THE ISOLATION AND SELECTED PROPERTIES OF BLOOD MONOCYTES*

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Plates 23 to 26

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The blood monocyte is generally thought to be an immature member of the reticuloendothelial system (RES) (1). It is normally present in the peripheral circulation in small numbers and accounts for 3 to 7% of the total leukocytes (2). Its origin and life history remains unclear, but from recent studies (3, 4) it appears that the bone marrow is the major source of blood monocytes. It has a short half-life in the circulation (4) and then apparently emigrates throught the capillary wall and enters the tissues in response to inflammatory stimuli (5). Once in extravascular sites, it may mature in size and complexity and become a macrophage or histiocyte (7). It is this cell which is then intimately involved in the functions ascribed to the RES (8–12).

The developmental potential and functional capacity of the monocytes has been the source of continued research (13–18). Many of the difficulties encountered in these investigations are related to the problem of obtaining homogeneous populations of monocytes in sufficient quantity for detailed analysis. This article will describe a relatively simple method for the quantitative recovery of pure populations of monocytes from the blood as well as selected functional and biochemical attributes of these cells upon cultivation in vitro.

Materials and Methods

Methods for the Maintenance and Harvest of Cultured Cells.—Monocytes isolated from circulating blood and suspended in culture medium were maintained as monolayers in Leighton tubes and T flasks for periods up to 72 hours. The culture medium routinely employed consisted of medium 199 with 40% newborn calf serum (NBCS) and 200 units of penicillin per ml. All cultures were gassed with 5% CO_2-air prior to incubation at 37°C.

Following varying periods of in vitro maintenance, cultures were processed for morphological, cytochemical, or quantitative biochemical studies as described below.

Leighton tubes: Flying cover slips from Leighton tube cultures were used for microscopy and cytochemistry. These methods have been described previously (19) and will be briefly summarized.

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After appropriate periods of culture, cover slips were washed in medium 199 to dislodge unattached cells. Preparations for light microscopy were dipped in No. 199 with 5% serum and fixed wet in cold absolute methanol for 4 minutes. They were then stained by the Giemsa method. Intracellular detail, however, was best appreciated with phase-contrast illumination of osmium-fixed cells. Washed cover slip preparations in Leighton tubes were overlayed for 10 minutes at 4°C with 0.5 ml of 1% OsO_2 in phosphate-saline. Following fixation, cover slips were thoroughly washed with distilled water, mounted wet, and sealed. On occasion, cells were postfixed in 1.25% glutaraldehyle for 10 minutes prior to mounting.

T flasks: Mass cultures for quantitative studies were maintained in flasks and harvested in the following manner: vessels were first agitated to suspend nonadherent cells, the supernatant aspirated, and the monolayers washed three times with 15 ml of saline. 3 ml of distilled water were then added to a culture and 3 minutes allowed for hypotonic lysis. The cell ghosts were removed by scraping and this harvest was transferred to a second washed monolayer. The process was repeated another time so that the contents of three T flasks were contained in the initial volume of water. Aliquots of pooled samples were then stored at −20°C. The yield from three flasks sufficed for a DNA and two enzyme determinations. Samples for acid hydrolase assays were frozen-thawed six times before use.

Cytochemistry: Acid phosphatase was demonstrated on cover slip monolayers by a modification of the Gomori method (19). Monolayers were washed by gentle agitation in unbuffered saline and fixed for 20 minutes at 4°C in 1.25% glutaraldehyde, pH 7.4. They were next placed in cold 0.88 M sucrose for 15 minutes and subsequently incubated in the β-glycerophosphate substrate for 40 minutes at 37°C. After washing, the cover slips were consecutively placed in 1% acetic acid and dilute ammonium sulfide for a period of 1 minute each. Cells were then mounted in distilled water.

Biochemical Assays.—

Deoxyribonucleic acid determination: All metabolic and enzymatic data have been expressed in terms of the deoxyribonucleic acid (DNA) content of the cultures. The method was an adaptation of the Bonting and Jones indole-HCL technique (20), employed previously (21). The DNA content was calculated from a standard curve prepared with highly polymerized calf thymus DNA (Sigma Biochemical Corp., St. Louis).

Acid phosphatase: Assays were performed using a modification of the method of Seligman et al. (22). 2 ml of the substrate β-naphthol acid phosphate (0.2 mg in 0.1 M acetate buffer, pH 5.0) were added to 1.0 ml of frozen-thawed cells and the mixture incubated for 2 hours at 37°C with occasional shaking. Samples were then brought to room temperature and adjusted to pH 7.4 with 0.05 M veronal buffer. Free β-naphthol was coupled by addition of 1.0 ml of the azo dye naphthanil diazo blue B (NDBB, 4 mg/ml), prepared just before use. After 3 minutes, 1.0 ml of 30% TCA was added and the colored complex extracted with 7.0 ml of ethyl acetate. The tubes were centrifuged and the organic layer transferred to cuvettes. Optical densities were read against a water control using the Coleman colorimeter at 540 mμ. The quantity of complexed β-naphthol was then obtained from a standard curve and the activity of 18- and 72-hour cultures expressed in units per mg DNA. The enzyme content of cells after 18 hours of cultivation was compared to that of 72-hour samples and reported as the per cent difference.

BPN hydrolase: (Hydrolysis of the substrate benzoyl d, *l*-phenylalanine β-naphthol ester). The BPN hydrolase of cells cultured for 18 and 72 hours was evaluated by a modification of the technique previously reported (21) and developed by Ravin et al. (23). 1 ml of cell sample was incubated with 2.0 ml of substrate in 0.1 M acetate buffer, pH 5.7 for 1 hour at 37°C.

Aryl sulfatase: The aryl sulfatase of cultured monocytes was measured by the method of Tanaka et al. (24). Both the limited enzyme in monocytes and its dilution in the harvest procedure required that the reaction mixture contain equal volumes (1.0 ml) of cell sample and substrate (2 hydroxy 5-nitrophenyl sulfate, 0.007 M) in acetate buffer, pH 5.7. Following 1

hour of incubation at 37°C, 0.6 ml of 30% TCA was added to stop the reaction. 2 ml of supernatant and 3.5 ml of alkaline quinol reagent were mixed and the resulting color read at 520 mμ. The extent of substrate hydrolysis was read from a standard curve prepared with 4-nitrocatechol.

Cytochrome oxidase: Cytochrome oxidase was determined by the method of Nielsen and Lehninger (25) employing cytochrome C which was reduced with borohydride according to the procedure of Martin (26). Monocytes maintained in culture for 4 and 72 hours were harvested and frozen-thawed twice. A sample of 1.5 ml was added to a cuvette that contained 1.0 ml of water, 0.3 ml of 10 × concentrated buffer and 0.2 ml of the dialyzed cytochrome C. Reaction components were mixed and the rate of substrate oxidation measured on the Beckman DU Spectrophotometer at 550 mμ. Zero order kinetics were maintained for 7 to 8 minutes and specific activity expressed on a DNA basis. All determinations were made within a few hours of cell harvest.

