Distribution of Leukocytes Labeled with In-111 Oxine in Dogs With Acute Inflammatory Lesions

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The biodistributions of In-111 oxine (with and without leukocyte labeling) of Ga-67 citrate and of In-111 chloride were compared in 30 dogs with chemical and bacterial abscesses and acute joint inflammation. Serial blood samples were taken and tissues radioassayed at 24 hr. The concentration of In-111-oxine leukocytes in all three types of inflammatory lesion was invariably much higher than that of Ga-67 injected simultaneously. For bacterial abscesses, the mean abscess-to-muscle concentration ratio was 3,000 for labeled leukocytes and 72 for Ga-67.

Aqueous buffered In-111 oxine sulfate solution appeared better for labeling leukocytes than In-111 oxine in ethanol. When In-111 oxine was not incubated with leukocytes before injection, or if the cells were poorly labeled or damaged, the abscess localization was often inferior to that of gallium. Localization of In-111 chloride also appeared inferior to that of gallium. No significant difference in distribution in the major organs or inflammatory lesions was demonstrable between labeled suspensions of "pure" neutrophils harvested by elutriation and "mixed" cell suspensions of leukocytes after erythrocyte sedimentation with hydroxyethyl starch.

For both types of leukocyte suspension labeled with In-111 oxine, the average recovery of cell-bound activity in the circulating blood at 4 hr was 32% of the administered activity, inferior to that of DFP-32. It is concluded, therefore, that In-111 oxine is a more effective agent than Ga-67 for the detection of acute focal inflammatory lesions if leukocytes are properly labeled, but current techniques are unsatisfactory for the study of neutrophil kinetics.

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Since the development of In-111 8-hydroxyquinoline (oxine) as a gamma-emitting agent for labeling leukocytes (*1*), several centers have used it extensively for the clinical detection of abscesses, whereas others have discontinued their initial trials because of technical problems. Many variations in the techniques of harvesting and labeling cells have been tried (*2*). Several important questions have been raised about the method. Is abscess-imaging better with labeled leukocytes than with Ga-67 citrate or In-111 transferrin, or with In-111-oxine-labeled plasma or whole blood, or with In-111 oxine injected directly intravenously? Is a mixed leukocyte suspension with some red-cell contamination adequate for this purpose, or must suspensions of "pure" neutrophils be used? Many dyes and radioactive agents, including even colloids, localize at inflammatory sites merely because of increased capillary permeability induced by cationic proteins released from platelets as well as exudative neutrophils (*3*). For ethical reasons one cannot conduct comparative crossover studies with Ga-67 and In-111 agents in patients with abscesses. Hence, the biodistributions of In-111 oxine with and

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without leukocyte labeling, of In-111 chloride, and of Ga-67 citrate were compared in dogs with induced inflammatory lesions.

MATERIALS AND METHODS

Induction of inflammatory lesions. Three types of inflammatory lesions were induced in each of 30 mongrel dogs of either sex (mean 24.5 kg, range 17.8–29.0 kg). A turpentine emulsion was prepared by mixing 2.0 ml of turpentine, 0.1 ml distilled water, and 0.1 ml Tween 80 and filtering through a 0.22-μ Millipore filter in a glass syringe. To induce a chemical abscess, 0.1 ml of this emulsion was injected with a 16-mm, 25-gauge needle and glass syringe into the right gastrocnemius muscle to a depth of 13 mm. To prepare a bacterial suspension, a loop carrying *E. coli* was incubated in 10 ml of thioglycolate at 37°C for 18–20 hr. To concentrate the bacteria, the culture was spun at 10,000 rpm (12,500 *g*)* for 10 min. The supernatant was removed and the bacteria resuspended in 3 ml of preservative-free physiological saline. To produce a bacterial abscess, 0.5 ml of this suspension (1.7×10^{10} organisms) was injected with a 16-mm, 25-gauge needle into the lateral head of the right triceps muscle.

On the following day, the dog was anesthetized with sodium pentobarbital 30 mg/kg i.v. After blood was withdrawn for leukocyte harvesting and labeling, two intracaths were inserted into subcutaneous veins for serial blood samples and injection of the radioactive preparations.

A third type of acute inflammatory lesion was induced in the left knee joint (stifle) by direct injection (38-mm needle, 18 gauge) of 30 mg of sodium urate crystals (10 to 15 μ long) suspended in 2 ml of sterile water according to the method of McCarty et al. (*4*). This induced a predictable leukocyte response with 92–96% neutrophils, maximal at 4 hr, simulating the lesion of gout, in which urate crystals are found in the synovial fluid. Unlike pyogenic abscesses, where neutrophils are destroyed, many of the cells migrating into the joint lesion were intact, permitting quantitation of the cellular exudation (*4*).

Preparation of In-111 oxine. Ground-glass-stoppered conical-bottom centrifuge tubes† were cleaned serially with acetone, concentrated HCl, sterile water, and absolute ethanol in that order, and the process repeated twice. No metal objects, including needles, were allowed to touch any of the solutions used. In-111 chloride‡ (1.5 mCi, 0.75 ml) was transferred to a centrifuge tube along with 50 μl of oxine‖ solution (50 μg in absolute ethanol), followed by 0.2 ml of sodium acetate solution (250 mg/ml water)¶ and mixed well using a vortex mixer. One milliliter of methylene chloride§ was then added and mixed well, and the tube centrifuged for one minute at 3,000 rpm (2,800 *g*) to produce a fast-phase separation. The methylene chloride layer was transferred to another similar tube and evaporated over a heating mantle using a stream of filtered nitrogen. The dry In-111-oxine complex was redissolved in 50 μl of absolute ethanol using a vortex mixer. The extraction yield was 96 ± 2% of the initial indium activity.

