m, nucleolar deposits; V, contractile vacuole; m, mitochondrion; R, small fragments of reticulum. × 30,000.
(Mercer and Shaffer: Slime mould cells)
Plate 184

Fig. 5. Similar to Fig. 4. Lettering as in that figure. F, food vacuoles showing whorls; G, a cluster of vacuoles. × 27,000.
(Mercer and Shafer: Slime mould cells)
**PLATE 185**

**Fig. 6.** The nature of the intercellular contact. Notice the triple-layered cell membranes (M) and the intercellular gap (G). m is a mitochondrion. Small dense particles crowd the cytoplasm. × 120,000.

**Fig. 7.** A well formed example of reticulum (R). m, mitochondrion; F, food vacuole; V, a contractile vacuole. Stain: uranyl acetate. × 31,000.
(Mercer and Shaffer: Slime mould cells)