RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

Imaging of Inflammation with Indium-111 Tropolonate Labeled Leukocytes

A. Michael Peters, Sethna H. Saverymuttu, Helen J. Reavy, Heather J. Danpure, Safiye Osman, and J. Peter Lavender

Hammersmith Hospital, London W12 OHS UK

Indium-111 tropolonate has recently been introduced as a new cell-labeling agent. It has the interesting property of labeling cells in plasma with high efficiency, and may therefore promote an improvement in viability of labeled cells. This paper describes our initial experience with In-111 tropolonate as a leukocyte label for abscess imaging. Pure populations of separated granulocytes, as well as crude leukocyte preparations, have been labeled. Of 101 studies performed, 51 were positive (no false positives) and 50 negatives, of which only two were false negatives. Localization in sites of inflammation was prominent and rapid. Of 36 positive studies, 27 were already positive at 40 min following injection and an additional nine at 3 hr. Of the other 15 positive studies, 11 were scanned for the first time at 3 hr, when they were positive. Granulocytes labeled with this agent in plasma showed minimal sequestration in lungs and liver, interpreted as indicating improved viability in comparison with cells displaying prolonged lung sequestration.

J Nucl Med 24: 39–44, 1983

Leukocytes have been labeled with a variety of gamma emitters in an attempt to locate inflammatory lesions (*1–3*). The introduction of the lipophilic complex In-111 oxine was an important advance in this field (*4,5*). Removal of cells from plasma is essential during labeling with In-111 oxine because of the affinity of this complex for transferrin. Recently a method of cell labeling with In-111 in plasma has been developed using the chelating agent tropolone (*6–8*). In this paper, we report initial clinical studies on the use of this agent in the diagnosis of inflammatory conditions.

METHODS

Patients. One hundred and one studies have been performed in 90 patients with suspected inflammatory disease.

Leukocyte labeling. Two types of autologous cell preparations have been used: 'mixed' buffy-coat cells and 'pure' granulocyte preparations. 'Mixed' cells were obtained from patients who were neutrophilic (leukocyte count of at least 12,000/mm^3 of which more than 80% were neutrophils). Patients in whom pure granulocyte preparations were used had neutrophil counts ranging from 2,000 to more than 12,000/mm^3. Apart from the use of pure preparations in patients who were not neutrophilic, the choice of preparation was random.

Cell separation. All procedures were carried out aseptically, in a laminar flow cabinet.

1. '*Mixed*' (51 studies). Eighty ml of venous blood were drawn into two sterile disposable plastic syringes, each containing 6.5 ml ACD (N.I.H. Formula A), then dispensed into 25-ml polystyrene tubes.¶ Hydroxyethyl starch in 0.9% sodium chloride* was added to give a final concentration of 0.6% v/v and the blood was allowed to stand at 37°C for 60 min. The supernatant containing the leukocytes was centrifuged at 100 *g* for 5 min to yield a 'mixed' cell pellet. The platelet-rich plasma was removed and centrifuged at 2000 *g* for 5 min. One ml of cell-free plasma was then added to the cell pellet, which was resuspended in it. One hundred μl tropolone,† at a concentration of 4.4 m*M* in Hepes-saline buffer (pH 7.6) containing 20 m*M* Hepes in 0.8% v/v sodium chloride, was added followed by about 300 μCi In-111 in less than

Received Feb. 11, 1982; revision accepted Aug. 24, 1982.

For reprints contact: Dr. A. M. Peters, Dept. of Diagnostic Radiology, Hammersmith Hosp., Du Cane Raod, London W12 OHS UK.

50 μl 0.04 *M* HCl. After 5 min incubation at room temperature, 5 ml cell-free plasma were added and the mixture centrifuged at 100 *g* for 5 min. After resuspension in more plasma, the cells were ready for reinjection. The number of cells labeled varied according to the size of the cell pellet, and was not routinely counted. The labeling efficiency was 50–80%, depending on the number of cells obtained (*6*). The injected dose was 150–250 μCi. The fraction of the dose present on cells other than granulocytes was not known but was assumed to be small, since all the patients receiving 'mixed' cells were neutrophilic. Erythrocyte contamination varied. On the basis of in-vitro studies indicating that the relative uptakes of In-111 tropolonate by equal numbers of erythrocytes and granulocytes in mixed suspensions was approximately 1:10, it was estimated that the activity on erythrocytes was less than 5% of the dose.