Glycolysis: The glucose uptake and lactate production of mass cultures was measured at 24 hour-intervals during the first 3 days of in vitro cultivation. Immediately after adherence to the culture vessel (2 hours), or following 24 and 48 hours in vitro, cell monolayers were washed with phosphate-saline and 6.0 ml of an indicator-free medium were added to the flasks. The new medium had the following composition: Basal medium (Eagle) with Earle salts (Grand Island Biological, Grand Island, New York), 0.7 mM glutamine, penicillin, and 40% NBCS. For the zero hour glucose and lactate samples, duplicate 0.1 and 0.5 ml aliquots were immediately stored at −20°C. Following 24 hours of incubation the medium was withdrawn, centrifuged, and duplicate samples frozen until the time of assay. Preliminary studies had demonstrated that monocytes cultured in this medium for 24 hours were morphologically similar to those maintained in the routinely employed system.

Glucose was determined by the oxidase method (27). Lactate was measured enzymatically by the method of Horn and Bruns (28). Values are expressed as μmole/24 hours/mg DNA.

Particle Ingestion.—

The uptake of bacteria: A stock laboratory strain of *Staphylococcus albus* "air" was employed as the test particle. Bacteria were cultured overnight in penassay broth on a rotatory shaker, collected by centrifugation, and washed twice with phosphate-saline. Bacteria were heat-killed at 80°C for 10 minutes, washed twice, and diluted with saline to an optical density of 0.200 (13 mm Coleman tube at 650 mμ against water). Phagocytosis test medium (PTM) contained a 1:200 dilution of this stock suspension in medium 199 with 20% NBCS. The addition of 1.0 ml PTM to Leighton tube cultures at T-24 resulted in the phagocytosis of bacteria by 50% of the monocytes after 30 minutes at 37°C. This represented an optimal multiplicity of bacteria/leukocyte at which to measure phagocytosis. This system was therefore used to evaluate both the temporal development of phagocytic activity in cultured monocytes and the influence of endotoxin on their rates of ingestion. Phagocytic activity of cells was evaluated following 2, 24, 48, and 72 hours of culture. Two hundred cells were scored per cover slip and the results expressed both as the per cent monocytes containing bacteria and the number of bacteria per 100 cells. The endotoxin employed was a purified lipopolysaccharide (LPS) isolated by Dr. Otto Westphal from *Salmonella abortus-equi.* A stock of 1.0 mg LPS/ml phosphate-saline was stored at −20°C. 18 hours prior to assay, LPS or an equivalent volume of saline was added to Leighton tube cultures. Following this preincubation, monolayers were washed with medium 199 and 1.0 ml of PTM containing LPS or saline was added to the tubes. These were gassed and maintained at 37°C for 30 minutes with occasional agitation. Following incubation, flying cover slips were removed, washed, and stained by the Giemsa method. When 2-hour cultures were to be examined, LPS was present only during the 30 minute incubation of monocytes with bacteria.

The uptake of colloidal gold.—The quantitative uptake of colloidal gold by monocytes was determined by a previously described method (29). Following maintenance of monocytes in

T flasks for 2, 24, and 48 hours, the supernatant fluid was aspirated and replaced by 8 ml of culture medium containing 10 μg/ml of dialyzed colloidal gold. The influence of LPS on colloid ingestion was simultaneously assayed on duplicate cultures which contained 1.0 μg LPS/ml. Control and treated cultures were gassed and incubated over 24 hours at 37°C. The monolayers were then washed three times with 15 ml of phosphate-saline, digested in 1.5 ml of 0.5 N NaOH, and the optical density determined at 550 mμ. The influence of LPS was most readily demonstrated when control and treated cells were obtained from the same harvest.

RESULTS

Monocyte Isolation from Peripheral Blood.—Horse blood was obtained by jugular puncture and collected in flasks containing $\frac{1}{10}$ volume of 0.1 M citrate-saline. Aliquots were dispensed to large tubes and the erythrocytes allowed to sediment spontaneously for 45 minutes. The leukocyte-rich plasma was removed and centrifuged for 12 minutes at 100 *g* (International, type I). This force sedimented the leukocytes leaving the platelets in suspension. The supernatant fluid was carefully decanted and the tubes allowed to drain in an inverted position. The packed cells were then suspended in 35% albumin (sterile, preservative-free, Pentex, Inc. Kankakee, Illinois) and diluted with phosphate-saline to a final albumin concentration of 27%. A white cell count was performed and 27% albumin added to give a final leukocyte concentration of 75×10^6 WBC/ml. 5 ml aliquots were placed in lusteroid tubes and centrifuged in the swinging bucket rotor (model SCR, Lourdes Instrument Corp., Brooklyn, New York) at 2400 *g* for 36 minutes at 12°C. This yielded a compact pellet containing erythrocytes and granulocytes. The less dense monocytes however, were found concentrated in a surface pellicle along with varying numbers of lymphocytes.

The pellicles were removed by means of a curved Pasteur pipette and the cells pooled in phosphate-saline containing 0.05 mg/ml of heparin. The pooled mononuclear cells were then collected by low speed centrifugation and resuspended in 2.0 ml of medium 199. Differential smears were made from this concentrated suspension and the sample quickly diluted to 25 ml with the complete tissue culture medium. Total and differential cell counts were performed in a hemocytometer and the cells suspended in tissue culture media to a density of 1.2×10^6 monocytes/ml. Since the number of trypan blue positive cells never exceeded 3%, cell counts were not corrected for nonviable cells. The appearance of isolated monocytes did not differ from those observed in smears of whole blood (Fig. 1 *a*). Table I presents the data from ten experiments on the per cent recovery and total yield of monocytes. The average yield from 450 ml of whole blood was 100×10^6 monocytes. This represents an average recovery of 65 ± 15% of the starting monocyte population.

To obtain pure preparations, the differential adhesive property of lymphocytes and monocytes was exploited. Albumin isolated cells suspended in tissue culture medium were placed in culture vessels, 1.0 and 10 ml to Leighton tubes

and T flasks, respectively. The preparations were gassed and cells preincubated at 37°C for 2 hours. During this period, monocytes, but not lymphocytes, adhered to the surface of the culture vessel. Unattached cells were then removed by washing with medium 199, the complete tissue culture medium replaced, and the preparations incubated at 37°C. Examination of cover slip monolayers revealed homogeneous populations of monocytes, (Fig. 1 *b*).

