Joint Scintigraphy Using Technetium-99m Pyrophosphate in Experimental Hemarthrosis

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To determine the validity of a method for induction of experimental hemarthrosis in dogs and for the nuclear imaging of hemarthrosis, serial technetium-99m pyrophosphate ([^{99m}Tc]PYP) flow and blood-pool scans were performed monthly in eight dogs who received bi-weekly injections of autologous blood into their femoro-tibial joints (also called stifle joint). In four control dogs, one joint was injected with saline while the other joint received only a sham injection. In addition, two dogs received intra-articular injections of autologous blood into their right stifle joint and saline into their left stifle joint. These dogs were studied with ^{99m}TcO4 joint scintigraphy at monthly intervals. The dogs were periodically taken out of the study and explored surgically. Pathologic examination of synovial tissue was performed. Serial radiographs were also obtained and correlated with the scan and surgical findings. There was a striking abnormal increase in blood-pool activity of [^{99m}Tc]PYP in the treated stifle joints, commencing at the first examination after 1 mo of blood injections and continuing for the length of the study. All radiographs showed only minimal joint space widening and some soft-tissue swelling. On pathologic examination, both grossly and microscopically, there was profuse pannus formation, with intense inflammatory infiltrate replacing much of the subsynovial fat. The scintigraphic findings correlated well with these pathologic findings. This study not only validates this method for simulating hemophilic hemarthrosis but also suggests that [^{99m}Tc]PYP joint scintigraphy is a simple, and noninvasive method for monitoring the early changes in hemophilic arthropathy and is superior to pertechnetate imaging for this disease process. Instead of the previously recommended delayed bone images, we recommend, in addition, flow studies to assess joint hypervascularity and immediate static images to visualize the synovium and joint capsule.

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Hemophilic arthropathy causing progressive destruction of the involved joints is one of the most common lesions in hemophilia. From the time of the first intra-articular hemorrhage the synovial membrane undergoes typical pathologic changes. In the early stage of the disease the synovial membrane is covered with a network of highly fragile, thin-walled varicose veins often forming plexuses. The cartilage at this stage is normal. These characteristics in stage 1 suggest that minimal trauma is sufficient to cause bleeding. Surgical synovectomy has been used as a hemostatic procedure in hemophiliacs (*1*). Synovial ablation by radioactive material has been suggested for the treatment of synovial inflammation in rheumatoid arthritis (*2*). Obviously, it is preferable to apply these treatments in the early stages of the disease when the synovium is inflamed but the cartilage or bony structures are not affected. Therefore, early detection of synovial involvement is crucial. The use of radionuclide joint imaging for detection of synovial inflammatory disease has been suggested in the past (*3–5*); however, the currently available radionuclide joint imaging techniques are not sensitive for detection of synovial inflammation in its early stages.

We describe a different radionuclide joint imaging technique that offers an accurate qualitative and quantitative analysis of synovial and capsular activity in experimentally induced hemarthrosis in a canine model.

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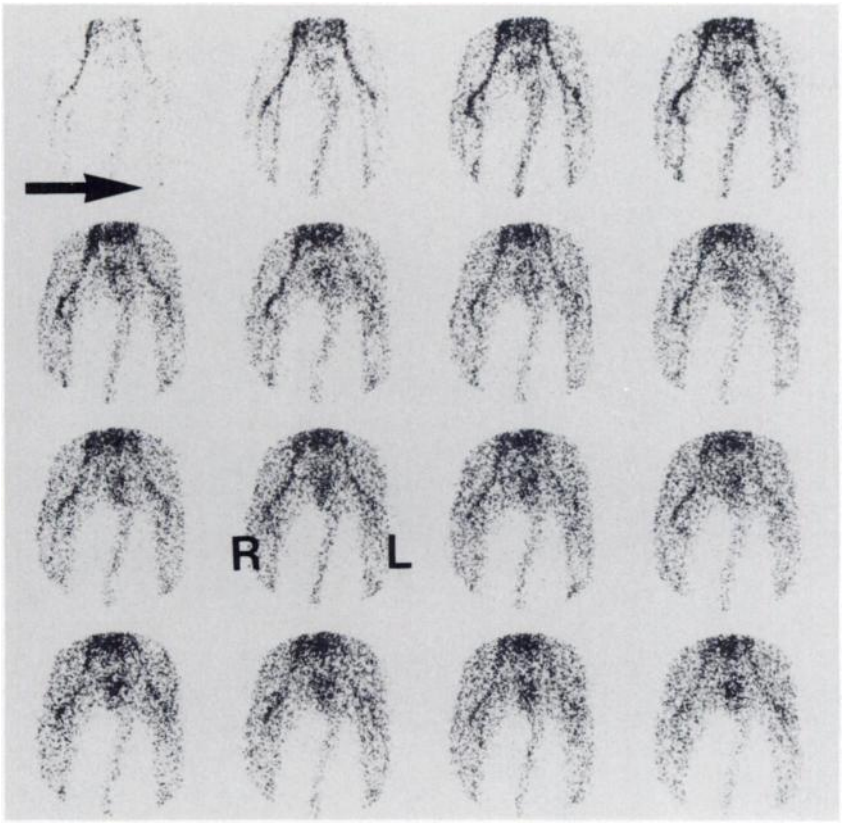

FIGURE 1
Baseline flow study of control dog shows good visualization of superficial femoral artery and its major branches

