THE ASSESSMENT OF MYOCARDIAL PERFUSION USING A NEW SCANNING AGENT

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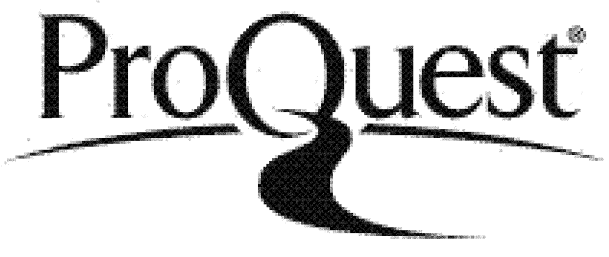

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List of Publications

1. Technetium-99m Isonitrile Complex As A Potential Myocardial Imaging Agent. M N KHALIL, J THORNBACK, M Y EARLY, D B MORTON, L PARTON, J M BERRY, P J B HUBNER. Nuclear Medicine Communications. 5: 238 1984 (abstr)

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3. The Use Of Tc-99m Tertiary Butyl Isonitrile (BIN) As A Myocardial Imaging Agent In Man. M N KHALIL, K M PATEL, M Y EARLY, G HARTLEY, J THORNBACK, J M BERRY, P J B HUBNER. Eur J Nucl Med II: A 22 1985 (abstr)

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5. Tc-99m Butyl Isonitrile (BIN) As A Potential Myocardial Imaging Agent. M N KHALIL, J THORNBACK, M Y EARLY, D MORTON, J M BERRY, P J B HUBNER. Second European Symposium on Radiopharmacy and Radiopharmaceuticals. Cambridge, England. March 24th-27th, 1985. (Oral presentation)

6. The Clinical Value Of A New Myocardial Imaging Agent;Technetium-99m Tertiary Butyl Isonitrile (Tc-99m BIN). M N KHALIL, J R THORNBACK, K M PATEL, M Y EARLY, G HARTLEY, D N TAYLOR, J M BERRY, P J HUBNER. British Cardiac Society Annual General Meeting, York, U.K. April 2nd and 3rd, 1986. (Oral presentation)

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DEDICATION

This work is dedicated to my wife and children.

CONTENTS

CHAPTER 1 REVIEW OF LITERATURE

Introduction

The diagnosis of angina due to coronary artery disease (CAD) is made in the majority of patients from the history and electrocardiogram(ECG) changes either at rest or at myocardial stress. Unfortunately, in some cases, uncertainty as to whether a patient has CAD causing angina will remain, and angiography is indicated in some patients (Julian-Cardiology). A less invasive technique for assessment of CAD would be of benefit in these patients.

The role of radionuclides in the assessment of myocardial ischaemic defects has been both clinical and research orientated. The major research goal of myocardial perfusion imaging would be the clarification of the functional significance of coronary stenosis. The main clinical value of a validated perfusion technique would be in non-invasive screening of apparently mild CAD lesions (Gould 1978). Further uses might be in the selection of patients for coronary artery bypass grafting (Pfisterer et al, 1982) or for percutaneous transluminal coronary angioplasty (Scholl et al, 1982) and in the assessment of the success of these procedures.

Although myocardial perfusion imaging at rest is useful in detecting areas of myocardial infarction and severe ischaemia, it is often inadequate in detecting narrowing of the coronary arteries without infarction. Studies have shown that a coronary artery may be narrowed to 70 percent of its diameter without altering the resting flow. However, the effect of stenosis of this severity can be detected by using radioisotope tracers during stress (Zaret et al, 1973). On exercise areas of the myocardium served by normal coronary arteries receive 3-

4 times the resting blood flow compared to areas supplied by a critically narrowed coronary artery (Zaret et al, 1973). These cannot sustain such an increase in flow and, therefore, appear as cold areas relative to the rest of the myocardium. A cationic tracer with rapid blood clearance such as potassium-43, rubidium-81 or thallium-201 administered during the peak of stress will show the myocardial perfusion at the time of tracer administration (Saperstein, 1964). Since the distribution of the tracer during the first hour after administration mainly reflects the initial flow-related distribution rather than cell loss, imaging may be performed after termination of exercise. If a myocardial perfusion defect is detected during stress the scan may be repeated later during rest (Berman et al, 1978). If a perfusion defect persists this suggests an infarction, whereas a normal resting scan suggests that the perfusion defect seen during stress is due to transient myocardial ischaemia. Myocardial perfusion imaging during exercise may be of special importance in detecting ischaemia in patients with nondiagnostic stress ECG either due to inadequate exercise or uninterpretable stress ECG e.g. left bundle branch block (McCarthy et al, 1979).

Over the last twenty five years, a number of radionuclear methods have been tried to assess myocardial perfusion. These include:

(A) Intra coronary measurements.

(B) Hot spot imaging.

(C) Fatty acids and analogues.

(D) Positron emission tomography.

(E) Myocardial imaging using ionic tracers.

Group (A) requires cardiac catheterization. Its main use has been in research to evaluate the agents in group (E) for coronary stenosis detection, or to understand the perfusion consequences of stenosis (Gould 1978). Groups (B), (C), (D) and (E) use an intravenous injection and, therefore, their non invasiveness would make them suitable for screening diagnostic tests.

(A)Intra coronary measurements

Myocardial perfusion can be defined in the catheterization laboratory by direct intra coronary injection. Either an inert gas such as Xenon-133 (Ross et al, 1964) or particulate agents such as macroaggregated albumin (Jansen et al, 1974) can be used. Previous toxicological studies in dogs failed to demonstrate significant changes in blood pressure, heart rate, electrocardiogram, or serum creatine phosphokinase activity following intracoronary injections of Tc-99m labelled human albumin microspheres in doses up to 200 times that required for adequate myocardial scintigraphy (Weller et al, 1972). This technique detected myocardial infarcts, as small as 1.5cm, resulting from microembolization however, preliminary results in patients substantiated its safety for clinical use (Weller et al, 1972).

Myocardial imaging following the intracoronary injection of particulate radiopharmaceuticals (microspheres) is based on the principle that the microspheres flow with the blood and are trapped in the first perfusion bed they encounter (Kirk et al, 1977). They are distributed in proportion to blood flow within each organ of the body and within tissue regions of each organ (Kirk et al, 1977). These investigators used selective left

and right coronary injection of two differently labelled microspheres to show the perfusion territory of each coronary artery and they were able to predict areas of viable myocardium distal to stenotic lesions which would benefit from grafting.

Among patients with coronary astherosclerosis, the state of the myocardium is directly related to regional blood flow, hence, the potential of the labelled particle technique in the direct assessment of regional myocardial integrity. Since all of the injected particles are trapped within the myocardium, the problem of adjacent organ uptake is eliminated and imaging can be performed several hours following injection (Miller et al, 1985). Additionally, the radiation burden to the myocardium is low, as only 0.1 to 0.2 m Ci of radiolabel is required to provide an image of very high quality in a short interval of imaging (Miller et al, 1985). This contrasts with many currently employed intravenous imaging agents such as thallium-201 in which both high background radiation and less than ideal photon energies degrade image quality, and is a major advantage of the intra coronary technique. However, the major disadvantage of the technique is that it is very invasive and hence does not lend itself easily to repeat studies.