2. *'Pure' granulocytes.* 'Mixed' leukocytes were isolated from 80 ml whole blood as described above. The granulocytes were then isolated by sedimentation on discontinuous density-gradient columns made up of mixtures of autologous plasma and either (a) Percoll‡ (28 studies) or (b) Metrizamide§ (22 studies). Full details of the Percoll/plasma separation are described elsewhere (*6*). Briefly, three densities were prepared by diluting iso-osmolar Percoll (9 volumes Percoll to 1 volume 1.5 *M* NaCl, sp. g. 1.12 with cell-free plasma to give 65%, 60%, and 50% v/v Percoll/plasma solutions. Two ml of each solution was then carefully layered into 10-ml polystyrene test tubes¶ (up to four, depending on the size of the mixed-cell pellet) with 'mixed' white cells in about two ml plasma layered on top of each. The gradients were spun at 200 *g* for 5 min and the majority of the granulocytes recovered from the interface between the 60% and 65% bands. For Metrizamide/plasma, a stock of 35% w/v Metrizamide in water, specific gravity 1.19 (at 20°C) was diluted with plasma to give 50% and 40% v/v Metrizamide/plasma solutions. Two ml of each solution was layered into 10 ml polystyrene test tubes¶ and followed by 2 ml of the 'mixed' white cells per tube, then centrifuged at 200 *g* for 5 min. The granulocytes were recovered from the interface between the two densities. The granulocytes isolated by both procedures were washed once with excess plasma and labeled in 1 ml plasma as described above for mixed cells. 'Pure' granulocytes isolated by both methods contained 2–20% erythrocytes but no platelets or mononuclear cells. Again, the number of cells labeled was variable, depending on the patient's neutrophil count. The fraction of the dose present on erythrocytes was estimated to be less than 3%.

Imaging. Scans were made with a gamma camera fitted with a parallel-hole, medium-energy collimator and interfaced on-line to a computer. In 68 studies, (19 using 'mixed' cells and 49 with 'pure' cells) dynamic imaging was performed for 40 min following reinjection of the labeled cells. The camera was positioned first over the chest; then after about 10 min it was moved caudally over the abdomen to include the site of suspected inflammation. Time-activity curves were constructed over the liver, lungs, spleen, and site of inflammation. Static scans were obtained from 40 min to 24 hr on 68 occasions, from 3 hr to 24 hr on 27, and at 24 hr only on 6.

RESULTS

The stability of the label was established by repeated washing (×3) of labeled 'pure' cells following incubation at 37°C for 1 hr in autologous plasma, when no appreciable loss of activity from the cells was demonstrable. In eight subjects receiving 'pure' cell preparations, the mean recovery at 10 min was 39% (s.d. 15; range 25–65) and the half-time of survival was 9 hr (s.d. 2.5; range 5.5–13). Plasma activity in these subjects was 5.9% (s.d. 2.9; range 2.9–9.9) of total blood activity at 40 min. The absolute activity present in plasma did not change appreciably thereafter.

Following reinjection of the labeled cells, there was an immediate lung image, which, in 60 of the 68 dynamic studies, faded substantially by about 5 min; i.e., lung retention was minimal. In contrast, in the eight showing lung 'hold-up', clear diffuse lung images, with little activity detectable elsewhere, persisted beyond 5 min and yielded time-activity curves showing an initial plateau longer than 3 min. Of the eight showing lung 'hold-up' (4 'mixed', 4 'pure'), three (2 'mixed', 1 'pure') were in patients with pulmonary disease (shock lung, pulmonary TB, interstitial pneumonitis) and one ('mixed') in chronic granulocyte leukemia; i.e. one of 19 'mixed' preparations, and three of 49 'pure', showed lung hold-up for which no explanation was apparent. One of the latter three studies was only faintly positive on subsequent scanning; when repeated using a similar preparation of 'pure' cells it showed no lung hold-up and was clearly positive.

