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Survey of Radioactive Agents for In Vitro Labeling of Phagocytic Leukocytes. I. Soluble Agents

John G. McAfee and Mathew L. Thakur

MRC Cyclotron Unit, Hammersmith Hospital, London, England

Twenty-three soluble (nonparticulate) radioactive agents were screened for their ability to label leukocytes in vitro in the presence or absence of plasma and red cells. The degree of cell binding was compared with that of other agents previously reported. Leukocyte binding of 2% or more of the added radioactivity was obtained with ten of these agents. High labeling yields were obtained only with nonpolar lipid-soluble complexes capable of penetrating lipid cell membranes. Unfortunately, this type of labeling tends to be reduced by plasma protein. Some lipophilic radioactive agents tend to elute from the cells after labeling. Compared with radioactive particles, soluble radiopharmaceuticals offer the advantage of easy centrifugal separation of the cell-bound from the residual activity in the suspension fluid. However, the labeling yield tends to be lower than for radioactive particles, and other cellular elements are labeled in addition to the phagocytic leukocytes. In this survey, the most promising soluble agents were ^{111}In or ^{99m}Tc complexes of oxine, ^{111}In-tetraphenylporphyrin, DNA labeled with radioactive iodine, and DNA labeled with ^{77}Br and complexed with adriamycin. The ultimate value of any of these agents must await adequate in vivo testing in experimental animals and clinical trials.

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If the phagocytic cells of the peripheral blood could be selectively labeled with a gamma-emitting agent without loss of viability, several clinical applications would be possible. Leukocyte survival and localization in various organs could be studied in normal individuals and in patients. Focal inflammatory lesions could be detected by imaging. Similarly, intravascular thrombi could be localized, since neutrophils as well as platelets are a major cellular component of thrombi. The peripheral blood may be regarded as the most convenient "biopsy" performed on humans, and leukocytes are the most accessible nucleated cells for examination under living conditions. Thus the cellular penetration of various radiopharmaceuticals may be conveniently examined in vitro, providing clues concerning their intracellular localization in other cell types in vivo. The more plentiful erythrocyte does not reflect the penetrability of nucleated cells, for many ions and compounds—including even pertechnetate—readily diffuse through its cell membrane.

This paper (Part 1) summarizes the current knowledge on neutrophil labeling and describes in vitro labeling experiments with a variety of soluble agents. Phagocytosis of radioactive particles will be discussed in Part 2. In one review, Cline (*1*) states that no truly satisfactory procedure has been described to obtain unmodified neutrophils free of

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For reprints contact: John G. McAfee, Div. of Nuclear Medicine, State University of New York, Upstate Medical Center, 750 Adams St., Syracuse, N.Y. 13210.

other cellular elements and plasma proteins. This is still the major difficulty in labeling neutrophils.

MATERIALS AND METHODS

Unless otherwise stated, the radioactive materials were prepared in the MRC fixed-energy cyclotron. The methods of preparation of some of the compounds have been reported previously (*2,3*); the methods for other agents used will be furnished on request.

Fifty milliliters of fresh human venous blood were obtained in heparin (5–50 IU/ml) without preservative (*4*). Red-cell sedimentation by gravity was carried out for 1 hr in two 16-mm-i.d. siliconized glass tubes after adding to each 2 ml of 2% methylcellulose (Methocel, 25 cP, Dow Chemical, Midland, Mich.) in 0.9% saline (*5*). Two milliliters of the supernatant were used for incubation with the various radiopharmaceuticals; this amount contained about 2×10^7 leukocytes (53% neutrophils) and $1.2–2 \times 10^8$ erythrocytes.

To obtain cell suspensions free of plasma and platelets, after red-cell sedimentation aliquots of supernatant were centrifuged gently at 100 g for 15 min. After the supernatant was removed, the cells were washed twice and then resuspended in Hank's balanced salt solution (HBSS) (*6*). Aliquots of supernatant cells were subjected also to hypotonic lysis in 0.22% saline twice for 30-sec intervals (*7*) to eliminate residual erythrocytes.

