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Indirect Caudal Lymphography using a New Water-Soluble Contrast Agent – Animal Experimental Studies in Pigs

D.E. Apitzsch, H.-U. Kroll, H.V. Zühlke

Department of Radiology, Nuclear Medicine and Physical Medicine (Acting Head of X-Ray Diagnosis: Professor R. Felix), and Department of Surgary (Head: Professor R. Häring), of the Steglitz Clinic, Free University of Berlin)

Summary

Animal experiments on caudal lymphography in pigs are presented, using a new water-soluble contrast medium which is renally excreted. Indirect cutaneous administration renders possible the radiological visualization of the entire lymphatic drainage system of the lower limb, the retro-peritoneal space and the thoracic duct. Visualization of the lymphatic system is rapid, homogeneous and can be repeated as often as desired. The quality of the lymphogram is as good as that obtained by the current methods in common use, employing iodized oils.

Since the introduction of direct lymphography into clinical practice in 1952 by the English surgeon, *J.B. Kinmonth* (13–16), first with water-soluble iodine-containing x-ray contrast media, and subsequently with iodized oil, which to date is the usual method for the visualization of lymph-vessels and lymph-nodes (3, 10, 19), there has been a need for a water-soluble lymphotropic substance which would produce diagnostically informative, rapid and reproducible visualization of the lymphatic system without the need for surgical dissection of the lymph-vessels, and which would be free from the well-known complications of fat emulsions (1, 4, 5, 7, 8, 9, 17, 18, 20).

This requirement appears to have been brought nearer to achievement, at least in animal experiments, with Iotasul*, a new, iodine-containing contrast medium wich is renally excreted.

*Schering AG, Berlin

Material and Methods

(1) The Contrast Medium (Iotasul)

The investigation was carried out using a new contrast medium which is renally excreted, and which in aqueous solution is a non-ionic, dimeric substance with an iodine content of 275 or 300 mg/ml. The molecular weight is 1608, and the iodine content 47% (2 triply-iodinated benzol rings, compound formation via a sulphide linkage). For chemical structure, empirical formula and chemical name see next page.

At a temperature of 37 °C of the viscosity of Iotasul is 19.8 or 32.9 MPas, at iodine contents of 275 and 300 mg/ml, respectively. In body fluids (blood, lymph and urine) (see Fig. 2), Iotasul breaks up into two phases with different molecular association (22). The more strongly associated phase has an iodine content of about 450 mg/ml and a "molecular weight" of over 100,000. On gentle agitation it passes into a clear, monophasic solution.

(2) The Investigations

The investigations were carried out in 22 healthy piglets and minipigs of both sexes, weighing 8–15 kg. All the animals were anaesthetized with Nembutal® following premedication with Stresnil®. The X-ray films were taken under fluoroscopic control with an overhead tube. The first investigation was of the possibility of using the substance in

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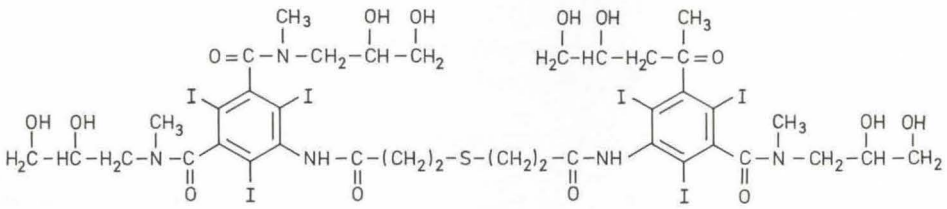
Chemical structure of Iotasul



Empirical formula: $C_{38}H_{50}I_6N_6O_{14}S$

Chemical Name: 5,5'-(4-thiaheptanedioyldiimino)-bis(2,4,6-triiodoisophthalic acid-bis(2,3-dihydroxypropyl-N-methyl) diamide)

indirect caudal lymphography. The following sites and methods of administration were tested:

(A) At the level of the distal part of the femur:

(1) Intra-cutaneous blebs of various volumes (1–5 ml)

(2) Blebs introduced at defined time-intervals (e.g., 2 ml of contrast medium intra-cutaneously at 3-minute intervals)

(3) Sub-cutaneous injection of different volumes (1–10 ml)

(4) Sub-cutaneous infusion of different volumes and at different rates

(5) Intra-cutaneous infusion of different volumes and at different rates.

(B) In the hind foot:

Inter-digital injection, similar to (A) above.