Several steps in the above procedure differed from those of other workers. The oxine-ethanol solution was added to the In-111 chloride at an acidic pH before, rather than after, the addition of the sodium acetate buffer solution to avoid the formation of colloidal indium hydroxide. The solvent extraction was performed with methylene chloride rather than chloroform because its lower boiling point (40°C as opposed to 62°C) hastened the evaporation step. After evaporation, the In-111-oxine complex dissolved in 50 μl of ethanol was added dropwise directly to the cell suspension. The common practice of diluting the In-111 oxine in ethanol to 0.2 ml with saline (*5*) was used only in three additional experiments for comparison. Only freshly prepared In-111 oxine was used, because satisfactory leukocyte labeling could not be achieved with batch or commercial preparations.

Preparation of In-111-oxine sulfate. The method of Ducassou et al. (*6*) for cell labeling was modified as follows. Tris buffer solution was prepared from 0.2 *M* Tris base by adding 0.2 *M* Tris-HCl to a pH of 7.4, followed by sodium chloride to a concentration of 2.6 mg/ml to adjust the osmolarity. 8-Hydroxyquinoline sulfate** was recrystallized from 50% ethanol in water and dissolved in double-distilled water to a concentration of 1 mg/ml. The solutions were sterilized by Millipore filtration. Fifty microliters of oxine sulfate solution were mixed with 0.75 ml of In-111 chloride (1.0–1.5 mCi in 10–50 m*M* HCl), then 0.75 ml of Tris buffer was added, resulting in a final pH of 7.0–7.2 and an osmolarity of 320 mOsm. This preparation, without either ethanol or solvent extraction, was added to cell suspensions for labeling.

HES method for harvesting "mixed" cell suspensions. Intravenous blood was withdrawn in a 60-cc plastic disposable syringe containing 3.0 ml of ACD (NIH-A) solution, to a volume of 30 ml. ACD was preferred to heparin as the anticoagulant, since this rendered neutrophils less adherent to the surfaces of plasticware. Six milliliters of 6% hydroxyethyl starch†† (HES) in 0.9% sodium chloride were added to the syringe, resulting in a final concentration of 1% HES. After thorough mixing, the inverted syringe was placed in a vertical position for 1 hr for gravity sedimentation of erythrocytes. With a needle bent to 90°, the supernatant plasma, containing platelets and approximately equal numbers of erythrocytes and leukocytes, was transferred in equal aliquots into two sterile 17- × 100-mm polyethylene tubes and spun at 150 *g* for 5 min. Although some suspensions were centrifuged at 450 *g* for 5 min, the slower spin speed was preferred. The supernatant was removed from each tube.

Each button was resuspended in 2.5 ml of physiological saline by alternating gentle aspiration and discharge with a plastic pipet, and the resuspensions were combined in one tube. After another similar centrifugation, the supernatant was removed and the button resuspended in 5 ml of physiological saline. The average leukocyte yield with this technique was 68% of the whole-blood leukocyte content. Hydroxyethyl starch was preferred as an erythrocyte-aggregating agent (*7*) because it, rather than methylcellulose, has been approved by the FDA for human use.

Harvesting neutrophils by elutriation. A relatively pure suspension of neutrophils was harvested by elutriation before labeling. The centrifuge[††] used a standard 4.5-ml elutriation chamber, a nonpulsatile, continuous-flow pump,[‖‖] and appropriate tubing configuration. The system was sterilized by flushing with 50 ml of 5% sodium hypochlorite, followed by 250 ml of sterile distilled water for injection, then blown dry with filtered (0.22 μ Millipore) compressed air. A 0.5% solution of hydroxyethyl starch was used as the elutriation medium. It contained 12 g Na citrate·$2H_2O$, 0.07 g citric acid ·H_2O, 107.6 g dextrose·H_2O, 250 ml 6% HES in saline, and 750 ml 0.9% saline. Sterile distilled H_2O was added to a total volume of 2 l, resulting in a final pH of 7.2, 320 mOsm. The dextrose content, citrate buffer, pH, osmolarity, and viscosity were critical parameters influencing cell separation and viability. A dilute solution of hydroxyethyl starch was used instead of serum albumin because the transferrin impurity in albumin prevented cell labeling with In-111 oxine. Such a macromolecular solution proved essential to prevent cell aggregation within the elutriation chamber. A 60-cc plastic disposable syringe containing fresh whole blood in ACD, drawn to 30 ml, was spun with the syringe tip outwards at 1,200 *g* for 10 min. A fraction of the lower layer was discarded, retaining the upper 7 ml of packed erythrocytes. This preliminary (buffy coat) spin was recommended previously (*8, 9*) to remove the excess erythrocytes. The remaining cell suspension was thoroughly mixed in the syringe and injected slowly into the loading chamber over a 3-min period, with the elutriation pump running at 8.0 ml/min, a centrifuge speed of 2,000 rpm (400 *g*), and temperature 10°C. After 1 hr, the loading chamber was bypassed and the flow rate was increased to 8.5 ml/min for 5 min, to 9.0 ml/min for 5 min, and to 9.3 ml/min for 15 min. Hence, the average separation time was 85 min. For labeling, the neutrophils were removed from the chamber by aspiration with a syringe and needle entering the septum at the top of the rotor (*10*) immediately after clamping the inflow tubing. In agreement with previous workers (*8, 9*) we found the elutriated cell suspensions to contain only 2–5% erythrocytes, no platelets, and 3–4% monocytes or large lymphocytes. The neutrophil recovery averaged 47% of the total leukocyte content of the whole-blood sample withdrawn.

