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Particle characterization, experimental studies, clinical applications and dosimetric considerations

Lennart Bergqvist

Lund 1987

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Abstract

Different techniques for the characterization of radioactive colloids, used in nuclear medicine, have been evaluated and compared. Several radioactive colloids have been characterized in vitro and in vivo and tested experimentally. Colloid biokinetics following interstitial or intravenous injection were evaluated with a scintillation camera technique. Lymphoscintigraphy with a Tc-99m-labelled antimony sulphur colloid was performed in 32 patients with malignant melanoma in order to evaluate the technique. Based on the biokinetic results, absorbed doses in tissues and organs were calculated. The function of the reticuloendothelial system has been evaluated in rats after inoculation with tumour cells.

Microfiltration and photon correlation spectroscopy were found to be suitable in determining activity-size and particle size distributions, respectively. Maximal lymph node uptake following subcutaneous injection was found to correspond to a colloid particle size between 10 and 50 nm. Lymphoscintigraphy was found to be useful in the study of lymphatic drainage from the primary tumour site in patients with malignant melanoma on the trunk. Quantitative analysis of ilio-inguinal lymph node uptake in patients with malignant melanoma on the lower extremities was, however, found to be of no value for the detection of metastatic disease in lymph nodes. High absorbed doses may be received in lymph nodes (up to 1 mGy/MBq) and at the injection site (about 10 mGy/MBq). In an experimental study it was found that the relative colloid uptake in bone marrow and spleen depended on the total number of intravenously injected particles. This may considerably affect the absorbed dose in these organs.

Key words colloids, compartments, CRE, dosimetry, filtration, lymphatics, lymphoscintigraphy, malignant melanoma, phagocytosis, radioactive, radio-labelled, radiopharmaceutical, RES, scintigraphy, Tc-99m

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CONTENTS

1. INTRODUCTION..... 1
2. AIMS OF THE PRESENT WORK..... 4

3. RADIO-LABELLED COLLOIDS..... 5
   3.1. Types of colloids..... 5
   3.2. Physical, chemical and radiochemical parameters..... 5
   3.3. Characterization techniques..... 5
      3.3.1. Number of particles..... 5
      3.3.2. Stability..... 6
      3.3.3. Particle size..... 6
   3.4. Characterization of colloids in vitro..... 10
   3.5. Characterization of colloids in vivo..... 10

4. STUDIES OF THE LYMPHATIC SYSTEM..... 12
   4.1. The lymphatic system..... 12
   4.2. Experimental studies..... 14
   4.3. Clinical applications..... 16

5. STUDIES OF THE RETICULOENDOTHELIAL SYSTEM..... 21
   5.1. The reticuloendothelial system..... 21
   5.2. Experimental studies..... 23
      5.2.1. Colloid properties influencing phagocytosis.... 23
      5.2.2. Biological factors influencing phagocytosis.... 27
   5.3. Clinical applications..... 29

6. DOSIMETRY OF INJECTED RADIOACTIVE COLLOIDS..... 31
   6.1. Interstitial injection..... 31
   6.2. Intravenous injection..... 35

7. FUTURE INVESTIGATIONS..... 36
8. SUMMARY..... 37
9. ACKNOWLEDGEMENTS..... 39
10. REFERENCES..... 40

Appendix: Papers I - V

1. INTRODUCTION

Colloidal particle preparations have been used for more than 60 years for studies of the reticuloendothelial (RE) function. Thorium dioxide (1) and colloidal carbon (2) were found to be suitable for vascular clearance studies in the 1940's. These colloids were succeeded by different radioactively labelled (radio-labelled) colloids in the 1950's (3). Colloidal ^{198}Au was widely used for imaging the reticuloendothelial system (RES) with scintillation detectors during the 1960's (4). $^{99}Tc^{m}$-labelled colloids were introduced in 1964 (5) and gradually replaced colloidal ^{198}Au for diagnostic purposes. $^{99}Tc^{m}$-labelled colloids are nowadays frequently used in most nuclear medicine centres since the radionuclide $^{99}Tc^{m}$ gives comparatively low absorbed doses to the patients and staff, has an optimal photon energy for scintillation camera imaging, and is easily available from ^{99}Mo-$^{99}Tc^{m}$-generators. By using a scintillation camera, or solid-state detector, connected to a computer it is now possible to carry out dynamic studies and thus evaluate the RE function in a non-invasive way.

Radio-labelled colloids are used in many diagnostic applications in nuclear medicine, such as the investigation of the RES (liver, spleen, bone marrow and lymph nodes), gastro-intestinal examinations, catheter controls, and shunt controls. Radio-labelled colloids are also used for therapy, e.g. ^{90}Y-citrate and ^{90}Y-silicate colloids for the treatment of pleural and peritoneal carcinosis (6) and intracavitary treatment of knees (7), and colloidal ^{198}Au for intrathecal treatment of leukaemia (8).

Particle classification is mainly based on the particle size. Particles in the submicrometre size range (10-1000 nm) are called nanoparticles whereas larger particles are called microparticles or microspheres. The term "colloid particle" is, in nuclear medicine, often used for both nanoparticles and small microparticles (less than a few micrometres). By definition, colloid particles in a suspension are small enough not to form a sediment, but large enough to scatter incoming light.

The distribution in the body of administered particles depends strongly on the particle size and the mode of delivery. After interstitial or intraperitoneal injection, particles in the size range of a few nanometres up to about 100 nm may enter the lymphatic capillaries and be transported with lymph to lymph nodes where phagocytosis may occur (9). After intravenous or intra-arterial injection, particles in the size range of about 5 nm to 2 μm will be rapidly cleared from the blood stream by macrophages of the RES. Particles larger than 7 μm will be mechanically entrapped in the lung capillaries (10). Particles between about 2 and 7 μm may pass the smallest lung vessels and be entrapped in the capillary network of the liver and spleen (10). Figure 1 shows schematically the biological behaviour after intravascular injection of particles with different size ranges.

Quality control of radio-labelled colloids is essential in many clinical applications. Furthermore, a good knowledge of the physical and chemical properties of different colloids may ease the search for optimal colloids, and possibly provide explanations of differences in biological behaviour between colloids. New techniques for colloid particle characterization and further improvements of older techniques are appearing constantly and should be evaluated. Comparatively few articles have, however, been published in these fields.

It is important to make dosimetric calculations for administered radio-labelled colloids in order to estimate the probability of inducing somatic or hereditary effects from irradiation. Tissue necrosis may also occur at the injection site following an interstitial injection. Most dosimetric calculations are based on studies of tissue uptake of colloids. The colloid distribution between different types of cells and the subcellular distribution has only been studied by a few investigators (11-13).

In the future, colloids may also be used in new imaging techniques, such as nuclear magnetic resonance (NMR) and magnetometry.

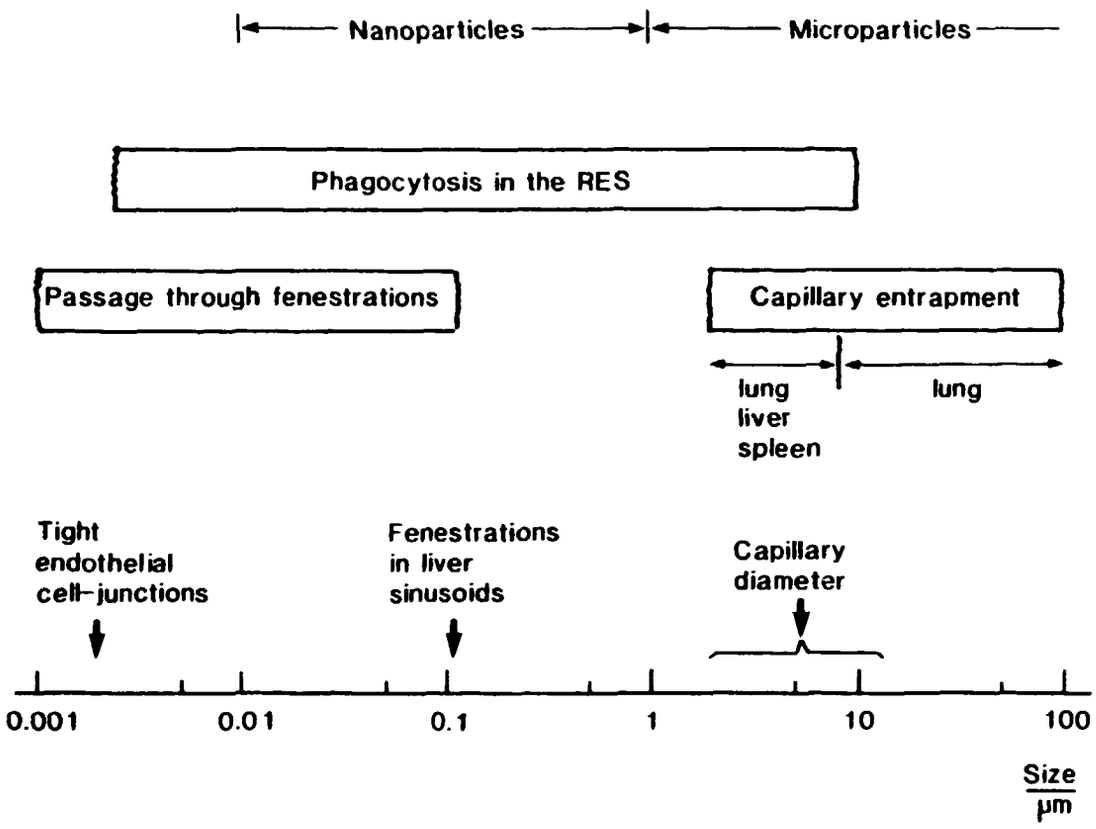

FIGURE 1. Size dependence for particle localization after intravascular injection. The size intervals for nano- and microparticles are also indicated.

