MORPHOLOGY AND PEROXIDASE CYTOCHEMISTRY OF MOUSE PROMONOCYTES, MONOCYTES, AND MACROPHAGES*

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(Received for publication 27 April 1970)

Previous studies on the life history of mouse mononuclear phagocytes have demonstrated that many types of macrophages are derived from blood monocytes and that the progenitors of these cells are situated in the bone marrow (1, 2). Identification of the progenitor of the monocyte, the promonocyte, has been difficult because of the variety of immature and mature cell types present in this tissue. We have devised a procedure for separating promonocytes and monocytes from the other cells of the bone marrow. This report describes the morphology and the peroxidase cytochemistry of these progenitors of mononuclear phagocytes; the accompanying report presents the results of studies on the kinetic behavior in vitro and in vivo of the promonocytes and bone marrow monocytes (3).

Materials and Methods

Animals.—In this study specific pathogen-free Swiss mice from the Rockefeller strain and from the Central Institute for the Breeding of Laboratory Animals TNO, Bilthoven, The Netherlands, were used.

Cell Cultures.—The techniques for harvesting and culturing mouse bone marrow cells and peritoneal cells have been described previously (2). All incubations were in a culture medium consisting of medium 199 (Microbiological Associates, Inc., Bethesda, Md.), 20% newborn calf serum (Colorado Serum Co, Denver, Colo.) with 200 units/ml penicillin G and 50 μg/ml streptomycin.

For light microscopic observations, a cell suspension made from the bone marrow of one femur was incubated in a Leighton tube provided with a flying cover slip. For electron microscopic investigations pooled bone marrow samples of 12 femurs (6 mice) were cultured in a glass Petri dish in a humidified incubator with 5% CO_2 in air as the gas phase. Before fixation of the cells adhering to the glass surface, the supernatant culture fluid was removed and the cultures were washed thoroughly with medium 199 to remove all cells not firmly attached to the glass.

* Supported by Research Grants AI 01831 and AI 07012 from the U.S. Public Health Service.

‡ Recipient of a travel grant from the Netherlands Organization for the Advancement of Pure Research (ZWO).

Peripheral blood monocytes were obtained from blood collected by puncture of the retro-orbital venous plexus. A leukocyte suspension was obtained by mixing equal volumes of pooled blood and 2% dextran solution (mol wt 250,000, Pharmacia Fine Chemicals, Uppsala, Sweden) in phosphate-buffered saline (pH 7.4) and 10 units/ml heparin, and letting it stand 20–30 min at room temperature until the red cells were agglutinated and sedimented. The leukocytes in the supernatant fluid were then washed with buffered saline containing 10 units/ml heparin and suspended in culture medium. This suspension, containing 10–15 $\times$ 10^3 leukocytes per mm^3, was incubated in a Leighton tube or glass Petri dish.

Light and Electron Microscopy.—Phase-contrast microscopy was done on cover slip preparations of living cells soon after they were collected. Rapidly air-dried cell preparations were stained with Wright-Giemsa (3 min undiluted Wright's stain, 6 min diluted Wright's stain, 9 min Giemsa stain), or fixed in absolute methyl alcohol for 10 min and stained with Giemsa stain (10 min). Photographs were taken with a Zeiss photomicroscope (Carl Zeiss, Inc., N.Y.).

For electron microscopy, the cells attached to the bottom of the Petri dish were chilled on ice and fixed with a mixture of 2% glutaraldehyde and 1% osmium tetroxide, both in 0.1 M cacodylate buffer (pH 7.4) (4). After 2–3 min the cells were gently scraped from the glass surface with a rubber policeman, collected in a conical test tube, and centrifuged for 4 min at 300 *g*. The pellet was suspended in 2.5 ml of the mixture of glutaraldehyde and osmium tetraoxide and kept in ice for 10 min. After a total fixation time of 15 min, the cells were concentrated by centrifugation (2 min, 300 *g*), washed with ice-cold phosphate-buffered saline, and then postfixed in 0.25% uranyl acetate for 15 min. The cells were then dehydrated and embedded according to Luft (5). Thin sections on Formvar-carbon coated grids were stained with lead citrate and uranyl acetate (6), and examined in a Siemens Elmiskop I (Siemens America, Inc., N.Y.) using a 50 μ objective aperture.

Peroxidase Reactivity.—Air-dried cells were fixed with ethanolic formalin and exposed briefly to benzidine dihydrochloride and hydrogen peroxide according to Kaplow (7).

Phagocytosis and Pinocytosis.—The phagocytic activity of the cells attached to the glass surface was studied by exposing them to culture medium containing about 1 $\times$ 10^7 *Staphylococcus albus* per ml; the bacteria to leukocyte ratio was at least 10:1. After a 60 min incubation, the cells were washed thoroughly with medium 199 to remove all noningested bacteria; they were then fixed and stained. The percentage of cells which had ingested bacteria was determined by microscopic counts.

The pinocytic activity of the mononuclear phagocytes was studied by incubating the cells with culture medium containing 0.25 mg/ml colloidal gold (Abbott Laboratories, Chicago, Ill.) for 1 hr (peripheral blood monocytes and peritoneal macrophages), or 4 hr (bone marrow cells). The uptake of colloidal gold was determined by electron microscopy.

RESULTS

Identification of Promonocytes.—In the previous study (2), marrow cells of one mouse femur, estimated to contain approximately 1.2 $\times$ 10^7 nucleated cells (3), were suspended in 2 ml culture medium and allowed to settle onto a glass cover slip for 2 hr at 37°C. After vigorus washing to remove nonadherent cells, the cover slips were examined or were recultured for 48 hr in fresh medium. After the first 2 hr of incubation the population of adherent cells was very heterogeneous, with a preponderance of granulocytic cells (Table I); after 48 hr of reculture, however, all adherent cells were macrophages, thus indicating that the granulocytic and other cell types had detached or died, and that the mononuclear phagocytes had matured into macrophages (Table II) (2).

Observations on cover slips were therefore made at various time periods in order to determine the earliest time at which the population consists predominantely of mononuclear phagocytes. This situation was found to exist at 6 hr of total culture: 2 hr initial settling and culture, and 4 hr of subsequent reculture of the washed adherent cells. As is seen in Tables I and II, at 6 hr the bone marrow cultures had about 70% mononuclear phagocytes, approximately 85% of which were morphologically identical with blood monocytes (see Figs.

TABLE I

Percentage Distribution of Cultured Bone Marrow Cells

Duration of culture*	Approximate cell density	Type of cell		
		Mononuclear phagocytes	Granulocytes	Others‡
hr	*cells per cover slip*	%	%	%
2	3.7×10^5	40	46	14
6	2.5×10^5	69	20	11
24	1.4×10^5	92	4	4
48	1.2×10^5	99	<1	<1

* Incubated in Leighton tubes with medium 199 and 20% newborn calf serum.

‡ Mainly cells with doughnut nuclei, granulocyte precursors, and some erythroblasts.

TABLE II

Composition of Population of Mononuclear Phagocytes in Bone Marrow Cultures

Duration of culture*	Promonocytes	Monocytes	Macrophages
hr	%	%	%
2	15.3	84.7	<0.1
6	15.9	84.1	<0.1
24	12.1‡	80.5‡	7.4
48	<1	<1	99

* Incubation on cover slips in Leighton tubes medium 199 and 20% newborn calf serum.