Two-milliliter aliquots of cell-rich supernatant and cell suspensions, as described above, were incubated with the radiopharmaceuticals for 1 hr. Control incubation tubes were also prepared by suspending 0.1 ml of outdated blood in 2 ml of pooled plasma, and 2-ml aliquots of cell-rich supernatant, 48 hr old, passed through a nylon-fiber column to remove any residual phagocytic cells.

The degree of binding of the radioactivity to the cellular elements was measured in three ways: (A) by measuring the loss of radioactivity from the suspension after centrifugation at 500 g for 5 min, followed by serial washing, centrifugation, and counting of the cell sediment; (B) by iron-particle* phagocytosis and magnetism (*8*); and (C) by passage through a 300-mg nylon-fiber column† and washing with 20 ml HBSS. For small volumes of blood, these fiber columns retain 85–97% of the neutrophils, 80% of the monocytes, and the majority of platelets (*9,10*) in the presence of calcium and magnesium ions (*11*).

Some radiopharmaceuticals suspended in plasma were partially retained on the nylon columns; in that event, this method could not be used for assessing the degree of cell binding. The nylon column method was effective, however, in separating phagocytic cells from plasma proteins and red cells. For ^{111}In-transferrin suspended in plasma, less than 0.1% of the added radioactivity was retained on the nylon column. For ^{203}Pb-labeled washed red cells resuspended in plasma, only 0.06% of the total added activity was retained on the column.

RESULTS

Agents binding to the cellular elements by no more than 1% of the added radioactivity are listed in Table 1. Those exhibiting a higher degree of cell binding are listed in Tables 2 and 3; these were arbitrarily divided into two categories: "indiscriminate" labels that bind to erythrocytes to a high degree in addition to leukocytes, and "preferential" agents that label predominantly the leukocytes.

Radioactive elements. Carrier-free ^{203}Pb-chloride, when added to whole blood, labeled the erythrocyte fraction with a yield of 93–94%. In cell-rich supernatant after red-cell sedimentation, 4% of the total radioactivity added was cell bound, of which about half was leukocyte bound. The nylon column method also produced about 2% binding to phagocytes. Similar leukocyte binding was obtained with ^{43}K-chloride, but the tagging was less stable as judged by elution on repeated washing of the cell sediment.

With carrier-free ^{67}Ga-citrate (1.7 pg/μCi), cell binding was only about 1% and no difference was noted at the incubation temperatures of 20° and

TABLE 1. SOLUBLE RADIOACTIVE AGENTS SHOWING NO SIGNIFICANT LEUKOCYTE LABELING (1% OR LESS)

Agent	Quantity added/2 ml cells
^{111}In-chloride (transferrin)	Carrier-free
^{111}In-bleomycin	15 μg
^{111}In-tetracycline	30 μg
^{111}In-nitrilotriacetic acid (NTA)	10 μg
^{99m}Tc-pertechnetate	Carrier-free
^{99m}Tc-dihydrothioctic acid	0.005 μl in buffered saline
^{99m}Tc-imidodiphosphate	50 μg
^{99m}Tc-adenylyl-imidodiphosphate	5 μg
^{131}I-2,4-diiodo-oestradiol	1 μg
^{131}I-rose bengal (aqueous and ethanolic)	4 μg
^{75}Se-selenomethionine	0.25 μg
^{18}F-sodium fluoride	Carrier-free to 25 μg
^{77}Br-DNA	0.5 μg

* Iron carbonyl particles, type SF, 3 μm, 1.5 mg in 0.1 ml of saline (General Analine Film Corp., Linden, N.J.).

† Dupont nylon fibers denier 1.5 in., type 200, scoured with "Duponal" RA, supplied in "Leukopaks" Fenwal Laboratories, Morton Grove, Ill.

TABLE 2. SOLUBLE RADIOACTIVE AGENTS SHOWING "INDISCRIMINATE" LEUKOCYTE LABELING

Agent	Quantity added/2 ml cells	Percent binding at 1 hr, room temp. RBC 10^6/2 ml	RBC In plasma	WBC (2×10^7/2 ml) Without plasma	WBC (2×10^7/2 ml) Without plasma RBC
^{51}Cr-chromate (23)		2	2		
^{203}Pb-chloride	Carrier-free	2	2		
^{42}K	Carrier-free	2	2		
^{111}In-acetylacetone	2 μl + 2 μl ethanol	0	0	51	20
^{111}In-tetraphenylporphyrin	2 μg in 10 μl ethanol	6	11	11	12
^{111}In-8-hydroxyquinoline	0.5 μg in 50 μl 50% ethanol	6	5	27–37	16–45
^{99m}Tc-reduced + Sn^{+2} (35)	—	—	—	—	—
^{99m}Tc-8-hydroxyquinoline	0.5 μg in 50 μl 50% ethanol	4	5	14	16