Labeling and reinjection of leukocytes. Whether harvested after HES gravity sedimentation or by elutriation, cells were suspended in 5 ml of saline or elutriating medium and were labeled by adding one drop (10 μl) of In-111 oxine in ethanol every 2 min, with gentle agitation, until a total of 50 μl were added. For the In-111 oxine sulfate solution, 1.5 ml were added all at once to cells suspended in a volume of 2.2 ml of elutriating medium. The suspensions were incubated at room temperature for an additional 15 min. After spinning the cells at 150 *g* for 5 min, the supernatant was removed and the cells resuspended in 5 ml of saline or elutriating medium. Five milliliters of cell-free autologous plasma were added and the labeled washed cells reinjected intravenously. Cell suspensions after HES sedimentation contained an average of 380 million cells, and the mean labeling yield with In-111 oxine was 96%. The elutriated cell suspensions contained an average of 280 million cells and the labeling yield was 93%. For In-111 oxine sulfate preparations the labeling yield was 80–92%. An average of about 900 μCi of In-111 activity was injected, immediately followed by 100 μCi of Ga-67 citrate. Standards of these injected doses were made for comparative radioassay measurements.

Camera imaging. Gamma images were made at 1, 3–4, and 24 hr after dose, using the 245-keV peak of In-111 and a 280-keV, medium-energy collimator. With 100,000 counts each, anterior and posterior images were obtained of the chest, abdomen, and pelvis, together with 50,000-count anterior images of the four limbs.

Tissue and organ assays. Blood samples in ACD solution were withdrawn at 2 min, and at 1, 2, 3, 4, and 24 hr, and were assayed for In-111 and Ga-67 activity in whole blood and cell-free plasma. The 2-min and 1-hr samples were elutriated to determine the fraction of the injected radioactivity remaining bound to the granulocyte fraction in vivo. The exudate from the left knee joint was aspirated at 4 and 24 hr. The aspirate was centrifugated at 3,500 *g* for 10 min, and both the cell button and supernatant were radioassayed. WBC and RBC counts were obtained[¶¶] on samples of whole blood before labeling, on blood samples before and after elutriation, and on the joint aspirates. Microhematocrits were obtained on the blood samples.

After the 24-hr blood sample, the animals were killed. After weighing the whole organs, three samples were obtained of the lungs, liver, and spleen. Samples were also taken of the left gastrocnemius muscle, bone, turpentine abscess, bacterial abscess, and the intra-articular debris and synovium of the left knee joint. Both the percent administered activity in the whole organ and percent administered activity per gram or ml were calculated. The total skeletal muscle mass was estimated as 54.45% of body weight (*11*). Since the estimated blood volume in dogs has varied from 7.07 to 10% of body

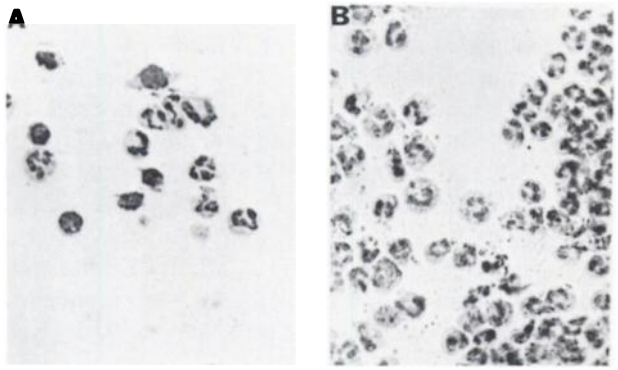

FIG. 1. Photomicrographs of cell suspensions used for labeling with In-111 oxine. (A) after HES erythrocyte sedimentation, supernatant contains polymorphs, mononuclear leukocytes, and erythrocytes; (B) after elutriation, cells are predominantly polymorphs. Cells were concentrated in a cytocentrifuge and stained.

weight (*12*), or 7.5% of body weight (*13*), a "middle" figure of 8.0% of body weight (*12*) was chosen for our calculations.

RESULTS

In the 30 dogs with inflammatory lesions, the mean venous white-cell count was $20.9 \times 10^3/mm^3$. In 18 dogs, this white blood count was above the normal range and in the remainder the count was toward the upper limits of normal (normal range 8.0–18.0; mean $12.0 \times 10^3/mm^3$) (*14*). Photomicrographs of the two different leukocyte suspensions just before labeling showed mononuclear leukocytes and erythocytes as well as polymorphs after HES sedimentation (Fig. 1A). These can be compared with the relatively pure polymorph suspensions obtained by elutriation (Fig. 1B). The mean recovery of In-111-oxine-labeled cells in whole blood (expressed as a percentage of administered radioactivity up to 24 hr) is illustrated in Fig. 2A, for comparison with Ga-67 citrate administered simultaneously, and In-111 chloride (transferrin). At early intervals, the recovery of elutriated labeled neutrophils was somewhat lower than that for the mixed cells after HES sedimentation, but at 4 hr they were identical (34%), and by 24 hr more of the elutriated cells remained in the circulation.

Elutriated cells labeled with In-111 oxine sulfate yielded a similar recovery of 38% at 4 hr and a high level of 17% at 24 hr. Figure 2B illustrates the cell-bound activity (whole blood minus plasma activity) for both "mixed cell" and elutriated-cell preparations. This cell-bound activity was similar to the whole-blood activity, since the mean recovered from the plasma volume was only 1.4% of the administered dose at all intervals. Only two of 12 animals had plasma activity exceeding 2% of the administered dose in some samples. In a previous study in humans (*15*), 4.0–9.8% of the total blood activity was found in the plasma. If one assumes that the marginating pool of granulocytes represents 50% of the total intravascular granulocytic pool, the actual cell-bound activity would be double the measured values.

