Imaging Experimental Infective Endocarditis with Indium-111-labeled Blood Cellular Components

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SUMMARY The capability of radionuclide imaging to detect experimental aortic valve infective endocarditis was assessed with indium-111 (111In)-labeled blood cells. Sequential cardiac imaging and tissue distribution studies were undertaken in 17 rabbits with infective endocarditis after administration of 111In-platelets and in five after 111In-polymorphonuclear leukocytes.

Forty-eight to 72 hours after platelet administration, in vivo imaging demonstrated abnormal 111In uptake in all animals in the region of the aortic valve in an anatomically distinct pattern. Images of the excised heart showed discrete cardiac uptake conforming to the in vivo image and gross pathological examination. 111In platelet uptake in vegetations from the 17 animals averaged 240 ± 41 times greater than that in normal myocardium and 99 ± 15 times greater uptake in blood. In contrast, 111In-leukocyte cardiac imaging showed no abnormal aortic valve uptake 24 hours after tracer administration and the lesion myocardium activity ratio was only 5 ± 2 (3 ± 1 for lesion/blood activity). Four normal rabbits demonstrated neither positive 111In platelet scintigraphs nor abnormal cardiac tissue uptake. Likewise, noncellular 111In was not concentrated to any significant extent in three animals with infective endocarditis.

This study demonstrates that 111In platelet, but not leukocyte cardiac imaging, is a sensitive technique for detecting experimental infective endocarditis. The imaging data conform to the cellular pathology of the infective endocarditis vegetation.

THE VEGETATIVE LESION of infective endocarditis is predominantly bacteria, platelets and fibrin adherent to damaged valvular endothelium. In contrast to the marked accumulation of platelets, the lesion contains relatively few neutrophils and erythrocytes. Recent studies have demonstrated that platelets promote bacterial adherence and propagation of the endothelial damage. The large numbers of platelets in the lesion and their crucial role in the pathogenesis of acute infective endocarditis led us to consider that radionuclide-labeled platelets might be an appropriate agent for imaging this condition. Studies were undertaken with indium-111 (111In)-labeled platelets in an experimental model of infective endocarditis. Both in vivo cardiac imaging and tissue distribution studies were performed, and the results obtained with platelets were compared with similar studies carried out with 111In-labeled polymorphonuclear leukocytes. The data indicate the potential of radionuclide cellular imaging techniques for detecting infective endocarditis. The technique also provides a means of directly observing the pathogenic role of blood cellular components in the formation of infective endocardial vegetations.

Methods

Induction of Experimental Infective Endocarditis

Aortic valve infective endocarditis was established in 25 New Zealand white rabbits using a modification of the standardized technique. Each rabbit was anesthetized with an intramuscular injection of 200 mg ketamine. An incision was made in the midline of the neck and either the right or left carotid artery was isolated. A 1-mm-diameter polyethylene catheter filled with sterile saline and connected to a Statham physiological pressure transducer was placed in the carotid artery and then advanced into the left ventricle under pressure monitoring. The catheter was then passed back and forth across the aortic valve approximately five times to abrade the valvular endocardium. The catheter was then pulled back above the aortic valve cusps, tied in place, and buried in the subcutaneous tissues by closing the incision.

Twenty-four to 48 hours later, each rabbit was injected via a marginal ear vein with approximately 10 million colony-forming units of *Streptococcus sanguis*. The bacteria were maintained on blood agar and transferred daily in a candle extinction jar maintained at 37°C. Before administration, the microorganisms were suspended in Brain Heart Infusion Broth supplemented with 5% sucrose. After injection of bacteria, the animals were isolated.

Preparation of 111In-Platelets

Platelets were isolated and labeled by the method of Thakur. From a healthy adult New Zealand donor rabbit, 43 ml of arterial blood was drawn into a 50-ml disposable plastic syringe containing 7 ml acid citrate dextrose (ACD) solution (Squibb). This amount of blood provided enough heterologous platelets for administration to three animals. Heterologous platelets were used to obtain sufficient blood to provide cells for

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multiple animals at one time. The blood was transferred into two 50-ml conical (Falcon) plastic test tubes and centrifuged at 180 g at room temperature for 15 minutes using a calibrated horizontal swing rotor table top centrifuge (IEN). The platelet-rich plasma was transferred with a glass Pasteur pipette, pooled into another plastic 50-ml tube and centrifuged again at 1500 g for 10 minutes. The platelet-poor plasma was removed by pipette and the platelet button was suspended in 5 ml 0.9% NaCl and centrifuged at 1000 g for 10 minutes. This step was repeated and the platelets were finally suspended in 5 ml normal saline. Platelet suspension was accomplished by gentle mixing with a Pasteur pipette. Any visible clumps that may have aggregated with contaminating red cells (one or less in every 1,000 platelets) were eliminated by sedimentation. The platelet suspension was then ready for radionuclide labeling, and was transferred into a 15-ml Falcon round bottom polyethylene test tube.

