A QUANTITATIVE RADIONUCLIDE TECHNIQUE FOR INVESTIGATING THE LYMPHATIC SYSTEM WITH PARTICULAR REFERENCE TO LYMPHOEDEMA

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Labor omnia vincit

Improbus, et duris urgens in rebus egestas

Virgil.


GASPAR ASELLIVS CIVIS CREMONENSIS ANATOMICVS TICINENSIS ANNO ÆTATIS XLII.
GASPARIS hæc facies. Est cuius dextra? GALENE
Cultior hæc; et non certior illa fuit.

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ABSTRACT

Chronic swelling of the lower limb presents a special diagnostic challenge. When the phlebograph is normal lymphography may be very helpful, but it is invasive, technically difficult and does not provide quantitative dynamic information about lymphatic function.

This thesis describes the development of an alternative method to lymphography for investigating the lymphatic system by examining the clearance from the interstitial space of large molecules labelled with radionuclides.

Two methods of investigating the lymphatic system by radionuclides in patients with chronic lower limb oedema are described and their results compared.

The second/revised technique proved to be more accurate than the first method with a greater sensitivity and specificity.

The revised technique consisted of measuring the clearance of 99m Tc Rhenium sulphide colloid from the inter-digital space and its arrival in the ilio-inguinal lymph nodes 30 minutes, 1, 2 and 3 hours later using a gamma camera in patients with the clinical and radiological features of primary lymphoedema, venous oedema, a group of patients with miscellaneous causes of oedema and a control group.

The serial images were assessed visually by an independent observer and measurement of lymph flow was carried out by calculating the percentage uptake of radioactivity in the ilio-inguinal nodes.

Simple visual interpretation of the images following an inter-digital space injection will clearly differentiate between lymphoedema and other forms of lower limb swelling (sensitivity - 95%; specificity 100%); but does not provide quantitative physiological information about lymph flow in these limbs. Estimation of lymph flow by calculation of the percentage uptake of the radionuclide has, however, shown a difference in the flow of lymph from the periphery in the groups of limbs studied.

The lymphoedematous limbs had a persistently lower percentage uptake of colloid throughout the three-hour study when compared to the percentage uptake of injected activity in the ilio-inguinal lymph nodes at half, 1, 2 and 3 hours in the normal limbs, those with venous oedema and those with miscellaneous oedema, the difference being more marked as time progressed.

The degree to which lymph flow was reduced in the lymphoedematous limbs correlated with the clinical degree of severity of lymphoedema. The results suggest that most severe lymphoedemas have a marked degree of impairment of lymph transport, although colloid clearance from the periphery may also be severely diminished even when the degree of lymphoedema is mild.

The 55 limbs with lymphoedema were subdivided by radiological appearance and calculation of the percentage uptake has shown that lymph flow was more severely impaired in the limbs with proximal lymphatic abnormalities. Furthermore, both visual interpretation and calculation of the uptake have detected hidden lymphatic deficiences in the clinically normal limb.

There was a consistently increased uptake of colloid in the ilio-inguinal region of the limbs with venous disease. These findings provide further evidence that there is an increased flow of lymph in limbs with venous oedema.

Calculation of the percentage uptake of the colloid of the limbs with miscellaneous oedemas showed only a slightly increased lymph flow when compared to normal limbs. However, the technique was clearly able to differentiate this group of limbs from the mild lymphoedemas for which they can often be clinically mistaken.

This technique has clearly differentiated between lymphatic and other causes of chronic lower limb oedema. There were no false positives using the iner-digital space injection technique, although 3 scans of patients with lymphoedema were interpreted as normal, i.e. 3 false negatives. Calculation of the percentage uptake of the radiocolloid in these studies at 30 minutes, however, showed that they were below the range for the control limbs and, by definition, would be classified on the borderline of abnormality.

Examination of the lymphatic system using this technique has certain advantages over lymphography. It is almost non-invasive, technically simple to perform and diagnostic. It has also provided pathophysiological information about lymph flow, not hitherto available. Its main disadvantage is the lack of anatomical definition of the lymphatics and the lymph nodes.

The results presented in this thesis suggest that this technique provides a new and simplified method of investigating the lymphatic system in patients with chronic lower limb oedema reserving X-Ray lymphography for those patients with lymphoedema who are being considered for direct lymphatic surgery and in whom a high degree of anatomical information is required.

ORIGINALITY

This thesis describes a quantitative technique for investigating the lymphatic system in varying disease states. Two techniques have been described and their results compared.

The second technique has been able to successully and accurately diagnose lymphoedema clearly differentiating lymphatic from venous and other causes of chronic lower limb oedema. The technique has also been able to measure lymph flow in normal limbs lymphoedema, venous oedema and idiopathic/cyclical oedema, and has shown differences in lymph flow between different clinical and radiological types of lymphoedema.

No such studies have been reported before. This thesis is therefore original and has contributed new physiologic knowledge about lymph flow and the lymphatic system to the science and practice of surgery.

PUBLICATIONS

Reprints of published work enclosed within the back cover:

The value of lymphoscintigraphy in the investigation of primary lymphoedema. G. Stewart, J. Gaunt, D. N. Croft, N. L. Browse, Immunology and Haematology Research, Monograph No. 4 1984 209-213.

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The study of lymph flow abnormalities in chronic limb oedema by lymphoscintigraphy. G. Stewart, J. I. Gaunt, D. N. Croft, N. L. Browse. Nuclear Medicine Communications 1984 5 229-230.

The use of radio-active colloid clearance in the diagnosis of lower limb oedema. G. Stewart, J. I. Gaunt, D. N. Croft, N. L. Browse. British Journal of Surgery 1984 71 393.

Manuscripts in press:

Isotope lymphography: a new method of investigating the role of the lymphatics in chronic limb oedema. G. Stewart, J. I. Gaunt, D. N. Croft, N. L. Browse. British Journal of Surgery.

CHAPTER 1

LYMPH AND THE LYMPHATIC SYSTEM

Ars inviendi
adolescit cum inventis

Francis Bacon (1561-1626)

Introduction

The lymphatic system has two main functions, namely immunological and absorbent. The latter aspect (of the lymphatic system) consists essentially of a series of channels pari passu the arterial and venous systems whose main function is to return to the circulation from the interstitial space fluid and large molecules which do not or cannot return via the venular capillaries. This thesis is devoted to describing a new method of investigating this particular aspect of the lymphatic system.

It is a system which in the past has often been neglected being the "Cinderella" of the vascular system; probably because the lymphatic system has always been technically difficult to investigate in comparison to her more obvious sisters. It has almost certainly been recognised as a separate system for over 2500 years although the term "lymphatics" was first used by Bartholin in 1653 when he described them as a separate system of vessels which carried watery fluid (Figure I).

Bartholin's findings resulted from work done by many eminent scientists and "ancient medicine men". Starting with the Ancient Hittites and Egyptians who recognised the pathology of the system if not the anatomy, there are many references in Ancient Literature to "swelling of the feet" and other parts of the body which probably correspond to what we now call "lymphoedema" and "elephantiasis".

Furthermore there is evidence that structures corresponding to lymph vessels were observed by the Ancients. Hippocrates referred to "white blood" and Aristotle is cited as having described anatomical structures containing colourless fluid,

Figure 1

Thomas Bartholin (1616-1680). Professor of Anatomy, Copenhagen. He introduced the term "lymphatics" publishing his findings in a book called "Vasa lymphatica" in 1653.

while Herophilus (335-280 BC) and Eristratus (310-250 BC), and other members of the Alexandrian School were probably the first anatomists to describe the structures we now know as mesenteric lacteals (Fulton).

We see that the Ancients obviously recognised the existence of a system of vessels carrying colourless fluid but real progress following these scanty early observations awaited the resurgence of scientific enquiry which followed the "Dark Ages".

There are few systems in the body which can truly be said to have been "discovered" at a definable time. The lymph system, however, is one of those and credit for this discovery belongs unreservedly to Gaspar Aselli, Professor of Anatomy at Pavia near Milan, who described the mesenteric lacteal vessels in 1622; almost simultaneously with the "discovery" of the circulation by Harvey in England.

On 23rd July, 1622, Aselli demonstrated the lacteals in the mesentery of a well fed dog. These vessels contained a white fluid which "gushed forth" when the vessel was cut. On repeating his observations on a dog which had been starved no such vessels were found and Aselli therefore ascribed an "absorptive" function to the lacteals. These and similar observations made on other animals are recorded in "De Lactibus Sive Lacteis Venis" published in 1627. In line with the "Galenic tradition" Aselli thought the lacteals transported lymph to the liver and this error is depicted pictorially on the frontispiece of "De Lactibus". In this book, Aselli inferred the existence of lymphatics in man although he did not confirm their presence by direct observation. This was left to Brechet (1628) who was the

first to observe lymphatics in man.

Knowledge of the lymphatic system was further developed in 1651 by Pecquet who as a medical student in Montpelier accurately described the cysterna chyli ("Pecquet's Cistern"), its connection with the thoracic duct (vena alba thoracis) and the communication of the thoracic duct with the venous system at the junction of the left jugular and subclavian veins.

This early work provided the inspiration for Thomas Bartholin (1616-1680), Professor of Anatomy in Copenhagen, to discover other vessels, distinct from the lacteals, which carried watery fluid and to which in 1652 he gave the name "lymphatics". He published his findings in a book called "Vasa Lymphatica" :- dated 1653. He was not alone as Olaus Rudbeck (1630-1702) independently had made similar observations in Sweden (Figure 2). However, Rudbeck went further than Bartholin describing a more comprehensive system than had previously been recognised and by an elegant series of ligature experiments he disproved the prevailing view that chyle passed to the liver. Rudbeck also demonstrated the presence of valves within lymphatics and recognised the special system of lymphatics in many viscera.

A violent dispute for priority took place between Rudbeck and Bartholin, and while Rudbeck was first to begin his investigations and most likely added more to detailed knowledge of the anatomy, Bartholin probably established the discovery by better documentation while postulating that it may have physiological significance. It seems, however, that Rudbeck lectured publicly on his findings in 1652 (Bartels 1909) and thus his claim for priority may be incontestable. The controversy

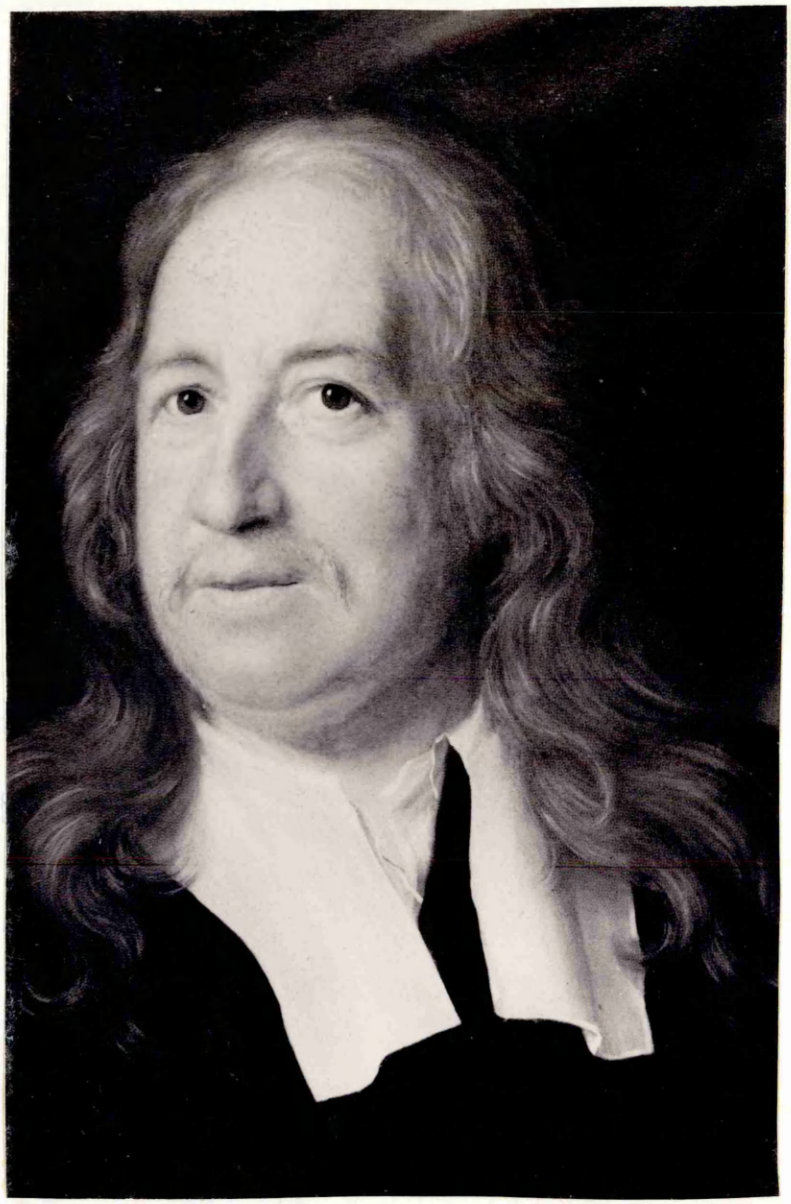


Figure 2

Olaus Rudbeck (1630 -1702). He made his first observations of the lymphatic system while a medical student in Upsala, Sweden.

over who made the greater contribution however lasted long after the deaths of the original postulants.

In the 18th century the debate continued as further progress in defining the anatomy, and understanding the function of the lymphatic system was achieved. This was accomplished principally by William Hunter (1718-1783) in London.

William Hunter and his pupils John Hunter, Hewson and Cruikshank used the innovative anatomical technique of fine injections discovered in the latter part of the 17th century by the Dutch Anatomist Ruysch and by Anton Nuch of Leyden. Using mercury injections in corpses they made previously imperceptible vessels easily visualised and with this technique Hewson carried out the most systematic study of the anatomy of the lymphatics in this era.

While Bartholin in the 17th century had postulated a physiological role for the lymphatics, William Hunter, from work done in collaboration with Cruikshank, actually suggested that this system may be an absorbent system. He stated that "the Lymphatics are like lacteals; lacteals are absorbents; it is highly probable that the lymphatics are the general absorbents of the body and carry the juices to the duct, receptaculum and finally to the jugular vein". Alexandra Monro, Secundus, claimed that William Hunter had obtained the idea from his inaugural lecture in 1755 but in fact Hunter had preached the "absorbent theory" from 1746 attested to by the lecture notes of several of his pupils (White 1752).

The Hunterian School made a great and lasting contribution to our knowledge of the lymphatic system by their systematic and

detailed studies in animals and man. By the end of the 18th century they, and others in the European Continent, had largely delineated the wide extent of the lymphatic system. This era is best illustrated by Paolo Mascagni's marvellous atlas published in Italy in 1787 and containing 41 plates of the human body elegantly drawn by Ciro Santi (Figure 3).

The works of the Hunterian School and Mascagni mark the completion of the early anatomical researches on the Lymph System. The distribution and characteristics of the lymphatic supply of various organs delineated by these anatomical studies thus formed the basis for the physiological investigation of the 19th and early 20th centuries.

The problems which were now uppermost concerned the relationship of the lymph system to the blood vascular system and the precise function of the lymphatics. Throughout the 19th century a wide variety of theories were proposed and towards the end of the century, as methods for physiological investigation became more refined, large volumes of experimental data were produced by different workers in support of the leading theories of the day.

Although the Hunterian School (Hunter 1762) held that the lymphatics formed a discrete system of blind ending absorbing vessels only communicating with the blood vascular system in the neck, the general 18th century view was that the lymphatics communicated with veins by very fine tubular connections - "vasa serosa". Lippi of Florence in 1825 went further than this and postulated that actual peripheral lympho-venous communications existed (Pannizzi, 1833).

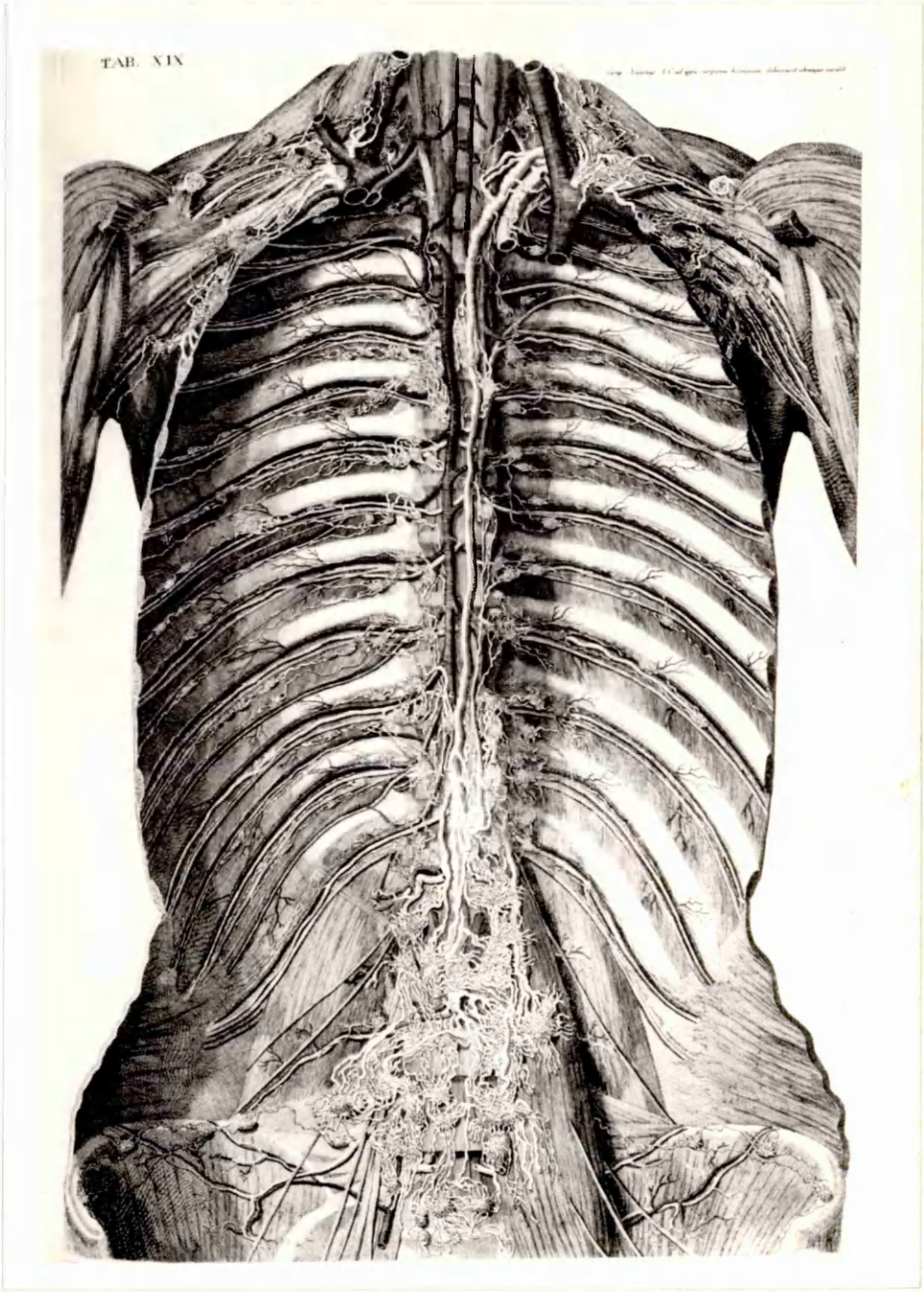


Figure 3

The Cysterna Chyli and Thoracic Duct from Vasorum lymphaticorum Corporis Historia et Ichnographia by P. Mascagni. Sienna 1787.

Virchow in "Cellular Pathology" 1860, doubted the existance of "Vasa Serosa" but still held the view that a communication between the lymph and blood system existed by way of "intercellular cannaliculi". In concept these seem to be little different from "Vasa Serosa". Von Recklinghausen proposed in 1862 that lymphatic endings open directly into the interstitial space by "stomata" (Von Recklinghausen 1872), but His in 1863 was probably the first to claim that the lymphatic system was formed from a closed system of tubes, a fact which could not be proved until much later.

In the 19th century and early 20th century there were many contributions to ideas regarding the origin and formation of lymph and the function of the lymphatic system. However, the major contributions to our knowledge were made through the efforts of four great physiologists.

Karl Ludwig (1816-1895), probably the greatest teacher of physiology ever, developed techniques, initially in Zurich and Vienna, and latterly in Liepzig, for the collection of lymph from sources other than the thoracic duct. He studied the function of the lymphatic system and postulated that lymph was a filtrate derived from blood, the rate of its formation being dependent on blood pressure (cited by Rusznyak, Foldi and Szabo 1960). As lymph formation is not always directly related to blood pressure, however, there were loopholes in Ludwig's "Theory of Filtration".

Heidenham (1891), Professor of Physiology at Breslau and Ludwig's great rival, contested this theory having failed to show a consistent relationship between blood pressure and the rate of

lymph flow. He proposed his own "Secretion Theory" maintaining that lymph was produced by an active secretory process by the lymphatic epithelium aided by various "lymphogoga". Although Heidenham's theory initially gained almost universal acceptance it was later criticised on the basis of certain internal inconsistencies and of his not wholly convincing experimental evidence.

Starling (1896) at the University College in London discovered the missing link in support of Ludwig's hypothesis - the colloid osmotic pressure of the plasma proteins - thus settling the controversy. He described the relationship between the capillary bed, the interstitial fluid and the lymphatic system, concluding, on the basis of measurements of the osmotic pressure of the colloids in blood plasma, that these colloids were paramount in promoting the intravascular migration of fluid. Starling's concepts of the fundamental physiology of capillary interstitial fluid and lymph formation are still generally applicable today.

Drinker (1887-1956), from the Harvard Medical School, made many contributions in the 1930's and 1940's including pioneering classics such as those on the composition of lymph from the lungs and heart. He demonstrated that a considerable amount of protein leaks from the blood capillaries and that the principle function of the lymphatic system is the absorption of protein from the interstitial tissues, the lymphatics returning the protein molecules to the blood stream, and therefore maintaining the colloid osmotic pressure of the blood and thereby the blood volume.

Newer techniques and equipment have significantly advanced our knowledge of the lymphatic system over the past thirty years. For example, the intradermal injection of dyes, in particular patent blue violet, permitted visualisation of peripheral lymphatics for anatomical studies and cannulation.

With the aid of patent blue violet, Kinmonth introduced lymphangiography in man in 1952 and this technique has undoubtedly led to considerable advances in our morphological knowledge of the lymphatic system in vivo.

More recently, the advent of the electron microscope has allowed the study of the microstructure of lymphatics and to a lesser extent the study of the passage of molecules and large particles through the lymphatic capillary walls.

Definitive studies of the exchange of substances between the capillaries and the lymphatics have been made possible with the discovery of radioisotope techniques while innovative techniques such as the chronically implanted capsule (Guyton 1963) and the wick catheter (Scholander, Hargans and Muller 1968) have produced considerable information about the composition of and the exchange of molecules through the interstitial fluid.

Despite these advances, however, the original concepts of Starling in 1896 refined by Drinker in the mid part of this century remain unchallenged and to some extent our knowledge of the physiology of the lymphatic system and how to investigate its abnormalities remains crude.

A better understanding of the potential abnormalities of the lymphatic system can only be achieved through a detailed knowledge of the known normal physiology and in the remainder of

this chapter, I will outline the modern concepts of the composition, formation and transport of lymph.

(I) Physiological Importance of Lymph

The lymphatic system, from a physiological point of view is primarily a drainage system. Its need arose phylogenetically with the development of a high pressure circulation. The latter development, designed to ensure an adequate supply of oxygen to tissues, created a situation favouring transudation of fluid and other substances from the capillaries. An increase in plasma protein served to partially counteract this leakage since the plasma proteins exert an osmotic pressure. There still remained the problem of clearing the tissue spaces of substances which had leaked out of blood capillaries and which were not absorbed into the blood stream. A system therefore developed whose prime function was to clear the interstitial spaces of excess water, large molecules and particles, and to transport them from the tissues back to the intravascular compartment (Figure 4). In this sense the lymphatic system can be regarded as a homeostatic mechanism which is important in maintaining the constancy of the "milieu interieur".

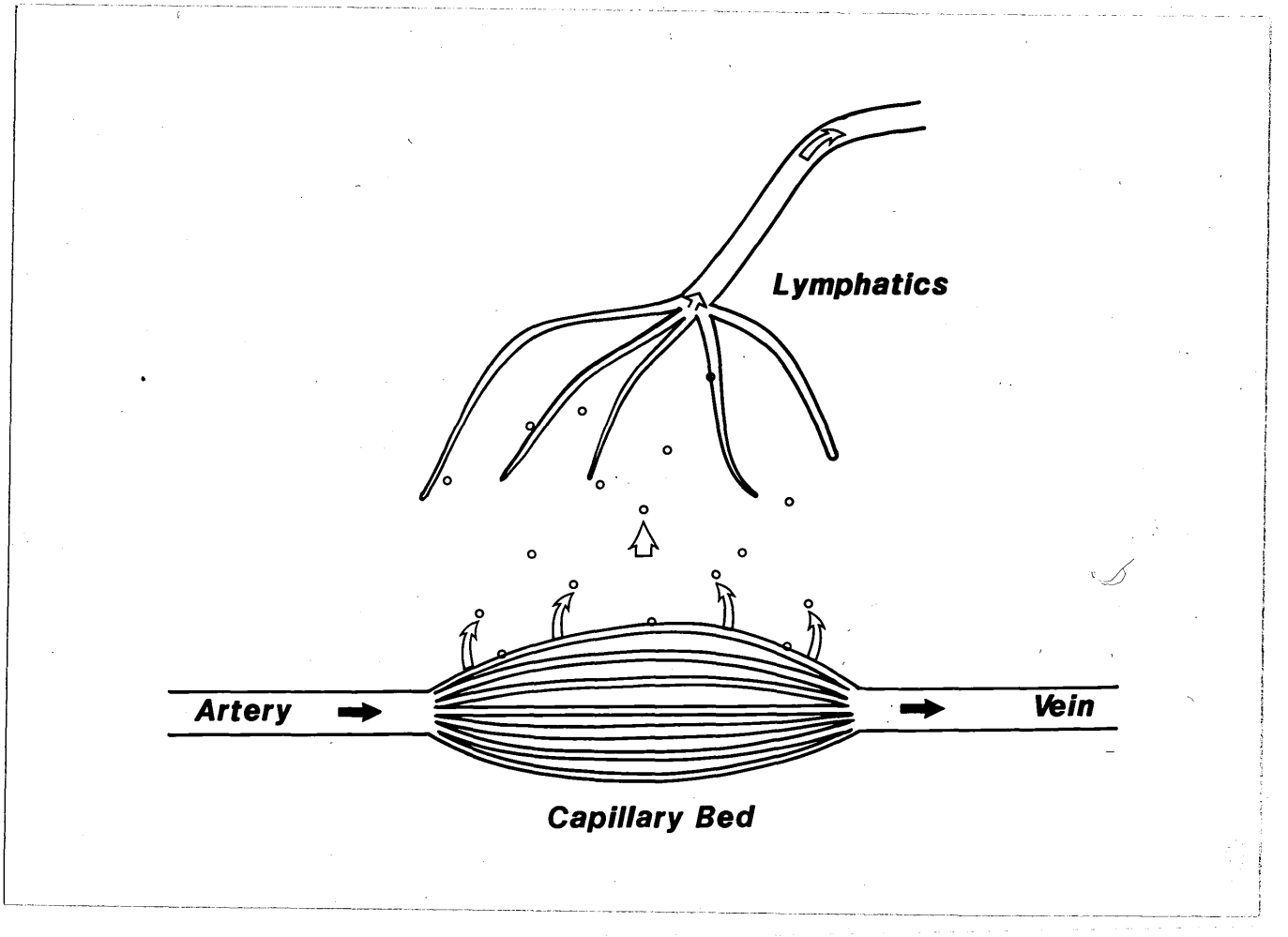


Figure 4

Simplified representation of the passage of large molecules from capillary - interstitium - lymphatics.

Structure of Lymphatics

The lymphatics may be considered as endothelial tubes resembling blood capillaries but thinner. However, it is necessary to start consideration of the lymphatic system in the blood capillaries and the tissues. The capillary - tissue - lymphatic complex really forms an entire system with each part affecting the other.

The interstitial matrix is largely composed of mucopolysaccharides to which most of the tissue water is absorbed. In normal circumstances the amount of actual water free is remarkably small and appears to be present in channels which are narrow, (100nm), sparse (1/um) and relatively short (Casley-Smith 1980a). Most of these form a "free circulation" from the arterial to the venous ends of capillaries but some pass to the initial lymphatics and may be termed "prelymphatics".

The initial lymphatics are the most peripheral elements, and it is here material is taken up. The endothelial intercellular junctions are the single most important feature of the initial lymphatics. They differ from blood capillaries by having 1-6% of the total junctional length open with gaps of 0.1 to several um. If the tissues are active as in oedemas or inflammation these lengths increase up to 50% (Casley Smith 1980a). This opening of the intercellular 'pores' is possible because of the lack of adhesion between the cells lining the lymphatics, tenuous basement membranes and tissue filaments which only adhere to the outer layer of the cells; the inward flow of the fluids from the tissues pulling the cells apart. Myofibrils have been described in the lymphatic capillaries in the wing of the American Bat

(Hogan and Nicoll 1979) but have not been found in any other species and probably do not exist in human lymphatic capillaries.

The collecting lymphatics are medium size vessels (100-200mm) and have muscle fibres, whereas the larger lymphatics are composed of an endothelial layer covered by a diffuse connective tissue sheath in which elastic and muscular elements are irregularly scattered. Amyelinated nerves can be traced to the muscle fibres in both types of vessels, valves are present in both the collecting and larger lymphatics and may be unicuspid or bicuspid. These valves are undirectional and are important in determining the central direction of flow.

It is now generally accepted that the lymphatics form a closed system (MacCallum, 1903; Sabin, 1908,), starting with the lymphatic capillaries in the periphery through lymphatics of increasing size and the intervening lymph nodes to the thoracic duct and hence the blood circulation.

Composition of Lymph

The composition of lymph and the amount of fluid and macromolecules presented to the lymphatic system has been shown to be determined more by the permeability of the blood capillaries in an area and the consequent pericapillary filtrate than by the metabolism of tissue cells (Mayerson, 1963). Thus lymph originates as an ultra-filtrate of plasma modified and added to in its course through the tissue before entering the lymphatic system.

Lymph can therefore be defined as "the pericapillary filtrate mixed with the interstitial fluid which has entered the closed lymphatic system".

The composition of lymph will be discussed under various headings and in most sections compared to the composition of the substance in plasma.

Proteins

Starling (1896) originally thought that the capillary bed was relatively impermeable to protein but Drinker and Field (1931) showed there was considerable protein leak from the capillaries and elaborated the concept of the lymphatic system as one of the absorbing vessels, whose main function was to return those protein molecules to the intravascular compartments. This concept of a continuous exchange of plasma protein between extravascular and plasma compartments has been confirmed repeatedly in radioactive labelled plasma protein experiments. Iodine labelled albumin studies in man indicate a daily exchange rate between the intravascular and extravascular pools of 140% of the total body albumin and Mayerson (1963) showed that between

half and all of the circulating proteins leave the intravascular compartment every twenty four hours. Szabo (1978) however, has shown in animal experiments that only 38% of the proteins that escape from the blood system are actually cleared by the lymphatics, the remainder returning via the venular capillaries.

The protein content in lymph has been analysed by many workers both in animals and humans. The full spectrum of plasma proteins is usually present although some are in reduced concentration. The ratio of lymph concentration to serum concentration of a given protein is thought to represent the permeability of the capillary to that protein. Grotte (1956) and Mayerson, Wolfram Shirley et al. (1960), using different sized dextran molecules, showed a lower permeability of larger molecules through blood capillary walls and hence lower lymphatic concentrations. The bulk of plasma protein have molecular weights ranging from 40,000 to 300,000 including the two major species, albumin (70,000) and gamma G immunoglobulin (155,000). Molecules such as fibrinogen and IgG have a lower permeability coefficient and the concentration of the various plasma proteins in lymph is, in general, inversely related to their molecular size.

The presence of nearly all plasma proteins in lymph has been verified (Szabo, Gerzely and Magyar, 1963) although quantitative data on individual protein species is conspicuously absent. Studies of the passage rates of very large molecules such as alpha-2 macroglobulin, IgM and low density lipoproteins to define an upper limit of molecular size would be useful.

Non Protein Constituents

Lipids

Lymph from all regions contain the lipid components of the lipoproteins present in plasma but in lower concentrations. The various lipids in lymph are present not in solution as such but as components of large complex lipoproteins. The interpretation of lipoprotein concentrations in lymph in relation to transport throughout the whole body is difficult, because of the very active metabolic role of the long chain fatty acids. Concentrations vary depending on the area of the body being drained. For example, the lipoprotein concentration of leg lymph is much less than hepatic lymph which in turn has a lower concentration than plasma (Adams 1964, Yoffey and Courtice, 1970); the lipids in plasma consisting mainly of fatty acid esters, cholesterol esters, phospholipids and triglycerides present as components of a broad spectrum of complex lipoprotein molecules with small amounts of free cholesterol and free fatty acids.

Non Lipids

The readily diffusible non protein constituents of plasma, e.g. glucose and urea, are present in lymph in concentrations which are approximately the same as plasma. With rapid changes in the levels in plasma the levels in lymph may lag behind. For example, the concentration of glucose in lymph will take sometime to reach equilibrium following a carbohydrate meal or an intravenous infusion of glucose which rapidly increases the plasma glucose concentration (Robertson and Williams, 1939). The pCO_2 pH and pO_2 in lymph generally reflect the values in the

venous blood draining the tissue from which the lymph is derived (Witte, Clauss & Dumont, 1967; Witte, Cole, Clauss et al., 1968).

Electrolytes

The ionic pattern of lymph is not essentially different from plasma. In general the total cations are slightly lower whereas anions, chloride and bicarbonate are higher in lymph than plasma. The direction of the differences in the concentrations of these constituents is consistent with the existence of a Gibbs-Donnan equilibrium operating on two phases whose concentration of non-diffusible ions (protein ions) differ.

Enzymes

The concentration of enzymes is usually lower in lymph than in plasma although in certain circumstances their concentration may be higher such as the increased GOT, GPT and LDH in the post thermal injury limb (Lewis 1967,1969). Renin has been shown to be present in renal lymph in much higher concentrations than in plasma suggesting that this enzyme at least to some extent enters the blood stream via the lymph system (Yoffey & Courtice, 1970).

Although in many cases the method of entrance of enzymes into the blood stream has not been established, it would seem that some enzymes at least enter via the lymphatic system. Once in plasma these enzymes will take part in the continuous circulation through the tissue fluid and lymph just as other protein molecules do.

Coagulation Factors

Lymph from all parts of the body clots but, as a rule, less rapidly than plasma. The concentrations of fibrinogen and

prothrombin in lymph are always less than plasma and vary from region to region. The concentration of calcium in lymph is slightly lower and platelets are almost absent. Lymphocytes, which are present, have a poor thromboplastic activity.

Little is known about the concentration of plasminogen, plasminogen activators and inhibitors and considerable work is still necessary on the coagulation properties of lymph.

The composition of lymph is important particularly with respect to proteins and will be discussed in the next chapter in the context of the aetiology and pathogenesis of lymphoedema.

(II) Lymph Formation and Lymph Flow

As mentioned earlier most physiologists agree that lymph originates as an ultrafiltrate of plasma, modified and added to in its course through the tissues before entering the lymphatic system. The energetics of lymph formation however, have yet to be solved.

The Starling "Hypothesis"

The factors governing the transfer of fluid across the capillary membranes are embodied in the Starling "hypothesis" of capillary exchange which states that " the exchange of water and small molecules across the capillary membranes is largely governed by the balance between the hydrostatic and colloid osmotic pressures in the intravascular and extravascular compartments, the colloid osmotic pressure being dependent upon the relative impermeability of the capillary membrane to plasma proteins.

Thus the movement of water and crystalloids across the capillary wall is essentially a hydrodynamic process dependent on the capillary hydrostatic pressure and the tissue osmotic pressure which favour extravasation and the colloid osmotic pressure which favours reabsorption (Figure 5).

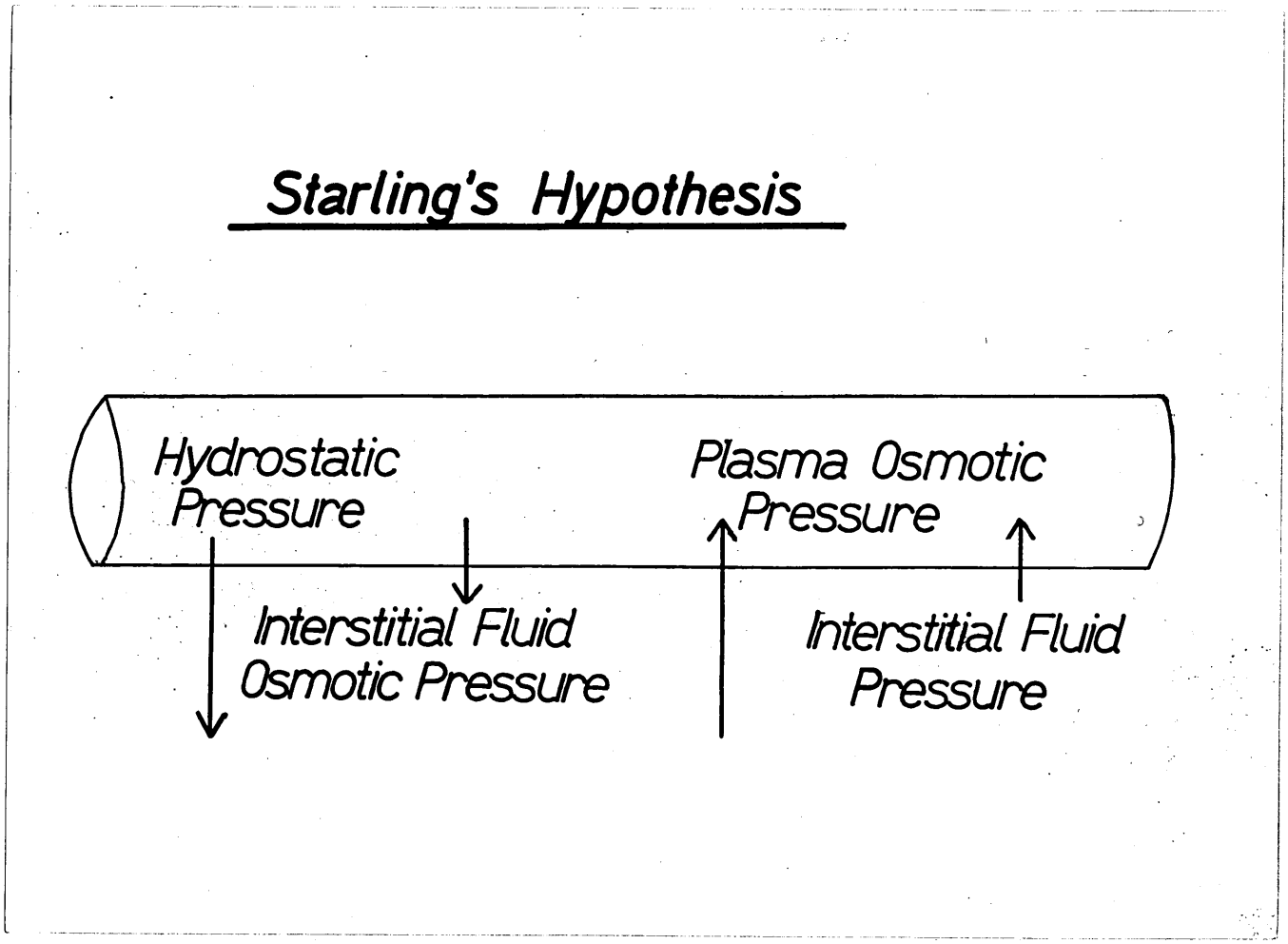


Figure 5

Starling's Hypothesis

Mathematically the hypothesis can be expressed as

$$F = k\,(Pc - Pi) + (TTi - TTc)$$

Where k = Filtration coefficient

Pc = Blood Capillary pressure

Pi = Pericapillary Interstitial pressure

TTi = Pericapillary colloid osmotic pressure

TTc = Plasma colloid osmotic pressure

Because the capillary hydrostatic pressure falls from the arterial to the venous end of the capillary there is a net tendency for extravasation at the arterial end and for reabsorption at the venous end. Furthermore, loss of water from the capillary causes the colloid osmotic pressure of the plasma to rise, and dilution of the interstitial fluid and proteins causes a reduction in the tissue osmotic pressure. These osmotic changes militate further in favour of reabsorption at the venous end of the capillary.

Starling believed that the slight excess of fluid filtered over that reabsorbed was removed by the lymphatics, this being the major function, in his view, of the lymph system. This theory, however, takes no account of the fact that "capillaries practically universally leak protein" (Drinker and Field, 1931) and do so in relatively large amounts (Asscher and Jones, 1965).

Pappenheimer, Renkin and Borrero (1951) reviewed evidence concerning capillary exchange, and on the basis of known facts regarding the movement of protein molecules across capillary membranes, gave support to the concept of "pores" through which such molecules would move.

In a largely mathematical treatment, Pappenheimer (1953) introduced the concept of "selective diffusion" or "molecular sieving" by which the facility of a molecule to pass through the membrane is governed by the ratio of its size to the total pore area. As the size of a molecule approached the pore size so its diffusion was restricted. From evidence then available Pappenheimer calculated the probable size of such pores. Confirmation of their existence awaited the development of the electron microscope when intercellular junctions were recognised. In addition, current evidence indicates that proteins almost certainly pass into the tissues probably via small vesicles in the endothelial cells (Casley-Smith, 1973; Carter, Joyner and Renkin, 1974).

In summary, it appears that diffusion rather than hydrodynamic flow is the chief means of exchange of protein molecules across the capillary membrane.

The Role of the Lymphatics.

The lymph system plays a central role in the regulation of the volume and composition of the interstitial fluid, by the extraction from it of protein, water, other large molecules and particulate matter. Removal of protein by this route preserves the constancy of the tissue osmotic pressure.

Field and Drinker (1931) were the first to show that the lymph system is the chief route by which protein is removed from the extracellular space. They found by a serological method that horse serum injected into the tissues first appeared in the thoracic duct lymph and later in the blood. Further, its appearance in the blood could be considerably delayed by ligating the thoracic duct.

Using radio-iodinated human serum albumen, Taylor and his colleagues again showed that the lymph system is the major pathway by which protein reaches the systemic circulation (Taylor, Kinmonth, Rollinson et al., 1957). On the other hand Jepson, Simeone and Dobyns (1953) using a similar technique concluded that most extravascular protein passed directly back into the bloodstream.

Recently, Foldi (1977) has shown that the data produced by Jepson et al in their paper is capable of an alternative interpretation which would support the original claims of Field and Drinker (1931).

Collection of lymph

Until the 1960's physiologists thought that the movement of fluid into the terminal blind ends of the lymphatics occurred automatically whenever excess fluid accumulated because this caused a positive interstitial pressure. In fact, Zweifach and Silberberg (1979) still consider the positive pressure gradient explanation for flow from capillary, to interstitial, to lymphatic to be the correct one.

There is however, strong evidence confirming the discovery by Guyton (1960, 1963) that the pressure in the tissue spaces is usually negative. (Scholander et al., 1968; Stromberg and Weiderhielm, 1970; Snashall and Boother, 1974).

This observation makes it difficult to explain how fluid and protein pass into the lymphatic system because the intralymphatic pressure is not usually negative and is therefore higher than the interstitial pressure (Weiderhielm and Weston, 1973; Zweifach and Prather, 1975). As yet no evidence has been presented to show that any active force, selective absorption being the best example, is involved, and an osmotic pressure which could draw water into the lymphatics can only exist after the entry of the protein into the lymphatics.

Guyton (1971) and others have suggested that the passage of fluid and macromolecule into the lymphatics is an active

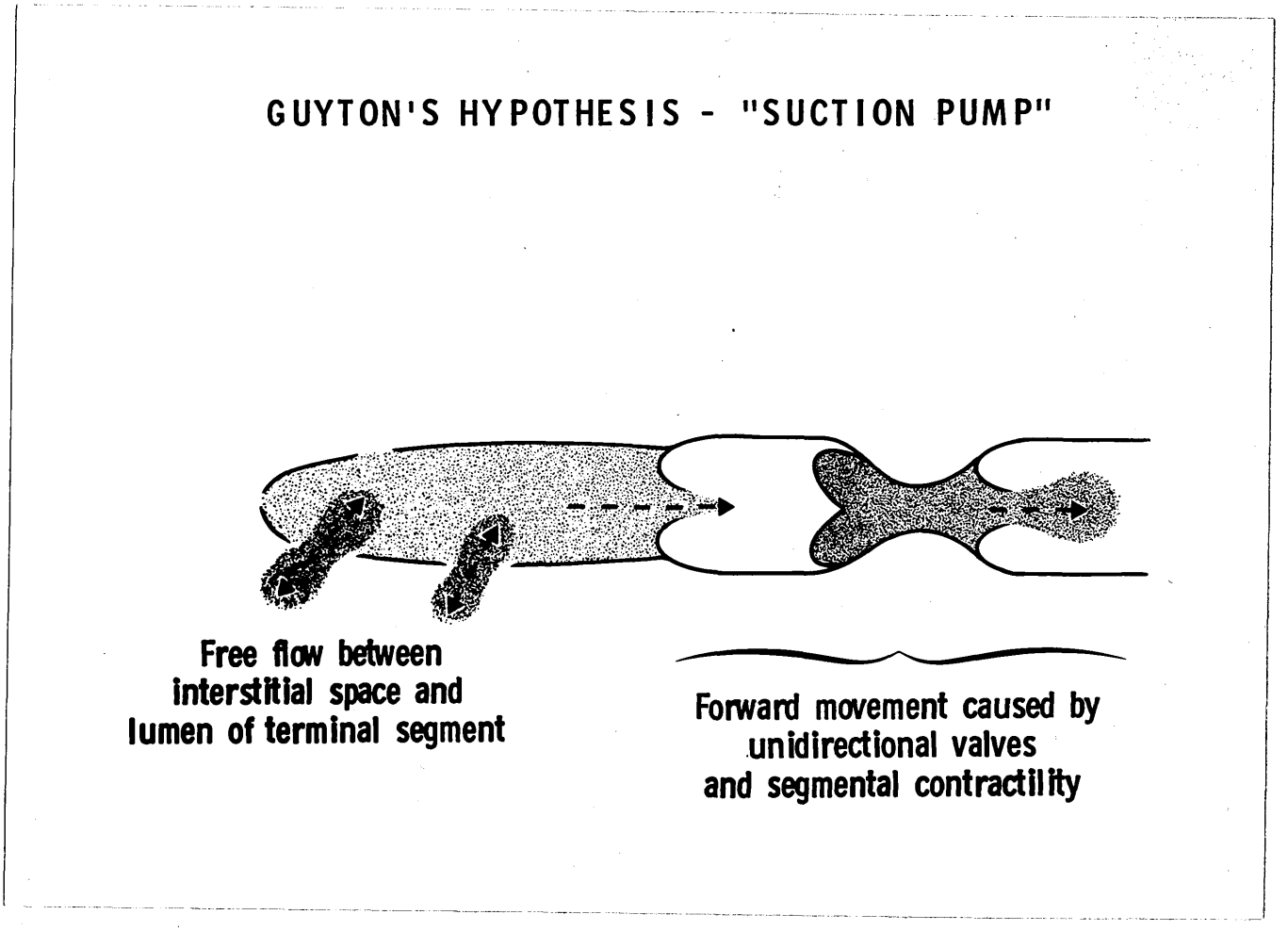


Figure 6

Guyton's Suction Pump Hypothesis.

mechanical process, achieved by the terminal buds of the lymphatics acting as a suction pump (Figure 6). This could occur if the lymph vessels are contractile and have competent valves. Contractions have been seen in the lymphatic capillaries in the wing of the American Bat and myofibrils seen in electron-microscopic studies of their walls (Hogan and Nicoll, 1979) but spontaneous activity has not been observed in the terminal buds of any other species.

Casley-Smith (1980b) has expanded this hypothesis by suggesting that the movement of fluids into the lymphatic capillaries is caused by a combination of filtration and osmosis. In the normal resting situation macromolecules and water enter the terminal segments of the lymphatics because there are large openings ("pores") between their endothelial cells which make the lumen of the terminal segment and the interstitial space, in effect, a single space.

Forward flow is caused by spontaneous contractions of the lymph trunks above the first valve which convert the lymphatic network into a series of force pumps, one between each pair of valves, the active emptying of the segment immediately upstream of the terminal segment reducing its intraluminal pressure to a level below that in the terminal segment.

If the terminal capillaries were freely permeable to molecules of all sizes in both directions, subsequent external compression would force some of their contents forwards into the next segment of the lymphatics but some would flow backwards into the interstitial space. The valves within the lymphatics would ensure some forward flow but a simple system such as this would

be relatively inefficient - a "leaky pump".

In the presence of a negative tissue fluid pressure a lymphatic "force pump" system such as this could only be efficient if the inflow through the pores of the terminal segments is unidirectional. When the terminal segment of the lymphatic system is empty and the surrounding tissues lax, the intercellular pores are open because there is little adhesion between adjacent cells, the basement membrane is poor and the tissue filaments, which adhere to the outer layer of these cells, pull them apart. Again, Casley-Smith suggests that, although the pores are open when the tissues are lax, they are closed by the increase of intralymphatic pressure that accompanies tissue compression during exercise. Thus, they act as unidirectional flap valves and prevent the reflux of water and large molecules back into the tissue space during external compression.

Casley-Smith (1980b), also postulates that although the closed pores of the terminal segments are impermeable to large molecules they remain permeable to small molecules such as water. If water can move out of the terminal segments when they contract or are compressed, their contents will be concentrated. The osmotic pressure will increase and assist the movement of water into the vessels during the next spell of tissue relaxation when the pores re-open. This implies that there is a cyclical dilution and concentration of lymph as a result of osmosis and the selective passage of large molecules through the pores, with the balance in favour of concentration (Figure 7). A mathematical model has shown this mechanism to be feasible and suggests that it would also possess the property of negative

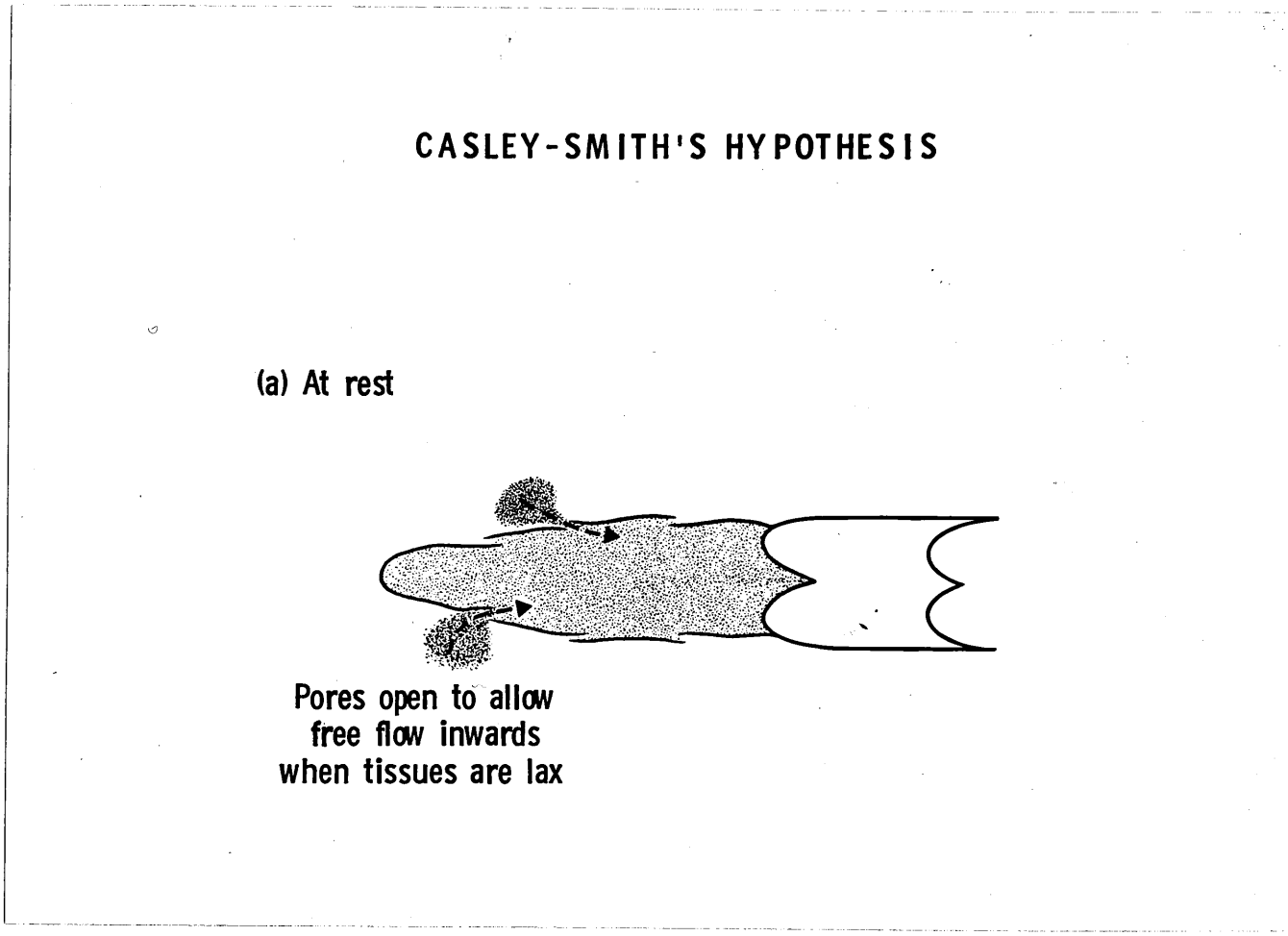


Figure 7a

Casley-Smith's "Osmotic" Hypothesis.
(a) at rest.