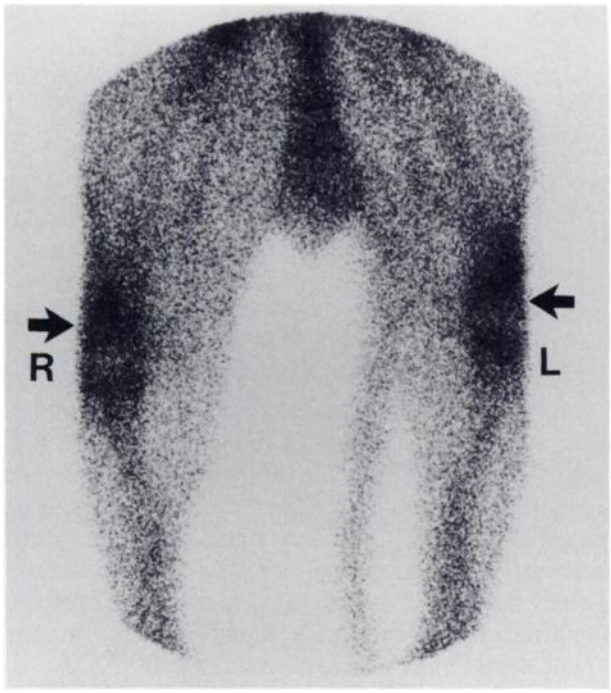

FIGURE 2
Baseline static images of control dog in anterior view shows blood-pool activity of soft tissue and bone. Intensity of epiphyseal blood-pool activity is well demonstrated (arrows)

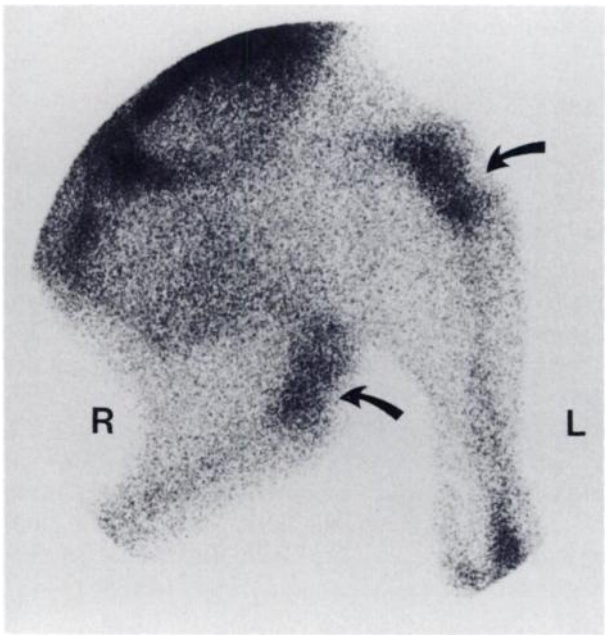

FIGURE 3
Immediate blood-pool activity of control dog in lateral view. Note: articular space is clear (arrows)

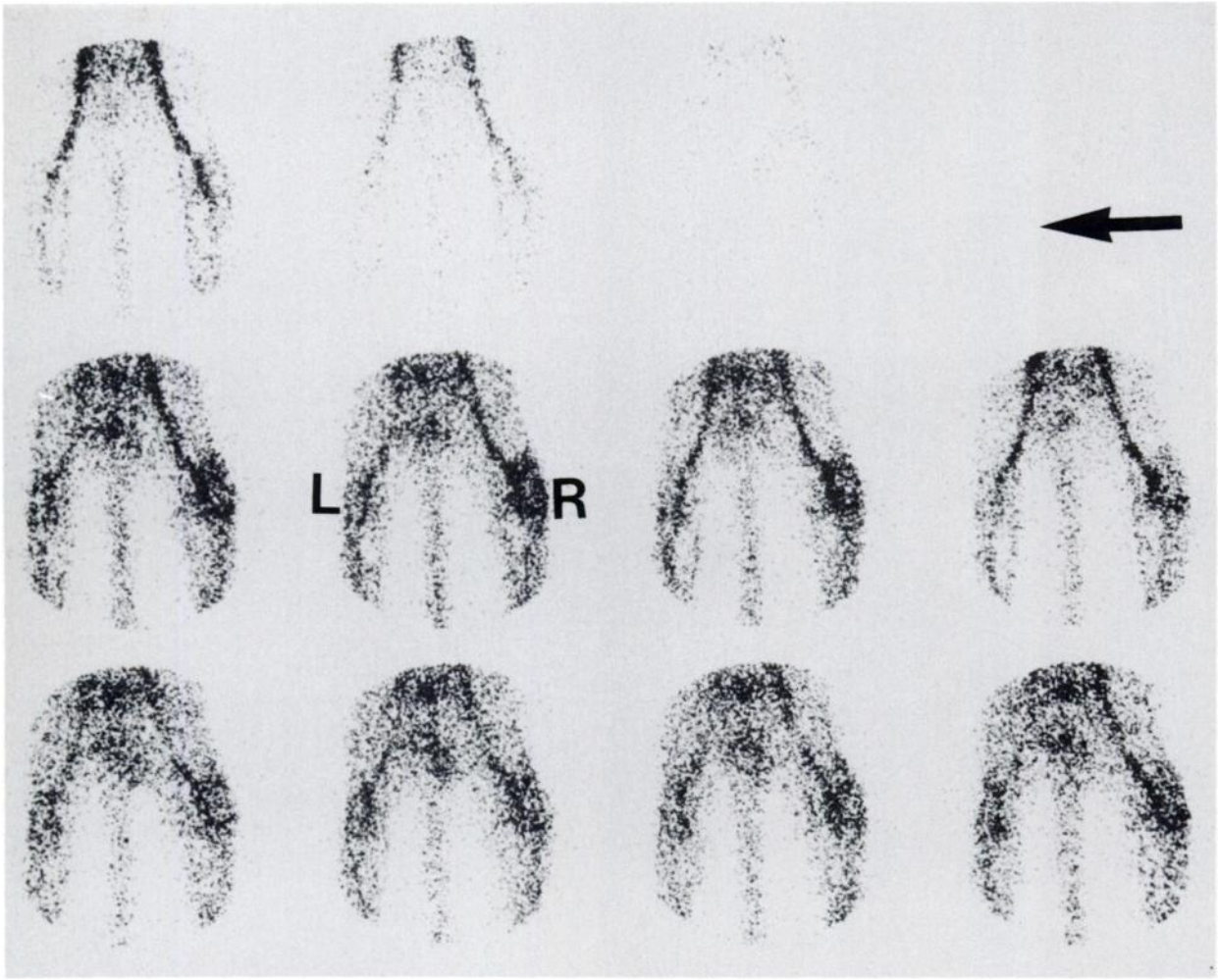

FIGURE 4
Follow-up flow study of control dog at 4 wk following bi-weekly injection of right stifle joint with saline and sham injection of left joint shows mild degree of hypervascularity of stifle joints bilaterally, slightly more on right side