A variety of inert gasses have been used for the measurement of blood flow in the myocardium, most commonly Krypton-81 (Selwyn et al, 1979) and xenon-133 (Cannon et al, 1972). In the concentration used for tissue flow measurements, the gases are chemically inert and physiologically inactive. Following intra-arterial administration, the inert gas tracers leave the capillary and enter tissue in direct proportion to their relative solubility in the tissue compared with that of

blood (L'Abbate and Maseri, 1980). Thereafter, the clearance of the tracer from the tissue is dependent on the rate of perfusion (Holman et al, 1974 and L'Abbate and Maseri, 1980). Because the myocardial clearance of Xenon usually has a half-time of less than one minute, a scintillation camera must be present in the catheterization laboratory to measure the myocardial clearance. The rapid clearance of Xenon makes it possible to record myocardial perfusion under several different circumstances in rapid succession (Cannon et al, 1977). However, the technique is invasive and subject to many theoretical problems due to non-homogeneous perfusion (Cannon et al, 1975). In addition the sensitivity of this technique to detect the degree of arterial stenosis is not known.

(B)Hot spot imaging

Radiopharmaceuticals that are extracted by the normal myocardium such as potassium analogues and thallium-201 (Holman et al, 1982), offer additional help by outlining poorly perfused tissue as regions of decreased tracer concentration in the myocardial scan. However, these agents have some limitations (Holman et al, 1982). These include: (1) acute and old myocardial infarction cannot be distinguished, (2) the sensitivity of the technique decreases with time after the onset of symptoms, (3) right ventricular infarction cannot be diagnosed, since the right ventricle is not visualized by thallium-201 under resting conditions and (4) unstable angina may result in perfusion defects that are indistinguishable from those of acute myocardial infarction. To overcome these difficulties, researchers have sought agents that are taken up

selectively by the damaged myocardium. (Hot spot imaging). This technique would be useful to detect acute infarction and potentially allow assessment of methods to reduce infarct size.

A number of radiopharmaceuticals have been suggested mainly in experimental infarcts. These included 203 Hg chlomerodin (Carr et al, 1962), 203-Hg fluorescein (Hubner, 1970), Tc-99m tetracyclin (Malek et al, 1963), Tc-99m gluco-heptonate (Alonso et al, 1978) and the Tc-99m phosphates. The best images have been with Tc-99m stannous pyrophosphate (Parkey et al, 1977) and they have been successfully used in patients. The mechanism of Tc-99m pyrophosphate uptake into the acute infarct is poorly understood. Studies suggest that the pyrophosphate is attached to a crystaline structure within the mitochondria of necrotic myocardial cells. In acute M.I., pyrophosphate accummulation appears to temporarily parallel calcium deposition (Buja et al, 1976). The periphery of the infarct is the site of maximal pyrophosphate and calcium accummulation. However, there must be other factors than calcium deposition. Detailed studies have demonstrated a poor correlation between the amount and rate of calcium deposition in a given area of ischaemic necrosis and that of pyrophosphate deposition (Buja et al, 1977).

Pyrophosphate accummulation in the zone of necrosis requires a certain level of blood flow. Maximal accummulation occurs in myocardial regions where flow has fallen to 30 to 40 percent of normal (Zaret et al, 1976). In regions with flow below this level, pyrophosphate uptake falls, even though the degree of myocardial necrosis is greater.

The sensitivity for the detection of acute M.I.,based on

24 studies with a total patient population of 1,143, was 89 percent (Holman et al, 1980). In a similar group of 19 different studies and 1,482 patients with no evidence of acute infarction, the Tc-99m pyrophosphate scans were normal in 86 percent of patients (Holman et al, 1980). Hence the false positive rate was 14 percent. These results may be deceptive, however. The diagnostic problem usually involves the distinction of unstable angina pectoris from acute infarction. In 374 patients with a clinical diagnosis of infarction, 152 patients (41 percent) had positive Tc-99m pyrophosphate scans (Holman et al, 1980). Patients with clinical evidence of unstable angina pectoris who were studied with both Tc-99m pyrophosphate and with sequential determination of serum MB creatine kinase (CK) activity, 22 of 36 patients with abnormal images had elevated total plasma CK and MBCK activity (Jaffe et al, 1979). These studies showed that at least in some patients with unstable angina and abnormal scintigrams, there is underlying tissue necrosis accounting for the pyrophosphate uptake.

Pyrophosphate scans are likely to demonstrate infarction when injected between 16 hours and 6 days after onset of symptoms. Acute infarction can be detected as early as 4 hours after the onset of symptoms (Holman et al, 1978), but with variable success. Patients with uncomplicated acute MI show peak Tc-99m pyrophosphate uptake between 48 and 72 hours after the onset of symptoms (Holman et al 1982). At this time, the intensity of uptake decreases, reaching normal levels after 1 to 2 weeks. There are many patients in whom the scintigraphic pattern returns to normal very very slowly, however, Olsen et

al (1977) observed a return to normal in only 43 percent of patients 6 to 37 weeks after acute infarction. In these patients with persistently abnormal scintigrams, the pattern was usually mildly diffuse and only 20 percent of those patients had focal activity.

Although the diagnostic significance of myocardial scintigraphy with pyrophosphate is at times unclear, the scintigraphic pattern of myocardial uptake provides clues to the patient's future course, both in hospital and long term (Holman et al 1978). The doughnut pattern of increased uptake is associated with extensive MI, and is also generally associated with a poor prognosis (Holman et al, 1978). A high proportion of patients with acute MI demonstrate persistently positive Tc-99m pyrophosphate scintigrams that are associated with an increased incidence of complications, including sudden death, recurrent myocardial infarction, new angina pectoris, and the development of congestive heart failure (Olsen et al, 1977). In fact, patients with clinical evidence of infarction and small foci of pyrophosphate myocardial uptake have complication rates comparable with those of patients without acute MI (Holman et al, 1978).

Tc-99m pyrophosphate myocardial scintigraphy is also of value in the diagnosis of (1) Intra-operative MI where chest pain, serum enzyme elevation and electrocardiographic changes may result from the operation itself (Righetti et al, 1977), (2) right ventricular infarction (Sharpe et al, 1978), (3) infarct extension or re-infarction provided that a base line scintigram is available (Holman et al, 1978). Also infarct size could be assessed. A significant relation has been

reported between the area of Tc-99m pyrophosphate uptake and the maximal serum CK elevation in patients with acute anterior MI (Willerson et al, 1975). However, other investigators found a poorer correlation between scintigraphy and infarct size in inferior wall MI (Henning et al, 1977).