Morphological Observations on Cultured Monocytes.—The appearance of adherent cells after 2 hours of incubation did not differ from that of monocytes in whole blood smears. The cells possessed moderate quantities of cytoplasm which contained occasional vacuoles and a few phase-dense bodies located in

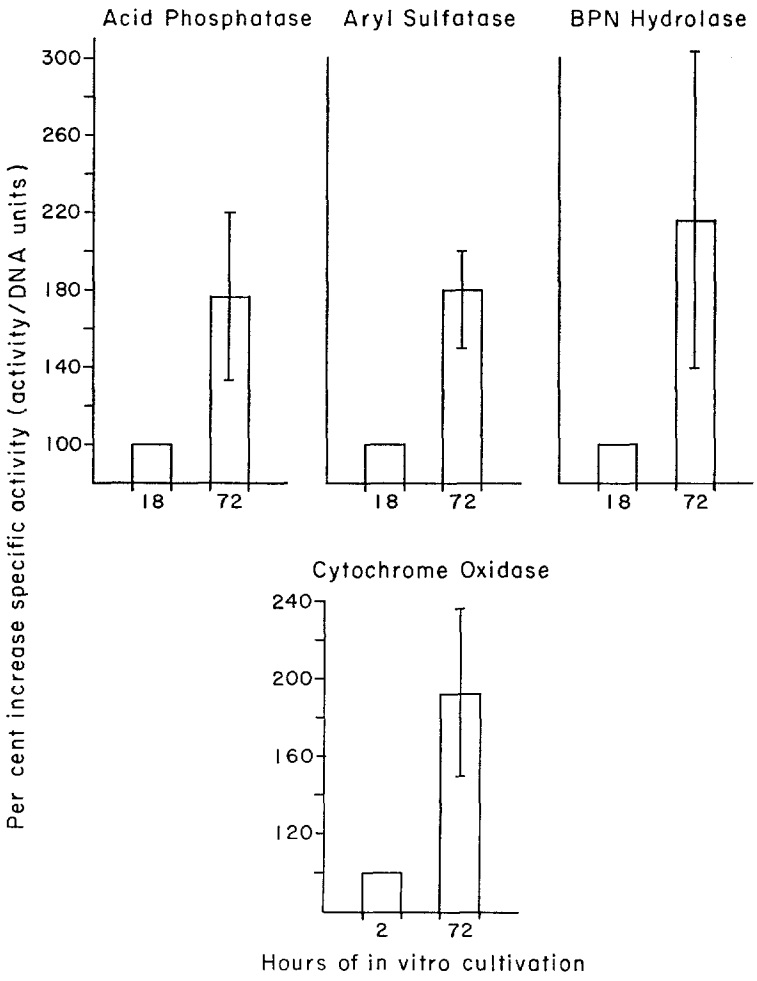
TABLE I

Yield of Monocytes from Whole Blood

Experiment No.	Whole blood				Postgradient pellicle		Yield
	Blood	Total WBC $\times 10^6$	Monocytes	Monocytes $\times 10^6$	Monocytes	Monocytes $\times 10^6$	$\frac{MN - Pellicle}{MN - Blood} \times 100$
	ml		%		%		
1	460	3220	4	129	75	120	92
2	450	3150	6	189	70	125	66
3	480	2400	4	96	50	56	58
4	490	3675	5	184	80	125	68
5	460	3588	4	143	85	132	92
6	450	3150	3	94	85	79	84
7	350	2520	4	101	70	56	56
8	490	3577	2.5	89	60	76	85
9	500	3550	2	71	80	69	97
10	510	3060	4	122	50	130	107

the nuclear "hof". The cytoplasm was free of both ingested material and lipid droplets. The centrally located nuclei were either oval or reniform in shape with a lacy chromatin pattern and distinct nucleoli. No binucleate cells were observed and only an occasional cell had extended blunt pseudopods. Cell spreading was minimal at this time period. Mitochondria were present as short, thick rods and were more readily distinguished when stained with Janus green. Fig. 1 *b* shows the appearance of such cells when photographed with oil immersion phase optics.

After maintenance in culture for 24 hours, monocytes demonstrated variable numbers of lipid droplets, increased numbers of spherical dense bodies and Janus green positive elongated mitochondria. Cell diameters were increased either by uniform cytoplasmic spreading or by extensions of pseudopods. Many nuclei were eccentrically located and some consisted of two or more lobes. A



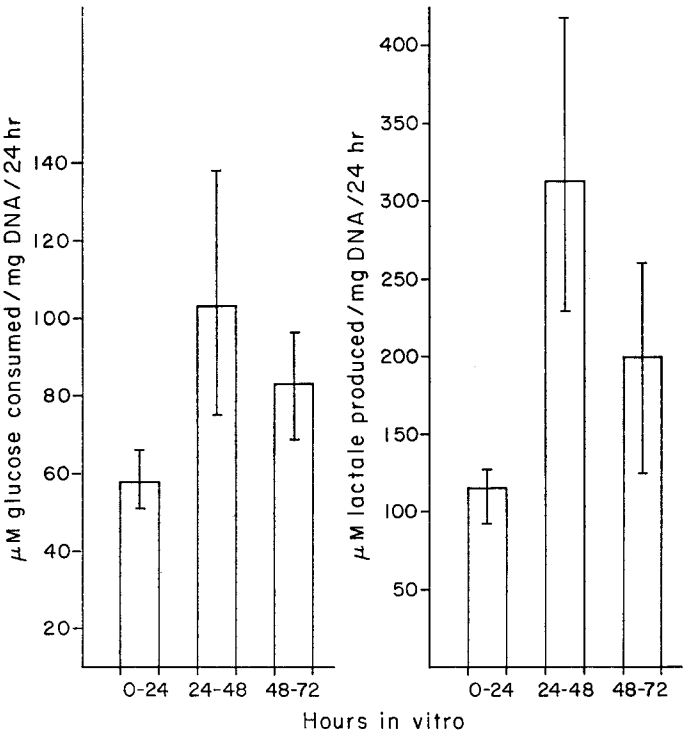
TEXT-FIG. 1. Upper section, the activity of three hydrolytic enzymes during the in vitro cultivation of blood monocytes. Lower section, cytochrome oxidase levels of cultured monocytes.

centrosphere was now present in the perinuclear region. Fig. 2 *a* illustrates a representative cell following 18 hours of culture.

The above changes were more uniformly present and accentuated at the 48 hour period. Some variation in the rapidity of overall cell development was noted as well as individual variation in cells from the same harvest.