MATERIALS AND METHODS

A total of 14 adult mongrel dogs were used for this study. Both stifle joints of eight dogs were used for induction of experimental hemarthrosis and four dogs were used as controls. These 12 dogs were scanned using technetium-99m pyrophosphate [^{99m}Tc]PYP. The remaining two dogs were scanned using ^{99m}TcO4.

In the eight study dogs, autologous blood drawn from the jugular vein was injected into each stifle joint bi-weekly for a total of 12 wk. In the first 15 injections, 7.5 cc of blood were injected each time into each stifle joint and then the volume of injected blood was increased to 15 cc for the last nine injections. The total volume of injected blood into each stifle joint was 247 cc. One dog was killed each month for pathologic evaluation of the stifle joints.

Serial scintigrams of both knee joints were performed every 4 wk, using a large field-of-view gamma camera interfaced with a computer and fitted with a low-energy, parallel hole collimator. Fifteen millicuries (555 MBq) of [^{99m}Tc]PYP was injected into the jugular vein as a bolus. Sequential images of both stifle joints at 2 sec/frame were obtained for 12 frames. Simultaneous computer pictures were taken for a total of 50 frames at 1 sec/frame. Immediate static images of both stifle joints were obtained in the anterior and lateral views, using 44 sec exposure time for each image. Two-hour delayed bone images were taken randomly in some of the studies in all dogs for correlation using the same technique. The computer images were compressed. A 40 × 60 pixel rectangular area was drawn around each stifle joint on the compressed computer images and area count of each stifle joint was quantitated following background subtraction. The calculated number was used as an index for degree of joint activity.

In the four control dogs the right stifle joint was injected with 7.5 cc of sterile normal saline and the left joint received only a sham injection with the same size

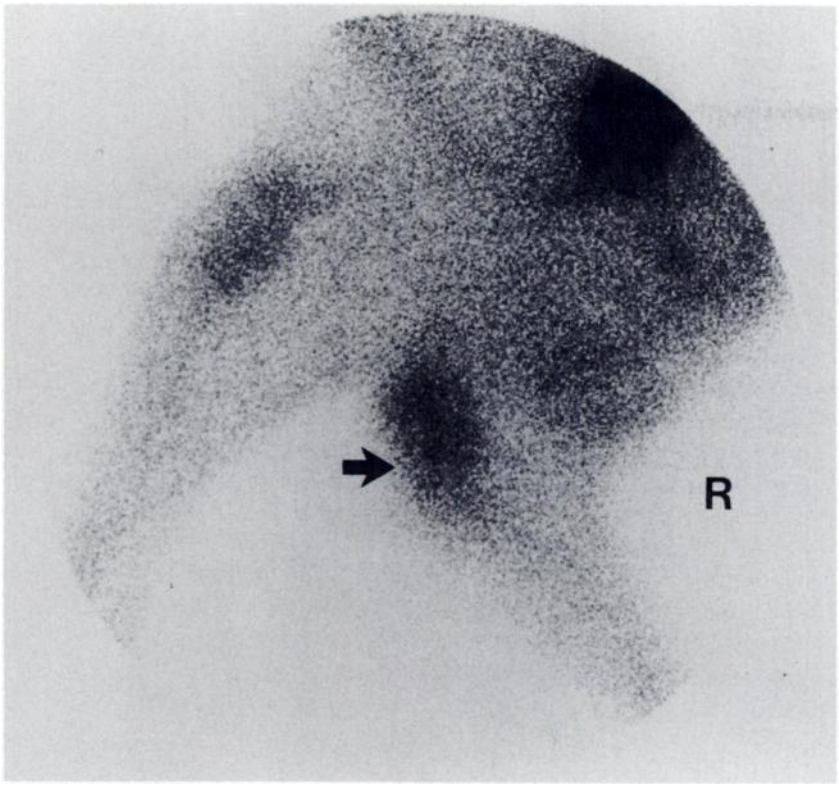

FIGURE 5
Immediate blood-pool static images of control dog in lateral view at 4 wk shows only slight degree of obliteration of stifle joint spaces bilaterally (arrows)

needle. This was done bi-weekly for a total of 4 mo. Baseline scintigram of both joints as well as monthly follow-up studies were performed using the same technique as in the study dogs. Serial radiographs of both stifle joints were also obtained in the AP and lateral views.

To evaluate the results of joint scintigraphy using ^{99m}TcO4 and to compare them with the [^{99m}Tc]PYP joint imaging, we used ^{99m}TcO4 for joint imaging in two dogs who received bi-weekly intra-articular injection of autologous blood into their right stifle joint and saline into their left stifle joint. The total volume of injected blood and saline into the right and left joint, respectively, was the same as in the study dogs. Stifle joint scintigrams were obtained at monthly intervals with injection of 15 mCi (555 MBq) ^{99m}TcO4 as a bolus. Flow and immediate static images of the stifle joints were obtained and the results were compared with[^{99m}Tc]PYP imaging.

