Macrophage Membranes Viewed through a Scanning Electron Microscope

Abstract. *When rabbit peritoneal macrophages were cultured in serum and Tyrode medium, numerous membranous structures varying in size and transparency were observed to cover the surface in complex cascading patterns. Modifications in the methods of fixation and drying probably accounted for the new perspective of macrophage surfaces gained in this study.*

Visualization of the macrophage surface in the electron microscope (*1, 2*) showed the roughness of the surface and the presence of microvilli which had been sketched in the pioneering studies on this cell (*3, 4*). The first views of the macrophage surface in the scanning electron microscope (*5*) provided a new perspective of the morphology and function. The possibility of lack of detail and of distortion commented upon by Carr *et al.* (*5, 6*) suggested that modifications in the fixation and drying of the cells might provide greater clarity in the pictures from the instrument recently described (*7*) and made available to us (*8*). After fixing and washing the macrophage suspension, Carr *et al.* (*5*) had allowed the cells to air dry. We cultured the macrophages onto glass cover slips before fixation, and removed the water by either sublimation or drying with Freon.

White, male New Zealand rabbits (1.8 to 2.3 kg) were injected intraperitoneally with 50 ml of sterile mineral oil. Five days later the peritoneal cavity was washed several times with chilled Tyrode's solution. The tissue culture medium was composed of 20 percent normal rabbit serum and 80 percent Tyrode's solution, *p*H 7.4. The mononuclear cells were adjusted to 5×10^5 cells per milliliter, and 1-ml amounts were cultured for 18 to 24 hours in neoprene-stoppered flat-bottomed glass vials containing flying cover slips (*9*).

The macrophages were fixed in glutaraldehyde (Polysciences, Inc.) (2.5 percent by volume in 0.066*M* phosphate buffer, *p*H 7.4) for 30 minutes, washed three times in water distilled three times, treated with OsO_4 (1 percent weight per volume in phosphate buffer, *p*H 7.4), and washed three times in water distilled three times. The cells were frozen in liquid nitrogen, after which they were transferred to the cold (−60°C) stage of a Pearse tissue dryer (Edwards High Vacuum Ltd.). Under vacuum and cold temperature, the specimens were dried for 2 to 17 hours, after which the stage was warmed slowly (1 to 2 hours) to room temperature, and the specimens were removed. An alternate method of drying the cells was that of Cohn *et al.* (*10*) in which Freon (E. I. du Pont de Nemours and Co.) is used. The glass cover slips with the dried macrophages were then attached to metal disks with silver paint and subsequently coated with a thin film of gold in a vacuum evaporator. The specimens were then stored at room temperature until examined. A Cambridge Stereoscan Electron Microscope (Cambridge Instruments Company Ltd., London, England) operating at 10 or 20 kv was used to study the specimens. The pictures were taken with Polaroid film, type 107.

A variety of shapes and positions of the macrophage population, especially the variance in the degree of spreading on the surface of the glass, was observed. Functional variation among such spread cells has been reported (*11*). All of the cells appeared to have a rough surface composed of processes of various sizes. Although the cell in Fig. 1 was not extensively spread, the outline of a number of apparently intracellular spherical bodies (about 0.64 μm in diameter) of the same general size range and shape as lysosomes may be seen.

The macrophages appear to be attached to the glass by an extension of the membrane. The surface of this extension is smooth compared to that of the main body of the cell (Figs. 1 and 2). This footlike process may remain smooth-edged or it may send out microprojections (Fig. 2). The main body of the macrophage has an extensive series of membranes of various sizes and shapes on its surface (Figs. 2 and 3). These membranes appear to be identical to the "veil-like membranous pseudopodia" and "undulating membrane" described by Lewis (*3*) and Carrel (*4*), respectively. Carr *et al.* (*5*) referred to "flange-like processes" on the surface of the macrophage which may indeed be collapsed versions of the membranes seen in Figs. 2 and 3.

