

## **Technetium-99m Labeling of Human Monocytes for Chemotactic Studies**

Cynthia K. Papierniak, Raymond E. Bourey, Roberto R. Kretschmer,  
Samuel P. Gotoff, and Lelio G. Colombetti

*Michael Reese Hospital and Medical Center, Pritzker School of Medicine,  
University of Chicago, Chicago, Illinois*

*Although the current use of  $^{51}\text{Cr}$  for the evaluation of monocyte chemotaxis has yielded encouraging results, certain properties of this radionuclide leave room for improvement. Technetium-99m was evaluated as an alternative label for this purpose. A cell loss of 50% was found, but the recovered cells showed excellent viability and function. Chemotaxis was measured using a modified Boyden's chamber and a lymphocyte-derived chemotactic factor. The chemotactic properties of  $^{99\text{m}}\text{Tc}$ -labeled human monocytes were preserved, and an excellent correlation between radioactive measurement and microscopic counting of migrating cells was observed.*

**J Nucl Med 17: 988-992, 1976**

The role of the human mononuclear phagocyte system in the defense against infection has received increased attention, leading to the identification of clinically significant chemotactic defects of monocytes in patients with tumors (1), primary immunodeficiencies (2), Chediak-Higashi syndrome (3), viral infections (4), and steroid therapy (5). This growing interest calls for new techniques to facilitate the study of monocytes and macrophages (6). Radio-labeled mononuclear phagocytes have been used for studies of migration, distribution, and survival (7,8). Labeling is usually achieved using radioactive colloids that are phagocytized by these cells (9). Chemotaxis, however, is more appropriately evaluated with cells that have not been labeled by phagocytic activation (10). Labeling of the cells eliminates the staining and microscopic counting of migrating cells in chemotaxis, thereby enhancing the efficiency and objectivity of the test. Chromium-51 has been used for this purpose (3), although its low specific activity, its low available gamma photon flux per microcurie, and its high spontaneous release from cells (11) are disadvantages that leave room for improvement.

Technetium-99m is the most widely used radio-

nuclide in nuclear medicine today as a result of its short half-life, high specific activity, pure gamma emissions, and high photon flux (12). Once certain types of cells have been labeled with  $^{99\text{m}}\text{Tc}$  in the presence of stannous ion, the spontaneous loss of radioactivity is low (13). Both in vivo and in vitro functions of erythrocytes and lymphocytes have been studied with  $^{99\text{m}}\text{Tc}$  (11,14). We present data showing that  $^{99\text{m}}\text{Tc}$  may serve as a convenient alternative to  $^{51}\text{Cr}$  as a radioactive label in the study of monocyte chemotaxis.

### **MATERIALS AND METHODS**

**Isolation of mononuclear cells.** Sixty milliliters of heparinized venous blood was obtained from healthy adult donors and the mononuclear cells were separated by Ficoll-Hypaque density-gradient centrifugation (15). The mononuclear layer was washed once with RPMI-1640 medium,\* and the cells were re-

Received Jan. 5, 1976; revision accepted June 17, 1976.