RESULTS OF [^{99m}Tc]PYP JOINT IMAGING

The baseline flow study of the control dogs showed good visualization of the superficial femoral artery which branches above the stifle joint in most dogs (Fig. 1). Immediate static images showed the blood-pool activity of the soft tissue and bone. The intensity of the blood-pool activity in the epiphyses was well demonstrated. The articular space was clear and epiphyseal borders were sharp due to low activity of the articular and para-articular soft tissue (Figs. 2 and 3). Delayed images showed normal bony structures.

On the first follow-up study 4 wk later (following bi-weekly injection of saline in the right joint and sham injection into the left joint), the flow study showed a mild degree of hypervascularity of the stifle joints bilaterally and immediate static images showed only a mild degree of obliteration of the joint spaces also seen bilaterally (Figs. 4 and 5). Two-hour delayed images of the bony structures showed no interval changes. Further follow-up studies were carried out for 3 mo and showed no significant changes.

The baseline examinations in the study dogs showed similar findings to those described in the control dogs. The first follow-up scan (obtained after 4 wk following bi-weekly injections of 7.5 cc of autologous blood into each stifle joint) showed a marked degree of hypervascularity in the joints on the flow study to a much greater degree than was demonstrated in control dogs and immediate blood-pool scans showed intense increased uptake in the epiphyses and periarticular soft tissue (Figs. 6 and 7). The joint space was obliterated and there was loss of epiphyseal outline. Area counts of both knees showed a marked rise in the number of counts bilaterally. Monthly follow-up studies were carried out for 3 mo with continued injections of blood and these showed a gradual rise in the number of counts as well as in the intensity of joint uptake. Delayed bone images were performed randomly and showed no significant

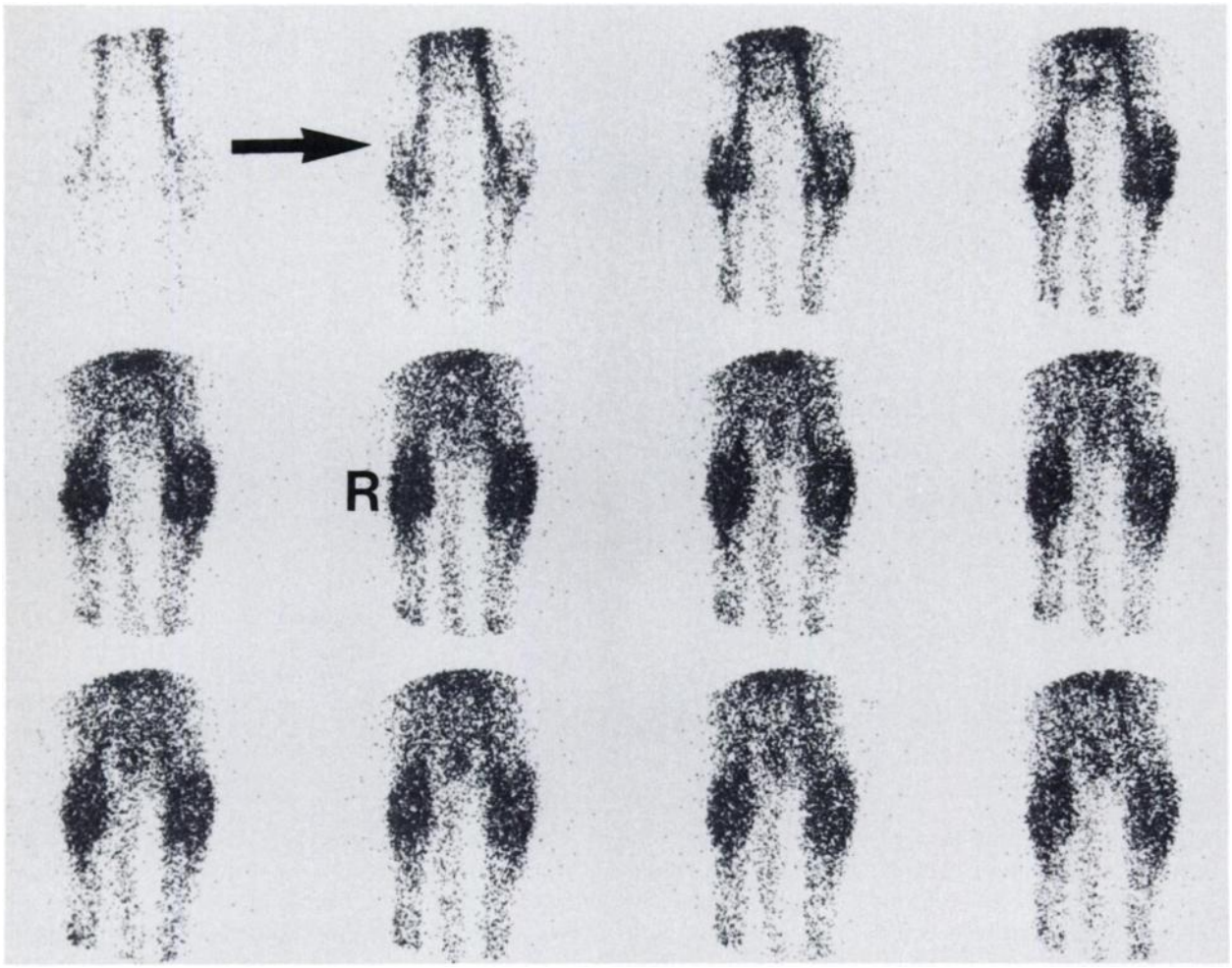

FIGURE 6
Follow-up flow scan in study dog shows marked degree of hypervascularity in stifle joints bilaterally, much greater than in control dogs

changes (Fig. 8).

Antero-posterior and lateral stifle joint radiographs demonstrated only minimal joint space widening and soft tissue swelling around the joints throughout the course of this study.

In the last two dogs, $^{99m}TcO4$ stifle joint scintigrams at 4 wk showed a mild degree of increased uptake in both joints which appeared to be bilaterally symmetrical in intensity. In addition, the intensity of uptake did not progressively increase with the number of blood injections and there was a higher degree of background uptake (Fig. 9).