‡ The differentiation between promonocytes and monocytes can not always be made in 24 hr cultures.

2, 4, and 12); the remaining 15% were larger round cells with a large indented or folded nucleus and prominent nucleoli (see Figs. 1, 4, and 10). Both types of mononuclear phagocytes in these 6 hr marrow cultures exhibited phagocytic and pinocytic activity (see below). It is worth noting that during the first 24 hr of incubation the ratio between promonocytes and monocytes remained almost constant, and that maturation into macrophages occurred mainly during the 2nd day of incubation (Table II). However, in some cultures the differentiation into promonocytes and monocytes could no longer be made with confidence after 24 hr of incubation. After these results were obtained, all studies on

the morphology and kinetics of mononuclear phagocytes of the bone marrow were done in 6 hr cultures. The accompanying paper (3) presents radioautographic studies done on these bone marrow cultures following exposure of the mice or the cultures to tritiated thymidine. After in vitro incubation with tritiated thymidine, the large mononuclear phagocytes in the 6 hr cultures were labeled, whereas the smaller monocytes were not. In vivo labeling studies demonstrated that the large labeled cells in 6 hr cultures are multiplicative cells from which monocytes derive. The above results permit the conclusion that the large mononuclear phagocytes in 6 hr bone marrow cultures are promonocytes, the progenitors of the monocytes.

Morphology of Promonocytes and Related Cells.—The promonocyte, which has a diameter of 14–20 μ, is larger than both the monocyte (diameter 11–14 μ) and the noncultured peritoneal macrophage (diameter 11–18 μ), but smaller than a peritoneal macrophage cultured for 24 hr. All these cells show nuclear indention or folding; the reniform or horseshoe shape of the nucleus is more prominent in the more mature cells. The nucleus to cytoplasm ratio is >1 in the promonocyte, ~ 1 in the monocyte, and <1 in the macrophage.

Phase-contrast microscopy of living promonocytes, monocytes, and peritoneal macrophages (Figs. 4 and 5) reveals several features not seen in the fixed and stained preparations. Surface membrane ruffling is slight in the promonocytes, whereas it is marked in the monocytes and macrophage. The promonocyte nucleus is generally phase lucent, and prominent nucleoli are present. Both promonocytes and monocytes show a few dense granules, clear vesicles, and mitochondria in the cytoplasmic area adjacent to the nuclear hilus or indentation. The macrophages show extensive perinuclear accumulations of clear vesicles and small dense granules and also variable numbers of refractile lipid droplets.

The features of promonocytes, progranulocytes, monocytes, and macrophages observed under the electron microscope are illustrated in Figs. 10–13. Detailed descriptions of the various cell types are presented in the legends and need not be repeated here. We shall confine our comments to the ultra-structural features that are especially useful in identifying the various cell types. The features of a bone marrow promonocyte and a neutrophil myelocyte, as viewed under the electron microscope, are shown in Figs. 10 and 11.

The surface of the promonocyte exhibits prominent finger-like projections, whereas the myelocyte surface is generally smooth. There are many electron-opaque granules distributed peripherally as well as centrally in the myelocyte, in contrast to the small number of dense granules in the centrosomal region of the promonocyte. Dilated rough endoplasmic reticulum is more prominent in the myelocyte than in the promonocyte.

Ultrastructural comparisons between promonocyte and marrow monocyte

are illustrated in Figs. 10 and 12. These cells are very similar in appearance under the microscope, but generally the Golgi apparatus is larger in the promonocyte than in monocytes, whereas granules and vesicles are seen somewhat more frequently in the monocyte than in the promonocyte cytoplasm. Ribosomal aggregates are common in both promonocytes and marrow monocytes. Blood monocytes display an ultrastructure identical with that of marrow monocytes, except for the fact that ribosomal aggregates are infrequently seen in the blood cells.

An electron micrograph of a typical peritoneal macrophage fixed promptly after harvesting is shown in Fig. 13. The Golgi apparatus and ribosome aggregates are more prominent in the promonocyte than in the macrophage, whereas cytoplasmic granules and vesicles are more abundant in the macrophage than in the promonocyte. Extensive surface convolutions in the macrophage produce in the thin section peripheral vacuolar profiles of irregular shape; these invaginations are not seen in the promonocyte.

TABLE III

Peroxidase Positive Mononuclear Phagocytes

Duration of culture	Bone marrow		Peripheral blood monocytes	Peritoneal macrophages				
	Promonocytes	Monocytes		Normal	Hr after serum i.p.*			
					12	24	48	72
hr	%	%	%	%	%	%	%	%
2	95.0	87.0	87.0	1.0	87.9	49.6	43.5	5.8
6	96.3	90.9	86.5					
24	93.6	87.0	59.8	0.4	82.4	52.7	30.3	5.4
48	89.0‡			0.1	28.7	27.4	8.8	4.5

* 1 ml newborn calf serum intraperitoneally.

‡ At this time-point all cells are morphologically macrophages.

Peroxidase Cytochemistry of Promonocytes, Monocytes, and Macrophages.— We found it difficult to distinguish between promonocytes and neutrophil myelocytes by light microscopy. Generally, the neutrophil and eosinophil leukocytes, whether immature or mature, gave strong reactions for peroxidase, whereas monocytes reacted weakly and macrophages not at all. This led us to study the peroxidase reactivity of promonocytes, and somewhat to our surprise these cells gave a moderately strongly positive reaction for peroxidase. As illustrated in Figs. 6–9 and Table III, the promonocytes showed numerous peroxidase-positive granules, almost all bone marrow and peripheral blood monocytes exhibited a small number of positive granules, and virtually all normal peritoneal macrophages were peroxidase negative (Table III). Also, no peroxidase reactivity was seen in alveolar macrophages obtained by tracheobronchial lavage. On blood smears, 92% of the monocytes were peroxidase positive.

The peroxidase reactivity of promonocytes and monocytes was validated by the following observations: it was not inhibited in the presence of 0.02 M 3-amino-1-2-4-triazole, it was partially blocked by 0.01 M sodium cyanide, and it was negative if either benzidine dihydrochloride or hydrogen peroxide were omitted from the reaction mixture. The peroxidase reactivity of promonocytes and monocytes was also studied by electron microscopy using the Graham and Karnovsky technique (8); the reaction product was localized in the granules.

Peritoneal macrophages stimulated by culture in vitro 1–4 days in a serum-rich medium (20% or 50% newborn calf serum) also remained peroxidase negative. In contrast to this complete absence of demonstrable peroxidase in normal macrophages, 12 hr after an intraperitoneal injection of newborn calf serum about 88% of the peritoneal macrophages were peroxidase positive; this value declined, however, to about 6% 72 hr after the serum injection (Table III). In these preparations the macrophages with clumps of positive granules in their cytoplasm, which are evidently parts of ingested granulocytes, were not counted as positive cells. Since an intraperitoneal injection of newborn calf serum evokes a short-lasting influx of mainly mononuclear phagocytes (peroxidase positive) from the peripheral blood into the peritoneal cavity (2), and glucocorticosteroids cause a diminished influx of mononuclear phagocytes into the inflammatory exudate (9), the effect of hydrocortisone was studied. In mice treated with 15 mg hydrocortisone and given 1 ml newborn calf serum 24 hr later, only 36.6%, 17.0%, and 16.5% peroxidase-positive peritoneal macrophages were found 12, 24, and 48 hr after the serum injection, respectively. These results indicate that peroxidase-positive macrophages in the inflammatory exudate are recent emigrees from the blood; this is in agreement with previous labeling studies (2, 9).