Another approach to the detection of acute necrosis has been the use of radio labelled antibody directed against the heavy chain of human cardiac myosin, anti myosin. This antibody can be labelled with iodine-131 (Khaw et al, 1978), Tc-99m (Khaw et al, 1982), iodine-125 (Khaw et al, 1983) or In-111(Khaw et al, 1984). The antibody localizes only in those cells where membranes have been sufficiently damaged to permit the development of large holes clearly visible on electron microimages. When cells reach this phase of damage, cell death is inevitable. Since exposure of the antigen can only occur when the membrane is disrupted, localization of antimyosin in the cell is associated only with that tissue that has undergone irreversible necrosis. To maximize the rate of blood clearance and to facilitate diffusion of the antibody into the injured area, a fragment of the antibody, antimyosin-Fab, has been used for studies in human subjects (Khaw et al, 1978 and 1982). Coupling of the radionuclide to the antibody was achieved via a "bifunctional" chelate by which one of its functional groups is coupled to a lysine on the protein, while the remaining sites are available to couple the radiolabel. The bifunctional chelate approach permits labelling with either Tc-99m or In-111 (Khaw et al, 1984). Studies of experimental myocardial infarction determined that the zone of necrosis could be visualized as early as 6 hours following acute coronary

occlusion with radio labelled antimyosin Fab and a positive scan can be obtained up to 5 days. Human studies with antimyosin demonstrated a similar time course of uptake (Khaw et al, in press). In contrast to Tc-99m pyrophosphate, which has a rapid blood clearance, antimyosin Fab has a blood clearance half-time of about 3 hours. As a result, the earliest practical time to image a zone of necrosis following intravenous injection is 6 hours. Small zones of necrosis may not become apparent for 18 to 24 hours after injection, when blood pool concentration has reached a nadir. As a result, it may be desirable to wait until 12 to 18 hours to image patients with the antibody.

Other "hot spot" scanning agents of note are indium-111-labelled leukocytes (Davies et al, 1980). These have been used successfully to detect infarction in patients by means of the accummulation in the region of the inflammatory response to the infarction process. However, the value of this technique has yet to be assessed.

In summary, although many agents have been demonstrated to concentrate in areas of acute myocardial necrosis, Tc-99m pyrophosphate continues to have the best practical combination of concentration in the abnormal zone and relatively rapid blood clearance leading to the development of a high target to background ratio between the infarct and the surrounding structures in the chest.

(C) Fatty acids and analogues

Fatty acid oxidation is the primary source of energy for the heart under aerobic conditions (Neely et al, 1972). In the blood fatty acids are transported bound to albumin. After

diffusion into the cell, they are activated to a fatty-acyl COA at the outer mitochondrial membrane. The acyl unit is then transferred to carnitine, which transports the activated fatty acids into the mitochondrial matrix where it either undergoes beta oxidation or is utilized in the synthesis of phospholipids and triglycerides. Beta oxidation of fatty acids predominates during aerobic metabolism, whereas during anaerobic conditions, synthesis of lipids predominates (Goldstein et al, 1980).

Evans and coworkers (1965), proposed the use of labelled free fatty acid (FFA) for myocardial imaging. They produced the first scintigrams of the heart with iodine-131 labelled stearic acid, prepared by the addition of iodine to the double bond of oleic acid. Further studies, however, mainly by Poe et al (1977) and by Machulla et al (1978) have demonstrated that iodination of FFA at double bonds strongly influences myocardial extraction and elimination of the labelled compound. More recently, palmitic acid has been labelled with carbon-11, a procedure that does not alter the biological behaviour of this fatty acid and one that has been used extensively for tomographic imaging of the heart by positron computerized tomography (Hoffman et al, 1977, Ter-Pogossian et al, 1980, Lerch et al, 1982 and Schon et al, 1984). As well as its use for myocardial imaging, this agent might allow quantification of regional myocardial fatty acid metabolism and its uptake in the myocardium or its turnover rates should be related to the rate of fatty acid utilization (Goldstein et al, 1978 and Klein et al, 1979). Hence labelling with carbon-11 should be ideal, but optimal imaging using 511-KeV annihilation radiation

from carbon-11 needs special equipment, which currently limits the wider use of carbon-11 labelled compounds (Freundlieb et al, 1980).

Initial animal experiments with various labelled FFAs have shown that (17-iodine-123) iodoheptadecanoic acid (I-123 HA), labelled in the omega position with I-123, is taken up by the myocardium almost exactly as is palmitic acid labelled with carbon-11 (Machulla et al, 1978). Labelling with I-123 permits an excellent external detection of this tracer by any conventional gamma camera, and the radiation exposure of the patient can be kept reasonably low (Poe et al, 1977). After intravenous injection of I-123 HA there is a rapid tracer accummulation in the myocardium and liver and a rapid disappearance of the tracer from the blood with a half-time of about 2 minutes (Freundlieb et al, 1980). The decrease of total I-123 activity in the blood is accompanied by a rise of the inorganic I-123 fraction. Beyond 15 minutes after administration, an average of 55-70 percent of the blood activity is attributed to inorganic I-123 (Freundlieb et al, 1980). Due to the metabolic degradation of FFA in the myocardium, the activity over the heart decreases with time. The period during which optimal myocardial images are recorded is limited to 5-30 minutes after injection (Freundlieb et al, 1980). Studies have shown that in normal subjects the average washout half-time for the whole heart is 24.0 $\pm$ 5 minutes at rest and 21.7 $\pm$ 2 minutes after exercise (Hock et al, 1983).

As with other suitable radiopharmaceuticals, such as potassium and its analogues, I-123 HA myocardial scintigraphy shows old infarct scars as photon-deficient areas. Moreover,

in patients with CAD, ischaemic regions may be clearly recognised even in the resting state; in these regions tracer accummulation is also reduced (Freundlieb et al, 1980 and Weiss et al, 1976). These results indicate that reduced coronary perfusion is accompanied by a marked diminution of myocardial FFA uptake. This decreased uptake is not only due to reduced FFA delivery, but also due to reduced uptake (Opie, 1976). This finding is different to that with glucose, where a decrease of delivery is partially offset by an increase of the extraction rate (Weiss et al, 1976). To increase the residence time of the fatty acids in the myocardium, analogues were synthesized with a phenyl ring at the omega position of the molecule (Rellas et al, 1983 and Reske et al, 1983). The substitution served both to stabilize the iodine on the molecule and to prevent terminal beta oxidation. The residence half-time of the radiolabel was increased from approximately 10 minutes with straight chain iodinated fatty acids, to approximately 20 minutes with the addition of the phenyl group (probably by diffusion after the fatty acid is catabolized by beta oxidation and the phenyl group remains with its iodine radiolabel). Substitution of a methyl group at the omega position of the fatty acid eliminated normal beta oxidation and caused a marked prolongation of the residence time of fatty acids in the myocardium to over five hours (Livni et al, 1982 and Miller et al, 1985). The very slow clearance of these agents is probably the result of an alternative pathway, alpha oxidation, which can slowly catabolize fatty acids one carbon at a time. These agents have been used to identify the altered use of substrate in cardiomyopathy (Hock et al, 1983), myocardial hypertrophy

(Yonekura et al, 1985) and during recovery from ischaemia (Miller et al,1985).

Although labelled fatty acids were among the first isotopes used to study myocardial perfusion, these agents have not been found to be successful in clinical practice. The difficulties which have been met with the labelling process to prepare these agents restricted their use to research. This has also been the author's experience in a small number of patients.

(D)Positron emission tomography (PET)

Some radionuclides used in nuclear cardiology emit positrons. When a positron collides with an electron, their mutual annihilation produces a pair of photons which are emitted in opposite directions and with equal energy (511 KEV) (Hoffman et al, 1979). It is possible, using specific instrumentation, to detect both photons in a pair simultaneously. This results in an accurate topographic localization of a regional event in an organ, and more accurate quantitation of tracer concentration in organ beds than is readily achieved by other methods (Phelps et al, 1980). This is due to the accompanying high photon energy emission and, therefore, less tissue attenuation (Phelps et al, 1980).

