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ORIGINAL ARTICLE

Indirect joint lymphoscintigraphy with 99 mTc-labelled dextran 70,000 in the dog

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SUMMARY

In a search for standardization of the technique of joint lymphoscintigraphy, an experimental study was made in 14 dogs in which the ankle joint was injected with 2,6 mCi of dextran 70,000 marked with 99 mTc. With this method, good scintigraphic images of the lymphatic system were obtained.

After having taken samples of blood during the examination and from the organs and tissues of the lymphatic tissues at necropsy of the animal, it was seen that 99 mTc-labelled dextran 70,000 was suitable for the performance of quantitative studies of lymphatic drainage of joints. The activity found in the lymph nodes draining the injected joint was 152 times greater than that obtained from the kidney and one gram of popliteal lymph node concentrates 8,929 times as much activity as one gram of blood.

Dextran 70,000, which is widely used clinically and is administered in humans without any risk, can be the marker capable of making studies of the lymphatic drainage of joints possible and allows lymphoscintigraphy to be used in the study of joints.

INTRODUCTION

Studies by lymphography or on the lymphatic circulation in orthopaedics are practically non-existent. The lymphatic circulation of joints, even though it was described morphologically by Davies (4) in 1946, has been the subject of rare and vague references in the literature, mainly from the point of view of its clinical application.

In spite of the lack of interest manifested in orthopaedics towards the lymphatic system, we consider that it plays an important role in joint function and its study might clarify some pathological findings. This is why we have tried to find more satisfactory means of displaying possible abnormalities of joint function.

We have been interested for a long time in research on the lymphatics of joints. In 1967, Canha, Branco *et al* (3) published a study using radio-isotopes such as micro-aggregates of albumen and colloidal gold.

This author obtained, by injection of these substances subcutaneously, good images of lymph nodes but the same results were not obtained when they were injected into joints and evidence of the lymphatic circulation in joints was not possible. The appearance of dextran 99 mTc, marked by the Henze technique, made it possible, in 1984, in work involving collaboration between the Servies of Orthopaedics and Nuclear Medicine at the University of Coimbra, to obtain confirmation of the existence of the lymphatic drainage of joints and the acquisition of a method of unquestionable value in the study and understanding

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of changes in lymphatic function which are present in many chronic joint lesions.

A large number of radio-colloids marked with technetium have been suggested for lymphoscintigraphy. However, there are limitations in the use of colloids, such as the small percentage of absorption of tracer at the site of injection, the effect of the size of the particles and the functional state of the reticulo-endothelial system.

As a possible substitute for colloids, Henze (6) suggested, in 1982, the use of dextran marked with 99 mTc, which is soluble in lymph and is of sufficiently high molecular weight not to penetrate the capillary membrane after interstitial administration. This author used dextran with a molecular weight of 110,000. Subsequently, Ercan, Schneidereit *et al* (5) in 1985, studied the subcutaneous use of 99 mTc dextran of smaller molecular weight (82,000) using the method modified by Henze, in the rabbit and, with this molecule, they were able to show smaller lymph nodes.

In the present study, we have used a dextran whose mean molecular weight was 70,000, injected into the joints of the dog.

MATERIAL AND METHOD

In the preparation of dextran 99 mTc, the Henze technique was used. The dextran had a mean molecular weight of 70,000 ; the technetium was obtained by an « Amersham » generator. In this study, 14 dogs, eight male and six female, with a mean weight of 8.35 kg, were used. Anaesthesia was obtained with Droleptan 0.1 mg/kg, followed by ketamine chlorhydrate 10 mg/kg and subsequently maintained with fractional doses of ketamine chlorhydrate as long as the examination lasted. The dorsal vein of the dog's forefoot was kept open with an angiocath 19.

After anaesthesia and venous catheterization, the dog was placed prone on the surface of the collimator. An injection of the marked product was made into the ankle joint, using an insulin syringe and a fine 16 mm needle. An injection of 2.5 mCi of 99 mTc-labelled dextran diluted in physiological saline was made so that a total volume of 0.3 cc of total volume was not exceeded.