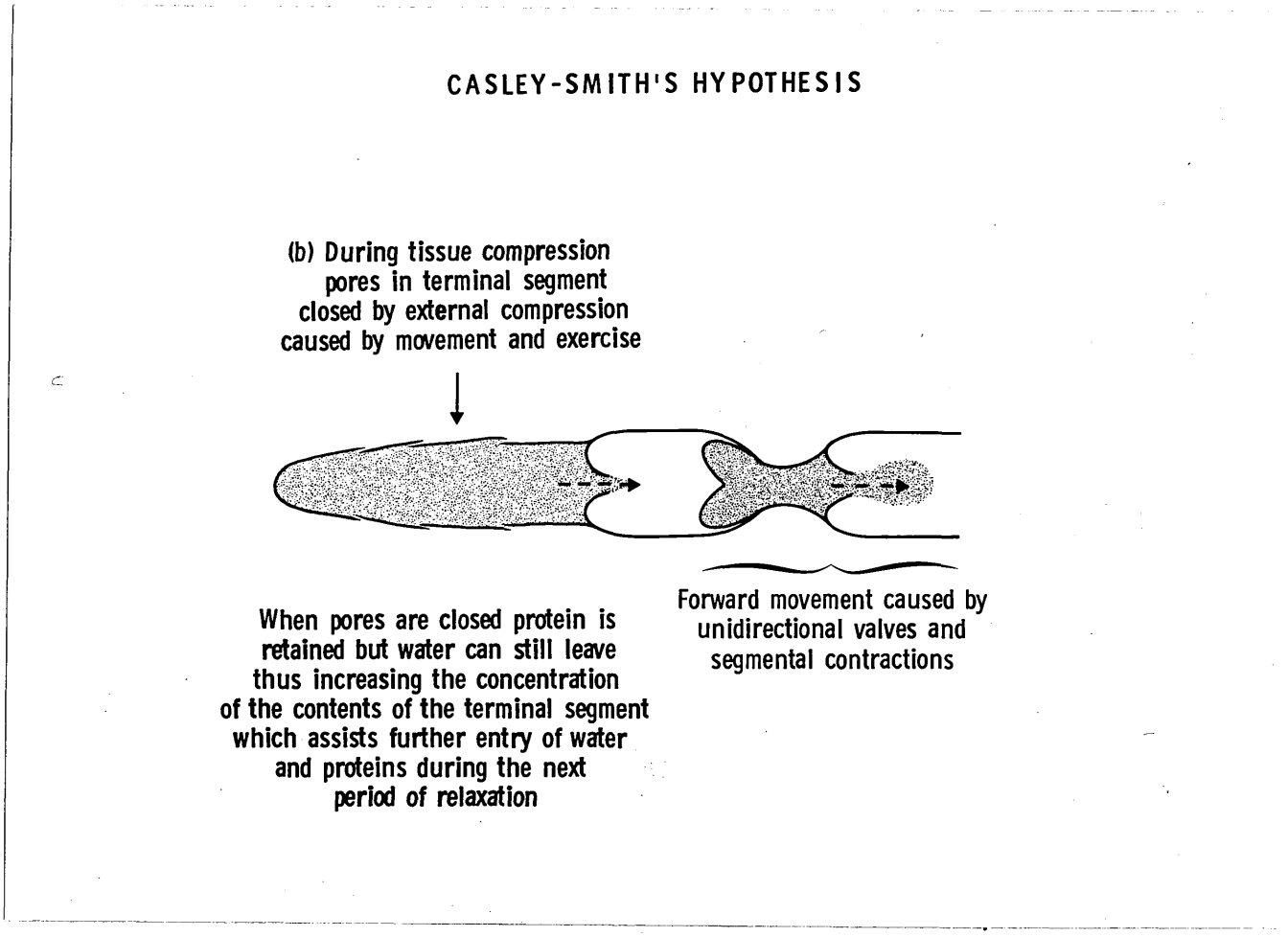


Figure 7b

Casley-Smith's "Osmotic" Hypothesis.
(b) During the active phase

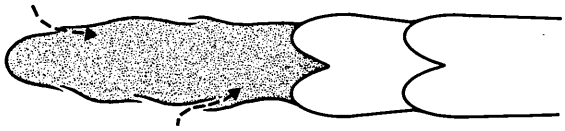
feedback (homeostasis) (Elhay and Casley-Smith 1976; Casley-Smith 1980a).

The movement of water and macromolecules from the interstitial space into the terminal lymphatics remains difficult to understand particularly in the presence of a negative interstitial fluid pressure. I have attempted to explain the modern hypotheses of this collection of lymph despite their complicated mechanisms. As yet, neither the existence of a lymphatic suction pump (Guyton) nor of an intermittent osmotic effect (Casley-Smith) has been proven.

The flow of interstitial fluid into the lymphatics may be explained by the suction effect of upstream segmental contraction and open pores in the terminal segments, whereas the high protein concentration of lymph is explained by the selective retention of protein by the terminal segments when their pores become semi-closed during tissue compression (Figure 8).

"AN HYPOTHESIS" FOR THE "COLLECTION OF LYMPH"

(a) Resting phase



Free flow inwards when tissues are lax due to temporary negative pressure within the terminal segment relative to interstitial pressure.

This leads to an increase of volume of contents and therefore pressure within the terminal segment combined with external compression by tissues and muscles on exercise and movement. This closes the 'pores' and thus fluid and molecules will move upstream.

Figure 8a

An hypothesis for the formation and collection of lymph.
a) At rest.

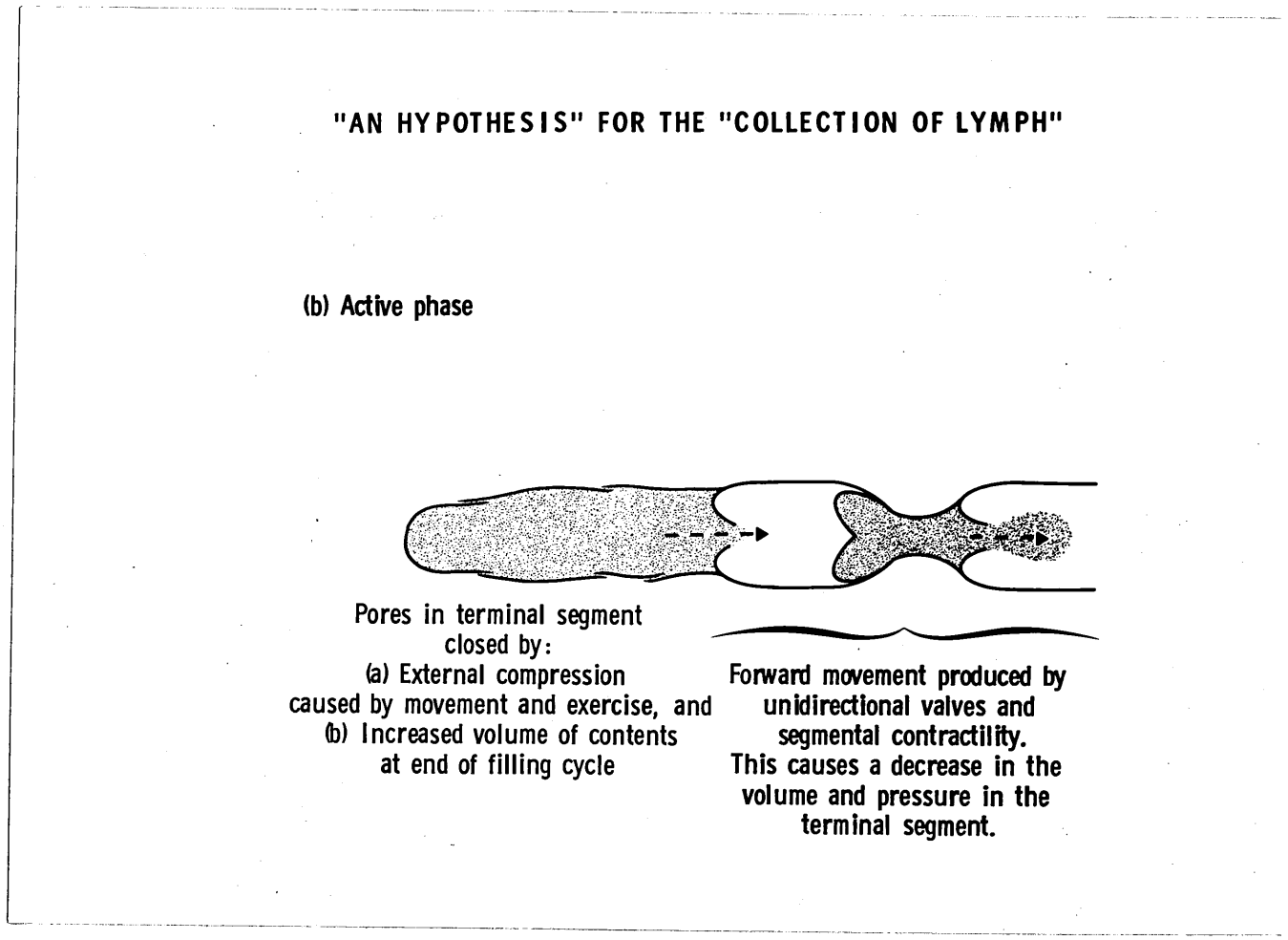


Figure 8b

An hypothesis for the formation and collection of lymph.
b) During the active phase.

Lymphatic Contractility

The walls of all lymphatics, except the terminal segments contain smooth muscle cells and nerves (Boggan and Palfrey, 1973). Spontaneous contractions of lymphatics were first noticed in birds, by Hewson in 1774, and in the mesenteric lymphatics of the guinea pig by Heller in 1869. In 1910 Lieben demonstrated a rhythmic contractility of 15-18 contractions per minute in the mesenteric vessels of the rat and in 1926 Carier observed similar contractions in the wing of the bat while Florey (1927 a/b) recorded them in the lacteals of the rat and guinea pig. Browse & Taylor, in 1969 studying the forward movement of Lipiodol in the thoracic duct of anaesthetised dogs with radiographic screening suggested that it may in part be caused by intrinsic contractions of the duct, but in subsequent studies of conscious dogs, reported in 1971, could find no physiological (manometric) evidence of intrinsic contractions. Roddie, McWhinney, McHale et al., (1980), studying the contractility of bovine mesenteric lymphatics in-vitro, observed spontaneous contractions every 30 seconds and increased their frequency by adding Norepinephrine or by stretching.

Spontaneous rhythmic contractions have been seen in the cysts of human retroperitoneal lymphangiomata (Kinmonth and Taylor 1956; Kinmonth and Sharpey-Schafer, Taylor, 1963; Olsewski, Kruszewski and Zeliczynski 1968), and Whimster and Browse (1976) have recorded spontaneous pressure waves of 5-15 mm/Hg at a frequency of 3 - 5 min in the closed cysts of lymphangiomata. In 1963 Szegvari, Lakos, Szontagh et al claimed to have seen spontaneous contractions in lymphatic vessels in man

during lymphography of the feet although Kinmonth and his colleagues (1982) have never seen such contractions in over 2000 lymphograms.

Olsewski and Engeset (1979 a/b) have attempted to measure the contractility of human foot lymphatics with indwelling catheters and reported regular pressure waves at 20 mm/Hg which sometimes rose to 50 mm/Hg. They postulate that external forces such as muscle contraction, arterial pulsation and the respiratory "vis a fronte" are not directly related to lymph flow (Engeset and Olsewski 1980). Their experiences in man suggest, but do not conclusively prove, that there are rhythmic intrinsic contractions of the lymphatics at rest and that lymph flow occurs during the waves of lymphatic contractions. They also claim that skeletal muscle contractions per se do not increase lymph flow but that muscular activity is associated with an increased number of intrinsic contractions. However, it could be that the increased amount of fluid forced into the lymphatics by the increased tissue pressure caused by muscular activity may itself stimulate lymphatic contractions. Massage may have a similar effect.

The sympathetic nervous system plays an important controlling role in the rest of the vacular tree. In 1968 Browse, in animal experiments, showed that the limb lymphatics contract in response to sympathetic nerve stimulation, so raising the possibility that lymphatic contractility and lymph propulsion may be under nervous control. Roddie and his co-workers (1980) have shown the lacteals respond to many pharmacological agents and Casley-Smith (1980b) suggests that intrinsic contractility may be auto-regulated to allow for variations in input. Although

there is no known mechanism through which such auto-regulation may be controlled, suitable receptors such as tissue pressure or stretch receptors and an effector muscle coat under sympathetic control both exist.

The many demonstrations by different techniques of spontaneous contractility of non-human lymph vessels make it likely that this property does exist in man and recent experimental work in man may confirm this.

Extrinsic Factors

Muscular Activity

It was originally thought that the motive power of flow through lymph vessels came chiefly from compression by voluntary muscle although the movement of other tissues including the pulsation of nearby blood vessels may play a part. McMaster (1937) showed that there is slow movement of lymph at rest by injecting very small volumes of dye (0.1 ml intradermally). The considerable increase in lymph flow during exercise is well recognised and some of the earliest work was performed on an unanaesthetised dog by White, Field and Drinker (1933).

It would be assumed that as muscular contraction raises tissue pressure, that this would increase lymph pressure. Hence lymph would move from this region of high pressure in the periphery to the thoracic duct and the blood circulation with the undirectional valves within the lymphatics directing the flow centripetally. Recent work, however, by Engeset and Olsewski (1979b) in man has shown that muscle contraction increases lymph flow by presenting more fluid to the system and increasing the frequency of lymphatic contractility rather than directly increasing lymph flow.

In summary, although the precise mechanism is not entirely clear there appears to be good evidence that muscular activity aids the central flow of lymph.

Thoracic and Abdominal Pressure

Changes in intrapleural pressures are transmitted to the mediastinal tissues (Meltzer 1892) and oesophagus (Fry, Stead, Ebert et al 1952). The negative intrathoracic pressure will act

as a suction pump and the phasic changes in pressure as a pumping mechanism, both propelling lymph through the mediastinum.

Yoffey and Courtice (1970) have shown that pressure on the abdominal wall over the cisterna chyli will accelerate flow. Any rise in intra-abdominal pressure due to straining, coughing or lifting will compress the lymphatics and therefore propel lymph towards the thoracic duct.

Pulsation of Blood Vessels

Surgical dissection of major arteries reveals a rich network of lymphatics around the vessels and it has been suggested that the pulsation of the blood massages the lymphatics and aids lymph flow. Parsons and McMaster (1938) demonstrated this using dye on the rabbit ear. A pulsatile perfusion of the blood vessels produced a much more rapid flow of the dye in the lymphatics than a constant pressure perfusion. The cisterna chyli, situated close to the aorta, is ideally placed to receive pulsation (Cressman and Blaloch 1939) and would support this view.

Venous Pressure

An acute rise in venous pressure increases resistance to the entrance of lymph into the left innominate vein and thus decreases flow in the thoracic duct (Wegria, Zekert, Walter et al., 1968). A localised venous obstruction to a limb will however lead to a considerable increase in lymph flow. This has been well recognised for a long time and the findings have been verified in many experiments (Field and Drinker, 1931; White, et al., 1933; Szabo, Magyar and Papp, 1963).

Gravity

The effect of gravity in the pooling of venous blood in the lower extremities and the effects of G forces es well known. In a low flow system such as the lymphatic system it would be surprising if gravity did not affect flow rates. Experimental evidence of the effect of gravity was produced by Entrup, Paiewonsky, Hughes et al., (1966). They showed that when a dog stood on its hind legs lymph flow in the thoracic duct was reduced and when the animal returned to the horizontal position lymph flow increased returning to the control level.

Valves and Lymph Flow

I have discussed so far the various mechanisms which may propel lymph; intrinsic contractility, muscle activity, blood vessel pulsation, thoracic and abdominal pressure. It must be stressed however, that none of these mechanisms would be particularly efficient without an adequate valvular system directing the flow centrally. The more phylogenetically developed the species the more valves that are present within the system and their presence in the human species is vital to the centripetal flow of lymph (Kampmeir, 1927-1928).

Lymph Nodes and Lymph Flow

Lymph flow at rest is sluggish and at a low pressure. Under these circumstances the lymph nodes may produce a significant resistance to flow. Virchow (1860) was the first to postulate that lymph nodes act as a barrier; he stated "the elements lie crowded together like the particles of a charcoal filter, so that the lymph trickles out again on the other side in a more or less

purified state". Thus particle or bacterial filtration appears to be very efficient. Drinker, Field and Homans (1934) perfused a node with a stream of haemolytic streptococcus and found, by sampling efferent lymph, that the filter was 99% effective.

The lymphatic system is more complex in the more highly developed vertebrates. Amphibians and reptiles have only unvalved vessels but birds, which are phylogenetically more developed, have simple lymph nodes interrupting the vessels, although these do not interrupt all the lymphatic channels before they enter the blood circulation.

The number of nodes in a bird is very small and, generally speaking, the higher the vertebrate is up the phylogenetic scale the more lymph nodes are present. For example, the dog, cat and rabbit have only a single node along the course of the deep cervical ducts, whereas the monkey has five or six and man even more.

In mammals, the lymph draining from the periphery always passes through at least one lymph node (Drinker et al., 1934) and there is only a single major connection between the lymphatic and the venous systems, namely the termination of the thoracic duct.

The pathway is completely interrupted at each node, which must lead to greater filtering capacity but may also lead to a greater resistance to flow. Certainly Vialleton (1903) observed that valves in the lymphatic vessels and interruption to flow by lymph nodes appear, phylogenetically, at the same time.

More recently Browse, Doig and Sizeland, (1970) and Papp, Makara and Hajtman, (1971) measured the lymph node resistance to

flow in dogs. They found that the lymph node produced a considerable resistance to flow which decreased as lymph flow increased. The node became swollen and water logged at high flow rates, suggesting that the decreased resistance was due to the dilated sinuses. At high flow rates the resistance to flow is reduced. This fact should be remembered when considering the functional significance of visualising the free flow of Lipiodol through lymph nodes as this is infused at an unphysiological rate and therefore any measurement of function of the lymphatic system must avoid nodal pressure changes.

Browse et al (1970) also found that node resistance rose with venous engorgement and fell with a reduced arterial pressure. They attributed these changes to the altered blood volume within the node affecting the space available for lymph. At low flow rates these effects on lymph flow will be enhanced.

Lymph nodes in mammals arise from lymphatic plexuses by the proliferation of mesenchymal elements into the meshes of the vascular plexuses (Sabin 1902) and thus lymph nodes derive originally from a lymphatic vessel. It appears that once an organism is sufficiently complex to require a lymphatic system for the circulation of tissue fluid, it becomes necessary to halt the rapid dissemination of a single focus of a noxious agent by filtering the lymph at lymph nodes. As the organism becomes highly developed it will require a greater filtering capacity and thus more lymph nodes. This effective filtration of lymph however occurs at the expense of increasing the resistance to flow.

The preceding review of the historical and physiological aspects of the lymphatic system forms the basis for interpretation and investigation of the pathology of the lymphatic system and in particular lymphoedema. The next chapter will outline the past and current classifications, known pathology, and aetiology of lymphoedema.

CHAPTER 2

THE PATHOPHYSIOLOGY AND CLASSIFICATION OF LYMPHOEDEMA

Chance is a word devoid of sense;
Nothing can exist without a cause

Voltaire

Lymphoedema is a pathological condition caused by the accumulation of lymph in the interstitial spaces, principally of the subcutaneous fat, caused by a fault in the lymphatic system.

Rudbeck and Bartholin, already referred to, ascribed oedema and ascites to lymphatic occlusion. Indeed throughout the 17th and 18th centuries the idea that accumulation of lymph was due to interruption of the lymphatic pathways was generally agreed. Elephantiasis was counted amongst a wide range of conditions held to be due to lymphatic disorders.

Actual proof that interruption of the lymph drainage of a part leads to oedema awaited the classical experiments of Drinker, Field and Homans (1934) which involved repeated injections of a suspension of fine silica and quinine hydrochloride into the lymphatics of a dog hind limb. They eventually produced a prolonged brawny oedema of the limb.

They noted that the protein content of the oedema fluid steadily increased and also that the limb was particularly susceptible to Streptococcal infections. Almost certainly Drinker and his colleagues had indeed produced lymphatic obstruction. However, the blockage would have been due to a chemical endolymphangitis, a process not comparable with primary or most types of secondary lymphoedema.

The difficulty in producing lymphoedema by simple division of the lymphatic pathways was illustrated by Reichert (1926) who found that the extreme capacity for lymphatics to regenerate frustrated his attempts at the production of a permanent oedema. However, recent attempts to produce complete lymphatic blockade by surgical means have been more successful (Olszewski,

Machowski, Sokolowski et al., 1980; Clodius and Wirth, 1974; Clodius, 1977) and provide better evidence that lymphatic obstruction and clinical oedema may be related.

The aetiology of primary lymphoedema however remains obscure and while there are many descriptive classifications of the causes of lymphoedema, none are based on the disordered physiology.

Current Classifications

Matas produced the first definition of lymphoedema in 1913. He stated that it was "a progressive pathologic state or condition characterised by chronic inflammatory fibromatosis and hypertrophy of the hypodermal and dermal connective tissues". He commented that "the high protein concentration within the tissues and the disruption of the immunoactive functions of the lymphatic system make the lymphoedematous extremity increasingly susceptible to infections".

In the 1930's Allen suggested that primary lymphoedema was caused by a congenital under-development of lymph vessels and he described two clinical varieties: Congenital - present at births: and Praecox - presenting in early life. In 1946 he reviewed the 300 cases of lymphoedema he had seen at the Mayo Clinic and classified them as inflammatory and non-inflammatory and further subdivided them into primary and secondary.

This was neither a logical nor practical classification and was replaced by Kinmonth in 1957 by a clinical classification which divided all cases into primary and secondary, but gave no indication of the cause of the primary variety although it did

emphasise that primary lymphoedeam had three ages of onset - praecox; and tarda - after 35 years of age.

Between 1950 and 1960 Kinmonth introduced X-Ray lymphangiography and subsequently devised a radiological classification based on 100 lymphographs (1969). He described three radiological appearances:

No vessels visualised - which he called aplasia.

A reduced number of vessels visible - hypoplasia.

An increased number of dilated vessels visible - hyperplasia.

In 1967 Kaindl, Mannheimer, Pfleger et al suggested various descriptive terms as substitutes for the term "hypoplasia", based on the histological appearance of the lymphatics. Their classification of lymphoedema was:

Hypoplasia and aplasia;

Lymphangiopathia Obliterans;

Ectasia (Kinmonth's hyperplasia group);

Milroy's disease;

Lymphangitis;

This classification has not been generally adopted as it combines in a confusing manner clinical, radiological and pathological features.

In 1970 Craig produced yet another classification based on the lymphograms of 40 patients with lymphoedema. He combined hyperplasia and megalymphatics under "lymphangiectasias" and suggested as a new cause of lymphoedema "leaking lymphatics" ("lymphatica porosa") in which the only finding was extravasation of contrast medium through the lymph vessel wall. As his

lymphograms were usually performed with a water soluble contrast medium the extravasation was almost certainly an artefactual appearance as these media diffuse easily through the lymphatic wall.

"Lymphatica porosa" is a term originally used by Buoncone and Young (1965) to describe the radiological appearance, seen in both normal and abnormal lymphatics, when they are filled with a water soluble contrast medium or with Lipiodol at an excessively high pressure. Further proof is needed before accepting this appearance as a true causative abnormality of lymphoedema and not an artefact.

Kinmonth's radiological classification of primary lymphoedema was recently expanded and is widely used. However, the suffix "plasia", with its various prefixes means growth. The use of these terms as adjectives describing an anatomical state is confusing because they imply the type of growth that caused the lymphatics to reach their existing state. It may also be confusing to use the term aplasia when the radiological absence of the vessels might have been caused by technical failure or quirks of filling related to the site and nature of the injection during lymphography. For example, when Kinmonth (1977) started exploring the subcutaneous tissues at the level of knee or groin when no vessels were found in the foot, they often found vessels in the thigh and always found iliac vessels and nodes. As Kinmonth's classification is based on pedal lymphography these cases would have been called aplasia - no lymphatics - but this would have been a false description. True aplasia does occur but is very rare and likely to be associated with those lymphoedemas

that are present at birth.

These various technical advances associated with contrast materials, methods of injection, and the use of an operating microscope have led to continuous adjustments in the proportions of patients assigned to each group in Kinmonth's radiological classification. Table 1 shows the results of lymphography in five reported series between 1958 and 1982.

Table 1

The Proportions of Patients within the separate Lymphographic Groups in five reported series 1958-1982

Author	Hypoplasia	Aplasia	Hyperplasia	Total no. of patients
Kinmonth, Taylor Tracy & Marsh (1957)	61%	14%	24%	107
Kinmonth (1960)	71%	15%	14%	192
Gough (1966)	74%	20%	6%	66
Kinmonth (1972)	87%	5%	8%	100
Kinmonth (1982)	90%	NIL	10%	562

The most striking feature on review of these five series is that the "hypoplasia" group has been expanded at the expense of both of the other groups. This reflects the increasing ability to demonstrate very fine attenuated vessels which may formerly have been unrecognized. Additionally, the true incidence of hyperplastic or magalymphatics was undoubtedly over-estimated in the early days on account of the relative ease with which they could be identified in comparison with the "hypoplastic" vessels.

A further reason for the decline in the incidence of failure to demonstrate lymphatics (aplasia) has been the recent practice of obtaining visualization of the proximal lymphatics by means of direct node puncture in those cases where no vessels could be found in the foot (Kinmonth, 1977). If lymphangiograms are successfully produced by this means, a classification of (distal) hypoplasia would be generally made, rather than aplasia as would have applied if the node puncture had not been made. In this study the term "aplasia" has been considered inappropriate and the patients classified as hypoplasia.

Another problem with a radiological classification is the observation that X-Ray appearances may change with time. Sequential lymphography has recorded the progressive disappearance of lymphatics in long-standing lymphoedema in the lower limb following block dissection (Danese and Howard, 1965; Jackson, 1966) and careful dissection during lymphography often reveals thrombosed lymphatics in the feet of patients with "radiological aplasia". This progressive disappearance of

lymphatics has also been termed the "die-back" phenomenon, (Fyfe, Wolfe and Kinmonth, 1982) another term which unjustifiably implies the causative mechanism. According to the radiological classification such cases change from "radiological hypoplasia" to "radiological aplasia" an incorrect use of English.

The above review of the current classifications of lymphoedema reveals the confusion surrounding the disease. There is no problem in understanding the way in which neoplastic infiltration in lymph nodes or lymph node excision causes lymphoedema but the lymphographs of primary lymphoedema usually give little or no clue to its aetiology.

The Pathophysiology of Lymphoedema

The prime function of the lymphatic system is to clear the interstitial spaces of excess water, large molecules, and particles, and to transport them from the tissues back to the intravascular circulation.

The hypothesis of Starling (1896) already referred to states that the exchange of water and small molecules across the capillary membrane is largely governed by the transmural capillary hydrostatic and colloid osmotic pressures, the colloid osmotic pressure being dependent upon the relative impermeability of the capillary membrane to plasma protein. This concept has been verified by direct experimental measurements (Landis, 1927 a,b; Pappenheimer and Soto-Riviera, 1948).

It has been known since 1896 that a proportion of the plasma proteins passes through the capillary wall but not all of it returns directly to the circulation. The concept of the lymphatic system as absorbing vessels, whose main function is to return to the bloodstream those protein molecules that fail to return return via the venular capillaries was first elaborated by Drinker and Field in 1931; a concept that has been confirmed repeatedly with experiments using radio-active labelled plasma proteins (Wasserman and Mayerson, 1951; Yoffey and Courtice, 1956).

Iodine labelled albumin studies in man indicate a daily exchange between the intra and extravascular pools of 140% of the total body albumin. Mayerson (1963) showed that between one half and all of the circulating proteins leave the intravascular compartment every 24 hours although Szabo (1978) showed in

animal experiments that only 38% of the proteins that escape from the blood are actually cleared by the lymphatics, the remainder returning via the venular capillaries.

Failure of any part of the lymphatic system will inevitably result in the accumulation of plasma proteins in the interstitial fluid and therefore lead to an increase in interstitial fluid colloid osmotic pressure and the movement of more water into the interstitial space, i.e., oedema.

Foldi (1972) called the task of the lymphatics of removing the plasma proteins from the interstitial space and returning them to the blood "the lymphatic transport function". He defined its capacity as the product of two factors, (i) the total cross sectional area of the vessels and (ii) the lympho-kinetic forces, i.e. those forces that produce the movement of lymph: the lymphatic valves, segmental lymphatic contractility and external factors such as muscle compression, respiration and negative intrathoracic pressure.

Clodius (1977) postulated that lymphoedema was caused by either a block in the lymphatics (a reduced cross-sectional area) or a malfunction of the pumping mechanisms in the collecting lymphatics (a reduced lymphokinetic force) and that either would cause an increase in the intralymphatic pressure so reducing the transmural pressure gradient in the terminal lymphatics that was originally thought to be responsible for the inflow of the water and protein into them.

Thus, lymphoedema may be defined as a disturbance of the equilibrium between the load to be cleared and the transport capacity of the clearing system, the load being the protein and

Potential abnormalities in the collection and transport of lymph

The mechanisms controlling the collection and passage of lymph, from the interstitial spaces to the blood system, which has been described by Marlys Witte as "the fantastic voyage", remain largely unknown. The various stages of lymph collection and transport have been discussed earlier in this chapter, and their potential abnormalities will be discussed in the following section, under separate headings (Figure 9).

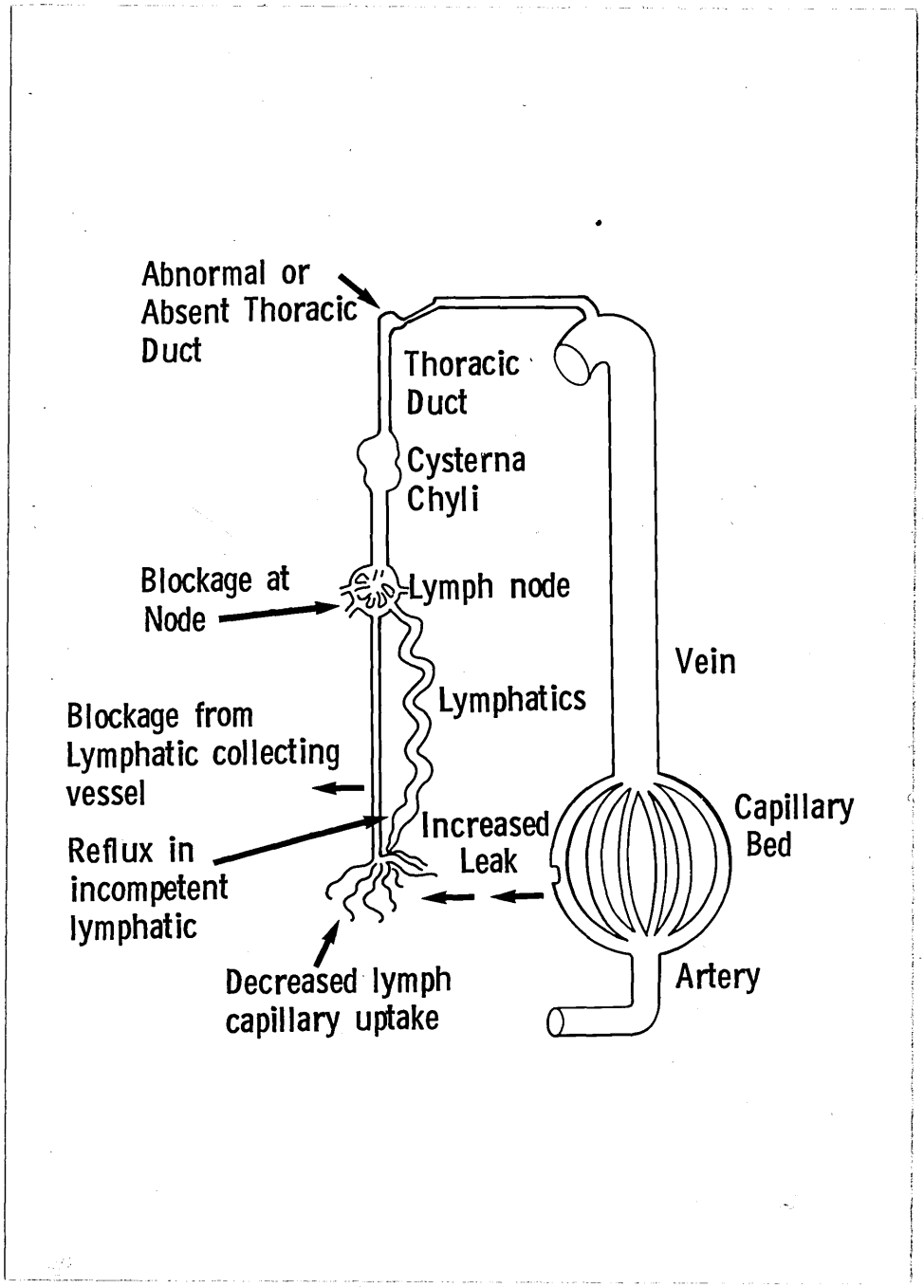


Figure 9

Potential abnormalities in the collection and the transport of lymph.

1) Overload of the System

It is now generally accepted that the composition of lymph is principally determined by the permeability of the blood capillaries and that lymph originates as an ultrafiltrate of plasma which is modified in its course through the tissues before it enters the lymphatic system. Lymph flow will therefore be closely related to the formation of tissue fluid. When the formation of tissue fluid is increased lymph flow will normally increase to restore the balance. If the rate of formation of tissue fluid is greater than the rate of its removal, oedema may result even when the lymphatic system is normal.

The capillary wall becomes more permeable in several pathological conditions. Injury by trauma, heat, irradiation, and infections all cause oedema secondary to increased capillary permeability. Venous congestion causes oedema by preventing reabsorption in the venular capillaries. Provided the lymphatic pathways are normal this type of oedema is eventually cleared, although it may take longer to resolve than the initial injury.

2) Inadequate Collection by the Lymphatic Terminal Buds

It has been suggested (McKendry, Lindsay and Gerstein, 1957) that the connections between the initial lymphatics and the collectors may be deficient although Casley-Smith (1983) has shown that the initial lymphatics gradually fade into the collecting lymphatics without any clear demarcation. A further possibility is that the connective tissue channels that Casley Smith suggests may carry interstitial fluid from the blood to lymphatic capillaries may be fewer in number or narrower than normal.

Casley-Smith believes that the minor changes of tissue pressure that are said to effect the opening and closing of the pores are absent because the interstitial pressure is both positive and relatively constant in lymphoedema. The pores may be kept permanently closed by the increased tension in the filaments attached to the endothelial cells caused by the increased tissue pressure and tissue fibrosis, a situation which will prevent their alternative opening and closing and so make the collection system inadequate or incompetent.

3) Abnormal Lymph Transport

The many demonstrations of spontaneous contactility in animal lymph vessels and the recent experimental work in man discussed previously make it likely that autonomous lymphatic contractility does exist in man. Studies of this spontaneous rhythmic activity of lymphatics and of the functional organisation of their co-ordinated mechanisms suggest a remarkably efficient system for the transport of lymph (Mislin 1983). Any abnormality or derangement of this intrinsic "pump" would therefore result in an inefficient transport of lymph although such a deficiency has not yet been demonstrated to be a cause of lymphoedema.

Lymphatics react to distension by increasing the force of frequency of their pumping but if the vessels are overdistended the valves adjacent to the distension will become incompetent and thus the lymphatic units or "lymphangions" peripheral to it will become progressively more distended as each downstream valve becomes incompetent. This will obviously contribute to the "failure" of the transport of lymph where there is partial or

complete proximal obstruction to flow but incompetent valves occur independent of proximal obstruction in megalymphatics seen in lymphography. This disorder is thought to be congenital in nature (Kinmonth, 1982) and one in which there is a primary valvular incompetence.

4) Insufficient Lymphatics

In 1934 Allen suggested that primary lymphoedema was caused by the underdevelopment of lymphatic vessels. X-Ray lymphographic assessment undoutedly confirms that the majority of patients with lymphoedema have a reduced number of lymphatics. Whether the patient is born with this reduced number which manifests itself as lymphoedema in later life or whether it reflects an acquired occlusion following some form of damage to the lymph vessels is often unclear. Whatever the cause, the reduced number of lymphatics are eventually inadequate, particularly following incidents which cause oedema such trauma or inflammation.

It would be of great value if we could decide whether a patient with a reduced number of lymphatics had a true congenital abnormality or an acquired obliteration. Tissue biopsy and serial sections might reveal blocked lymphatics but any such technique runs the risk of damaging any remaining normal lymphatics and making the clinical problem worse. Biopsies of tissue strands removed at lymphangiography because they are thought to be obliterated lymphatics are not very helpful. Thus at present it is not possible to clearly differentiate between a congenital deficiency and an acquired occlusion.

5) Lymph Node Obstruction

The lymphoedema that follows a block dissection of the groin or axilla is well recognised. However, in 1966 Gough suggested that some forms of "primary" lymphoedema were caused by proximal obstruction to flow and in recent years some cases of primary lymphoedema have been found to have lymph node abnormalities. Histological studies of the lymph vessels in primary lymphoedema have failed to reveal any primary abnormality but have detected changes that could be attributed to obstruction and lymph stasis changes (Boggan and Palfrey, 1973), similar to those seen in known secondary obstructive lymphoedema such as that caused by filarial tropical elephantiasis (Manson, 1898).

In 1976 Price attributed non-filarial tropical primary lymphoedema to the deposition of silica particles from the soil in the lymph nodes after finding a relationship between the amount of silica in the nodes, the prevalence of lymphoedema and the concentration of silica in the soil (Heather and Price, 1972; Price and Pitwell, 1972). Kinmonth and Wolfe (1980) have shown that some patients with unilateral whole leg lymphoedema have small shrunken lymph nodes with an increased amount of fibrous tissue in the hilum. They described a radiological appearance which suggests that the hilar fibrosis causes obstruction to lymph flow. They argued that obstruction would cause dilatation of the lymphatics which would spread peripherally from the obstructing site as each downstream valve becomes incompetent until finally the most peripheral terminal lymphatics become dilated and damaged. The vessels longest distended and most damaged would be the first to be occluded by

epithelial proliferation or fibrin thrombus and explain the "die back" phenomenon following block dissection described by Jackson (1966). Lesser degrees of node obstruction might cause a slower obliteration without much distension.

The frequent lymphographic finding of slightly distended peripheral lymphatics and a reduced number of proximal vessels supports this hypothesis. The other two common radiological appearances (a) small fibrotic nodes with distal vessel distension, and (b) small fibrotic nodes with a reduced number of distal vessels may also reflect opposite ends of the spectrum of the same disease process rather than two different processes, but as these appearances are totally different from the dilated tortuous vessels and collaterals seen in secondary lymph node obstruction these causal hypotheses must be viewed with caution and their acceptance await further study and better supporting evidence.

6) Central Vessel Defects

Congenital or acquired abnnormalities of the central abdominal or thoracic collecting ducts may cause simple lymphoedema or chylous reflux. If chyle refluxes into the limb lymphatics it may appear at various sites commonly in cutaneous vesicles in the proximal part of a lymphoedematous limb but may also appear in other regions such as the peritoneum (chylous ascites), the pleura (chylothorax), the kidneys (chyluria) and the uterus and vagina(chylo-metrorrhagia). Lymphography shows dilated incompetent "mega-lymphatics" in the abdomen and limb.

This disorder was summarised by Kinmonth (1982) "There are large tortuous incompetent lymph trunks extending from the area

of the cysterna chyli down through the retroperitoneal lymphatics into the root of one limb and often lower. The association with congenital vascular anomalies, e.g. cutaneous angiomata and the early age of onset, suggest an embryonic developmental origin of the lymphatic obstruction. The basic lesion is an obstruction to lymph conduction caused by either a primary valvular incompetence or a mechanical blockage above the point where the mesenteric lymphatics join the pre-aortic lymph vessels. In some patients this obstruction may be in the intrathoracic or even the cervical part of the thoracic duct."

Although congenital abnormalities of the thoracic duct may be symptom-less, they usually cause bilateral lymphoedema with moderate dilatation of the vessels and nodes but without chylous reflux, or the full-blown picture of chylous reflux described above. Acquired obstruction of the thoracic duct, in children or adults, through trauma, mediastinitis, tumours, venous thrombosis or surgery is often symptom-less. It may occasionally cause chylothorax but hardly ever causes peripheral lymphoedema. Surgical ligation of the termination of the thoracic duct rarely causes clinical problems because there is frequently a smaller duct on the right side draining into the right jugular vein and other distal lymphovenous anastomoses.

Secondary Changes in Lymphoedematous Tissues

The oedema of lymphatic origin is associated with a high interstitial fluid protein concentration (Crocket, 1956). Taylor, Kinmonth and Dangerfield (1958) found a direct relationship between the severity and durationof the oedema and the protein concentration in the tissue fluid and Casley-Smith and Piller (1974) have shown that failure to remove proteins from the interstitial spaces steadily increased its colloid osmotic pressure.

The exchange of protein and fluid between the tissues and the blood continues even in a lymphoedematous limb, but a state is reached when there is no net removal and then a net increase in the retention of protein and fluid. As early as 1934 Drinker et al suggested that the fibrosis and the overgrowth of interstitial connective tissue in a lymphoedematous limb was the reaction to the excess interstitial protein particularly the fibrinogen.

Piller (1980) postulated that macrophages also play a role in the pathogenesis of lymphoedema. He suggested that lymphoedema is a progressive pathophysiological process in which the excess accumulation of protein and fluid stimulate inflammation and macrophage activity. The presence of excess protein undoubtedly causes a reaction similar to chronic inflammation and in their attempts to lyse proteins, macrophages can produce substances that are toxic to other cells. Piller also postulated that macrophage and general phagocytic function are depressed in lymphoedema, a hypothesis that forms the rationale for the treatment of lymphoedema with benzpyrone

drugs (Piller and Clodius, 1976; Clodius and Piller 1978).

The presence of nearly all the plasma proteins in lymph and therefore interstitial fluid, has been verified, (Szabo et al., 1963) although quantitative data on individual protein species is conspicuously absent.

The fact that lymph will clot despite an absence of platelets is well known. Langdell, Bowersox, Weaver et al (1960) have shown that the fibrinogen concentration of lymph is 40-60% that of plasma. Lymph clots more slowly than plasma but contains little or no fibrinolytic inhibitors (Blomstrand, Nilsson and Dahlback, 1963). The coagulative properties of lymph and its possible role in the pathogenesis of patients with the thrombosed/obliterated type of lymphoedema is unknown. The investigation of the composition of lymph, particularly its protein elements, may improve our knowledge of the aetiology of primary lymphoedema.

Summary

The foregoing review of the pathophysiology and the current classification of primary lymphoedema reflects the confusion surrounding this disease. In this study I have based the subdivision of patients with primary lymphoedema on Kinmonth's classification (1982) with some modifications described below.

It is helpful to begin by defining primary lymphoedema as lymphoedema caused by a primary abnormality or disease of the lymph conducting elements of the lymph vessels or lymph nodes.

Secondary lymphoedema is lymphoedema caused by disease in the nodes or vessels that began elsewhere (eg neoplastic or filiarial infiltration of the lymph nodes) or began in the cellular non-conducting elements of the nodes (eg the lymphocytic proliferative disorder) or following surgical extirpation of the lymph nodes or vessels. Patients with any form of secondary lymphoedema were excluded from this study.

There are three groups of primary lymphoedema in which the functional abnormality and its cause is known; lymphoedema caused by large vessel abnormalities such as congenital obstruction or aplasia of the thoracic duct and cysterna chyli, lymphoedema caused by congenital lymphatic valvular incompetence or congenital aplasia, and lymphoedema caused by fibrosis in the hilum of the lymph nodes.

The remaining groups, which constitute the majority are those lymphoedemas in which lymphography reveals a reduced number of lymphatics. The age of onset helps clarify some of these. Patients that have this abnormality present at or within a few

years of birth can be considered to have been born with too few lymphatics i.e., they probably have true congenital aplasia or hypoplasia. Those who present at a later age often have histological evidence of "thrombosed" or obliterated lymphatics and, therefore, may have an acquired not a congenital disease.

Lymph vessel obliteration may affect the distal or the proximal vessels of the limb. When the proximal vessels are involved we cannot say whether the vessels have become occluded secondary to lymph node disease or independently, but whatever the mechanism there are likely to be secondary changes in those vessels that remain patent distal to the obstruction.

In summary, primary lymphoedema is lymphoedema caused by abnormalities or disease originating in the lymphatics or the lymph conducting elements of the lymph nodes and can be classified as follows:

A. Congenital

1. Congenital aplasia or hypoplasia of peripheral lymphatics (Figure 10). (Lymphographic abnormality and/or oedema present at or appearing within 2 years of birth).
2. Congenital abnormalities of the abdominal or thoracic lymph trunks (Figures 11 and 12).
3. Congenital valvular incompetence. This is always associated with megalymphatics and often with chylous reflux (Figure 13). Bilateral hyperplasia is a subdivision of this group.

B. Acquired

These form the majority of the primary lymphoedemas.

4. Lymphatic obliteration (Kinmonth's Hypoplasia Group)

   (a) Distal Hypoplasia - Acquired obliteration of the distal limb lymphatics, (Figure 14).

   (b) proximal hypoplasia - acquired obliteration of the lymphatics in the proximal part of the limb, usually associated with distal dilatation, (Figure 15).

   (c) Combined - Acquired obliteration of all the lymphatics of the limb (Figure 16).

5. Obstruction by the Lymph Nodes (Figure 17)

   Obliteration of the lymph conducting pathways through the node by hilar fibrosis. This may cause the changes classified above as hypoplasia, i.e., types 4 and 5 often co-exist. Acquired valvular incompetence may follow any form of obstruction.

As methods of investigating the possible deficiencies in lymphatic function and tissue proteolysis improve, it may be possible to redefine the cause of lymphoedema in a more physiological way rather than by radiological appearances and therefore to devise better methods of treatment.

This thesis is devoted to establishing a new method of investigating the lymphatic system, with particular reference to the primary lymphoedemas. In the next chapter I will outline the development and current role of lymphography which has been the accepted method of investigating the system for the past 30 years and introduce the concept of radionuclide investigation of the lymph system.

Figure 10

Congenital Lymphoedema of right lower limb due to hypoplasia of main lymph trunks and inguinal and iliac nodes causing distension and tortuosity of dermal lymphatics.

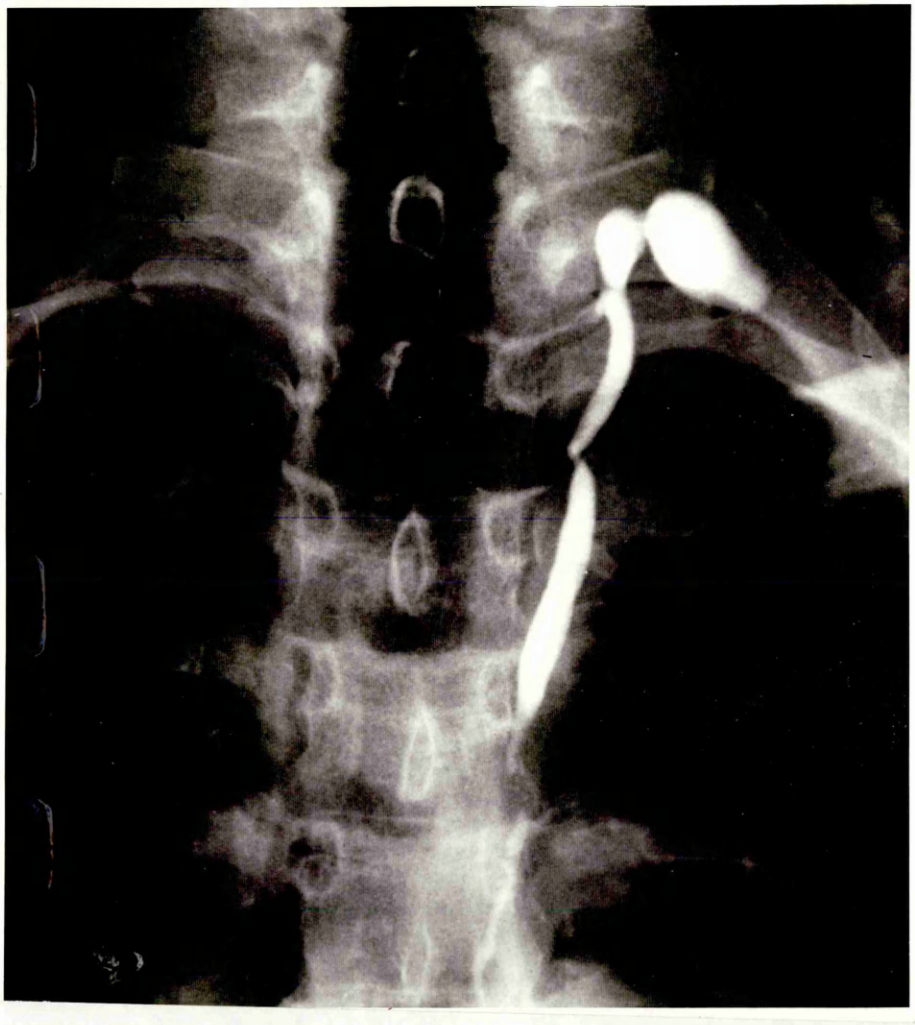


Figure 11a

Lymphographs of the thoracic duct:
a) Normal thoracic duct.

Figure 11b

Lymphographs of the thoracic duct:
b) Abnormal thoracic duct with fragmented appearance and diversion of lipiodol into collateral vessels in the supraclavicular fossa.

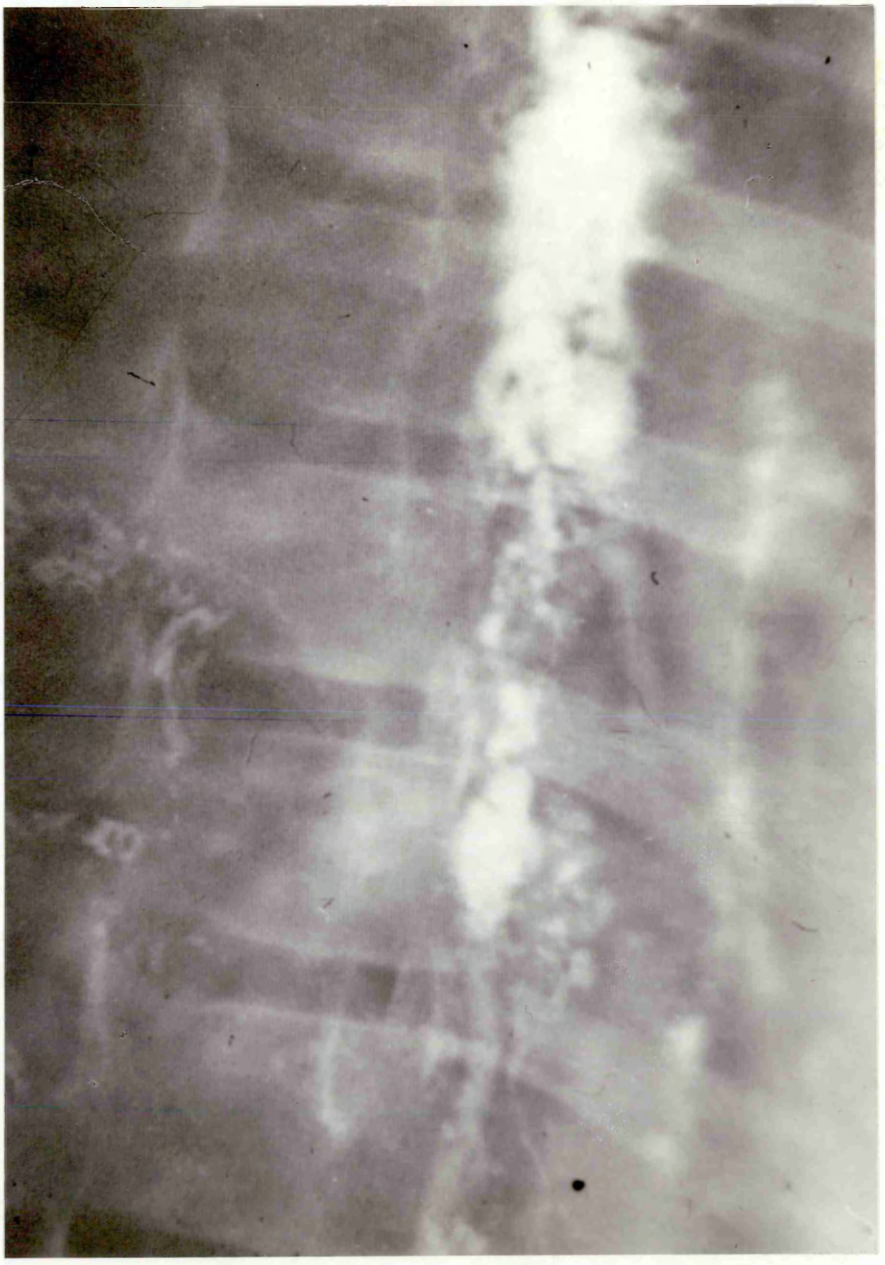


Figure 12

Marked congenital abnormality of lower thoracic duct in young boy who presented with chylothorax and chylous ascites.

Figure 13

Congenital incompetent megalymphatics of left lower limb in patient who presented with swelling of the whole limb.

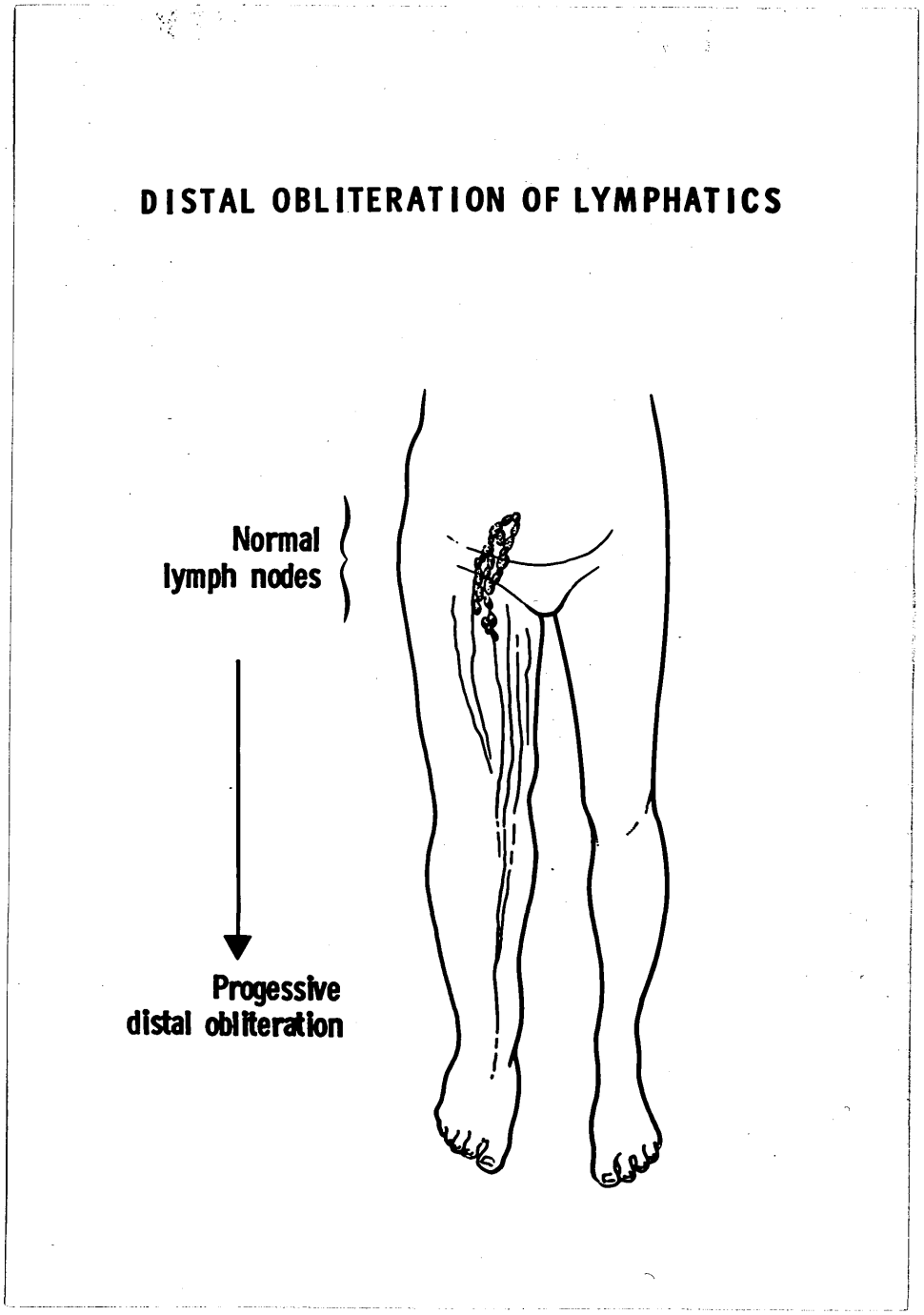


Figure 14a

Acquired obliteration of distal limb lymphatics:
a) Diagrammatic representaion

Figure 14b

Acquired obliteration of distal limb lymphatics:
b) Lymphograph of thighs, 1 vessel on right, 4 vessels on left.

Figure 14c

Acquired obliteraion of distal limb lymphatics:
c) Lymphograph of both lower legs, only one vessel visualized.