Fig. 2 *b* illustrates a cell which has been maintained in culture for 72 hours. The increased diameters of cells at this time are readily apparent. An inner, dense zone of cytoplasm is surrounded by a relatively clear cytoplasmic veil

best appreciated with phase optics. An increased number of rod shaped and pleomorphic mitochondria were found in the cytoplasm as well as dense bodies in the now enlarged centrosphere. Some of these bodies, however, were less phase dense than those observed at the 24 hour period. At 72 hours, varying numbers of multinucleate cells were observed. The nuclei of such cells were oriented in a rosette about the centrosphere and increased chromatin clumping

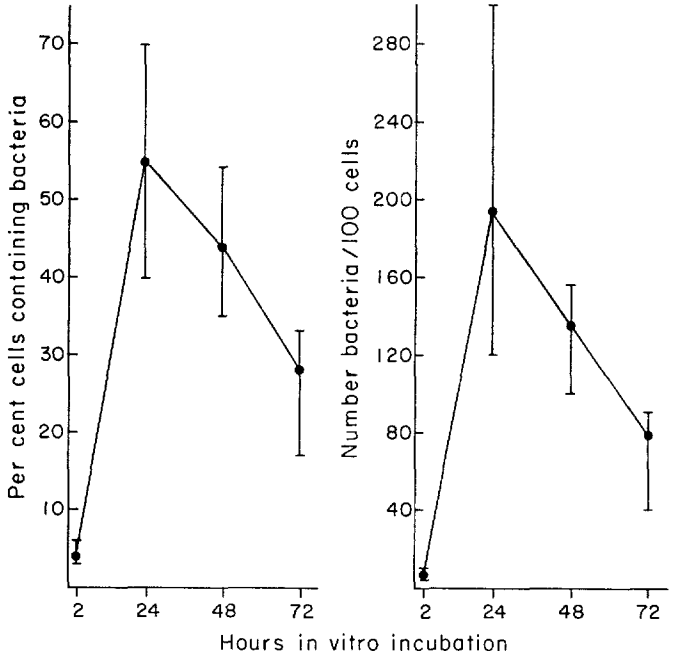


TEXT-FIG. 2. The glucose utilization and lactate production of monocytes during in vitro cultivation.

was noted. Many lipid droplets were present in a zone surrounding the dense granules. Fig. 2 *c* illustrates a multinucleate giant cell. At no time during the cultivation of blood monocytes was there an indication of mitotic activity.

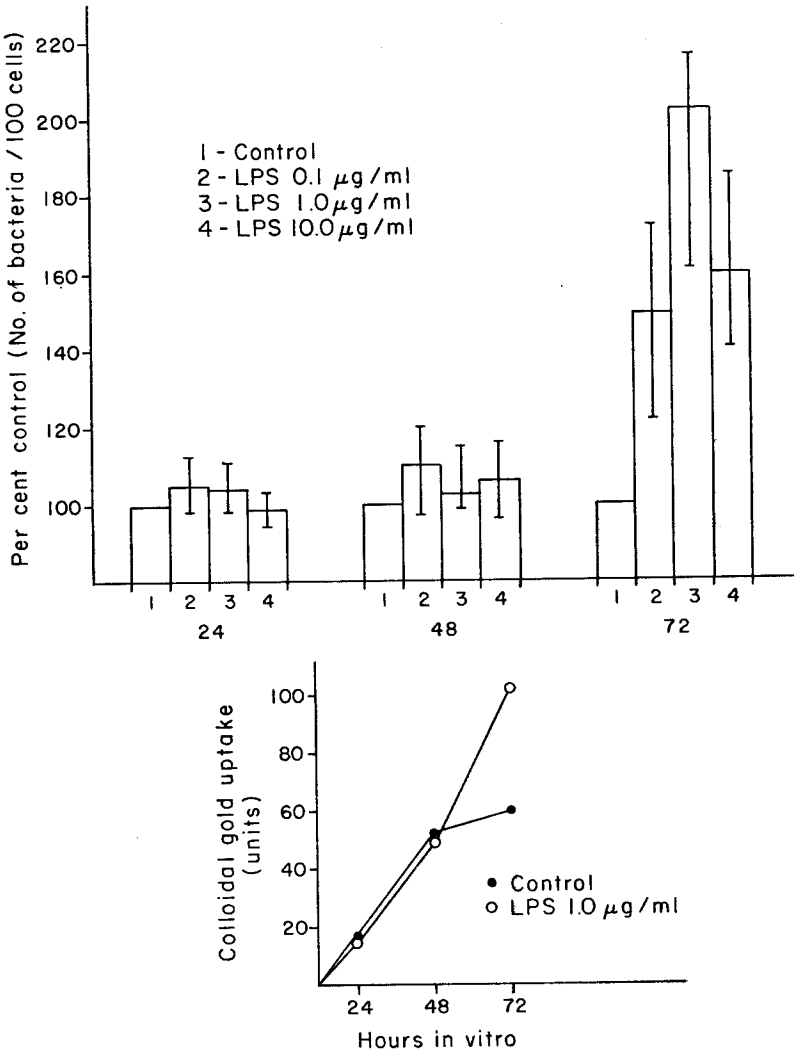
Acid Phosphatase Activity of Cultured Monocytes.—Prior studies had indicated that avian blood monocytes as well as mouse peritoneal phagocytes (18, 19), developed increasing levels of cytochemically demonstrable acid phosphatase upon in vitro cultivation. In the latter case, the reactive sites were localized to the phase-dense, osmiophilic organelles present in the perinuclear region. It was therefore indicated to examine the level and localization of this enzyme in cultures of horse blood monocytes.

The appearance of cells stained by the Gomori procedure is illustrated in Figs. 3 *a* and 3 *b*. Fig. 3 *a* shows a cell after 24 hours of cultivation in which little or no reaction product is apparent. In contrast, cells stained at 72 hours (Fig. 3 *b*) exhibit much more reaction product, which appears localized to the phase-dense granules of the perinuclear region. Though not all cells in a preparation were positive at 72 hours, the total number of such cells exceeded those present at the earlier interval by severalfold. The lead sulfide reaction product was not associated with nuclei, mitochondria or lipid droplets.



TEXT-FIG. 3. The phagocytic activity of monocytes in culture.

The Production of Three Acid Hydrolases by Cultured Monocytes.—The sequential increase in cytochemically demonstrable acid phosphatase was related to the time of in vitro cultivation and suggested the desirability of more quantitative biochemical analyses. It was decided, therefore, to study the levels of acid phosphatase as well as two other acid hydrolases, i.e. aryl sulfatase and BPN hydrolase. The results of these assays are found in the upper section of Text-fig. 1. It is seen that the average activity of cells in culture for 72 hours was 80 to 120% greater than that observed for cells in culture for only 18 hours. When specific activity is based on the content of cell protein, this relationship is less apparent and reflects a concomitant increase in the protein of cultured



TEXT-FIG. 4. Upper section, the influence of lipopolysaccharides (LPS) on the phagocytosis of bacteria by monocytes. Lower section, the influence of lipopolysaccharides (LPS) on the uptake of colloidal gold by monocytes.

cells. This was supported by a progressive increase in the ratio of protein/DNA in cells maintained up to 72 hours. The stability of the DNA content of culture monocytes was, therefore, a more meaningful basis on which to express cell activity.