Activity appeared rapidly in the liver and spleen, although at 40 min it was much more concentrated in the

TABLE 1. RESULTS OF IMAGING—SUMMARY

Site of suspected inflammation		True pos.	True neg.	False pos.	False neg.
Abdominal sepsis	37	19	16	0	2
Inflammatory bowel disease	54	29	25	0	0
Pulmonary sepsis	6	1	5	0	0
Bone/joint sepsis	2	2	0	0	0
Cerebral abscess	2	0	2	0	0
Total (90) patients	101	51	48	0	2

Specificity 100% Sensitivity 96%

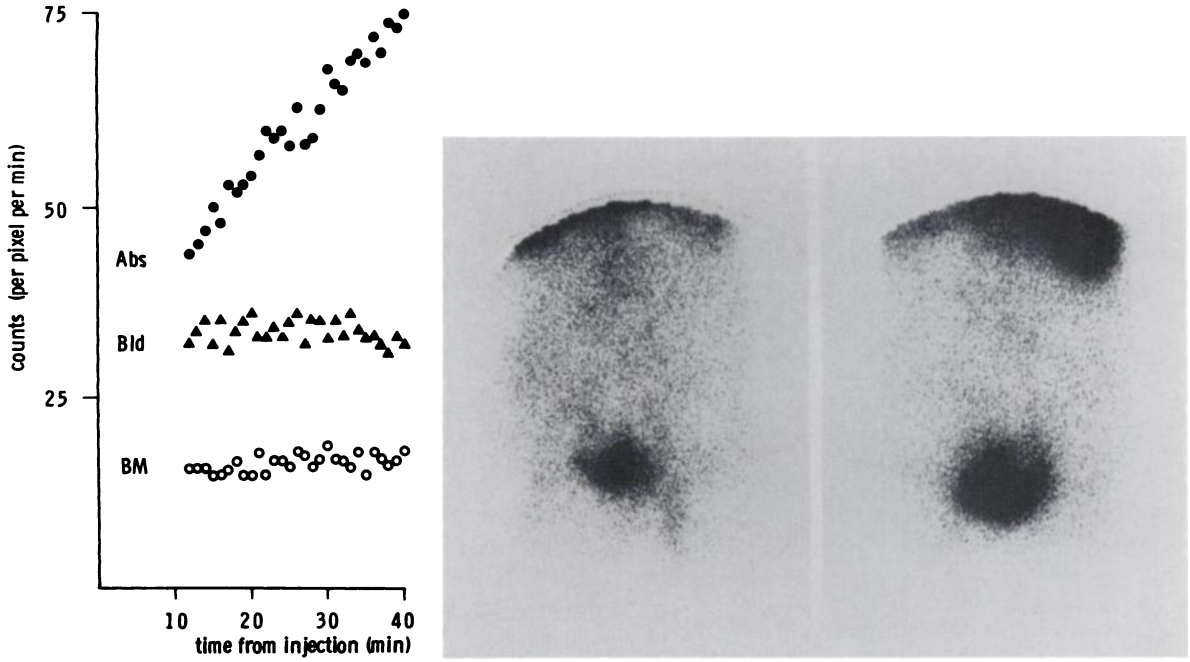

FIG. 1. Time-activity curves (left) recorded over an abscess (Abs), blood pool (Bld; iliac vessels), and bone marrow (BM; iliac crest) following injection of granulocytes separated on a Metrizamide-plasma gradient and labelled in plasma with In-111 tropolonate. Anterior abdominal scans show abscess at 40 min (center) and 3 hr (right).

spleen. The kinetics in relation to the lungs, liver, and spleen of cells labeled with this technique are described in detail elsewhere (*9*). Since absolute organ quantitation was not performed, radiation doses have not been calculated. However, apart from a higher bone-marrow uptake, the distribution visually was very similar to that in indium platelet studies. Compared with previous reports on In-111 oxine (*10*), cells labeled with In-111 tropolonate appear to result in a lower liver activity level, although a quantitative comparison has not been made. Bowel activity was seen only in patients in whom lung disease (from swallowed granulocytes) or inflammatory bowel disease was known to be present.

Early accumulation of abnormal activity in sites of inflammation was demonstrated by dynamic imaging (Fig. 1) in 25 studies. In these, count rates in regions over the blood pool remained static or fell. Sixty-eight static scans were made at 40 min, of which 27 (18 in inflammatory bowel disease) showed abnormal activity; of the 41 studies that were negative at 40 min, only nine (of which five involved inflammatory bowel disease) subsequently became positive. Four of these showed lung hold-up of cells following reinjection. The other four studies showing lung hold-up were negative on subsequent static scanning. No scans that were negative at 3 hr subsequently became positive. An analysis of the timing of the scans is shown in Fig. 2. Figure 3 shows a comparison of positive images at 40 min, 3 hr, and 60 hr in a splenectomized patient with a left subphrenic abscess.