36°C. Binding was slightly increased by suspending the cells in HBSS free of plasma protein. A further increase was obtained by adding 5 μg/ml of carrier gallium, zinc, or ferric ions to the medium. Assuming a normal plasma transferrin concentration of 200 mg per 100 ml, only 5.5 μg of ferric ion should be required to saturate the transferrin in 2 ml of plasma, since 2 moles of ferric ion bind to 1 mole of transferrin. No further increase in cell binding was obtained with larger amounts of carrier iron or gallium (i.e., 10 μg/ml alone or in combination). The most cell binding was obtained with 5 μg/ml of carrier ions with the cells suspended in HBSS.

^{131}I-labeled lipids. Some degree of leukocyte labeling was obtained with ^{131}I-2,4-diiodocholesterol by adding small amounts to the cell-rich supernatant (20 μg in 5 μl per 2 ml). This result was surprising in view of the presence of considerable free and esterified cholesterol in normal plasma. The cell-binding yield was increased from 5% to 12% when the incubation temperature was 37°C instead of 20°C. This binding increase with the higher temperature was unusual for soluble radiopharmaceuticals, except for lipids. For ^{131}I-oleic acid, cell binding was only 3% even at 37°C.

Complexes of ^{111}In. No significant cell binding occurred with the water-soluble complexes: ^{111}In-bleomycin, ^{111}In-tetracycline, or ^{111}In-indium chloride, which become bound to transferrin in plasma. Cell labeling, however, did occur with several lipid-soluble chelating agents. Tetraphenylporphyrin complex in 95% ethanol, incubated with whole blood, had a retention of 7% on nylon columns. In cell-rich supernatant, the column retention was 11% at 1 hr, increasing to only 13% at 3 hr. Little difference in labeling was observed between room temperature and 36°C. The leukocyte-labeling yield was apparently not influenced by the presence or absence of plasma protein. Labeling of the erythrocyte fraction occurred but did not appear to be as frequent as for the leukocyte fraction. Relatively little radioactivity was lost with repeated washing of labeled leukocyte suspensions with HBSS.

TABLE 3. SOLUBLE RADIOACTIVE AGENTS SHOWING "PREFERENTIAL" LEUKOCYTE LABELING

Agent	Quantity added/2 ml cells	Percent binding at 1 hr, room temp. WBC (2×10^7/2 ml) With plasma	WBC (2×10^7/2 ml) Without plasma
^{32}P-DFP (20)		99	
^{125}I-acridine (25)		46	
^{125}I-quinoline (25)		46	
^{131}I-cholesterol	8 μg in 2 μl ethanol	5	12
^{3}H-adriamycin (42)	0.6 μg/ml	14	—
^{77}Br-DNA–adriamycin	0.4 μg of each	16	
^{125}I-DNA	1 μg	0	9–20
^{67}Ga-citrate	Carrier-free	1	2
^{67}Ga-citrate	10 μg of Ga, Zn, or Fe	3	4–13

The ^{111}In complex of acetylacetone dissolved in ethanol did not label cells in the presence of plasma protein. With supernatant cells resuspended in HBSS (free of protein), however, cell binding ranged from 45% to 64% (average 51%). After hypotonic lysis, 20–26% leukocyte labeling was obtained. This agent became bound to nylon columns when suspended in saline without cells, but did not bind when suspended in plasma. When using the column method, therefore, plasma had to be re-added to cell suspensions before passage through the column. With this method, leukocyte labeling reached 20%. When cell-rich supernatant was used, the percentage of cell tagging could be increased up to 94% by thorough washing of the cells in HBSS. The leukocyte binding

appeared labile. The ratio of leukocyte to erythrocyte activities was only 4; hence, the cell labeling appeared indiscriminate.