PATHOLOGIC RESULTS

Gross and microscopic examination of the resected adult dog stifle joint specimens was performed at monthly intervals following experimentally produced hemarthrosis. On gross examination after 4 wk of intra-articular injections, the articular cartilage appeared smooth and glistening. The synovial tissues, especially within the intercondylar notch region, were discolored and thickened (Fig. 10). Microscopically, the synovial membrane was thickened when compared with the normal stifle joint (Fig. 11).

The lining cuboidal cells rested on loose, edematous, fibrous tissue. The synovial membrane demonstrated blunt villous projections with infiltration by mononucleated inflammatory cells, siderophages, and excess capillaries. The capsule appeared thicker while the adjacent connective tissue between the synovial membrane and dermis was fibrotic with thickened blood vessels.

The gross examination of joints following 8 wk of blood injections demonstrated a granulation tissue membrane on the periphery of the femoral condyle. The intercondylar notch was filled with reddish-brown synovial tissue and residual blood elements. Microscopically the synovial membrane was covered with an irregular layer of fibrin and blood elements that had begun

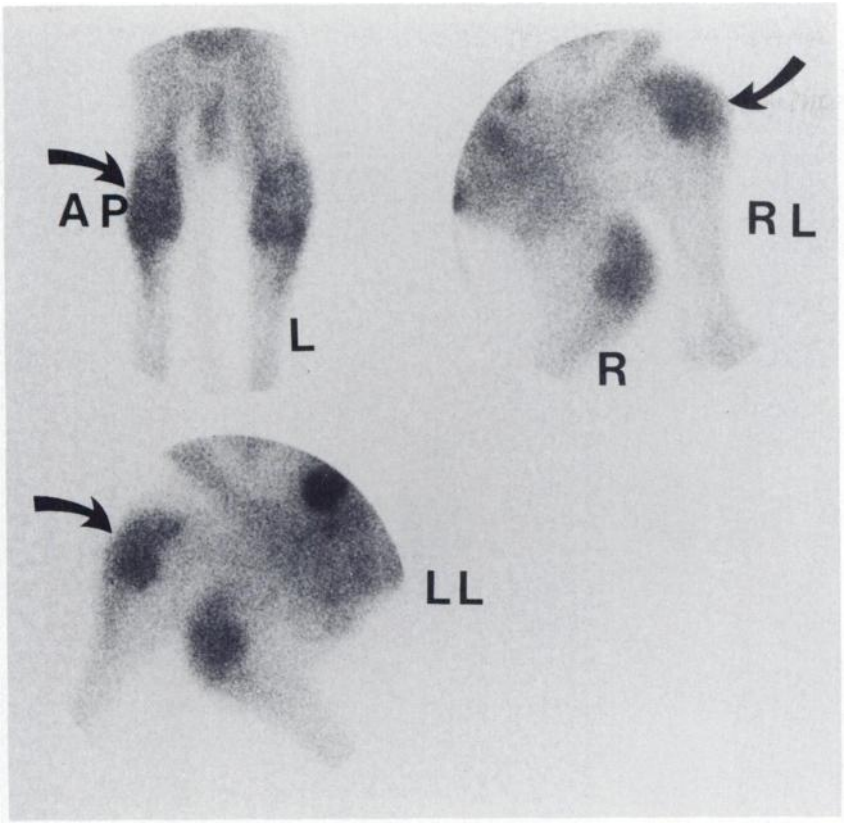

FIGURE 7
Follow-up immediate blood-pool scan in study dog in three different views shows intense increased uptake in epiphysis and periarticular soft tissue. Joint space is obliterated (arrows)

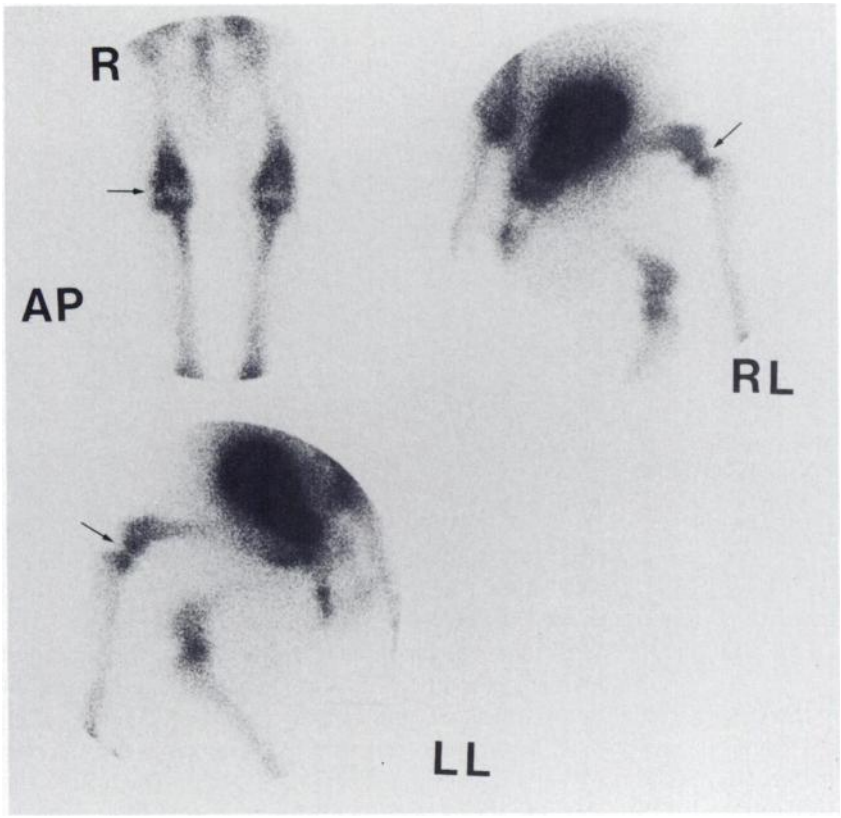

FIGURE 8
Three views of delayed bone images on same exam in study dog shows no interval change from baseline bone images