Comparison was also made with another watersoluble contrast medium (Angiografin®). Two comparative studies were carried out with Iotasul (intra-cutaneous infusion and direct visualization using the usual technique of caudal lymphography (Fig. 3). Finally, a comparative trial was carried out with Lipiodol UF, administered with the usual technique, and the intra-cutaneous infusion of Iotasul.

Results

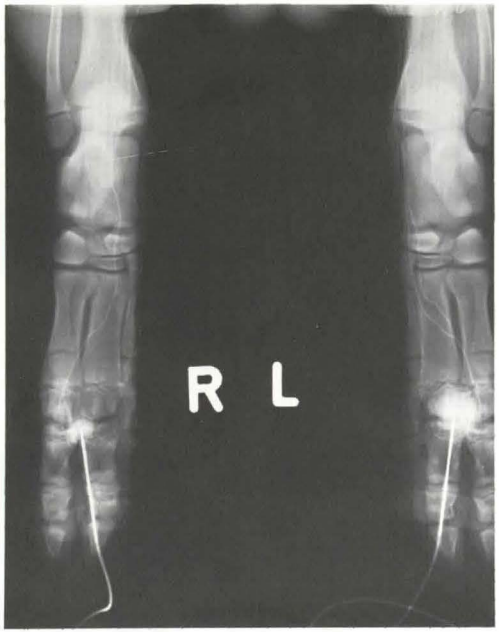
(1) Interdigital intra-cutaneous infusion of Iotasul in the pig's hind foot yielded a rapid diagnostically informative visualization, capable of reproduction as often as desired, of the inguinal and para-aortic lymph nodes and lymph vessels, as well as visualization of the thoracic duct. The volume of contrast medium required in animals of this size was between 5 and 10 ml. An infusion rate of 3.3 ml/hour as a minimum is adequate to show the lymphatic structures: optimal rates are 6.6 ml/hour and 16.5 ml/hour (Fig. 1).

(2) The detailed definition of lymph-node structure with intracutaneous infusion of Iotasul is equal to that obtained with the currently used oily contrast media (Fig. 2).

(3) In animal studies (relatively short distances!), the indirect administration of Iotasul provides a more rapid visualization of the lymphatic structures than does direct injection into a dissected-out lymph vessel (Fig. 3). Lymph-node contrast by this method is either as good as or better than that obtained by intra-lymphatic injection.

(4) Adequate visualization of the lymphatic system largely depends on the use of the correct mode of administration (intracutaneous), a continuous vis a tergo (infusion), and the site of the injection (inter-digital). Clearly, the number of lymph-clefts in the cutis, and the tissue pressure, are essential factors here (2, 6, 8).

(5) Complete visualization of the lymph-vessels, lymph-nodes and thoracic duct occurs in sequence, and in the animal takes about 30–40 minutes. Hence an investigation lasting over two days is avoided. A short time



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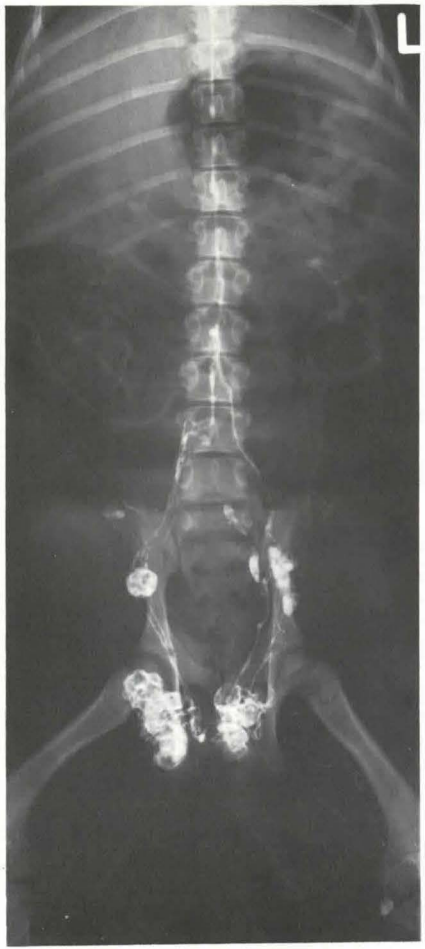