The indium complex of 8-hydroxyquinoline (oxine) was dissolved in 50% ethanol–50% 0.9% saline. Some labeling occurred with supernatant cells suspended in plasma, but it was much greater when the cells were suspended in HBSS. Labeling reached 45% after hypotonic lysis. The method of iron-particle phagocytosis produced cell binding of 24%. No significant difference in cell labeling was obtained between 20° and 36°C. Red cells thoroughly washed in saline and free of plasma proteins were labeled with this agent, but to a lower degree than with acetylacetone.

Complexes of ^{99m}Tc. No cell labeling was obtained with ^{99m}Tc as pertechnetate or as the three complexes listed in Table 1. These were selected because their lipid solubility was somewhat greater than that of most ^{99m}Tc radiopharmaceuticals; nonetheless, their oil-to-water partition coefficients were still much less than unity. On the other hand, the lipophilic ^{99m}Tc-oxine complex labeled the cells well when they were suspended in HBSS. The percentage of labeling was not as high as for the ^{111}In-oxine complex. With a high concentration of leukocytes, however, the labeling yield could be at least doubled. Cell tagging appeared relatively stable.

Radioactive deoxyribonucleic acid (DNA). Calf-thymus DNA (type V, Sigma Chemical Corp., St. Louis, Mo.) with an average molecular weight of about 1 million could not be labeled successfully with ^{99m}Tc. When stannous ions in ethanol were used, some labeling did occur, but gel chromatography revealed that a breakdown product with a molecular weight of about 5,000 was being tagged, rather than the intact DNA. Calf-thymus DNA was successfully labeled with ^{77}Br. No significant cell labeling occurred at either room temperature or 36°C with or without plasma in the supernatant fluid. In contrast, when an equal amount of adriamycin was added [a drug known to intercalate DNA molecules (*12*)], about 16% of the added radioactivity became bound to the leukocyte fraction with or without plasma. For this type of DNA, therefore, the presence of a cell-permeable intercalating drug appeared essential for the labeled DNA to gain entrance into the cells.

Another type of DNA obtained from HeLa cells (molecular weight about 10 million) was labeled by adding ^{125}I-iododeoxycytidine to the growth medium. No binding to the cell fraction occurred when minute amounts of plasma were present in the incubation medium, even when the intercalating drugs adriamycin, proflavine, or actinomycin D were bound to the DNA before addition to the incubation tubes. Significant cell binding of this DNA occurred, however, with or without intercalating drugs, when plasma was eliminated from the suspending medium. With suspensions of red cells from 0.1 ml of whole blood alone, binding was about 5%. With suspensions of leukocytes obtained after the hypotonic lysis of cell-rich supernatant, cell binding ranged from 9% to 20%. Varying the incubation temperature between 20°C and 36°C produced no difference in tagging; neither did varying the amount of labeled DNA from 0.2 to 1 μg per incubation tube. Increasing the leukocyte concentration by a factor of 4, however, increased the cell binding to 40–60%. Thus for leukocyte tagging with this type of DNA, complete elimination of the plasma protein appeared important and intercalating drugs were not needed.

DISCUSSION

The present study was limited to screening various soluble gamma-emitting radiopharmaceuticals for their ability to tag suspensions of leukocytes in the presence or absence of other blood components, using small quantities of blood and incubation times generally limited to 1 hr. Cell viability was not assessed, but the chemical doses of the agent were kept low to minimize cell damage.

Although the total life span of the granulocyte is about 11–12 days, its half-time in the blood stream is only 6–7 hr (*13,14*) and its tissue localization is rapid compared with that of most other blood components. If a granulocyte tagging agent also indiscriminately labels serum albumin, red cells, or lymphocytes, whose biologic half-times are long, a high blood level will result. The plasma proteins are most easily separated from granulocytes. These cells, however, when deprived of their protective protein coating, tend to clump and undergo reversible morphologic changes within 10 min, as observed by phase microscopy (*15*). Hence, if the plasma component must be removed to achieve cell labeling with a high yield, incubation times must be kept to only a few minutes before replacing plasma, to preserve cell viability. Monocytes, like granulocytes, have a short half-time in the blood. Their localization in focal inflammatory lesions, however, is not as dramatic. Quantitative studies by Spector et al (*16*) showed that, for 1 week after the induction of small inflammatory granulomata in rats, 10% of circulating neutrophils migrated into the lesion every 24 hr, whereas the daily migration was only 1% for circulating monocytes.

