Results. The results of metaphase counts are presented in figure 1, their significance was examined using Wilcoxon's test. Differences between control and exposed animals are significant at the 0.05 level in each instance following exposure to 0.1 mW/cm^2.

The results of qualitative analysis of MI are shown in the table. Aneuploid metaphases were encountered. Hypoploid metaphases were seen both in control and experimental animals, no significant differences were, however, noted in their frequency. In control animals, the presence of quadrivalents was noted in 3 metaphases out of 175. In 2 metaphases besides bivalents, 2 and 4 univalents were seen.

Following exposure to 0.1, 1.0 and 10.0 mW/cm^2, the incidence of metaphases with quadrivalents (and in some instances of hexavalents) increased significantly. The chromosome associations (translocations) occurred at random and no particular chromosome pairs demonstrated a tendency for translocations. Metaphases with one or more (up to 6) chromosome pairs remaining at MI as univalents were significantly (chi^2 test) more frequent in all groups of exposed animals. The largest proportion of such metaphases was seen following exposure to 0.1 and 10.0 mW/cm^2, 15.2% and 30.4% respectively. Figures 2–4 illustrate the MI findings.

Discussion and conclusions. The findings presented above obviously need confirmation on larger number of animals, other mice strains and other animal species. However, the data obtained seem to indicate that repeated microwave exposure at incident power levels equal to or lower than values accepted as maximum exposure levels[3] may interfere with the normal course of meiosis. Similarly as ionizing radiation[10,11] or chemical mutagens[12,13] microwave exposure may lead to an increase in translocations and the occurrence of chromatid breaks, in spite of the difference in the primary mode of interaction with living matter. The high incidence of metaphases with the presence of univalents, instead of bivalents, may indicate that microwave exposure interferes somehow with chiasma formation and/or behaviour.

Possible consequences of the observed phenomena in terms of reduced fertility or hazards to offspring, remain to be verifield in specially designed experiments.

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Quantitative ultrastructural features of maturing mononuclear phagocytes in rat peritoneal fluids[1]

T.M. Mayhew

Department of Human Biology and Anatomy, The University, Western Bank, Sheffield S10 2TN (England), 27 June 1978

Summary. Ultrastructural features of the in vivo transformation of macrophage congeners in resident and adjuvant-induced peritoneal populations are evaluated by stereological methods. Maturation involves an increase in cell size by the differential hypertrophy of subcellular compartments, notably remaining cytoplasm, nucleus and lysosome-like granules. Larger cells have more and larger granules, more mitochondria and a greater plasmalemmal surface. In contrast, adjuvant activation tends to produce fewer granules and a nett loss of surface membrane.

Studies of the morphology, functional properties, cytochemistry and life history of mononuclear phagocytes have demonstrated that monocytes can transform in vivo and in vitro into cells resembling tissue macrophages[2–4]. The process is referred to as a maturation or differentiation of cells and is characterized by increases in cell size, complexity and functional activity. The changes can be viewed in the context of an overall sequence which begins with a haemopoietic stem cell in bone marrow and proceeds, via the circulating monocyte, to an activated macrophage at an inflammatory locus[5–8].

Large numbers of cells at various stages of maturity may be obtained by peritoneal lavage, particularly after administration of an inflammatory agent. The functional maturity of these cells may be assessed by studying their morphology. In the resident population and in inflammatory exudates, smaller cells may be distinguished from larger, more mature forms containing many lysosomal granules and mitochondria and with a more extensive cell surface[4,9–12]. Many small cells resemble blood monocytes but have more granules and surface processes, presumably reflecting prior endocytic activity in their new environment. Similar differences exist between resident and induced cells following activation by certain stimuli[9,10] but not by others[13,14].

