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PREPARATION AND EVALUATION OF Tc-99m HYDROXYETHYL STARCH AS A POTENTIAL RADIOPHARMACEUTICAL FOR LYMPHOSCINTIGRAPHY:

Comparison with Tc-99m human serum albumin, Tc-99m dextran, and Tc-99m sulfur microcolloid

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ABSTRACT

Tc-99m hydroxyethyl starch (Tc-99m HES) prepared with a labeling efficiency greater than 95% was evaluated in rabbits for visualization of lymphatic channels and lymph nodes, and the findings compared with Tc-99m human serum albumin (Tc-99m HSA), Tc-99m dextran (Tc-99m DXT), and Tc-99m sulfur microcolloid (Tc-99m SMC). Tc-99m HES showed good visualization of lymphatic channels and regional nodes and it had the highest clearance rate from the injection site ($p<0.01$). Tc-99m HES showed greater uptake by the nodes than Tc-99m DXT ($p<0.001$) at 90 minutes post-injection. The concentration of Tc-99m HES in the lymphatic channels was higher than that of Tc-99m SMC at 90 minutes post-injection ($p<0.001$). Preliminary clinical studies of Tc-99m HES yielded high quality lymphoscintigrams of the leg, and the pelvic and para-aortic lymph nodes in less than 10 minutes post-injection. In addition, partially and completed obstructed lymphatics could be differentiated from normal lymphatic pathways. In conclusion, Tc-99m HES is a promising radiopharmaceutical for imaging of lymphatic channels and nodes.

The lymphatic system has conventionally been imaged by direct lymphography but this technique has several shortcomings. The most important is tedious cannulation of a peripheral lymphatic vessel which entails incising the skin. Other disadvantages include pulmonary oil embolism, and hypersensitivity reactions to the oily contrast medium which is usually administered by constant infusion using a perfusion pump directly into a small lymphatic (1). To overcome most of these problems, a simpler and easily performed technique namely radionuclide imaging is recommended and for this purpose several radiopharmaceuticals have been evaluated (2-7). Interstitial injection of Au-198 colloid was abandoned early on because of unacceptably high absorbed radiation doses at the injection site (8). Among the various Tc-99m labeled colloidal particles, only microcolloidal antimony sulfide has satisfactory properties (4-7). There are, however, two major limitations to the use of colloidal particles: (a) less than 35% of the interstitially injected dose is absorbed in 24 hours (9) and (b) clearance from the injection site and trapping in draining lymph nodes depends mainly on particle size and on the functional status of the reticuloendothelial system (4,7,10). Accordingly, these radiocolloids do not accurately reflect the status of lymphatic flow and patients often have to wait several hours before draining lymph nodes are depicted. In