After culturing in vitro for 24 or 48 hr the enzyme activity of peroxidase-positive promonocytes and bone marrow monocytes remained constant, but the peroxidase reactivity of the peripheral blood monocytes and serum-induced peritoneal macrophages decreased (Table III). When serum-induced peritoneal macrophages were allowed to ingest *Staphylococcus albus* the percentage of peroxidase-positive peritoneal macrophages was not altered 2 hr after phagocytosis.

Phagocytosis and Pinocytosis by Promonocytes, Monocytes, and Macrophages.— Both promonocytes and bone marrow monocytes exhibited phagocytic activity (see Table IV and Fig. 14) when 6 hr cultures were exposed to about 1×10^7 *Staphylococcus albus*. However, the phagocytic activity of the mononuclear phagocytes of the bone marrow in 6 hr cultures was lower than that of the peripheral blood monocytes and of the peritoneal macrophages exposed to the same concentration of organisms (Table IV). Incubation of the bone marrow cells with 10 times more bacteria revealed no greater phagocytic activity of the

mononuclear phagocytes. In 48 hr cultures, when all mononuclear phagocytes of the bone marrow had the morphology of macrophages, almost all cells were capable of phagocytosis (Table IV and Fig. 15).

Incubation of mononuclear phagocytes in the presence of colloidal gold showed that gold particles entered these cells and were localized in pinocytic vesicles and dense bodies. Peritoneal macrophages were most active in pinocytosis, but the promonocytes also showed pinocytic activity (see Fig. 16). Bone marrow and peripheral blood monocytes displayed pinocytic activity greater than that of promonocytes, but less than that of macrophages. Neutrophil granulocytes and myelocytes exhibited no endocytosis of gold under the conditions employed.

TABLE IV

Phagocytic Activity of Mononuclear Phagocytes

Duration of culture*	Phagocytic bone marrow cells			Phagocytic peripheral blood monocytes	Phagocytic peritoneal macrophages
	Promonocytes	Monocytes	Macrophages		
hr	%	%	%	%	%
6	69	72		78	99
48			98		99

* Cells cultured on cover slips in medium 199 and 20% newborn calf serum for 6 or 48 hr and then allowed to phagocytize *Staphylococcus albus* (1×10^7 organisms per ml) for 1 hr.

DISCUSSION

It will be well to begin by pointing out the inadequacy of light microscope observations of stained preparations for precise morphologic classification of the various stages of mouse mononuclear phagocytes. As is seen in Figs. 1–3, promonocytes, monocytes, and macrophages show differences in size, nuclear shape, nuclear to cytoplasmic ratio, and in amount and granularity of cytoplasm, but little more can be seen in these stained preparations. In contrast, electron micrographs of these three cells (Figs. 10, 12, and 13) reveal a rich assortment of organelles and ultrastructures, allowing comparison at an extraordinary level of detail. However, the electron microscope method also has some important limitations, since one is able to view only a very thin slice of the cell after it has been fixed, processed, and stained; thus, it can be difficult to recognize artefacts related to these manipulations, and it is necessary to examine serial sections to achieve reliable quantitation. Examination of living cells under phase-contrast conditions provides a reasonably detailed view of these cells free of fixation arefacts; as seen in Figs. 4 and 5, the general cell structure and many of the larger organelles are well visualized by this method. We find the combination of phase and electron microscopy on these or other

cells especially useful and reliable for morphologic observation, and especially for evaluation of structure–function relationships.

The morphology of the promonocyte, as revealed in these studies, presents no surprising features. The nucleoplasm is generally phase and electron transparent, and nucleoli are prominent, as is to be expected in cells dividing and engaging in various synthetic activities. The cytoplasm of the promonocyte has the general organization and constituents common to all mononuclear phagocytes: Golgi apparatus; vacuoles and vesicles surrounding the centrioles in the cytoplasm adjacent to the nuclear hilus or indentation; granules and mitochondria situated around the Golgi area; strips of rough endoplasmic reticulum and, variably, ribosome aggregates, lipid deposits, and chains of pinocytic vesicles in the peripheral cytoplasm; and an irregular contour of the surface membrane, with many finger-like projections. The promonocyte is especially richly endowed with Golgi saccules and with polyribosomes, findings which accord well with the young, developing nature of the cell. Also notable is the presence, albeit at a lower level than in macrophages, of phagocytic and pinocytic activity in promonocytes.

Promonocyte seems an apt name for this cell, since it is the progenitor of the monocytes, as will be documented further in the following paper (3). It should be noted here that in a previous paper (2) all mononuclear phagocytes of the bone marrow studied in 48 hr cultures were called promonocytes, because of their labeling characteristics with tritiated thymidine. After that duration of culture, all mononuclear phagocytes have the morphology of macrophages; only with the shorter incubation periods used in the present study could differentiation between the progenitor of the monocytes, i.e. the promonocyte, and the monocyte be achieved (Table I). There no doubt exists an earlier bone marrow cell that gives rise to promonocytes, but we have not seen a population of more primitive precursors of mononuclear phagocytes; perhaps they do not persistently adhere to glass in our culture system.

The morphologic and cytochemical observations reported here deal only with mouse cells. A preliminary report of studies on monocyte precursors in several species by other workers (10) appears to be in general agreement with our results. However, studies on peroxidase in guinea pig macrophages and Kupffer cells, using the Graham and Karnovsky method (8), reveal activity in the endoplasmic reticulum and granules of some of these cells according to another preliminary report (11), in contrast to the absence of peroxidase in mouse peritoneal macrophages in our observations using the Kaplow technique. The peroxidase reactivity of rat blood monocytes has also been noted (12). Convincing evidence has been presented that a nonspecific esterase serves as a marker for mature and immature monocytes in human blood (13, 14).

In terms of general appearance in stained preparations, promonocytes may resemble closely the neutrophil melocyte. Promonocytes and neutrophil

myelocytes are approximately the same size, have similar nuclear and cytoplasmic staining characteristics, and both are also peroxidase reactive; consequently, certain identification by light microscopy is difficult. Two ultrastructural differences are helpful in distinguishing between promonocytes and neutrophil myelocytes: promonocytes have many finger-like surface projections, whereas the myelocyte surface contour is generally smooth, and the promonocyte has only a few granules located in the centrosomal region, in contrast to the large number of granules widely distributed in the myelocyte cytoplasm. Functional properties also assist in distinguishing between these two cell types, since promonocytes engage in phagocytosis and pinocytosis, whereas under most conditions myelocytes exhibit very little or none of these activities.

Another difference between promonocytes and myelocytes is that the mononuclear phagocytes of the bone marrow attach firmly to a glass surface and survive and remain constant in number during an incubation period of 48 hr (Table I); myelocytes, however, adhere much less firmly and detach or die within the first 24 hr of incubation. This characteristic made it possible to obtain from the bone marrow, which contains only about 1.5% mononuclear phagocytes, an enriched population of promonocytes and monocytes on cover slips, so that the kinetics of these cells in the bone marrow could be studied (3).