The percent of injected radioactivity recovered from the neutrophil fraction harvested by the elutriator from

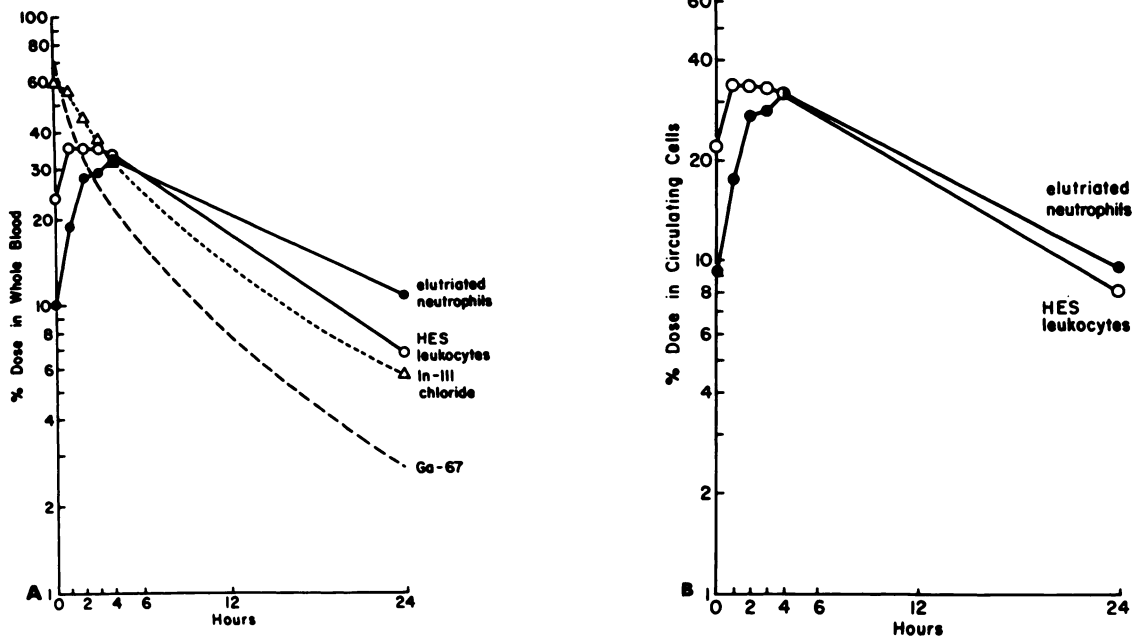

FIG. 2. Recovery of administered activity. (A) in canine whole blood for In-111-oxine-labeled, elutriated neutrophils and "mixed cells" after HES sedimentation, compared with Ga-67 and In-111 chloride. (B) Recovery in circulating cells (whole blood minus plasma activity).

TABLE 1. ORGAN DISTRIBUTION AT 24 HR (MEAN ± s.e.m., % ADMINISTERED ACTIVITY PER ORGAN)

	In-111 chloride	In-111-oxine leukocytes*	Ga-67 citrate
Blood	5.6	8.5 ± 1.6	2.7 ± 0.5
Lung	3.0	7.9 ± 1.8	1.1 ± 0.1
Liver	22.2	48.5 ± 5.9	13.0 ± 1.6
Spleen	2.5	11.0 ± 1.0	1.3 ± 0.2
Muscle	8.7	2.4 ± 0.4	2.5 ± 0.8
No. of dogs	4	12	12

* Mean and range.

2-min blood samples was only (11 ± 4)%, but at 1 hr was (28 ± 4)%. No significant difference in the recovery of injected radioactivity in the neutrophil fraction was demonstrated between cells labeled after elutriation, compared with mixed-cell suspensions after HES sedimentation. For In-111 oxine, incubated with plasma or injected in saline, no activity was recovered in the circulating granulocyte pool; therefore, there was no evidence of in vivo granulocyte labeling. Likewise, no in vivo labeling occurred following the intravenous injections of In-111 chloride. With Ga-67 citrate, only 0.7% of the injected radioactivity was recovered in the circulating neutrophil pool in 2-min samples, and only 2% in 1-hr samples. Hence, in vivo labeling of granulocytes with Ga-67 citrate was negligible.

Table 1 compares the radioactive contents of major organs at 24 hr for In-111-oxine leukocytes with those of Ga-67 citrate and In-111 chloride. In these dogs with inflammatory lesions, the spleen appeared considerably larger than normal, weighing an average of 1.03% of the body weight compared with the normal mean of 0.26% (*11*). The liver was slightly larger than normal, representing 3.34% of the body weight (normal 2.32%). The lungs comprised an average of 1.24% of the body weight, not significantly different from normal. In the dogs injected with In-111-oxine-sulfate labeled cells, the average skeletal content at 24 hr was 12% of the administered dose, of which 1.5% was estimated in the bone itself, the remaining 10.5% in the marrow.