2. AIMS OF THE PRESENT WORK

The aims of this work were to evaluate techniques for particle characterization, to increase the knowledge of colloid characteristics *in vitro* and *in vivo*, to study the kinetics of colloids after interstitial and intravenous injection, and to perform dosimetric calculations based on biokinetics. Consequently, the following will be presented in this thesis:

a) the evaluation of four techniques for the characterization of colloids

b) investigations of the effect on colloid particle size after contact with plasma proteins

c) data and kinetics of colloids used for studies of the RES

d) data and kinetics of colloids used for studies of the lymphatic system

e) dosimetric calculations after interstitial injection of colloids

It is of great interest to find simple and non-invasive diagnostic methods for functional studies of the reticuloendothelial and lymphatic systems. By using radio-labelled colloids we - colleagues at the Departments of Radiation Physics and Surgery, Lund, - wished to elucidate the following:

f) can functional studies of the RES be used to demonstrate tumour growth?

g) can lymphoscintigraphy be used for detection of metastatic disease in lymph nodes in patients with malignant melanoma?

3. RADIO-LABELLED COLLOIDS

3.1. Types of Colloids

In the development of radio-labelled colloids emphasis has often been placed on rapidity and efficiency of labelling, stability, and reasonable particle size (4). Considerations of physical and chemical characteristics of the colloids have thus been less stressed.

The radio-labelled colloids used at present can be divided in two categories: a) inert colloids, such as $^{99}Tc^{m}$-labelled sulphur, antimony sulphide, stannous sulphur, and rhenium sulphur colloids, and b) biodegradable colloids, such as $^{99}Tc^{m}$-labelled microaggregated albumin colloid and liposomes.

3.2. Physical, Chemical and Radiochemical Parameters

The characterization of radio-labelled colloids includes several physical, chemical and radiochemical parameters. Physical parameters are particle size and activity-size distributions, particle shape, particle concentration and stability. Chemical parameters comprise chemical composition and purity, surface charge, pH and osmolarity. The radiochemical purity (fraction of radioactivity in the desired chemical composition) and the analysis of different impurities are included in radiochemical characterization.

3.3. Characterization Techniques

Several physical and chemical parameters are considered to affect the biodistribution of administered colloids, such as particle size, shape, number of particles injected, stability, particle surface charge and surface characteristics (9,14).

3.3.1. Number of Particles

The number of particles in a preparation may be determined with several methods. The number of particles with diameters above 0.2 μm can be determined with autoradiography or phase-contrast

microscopy, if the sample is placed on a haemocytometer grid (15). For smaller particles, transmission electron microscopy (TEM) may be used (15). It is also possible to calculate the number of particles if the amount of particle-forming material, the particle density, and the particle sizes are known (15,16).

3.3.2. Stability

Since the particle size is of great importance for the biological behaviour, the preparation must be stable until the time of administration. The long-term particle size stability for colloids has been checked with gel filtration (16), photon correlation (PC) spectroscopy (16,17) and microfiltration (17). The radiochemical stability can be checked with, e.g., paper chromatography, thin-layer chromatography, high-pressure liquid chromatography (HPLC), gel filtration and electrophoresis.

3.3.3. Particle Size

The particle size of the colloid is a very important factor for the distribution after intravenous or interstitial injection (9,14,16). Since batch-to-batch differences in particle size sometimes occur for colloid preparations, routine quality control of radio-labelled colloids is mainly focused on the particle size together with checking the radiochemical purity. When sizing radio-labelled colloids, either the particle-size or the activity-size distribution is obtained. Several sizing techniques and their applications are given in Table 1.

All sizing techniques have their advantages and disadvantages. Ideally, a sizing technique should be accurate, fast, cheap, and cover a large size interval. The techniques listed in Table 1 will be presented in more detail below.

TABLE 1
Techniques for Sizing Radio-Labelled Colloids

Technique	Size interval	Measured characteristic	
		Particle-size distribution	Activity-size distribution
Electrophoresis	Molecules -		Y
Gel filtration	Molecules - 100 nm		Y
TEM	0.2 nm -	Y	
SEM	10 nm -	Y	
PC spectroscopy	3-3000 nm	Y	
Ultrafiltration	3-18 nm		Y
Microfiltration	10 nm - 12 µm		Y
Ultracentrifugation	0.1 µm -		Y
Microscopy	0.2 µm -	Y	
Coulter® counter	0.4 - 800 µm	Y	

Electrophoresis: Colloid particles carrying charge may be separated by electrophoresis, e.g. in sucrose density gradients. In 1977, Lim et al. (18) developed an elaborate technique to measure the size and charge distributions of colloid particles by combining electrophoresis and laser light scattering measurements.

Gel Filtration: Colloid preparations may be eluted through a chromatographic bed in a column in order to separate particles of different sizes. In 1978, Persson et al. (19) suggested a filtration technique for colloids where the colloid sample is applied at the top of the column and eluted with 10 ml of isotonic saline. The column is then sealed and scanned with a slit-collimated NaI(Tl) detector. The obtained scanning profile gives qualitative information on the size distribution below 100 nm and also indicates the presence of radio-labelled impurities. We have found the technique to be well-suited for routine quality control of colloids and long-term stability tests (16). In 1979, Billinghurst and Jette (20) demonstrated a technique for determining the activity-size distribution of colloids (<100 nm) by fraction collection from calibrated gel columns.

Transmission Electron Microscopy (TEM): An electron microscope, equipped with X-ray fluorescence apparatus and an image-analyser system, can be used to determine the size distribution, particle shape, particle concentration, and chemical composition. Before analysis, the colloid sample is spotted onto or nebulized on a plastic-coated grid and allowed to dry or partially dry. Unfortunately, the particles may change or sublime due to the vacuum in the microscope and the heat of the electron beam. Furthermore, it is difficult to analyse preparations containing stabilizers and contaminants (21). However, after the evaluation of several sizing techniques, Warbick et al. (21) found TEM to be their sizing-method of choice.

Scanning Electron Microscopy (SEM): As for TEM, SEM can also be used to determine the size distribution, particle shape, concentration, and chemical composition. The colloid sample is usually spread on a polycarbonate filter and allowed to dry (16). Low-Z particles are then coated with a thin layer of e.g. a gold-palladium alloy, by sputtering, in order to increase the image contrast. The layer also prevents heat effects, but may in turn prevent close studies of the surface structure of small particles. With a freeze-fracture technique it is possible to eliminate the risk of volatilization of particles (20).

Photon Correlation Spectroscopy: Pedersen and Kristensen (17) and Bergqvist et al. (16) evaluated the size and stability of ten and six colloids, respectively, with photon correlation spectroscopy (Coulter® Nano-Sizer). This technique involves the illumination of a particle solution with a laser. The light scattered at 90^{o} is detected in a photomultiplier. As the particles move and diffuse in the solution due to Brownian motion, the scattered light will give rise to a diffraction pattern. The rate at which this intensity pattern changes is inversely proportional to the particle size. A computer is used to calculate the average particle size and a polydispersity index, which is an indication of the width of the size distribution. In 1985, a new instrument was introduced (Coulter® Model N4) which can analyse several peaks simultaneously and graphically display the particle-size distributions (22). I have found this instrument to be well-suited for sizing and stability-testing of radio-labelled colloids (unpublished data).

Ultrafiltration: Ultrafiltration is a pressure-driven process which is based on liquid flow through a membrane (23). The technique separates particles and molecules according to the molecular weight cut-off (MWCO) value of the membrane. A membrane thus retains most particles above its retention rating and allows most smaller particles, along with the solvent, to flow through it. Some percentage of the retained particles can, however, adsorb on the surface of the membrane and create a "gel layer" which in turn may have a higher retention than the membrane itself. For a $^{99}Tc^{m}$-antimony sulphide colloid, at least 1 ml pre-filtration of the colloid was needed to establish the "gel-layer" before consistent results could be obtained (own unpublished data).

Microfiltration: In 1974, Davis et al. (24) introduced microfiltration with polycarbonate membrane filters of well-defined pore sizes. The technique has unfortunately been misnamed "ultrafiltration" by several investigators (16,20,21). The frequently used Nuclepore® polycarbonate membranes are available with 17 different pore sizes, ranging from 0.01 µm to 12 µm. A sample of colloid is passed through a membrane (held in a filter holder) followed by a 2 ml distilled water rinse. The fraction of activity passing the filter can then be determined by radioactivity measurements on the filter and filtrate in, e.g., a radioisotope calibrator.

Ultracentrifugation: Warbick et al. (21) have described an ultracentrifugation technique for sizing colloids. A small amount of colloid was layered on a sucrose gradient which was then spun in an ultracentrifuge in order to separate the gradient into fractions. The fractions were then measured for activity. The technique demands careful calibration of the apparatus. Its use for sizing colloids is, however, limited due to several serious drawbacks (21).

Microscopy: Phase contrast microscopy or light microscopy may be used for sizing larger colloid particles. It is a fast and simple technique and it gives a rough estimate of the particle size of these types of colloids.