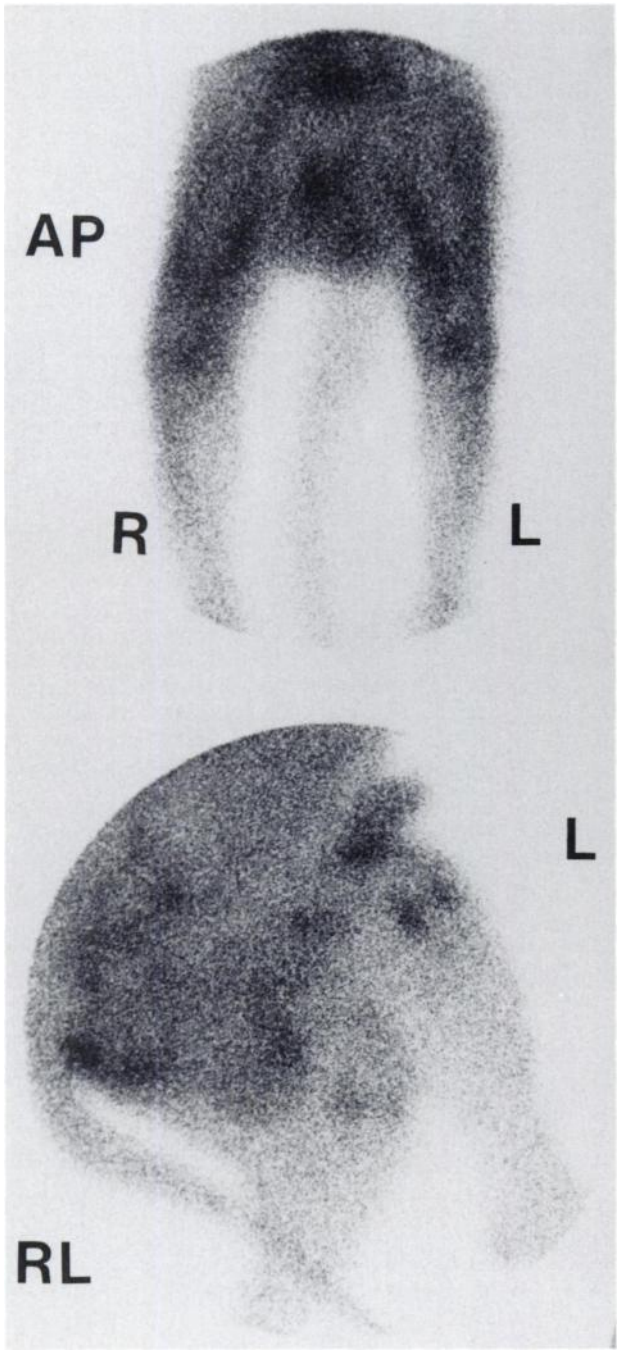

FIGURE 9
$^{99m}TcO4$ stifle joints scintigrams (AP and lateral views) of dog who received 12 wk of bi-weekly blood injection into right stifle joint and same volume of saline into left joint shows high background activity and poor synovial uptake

to organize. The synovial lining was increasingly more prominent because of taller synovial cells and villous projections.

The synovial membrane as well as the subsynovial tissue contained increased numbers of siderophages and small blood vessels with inflammatory cells. The capsule appeared thicker with fibrosis that extended to

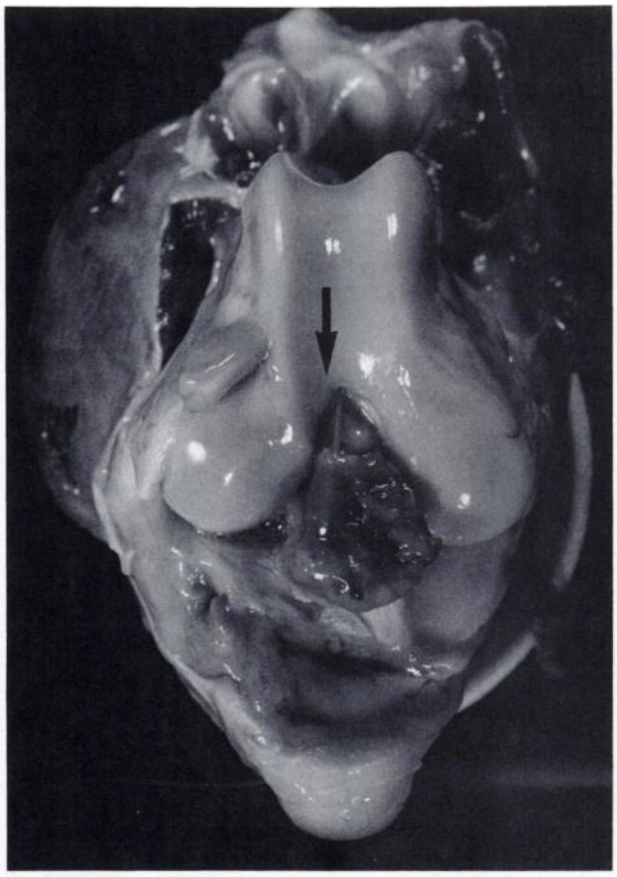

FIGURE 10
Gross photograph of dog stifle joint following 4 wk of blood injections shows intercondylar notch to be filled with hypertrophied synovial tissue (arrow)

the subcutaneous tissue. Prominent thick-walled vessels were present (Fig. 12).