The localization of In-111-oxine leukocytes, Ga-67 citrate, and In-111 chloride in the three types of inflammatory lesion is compared in Table 2. There was no significant difference in the localization of elutriated neutrophils and "mixed" cell suspensions after HES sedimentation, by either Student's unpaired t-test or Wilcoxon's two-sample test. The abscess-to-blood concentration ratios were somewhat lower for elutriated cells, because the blood levels were higher at 24 hr. The concentrations in the lesions were much higher for labeled cells than for Ga-67: by a factor of 52 for bacterial abscesses, 32 for the joint lesions, and 21 for the turpentine abscesses. Abscess concentrations and abscess-to-muscle ratios for labeled cells were not significantly different for *E. coli* as opposed to chemical abscesses. In contrast, the concentration of Ga-67 was significantly better in chemical than in *E. coli* abscesses ($p < 0.05$ by the Wilcoxon signed rank test). In three experiments, the Ga-67 concentration in the bacterial abscess was negligible, despite good localization of labeled cells and de-

TABLE 2. ABSCESS CONCENTRATION AT 24 HR BY TISSUE ASSAY* (MEANS ± STANDARD ERROR OF THE MEAN)

		In-111-oxine leukocytes				
		elutriated cells	HES cells	combined series elutriated & HES cells	Ga-67 citrate	In-111 chloride
No. of dogs		6	6	12	12	4
Abscess assay	chem	400 ± 142	389 ± 83	394 ± 78	21 ± 7.3	8.2
1000 × % dose/g	*E. coli*	543 ± 102	423 ± 79	483 ± 64	8.9 ± 2.4	5.5
	joint	299 ± 76	489 ± 129	385 ± 74	13.7 ± 4.3	11.4
% Dose/1% body wt	chem	108 ± 38	95 ± 20	101 ± 21	4.9 ± 1.7	1.7
	E. coli	147 ± 29	103 ± 19	125 ± 18	2.4 ± 0.76	1.2
	joint	81 ± 21	149 ± 23	108 ± 18	3.4 ± 1.1	2.4
Abscess-to-muscle	chem.	2444 ± 977	2575 ± 142	2509 ± 471	182 ± 70	10.7
ratio	*E. coli*	3293 ± 602	2895 ± 286	3094 ± 323	72 ± 19	7.4
	joint	1468 ± 615	3112 ± 1086	2290 ± 649	100 ± 31	14.9
Abscess-to-blood	chem.	72 ± 24	217 ± 103	144 ± 55	25 ± 9.1	2.3
ratio	*E. Coli*	95 ± 14	253 ± 127	174 ± 65	8.4 ± 2.5	2.0
	joint	86 ± 46	287 ± 142	177 ± 72	17 ± 7.1	3.8

* At the time of sacrifice, the chemical and *E. coli* abscesses were 48 hr old, the joint lesions 26 hr old.

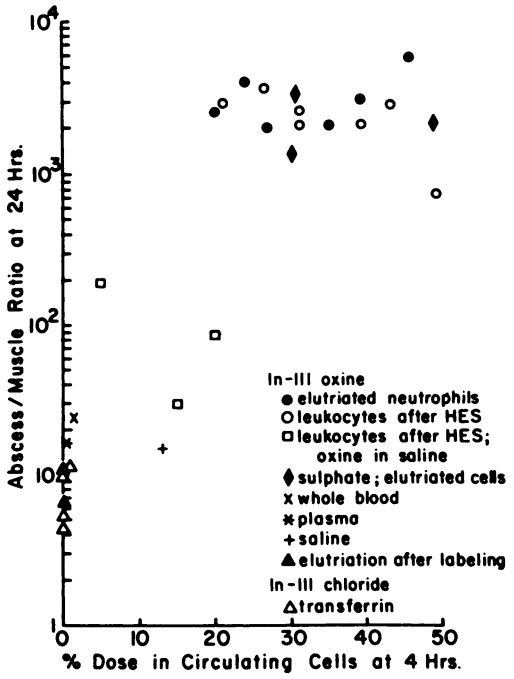

FIG. 3. *E. coli* abscesses in dogs: relation between cell-bound In-111 activity in blood at 4 hr, and abscess-to-muscle concentration ratio at 24 hr.

spite good localization of gallium in other inflammatory lesions in the same animal. In four animals, the localization of In-111 chloride in the inflammatory lesions was somewhat inferior to that of gallium.

Fourteen additional canine experiments (not listed in Table 2 but included in Fig. 3) explored several variations in procedure. In three dogs, elutriated cells labeled with In-111-oxine sulfate showed the same excellent localization in the inflammatory lesions as similar cell suspensions labeled with In-111 oxine in ethanol. In one experiment, a "buffy coat" centrifugation to remove a layer of packed erythrocytes preceded HES sedimentation, as recommended by Roy et al. (*7*), but the biodistribution was no better than with HES sedimentation alone. In another, with inflammatory lesions 72 hr old, the localization of labeled cells and Ga-67 was similar to that of lesions 48 hr old. In another animal, radioassays 3 hr after injection showed poor localization, but the concentration of labeled cells in the lesions was still greater than that of gallium. In three experiments, the In-111 oxine in 50 μl ethanol was diluted to 0.2 ml with saline for cell labeling, as recommended by others (*5*). In all instances the in vivo cell recovery and localization in lesions were not as good as with the direct dropwise addition of the In-111 oxine in ethanol to the cell suspensions. In two experiments the combination of HES sedimentation followed by elutriation after labeling resulted in poor recovery and survival of the labeled cells in the blood stream. When In-111 oxine was incubated with cell-free plasma or whole blood, or injected directly intravenously in saline (one experiment each), the localization in the inflammatory lesions was poor. Thus, in a total of eight experiments in which neutrophils were not labeled, or were poorly labeled, or were damaged, the concentration of radioactivity was inferior to that obtained with labeled cells.

Figure 3 demonstrates a relationship between the recovery of cell-bound radioactivity in the circulation at 4 hr after injection, and the localization in bacterial abscesses (as abscess-to-muscle ratios) at 24 hr. When the recovery of cell-bound activity was <20% of the administered dose, the abscess-to-muscle ratio was <200. Conversely, when the cell-bound activity was >20%, the abscess-to-muscle ratio was usually >2,000.