Cytochrome Oxidase Activity of Cultured Monocytes.—Since monocytes main-

tained in vitro demonstrated an increase in the number of structures identified as mitochondria, it was of importance to examine the level of a structurally bound mitochondrial enzyme. Cells maintained in vitro for 4 and for 72 hours were simultaneously harvested and their cytochrome oxidase activity evaluated. The composite results are shown in the lower section of Text-fig. 1. It is seen that during this 3 day interval there was a 100% increase in the level of enzyme activity. This finding was consistant with an increase in mitochondrial mass during in vitro cultivation.

Glycolytic Activity in Cultured Monocytes.—The glucose uptake and lactate production by monocytes in culture is presented in Text-fig. 2. It is apparent that in vitro maintenance was associated with a significant increase in glycolytic activity. The maximal values occured during the 2nd day of culture. During this period, glucose utilization and lactate production increased 40 and 200% respectively. Thereafter, some diminution in activity occured which, nevertheless, remained elevated over that of the first 24 hours. It was of interest that the lactate/glucose ratio was initially 2 but increased to a value of 3 at 48 hours and remained elevated at 72 hours.

Particle Ingestion by Cultured Monocytes.—In view of the marked morphological and biochemical alterations just described, it was of interest to examine the influence of in vitro cultivation on a functional property of the monocytes, i.e., phagocytosis. The influence of in vitro cultivation as well as that of a purified lipopolysaccharide was studied in detail.

The data concerning the uptake of bacteria are presented in Text-fig. 3. Shortly after cell adherence (T-2), the monocytes demonstrated low levels of phagocytic activity expressed either as the percentage of active cells or as the number of particles per cell. After 24 hours' cultivation, there was a marked and consistent enhancement of phagocytosis. Continued cultivation was associated with a progressive loss in activity, which remained severalfold greater than that of recently isolated cells.

The influence of various dilutions of LPS on the uptake of both bacteria and colloidal gold is presented in Text-fig. 4. The upper section of the figure represents the data for the uptake of bacteria. It is seen that LPS did not significantly stimulate phagocytosis by monocytes during the first 48 hours of in vitro maintainence. At the 72 hour period, however, all dilutions were found to be stimulatory and the activity was enhanced more than 100% in the presence of 1.0 μg LPS/ml. This level of activity, however, was not appreciably greater than the maximal level reached by untreated cells at 24 hours.

The uptake of colloidal gold by control and LPS-treated cells is illustrated in the lower section of Text-fig. 4. Unlike the phagocytosis of bacteria, untreated monocytes in culture for 24, 48, and 72 hours demonstrated a progressive increase in the rate of colloidal gold ingestion. It was only following 48 hours in vitro, however, that endotoxin had a demonstrable effect and resulted in a twofold increase in gold uptake.

The Influence of Lipopolysaccharides on the Morphology and Pinocytic Activity of Cultured Monocytes.—During the course of studies on particle uptake, it was noted that LPS had striking effects on the shape and cytoplasmic organization of cultured monocytes. Exposure of cells to 1 μg/ml of LPS for periods of 24 hours led to the development of larger numbers of phase-dense granules in the centrosphere region. Fig. 4 *b* shows a typical cell which had been incubated in the presence of LPS for the first 24 hours, and contains many more dense bodies than do control monocytes, (see Fig. 2 *a*). The formation of granules could also be stimulated by LPS at later stages of cultivation. Fig. 4 *c* illustrates a cell exposed for the final 18 hours of cultivation and fixed with osmium at T-72.

Prior studies on the formation of dense granules in cultivated mouse mononuclear phagocytes (30) had suggested that they arose from clear pinocytic vacuoles. This prompted a comparison between normal and LPS treated viable monocytes in warm chambers.

Isolated blood monocytes were cultured on cover slips in Leighton tubes. At various intervals (2 to 48 hours) 1 μg/ml LPS was added and the cells exposed for 18 hours. The cover slips were then inverted over shallow chambers (30), sealed, and both control and treated cells observed under oil immersion phase optics at 37°C.

Under these conditions, control cells exhibited little or no discernable pinocytic activity for the first 20 hours. Thereafter, the formation of vesicles and their subsequent fusion and movement towards the centrosphere became progressively more active. Cells treated with LPS and observed at 24 to 72 hours in vitro demonstrated considerable more vesicle formation than identically incubated controls. The pinocytic response to LPS became more prominent as the time of incubation increased. The appearance of a living LPS-treated cell is shown in Fig. 4 *a* and illustrates the marked ruffling of the cytoplasmic membrane and the presence of clear vesicles in the cytoplasm.

DISCUSSION

The lack of information concerning the intrinsic properties of the blood monocyte stimulated efforts to improve the methods of isolation. In the present study, these were applied to horse blood which repeatedly yielded large numbers of cells from the same donor. The methods of isolation depended on two properties of the monocyte. The first was its lower density when compared to the other blood leukocytes and allowed the use of flotation techniques, which resulted in the concentration of monocytes in a surface pellicle. Varying numbers of medium and large lymphocytes also shared a similar density and were collected in the pellicle. The separation of these cells from the monocytes required the exploitation of the second property of monocytes, i.e., their rapid adherence to glass surfaces. When the cells were placed in a glass vessel, the monocytes attached, whereas, the lymphocyte population remained in suspension and was easily removed by a change of the medium. This resulted in

a homogeneous monolayer of viable monocytes. The use of cell elution techniques for the isolation of blood monocytes from variously packed columns results in either neutrophil contamination or samples with a limited yield of monocytes (13). Since the monocytes of other species including man share the properties (31) exploited in the isolation method of the present study, it is likely that the albumin flotation technique could be readily adapted.

The in vitro cultivation of the monocytes resulted in a sequence of morphological alterations in which the cells matured into typical macrophages. This consisted in the formation of increasing numbers of phase-dense granules as well as clear vesicles. The dense granules were localized to the perinuclear or centrosphere region and reacted positively for acid phosphatase. These organelles thereby satisfy one important criterion for being identified as lysosomes (32). This impression was strengthened by a concomitant increase in the assayable levels of acid phosphatase, arylsulfatase, and BPN-hydrolase.

Another change in the cytoplasmic organization of cultured monocytes was the apparent increase in the number and size of mitochondria. This was consistant with the macrophagelike quality of the cells (33) and was corroborated by a twofold increase in the cellular content of cytochrome oxidase. Whether this change is related to the dependence of alveolar macrophages for aerobic processes in phagocytosis is not clear (34). It is of interest, however, that the phagocytic activity of cultured monocytes became increasingly susceptible to the action of 2, 4 dinitrophenol (35). These data suggest that the monocyte-macrophage transition is normally accompanied by an increase in mitochondrial mass and perhaps by a shift to a greater dependence on respiration. The problem is, however, more complex since the glycolytic activity of these cells is also stimulated in vitro. This however, may merely reflect the increased size and cytoplasm of the cells.