Commercially available 111In-8-hydroxquinoline (supplied as 111In-oxine, Diagnostic Isotopes, calibrated as 1 mCi in 50 μg oxine and 50 μl ethanol) was used for labeling. A suitable volume containing approximately 450 μCi 111In radioactivity was withdrawn, diluted to fourfold with normal saline and added dropwise to the platelet suspension with a Pasteur pipette. The mixture then was incubated at room temperature for 15 minutes. With this labeling technique, greater than 90% of the radioactivity is found in association with the platelets.5 The suspension of the labeled platelets then was divided into three equal portions, each with approximately 150 μCi 111In activity, and administered intravenously to three experimental animals. Each animal received approximately 3 × 10⁹ radiolabeled platelets.

111In-oxine is a suitable radionuclide for labeling blood cellular components.6,7 111In emits two photons per disintegration with energies of 173 and 247 keV, well-suited for detection by conventional scintigraphic cameras. It has a physical half-life of 2.8 days, permitting imaging over several days after a single injection. 111In-oxine is a lipid soluble compound which labels platelets and leukocytes efficiently without altering their functional properties.5,6,8,9 Labeled platelets clear from the circulating blood with a life span of 8 days.8 The half-life of circulating labeled polymorphonuclear leukocytes is 7.5 hours.8

Preparation of 111In-Polymorphonuclear Leukocytes

Heterologous rabbit polymorphonuclear leukocytes were separated and labeled using a modification of a previously described method.8 Thirty milliliters of venous blood from a normal donor rabbit were drawn into a disposable plastic syringe containing 5 units/ml preservative-free heparin. Methyl cellulose solution then was added (3% vol/vol of 2% weight volume solution) and the mixture was gently agitated. The blood was allowed to sediment spontaneously for 60 minutes, removing the bulk of the erythrocytes. The leukocyte-rich plasma was separated with a Pasteur pipette and gently layered over a Ficoll/Hypaque gradient (Pharmacia) contained in a 50-ml round bottom disposable plastic tube (7.5 ml gradient for each 10 ml of leukocyte-rich plasma). The test tube then was centrifuged at 400 g for 30 minutes. The plasma layer was separated with a Pasteur pipette and saved. The remaining supernatant was discarded. The resulting cellular button was suspended in 2.5 ml sterile water for 60 seconds to lyse hypotonically the erythrocytes. The cell suspension was then made isotonic by the addition of 2.5 ml of 1.8% NaCl. This was then centrifuged at 450 g for 5 minutes and the cells were washed with 5 ml of 0.9% NaCl. They were finally suspended in 5 ml 0.9% NaCl. Approximately 150 μCi 111In-oxine solution in ethanol (Diagnostic Isotopes) was diluted fourfold with 0.9% NaCl and added dropwise. The mixture then was incubated for 15 minutes at room temperature. This usually results in a labeling efficiency of at least 90%.8 An equal volume of the previously retained plasma was added to the cell suspension and administered intravenously to the animal being studied.