Three positron emitting radionuclides have been tried for myocardial perfusion imaging: cyclotron-produced nitrogen-13 (N-13) ammonia (Bergmann et al, 1980) which has a physical half-life of 10 minutes, cyclotron produced potassium-38 (Phelps et al, 1983) with a physical half-life of 7.6 minutes and generator produced rubidium-82 (Bergmann et al, 1982 and

Mullani et al, 1984) with a physical half-life of 75 seconds.

Studies by Gould and associates in dogs suggested that stenosis of less than 50 percent of luminal diameter can be detected when these radionuclides are given with dipyridamole vasodilatation (Gould et al, 1979) and the regional distribution of both ammonia and rubidium is very similar to that obtained with microspheres. More recent studies showed that PET can detect and quantify regional myocardial uptake of tracers such as rubidium-82 and N-13 labelled ammonia (Mullani et al, 1983 and Goldstein et al, 1983). These investigators recorded the rapid vascular transit of rubidium-82 to make calculations of uptake, flow and extraction in the myocardium, permitting measurements of disturbances of extraction with changes in pH. The advantages would be considerable if this approach could be used in man, particularly for early detection of noncritical coronary artery stenosis (Schelbert et al, 1982). As discussed before in this chapter, the uptake of monovalent cations is influenced by delivered concentration, regional flow rates and fractional extraction which may be influenced by cellular integrity. Under some circumstances, it may be important to obtain a straightforward measure of regional myocardial perfusion, rather than an admixture of local flow and metabolic state.

There are problems limiting the use of PET. The short half-lives of positrons require cyclotron production in close proximity to the imaging site. This involves a large initial capital expense (Wolf, 1984). In addition, a trained operating staff, shielded radioctivity handling areas, and facilities for safe, automated radiopharmaceutical synthesis are required.

However, the emerging generation of high resolution PET imaging devices should make the use of PET more widespread in the near future.

(E) Myocardial imaging using ionic tracers

(i)General review.

According to the Saperstein principle (1964), the initial distribution of radiopharmaceuticals that have a high extraction will be proportion to relative perfusion. The principle does not require a radionuclide to belong to a particular class of tracers to serve as an indicator of regional perfusion but instead defines a kinetic pattern, for example, high extraction by the organ of interest and rapid clearance from the blood. If a radiopharmaceutical meets this criterion, it will be distributed in proportional to regional perfusion. If the regional extraction of an agent falls below 50 percent or if the residence time of the agent in the blood is prolonged such that the half-time clearance is greater than 5 minutes, the agent will not follow the definition of a Saperstein tracer and cannot be employed to define regional perfusion (Miller et al, 1985).

The determination of regional perfusion with ionic tracers, such as the monovalent cations, is dependent on their maintaining a high extraction in the myocardial bed. Under reasonable physiological circumstances, this is true, but when myocardial perfusion is markedly increased by vasodilators, the extraction of the indicator may be reduced (Weich et al, 1977). In experimental animal, regional myocardial uptake of thallium (Tl)-201, one of the monovalent cations, has been shown to be

linearly related to regional myocardial blood flow at flow levels from resting control down to zero (Mueller et al, 1976). Similarly, increasing coronary blood flow above the resting level increases myocardial uptake of Tl-201. It is not yet clear whether at these high flow levels the relationship between coronary blood and Tl-201 uptake is dependent on the type of stimulus for increased flow. It appears that at increased flow levels associated with increase in myocardial oxygen demand, for example, pacing (Weich et al, 1977) or exercise (Nielson et al, 1979), the linear relationship between blood flow and Tl-201 uptake is preserved. However, at exceedingly high flow levels that are in excess of the myocardial needs, which may be induced by coronary vasodilators, myocardial uptake of Tl-201 is not linearly related to increasing blood flow (Strauss et al, 1977 and Gould, 1978), implying a progressive decrease in extraction fraction. At these high flow rates Tl-201 uptake increases by a mean value of approximately one-half of the increase in coronary flow (Weich et al, 1977 and Gould, 1978).

Monovalent cations potassium, rubidium, caesium and thallium concentrate in the myocardium to a sufficient degree to permit myocardial imaging (Strauss et al, 1975). These agents enter the myocardium by the energy requiring sodium-potassium-ATP ase pump mechanism (Pohost et al, 1980 and Strauss et al, 1975). They differ in their physical half-lives, gamma emissions and time course of myocardial concentration and release. In a single transit through the coronary bed, the extraction of thallium averages 88 percent, that of potassium and rubidium is between 65 and 75 percent and

that of caesium is only 33 percent (Strauss and Pitt, 1978). Because the myocardial extraction and the total body extraction are usually similar, the time courses of blood clearance and myocardial concentration will be different for each of the potassium analogues. The most rapid concentration in the myocardium occurs with thallium, followed by potassium and rubidium whereas that for caesium is far slower (Miller et al, 1985). The importance of the time for localization stems from the need to maintain a "steady state" during the interval of radionuclide concentration in the myocardium. Within three circulation times, the majority of monovalent cationic myocardial tracers with high extraction fraction (Tl-201, rubidium-81, rubidium-82, potassium-43 and potassium-38) have concentrated in the myocardium (Miller et al, 1985). Once in the myocardium, the ionic radionuclides slowly leak out, with half-times ranging from 1.5 hours for potassium-43 (Strauss et al, 1975) to 4-6 hours for Tl-201 (Pohost et al, 1980). The factors controlling the rate of loss from the myocardium are not fully understood but include (1) the presence of regional ischaemia (slower loss from ischaemic area) (Pohost et al, 1980), (2) level of exercise at the time of tracer administration (increased loss from patients achieving higher levels of exercise) (Massie et al, 1982) and (3) the plasma insulin levels (increased rate of loss from both ischaemic and normal segments in animals infused with a glucose-insulin-potassium solution) (Wilson et al, 1983).

Although the initial distribution of Tl-201 reflects regional flow, absolute quantitation of this distribution, and thus of regional flow, has not been possible. Following its

initial distribution, myocardial Tl-201 deposition is dynamic. After an injection of Tl-201 during exercise there is both an immediate and prolonged period of so called "redistribution". As coronary perfusion returns to base line levels after stress testing, myocardial wash-in and washout of Tl-201 continue to occur, and over time a new equilibrium reflecting basal flow is reached (Ritchie, 1982). Delayed or late imaging, typically performed 3 to 6 hours after isotope injection, provides additional information on the state of basal perfusion (Pohost et al, 1977 and Beller et al, 1980). However, this redistribution phenomenon limits stress imaging in several respects. Because a clinical observation period after exercise testing is required, isotope redistribution may start before imaging begins and continue as multiple images are acquired. Some perfusion abnormalities may be masked if imaging is delayed more than a few minutes after stress, or they may change during the course of imaging. Additionally, the speed and amount of redistribution depend on the blood concentration of Tl-201; when blood levels of isotope are relatively high, as they might be if the period of exercise maintained after Tl-201 injection is brief, the rapidity and extent of redistribution is relatively greater (Wharton et al, 1980).