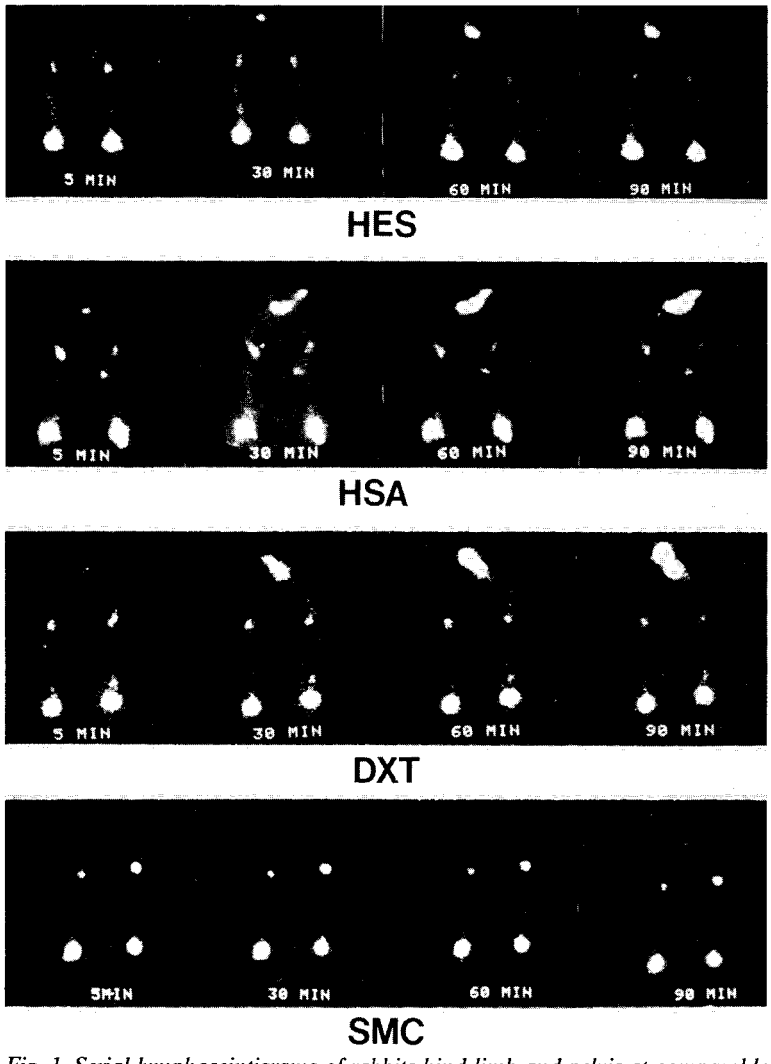

Fig. 1. Serial lymphoscintigrams of rabbits hind limb and pelvis at comparable intervals after intradermal injection of 200-250μCi of Tc-99m hydroxyethyl starch (HES), Tc-99m human serum albumin (HSA), Tc-99m dextran (DXT), and Tc-99m sulfur microcolloid (SMC).

addition, the radiation dose to the injection site is still considerable. These shortcomings in visualizing lymphatics using a radiocolloid has led some investigators to suggest use of a noncolloidal, nonparticulate tracer soluble in lymph fluid for lymphatic imaging (11,12). Polymers with molecular weight high enough (>40,000 daltons) would be unable to penetrate blood capillary membranes after intradermal administration, and would seem ideal agents for this purpose. Dextran (DXT) with an average molecular weight (MW) of 70,000 daltons, human serum albumin (HSA) with an average MW of 69,000 daltons, and hydroxyethyl starch (HES) (average MW - 450,000 daltons) each remains in the vascular space for some time after intravenous injection. These substances, therefore, should probably also drain from the injection site entirely by the lymphatic system after intradermal injection and not cross blood capillaries (13).

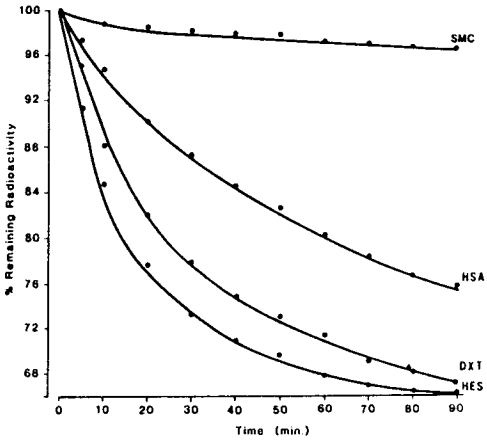

Fig. 2. Time-activity curves obtained from regions of interest over two injection sites of five rabbits after intradermal injection of 200-250μCi of Tc-99m hydroxyethyl starch (HES), Tc-99m human serum albumin (HSA), Tc-99m dextran (DXT), and Tc-99m sulfur microcolloid (SMC).

Tc-99m dextran (Tc-99m DXT) has previously been evaluated in both experimental animals and man as a lymphoscintigraphic agent and the results seem satisfactory (11,12,14,15). The use of Tc-99m human serum albumin (Tc-99m HSA) has previously been described by us in patients with benign lymphedema (16). Even though the results obtained with Tc-99m DXT and Tc-99m HSA were satisfactory, even less urinary bladder and liver background activity would be better. Hydroxyethyl starch (HES) is a plasma substitute that in contrast to DXT causes no recognizable histamine release or allergic reaction (17). HES has a long intravascular half-time (18); therefore, its Tc-99m labeled derivative can be expected to clear from interstitial tissue through lymphatics without crossing the blood capillaries. Tc-99m HES has not as yet been evaluated for imaging lymphatic channels and nodes. In this report, we describe in rabbits the labeling of HES with Tc-99m, its evaluation and comparison with other radiopharmaceuticals for visualization of lymphatic channels and lymph nodes as well as its preliminary use in man.