It also can be difficult to distinguish between promonocytes and small macrophages in stained preparations, although usually the nuclear–cytoplasmic ratio is much higher in promonocytes than in macrophages and the promonocyte cytoplasm is much more basophilic than is the macrophage cytoplasm. From the ultrastructural point of view, macrophages have more cytoplasmic granules, vesicles, and vacuoles than promonocytes, whereas polyribosomes are usually more numerous in promonocyte than in macrophage cytoplasm. Peroxidase cytochemistry is most helpful in distinguishing between these cells, since the promonocyte is distinctly positive whereas the macrophage is negative in this reaction. Finally, although both of these cells exhibit pinocytic and phagocytic activities, the macrophage is much more active than is the promonocyte in these respects.

Granulogenesis in promonocytes has much in common with the production of azurophil granules in progranulocytes (15–18). Apparently in both cases the early progenitor cells manufacture cytoplasmic granules rich in various hydrolases and in peroxidase. In the mid-stage of the maturation process the production of these primary lysosomes ceases, and the number of granules per cell is reduced at the time of subsequent cell division. In the later stages of granulocyte maturation, the cells produce another type of granule, the so-called "specific" granule, which does not contain hydrolases or peroxidase. Late monocyte progenitors do not manufacture such specific granules. However,

the mature macrophage can be stimulated to produce new hydrolases and granules lacking peroxidase. These variations in lysosomal hydrolase and peroxidase production and packaging in the same cell line at different stages of maturation are of interest in terms of gene expression and the precision of cellular regulatory mechanisms.

As mentioned above, the diminution in numbers of peroxidase-positive granules accompanying maturation of promonocytes may be, at least in part, a reflection of cell division following cessation of granule production. This reduction, as well as the subsequent disappearance of all peroxidase activity as the monocyte becomes a macrophage, may also be due to catabolism of the enzyme as a result of endocytic activity, fusion of primary granules with pinocytic vacuoles, and digestion. The decrease of enzyme activity of peroxidase-positive mononuclear phagocytes cultured in vitro should be considered in relation to their age. Promonocytes and monocytes of the bone marrow, which are relatively young cells, retain peroxidase activity when cultured for 48 hr in vitro. However, the peroxidase activity of the peripheral blood monocytes and serum-induced peritoneal macrophages, cells which have already sojourned 1–2 days in the bone marrow and peripheral blood compartments (2, 3), decreases during incubation for 24 or 48 hr. These results indicate that in 4-5–day old mononuclear phagocytes the enzyme activity has disappeared.

The absence of peroxidase in all of the mature or in vitro-stimulated mouse macrophages is worthy of discussion in relation to the proposed role for this enzyme in an intracellular bactericidal system. Microorganisms exposed to low concentrations of peroxidase in the presence of hydrogen peroxide and iodide or chloride salts are rapidly halogenated and killed (19). Macrophages are highly effective in killing engulfed microbes, and therefore antibacterial systems other than the peroxidase system are presumably at work. Antibacterial cationic proteins of the type found in polymorphonuclear leukocyte granules (20) are not demonstrable in macrophages. Thus, the mechanism of bactericidal action within macrophages remains a mystery.

SUMMARY

Mouse promonocytes have been identified and studied in cultures of bone marrow cells. These cells have a diameter of 14–20 μ, and in stained preparations reveal a large, indented or folded nucleus, and basophilic, finely granular cytoplasm. The living promonocyte viewed by phase contrast shows additional features: nucleoli, small dense bodies, and vesicles in the cytoplasm adjacent to the nuclear hilus, and slight membrane ruffling. Prominent ultrastructural components of promonocytes include a well developed Golgi apparatus, small numbers of centrosomal granules and vacuoles, extensive ribosomal aggregates, and finger-like projections of the cell surface.

Promonocytes engage in pinocytosis and phagocytosis, but they are less

active in these functions than are peripheral blood monocytes of peritoneal macrophages.

Promonocytes are positive for peroxidase, the reaction product being localized to granules most of which are centrally situated in the cell. Monocytes in blood or in inflammatory peritoneal exudates display much smaller numbers of peroxidase-positive granules, and various types of mature mouse macrophages are peroxidase negative.

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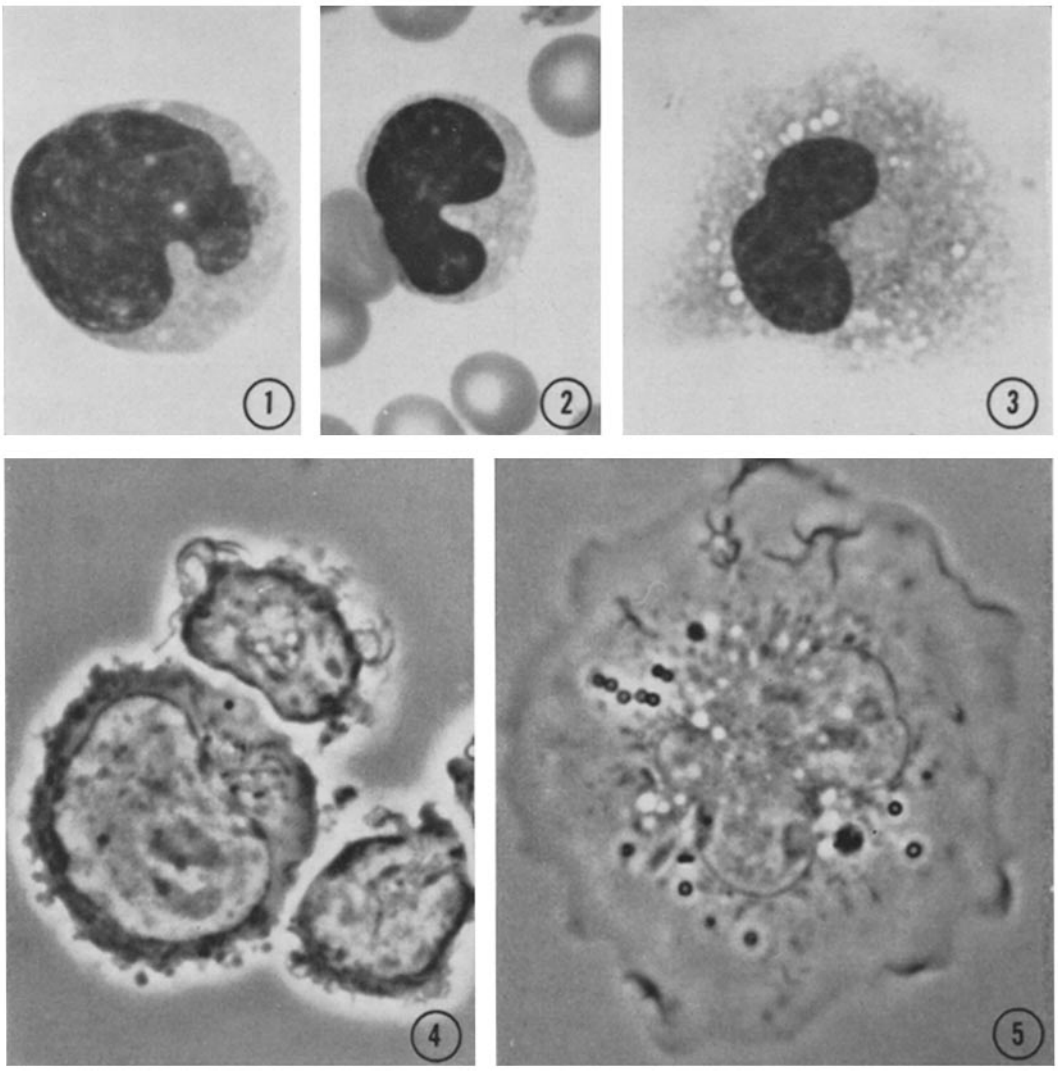


FIGS. 1–3. Wright-Giesma stains of air-dried mouse mononuclear phagocyte preparations.