Coulter® Counter: In a Coulter® counter, particles suspended in an electrolyte are made to pass through a small aperture, across which an electric current flows. Each particle displaces electrolyte in the aperture and thus produces a pulse proportional to its displaced volume. Each pulse is counted and sized in order to obtain the size distribution.

3.4. Characterization of Colloids In Vitro

Few articles concerning the physico-chemical properties of different colloids have been published. This is unfortunate, as a good knowledge of the physico-chemical properties will facilitate the search for optimal radio-labelled colloids as well as possibly providing explanations of differences in biological behaviour between colloids.

Most investigators have focused on the activity-size distribution, particle-size distribution, and/or stability of the colloid preparation (15-21,24). Only a few investigators have evaluated and compared different techniques for particle characterization *in vitro* (15-17,20,21).

An interesting aspect of colloid properties is the formation mechanism. It is generally believed that the radioactivity associated with a colloid particle is proportional to the volume or the surface area of the particle (20). Steigman et al. (25) have demonstrated, in a gelatin-stabilized $^{99}Tc^{m}$-sulphur colloid, that the sulphur distribution followed the technetium distribution for different particle size intervals, which indicates a homogeneous colloid formation. Frier et al. (15) have, however, found indications that in another gelatin-stabilized $^{99}Tc^{m}$-sulphur colloid, the technetium was localized in the central part of the particles.

3.5. Characterization of Colloids In Vivo

When characterizing radio-labelled colloids for biokinetic studies, it is also important to know what happens to the particles *in vivo* before making contact with fixed macrophages of the RES. Very few studies have, however, been carried out in this field and much still remains to be done.

It has been found in some studies that particles may increase or decrease in size after incubation in serum. An increase in particle size may be due to the fact that some materials require a coating of specific serum proteins (opsonins) before they are recognized by the macrophages (26). For $^{99}Tc^{m}$-sulphur colloids, it has been documented that deficiencies in certain opsonins will affect the rate of phagocytosis in, e.g., patients with carcinoma (26). Another reason for increased particle size may be rapid aggregation of particles prior to clearance. This was found by Dornfest et al. (26) when incubating a gelatin-stabilized $^{99}Tc^{m}$-sulphur colloid in normal rat serum (the median activity-size, as obtained by microfiltration, increased from 0.17 μm to 0.22 μm). A decrease in particle size was, however, observed by Frier et al. (15) for another gelatin-stabilized $^{99}Tc^{m}$-sulphur colloid when incubated in normal rat serum. By incorporating ^{35}S in the colloid, they were able to show that it was the sulphur that dissolved from the surface. In a study by Bergqvist et al. (27), the median activity-size of a gelatin-stabilized $^{99}Tc^{m}$-sulphur colloid increased by about 45% in three cases out of ten after incubation in normal rat plasma. In this study, the influence of plasma incubation on different particle concentrations of the colloid was also investigated by microfiltration. No distinct differences were observed.

In the study by Dornfest et al. (26), inconsistent effects on particle size of the $^{99}Tc^{m}$-sulphur colloid were observed when the colloid was incubated in sera from rats with leukaemia. In half of the tests there was a significant increase, whereas in the other half there was a significant decrease in particle size. These findings indicate that alterations in opsonins may play an important role in regulating the particle size. It is well known that quantitative and qualitative changes in serum proteins may occur in connection with shock, septicaemia, trauma and malignancy (26). In these conditions a disturbed RE function could be expected and is therefore interesting to monitor. It is thus important to bear in mind that biokinetic studies may be affected by alterations in serum proteins.

4. STUDIES OF THE LYMPHATIC SYSTEM

4.1. The Lymphatic System

The lymphatic system has been comprehensively reviewed by Yoffey and Courtice (28). The short description below is based on their review.

Large proteins and lymphocytes are known to pass from plasma to tissue fluid. The main function of the lymphatic system is to return these components to the blood system. Furthermore, large molecular complexes and particles that enter the tissue fluid will generally also be taken up by the fine network of lymphatic capillaries that is present in most tissues in the body. As shown in Figure 2, the molecules and particles are transported with the lymph, which is part of the extracellular fluid, to larger afferent vessels (collecting ducts). The lymph passes through one or more lymph nodes and is further transported, via the efferent vessels, to the great veins at the base of the neck.

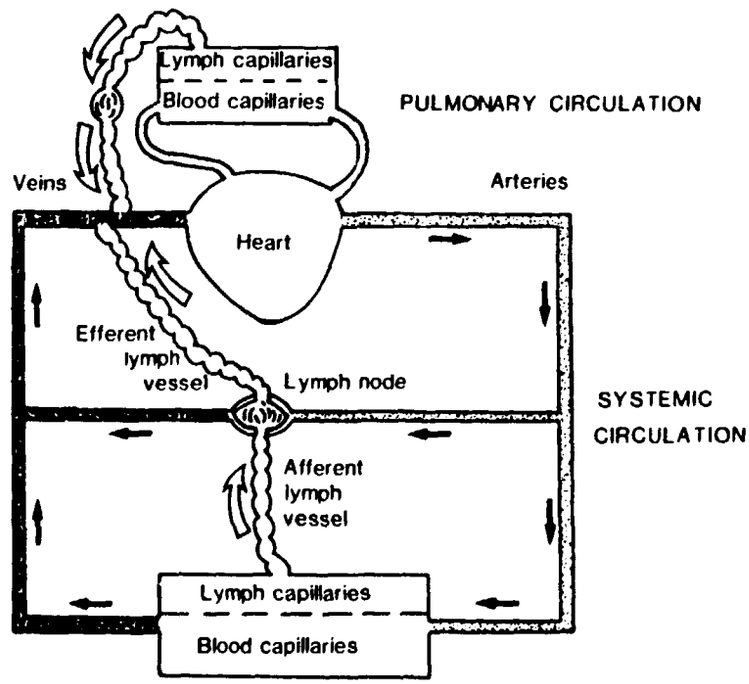

FIGURE 2. Schematic presentation of lymph and blood circulation in man. (Redrawn from Yoffey and Courtice (28).)

The ultrastructure of lymphatic capillaries resembles that of the blood capillaries. The capillaries are lined with thin-walled endothelial cells. The endothelial cell junctions have been found

to be open in some tissues and closed in others. The junctions are probably continuously changing depending on the local circumstances at the time.

The thicker-walled collecting ducts consist of an inner endothelial layer, with few open junctions, covered by a connective tissue sheath in which elastic and muscular cells are scattered. The muscle cells enable the vessels to contract and relax rhythmically for the propulsion of the lymph.

Lymph entering a lymph node flows through the cortical and medullary sinuses. The sinuses are either open channels or are traversed by a reticulum, consisting of cells which can both mechanically trap particles and act actively by phagocytosis. Phagocytic cells also line the sinus walls and are scattered throughout the lymphoid tissue.

Besides the rhythmical contractions and relaxations of the smooth muscle cells in the capillary walls, several other factors may also influence the propulsion of the lymph. Muscular activity will increase the lymph pressure thus increasing the lymph flow. Respiratory movement has a considerable effect on lymph propulsion from the abdominal and thoracic cavities. Slight movement, such as massage, also stimulates lymph propulsion. Anaesthesia may affect the flow of lymph and the factors responsible for lymph propulsion. Anaesthesia often depresses the lymph flow but the magnitude of depression can vary considerably, depending on the anaesthetic used.

Obstruction of the lymphatic flow in a region results in lymphoedema which will persist until the regeneration of new vessels has been re-established. In most surgical procedures acute lymphoedema is a common occurrence, but often passes unnoticed because of the relatively fast re-establishment of lymph flow. Chronic lymphoedema may arise without any known reason, or may be secondary to some known cause, such as malignant disease or surgical removal of lymph nodes.

The lymphatic system plays an important role in the dissemination of tumours. Malignant cells may enter the lymph stream and be captured in the closest lymph node and produce secondary growths. The whole lymph node chain may be invaded after some time, although some lymph nodes may be by-passed. Tumour cells may thereafter reach the veins and be deposited in lungs or other organs.

4.2. Experimental Studies

The behaviour of colloids injected interstitially has been found to be strongly dependent on the particle size (16,29-34). The number of injected colloid particles has also been reported to influence the rate of outflow from the injection site (35) and the phagocytosis in lymph nodes (36).

The particle size dependence on the migration rate at the injection site and the rate of uptake in lymph nodes has been thoroughly investigated in animal studies for different radiopharmaceuticals at the Department of Radiation Physics in Lund (16,30,37). The experiments were carried out in anaesthetized rabbits that were fixed in supine position under a scintillation camera. A bilateral subcutaneous (s.c.) injection of colloid just below the xiphoid process was performed in each experiment. Sequential images were taken during the initial 1-2 hours and static images after about 4-5 hours.

The optimal colloid particle size for lymphatic studies has been found to be about 5 nm (30). Colloidal ^{198}Au has this particle size and was used clinically for many years to study the lymphatic system. Unfortunately, tissue necrosis at the injection site was sometimes observed due to the high absorbed dose from electrons (38). Furthermore, the photon energies of ^{198}Au are unsuitable for scintillation camera measurements. Strand and Persson (30) found that a $^{99}Tc^{m}$-labelled antimony sulphide colloid ($^{99}Tc^{m}Sb_2S_3$) with a particle size range of 3-30 nm was better suited for lymphoscintigraphy. This colloid has been the most widely employed in the last decade for clinical applications (39).