From the joint inflammatory lesion, an average of 3 ml was aspirated at 4 and 24 hr. The 4-hr leukocyte count of the aspirate was 105,000/mm^3 ± 1130 s.e.m. and the 24-hr leukocyte count 70,000/mm^3 ± 400. (A dog's knee joint normally contains about 700 leukocytes/mm^3 with about 32% neutrophils (*4*)). The leukocyte count in the aspirate from the joint at 4 hr averaged 6.7 times the leukocyte count of whole blood, and at 24 hr 4.4 times. In most experiments, 95% or more of the radioactivity in the joint aspirate was associated with the pellet on centrifugation, whereas the radiogallium activity was in the supernatant fluid after centrifugation. This finding again indicated that the localization of radiogallium activity in inflammatory lesions is not due to in vivo leukocyte binding. If one assumes that all of the In-111 activity recovered from the knee joint was associated with intact leukocytes or cellular debris, an average of 5.2% of the circulating leukocyte pool migrated into the joint in 24 hours.

Camera images of the dogs after the injection of In-111-oxine neutrophils (Fig. 4) show a higher concentration in the bacterial and chemical abscesses of the right limbs at 24 hr than at 3 hr. During this interval, cardiovascular activity decreases, marrow activity increases, and the hepatosplenic activity persists. The abscesses are not as well demonstrated with In-111-oxine-labeled whole blood (Fig. 5), In-111 chloride, or Ga-67 citrate (Fig. 6).

DISCUSSION

The highest recovery of labeled neutrophils in the circulation in man (58%)(*16*) has been achieved following the labeling of marrow precursors with tritiated thymidine, with a disappearance half-time of 7.6 hr. It has been assumed, therefore, that the remaining 42% of intravascular neutrophils are in a marginated pool and do not contribute to withdrawn blood samples. The recovery of neutrophils labeled in vitro with DFP-32 has been somewhat lower (48%) and the disappearance half-time somewhat shorter (5.4 hr). Neutrophil kinetics

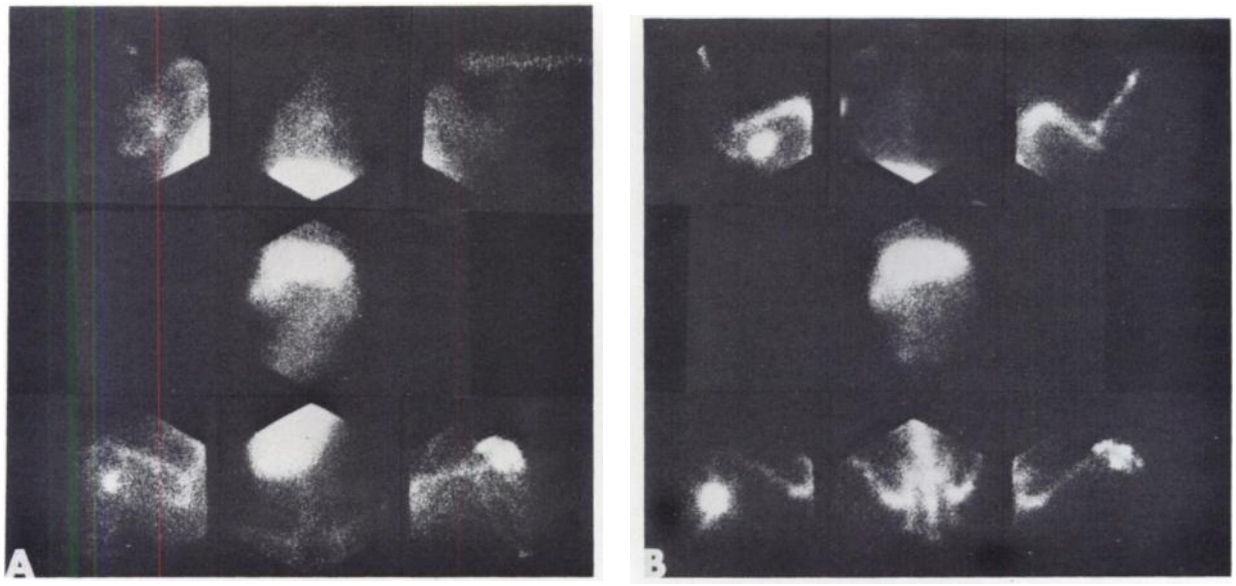

FIG. 4. Camera images of dog at 3 hr (A) and 24 hr (B) after i.v. injection of In-111-oxine-labeled elutriated neutrophils. Progressive concentration in *E. coli* abscess (left upper frame) and chemical abscess (left lower). Knee-joint exudate (right lower) was aspirated after 3-hr images. Note decrease in activity in heart, limb vessels, and lungs after 3 hr, increasing activity in marrow, and persistent activity in liver and spleen. At 24 hr, bacterial abscess-to-muscle ratio = 3,100:1 and chemical abscess-to-muscle ratio = 2,800:1.

in the normal dog are similar to those in normal man—the recovery is 65% and the half-time 6.7 hr with tritiated thymidine, and with DFP-32 the recovery is 51% and the half-time 5.4 hr (*17*). The kinetics are markedly altered with acute infection (*18*). Within the first few hours in dogs, the half-time of granulocytes in the circulation decreases and an elevation in the marginated pool sometimes precedes the elevation in the circulating pool ("masked" granulocytosis). The granulocytosis is due to an accelerated release of cells from the marrow, since extravascular cells in other tissues do not return to the circulation. One day after infection, the total and circulating granulocyte pools are usually greater than normal, but the circulating pool may represent only about 30% of the total pool and the marginating pool is variable. Hence, the ratio of circulating to marginating granulocytes varies with different stages of infection.