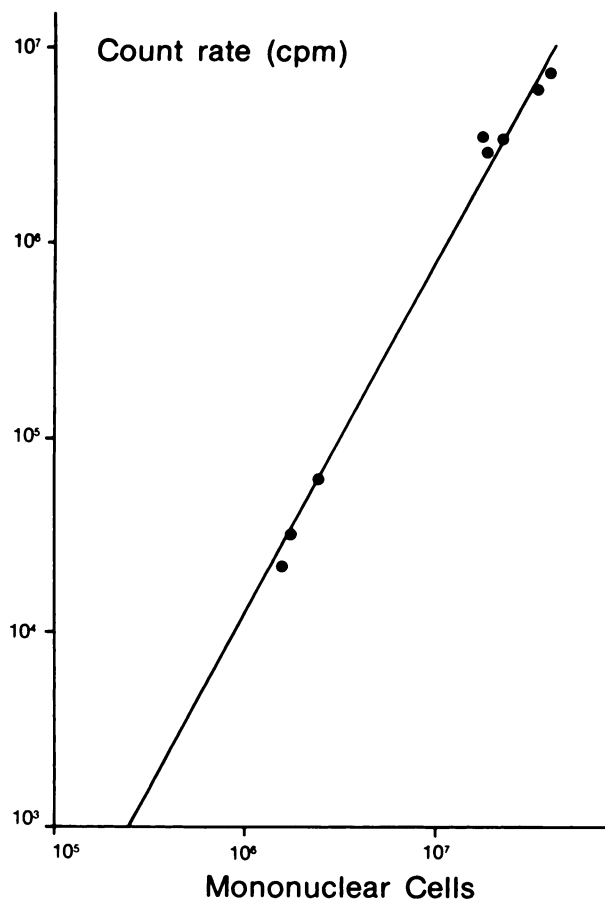
For reprints contact: R. R. Kretschmer, Div. of Immunology, Dept. of Pediatrics, Michael Reese Medical Center, 29th St. and Ellis Ave., Chicago, IL 60616.

suspended by gentle Pasteur pipette aspiration in the same medium to a concentration of  $25 \times 10^6$  mononuclear cells per milliliter in  $16 \times 150$ -mm screw-cap Falcon round-bottom tubes. Differential cell counts revealed 25–35% monocytes, 65–75% small lymphocytes, and less than 3% polymorphonuclear leukocytes. The average yield of monocytes was  $0.45 \times 10^6$  per milliliter of heparinized blood. Viability was 90–95% by trypan blue exclusion (16).

**Technetium-99m labeling.** The method of Barth (17) was followed with minor modifications, primarily the avoidance of chromate, since the efficiency and strength of labeling were not improved by its addition. One to five millicuries of pertechnetate eluted with 0.9% NaCl from a  $^{99m}\text{Tc}$  generator† was added to 2 ml of the cell suspension and incubated for 15 min in a 37°C water bath. The technetium was then reduced by slowly dispensing 0.3 ml of sterile freshly prepared 0.2%  $\text{SnCl}_2 \cdot \text{H}_2\text{O}$  dissolved in freshly prepared acid citrate dextrose–phosphate buffer.‡ The mixture was incubated for an additional 15 min in the 37°C water bath. The tubes were inverted several times to allow equilibration of the reducing solution. Cells were immediately washed three times with RPMI-1640, centrifuging each time at 400 g at room temperature for 15 min. The supernatants were counted to measure the rate of removal of unbound  $^{99m}\text{Tc}$ . After labeling and washing with RPMI-1640, cell counts of the resuspended pellet revealed 45–60% recovery of the cells, unchanged white-blood-cell differential count, and 90–95% viability by trypan blue exclusion. In some experiments six washings with RPMI-1640 were performed in order to ascertain whether all the free  $^{99m}\text{Tc}$  had been removed. No significant removal of unbound nuclide was found, but the recovery of cell-free radioactivity attributable to cell loss was considerable (20–25% for each subsequent washing). Therefore, only three washings were used throughout this experiment to remove unbound  $^{99m}\text{Tc}$ .

**Technetium-99m activity measurement.** Counts per minute were obtained in an automatic gamma counter|| and normalized to 1 mCi of  $^{99m}\text{Tc}$ , since varying amounts of the nuclide were added in each experiment. All samples were counted at exactly the same time after labeling (usually 24 hr) to simplify calculations. Uptake curves were corrected for decay and expressed as counts per minute (cpm) at time zero.