Radionuclide Imaging

Seventeen rabbits each received approximately 150 μCi 111In-labeled heterologous platelets, and five rabbits received approximately 150 μCi 111In-labeled heterologous leukocytes 3–5 days after the induction of infective endocarditis. Cardiac images were obtained on Polaroid film using a Searle Radiographics Pho Gamma HP scintillation camera fitted with a 4 mm pinhole collimator. Images were obtained in the left anterior oblique position with the pinhole located over the precordial impulse. A standard field of view included the cardiac region as defined by a blood pool image and the superior border of the liver. Twenty-five thousand-count images were obtained using a 20% window centered on the 247 keV photopeak of 111In. Cardiac images were obtained 24, 48 and 72 hours after administration of 111In-platelets and 24 hours after 111In-leukocytes. Before imaging, the animal was anesthetized with 200 mg ketamine. The 24-hour image in rabbits receiving 111In-platelets demonstrated a cardiac blood pool. By 72 hours, the platelets had substantially cleared from the blood, with normal uptake predominantly within the reticuloendothelial system. In contrast, by 24 hours there was only faint residual blood pool activity in those animals receiving 111In-leukocytes. Blood pool and hepatic activity in the 24-hour 111In scintigraph provided anatomic references for localization of any abnormal focal cardiac activity. All sequential imaging was performed with the animal and collimator in the same positional relationship. Abnormal 111In uptake was considered present in the region of the aortic valve if the localization had an intensity greater than any residual blood pool and was equal or greater to that noted in the liver. Furthermore, when correlated with the 24-hour cardiac blood pool image, abnormal focal uptake had to be anatomically distinct and located in the region of the aortic outflow tract.

Four healthy, noncatheterized and noninfected animals served as controls for 111In-platelet cardiac
scintigraphy. Three additional control animals with infective endocarditis each received 150 μCi free ¹¹¹In-oxine to assess whether uptake was independent of cellular binding of the radioactive label. All cardiac scintigraphs were interpreted independently as either positive or negative by at least two of the authors, unaware of the experimental conditions.

Tissue Radionuclide Distribution, Excised Heart Imaging

After in vivo imaging, each animal was sacrificed with a lethal dose of intravenous sodium pentobarbital (72 hours after ¹¹¹In-platelets and 24 hours after ¹¹¹In-leukocytes). The heart was removed immediately after sacrifice and washed free of blood and clot. Weighed samples of blood were taken. The anterior wall of the aorta and left ventricle were excised and the dissected heart was examined grossly for the presence of vegetations on the aortic valve, aorta or left ventricle. The excised hearts were imaged again using the same gamma camera and pinhole collimator. The excised heart images were directly compared to both the in vivo cardiac scintigraphs and gross pathology.

In each heart, the vegetative material was carefully dissected free from its valvular endocardium and aortic endothelium. Samples of the vegetation and normal left ventricular myocardium were washed, dried and weighed. In the normal animals, samples of normal aortic valvular structures and left ventricular myocardium were similarly handled. The weighed samples were then counted in a well-type scintillation counter (Beckman Gamma 8000). Radioactivity concentrations of each sample were expressed as counts/min per gram of tissue. A normal radioactive uptake for each heart was calculated by averaging at least three samples of the normal appearing left ventricular myocardium. The uptake of ¹¹¹In platelets or ¹¹¹In leukocytes by the vegetative lesion was determined by averaging two or three samples from each lesion and comparing it directly to that in normal myocardium and blood from the same animal. The results were then expressed as lesion/myocardial and lesion/blood radioactivity uptake ratios.

**Figure 1.** Photomicrograph (× 250) of an experimentally induced aortic valve infective endocarditis lesion. The darkly stained masses to the right are clumps of bacteria which are surrounded by a fibrin-collagen mesh. On the surface of the lesion is a dense cellular infiltration consisting predominantly of platelets and, to a lesser extent, leukocytes. The microscopic appearance is identical to the naturally occurring lesion in humans.
Pathology and Microbiology

The vegetative material from 15 randomly selected animals with infective endocarditis was analyzed by standard histopathological and microbiological techniques. A small sample of the lesion was excised using sterile techniques and placed in Brain Heart Infusion Broth supplemented with 5% sucrose. The culture was incubated in an atmosphere of carbon dioxide maintained at 37°C for at least 5 days or until turbidity became evident. A sample of the growth was plated out on blood agar, cultured and the organism subsequently confirmed with appropriate biochemical tests. Samples of the lesion and their supportive endocardium were placed in formalin and submitted for histological analysis. Normal-appearing left ventricular myocardium remote from the lesions was similarly handled. Microscopic examination was performed on samples stained with hematoxylin and eosin and Brown-Brenn dyes.