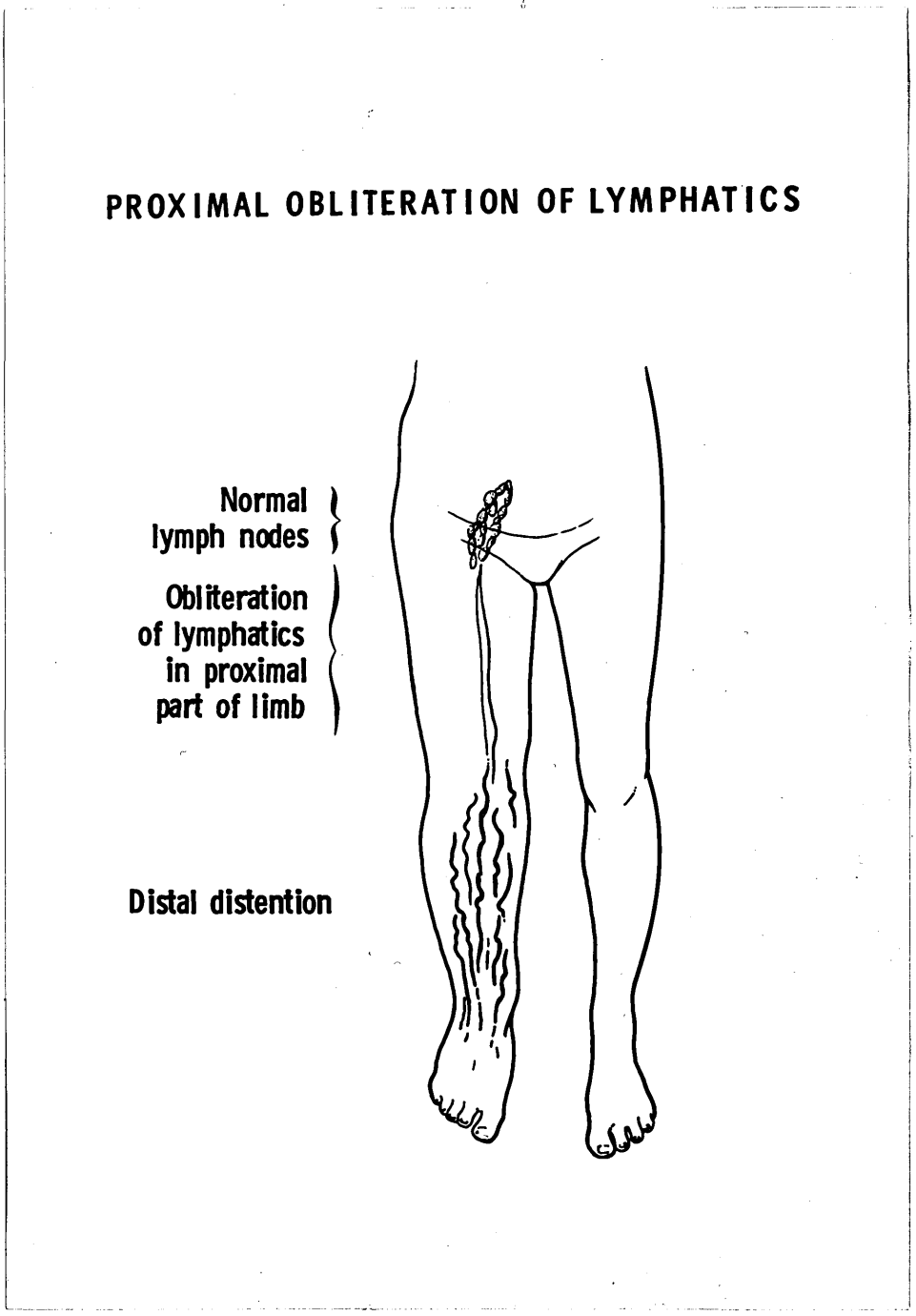


Figure 15a

Acquired obliteration of the lymphatics in the proximal part of the limb associated with distal distension:
a) Diagrammatic representation.

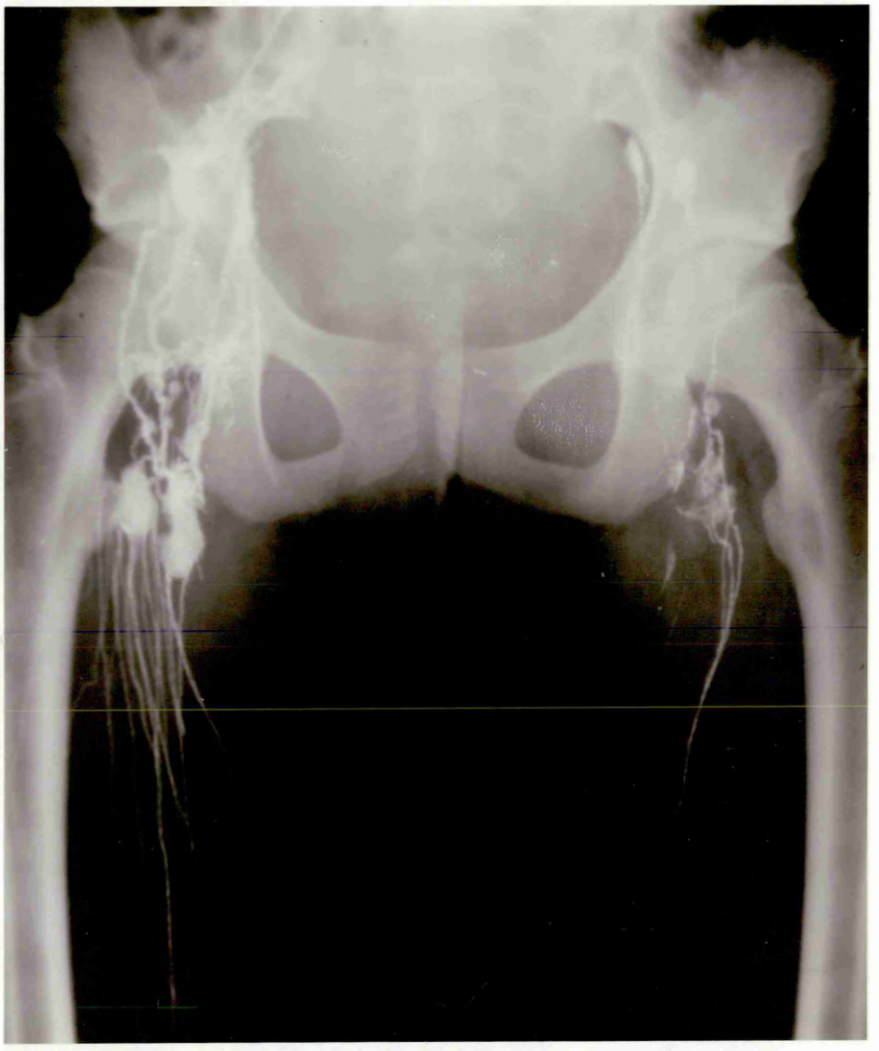


Figure 15b

Acquired obliteration of the lymphatics in the proximal part of the limb associated with distal distention:
b) Lymphographs showing obliteration of the left inguinal lymphatics and no filling of the iliac segment. Right side normal.

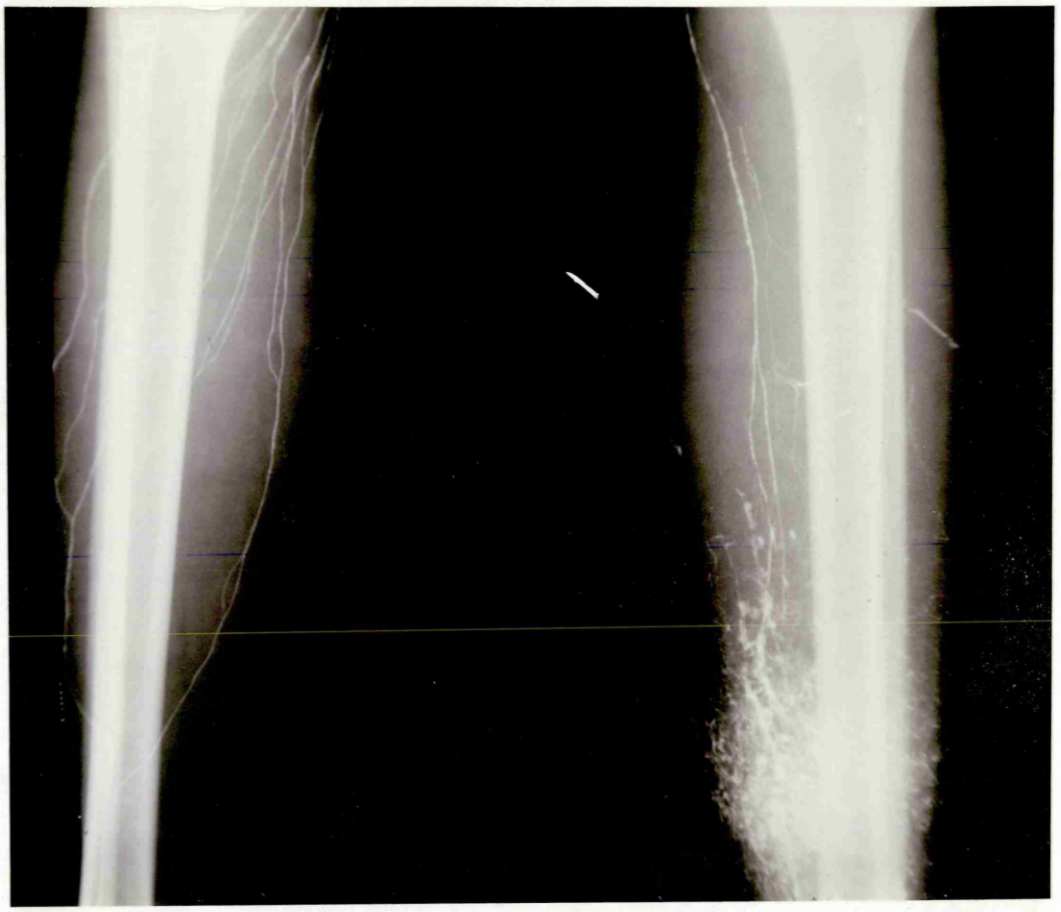


Figure 15c

Acquired obliteration of the lymphatics in the proximal part of the limb associated with distal distension:
c) Left lower leg showing distal distention and dermal backflow.

Figure 16

Acquired obliteration of all the lymphatics of the limb. No lymphatics could be found in the right foot but the injection of lipiodol into a normal right inguinal lymph node revealed normal iliac lymphatics.

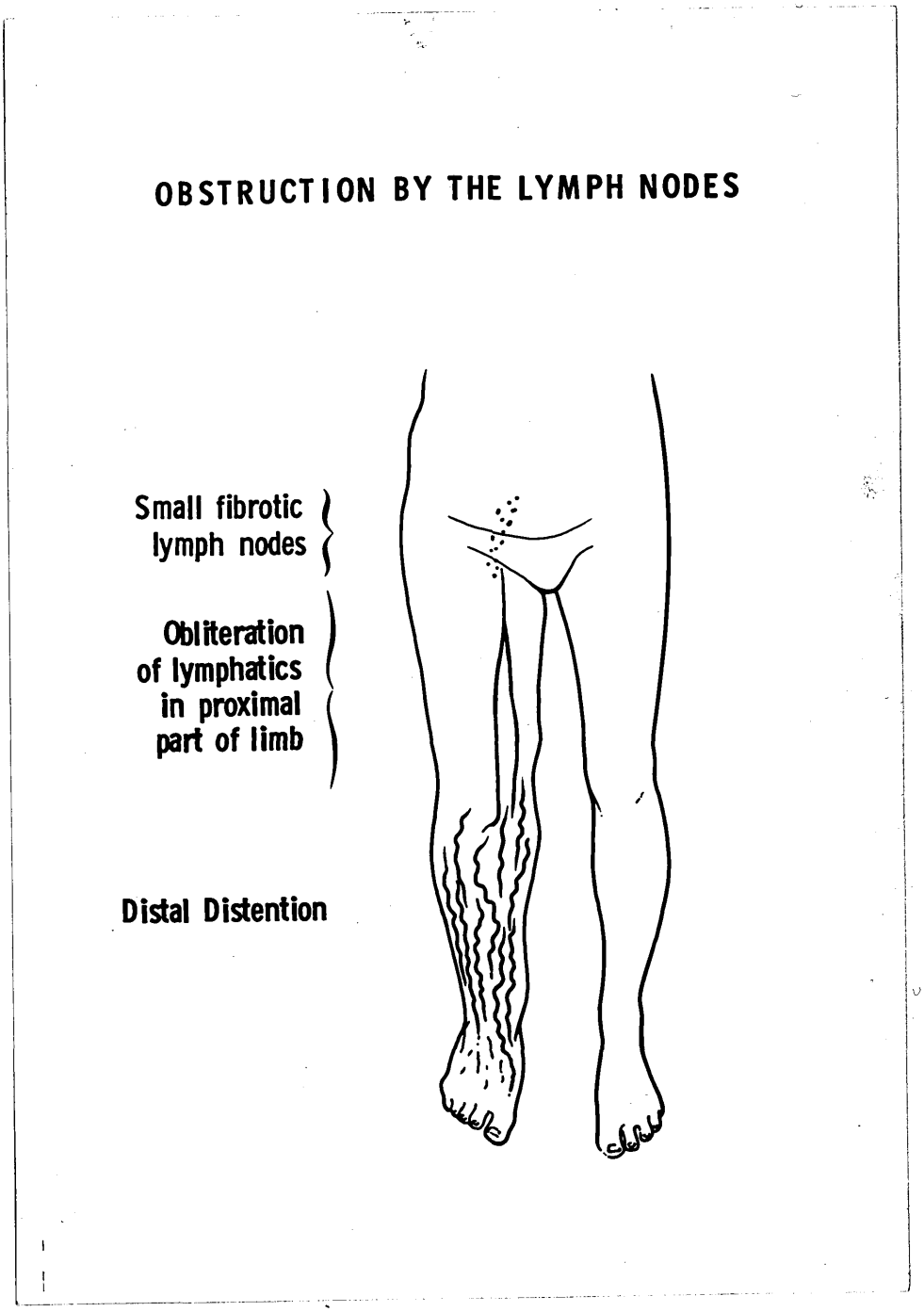


Figure 17a

Obliteration of the lymph conducting pathways through the node by hilar fibrosis:
a) Diagrammatic representation.

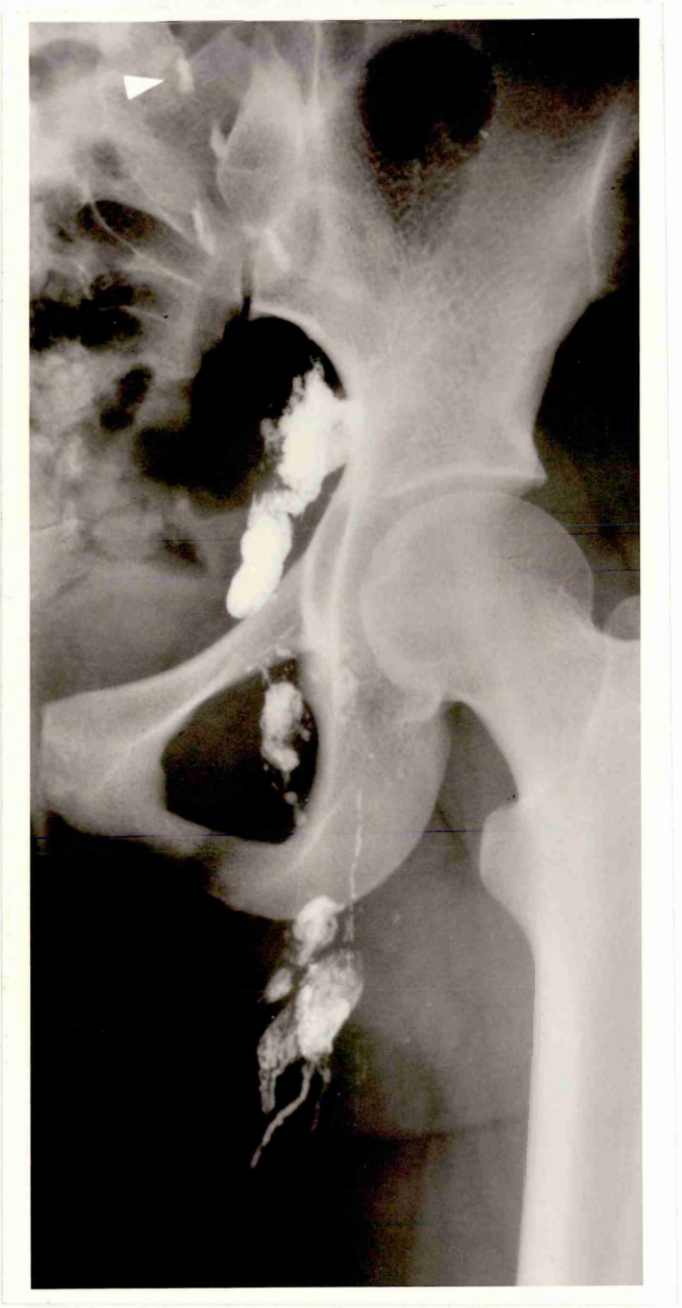


Figure 17b

Obliteration of the lymph conducting pathways through the node by hilar fibrosis:
b) A lymphograph of small fibrotic left iliac lymph nodes (arrowed).

Figure 17c

Obliteration of the lymph conducting pathways through the node by hilar fibrosis:
c) The vessels of the lower leg of the patient with the fibrotic nodes shown in (b).

CHAPTER 3

THE INVESTIGATION OF THE LYMPHATIC SYSTEM

Let us blush in this so ample and so
wonderful a field of nature,
to credit other men's traditions only,
and thence coine uncertain problems,
to spin out thorney and captious questions

William Harvey, 1653

(I) Development and Current Role of Lymphography

With regard to the primary lymphoedemas little understanding of the nature of the lymphatic defect was possible before the advent of effective methods of lymphography. The condition had been generally regarded as a congenital underdevelopment of the lymph system as evidenced by the high incidence of a family history and the not infrequent occurrence of other associated congenital defects but only lymphography could locate and define a lymphatic fault.

The injection studies of Mascagni and the Hunterian School in the 19th Century, already referred to, were the foundation of further studies. Experimental animal and human post-mortem experiments continued throughout the 19th and 20th centuries, using modifications of the injection technique with a variety of substances such as gelatine, oil, chinese ink, prussian blue, carmine and mercury, and late in the 19th century further studies injecting mercury into lymph nodes and lymphatics following interstitial injections of coloured dyes continued the detailed post-mortem anatomical study of the human lymphatic system (Sappey, 1874; Poirier, Cuneo and Delamere, 1903; Bartels, 1909).

In 1895, Conrad Roentgen discovered X-Rays, fundamental to the technique of lymphography and other forms of contrast radiography although another 30 years elapsed before direct puncture of blood vessels and injection of radiopaque media was carried out. Iodised poppy seed oil (Lipiodol) was one of the first substances used, but because intra-arterial and intravenous

injections of Lipiodol precipitated vascular thromboses in both animals and man, its use as a contrast agent was abandoned until it was re-introduced in the late 1950's for lymphography.

Early attempts at radiologic lymphography in experimental animals involved a variety of radio-opaque materials injected interstitially. These included iodine, mercury, lead, connabar colloidal bismuth and sodium bromide. All of these substances proved too toxic for clinical use (Fischer and Zimmerman, 1959).

The first pictures of the lymphatics in man were seen inadvertently during other radiological investigations in the 1930's, e.g. in myelography (Kruchen, 1934) and retrograde pyelography (Abehouse, 1933). This could be described as an early form of indirect lymphography and the concept of an interstitial injection of a contrast agent which enters the lymphatic system and outlines it radiologically, i.e. indirect lymphography, had considerable attractions. The definition of these early accidental studies in man was however very poor.

In 1932 Menville and Ane reported what in their view was the first radiological demonstration of lymph vessels and nodes by use of interstitial injections of thorium dioxide in animals. However, the films shown in their first report and in a series of subsequent papers show that very poor definition was obtained. Moreover, these authors were clearly unaware of a previous report by Carvalho, Rodrigues and Pereira (1931) in which excellent visualisation of iliac and lumbar lymphatics and the thoracic duct had been obtained by direct infusion of contrast into an inguinal node in dogs. In this latter paper, the authors did not state the nature of the contrast medium used; however, in a

later publication (Carvalho, Rodrigues and Pereira, 1934) this was revealed as being Thorotrast. Again, studies on dogs were described and in a tantalising final paragraph the authors make reference to their use of Thorotrast for lymphography in man, concluding that: "les resultats obtenus.....ne sont pas de nature a nous permettre de presenter".

Although it was later confirmed that Thorotrast produced good lymphograms in experimental application in animals such as the dog (Arnulf, 1958) its use in man was very limited owing to its poor absorption by the lymphatics following interstitial injection. This, and the oncogenic properties of Thorotrast, led to its abandonment and the search for other suitable contrast media continued.

Various oil-based substances emerged including sesame and emulsion, ethyl di-iodo stearate (Angiopac) and Lipiodol (an iodinated glyceryl ester of poppy seed oil). Gelhorn (1934) injected oidised oil into parametrial tissues in females and Gilbride (1938) used several oil and water based agents to outline lymphatics in breast carcinoma although neither were particularly successful.

In a review article in 1959 Fischer looked at other agents including Diodrast and Urokon but found they produced marked oedema in the dog foot pad and in some dogs marked soft tissue necrosis after several days. He found Hypaque produced little local toxicity and volume for volume produced the best quality pictures when compared to Diodrast and Urokon. However, the results in man were unsatisfactory due to inadequate absorption by lymphatics and diffusion of the water soluble agent out of the

lymphatics.

In addition to the subcutaneous routes for administration of radio-opaque substances, intracavitary routes such as intrapericardial, intraperitoneal and intra-articular injections were tried. It is however apparent that progress in the development of indirect lymphography was severely hindered by the failure to find a contrast medium which would be selectively absorbed from the tissues by the lymph system.

Visual Lymphography.

One of the more important advances in lymphography was the discovery of coloured dyes which facilitate the visualisation of the lymphatics (visual lymphography). The first recorded experiments of visual lymphography (Braithwaite 1923) used indigo carmine interstitially in investigating lymph flow in cats. It was, however, in 1933 that the major advance was made when Huddack and McMaster used intra-dermal injections of Patent Blue Violet to show the minute lymphatics of the skin.

Patent blue violet is a highly diffusible dye which rapidly finds its way into the deep lymphatics when injected into the tissues. It has a molecular weight of 1158 in the form of a calcium salt.

Its extreme diffusibility probably depends on its not combining with protein to the extent that other dyes do, thereby increasing their molecular size. It is made as an isotonic solution (1%) and enters the lymphatics rapidly after injection. It is also absorbed, however, by the blood vascular system the patient turning a nasty bluish-green colour and as it is excreted by the kidneys their urine also turns blue. This dicolouration disappears within 24 to 48 hours. It also colours the site of injection locally for a much longer and variable period from weeks to months.

A more modern application of patent blue violet was described by Leaper, Evans and Pollock in 1979 to delineate the lymphatics in an attempt to avoid post-operative lymphoceles and their subsequent problems, when dissecting the groin to expose the femoral artery in arterial surgery.

Although patent blue violet was first used by Huddack and McMaster in 1933 its value in clinical lymphography was not fully appreciated until the early 50's when the late J. B. Kinmonth pioneered direct lymphography; its main use remaining in this field.

Direct Lymphography.

While Walker in 1950 directly cannulated lymphatics and infused radioactive colloids for therapeutic purposes, J.B.Kinmonth (1952, 1954, 1955) developed the technique of "direct" contrast lymphography in London.

In 1952, Kinmonth "introduced" direct lymphography by cannulating the small lymphatics in the peripheral tissues and injecting a contrast medium directly into the lumen of the vessel. Initially this was done without the aid of "visual" lymphography but later he used patent blue violet which greatly improved the detection of the lymphatics.

The contrast agent used was a water soluble substance "diodone" which diffused rapidly out of both the lymph vessels and nodes giving short-lived but reasonable radiological definition of the lymphatic vessels. However, it was difficult to obtain good visualisation of the lymph nodes and the efferent post nodal vessels were especially poorly demonstrated (Lamarque, Romieu, Colin et al., 1956).

Bruun and Engeset (1956) used percutaneous injections into lymph nodes in humans and found a thin iodised oil (Iodipin) gave satisfactory radiological definition of the lymph nodes. Shanbrom and Zheutlin (1959) confirmed these findings using

Ethiodol and Fischer (1959) concurred although he pointed out two potential drawbacks: when mixed with lymph Ethiodol formed globules which occasionally produced a confusing radiological appearance, and it also found its way to the lungs and thus could potentially cause fat emboli.

The use of Ethiodol as the contrast medium in clinical lymphography became established in the early 1960's on both sides of the Atlantic. With this compound the lymphatics can be visualised for hours after the injection and the nodes for months. The technique offers considerable scope for studying many conditions including lymphovascular abnormalities, evaluation of oedema of the extremities, chylous disorders, lymphomas, inflammatory conditions of the lymph nodes and metastatic carcinoma.

Today, ultrafluid lipiodol, formed by replacing the glyceryl moiety of the ester with an ethyl group thereby lowering its viscosity, is used almost universally for clinical lymphography. By decreasing the viscosity the likelihood of producing confusing globules with lymph is much reduced. This material gives excellent visualisation of the lymph trunks and long lasting opacification of the lymph nodes. The technique itself basically remains the same, with minor modifications, as that described by Kinmonth in the early 1950's.

The initial enthusiasm in the fifties and sixties was replaced to a certain extent by scepticism in the seventies. Goonerante, reviewing lymphography in 1975, stated that it was a time consuming and often technically difficult procedure which usually provided less definite information than expected although

it had some value in the diagnosis of disease. Furthermore, in the presence of oedema it becomes technically a demanding procedure which requires general anaesthesia and special equipment such as an operating microscope (Kinmonth, 1977).

A recent report from Germany (Siefert, Mutzel, Schobel et al. 1980) described a promising development in contrast lymphography. A non-ionic dimeric water soluble contrast agent "Iotasul" was shown to produce good quality lymphangiograms and lymphadenograms by both direct and "indirect" (subcutaneous) methods in animals (Figures 18). Although the medium was eliminated almost completely within 24 hours, it was nevertheless retained within the lymph system for a sufficiently long period to give adequate visualisation of the nodes and vessels. This prolonged retention compared with other water soluble media was attributed by the authors to the higher molecular weight and lower osmotic pressure of Iotasul.

The fact that Iotasul is taken up by the lymphatics following a subcutaneous injection potentially gives it a useful place in lymphography. However, the initial clinical trials have been disappointing partly because the stability of the compound has been questionable but mainly because of the poor quality of lymphographs produced and as yet no lymph nodes have been visualised in man. Either, Iotasul is handled differently in man than in animals or, the amounts of Iotasul required for interstitial injection are prohibitive.

Figure 18a

Indirect lymphography in the rabbit using a subcutaneous infusion of Iotasul showing :
a) the popliteal lymph node with its afferent and efferent lymphatics.

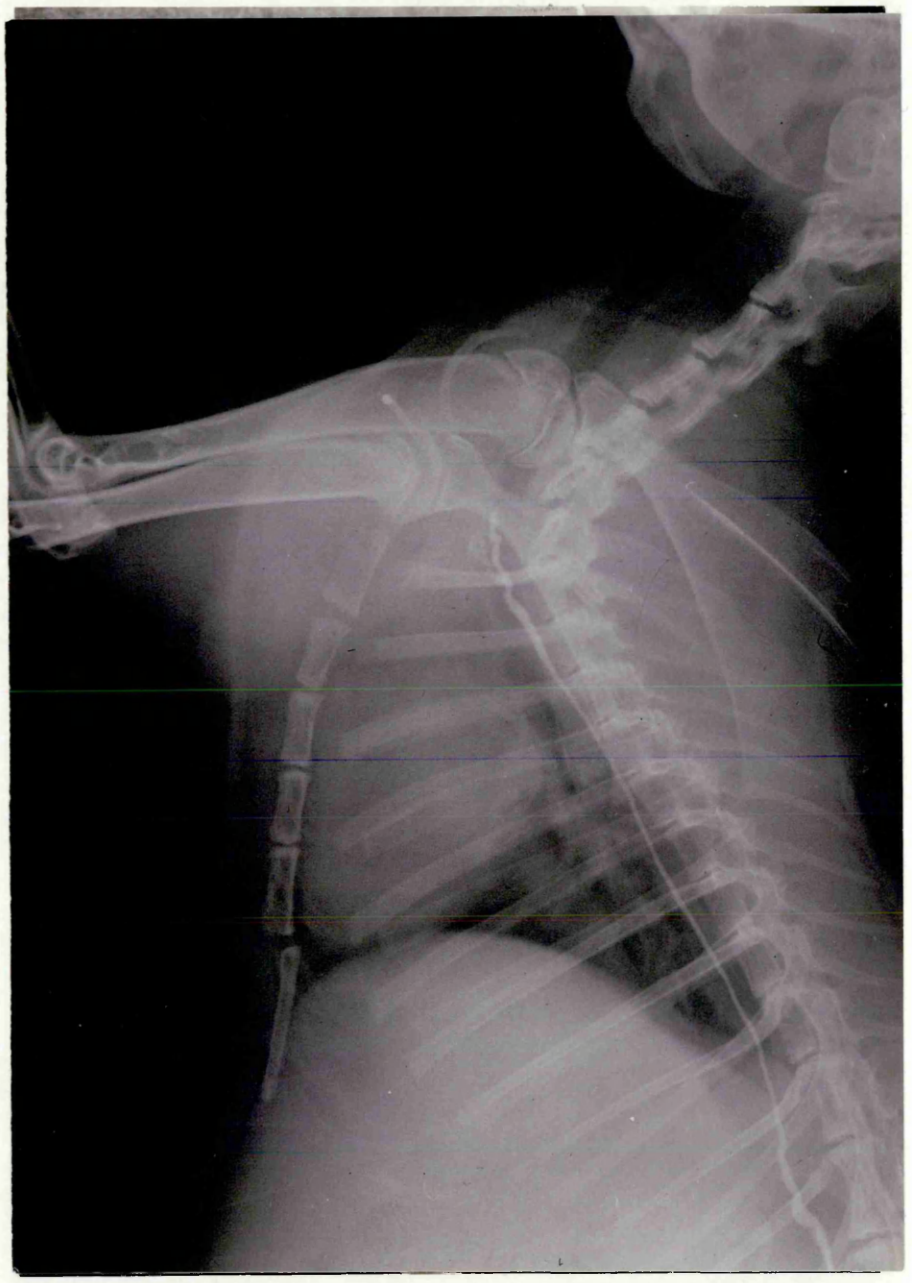


Figure 18b

Indirect lymphography in the rabbit using a subcutaneous infusion of Iotasul showing :
b) the retroperitoneal lymphatics and the thoracic duct.

The human lymphatic system, therefore, has until now been conventionally imaged for diagnostic puposes by X-Ray lymphography. Standard ethiodol lymphangiography, however, suffers from several acknowledged shortcomings (Stechel, Furmanski, Dunham et al., 1975):

1. Technical expertise is required to cannulate a lymph vessel, a technique which may be time consuming, tedious and unsuccessful and may also require a general anaesthetic.

2. Pulmonary oil emboli may occur and while the vast majority are asymptomatic, occasional complications including pulmonary infarction and cerebral oil embolism are reported (Stechel et al. 1975) although Kinmonth (1982) has shown that these can be minimised by careful technique.

3. Hypersensitivity reactions to the iodine component of the contrast medium.

4. Failure to provide kinetic physiological and quantitative studies since the contrast medium is administered by a perfusion pump.

5. Certain lymph node groups are technically inaccessible to this technique e.g. the internal mammary lymph node chain.

These drawbacks remain sufficiently great to initiate and encourage assessment of other techniques to investigate the lymphatic system, either to supplant or supplement lymphography.

(II) Radionuclides in the Investigation of the Lymphatic System

The search for alternative methods to lymphography for investigating the lymphatic system has led over the last 30 years to the development of compounds labelled with radionuclides which have been used in a variety of techniques in an attempt to diagnose lymphatic abnormalities.

The first published series measured the clearance of 131 Iodine labelled albumin (half-life 192 hours) from the sub-cutaneous tissues in animals using an external scintillation counter (Jepson et al., 1953). Since then the clearanceOF $I^{(131)}$ labelled albumin has been used to study the lymphatic system in man by several workers in an attempt to differentiate between various forms of oedema (Taylor et al., 1957; Hollander, Reilly and Burrows, 1961; Battezzati and Donini, 1964).

Although they were relatively successful, they found the technique clinically impractical for several reasons; the investigation took several days to complete, inaccuracies occurred because of variations of diffusion of the tracer within the tissue fluid, particularly in the presence of oedema, and the slow removal of the radionuclide from the site of the injection made the calculation of clearance difficult.

Further experimental work was carried out by Seki in Japan (Seki, Yamane, Shinoura et al., 1968, Seki, Yabuki and Ishida, 1976) and Miller in the United States (1980), but as long as 1957 Taylor et al. were suggesting that, while useful for research, labelled albumin clearance was not suitable for clinical application.

Lymphoscintigraphy was introduced in the 1950's following a report by Sherman and Ter-Pogassian that lymph glands could be visualised following the interstitial injection of radioactive colloidal gold in 1953. This was a spin-off of their work on the use of radioactive colloid gold in the treatment of cancer in pelvic nodes (1950), a concept introduced by Hahn, Goodall & Shepherd in 1947.

Hultborn, Larson & Ragnholt (1955) and, several months later, Seaman & Powers (1955) both confirmed the potential of Sherman's observation that a colloid injected into the interstitial tissues was transported to, and concentrated in, the lymph nodes via the lymphatics, where It could be detected with external scintillation counting.

Seaman and Powers (1955) both showed that malignant lymphatic tissue lost its ability to accumulate radioactive gold colloid and because of this, concentrated on its use in the diagnosis rather than the treatment of malignant disease. The technique, however, was not widely utilised until Zum Winkel and Scheer (1965) in Heidelberg, used it to visualise pelvic and para-aortic nodes, while Rossi and Ferri (1966) and Schenck (1966) independently reported the visualisation of the parasternal lymph node groups for the first time.

There were several publicaions following this (Kazem, Brady & Croll, 1969, Matsuo, 1974) but while colloidal gold gave high quality scintigraphic pictures of the lymph node because of its optimum particle size of 5-10 nm (Anghileri, 1967), it presented an unacceptably high dose of Beta radiation to the patient (Zum Winkel & Hermann, 1977). The advent of a new group of Technetium

labelled colloids with no Beta radiation and a better gamma camera imaging potential thus heralded its decline as a tracer.

The early clinical work with these new colloids was carried out by Ege (1976) who used Technetium labelled sulphur colloid to detect internal mammary lymph nodes in patients with carcinoma of the breast. Ege reported that this colloid gave inconsistent results because of its large particle size (Warbrick, Ege, Henkelman et al., 1977) but subsequently found the small microcolloid - antimony sulphur colloid - more reliable and has now used this colloid extensively (Ege, 1978).

There have been many publications since 1978 describing the detection of metastic disease particularly in the internal mammary lymph nodes although other areas such as the axillary lymph nodes in breast cancer (Agwonobi & Boak 1978; Osborne, Payne, Richardson et al., 1984) and the pelvic lymph nodes in prostatic testicular and cervical cancer (Ege, 1982; Kaplan, 1983) have also been studied.

The results published have, however, been conflicting. As a result evaluation of lymphoscintigraphy for detecting metastatic disease has been difficult and the technique has not yet gained wide acceptance in surgical practice.

There have been no major studies using the technique to evaluate the lymphatic function of a swollen limb, for example in lymphoedema. There have been several case reports using the technique of static lymphoscintigraphy (Vieras & Boyd, 1977; Bowen & Lendle, 1978; Gates & Dore, 1971) to assess lymphoedema but no prospective study comparing lymphoscintigraphy with the clinical and lymphographic findings in patients with lymphoedema

has yet been carried out.

I have therefore chosen lymphoscintigraphy (radionuclide imaging) to investigate the lymphatic system of swollen limbs. The remainder of this thesis describes the development of the techniques used and reports their results in the assessment of the lymphatic system of patients with swollen limbs.

CHAPTER 4

METHODS

Tasks in hours of insight willed
may bethrough hours of gloom fulfilled

Matthew Arnold

(I) Principle of Lymphoscintigraphy or Radionuclide Lymph Node Imaging

Lymphoscintigraphy is based upon the mechanism of transport of a radioactive colloid following its injection into the subcutaneous tissues. If a colloidal substance is injected intravenously it is localised within the reticulo-endothelial system of the liver, spleen and bone marrow. When radio-active colloidal substances are injected into the intradermal and subcutaneous tissues the flow is through the lymphatic channels to the regional lymph node groups. This is carried out by phagocytosis as well as by direct transport through the lymphatic channels (Croll, Brady and Dadparvar, 1983).

The localisation of the colloidal particles in the lymph node areas therefore depends upon both the patency of the lymphatic channels peripherally and within the lymph nodes, and thus radionuclide imaging using a gamma camera assesses not only peripheral lymph clearance but also the integrity of the larger collecting lymphatic vessels and lymph nodes. Two different methods will be described and the results of each method discussed.

(II) The Radionuclide

The particle size of the radioactive colloid is critical as it affects both its biological behaviour and the eventual quality of the diagnostic study (Anghileri, 1967; Warbick, Ege, et al., 1977). Colloidal gold gave high quality scintigraphic pictures of the lymph nodes following interstitial administration because of its optimum particle size (5 - 10nm) (Anghileri, 1967), but its use was abandoned because it presented an unacceptably high dose of Beta radiation at the injection site (Zum Winkel and Hermann, 1977).

It was, therefore, replaced by Technetium labelled colloids which have no Beta radiation and better gamma camera imaging characteristics. Technetium labelled sulphur colloid with a mean particle size of 300nm was used initially but gave inconsistent results because of its large particle size (Ege, 1976; Warbick et al, 1977). The smaller micro-colloids such as rhenium sulphide or antimony sulphide, labelled with Technetium, have a smaller particle size of 4 - 12 nm and 3 - 30 nm respectively. They provide a more consistent visualisation of the lymph nodes (Ege, 1978), and are therefore more suitable.

It was decided to use rhenium sulphide colloid, labelled with Technetium, for this study. Both antimony sulphide colloid and rhenium sulphide colloid were considered but the former was rejected for the following reasons.

1. Rhenium sulphide colloid has a narrower particle size range, and, therefore, theoretically should be more suitable and provide more consistent visualisation of the lymph nodes.

2. In a small preliminary study in which both colloids were used in the same subjects, rhenium sulphide colloid appeared to have two advantages - based on subjective assessment:

(a) As theoretically suggested the lymph node images produced by rhenium sulphide colloid appeared slightly better.

(b) Injections in both calf and interdigital space were less painful with rhenium sulphide colloid - the pH of the substance being 6 - 7 and that of antimony sulphide colloid 5.2 - 5.4.

It was, therefore, possible to inject rhenium sulphide colloid without any local anaesthetic which is normally recommended for lymph node imaging (Ege, 1976). Theoretically, the volume of local anaesthetic may interfere with the local diffusion of the colloid.

3. Rhenium sulphide colloid was more readily available through the London based office of International CIS whereas antimony sulphide had to be imported from Holland at the start of the study.

4. Manufacturers data showed that rhenium sulphide colloid could be labelled to Technetium to greater than 95% with stability as opposed to 90 - 95% for antimony sulphide colloid.

(III) TECHNETIUM - Chemical and physical characteristics.

Chemically technetium belongs in group VII-A along with manganese and rhenium, the resemblance to the latter being particularly close. Physiologically, when reduced to a lower valency, technetium appears to combine avidly with a variety of materials to form stable compounds or complexes, i.e. it has flexibility.

Technetium (99mTc), a radioactive derivative of molybdenum, has almost no beta emission but emits gamma rays, rendering it sutable for gamma-camera scanning.

The favourable physical characteristics as a clinical tracer material are:

1. A short physical half life of 6 hours.
2. Emission of clean 140 Kev gamma ray.
3. Absence of primary particle radiation.
4. Availability as the daughter of 2.8 days molybdenum.

The short half-life obviously reduces radiation dosage and the absence of primary particle radiation ensures that much of the more penetrating photon radiation leaves the body without being absorbed.

An agent suitable for clinical use has been prepared by combining 99mTc with sulphur colloids and the agent has been used to visualise malignancy within many tissues including abdominal lymph nodes, axillary and internal mammary nodes.

10 mCi more can be given without undue radiation hazard and there is therefore less risk involved in performing or repeating a scintiscan in a non-pregnant female of any age than may be associated with mammography (Editorial - Brit. Med. J., 1977).

(IV) Rhenium Sulphide Colloid

The colloid is produced by INTERNATIONAL CIS St-Quentin-Yvelines Cedex France. It consists of two components: a rhenium sulphide suspension and a lyophylisate of sodium phosphate and stannous chloride both contained in vials under nitrogen atmosphere. The rhenium sulphide suspension (Vial A) contains 1 ml of colloidal rhenium sulphide as:

- rhenium (as sulphide) 0.15 mg
- gelatin 9.6 mg
- ascorbic acid 7.0 mg
- water for injection 1.0 mg

The lyophylisate (Vial B) contains:

- sodium pyrophosphate 3.0 mg
- stannous chloride 0.5 mg

Preparation of and labelling of rhenium sulphide colloid with Technetium

1. Measure the activity of the Technetium generator and calculate the volume of Technetium eluate containing 925-1110 Mega Bequerels (MBq) - 25-30 milliCuries (mCi): usually 1.0 to 2.0ml.

2. Introduce 2ml of Water for Injection B.P. into vial B (without a breather needle). The vial is shaken until the product lyophylisate is completely dissolved.

3. 0.5ml of this solution is added to Vial A which is shaken to mix the rhenium sulphide suspension and the lyophysilate. This produces a volume of 1.5mls.

4. The calculated volume of Technetium eluate (1-2ml) is withdrawn from the eluate vial and the activity introduced into vial A. and shaken for 15 seconds to mix the contents.

5. Vial A is put in a boiling water bath for 30 minutes and is afterwards cooled under running water.

6. The activity in vial A is then measured and the activity in MBq/ml recorded on a label which is attached to the vial. The activity required is 375MBq/ml (10mCi/ml) and the volume can be dilute with normal saline until this is achieved. This allows injection of 75MBq (2mCi) of activity in a volume of 0.2ml.

7. A sample is taken from the vial for quality control using chromatography (see page 128).

N.B. The contents of Vial A and B are kept under nitrogen atmosphere and an equivalent volume of nitrogen is removed in stages 2, 3 and 4 to equalise this pressure.

The preparation time is about forty minutes and the colloid is ready for immediate use. It is stable for up to 24 hours if kept at room temperature. The "kit" itself (Vial A and B) is stable for 6 months if kept at 4 degrees centigrade.

Properties of the labelled colloid.

Tc99m binding	: 95% +
particle size	: 4-12 nm
pH	: 6-7

Chromatography

Chromatographic separation is the commonest form of analysis for the determination of radio-chemical purity. Rapid assay of 99mTc labelled pharmaceuticals may be accomplished by the use of small chromatographic strips allowing very short development times.

i) A strip 1 cm. wide and 5 cm. long is adequate. Duplicates should be run for each sample. Care should be taken not to handle the surface of the strip when marking or spotting it, and ideally forceps should be used. Each strip is marked 0.8 cm. (O) from the bottom and 0.5 cm. (F) from the top. Whatman No. 1 paper is used as the support medium (Figure 19).

ii) Sample application. A very small drop of the sample (from a 25g needle) is placed on the origin ('O') line and allowed to dry thoroughly.

iii) Development. Chromatography is carried out at room temperature ($20^{2}o^{3}C$). The strip is place in a tank containing 95% acetone as the solvent and left until the latter has reached ('F'). The strips are then taken out and dried. Once the strips are dry they are ready for counting.

iv) Counting the samples. For detection of free Technetium only, the strips are cut across Rf 0.4, activity corresponding to the bound Technetium being on the bottom half (O) of the chromatograph. Each half is placed in a separate counting vial and counted in a well counter calibrated for 99mTc. The geometry of the strip in the vial should be kept constant. A background reading is taken to correct the sample counts.

v) The percentage of free Technetium is calculated by dividing the activity on the top portion by total activity and multiplying by 100.

vi) Ideally, the level of radio-chemical impurity (free Technetium) should be negligible but an acceptable level for rhenium sulphide colloid is less than 5% as was discussedearlier.

vii) Each sample was subjected to further quality control of the initial labelling of the colloid by measuring the distribution of technetium in the labelled colloid sample and comparing it to the distribution of free Technetium in the original technetium eluate. Both samples are analysed by the technique described above. However, the paper strips are divided in 1 cm. strips onthis occasion and each strip counted separately. An example of the chromatograph obtained is shown in Figure 20 in which only 3% of the Technetium in the colloid sample falls within the distribution for the Technetium from the free Technetium eluate.

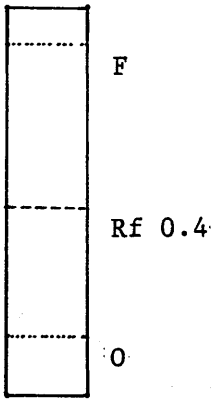


Figure 19

Diagrammatic representation of a Whatman No. I chromatographic strip.

	Cm from Origin	Mean counts	% total Activity
ELUATE	01	18576	-
	02	4119	-
	03	6361	-
	04	10709	-
	05	29050	-
	06*	5996985	41
	07*	8404376	57
	08	208344	1
	09	3744	-
	10	2267	-
RSC	01	5394237	88
	02	239074	4
	03	54425	-
	04	79487	1
	05	106207	2
	06*	154721	3
	07*	27819	-
	08	17035	-
	09	16280	-
	10	6467	-

Figure 20

Diagrammatic representation of chromatograph comparing Technetium eluate with the labelled 99m Tc RSC. This shows that only 3% of 99m Tc in the colloid sample falls with distribution of activity of free Technetium eluate i.e. 6-7cm from the origin.

Toxicology.

Toxicology has been studied in male mice 20g ± 2 in weight and a detailed report is registered at the French Ministry of Health. For 2 milli Curies (mCi)-75 Mega Bequerels (MBq) of Technetium labelled rhenium sulphide colloid (99mTc RSC) injected subcutaneously the rhenium dose injected is 0.08 mg/kg i.e. 187 times less than the L.D.50 of colloidal rhenium sulphide determined intravenously in mice, which means an enormous toxicity ratio following a subcutaneous injection in man. No death occured in mice after injection of 1 ml of the product per 20 gm of animal body weight, equivalent to an intravenous injection of 3500 mls for a 70 kg man.

For a 5mCi (185 MBq) subcutaneous injection of 99mTc RSC in a 70 kg human adult the quantity of sodium pyrophospate administered is approximately 0.007 mg/kg i.e. 12,500 less than the corresponding L.D.50 in mice if injected intravenously, the ratio being 23,000 for chloride.

Pharmokinetic Studies (data obtained from manufacturers)

1) Organ distribution in animals

The organ distribution of Technetium labelled Rhenium sulphide colloid at 15 minutes and 5 hours following a subcutaneous injection has been studied in mice and is expressed below in Table 2 as a percentage of the migrating activity.

TABLE 2

Organ	Percentage of migrating activity at	
	15 minutes	5 hours
Popliteal lymph glands	11.3 ± 5.5	9.9 ± 3.8
Inguinal lymph glands	18.9 ± 6.5	16.3 ± 3.7
Liver / Kidneys	8.8 ± 11.4	27.9 ± 18.9
Urine	0.5 ± 3	19.9 ± 11.8

II) Behaviour in healthy man

a) The percentage uptake of the radioactivity in the liver following a subcutaneous injection of 99mTc RSC in man is shown in the graph below (Figure 21), expressed as a percentage of the total injected activity:

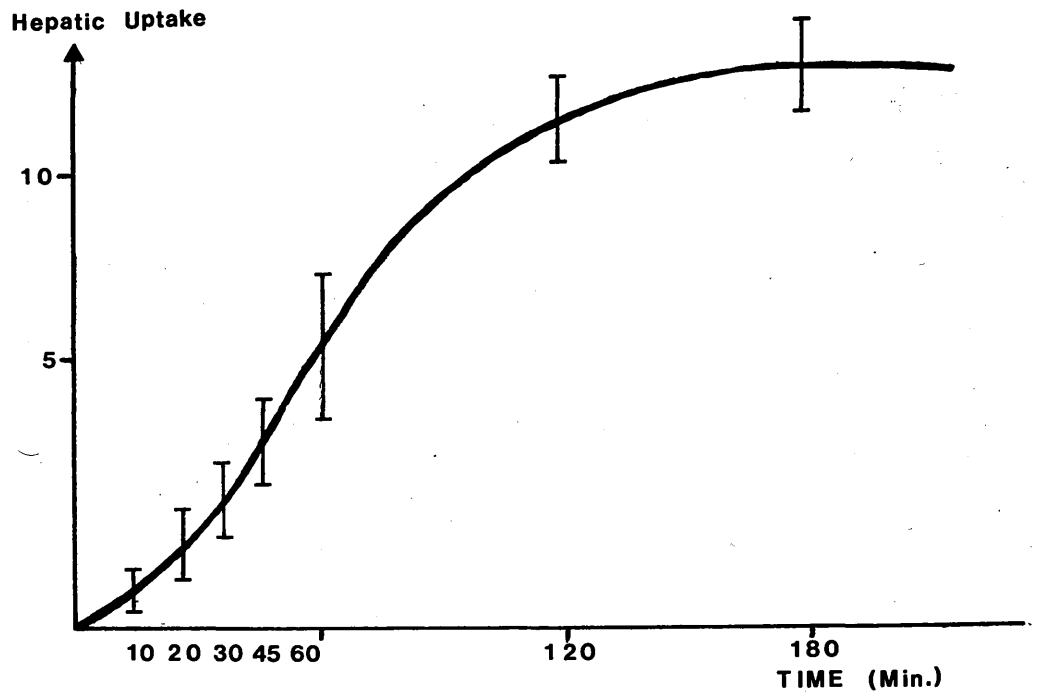


Figure 21

This data was obtained from the manufacturers own pharmokenetic studies.

b) Urinary clearance of the labelled colloid following a subcutaneous injection of 99mTc RSC is shown in Figure 22. This data was obtained from the manufacturers own pharmokinetic studies. In order to confirm these results urine samples were taken at 30 minutes from a group of control subjects (Figure 23) and from 20 patients with primary lymphoedema (Figure 24). Each urine sample was subjected to chromatography (described later in this Chapter) to assess the amount of activity present in the bladder as labelled colloid. This was expressed as a percentage of the injected activity.

The mean ($\pm$SD) for the control group was 3.92% $\pm$ 1.79 similar to that found in the manufacturers pharmokinetic study (Figure 22). The urinary clearance for 20 limbs with primary lymphoedema at 30 minutes was 1.27% $\pm$ 1.42. This value was statistically much lower (Figure 22) than that obtained for the control group ($p < 0.001$) and suggests that urinary clearance may be related to the clearance of the colloid from the injection site.

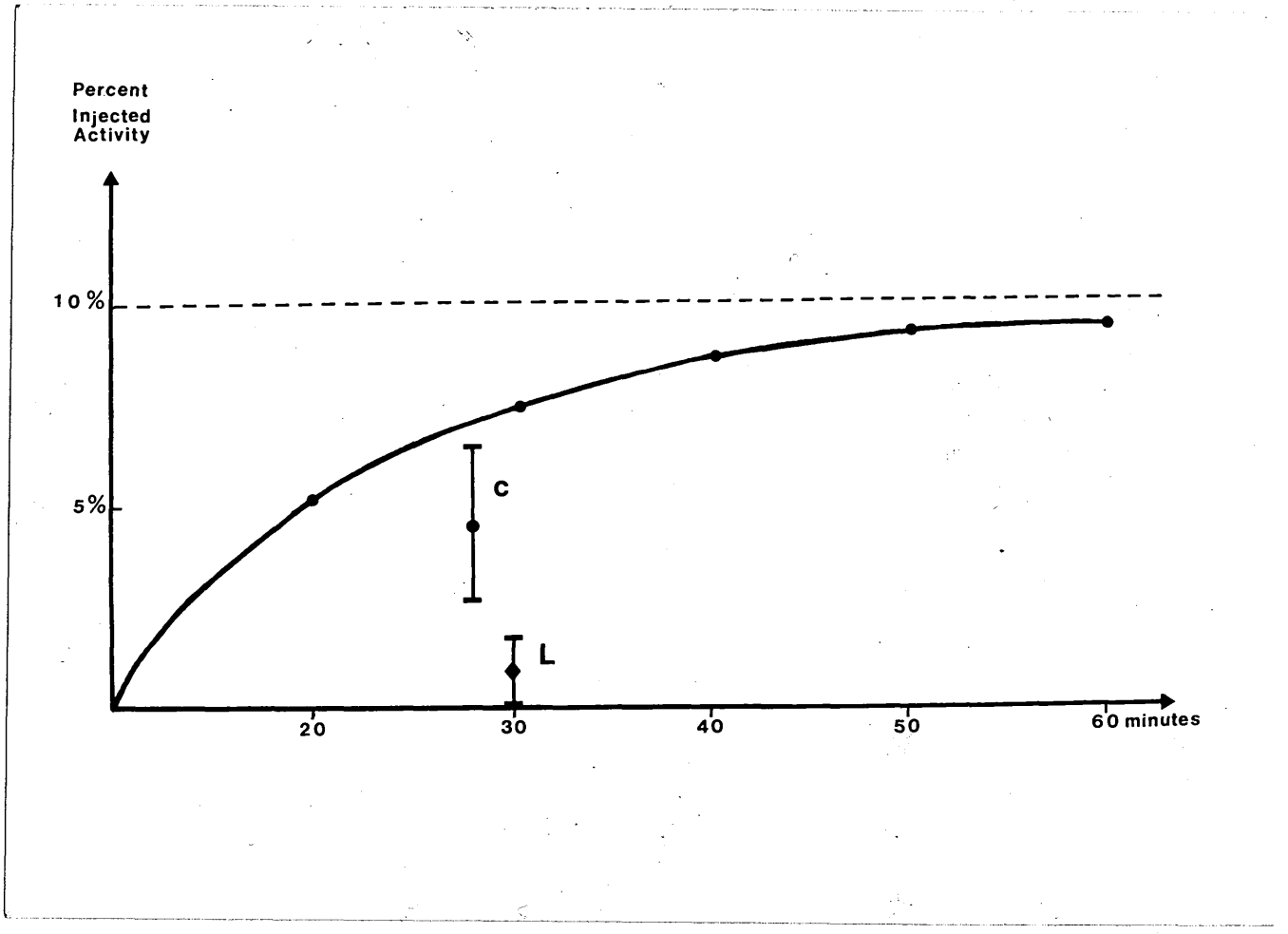


Figure 22

Urine clearance of 99m Tc RSc in man. The urine clearance of the radioactivity at 30 minutes in 12 control subjects (C) and 20 patients with primary lymphoedema (L) has been compared to the manufacturers own data.

Study No.	Percentage of injected activity
1	4.5
2	5.6
3	3.3
4	1.61
5	2.4
6	7.3
7	3.0
8	6.0
9	3.5
10	4.0
11	1.27
12	4.5

NB. Injections were made in both limbs in all studies.

Figure 23

The amount of Technetium labelled Rhenium Sulphide Colloid present in the urine of twelve control subjects at 30 minutes expressed as a percentage of the injected activity.

Study No.	Percentage of injected activity
1	6.0
2	1.27
3	4.5
4	0.8
5	0.74
6*	0.96
7*	0.53
8	0.8
9*	0.41
10	0.61
11	0.61
12	0.99
13	1.7
14	1.2
15	0.71
16	0.94
17*	0.45
18	0.55
19*	0.44
20	1.28

* Patients with bilateral lymphoedema.
NB. Injections were made in both limbs in all studies.