After injection of the labelled material into the joint, scintigraphic images were obtained 1, 5, 15, 30, 60 and 180 minutes later at the level of the injection and in active zones corresponding to the lymph nodes of the area of lymphatic drainage. These images were obtained with the help of a gamma camera (Maxi Camera GE II, 400 T) and after accumulated counts between 200,000 and 500,000. Simultaneously, the frames were treated by a computer (Data General Dasher D II). The detector was positioned and the sequential information was registered so as to obtain 30 images of 60 seconds. At 60 and 180 minutes, the frames were each made for 180 seconds. Samples of blood were taken 1, 15, 30, 60 and 180 minutes after injection.

The time of radioactive migration to the popliteal and inguinal nodes and the visualization time of the liver and kidney were recorded. The levels of activity present in the thyroid, stomach and bone marrow were also recorded.

The dogs were anaesthetized again and sacrificed by an intracardiac injection of 5 cc of potassium chloride about 22 hours after injection. A further sample of blood was taken and lymph nodes and organs considered to be of interest were removed. The organs were weighed and preserved so as to obtain a quantification of activity in each of them.

RESULTS

The effectiveness of the labelling was tested by chromatography, using several solvents and was always greater than 95 per cent.

In the majority of dogs it was possible to vizualise the lymphatic channels from the injected joint and it was sometimes possible to visualize their detailed structure *(figs. 1 and 2)*.

The demonstration of the popliteal and inguinal lymph nodes was confirmed between 30 and 180 minutes after intra-articular injection with good definition *(fig. 1)*. Activity appeared in the liver, the kidney and the bladder 30 to 60 minutes after injection *(fig. 2)*. No activity of any kind was seen in the thyroid or the stomach.

Activity in the blood and lymph glands

The various samples taken during the examination and after necropsy were able to verify the level of activity in the blood in the course of the study. After the necropsy of the dogs, the popliteal and inguinal lymph nodes were removed, weighed and their activity measured with the help of a computer *(Table I)*. The figures concerning the right lymph nodes on the injected side were significantly higher than those on the left which were not injected. There was a significant correlation between the level of activity in the lymph nodes and in the blood. The regression equation is as follows: $Y = 6{,}100\ X + 11{,}986{,}000$ *(fig. 3)*.

Activity in the viscera

The most significant organs were removed at the necropsy and their activity measured by computer. The relationship between the activity per gram of

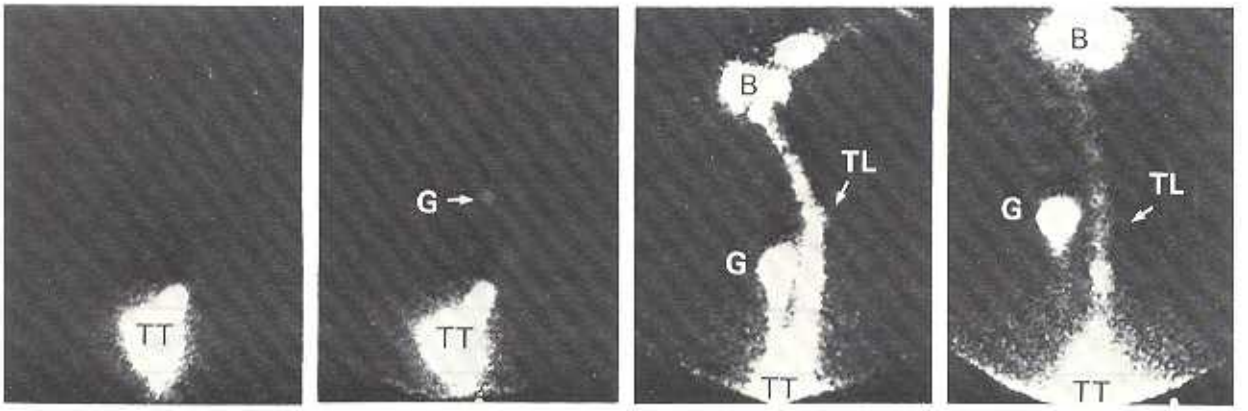
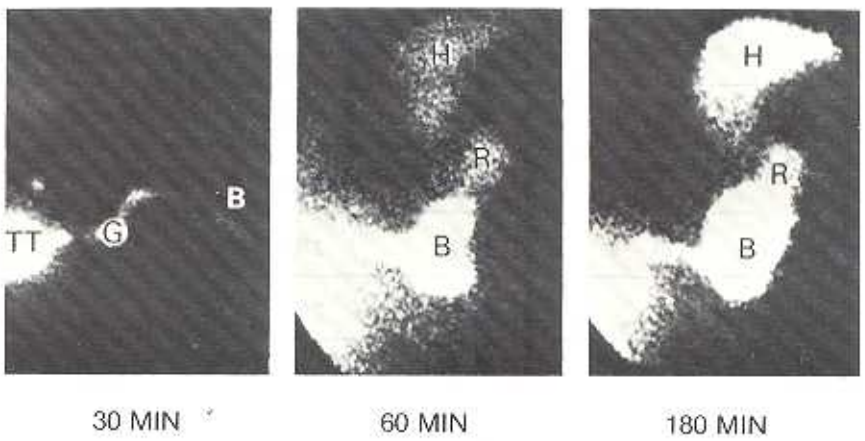