In addition to the morphological and biochemical changes which characterize the maturation of the monocyte, there were also alterations in the functional properties of the cells. These were characterized by an increased capacity to ingest particles and differed somewhat depending upon the particle presented to the cell. In the case of bacteria, there seemed to be no correlation with the size of the cell and maximum activity occured at 24 hours. In view of the system in which a limited number of bacteria were allowed to settle on the monocyte, such factors as the "stickiness" of the monocyte-limiting membrane could play an important role for the initial adherence of the bacteria. Unfortunately, our knowledge of the limiting membrane of the cell is extremely fragmentary and factors influencing its properties are unknown. In contrast, monocytes exposed to large numbers of colloidal gold particles demonstrated a progressive uptake of particles which increased with the age of the cells in vitro.

Prior studies from this and other laboratories have indicated that lipopoly-

saccharide endotoxins, in microgram quantities, may stimulate the phagocytic activities of both polymorphonuclear leukocytes and macrophages (36, 37). A somewhat similar finding is reported in this article. It appears that LPS not only activates the phagocytic process but also stimuates the limiting membrane so that more active pinocytosis occurs. This is correlated with the increasing number of phase-dense granules which accumulate in LPS-treated cells. Since these granules probably arise from pinocytic vesicles as they do in mouse phagocytes (30), the treatment with LPS may have also stimulated the formation of lysosomal hydrolases. This influence of LPS has been noted previously in studies on endotoxin-treated animals (19).

SUMMARY

A technique is described for the quantitative recovery of monocytes from horse blood by means of flotation on dense albumin solutions. Monocytes are concentrated in a surface pellicle along with a few lymphocytes which are then removed when the monocytes adhere to a glass surface.

The in vitro cultivation of homogeneous populations of monocytes results in an increase in (*a*) cell size, (*b*) number of mitochondria, and (*c*) phase-dense granules of the centrosphere. The phase-dense granules are osmiophilic and acid phosphatase positive.

Quantitative biochemical analysis during cultivation have revealed increased levels of cytochrome oxidase, acid phosphatase, arylsulfatase, and BPN hydrolase. In addition, glucose utilization and lactic acid production are stimulated under the same conditions.

The uptake of both bacteria and colloidal gold is stimulated during in vitro cultivation. The phagocytic activity of cultured monocytes may be enhanced by a purified bacterial lipopolysaccharide.

These data are consistant with the in vitro maturation of monocytes to macrophages, a cell with greater metabolic and functional potentional.

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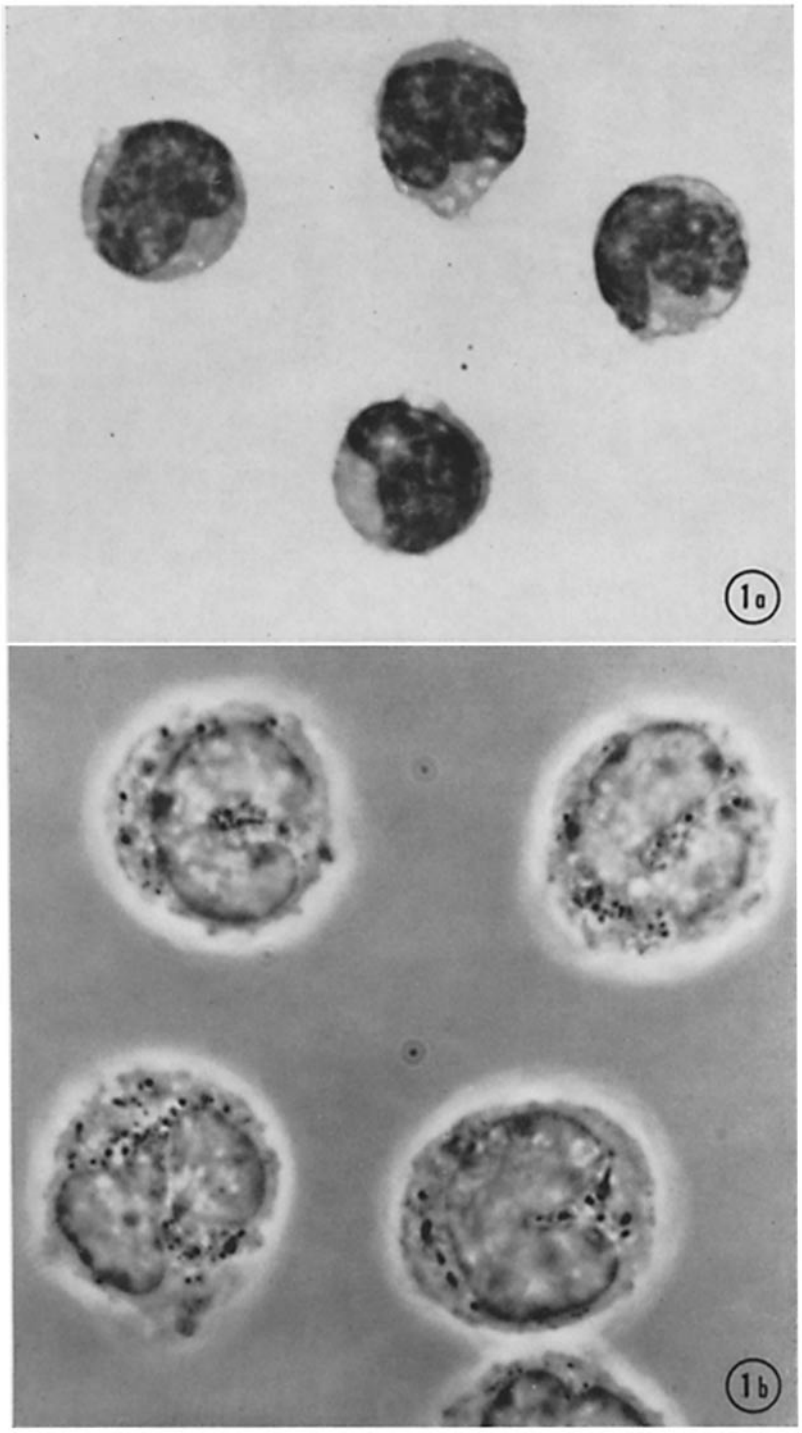
EXPLANATION OF PLATES

PLATE 23

FIG. 1 *a*. Giemsa-stained smear of cells obtained from the surface pellicle. × 1625.

FIG. 1 *b*. Osmium-fixed monocytes shortly after attachment to a glass surface. Short mitochondria and occasional spherical dense bodies are apparent. Phase contrast. × 1625.