**Quantitative nitrobluetetrazolium (NBT) reduction.** The function of labeled cells was measured by their ability to reduce NBT after ingestion of latex particles. Baehner and Nathan's method was used with minor modifications (18). A prefiltered 0.2% solution of NBT¶ in normal saline was used instead



**FIG. 1.** Relationship between number of mononuclear cells recovered after labeling with  $^{99m}\text{Tc}$  and absolute count rate (cpm) corrected for decay back to time zero.

of the 0.1% NBT solution recommended for polymorphonuclears, since the latter yielded reactions too weak for accurate measurement. Parallel radio-labeled and unlabeled cells from the same donor were assayed, and the optical density (OD) at 515 nm for resting cells and latex-phagocytizing cells were measured; also the individual differences ( $\Delta\text{OD}_{515}$ ) of these two measurements were noted.

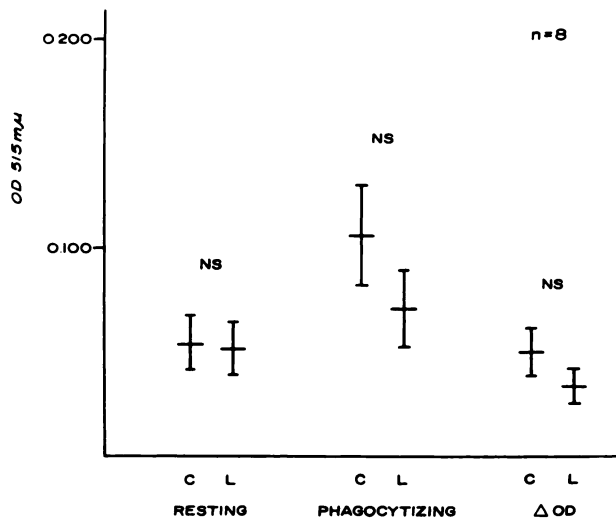
**Spontaneous release of  $^{99m}\text{Tc}$ .** Washed labeled cells were resuspended in RPMI-1640 medium to a concentration of  $3\text{--}5 \times 10^6$  cells per ml. A spontaneous gravity-ultrafiltration system with a  $5.0\text{-}\mu\text{m}$  Nucleopore filter§ was set up using 10 ml of this cell suspension in an inverted syringe attached to a Swinex filter. The filtrate (approximate volume, 0.5 ml) was collected every 30 min for 4 hr and counted. Since random migration of monocytes may occur under these conditions, ultrafiltrates were assayed for the presence of detached cells by centrifugation at 500 g for 45 min at room temperature. The count rate in these supernatant fluids was determined and compared to the count rate in the original ultrafiltrate. The putative cell pellet was also counted.

**Chemotaxis of monocytes.** The method of Snyderman with Boyden chambers\*\* (19) and the double-filter modification (20) was used. All experiments were done in duplicate or triplicate. The top filter was a 5- $\mu$ m Nuclepore and the bottom an 8- $\mu$ m cell-impermeable cellulose nitrate filter.†† The upper compartment was filled with  $0.8 \times 10^6$  monocytes suspended in 0.5 ml of modified Gey's solution (21). The bottom compartment contained 0.4 ml of the chemotactic attractant dissolved in 1.1 ml of Gey's veronal-gelatin buffer (22). The attractant used was a lymphocyte-derived chemotactic factor obtained from phytohemmagglutinin (PHA) stimulated adult lymphocytes (LS), and appropriate controls (LC and O) as described by Altman (23). Chemotaxis was allowed to proceed for 3 hr at 37°C in a 5% CO<sub>2</sub> atmosphere, after which the compartments were emptied, the filters removed, and the bottom filters placed in counting vials with 95% ethanol for fixation. Chemotaxis was expressed as cpm/mCi in the bottom filter 24 hr after labeling. After counting, the filters were stained with Harris' hematoxylin (24). Monocytes present on the upper surface of the bottom filter were counted by microscopy and expressed as monocytes per high-power field.