Imaging with Tl-201 is performed from the 80 KeV mercury X-ray emission. Although clearly favourable compared with the very high energy tracers, the energy spectrum of this radionuclide is less than ideal. A photon energy less than 100 KeV will be too low to be optimum for passage through tissue, and many of the photons, particularly those from the back of the heart, are absorbed without reaching the scintillation

detectors in the gamma camera. This will result in a low resolution image which can make its interpretation difficult. On the other hand, high energies of more than 200 KeV, for example the energies of rubidium and potassium nuclides, are too high to permit efficient detection with the anger camera and require high energy collimators to reduce scattered photons. These collimators generally have a lower sensitivity and lower resolution than do the lower energy collimators (Miller et al, 1985).

While Tl-201 is currently the isotope of choice for myocardial imaging, it has a number of limitations as a radiopharmaceutical. Because of its low energy, Tl-201 is substantially absorbed and scattered by soft tissue or contiguous myocardium interposed between the gamma camera and the region of interest. Mueller et al (1976) employing a canine model, showed that 4 to 6 gm ischaemic ventricular segments with flow reductions of 40 to 60 percent were inconsistently detected by qualitative or visual image analysis, whereas larger perfusion defects were regularly seen. Artifactual perfusion defects can also be caused by attenuation that is due to the hemidiaphragm, overlying soft tissue, or breast (Botvinick et al, 1980). Additionally the gamma camera employs multiple views or two dimensional images to assess the three dimensional distribution of isotope within the body. Because some isotope is taken up by the non myocardial structures overlying the heart, and because the region of myocardial defect may be superimposed over a region of normal myocardium, planar or standard imaging cannot be quantitative. Tomographic approaches to imaging may partially resolve this

problem (Holman et al, 1979 and Ritchie et al, 1980). This will be discussed in more detail later in this chapter.

Interpretation of Tl-201 studies should involve visual analysis of analogue images as well as computer-enhanced images. Analogue images should be included in the interpretation because they can often help one judge the quality of the raw data (Berger and Zaret, 1981). For visual interpretation, computer processing may include background correction and contrast enhancement of the image data (Berger et al, 1978). Image enhancement may result in increased detection of perfusion abnormalities, but the trade off may be in decreased specificity (Berger and Zaret, 1981).

The radiation dose delivered to the patient per mCi (37 MBq) of Tl-201 has been estimated to be 0.34 rad to the heart and 0.24 rad to the total body (New England Nuclear Thallous Chloride package insert, 1977 and Atkins et al, 1977). The largest dose, 1.2 rads, is delivered to the kidneys (Atkins et al, 1977). The long half life of Tl-201 (72 hours) and its high concentration in the kidneys limit the administration dose to 2 mCi per study. Thus prolonged imaging time is required to obtain high counts. Tl-201 is cyclotron produced and is relatively expensive.

Hence, the initial myocardial distribution of Tl-201 parallels regional coronary perfusion. Because of overlapping of structures, limited resolution of the gamma camera, and some uncertainties in the timing of isotope injection and the subsequent dynamic state of myocardial isotope wash-in and washout, only qualitative or semiquantitative indices of perfusion are obtained from external gamma camera images. The

optimal gamma emitting radioactive potassium analog has not as yet become available for routine clinical use. To be used optimally with conventional scintillation cameras, this radionuclide should have its major photopeak in the range of technetium-99m, that is, 140 KeV photon. Ideally, like technetium, it should be generator produced so that problems associated with tracer cost and logistics of shipment from commercial reactors would be eliminated. For these reasons, the successful imaging of the heart with a Tc-99m labelled compound would be very valuable. The work in this thesis will be mainly directed towards achieving this purpose.

(ii)Sensitivity and specificity of exercise thallium-201 myocardial scintigraphy

Given the experimental and theoretical limitations inherent in Tl-201 perfusion imaging, certain clinical uses have evolved. Specifically Tl-201 perfusion imaging may help answer the following clinical questions;

(1) Among patients with a chest pain syndrome, is CAD present or absent?

(2) Among patients with known CAD, what is the extent and the site of disease?

In addressing the first of these questions, Tl-201 perfusion imaging has been extensively compared with exercise electrocardiographic testing in patients with a chest pain syndrome and suspected CAD. In general, these studies have shown moderate and statistically significant improvement in the detection of disease when present (sensitivity) and exclusion of disease when absent (specificity) - compared with those of exercise electrocardiography alone (Ritchie et al, 1977 and

1978, Bailey et al, 1977 and Verani et al, 1978). In a more recent review, the results from 2,048 patients were collected from 22 studies reported in the literature (Gibson and Beller, 1982). In these patients, the overall sensitivity and specificity of Tl-201 exercise scintigraphy were 83 percent and 90 percent respectively, compared with 58 percent and 82 percent, respectively for the exercise ECG. The higher sensitivity of Tl-201 imaging over that of exercise electrocardiography derives in part from that subset of patients whose exercise tests are non diagnostic with either (1) an abnormal resting electrocardiogram in which ischaemic S-T segment changes cannot be defined or (2) failure to achieve 85 percent of predicted heart rate when no ischaemic S-T segment changes are noted. In a study by Ritchie et al (1978), 13 of 16 patients with CAD and left bundle branch block or digitalis effect in the base line electrocardiogram were identified with Tl-201 exercise testing, whereas ischaemic S-T segment changes were uninterpretable. In four other series (Verani et al, 1978, Berger et al, 1979, Iskandrian et al, 1980 and McCarthy et al, 1979), a total of 163 patients with non diagnostic exercise tests were reported on. In each, the sensitivity and specificity of Tl-201 imaging in this subset were not significantly different from those of patients with exercise tests and diagnostic end points. Sensitivity ranged from 70 to 100 percent and specificity from 69 to 100 percent. Thus among patients with an exercise test that is not diagnostic, the Tl-201 exercise study can provide an extra aid to reach a diagnosis.

Tl-201 study is relatively poor at assessing the location

and severity of CAD (Rigo et al, 1980). The authors found that the detection rate of CAD for individual vessel was 63 percent for the left anterior descending coronary artery, 50 percent for the right coronary artery and 21 percent for the left circumflex coronary artery. Similarly, Lenaers (1979) detected disease of the left anterior descending coronary artery in 83 percent of cases, the right coronary artery in 63 percent of cases, and the left circumflex artery in 48 percent of cases. Additionally, in each of these series, high grade stenoses of 90 to 100 percent were more commonly detected than were stenoses of lesser magnitude. Dash et al (1979), in a small series of patients with left main coronary disease and a larger series with three vessel CAD, found that either left main or three vessel disease was identifiable in only 43 percent. Similarly, Leppo et al (1979) studied a group of 30 patients with three vessel coronary disease and exercise electrocardiographic signs of ischaemia and showed that only two thirds of this subset had perfusion abnormalities.