FIG. 1. A marrow promonocyte after culture on glass for 6 hr. Note the large nucleus (nuclear-cytoplasmic ratio >1) and finely granular cytoplasm. In the original the cytoplasm was a deep blue color. × 1500.

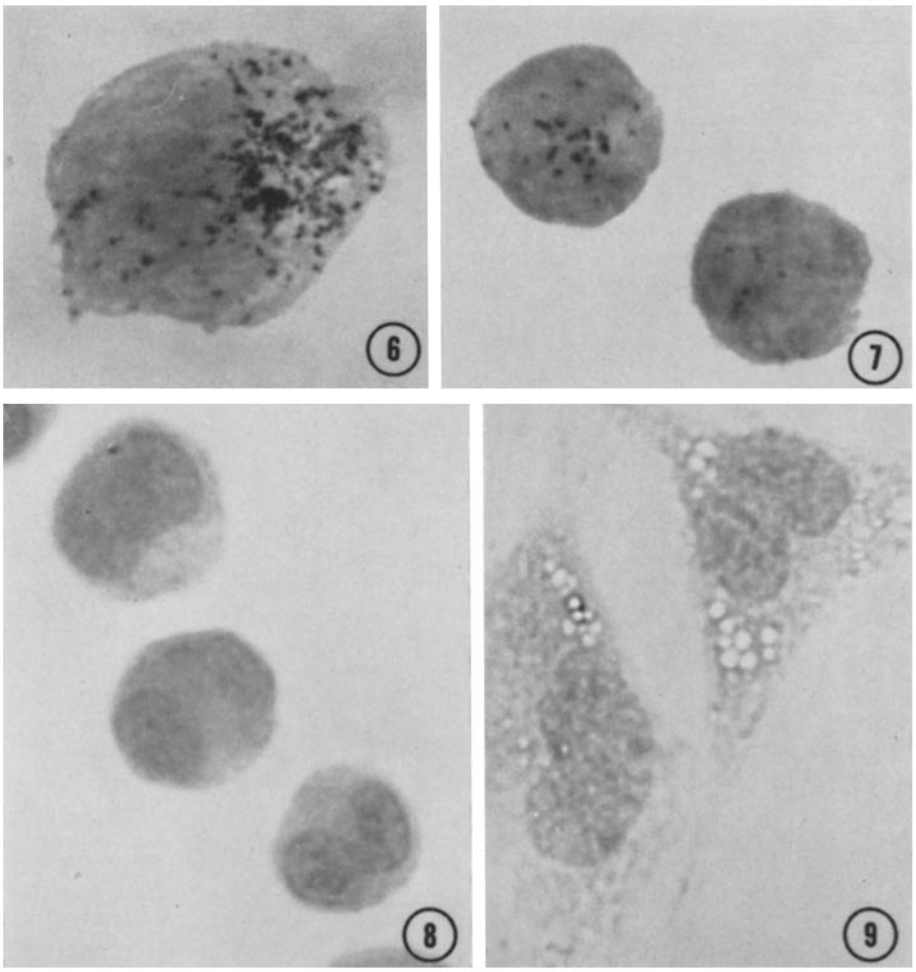
FIG. 2. Blood monocyte. The nucleus stains intensely and is deeply indented (nuclear-cytoplasmic ratio approximately 1). Cytoplasm is finely granular (pale bluish-gray in the original). × 1500.

FIG. 3. A stained mouse peritoneal macrophage after culture on glass for 24 hr. The nucleus is reniform in shape and intensely stained (nuclear-cytoplasmic ratio much less than 1). The cytoplasm is coarsely granular with small dense bodies and empty round areas. In the original the cytoplasm was a pale bluish-grey. The cell margin is irregular and indistinct. × 1500.

FIGS. 4 and 5. Phase-contrast photomicrographs of living mouse mononuclear phagocytes.

FIG. 4. Cells in a 6 hr culture of mouse bone marrow. The large cell at lower left is a typical promonocyte. Note the large transparent nucleus and prominent nucleoli. Small phase-dense round bodies, elongated mitochondria, and a few small clear vesicles are seen in the cytoplasm adjacent to the nuclear hilus. The cell margin shows many small projections. The two smaller cells are marrow monocytes. The nuclei are large and horseshoe shaped. A few dense bodies, mitochondria, and vesicles are visible in the cytoplasm. The plasma membrane is ruffled. × 1500.

FIG. 5. Mouse peritoneal macrophage after culture for 24 hr in vitro. The cell is well spread, and is considerably larger in diameter than monocytes or promonocytes. Many small phase-dense granules, clear vesicles, and refractile lipid droplets are seen in the perinuclear cytoplasm. The cell margin is irregular and membrane ruffling is visible in some areas. × 1500.



FIGS. 6–9. Peroxidase cytochemical reactions of mouse mononuclear phagocytes.

FIG. 6. Promonocyte after culture in vitro for 6 hr. The peroxidase reaction is positive with reaction product in small granules most prominently distributed in the central cytoplasm adjacent to the nuclear hilus. × 1200.

FIG. 7. Two marrow monocytes maintained in culture on glass for 6 hr. A few peroxidase-positive granules are seen centrally located in these cells. Blood monocytes gave an identical peroxidase reaction. × 1200.

FIGS. 8 and 9. Peroxidase reactions are negative on freshly harvested mouse peritoneal macrophages (Fig. 8) and on peritoneal macrophages stimulated by culture in a serum-rich medium for 48 hr (Fig. 9). × 1200.