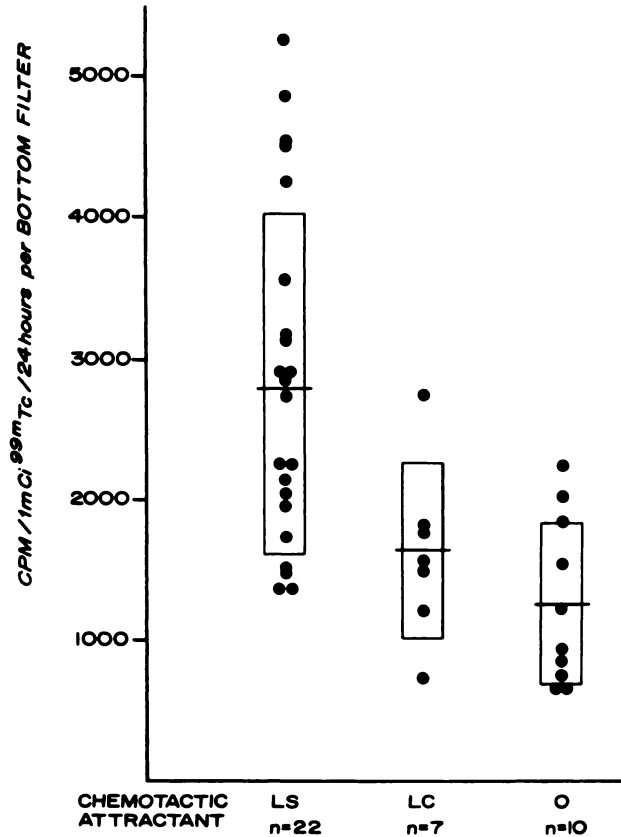
**Statistical analysis.** Results were analyzed by the Student's t-test and the r test for linear correlation (25).

RESULTS

**Technetium-99m uptake by mononuclear cells.** Of the <sup>99m</sup>Tc added, 0.5–2.0% remained bound to the mononuclear cells after three washes. Over 90% of



**FIG. 2.** Comparative quantitative NBT reduction test in unlabeled (C) and <sup>99m</sup>Tc-labeled (L) cells. Geometric means and standard errors are shown: (NS) not significant; (ΔOD) difference in optical densities between corresponding resting and phagocytizing cells.



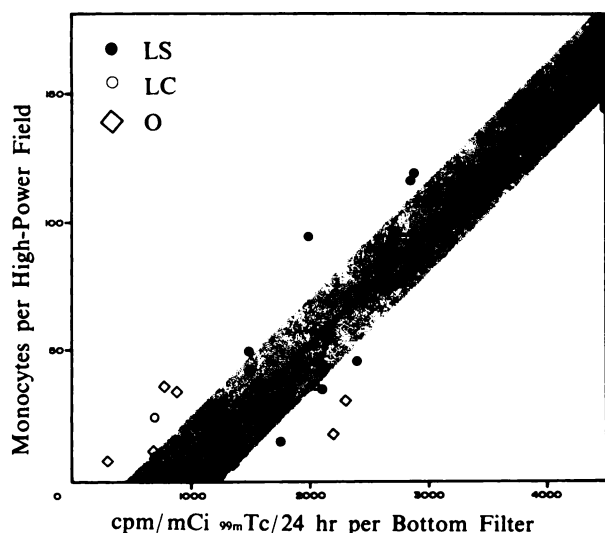
**FIG. 3.** Chemotaxis of <sup>99m</sup>Tc-labeled monocytes. Attractants: (LS) supernatants of PHA-stimulated lymphocytes; (LC) supernatant of unstimulated lymphocytes, i.e., PHA added just prior to harvesting; (O) culture medium alone, random migration. Geometric means  $\pm$  1 s.d. are shown.

the pertechnetate was removed with the first washing. Within the limits of the uptake range, there was a linear relationship between the number of cells recovered and cell-bound <sup>99m</sup>Tc (Fig. 1).