Results

Gross and Microscopic Pathology, Bacteriology

Bulky, friable, greyish-white vegetations ranging in size from 2–6 mm were present in the region of the aortic valve in all animals injected with bacteria. In most cases, at least one aortic valve cusp was destroyed by vegetations. Lesions frequently extended into the proximal portions of the ascending aorta and occasionally into the subvalvular left ventricular myocardium. In one case, vegetations appeared on the anterior mitral leaflet and papillary muscle. Despite the presence of occasional left ventricular endocardial lesions, there did not appear to be any grossly visible abscess cavities or necrotic areas of the left ventricular myocardium. In the majority of cases, the catheter had endothelialized into the proximal ascending aorta, with occasional vegetative material at points of contact between the catheter and endothelium. However, the majority of vegetations appeared on the aortic valve or contiguous proximal ascending aorta.

Microscopic analysis of vegetative material and its supporting tissues confirmed the diagnosis of infective endocarditis. Each lesion consisted of a diffuse cellular infiltrate of platelets, mononuclear and polymorphonuclear leukocytes, enmeshed within a collagen fibrin framework (fig. 1). The endocardial surfaces were covered with a layer of fibrin, platelets and a focal infiltration of leukocytes. Despite the normal appearance of left ventricular myocardium remote from the lesion, microscopic analysis revealed focal infiltration of leukocytes and spotty areas of necrosis. Nests of streptococcal organisms permeated the entire vegetation, and were most prominent beneath the fibrin platelet surface layer. Pure Streptococcus sanguis was recovered from each of the samples submitted for bacteriologic analysis.

**111**In-Platelet Imaging

During the first 24–48 hours after administration of **111**In-platelets, cardiac imaging revealed a radioactive blood pool (fig. 2). In some animals focal-enhanced activity in the aortic outflow tract, with an intensity greater than the blood pool, began to appear by 48 hours. By 72 hours, blood pool activity had cleared. At this time, abnormal **111**In uptake was visualized in the region of the aortic valve in all animals with endocarditis (fig. 2). Uptake in the region of the aortic valve was either focal or multifocal; either pattern was anatomically distinct from the general blood pool as defined by the 24-hour study (fig. 3). At 72 hours, no abnormal aortic valve uptake was demonstrated in normal control animals, in whom only faint residual uptake in the aortic root was seen. The image of the excised heart confirms that only a single hot spot is present in the region of the aortic valve.

**Figure 2.** Serial **111**In-platelet cardiac scintigraphs in the left anterior oblique position from a rabbit with infective endocarditis. Twenty-four hours after the administration of **111**In-platelets cardiac imaging demonstrates radioactivity within the blood pool (BP) and liver (shown in the bottom of BP scan). In this animal, abnormal **111**In activity began to appear in the aortic outflow tract with an intensity greater than the blood pool and equal to the liver by 48 hours after platelet administration. The 72-hour image shows almost total clearance of the blood pool, with intense aortic valvular uptake. Both the initial blood pool activity and hepatic uptake define and localize the abnormal valvular radioactivity. This and all other scintigraphs were obtained with a pinhole collimator which magnifies the image. The image of the excised heart confirms that only a single hot spot is present in the region of the aortic valve.
proximal aorta. The extent of heart conformed the aortic both the excised heart showed and excised vivo 5). Those animals with a single focus of uptake demonstrated a contiguous lesion on and around the aortic valve. Those animals with more than one focus of uptake on in vivo imaging invariably demonstrated noncontiguous endocardial lesions in the aortic outflow tract. The top two scans are taken from two animals with single large lesions in the aortic valve and proximal aorta. The third scintigraph is a 72-hour 111In platelet cardiac image from an animal with noncontiguous lesions of the distal ascending aorta and aortic valve. The fourth scintigraph is from an animal with three separate noncontiguous lesions present on the proximal ascending aorta, aortic valve and subaortic left ventricular endocardium.

blood pool activity and intense hepatic uptake were present (fig. 4).

The abnormal focal uptake in each case of infective endocarditis correlated well with pathological evidence of either isolated aortic valve or contiguous aortic endothelial involvement. Those animals with a single discrete area of uptake on in vivo imaging demonstrated only a single mass lesion in and around the aortic valve. Animals with more than one focus of abnormal uptake on in vivo imaging demonstrated at least two noncontiguous vegetative lesions on gross pathological examination. In every animal, images of the excised heart showed an excellent correlation with both the grossly observed lesions and in vivo imaging (fig. 5). In each case, the areas of uptake on both in vivo and excised heart imaging, accurately defined the extent of vegetative lesions on the aortic valve and proximal aorta. The “hot spot” images of the excised heart conformed exactly to the anatomical extent of the lesion and confirmed that only the lesion accumulated significant amounts of the tracer. Despite the presence of the catheter containing clotted blood within the endothelium of the proximal aorta, uptake was only seen at points of contact containing vegetations. Imaging in the three animals with infective endocarditis receiving 111In-oxine demonstrated only residual blood pool activity after 72 hours.

