# The Separation of Human Monocytes from Blood Including Biochemical Observations[1,2] (37716)

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The importance of the monocyte and its derivative, the macrophage, is becoming increasingly apparent in many areas of biologic research. In addition to its well-known role in phagocytosis, it likely plays a part in processing antigen in cellular immunity (1) and facilitating the response of lymphocytes to antigen (2), pokeweed mitogen (3), and phytohemagglutinin (4). It is a rich source of colony-stimulating factor for the *in vitro* growth of colonies of granulocytes and mononuclear cells from human and murine marrow (5). This cell may of use in the immunotherapy of human neoplastic processes. The monocyte is produced in the bone marrow (6), but its precursor cell is yet to be identified. Measurement of the physiological, immunological, and biochemical activities of the normal human monocyte has been hampered by the difficulty of obtaining pure samples in sufficient quantity. Most methods have resulted in monocyte monolayers adherent to a surface where they cannot be accurately counted or easily handled for study. The present study reports the separation of monocytes in high purity and large quantities from normal human blood. It results in cells that are in suspension and, therefore, can be easily quantitated. They can be easily transferred for radioisotopic, morphologic, and immunologic investigations.

*Materials and Methods.* Two hundred and fifty milliliters of venous blood from normal volunteers was anticoagulated with heparin (12 U/ml of blood) and the erythrocytes sedimented with 5% dextran (Sigma Chem. Co., St. Louis, MO) for 40 min at 37°. The cells in the supernatant were harvested by centrifugation at 190*g* for 8 min and washed in sterile phosphate-buffered saline (PBS, pH 7.25). The mononuclear cells were initially separated by the method of Bennett and Cohn (7). The cells were suspended in 27% bovine albumin (Cohn fraction V) (Sigma Chem. Co.) in PBS at a cell concentration of $75 \times 10^6$ leukocytes/ml. The preparation was centrifuged at 2400*g* at 12° for 36 min. The pellicle of cells formed at the surface was carefully removed with a pasteur pipet.

Further steps were carried out to improve the percentage of monocytes. The leukocytes from the albumin flotation were suspended in medium 199 (Grand Island Biological Co., Grand Island, NY) containing 30% fetal calf serum (Grand Island Biological Co.), penicillin, 200 U/ml, and streptomycin, 0.2 mg/ml, at a cell concentration of $1.2 \times 10^6$ leukocytes/ml. This leukocyte suspension was incubated at 37° in 250-ml Falcon Plastic Culture Flasks (Falcon Plastics, Oxnard, CA) with a constant 5% $CO_2$ environment. After 2 hr, the supernatant and nonadherent cells were decanted and the medium reconstituted. Then, the adherent cells were suspended by gentle scraping with a rubber policeman. Sterile procedures were used throughout.

Cell counts were done in a hemocytometer after staining with Turk's stain and viability of cells was determined by dye exclusion (0.4% erythrosin B). Monocyte purity was

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[2] A preliminary report of this work has appeared in abstract form in Clin. Res. **21**, 547 (1973).

TABLE I. Recovery and Purity of Monocyte Preparations.

| | Monocyte yield | Purity[a] | Recovery[b] | $n$ |
|---|---|---|---|---|
| A.F.[c] | $52.9 \pm 6.4 \times 10^6$[d] (25–166 $\times 10^6$) | 68 ± 4% (54–82%) | 56 ± 3% (34–66%) | 11 |
| A.F.–S.A.[e] | $25.2 \pm 3.6 \times 10^6$ (19–45 $\times 10^6$) | 88 ± 3% (70–95%) | 27 ± 4% (10–49%) | 7 |

[a] Percent monocytes after indicated procedure.
[b] Monocytes harvested as percent of number after dextran sedimentation.
[c] A.F. = albumin flotation alone.
[d] Mean and standard error. Range is shown in parentheses.
[e] A.F.–S.A. = albumin flotation followed by surface adherence (see *Methods*).

assessed initially by Wright's stained smears of the cell preparations and confirmed by nonspecific esterase stains (8). Thin-section electron microscopy was done by the method of Hirsch and Fedorko (9). Macrophage transformation was assessed by light microscopy after culturing the cells for 5 days in the medium 199 solution described above. The methods for thymidine and uridine incorporation and glucose utilization have been previously described (10, 11). Thymidine and uridine incorporation studies were done in triplicate. Isotopes used were 5 μCi thymidine-methyl-$^3$H, 5 μCi uridine-5-$^3$H, and 1 μCi glucose-1-$^{14}$C (New England Nuclear Corp., Boston, MA). Leukocytes ($2 \times 10^6$) from the monocyte preparations were used for the thymidine and uridine incorporation studies and $25 \times 10^6$ for glucose utilization. The culture experiment and biochemical studies were performed only on the cells carried through the entire procedure.