None of the gamma-emitting agents evaluated thus far label granulocytes as selectively as ^{32}P-diisopro-

pylfluorophosphate (^{32}P-DFP) (*9,17–19*). In whole blood with acid citrate dextrose anticoagulant, the relative labeling is 84% granulocytes, 12.5% monocytes, 2.5% lymphocytes, 0.4% erythrocytes, and 0.3% platelets (*20*). Unfortunately, this compound has not been tagged with a gamma-emitter.

The first gamma-emitting leukocyte label, ^{51}Cr-chromate, has a low tagging efficiency for granulocytes and indiscriminately labels all major blood components. It has been used effectively in leukemic patients (*21,22*), in whom the granulocyte count is high. The data of Eyre et al (*23*) give the relative labeling of components of normal blood as: 81.5% red-cell fraction, 15% plasma fraction, 1.3% platelets, 1.0% monocytes, 0.6% granulocyte fraction, and 0.6% lymphocyte fraction. In a cell suspension of isolated granulocytes in a medium containing serum, Burleson (*24*) obtained a labeling yield of only 2.2%. Similar yields were obtained in the present study with ^{203}Pb-chloride and ^{43}K-chloride, though the cell tagging with the latter appeared labile.

Gallium-67-citrate injected intravenously is now used clinically for localization of abscesses by imaging, despite the disadvantage of high concentration in the gastrointestinal contents and liver. Experimentally, chemical and bacterial abscesses can be localized by injecting only the cellular component after incubating whole blood with ^{67}Ga (*24,25*). Nonetheless, the labeling yield of the cellular fraction in whole blood is extremely low (*28*), because of the high degree of binding to plasma proteins. In a cell suspension of 90% granulocytes in a medium containing serum, the labeling yield was still only 2.8% (*24*). In this study of supernatant cells in plasma after red-cell sedimentation, the yield was 1%. With removal of most of the plasma protein and the addition of minute amounts of carrier gallium, zinc, or ferric ions, the yield was improved to some extent.

The radioiodinated compounds that have so far tagged granulocytes with the highest labeling yield are acridine and quinoline analogs of atabrine and chloroquin, respectively (*25*). These drugs have limited aqueous solubility and penetrate cell membranes readily. In chemical doses, their fluorescence has been observed concentrated in the cytoplasmic granules of neutrophils, and they are thought to intercalate nuclear DNA (*26,27*). In carrier doses, they impede the degranulation of neutrophils during phagocytosis (*14*). Yeates et al (*25*) obtained a labeling yield of 46% with ^{125}I-labeled analogs in supernatant cells after red-cell sedimentation. Unfortunately, with carrier doses of these compounds, the labeled cells disappear from the bloodstream within about 90 min (*28*).

In this survey, ^{131}I-2,4-diiodoestradiol did not label the cellular fraction to any significant extent. This agent was tried because the anti-inflammatory effects of estrogens have been attributed partly to a direct effect on granulocytes. Estradiol added in vitro to polymorph suspensions did not inhibit phagocytosis but reduced glucose oxidation and endogenous pyrogen release from these cells (*29*). It would be of considerable interest to study other types of membrane-localizing steroids, such as radioiodinated glucocorticoids, since a saturable uptake of tritiated cortisol by polymorphs has already been shown (*30*) and since the glucocorticoids are known to decrease granulocyte adherence (*31*).

Radioiodinated cholesterol labeled leukocytes to some extent even in the presence of normal plasma cholesterol. Among other lipid compounds, Elsbach observed that neutrophils effectively incorporate ^{14}C-labeled free fatty acids but do not pick up other lipid constituents, such as glycerol esters or chylomicrons, beyond nonspecific surface adsorption. In contrast to an uptake of about 60% in 1 hr at 37°C for ^{14}C-fatty acids, only 3% binding of ^{131}I-oleic acid was obtained in the present study.

Although glucose and fatty acids are actively metabolized by mature neutrophils (*33*), their synthesis and metabolism of protein is not significant. Hence, the lack of uptake of ^{75}Se-selenomethionine after 3 hr of incubation was not unexpected. Cavalieri et al (*34*) reported some leukocytic binding with ^{75}Se-selenite: the labeling yield at 1 hr, however, was only about 0.5%. With ^{14}C-leucine, their labeling percentage was even lower.