Fifty-one scans were abnormal, with no false positives (specificity 100%). All positive scans in patients with inflammatory bowel disease were confirmed either by diagnostic radiology or histology. In other patients with positive scans, the abnormality found on the scan was confirmed by surgery, post mortem, or the spontaneous discharge of pus. There were two false negatives (sensitivity 96%). The other 48 patients with negative scans had no source of sepsis found on full clinical evaluation. One false negative was recorded in a patient with multiple intrahepatic fungal collections complicating chronic granulocytic leukemia. Histology, however, showed no evidence of neutrophilic infiltration. The other was in a patient who was on antibiotics at the time of the study. Further examples of images obtained with 'mixed' cells, 'pure' cells isolated using Percoll, and 'pure' cells isolated

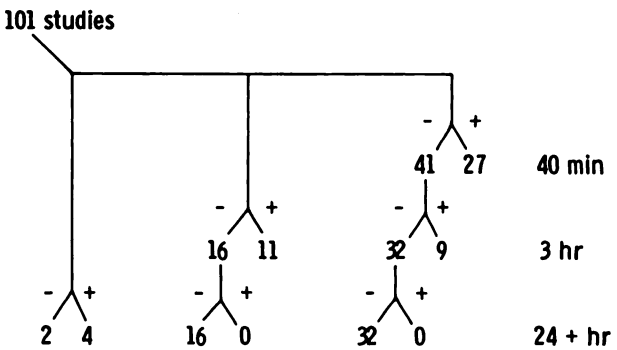

FIG. 2. Scan timing. Of 101 studies, some were scanned from 40 min, some from 3 hr, and some for first time at 24 h. Subsequent appearance of positivity in previously negative scans is demonstrated.

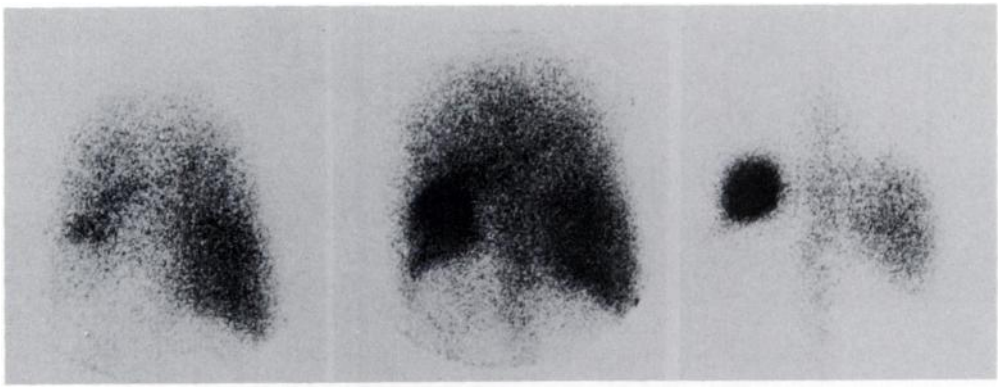

FIG. 3. Posterior scans, taken at 40 min (left), 3 hr (center), and 60 hr (right), following injection of "mixed" cells, in an asplenic patient with a left subphrenic abscess. Although chest activity appears high in early scans, much of this activity presumably represents blood pool, since there are no lung images as such. Note that more counts were collected on 3-hr than on 40-min image.

using Metrizamide are shown in Figs. 4, 5, and 6 respectively.

DISCUSSION

This study demonstrates that leukocytes labeled with In-111 tropolonate in plasma are capable of localizing in inflammatory lesions with an accuracy equal to that in series reported using leukocytes labeled with In-111 oxine (*10*) or In-111 acetylacetonate (*11*). No false positives were observed. One false negative might be interpreted as a true negative, since granulocyte infiltration was not seen histologically. A rather high proportion of this series involved inflammatory bowel disease. This is because of the great usefulness that we and others have found for labeled leukocyte studies in this group of disorders (*12*).