MATERIALS AND METHODS

Tc-99m hydroxyethyl starch (Tc-99m HES)

To 10ml of 6% hydroxyethyl starch solution, 50μl of concentrated HCl containing 1.5mg $SnCl_2$:$2H_2O$ was added and the solution mixed and kept at room temperature for 5 minutes. Technetium-99m pertechnetate (20mCi) was added to 1ml of the stannous-HES solution. After mixing, the solution was left at room temperature for 5 min and passed through 0.22μ millipore filter. The labeling efficiency was determined by paper chromatography using acetone as a solvent and 3MM paper.

Tc-99m dextran (Tc-99m DXT)

This tracer was prepared according to the method described by Henze et al (11). Briefly, one gram dextran (average MW 70,000 daltons) was dissolved in 10ml of 0.9% saline. This solution was mixed with 1.5mg $SnCl_2$:$2H_2O$ dissolved in 50μl of concentrated HCl. Technetium-99m pertechnetate (30mCi) was added to 1.0ml of the stannous-dextran solution and passed through 0.22μ millipore filter

Table 1

The percentage of administered dose* at the injection site, lymphatic channel,** and regional lymph nodes at 90 minutes post-injection of Tc-99m SMC, Tc-99m HSA, Tc-99m DXT, and Tc-99m HES in rabbits.

	Tc-99m SMC	Tc-99m HSA	Tc-99m DXT	Tc-99m HES
Injection site	95.1±0.88	75.6±1.28	67.2±0.43	65.6±2.79
Lymph nodes	3.4±0.44	2.5±0.29	0.85±0.19	3.1±0.33
Lower limb lymphatic	1.8±0.31	4.2±0.45	2.7±0.47	3.9±0.60
Pelvic lymphatic	1.0±0.31	3.6±0.4	2.1±0.27	3.2±0.30

* The values represent the mean and standard error of 10 determinations.
** The area assigned to the lymphatic channel was normalized to 1000 pixels.

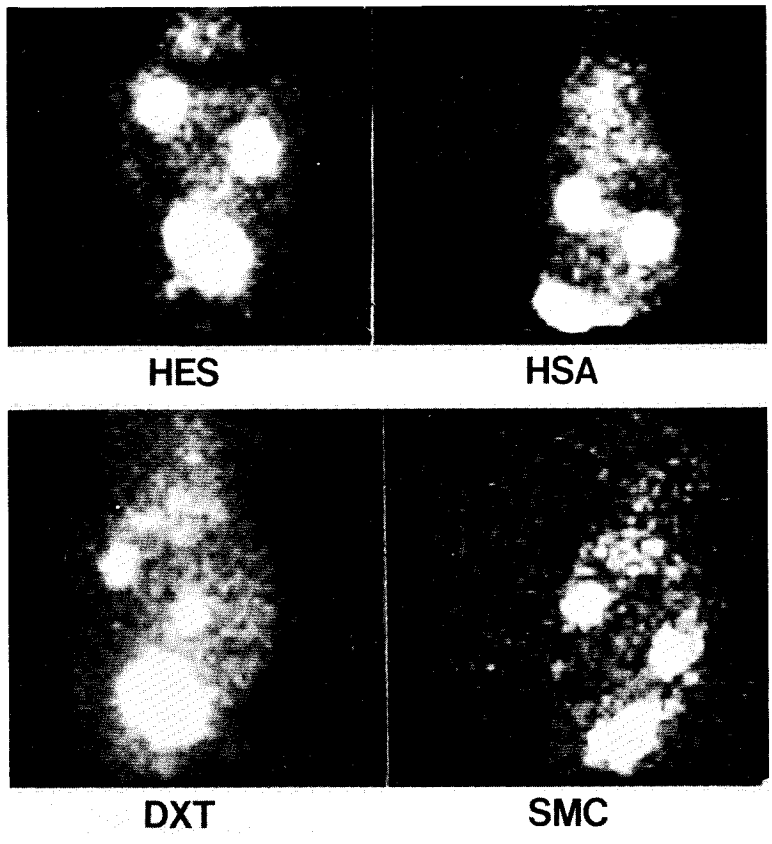

Fig. 3. Abdominal region of rabbits showing the accumulation of radioactivity in liver, kidney, and urinary bladder at 90 minutes after intradermal injection of 200-250μCi of Tc-99m hydroxyethyl starch (HES), Tc-99m human serum albumin (HSA), Tc-99m dextran (DXT), Tc-99m sulfur microcolloid (SMC).

and left at room temperature for 5 minutes. The labeling efficiency was determined by paper chromatography using acetone as a solvent and 3MM paper.

