Thus, a quick insight into anti-platelet and vasomotor activities of prostacyclin analogues, searching out their prostaglandin-like and thromboxane-like properties as well as evaluation of their in vivo de-aggregatory potency, are the main features of the proposed triple test.

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Membrane interiorization by phagocytosing macrophages – an ultrastructural morphometric approach[1]

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Summary. Stereological methods have been used to quantify selected membrane compartments of normal and activated rat peritoneal macrophages, before and after phagocytosis of latex beads. Despite being rounder, activated cells are more efficient phagocytes: 30 min after latex challenge they suffer a greater nett depletion of plasma membrane and sequester more and larger phagocytic vacuoles. However, phagocytosis of latex is not the major route of membrane interiorization.

A wide range of substances with different physical and chemical properties is known to induce alterations in the morphological appearance of peritoneal macrophages. The changes reflect a functional 'stimulation' or 'activation' of cells and this includes an enhanced endocytic ability[2]. At the ultrastructural level, activation involves an increase in cell size characterized by differential hypertrophy of certain intracellular compartments, particularly of lysosomes. Alterations in the number and dimensions of cell surface features are also reported[3-6].

Structural differences between normal and activated, resting and endocytosing cells may be estimated by morphometry. In a series of studies undertaken in this laboratory, stereological techniques[7] were used to quantify ultrastructural variations between resident rat peritoneal macrophages and cells stimulated with Freund's adjuvants[5,6]. Amongst other changes, it was revealed that induced peritoneal exudates contained a population of activated macrophages which had substantially less plasma membrane surface (on average, roughly 30% less than normal). In addition, the cells were rounder than normal and this rounding-up was partly due to the sequestration of adjuvant ingredients within membrane-bound vacuoles (phagosomes)[5].

In this report, we present the results of a preliminary stereological investigation designed to assess the effect of adjuvant-induced membrane depletion on the subsequent phagocytic potential of these cells. At the same time, we have attempted to evaluate changes in plasmalemma surface area that accompany phagocytosis. For this purpose, latex beads afforded a useful means of following phagocytic activity.

Materials and methods. Full particulars of all preparative stages will be found in our earlier reports[5,6,8]. Macrophages were harvested from an inbred strain of rats by peritoneal lavage. Normal animals were used, together with rats challenged 5 days previously by an i.p. injection of Freund's complete adjuvant (FCA) emulsified in Hank's solution. These 2 groups were controls. In addition, experimental groups of normal and FCA-induced cells were challenged in vivo by a single i.p. injection of latex beads (EMscope; mean diameter 0.481 ± 0.002 µm) diluted 1:25 in balanced Hank's solution. The dose (0.2 ml for normal animals and 1.4 ml for FCA-challenged rats) was according to predicted macrophage yields obtained from differential cell counts and haemocytometry. Cells were harvested 30 min later.

Following centrifugation, the 4 groups of cells were fixed in 3% buffered glutaraldehyde (pH 7.4) and postfixed in 2% aqueous osmium tetroxide. After dehydration, cell pellets were embedded in araldite. Ultrathin sections were taken for electron microscopy.

A systematic random sample of cell profiles was taken using an AEI-Corinth Electron Microscope. Only cell profiles in which the nucleus was present were recorded[8]. Final print magnification was ×11,230 as determined from micrographs of a grating replica. Morphometric data were recorded with the aid of a simple quadratic test lattice (spacing between lines 1 cm) superposed on to each micrograph. A total of 100 micrographs per group was analyzed and this sample size was larger than estimated minimal requirements using cumulative mean plots[5].

Standard stereological relationships[5-7] were invoked to estimate morphological parameters we considered important. Membrane surface areas (of plasmalemma and of latex-containing vacuoles) were estimated by counting intersections between the sectioned membranes and the test lines on the lattice. Numerical densities of (latex) vacuoles were calculated from the number and size of vacuole profiles. Cell volume-to-surface ratio was determined using the method described by Chalkley et al.[9]. Estimates of mean cell diameter were derived from measured cell profile areas (assuming circularity) and mean cell volume computed for a sphere of equivalent diameter. Finally, a measure of how much more surface area the average cell had than an equivalent sphere was made by comparing cell volume-to-surface ratio with that of a sphere of equivalent diameter. This surface amplification factor provides a useful indication of cell roundness.