(Bennett and Cohn: Isolation and properties of monocytes)

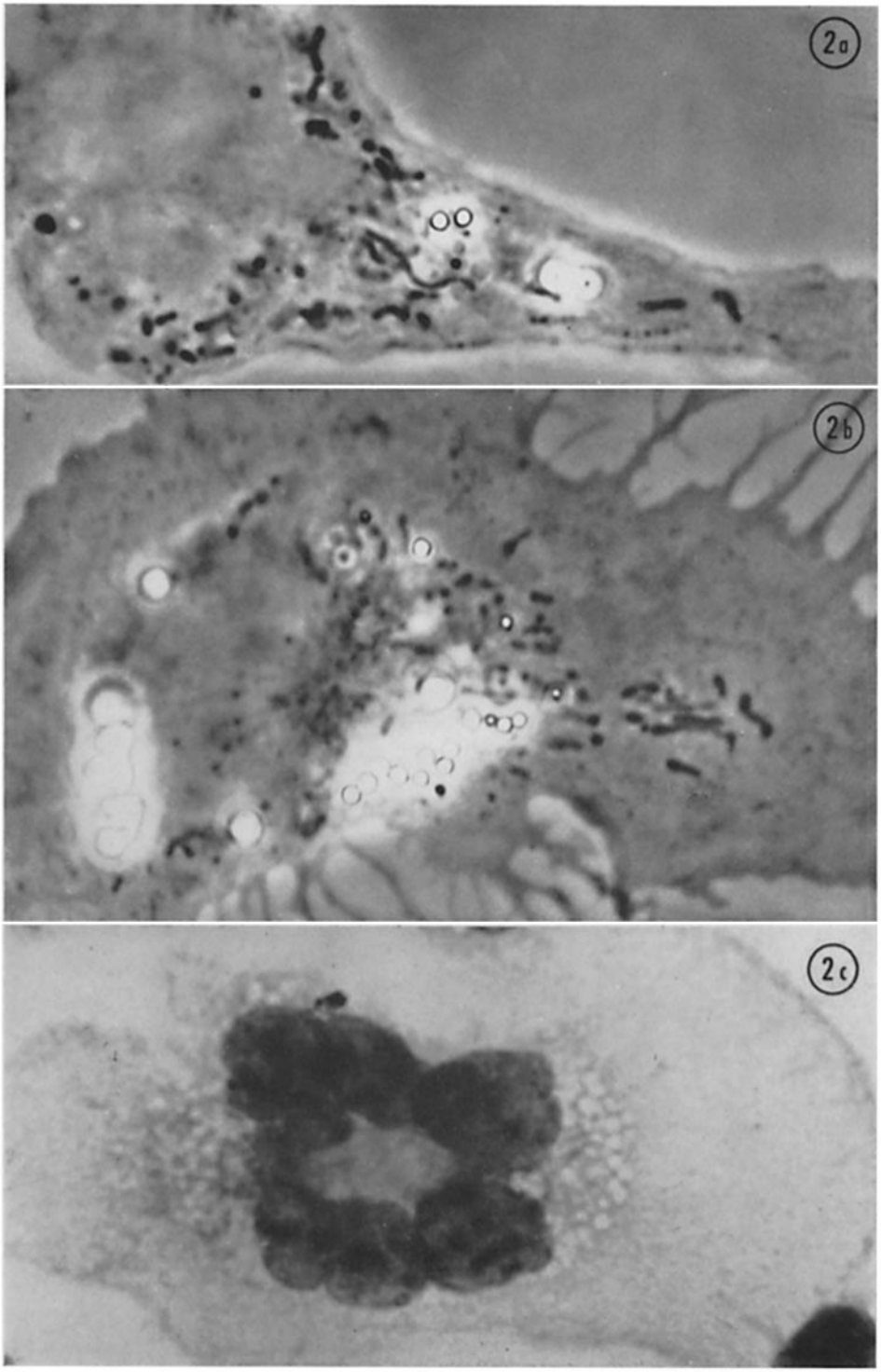
PLATE 24

FIGS 2 *a* to 2 *c*. The appearance of blood monocytes after in vitro maintenance.

FIG. 2 *a*. Appearance of an osmium-fixed cell after 18 hours of cultivation. The mitochondria are somewhat longer and perinuclear dense bodies are seen. This cell illustrates the upper limit of cytoplasmic spreading at this time period. Phase contrast. × 2500.

FIG. 2 *b*. Osmium-fixed cell after 72 hours of cultivation. It illustrates the appearance of a typical macrophage with increased cytoplasmic diameter and elongated pseudopods. The nucleus is eccentrically located and osmophilic granules are present in the centrosphere. The mitochondria are rod-shaped and two clusters of highly refractile lipid droplets are present. Phase contrast. × 2500.

FIG. 2 *c*. A multinucleate giant cell in a 72 hour culture. The granules of the centrosphere are not apparent with methanol fixation. Giemsa stain. × 2500.



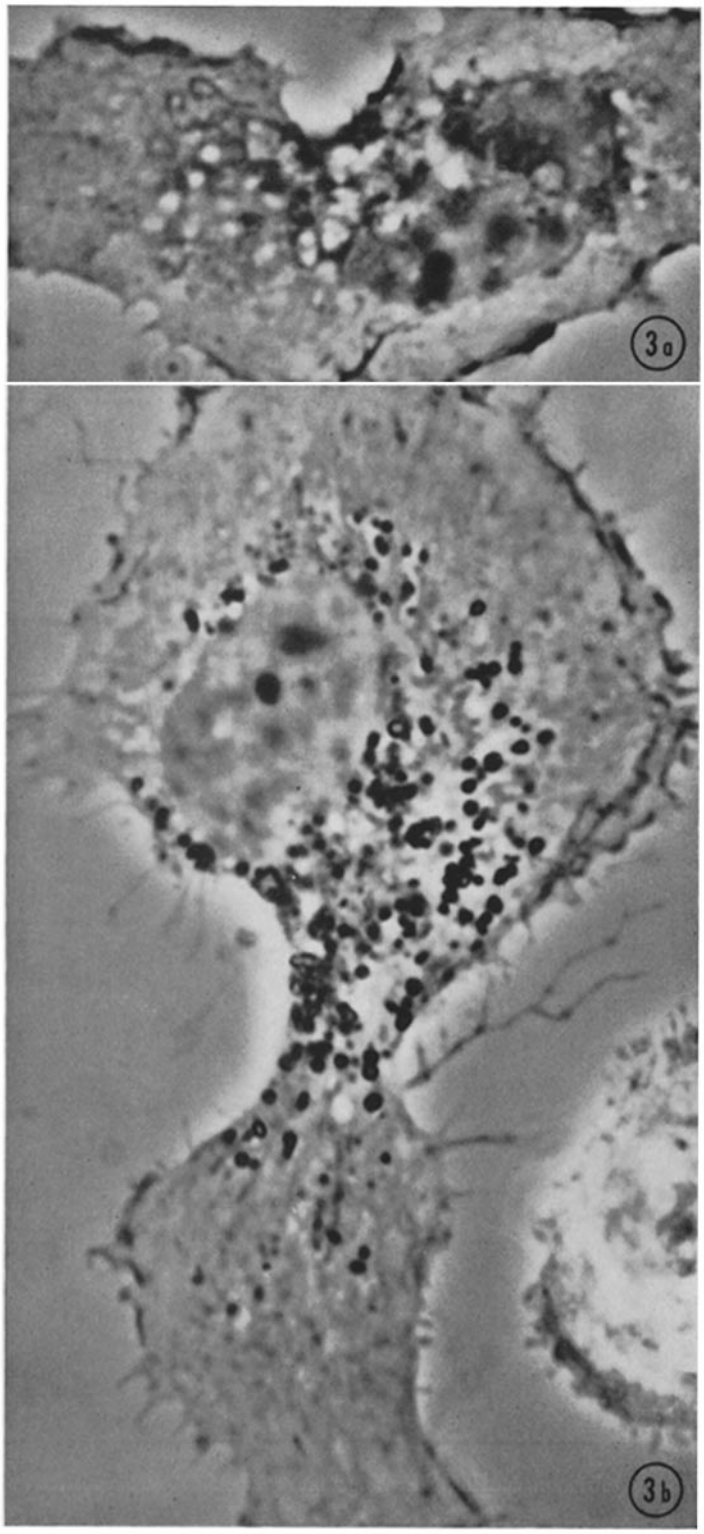
(Bennett and Cohn: Isolation and properties of monocytes)