Tc-99m sulfur microcolloid (Tc-99m SMC) and Tc-99m HSA

Both tracers were prepared according to the manufacturer's recommendations. Their labeling efficiency was also determined by paper chromatography.

Animal studies

New Zealand white rabbits with an average body weight of 3.5-4.0kg were used to test the radiopharmaceuticals under investigation. Anesthesia was induced by intraperitoneal injection of urethane/sagatal mixture. Each radiopharmaceutical (200-250μCi in 0.05ml) was injected intradermally using a tuberculin syringe and a 27 gauge needle into a web space between the second and third toes in both hind legs. Five rabbits were used for each radiopharmaceutical. The injection sites and hind legs were massaged by one person for two minutes immediately after injection. Dynamic imaging from the anterior position with the rabbit lying prone on the collimator surface was performed for 90 minutes using a GE gamma camera interfaced with a Star Computer. Data were acquired in a dynamic byte mode, matrix size 128x128 and were stored every 30 seconds. Static images of the abdomen were obtained 90 minutes

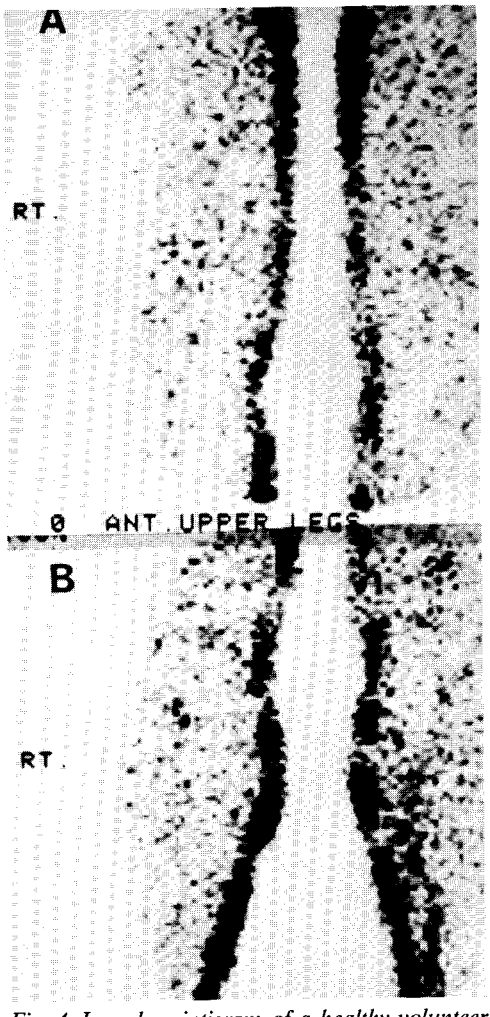

Fig. 4. Lymphoscintigram of a healthy volunteer injected intradermally with 1.0mCi of Tc-99m HES in each foot. Lymphoscintigram of reframed images (A) upper legs, (B) lower legs.

post-injection. Equal regions of interest were assigned over the injection sites, lymphatic channels and lymph nodes. The amount of radioactivity in the assigned regions of interest were recorded at different time intervals.

Preliminary studies in humans were performed. Three normal volunteers and ten patients with swelling of the lower extremities were referred for dynamic lymphoscintigraphy. After the procedure was carefully explained and before injection, the feet were washed with soap and water. Under aseptic conditions 1mCi of Tc-99m HES was injected intradermally using tuberculin needles on the dorsum of each foot in the medial web in the space between the first and second toes. Patients were positioned within 5 minutes under an extra-large field-of-view gamma camera interfaced to a computer. Using a low-energy, general-purpose collimator, the fields of view covered the area from the lower pelvis to the lower thighs. Data were acquired in dynamic-byte mode, matrix size 128x128 pixels, and were stored every minute for up to 40 minutes. Delayed images for the same regions and for the legs were acquired at 90 to 120 minutes from the time of injection. All studies were stored on floppy discs. Data were displayed by reframing the dynamic part to have cumulative images of 10 minutes each. Equal regions of interest were assigned over each inguinal region. Background-corrected smoothed time-activity curves were generated.