Figure 24

The amount of Technetium labelled Rhenium Sulphide Colloid present in the urine of 20 patients with primary lymphoedema at 30 minutes expressed as percentage of the injected activity.

(V) Labelling of Radionuclide

The technique of labelling rhenium sulphide colloid with Technetium has been described earlier in this chapter. While the physical characteristics of the colloid determines its transport via the lymphatics, the element of the labelled compound which produces the gamma camera image is Technetium. Free Technetium will enter the blood stream freely from the subcutaneous space and will be picked up by the thyroid and the gastric mucosa:- the greater the percentage of free Technetium the poorer the study of the lymphatic system.

Animal experiments have shown that once the radio colloid is less than 90% labelled the study begins to lose credibility, Bergqvist, Strand and Persson (1983), and our own experience in three patients in whom the colloid was found to be less than 85% labelled and the definition of the study was poor, would support this view. It is thus important to ensure that the rhenium sulphide colloid is correctly labelled to the technetium and also that it does not separate from the colloid once injected into the patient.

To verify this labelling, each sample of technetium labelled rhenium sulphide colloid was measured for free technetium. To verify the continued labelling of the colloid in the body, twenty patients had blood and urine samples taken 30 minutes following injection of the colloid. (Appendix I)

The results of this part of the study show that there was only a trace of free technetium in the blood of this group of patients with the exception of 4 studies, showing that the colloid remains well labelled when it is present in blood.

However, the amount of free Technetium is much greater in urine - 8.11% of the Technetium present.

The explanation is not entirely clear although it may be that any free Technetium that is in the blood is excreted readily by the kidneys whereas the colloid itself has a much larger particle size which prevents it from freely crossing the renal tubule.

Conclusions

The studies previously referred to, in which the free Technetium element in the injected sample was more than 5% underline the need to label the colloid properly. Thus, it was ensured that the colloid was labelled at 95% or greater prior to each study being carried out.

(VI) Development of the Technique of Radionuclide lymph node imaging

Introduction

Radionuclides have been used to investigate the lymphatic system in two ways:

a) to demonstrate the lymph nodes and,

b) to estimate or measure the rate of lymph flow.

They were originally assessed using Au 198 colloid for lymph node imaging and labelled albumin to measure lymph clearance, the shortcomings of which have been discussed previously.

With the advent of newer Technetium labelled microcolloids which allow better gamma camera imaging, it was decided to re-examine their value in the investigation of the lymphatic system and to re-examine the clearance studies described previously by Taylor et al (1957).

A preliminary study was set up therefore to look at the two aspects mentioned at the beginning of this section namely; lymph node imaging and lymph flow.

Preliminary Study

0.2 ml (75MBq) of Technetium labelled rhenium sulphide colloid was injected into the subcutaneous tissues of the lower leg (either mid-calf or inter-digital cleft) using a 25 gauge orange needle on a 1 ml syringe with the patient/subject lying supine. The patient/subject was at rest for as much of the study as was feasable.

Measurement of the rate of lymph flow was carried out by

externally counting the clearance of radioactivity from the injection site and its arrival at the inguinal lymph nodes at 30 minute intervals up to 180 minutes, using a scintillation counter with a sodium iodide 2.5 cm collimator head (Figure 25), linked to an MS 310 ratemeter.

At the same time, gamma camera images of the ilio-inguinal lymph nodes were accumulated for 5 minutes at 2 and 3 hours.

This preliminary study was undertaken in 9 patients with the clinical diagnosis of primary lymphoedema in 11 limbs - subsequently confirmed by lymphography in all eleven limbs.

In all eleven lymphoedematous limbs the gamma camera image at 2 hours showed a poor or absent lymph node activity pattern suggesting that a simple 5 minute image taken by the gamma camera positioned over the groins and lower abdomen following a peripheral injection of a radiocolloid may provide an accurate diagnostic test.

The scintillation studies, however, were less easy to interpret. A successful study is illustrated in Figure 26. This patient had primary lymphoedema of the left leg. The poor clearance of the radionuclide activity from the lymphoedematous leg and the poor uptake of activity in the inguinal lymph nodes suggest that there is a diminished flow of lymph from the periphery compatible with a diagnosis of lymphoedema. In comparison there is better clearance of the activity from the non affected limb and better uptake in the inguinal lymph nodes.

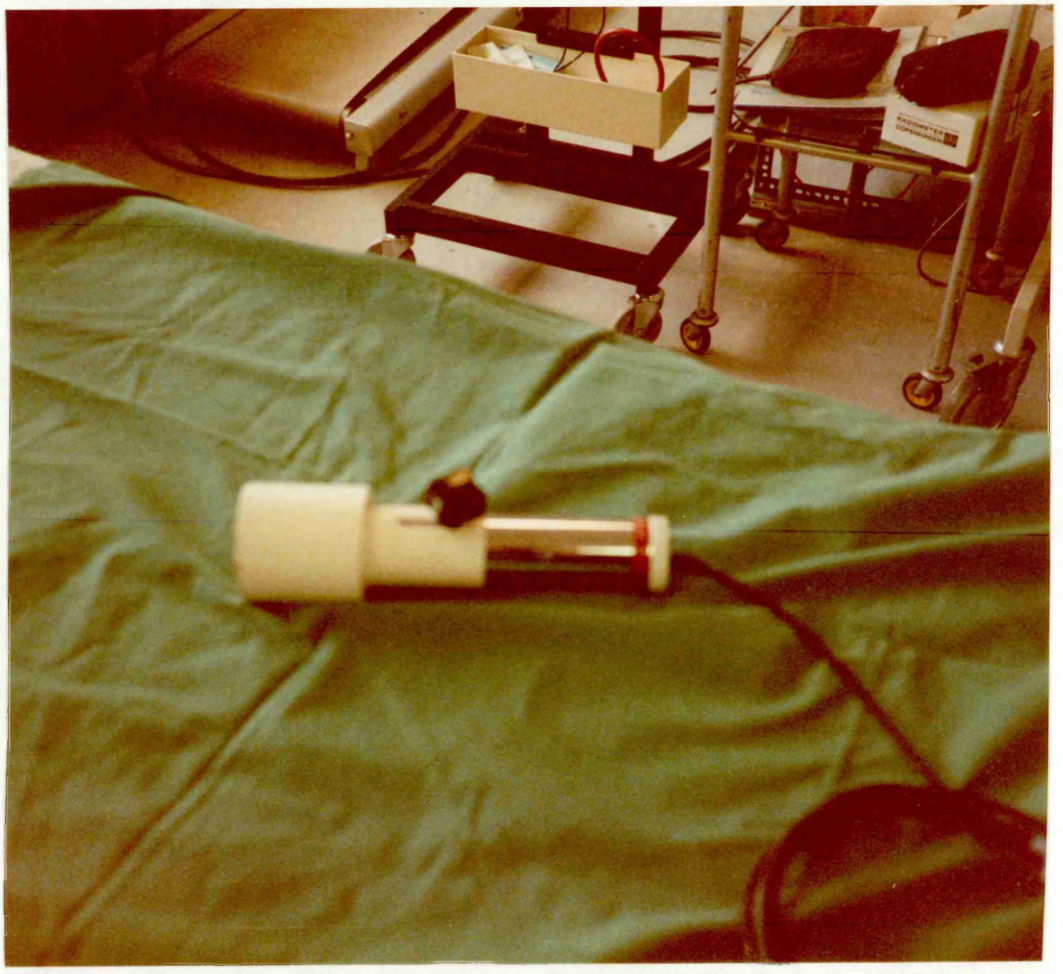


Figure 25

Photograph of sodium iodide scintillation counter which was used to externally count the clearance of radioactivity from the injection site and its arrival in the inguinal nodes.

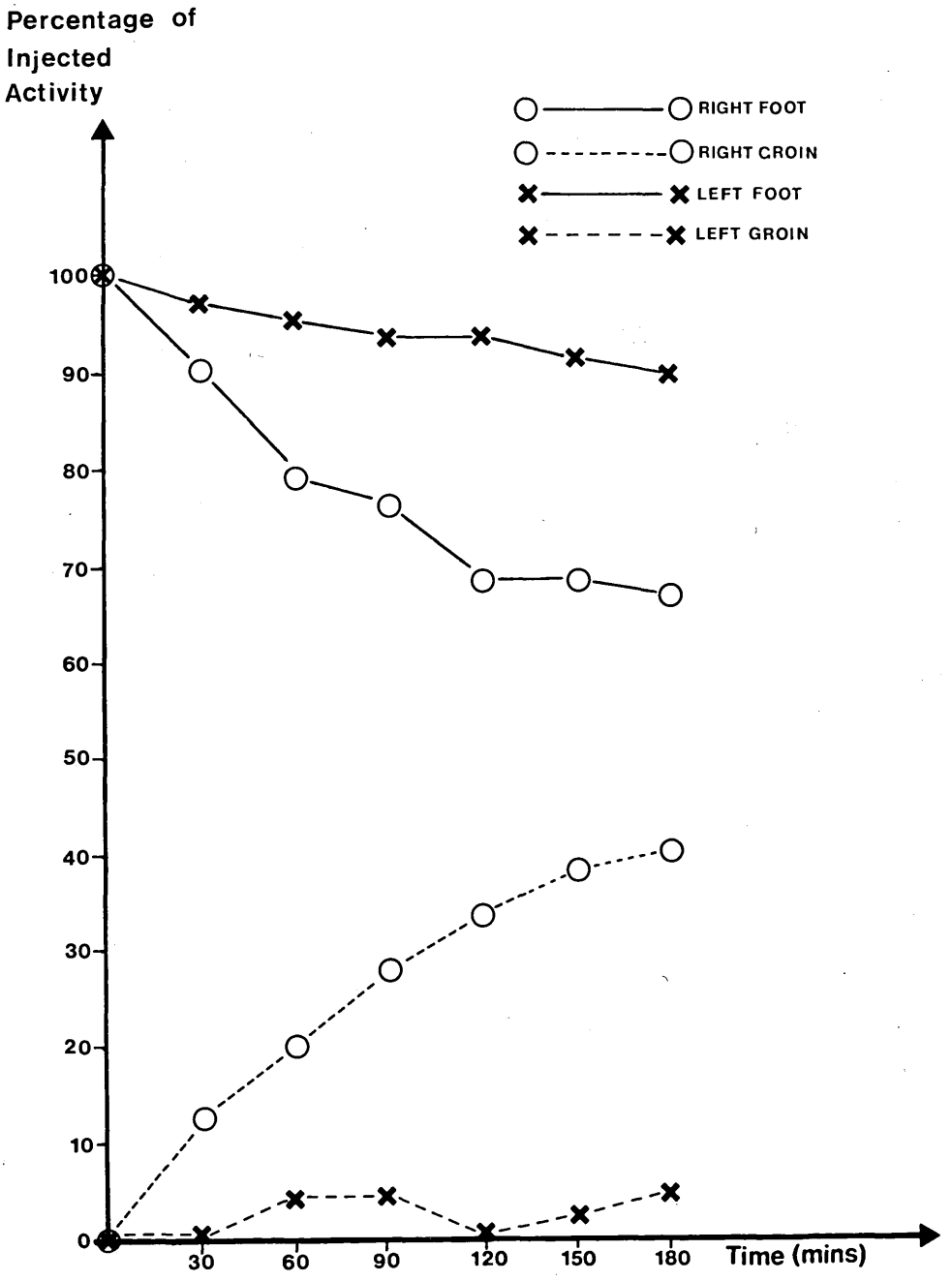


Figure 26

Graph showing clearance of 99m Tc RSC from injection site and arrival in the inguinal lymh nodes, measured externally by a scintillation counter in a patient with primary lymphoedema of the left leg.

A second study (Figure 27) in a patient again with primary lymphoedema of the left leg, however, illustrates some of the technical problems of this particular technique. Firstly, there was poor arrival of the activity in the early part of the study not only in the affected limb but also in the other limb (clinically and lymphographically normal), whilst in the latter part of the study (150 to 180 minutes) there was an apparent decrease in the amount of activity accumulated in the lymph nodes. Secondly, there appeared to be a greater amount of activity present at the injection site at 60 minutes in the lymphoedematous limb than was originally injected.

Three other studies revealed a similar problem in that there was an apparent increase in activity at the injection site and inconsistent uptake of activity in the inguinal lymph nodes from one time interval to the next.

These studies illustrate several problems in using this technique to measure lymph flow:

a) When the vast majority of the injected activity remains at the injection site the readings tend to be inaccurate.

b) Local diffusion of the radionuclide particularly in the presence of oedema further adds to the inaccuracy of measuring clearance of the isotope.

c) Positioning of the collimator head was extremely important as the readings in absolute counts/minute varied considerably with the angle at which the collimator was held against the skin. This was particularly true when assessing the arrival of the colloid in the inguinal lymph nodes as a slight change in the position may lose or add part of a lymph node or a complete lymph

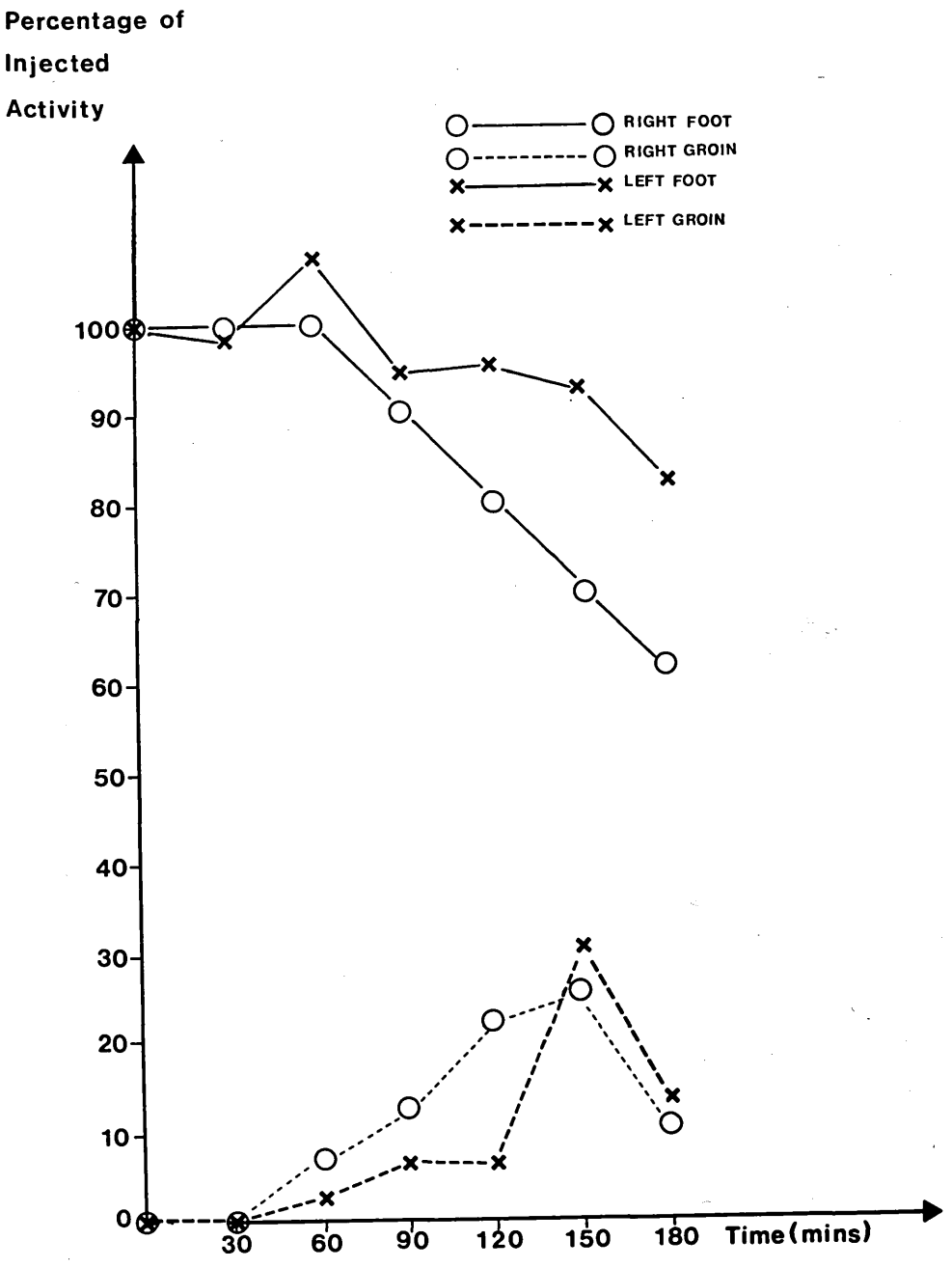


Figure 27

Graph showing clearance of 99m Tc RSC from injection site and arrival in inguinal lymph nodes measured externally by a scintillation counter in a second patient with primary lymphoedema of the left leg.

node to the collimator's field.

d) There appeared to be no difference in the studies following either an inter-digital space injection or a mid calf injection and as the latter was less painful - presumably because there is greater tension in the tissues in the inter digital space, the mid calf injection appeared advantageous.

The conclusions from this preliminary study were:

1. Gamma camera imaging of the ilio-inguinal lymph nodes may be of sufficient diagnostic accuracy to be useful and this was therefore pursued in the rest of the study.

2. The measurement of the clearance of the radioactivity of the colloid form the injection site using a scintillation counter tended to be inaccurate particularly in the presence of oedema.

3. The measurement of the amount of radioactivity arriving in the inguinal lymph nodes was also inaccurate. However, it is possible to calculate the amount of activity present in the ilio-inguinal lymph nodes using the gamma camera in a much wider area than by using the scintillation counter - because the gmma camera has a much wider collimaor - without the positional problems encountered with the narrow collimator head of the external scintillation counter.

4. An interdigital space injection was more painful and therefore tolerated less well than an injection in the subcutaneous tissues of the calf.

The technique was therefore modified, and the use of the scintillation counter abandoned.

(VII) Radionuclide lymph node imaging following a mid-calf injection - Method 1

Introduction

In designing this part of the study the original two aims were accommodated by using the gamma camera with computer link up for accumulating the data.

The flow of lymph was assessed by looking at the rate of arrival of the colloid in the ilio-inguinal lymph nodes for the first 30 minutes following a peripheral injection of the radioactive colloid. Initially, this was carried out for 60 minutes but this was reduced to 30 minutes for two reasons:

(i) The data appeared relatively linear in nature and the data obtained during the first 30 minutes appeared to reflect that being accumulated over the longer period.

(ii) It proved impractical to keep the subject in the same position under the gamma camera for that length of time without some movement.

Technique

0.2 mls (75 MBq) 99mTc Rhenium sulphide colloid was injected into the subcutaneous tissues over the centre of the anterior tibial compartment of each leg with the patient lying supine using a 25 gauge orange needle on a 1ml syringe (Figure 28).

The arrival of the colloid in the ilio-inguinal lymph nodes was imaged externally by a large field of view gamma camera (International General Electric), fitted with a low energy high resolution parallel hole collimater, 40 cm in diameter, with on-line computer facilities, positioned over the lower abdomen and groins for each study (Figure 29). The upper limit of the field of view was the umbilicus, the lower limit being the mid-thigh region (Figure 30).

The subject remained at rest during the first 30 minutes but was allowed to get up and about during subsequent images. Data was accumulated continuously in 300 second frames for 30 minutes following the injection and then 300 second images were obtained at 1 hour, 2 hours and 3 hours. The data was stored in the computer for later analysis.

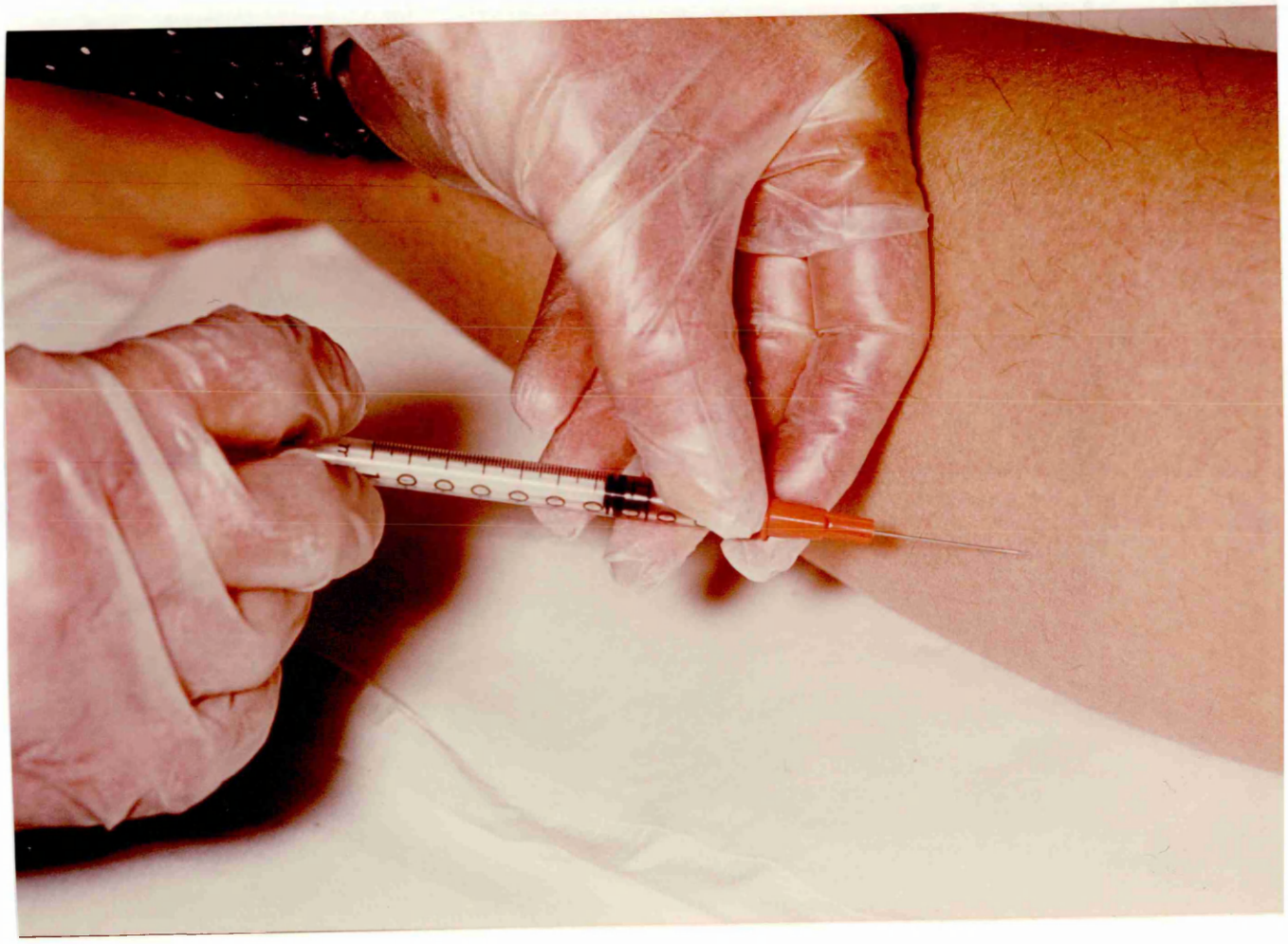


Figure 28

Photograph of an injection of 99m Tc RSC into the subcutaneous tissues of the calf over the anterior tibial compartment in a control subject. The angle of the needle was kept at 45° to the skin and was introduced approximately 1 cm into the subcutaneous tissues.

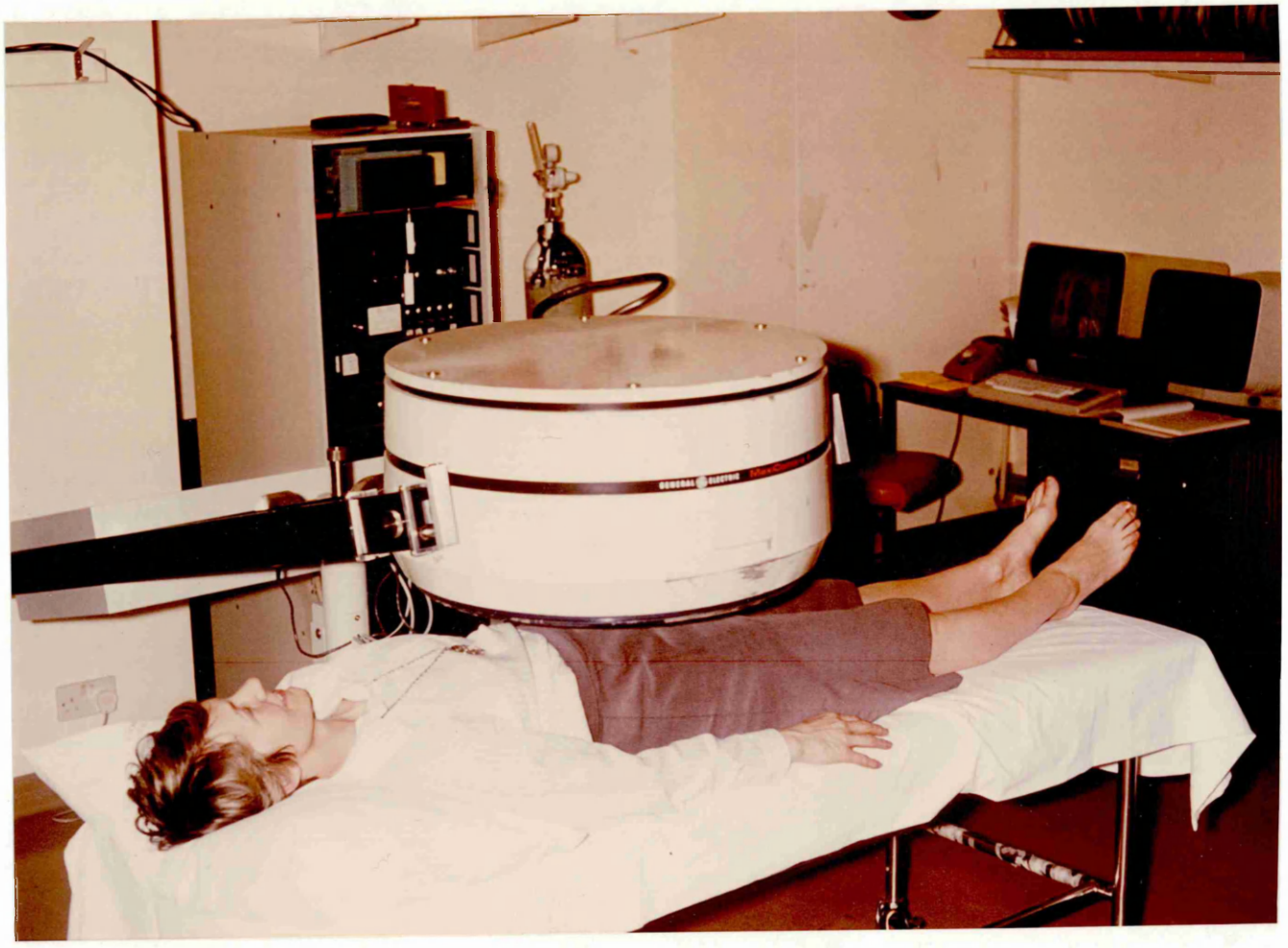


Figure 29

Photograph of the gamma camera in position over the lower abdomen and groins of a control subject.

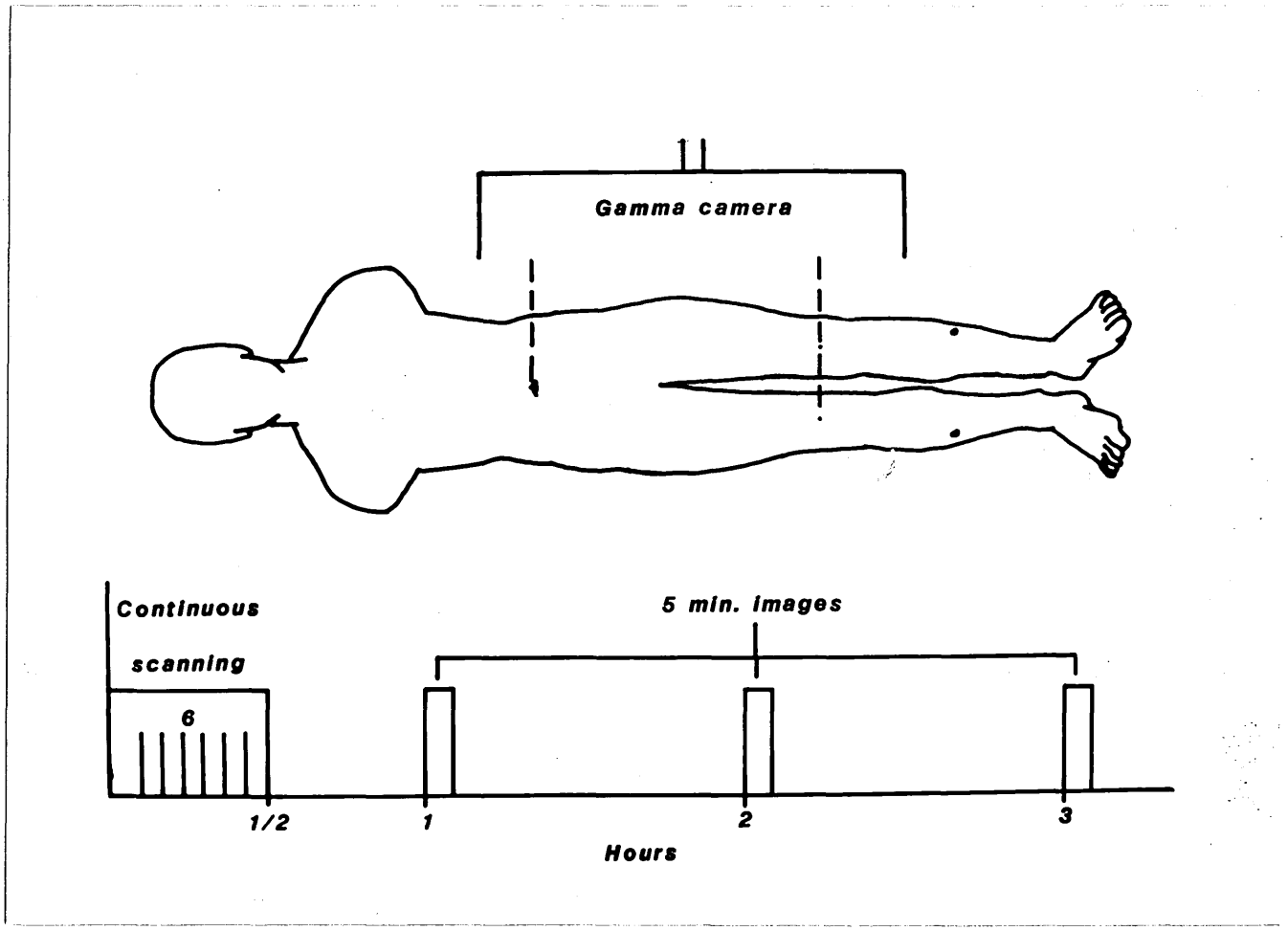


Figure 30

Diagrammatic representation of the technique showing the position of the gamma camera and the timing of the images following the injection of 99m Tc RSC.

Analysis of data obtained in the first 30 minutes

A region of interest was drawn around each ilio-inguinal lymph node chain using the data obtained over the first 30 minutes (Figure 31) and time/activity curves plotted for each study. These curves were corrected for background activity and the decay of the isotope. A linear regression curve was then calculated from the time /activity curve so obtained using a least squares approximation analysis, to provide a gradient of this curve for each limb (Figure 32); this gradient being taken as a measure of the rate of arrival of the colloid.

Analysis of the images obtained

The serial images were also observed visually by an independent observer who had no knowledge of the clinical or radiological features of the legs for the presence or absence of activity in the ilio-inguinal lymph nodes for all images taken in each study.

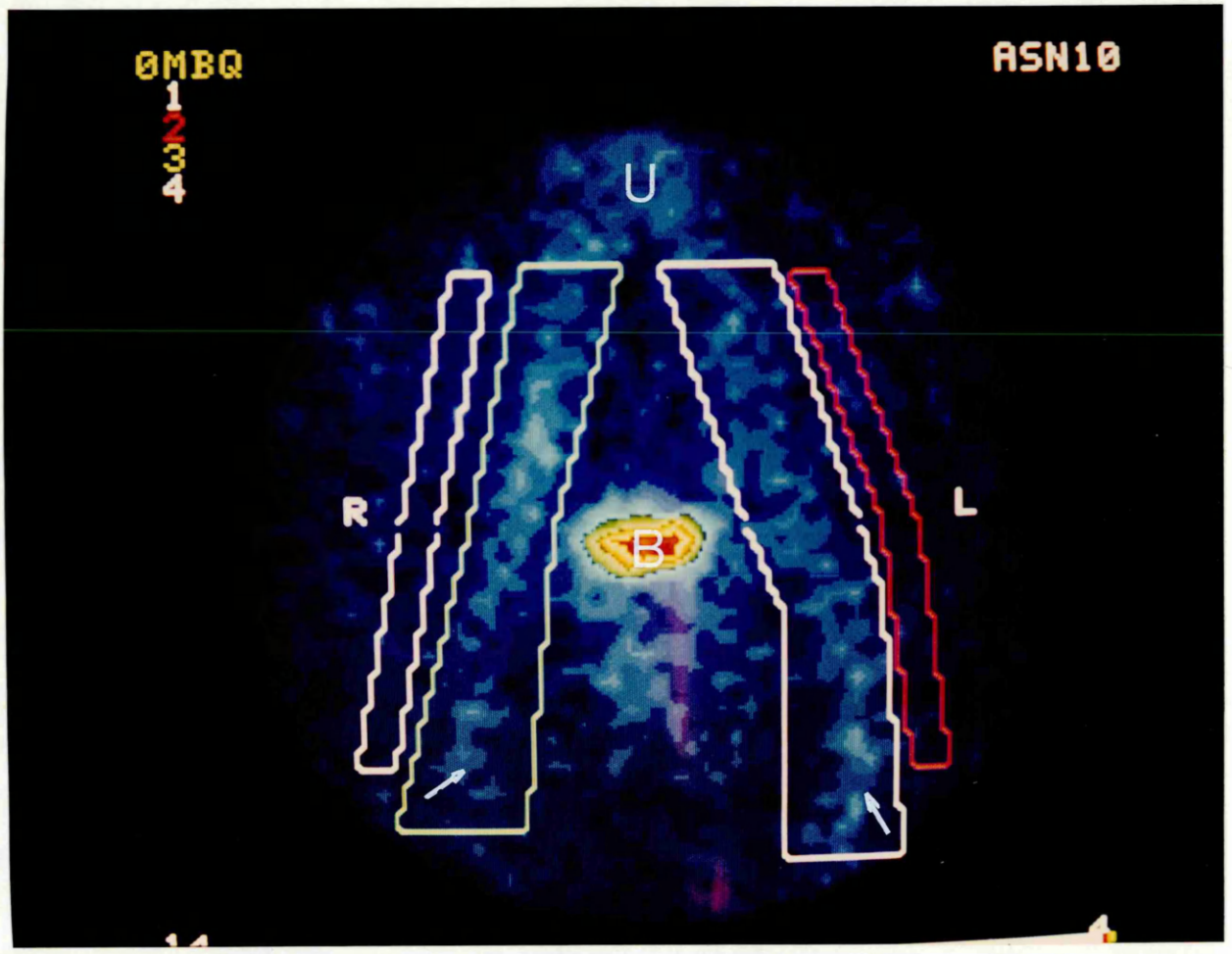


Figure 31

Image of the ilio-inguinal lymphatic chain taken at 10 minutes in a control subject showing a normal pattern of activity in the ilio-inguinal lymphatic chains on both sides (arrowed). A region of interest has been drawn around each chain and over the background on each side in order to calculate the time/activity curves.
The position of the umbilicus is arrowed.
B = position of bladder.

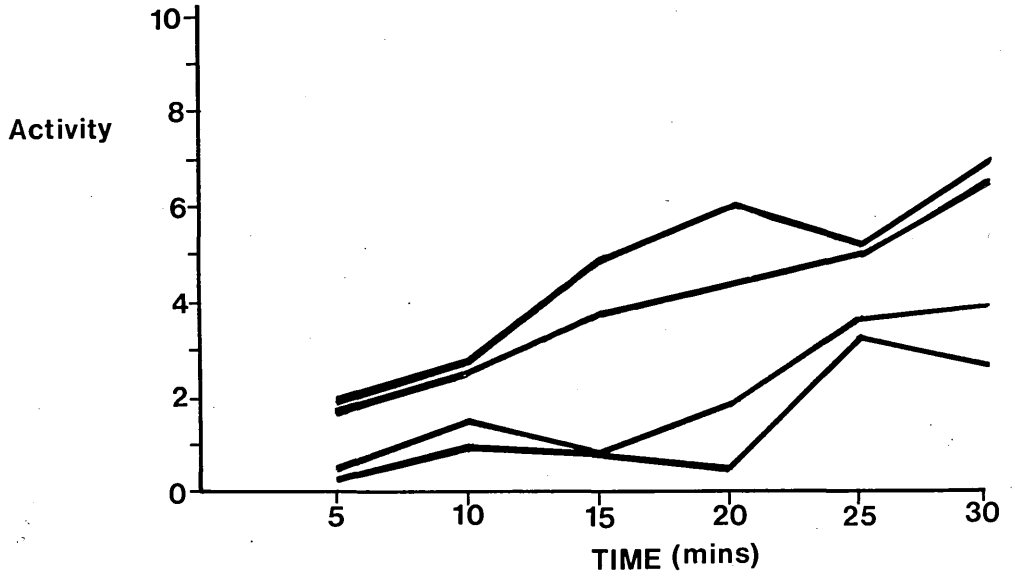


Figure 32a

Time/Activity curves obtained in a control subject
a) corrected for decay of the isotope and background activity.

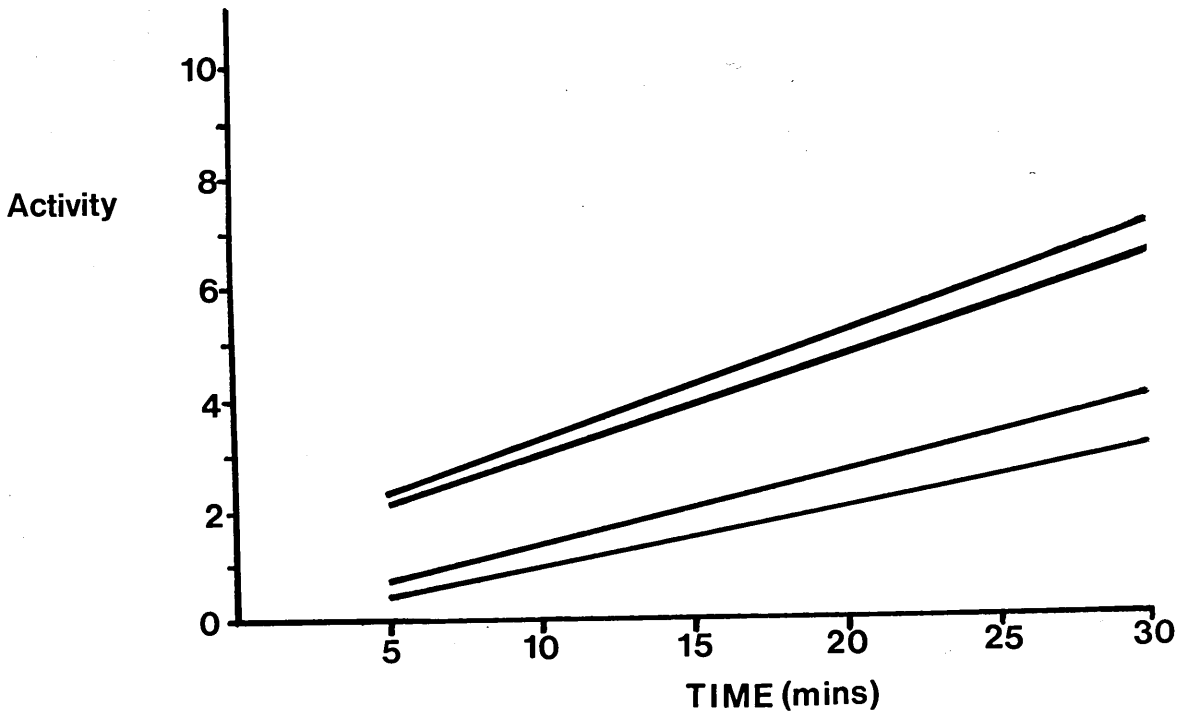


Figure 32b

Time/Activity curves obtained in a control subject
b) the linear regression curves obtained from these time/activity curves.

Reproducibility of the technique

A study was set up to evaluate the reproducibility of the method. Six control limbs were studied twice by the above method. The linear regression curves i.e. the rate of arrival of the colloid obtained in each was compared (Figure 33).

Study No.	Rate of arrival of the colloid	
	Study 1	Study 2
1	0.92	1.23
2	0.64	0.72
3	1.16	1.41
4	1.14	1.10
5	1.40	1.34
6	1.05	1.20

Figure 33

Reproducibility (Method 1):- Rate of Appearance of the Isotope from 0 - 30 minutes (studies repeated in 6 control limbs)

Analysis of these results shows that the likelyhood of being able to repeat this test in the population as a whole within 10% and 20% of the original result is 4-78% and 22-96% respectively (confidence limits of 95%). Although the numbers are small, the reproducibility of this method appears moderate to reasonable.

Summary

The technique of continuous data accumulation was employed in an attempt to provide functional information about lymph flow in a particular limb. The mid calf injection site was used because it was less painful than an interdigital space injection, and also to provide functional information about the whole limb rather than it most peripheral part.

The technique, however, had certain drawbacks, paramount being the inability to differentiate clearly between venous and lymphatic oedemas. Furthermore, the calculation of the rate of arrival of the colloid produced a considerable scatter of results with no clear differentiation between the 3 groups studied. Both these points will be discussed in more detail in Chapter 7.

(VIII) Radionuclide lymph node imaging following an interdigital space injection - Method 2

Introduction

In designing this part of the study several factors were taken into account. Firstly, it was decided to revert to an interdigital space injection despite the fact it is slightly more painful, for three reasons:

1. To diminish the effects of local diffusion in the presence of oedematous calf tissues particulary in lymphoedema.

2. To prevent the potential effects of the local problems of fibrosis known to be present in the calf skin of patients with chronic venous disease (Browse and Burnand, 1982).

3. Patients with mild lymphoedema often have normal lymphatics from the knee upwards with only distal lymphatic abnormalities (Kinmonth, 1982), and hence in this group of patients a radionuclide study following a calf injection may be normal.

It was, therefore, hypothesised that the use of an interdigital space injection may prove more accurate in differentiating lymphoedema from other causes of chronic lower limb swelling.

It was also decided to abandon continuous data accumulation and to base the assessment of lymph flow on the amount of activity present in the ilio-inguinal lymph nodes at four specific times following injections for 2 reasons:

1. The technique of uptake estimation is already established in thyroid uptake scans (Maisey, 1980) and has proven to be accurate, and it was hypothesised that this may be the case in the measurement of lymph flow.

2. Calculation of the percentage uptake of activity in the ilio-inguinal lymph nodes has two potential advantages as a quantitative measurement of lymph flow over continuous data accumulation :

a) The technique was simpler and did not require the patient to lie immobile under the gamma camera for 30 minutes.

b) It allowed quantitative assessment of lymph flow over a longer period of time (3 hours) than that obtained with continuous data accumulation (30 minutes).

Technique

0.2 ml (75mBq) of Technetium labelled rhenium sulphide colloid was injected into the subcutaneous tissues in the second interdigital cleft of both feet using a 25 gauge orange needle on a lml syringe with the patient lying supine (Figure 34).

The ilio-inguinal lymph nodes were externally imaged by a large field of view gamma camera (International General Electric), fitted with a low energy high resolution parallel hole collimator, 40 cms in diameter, with on line computer facilities (Nodecrest). The gamma camera was positioned over the lower abdomen and groins for each study (Figure 29), the upper limit of the field being the umbilicus and the lower limit the mid thigh region, similar to that in Method I.

Three hundred second images of the ilio-inguinal lymph nodes were obtained at 30 minutes, 1 hour, 2 hours and 3 hours following the injection (Figure 35) and the data stored in the computer for later analysis. To keep the subject's activity similar to that of Method I and to each other, each subject/patient was kept at rest during the first 30 minutes of the study but was allowed to get up and move about between subsequent images.

Figure 34

Photograph of an injection of 99m Tc RSC into the subcutaneous tissues of the 2nd inter-digital space in a control subject, the needle being introduced approximately 1 cm into the subcutaneous tissues.

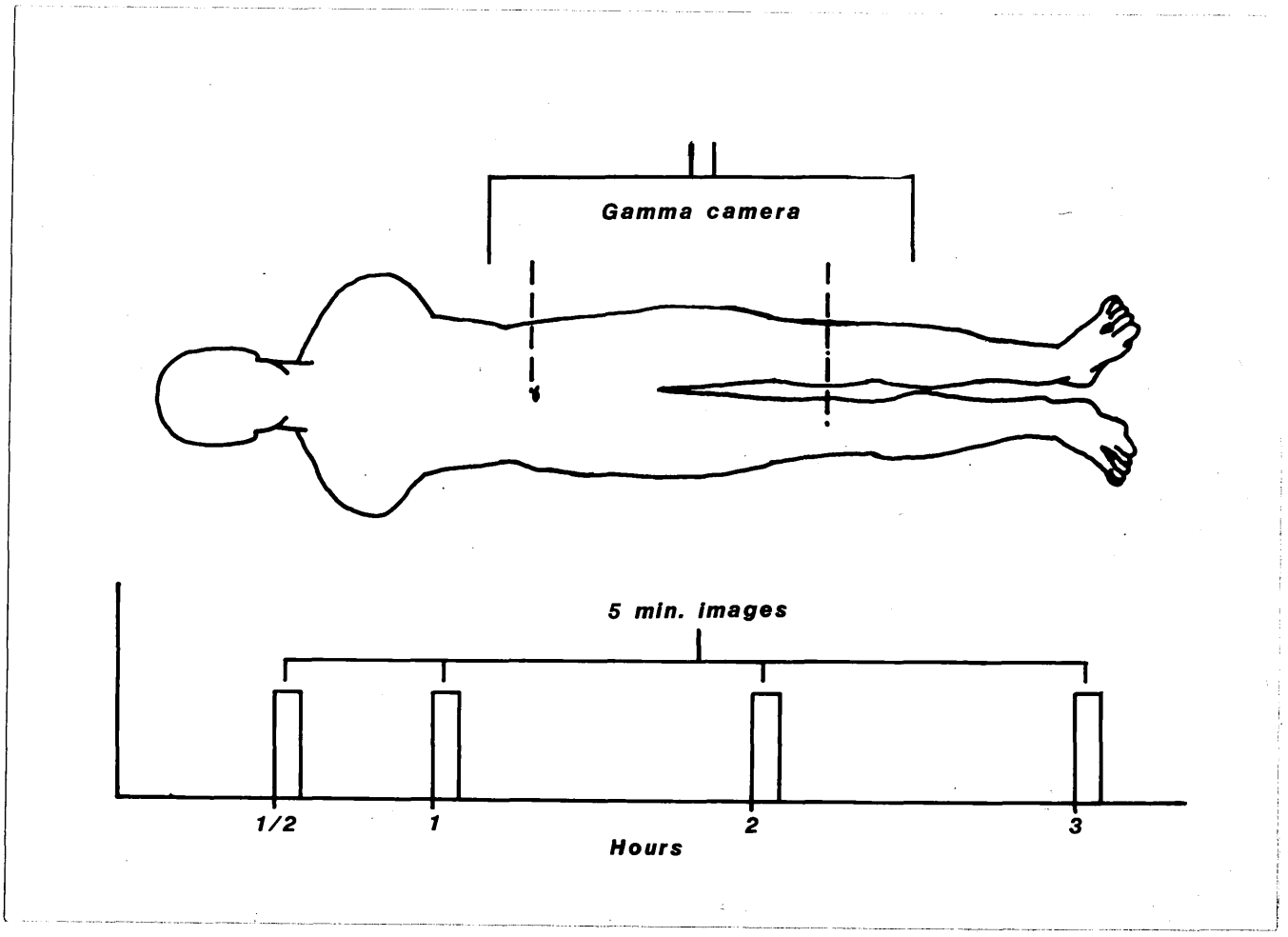


Figure 35

Diagrammatic representation of Method 2, showing the position of the gamma camera and the timing of the images following the injection of 99m Tc RSC.

Calculation of the percentage uptake of the radiocolloid in the ilio-inguinal lymph nodes

The activity of the injected dose was determined by measuring the activity of the colloid in the syringe place in a ghost model on top of the gamma camera collimator (Figure 36) before and after the injection in absolute counts over a ten second period. This was repeated on 3 occasions before and after for each injection and the mean of these readings taken as the injected activity. This ensured that the activity present in the injected site was measured at the same distance from the gamma camera for all studies.

A region of interest was drawn around each ilio-inguinal lymph node chain on each of the half, one, two and three hour images (Figure 37). The activity within this region was calculated in counts per minute and corrected for background activity and decay of the isotope. This value was compared with the activity of the initial injection in counts per minute to obtain a percentage uptake of the colloid in the ilio-inguinal lymph nodes at half, one, two and three hours.

An example of this calculation is shown in Figure 38.

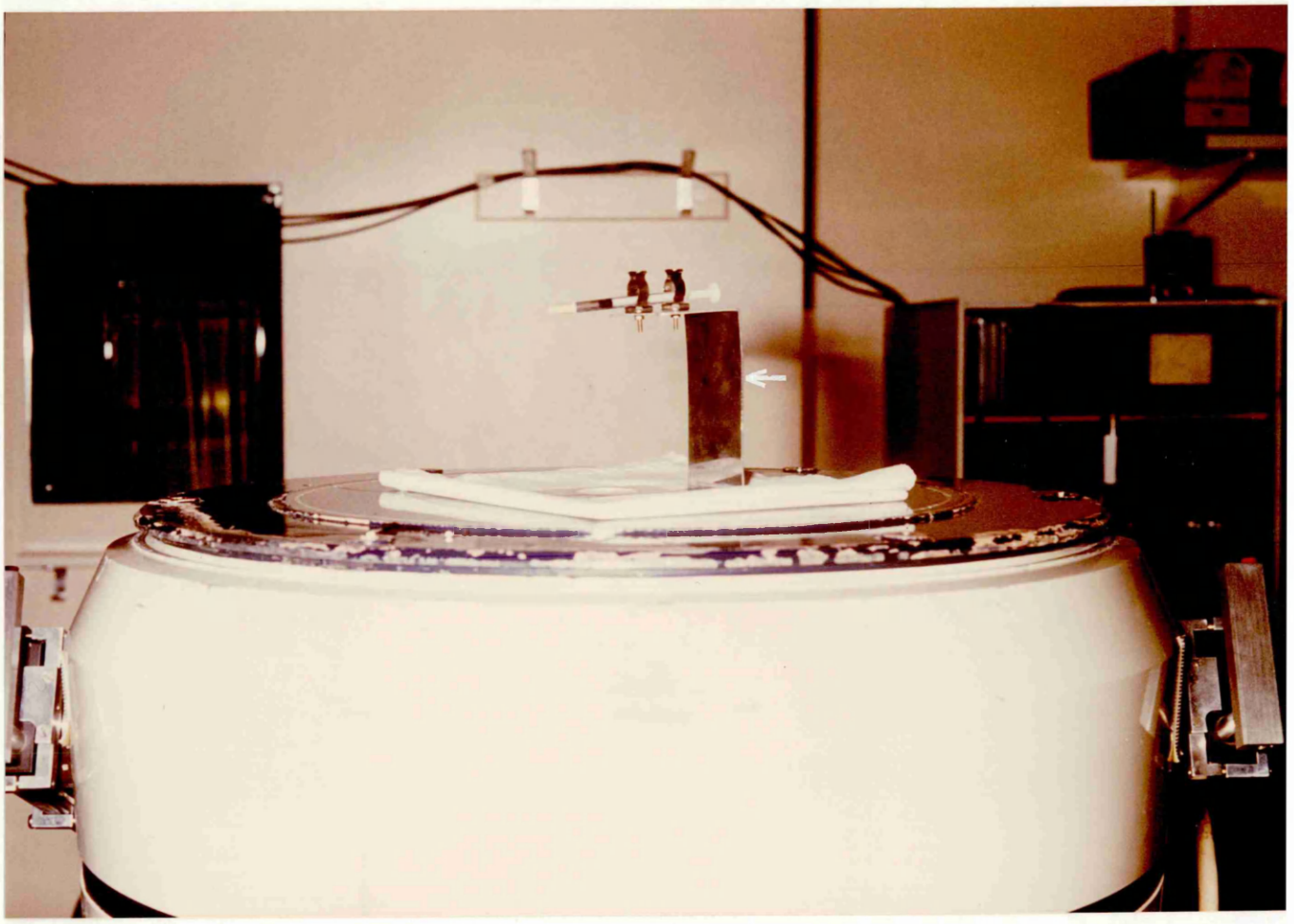


Figure 36

The syringe was placed on the top of the gamma camera in the ghost model/jig (arrowed) before and after each injection. This was designed to hold the syringe at the same distance from the gamma camera for each study.

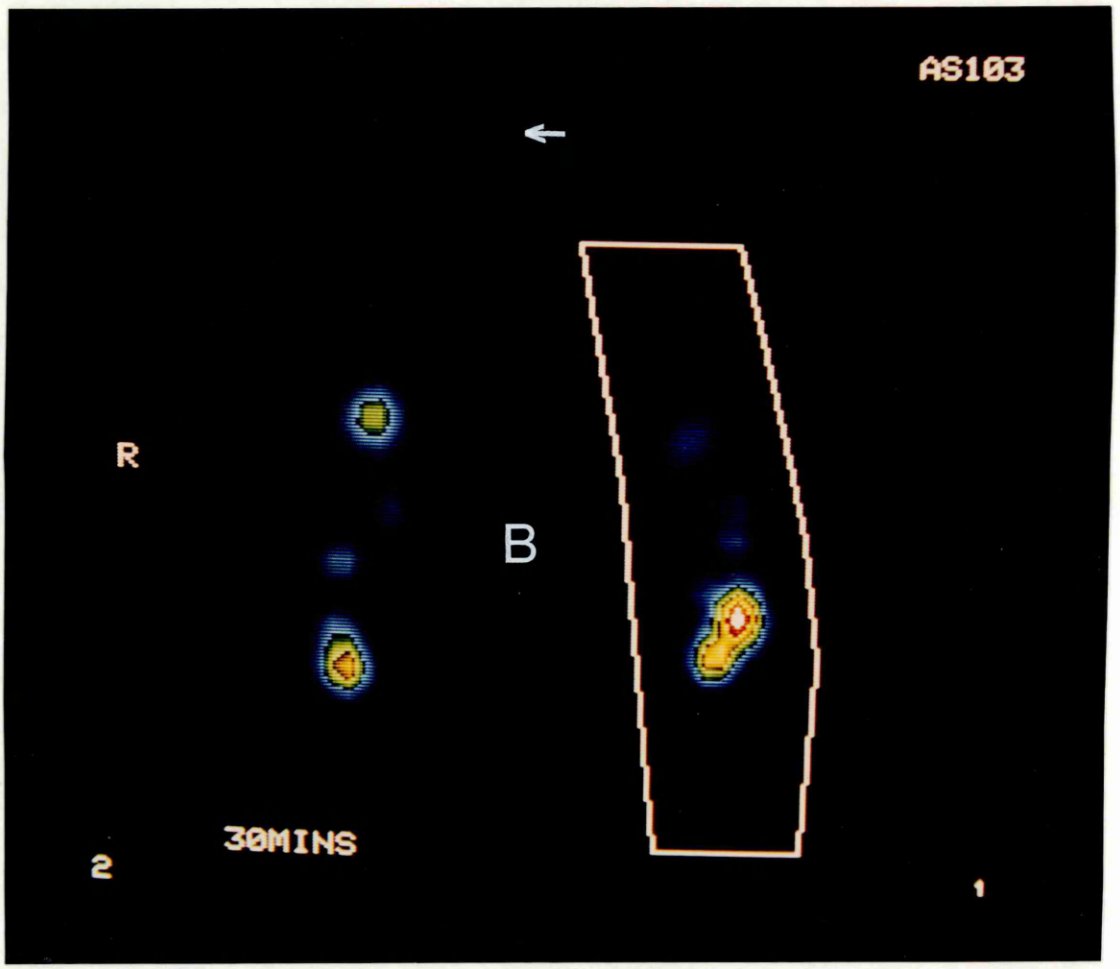


Figure 37

Radionuclide image taken at 30 minutes in a control subject showing radioactivity clearly visible in both ilio-inguinal lymph node chains. A region of interest has been drawn around the left ilio-inguinal lymph nodes. Another region of interest is drawn over the background (see Figure 31) to calculate the amount of background activity within a similar area. The position of the umbilicus is arrowed.
B = position of bladder.