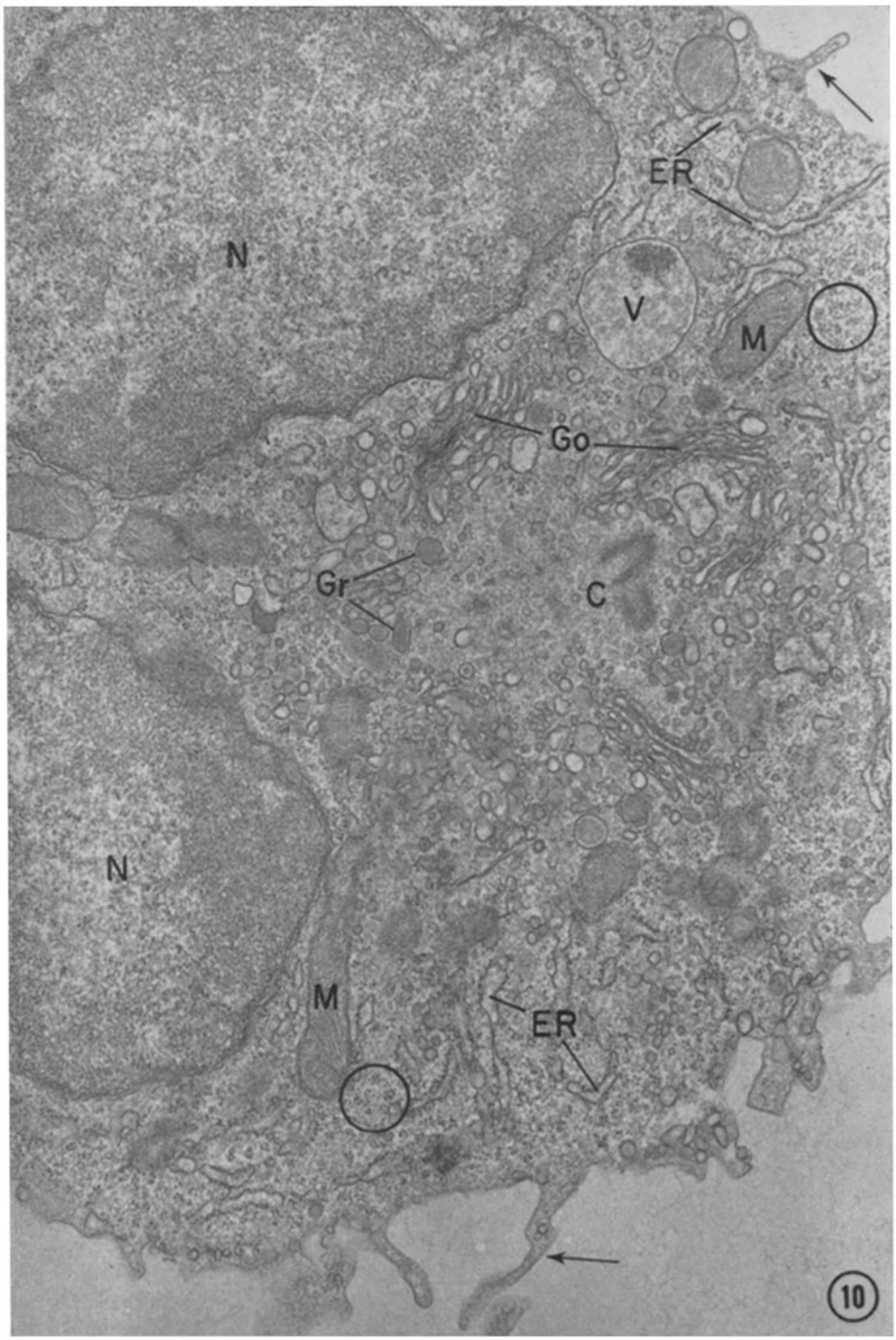


FIG. 10. Electron micrograph of a mouse promonocyte cultured on glass for 6 hr. The reniform nucleus (*N*) appears as two lobes in this thin section. Nucleoli were frequently seen in these cells, although none is present in this section. The centrioles (*C*) are surrounded by small vesicles and flattened saccules of the Golgi apparatus (*Go*). A few small, electron-opaque, membrane-bound granules (*Gr*) are also present in and about the centrosomal region. Mitochondria (*M*) are situated at the periphery of the Golgi region and in the peripheral cytoplasm. A large vacuole (*V*) contains amorphous material and a few vesicles. The peripheral cytoplasm contains scattered strips of rough surfaced endoplasmic reticulum (*ER*). Aggregates of free ribosomes are prominent (circled areas). Many finger-like projections (arrows) are seen at the cell surface. × 20,800.

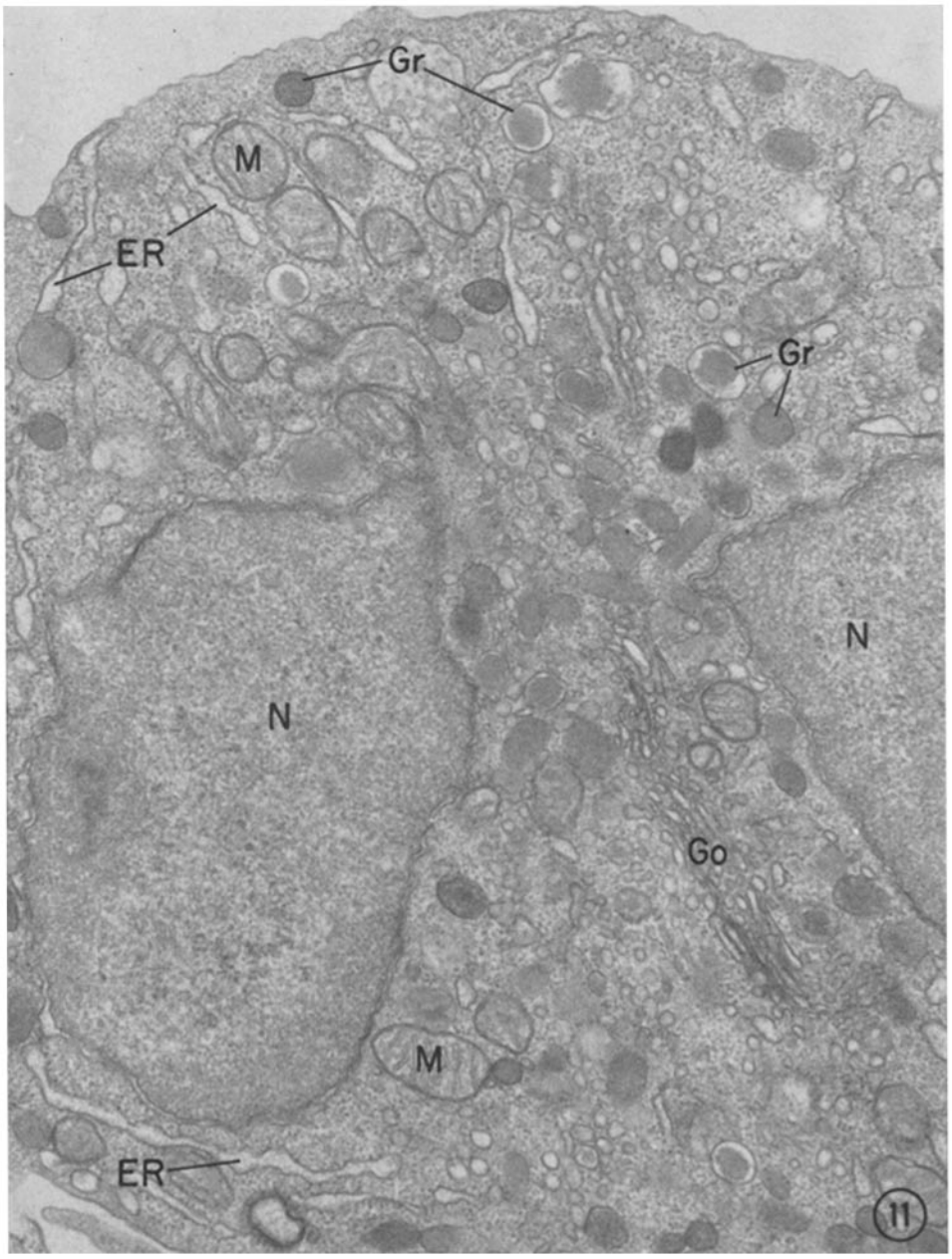


FIG. 11. Electron micrograph of a neutrophil myelocyte present in a 2 hr culture of mouse marrow, shown for comparison with the promonocyte illustrated in Fig. 10. Ultrastructural features of the myelocyte useful in distinguishing this cell from the promonocyte are as follows. The surface is generally smooth and devoid of finger-like projections. The granules (*Gr*) are quite numerous, are large, and are distributed in the peripheral as well as the central cytoplasm. In some of the granules the electron-opaque content appears to have retracted from the limiting membrane. Rough endoplasmic reticulum (*ER*) is abundant and dilated. Mitochondria (*M*) are large and numerous, and show a prominent transverse cristal pattern. × 24,000.