RESULTS

The labeling efficiency of each radiopharmaceutical preparation was greater than 96% as determined by paper chromatography using acetone as a developing solvent. The pertechnetate migrated with the solvent front while the radiopharmaceutical stayed at the origin.

In rabbits, intradermal administration of Tc-99m radiopharmaceuticals under investigation visualized the lymph nodes. In all the studies, the popliteal lymph nodes were visualized in the first 5 minutes and the external iliac lymph nodes within 10 minutes post-injection *(Fig. 1)*. With Tc-99m SMC, the lymph nodes were well visualized but the lymphatic vessels were less well-defined. On the other hand, both lymph vessels of the hind legs and regional lymph nodes were well visualized using Tc-99m-HSA and Tc-99m HES *(Fig. 1)*.

The images show the injection sites, lymph channels and the popliteal lymph nodes imaged at 5, 30, 60, and 90 minutes post-injection. In all studies as time pro-

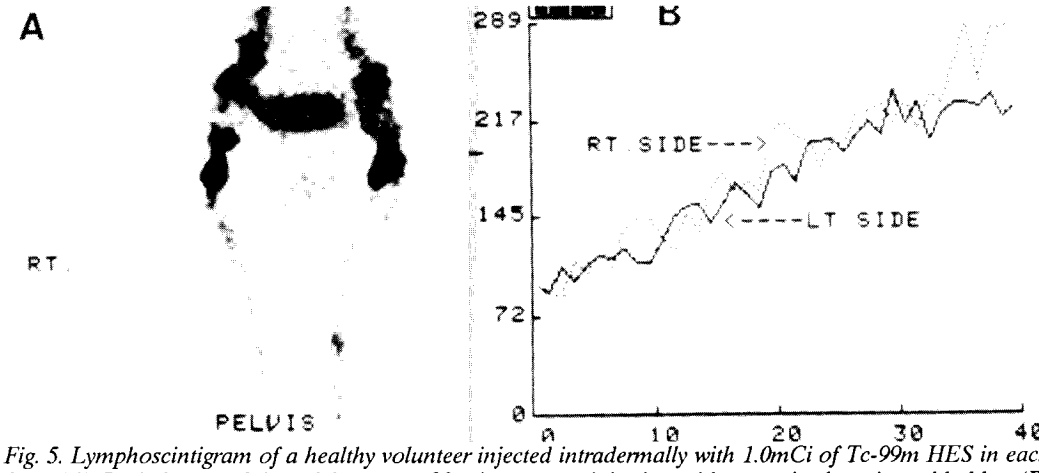

Fig. 5. Lymphoscintigram of a healthy volunteer injected intradermally with 1.0mCi of Tc-99m HES in each foot. (A) Static image of the pelvis area at 90 minutes post-injection with tracer in the urinary bladder. (B) Time activity curves obtained from regions of interest over the inguinal lymph nodes of left and right side.

gressed, more radioactivity appeared in the urinary bladder which obscured the external iliac lymph nodes.

The amount of radioactivity at the injection site plotted as a function of time for each radiopharmaceutical is shown in *Fig. 2*. Tc-99m HES showed a fast migration rate; about 35% of the initial radioactivity had left the injection site within 90 minutes. With Tc-99m DXT, Tc-99m HSA, and Tc=99m SMC the values were about 33%, 20%, and 5%, respectively.

The amount of radioactivity at 90 minutes post-injection in the assigned areas of injection site, lymphatic channels, and lymph nodes are shown in *Table 1*. The area of lymphatic channels were normalized to 1000 pixels. Tc-99m HES showed higher uptake by the nodes than Tc-99m DXT ($p<0.001$) whereas the uptake of Tc-99m HSA and Tc-99m SMC were equivalent to that of Tc-99m HES ($p>0.05$). Tc-99m HES showed a higher concentration in the lymphatic channel than Tc-99m SMC ($p<0.001$) at 90 minutes post-injection while that of Tc-99m HSA and Tc-99m DXT were equivalent to Tc-99m HES ($p>0.05$).