Calculation of percentage uptake of 99m Tc RSC at 30 minutes

1. Total injected activity (calculated by subtracting the amount of activity in the syringe before and after injection).
= 8900 counts/second

2. Activity within region of interest around ilio-inguinal lymph nodes at 30 minutes.
= 78219 counts/300 seconds

3. Background activity in equivalent area at 30 minutes.
= 6469 counts/300 seconds

The percentage uptake of activity at 30 minutes (corrected for background)

$$= \frac{\text{(Total Activity - Background Activity) counts/300 secs}}{\text{Injected activity counts/sec}} \times 100$$

$$= \frac{\text{Total Activity - Background Activity} \times 100}{\text{Injected Activity} \times 300}$$

This calculation needs correction for decay of the isotope, the correction factor being calculated from the decay curve of the Technetium and for 30 minutes is 0.92

Therefore, the percentage uptake of activity at 30 minutes:

$$= \frac{\text{Total activity - Background activity} \times 100}{\text{Injected Activity} \times 300 \times 0.92}$$

$$= \frac{78219 - 6469}{8900 \times 300 \times 0.92} \times 100$$

$$= 2.92\%$$

Figure 38

Specimen calculation of the percentage uptake of the injected activity in the ilio-inguinal lymph nodes in the left limb of a control subject.

Initially these calculation were done by hand but later a computer programme was devised for this calculation thus providing the results of the percentage uptake without laborious calculation.

Analysis of the images obtained

The images obtained were also observed visually by an independent observer, who had no knowledge of the clinical or radiological features of the legs, for the presence or absence of activity in the ilio-inguinal lymph nodes.

Reproducibility of the technique

A study was set up to determine the reproducibility of the technique. Twelve limbs (7 controls and 5 with lymphoedema) were studied on two occasions and the percentage uptake of the radioactive colloid at 30 minutes compared.

No.	Study 1	Study 2
1	1.2	1.4
2	0.96	1.05
3	1.6	1.56
4	1.4	1.34
5	0.8	0.86
6	1.4	1.46
7	1.0	1.2
8	0.06	0.08
9	0.0	0.0
10	0.0	0.0
11	0.003	0.0
12	0.02	0.0

Figure 39

Reproducibility Method 2. Percentage uptake of the isotope measured at 30 minutes (studies repeated in 7 control limbs and 5 limbs with primary lymphoedema).

Analysis of these results shows that the likelihood of being able to repeat this test in the population as a whole within 10% and 20% of the original result is 27-85% and 52-98% respectively. (Confidence limits of 95%). Furthermore, the likeihood of repeating the result within 0.1% - 0.2% of the original percentage uptake is 62 - 99% (Confidence limits of 95%).

Comparison of the results of this study with those of Method 1 suggests that this technique is more easily reproducible and although the numbers are small, the reproducibility of Method 2 appears good.

(IX) Technique of Lymphography.

The technique of lymphography is now well established and is based on the description by Kinmonth (1952, 1982). The investigation is performed with a general anaesthetic which has several advantages over local anaesthetic: a more radical exploration for lymphatic trunks in the foot can be carried out and if necessary a groin injection can be performed. Injections of patent blue violet are painful which may limit the number of injection sites if the patient has a local anaesthetic and since the procedure may continue for some time problems of patient discomfort and movement (with consequent dislodging of the cannula and blurring of the radiographs) will be avoided.

Bipedal Lymphangiography.

(a) Preparation

The patient lies supine with a 15^{o} head down tilt and the feet and groins are prepared with a standard antiseptic solution and draped with sterile towels. A subcutaneous injection of 0.2 ml of 1% patent blue violet (isotonic solution) is made into each web space of the toe, and the foot is then massaged vigorously to fill the subcutaneous lymphatics with the dye. These are clearly visible through normal skin if they are of normal calibre (Figure 40). Both feet are injected as both limbs are always examined.

(b) Exposure and Cannulation of Lymphatic.

A transverse incision is made on the dorsum of the foot and a lymphatic dissected free with the aid of the Zeiss operating microscope. It is important not to cannulate a vein as this

will result in pulmonary oil embolism. The veins appear a darker blue colour and are thicker walled. If a vein is cannulated an early radiograph will show characteristic droplets of oil in the vein, known as the 'caviare sign'.

The contrast medium employed was ultrafluid Lipiodol UFL (May & Baker Ltd.). This is an iodinated ethyl ester of certain fatty acids from poppy seed oil (Papaver Somniferum). The iodine content of the medium is 38% w/v and it has a viscosity of 55 centipoises at 20°C.

Handling of the lymphatic results in spasm and should therefore be kept to a minimum. If it occurs, a little procaine solution should result in dilation of the vessel. The lymphatic is distended by means of temporary occlusion using proximal pressure and massage of dye towards the occlusion. The exposed lymphatic is then cannulated (Figure 40) using a St. Thomas' lymphangiogram set (Rutt, Gough and Kinmonth, 1964).

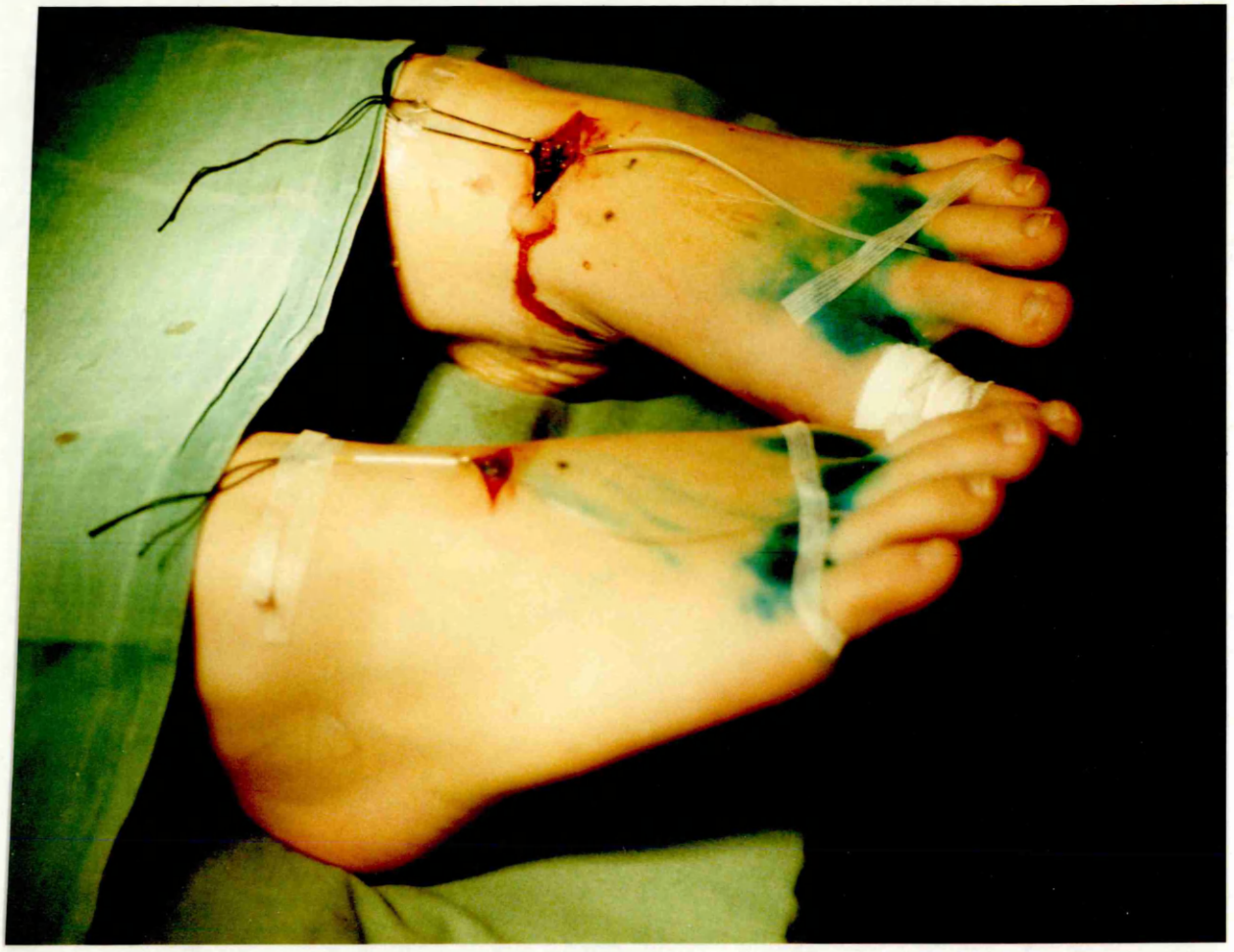


Figure 40

Injection of patent blue violet into the inter-digital space during lymphography outlines the normal lymphatics in the dorsum of the clinically normal right foot and allows cannulation of the lymphatics in the dorsum of both feet. This patient had mild lymphoedema of the left leg.

Injection.

A little air is injected into the lymphatic to ensure that the needle is in the lumen, and the contrast medium then injected.

This oily radio-opaque contrast medium will remain in the lymph nodes for many weeks. The dye that is not retained in the lymphatic system enters the circulation and is trapped in the pulmonary capillaries. If too much oil is injected, the patient may become pyrexial and short of breath due to pulmonary oil embolism. Consequently a maximum of 7 ml is injected into each side making a total infusion not exceeding 14 ml.

A Lund pump (Clementz and Olin, 1961) is used to deliver the infusion at a constant volume at the set infusion rate which can be altered on the ten speed gearbox. The infusion is started at rate 8 (1 ml in 8 minutes) and adjusted according to the resistance of the lymphatic system. If the dye is pumped into an obstructed system too fast it will extravasate and useful information will be lost. To avoid this, a radiograph is taken of the lower legs five minutes after the infusion has started, to ensure that the contrast material is passing up the lymphatic statisfactorily. If there is extravasation the rate of infusion is slowed.

When the infusion is complete the needle is removed and the skin sutured with interrupted nylon sutures that are removed between seven and ten days later according to the degree of oedema in the foot. Any leakage of Lipiodol should be cleared from the wound and meticulous care taken with the suturing since these wounds can heal poorly in lymphoedematous feet.

Inguinal Node Injection.

It is sometimes impossible to obtain a full lymphangiogram from the pedal route for the following reasons:

1. No visible lymphatic in the foot.
2. Lymphatics are fibrotic or too small for cannulation.
3. Cannulation is possible but proximal flow up the main lymphatics is not achieved.

Groin lymphography should then be performed.

The patient has been previously prepared and draped for groin incision, and only a very small groin incision is necessary. On some occasions a superficial inguinal node is clinically palpable but otherwise one is usually found following the incision. The dissection should be minimal and done with great care to avoid cutting or avulsing the lymphatics of the node. An afferent lymphatic is then cannulated as it enters the node and Lipiodol infused. The subsequent lymphangiograms and lymphadenograms are taken in the usual way.

There is usually more extravasation of Lipiodol using this method, especially if there is proximal obstruction to flow or if lymphatics have been avulsed from the node. Also little accurate information can be achieved about the inguinal nodes theselves. Nevertheless, useful information on the proximal lymphatic channels may be obtained. Sometimes, with the aid of patent blue violet injected subcutaneously into the thigh, it is possible to cannulate an afferent lymphatic in the groin. Using this method there is less extravasation and the lymphatic network is better shown.

Radiography.

Serial antero-posterior supine lymphangiograms of the lower limbs, inguinal region, pelvis, abdomen and chest are taken during the procedure. The time of commencing the infusion and the exposure of each radiograph is noted so that a 'transit time' of the flow of Lipiodol from the limb can be calculated. This department uses the sacro-iliac joint as a marker and the transit time to this point should be less than 35 minutes.

If the contrast medium has not reached the thoracic duct before the patient leaves theatre, further thoracic radiographs should be taken in the recovery room. The flow of Lipiodol into the thoracic duct is assisted by vigorous flexion and massage of the legs before the patient is woken up from the anaesthetic. Anterior right oblique and antero-posterior radiographs of the chest are then taken to show the thoracic duct.

The contrast medium should clear from the lymphatics within a few hours but remains in the lymph nodes for several weeks. Subsequent lymphadenograms are taken at 24 hours of the inguinal, lumbar, mediastinal and supra-clavicular nodes. Radiographs are also taken of the legs to see if any Lipiodol remains in obstructed lymphatics. If there is gross lymphatic obstruction in the pelvis the axillary nodes may be filled through collaterals and these should be specifically looked for on chest X-Ray.

(X) Technique of Bipedal Ascending Phlebography

This is a well established technique carried out in most X-Ray departments as a routine procedure, the aim of the examination being to demonstrate the deep venous system from the foot to the lower inferior vena cava. As the technique of ascending phlebography is well known and is well described elsewhere (Lea Thomas, 1982), I therefore will not go into the technique in any further detail in this thesis.

In this study the phlebography was carried out in the Department of Radiology, St. Thomas' Hospital by the technique described by Lea Thomas and the phlebographs interpreted by an independent observer who had no knowledge of the clinical features of each patient.

(XI) Radiation Dose - Ethical permisssion.

The maximum radiation dose was locally at the injection site. This was 2100 m Rem and the dose to the ovaries from all sources of radiation was 100 m Rad. These doses are within Category I (the lowest range) of the classification used by ARSAC* (DHSS). Ethical approval for the study was obtained from the St. Thomas' Hospital Research (Endowments) Committee and informed consent obtained before the procedure.

* ARSAC - Administration of Radioactive substances Advisory Committee.

(XII) Statistical Analysis

This was performed using a student's t test both paired and unpaired and where non parametric analysis was indicated the Mann Whitney U Test was performed.

CHAPTER 5

RESULTS

If the mathematicians are right,
the biologists cannot have what they demand

Lord Salisbury 1894

Introduction

The original aim of this thesis was to assess the value of radionuclides in the investigation of the lymphatic system, in patients with primary lymphoedema, a specific interest of the Department of Surgery at St. Thomas' Hospital. Early in the study it was suggested that I was measuring the effect of simple oedema on lymph flow and not the slow lymph clearance associated with lymphoedema. Therefore, as well as a control group of limbs, a group of limbs with venous oedema were studied. In the latter part of the study a further group of limbs with non cardiac, non renal causes of lower limb oedema were also studied. The next section deals with the clinical and radiological features of these groups.

(1) Assessment of Control Groups

Sixteen volunteers were studied with the isotope techniques. These volunteers acted as the control limbs in the study. They were assessed clinically and were included as controls if there was no evidence of swelling of the legs, no clinical evidence of primary lymphoedema, no family history of primary lymphoedema, no history of deep venous thrombosis and no stigmata of venous disease.

It was not felt ethical to ask them to undergo lymphography or phlebography and therefore their inclusion as control limbs was made on clinical grounds. Ethical approval was obtained to study this group of volunteers.

(II) Assessment and diagnosis of patients with primary lymphoedema.

78 patients with primary lymphoedema were studied. Each patient was assessed clinically and lymphographically. The isotope study was carried out in all cases prior to lymphography to obviate any effects that Lipiodol may have on the lymph nodes and lymph flow.

Clinical assessment

Each patient had a careful clinical assessment and the previous case notes were studied. Particular attention was paid to following points which were transposed onto a standard form.

(a) History

1. Family history of primary lymphoedema.
2. Age of onset of clinical lymphoedema.
3. Factors precipitating clinical lymphoedema.
4. Visits to countries where filariasis is endemic.
5. The original extent and subsequent progress of clinical lymphoedema.
6. Complications of the lymphoedema such as erythema, cellulitis or weeping lymphatic vesicles.

(b) Examination (Figure 41)

1. Measurement of limb girth and length
2. Associated genital lymphoedema, hydroceles, upper limb lymphoedema, or facial oedema.
3. Evidence of other congentital abnormalities asociated with lymphatic disease, especially capillary naevi on the

affected limb.

5. Evidence of dermal infection on the limb or in the inter-digital clefts.

(c) Initial Investigations

1. Full blood count.
2. Plasma proteins.
3. Culture swab of any infected sites.
4. Urine test for proteinuria.

The most significant feature of the history is the length. Lymphoedema develops very slowly. It may be familial and/or congentital and is often exacerbated by minor injuries to the limb, such as a sprained ankle. Perhaps the most noticeable feature of the swelling is that it affects the whole foot as well as the leg including the toes which often look 'square' in transverse section.

Lymphoedema is sometimes associated with vascular abnormalities; the commonest but most minor is a pale cutaneous naevus - the 'vin-rose' patch.

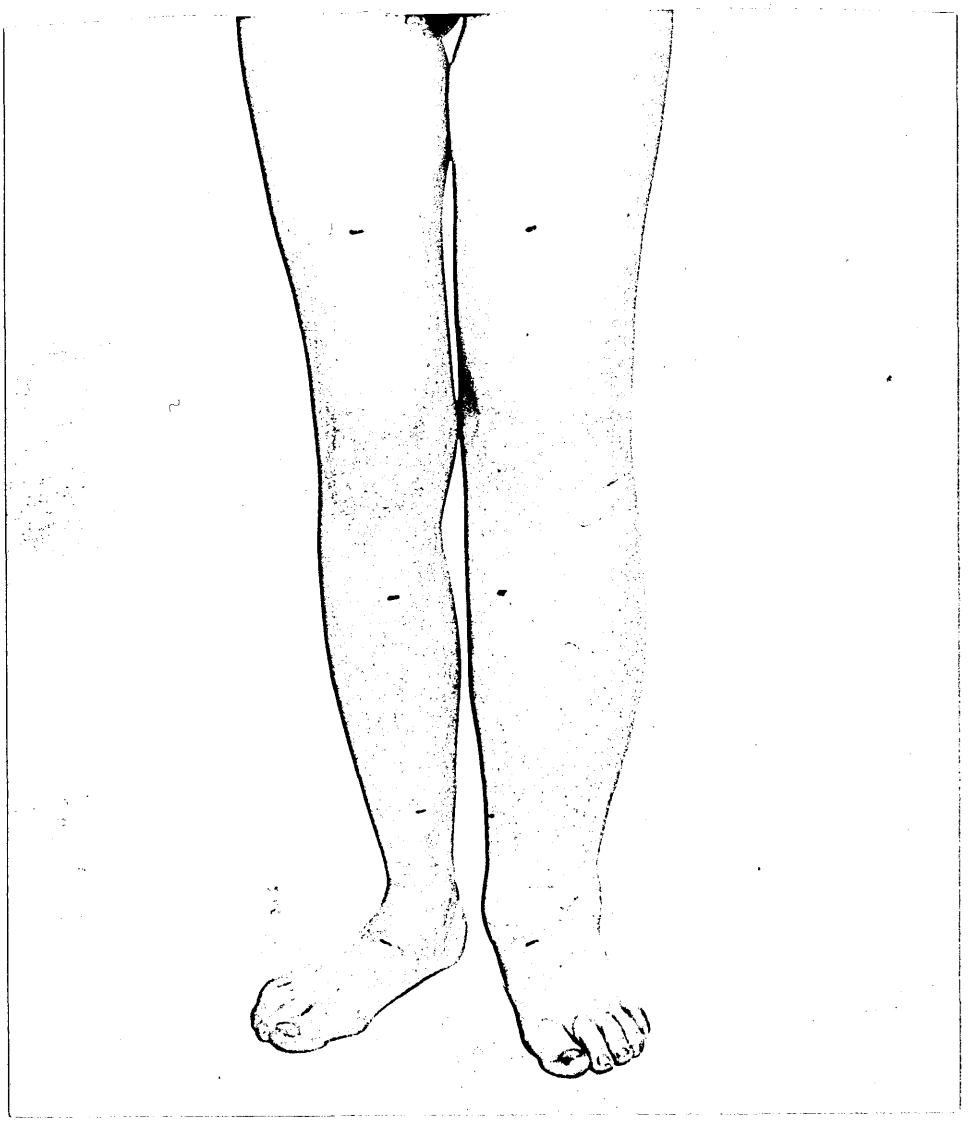


Figure 41

Patient with primary lymphoedema of the left leg showing the typical clinical features of hyperkeratosis, square toes and oedema.

Visual lymphography

When a highly diffusible dye (1% Patent Blue Violet) is injected subcutaneously it is, under normal circumstances, rapidly carried to the large lymphatic vessels and away from the site of injection. These major vessels become readily visible through the skin (Figure 40) and are subsequently demonstrated by radiological lymphography. If these collecting vessels are inadequate or obliterated the dye often diffuses through the dermal plexus of lymphatics and produces a blue marbled appearance to the skin (Figure 42), described as 'dermal backflow' (Kinmonth, 1982).

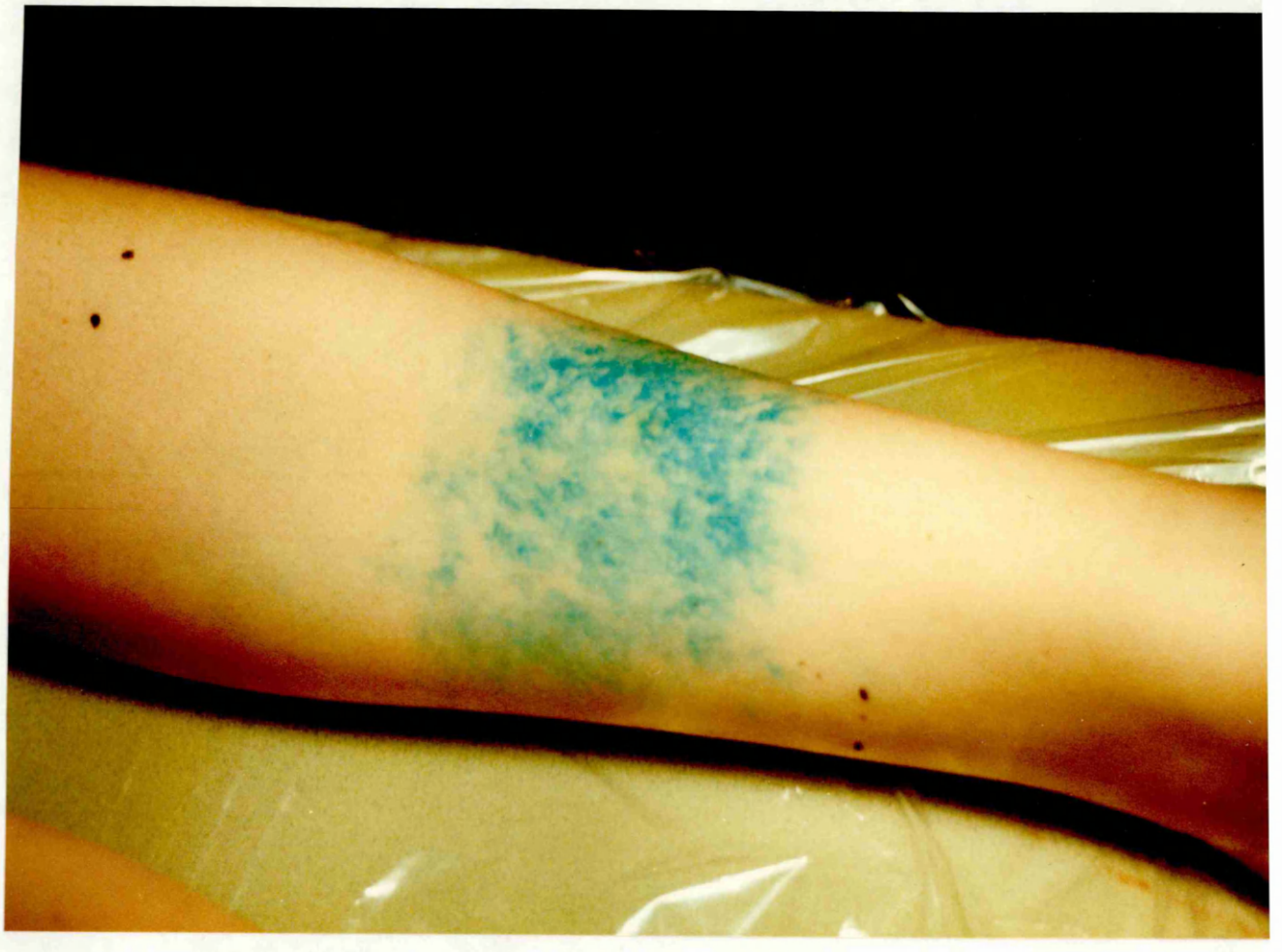


Figure 42

"Dermal backflow" in the calf of a patient with primary lymphoedema. Patent blue violet diffuses back into the dermal lymphatic plexus because of obliteraion of the main lymphatic trunks.

Radiological lymphography

The features of radiological lymphography in primary lymphoedema have been described in detail in Chapter 2. In summary, X-Ray lymphography may reveal no lymphatics, a reduced number of lymphatics in the leg or lymphatic dilatation caused by proximal obstruction or valvular incompetence. It also shows the architecture of the lymph nodes, and may reveal abnormalities of the lymph nodes or major lymph trunks.

A normal lymphangiograph is illustrated in Figure 43 and can be compared to the lymphangiograph of a patient with primary lymphoedeama - Figure 44.

Figure 43a

a) lymphangiograph showing normal lymphatics in the lower limb.

Figure 43b

b) Lymphangiograph showing the normal lymphatic appearance of the ilio-inguinal lymphatic chain.

Figure 44

Lymphangiograph showing rediological "hypoplasia". There is obliteration of the distal lymphatics showing only one lymphatic in the lower leg and thigh. The iliac lymphtics are normal.

Clinical findings in patients with primary lymphoedema

Patients were included in the study providing they had the clinical features of primary lymphoedema and that the lymphogram was abnormal. Patients were then sudivided into radiological subgroups according to Kinmonth's recent classification (1982) with the modifications described in Chapter 2.

Age distribution

The median age of onset was 24 years (range-congenital to 70). The age distribution is shown in Figure 45 and confirms that the majority of the patients have lymphoedema praecox (75% in this series). The median age at the time of the radionuclide study was 39 (range 13 to 72).

Factors relating to the aetiology of lymphoedema

These are summarised in Table 3. Eighteen patients had a close relative with primary lymphoedema. A firm diagnosis of lymphoedema was required and the majority of patients seen in our department. A vague history of a relative with swelling of the legs was not considered as evidence of primary lymphoedema.

Associated congenital anomalies were present in 5 patients but in 3 of these the only abnormality was a cutaneous angioma.

One male patient had congenital lymphoedema of both limbs associated with the Klippel Trenaunay Syndrome (Baskerville, Ackroyd, Lea Thomas et al., 1985) and none of the patients had Milroys disease (Congenital hereditary lymphoedema).

Twelve patients (15%), one male and eleven females, related the onset of the oedema to minor trauma, in most cases a sprained ankle. A further five patients related the onset of oedema to a minor cutaneous infection. In one patient this followed an insect bite.

Seventeen of the patients (2 males and 15 females) therefore related the oedema to a local factor. If, however, the patient who subsequently developed bilateral oedema (despite unilateral trauma) is excluded, 23% of the females related the onset of oedema to a local cause.

Three female patients developed oedematous ankles in pregnancy which left residual oedema following delivery. Each succeeding pregnancy exacerbated the swelling. In 24 (37%) of the female patients the onset was during the two years following puberty while this was the case in only one of the male patients.

Thus, in 28 of the patients (36%) it was possible that the onset of lymphoedema was related to hormonal changes at puberty or in pregnancy.

In none of the patients who related the oedema to a particular cause was the precipitating factor severe enough to cause oedema without a serious underlying defect in the lymphatics and in 33 (42%) patients there was no precipitating factor.

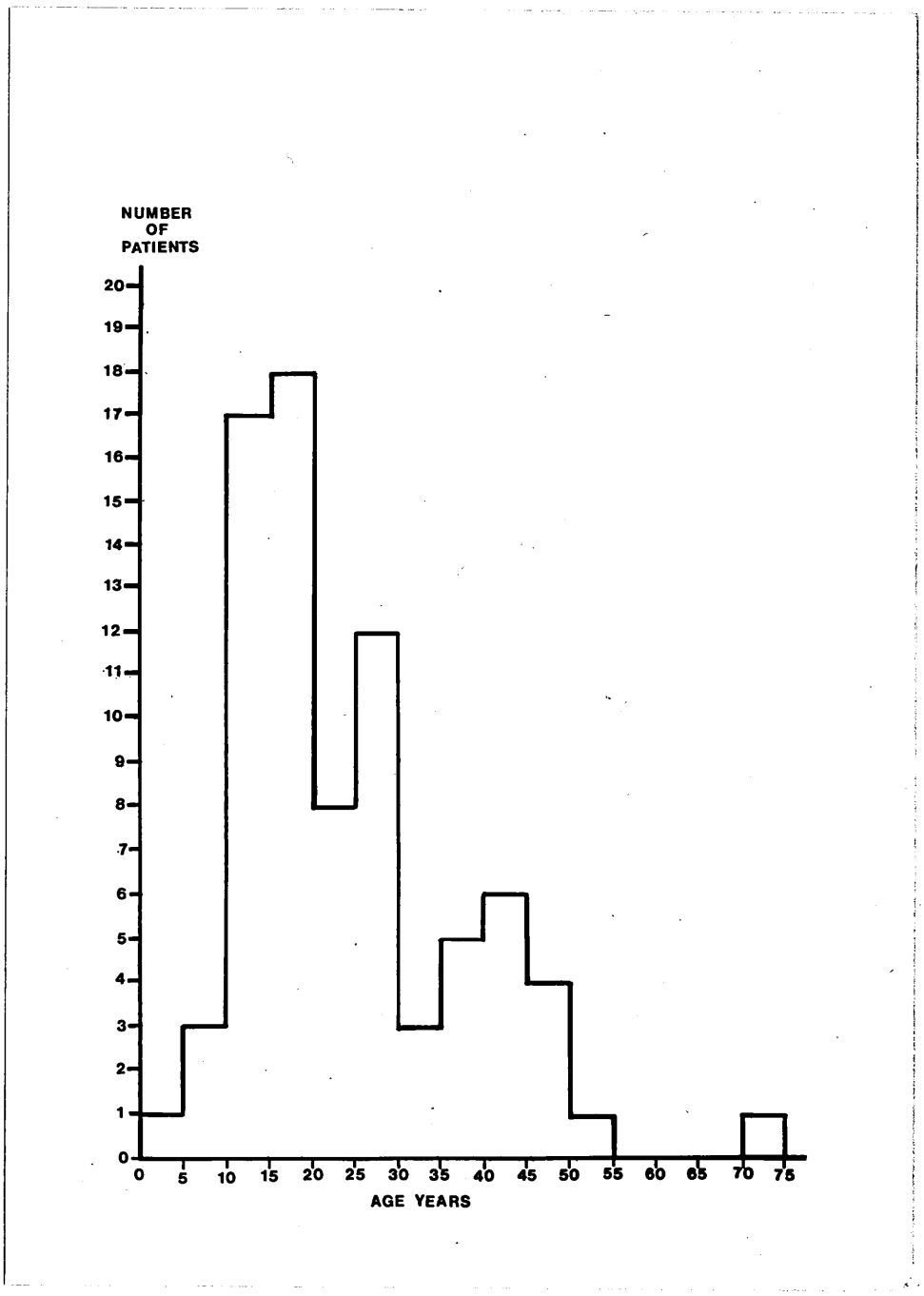


Figure 45

Distribution of the age of onset of lymphoedema.

TABLE 3

FACTORS ASSOCIATED WITH THE ONSET OF PRIMARY LYMPHOEDEMA IN 78 PATIENTS

		Males	Females	Total	% of Patients
Associated Congenital/ Hereditary Factors	Congenital lymphoedema	1	0	1	1.3%
	Family history of lymphoedema	1	17	18	16.7%
	Associated congenital abnormalities (excluding cutaneous angiomata)	1	4	5*	6.4%
	Associated cutaneous angiomata	0	3	3	4%
Associated Hormonal Changes	Puberty	1	24	25	32%
	Pregnancy	0	3	3	4%
Associated local factors	Trauma	1	11	12	15%
	Minor cutaneous Infection	1	4	5	6.4%

TOTAL 78 patients (65 female 13 male)

* Patient ductus arteriosus, atrial septal defect and deformed chest, Klippel-Trenaunay Syndrome.

Distribution of lymphoedema

These results are tabulated in Table 4.

Fifty-seven patients (8 male and 49 female) had unilateral lymphoedema. The extent of the oedema was usually more extensive in those patients with unilateral lymphoedema. 21 patients (37%) with unilateral disease had lymphoedema of the whole limb. Males were more prone to have extensive lymphoedema. Seven males (54%) and 18 females (27.5%) had lymphoedema affecting the leg and thigh.

These results suggest that there is a mild bilateral form of lymphoedema that usually affects females. Conversely unilateral lymphoedema is usually more severe and affects both sexes equally. Males with lymphoedema thus appear to have a worse prognosis than females.

Only three patients had lymphoedema affecting other areas; one female patient had genital oedema, one had noticed swelling of the hands and face and the third patient had lymphoedema of both arms and the face.

TABLE 4

The distribution of lymphoedema

	MALE	FEMALE	TOTAL
BILATERAL	5	16	21
WHOLE LIMB	2	2	4
UNILATERAL	8	49	57
WHOLE LIMB	5	16	21
OTHER PARTS AFFECTED	1	2	3*

* 1 genital, 1 hands and face, 1 both arms, hands and face

The severity of lymphoedema

The severity of lymphoedema was assessed both on clinical grounds and by considering the number of patients requiring surgery.

Patients were placed in three groups on subjective clinical assessment namely mild lymphoedema, moderate lymphoedema and severe lymphoedema (Figure 46).

(a) Mild Lymphoedema - swelling of the dorsum of the foot and ankle only.

(b) Moderate Lymphoedema - swelling of the lower leg below the knee, but no or minimal swelling of the knee and thigh and no previous surgery to reduce the size of the limb.

(c) Severe Lymphoedema - swelling of the whole of the leg or patients who had undergone any form of surgery either a reducing operation or a physiological procedure even if the swelling affected only the lower part of the limb.

The severity of lymphoedema (Table 5) was much greater in males with lymphoedema (39%) as compared to females (21%) and females tended to have a milder form of the disease (44% of limbs) than males (1 out of 18 limbs). These results again confirm the finding in the previous section that females tend to have a milder form of lymphoedema.

TABLE 5

The severity of lymphoedema

Severity	MALES (18 LIMBS)	FEMALES (81 LIMBS)	TOTAL (99 LIMBS)
Mild	1	36	37
Moderate	10	28	38
Severe	7	17	24

Figure 46a

Photographs of patients with lymphoedema showing:
a) mild swelling ot the lower left leg.

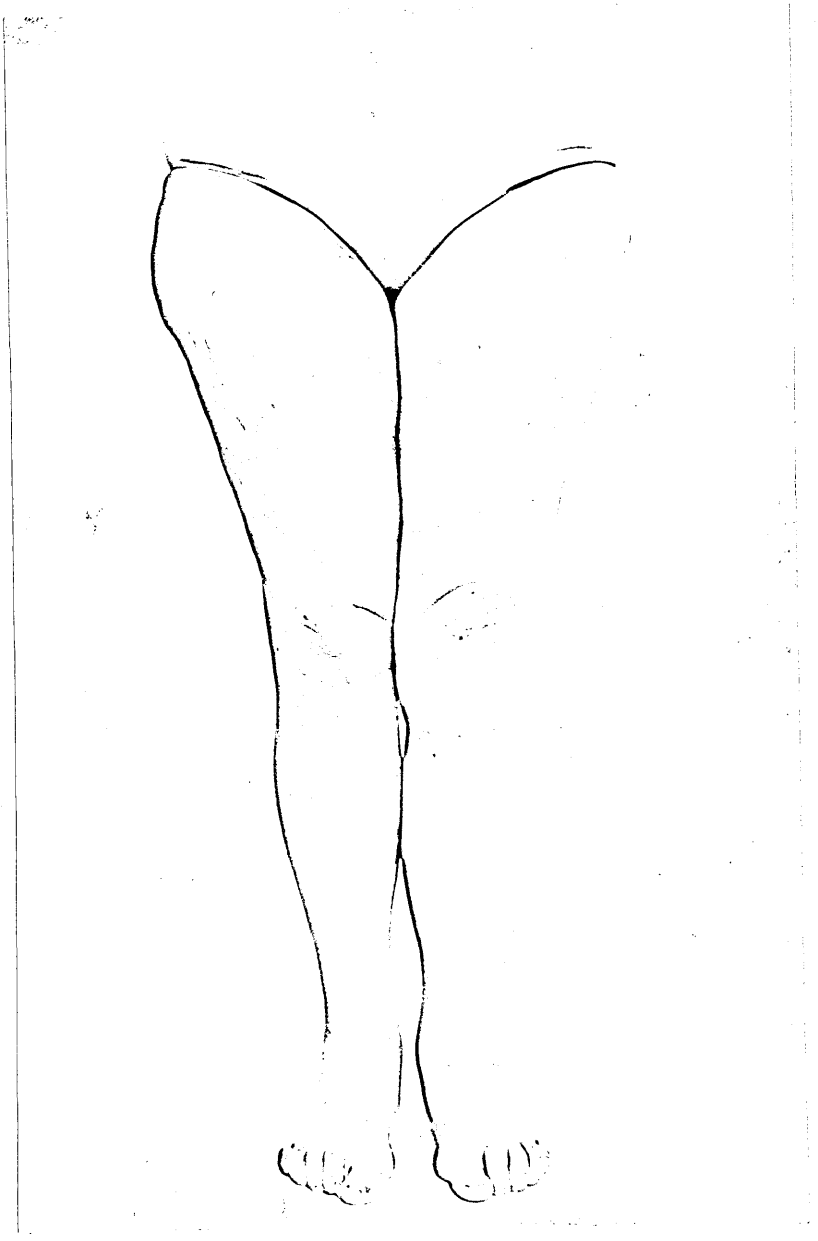


Figure 46b

Photograph of patients with lymphoedema showing:
b) moderate swelling of lower left leg involving the knee.

Figure 46c

Photographs of patients showing lymphoedema showing:
c) severe lymphoedema involving the whole of the right leg.

Lymphoedema Complicated by Episodes of Cellulitis

As was expected patients with severe lymphoedema were prone to episodes of cellulitis but the differences were not as marked as might be expected. Seven patients (28%) with lymphoedema of the whole leg were troubled by one or more attacks of cellulitis and 7 patients (28%) with below knee lymphoedema while only 4 patients of those with ankle oedema developed cellulitis.

Summary

These results confirm that there are marked clinical differences between the patients presenting under the umbrella diagnosis of primary lymphoedema.

In the next section, the clinical findings are therefore reviewed in relation to a lymphographic classification.

Clinical findings related to lymphographic classification

Lymphography has shown that there are anatomical variations of lymphatic abnormalities and a pattern of lymphoedema emerges when the patients are grouped according to the lymphographic findings (Table 6). The most striking difference is the overwhelming preponderence of females in the group with distal hypoplasia alone and many of these patients had mild oedema. Certainly the young females were more likely to present with an unsightly ankle than the male but this is unlikely to be the whole explanation.

Factors associated with the onset of lymphoedema

When comparisons are made between distal hypoplasia and proximal obstructive hypoplasia (the other extreme of lymphatic obliteration) other differences are apparent. Known hereditary associations were present in 31% of the patients with distal hypoplasia and only 8% of those with proximal hypoplasia. Onset related to the time of puberty was more common in those with distal hypoplasia than those with proximal obstructive hypoplasia. Conversly, local factors that the patient related the onset of lymphoedema were more common in those with proximal obstructive hypoplasia (40%) than those with distal hypoplasia (27.5%). This may merely be an indication of the rapidity with which the oedema developed, a rapid onset being more likely to be related by the patient to any particular event.

Thus, distal hypoplasia usually occurs in the female, appears to be a mild form of lymphoedema and often presents in the years following puberty. Whereas, proximal obstructive hypoplasia does not have the same female sex predominance and is

often associated by the patient with a minor local injury.

The clinical features of patients with both proximal and distal hypoplasia suggest that this is a form of hypoplasia that has the clinical facets of both the extreme forms.

Only two of the patients in the study had hyperplasia, one of them having associated cutaneous angiomata. Neither of the patients had a family history of lymphoedema and both developed lymphoedema within two years of puberty. Hyperplasia is almost always associated with bilateral lymphoedema and both these patients had bilateral swollen limbs.

No patient in this study had megalymphatics and none of them had chylous reflux.

Mode of onset of lymphoedema

In the majority of patients, as one might expect, lymphoedema started distally and progressed proximally but in 15 patients lymphoedema started as a swelling on the medial aspect of the upper thigh and groin and spread down the leg. Eight of these patients had severe whole leg swelling with proximal obstructive hypoplasia while only one patient with distal hypoplasia presented in this way.

TABLE 6

Factors associated with the onset of lymphoedema related to lymphangiographic findings

	DISTAL HYPOPLASIA	PROXIMAL + DISTAL HYPOPLASIA	PROXIMAL OBSTRUCTIVE HYPOPLASIA	PROXIMAL HYPERPLASIA
No. of patients	51	15	10	2
Male : Female	7.44	3.12	3.7	0.2
Hereditary factors	16	2	0	0
Congenital abnormalities (including angiomata)	2	0	2	1
Associated Hormonal Factors				
- Puberty	15	6	2	2
- Pregnancy	2	1	0	0
Local Factors				
- Trauma	12	3	2	0
- Infection	2	1	2	0

The duration and distribution of lymphoedema (Table 7)

The distribution of lymphoedema varied in different groups. Table 7 shows that the median duration of the disease at the time of lymphography was similar in all patients with hypoplastic lymphatics so it can be concluded that distal hypoplasia is a milder form not an earlier form of the disease. Distal hypoplasia was associated with bilateral lymphoedema in the majority of patients. But the majority of patients with proximal obstructive hypoplasia had unilateral clinical lymphoedema.

Whole limb oedema was present in 90% of those patients with proximal obstructive hypoplasia and in 16% of those with distal hypoplasia alone.

Summary of the lymphographic classification suggests that at one end of the spectrum of primary hypoplastic lymphoedema there is a mild bilateral disease almost completely confined to females (distal hypoplasia) and at the other end in a severe form of unilateral disease which may affect both males and females (proximal obstructive hypoplasia).

Furthermore the mild disease does not appear to progress to the severe form of the disease although there is a small group of patients with proximal and distal hypoplasia who seem to provide a mixture of both mild and severe forms of lymphoedema. Are we therefore dealing with the same disease process or several different types known collectively as the primary lymphoedemas.

TABLE 7

Distribution related to lymphangiographic findings

	DISTAL HYPOPLASIA	PROXIMAL + DISTAL HYPOPLASIA	OBSTRUCTIVE HYPOPLASIA	HYPERPLASIA
No of patients	51	15	10	2
Bilateral swelling	17	2	0	2
No of limbs	68	17	10	4
Severity of lymphoedema				
- mild	32	3	0	2
- moderate	28	7	1	2
- severe	8	7	9	0
Proximal mode of onset	1	6	8	0
Peripheral lymphatics absent (nodogram)	17	7	0	0
Lymphatic abnormalities in "normal" limb	8	0	0	0
Median duration* of lymphoedema	14	13.0	10	14

* At time of presentation to St. Thomas' Hospital

Results of lymphography

Visual lymphography using an inter-digital subcutaneous injection of patent blue violet revealed dermal backflow in most, but not all lymphoedematous limbs. Interestingly, 4 patients with distal hypoplasia had dermal backflow in the contralateral non-lymphoedematous limbs. One patient developed an anaphylactic reaction to the injection of patent blue violet which caused multiple skin wheals. This required no treatment and settled within 24 hours. There was no other reactions to the injections of patent blue violet.

An interesting point of note is that rather unexpectedly dermal backflow was present in only 80% of patients and in 25% of these only mild dermal backflow was seen.

Radiological Lymphography

The results of the lymphangiographic classification of patients with primary lymphoedema is shown below in Table 8.

TABLE 8

Radiological classification of patients with primary lymphoedema

	No. of limbs
Distal Obliteration (Hypoplasia)	68
Proximal and Distal Obliteration (Hypoplasia)	17
Proximal Obstructive Hypoplasia	10
Hyperplasia (Varicose or megalymphatics)	4

a) Distal lymphatic obliteration (hypoplasia) and normal proximal pathways

Both limbs were examined in all 51 patients. It was not possible to find a patent lymphatic in the dorsum of the foot in 17 limbs but the subsequent inguinal lymph node injection revealed normal proximal lymphatics. Seventeen of the patients had bilateral clinical lymphoedema, 34 unilateral lymphoedema. Eight patients had hypoplastic lymphatics in the clinically normal limb. Thus, in this group 49% of the patients had lymphographic evidence of bilateral lymphatic abnormalities.

The subsequent lymphadenograms were normal in the majority of patients but in 7 patients there were minor inguinal and iliac lymph nodes abnormalities which were difficult to categorise, but did not obstruct the flow of lipiodol.

In all cases a normal thoracic duct was visualised.

b) Proximal and distal lymphatic obliteration (hypoplasia)

Thirty limbs were examined in the 15 patients of this group. Thirteen patients had unilateral clinical lymphoedema and none of these patients had evidence of hypoplasia in the normal limb. Therefore, only 13% had lymphographic evidence of bilateral abnormalities of the lymphatics compared with 49% of those with the distal hypoplasia alone.

Unfortunately, it is particularly difficult to obtain an adequate lymphogram in these patients. In only 8 patients was itpossible to cannulate a lymphatic in the foot of the abnormal limb. Even following a nodal injection only a small part of the

lymphatic system was shown in many cases.

It was only possible to visualise the thoracic duct in 9 patients and in all it was normal. No ectopic nodes or collateral pathways were seen in the remainde but the thoracic duct was not visualised. This suggests, but does not confirm, that the failure to opacify the duct was due to inadequate lipiodol reaching it and not to any abnormality of the duct itself.

The lymphadenograms in the clinically abnormal side showed small dense nodes and very few were visualised in most patients. In three patients who had normal distal lymphatics on the clinically normal side the lymph nodes were abnormally small dense and rounded.

c) Proximal obstructive Hypoplasia

Both limbs were studied in all 10 of the patients with proximal obstructive hypoplasia and all of these patients had unilateral clinical lymphoedema. Little difficulty was encountered in obtaining a pedal lymphogram in most patients because dilated lymphatics were present, and in no patient was it necessary to resort to an inguinal node infusion. The lymphangiogram transit timeswere normal in the clinically normal contralateral limb but the lymphadenograms of the unaffected limb were abnormal in 21% of these patients.

It was difficult to obtain good views of the thoracic duct but it was normal in the 80% of the patients in whom it was shown. No axillary or mediastinal nodes were visualised in these

patients which suggests that the thoracic duct was present but not visualised.

d) Hyperplasia (Congenital valvular incompetence)

Four limbs were examined in the 2 patients with hyperplasia and lymphangiography showed an increase in the number of lymphatics in all four limbs. Both patients had bilateral limb swelling. The thigh lymphatics in these patients were slightly dilated and the lymphatics in the lower leg were often tortuous and grossly dilated. The transit time of lipiodol was usually recorded as very slow because the sacro-iliac joint was not reached in the course of the examination.

The pelvic lymph nodes were hypertrophic in both patients and the thoracic duct was normal in one of the patients. In the other patient the thoracic duct was not visualised but numbers of mediastinal nodes were seen in the lymphadenograms strongly suggesting that the thoracic duct or the cysterna chyli was abnormal. These lymph nodes do not fill under normal circumstances since lipiodol preferentially fills the thoracic duct.

Summary

With the exception of four limbs with hyperplasia all the limbs with primary lymphoedema were classified as radiological "hypoplasia". There were 3 categories:

a) Limbs with obliteration of the distal limb lymphatics.(68 limbs)

b) Limbs with proximal obliteration of lymphatics with distal distension of the lymphatics (10 limbs).

c) Limbs with the radiological appearance of both proximal and distal obliteration of lymphatics (17 limbs).

(III) Assessment and diagnosis of patients with venous oedema

Sixteen patients with venous oedema were studied. Each patient was assessed clinically and with phlebography, the isotope study being carried out after the diagnosis of oedema due to venous causes had been made.

Clinical assessment

Each patient had a careful clinical assessment and previous case notes were studied. Particular attention was paid to the following points which were transposed onto a standard form.

a) History

Past history of deep vein thrombosis.

Age of onset of venous oedema.

Factors precipitating clinical oedema.

Original extent and subsequent progress of clinical oedema.

Complications of deep venous damage, such as pulmonary emboli.

b) The examination (Figure 47)

1. Measurement of limb girth and length.

2. Associated lipodermatosclerosis or venous ulceration.

c) Initial investigations

1. Full blood count.

2. Plasma proteins.

3. Urine test for proteinuria.

Figure 47

Patient with venous oedema of the right leg showing lipodermatosclerosis of the gaiter region.

Phlebography

Bilateral ascending phlebography was carried out by the technique described by Lea Thomas (1982). Investigations were assessed as abnormal if there was damage to the deep veins and/or obvious venous collaterals in the calf or thigh (Figure 48). All the patients in this study had had a previous history of a deep venous thrombosis which was the precipitating cause of their long standing venous oedema. Deep venous reflux is an uncommon cause of venous oedema without previous venous damage from deep venous thrombosis and is usually due to congenital lack of valves in the deep venous system. None of these patients exhibited this type of deep venous reflux.

Figure 48a

Phlebographs showing extensive post thrombotic changes in the calf. There are occlusions of parts of the stem veins of the claf and also of the popliteal veins. A very large number of collateral veins are present arising from the deep and superficial venous systems and also the communicating veins.

Figure 48b

b) Phlebograph showing severe recanalisation affecting the leg veins. No valves can be identified and can be presumed to have been destroyed in the thrombotic process.

The Clinical finding in patients with venous oedema (Table 9)

Patients were included in this study provided that their phlebographs were abnormal showing evidence of deep venous damage or deep venous reflux as a cause of their leg swelling.

Factors relating the aetiology of venous oedema.

All 16 patients had had a previous deep venous thrombosis and were therefore post-phlebitic limbs with associated swelling. Age of onset of the oedema was 28 years (range 20-60) and the median age at the time of the study was 37.

Thirteen of the 16 patients had clinical stigmata of deep venous disease namely lipodermatosclerosis and ulceration (11 and 3 respectively) and 3 patients had whole limb swelling attributable to a venous cause.

Phlebographic findings

All limbs were assessed with bilateral phlebography. Fourteen limbs showed marked damage of the calf and popliteal veins with considerable collateral circulation and 6 of the limbs had evidence of previous deep venous damage to the ilio-femoral segment. None of the patients had inferior vena cava obstruction.

TABLE 9

Clinical and phlebographic features of patients with venous oedema

	Males	Females	Total
No. of patients	5	11	16
History of deep venous thrombosis	5	11	16
No. of limbs	7	13	20
Liposclerosis	4	7	11
Liposclerosis and Ulceration	2	3	5
Phlebographic abnormalities			
- femoro - popliteal	5	9	14
- ilio - femoral	2	4	6

(IV) Assessment and diagnosis of patients with other causes of lower limb oedema

Nine patients in this category were studied. Each patient was assessed clinically and with phlebography and venous function tests prior to the radionuclide study. Lymphography was carried out in 7 of these patients, 2 patients refusing lymphography as a method of investigation.

Clinical assessment

Each patient had a careful clinical assessment and the previous case notes were studied. Particular attention was paid to the following points and transposed onto a standard form.

a) History

1. Age of onset of oedema.
2. Factors precipitating clinical oedema.
3. History of any previous deep vein thrombosis.
4. The original extent and subsequent progress of the clinical oedema.

b) Examination (Figure 49)

1. Measurement of limb girth and length.
2. Associated evidence of the clinical features of primary lymphoedema and the stigmata of a venous disease such as liposclerosis.

c) Initial investigations.

1. Full blood count.

2. Serum urea and electrolytes.

3. Plasma proteins.

4. Urine test for proteinuria.

d) Other investigations.

Patients were included in this particular group of oedemas if there was no clinical evidence of primary lymphoedema including a normal lymphogram where feasable and no clinical evidence of venous oedema, a normal phlebogram and normal venous function tests. No patients with cardiac or renal causes of peripheral oedema were included in this study.

Figure 49

Photograph of a patient with swelling of the left ankle and calf due to pretibial myxoedema.

Clinical and radiological findings in patients with oedema due to miscellaneous causes

Six patients had bilateral swelling and eight of the 9 patients were female. The age of ouset of the oedema was 28.5 (range 16 -46) and the median age at the time of the study was 36.

All 9 patients had normal phlebography and venous function tests - isotope phlethysmography (Whitehead, Clemenson and Browse, 1983) and were assessed as having a normal deep venous system.

Seven of the 9 patients underwent lymphography which showed normal peripheral lymphatics, normal lymph nodes and normal thoracic ducts. Two of the patients refused lymphography. These two patients had the clinical features of lipodystrophy and erythrocyanosis frigida respectively. They had no clinical evidence of lymphoedema and were therefore included in this study.

The causes of swelling are listed in Table 11. Ten of the fifteen limbs had idiopathic cyclical oedema, 2 limbs erythrocyanosis frigida, one limb pretibial myxoedema and one patient bilateral lipo-dystrophy.

TABLE 10

Causes of miscellaneous group of limb oedema

Diagnosis	No. of patients	No. of limbs
Idiopathic/cyclical oedema	6	10
Erythrocyanosis Frigida	1	2
Lipodystrophy	1	2
Pretibial myxoedema	1	1
Total	9	15

Male : Female 1.8

Age of onset 28.5 (Range 16-46)

Age at examination 36

(V) The results of the Radionuclide studies following a calf injection (Method 1)

Ten control subjects (20 limbs), 27 patients (38 limbs) with primary lymphoedema and 6 patients (8 limbs) with venous oedema have been investigated by this method.

The results of this part of the study were assessed in two ways:

1. The time of arrival of the colloid in the ilio-inguinal lymph nodes was assessed by an independent observer and the time at which the ilio inguinal lymph nodes could be visualised was noted.

2. The rate of arrival of the radioactive colloid over the first 30 minutes of the study was measured using the method described in Chapter 4.

Time of arrival of the colloid in the ilio-inguinal nodes

(Table 11)

Although radioactivity can be seen in the ilio-inguinal lymphatic chain in the early part of the study in the control limbs (Figure 50), the ilio-inguinal lymph nodes were not usually clearly visible until at least 25 to 30 minutes after the injection of 99mTcRSC.

Nine of the 20 control limbs showed radio-activity present in the ilio-inguinal lymph nodes 30 minutes after the injection and the other 11 showed activity by 1 hour. There was no radio-activity seen in the ilio-inguinal region at 1 hour in 34 of the 38 lymphoedematous limbs; 14 showed poor uptake at 2 hours but good uptake at 3 hours and 20 no activity in the ilioinguinal region by 3 hours.