Rotation of the cell in Fig. 3 by approximately 90° counterclockwise revealed that the ridgelike areas on the

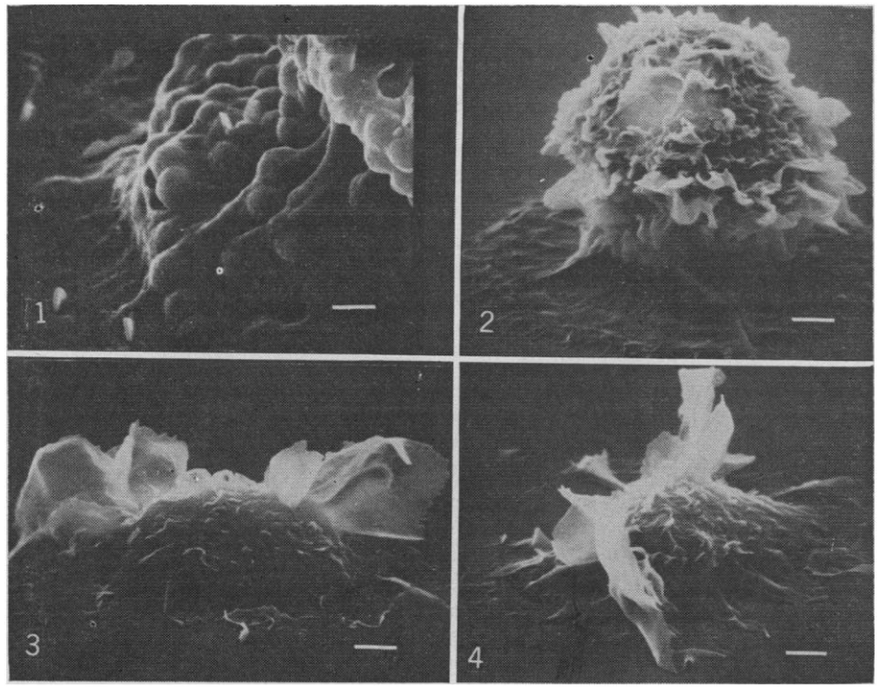

Fig. 1. Macrophage. Pearse dried. Sample at 55° tilt, × 6000 (scale, 1 cm). Fig. 2. Macrophage, Pearse dried. Sample at 55° tilt, × 3480 (scale, 1 cm). Fig. 3. Macrophage, Pearse dried. Sample at 55° tilt, × 3030 (scale, 1 cm). Fig. 4. Macrophage, Pearse dried. Same cell as in Fig. 3. Sample at 55° tilt, rotated approximately 90° counterclockwise, × 3240 (scale, 1 cm).

surface (Fig. 3) were actually small membranous structures (Fig. 4). Portions of the broad membranes were resting on the surface of the cover slip (Fig. 4) and were continuous with the main body of the cell.

Although the membranous structures on the surface of the cell appear well preserved and intact, there are areas at the edge of the cell, where the membrane is attached to the cover slip, that appear to have been torn from the cell. The cause of this tearing is unknown, but it is presumed to be the result of the method of preparing the cells.

If time-lapse microcinematography of the cells in the living state had been performed (Figs. 2 and 3), results very similar to the picture of the macrophage in figure 2.3 in (*1*) would probably have been obtained. Our findings are compatible with the electron micrographs of other workers (*1*, *2*) who have shown the macrophage to be rough-surfaced with microprojections. If the cells in Figs. 2 and 3 had been sectioned in the proper plane, they would undoubtedly look very similar to the cell in figure 2.6 in (*1*). The microvilli mentioned by Nelson (*1*) may be the result of sectioning through the surface membranes.

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Specific Inhibition of Nuclear RNA Polymerase II by α-Amanitin

Abstract. *α-Amanitin, a toxic substance from the mushroom* Amanita phalloides, *is a potent inhibitor of DNA-dependent RNA polymerase II (the nucleoplasmic form) from sea urchin, rat liver, and calf thymus. This compound exerts no effect on the activity of polymerase I (nucleolar form) or polymerase III (also nucleoplasmic). The inhibition is due to a specific interaction with polymerase II or with a complex of DNA and polymerase II.*