Figure 3, a summary of our results, shows the uptake in the parasternal lymph nodes two hours after injection of different radiopharmaceuticals. The size ranges are also given for most of the particle preparations. Some of the colloids with median particle sizes around 40 nm showed large differences in uptake in lymph nodes. This might be explained by differences in physico-chemical factors of the colloids which may influence the uptake (see chapter 5.2.). Furthermore, only a few experiments were carried out for several of the colloids. The results,

however, indicate that there is a relation between particle size and activity uptake. Molecules and particles smaller than a few nanometres will probably leak into the blood capillaries. Larger particles, up to about 100 nm, will be preferably absorbed into the lymph capillaries and phagocytized in the lymph nodes. Still larger particles will, for a long time, be trapped in the interstitial space. The shaded area in Figure 3 shows a maximal lymph node uptake for colloids with particle sizes between about 10 and 50 nm.

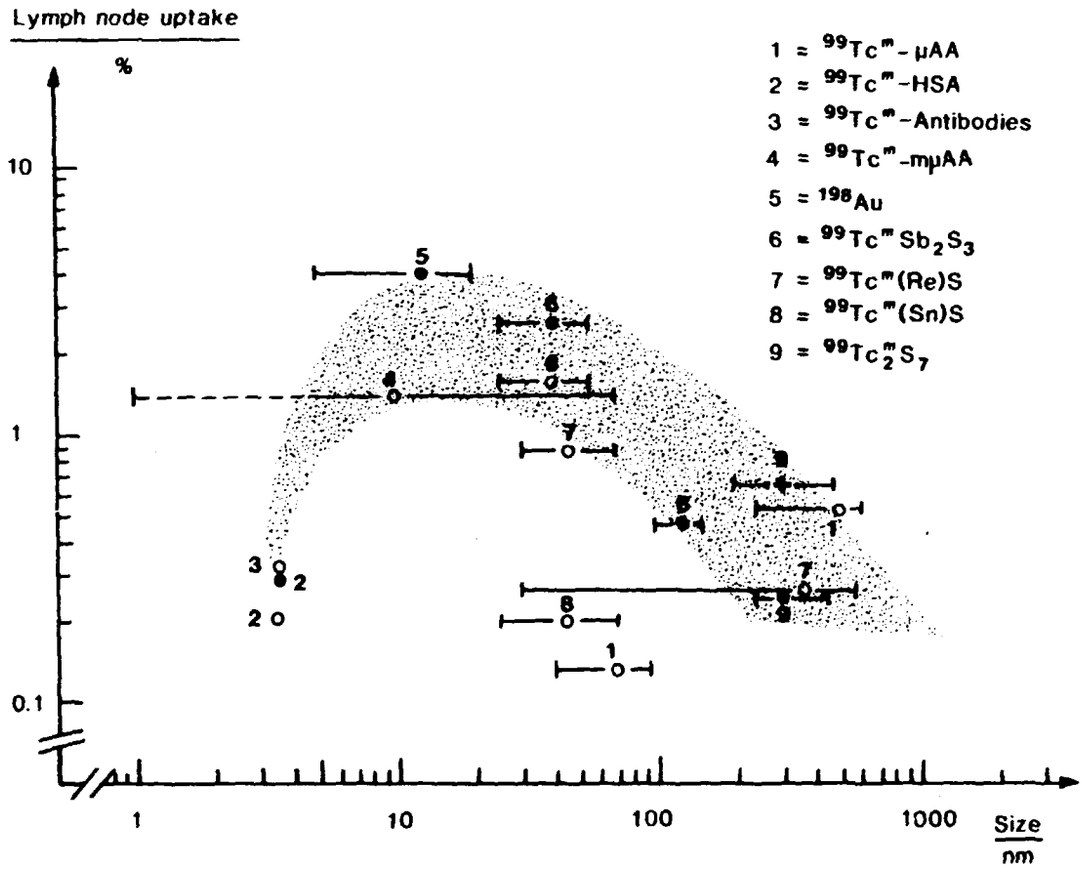

FIGURE 3. Parasternal lymph node uptake of radiopharmaceuticals at two hours after s.c. bilateral injection near the xiphoid process in rabbits. The horizontal lines indicate the size ranges of the particles. The symbols o, • and +, placed at the median particle size, represent results from references 16, 30 and 37, respectively.

4.3. Clinical Applications

The lymphatic system in man has been much less studied in comparison to the vascular system. It is, however, important to study the lymphatic system since it is considered to be very important in the spread of cancer. Furthermore, lymphoedema is a not uncommon complication after breast surgery in patients with cancer. A technique for the evaluation of the lymphatic function in these patients is of utmost interest. The morphology of the lymphatic system may be studied with lymphography, computerized tomography (CT), ultrasound, NMR and lymphoscintigraphy (40-43). Lymphoscintigraphy is the only technique which may, in addition, provide information on the lymphatic function since kinetic, physiologic and quantitative studies can be performed.

Radio-labelled colloids for the evaluation of the lymphatic system were first reported by Sherman and Ter-Pogossian in 1950 (44). They demonstrated the migration of colloidal ^{198}Au to regional lymph nodes after interstitial injection. They also found that the colloid migrated to lymph nodes containing cancer. These findings initiated studies on whether or not colloidal ^{198}Au could have therapeutic effects on lymph node metastases. Several investigators found, however, that not all cancer-bearing lymph nodes exhibited colloid uptake (45-47). Furthermore, using autoradiography they showed that the radioactivity (the colloid particles) was restricted to the residual normal tissues in the cancer-invaded lymph nodes. Similar results were obtained by Sullivan et al. (48) and Bergqvist et al. (49) when using $^{99}Tc^{m}Sb_2S_3$.

During the 1950's and 1960's, methods for morphological and functional studies were developed for axillary and parasternal lymph nodes (45,50), cervical lymph nodes (51), and ilio-inguinal and para-aortic lymph nodes (38,52). More thorough evaluations of the potential of lymphoscintigraphy have been carried out during the 1970's and 1980's.

In general, the colloid is injected interstitially either at the most distal anatomic site at the origin of the lymphatics under study (53), or close to the site of the primary lesion (54).

Usually the injected activity of $^{99}Tc^{m}$-labelled colloids is between 5 and 100 MBq in a volume of 0.1 to 1.0 ml. Sequential scintillation camera images can be registered during the initial 1-2 hours to study the migration rate of the colloid. Scintigrams after about 2-4 hours are generally obtained for quantitative and morphological studies of the lymph nodes. Muscular motion, massage over the injection site, and administration of local anaesthesia are sometimes carried out in order to improve the migration rate of colloid from the injection site (38,49,55).

A large number of radiopharmaceuticals have been used in man for lymphoscintigraphy. Most of these are listed in Table 2.

TABLE 2

Radiopharmaceuticals Used for Lymphoscintigraphy

Radiopharmaceutical	Investigators (reference No.)
^{198}Au colloid	38,52,56-58
^{197}Hg-sulphide colloid	59
$^{99}Tc^{m}$-sulphur colloid	29,55,58,60
$^{99}Tc^{m}$-antimony sulphide colloid	48,54,61-72
$^{99}Tc^{m}$-hydrogen sulphide colloid	39
$^{99}Tc^{m}$-rhenium sulphur colloid	73
$^{99}Tc^{m}$-stannous sulphur colloid	74-77
$^{99}Tc^{m}$-labelled liposomes	78
$^{99}Tc^{m}$-labelled serum albumin	50,79-81
$^{99}Tc^{m}$-stannous phytate	31,32
$^{99}Tc^{m}$-labelled dextran	82
^{67}Ga-citrate	83
^{131}I-labelled antibodies	84,85
^{111}In-labelled antibodies	86,87

A large number of potential clinical applications for lymphoscintigraphy have been tested throughout the years. A summary of the results is given in Table 3.

TABLE 3

The Reported Values of Lymphoscintigraphy in Clinical Practice

Application	Type of disease	Injection site(s)	Reported value x)	Investigators (reference No.)
Staging	Breast cancer	Bilateral subcostal	***	61
	-"-	Periareolar	*-***	62,63,65,78,81
	-"-	Periareolar and interdigital	**	88
	Malignant melanoma	Dorsum of both feet	*	49
	Cervical cancer	Labium majus	*	75
	-"-	Bilateral mastoid process	***	82
	Pelvic neoplasms	Perianal	**-***	42,76,89
	-"-	Dorsum of both feet	***	38,56
	Lymphoma	Bilateral subcostal	***	61
Before lymph-adoenectomy	Malignant melanoma	Peritumoural	***	48,54,55,57,66,70,72
	Oesophageal cancer	Submucosal layer	***	73
During lymph-adoenectomy	Breast cancer	Periareolar	***	90
	Cervical cancer	Between toes of both feet	***	69
After lymph-adoenectomy	Breast cancer	Bilateral subcostal and hands	***	91
	Malignant melanoma	Peritumoural	*	49,58
Follow-up	Breast cancer	Bilateral subcostal	***	92
Radiotherapy planning	Breast cancer	Bilateral subcostal	***	61,64,68,74,93
lymphatic function studies	Oedema	Dorsum of both feet	***	60,67,94
	-"-	Web spaces of fingers or toes	***	71,95,96
	Lymph transplantation	Web spaces of fingers or toes	***	77

x) *** = valuable, ** = possible value, * = no value, according to the investigators.