The 17 clinically and lymphangiographically normal limbs showed radioactivity present in the lymph nodes by 1 hour, but the time of the arrival of the colloid in the one clinically normal limb with lymphangiographic abnormalities studied by this method was delayed until 2 hours.

In the group of patients with venous oedema (8 limbs) the isotope had appeared in the ilio-inguinal lymph nodes after one hour in 5 limbs but was delayed until three hours in 3 limbs.

In summary, all 20 control limbs showed a radioactivity clearly visible with in the ilio-inguinal lymph nodes at 1 hour and therefore it was decided to base the interpretation of the radionuclide images obtained in this part of the study on the images taken at 1 hour.

TABLE 11

Time of arrival of the colloid in the ilio-inguinal lymph nodes

	Number of legs				
Time of arrival of the colloid in the ilio-inguinal lymph nodes (Hrs)	½	1	2	3	>3
Controls (20 limbs)	9	11	0	0	0
Primary Lymphoedema (38 limbs)	0	4	0	14	20
Venous oedema (8 limbs)	3	2	0	3	0

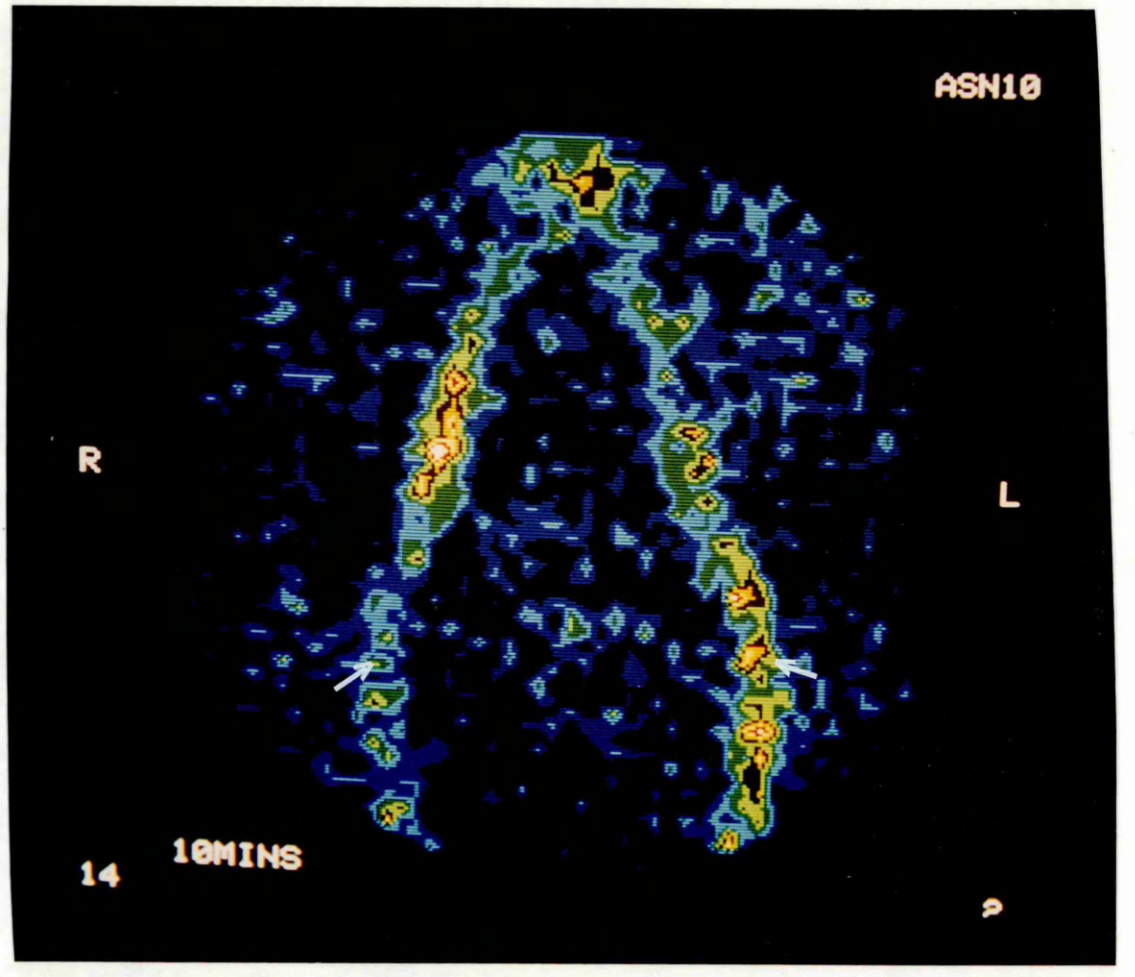


Figure 50a

Radionuclide image of control subject taken at 10 minutes showing radioactivity present in both ilio-inguinal lymphatic chains (arrowed). This image can be compared to the image shown in Figure 50b.

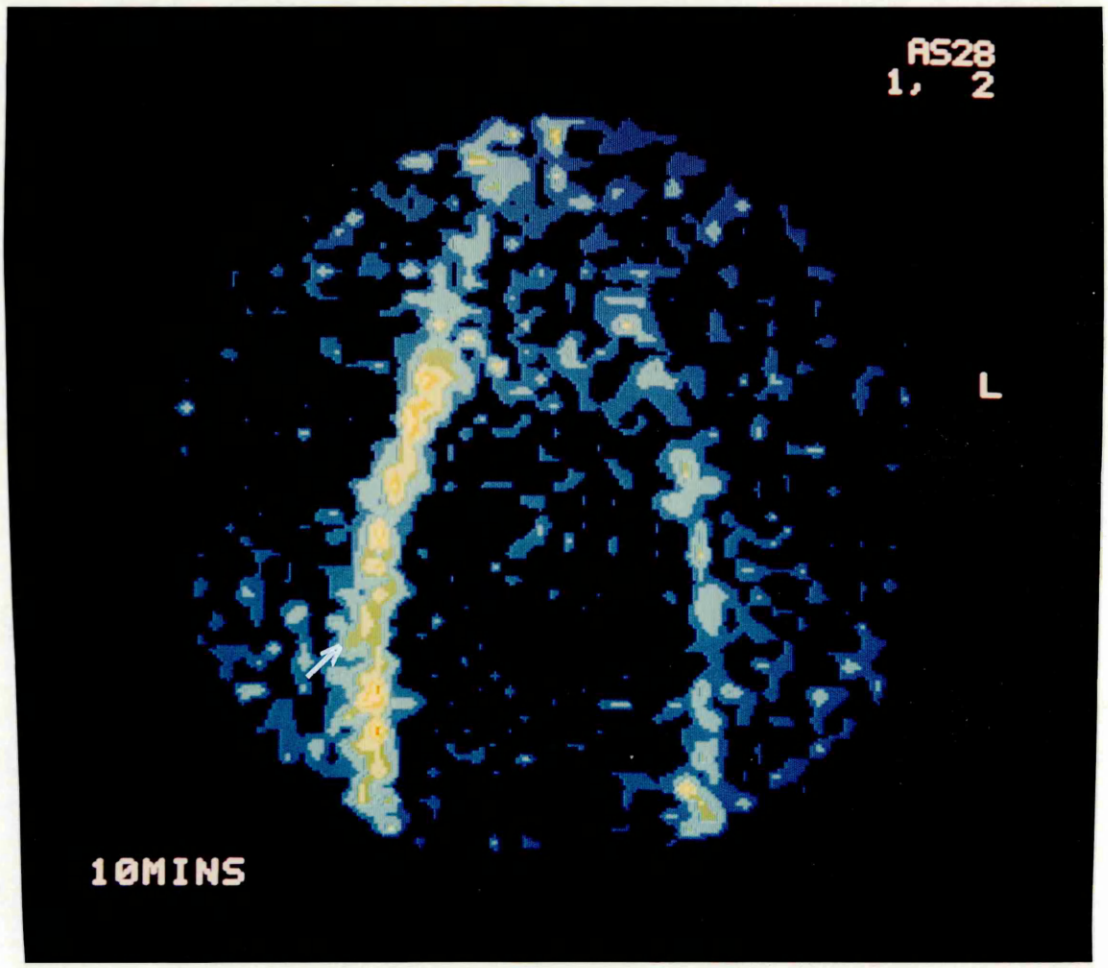


Figure 50b

b) An image taken at 10 minutes in a patient with lymphoedema of the left leg showing a good pattern of radioactiviy on the clinically and lymphographically normal side (right - arrowed) but a poor pattern on the affected side.

Visual interpretation of the radionuclide images (Table 12)

The criteria for interpreting these studies was based on the data in the control group and an investigation was classified as normal when the ilio-inguinal lymph nodes were clearly visible at 1 hour or less (Figure 51a). An investigation was interpreted as abnormal when the regional lymph nodes were either not seen or only faintly visualised because of inadequate uptake of the radio-active colloid in the ilio-inguinal lymph nodes at one hour (Figure 51b).

Using these criteria 34 of the 38 lymphoedematous limbs had abnormal scans and 4 were within normal limits. Three of the 8 venous limbs were also abnormal providing a sensitiviy of 89% for the diagnosis of lymphoedema but only a 62.5% specificity when attempting to differentiate between lymphoedema and venous oedema. Thus, visual interpretation of this technique failed to differentiate between lymphoedema and venous oedema in 7 out of 46 cases.

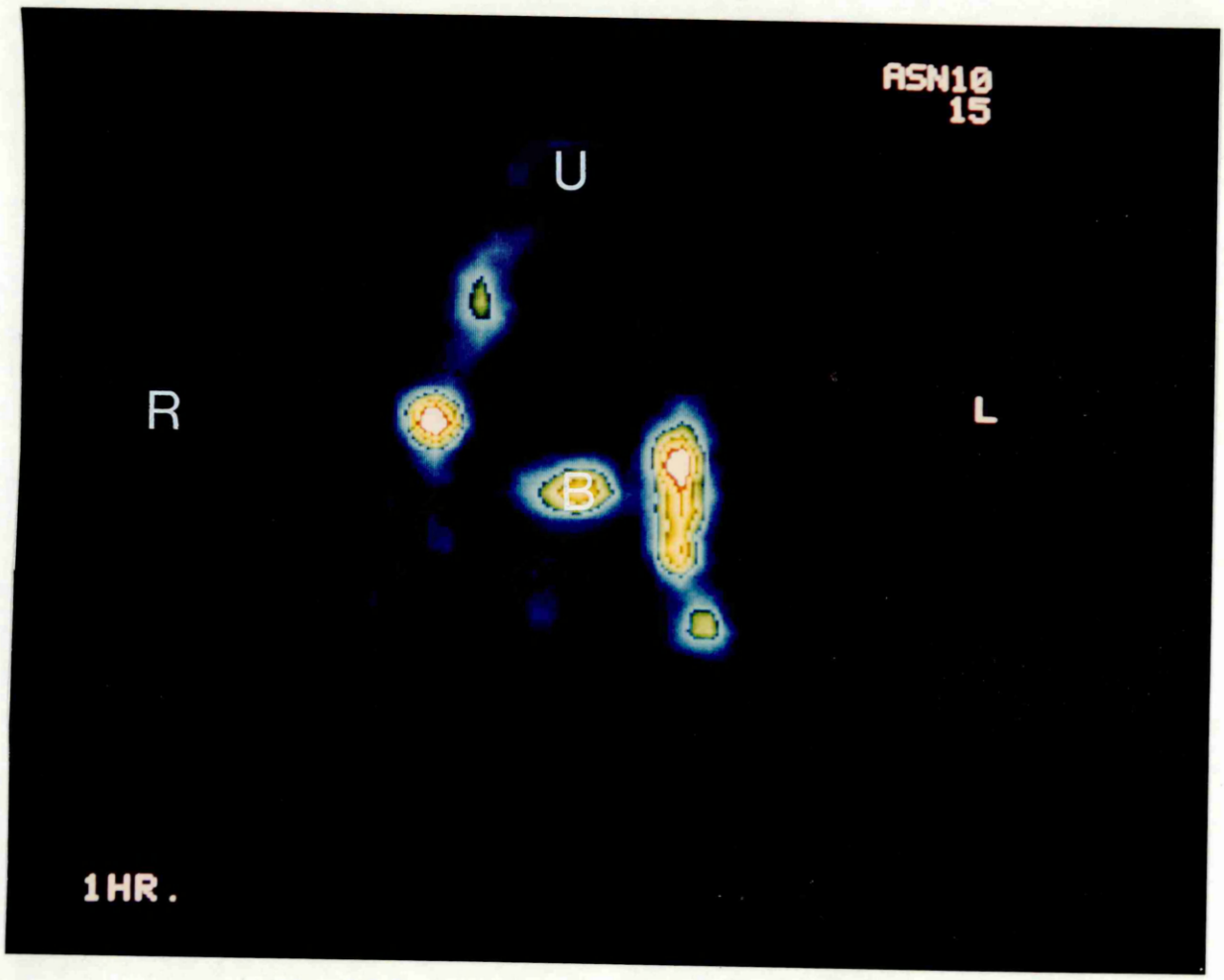


Figure 51a

Radionuclide image taken at 1 hour in same control subject as in Figure 50a showing a normal pattern of activity in the ilio-inguinal lymph nodes both sides.
U = position of umbilicus
B = position of bladder

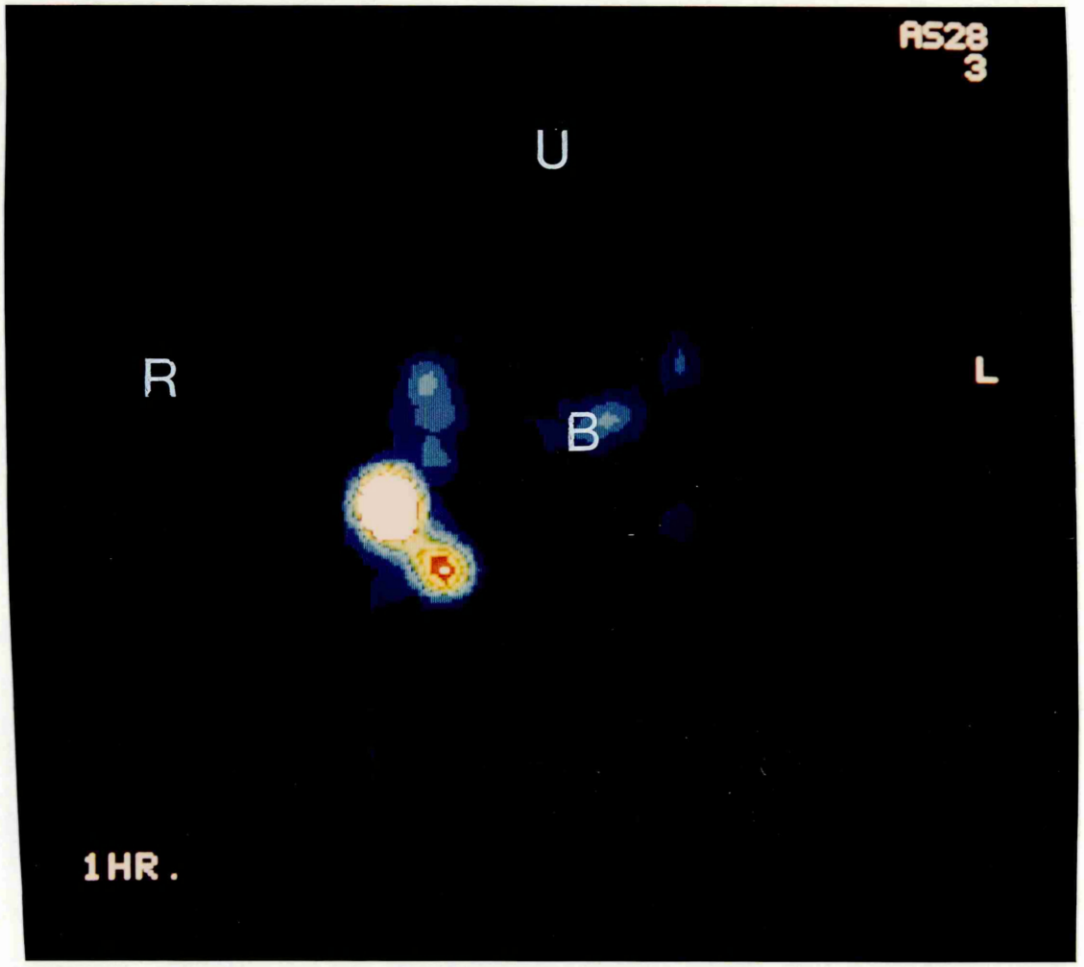


Figure 51b

b) The 1 hour image of the patient with lymphoedema in Figure 50b showing a normal ilio-inguinal lymph node pattern on the clinically and lymphographically normal side and almost negligible uptake on the affected side, compatable with the diagnosis of lymphoedema. There is considerable variation in the normal lymph node pattern seen in the control subject and in the right side of the patient with lymphoedema.
U = position of umbilicus

TABLE 12

Visual assessment of the uptake of radioactivity in the ilio-inguinal lymph nodes at 1 hour

	Activity at 1 hour	
	Present	Absent
Controls (20 limbs)	20	0
Primary Lymphoedema (38 limbs)	4	34
Venous oedema (8 limbs)	5	3

The Rate of arrival of the colloid

The gradient of the time activity curves created from the data accumulated during the first 30 minutes of continuous scanning was used to express the rate of appearance of the isotope in the ilio-inguinal lymph nodes (Table 13).

The mean value of this gradient in the 20 control limbs was 1.12 $\pm$ 0.27 with a range of 0.84 to 1.67. The mean value of the rate of appearance of the radio-isotope in the ilio-inguinal lymph nodes of the 30 lymphoedematous limbs was 0.64 $\pm$ 0.34 (range 0.1 to 1.61) significantly lower than that of the control limbs. The mean value and range of this gradient for the patients with venous oedema was significantly different from those with lymphoedematous limbs but was similar to the value for the control limbs (Figure 52).

Thus, the measurement of the rate of appearance of the colloid in the ilio-inguinal lymph nodes showed that there was a faster clearance of the colloid to the lymph nodes in both normal limbs and limbs with venous oedema compared to lymphoedematous limbs. There was no statistical difference between the control limbs and limbs with venous oedema although the control limnbs appeared to have a slightly faster clearance of the colloid. Analysis of the range of results for all 3 groups showed a considerable overlap between the lymphoedematous limbs and the other 2 groups studied.

TABLE 13

Rate of appearance of radioactivity in the ilio-inguinal nodes from 0 - 30 minutes in controls, primary lymphoedema and venous oedema

	Mean & S.D.	Range
Controls (20 limbs)	1.12 (0.27)	0.84 - 1.67
Primary lymphoedema* (30 limbs)	0.64 (0.34)	0.1 - 1.61
Venous oedema (8 limbs)	1.0 (0.32)	0.6 - 1.42

Controls v Lymphoedema	$p < 0.001$
Venous Oedema v Lymphoedema	$p < 0.001$
Controls v Venous oedema	n.s.

* The rate of arrival was measured in only 30 out of the 38 limbs.

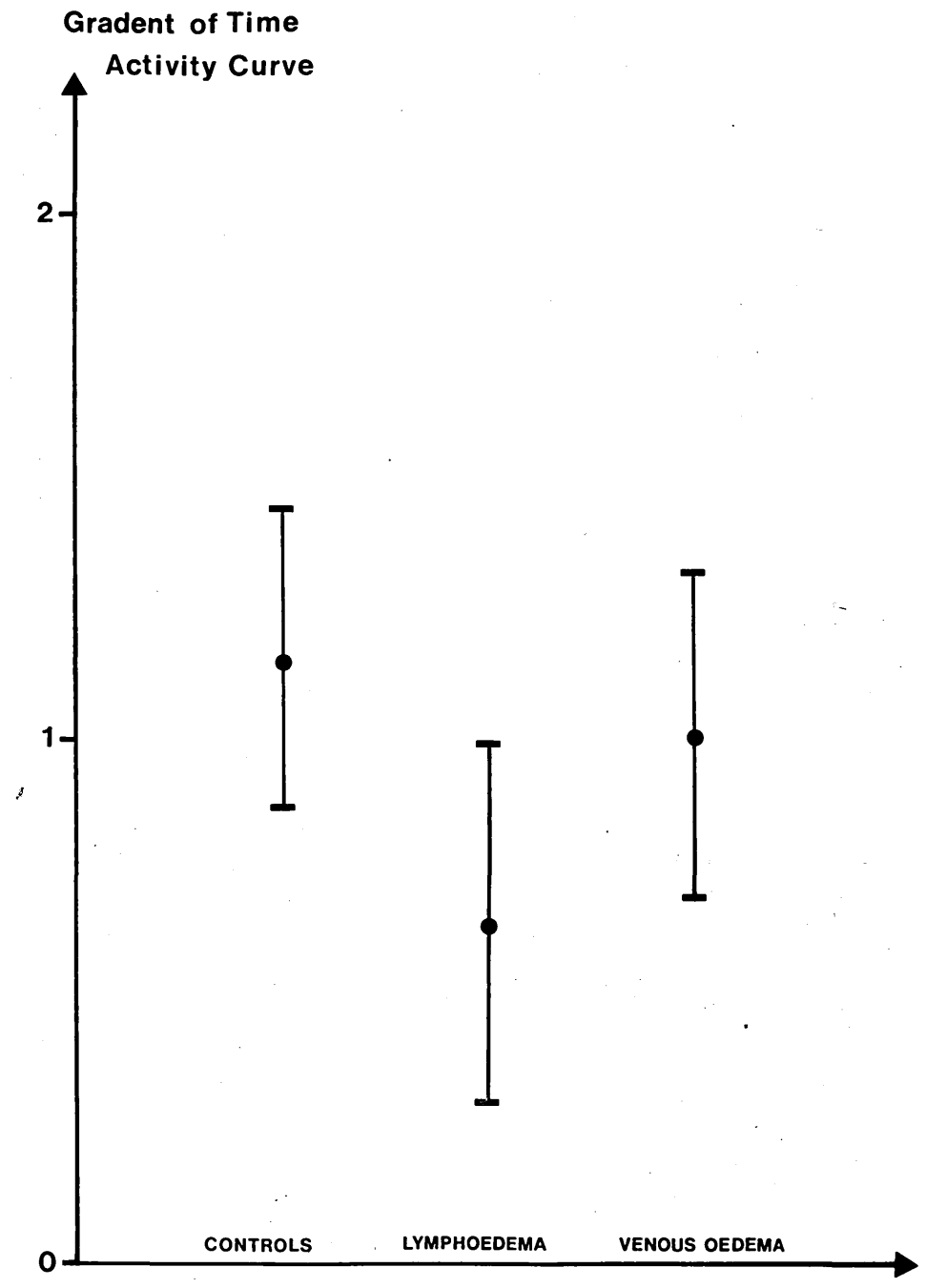


Figure 52

Rate of appearance of the colloid (mean and standard deviation) in the ilio-inguinal lymph nodes in controls, lymphoedema and venous oedema (0 - 30 minutes)

The effect of bed rest

The study was repeated in 6 patients (7 limbs) before and after 6 days bedrest (Table 14). This showed that there was a significant increase in the rate of arrival of the colloid following a period of bedrest. These results, although not conclusive, suggest that the presence of oedema may affect the clearance of colloid from the subcutaneous tissues of the mid calf region.

TABLE 14

Rate of Appearance of the radioactive colloid from 0 - 30 minutes before and after bedrest in six patients (7 limbs)

Number	Before	After
1	1.20	1.61
2	0.50	0.72
3	0.67	1.12
4	0.1	0.15
5	0.21	0.25
6	0.40	0.55
7	0.35	0.60

$t = 3.68$ $p = 0.015$ (paired t-test)

SUMMARY

It was the aim of this thesis to evaluate radionuclides as a method of investigating the lymphatic system. The relevant merits of Method 1 and Method 2 are discussed in complete detail in Chapter 7 but it is already apparent from the results presented in this section that this particular technique using a calf injection was not accurate enough.

Visual interpretation of the time of arrival of the colloid failed to differentiate between lymphatic and venous oedema in 7 out of 46 cases, (specificity 62.5%) and, although the measurement of the rate of arrival of the colloid showed a statistical difference between the controls and the lymphoedematous limbs, there was a considerable overlap of individual results between the controls, lymphoedematous limbs and limbs with venous oedema. Furthermore, it did not distinguish the controls from the venous limbs.

It was these results that lead to the development of the second method using an interdigital space injection which despite its drawback of being more painful for the patient proved to be more accurate. It was also decided at this time to measure the amount of activity present in the ilio-inguinal lymph nodes at each time interval and compare it with original injected activity to see if this would provide an accurate method of measuring the flow of lymph from the periphery to the ilio-inguinal lymph nodes.

(VI) Results of the Radionuclide Studies following an inter digital injection (Method 2)

Six control subjects (12 limbs) 47 patients with lymphoedema (55 limbs) 10 patients (12 limbs) with venous oedema and 9 patients (15 limbs) with oedema due to other causes were investigated by this method.

The results of this part of the study were assessed in two ways:

1. The serial images obtained were assessed by an independent observer and the time of arrival of the colloid in the ilio-inguinal lymph nodes was noted.

2. Estimation of the percentage uptake of activity of the radiocolloid by the ilio-inguinal lymph nodes was carried out by the method described in Chapter 4.

The latter will form the basis on which the pathophysiology of lymph flow will be discussed.

Time of arrival of the colloid in the ilio-inguinal lymph nodes

All 12 control limbs showed radio-activity present in the ilio-inguinal lymph nodes within 30 minutes of the inter-digital space injection. Only 3 of the 55 lymphoedematous limbs showed ilio-inguinal uptake 30 minutes after the injection. Ten lymphoedematous limbs showed good uptake at 1 hour; 7 at 2 hours; 9 at 3 hours and 26 showed no activity in the ilio-inguinal region by 3 hours (Table 15).

The clinically normal limbs of the patients with unilateral lymphoedema were subdivided into two groups, those with normal and those with abnormal lymphangiograms. Seven of these limbs were excluded for technical reasons leaving 32 limbs available to study.

In the seven clinically normal limbs with lymphangiographic abnormalities ("hypoplasia") activity appeared in the ilio-inguinal region at 30 minutes in four, 1 hour in two and in one no activity was seen by 3 hours. The 25 limbs which were clinically and lymphangiographically normal all showed good ilio-inguinal uptake by 30 minutes.

Twelve limbs with venous oedema all showed a good uptake of the radionuclide in the ilio-inguinal lymph nodes as did the 15 limbs with miscellaneous oedemas although there was one study with bilateral idiopathic oedema which showed a slightly diminished uptake of activity on the left side when compared to the right side.

In summary, all 12 control limbs showed a clearly visible pattern of activity within the ilio-inguinal lymph nodes at 30 minutes and it was therefore decided to base the interpretation

of the serial radionuclide images obtained in this part of the study on the image taken at 30 minutes.

TABLE 15

Time of arrival of the colloid in the ilio-inguinal lymph nodes

	Number of legs				
Time of arrival of the colloid in the ilio-inguinal lymph nodes (Hrs)	1/2	1	2	3	3
Controls (12 limbs)	12	-	-	-	-
Primary Lymphoedema (55 limbs)	3	10	7	9	26
Venous Oedema (12 limbs)	12	-	-	-	-
Miscellaneous oedemas	15	-	-	-	-

Visual interpretation of the serial images (Table 16)

The criteria for interpreting these studies was based on the data of the control group. An investigation was classified as normal when the ilio-inguinal lymph nodes were clearly visible at 30 minutes (Figure 53, 54) and as abnormal when the regional lymph nodes were either not seen or only faintly visualised because of an inadequate uptake of the radio-active colloid in the ilio-inguinal lymph nodes at 30 minutes (Figure 55 and 56).

Using these criteria 52 out of 55 of the lymphoedematous limbs had abnormal scans and all 12 limbs with venous oedema had normal scans. Furthermore, all 15 limbs in patients with oedema due to other causes such as idiopathic oedema had normal scans.

Thus, simple visual interpretation of the gamma camera images has a sensitivity of 95% in diagnosing lymphoedema and a specifity of 100% and clearly differentiates lymphoedema from lower limb swelling due to venous and other causes.

Conclusion

The sensitivity of 95% showed that this technique was more sensitive than Method 1 (86%). The specifity of Method 1 was only 62.5 ~~58~~% whereas that of this method was 100%. Therefore, on simple visual assessment this technique was considerably more accurate than Method 1.

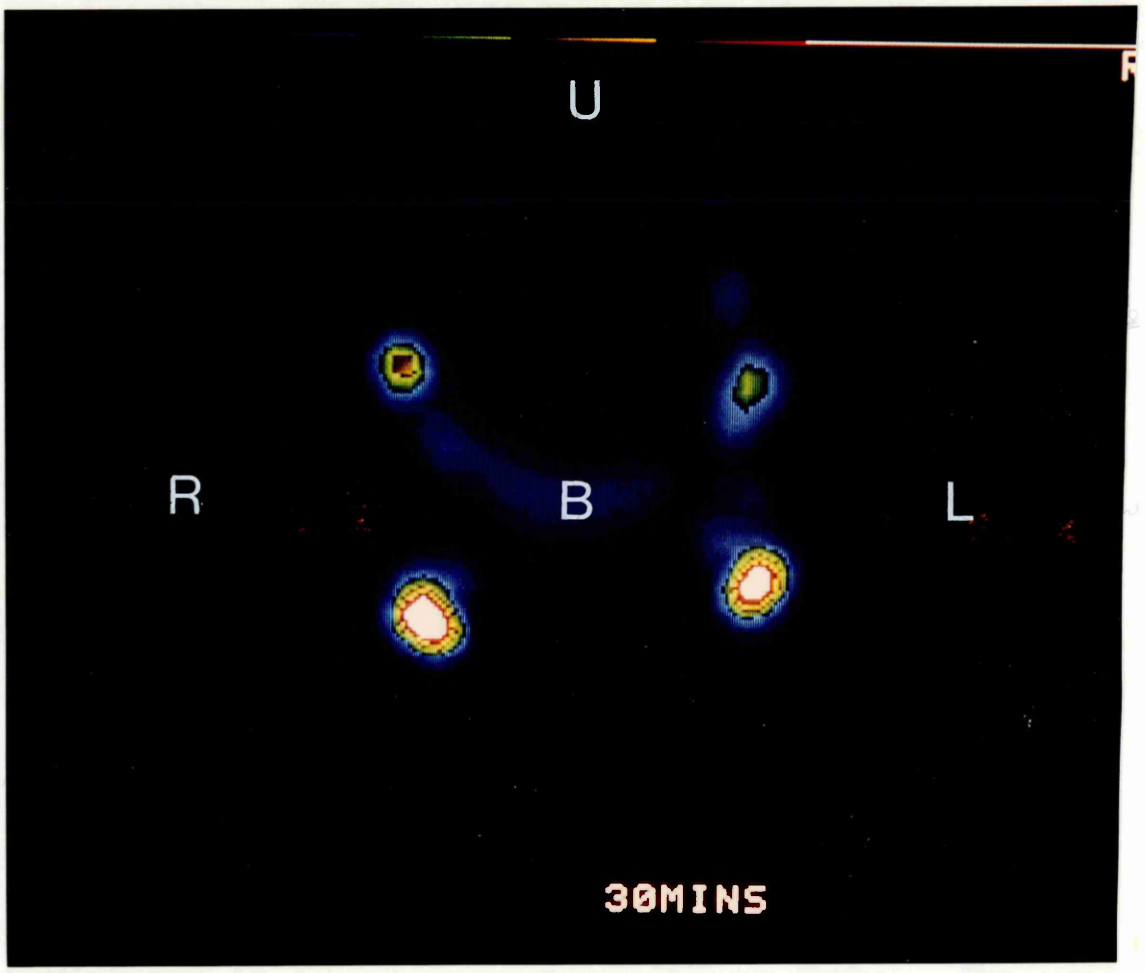


Figure 53

Radionuclide image taken at 30 minutes in a control subject showing a clerly visible pattern of activiy in the ilio-inguinal lymph nodes on both sides. All subsequent images are orientated in a similar manner.
R = right, L = left, U = position of umbilicus, B = position of bladder.

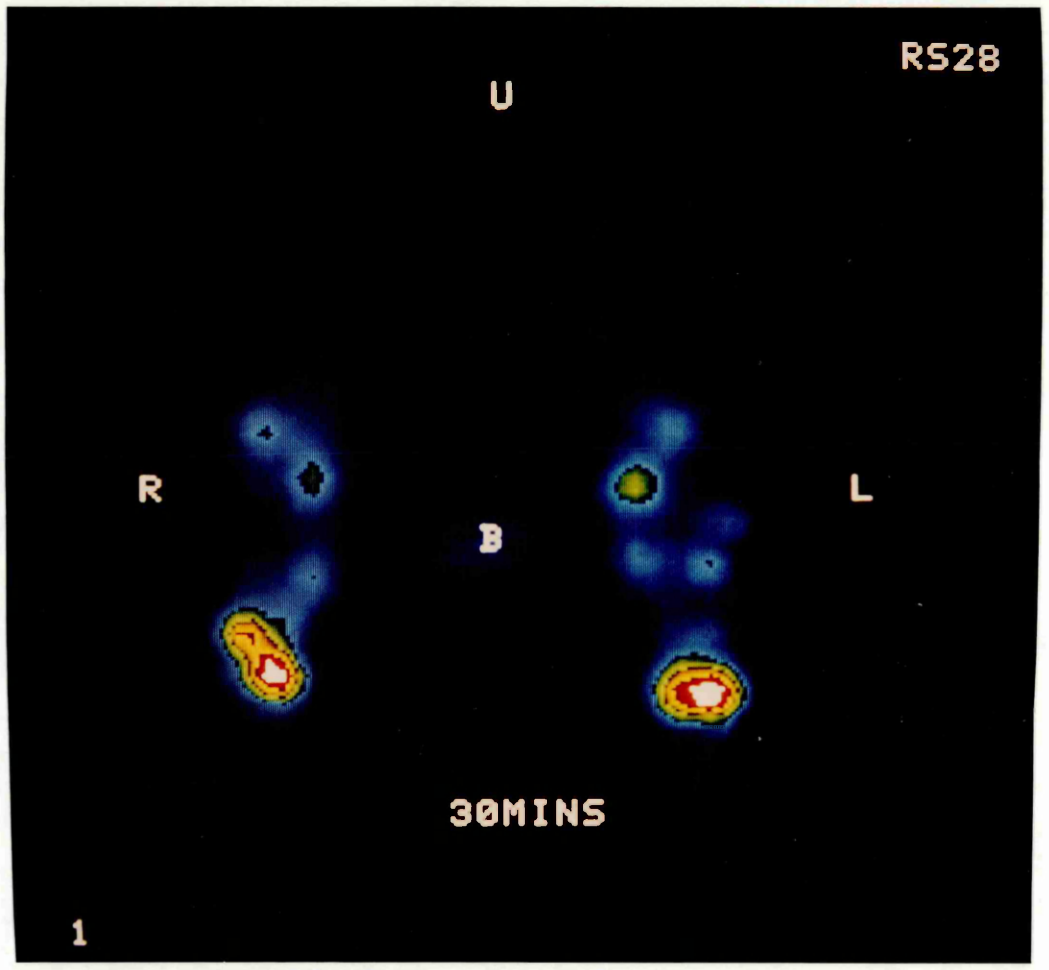


Figure 54

Radionuclide image taken at 30 minutes in a patient with bilateral idiopathic oedema showing a clearly visible pattern of activity in the ilio-inguinal lymph nodes on both sides.
R = right, L = left, U = position of umbilicus, B = position of bladder.

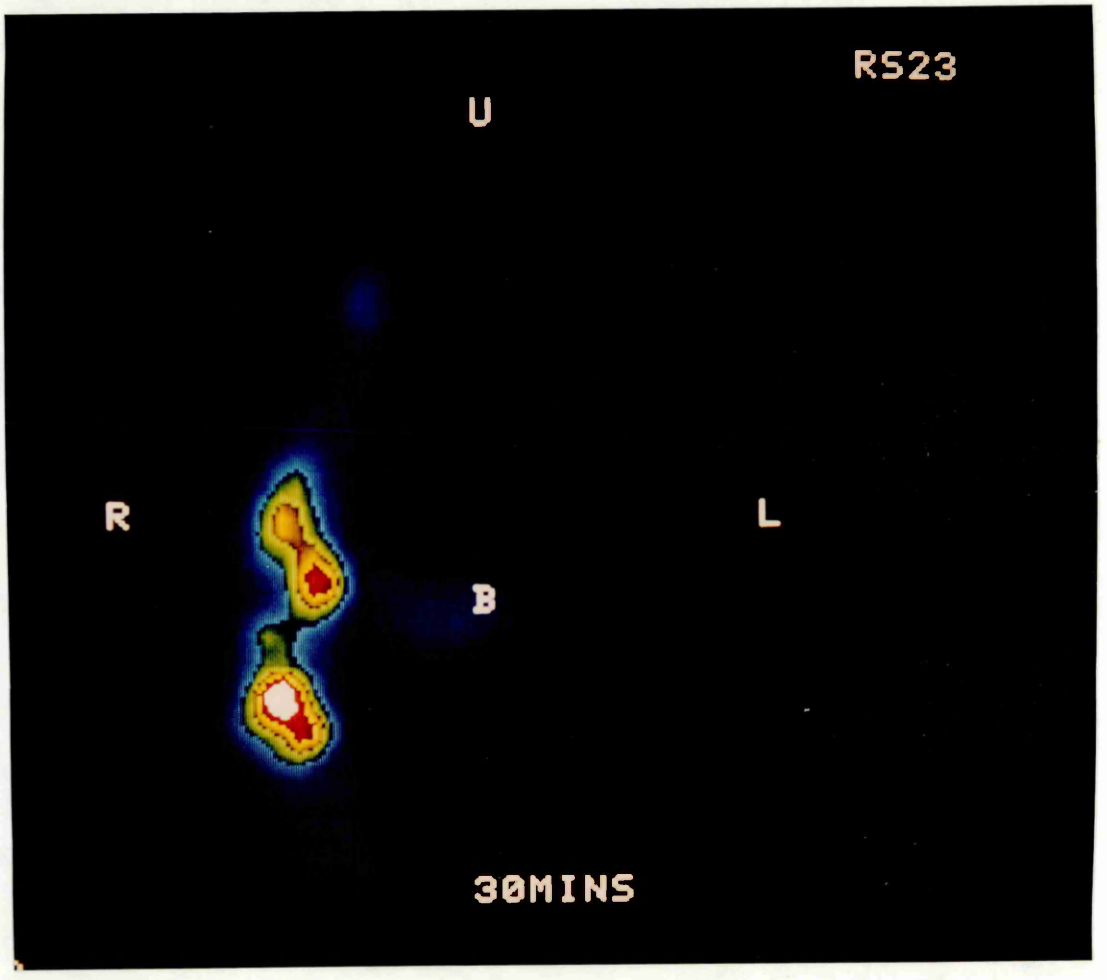


Figure 55

Radionuclide image taken at 30 minutes in a female patient with moderate lymphoedema involving the lower left leg. The ilio-inguinal lymph nodes are clearly visible on the unaffected right side but there is no activity seen at 30 minutes on the left side, compatible with the diagnosis of lymphoedema.

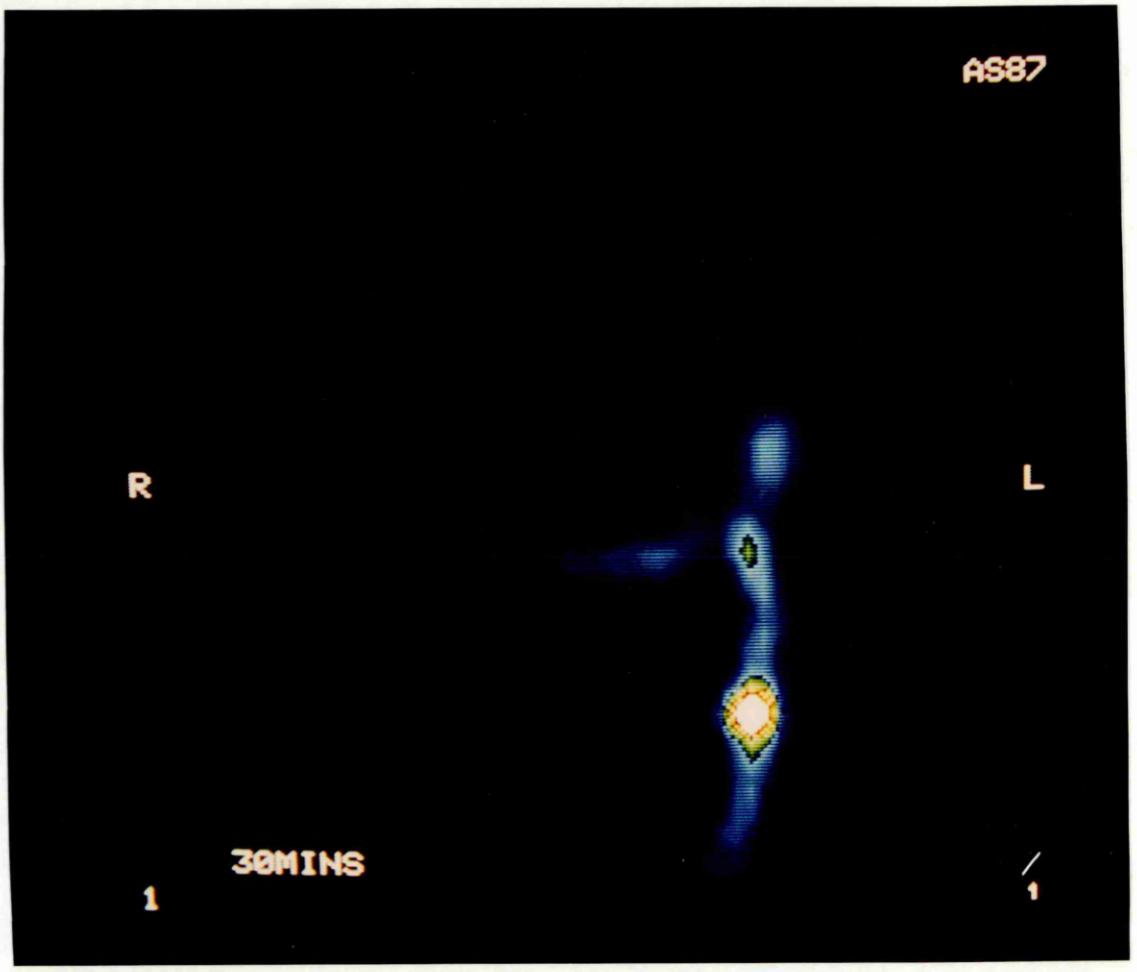


Figure 56

Radionuclide image taken at 30 minutes in a female patient with moderate lymphoedema involving the right lower leg. The ilio-inguinal lymph nodes are clearly visible on the unaffeted left side (note the difference in the lymph node pattern compared to Figures 53, 54, 55). There is no activity seen on the right side at 30 minutes, compatible with the diagnosis of lymphoedema.

TABLE 16

Visual assessment of the uptake of radioactivity in the ilio-inguinal lymph nodes at 30 minutes

		Activity at 30 minutes	
	No. of limbs	present	absent
Controls	12	12	Nil
Primary Lymphoedema	55	3	52
Venous oedema	12	12	Nil
Miscellaneous			
Oedemas	15	15	Nil

Percentage uptake of activity in the ilio-inguinal lymph nodes

The percentage uptake of the radioactive colloid (99mTcRSC) in the ilio-inguinal lymph nodes was calculated for each study at half hour, one hour, two hours and three hours following the interdigital space injection, by the method described in Chapter 4. From these results the mean, standard deviation and standard error were calculated for each group : controls, lymphoedema, venous oedema and miscellaneous oedemas.

Control Limbs

Calculation of the percentage uptake of activity in the ilio-inguinal lymph nodes at half, one, two and three hours was carried out in 12 control limbs (Appendix II).

The mean percentage uptake at 30 minutes for the 12 control limbs was 1.40 $\pm$ 0.70 (range 0.46 to 3.00). This increased with time and at 1 hour was 2.90 $\pm$ 1.12, two hours 5.25 $\pm$ 1.76 and 3 hours 9.87 $\pm$ 5.47. Figure 57 shows the increasing uptake of the radioactive colloid in the ilio-inguinal lymph nodes during the 3 hours of the study. This increase appears linear in nature suggesting that lymph flow in the control limbs is more or less constant throughout the 3 hour study.

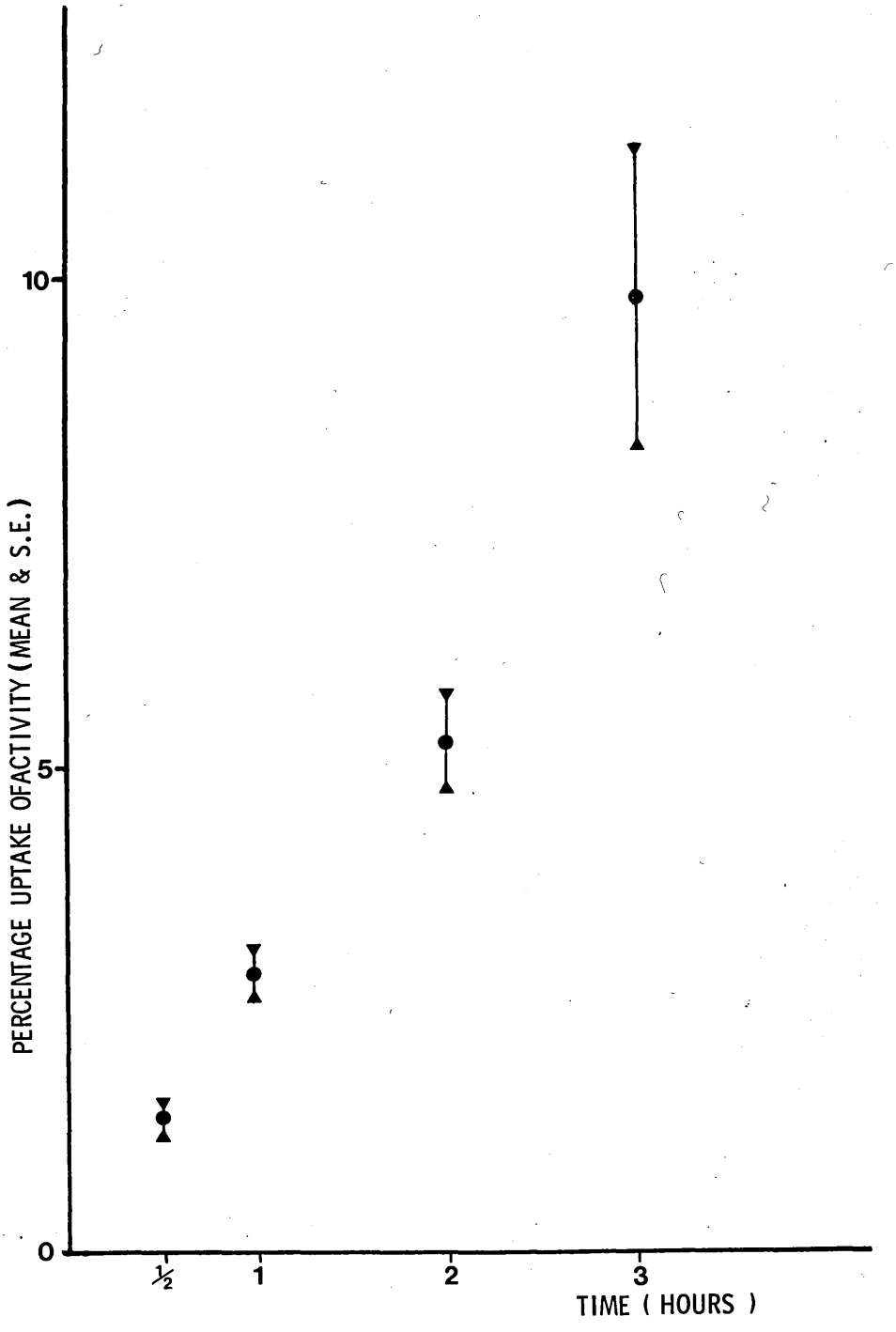


Figure 57

The percentage uptake of activity in the ilio-inguinal lymph nodes in 12 control limbs (0 - 3 hours).

Lymphoedema

Calculation of the percentage uptake of activity in the ilio-inguinal lymph nodes at half, one, two and three hours was carried out in 55 limbs with lymphoedema (Appendices III - VI).

The mean percentage uptake for the lymphoedematous limbs at 30 minutes was 0.053 $\pm$ 0.079 with a range 0.00 to 0.3 percent. Throughout the three hour study the uptake of activity remained low with a mean percentage uptake at 3 hours of 0.78 $\pm$ 1.25 (range 0 to 4.5) 20 of the limbs having no uptake of activity in the ilio-inguinal region nodes at 3 hours (Figure 58).

The three lymphoedematous limbs which were visually classified as normal at 30 minutes had percentage uptakes of 0.2%, 0.3% and 0.3% at 30 minutes; less than the range of values of the control group of 0.46 to 3.0. Seven limbs which had lymphographic abnormalities but were clinically normal had uptakes of between 0.0% to 0.9%. On visual interpretation the 30 minute image of three of the limbs was interpreted as abnormal but that of the other four limbs as normal.

The 55 lymphoedematous limbs were divided on subjective clinical grounds into mild, moderate or severe lymphoedema. This showed that there was a correlation between the percentage uptake of activity and the clinical severity of the lymphoedema. These results are presented in Figure 59 and Table 17.

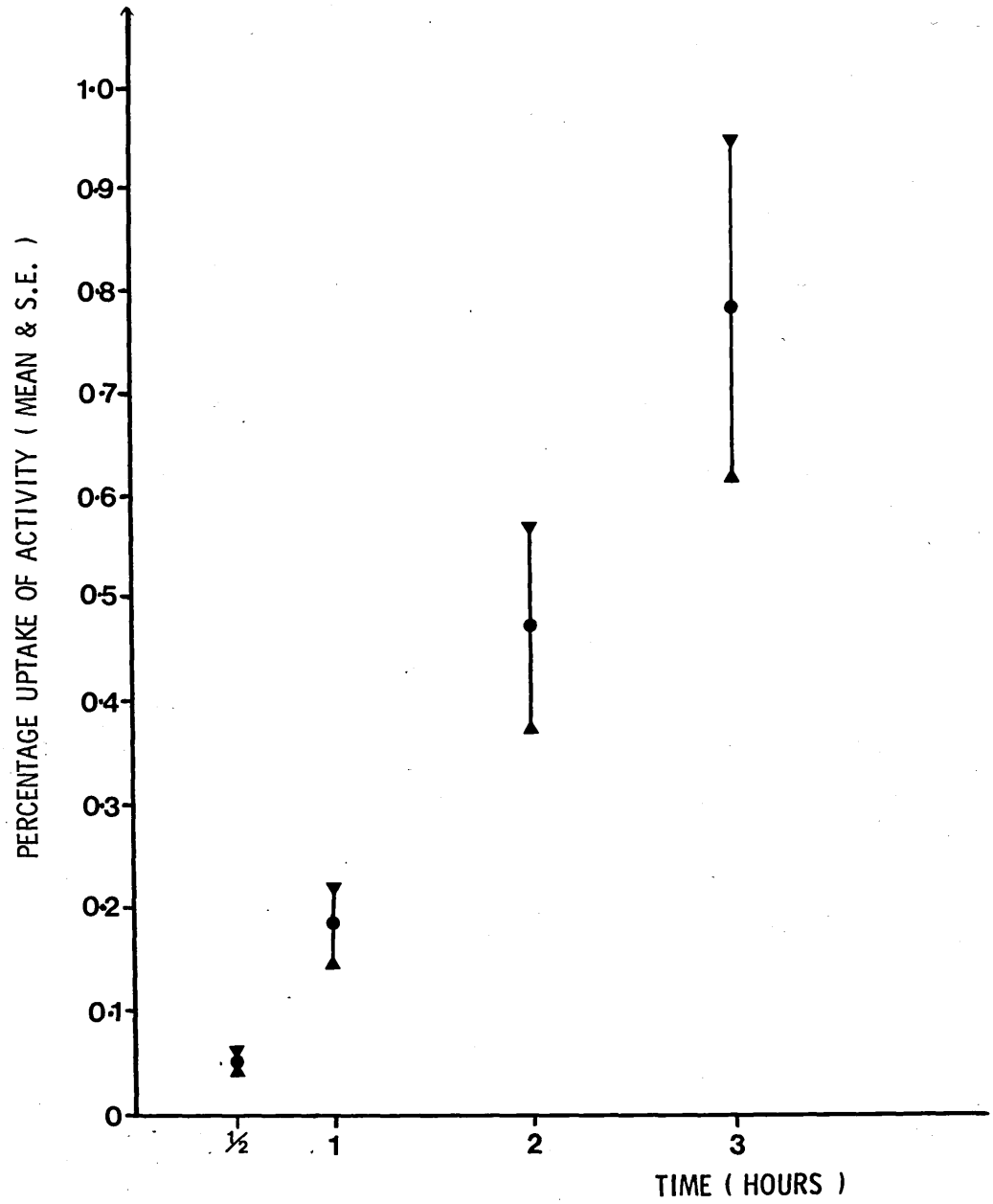


Figure 58

The percentage uptake of activity in the ilio-inguinal lymph nodes in 55 lymphoedematous limbs (0 - 3 hours)

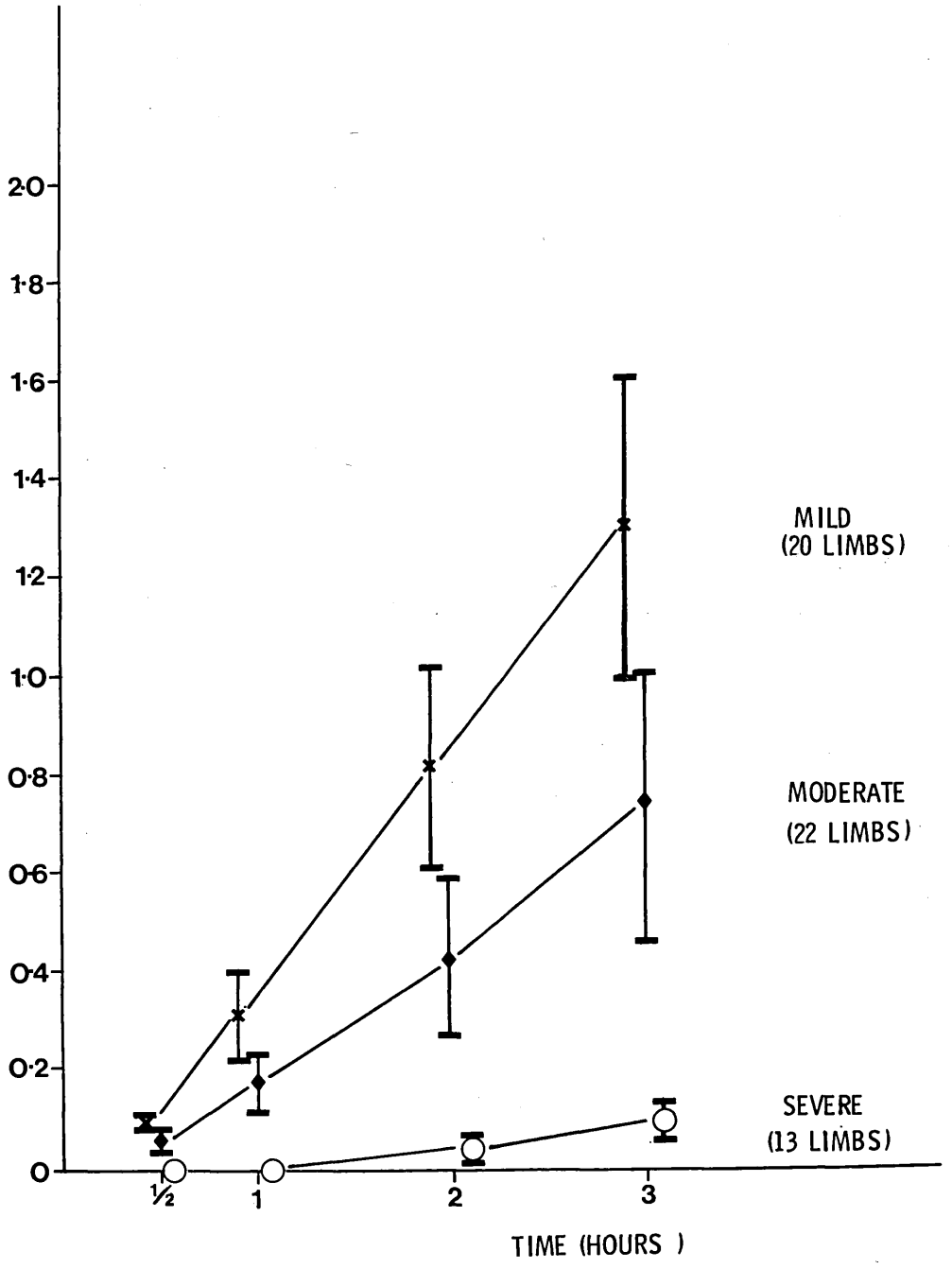


Figure 59

The percentage uptake of activity (mean and standard error) in the ilio-inguinal lymph nodes of 55 lymphoedematous limbs subdivided by severity (0 - 3 hours).