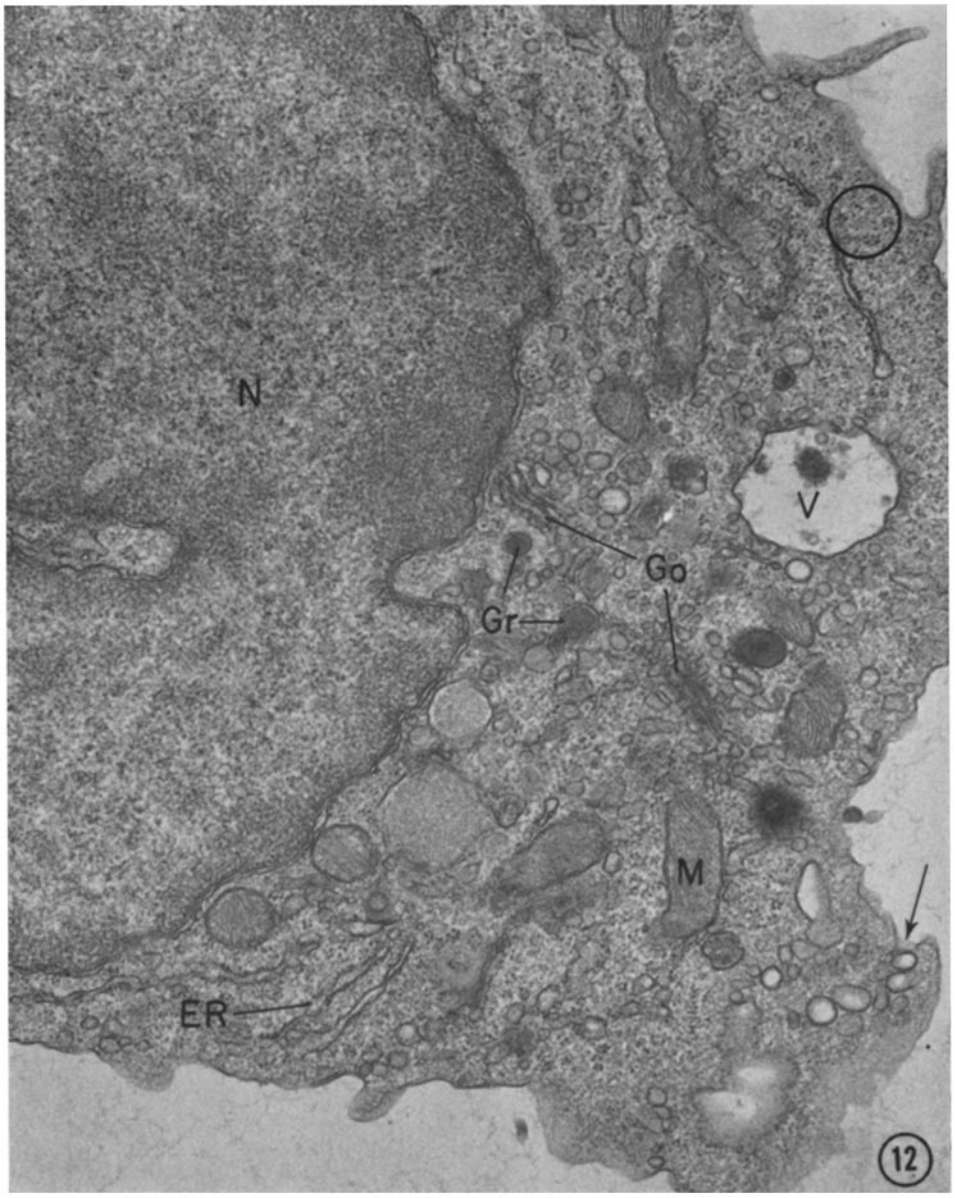


FIG. 12. Electron micrograph of a mouse marrow monocyte cultured on glass for 6 hr. The nucleus (*N*) exhibits a central constriction. Golgi saccules (*Go*) are less prominent than in the promonocyte. Electron-opaque granules (*Gr*) are distributed in the central cytoplasm. A large vacuole (*V*) appears empty except for an amorphous deposit and tiny vesicles. Mitochondria (*M*) and strips of endoplasmic reticulum (*ER*) are distributed in the peripheral cytoplasm. Polyribosomes are present (circled area). The cell surface shows finger-like projections; at lower right (arrow) a chain of clear vesicles may reflect pinocytic activity. × 24,000.