Ninety minutes after intradermal injection, the images of the abdomen, uniformly showed accumulation of radioactivity in the kidneys and urinary bladder *(Fig. 3)*. The liver was also faintly visualized with all four radiopharmaceuticals.

The initial experience with Tc-99m HES in humans suggested that Tc-99m HES was a potentially useful radiopharmaceutical for lymphatic channel and lymph nodal imaging. High-quality images were obtained and the lymphatics were clearly seen in healthy volunteers *(Fig. 4A,B)*. The inguinal nodes were well visualized at 90 minutes post-injection along with the urinary bladder *Fig. 5A)*. The time-activity curves on both limbs were equal, and were characterized by a step-ladder rising pattern, with pulses appearing every 3-4 minutes *(Fig. 5B)*.

An obstructed pattern was seen in some patients and was characterized by an absence of medial bands along the leg and thigh on the affected side and nonvisualization of lymph nodes in the inguinal and pelvic regions in the delayed image *(Fig. 6A,B)*.

An enhanced pattern was seen in some patients and was characterized by: (a) rapid flow of lymph from the injected site through markedly dilated lymphatics. There was an accelerated appearance of single or double medial bands along the medial side of the leg or thigh *(Fig. 7A,B)*. (b) An increase in the size and number of visualized lymph nodes on the affected side *(Fig. 8A)*. (c) The time-activity curves over the inguinal regions were also several times higher than normal *(Fig. 8B)*.

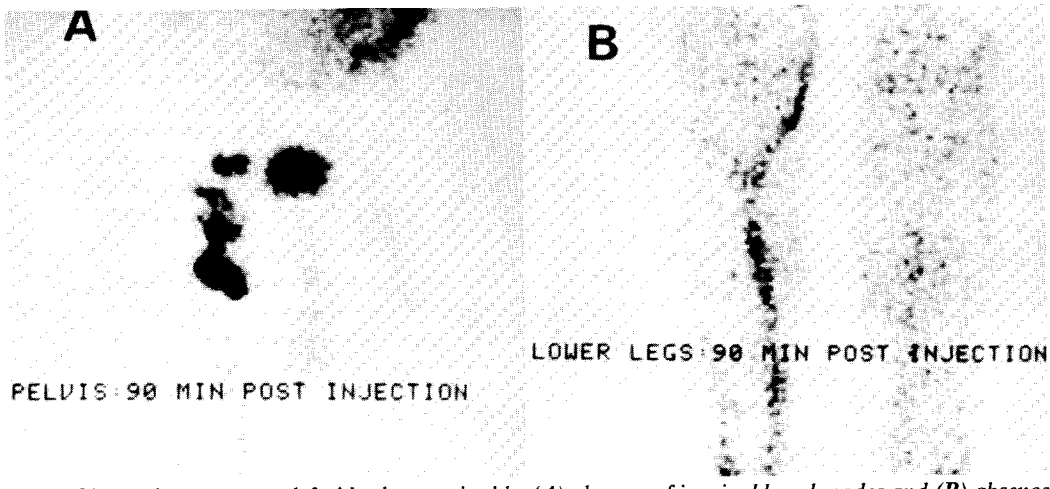

Fig. 6. Obstructive pattern on left side characterized by (A) absence of inguinal lymph nodes and (B) absence of medial band.