The stifle joints that received 12 wk of blood injections grossly demonstrated further progression of the pannus upon intact articular cartilage. The synovial and blood elements appeared more organized and were contracted within the intercondylar notch. Microscopically, the synovial and capsule elements showed further cicatrization and organization. The inflammatory cells and small blood vessels were diminished (Fig. 13). Histologically, the articular cartilage remained normal.

DISCUSSION

Recurrent joint bleeds in patients with blood dyscrasias lead to progressive and crippling arthropathy. One of the earliest, most frequent and most disabling manifestations of hemophilia is hemarthrosis, the knee joint being most frequently affected. In hemophiliacs, as few as two to three clinically detectable episodes of hemarthrosis have been reported to cause severe arthritis (*6*). In acute hemarthrosis it is believed that bleeding starts in the synovial and subsynovial tissues, forming

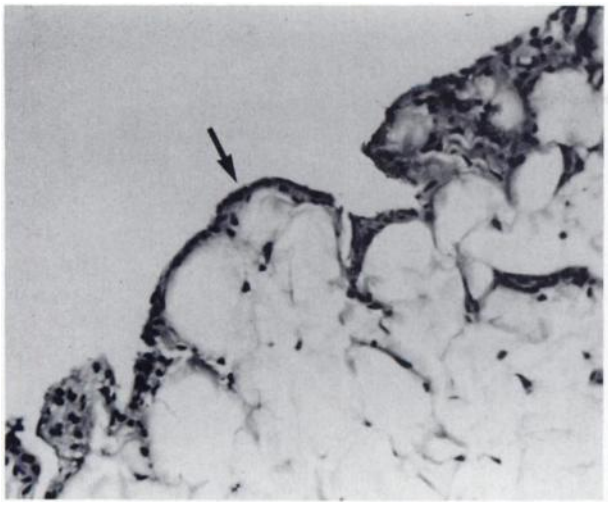

FIGURE 11
Photomicrograph of normal dog synovium showing thin layer of synovial cells overlying normal fat (arrow). (H & E, 400 x)

small hematomas which become confluent and rupture into the joint cavity to produce sudden severe pain and immobility (*7*). Likewise, it is believed that it is recurrent intra-articular hemorrhage that lead to chronic arthropathy, hypertrophy of the synovium, and adherence of adjacent villi progressing to produce joints with thickened hyperemic synovium and fibrosis of the synovial tissue. After these changes are established, degenerative changes occur in the adjacent cartilage and bone.

Surgical synovectomy has been used in the treatment of recurrent hemarthrosis when prophylactic replacement therapy is not effective. Although that technique has demonstrated a significant reduction in joint bleeds, postoperative complications are not uncommon (*1*). Radiation synovectomy, utilizing an intra-articular radionuclide has been recommended for treatment of severe inflammatory synovitis associated with chronic rheumatoid arthritis (*2*).

Certainly if these modern therapeutic modalities are to be of value they must be initiated before radiographic evidence of joint destruction is present. Radiographs are well known to be insensitive for early detection of synovial disease as was also demonstrated in our study. Radionuclide joint imaging has been described previously by many authors. There are two different approaches for detection of synovial disease by radionuclide imaging. In the first of these, images are obtained 5 to 20 min after i.v. administration of a radiopharmaceutical which localizes in the blood pool and/or extracellular fluid compartment, e.g., [^{99m}Tc]pertechnetate (*8*). The images obtained with these radiopharmaceuticals demonstrate active synovitis as foci of increased activity due to hyperemia and increased capillary permeability accompanying the inflammatory process (*9,10*). Most recent studies have focused on the second type of radiopharmaceutical joint imaging employing [^{99m}Tc]phosphate compounds.

The mechanism of action of [^{99m}Tc]phosphate compounds has not yet been elucidated. These complexes may become attached to the calcium of hydroxyapatite in bone, they may be concentrated in immature collagen as a consequence of increased osteoblastic activity, or technetium in its reduced state may be directly incorporated into bone. These radiopharmaceutical compounds clear from blood exponentially with a rapid, early phase that distributes through the extracellular space and a slower later phase that goes to the bone. The two features of radionuclide scans that may be of value to the clinician are: (a) early extracellular flow phase indicating hyperemic tissue (e.g., synovium), and (b) the later activity in bone demonstrating osteoblastic activity (*11,12*). In the early phase it has been shown that isotope is not concentrated by synovial cells but that any uptake in the early phase is a reflection of the vascularity of the synovium (*13*). When [^{99m}Tc]PYP was suggested for joint imaging, the recommended imaging time was 2 hr postinjection time, based on the idea that increased localization of these agents in the periarticular bone occurs due to increased blood flow to the juxta-articular bone that accompanies synovial inflammation (*9*). This stems from the fact that the blood supply to the epiphyseal and metaphyseal bone adjacent to the joints arises from the synovial vessel network. Therefore, factors that increase vascularity of the synovium also increase the blood supply to the adjacent bone (*4*). Although this may be true in advanced cases

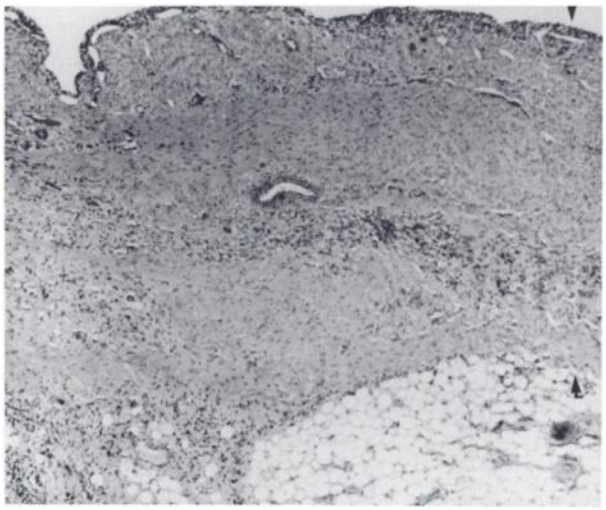

FIGURE 12
Photomicrograph of stifle joint capsule after 8 wk of blood injections shows thickened synovium and dense subsynovial fibrosis replacing normal fat tissue (arrows). (H & E, 57 X)