Staging of Cancer: Several studies have been performed in order to establish lymphoscintigraphy as a staging procedure, i.e. determining the presence or absence of regional lymph node metastases. Ege has performed internal mammary lymphoscintigraphy using bilateral subcostal injections in more than one thousand patients and found this technique to be of great value (97). Axillary lymphoscintigraphy with periareolar subcutaneous injections to demonstrate axillary lymph node metastases has been performed in a rather limited number of patient studies. Most authors found this technique either to be of possible value (62,78,88) or of no value (63,65). Mazzeo et al. (81), however, found the technique to be highly reliable when using colloidal albumin. Iliopelvic lymphoscintigraphy in staging genito-urinary cancers with bilateral perianal injections has been reported to be promising by some authors (42,89) and valuable by others (76). Ilio-inguinal lymphoscintigraphy with bilateral dorsopedal s.c. injections in patients with malignant melanoma on the lower extremities has been found by Bergqvist et al. (49) to be of no value for the demonstration of lymph node metastases.

Surgical Guidance and Follow-up: Frequent reports have been made on the great value of lymphoscintigraphy in patients with malignant melanoma. Subcutaneous injections close to the primary tumour site can demonstrate the route of lymphatic drainage from the tumour, as shown in Figure 4. Lymph nodes at risk for metastatic disease may thus also be identified. This information is important for the follow-up of patients and when regional lymph node dissections are planned. Intraoperative lymphoscintigraphy has been used during lymphadoenectomy as guidance in localizing regional lymph nodes (69,90). Post-operative lymphoscintigraphy can also be carried out to verify that no residual lymph nodes are present (91).

Radiation Therapy Planning: Internal mammary lymphoscintigraphy when planning radiation therapy for patients with breast cancer has been found to be valuable in establishing the exact location of the lymph nodes and thus optimizing the treatment (64,68,74,93). Bronskill et al. (98) and Siddon et al. (93) have described methods of superimposing the treatment field(s) onto the lymphoscintigram(s).

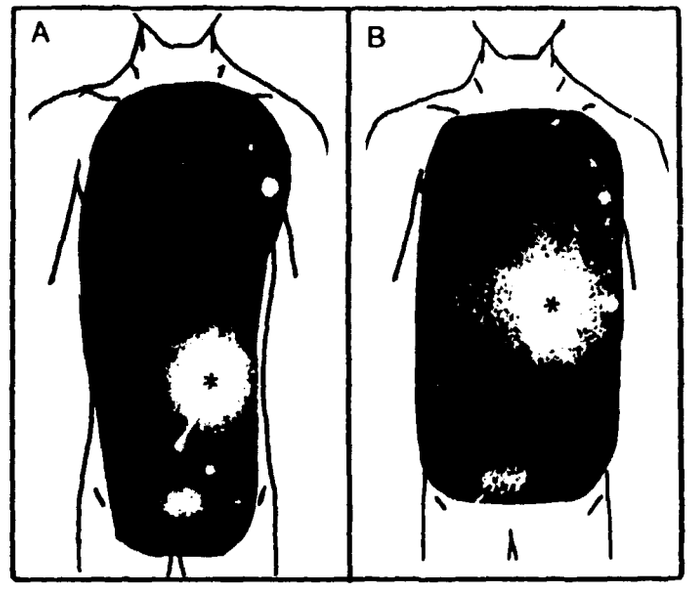

FIGURE 4. Lymphoscintigraphy with peritumoural injections (*) in two patients with malignant melanoma. (A) Injection 6 cm left and 2 cm above the umbilicus demonstrating lymph flow to the left axilla and groin. (B) Injection 6 cm left of the midline of L2 showing lymph flow to the left axilla and flank. (From Bergqvist et al. (49).)

Edema: Several studies have indicated the value of lymphoscintigraphy in determining the aetiology of lymphoedema (60,67,71,94,96) and in selecting patients for microvascular operation (95,96).

In conclusion, many lymphoscintigraphic procedures have been found to be clinically valuable. More studies of the lymphatic system with lymphoscintigraphy are, however, needed in order to further increase our knowledge concerning this complicated system and its function.

5. STUDIES OF THE RETICULOENDOTHELIAL SYSTEM

5.1. The Reticuloendothelial System

The reticuloendothelial system (RES) consists of a population of mononuclear, highly phagocytic cells, called macrophages, that spreads throughout the body (99). The cells are derived from the bone marrow and have membrane receptors. Both stationary (sessile) and wandering macrophages are present in the RES, as shown in Figure 5. Perhaps more appropriate, but less used, names for this system are 'macrophage system', 'mononuclear cell system' or 'histiocyte cell system' (9).

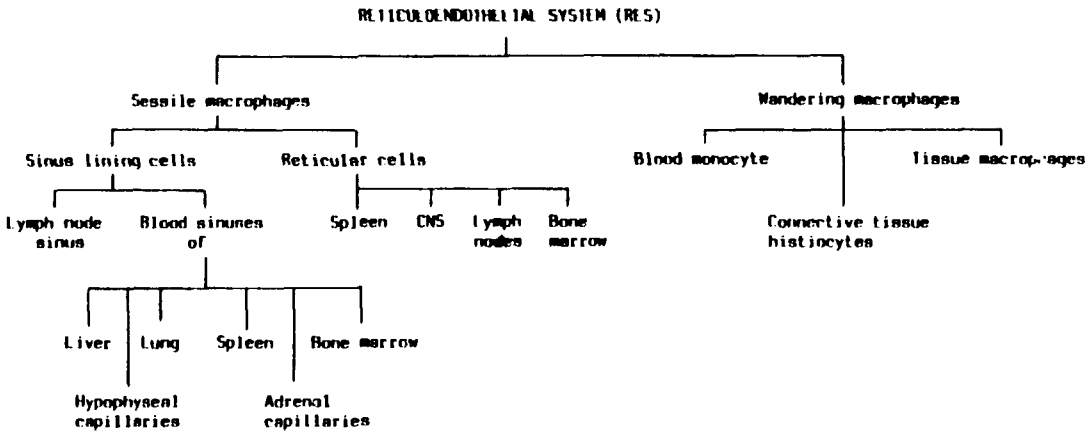

FIGURE 5. Schematic presentation of the RES according to Saba (100). (From Rydén (14).)

The clearance of foreign particulate material from the blood stream is confined to the macrophages that line the vascular channels (99). Macrophages in the body not lining blood channels, e.g. in lymph nodes and lungs, can thus not contribute to blood clearance.

The blood-vessel-lining macrophages in the liver play an essential role in the clearance of particulate matter. They are generally considered to account for about 80-90% of the total phagocytic activity (100). The capillaries in the liver (sinusoids; diameter 3-12 μm) play an important role in the hepatic microcirculation

(101). The capillary walls are formed by endothelial cells and underlying fat-storing cells. Macrophages in the liver, among which are the Kupffer cells, are found on or within the endothelial lining of liver sinusoids and have a high phagocytic and a considerable pinocytic capacity (101). Endothelial cells have a high pinocytic capacity. The endothelial walls have open fenestrations ("windows") with diameters around 0.1 μm (101). Small particles and solutes in the sinusiods can pass the fenestrations and enter a tissue space called the space of Disse. Numerous microvilli are found on the parenchymal cells in the space of Disse.

Red blood cells entering a small sinusoid will rapidly adapt their diameter. When passing through a sinusoid, particles and solutes may enter the space of Disse through the fenestrations by a mechanism called "forced sieving" (101), as shown in Figure 6. White blood cells are less flexible in adapting their size when entering a small sinusoid. This may cause interrupted flow and compressions of the endothelial wall and the space of Disse. The compressed parts will draw particles and solutes into the space of Disse as they return to their original position, as shown in Figure 6. This mechanism is called "endothelial massage" (101).

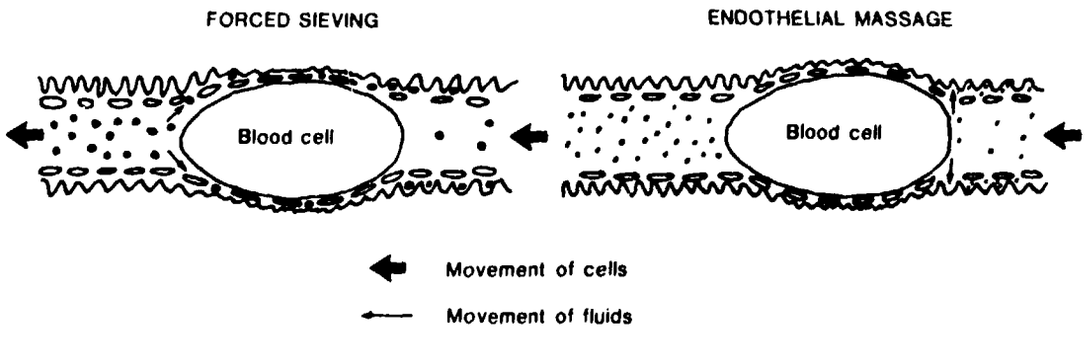

FIGURE 6. Small particles are passed with the fluids through the endothelial fenestrations to the space of Disse by "forced sieving" or "endothelial massage". (Redrawn from Wisse and DeLeeuw (101).)

5.2. Experimental Studies

From a physiological point of view, the RES can be characterized by its phagocytic behaviour. This has led to the development of numerous techniques using different types of colloids for the measurement of the RE function. After intravenous injection, most types of colloids are rapidly cleared from the blood stream by the macrophages. The kinetics of radio-labelled colloids can be quantitatively measured with a scintillation camera, equipped with an on-line computer system.