TABLE 17

Mean and S.D. of percentage uptake of activity in the ilio-inguinal lymph nodes lymphoedematous limbs subdivided by severity

	1/2hr	1hr	2hr	3hr
Mild lymphoedema (20 limbs)	0.087 ± 0.094	0.312 ± 0.4	0.814 ± 0.944	1.295 ± 1.374
Moderate Lymphoedema (22 limbs)	0.053 ± 0.074	0.17 ± 0,28	0.43 ± 0.77	0.73 ± 1.32
Severe lymphoedema (13 limbs)	0.003 ± 0.01	0.006 ± 0.22	0.025 ± 0.083	0.095 ± 0.192

Mann Whitney U test

Mild v Moderate	p =	ns	0.05	0.02	0.02
Moderate v Severe	p =	0.002	0.002	0.006	0.004
Mild v Severe	α =	0.001	0.001	0.001	0.001

The percentage uptake of the colloid was lowest throughout the 3 hour study in the 13 limbs with the most severe disease, the mildly and moderately affected limbs having a statistically higher uptake at half, 1, 2 and 3 hours. The 30 minute ilio-inguinal uptake in the limbs with mild lymphoedema was not significantly different from the limbs with moderate lymphoedema, but was significantly greater at 1, 2 and 3 hours.

Although 4 limbs with mild lymphoedema and 6 limbs with moderate lymphoedema showed no uptake throughout the whole study, in general the value of the percentage uptake reflects the severity of the lymphoedema.

Figure 60 shows radionuclide images taken at 30 minutes and one hour in a patient with lymphoedema of the left leg. These images show a normal pattern of lymph nodes clearly visible on the unaffected right side at 30 minutes. The 30 minute image shows no visible lymph node pattern on the left side compatible with the diagnosis of lymphoedema. It also shows that there is some lymph flow on this side as at 1 hour a faint pattern of activity in the lymph nodes can be seen, the percentage uptake increasing from 0.03% at 30 minutes to 0.1% at 1 hour.

The 55 lymphoedematous limbs were also subdivided by lymphographic classification. Although there are wide anatomical variations in the 55 limbs there was some significant differences between the groups and in particular the proximal obstructive hypoplasias tended to have a much poorer uptake probably because they had the severer type of lymphoedema. These results will be presented in greater detail in the next chapter.

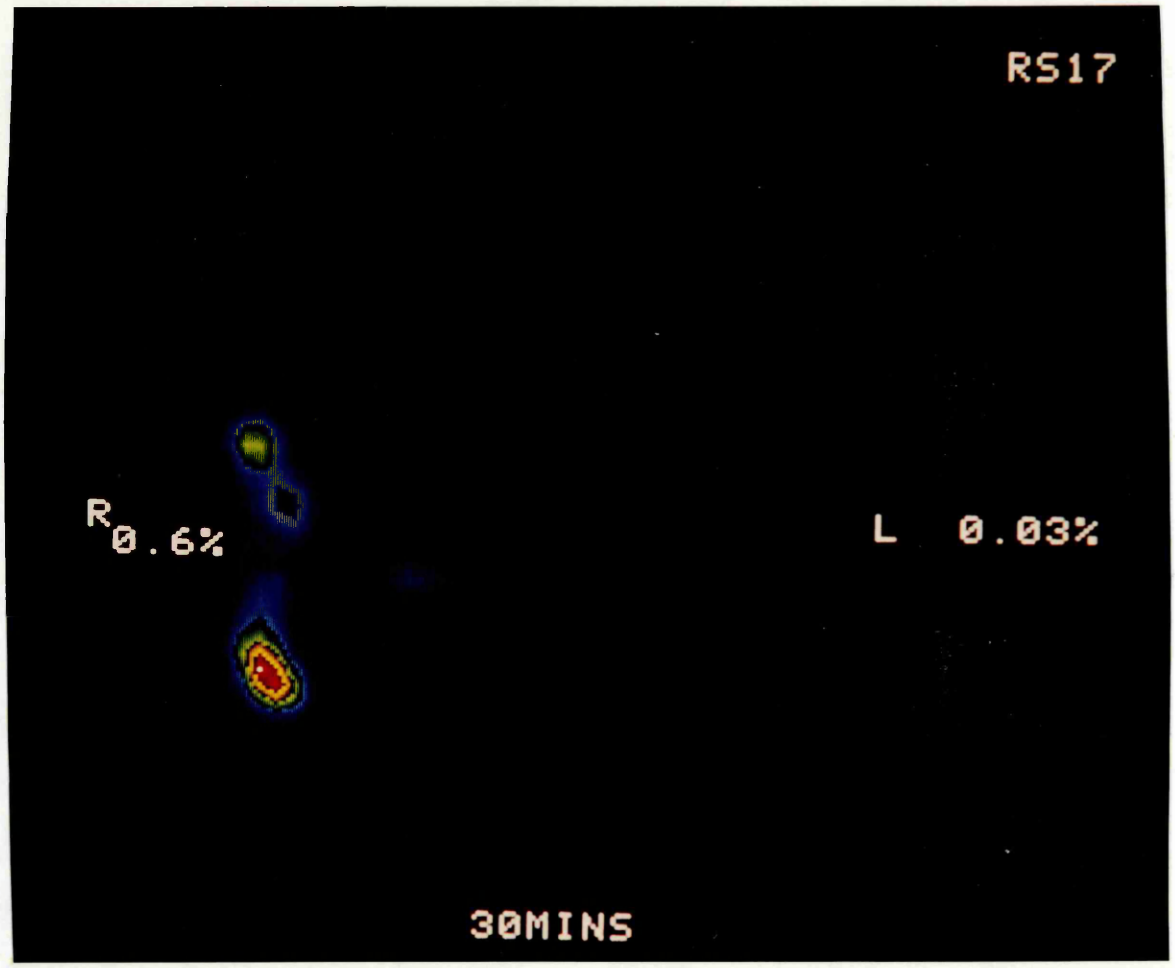


Figure 60a

Radionuclide images taken at a) 30 minutes and b) 1 hour in a patient with lymphoedema of the left leg. This shows a clearly visible pattern of activity in the lymph nodes on the right side with a normal uptake percentage. A faint pattern of activity is becoming visible on the affected left side at 1 hour.

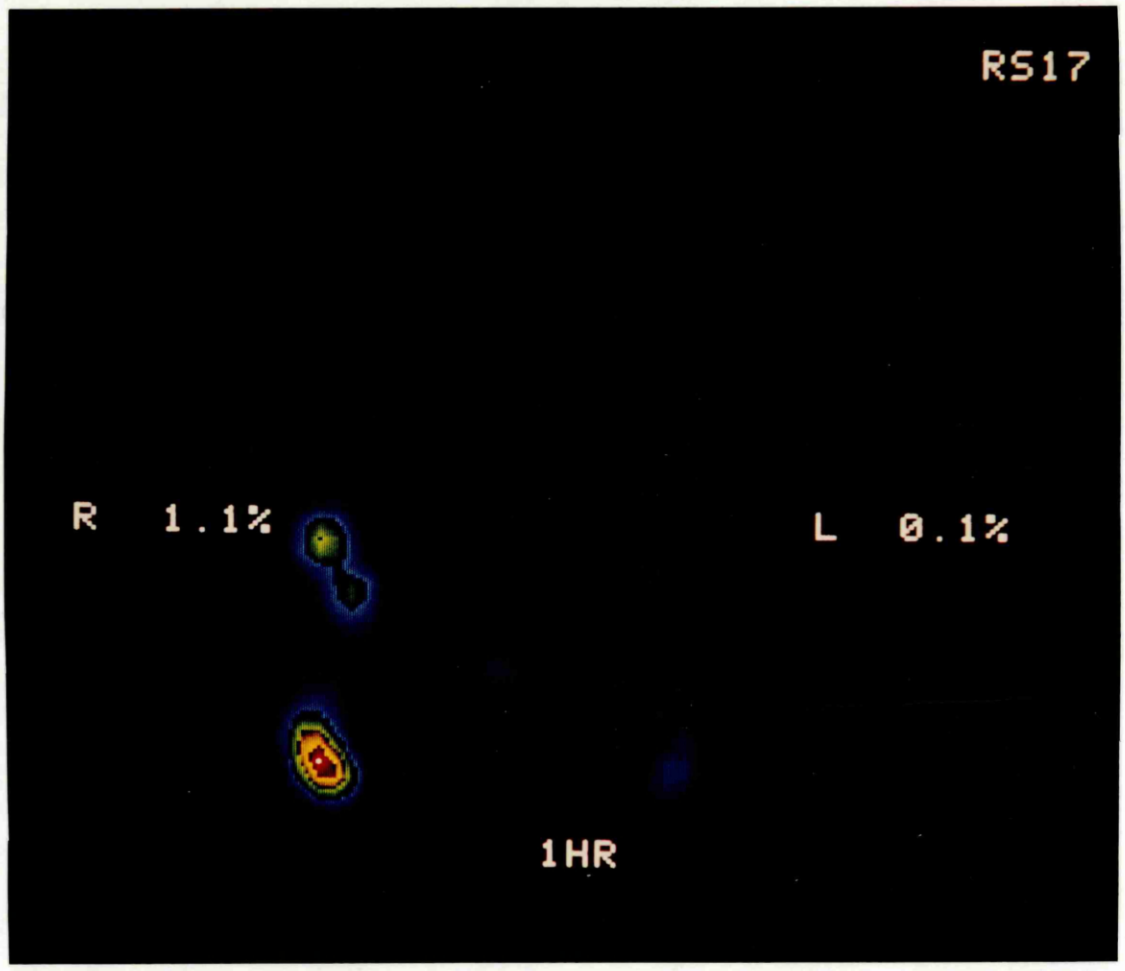


Figure 60b

Radionuclide image taken at one hour in the same patient with lymphoedema of the left leg whose 30 minute image is shown on the previous page.

Conclusion

These results show that there is a low uptake of activity in the ilio-inguinal lymph nodes following an interdigital space of radio active colloid in limbs with proven lymphoedema and that the more severe the swelling the poorer the flow of lymph from the periphery to the ilio-inguinal lymph nodes.

Venous Oedema

Calculation of the percentage uptake of activity in the ilio-inguinal lymph nodes at half, one, two and three hours was carried out in 12 limbs (Appendix X).

The mean percentage uptake for limbs with venous oedema at 30 minutes was 2.27 $\pm$ 1.17 (range 0.46 to 4.6). This uptake of activity increased throughout the study and was 5.02 $\pm$ 2.83 at 1 hr, 10.76 $\pm$ 5.25 at 2 hours and by 3hours the percentage uptake was 15.64 $\pm$ 6.87 with a range of 5.8 to 28 (Figure 61).

In summary, there is a high uptake of the radionuclide in limbs with venous oedema particularly when compared to lymphoedematous limbs but also when compared to the control limbs.

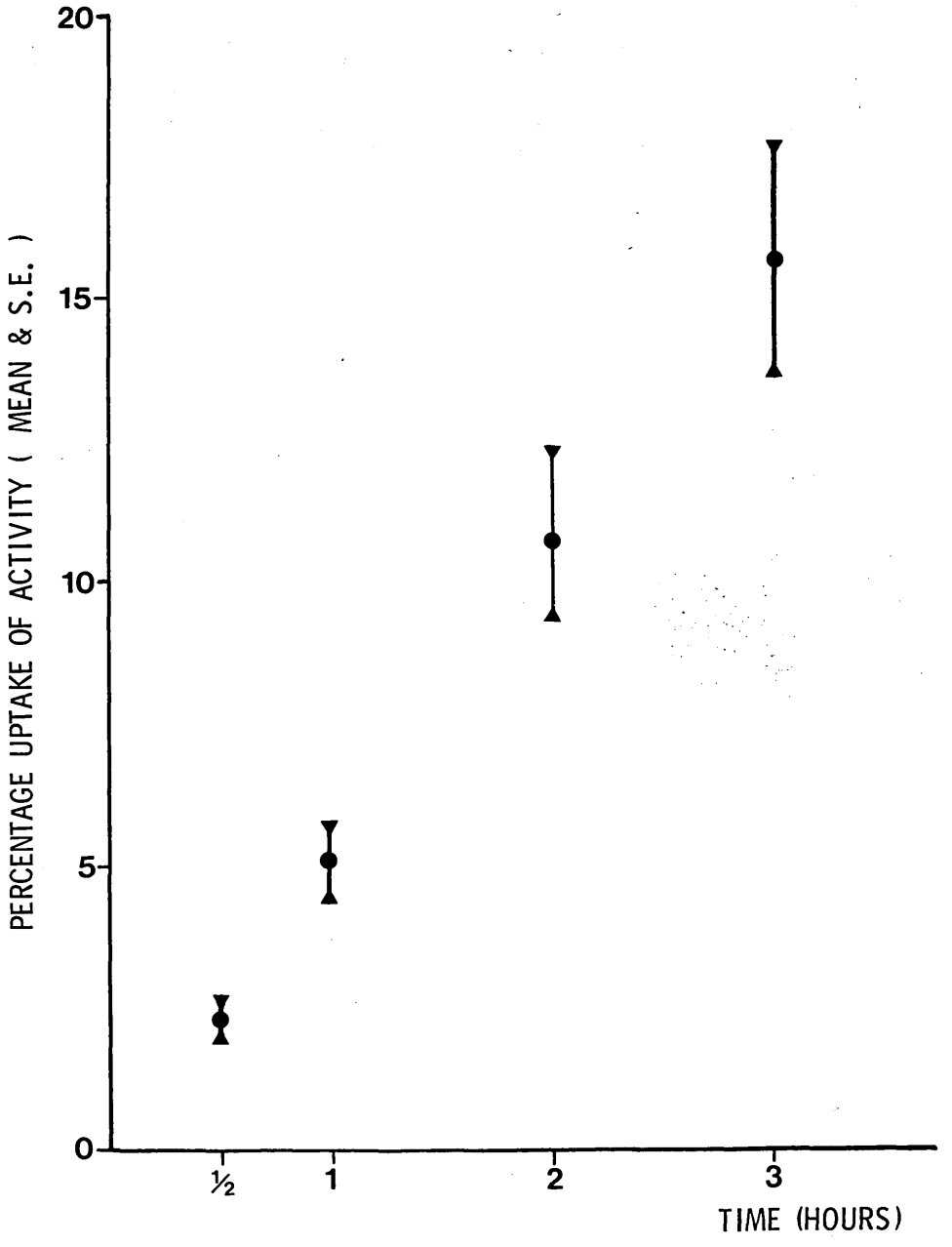


Figure 61

The percentage uptake of activity in the ilio-inguinal lymph nodes in 12 limbs with venous oedema (0 - 3 hours).

Miscellaneous oedemas

Calculation of the percentage uptake of activity in the ilio-inguinal lymph nodes at half, one, two and three hours was carried out in 15 limbs with miscellaneous causes of chronic lower limb oedema (Appendix XI).

The mean percentage uptake of activity at 30 minutes was 2.03 $\pm$ 1.15 with a range of 0.46to 4.7 and once again the percentage uptake of activity increased as time progressed until at 3 hours the mean percentage uptake was 12.01 $\pm$ 7.52 with a range of 3.3 to 27 (Figure 62).

Referring to Appendix XI it can be seen that there is a much wider range of uptake of activity in this group when compared to the other groups, and although there appears to be a higher uptake in this group of limbs in comparison to the control group at all 4 time intervals, there is a considerable overlap of individual results and the difference does not reach statistical significance.

Figure 63 shows radionuclide images taken at 30 minutes and 1 hour in a patient with bilateral idiopathic/cyclical oedema. This illustrates the importance of the calculation of percentage uptake of the rdionuclide, as there is very little difference in the visual appearance of the ilio-inguinal lymph nodes between 30 minutes and 1 hour, despite an increase in percentage uptake from 1.7% to 5.0% on the right and 2.2% to 6.4% on the left.

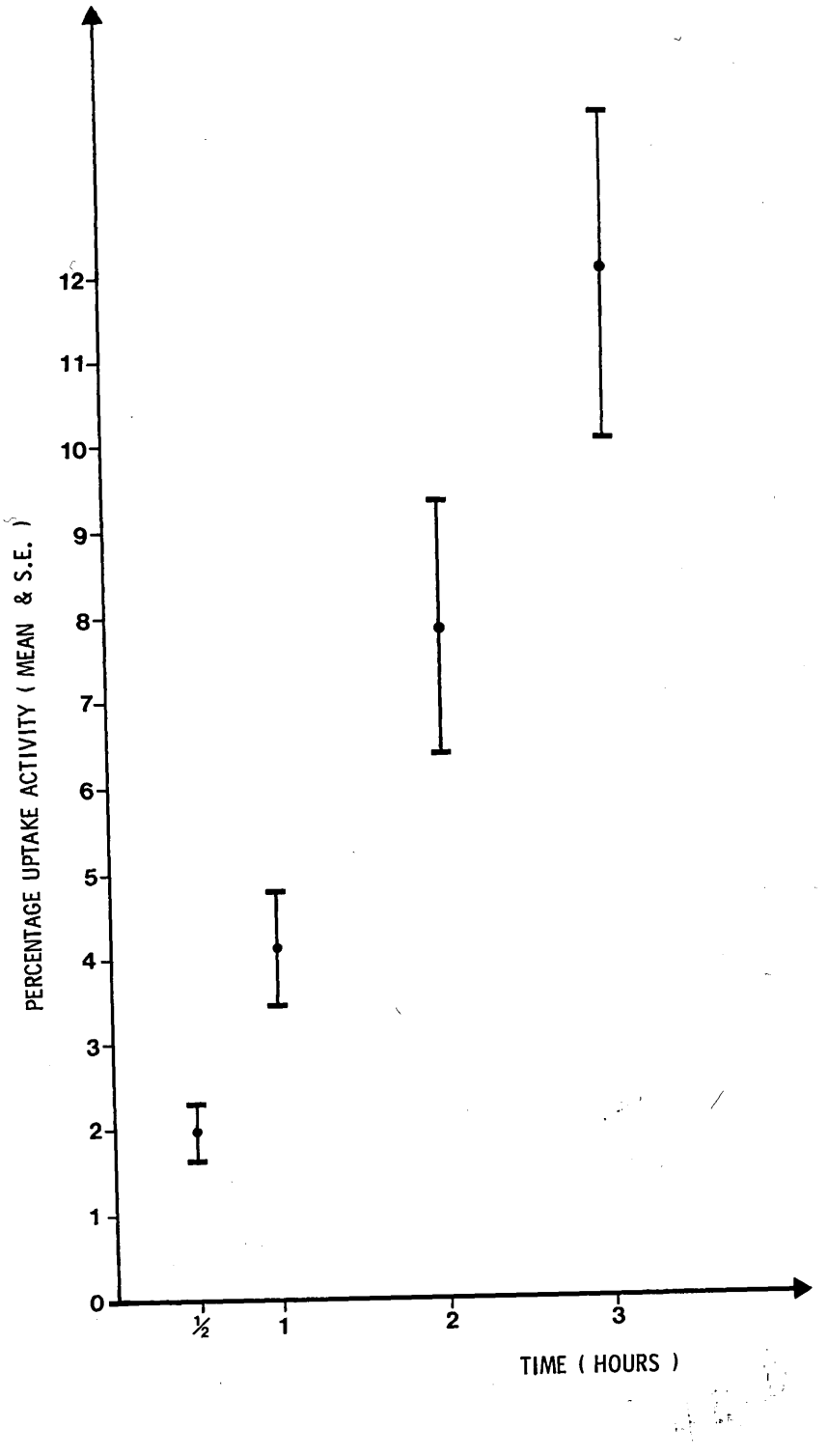


Figure 62

The percentage uptake of activity in the ilio-inguinal lymph nodes in 15 limbs with miscellaneous causes of chronic lower limb oedema (0 - 3 hours).

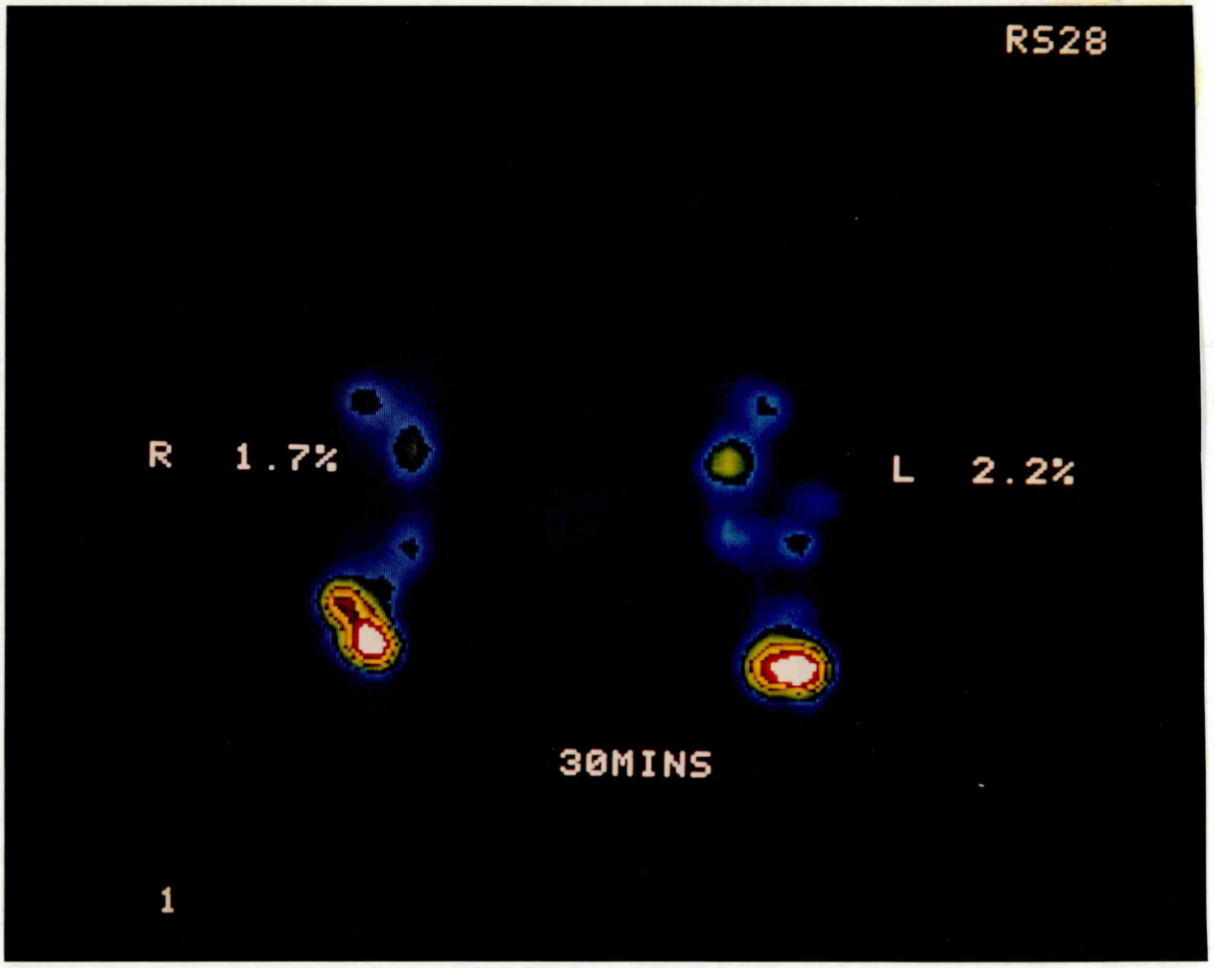


Figure 63a

Radionuclide images taken at 30 minutes (a) and 1 hour in a patient with cyclical oedema of both legs show the increase in % uptake on both sides from 30 minutes to 1 hour despite the similarity in the visual appearance.

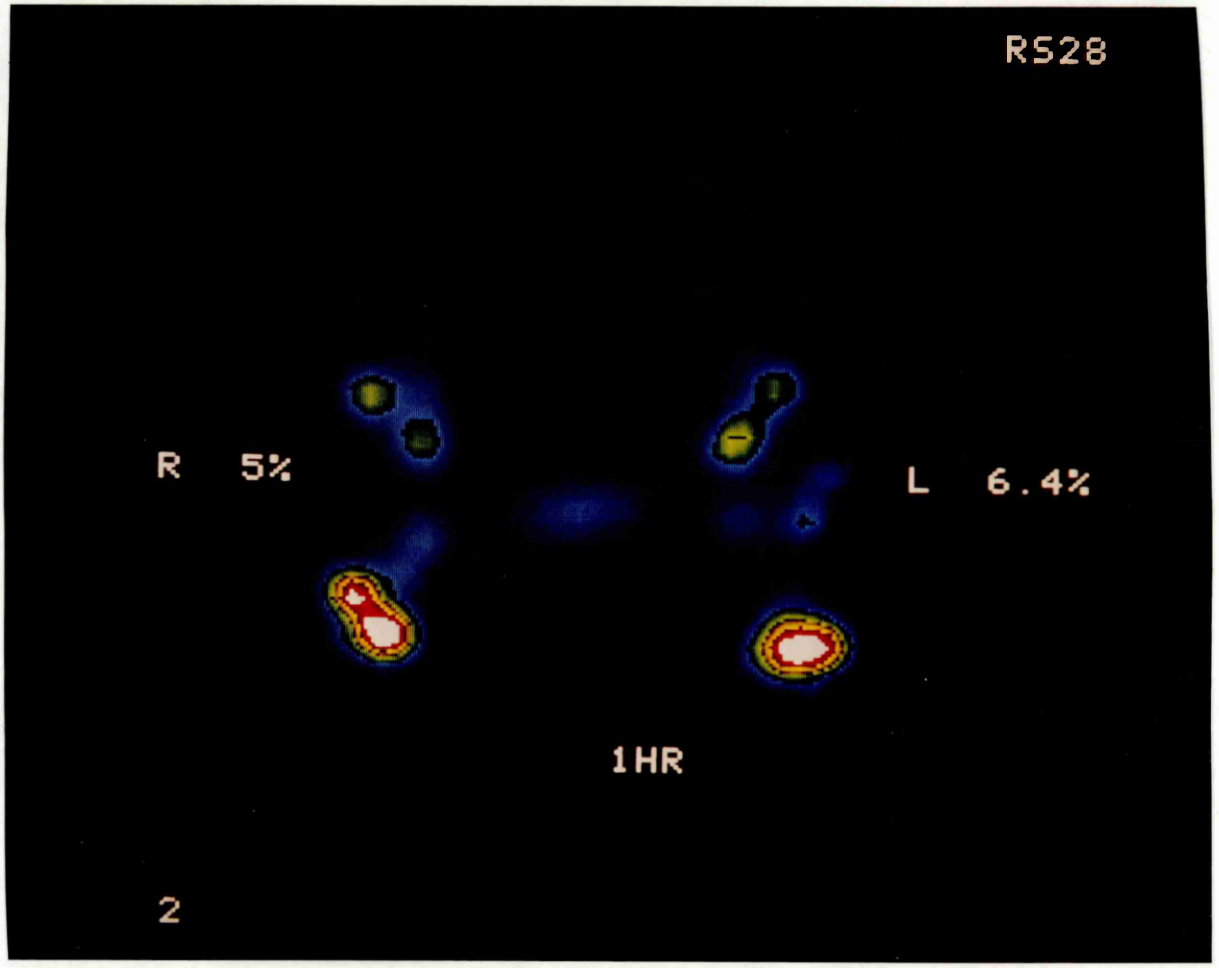


Figure 63b

The radionuclide image taken at one hour in the same patient whose 30 minutes image is shown on the previous page.

Percentage uptake of activity in the ilio-inguinal region in all 4 groups

The lymphoedematous limbs had a persistently lower percentage of uptake of the colloid throughout the three hour study when compared to the percentage uptake of injected activity in the ilio-inguinal lymph nodes at half, 1, 2 and 3 hours in the normal limbs and those with venous oedema, the difference becoming greater as time progressed (Figure 64).

A comparison of the limbs with venous oedema with the normal limbs showed that the former maintained a significantly higher uptake in the ilio-inguinal lymph nodes at half, 1, 2, and 3 hours suggesting that they had an increased lymph flow.

Both simple visual interpretation and calculation of the percentage uptake will, therefore, clearly differentiate venous and lymphatic oedemas. This fact is particularly well illustrated by the radionuclide images taken at 30 minutes and 1 hour (Figure 65) in a male patient who presented with primary lymphoedema of the left leg (confirmed by lymphography) and venous oedema of the right leg (normal lymphograph and post-phlebitic venous changes on phlebography).

The group of limbs with miscellaneous oedema also maintained a much higher percentage of activity at half, 1, 2 and 3 hours when compared to the lymphoedematous limbs.

The majority of these limbs (10) are due to idiopathic/ cyclical oedema which are often difficult to differentiate clinically from mild lymphoedema. Calculation of the percentage uptake activity will, however, clearly differentiate between these two groups (Figure 66).

A comparison between the limbs with miscellaneous oedema and the normal limbs showed that the former maintained a slightly higher uptake of activity in the ilio-inguinal lymph nodes throughout the 3 hour study (Figure 66), but this difference does not reach statistical significance. There was no statistical difference between the group of limbs with venous and those with miscellaneous oedemas although the venous limbs had a slightly higher uptake at half, 1, 2 and 3 hours.

The results presented so far show a considerable difference in uptake between the limbs with lymphoedema and the other 3 groups. It was, however, difficult to standardise the amount of activity each subject/patient underwent after the 30 minute study when the subject/patient was allowed to get up and move about. In order to compare the percentage uptakes of each group and attempt to define limits of uptake for each group, I have therefore, used the results obtained at 30 minutesas each subject/patient remained at rest during the first 30 minutes of the study.

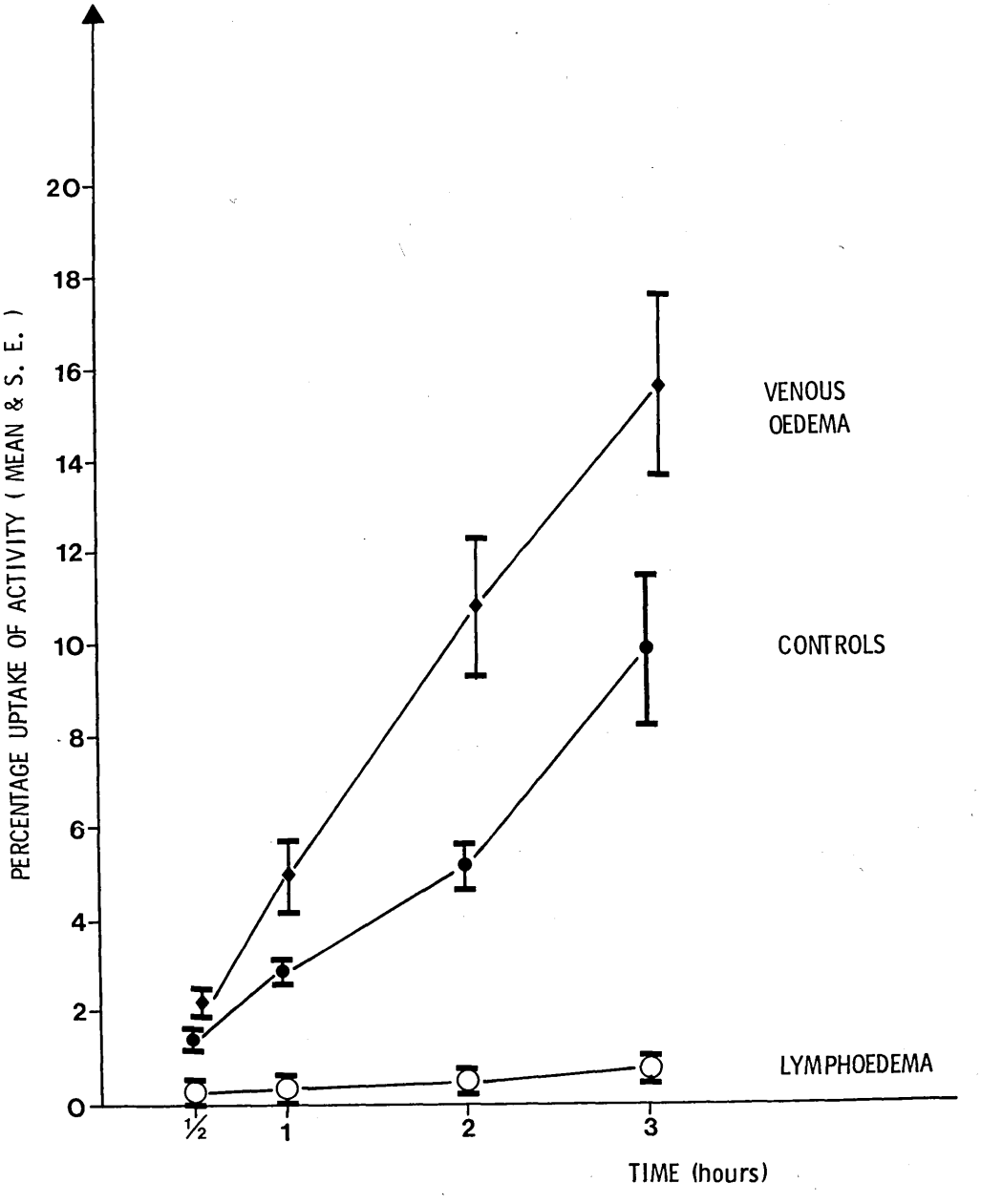


Figure 64

The percentage uptake of activity in the ilio-inguinal lymph nodes in 12 limbs, 55 lymphoedematous limbs and 12 limbs with venous oedema (0 - 3 hours).

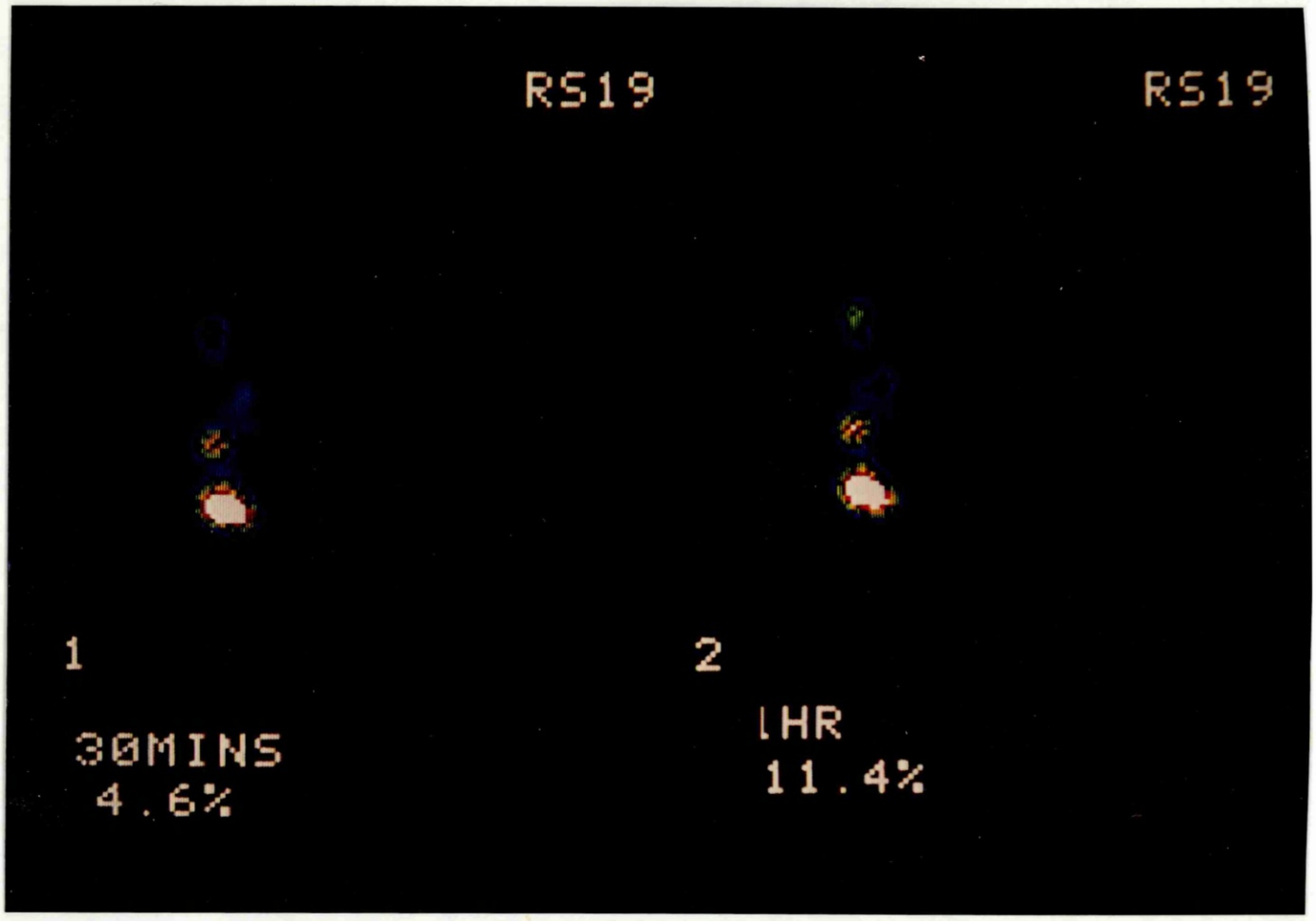


Figure 65

Radionuclide images at 30 minutes and 1 hour in a patient with lymphoedema of the left leg and venous oedema of the right showing a clearly visible pattern of ilio-inguinal lymph nodes on the right side with a high percentage uptake at 30 minutes and 1 hour. There is no uptake in the ilio-inguinal region on left side.

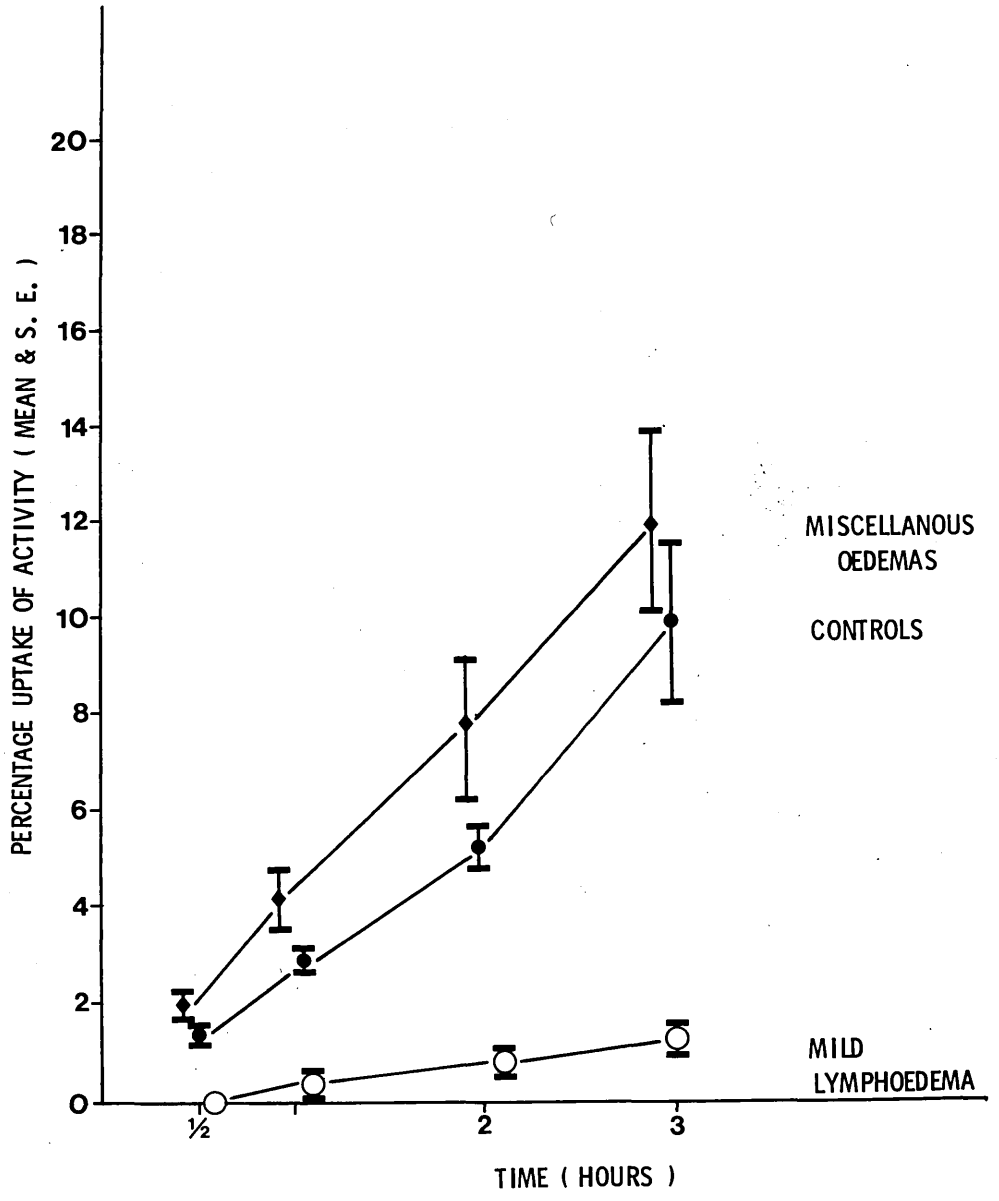


Figure 66

The percentage uptake of activity in the ilio-inguinal lymph nodes in 12 control limbs, 15 limbs with miscellaneous oedema and 20 limbs with mild lymphoedema (0 - 3 hours).

Comparison of percentage uptake of activity at 30 minutes in controls, lymphoedema, venous oedema and miscellaneous oedema

The mean (± S.D.) percentage uptake of activity in the ilio-inguinal lymph nodes calculated at 30 minutes for each group showed the uptake for the lymphoedematous limbs was 0.054% ± 0.079 with a range of 0 - 0.3% (Table 18). This was significantly lower than the uptake in the 12 control limbs of 1.40% + .70 (range 0.46 - 3.0) ($p < 0.001$) and the limbs with venous oedema of 2.27% + 1.16 (range 0.46 - 4.6) ($p < 0.001$). The amount of activity present in the ilio-inguinal lymph node chain in the limbs with venous oedema was significantly greater than that of the control limbs ($p = 0.04$).

The percentage uptake of the radioactive colloid in the 25 limbs of the patients with unilateral lymphoedema that were clinically and lymphangiographically normal was 1.56% + 0.93 (range 0.3 - 3.4) similar to that of the control group.

I would therefore define the limits of uptake at 30 mins. compatible with the diagnosis of lymphoedema as 0.30% which is the mean % uptake observed in the 55 legs with lymphoedema plus 3 standard deviations and is below the lowest level of uptake observed in any of the normal limbs (0.46%) and 1.5 standard deviations below the mean of the normal limbs.

The three lymphoedematous limbs whose images were classified on visual interpretaion as normal had a percentage uptake of 0.2, 0.3 amd 0.3% at 30 minutes; at the limits of uptake compatible with lymphoedema and less than the range of values of the normal group of 0.46% - 3.0%

It is more difficult to define the limits within in which venous oedema may be said to occur as there is an overlap between the control limbs and venous oedema, and I feel that these results do not indicate such a definition is valid.

TABLE 18

Uptake of activity in the ilio-inguinal lymph nodes at 30 mins

(mean S.D and range of percentage of injected activity)

Controls (12 limbs)	1.40	(0.70)	0.46 - 3.0
Primary lymphoedema (55 limbs)	0.054	(0.08)	0 - 0.3
Venous Oedema (12 limbs)	2.28	(1.16)	0.46 - 4.6
Miscellaneous oedema (15 limbs)	2.03	(1.15)	0.46 - 4.7

Controls v lymphoedema	$p < 0.001$
Controls v venous oedema	$p = 0.04$
Controls v miscellaneous oedema	n.s
Venous oedema v lymphoedema	$p < 0.001$
Miscellaneous oedema v lymphoedema	$p < 0.001$
Venous oedema v miscellaneous oedema	n.s.

Conclusion

The results presented in this section have shown that

1. Visual interpretation of the serial radionuclide images following an inter-digital space injection can accurately diagnose lymphoedema and clearly differentiate it from venous oedema and other causes of chronic lower limb swelling.

2. The calculation of the percentage uptake provides a quantitiative assessment of lymph flow in these limbs which simple visual interpretation is not capable of.

This technique is much more accurate than that described in Method 1 and in the next chapter I will compare the results of radionuclide lymph node imaging following an inter-digital space injection (Method 2) with the results of the X-Ray lymphography in the 55 lymphoedematous limbs studied by this technique.

CHAPTER 6

COMPARISON OF RADIONUCLIDE LYMPH NODE IMAGING (ISOTOPE LYMPHOGRAPHY) AND X-RAY LYMPHOGRAPHY

Introduction

The results presented in the last chapter show that isotope lymphography following an interdigital space injection can accurately diagnose lymphoedema and clearly differentiate primary lymphoedema from other forms of chronic lower limb swelling. In this chapter I will compare the results of isotope lymphography with those of X-ray lymphography in 55 patients with primary lymphoedema.

X-Ray lymphography in primary lymphoedema

X-Ray lymphography has given rise to a radiological classification originally based on the belief that there is a fundamental genetic defect which underlies the structural malformations seen. On the basis of X-Ray lymphography patients were thus assigned to one of a number of categories: there may be a complete failure to demonstrate any vessels (aplasia) - now accepted to be an extreme degree of hypoplasia; the vessels may be too few or too small (hypoplasia) or in a small percentage of cases may be excessively numerous (hyperplasia - usually bilateral) or dilated and varicose (megalymphatics).

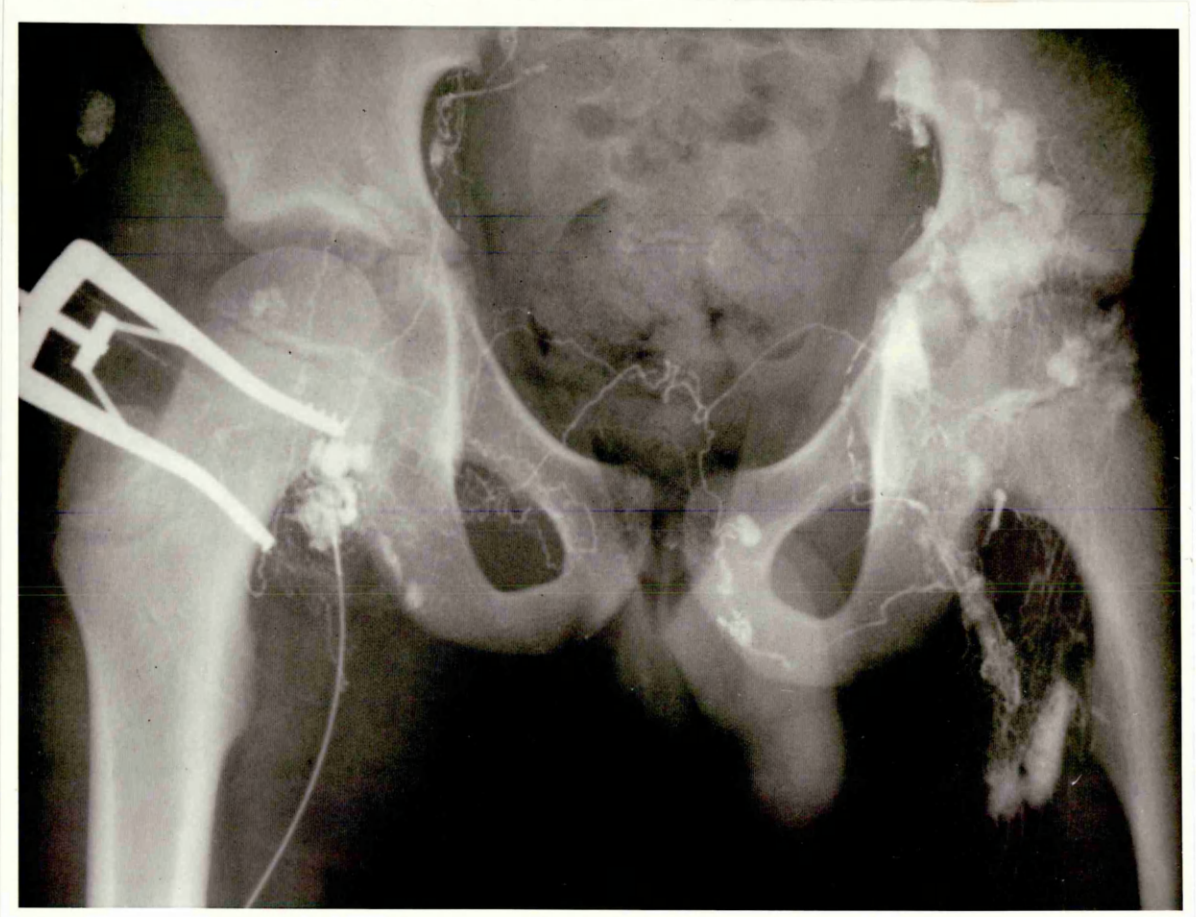


Figure 67

Lymphograph showing obliteration of both the distal limb lymphatics and the ilio-inguinal lymphtic chain on the right with hairpinning of vessels and collateral vessels linking with the left side. The left side is also abnormal.

In this part of the study, all the 55 limbs were categorised as hypoplasia (the vast majority of lymphoedemas) and subdivided into three distinct groups (Kinmonth 1982).

1. Distal obliteration (hypoplasia) of the limb lymphatics with normal proximal lymphatics (40 limbs).

2. Proximal and distal obliteration (hypoplasia) of both the limb lymphatics and the ilio-inguinal lymphatics and lymph nodes. In this group, there are few if any lymphatics in the leg and extremely poor or no forward flow following a lymph node puncture (Figure 67). There were 10 limbs with this radiological appearance.

3. Proximal node obstruction (hypoplasia) i.e. obliteration of the lymphatics and lymph nodes in the ilio-inguinal region with distension distally of the limb lymphatics secondary to this proximal block (5 limbs)

Kinmonth and Wolfe (1980) suggest that proximal lymph node obstruction will produce distension of the distal lymphatics of the limb. Lesser degrees of node obstruction may however cause slower obstruction of these lymphatics without distension.

The frequent lymphographic findings of patent slightly distended peripheral lymphatics and a reduced number of peripheral vessels support this hypothesis. The other two common radiological appearances, namely (a) small fibrotic nodes with distal distension and (b) small fibrotic nodes with a reduced number of distal vessels, may also reflect the opposite ends of the spectrum of the same disease process rather than two different processes.

Therefore for the purposes of this study these two groups have been combined and the limbs have been sub-divided into:

(a) 40 limbs with distal lymphatic obliteration

(b) 15 limbs with a combination of proximal obliteration of lymphatics with either distal distension or distal obstruction of lymphatics.

Serial Images

Three of the 40 limbs with distal hypoplasia showed radioactivity in the ilio-inguinal lymph nodes within 30 minutes of the inter-digital cleft injection. Nine of the 40 limbs showed some uptake by 1 hour, 7 at 2 hours, and 4 at 3 hours leaving 17 limbs showing no activity in the ilio-inguinal regions after 3 hours. In comparison, none of the 15 limbs with proximal lymphatic abnormalities had radioactivity present in the ilio-inguinal region at 30 minutes, one limb showed radioactivity present by 1 hour, 3 at 3 hours and the remaining 11 limbs showed no activity present in the ilio-inguinal region after 3 hours.

Using the criteria for visual interpretation of the gamma camera images at 30 minutes, based on the control group (Chapter 5), 3 of the 40 limbs with distal hypoplasia had normal studies at 30 minutes whereas all of the limbs with proximal hypoplasia were abnormal. Furthermore, 73% of the limbs with proximal hypoplasia showed no activity by 3 hours compared to 42.5% of the limbs with distal hypoplasia. These results suggest that lymph flow was more severely diminished in the group of limbs with proximal lymphatic abnormalities.

The 32 clinically normal limbs of the patients with unilateral lymphoedema were subdivided into two groups: those with normal and those with abnormal lymphangiograms. The seven clinically normal limbs with lymphangiographic abnormalities all had distal hypoplasia. Radioactivity appeared in the ilio-inguinal lymph nodes at 30 minutes in 5 and at 3 hours in 1; no activity was seen in the ilio-inguinal region after 3 hours in

the seventh. Four of the seven limbs were visually assessed as normal, one as equivocal and two as abnormal. The 25 limbs which were clinically and lymphangiographically normal all showed good uptake at 30 minutes.

Further analysis of the lymphangiographic findings showed that in 8 limbs no peripheral lymphatics could be found while an inguinal lymph node injection showed normal ilio-inguinal lymphatics in 7 of the 8 limbs. In contrast radioactivity could be seen in the ilio-inguinal lymph nodes in 4 out of the 8 limbs at 1 hour, 2 hours and 3 hours (in 2) respectively (Figure 68).

This provides further evidence that the radiological absence of vessels may be caused by technical failure or quirks of filling related to the site and nature of the injection during lymphography.

There were 20 radionuclide studies in which no activity was seen in the ilio-inguinal regions at 3 hours. Peripheral lymphatics could not be found in 4 of the 20 limbs. Eight of the other 16 limbs in which cannulation of a lymphatic was possible had proximal lymphatic abnormalities.

TABLE 19

Time of arrival of the colloid in the ilio inguinal lymph nodes in 55 limbs with lymphoedema subdivided by radiological appearance.