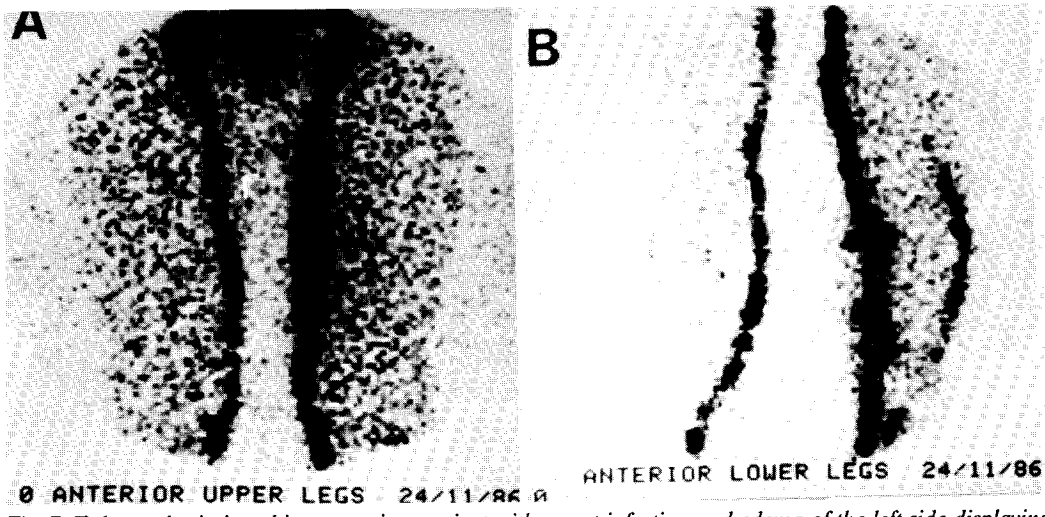

Fig. 7. Enhanced scintigraphic pattern in a patient with recent infection and edema of the left side displaying fast transport of tracer and dilated lymphatics; (A) upper legs, (B) lower legs.

DISCUSSION

These results suggest that Tc-99m labeled noncolloidal, nonparticulate polymers are superior to Tc-99m labeled colloids for imaging lymphatic channels. Any agent for visualizing the lymphatic system should be evaluated using the following criteria: clearance rate from the injection site, quality of images of the lymphatic channels and lymph nodes, and possible limitations associated with the labeling procedure. The molecular size of the four radiopharmaceuticals tested are large enough not to penetrate the blood capillary membranes and thereby remain within the interstitial space. Technetium-99m sulfur colloid with particle size of 5-15nm was chosen for comparison as an example of a colloidal substance insoluble in lymph fluid. As anticipated, Tc-99m SMC showed a comparatively slow clearance from the injection site. About 5% of the initial activity was cleared from the injection site and 3.4% was trapped in the nodes at 90 minutes post-injection *(Table*

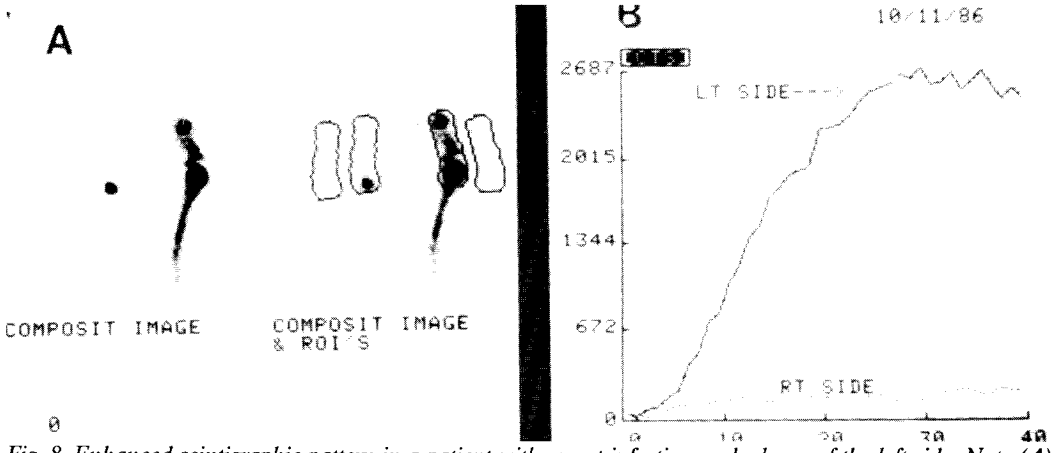

*Fig. 8. Enhanced scintigraphic pattern in a patient with recent infection and edema of the left side. Note (**A**) early imaging of inguinal lymph nodes and (**B**) the time activity curve over the diseased (left) side is several-fold greater and faster than the normal (right) side.*

1). On the other hand, Tc-99m HES, which is soluble in lymph, cleared much faster. Thus, about 35% of the initial radioactivity of Tc-99m HES was released from the injection site and 3.1% was trapped in the nodes at 90 minutes post-injection.