Many of the earlier reports are subjective studies, concentrating on the differences between resident and induced cells, and there has been no detailed attempt to quantify the morphological variation which exists within a given population. The present report tries to fill this gap and describes a stereological analysis[13–15] of mononuclear phagocytes in rat peritoneal fluids. Cells are classified as small or large on the basis of profile area and the 2 groups

Table 1. Morphometric differences between resident mononuclear phagocytes

Parameter	All cells	Small cells	Large cells	Increment index
Volume (μm^3)				
Cell	119.8±4.95	88.3±1.79	166.8±2.42	1.89
Nucleus	29.6±0.26	22.9±1.28	39.5±3.47	1.72
Granules	5.7±0.21	3.7±0.36	8.8±0.52	2.41
Mitochondria	7.7±0.73	5.7±0.25	10.7±1.07	1.87
Rough ER	4.2±0.06	3.0±0.23	5.8±0.23	1.94
Remaining cytoplasm	72.6±4.03	53.0±0.92	101.9±2.05	1.92
Surface area (μm^2)				
Plasmalemma	544.8	420.5	725.1	1.72
Nuclear envelope	92.6	76.3	112.9	1.48
Surface amplification (cf. equivalent sphere)	4.6	4.4	4.9	-
Organelle number per cell				
Granules	1593	1212	2155	1.78
Mitochondria	132	99	182	1.84

Overall means ± SE.

equated with relatively immature and mature stages respectively. This approach provides a more objective picture of the ultrastructural differences between the 2 functional states.

Materials and methods. Details of all preparative stages will be found in an earlier report[13]. Cells were harvested from an inbred strain by peritoneal lavage of 3 groups of normal rats and 3 groups challenged 5 days previously by a single i.p. injection of Freund's complete adjuvant emulsified in balanced Hank's solution.

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

A systematic random sample of cell profiles containing nuclei was taken using an AEI-EM6B microscope[13]. The geometry of these cells is such that nucleate profiles give a more reliable indication of cell size than do purely random transections. Final print magnification was ×28,200 as calculated from micrographs of a grating replica. Morphometric information was recorded by confronting each micrograph with a simple quadratic test system bearing lines 1 cm apart. The relative SE[13] of component volume densities afforded a check on the suitability of micrograph sample sizes.

Standard stereological relations[13,15] were invoked to quantify interesting ultrastructural features. Cell profile areas were estimated by point counting (one test point represented 0.13 μm^2). The population mode of the profile size distribution of resident cells was adopted as a convenient boundary area with which to classify profiles as small (less than 24.7 μm^2 in area) or large (greater than 24.7 μm^2).

Compartment volume densities were also estimated by point counting. The following compartments were selected: nucleus, granules (primary and derived lysosomes), mitochondria, rough ER cisternae and remaining cytoplasm. Granule and mitochondrion numerical densities and cell volume-to-surface ratios were also computed[13]. Estimates of cell surface amplification were obtained by comparing each average cell with its equivalent sphere.

Converting relative data into absolute dimensions (volumes, surface areas, numbers) required determination of a reference volume and the nucleus was chosen for this purpose. Mean nuclear profile area (A) provided an equivalent sphere diameter (D) since $A = (\pi/6)D^2$ if nuclear sphericity is assumed. This diameter was then used to approximate a mean nuclear volume (V) since $V = A.D$. The final data define the average 'small' and 'large' cell in each population. Values for total resident and induced populations are included for completeness and for comparison with earlier results[13].

Table 2. Morphometric differences between adjuvant-induced mononuclear phagocytes

Parameter	All cells	Small cells	Large cells	Increment index
Volume (μm^3)				
Cell	157.9±6.95	93.6±3.84	185.3±6.77	1.98
Nucleus	37.2±2.54	25.2±1.38	42.2±3.04	1.68
Granules	10.4±1.47	4.3±1.31	13.1±2.56	3.05
Mitochondria	9.6±0.73	6.7±0.36	10.8±1.30	1.60
Rough ER	4.7±0.82	2.9±0.27	5.5±1.07	1.87
Remaining cytoplasm	96.0±5.89	54.5±2.06	113.8±5.29	2.09
Surface area (μm^2)				
Plasmalemma	451.2	275.4	529.5	1.92
Nuclear envelope	79.1	62.9	86.1	1.37
Surface amplification (cf. equivalent sphere)	3.2	2.8	3.4	-
Organelle number per cell				
Granules	1126	888	1240	1.40
Mitochondria	182	134	201	1.50

Overall means ± SE.

Results and discussion. Mononuclear phagocytes in resident and induced populations present different stages of maturation but 2 extremes of cell morphology may be defined. The one corresponds to a small cell with relatively few surface processes and invaginations. It has few cytoplasmic granules and mitochondria and moderate amounts of rough ER. The other extreme is typified by a much larger cell with a more voluminous cytoplasm containing many organelles, abundant rough ER and a more extensive and irregular cell surface.

