be achieved only by minimal handling, minimal addition, and minimal ex-vivo time. The concept of labeling in plasma is a step in the right direction, but if cell concentration should be necessary the advantage is lost, especially if labeling efficiency is also reduced. We feel that it is not sufficient to quote biological handbook data based on LD 50 (*8*) but to plot ligand concentration against cell damage and labelling efficiency in order to select the best agents.

We believe that, of the three simple ligands currently reported, oxine is probably the most suitable and least toxic for normal cell labeling. The concentration of acetylacetone, combined with the very narrow concentration range to achieve high labelling efficiencies, limit its usefulness (*9*). Tropolone at optimal labeling concentrations in plasma can be shown to cause structural changes in cells (*10*).

We should like to see our commercial colleagues concentrating not on new nonspecific labeling agents, but upon cell-specific agents or on complete laboratory kits for the smaller hospital, thus making the cell-labeling technique more readily available and reproducible between research and diagnostic establishments.

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Reply

I find the comments of Dr. R. J. Hawker interesting. Some have been substantiated however, others are difficult to prove.

We have reservations concerning their remarks about the solubility of oxine in aqueous solutions. Oxine is soluble in acid solution, but we are interested in oxine at pH 6.5–7.4 for cell labeling. Without any proper solvent, oxine and In-oxine will be in the colloidal form. In nonphagocyte cell labeling, it is important to add alcohol and make In-111 oxine soluble. It is difficult to check colloid sizing with filter papers of different porosity. Most of the neutral In-111 complexes are very sticky and spread on millipore surfaces and our preliminary studies on In-111 oxine colloid sizing using the scanning electron microscope were not helpful either, though In-111 oxine is available without alcohol, this does not necessarily mean that In-111 oxine is water soluble. The true physical form of In-111 oxine is irrelevant as long as cell labeling can be performed uniformly and consistently. The uniform distribution of In-111 on a cell population might be more in doubt if In-111 oxine colloid were used. Since we are quantifying platelet thrombosis and expressing the interaction of platelets with a de-endothelialized vessel wall in terms of platelet density per unit surface area of artery, vein, or synthetic vascular graft (*1*,*2*), this uniformity is critical.

We consistently found 15–30% higher platelet (human) labeling efficiency with In-111 tropolone than with In-111 oxine. Platelet extraction in an ACD-plasma medium was never 100% with any of the ligands, tropolone, oxine, or acetylacetone (*3–6*), and labeling efficiency with human platelets is 5–10% lower than with canine platelets under identical conditions of cell number and ACD-plasma volume. Moreover, we do not like to use PGE_1 in incubation media. Drs. Lavender and Subramanian considered In-111 tropolone as the preferable label for platelets and white cells (*8*), although Thakur suggested that In-111 mercaptopyridine oxide might serve the same purpose equally well (*9*).

Partitioning of In-111 complexes was quantified by lipoproteins of dog and human plasma and no marked difference was observed. Lipoproteins do not extract In-111 complexes as efficiently as cell membranes. Lipoprotein binding of these three In-111 complexes in human plasma was studied by cesium chloride density-gradient separation and ultracentrifugation. Human plasma (18 ml) was incubated with 50 μCi each of In-111 oxine, In-111 acetylacetone, In-111 tropolone, I-131 iodocholesterol, and C-14 cholesterol. The fractions of very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), high-density lipoprotein (HDL), albumin, and gamma globulin (A + GG) are separated by spinning 4.5 ml at 210,000 *g* for 12, 16, and 20 hr using density adjustment with cesium chloride solution at 1.06 and 1.25 g/ml respectively. The radioactivity in four fractions was determined in a gamma well and liquid scintillation counter, and the percentage of activity with different lipoprotein fractions is quantitated and tabulated below.

	VLDL	LDL	HDL	A + GG
In-111 Tropolone	4.20	9.58	29.90	56.29
In-111 Oxine	6.63	14.91	26.45	52.00
In-111 Acetylacetone	4.99	24.99	40.00	28.33
C-14 Cholesterol	23.83	63.40	11.80	2.33
I-131 Cholesterol	28.69	55.25	10.23	5.84

In cell labeling with microgram amounts of oxine and tropolone, toxicity is only of theoretical interest, (*7*) since most of these ligands wash out during cell labeling. Of the three ligands, In-111 $(tropolonate)_3$ appears to be the best agent at this time.

After reviewing hundreds of transmission and scanning electron micrographs of platelets, polymorphonuclear leukocytes, and endothelial and smooth-muscle cells, we are convinced that definite conclusions need careful evaluation of a statistically significant number of cells by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The artifacts in micrographs must be properly scrutinized. Many changes in cells labeled with In-111 oxine/tropolone are observed by SEM or

TEM. These alterations might be due to manipulation of cells during harvesting, pelleting, or washing, mainly degranulations and some pseudo-pod formation. None of these changes could be attributed to labeling with In-111 oxine or In-111 tropolone.

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Re: Cardiac Lymphoscintigraphy Following Closed-Chest Catheter Injection of Radiolabeled Colloid Into the Myocardium of Dogs: Concise Communication

I read with interest this article (*1*) that describes an elegant technique for yet another application of lymphoscintigraphy and would appreciate further comment on the following points.

The authors selected $^{99m}TcSb_2S_3$ and In-III hydroxide colloid as their agents, but the majority of the data reported concerns $^{99m}TcSb_2S_3$ with only brief allusion to In-III hydroxide colloid as a cardiac lymphoscintigraphic agent. What influenced their choice of this agent in the first instance?

On post mortem examination and imaging, nodes located between the aorta and pulmonary outflow tract consistently were found to contain radiocolloid; however, there were also at least four to seven nonradioactive thoracic nodes harvested from each animal. I would be interested in the authors' interpretation of these findings. What was the precise location in the thorax of the nonradioactive nodes? I assume they are anterior mediastinal nodes receiving drainage from the peritoneal cavity via the phrenic lymphatics, which would be demonstrable with intraperitoneal radiocolloid and not necessarily lymph nodes draining the myocardium.

Incidentally, Ref. 24 has been in print since 1979 (*2*).

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Reply

The authors thank Dr. Ege for her thoughtful questions about our article on cardiac lymphoscintigraphy (*1*). In response to the first question, the data in Table 1 refer to studies with $^{99m}TcSb_2S_3$. The hypotheses approached by the investigation of cardiac lymphatics were that the sites of lymphatic drainage from the right and left ventricles were similar and that the rate of drainage from the heart was rapid. Our preliminary results in dogs studied sequentially suggested that this was indeed the case. We felt compelled, however, to investigate this process with simultaneous injections in both ventricles, which required the selection of a second colloid. The choice of In-III hydroxide colloid as the alternative material for these studies was based on the preliminary work of Castronovo (*2*). Although no definitive data comparing indium colloid and antimony sulfide were available, we felt that the crossover experiment would solve the problem of comparison.

There were typically four to seven nodes located in the superior mediastinum that did not contain radioactivity. The precise location of these nodes varied. We interpreted this observation, as suggested by Dr. Ege, to indicate that these nodes did not drain the myocardium.

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