In our experiments, the hepatic and extra-hepatic clearance rates of an injected $^{99}Tc^{m}$-labelled sulphur colloid ($^{99}Tc^{m}_{2}S_{7}$) were evaluated with a two-compartment model, as described by Palmer et al. (102). The median particle size of the colloid was about 300 nm and was prepared according to the procedure described by Persson and Naversten (103).

Many factors which may control the rate of phagocytosis and colloid distribution in organs have been investigated during the last four decades. Some factors are determined by colloid properties while other factors are related to the state of the investigated subject. This will be discussed below.

5.2.1. Colloid Properties Influencing Phagocytosis

The biokinetics of an intravenously injected colloid are affected by a number of physico-chemical properties of the colloid itself, such as particle size, number of particles injected, surface charge, and antigenic properties (9,104). Furthermore, the presence of carrier, stabilizer and competing substances in the preparation may also affect the extraction efficiency (9). Figure 7 demonstrates the considerable influence colloid properties may have on colloid uptake rate in the liver.

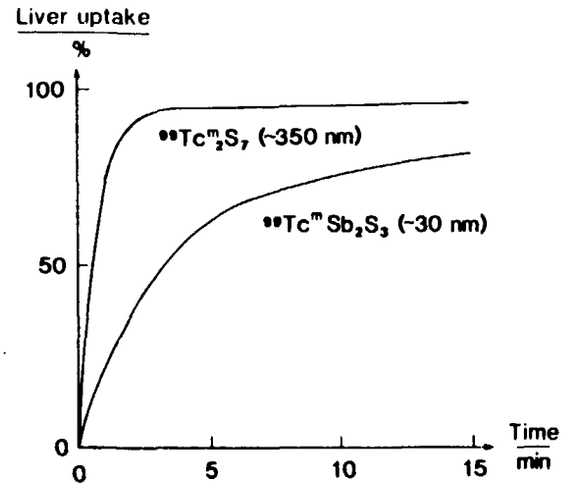

FIGURE 7. Uptake rate in the liver of rats following i.v. injection of two inert $^{99}Tc^{m}$-labelled colloids with different median particle sizes.

Particle Size: The particle size of injected colloid particles is considered to influence the clearance rate from the blood and the colloid distribution between organs (9,14,105). Small particles will generally be cleared more slowly and accumulate to a larger extent in the bone marrow in comparison with larger particles (9), as shown in Figure 8A. The results in Figure 8A may, however, also be influenced by differences in the number of injected particles. No studies of the RE function have, to our knowledge, been carried out where the particle size was the only variable.

The extraction mechanism of colloid particles is still not fully understood, but may partly depend on the particle size. Autoradiographic studies with large $^{99}Tc^{m}$-sulphur colloids (diameter >100 nm) performed by Chaudhuri et al. (106) and George et al. (11) indicate an exclusive uptake in the liver by the Kupffer cells. The latter study showed that extracted colloid particles were attached in groups to Kupffer cell membranes, but without any evidence of endocytosis (3 hours after injection). For a smaller-diameter $^{99}Tc^{m}$-sulphur colloid (about 40 nm), Schell-Frederick et al. (107) found indications of active ingestion of the colloid into the macrophages, i.e. endocytosis.

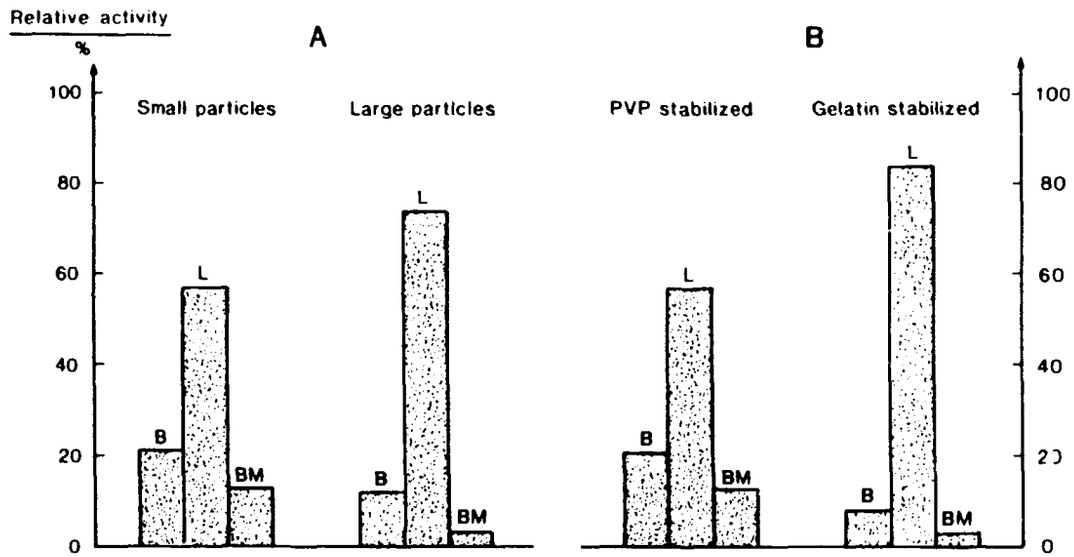

FIGURE 8. Colloid distribution in rats 15 minutes after i.v. injection of $^{99}Tc^{m}Sb_2S_3$. B = blood, L = liver and BM = bone marrow. A: Two colloid particle preparations with particle sizes <10 nm and 200-400 nm. B: The smaller-diameter colloid stabilized with PVP and gelatin, respectively. (Data from Frier (9).)

Number of Particles: The colloid distribution and uptake rate in RE organs will also be affected by the number of particles injected. During the 1950's, some investigators reported that very small amounts of colloid particles were taken up almost completely by the liver during their first passage through the organ (2,3). Blood clearance of colloids would, in such cases, mainly be a measure of the liver blood flow. Biozzi et al. (2) found a decreasing liver uptake and an increasing extra-hepatic uptake with increasing amounts of injected colloid particles. The authors pointed out the importance of a "critical colloid dose" for studies of RE function. Above the "critical colloid dose", the rate of blood clearance of colloid would be more of a measure of total phagocytic capacity.

The concept of the "critical colloid dose" has recently been challenged by some authors (108,109,110). We have found that the relative liver and spleen uptake of $^{99}Tc^{m}{}_{2}S_{7}$ was constant for varying numbers of injected particles between 10^{7} and 10^{10} (27). Saturation of the liver and spleen was not reached, but the blood elimination was significantly reduced after the injection of 10^{9}-10^{10} particles. A corresponding increase in relative lung uptake was also noted at these particle numbers. The relative bone marrow uptake decreased significantly with increasing number of injected colloid particles in our study. Atkins et al. (104), however, demonstrated hepatic saturation and increased relative bone marrow uptake with large amounts of particles of a small-diameter $^{99}Tc^{m}$-sulphur colloid. The results above indicate that bone marrow might be saturated by large colloid particles, such as $^{99}Tc^{m}{}_{2}S_{7}$, in an amount not sufficient to produce saturation of the Kupffer cells. Thus, the "critical colloid dose" may differ for the various RE organs.

Surface Characteristics of Particles: If the particles and macrophages have surface charges, either electrostatic repulsion or attraction may occur between them. Wilkins and Myers (111) have reported that negatively charged particles are mainly taken up by the liver while positively charged particles show a significant initial accumulation in the lungs and later an accumulation in the spleen. Arturson et al. (112) have found that phagocytosis is probably mediated by a receptor mechanism that makes it possible for the macrophages to discriminate between different surface characteristics and to estimate the extent of the foreign nature of the particles. Frier (9) has demonstrated that the stabilizing agent may affect the colloid kinetics (Figure 8B). In this study, the surface properties of the PVP stabilizer were probably similar to those of the colloid particles and thus suppressed the RE function.

5.2.2. Biological Factors Influencing Phagocytosis

A number of biological factors may influence the colloid distribution between organs and the rate of phagocytosis, such as blood flow to the various RE organs, plasma levels of opsonins, the presence of specific antibodies, and the presence of RE depression or activation (14,100).

Blood Flow: The blood flow to the RE organs plays an important part in the biokinetics of injected particles. Inadequate portal blood perfusion of the liver sinusoids has been found to affect the phagocytosis of Kupffer cells (113). We have recently studied the hepatic and extra-hepatic RE function in rats with different portosystemic shunts or total liver arterialization (114). Significant differences were found which, however, could not only be explained by differences in blood flow to the RE organs.

Opsonins: Plasma opsonins play a major role in promoting phagocytosis. Saba and Di Luzio (115) demonstrated that inert colloid particles could be phagocytized by Kupffer cells in vitro, even in the absence of plasma factors. Phagocytosis was, however, greatly enhanced by the addition of opsonins. Depression of the RE function has been associated with a decrease in plasma opsonins (115,116) which may occur in, e.g., patients with advanced malignancies.

Macrophage Activation: The macrophages in the body are normally in a resting state and must be activated to be able to perform their various tasks. Macrophages can be activated by, e.g., immunoglobulins, endotoxin, bacteria, and foreign particles (14). The stimulatory effects of zymosan on RE function have been tested in one of our studies (117). In certain diseases, such as infections and malignancies, reticuloendothelial clearance of injected particles has been shown to be increased, a finding probably attributable to macrophage activation (14).