Technetium-99m as pertechnetate alone did not label any blood cells. Leukocyte labeling has been reported (*35*) with cells suspended in saline after the addition of $SnCl_2$ reducing agent to pertechnetate, but the true labeling yield for granulocytes is questionable. Moreover, this technique labels any residual erythrocytes or serum albumin with a high yield. This direct labeling of leukocytes with ^{99m}Tc has already been used for thrombus localization in dogs (*36*), but with only partial success. The ^{99m}Tc complexes that are predominantly water soluble exhibited no significant cell binding in this study. The negative result obtained with ^{99m}Tc-adenylyl–imidodiphosphate is of particular interest because it is an analog of ATP. Leukocytes are known to utilize preformed purine bases, nucleosides, and nucleotides synthesized in the liver (*1*).

None of the water-soluble complexes of ^{111}In, including bleomycin, produced a significant degree of cell labeling. This antibiotic uncommonly produces pyrogenic reactions in man because of the release of endogenous pyrogen from interaction with leu-

kocytes. Pyrogen production has been induced experimentally by incubation of this drug with human leukocytes (*37*). Nevertheless, no leukocyte labeling was observed with the indium complex.

The lipid-soluble ^{111}In complexes labeled the cellular components of blood, in keeping with the Davson–Danielli principle that the ease of penetration of a substance through cell membranes is proportional to its lipophilic nature. Satisfactory cell labeling was achieved with the indium complex of tetraphenylporphyrin in the presence of plasma, but this preparation was difficult to make quickly on a daily basis. The organ distribution of this agent injected intravenously in rats has already been reported (*3*).

Indium-111-acetylacetone labels the cellular components of blood with high yield only in the absence of plasma. This complex, however, tends to elute from leukocytes. This substance has been used previously for labeling red cells (*38*). The cellular labeling yield in whole blood without plasma is as high as 98%. Because of the high degree of erythrocyte labeling, ^{111}In-acetylacetone appears unsatisfactory as a leukocyte label.

Oxine (8-hydroxyquinoline) is also highly lipophilic (oleyl alcohol-to-water partition coefficient 67) (*39*) and readily forms a complex with ^{111}In or ^{99m}Tc extractable in carbon tetrachloride which, following evaporation, is soluble in ethanol–aqueous solution. The tissue distribution of its ^{111}In complex in tumor-bearing rodents has been explored by Goodwin (*40*). Its copper and iron complexes have been evaluated extensively as antibacterial agents (*39*) but have proven ineffective in vivo because of their interaction with blood-cell metabolites. In bacterial suspensions, its metal complexes appear to localize intracellularly. According to Albert (*39*), oxine penetrates bacterial cells as a highly lipophilic 1:2 metal-to-oxine complex, whereas the 1:1 complex is ionized rather than lipophilic and cannot penetrate cell walls. Once inside the cell, the 1:2 complex is probably broken down to the 1:1 complex and ionized, and hence cannot diffuse out of the cell. The labeling of blood cellular components observed in this study with radioactive complexes of oxine may be due to the same mechanism. Because of the relatively high cellular labeling yield in the absence of plasma protein, the ^{111}In and ^{99m}Tc complexes appear promising as agents for leukocytic tagging, although they do not selectively label granulocytes. The labeling yield with the ^{99m}Tc complex was not as high as that obtained with ^{111}In complex. Indium complexes, however, have the inherent disadvantage that any free indium liberated in vivo promptly labels transferrin, causing a persistent high level of activity in the bloodstream.

Adriamycin (14-hydroxydaunorubicin) rapidly penetrates cells and interacts with the nuclei, inhibiting mitosis and synthesis of both DNA and RNA (*41*). When the tritiated drug is incubated with whole blood in vitro, 14% labels leukocytes (*42*) at minute doses of 0.6 μg/ml blood, and the leukocyte-to-erythrocyte concentration ratio is 500. Attempts to label this drug (free of carbohydrate-stabilizing agents) with ^{111}In and ^{99m}Tc were unsuccessful, probably because of the predominantly aromatic nature of this four-ringed compound. Moreover, acidification cannot be used for labeling, since this promptly splits the drug molecule into its two components, a water-insoluble aglycone (adriamycinone) and a water-soluble basic amino sugar (daunosamine). Radioiodination also has failed.