We have described the physical separation of three distinct DNA-dependent RNA polymerases from sea urchin embryos and two polymerases from rat liver (*1*, *2*). Polymerase I resides in the nucleolus, and polymerases II and III reside in the nucleoplasm (*2*). The enzymes exhibit distinctive variations in activity with magnesium, manganese, and salt concentration changes. It is inferred that the polymerases have different functions, but for definitive investigations on this point specific inhibitors would prove highly useful. We have tested the effect of α-amanitin [the toxic bicyclic octapeptide from the mushroom *Amanita phalloides* (*3*)] on the various nuclear polymerases. Administration of this compound to mice results in fragmentation of liver nucleoli (*4*) and an overall decrease in nuclear RNA content (*5*). Furthermore, in the presence of Mn++ and high concentrations of salt, α-amanitin partially inhibits the polymerase activity of mouse liver nuclei (*6*) and of crude nuclear preparations (*7*).

We now report that α-amanitin specifically inhibits polymerase II from the nuclei of several organisms although polymerase I and III activities are not affected. We also provide substantial

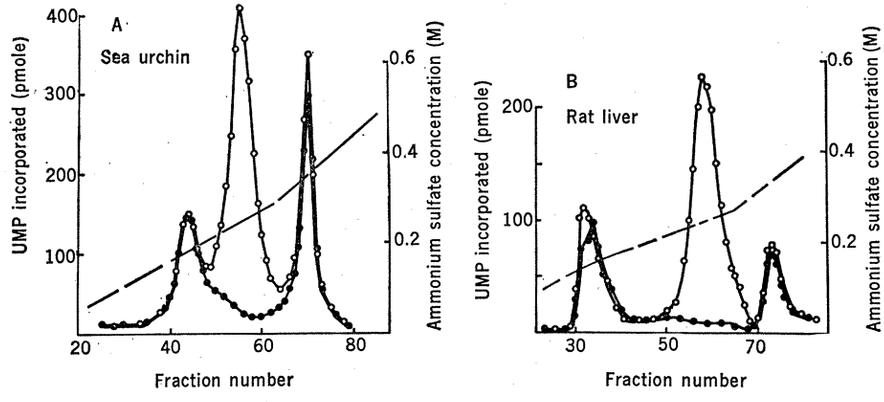

Fig. 1. (A) Inhibition of sea urchin (*Strongylocentrotus purpuratus*) and rat liver RNA polymerase II by α-amanitin. A sample containing 9.5 mg of protein was applied to a column (1 by 5 cm) of DEAE Sephadex (A-25) that was equilibrated with 0.05*M* tris-HCl (*p*H 7.9), 25 percent (by volume) glycerol, 5 m*M* $MgCl_2$, 0.1 m*M* EDTA, 0.5 m*M* dithiothreitol, and 0.02*M* ammonium sulfate. Polymerase activities were eluted with a gradient from 0.02 to 0.42*M* ammonium sulfate (40 ml total volume) with an additional 10-ml washing of 0.5*M*; 0.6-ml fractions were collected. Polymerase activities were assayed by the usual procedure and at $3.4 \times 10^{-6}M$ α-amanitin. The assay mixture contained 0.01 m*M* uridine triphosphate (UTP) (unlabeled) and sheared calf-thymus DNA, but otherwise it was identical to that of Roeder and Rutter (*1*). Reactions were stopped by pipetting 0.10 ml of this assay onto Whatman DE-81 filter disks (2.1 cm). Filters were washed six times, for 4 minutes each time, in 5 percent Na_2HPO_4, then twice in water, twice in 95 percent ethanol, and twice in diethyl ether, and air dried. Samples were counted after solubilization in a toluene-based scintillation fluid containing Ominifluor (4 g/liter) (New England Nuclear) and 2.5 percent NCS (Nuclear-Chicago solubilizer) in a Nuclear-Chicago Mark II at 60 percent efficiency (^3H). The RNA polymerase activity is expressed as the number of picomoles of uridine monophosphate incorporated into RNA per milliliter per 10 minutes. (B) Conditions were the same as those in (A) except for the following. The protein (7.4 mg) was applied to the column, and the column was washed with an additional portion of the equilibrating solution; then a gradient (0.1 to 0.5*M* ammonium sulfate) was applied in a total volume of 30 ml. Fractions of 0.3 ml were collected, and the assays were performed at 3 μ*M* UTP. Open circles, activity in the absence of α-amanitin; closed circles, activity in the presence of α-amanitin.