Suppression of RE Function: All of the compounds known to activate macrophages will, if administered in sufficient quantities, suppress RE function. This may also, as mentioned above, occur for colloids. Studies in Lund have shown that intravenous administration of gelatin significantly reduced the total uptake and uptake rate of $^{99}Tc^{m}_{2}S_{7}$ in the liver of experimental rats (118). The same was found for methyl palmitate when administered on two consecutive days before testing the RE function in normal and tumour-inoculated rats (119).

Organ Specific Factors: There are numerous conditions which may affect only parts of the RES. We have noted a compensatory increase of colloid uptake in the extra-hepatic RES in cases of liver disease, biliary obstruction, and after suppression of the RE function (119-122). It thus seems that the extra-hepatic RES has a reserve capacity which may partly compensate for decreased phagocytic capacity of the liver. This emphasizes the importance of a method for measuring the RE function, which allows for discrimination between the various RE organs. The development of scintillation camera techniques using radio-labelled colloids has provided the possibility of doing this.

RE Function in Tumour Growth: We have studied the effects on colloid uptake rate and colloid distribution in rats with inoculated experimental tumours of different sizes in different locations (120). Animals with small liver tumours or subcutaneous tumours showed an increased hepatic and extra-hepatic RE function in comparison with control animals. Animals with larger tumours, however, had a significantly depressed hepatic RE function. These findings are in accordance with earlier observations (123). In our study (120), it was also found that depression of the hepatic RE function at the time of tumour inoculation in the liver resulted in an accelerated growth of the tumour and an increased mortality.

5.3. Clinical Applications

Morphological Studies: Liver-spleen scintigraphy using radioactive colloids has been used clinically for many years to study the morphology of these organs. The method is still widely used, but other diagnostic methods (e.g. computerized tomography, ultrasound and NMR) are gradually replacing scintigraphy in this respect.

Functional Studies: Dynamic scintillation camera imaging following intravenous administration of radio-labelled colloids has been used clinically to study the RE function and the hepatic blood flow.

Dynamic imaging of colloid uptake in liver has been reported to be useful in determining the liver blood flow (124). Several studies have been performed in order to quantitatively assess the relative arterial and venous blood flow through the liver (125-129). This requires fast dynamic imaging of the colloid biokinetics for about 1-2 minutes following a bolus injection.

Studies of the RE function have been carried out using both inert and biodegradable radio-labelled colloids. Depending on the colloid used, sequential images are usually recorded for 15-90 minutes following the injection. Regions of interest may be selected over the liver, spleen and a blood pool for generation of time-activity curves. The colloid kinetics in the liver and extra-hepatic RES can then be quantitied by using a suitable compartment model.

A few investigators have studied the kinetics of biodegradable $^{99}Tc^{m}$-labelled colloidal HSA during a period of 45-90 minutes using a three-compartment model. Munz et al. (130) and Reske et al. (131) found a faster colloid uptake rate in the RE organs in patients with various untreated tumours in comparison with normal healthy adults. The most striking difference was, however, the much slower elimination rate(s) of the radioactivity (i.e. particle biodegradation) from the liver, spleen and/or bone marrow in the patients with tumours.

De Nardo et al. (132) have reported on a method employing an inert radio-labelled colloid (colloidal ^{198}Au). A three-compartment model was used to obtain hepatic, splenic and extra-hepatosplenic clearance rate constants. Promising results were demonstrated for differential diagnosis of liver disease in their limited patient material. Winkler and Skovgård (133) described a method using another small-diameter colloid ($^{99}Tc^{m}Sb_2S_3$) for the determination of hepatic and splenic RE function. The small-diameter colloid was chosen in order to minimize the influence of blood flow on RE colloid uptake. Only a few patients with liver cirrhosis and a small reference group were tested, but the method was considered to be well suited for clinical studies.

In conclusion, clinical studies of RE function using scintillation camera techniques have been rather sparsely reported. This may partly be due to uncertainties as to the proper way of measuring the RE function (e.g. choice of colloid and compartment model). Furthermore, problems may arise in the correct interpretation of the results which may be due to several biological factors that may affect colloid biokinetics. A combination of liver blood flow and RE function measurements, using a suitable scintillation camera technique, may possibly be found to be suitable for diagnosis of, e.g., patients with small liver tumours not readily observed in static scintigraphic images. We have recently initiated such clinical studies.

6. DOSIMETRY OF INJECTED RADIOACTIVE COLLOIDS

It is important to carry out dosimetric calculations in order to estimate the absorbed dose to tissues and organs when administering radiopharmaceuticals to patients. With the concept "effective dose equivalent", dosimetric calculations can also be used to evaluate the probability of inducing somatic effects (mainly malignant diseases) and genetic effects (134,135,136).

6.1. Interstitial Injection

Absorbed Dose and Effective Dose Equivalent: The absorbed doses in tissues and organs of subcutaneously injected $^{99}Tc^{m}Sb_2S_3$, in patients with malignant melanoma, have been calculated (137). In the 22 patients investigated, the maximum absorbed doses in the liver, gonads, whole body and single lymph nodes were found to be 4, 20, 5 and 1000 μGy per MBq injected activity, respectively.

The absorbed dose at the injection site must also be considered when performing interstitial injections. This also applies to interstitial extravasation following an intravenous injection (138). The clearance rate from the injection site of an administered radiopharmaceutical will, as mentioned earlier, strongly depend on the size of the particles or molecules. Colloids with a wide activity-size distribution may have an activity clearance pattern with several exponential components. Inert colloids, such as $^{99}Tc^{m}Sb_2S_3$, usually have a fairly narrow size distribution and should consequently be cleared almost mono-exponentially from the injection site. This has also been verified when studying the biokinetics in humans up to 6 hours, and in experimental animals up to 30 hours after colloid administration (16,37,137,139).

Interstitially injected radiopharmaceuticals will diffuse into the surrounding tissues. In our patient study (137), measurements of eight injection sites indicated that the radioactive tissue volume increased asymptotically with time to reach about 6-12 cm^3 (mean 9 cm^3) 5 hours after injection. The activity distribution at the injection site was estimated with scintillation camera measurements of the FWTM (full width at one tenth maximum) and an assumption

that the shape of the radioactive volume was semi-ellipsoidal (parallel to the skin layer). Bronskill (140) has studied the radioactive tissue volume up to 24 hours after subcostal intramuscular injections of $^{99}Tc^{m}Sb_2S_3$. He reported that the radioactive volume increased on average from about 1.5 cm^3 to 5 cm^3 in the time interval 5–24 hours after injection.

Assuming a mono-exponential outflow of $^{99}Tc^{m}$, the mean absorbed dose at the injection site will depend on the final radioactive tissue volume and the clearance rate, as demonstrated in Figure 9. If the final activity volume is 10 cm^3 and the clearance rate is 2 %/h, the mean absorbed dose at the injection site will be about 10 mGy per MBq $^{99}Tc^{m}$. Figure 9 can also be used for interstitially injected non-colloidal $^{99}Tc^{m}$-labelled pharmaceuticals.

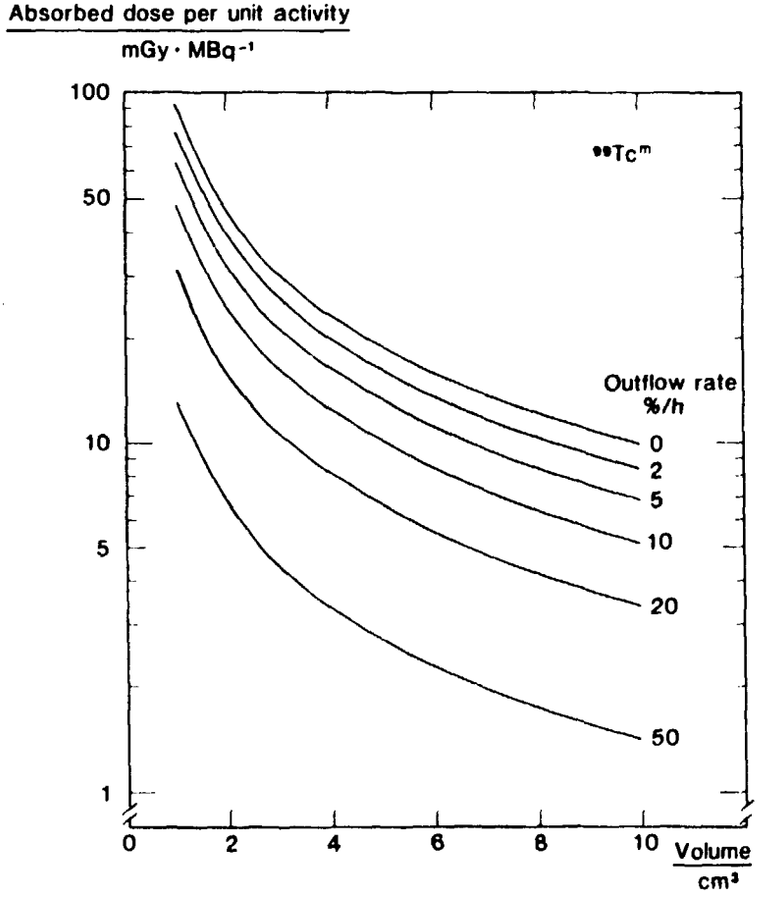

FIGURE 9. Mean absorbed dose per MBq $^{99}Tc^{m}$ at the injection site for different final activity-containing tissue volumes for clearance rates of 0, 2, 5, 10, 20 and 50 %/h, respectively.