**Spontaneous release of <sup>99m</sup>Tc from labeled mononuclear cells.** Leakage did not exceed 3% of the cell-bound activity at any time over 4 hr. No cell pellet was found after centrifugation of the filtrates, and there was less than background difference between the ultrafiltrate and the supernatant fluid of the centrifuged ultrafiltrate. This confirms the negligible detachment of monocytes from filter surfaces into the attractant fluid of the chemotactic chambers (26).

**Nitrobluetetrazolium reduction test.** An insignificant decrease in NBT reduction was observed in labeled monocytes when compared to unlabeled cells (Fig. 2). Since only monocytes, but not lymphocytes, have the phagocytic ability and enzymatic machinery to reduce NBT (27), the results establish the functional integrity of the labeled monocytes.

**Chemotaxis.** Results are shown in Fig. 3. Radioactivity in the bottom filter of chemotactically stimu-



**FIG. 4.** Linear correlation between chemotaxis evaluated microscopically (monocytes per high-power field and measured by radioactivity counting (cpm/mCi  $^{99m}\text{Tc}/24$  hr per bottom filter). Attractants: (LS) supernatants of PHA-stimulated lymphocytes; (LC) supernatants of unstimulated lymphocytes, i.e., PHA added just prior to harvesting; (O) culture medium alone. Shaded area represents mean  $\pm 1$  s.d.

lated chambers (LS) was significantly higher ( $p < 0.01$ ) than that of nonstimulated (LC) or random migration (O) chambers. The corrected ratios between stimulated and nonstimulated chambers was  $3.92 \pm 1.91$  (mean  $\pm$  s.d.). Since virtually only monocytes, as opposed to lymphocytes, migrate through  $5\text{-}\mu\text{m}$  filters (19), these results indicate uptake of the nuclide by monocytes. Finally, a strong linear correlation ( $r = +0.93$ , s.e.r. = 0.058,  $n = 40$ ) was found between radioactivity in the bottom filter and the number of monocytes per high-power field counted on the upper surface of the same filter (Fig. 4).

#### DISCUSSION

The results of this study indicate that  $^{99m}\text{Tc}$  can be used as a radioisotopic label to study human monocyte chemotaxis. This nuclide offers some advantages over  $^{51}\text{Cr}$  (11): lower spontaneous release from the cells and higher specific activity in the face of similar, albeit low labeling yields (28). Labeling with  $^{51}\text{Cr}$  (3) or  $^{99m}\text{Tc}$  causes approximately 50% cell loss. However, 95% of the cells recovered after labeling with either nuclide are viable (3), and in the case of  $^{99m}\text{Tc}$  labeling they displayed excellent phagocytic and enzymatic functions as revealed by their ability to reduce NBT (29). The disadvantage of the cell loss can be overcome by using chemotactic chambers that require smaller numbers of cells.

Not more than 3% of the  $^{99m}\text{Tc}$  leaks out spontaneously over 4 hr under the experimental conditions of chemotaxis, a figure that compares favorably

with the 10% leakage of  $^{51}\text{Cr}$  under similar conditions (11,30,31). Part of the leaked nuclide may go through the filter(s) used for chemotaxis and eventually appear in the lower (attractant) compartment. A fraction of this unbound radioactive material may be retained by the filters and thus constitutes a constant background (Fig. 4). Since an excellent correlation was found between the radioactivity of the lower filter and the monocytes counted microscopically on the upper surface of the same filter, no correction for this background seems necessary provided chemotaxis is expressed in the currently preferred form of corrected cpm per bottom filter (3,28). On the other hand, this background does affect the results if chemotaxis is expressed as a ratio and therefore has to be subtracted. The corrected monocyte chemotactic ratios observed in this study, and those using a  $^{51}\text{Cr}$  label (3), are smaller than those reported in studies using unlabeled cells (19,32). This difference may be attributed to a small loss of chemotactic vigor by the labeled monocytes, yet it seems a tolerable price to pay for the advantages offered by the labeled-cells method. First, a larger number of chemotactic chambers can be studied in the same period of time by avoiding the laborious staining and microscopic counting of the migrating cells. Secondly, the whole filter area is counted as opposed to the limited number of high-power fields evaluated microscopically. Finally, we have developed an objective method of measurement, eliminating investigator bias.