Since the introduction of In-111 as a leukocyte label, there has been concern that separation from plasma during the labeling procedure may damage the cell and alter its subsequent behavior. Indeed, leukocytes are so sensitive to in-vitro manipulation that there may be no satisfactory method of isolating unmodified leukocytes (*13*). Several groups have examined the viability of In-111-oxine-labeled cells (*14,15*) and found normal function by in vitro testing, though there is some doubt as to the relevance of this to in-vivo behavior (*16*). The latter would be a more meaningful test of cell function if it could be quantitated. In man, the quantitation of leukocyte localization in septic foci is difficult. In animals, however, it can be accomplished relatively easily. Thus, Issekutz et al. (*17*) found greater accumulation in experimental inflammatory lesions of leukocytes labeled in plasma than had been reported previously with leukocytes deprived of plasma (*18,19*). The early localization, in sites of inflammation, of granulocytes labeled in plasma with In-111 tropolonate is good evidence that these cells have a viability at least no less than that of previously reported oxine-labeled cells.

Pulmonary sequestration of labeled cells has been consistently observed previously (*2,3,14*) and been interpreted as due to physiological margination or to

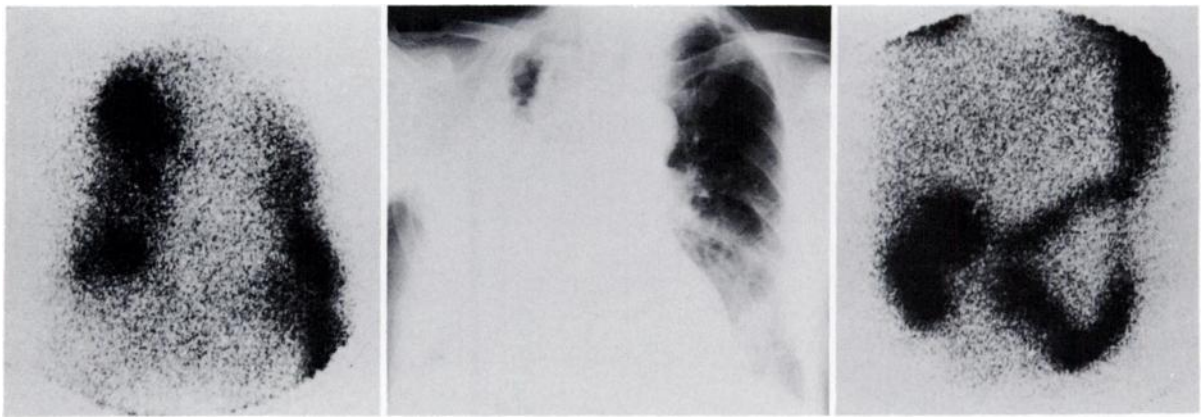

FIG. 4. Images from patient with an infected cavitating right-upper-lobe bronchial carcinoma who received "mixed" cells labelled with In-111 tropolonate. Abnormal activity is seen on 24-hr anterior chest image (left), especially in right upper, right lower, and left lower zones. On dynamic imaging, labelled cells were not retained in lung following injection, although at 40 min the count rate over right upper zone was increasing. Other areas abnormal at 24 hr were clear at 40 min, with static count rates. Splenic activity is also visible but there is minimal liver uptake. Chest radiograph is also shown (center). Extensive abnormal activity is seen in bowel at 48 hr (right), reflecting swallowed granulocytes.

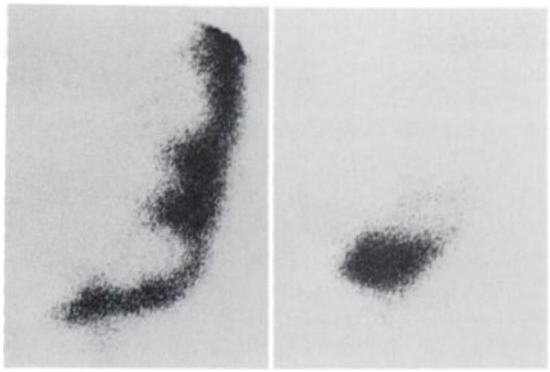

FIG. 5. Anterior abdominal scan in colectomized patient, made 40 min after injection of granulocytes labeled with In-111 tropolonate and separated on a Percoll/plasma gradient. Scan made in supine position (left) shows left-sided abnormal activity extending from lower pole of spleen into the pelvis. Erect scan (right) shows gravitation of pus into pelvis. Note almost complete absence of activity in liver.