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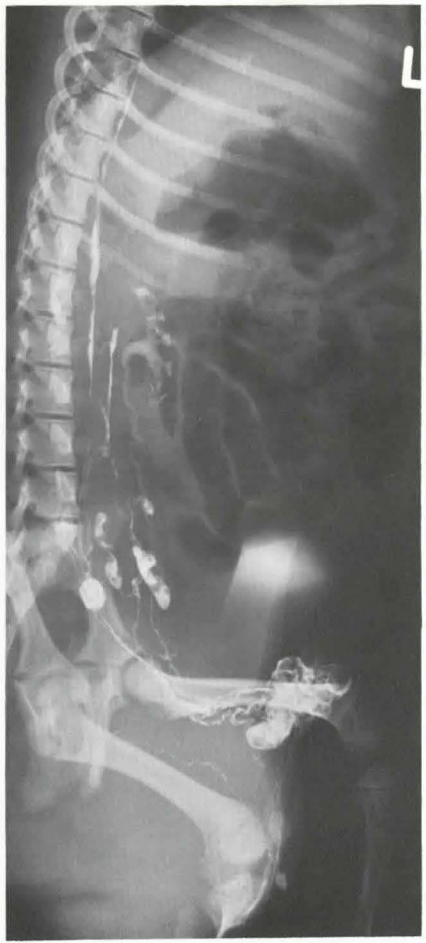

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Fig. 1 Bilateral intra-cutaneous infusion of hind-limbs. Rate of infusion 6.6 ml/hour. Lymphoflex cannulae.
(a) Both hind-limbs 10 minutes after beginning infusion
(b) View 27 minutes after beginning. (Visualization of kidneys!)
(c) View in RAD-projection 32 minutes after beginning. Full visualization of lymphatic system and of urinary bladder.

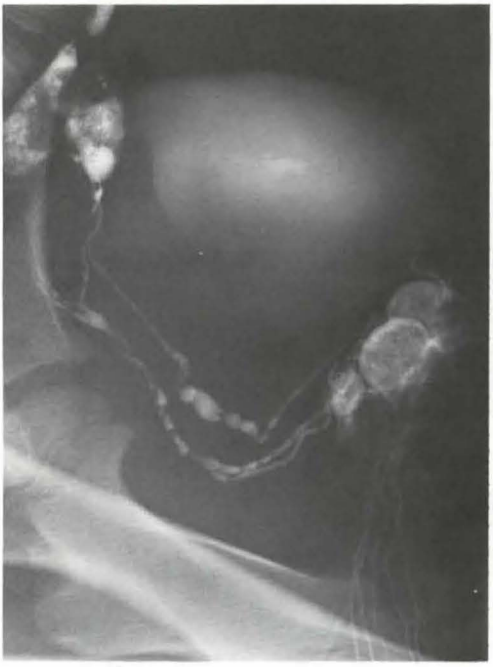

Fig. 2 Detailed view, 24 minutes after intra-cutaneous infusion in the left hind limb. Drop-like, markedly associated contrast medium in the otherwise homogeneously contrasted urinary bladder

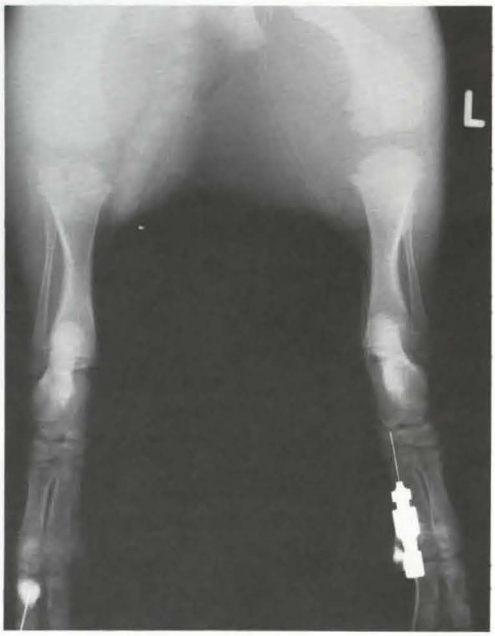

Fig. 3 Infusion of Iotasul in both hind limbs, intralymphatically following vessel preparation on the left, intra-cutaneously on the right.
Rate of infusion the same on both sides

after the end of the infusion (20–60 minutes), the contrast medium has diffused and been removed to such an extent that it can no longer be visualized radiologically. The lymphatic system can again be visualized, in a similar way, by repeating the infusion, even if the needle has been left in situ.