Large profiles in the resident sample accounted for only 43% of the total but the figure rises to some 72% after adjuvant challenge. This is taken to indicate that the resident population has relatively and absolutely fewer of the more mature cells since the largest cells must generate the largest profiles. Moreover, exposure to Freund's complete adjuvant evokes a florid peritoneal leucocytosis[13].

Morphometric differences between the 'average cell' in each (small and large) group are summarized in tables 1 and 2. Each value is an overall mean for 3 experiments. The data characterizing the total resident and induced populations are in very good agreement with earlier estimates[13].

The results suggest that cell maturation within a population involves the differential hypertrophy of all the compartments analyzed. Much of the increase in cell size (62% in resident, 65% in induced cells) results from expansion of the remaining cytoplasm which comprises free ribosomes, smooth ER, Golgi zones, phagosomes, centrioles and cytoplasmic ground substance. The rough ER contributes least (only 3–4%) to overall cell growth. The compartment which undergoes the greatest individual change is the granule: it increases by 141% in resident and by 205% in induced cells.

Mean organelle volumes were calculated from compartment volume and number. They reveal that the granule hypertrophy follows from increases in both number and size. In resident cells, the average granule is about 36% larger in 'large' cells (4.1×10^{-3} μm^3 versus 3.0×10^{-3} μm^3) and in induced cells the difference is even greater (10.6×10^{-3} μm^3 versus 4.8×10^{-3}). It would appear that number changes more than size in resident cells but the reverse obtains after adjuvant challenge and this may be an effect by certain adjuvant ingredients[13,14].

Expansion of the mitochondrial compartment is due solely to an increase in number since there is little, if any, change in size (mean volume about 5.5×10^{-2} μm^3). There was no evidence in the present model of any change in organelle length[4,11].

During maturation, cells acquire substantially more plasmalemma. Moreover, larger cells tend to have relatively more surface membrane than an equivalent sphere and are consequently less rounded[13] than smaller forms.

The overall picture of maturation given here validates earlier subjective impressions[4,9–12]. Smaller cells tend to have less plasmalemma, less rough ER, fewer granules and mitochondria. They therefore resemble more closely blood monocytes which typically have fewer surface features, reflecting their limited endocytic activity and membrane ruffling[4]. Differences in granule size may be determined by prior endocytosis and the fact that monocytes contain a peculiar type of granule not found in mature cells[6,16,17]. Changes in the amounts of ground cytoplasm and mitochondria may also reflect altered metabolic status and energy requirements[4].

Differences within a population are to be contrasted with those between resident and induced cells. Following adjuvant activation there is a considerable drop in mean granule content and a nett depletion of cell surface so that the average cell is rounder than normal[13,14]. Even those cells which are larger than the biggest cells in the resident fluid have, on average, fewer granules. However, they do have a more extensive surface. The affects of adjuvant probably result from the greater endocytic demands made on the cells, although the nature and degree of stimulation for resident cells are not yet clear.

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Asialoprotein uptake by liver cells: Immunofluorescence microscopy[1]

A.V. LeBouton and G. Lippman

Department of Anatomy, College of Medicine, University of Arizona, Tucson (Arizona 85724, USA), 3 July 1978

Summary. Uptake of asialoproteins by hepatocytes causes a change in the intracellular pattern of immunofluorescence. Control cells display a peripheral fluorescence which probably represents nascent proteins. Dark nonfluorescent areas, that presumably contain glycogen, are located around the nucleus. In contrast, liver cells from rats injected with asialoproteins display a pancytoplasmic fluorescence due to an influx of endocytotic vesicles.

A concept is currently emerging that certain highly metabolic organs, such as the liver, extend their influence within the body via interaction with mobile carrier structures such as plasma proteins[2–5]. The idea is exemplified by current studies on interactions between liver cells and certain glycoproteins that have had their terminal sialic acid residue removed (asialoproteins). Thus, asialo-ceruloplasmin[6], alpha-1 acid glycoprotein[7], interferon[8], fibrinogen[9] and alpha-1 antitrypsin[10] are rapidly cleared from the circulation and sequestered in the liver.

Additional biochemical findings show that hepatocytes are the target cells; receptors specific for asialoproteins have