damage sustained during isolation and labeling. The latter interpretation gains support from animal work in which it has been shown that cells damaged by heating show prolonged lung hold-up (*5*). The finding that this sequestration is largely prevented by maintaining the cells in plasma during the labeling procedure (*9*) suggests that lung holdup is artifactual rather than physiological, a conclusion supported by the earlier localization, in septic foci, of granulocytes not displaying lung retention (*20*). Furthermore, we report here that, considering those positive studies in which dynamic imaging was performed, the four showing lung hold-up were negative for white-cell localization at 40 min but positive at 3 hr, whereas 27 of the remaining 32 studies not showing lung hold-up were positive at 40 min.

FIG. 6. Anterior abdominal scan in patient with ulcerative colitis, made 3 hr after injection of labelled granulocytes separated on a Metrizamide/plasma gradient. Note minimal uptake in liver.

Leukocyte labeling in plasma offers several advantages over the existing labeling techniques. The early localization in inflammatory lesions overcomes one of the main drawbacks of abscess detection using labeled leukocytes, namely that of a later diagnosis as compared with ultrasound or computerized tomography. Although the time after injection for optimal imaging of abscesses is usually greater than 3 hr (e.g., Fig. 3), tropolone labeling in plasma gives images clear enough to provide a diagnosis well before 3 hr. Obviously, this may be important in some clinical circumstances. Early localization of In-111 labeled (homologous) granulocytes has been claimed previously (*21*), though others (*22*) have suggested that it represents blood-pool activity. The usefulness of dynamic imaging lies in its ability, as shown in Fig. 1, to identify neutrophil localization in a suspicious area, and to distinguish it from blood-pool activity. The apparent preservation of viability of cells labeled in plasma may be critical in patients with impaired neutrophil function—as, for example, in hypoglycaemia, hypocalcemia, and hyperalimentation—and so may avoid the necessity of using homologous leucocytes (*23*).

The use of Percoll/plasma or Metrizamide/plasma density-gradient separation should allow the use of In-111 leukocyte scanning in patients with low neutrophil counts, where homologous neutrophils have previously been used (*24*). Furthermore, this technique provides a "relatively pure" population of neutrophils suitable for the study of neutrophil kinetics and distribution both in normals and in special situations, such as 'shock lung' and hemodialysis neutropenia, where abnormal neutrophil kinetics are suspected (*25,26*).

FOOTNOTES

* Fresenius, "Plasmasteril".
† Fluka.
‡ Pharmacia Fine Chemicals.
§ Nyegaard (UK) Ltd.
¶ Sterilin.

ACKNOWLEDGMENTS

The authors thank Belinda Henderson and Shaughnan Dickie for radiographic assistance.

AMP is supported by the Cancer Research Campaign, SHS by the Wellcome Trust, and HJR by the Nuffield Foundation.

$^{111}In Cl_3$ was kindly supplied by Amersham International.

REFERENCES

1. DEYSINE M, ROBINSON RG, WILDER JR: Abscess detection by radioactive chromium labeled autologous white blood cells. *Surg Gynecol Obstet* 131:216-220, 1970
2. BURLESON RL, HOLMAN BL, TOW DE: Scintigraphic demonstration of abscesses with radioactive gallium labeled leukocytes. *Surg Gynecol Obstet* 141:379-382, 1975
3. ENGLISH D, ANDERSEN BR: Labeling of phagocytes from