#### ACKNOWLEDGMENTS

We thank Diane April for the preparation of this manuscript. This investigation was supported in part by NIH Research Grant RR5476 from the General Research Support Branch NIH/DHEW and in part by the Medical Research Institute Council of Michael Reese Medical Center.

#### FOOTNOTES

\* RPMI-1640 medium consists of 50 U penicillin and 50  $\mu\text{g}$  streptomycin per ml; for chemotactic studies 5% de-natured AB/Rh+ plasma was also added.

† Mallinckrodt Ultra TechneKow FM (St. Louis, Mo.).

‡ The buffer consisted of 3.00 gm of  $\text{Na}_2\text{C}_2\text{H}_3\text{O}_7$ , 0.20 gm of dextrose, and 0.02 gm of  $\text{NaH}_2\text{PO}_4$  to 100 ml of distilled water. The pH was adjusted to 7.4 with 10%  $\text{NaHCO}_3$ .

|| Searle Radiographics Series 1185 (Des Plaines, Ill.).

¶ Sigma Chemical Co., St. Louis, Mo.

§ Nuclepore Co., Calif.

\*\* Neuropore, Bethesda, Md.

†† Beckman-Sartorius, Chicago, Ill.

#### REFERENCES

1. BOETCHER DA, LEONARD EJ: Abnormal monocyte chemotactic response in cancer patients. *J Natl Cancer Inst* 52: 1091-1099, 1974
2. ALTMAN LC, SNYDERMAN R, BLAESE RM: Abnormalities of chemotactic lymphokine synthesis and mononuclear