111In-Leukocyte Imaging

Twenty-four hours after the administration of 111In-leukocytes, cardiac imaging revealed only faint residual blood pool activity in five animals with infective endocarditis. No abnormal or focal aortic valvular uptake was seen despite the presence of extensive vegetations. The most intense activity appeared

![Figure 3](http://circ.ahajournals.org/)

**Figure 3.** 111In-platelet cardiac scintigraphic patterns in animals with experimental infective endocarditis. Each image pattern was anatomically distinct from the initial blood pool image and localized to the aortic outflow tract. The abnormal uptake correlated well with the extent of endocardial involvement as defined by gross pathological examination. Those animals with a single focus of uptake demonstrated a contiguous lesion on and around the aortic valve. Those animals with more than one focus of uptake on in vivo imaging invariably demonstrated noncontiguous endocardial lesions in the aortic outflow tract. The top two scans are taken from two animals with single large lesions in the aortic valve and proximal aorta. The third scintigraph is a 72-hour 111In platelet cardiac image from an animal with noncontiguous lesions of the distal ascending aorta and aortic valve. The fourth scintigraph is from an animal with three separate noncontiguous lesions present on the proximal ascending aorta, aortic valve and subaortic left ventricular endocardium.

![Figure 4](http://circ.ahajournals.org/)

**Figure 4.** Comparison of imaging results in normal (NL) and infective endocarditis (IE) animals. Twenty-four hours after administration of 111In-platelets, imaging revealed diffuse cardiac uptake, reflecting the blood pool (BP) scan. Activity is also normally present in the liver, shown at the bottom of the scan. By 72 hours, the blood pool had cleared in normal animals due to sequestration of platelets within the reticuloendothelial system of the liver and spleen. In contrast, intense focal uptake of 111In activity appeared in the region of the aortic valve in the animals with infective endocarditis 72 hours after platelet administration.
under the diaphragm within the liver and spleen. Images of the excised heart showed very faint uptake in the region of the aortic valve in three animals conforming to the site of vegetation. This degree of uptake, however, was not sufficient to result in a positive in vivo scintigram.

Tissue Radioactivity Distribution

Uptake of $^{111}$In-platelets in the vegetative lesions from 17 animals with infective endocarditis was 240 ± 41 (mean ± SEM, range 27-532) times greater than that in normal left ventricular myocardium (table 1). When compared to radionuclide concentration of blood, the lesion had 99 ± 15 times greater activity. No significant abnormal cardiac tissue uptake was demonstrated in the healthy rabbits receiving $^{111}$In-platelets or in those animals with endocarditis receiving free $^{111}$In-oxine. The insignificant lesion uptake of $^{111}$In-oxine compared with blood indicates that platelet binding is essential for a positive image. In normal controls, the $^{111}$In-platelet radioactivity uptake ratio in aortic valve structures compared to left ventricular myocardium was 1 ± 0.3 (range 0.2-2.1). The lesions in those animals receiving $^{111}$In-leukocytes accumulated 5 ± 2 (range 1-15) times more activity compared with normal left ventricular activity and only 3 ± 1 times more activity than blood.

Discussion

This study demonstrates that heterologous $^{111}$In-labeled platelets, and not leukocytes, selectively localize in experimental aortic valve vegetations, allowing external detection of infective endocarditis by in vivo cardiac imaging. $^{111}$In-platelet cardiac scintigraphy in each of 17 animals with experimental infective endocarditis revealed focal areas of enhanced radioactivity uptake in the region of the aortic valve which conformed to the anatomic extent of the lesion demonstrated by gross pathology. Images of the excised hearts demonstrated “hot spot” scintigraphs with precise correlation to sites pathologically shown to be involved with vegetations. Tissue distribution studies supported these scintigraphic results. Although excised heart imaging in those animals studied with $^{111}$In-leukocytes revealed faint vegetation uptake, the extent of lesion accumulation did not allow identification by in vivo imaging. The vegetative lesion likewise did not significantly concentrate free $^{111}$In-oxine. Therefore, the high lesion activity ratio after the administration of labeled platelets represented intense platelet deposition.