A subcutaneous injection of $^{99}Tc^{m}Sb_2S_3$ near the umbilicus will give an effective dose equivalent of about 5 μSv per MBq $^{99}Tc^{m}$, equivalent to 0.2 mSv from an injection of 40 MBq (137).

Cumulative Radiation Effect: It is of particular interest to be able to predict the possible biological effects at the injection site after an interstitially injected radiopharmaceutical. The cumulative radiation effect (CRE) is a concept used in radiotherapy for assessing and comparing the biological effects of fractionated and continuous irradiation (141). Damage to the connective tissue of the skin ("skin tolerance") is likely to occur at a CRE value $\geq$20 and skin necrosis at a CRE value $\geq$27 according to data from Strandqvist (142).

This concept has, to our knowledge, not been used in nuclear medicine. In 1973, Kirk et al. (141) introduced the CRE concept for short-lived radionuclides, used in radiotherapy as implants or as external applicators, and recommended the following formula to be used for calculating the CRE (R_c) in the case of an infinite treatment time:

$$R_c = n \cdot q \cdot r_o \cdot (z/\lambda)^z$$

where n (=0.53) is a normalizing constant, q is the relative biological effectiveness (RBE) of the radionuclide with reference to ^{60}Co, z has the value 0.71, λ is the decay constant (in day^{-1}) of the radionuclide, and r_o is the initial dose rate ($r_o = D \cdot \lambda$; where D is the absorbed dose in Gy).

In 1975, Kirk et al. (143) revised the above formula in order to describe the dependence of the CRE on the volume treated. The normalizing constant n should now be written:

$$n = \mu \cdot \varphi$$

where μ is the new normalizing constant (=0.77) and φ is a volume factor ($\varphi = (V/1000)^{0.16}$; where V is the volume in cm^3).

We have applied this concept to interstitially injected radionuclides under the assumption that the final activity-containing tissue volume is uniformely irradiated. Other

biological factors that will affect the CRE at the injection site include the rate of activity expansion, the final activity volume and the clearance rate of the activity. In Figure 10, CRE values at the injection site are given for different subcutaneously injected radionuclides and final activity volumes. The value of q was set to 1 according to recommendations of the ICRP (134). The radionuclides in the figure have all been used for lymphoscintigraphy (Table 2).

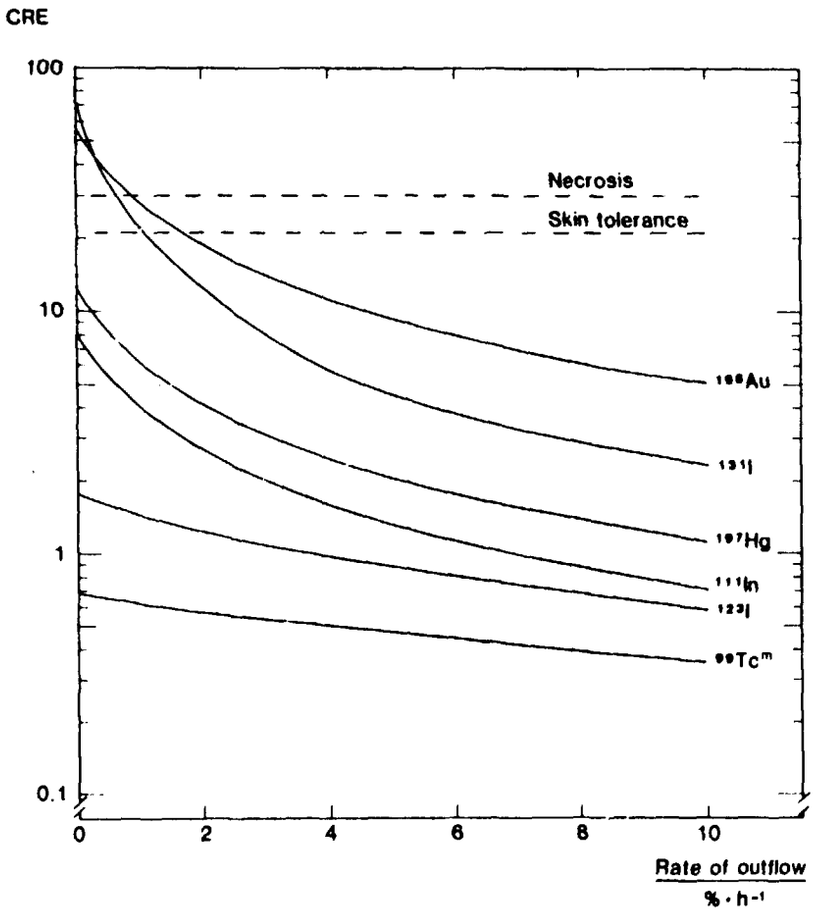

FIGURE 10. The CRE at the injection site following a subcutaneous injection of different radionuclides. The CRE values are approximately correct for the following final activity volume and injected activity combinations: 5 cm^3 and 40 MBq, 10 cm^3 and 65 MBq, and 15 cm^3 and 90 MBq.

6.2. Intravenous Injection

As pointed out in chapters 3 and 5, the colloid distribution in different tissues, following an intravenous injection, may vary considerably due to a number of colloid properties and biological factors. Furthermore, injection of different numbers of particles of a colloid may also affect the distribution (27). While increasing the number of injected colloid particles from 10^7 to 10^{10} in experimental rats, the lung uptake increased threefold, and the bone marrow uptake decreased threefold (27). The number of injected particles may thus highly affect the absorbed doses to these organs and also affect the effective dose equivalent.

The biokinetics of different radio-labelled colloids have been evaluated in several experimental and clinical studies (144-147). Dosimetric calculations have, however, seldom been carried out. Johansson et al. (136) have calculated the effective dose equivalent per unit of administered activity for various radiopharmaceuticals. A mean value of 13 μSv/MBq was obtained for intravenously injected $^{99}Tc^m$-labelled colloids.

7. FUTURE INVESTIGATIONS

A method of testing the reticuloendothelial function is desirable in many clinical situations. Scintillation camera techniques for biokinetic studies of administered radio-labelled colloids have the potential of becoming the method of choice. Clinical studies of the reticuloendothelial function with radioactive colloids have, however, not gained general acceptance. Further studies are needed in order to increase our knowledge of the biokinetics of administered colloids. This demands a thorough characterization *in vitro* and *in vivo* of the colloid particles. In order to find an optimal radiopharmaceutical for studies of the RE function, it is also important to study the partitioning of colloid particles between different types of cells as well as the subcellular distribution. This may, in addition, give valuable data for microdosimetric calculations. Most radionuclides used in nuclear medicine emit a cascade of low-energy electrons, i.e. Auger and Coster-Kronig electrons, when decaying (148). These electrons have a very short range in tissue and may have severe radiotoxic effects, especially if the radionuclides are incorporated into DNA molecules (148).

As shown in this thesis, colloids play an essential role in both research and clinical applications. In the future, radioactive colloids will probably continue to be important for diagnostic purposes in nuclear medicine. Furthermore, radio-labelled colloids may find wider applications for therapeutic purposes. Colloids have also been proposed for use in new diagnostic techniques, such as magnetometry and NMR. Further research in order to increase our knowledge of the physical and biological behaviour of radioactive colloids should therefore be encouraged.

8. SUMMARY

In the present thesis, different techniques for the characterization of radioactive colloids have been evaluated and compared (Paper I). Special attention was focused on techniques for the measurement of the size and activity-size distributions of the colloid particles. Several radioactive colloids have been characterized and tested in experimental animals. Their biokinetics following subcutaneous injection were evaluated with a scintillation camera technique. It was found that maximal lymph node uptake was obtained for colloids with particle sizes between about 10 and 50 nm.

Patients with malignant melanoma were investigated with lymphoscintigraphy using a $^{99}Tc^{m}$-labelled antimony sulphide colloid ($^{99}Tc^{m}Sb_2S_3$) (Paper II). Colloid biokinetics were thoroughly studied in these patients. Dissected regional lymph nodes were measured for activity and examined microscopically. Lymphoscintigraphy in patients with malignant melanoma on the trunk was found to be suitable for identification of the lymphatic drainage from the primary tumour site. This information is important for the follow-up of patients and when regional lymph node dissections are planned. Quantitative lymphoscintigraphy in patients with malignant melanoma on the lower extremities was, however, of no value for the identification of regional lymph node metastases.

Dosimetric calculations have been done on the basis of the biokinetics of $^{99}Tc^{m}Sb_2S_3$ in patients with malignant melanoma (Paper III). High absorbed doses may be received in regional lymph nodes (up to 1 mGy/MBq) and at the injection site (about 10 mGy/MBq). The effective dose equivalent may reach 5 μSv/MBq. Dosimetric comparisons between different radionuclides have also been made.

The reticuloendothelial function in experimental animals with inoculated tumours of different sizes in different locations has been studied with the aid of a compartment model (Paper IV). Animals with small liver tumours or subcutaneous tumours showed an increased hepatic and extra-hepatic RE function in comparison with control animals. Animals with larger tumours, however, had a

significantly depressed hepatic RE function.

The colloid particle size of a $^{99}Tc^{m}$-labelled sulphur colloid has been shown to be affected after contact with plasma proteins (Paper V). In Paper V, it was also demonstrated that the relative uptake in lungs and bone marrow of the sulphur colloid may depend on the number of intravenously injected particles. This may influence the absorbed dose to these organs considerably.

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