*Results.* Details concerning the recovery rate and purity of the monocyte cell preparations are tabulated in Table I. The entire method was used in separating the monocytes from seven donors. Also shown are the results after the albumin flotation step in those seven donors, as well as cells from an additional four donors where no surface adherence step was carried out. Of those cells obtained from the albumin flotation, 41 ± 17% (mean ± standard error) were recovered after the surface adherence step. The surface adherence resulted in lower monocyte yield but higher purity. The other leukocytes present in the final monocyte preparations were mostly lymphocytes with less than 1% granulocytes.

After scraping the adherent cells from the surfaces of the plastic flasks, the cell viability was 84 ± 4%, but never less than 79%. The entire procedure from venipuncture to enumeration of the cells after removing from surface took less than 8 hr. Attempts to remove the adherent cells from the plastic surface with 0.125% trypsin and 0.1% ethylenediaminetetraacetic acid did not remove the entire monolayer and, therefore, did not obviate the need for gentle scraping. Platelets were not eliminated by the method. The final preparations of monocytes contained 1.4 platelets per monocyte.

The cells appeared normal by Wright's stain. Thin-section electron microscopy revealed normal-appearing monocytes with no cytolysis or evidence of damage from the methods employed (Fig. 1). After culture for 5 days, the cells from the one case studied transformed to macrophages.

The monocyte preparations incorporated labeled thymidine at a rate of 2.2 ± 0.79 ($n = 6$) and uridine at 1.88 ± 0.81 ($n = 6$) picomoles/hr/$10^8$ cells, respectively. Glucose was utilized at 2.59 ± 0.2 μmoles/hr/$10^8$ cells ($n = 3$).

*Discussion.* Previous methods used to separate human monocytes have relied on column techniques with low yields (12) or have used gradient techniques followed by their study as monolayers after surface adhesion (2, 5, 13–16).

Rabinowitz reported a technique which separates monocytes from other blood cells and results in monocytes in suspension (12). Leukocytes were passed through a glass bead

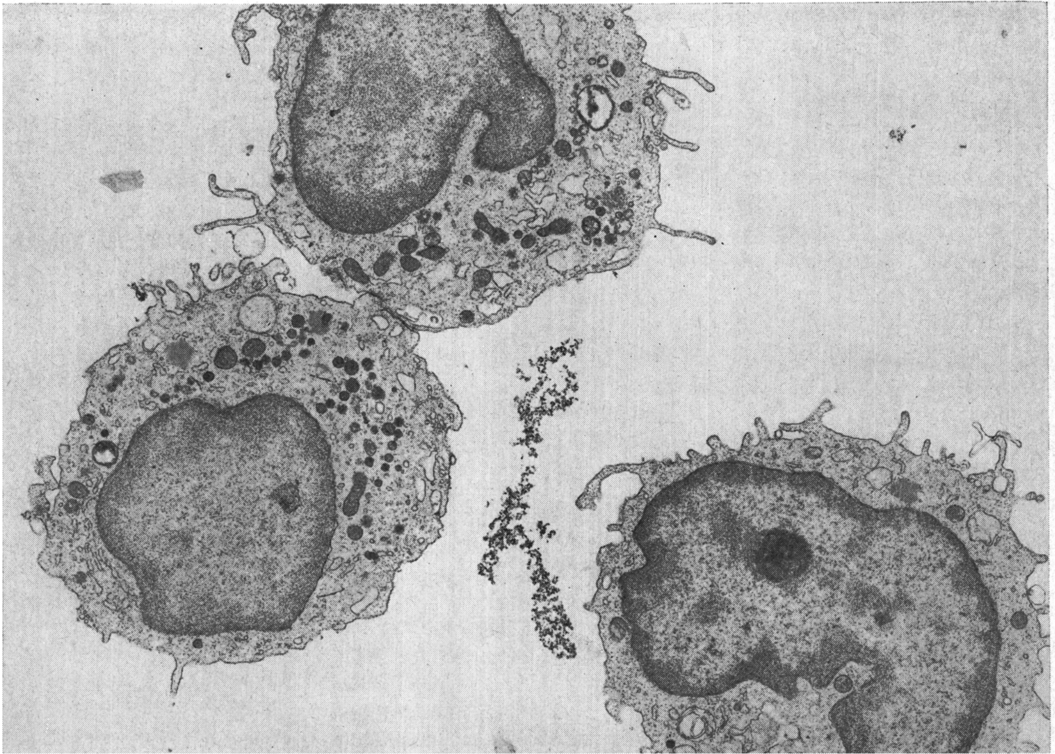

FIG. 1. Electron micrograph of cells obtained. ×9120.

column and the monocytes eluted with a chelating agent. The author reported yields of 5.9–24.2 × $10^6$ monocytes from 500 ml of blood, but less than 74% of the cells in the suspensions were monocytes. Passage of the cells through a second column improved the purity to greater than 95% monocytes, but the total cells obtained from the original 500 ml of blood was 6.1–9.2 × $10^6$. It is difficult to compare the results of other previously reported methods, since they resulted in monolayers of monocytes which could not be enumerated directly.