Although a molecular weight of 450,000 may seem large for fast migration, the highly branched hydroxyethyl starch molecule is more compact and globular than the linear dextran molecule (MW 70,000) (17). This feature may explain the higher clearance rate of Tc-99m HES from the injection site as compared with Tc-99m DXT.

The superior visualization of lymph nodes by Tc-99m HES probably is due to the presence of molecules with large weight which are trapped by the nodes. The mechanism of accumulation of labeled HES, DXT, and HSA in the lymph nodes is, however, still unknown.

HES is catabolized partially by α-amylase to fragments with lower molecular weight which in turn are subject to glomerular filtration and urinary excretion (19). This "breakdown" may account for the increased radioactivity in the urinary bladder with time, although we can not exclude that a small amount of low molecular weight HES (<40,000) present in the injected solution penetrated the blood capillary membrane and entered the bloodstream directly. Similarly, dextran which is oxidized in the liver into smaller fragments is subject to glomerular filtration (20). Radioactivity in the urinary bladder obscured the external iliac lymph nodes in rabbits but did not pose a similar problem in humans due to anatomical differences *(see Fig. 5A)*.

Because unlabeled HES and DXT are taken up by the liver (17), it is not surprising that the liver is faintly visualized with Tc-99m HES and Tc-99m DXT.

It has also been suggested that side-effects of anesthesia such as hypotension, decreased peripheral perfusion, and lack of motion promotes slow clearance of tracers from the injection site (12). Without applying massage to the injection site of anesthetized rabbits, lymph nodes and lymphatic channels were not able to be visualized at 60 minutes post-injection, a finding probably attributable to anesthesia. Similar observations were reported previously by others (12). To solve this problem, massage was applied to the injection sites and the hind legs for 2 minutes and as a result the lymph nodes and lymphatic channels were visualized in a few minutes. For human studies, massage of the injection site was carried out for only 10 seconds as the patients were not anesthetized.

Unlabeled HES has already been used clinically and shown to be safe (18). Therefore, it was appropriate to examine the use of this tracer as an imaging agent in patients. The initial experience with Tc-99m HES in healthy subjects indicated that it was a useful radiopharmaceutical for visualization of both lymphatics and lymph nodes *(see Figs. 4,5)*. Moreover, the edematous leg was differentiated from the normal side as lymphatic channels and inguinal nodes were not seen in the diseased leg of some patients, whereas they were clearly seen on the normal contralateral side *(see Fig. 6)*. In the delayed image of the pelvis, inguinal lymph nodes (right side) were clearly visualized after urination and elimination of most of the radioactivity from the urinary bladder *(see Fig. 6A)*.

An enhanced pattern is usually seen with increased lymph production such as patients with repeated attacks of cellulitis, erysipelas, early filarial infestation, or previous trauma. Increased production of lymph in the interstitial tissues leads to rapid lymphatic flow, dilatation of lymphatics and depiction of more than one band of lymphatics along the medial side of the leg and the thigh *(see Fig. 7)*. In these patients the number and size of inguinal and pelvic lymph nodes may also be increased compared to the normal contralateral side *(Fig. 8)*.

SUMMARY

Tc-99m HES was prepared easily and rapidly and in a high specific activity and high yield. The administration of small quantities of HES (3mg) in a minute volume (0.05ml) to rabbits and humans demonstrated its superiority over Tc-99m DXT and Tc-99m SMC in visualizing lymph channels and nodes. Among the radiopharmaceuticals studied, Tc-99m HES showed the highest clearance rate from the injection site ($p<0.01$). Tc-99m HES also showed greater uptake by regional nodes compared with Tc-99m SMC at 90 minutes post-injection ($p<0.001$), and the concentration of Tc-99m HES in the lymphatic channels was higher than that of Tc-99m SMC at 90 minutes post-injection ($p<0.001$). Preliminary clinical studies indicated that Tc-99m HES yielded good quality and high-contrast images of the peripheral lymphatics and regional nodes of the leg and pelvis in less than 10 minutes post-injection.

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