FIG. 1. — *Lymphoscintigraphic study of joints in the dog (usual appearance of lymphatic channels).* Images obtained 15, 30, 60 and 180 minutes after injection of the labelled product into the ankle joint. The image of the popliteal lymph node starts to appear after 30 minutes. The detailed structure of the lymphatic channels is not visualized until 60 and 180 minutes.
TT : Injected joint. G : Popliteal lymph node on the side of the injection. TL : Lymphatic channels. B : Bladder.

FIG. 2. — *Lymphoscintigraphic study of joints in the dog.* Images obtained 30, 60 and 180 minutes after injection of the labelled product into the ankle joint ; the image of the popliteal lymph node and the lymphatic channels is defined after 30 minutes. The last two images (60 and 180 minutes) show the activity of the liver, kidney and bladder. No activity was seen in the thyroid or the stomach.
B : Bladder. R : Kidney. H : Liver. TT : Injected joint. G : Popliteal node on the side of injection. TL : Lymphatic channels.



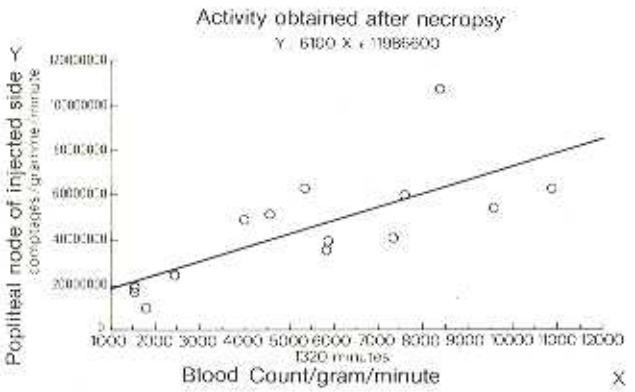


FIG. 3. — *Linear regression.* Represented by the straight line between the values of activity in the right popliteal lymph node (Y) and the blood (X) at the time of necropsy of the dogs studied.

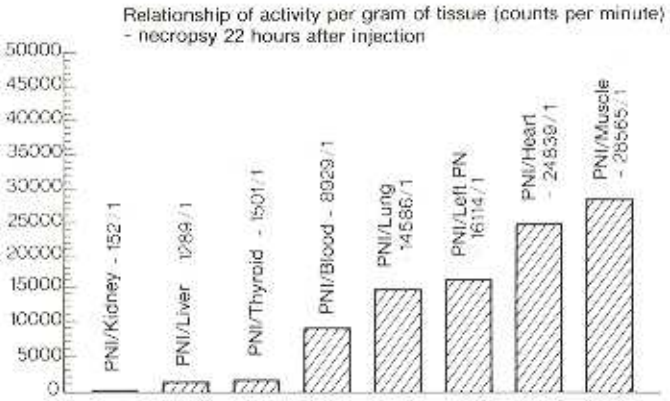


FIG. 4. — *Relationship of activity per gram of tissue.* A comparison of the activity obtained in several tissues from the various samples collected during necopsies made 1320 minutes after injection into the right ankle joint. A relationship was established between the popliteal node on the side of injection and the blood, the various organs and the tissues taken at the time of necropsy.