Discussion

As early as in 1953, *Malek* and *Kolc* (17) defined the conditions under which a (at that time hypothetical) contrast agent should be used indirectly to produce satisfactory visualization of the lymphatic system:

(1) The selection of the correct injection site, with a sufficiently dense lymphatic capillary network.

(2) Sufficient pressure at the injection site.

(3) Slow absorption of the substance into the blood capillaries and from the injection site, as well as from the surroundings of the lymph-vessels and lymph-nodes.

These requirements are still fully applicable. The cutis, with its rich lymphatic network, is much better suited for the injection of contrast medium than the sub-cutis, which is only poorly supplied with lymph capillaries (12). In the indirect route of administration (intra- and sub-cutaneous), as *Bellman* and *Oden* and other investigators have shown (2, 11, 21, 23), it is largely a question of directly forcing the substance into lymph clefts already present, or into ruptured interstitial lymph passages. Hence the concepts of "direct" and "indirect" lymphography cannot be sharply differentiated one from the other. The subcutaneous injection of Iotasul certainly results in the visualization of the lymph-vessels in the immediate vicinity, but does not yield any

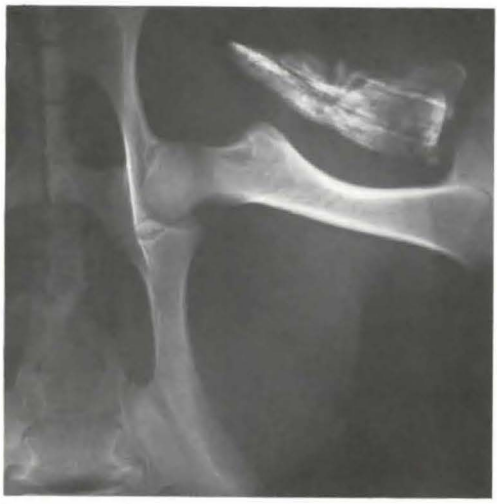

Fig. 4 Subcutaneous-intramuscular depot (2 ml) at the level of the distal part of the femur. Lateral spread of the contrast medium with only a suggestion of visualization of lymphatics, 10 minutes after injection

useful visualization of the rest of the system. This route results in a more widely-spread depot of substance, from which diffusion is evidently more rapid than lymphatic transportation (Fig. 4).

The speed of visualization of the lymphatic system cannot be usefully increased by more rapid infusion: this merely increases contrast density up to a certain limit. If this limit is exceeded, and the limit is clearly largely determined by the tissue pressure present (6), there is the formation of a sub-cutaneous depot, with the impairment in absorption referred to above.

In general, good visualization can be obtained with any infusion time, so long as the vis a tergo, on the one hand, and the lymph-flow and diffusion, on the other, are in equilibrium. For this reason, a single bleb of contrast medium yields only a certain range of contrast.

With injection (infusion) at more proximal sites in the pig's hind limb there is, as would have been anticipated, removal of the medium via the epigastric lymph-vessels. At the same time the contrast density is much poorer than with interdigital injection.

No histological studies were carried out in this trial. The extensive optical and electron microscopy studies of *Huth* et al. (not yet published), however, show no evidence of persistent change or reaction in the tissues involved. The contrast medium clearly passes through the lymphatic system without causing any morphologically evident cellular reactions, and after reaching the thoracic duct it is excreted through the renal tract within 24 hours, at which time the descending urinary tract can be visualized, depending on the concentration (Figs. 1, 2). Less than 2% is excreted via the gut.

The advantages of the contrast agent are obvious in animal trials. It avoids:

- surgical preparation of the lymph-vessels in the limb,
- the injection of vital dyes to demonstrate the lymph-vessels, and the possible complications of these,
- damage to the lymphatic system, in the form of the well-known tissue reaction when iodized oils are used.
- the possibility of fat embolism in the lungs, kidneys and brain,
- an investigation in two stages (over two days).

In contrast to this, a quick investigation yields a high-quality picture of the lymphatic system. The additional visualization of the urinary tract after the contrast medium has entered the venous circulation is regarded as providing further diagnostic information in animal studies (Figs. 1, 2).

The results of animal studies suggest that the use of this agent in man would be of value, both in direct and indirect lymphography. Further indications, in addition to caudal lymphography, may be considered.

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D.E. Apitzsch, M.D., Klinikum Steglitz FUB, Klinik für Radiologie, Nuklearmedizin und physikalische Therapie, Hindenburgdamm 30, D-1000 Berlin (West)-45