human blood with ^{99m}Tc sulphur colloid. *J Nucl Med* 16:5-10, 1975

4. MCAFEE JG, THAKUR ML: Survey of radioactive agents for in vitro labeling of phagocytic leukocytes. 1. Soluble agents. *J Nucl Med* 17:480-487, 1976
5. THAKUR ML, COLEMAN RE, WELCH MJ: Indium-111 labeled leucocytes for the localization of abscesses. Preparation, analysis, tissue distribution and comparison with Gallium-67 citrate in dogs. *J Lab Clin Med* 89:217-228, 1977
6. DANPURE HJ, OSMAN S, BRADY F: The labelling of blood cells in plasma with ^{111}In-tropolonate. *Br J Radiol* 55: 247-249, 1982
7. DEWANJEE MK, RAO SA, DIDISHEIM P: Indium-111 tropolone, a new high affinity platelet label: Preparation and evaluation of labeling parameters. *J Nucl Med* 22:981-987, 1981
8. BURKE JET, ROATH S, ACKERY D, et al: The comparison of 8-hydroxyquinoline, tropolone and acetylacetone as mediators in the labelling of polymorphonuclear leucocytes with Indium-111: A functional study. *Eur J Nucl Med* 7:73-76, 1982
9. PETERS AM, SAVERYMUTTU SH, DANPURE HJ, et al: Granulocyte kinetics: Isolation and labelling in plasma abolishes lung sequestration. *Clin Sci* 62:P7, 1982
10. THAKUR ML, LAVENDER JP, ARNOT RN, et al: Indium-111-labeled autologous leukocytes in man. *J Nucl Med* 18: 1012-1019, 1977
11. SAVERYMUTTU SH, PETERS AM, CHADWICK VS, et al: Imaging diseased bowel with indium-111 labelled leucocytes. *Br J Radiol* 54:707, 1981
12. SAVERYMUTTU SH, PETERS AM, CHADWICK VS, et al: ^{111}Indium autologous leucocytes in inflammatory bowel disease. *Gut* (in press)
13. CLINE MJ: Leukocyte metabolism in regulation of haemopoesis. In *White cell and platelet production.* A. S. Gordon, Ed. New York, 1970, Meredith Corp., Vol. II P 1045
14. WEIBLEN BJ, FORSTROM L, MCCULLOUGH J: Studies of the kinetics of indium-111-labeled granulocytes. *J Lab Clin Med* 94:246-255, 1979
15. ZAKHIREH B, THAKUR ML, MALECH HL, et al: Indium-111-labeled human polymorphonuclear leukocytes: Viability, random migration, chemotaxis, bacteriocidal capacity and ultrastructure. *J Nucl Med* 20:741-747, 1979
16. ECKELMAN WC: Technical considerations in labeling of blood elements. *Semin Nucl Med* 5:3-10, 1975
17. ISSEKUTZ AC, MOVAT HZ: The in vivo quantitation and kinetics of rabbit neutrophil leucocyte accumulation in the skin in response to chemotactic agents and Escherichia coli. *Lab Invest* 42:310-317, 1980
18. JONES DG, RICHARDSON DL, KAY AB: Neutrophil accumulation in vivo following the administration of chemotactic factors. *Br J Haematol* 35:19-24, 1977
19. PERPER RJ, SANDA M, CHINEA G, et al: Leucocyte chemotaxis in vivo. I. Description of a model of cell accumulation using adoptively transferred ^{51}Cr-labeled cells. *J Lab Clin Med* 84:378-393, 1974
20. SAVERYMUTTU SH, PETERS AM, REAVY HJ, et al: Avoidance of pulmonary sequestration of ^{111}Indium labelled leucocytes permits early diagnosis of inflammatory foci. *Eur J Clin Invest* (in press)
21. DUTCHER JP, SCHIFFER CA, JOHNSTON GS: Rapid migration of ^{111}Indium-labeled granulocytes to sites of infection. *N Eng J Med* 304:586-589, 1981
22. DOHERTY PW, GOODWIN DA: Migration of ^{111}In-labeled granulocytes to infection sites. *N Eng J Med* 304:1546-1547, 1981
23. ASCHER NL, FORSTROM L, SIMMONS RL: Radiolabeled autologous leukocyte scanning in abscess detection. *World J Surg* 4:395-402, 1980
24. MCDOUGALL IR, BAUMERT JE, LANTIERI RL: Evaluation of ^{111}In leukocyte whole body scanning. *Am J Roentgenol* 133:849-854, 1979
25. HAMMERSCHMIDT DE, WEAVER LJ, HUDSON LD, et al: Association of complement activation and elevated plasma-C5a with adult respiratory distress syndrome. *Lancet* 1: 947-949, 1980
26. CRADDOCK PR, DALMASSO AP, FEHR J, et al: Hemodialysis leukopenia: Pulmonary vascular leukostasis resulting from complement activation by dialyzer cellophane membrane. *J Clin Invest* 59:879-888, 1977