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LYMPH HEART SCINTIGRAPHY IN THE TOAD

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ABSTRACT

To our knowledge, there are no reports on the use of radionuclide labeled agents to image the lymphatic system of anuran amphibia. Lymphoscintigraphy was performed in 18 toads Bufo marinus L, after the injection of Tc-99m-serum albumin within the ventral lymph sac. Images were obtained with a rectilinear scanner immediately after tracer administration. Scintigrams were digitalized and densitometric profiles were obtained. One or more lymph hearts were demonstrated on all 18 scans. Scintigrams showed the four lymph hearts functioning simultaneously in only 17% of the toads. Lymphoscintigraphy with Tc-99m serum albumin is useful to study the movement of lymph from the lymph sacs or to study the function of lymph hearts in amphibia.

INTRODUCTION

Imaging methods have been rarely used to demonstrate the lymph hearts (LH) of anuran amphibia. Zolotukhin (1) injected bismuth carbonate suspension subcutaneously in frogs, and observing lymph hearts on roentgenograms. However, the quality of the images of these organs was poor. Foxon and Rowson (2), could not demonstrate lymph hearts on radiographs obtained after the injection of thorotrast into the dorsal lymph sac of the common frog.

For physiologists interested in the lymphatic system, the availability of radioisotopes has been a great boon (3). Scintigraphy with Tc-99m labeled agents has been used to detect lymph nodes in dogs (4,5) and rabbits (6). However, to the best of our knowledge, there are no reports on the use of radiotracers to image the lymphatic system of amphibia. This lack of interest may be due to the marked differences between the lymphatic system of mammals and anurans. For instance, there are no lymph nodes in toads and frogs (7) and no lymph hearts in mammals.

This paper reports on a scintigraphic method for detection of functioning LH in toads, using radioactive serum albumin as a tracer.

MATERIALS AND METHODS

Animals

The investigations were conducted with eighteen toads Bufo marinus (L) males, weighing 100-250 g. The toads were kept in tap water.

Radiopharmaceutical

Tc-99m-labeled human serum albumin (Tc-99m-HSA, Cintichem, Union Carbide) was employed. The radiotracer was used immediately after preparation. The final dosage was 400-500 μCi (14.8-18.5 MBq).

Imaging

Toads were lightly anesthetized with ether and placed on the back on a corkboard. The radiopharmaceutical, diluted in 0.9% saline in a volume of 0.5 ml, was injected into the ventral lymph sac. The site of injection was kept constant (xiphoid area) in each

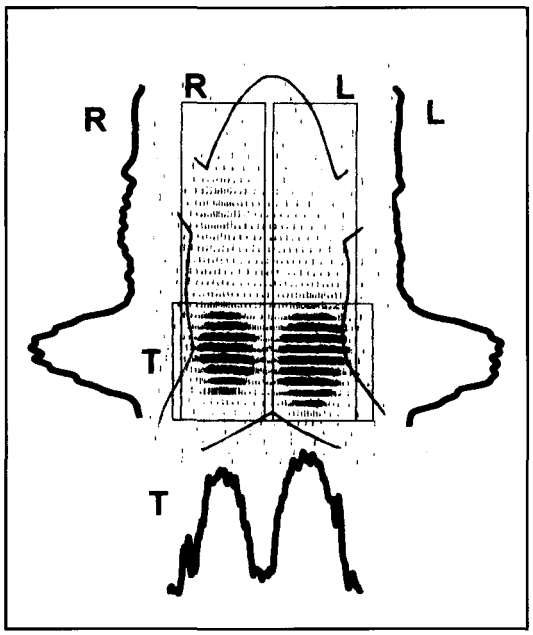

Fig. 1. Scintigraphy of posterior lymph hearts and normalized densitometric profiles. Tracer: Tc-99m-HSA. Thin rectangular boxes represent measured densitometric windows, R: Right side; L: Left side and T: Transverse.

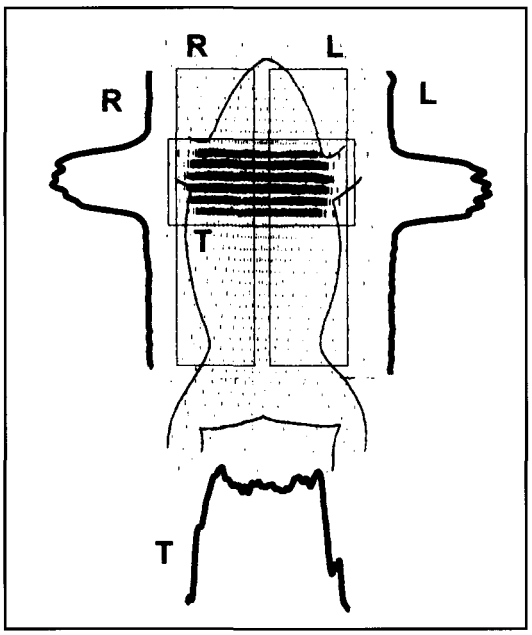

Fig. 2. Lymphoscintigram of anterior lymph hearts and normalized densitometric profiles. Tc-99m-HSA was injected in the ventral lymph sac. Thin rectangular boxes represent measured densitometric windows, R: Right side; L: Left side and T: Transverse.

toad. Scintigrams were perfomed immediately after tracer injection. A rectilinear scanner (Nuclear Chicago) with multihole collimator was used. The distance between the probe and the toads was about 5 cms.