Adriamycin added to native or heat-denatured DNA becomes firmly bound. Calf-thymus DNA has been used as a carrier for adriamycin in cancer chemotherapy to reduce the cardiac toxicity of the drug. According to Trouet (*12*), the adriamycin–DNA complex, unlike the free drug, penetrates only cells with endocytic activity; the free drug is liberated intracellularly by lysosomal digestion. Deoxyribonucleic acid also binds a variety of other drugs (*27*), including some acridines and quinolines that fulfill certain molecular requirements, by intercalating (skewering) the double helix, with uncoiling. In the present study, ^{77}Br-labeled calf-thymus DNA did not label blood cells in the presence or absence of plasma. When adriamycin was intercalated with this preparation of DNA, however, leukocyte labeling occurred even with the cells suspended in plasma. On the other hand, ^{125}I-labeled DNA of HeLa cell origin, with a larger molecular weight of 10 million, labeled leukocytes without an intercalating drug, but only in a plasma-free medium. For many years, it has been known that the DNA of bone marrow precursor cells can be labeled in vivo with either ^{32}P-phosphate or tritiated thymidine; this approach, however, is not applicable to mature circulating granulocytes, which do not undergo DNA synthesis or mitosis (*28*).

Through the process of "piggyback" phagocytosis (*43*), a soluble radioactive agent is incorporated into monocytic or granulocytic phagocytes as nonradioactive particles become engulfed. This approach has been used by Klebanoff et al (*44*) to label peritoneal or blood leukocytes with ^{131}I-iodide in the presence of heat-killed bacteria or yeast cells. The iodide ions are oxidized intracellularly by hydrogen peroxide and myeloperoxidase. As a result, the engulfed particles are radioiodinated within the phagocytes. Only the phagocytic leukocytes become labeled, and the leukocyte-to-erythrocyte concentra-

tion ratios may be as high as 1200. Unfortunately, however, the resultant labeling yields are low. Klebanoff obtained approximately 6% incorporation of the ^{131}I label into phagocytes. Using various carrier doses of iodide, Stolc (*45*) found that the optimum iodide concentration was 100 ng/ml for maximal cellular accumulation of iodide by human polymorphs at 37°C after 1 hr, but the observed cell labeling was only 5.5% of the added radioactivity. Charkes et al (*46*) used this method for labeling leukocytes for the localization of thrombi in dogs by rectilinear scanning.

Chang et al (*47*) studied leukocytic phagocytosis with ^{131}I-serum albumin in the incubation medium with starch granules. Although incorporation of the label into the cells was extremely low (estimated at 0.05%), this approach could nevertheless be used to quantitate the rates of phagocytosis of different particles. It is likely that other labeled proteins may be better than serum albumin for leukocyte labeling. The autoradiographic study of antemortem thrombi by Edwards and Haynes (*48*) suggests that at least part of the localization of ^{131}I-fibrinogen in thrombi may be due to surface adsorption of the protein to leukocytes.

In the present study, the leukocyte incorporation of ^{77}Br-DNA was negligible with or without the addition of phagocytosable particles at 36°C. The leukocytic labeling of ^{111}In-oxine and other lipid soluble agents could not be enhanced significantly by phagocytosis, because some plasma had to be added to the incubation medium as an essential condition for phagocytosis, which inhibited the cell binding of these agents. With ^{131}I-cholesterol suspended in plasma, the cell binding was increased slightly (by 2–3%) with the addition of yeast or iron carbonyl particles and incubation at 36°C. To date, therefore, "piggyback" phagocytosis has not labeled leukocytes with a high yield.

In conclusion, routine reproducible labeling of granulocytes is difficult, because there is no rapid and efficient method for separating them from small volumes of blood while preserving viability. However, ^{111}In or ^{99m}Tc complexes of oxine, ^{111}In-tetraphenylporphyrin, and radioactive DNA complexed with adriamycin appear to be promising agents for this purpose. Their in vivo evaluation in experimental animals is therefore warranted.

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