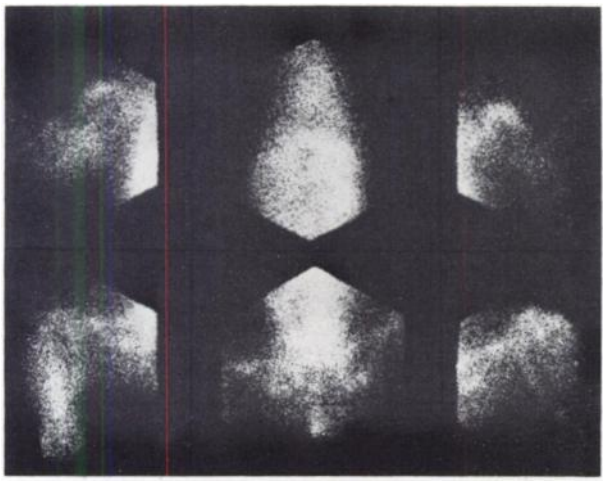

FIG. 5. Camera images of dog 24 hr after i.v. injection of In-111-oxine-labeled whole blood. Inflammatory lesions at same sites as in Fig. 4 are not as well demonstrated. Persistent activity in heart pool, limb vessels, liver, spleen, and marrow. Bacterial and chemical abscess-to-muscle ratios are 24:1 at 24 hr.

In the current series of dogs with inflammatory lesions, the mean recovery of leukocytes labeled with In-111 oxine was 32% at 4 hr. This compares with values of (30 ± 6)% at 1-2 hr obtained in ten normal human subjects in whom cells were separated by HES gravity sedimentation and double-density gradient centrifugation before labeling (*19*). In that study, as in the current one, the cell-bound intravascular recovery improved during the first few hours, presumably due to recovery of partially damaged cells, and the recovery at 24 hr was higher than expected for a half-time of 5-6 hr. With the current methods of harvesting and labeling neutrophils with In-111 oxine, the recoveries are not comparable with those for DFP-32 and, therefore, cannot replace the latter for kinetic studies.

Many steps in the harvesting and labeling procedures may contribute to neutrophil injury. Unfortunately, for labeling with In-111 oxine the protective plasma proteins must be removed from the suspending medium. Both oxine and ethanol are toxic to neutrophils in relatively low concentrations. As little as 0.125% ethanol induces structural changes (*20*). With current labeling techniques, 50 μl of ethanol containing 50 μg of oxine are usually added to 5 ml of cell suspension, resulting in a concentration of 1% ethanol. The radiochemical In-111 chloride may contain the trace metals Zn, Cd, Cu, or Fe

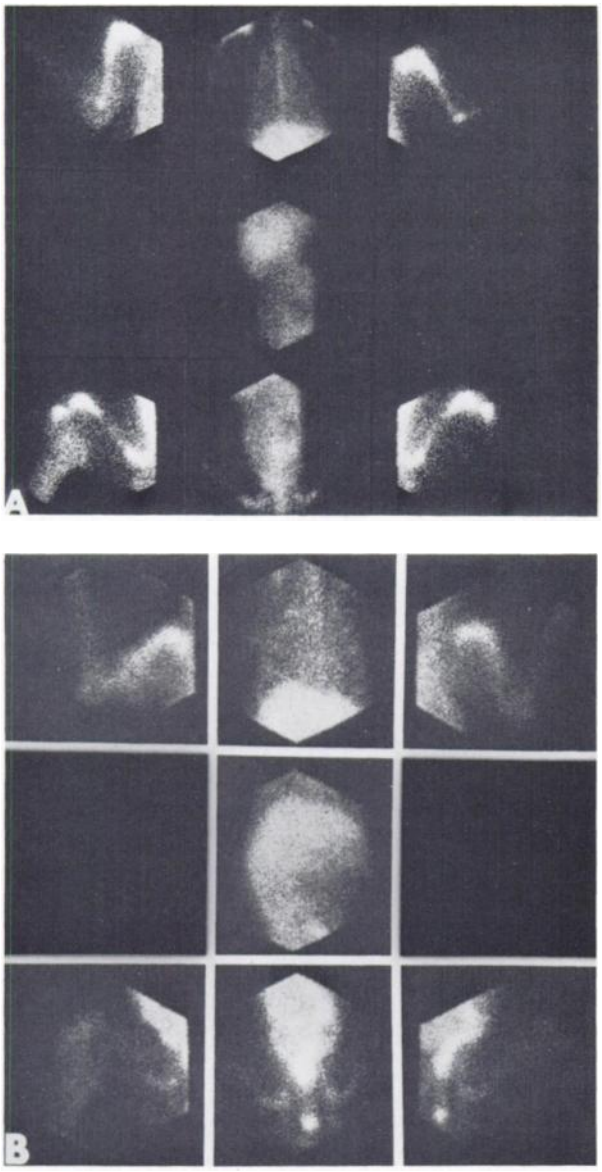

FIG. 6. Camera images of dog 24 hr after i.v. injection of (A) In-111 chloride, (B) gallium-67 citrate. Inflammatory lesions at same sites as in Fig. 4 are poorly demonstrated. Activity is seen in liver, spleen, and marrow; marked intestinal gallium activity. Abscess-to-muscle ratios 5:1 at 24 hr for In-111, 6 to 57:1 for Ga-67.

in a concentration of about 3 μg/ml, and these may influence cell viability. Chvapil (*21*) found that these cells are uniquely sensitive to Zn in concentrations as low as 0.3 μg/ml. Cell damage by the mechanical stress of pelleting during centrifugation also must be considered.

The newer technique of labeling leukocytes with In-111 oxine sulfate (*6*) offers several important advantages over the older method using In-111 oxine in ethanol. Oxine sulfate is soluble in neutral aqueous buffered media; hence the need for ethanol as an oxine solvent is eliminated. Likewise, the procedure of solvent extraction of the freshly prepared In-111-oxine complex is avoided, thus simplifying the method. In the current study, the recovery of labeled neutrophils from the blood stream, and their localization in inflammatory lesions, were at least as good as after the more laborious method with In-111 oxine in ethanol.