Whether exercise should be maximal (terminated only by severe symptoms, serious arrhythmias, or hypotension) or submaximal, defined by achievement of 85 percent of the maximal predicted heart rate, is controversial. Studies comparing Tl-201 imaging results with different levels of exercise have demonstrated, however, that assessment of the extent of disease is best with maximal exercise producing maximal disparity in blood flow between normal and abnormal zones (McLaughlin et al, 1979). Studies have also shown that the performance of stress Tl-201 imaging in patients on propranolol has reduced sensitivity for detection of CAD because of effect of this drug

on maximal exercise heart rate - blood pressure product (Pohost et al, 1979). Tl-201 imaging obtained after submaximal exercise appears to be useful in identifying patients at high risk after acute myocardial infarction (Turner et al, 1980). Residual jeopardized myocardium, as well as the extent of coronary artery disease, can be predicted with quantitative serial imaging (Gibson et al, 1981).

In summary, exercise Tl-201 scanning is useful in the diagnosis of absence or presence of CAD. Its sensitivity and specificity are higher than that of exercise electrocardiogram, however, more information can be obtained from the analysis of both tests. The technique cannot be accurately used to assess the site and severity of CAD.

(iii) ECG gated thallium-201 scintigraphy

In the usual method of acquisition, Tl-201 myocardial images contain data from all phases of the cardiac cycle, and are thus blurred to some extent by cardiac motion, with some possible loss of resolution of abnormalities (McKillop et al, 1981). ECG-gated acquisition of data, reduces the effects of cardiac motion on the scans. (Green et al, 1975). With this method an end diastolic image is obtained. Longer acquisition times become necessary, since an image contains data from only a fraction of a cycle (Hamilton et al, 1978). The increased acquisition time can be a problem with post exercise scans when redistribution is progressively taking place. This can produce false-negative results due to early redistribution of the tracer to ischaemic but viable areas of myocardium (Schwartz et al, 1978).

Although some studies using gated Tl-201 imaging have

shown improved edge definition and increased certainty of defect detection (McKusick et al, 1979, Hamilton et al, 1978 and Valette et al, 1985), others have demonstrated no improvement through the use of gating (McKillop et al, 1981). Hamilton and colleagues (1978) reported that when the acquisition was obtained from 50 msec segments of the cycle in diastole, the gated images differed significantly from the non-gated, with the former showing clearer resolution of the myocardium, a larger ventricular cavity, and easier appreciation of areas of decreased uptake. In addition to that systolic myocardial thickening and wall motion could also be analysed by visual inspection of a cine loop of the different segments of the cycle. McKillop and Coworkers (1981) examined the value of ECG-gated acquisition of static Tl-201 myocardial images by comparing gated and non-gated studies in three projections from 54 patients who also had coronary arteriograms. They found no significant differences in sensitivity, specificity, detection rate for myocardial ischaèmia, accuracy of prediction of extent of CAD, and inter-observer agreement. The authors concluded in their study that, at least when the images were subsequently processed by interpolative background subtraction, ECG-gated acquisition of static images had no advantages over non-gated acquisition. The sensitivity reported in their work on the gated studies (36 of 43) was significantly lower than the figure of 65 of 67 given by Buda and his associates (Buda et al, 1978) who also used interpolative background subtraction. The difference between the two studies was attributed to the use of a different method to derive the sensitivity figures by each group. While

Buda et al relied on review of the clinical reports on Tl-201 studies, the study by McKillop et al involved independent blind readings by four observers who had no clinical information available. When the clinical reports were analyzed by McKillop et al, the sensitivity for the gated images was not significantly different from that of Buda et al.

More recent reports have demonstrated favourable results for ECG-gated Tl-201 scintigraphy. Martin et al (1985) compared the accuracy of gated Tc-99m blood pool scanning at rest and during maximal supine exercise, and post exercise gated Tl-201 scintigraphy in the detection of patients with significant CAD. The authors showed with 50 patients undergoing coronary arteriography, that regional wall abnormalities occurring on either the rest or exercise Tc-99m scan provided a sensitivity of 63 percent, and a specificity of 73 percent in the detection of CAD. However, perfusion defects occurring on the gated Tl-201 scan detected significant coronary disease in the same patients with a sensitivity of 86 percent and specificity of 95 percent. Another study by Valette et al, showed that accurate ECG gated Tl-201 myocardial images can be routinely obtained; it did improve detection sensitivity of CAD extent, mostly in patients with two or three vessel disease (Valette et al, 1985). It has been also shown that ECG gated Tl-201 scintigraphy is a useful tool to detect right CAD and right ventricular ischaemia or infarction (Le Guludec et al, 1985).

Hence, ECG gated Tl-201 scintigraphy may increase the apparent resolution of the images which may be bought at the price of true diagnostic accuracy. The availability of a Tc-99m labelled myocardial imaging agent may change this

situation. Theoretically it should show better resolution due to the higher photon energy, therefore, less time will be required to obtain a high quality image.

(iv)Quantitative thallium-201 scanning

Visual interpretation of Tl-201 scans is often difficult and subject to significant inter and intra observer disagreement (Trobaugh et al, 1978). This may be, in part, due to the superimposition of one portion of the myocardium upon another, creating a source of confusion in interpretation. In addition, as discussed above, visual Tl-201 scanning is less accurate for predicting the number of involved vessels (McKillop et al, 1979) or for detecting disease in specific coronary arteries (Rigo et al, 1981). These are significant limitations, because coronary artery disease prognosis relates in part to the extent of disease (Harris et al, 1979). To improve the interpretation, semi automatic computer based techniques have been tried. These scans are usually called quantitative scans.

To address some of these shortcomings, some investigators have proposed quantitative methods for analysis of planar Tl-201 scans (Watson et al, 1981 and Garcia et al, 1981). Based on the assumption that the myocardial concentration of the isotope is proportional to local tissue blood flow, and can be determined by the counting rate at the camera face. The initial encouraging reports of quantitative thallium scanning (Berger et al, 1981 and Maddahi et al, 1981) have resulted in their widespread clinical use. This review points out some potential limitations of these methods based on the published reports.

The two main methods that have been tried are horizontal (Watson et al, 1981) and circumferential profile analysis (Garcia et al, 1981). In both methods, initial image processing involves background subtraction, which is designed to isolate "true" myocardial Tl-201 activity from non myocardial scatter. Once the background-subtracted myocardial image is obtained the two methods diverge. In the horizontal profile, the operator identifies the superior and inferior myocardial walls, and the computer automatically generates horizontal slices at those two levels and at two evenly spaced levels in between for a total of four horizontal slices that measure the radioactive counts at each specific level. In the circumferential profiles, the operator marks the centre and, in some programs, the apex of the left ventricle; the computer searches radially from the centre to the myocardial wall for the peak counts, which are then plotted, usually for sixty 6 degree sectors to comprise the entire circumference of the heart. Both techniques are performed on both the stress and redistribution scans, and the pattern of Tl-201 uptake and washout are assessed against normal established limits (Berger et al, 1981 and Maddahi et al, 1981). It has been reported that either of these methods is at least as accurate as visual analysis for detecting CAD, and significantly more accurate for determining the presence of multi vessel disease and lesions in specific coronary arteries (Berger et al, 1981 and Maddahi et al, 1981). In addition, these techniques are assumed to be more reproducible than visual scan interpretation. Makler et al (1983) recently studied the reproducibility of circumferential profile analysis and found that the intra

observer variability (1 observer calculating profile curves on the same scan) averaged 4.9 percent, whereas inter observer variability was 5.4 percent.