TABLE I. — *Activity per gram of lymphatic tissue and blood, expressed in counts per minute, obtained in the 14 dogs studied relative to the various samples of node tissue and blood received during necropsy taken 1,320 minutes after injection into the right ankle joint.*

No. of dog	*Nodes*				*Blood*
	Right popliteal	*Left popliteal*	*Right inguinal*	*Left inguinal*	
1	17073392	1296	294227	1176	1543
2	49119334	3992	621694	4974	3964
3	59997426	4234	738716	5257	7543
4	24086742	1438	272328	1491	2441
5	54701710	2725	411824	3358	9553
6	9687474	1035	644656	1143	1901
7	51972154	2062	337062	2513	4544
8	107542090	6177	658091	5888	8339
9	62927295	3167	506861	4159	5315
10	40416597	2700	478211	2566	7292
11	39083037	2373	372048	3854	5859
12	62950868	3178	816458	5098	10836
13	35545447	2651	394048	1548	5822
14	19064481	1468	281751	1414	1547

tissue, expressed in counts per minute was obtained in comparison with the popliteal nodes of the injected side, with other lymph nodes and with other organs *(fig. 4)*. The results allowed us to emphasize the high radioactive concentration obtained in the lymphatic tissues of the nodes studied.

Activity in the blood, which was 8,929 times less than that in the popliteal lymph nodes of the injected side, was not sufficient to represent a fundamental problem. In addition, the good quality of the images obtained in the lymphatic system with dextran 70,000 was confirmed.

DISCUSSION

Joint lymphatics, present in the lymphatic plexuses of the synovium, are inaccessible to conventional lymphography. Joint lymphoscintigraphy could form the means for studying possible abnormalities of lymphatic function in various joint conditions [Albuquerque, Canha *et al* (1)].

The capacity for articular lymphatic absorption being less than that of the skin, we think that the success obtained with the dextran used by us for the study of joints was due to the use of particles of molecular weight of the order of 70,000. Larger particles are absorbed with difficulty by the synovium and a major part of the radioactive material is retained in the joint. Particles of smaller size than 40,000 can pass across the capillary wall. This material, in contrast with other substances previously tested, once injected into the joint reveal not only the joint morphology, as has been shown by Canha, Branco *et al* (2), but also allows the demonstration

and quantification of lymphatic drainage. But our primary objective was not to demonstrate the morphology of the lymphatic system of the joints but to study its function, verifying the capacity of the system in the drainage of interstitial joint fluid and, in particular, the proteins of the chondro-synovial space.

Our present method is far from ideal. We hope, in future, to be able to use other markers or other molecules. However it can be said that 99 mTc-labelled dextran 70,000 injected into joints allows quantitative studies to be made in that it presents a rate of absorption and of lymph node impregnation which is sufficiently favourable.

Dextran 70,000, which is widely used clinically and is administered without risk in humans, is a marker which is able to make studies of joint lymphatic drainage possible and allows lymphoscintigraphy, which is a technique currently being expanded in other branches of medicine, to be used in the study of joints. Its future applications in rheumatoid pathology and in the surgical pathology of the knees are innumerable and will allow a better understanding of the pathology of chronic arthropathies with increase in pressure in the interstitial tissue of the joint structure. At the knee, our personal experience seems to show the value of this examination in joint stiffness, in chronic chondropathies and arthropathies and in chronic effusions.

CONCLUSIONS

Indirect isotopic joint lymphography of the ankle of the dog using dextran 70,000 is shown to be a simple method of obtaining images of the injected joints and of making an ideal assessment of the lymph nodes and lymphatic channels.

Since one gram of lymphatic tissue of the popliteal lymph node of the injected joint concentrated 8,929 as much activity as one gram of blood, dextran 70,000, used in the way described, can allow quantitative functional studies of the lymphatic system of joints.

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