Table 1. Morphometric differences between normal and FCA-induced macrophages before and after a phagocytic challenge with latex beads. Overall means ± SE

	Cell volume (μm^3)	Cell surface (μm^2)	Surface amplification
Normal Cells:			
Before challenge	208.2± 8.0	836.2±41.6	4.9
After challenge	201.4±27.8	729.6±53.0	4.7
Change	−6.8 μm^3 (3%)	−106.6 μm^2 (13%)	Rounding-up
FCA-induced Cells:			
Before challenge	288.0±11.5	816.7±49.0	3.9
After challenge	258.6±22.8	664.7±43.8	3.5
Change	−29.4 μm^3 (10%)	−152.0 μm^2 (19%)	Rounding-up

Table 2. Morphometric dimensions of latex-containing phagocytic vacuoles in challenged normal and FCA-induced cells

	Number/cell	Surface area/cell	Mean surface area
Normal cells	3.62±1.13	3.2 μm^2	0.88 μm^2
FCA-induced cells	6.07±2.90	11.2 μm^2	1.85 μm^2
Difference:	+2.45 (67%)	+8.0 μm^2 (251%)	+0.96 μm^2 (109%)

Results and discussion. The results of our morphometric analyses of cell volume and surface area are summarized in table 1, the values being the overall means for 2 (before challenge) or 3 (after challenge) experiments. Wide variations in these parameters account for the large SE. 30 min after an injection of latex beads, normal cells have less surface membrane with little if any change in cell size. In consequence, the average challenged cell is slightly rounder than before. The same sort of change occurs with FCA-induced macrophages although there are quantitative differences. In particular, following phagocytosis of latex beads the average FCA-induced cell exhibits a relatively and absolutely greater nett depletion of surface membrane (19% or 152 μm^2 versus 13% or 106 μm^2). Activated cells therefore interiorize more plasmalemma during the 30-min challenge.

To evaluate the contribution made by latex phagocytosis to this nett membrane influx, we have calculated the number and membrane surface area of intracellular vacuoles (phagosomes and phagolysosomes) containing latex beads. Our results are shown in table 2. Almost 3.5 times as much vacuolar membrane is sequestered by FCA-induced cells and this is due to the ingestion of more and larger vacuoles. The results therefore confirm the heightened phagocytic ability of activated cells.

If we assume that vacuoles are spheres, we can calculate their mean diameter (from their membrane surface area) and compare this with that of the average latex bead to approximate the mean number of beads per vacuole. The data permit a possible 1.10 beads/vacuole in normal and 1.59 beads/vacuole in induced macrophages. Moreover, if we set an upper limit of 2 beads in a single vacuole, we can estimate that in 1000 normal cells there are 3620 vacuoles of which 3247 could contain 1 bead and only 373 contain 2. By comparison, 1000 activated cells would sequester 6070 vacuoles of which only 2470 could contain 1 but 3600 could contain 2 beads. Clearly other possibilities exist, the important point being that FCA-induced cells have more beads per vacuole and per cell. These differences become even greater when we consider populations rather than individuals since the FCA-induced peritoneal exudate harbours considerably more macrophages[5].

Activated cells in these studies proved to be more efficient at phagocytosis of latex. However, an impressive incidental observation is that the amount of membrane taken in around vacuoles is considerably less than the nett loss of plasma membrane over the 30-min period. In fact, the amount corresponds to a mere 3% of the total in normal cells and 7% in activated cells. Therefore, the vast bulk (over 90%) of all membrane interiorized enters by some other mechanism and our impression is that this is mainly by pinocytosis of extracellular fluid. As yet, we have not analyzed this route specifically but the morphometric studies by Steinman et al.[10] demonstrate that it may be a major one for membrane influx. Their analyses indicate that during induced pinocytosis of horseradish peroxidase, cultured mouse peritoneal macrophages may interiorize the equivalent of their membrane surface every 33 min. Rates calculated from the present results are markedly lower than this: every 4 h in the case of normal macrophages but faster, every 3 h, in induced cells.

These results are open to several sources of error and the values should not be treated as absolute. Nevertheless, we regard them as valid comparative estimates of the changes involved. We conclude that despite being rounder, FCA-induced peritoneal macrophages are more efficient than normal cells at phagocytosing latex beads but that, under the conditions of our experiments, phagocytosis is very much the minor mechanism of membrane influx. Indeed most membrane appears to enter during pinocytosis but the rate at which this proceeds is also greater in activated cells.

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