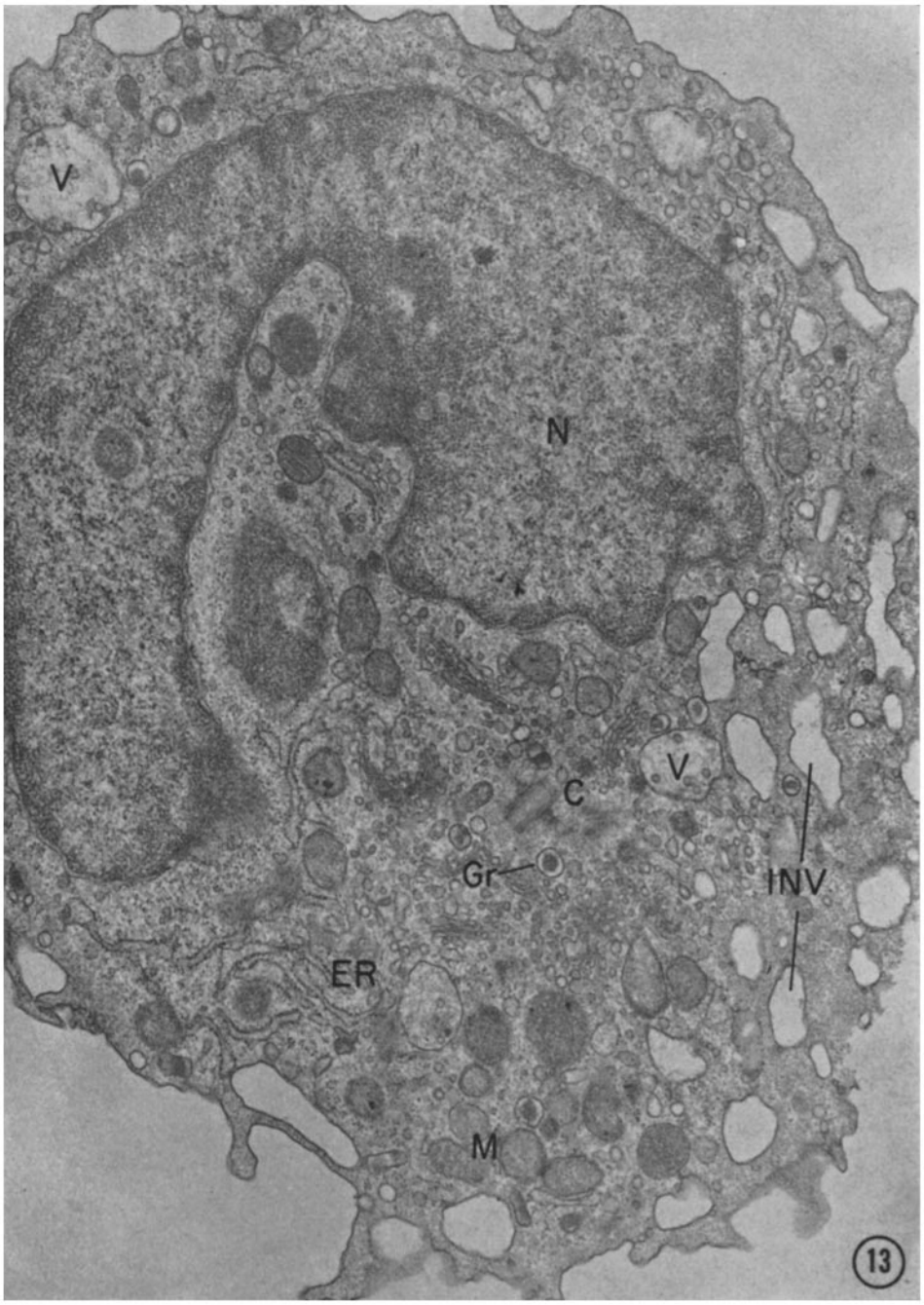
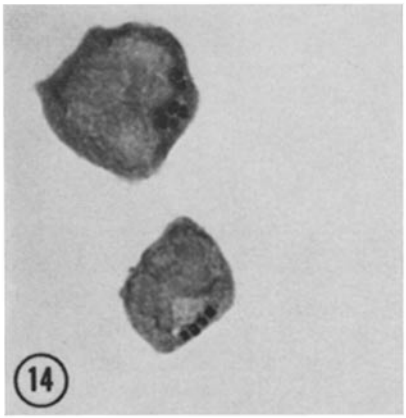
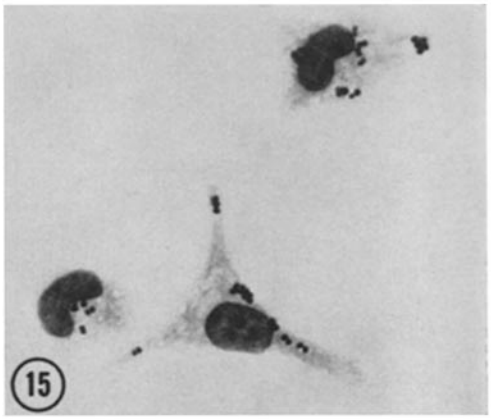
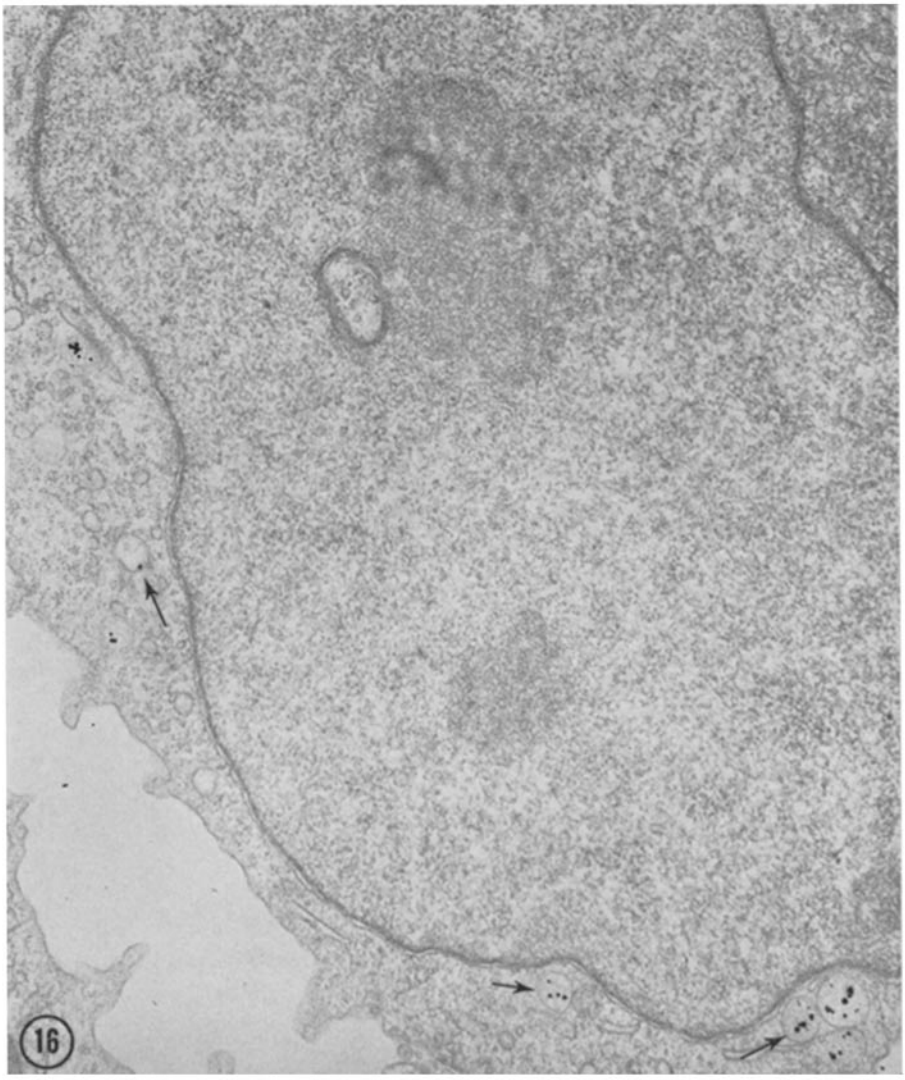


FIG. 13. Electron micrograph of a mouse macrophage fixed promptly after harvest from the peritoneal cavity. The nucleus (*N*) is horseshoe shaped. The centriole (*C*) is situated adjacent to the nuclear identation and is surrounded by numerous small vesicles and granules (*Gr*). Large vacuoles (*V*) contain amorphous material and vesicles. Mitochondria (*M*) and strips of rough endoplasmic reticulum are scattered in the central and peripheral cytoplasm. In some peripheral areas there are large, irregularly shaped empty vacuoles (*INV*), probably representing sections through invaginations of a highly convoluted cell surface. × 20,000.







FIGS. 14–16. Phagocytosis and pinocytosis by promonocytes and marrow monocytes.

FIG. 14. Promonocyte and monocyte cultured for 6 hr; subsequent ingestion of *Staphylococcus albus*. × 960.

FIG. 15. A 48 hr culture of mononuclear phagocytes of the bone marrow. All cells have the morphology of macrophages and all had phagocytized bacteria. × 400.

FIG. 16. Electron micrograph of a promonocyte which had been incubated in a medium containing colloidal gold as a marker for endocytic activity. The large pale nucleus occupies most of this section; a prominent nucleolus is present. Particles of gold are seen in cytoplasmic vesicles and granules (arrows). × 19,500.