- leukocyte chemotaxis in Wiskott-Aldrich syndrome. *J Clin Invest* 54: 486-493, 1974
3. GALLIN JI, KLIMERMAN JA, PADGETT GA, et al.: Defective mononuclear leukocyte chemotaxis in the Chediak-Higashi syndrome of humans, mink and cattle. *Blood* 45: 863-870, 1975
  4. KLEINERMAN ES, SNYDERMAN R, DANIELS CA: Depression of human monocyte chemotaxis by Herpes simplex and influenza viruses. *J Immunol* 113: 1562-1567, 1974
  5. RINEHART JJ, BALCERZAK SP, SAGONE AL, et al.: Effects of corticosteroids on human monocyte function. *J Clin Invest* 54: 1337-1343, 1974
  6. VAN FURTH R, LANGEVOORT HL, SCHABERG A: Mononuclear phagocytes in human pathology. In *Mononuclear Phagocytes*. Oxford, Blackwell, 1975, pp 1-13
  7. VAN FURTH R, COHN ZA: The origin and kinetics of mononuclear phagocytes. *J Exp Med* 128: 415-435, 1968
  8. MEURET G, BAMMERT J, HOFFMANN G: Kinetics of human monocytopoiesis. *Blood* 44: 801-816, 1974
  9. ROSER B: The migration of macrophages in vivo. In *Mononuclear Phagocytes*, van Furth R, ed. Philadelphia, F. A. Davis, 1970, pp 166-177
  10. KELLER HU, SORKIN E: Studies on chemotaxis. X. Inhibition of chemotaxis of rabbit polymorphonuclear leukocytes. *Int Arch Allergy* 34: 513-520, 1968
  11. BARTH RF, SINGLA O, GILLESPIE GY: Use of  $^{99m}\text{Tc}$  as a radioisotopic label to study the migratory patterns of normal and neoplastic cells. *J Nucl Med* 15: 656-661, 1974
  12. LEDERER CM, HOLLANDER JM, PERLMAN I: *Table of Isotopes*, 6th ed. New York, Wiley, 1967, p 47
  13. BARTH RF, GILLESPIE GY: The use of technetium-99m as a radioisotopic label to assess cell mediated immunity in vitro. *Cell Immunol* 10: 38-49, 1974
  14. ANGHILERI LJ, LEE JI, MILLER ES: The  $^{99m}\text{Tc}$ -labeling of erythrocytes. *J Nucl Med* 11: 530-533, 1970
  15. BOYUM A: Isolation of mononuclear cells and granulocytes from human blood. *Scand J Clin Lab Invest* 21: Suppl 97, 77-89, 1968
  16. BOYSE EA, OLD LJ, CHOUROULINKOV I: VI. Cytotoxic test for demonstration of mouse antibody. *Methods Med Res* 10: 39-47, 1964
  17. BARTH RF, SINGLA OM: Migratory patterns of technetium-99m-labeled lymphoid cells. I. Effects of antilymphocyte serum on the organ distribution of murine thymocytes. *Cell Immunol* 17: 83-95, 1975
  18. BAEHNER RL, NATHAN DG: Quantitative nitroblue tetrazolium test in chronic granulomatous disease. *N Engl J Med* 278: 971-976, 1968
  19. SNYDERMAN R, ALTMAN LC, HAUSMAN MS, et al.: Human mononuclear leukocyte chemotaxis: A quantitative assay for humoral and cellular chemotactic factors. *J Immunol* 108: 857-860, 1972
  20. KELLER HU, BOREL JF, WILKINSON PC, et al.: Re-assessment of Boyden's technique for measuring chemotaxis. *J Immunol Methods* 1: 165-168, 1972
  21. SNYDERMAN R, GEWURZ H, MERGENHAGEN SE: Interactions of the complement system with endotoxic lipopolysaccharide. Generation of a factor chemotactic for polymorphonuclear leukocytes. *J Exp Med* 128: 259-275, 1968
  22. RAPP HJ, BORSOS T: *Molecular Basis of Complement Action*. New York, Appleton-Century-Crofts, 1970, pp 75-80
  23. ALTMAN LC, SNYDERMAN R, OPPENHEIM JJ, et al.: A human mononuclear leukocyte chemotactic factor: Characterization, specificity, and kinetics of production by homologous leukocytes. *J Immunol* 110: 801-810, 1973
  24. PREECE A: *A Manual for Histologic Technicians*. Boston, Little, Brown, and Co., 1965, pp 155-156
  25. ARMITAGE P: *Statistical Methods in Medical Research*. New York, Wiley, 1973, pp 104-166
  26. KELLER HU, HESS MW, COTTIER H: The in vitro assessment of leukocyte chemotaxis. *Antibiot Chemother* 19: 112-125, 1974
  27. LIM SD, KIM WS, KIM CS, et al.: NBT responses of neutrophils and monocytes in leprosy. *Int J Lepr* 42: 150-153, 1974
  28. GALLIN JI, CLARK RA, KIMBALL HR: Granulocyte chemotaxis: an improved in vitro assay employing  $^{51}\text{Cr}$ -labeled granulocytes. *J Immunol* 110: 233-240, 1973
  29. KARNOVSKY ML: Metabolic basis of phagocytic activity. *Physiol Rev* 42: 143-168, 1962
  30. HARVEY WC, SILVA J:  $^{51}\text{Cr}$  labeling of concentrated phagocytes. *J Nucl Med* 14: 890-894, 1973
  31. RONAI PM: The elution of  $^{51}\text{Cr}$  from labelled leukocytes—A new theory. *Blood* 33: 408-413, 1969
  32. SNYDERMAN R, ALTMAN LC, FRANKEL A, et al.: Defective mononuclear leukocyte chemotaxis: A previously unrecognized immune dysfunction. *Ann Intern Med* 78: 509-513, 1974