	Number of limbs				
Time of arrival of the 99mTc RSC in the ilio-inguinal lymph nodes	1/2	1	2	3	3
Controls (12 Limbs)	12	0	0	0	0
Distal Hypoplasia (40 limbs)	3	9	7	4	17
Proximal lymphatic Abnormalities (15 limbs)	0	1	0	3	11

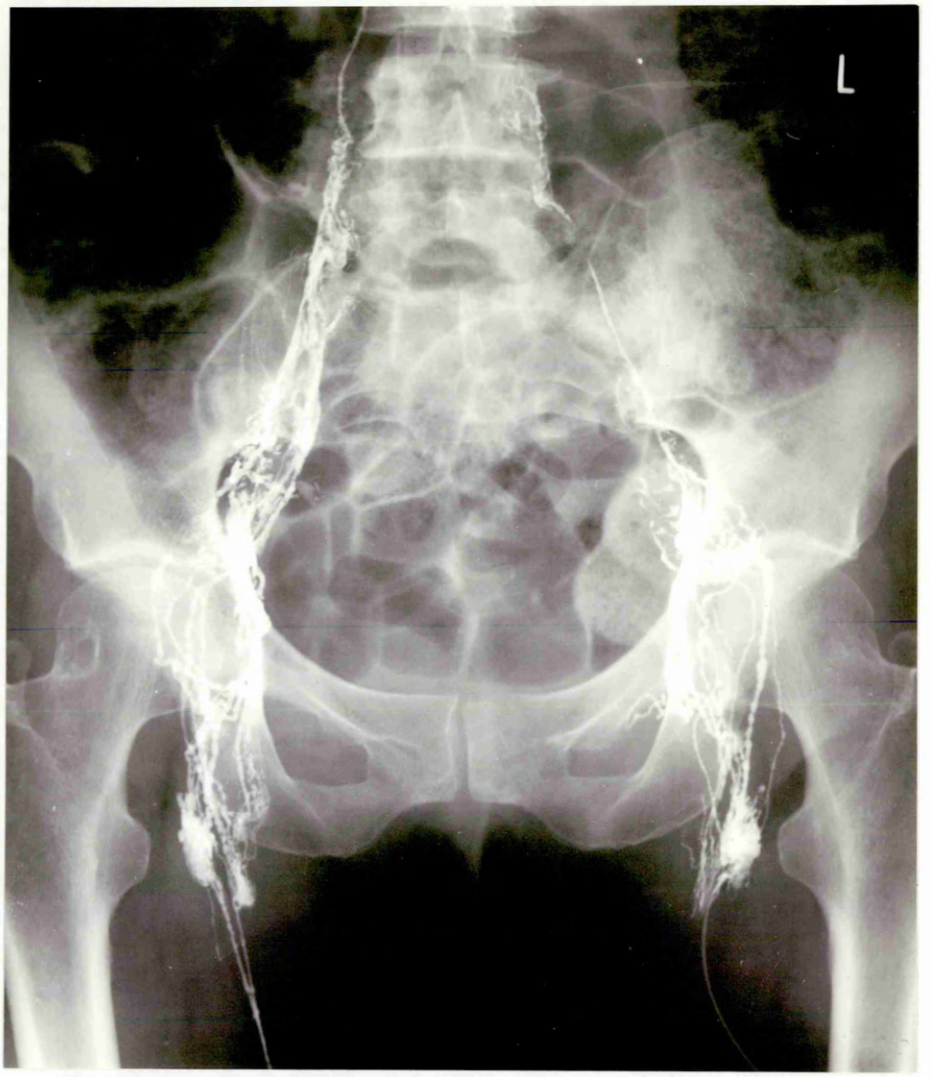


Figure 68a

Lymphograph showing obliteration of the distal lymphtics of both limbs. There is solitary hypoplasia on the right but no lymphatics could be found in the left foot. The iliac lymphtics are normal.

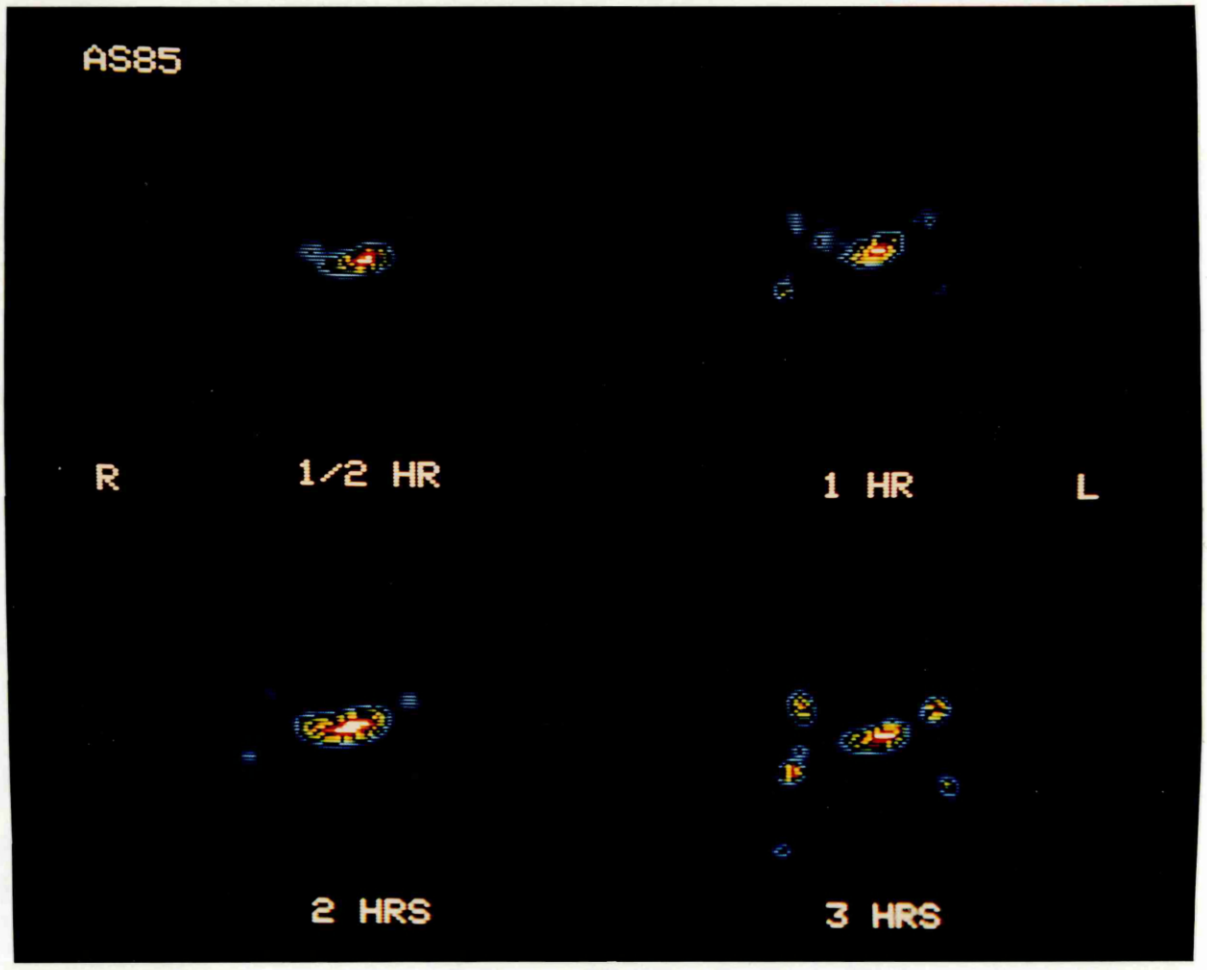


Figure 68b

Serial radionuclide images showing only a faint trace of activity on the right side at 30 minutes and at 1 and 2 hours both sides. A 3 hours the ilio-inguinal lymph nodes are becoming more clearly visible on both sides.

Mascagni's illustration of the lower limb lymphatics (Figure 69) depicts the numerous lymphatics available for cannulation in the foot and there are said to be many more than 50 lymphatics in the groin compared to Kinmonth's "normal classification" of between 5 to 15 lymphatics in the upper thigh. However, although there may be difficulty in finding lymphatics to cannulate in the foot in lymphoedema, the anatomical detail provided by lymphography is much better in comparison to the radionuclide images seen in Figures 68, 71, 72.

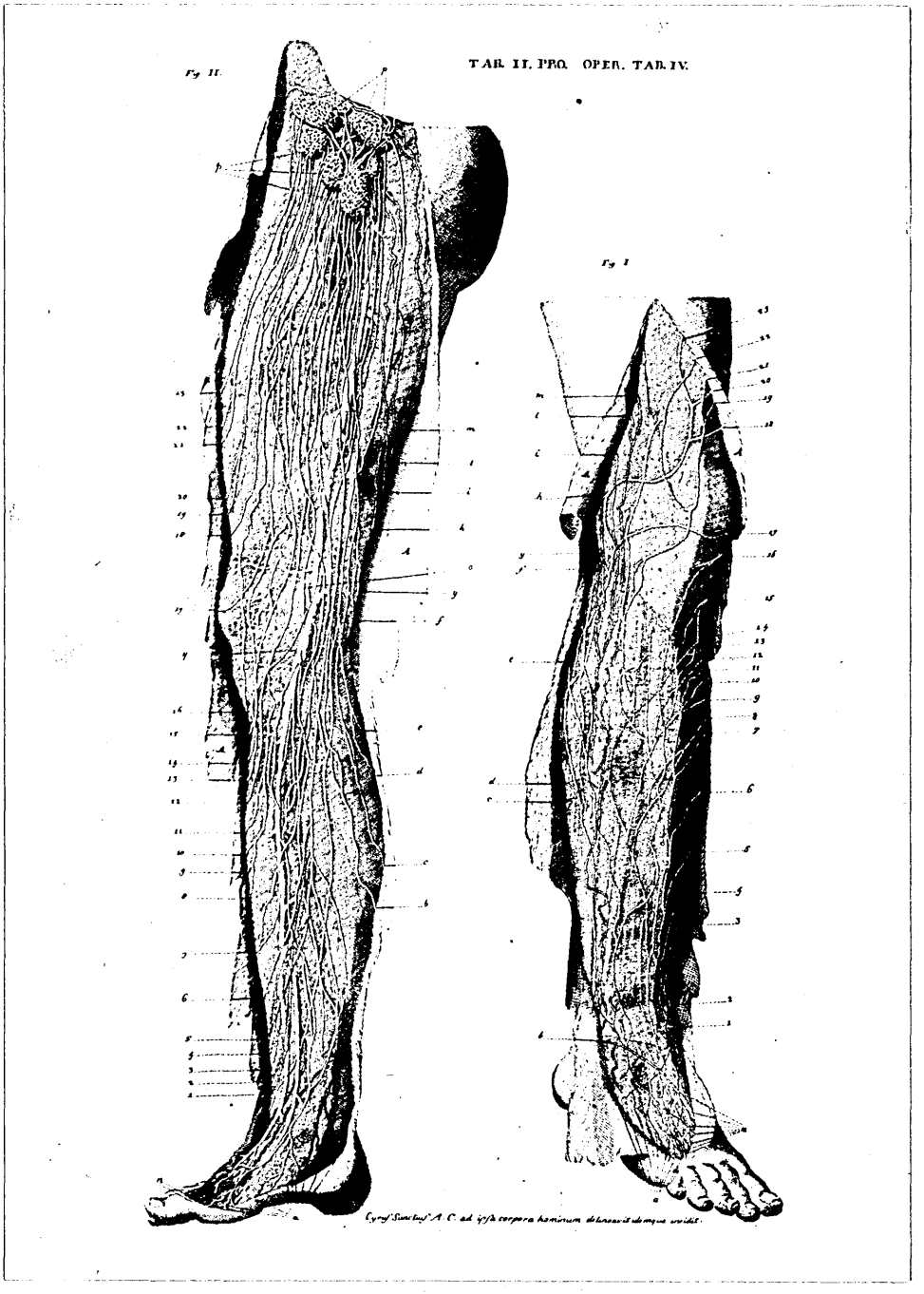


Figure 69

The lower limb lymphatics from Vasorum lymphaticorum Corporis Historia et Ichnographia by P. Mascagni, Sienna 1787.

Percentage uptake of activity in the ilio-inguinal lymph nodes

It has been shown in the previous chapter that the lymphoedematous limbs have a persistently lower percentage uptake throughout the 3 hour study when compared to the percentage uptake in the ilio-inguinal lymph nodes in the normal limb, those with venous oedema and those with miscellaneous oedemas.

The 55 lymphoedematous limbs were subdivided by lymphangiographic findings into the two groups discussed earlier in this chapter:

1. Distal lymphatic abnormalities (40 limbs)
2. Proximal lymphatic abnormalities (15 limbs)

The percentage uptake was lower throughout the study in the group of limbs with proximal lymphatic abnormalities, the limbs with distal hypoplasia alone having a statistically higher uptake at 30 minutes 1, 2, and 3 hours (Figure 70 and Table 20). These results again show that lymph flow is more severely diminished in limbs with proximal lymphatic abnormalities.

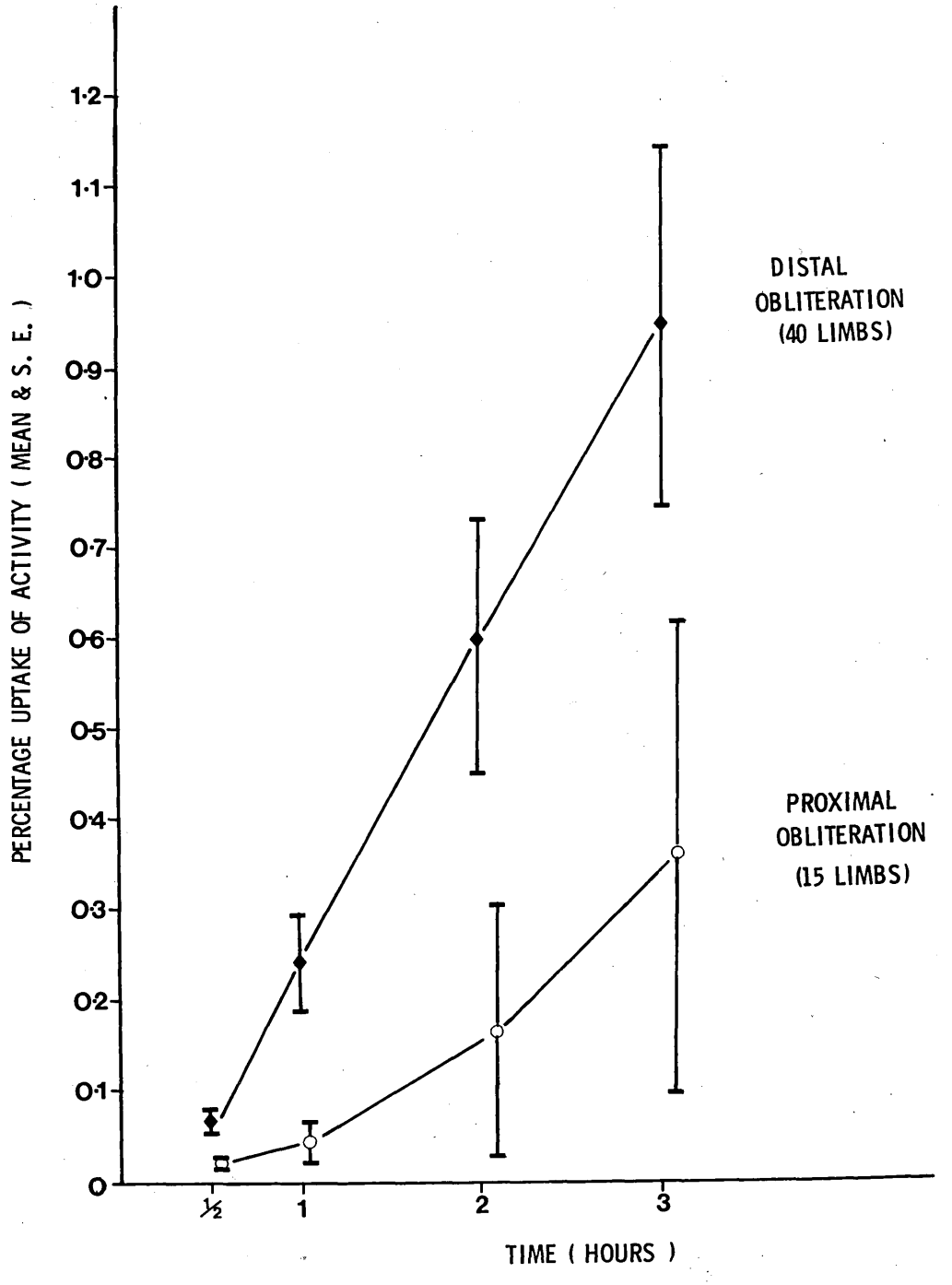


Figure 70

The percentage uptake of activity in the ilio-inguinal lymph nodes in 55 lymphoedematous limbs subdivided by radiological appearance (0 - 3 hours).

TABLE 20

The percentage uptake (mean and standard deviation) in the ilio-inguinal lymph nodes in 55 limbs with lymphoedema subdivided by radiological appearance

	Percentage uptake of Activity			
Time (hours)	1/2	1	2	3
Distal obliteration (40 limbs)	0.067 ± 0.086	0.24 ± 0.35	0.59 ± 0.85	0.94 ± 1.30
Proximal Lymphatic abnormalities (15 limbs)	0.02 ± 0.043	0.05 ± 0.10	0.17 ± 0.53	0.36 ± 1.02
Distal v Proximal *p =	0.004	0.004	0.002	0.002

*p values - Mann Whitney U test

The 32 clinically normal limbs with unilateral lymphoedema were subdivided into 2 groups, those with normal and those with abnormal lymphangiograms. The 7 clinically normal limbs with abnormal lymphangiograms had uptakes of 0.0 to 0.9% at 30 minutes (Table 21).

TABLE 21

The percentage uptake of radionuclide (0 - 3 hours) in the seven clinically normal limbs with lymphographic abnormalities

	Percentage uptake of injected activity			
No. of Study	1/2	1 hr	2hr	3hr
1*	0.003	0.1	0.16	0.3
2	0.3	0.6	2.1	2.9
3	0.6	0.94	2.0	3.4
4	0.9	2.9	4.1	6.2
5	0.3	4.3	5.6	7.2
6	0.3	3.0	4.8	5.4
7*	0	0	0	0

All limbs had distal hypoplasia. In studies 1* and 7* no peripheral lymphatics were found at lymphography although there were normal iliac lymphatics following an inguinal node injection.

Five of the 7 limbs had percentage uptake at 30 minutes of 0.3% or less; 0.3% being the border line of abnormality previously defined for lymphoedema. The images at 30 minutes of two were interpreted as normal, one as equivocal and the rest as abnormal. Thus both visual interpretation and the calculation of the uptake is able to detect a hidden lymphatic deficiency (Figures 71, 72).

The 25 clinically normal limbs with normal lymphangiograms had an uptake throughout the study (Figure 73; Appendix VII) similar to that of the control group of limbs (Figure 57; Appendix II).

Figure 71a

Lymphograph of patient with lymphoedema of the left leg showing no distal lymphatics and normal iliac vessels on the affected left side. On the clinically normal side there is a decreased number of distal lymphatics.

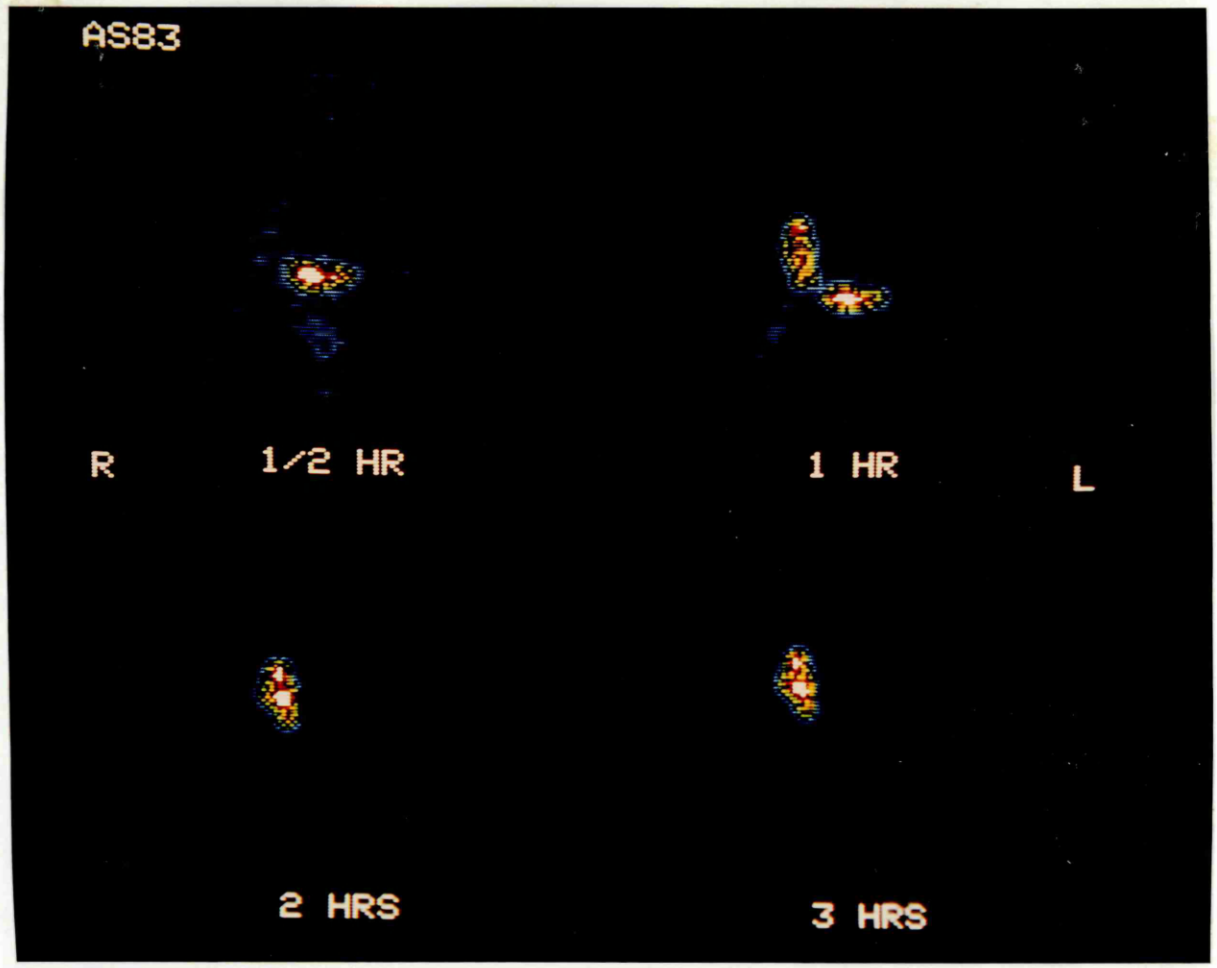


Figure 7 1b

Serial radionuclide images from the same patient showing no activity on the affected left side. The right side shows delayed uptake and shows a clearly visible pattern at 1 - 2 hours.

Figure 72a

Lymphograph of patient with mild lymphoedema showing distal obliteration of lymphatics on the swollen left side and a decreased number of lymphatics on the clinically normal side.

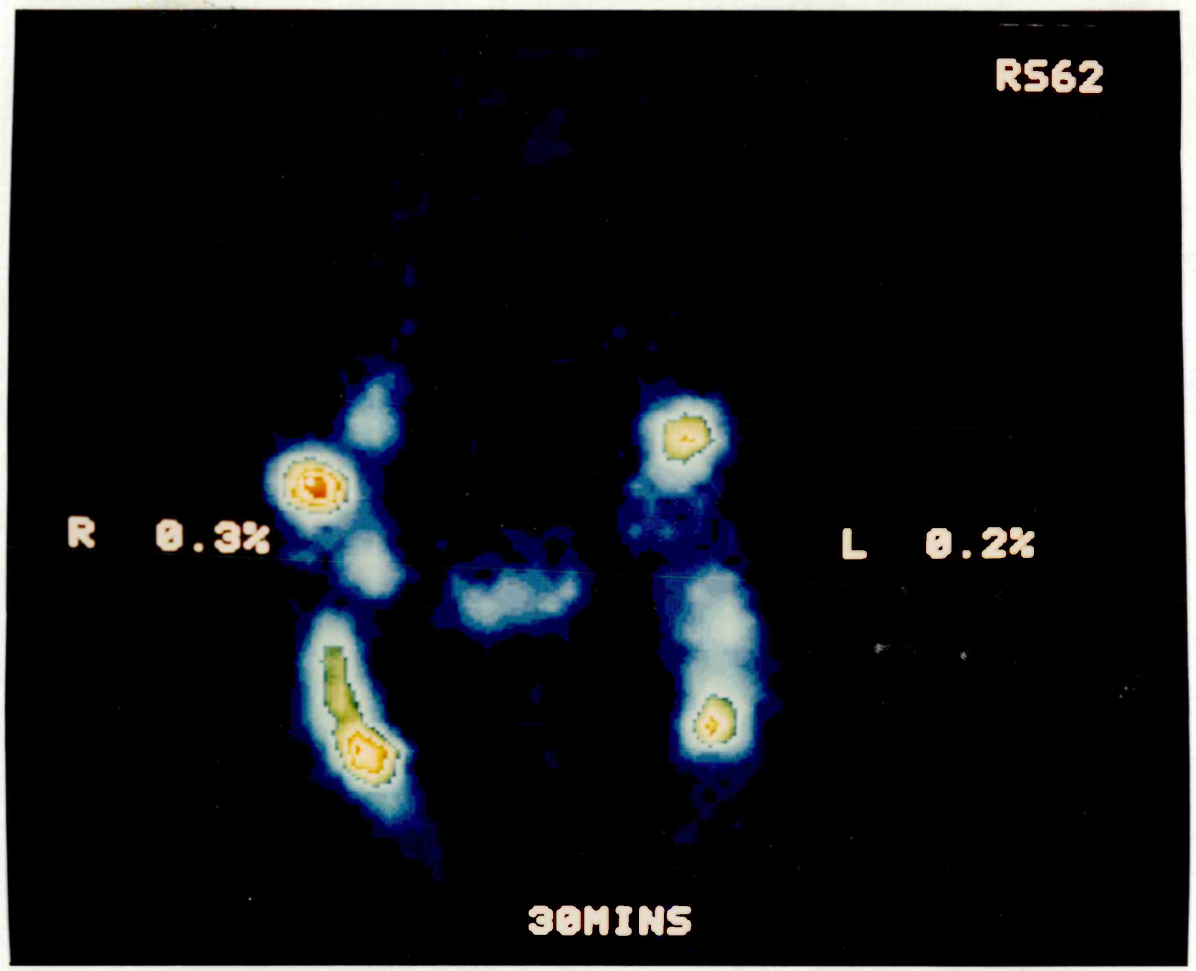


Figure 72b

Radionuclide image at 30 minutes in same patient visually assessed as normal on both sides but showing a decreased percentage uptake of 0.3% and 0.2% on the clinically normal and swollen limbs respectively.

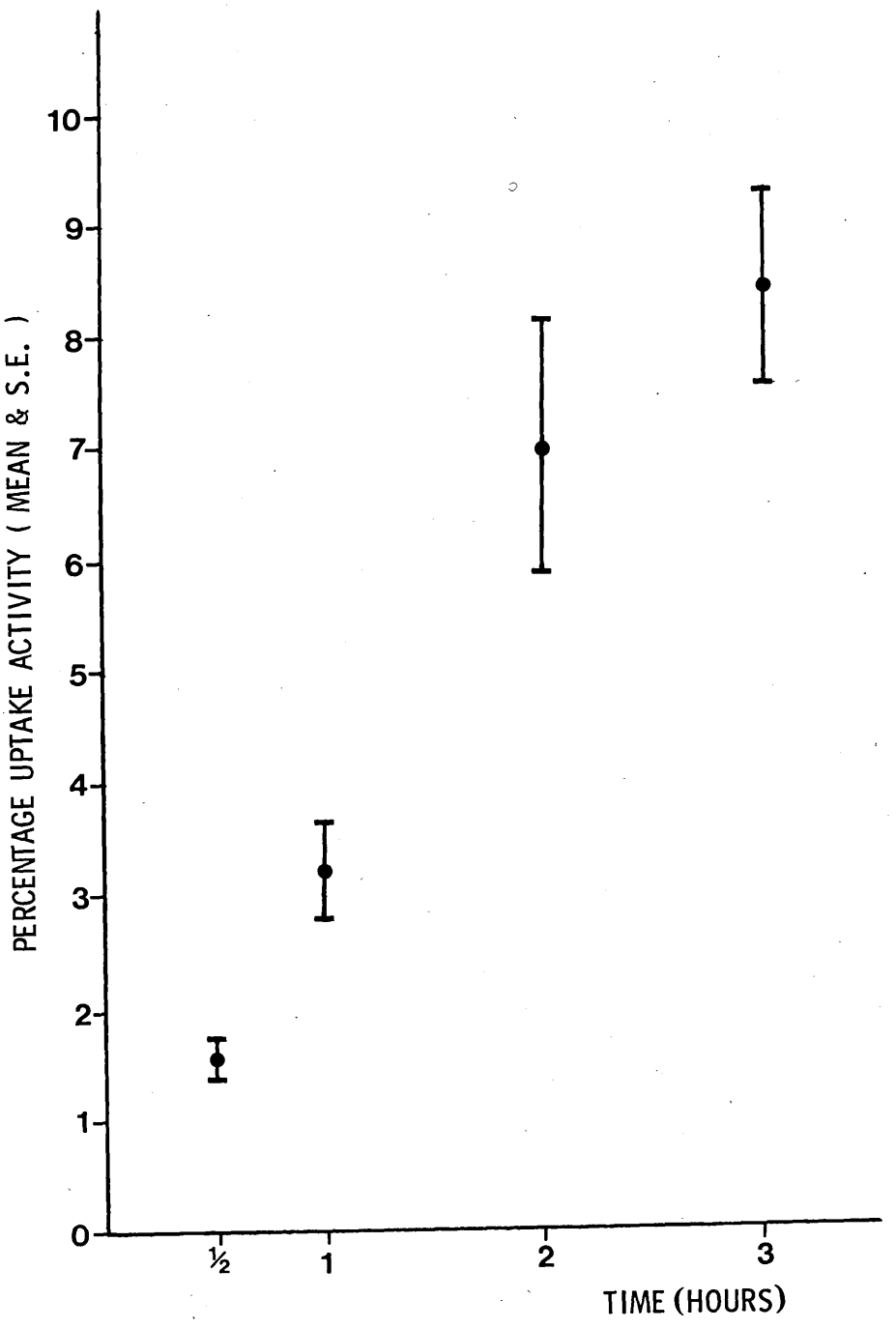


Figure 73
The percentage uptake of activity in the ilio-inguinal lymph nodes in 25 clinically and lymphographically normal limbs (0 - 3 hours).

Conclusions

1. Both visual interpretation and calculation of the percentage uptake of the colloid have shown marked differences between limbs with distal lymphatic abnormalities alone and those with proximal and distal abnormalities. These results show that the flow of lymph is more severely impaired in the latter group of limbs, suggesting that the two groups are separate and distinct entities within the umbrella diagnosis of primary lymphoedema.

2. The anatomical detail obtained from lymphography is greater than that from radionuclide imaging.

CHAPTER 7

DISCUSSION

If man is to think beyond what senses had directly given him,
He must first throw some wild guess work into the air,
And then, by comparing it bit by bit with nature
Improve and shape it into a truth.

William Smith, Thorndale 1859

The human lymphatic system has until now been conventionally imaged for diagnostic purposes by X-Ray contrast lymphography introduced by Kinmonth in the 1950's. It demonstrates a previously inaccessable system of the body with high resolution so that nodes and lymphatics can be seen in considerable detail. Its obvious advantages however hide its less obvious disadvantages which are sufficiently great to justify persuing radionuclide lymph node scanning as a possible alternative and indeed, it was these disadvantages, previously discussed, which provided the incentive for this study.

Radionuclide studies of the lymphatics in chronic lower limb oedema were first reported in the 1950's. Taylor et al., (1957) showed the clearance studies using labelled albumin could distinguish between lymphoedema and venous oedema. He pointed out however several practical problems with the technique which have been well illustrated by the preliminary study carried out in developing the radionuclide techniques described in Chapter 4.

The preliminary study showed that the technique of external counting was inaccurate and was particularly prone to positional errors of the external scintillation counter. The use of the gamma camera, however, has solved this problem because of the much wider collimator available for gamma camera imaging.

In the first study (Method 1) the radio-active colloid was injected into subcutaneous tissues over the anterior compartment of the lower limb and its arrival at the groin observed for 30 minutes using continuous data storage. Visual interpretation of the 1 hour images was able to diagnose lymphoedema in the

majority of cases (sensitivity 89%) but was unable to differentiate between lymphatic and venous causes of oedema in 7 out of 46 cases (specificity 62.5%).

Measurement of the rate of arrival of the colloid in the ilio-inguinal lymph nodes over the first 30 minutes showed that the colloid arrived at a statistically faster rate in normal limbs and limbs with venous oedema when compared to the slower lymph flow in lymphoedematous limbs, There was no difference, however, in the rate of arrival of the colloid between the normal limbs and the limbs with venous oedema and some of the limbs with venous oedema had a very slow lymph flow similar to that found in the lymphoedematous limbs.

This may be explained because the site of injection was in subcutaneous tissues which potentially could be the site of lymphatic abnormalities. Firstly, it is well recognised that the changes of lipodermatosclerosis in venous diseases produces fibrosis of the subcutaneous tissues which may therefore lead to poor local diffusion of the isotope and delay local clearance of the colloid (Browse and Burnand, 1982). Secondly, Bollinger, Isenring and Franzeck, (1982) have suggested that there is a micro-lymphangiopathy in the subcutaneous tissues of the calf in patients who have chronic venous hypertension. The combination of these two factors, or either one of them alone, may restrict the local diffusion of the isotope and delay its clearance from the injection site.

By contrast, the tissues in the interdigital space are rarely abnormal in patients with chronic venous disease and visual interpretation of the images obtained 30 minutes after

injections of the radio-active colloid in this site (Method 2) gave a diagnostic sensitivity of 95% and a specificity of 100%, providing a much more accurate method for diagnosing lymphoedema and differentiating between lymphatic, venous and other causes of lower limb swelling.

Calculation of the percentage uptake of activity in the ilio-inguinal lymph nodes following an 'inter-digital space injection clearly showed that lymphoedematous limbs had a persistently lower uptake of the colloid throughout the 3 hour study compared to normal limbs, limbs with venous oedema and limbs with miscellaneous causes of oedema.

Method 2, therefore, was a much more accurate technique for differentiating between lymphatic and other cases of chronic lower limb swelling. Thus, I will concentrate in the remainder of the discussion on the results of radionuclide imaging of the lymphatic system following an inter-digital space injection in varying disease states.

Simple visual interpretation of the images at 30 minutes following an interdigital space injection clearly differentiates between lymphoedematous and other limbs but does not provide any physiological information about lymph flow in a particular limb or group of limbs. Estimation of lymph flow by calculation of the percentage uptake of the radionuclide has, however, shown differences in the flow of lymph from the periphery in the groups studied, and has shown that there is a markedly reduced lymph flow in limbs with lymphoedema.

The degree to which lymph flow was reduced in the lymphoedematous limbs correlated with the clinical

degree of severity of lymphoedema. In all the limbs with severe lymphoedema the uptake of the colloid in the ilio-inguinal lymph nodes was minimal to zero. Nine limbs with moderate oedema and 4 limbs with mild oedema showed no uptake of the colloid by 3 hours. In contrast, one limb with moderate oedema had a study which was visually interpreted as normal although the percentage uptake at 30 minutes was 0.3%. These results suggest that the most severe lymphoedemas have a marked degree of impairment of lymph flow although the colloid clearance from the periphery may also be severely diminished even when the degree of lymphoedema is mild.

There is some evidence that some lymphographic abnormalities, such as hypoplasia, may subsequently lead to clinical lymphoedema (Kinmonth 1982, Fyfe et al. 1982). Five of the seven clinically normal limbs with lymphangiographic abnormalities had a reduced uptake of colloid (0.3% or less at 30 minutes) suggesting that there was an impaired lymph flow in these limbs in spite of the absence of clinical swelling at the time of the study. The images of three of the seven limbs were interpreted as abnormal. Thus, both the visual interpretation and the calculation of the uptake can detect a hidden lymphatic deficiency.

Over the past 3 decades, various tehnical advances in X-Ray lymphography have found feet lymphatics in a greater proportion of patients (Table 1) and the proportion in the category of "hypoplasias" has been expanded mainly at the expense of the "aplasias".

Four out of the eight studies in which no lymphatics could be

found in fact to cannulate , showed activity in the ilio-inguinal lymph nodes by 2 or 3 hours. This provides further evidence that failure to find lymphatics for cannulation in the foot is due to technical problems or quirks of filling related to the site and nature of the injection during lymphography.

In contrast, there were several studies (16) in which no activity was seen in the ilio-inguinal region by 3 hours. However, inguinal lymph nodes were visualised following infusion of lipiodol in a foot lymphatic. The explanation for this is unclear but may be that infusion of lipiodol which is carried out at an unphysiological rate, allowed the contrast to reach the groin; whereas, lymph flow was so severely dimminished that the colloid did not reach the groin's by 3 hours. Eight of these limbs had proximal lymphatic abnormalities.

Further work needs to be carried out in this group of limbs, but, initial radionuclide studies performed since the completion of this study have shown the presence of radioactivity in the calf tissues; suggesting that the distal lymphatics are present but that the flow of lymph more proximally is severely impaired.

In the second part of the study the 55 limbs with lymphoedema were subdivided by radiological appearance: those with distal lymphatic abnormalities (hypoplasia)and those with proximal lymphatic and lymph node abnormalities either with distal lymphatic distension or obliteration. Comparison of these two groups showed that the uptake of the colloid in the ilio-inguinal lymph nodes in patients with proximal lymphatic abnormalities was nil to minimal and very much less than the

patients with who had distal hypoplasias.

This provides further evidence that these two differing radiological appearances may form distinct and separate groups, the limbs with distal lymphatic abnormalities being the milder form of the disease. It also gives some support to the hypothesis that there are at least 2 different disease processes known by the umbrella diagnosis of primary lymphoedema: one in which there is progressive obliteration of the distal lymphatics and the other in which the disease process starts in the lymphatics/lymph nodes in the ilio-inguinal region producing distal lymnphatic abnormalities with the radiological appearance of either distal lymphatic distension or obliteration (Browse and Stewart, 1985). The findings in the second group in particular, challenge the original concept of primary lymphoedema as a genetic defect and suggest that this group may have an acquired cause for their lymphoedema.

Two of the five limbs with the lymphographic features of proximal obstruction and distal lymphatic distension showed no uptake of the colloid by 3 hours but the other threeshowed a small amount of uptake at 3 hours (0.06, 0.06 and 0.6%). The radionuclide studies of these patients suggest that lymph flow is severely diminished in this group but lymphographically there are lymphatics present in the lower limb. The presence of a small amount of activity in three of the studies suggest that this type of lymphoedema may be detected by imaging the ilio-inguinal lymph nodes and the upper thigh at times later than three hours.

The importance of this particular group is that they may be amenable to a by-pass procedure such as the mesenteric bridge

operation to by-pass the block in the lymphatics in the iliac region (Hurst, Kinmonth and Rutt, 1978). This is a small percentage of the patients with lymphoedema but it is thought at present that this is the only group of patients with lymphoedema in whom a physiological procedure may be successful.

In 1861 Ludwig and Tomsa observed that testicular lymphatics became grossly dilated almost immediately after ligation of the pampiniform veins and there is now considerable animal experimental evidence that acute venous obstruction in a limb will cause an increase in lymph flow (Field and Drinker, 1931; White et al. 1933; Szabo, 1963). In man, chronic venous obstruction secondary to portal hypertension has been shown to increase lymph flow in the thoracic duct (Dumont and Mulholland, 1960) and radio-active clearance studies (Taylor et al., 1957, Hollander et al., 1961) suggest that the same phenomenon exists in chronic venous obstruction of the lower limb caused by venous disease. The results presented in Method 2 have shown a consistently higher uptake of the colloid in the limbs with venous disease when compared to the control limbs. These findings provide further evidence that there is an increased flow of lymph in the limbs with venous oedema.

It is often difficult to differentiate between mild lymphoedema and other obscure causes of the swollen limb such as idiopathic/ cyclical oedema or lipodystrophy. The clinician may be guided by the clinical features of the particular type of oedema but further investigation of the venous and/or lymphatic systems may be necessary. The results presented in Chapter 5 show that radionuclide imaging can clearly differentiate mild

lymphoedema from these other causes.

The majority of the limbs with miscellaneous oedema (10) were due to idiopathic/cyclical oedema, a poorly understood entity. Several theories have been suggested to explain this condition but its exact cause remains unknown. There is no venous or lymphatic defect in this group of limbs. These limbs, however, appeared to have a slightly increased flow of lymph when compared to normal limbs. It has been shown that there is a positive correlation between increased filtration of fluid and lymph flow (Szabo et al. 1963); and it may be that the defect in this group of limbs is at the capillary level.

This technique has clearly differentiated between lymphatic and other causes of lower limb oedema such as venous oedemas. There were no false positives using the interdigital space technique although these images of 3 patients with lymphoedema were interpreted as normal, ie 3 false negatives. Calculation of the percentage uptake in these 3 studies at 30 minutes was 0.2%, 0.3% and 0.3% and showed these uptakes to be below the range for the control limbs of 0.46% to 3.1%. The limits of uptake at 30 minutes compatible with the diagnosis has been defined as less than 0.3% (ie the mean percentage uptake at 30 minutes in the 55 limbs with lymphoedema plus 3 standard deviations), and all 3 of these studies would, therefore, be classified on the border-line of abnormality.

Thus, a simple web-space injection followed by a 300 second image 30 minutes later provides an accurate diagnostic assessment of the lymphatic system usually capable of visual interpretation but occasionally needing the calculation

of the percentage uptake of the colloid in the lymph nodes. The calculated uptake and 1, 2 or 3 hour studies may prove useful in assessing the flow of lymph in a particular limb before and after treatment.

Previous radio-nuclide techniques have suffered from two main drawbacks namely the lack of a suitable radio colloid and lack of both anatomical definition and functional information about the lymphatic system. The introduction of the newer technetium labelled micro colloids has to a great extent solved the first. The technique described in Method 2 in this thesis, however, has shown that by calculating the percentage uptake of the injected activity, a quantitative measurement of the lymph flow in the particular limb can be made, providing functional information about the lymphatics in chronic lower limb swelling which has hitherto not been available.

Previous radionuclide investigators of the lymphatic system have used the terms lymphoscintigraphy, radionuclide imaging and clearance studies to describe these techniques. The technique described in Method 2 does not really fall into any of these categories. It provides visualization of the lymph nodes as in lymphoscintigraphy, but the calculation of the percentage uptake provides a measure of the flow of lymph in that particularlimb and the terminology previously mentioned seems inappropriate. It is probably best, at present, to term this technique isotope lymphography as it provides not only a measure of the anatomical presence of lymph nodes, when the colloid is able to reach the lymph nodes but also a quantitative assessment of lymph flow in that particular limb.

The results presented in this study together with its great merit of simplicity suggest that this technique is sufficiently accurate to be used as the first investigation of choice of the lymphatic system to confirm for example the clinical diagnosis of lymphoedema. X-Ray lymphography can therefore be reserved for those patients with lymphoedema who are being considered for direct lymphatic surgery and in whom a high degree of anatomical information is required.

CHAPTER 8

CONCLUSIONS

Conclusions

1. Comparison of method 1 and method 2 has shown that radionuclide lymph node imaging (isotope lymphography) following an interdigital space injection (Method 2) is a considerably more accurate method of diagnosing lymphoedema and differentiating it from other forms of lower limb oedema.

2. Visual assessment of images obtained 30 minutes after an interdigital space injection can accurately diagnose lymphoedema and differentiate it from normal limbs, limbs with venous oedema and limbs with other causes of lower limb swelling.

Calculation of the percentage uptake of the radioactivity in the ilio-inguinal lymph nodes has shown that:

4. Limbs with lymphoedema have a markedly reduced lymph flow.

5. The degreee to which lymph flow is reduced in the lymphoedematous limbs correlated with the degree of severity. The most severe lymphoedemas had the most marked degree of impairment of lymph flow although the colloid clearance from the periphery may also be severely diminished in a minority of the limbs with mild lymphoedema.

6. Five of the clinically normal limbs with lymphangiographic abnormalities showed an impaired lymph flow in spite of the absence of clinical swelling, showing that this technique can detect hidden lymphatic deficiencies.

7. Limbs with venous oedema had an consistently higher uptake of the colloid when compared to normal limbs providing evidence that there is an increased flow of limbs with chronic venous oedema.

8. Limbs with miscellaneous oedemas have showed a consistently higher uptake than normal limbs. This difference however did not reach statistical significance.

9. Comparison of isotope lymphography with X-ray lymphography has shown that the limbs with distal lymphatic abnormalities have a greater lymph flow than those with proximal lymphatic abnormalities.

10. Isotope lymphography is almost non invasive technically simple to perform and is diagnostic. In contrast to X-Ray lymphography, however, it provides poor anatomical definition of lymphatics and lymph nodes.

11. Isotope lymphography is sufficiently accurate to be used as the first line investigation of the lymphatic system in chronic limb oedema.

Life can only be understood backward

but it must be lived forward.

Kierkegaard.

APPENDICES

APPENDIX I

The percentage of free Technetium in the injected sample compared to the percentage of free Technetium in blood and urine in eighteen subjects.

Study No.	Percentage of free Technetium		
	Injected Sample	Blood	Urine
1	1-2	Trace	3.4
2	5	Trace	17
3	5	Trace	8.9
4	5	Trace	13
5	1	Trace	6
6	3.8	Trace	10
7	11	Trace	13
8	1.8	Trace	6
9	3.2	Trace	13
10	4.5	Trace	18
11	1	Trace	7
12	3	Trace	6
13	4.5	3%	17
14	5	Trace	4
15	7	3%	7
16	1.5	Trace	3.4
17	5	5.3%	9.3
18	3%	Trace	2.9

APPENDIX II

The percentage uptake of 99m Tc RSC in the ilio-inguinal lymph nodes in 12 control limbs.

No. of Study	Percentage uptake of 99m Tc RSC			
	$^1/_2$hr	1hr	2hr	3hr
1	1.1	1.5	2.7	4.6
2	1.37	2.3	3.05	4.89
3	0.46	1.03	2.29	3.30
4	1.0	3.2	6.7	16.4
5	1.5	3.7	6.7	21.4
6	1.6	3.8	6.1	13.6
7	3.0	4.5	7.5	13.8
8	2.3	4.2	7.5	11.1
9	1.3	2.5	6.0	7.7
10	1.2	3.74	6.1	8.1
11	0.49	1.9	3.7	6.7
12	1.5	2.4	5.4	6.9
Mean	1.4	2.90	5.25	9.87
S.D	0.7	1.12	1.76	5.47
S.E	0.2	0.32	0.51	1.58

APPENDIX III

The percentage uptake of 99m Tc RSC in the ilio-inguinal lymph nodes in 20 limbs with mild lymphoedema.

No. of Study	Percentage uptake of 99m Tc RSC			
	$^1/_2$ hr	1 hr	2 hr	3 hr
1	0.0	0.0	0.34	0.48
2	0.003	0.06	0.22	0.44
3	0.008	0.12	0.24	0.96
4	0.08	0.14	1.1	2.8
5	0.2	0.5	1.9	2.7
6	0.04	0.05	0.07	0.15
7	0.2	0.28	0.6	1.0
8	0.07	0.18	0.3	0.3
9	0.2	0.9	3.0	3.4
10	0.0	0	0	0
11	0.2	0.3	0.54	0.96
12	0.0	0	0	0
13	0.1	0.4	0.62	0.94
14	0.06	0.5	1.6	3.1
15	0.04	0.2	0.6	0.9
16	0.03	0.1	0.24	0.36
17	0.0	0	0	0
18	0.2	1.1	2.4	4.2
19	0.3	1.4	2.5	3.2
20	0	0	0	0
Mean	0.087	0.312	0.814	1.295
S.D.	0.094	0.397	0.944	1.374
S.E.	0.021	0.089	0.211	0.307

APPENDIX IV

The percentage uptake of 99m Tc RSC in the ilio-inguinal lymph nodes in 22 limbs with moderate lymphoedema.

No. of Study	Percentage uptake of 99m Tc RSC			
	1/2 hr	1 hr	2 hr	3 hr
1	0.08	0.05	0.18	0.48
2	0.06	0.2	2.06	4.0
3	0.08	0.15	0.4	0.56
4	0	0	0	0
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
8	0.13	0.14	0.27	0.43
9	0.11	0.35	0.17	0.2
10	0.06	0.65	2.7	4.5
11	0	0	0	0
12	0.02	0.02	0.10	0.4
13	0	0	0	0
14	0.17	0.3	0.4	0.41
15	0.3	0.9	1.3	2.2
16	0.05	0.05	0.1	0.2
17	0.02	0.04	0.09	0.16
18	0.01	0.03	0.05	0.06
19	0	0	0	0
20	0.08	0.9	1.7	2.4
Mean :	0.053	0.172	0.433	0.727
SD :	0.014	0.0284	0.77	1.16
SE :	0.018	0.06	0.16	0.25

APPENDIX V

The percentage uptake of 99m Tc RSC in the ilio - inguinal lymph nodes in 13 limbs with severe lymphoedema.

No of Study	Percentage of uptake of 99m Tc			
	$^1/_2$ hr	1 hr	2 hr	3 hr
1	0	0	0	0
2	0	0	0	0
3	0	0	0	0.6
4	0	0	0	0.1
5	0	0	0	0
6	0	0	0	0
8	0	0	0	0
9	0.4	0.08	0.3	0.43
10	0	0	0	0.06
11	0	0	0	0
12	0	0	0	0
13	0	0	0.02	0.05
Mean	0.003	0.006	0.025	0.095
S.D	0.01	0.22	0.083	0.083
S.E.	0.003	0.006	0.023	0.053

APPENDIX VI

The mean standard deviation and standard error of the percentage uptake of activity in the ilio-inguinal lymph nodes in all 55 limbs with primary lymhoedema.

Time	1/2 hr	1 hr	2 hr	3 hr
Mean	0.0535	0.103	0.475	0.779
S.D.	0.079	0.317	0.798	1.250
S.E.	0.011	0.043	0.107	0.169

APPENDIX VII

The percentage uptake of activity of 99m Tc RSC in the ilio-inguinal lymph nodes in 25 clinically and radiologically normal limbs.

No. of Study	Percentage uptake of 99m Tc RSC			
	1/2 hr	1 hr	2 hr	3 hr
1	1.1	1.5	2.7	4.6
2	2.9	5.36	8.91	13.9
3	2.5	4.1	6.7	12.6
4	0.79	1.45	4.19	7.0
5	1.42	2.1	3.5	4.7
6	0.3	1.0	3.1	5.4
7	1.4	4.6	10.3	15.4
8	1.7	2.75	3.9	4.5
9	2.3	4.2	7.5	11.1
10	2.5	3.9	5.7	7.3
11	0.5	1.6	3.4	5.2
12	2.6	4.9	9.6	12.8
13	1.5	2.4	5.4	6.9
14	2.4	8.8	15.8	21.0
15	1.6	3.6	6.9	7.6
16	0.32	0.68	1.3	1.73
17	2.8	3.9	6.9	7.8
18	1.1	2.2	7.8	9.8
19	1.8	5.0	7.1	9.8
20	0.9	2.0	3.6	5.1
21	0.6	1.1	2.5	4.1
22	0.14	2.7	4.1	6.3
23	0.5	1.2	3.1	4.2
24	1.8	4.2	6.8	10.2
25	3.4	5.6	7.9	10.9
Mean	1.56	3.23	7.06	8.39
S.D.	0.93	1.9	5.88	4.38
S.E.	0.19	0.38	1.18	0.88

APPENDIX VIII

The percentage uptake of activity of 99m Tc RSC in the ilio-inguinal lymph nodes in 40 limbs with distal obliteration of the lymphatics.

No. of Study	Percentage of activity			
	1/2 hr	1 hr	2 hr	3 hr
1	0	0	0	0
2	0.08	0.05	0.18	0.2
3	0.08	0.15	0.4	0.56
4	0.03	0.06	0.22	0.4
5	0	0	0	0
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0
9	0.008	0.12	0.24	0.96
10	0	0	0	0
11	0.08	0.14	1.1	2.8
12	0	0	0	0
13	0.06	0.65	2.7	4.5
14	0.04	0.08	0.3	0.43
15	0.2	0.5	1.9	2.7
16	0.02	0.02	0.1	0.4
17	0.04	0.05	0.07	0.15
18	0	0	0	0
19	0.17	0.3	0.4	0.41
20	0.2	0.28	0.6	1.0
21	0.3	0.9	1.3	2.2
22	0.05	0.05	0.1	0.2
23	0.02	0.04	0.09	0.16
24	0.01	0.03	0.05	0.06
25	0.07	0.18	0.3	0.3
26	0.2	0.9	3.0	3.4
27	0	0	0	0
28	0.2	0.3	0.54	0.96
29	0	0	0	0
30	0.1	0.4	0.62	0.94
31	0.06	0.5	1.6	3.1
32	0.03	0.1	0.24	0.36
33	0.04	0.2	0.6	0.9
34	0	0	0	0
35	0.08	0.9	1.7	2.4
36	0	0	0	0
37	0.2	1.1	2.4	4.2
38	0.3	1.4	2.5	3.2
39	0	0	0	0
40	0	0	0	0
Mean	0.067	0.24	0.59	0.936
S.D	0.086	0.354	0.85	1.30
S.E.	0.014	0.056	0.14	0.20

APPENDIX IX

The percentage uptake of 99mTc RSC in the ilio-inguinal lymph nodes in 10 limbs with Proximal and distal hypoplasia and 5 limbs with Proximal obstructive hypoplasia and distal lymphatic distension.

	Percentage uptake of 99m Tc RSC			
	$1/_2$ hr	1 hr	2 hr	3 hr
Proximal and distal hypoplasia				
1	0	0	0	0
2	0	0	0	0
3	0.06	0.2	2.06	4.0
4	0.13	0.14	0.27	0.43
5	0.11	0.35	0.17	0.20
6	0	0	0	0
7	0	0	0	0
8	0	0	0	0
9	0	0	0	0
10	0	0	0.02	0.05
Proximal obstructive hypoplasia				
1	0	0	0	0.6
2	0	0	0	0.06
3	0	0	0	0
4	0	0	0	0
5	0	0	0	0.06
Mean	0.02	0.046	0.168	0.36
S.D.	0.043	0.104	0.53	1.023
S.E	0.01	0.027	0.14	0.26

APPENDIX X

The percentage uptake of 99m Tc RSC in the ilio-inguinal lymph nodes in 12 limbs with venous oedema

No. of Study		Percentage uptake of 99m Tc RSC			
		$^1/_2$ hr	1 hr	2 hr	3 hr
1		2.5	4.0	9.4	14
2		3.9	7.4	16.5	24
3		2.0	3.4	7.2	11
4		1.9	4.0	8.2	14.4
5		1.2	3.74	6.1	8.1
6		2.4	8.8	15.8	21.0
7		1.94	3.24	9.6	14.9
8		3.18	6.09	14.2	21.4
9		1.89	3.2	11.3	16.8
10		4.6	11.4	21.4	28.0
11		1.25	3.1	5.8	8.3
12		0.46	1.9	36	5.8
	Mean	2.27	5.02	10.76	15.64
	S.D.	1.165	2.83	5.25	6.87
	S.E.	0.34	0.82	1.52	1.98

APPENDIX XI

The percentage uptake of 99m Tc RSC in the ilio-inguinal lymph nodes in 15 limbs with miscellaneous oedemas.

No. of Studies		Percentage uptake of 99m Tc RSC			
		$1/_2$ hr	1 hr	2 hr	3 hr
1		1.37	2.3	3.85	4.89
2		0.46	1.03	2.29	3.3
3		1.6	4.1	6.1	13.6
4		3.0	5.2	7.5	13.8
5		3.5	8.2	18.5	26.9
6		4.7	9.6	22.4	27.0
7		1.7	5.0	8.2	14.1
8		2.2	6.4	9.6	16.9
9		0.9	1.3	2.4	3.8
10		0.7	1.3	2.9	4.1
11		1.2	2.9	5.6	8.0
12		1.74	2.5	4.5	6.2
13		1.9	4.0	7.5	11.2
14		2.98	4.68	8.5	13.8
15		2.49	3.80	7.8	12.6
	Mean	2.03	4.15	7.84	12.01
	S.D.	1.147	2.49	5.67	7.52
	S.E.	0.296	0.64	1.46	1.94

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