The degree of uptake demonstrated in this study confirms that platelet imaging may serve as a sensitive label and physiological marker of acute infective endocarditis. Platelets are the blood components most prevalent in the vegetative lesion of infective endocarditis. Angrist and Oka have shown that the early lesion of infective endocarditis occurs in areas of prior endocardial stress or trauma, presenting an altered surface to which platelets and fibrin adhere. Pathological observations have shown that the platelet-fibrin mass contains few leukocytes and erythrocytes. During bacteremia the platelet-fibrin mass is colonized, and intravascular infection is established. This generally accepted theory of the pathogenesis of infective endocarditis is based upon detailed

Table 1. $^{111}$In Activity Ratios in Vegetations from Animals with Infective Endocarditis and Normal Controls

<table>
<thead>
<tr>
<th>Study group</th>
<th>No. of animals</th>
<th>$^{111}$In activity ratios</th>
<th>$^{111}$In activity ratios</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infective endocarditis</td>
<td>17</td>
<td>240 ± 41 (27-532)</td>
<td>99 ± 15 (13-238)</td>
</tr>
<tr>
<td>+ $^{111}$In-platelets</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infective endocarditis</td>
<td>5</td>
<td>5 ± 2 (1-15)</td>
<td>3 ± 1 (1-7)</td>
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<tr>
<td>+ $^{111}$In-leukocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Infective endocarditis</td>
<td>3</td>
<td>7 ± 2 (3-11)</td>
<td>3 ± 1 (1-6)</td>
</tr>
<tr>
<td>+ $^{111}$In-oxine</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal +</td>
<td>4</td>
<td>1 ± 0.3 (0.2-2.1)</td>
<td>0.1 ± 0.04 (0.02-0.25)</td>
</tr>
<tr>
<td>+ $^{111}$In-platelets</td>
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Values are mean ± SEM. Numbers in parentheses indicate range.

**Figure 5.** Relationship of in vivo imaging to the corresponding excised heart image and gross pathology in an animal with infective endocarditis. By 72 hours after $^{111}$In-platelet administration, cardiac imaging in the left anterior oblique position revealed abnormal uptake in vivo corresponding to the aortic valve and proximal aorta. Excised heart imaging demonstrated two hot spots which conform to two separate, noncontiguous vegetations (arrows, top of figure), located on the aortic valve and proximal aorta. BP = blood pool.
histopathological analysis and has been confirmed experimentally.\textsuperscript{10-12} Platelets have more than a passive role in the pathogenesis of the lesion by elaborating substances known to initiate coagulation and activate complement.\textsuperscript{13} Studies performed in vitro show that activated platelets release chemotaxins for leukocytes and monocytes.\textsuperscript{14} Lysosomal products released after platelet aggregation can cause endocardial damage.\textsuperscript{3} Furthermore, the presence of platelets within the fibrin-endocardial matrix enhances bacterial adherence to the damaged surface.\textsuperscript{2} Although platelet aggregates avidly attract leukocytes in vitro,\textsuperscript{14, 15} there are few leukocytes in the vegetation of infective endocarditis. The pathological role of platelets and deficiency of leukocytes in the vegetative lesion of endocarditis are consistent with our imaging results.

Cardiac uptake of \textsuperscript{111}In platelets was confined solely to the vegetation, since the excised heart images showed no uptake in regions other than those containing endocardial lesions. Bland clot contained within the catheters did not accumulate enough radioactivity to be detected by scintigraphy. Each lesion was meticulously dissected from its supporting endothelium and the tissue distribution studies showed significant uptake only in vegetative material. Focal uptake was not evident before 48 hours, and in most cases did not appear until the 72-hour cardiac scintigraph. The biological clearance of heterologous platelets initially resulted in blood pool activity during the first 2 days. The rapid and intense accumulation of the labeled platelets by the vegetation should allow identification of the lesion even with residual blood pool activity. Uptake in the vegetation was far greater than that reported for venous thrombosis or endarterectomized carotid arteries in dogs.\textsuperscript{16} This probably reflects the intense platelet aggregation induced by bacteria.\textsuperscript{16} The relationship of radioactively-labeled platelet uptake to the quantity and type of bacteria in infective endocarditis lesions should be further studied. Based on the differential aggregability potential of various bacteria, the magnitude of platelet uptake may depend on the type of infecting organism.