Quantitative Tl-201 scan analysis is based on several assumptions that may not always be true. This can lead to potential problems in interpretation, which will be discussed.

Background subtraction: The subtraction algorithm is generally some modification of the interpolative technique of Goris et al (1976), in which the non cardiac activity in a rectangular region surrounding the heart is used to estimate the non cardiac activity over - and underlying the heart, which is subtracted (pixel by pixel) from the heart tissue. Caution should be exercised when subtracting background from Tl-201 images, in particular when there is considerable activity in adjacent organs, for example, liver or spleen (Narahara et al, 1977), or when the lung uptake of Tl-201 is increased (Rothendler et al, 1983). High lung uptake with Tl-201 is sometimes observed on the stress images in patients with severe CAD, and is probably due to exercise-induced left ventricular dysfunction (Kushner et al, 1981). In these situations, erroneous regional perfusion defects can occur due to overestimation of background in the scans which results in considerably fewer myocardial counts in the background-subtracted image than are present in the raw myocardial scan (Narahara et al, 1977), and in significant errors in calculating regional washout rates of Tl-201 (Rothendler et al, 1983).

Reposition variability: Because quantitative Tl-201 analysis is performed on two images, one immediately after

exercise and one 2 to 4 hours later, another potential source of error is the position of the patient relative to the gamma camera despite the use of standard views and the adjustment of the camera head to the same angle. The contribution of variability in reposition has been studied recently in 19 patients who had 2 separate redistribution scans performed in the 40 degree left anterior oblique (Makler et al, 1983). The operators were instructed to duplicate the position as carefully as possible, and the redistribution scans were chosen for analysis, since there should be little change in Tl-201 activity between 2 scans performed several hours after injection. The 2 scans were analyzed using the circumferential profile method, and there was 12.9 percent difference in the curves. Even when the variability of re-analysis was taken into account, there was an implicit repositioning variability of 11.5 percent. Thus quantitative comparison of 2 scans will have a degree of variability that is independent of the operator or the software.

Level of exercise: Quantitative analysis of exercise and redistribution Tl-201 scans requires that the count profiles and curves be compared with "normal limits" of uptake and washout values determined in normal patients undergoing Tl-201 imaging. However, patients with CAD often cannot perform the same level of exercise as normal subjects, and the level of myocardial oxygen consumption, coronary blood flow and Tl-201 uptake may be different, not as the consequence of ischaemia but because of the different level of stress. Massie et al (1982) studied normal subjects on two occasions: The first test was a symptom-limited maximal stress test, and the second

test was stopped after only one-half of the exercise duration on the maximal test. Tl-201 exercise and 3-hours redistribution scans were obtained in conjunction with both stress tests. The investigators found that the washout rate (over 3 hours) was significantly greater on the maximal test than on the submaximal test. In some patients with CAD who exercised submaximally because they reached non ischaemic end points (e.g. claudication), the washout rates were abnormal in regions unaffected by diseased coronary vessels. The group concluded that the normal limits of washout, which have been established in normal subjects who have exercised maximally, cannot be used to evaluate Tl-201 washout in patients who exercise submaximally, stopping for non ischaemic reasons.

Redistribution kinetics: The redistribution phenomenon of Tl-201 can be used to calculate the "washout rate" which adds additional data in the detection of CAD. (Berger et al, 1981 and Maddahi et al, 1981). However, patterns of Tl-201 redistribution are variable. Early redistribution of stress-induced defects can be detected both by visual inspection (Makler et al, 1982) and by quantitative analysis (Gutman et al, 1983), findings that have important implications on when imaging immediately after exercise should commence. However, some defects demonstrate "late" (18 to 24 hours) redistribution; these are generally in regions supplied by severely stenosed coronary arteries, yet with normal wall motion and no evidence of myocardial infarction (Gutman et al, 1983). Thus, if Tl-201 uptake and washout rates are determined from two scans (i.e. exercise and 2-4 hour redistribution), possible errors in assessing tissue viability may result.

In summary, although quantitative planar Tl-201 scanning appears to improve the analysis ofTl-201 imaging, they have not eliminated the need for visual interpretation. Optimal results may be obtained by combining the interpretation of the images and the quantitative profiles.

(v) Thallium-201 myocardial tomography

The second major advance in myocardial perfusion imaging, is the use of single photon emission computerized tomography (SPECT) (Caldwell et al, 1982, Nohara et al, 1984, Garcia et al, 1985 and De Pasquale, 1986).

In SPECT, data are collected circumferentially around the subject's chest. These data are then reconstructed by a computer into a number of images, each of which represents a transaxial slice through the chest. SPECT allows a three rather than two dimensional image to be obtained (Keyes, 1982). Beside better visual assessment of the scans, the technique should also allow better quantification of myocardial perfusion than a planar scan (Keyes, 1982). On the other hand, the cost effectiveness of SPECT has not been established and furthermore, unless great care is taken with such factors as field uniformity, the centre of rotation determination and table-camera alignment, artefacts are likely to be common. SPECT can be performed in a variety of ways. These include the use of static gamma cameras with special collimator systems such as rotating slant hole collimator (Ratib et al, 1982) and the quadrant slant hole collimator (Chang et al, 1982), rotating gamma cameras (Maublant et al, 1982 and Ritchie et al, 1982), or with ring detector systems. Currently, rotating

gamma cameras are popular, but factors such as the relative merits of 180 degree or 360 degree rotations remain unresolved (Coleman et al, 1982, Tamaki et al, 1982 and Raymundo et al, 1985).

The specificity of SPECT in the detection of Tl-201 uptake defects seems to be identical to that of planar imaging but the sensitivity, both at rest and after stress, and the sizing and localization of such lesions seems to be superior with SPECT (Maublant et al, 1982 and Tamaki et al, 1982). In patients with 3 vessel disease, sensitivity of SPECT was higher than planar imaging, with a significant difference for the left circumflex coronary artery (Nohara et al, 1984). In a more recent study, it has been shown that exercise Tl-201 SPECT carried out with a slant hole collimator has a good sensitivity in the detection of individual coronary lesions but only a fair sensitivity in the detection of multi vessel involvement by perfusion defects only. The addition of the heart lung ratio increases the sensitivity for multi vessel disease and should be used in all Tl-201 SPECT myocardial scintigraphy (Schneider et al, 1985). Studies with SPECT in patients with myocardial infarction have shown that right ventricular infarction can be demonstrated (Kotter et al, 1982), infarct size estimates agree more closely than similar measurements from planar imaging with those provided by serial CK-MB levels (Tamaki et al, 1982), and measurements of myocardial blood flow agree closely with those made after injection of Tc-99m labelled macro aggregate particles (Caldwell et al, 1982).