PLATE 25

FIGS. 3 *a* and 3 *b*. Acid Phosphatase–stained monocytes, fixed in 1.25% glutaraldehyde.

FIG. 3 *a*. 18 hours in vitro. Little lead sulfide reaction product is apparent. Phase contrast. × 2500.

FIG. 3 *b*. 72 hours in vitro. Many granular reaction sites concentrated in the region of the centrosphere. Phase contrast. × 2500.



(Bennett and Cohn: Isolation and properties of monocytes)

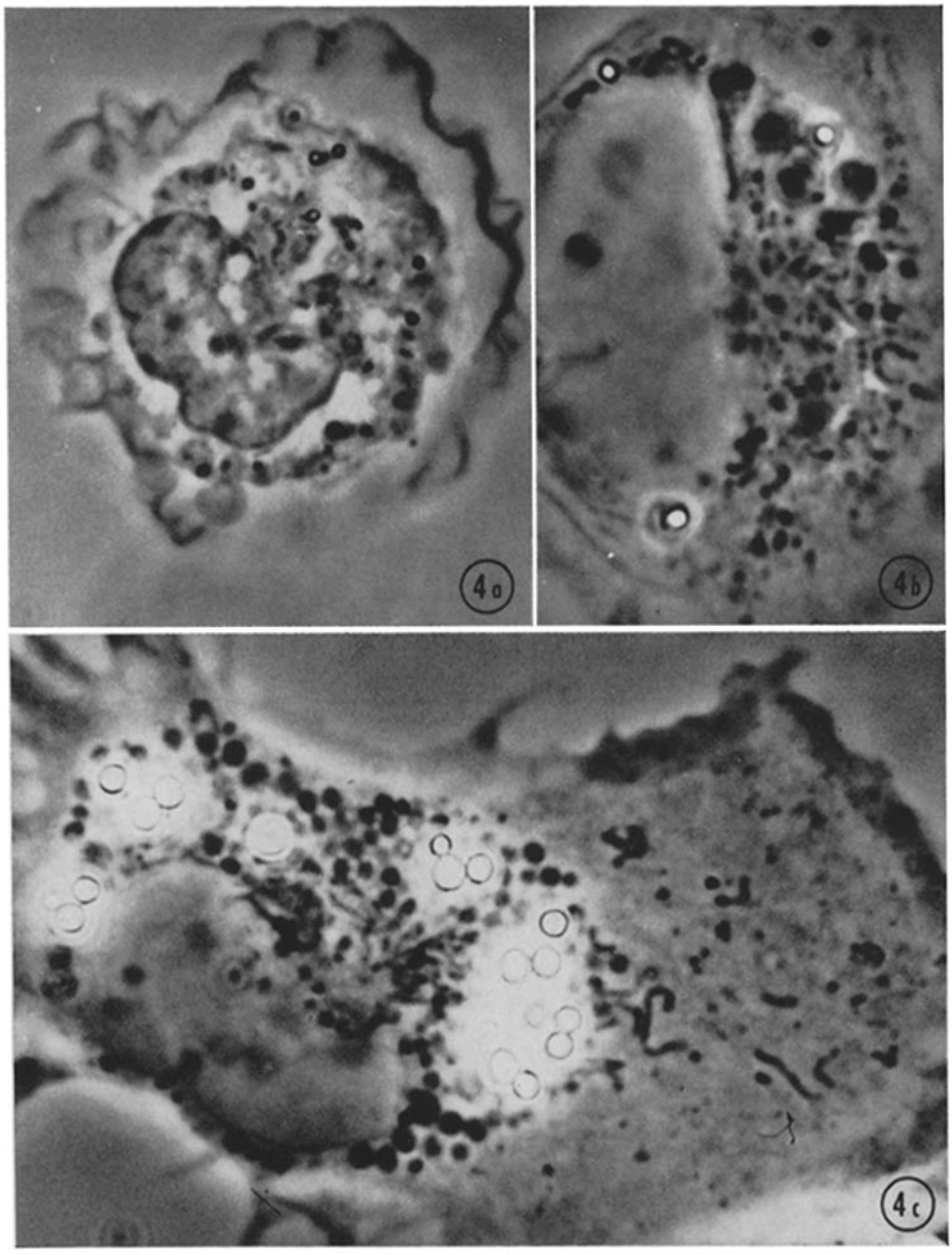
PLATE 26

FIGS. 4 *a* to 4 *c*. Monocytes exposed to 1 μg/ml lipopolysaccharide for 18 hours before fixation.

FIG. 4 *a*. Viable cell in a warm chamber preparation following 24 hours' cultivation. Very ruffled and active limiting membrane. The clear vacuoles arise from the coalescence of pinocytic vesicles. Phase contrast. × 2500.

FIG. 4 *b*. Osmium-fixed cell after 24 hours maintenance in vitro. An increased number of phase-dense, osmiophilic granules in the centrosphere following exposure to LPS. × 2500.

FIG. 4 *c*. Osmium-fixed cell after 72 hours' cultivation. Increased number of dense granules also observed when cells are exposed to LPS during the later phases of cultivation. × 2500.



(Bennett and Cohn: Isolation and properties of monocytes)