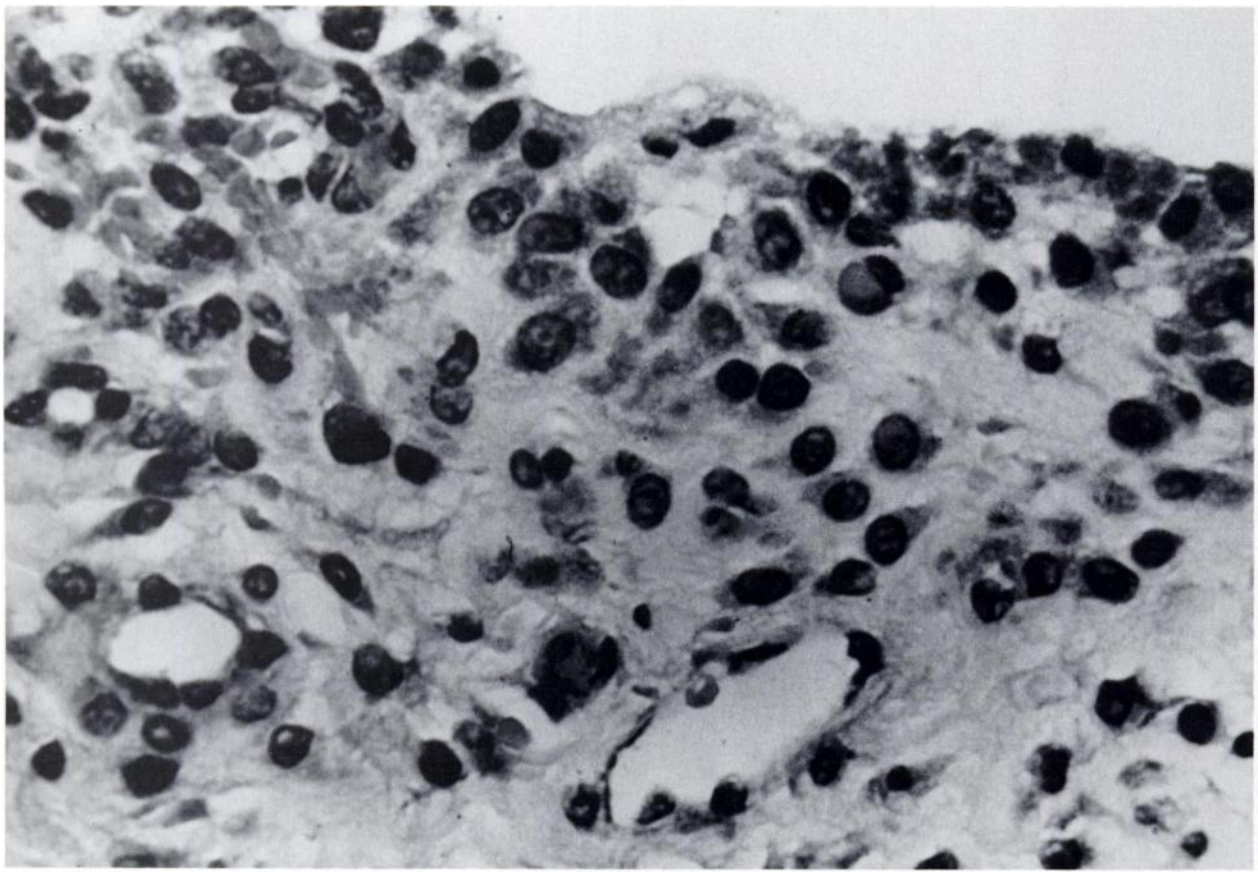

FIGURE 13
Following 12 wk of blood injections, thickened reactive synovium is more developed and has diminished number of inflammatory cells and blood vessels compared with 8 wk specimen. (H & E, 400 X)

of synovial disease, in early synovial inflammation, as we have demonstrated in our study, early images are primarily representative of synovial inflammation. Furthermore, in advanced synovial inflammatory disease, the early images are more specific since secondary bone degeneration and other types of bone lesions will affect the joint uptake on the delayed images. In this study, [^{99m}Tc]PYP was employed in order to provide both early (flow and blood pool) as well as late (bone stage) images.

The first joint scintigram at 1-mo intervals following bi-weekly intrarticular injections of blood showed marked hypervascularity of the joints in the flow study and increased uptake in the periarticular soft tissue on the immediate blood-pool images (Figs. 6 and 7). This was accompanied by a sudden rise in the number of counts. Delayed bone images, however, were unchanged from the baseline study (Fig. 8). Radiographs showed only a minimal degree of joint space widening and soft-tissue swelling.

When we compared the results of ^{99m}TcO4 joint scintigraphy with [^{99m}Tc]PYP early images we found ^{99m}TcO4 to be less sensitive and less specific than the early images of [^{99m}Tc]PYP since in the two dogs that received experimental hemarthrosis in the right stifle joint and only saline injection into their left joint the intensity of uptake with ^{99m}TcO4 was almost equal in both joints. In addition, there was a very high degree of background activity and low signal to noise ratio compared to [^{99m}Tc]PYP images, indicating the superior sensitivity of the latter technique (Fig. 9).

On the basis of this study, we recommend a three-phase bone scan with [^{99m}Tc]PYP. Flow and early blood-pool images represent synovial vascularity and delayed bone images will verify secondary degenerative bony changes. Our scan findings with [^{99m}Tc]PYP correlated well with the pathologic findings of marked hypervascularity, pannus formation, and inflammatory cell infiltration seen in the synovium and joint capsule. In addition, good imaging of the synovium and joint capsule was demonstrated on immediate static images.

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