Serial images after the administration of leukocytes labeled with In-111 oxine show an immediate concentration in the liver and spleen, reaching a plateau by about 1 hr, probably due to a combination of marginated or damaged, sequestered cells or noncellular activity. The initial lung activity, which tends to fade by about 4 hr, is probably due to recoverable damaged cells. In the previous study of Thakur (*15*), the lung activity decreased by one-half within 15 min. Some bone-marrow activity visible by 24 hr is presumably due to localization of In-111 transferrin. An immediate intense visualization of the reticuloendothelial organs, with a disappearance of the cardiac pool and major vessels by 4 hr, correlates with poor cell recovery in the bloodstream. With labeled cells, inflammatory lesions are better demonstrated at 24 hr than at 4 hr. The absence of gastrointestinal activity is an obvious advantage of In-111-labeled cells or of transferrin over radiogallium in the demonstration of abdominal abscesses. However, the relatively high concentration of In-111-oxine-labeled cells in the liver is an obvious disadvantage for the demonstration of hepatic abscesses. Previous estimates of concentration in the major organs, based on human camera images, varied widely (*15, 19*). The estimate of total liver activity at 24 hours, based on organ radioassay, is considerably higher than previously reported values.

The present work confirms the previous claims (*8, 9*) that elutriation is an effective means of harvesting relatively pure suspensions of granulocytes, since this technique separates cells of different diameter as well as different density without the stress of mechanical pelleting. The morphological integrity of the cells is preserved as assessed by transmission electron microscopy, scanning electron microscopy, flourescence microscopy, and measurement of cell volume. Moreover, functional activity of the cells is preserved, including enzyme activity, oxygen consumption, chemotaxis, and bacteriocidal and phagocytic capacity. This functional activity is similar to that of cell suspensions after simple HES sedimentation. Nonetheless, the present study demonstrates that in vivo cell recovery and abscess concentration is no better for elutriated cells than for cells after gravity HES sedimentation in the presence of relative or absolute granulocytosis.

With In-111-oxine-labeled leukocytes, the abscess concentrations and abscess-to-muscle concentration ratios obtained in the current study are much higher than those previously reported by Thakur (*5, 22*), probably due to variations in cell labeling, abscess induction techniques, and variations from one animal to another. Nonetheless, in both studies the localization was sig-

nificantly better than is achieved with radiogallium injected simultaneously. In a previous study (*23*) of canine turpentine abscesses with Tc-99m-oxine-labeled leukocytes, abscess concentration was also better than with radiogallium. However, this Tc-99m agent was partially eluted from the cells in vivo and was excreted to some extent in the gastrointestinal tract. The present work suggests that the abscess concentration of radiogallium is somewhat better than that of In-111 transferrin, in agreement with a previous study of pyogenic abscesses in rabbits by Yeates (*24*), but in conflict with another study of Tsan (*25*).

The intra-articular injection of microcrystalline urate appears to provide a suitable model for joint inflammation. In a variety of spontaneous joint inflammations, such as gout and Reiter's syndrome, there is continued neutrophil exudation, which occurs even in long-standing rheumatoid arthritis (*26*). DFP-32-labeled neutrophils from joint exudates in rheumatoid patients have a half-life of about 4 hr, indicating a rapid cell turnover. Enzymes secreted by these cells may contribute partially to the joint destruction. It has been estimated that an average rheumatoid joint effusion may consume 10^9 granulocytes (~3% of the circulating pool) each day (*26*). In the present study, about 5% of the circulating pool of labeled leukocytes migrated into a single joint in the dog in 24 hr.

We conclude that leukocyte suspensions localize much better in inflammatory lesions than gallium provided that the cells are well labeled and remain viable. If the cells are damaged or improperly labeled, localization is sometimes similar and sometimes inferior to that of radiogallium. Nevertheless, some degree of visualization of the lesion by camera imaging is usually achieved with In-111 in the absence of cell labeling.

Numerous variations in the techniques of harvesting leukocytes and labeling them with In-111 oxine have been used at various centers, many greatly influencing cell viability and the stability of the label. The only reliable means of assessing the validity of a particular technique is the measurement of the recovery and survival of the cells on reinjection into the bloodstream, because no one has devised an in vitro test that predicts the in vivo behavior of these labeled cells.

FOOTNOTES

* Sorvall SS-3 Centrifuge with SS-34 rotor.

† 8, hydroxyquinolone, Ultrex grade, J. T. Baker Chemical Co., Phillipsburg, NJ.

‡ Mediphysics, Emeryville, CA.

‖ Wheaton Co., Millville, NJ.

§ Burdick and Jackson Co., Muskegan, MI.

¶ Sodium acetate, anhydrous, J. T. Baker Co., 250 mg/ml water. Phillipsburg, NJ.

** Aldrich Chemical, Milwaukee, WI.

†† Volex, McGraw Laboratories, Newport Beach, CA.

‡‡ Beckman J-21C with JE-6 elutriator rotor.

‖‖ Minipuls Model HP-4 Pump, Gilson Medical Electronics Inc., Middletown, WI, with Tygon Medical Grade tubing manifold ($\frac{1}{8}$ in. ID, $\frac{1}{4}$ in. OD, Norton Plastics and Synthetics Div., Akron, OH.

§§ Dow Corning Silastic Medical Grade tubing $\frac{7}{64}$ in. ID, $\frac{3}{16}$ in. OD.

¶¶ Coulter Counter Model ZB1.

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