The application of \textsuperscript{111}In-platelet cardiac scintigraphy to the diagnosis of infective endocarditis in humans has potential limitations. The biological clearance of transfused platelets is such that scintigraphic diagnosis may require several days. Despite this, images became positive beginning 48 hours after platelet administration. The preparation of \textsuperscript{111}In-labeled blood cellular components requires laboratory facilities which are available in only a few medical centers. The specificity of labeled platelet cardiac uptake must be defined. It is likely that such uptake will be seen in ongoing surface microthrombosis of calcified valves.\textsuperscript{17} The lesion of non-bacterial thrombotic (marantic) endocarditis consists entirely of platelets and fibrin, which theoretically also will accumulate labeled platelets. In this condition the degree of uptake may not be as significant as seen in bacterial endocarditis because of the effect bacteria have upon enhancing platelet aggregation. The specificity of the present findings may also be limited by the occurrence of prosthetic valvular thromboses, making differentiation of an infected versus a clotted valve difficult. The intense platelet deposition seen in bacterial vegetations, however, may cause a greater degree of uptake than that seen in bland clot. Thrombosis accompanying left ventricular aneurysm and mitral stenosis are two additional conditions potentially capable of concentrating labeled platelets.

The sensitivity of \textsuperscript{111}In-platelets to detect experimental infective endocarditis is comparable to the results obtained with technetium-99m pyrophosphate (\textsuperscript{99m}Tc-PYP).\textsuperscript{18} Using the same model of infective endocarditis, we have demonstrated focal aortic valve uptake of \textsuperscript{99m}Tc-PYP readily discernible from the intense surrounding bone uptake. The advantage of \textsuperscript{99m}Tc-PYP is that it rapidly clears from the blood, allowing in vivo detection of the vegetation within 2 hours, compared with at least 48 hours with \textsuperscript{111}In-platelets. The specificity of cardiac \textsuperscript{99m}Tc-PYP uptake is variable,\textsuperscript{19} and this may be a major limitation to its applicability of detecting endocarditis. The major advantage of \textsuperscript{111}In-platelet imaging for detecting infective endocarditis is the much greater concentration of the tracer by the lesion, better quality of the images, and lack of competing uptake within the thorax.

Platelets have a crucial role in various pathophysiological processes. The lack of a suitable radioactive labeling agent for platelets has limited clinical and experimental investigations involving imaging.\textsuperscript{20, 21} The recent development of a platelet labeling technique using a lipid-soluble complex of \textsuperscript{111}In with 8-hydroxyquinoline has solved the problem.\textsuperscript{25} The usefulness of this agent for the scintigraphic detection of vascular thrombosis,\textsuperscript{17, 22, 23} arterial endothelial injury,\textsuperscript{4} and pulmonary embolization\textsuperscript{4} have recently been reported. An additional application of labeled platelet scintigraphy to cardiac diagnosis is the detection of platelet deposition associated with coronary artery atherosclerosis and thrombosis. In a preliminary investigation, Dewanjee et al. recently reported the results of \textsuperscript{111}In-platelet scintigraphy in dogs with saphenous vein bypass grafts.\textsuperscript{25} Enhanced uptake of \textsuperscript{111}In-platelets were seen in the proximal region of patent saphenous vein graft sites 8–32 hours after surgery. Excised heart imaging confirmed that the graft was the major site of platelet deposition in the heart and subsequent tissue analysis showed 23 times greater activity in the graft compared with blood. The reason for platelet deposition was not clear, since the grafts had not been occluded; however, the mechanism of uptake may have been related to endothelial damage induced by the surgery.

Our findings indicate that experimental infective endocarditis can be detected by radionuclide \textsuperscript{111}In-platelet imaging. However, the lesions produced were relatively large and represented acute fulminant endocarditis. The difference in lesion size between experimental and human endocarditis may not allow ready extrapolation to clinical imaging. In addition, it
is unclear whether healed or subacute infective endocarditis lesion will likewise concentrate platelets to a comparable degree, and what effect antibiotic therapy has on platelet deposition. The results of the present study, however, warrant clinical evaluation.

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