The present study reports the yield and purity of human monocytes in suspension obtained from 250 ml of normal peripheral blood. The cells obtained appeared normal by light and electron microscopy and were capable of excluding Erythrosin B, incorporating thymidine and uridine, and utilizing glucose. In addition, the monocytes transformed to macrophages after 5 days in culture *in vitro*. Their harvesting as cells suspended in liquid media allowed direct and accurate enumeration and easy aliquoting to flasks and tubes for biochemical incubations or immunologic observations. We feel that the study of suspended monocytes, rather than adherent layers of cells, more nearly approaches physiologic conditions.

Previous studies of the thymidine and uridine incorporation of separated human monocytes have generally demonstrated uridine incorporation into RNA, but negligible rates of thymidine incorporation into DNA (2, 14, 16–19). van Furth and Cohn found no labeled cells after the incubation of mice blood monocytes with thymidine-$^3$H (20). Thymidine incorporation by the human macrophage (21) and mouse peritoneal macrophage (22) have been reported. Monocytes separated by the present method are capable of small but measurable rates of thymidine incorporation. The rate is comparable to that of other unstimulated mature human leukocytes (23, 24). Low rates of thymidine incorporation may be for DNA repair, rather than for DNA replication prior to division

(21). Failure to appreciate low rates of thymidine incorporation may be explained by a lower sensitivity of the autoradiography generally used previously. Alternately, the handling of the monocytes by the present method might result in cells with the ability to incorporate thymidine by either a selective process or activation of the cells by some portion of the method. The values for glucose utilization are similar to reports of determinations performed on monocyte monolayers (15) and to monocytic leukemic cells in suspension [(25), Burns unpublished observations].

This study demonstrates the feasibility of obtaining morphologically intact and metabolically active normal human monocytes, in suspension, in reasonably large quantities and with good separation from other leukocytes. In addition, the rates of thymidine and uridine incorporation and glucose utilization by monocytes separated using these techniques are documented.

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1. Fishman, M. J., J. Exp. Med. **114**, 837 (1961).
2. Hersh, E. M., and Harris, J. E., J. Immunol. **100**, 1184 (1968).
3. Gajl-Peczalska, K., Meuwissen, J. H., and Good, R. A., Int. Arch. Allergy **36**, 546 (1969).
4. Levis, W. R., and Robbins, J. H., Exp. Cell Res. **61**, 153 (1970).
5. Chervenick, P. A., and LoBuglio, A. F., Science **178**, 164 (1972).
6. Volkman, A., and Gowans, J. L., Brit. J. Exp. Pathol. **46**, 62 (1965).
7. Bennett, W. E., and Cohn, Z. A., J. Exp. Med. **123**, 145 (1966).
8. Yam, L. T., Li, C. Y., and Crosby, W. H., Amer. J. Clin. Pathol. **55**, 283 (1971).
9. Hirsch, J. G., and Fedorko, M. E., J. Cell Biol. **38**, 615 (1968).
10. Burns, C. P., Armentrout, S. A., and Stjernholm, R. L., Can. Chemo. Rep. **56**, 527 (1972).
11. Stjernholm, R. L., J. Bacteriol. **93**, 1657 (1967).
12. Rabinowitz, Y., Blood **23**, 811 (1964).
13. Huber, H., and Fudenberg, H. H., Int. Arch. Allergy **34**, 18 (1968).
14. Horowitz, D. A., J. Clin. Invest. **51**, 760 (1972).
15. Para, M., Sagone, A., Balcerzak, S. P., and LoBuglio, A., Clin. Res. **20**, 742 (1972).
16. Levis, W. R., and Robbins, J. H., Blood **40**, 77 (1972).
17. Hanifin, J. M., and Cline, M. J., J. Cell Biol. **46**, 97 (1970).
18. Cline, M. J., and Swett, V. C., J. Exp. Med. **128**, 1309 (1968).
19. Cohn, Z. A., Advan. Immunol. **9**, 163 (1968).
20. van Furth, R., and Cohn, Z. A., J. Exp. Med. **128**, 415 (1968).
21. Cline, M. J., *in* "Formation and Destruction of Blood Cells" (T. J. Greenwalt and G. A. Jamieson, eds.), p. 222. J. B. Lippincott Company, Philadelphia, PA (1970).
22. Weiner, E., Exp. Cell Res. **45**, 450 (1967).
23. Burns, C. P., and Stjernholm, R. L., J. Reticuloendothel. Soc. **9**, 323 (1971).
24. Stjernholm, R. L., Burns, C. P., and Hohnadel, J. H., Enzyme **13**, 7 (1972).
25. Dimitrov, N. V., and Hansz, J., *in* "Cancer Chemotherapy II" (I. Brodsky and S. B. Kahn, eds.), p. 63. Grune & Stratton, New York (1972).

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