Image Processing

Scans were digitalized using the program Band Leader Application, V.2.01 (Israel, 1.995), 0-225 gray scale, user defined measure windows which were displayed on each image *(Figs. 1,2)*. The program gives profiles of densitometric units normalized to the maximum density value of each profile.

RESULTS

All 18 scintigrams showed from one to four areas of focally increased tracer accumulation *(Table 1)* located at the roots of the limbs *(Figs. 1,2)*. Increased optical density was noted in the densitometric profiles. These findings represent activity within and around functioning LH.

In each of 18 experiments the site of injection of the radiopharmaceutical within the ventral lymph sac and the volume of Tc-99m-HSA administered were kept constant. Nonetheless, there was a variable pattern of LH observed on lymphoscintigrams. Only in 17% of the toads were four LH detected simultaneously *(Table 1)*. Usually, two or three lymph hearts were depicted (12 toads). In this group, the anterior pair of LH was detected in five toads. The posterior pair was visualized in seven toads.

DISCUSSION

Radiolabeled albumin has been used as a tracer to visualize lymphatics (8). In the present study, Tc-99m-HSA as a radiotracer was used to detect LH in toads. In all of the scintigrams performed, one or more functioning LH were demonstrated. The

TABLE 1
Detection of Functioning Lymph Hearts on Lymphoscintigrams

Number of LH Detected	Number of Toads
One	3 (16.6%)
Two	6 (33.3%)
Three	6 (33.3%)
Four	3 (16.6%)

LH: Lymph heart

quality of the scintigraphic images were better than those obtained earlier (1).

The detection of LH on lymphoscintigrams performed immediately after the injection of Tc-99m-HSA confirms the fact that in Amphibia, there are cor lymphaticum and a rapid lymph circulation (7).

Most studies on lymph hearts have been performed on the posterior (coccygeal) pair located immediately under the skin. Scintigraphy allows visualization of the more elusive anterior pair, which are located under the scapulae.

Only in 17% of scintigrams were four lymph hearts visualized simultaneously. This lack of demonstration of one or more lymph hearts on scintiscans may be due to temporary pauses in the intrinsic contraction of these organs (9,10), variations in the amplitude of beats (10) or differences in drainage of lymph from the ventral lymph sac.

Colored dyes have been used to study the movement of lymph from the lymph sacs to the blood in anuran amphibia (11). Lymphoscintigraphy with Tc-99m-HSA may also be useful for this purpose but due to the rapid lymph circulation in amphibia, imaging with a computer-interfaced scintillation camera is recommended.

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REFERENCES

1. Zolotukhin, A: Roentgenologic method of examination of the lymphatic system in man and animals. Radiology 23 (1934), 455-462.
2. Foxon, G E, KE Rowson: The fate of "Thorotrast" (Thorium dioxide) injected into the dorsal lymph sac of the frog, Rana temporaria. Q. J. Microsc. Science 97 (1956), 47-57.
3. Mayerson, HS: The physiological importance of lymph. In: *Handbook of Physiology*, section 2. The Cardiovascular System, vol II. American Physiological Society. Washington, 1963, 1035-1073.
4. Dunson, G, J Thrall, J Stevenson, et al: ^{99m}Tc Minicolloid for radionuclide lymphography. Radiology 109 (1973), 387-392.
5. Miller, GE, JB Smathers, D Hightower, et al: Lymphatic clearance of radioactive sulfur colloid. Lymphology 13 (1980), 24-29.
6. Strand, SE, BRR Persson: Quantitative lymphoscintigraphy 1: Basic concepts for optimal uptake of the radiocolloids in the parasternal lymph nodes of rabbits. J. Nucl. Med. 20 (1979), 1038-1046.
7. Yoffey JM, FC Courtice: *Lymphatics, Lymph and Lymphoid Tissue.* Edward Arnold, London, 1956, 220-221.
8. Threefoot, SA: Tracers and lymph. Am. Heart J. 62 (1961), 425-426,
9. Braun-Menendez, E, VG Foglia: Biologie et pharmacologie des coeurs lymphatiques des batraciens. Arch. Int. Pharmacodyn Ther. 64 (1940), 273-307.
10. Flindt, R: Relative Koordination der lympherzbewegungen bei Anuren. Pflügers Archiv 290 (1966), 28-37.
11. Malvin G, Macias S., Sanchez M et al: Lymphatic regulation of hematocrit during hypoxia in the toad Bufo woodhousei. Am. J. Physiol. 269 (1995), R814-821.

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