The use of tomographic Tl-201 myocardial perfusion scintigraphy with intravenous dipyridamole stress has also been

reported (Francisco et al, 1982). Dipyridamole, a powerful coronary vasodilator, has been suggested as an alternative to physical exercise for myocardial perfusion imaging with Tl-201 (Leppo et al, 1982). The theory of its use is that the vasodilation induced by the drug will greatly increase myocardial blood flow in regions perfused by normal coronary vessels but not in myocardial zones supplied by stenotic vessels (coronary steal), which will result in an inhomogeneity of regional perfusion and can be detected by Tl-201 scintigraphy. Francisco et al (1982) using this method in 86 patients with chest pain, demonstrated 90 percent sensitivity for detecting CAD and a 96 percent specificity in patients with normal or non significant CAD by tomographic Tl-201 scintigraphy. The authors concluded that overall, the predictive accuracy of an abnormal scintigram with quantitative tomographic imaging (98 percent) was better than qualitative planar or qualitative tomographic image analysis.

SPECT can also be used in the assessment of myocardial perfusion prior to and after percutaneous transluminal coronary angioplasty (Kirsch et al, 1985, and De Puey et al, 1986) and after coronary thrombolysis following acute myocardial infarction (Spielmann et al, 1985). These studies confirmed that SPECT is more sensitive than planar images in the detection of perfusion defects particularly in the inferior wall of the myocardium which are missed by planar scintigraphy. They also showed that it provided an excellent means to quantify ischaemia and to assess the success of these therapeutic procedures.

The major limitation of SPECT, when quantitative analysis

is applied is the lack of an adequate photon attenuation correction. This problem with SPECT is particularly important with relatively low energy tracers such as Tl-201. Development of new radiopharmaceutical agents with better photon energies, like Tc-99m labelled compounds, should expand the applicability of SPECT in this field.

Hence, the implementation of radionuclide techniques has been partially successful in providing better diagnosis and assessment of cardiac pathology but the methods have not made a major impact. This can be explained by physical factors. The poor imaging properties of thallium-201 for example, have meant that the test has not been universally accepted. As it becomes increasingly clear that myocardial perfusion scanning has a great potential in the diagnosis of CAD, new agents have to be found. One such approach is the development of a compound with Tc-99m properties in order to overcome the current limitations of Tl-201 scintigraphy. Recent reports in animals have indicated that there are certain potential agents that can be studied to assess myocardial perfusion. However, many more experimental investigations need to be performed and, in particular, the clinical assessment of these agents in patients is required. The work described in this thesis is aimed at achieving this purpose.

CHAPTER 2 Tc-99m TERTIARY BUTYL ISONITRILE (T-BIN); DEVELOPMENT AND ANIMAL WORK

(A) Introduction

Thallium (Tl)-201 is currently the isotope of choice for myocardial imaging. However, relative to Tc-99m, it is a poor radionuclide for procedures in nuclear medicine. As discussed in chapter I, it has a photon energy that is too low for optimum imaging, and the absorbed radiation dose is high due to its long half life (Poe, 1977).

It has been suggested (Deutsch et al, 1981) that positively charged complexes of Tc-99m might mimic the in vivo behaviour of group I cations (e.g. potassium and rubidium), which accumulate in oxygenated myocardial cells by an active transport across the cell membrane determined by the Na+/K+ ATPase pump and are taken up by the normal myocardium of animals.

Deutsch et al (1981) reported an intensive investigation of the biological properties of several octahedral Tc (3+) compounds of the type (Tc-99m L_2 X_2 where L is the bidentate ligand o-phenylene-bis (dimethylarsine)(diars) or bis (1,2-dimethylphesphino) ethane (DMPE) and x = Fluorine, chlorine, bromine or iodine. These chemicals were taken up into the myocardium of a variety of animal species including higher primates. However, when one of these radio tracers , DMPE was tested in humans, the results were poor because of low myocardial uptake, high liver and background activity and visualization of the ribs and sternum which contributed to difficulties in defining the myocardium (Dudczak et al 1983). The group concluded that Tc-99m DMPE was of very little value as a myocardial imaging agent.

At the start of this project, there were reports of

several of the hexakis (alkylisonitrile) technetium (I) cations that had recently been synthesized and were taken up by the normal myocardium in a number of animal species; normal anaesthetized rats, rabbits, dogs and rhesus monkeys (Jones et al, 1982 and 1984). Tissue analysis in mice showed one of these compounds, Tc-99m tertiary butyl isonitrile (T-BIN) was taken up by the myocardium in 5-10 minutes after I.V. injection and the myocardial concentration was maintained for two hours. After intra coronary injection there was no measurable myocardial washout for up to one hour (Pendleton et al, 1984). These favourable results suggested that Tc-99m T-BIN might be suitable for myocardial scanning. This thesis reports the work that has been performed to assess the value of T-BIN as a myocardial scanning agent in patients. The studies were performed in conjunction with the following departments:-

1. Nuclear chemistry, Loughborough University (Dr. John Thornback).
2. Medical physics (Mr. Mike Early) and Radiopharmacy (Miss Gill Hartley), Leicester Royal Infirmary.
3. Biomedical studies, University of Leicester (Dr. David Morton).
4. Radiology (Dr. Jonathan Berry) and Cardiology (Dr. Peter Hubner), Groby Road Hospital, Leicester.

(B) Materials and methods

(i) Formulation of Tc-99m T-BIN(Given by Dr. John Thornback).

Details of the exact formulation proposed by Davison and co workers were not available at the time of commencement of this project but general details were established, namely, the use of dithionite solution as a reducing agent.

$$TcO_4^- + {}^tBuNC \xrightarrow{S_2O_4} Tc(CN^tBu)_6^+$$

Due to the insolubility of T-BIN, ethanol had to be present in the solution.

(ii) Preparation of Tc-99m T-BIN (Given by Miss Gill Hartley).

1. 120 mg of sodium dithionite ($Na_2S_2O_4$) was weighed into a clean plastic dine.

2. In a fume cupboard 0.1 ml of T-BIN was added to a tared insulin vial.

3. All the equipment used was cleaned and sprayed with 70 percent methylated spirit and placed in the transfer hatch.

4. Inside the clean room the equipment was sprayed into the laminar air flow cabinet. The following steps (5-8) were carried out using aseptic technique and all subsequent vials were sterile.

5. Sufficient sterile absolute alcohol (ethanol) was added to the weighed T-BIN to produce a 6 percent solution which was then filtered through a 0.22 micron hydrophobic filter into a sterile vial.

6. 0.1 ml aliquots of this sterile 6 percent T-BIN in ethanol solution were placed into 10ml vials.

7. 4 mls of sterile 0.04M sodium hydroxide were added to the sodium dithionite in the dine. Once the powder had dissolved it was filtered through a 0.22 micron filter into a sterile vial.

8. Finally 0.4 mls ethanol, 0.5 mls water for injection (Analar water), 0.5 mls sodium dithionite in 0.04M sodium hydroxide (from step 1 and 7) and 0.7 - 1.2 mls sodium

pertechnetate injection containing 200 MBq activity were added to the 0.1 ml of 6 percent sterile T-BIN.

9. The vial was mixed and placed in a boiling water bath for 15 minutes, then removed and cooled. The contents were then neutralized using 0.02 molar aqueous hydrochloric acid.

During the work at the nuclear chemistry department, Loughborough University, to prepare the compound, it was found by Dr. J. Thornback that Tc-99m T-BIN adhered to both glass and plastic. 60 percent of activity was lost with ordinary glass vials, but sterile nitrogen filled insulin vials supplied by Mallinckrodt Diagnostica U.K. only retained 10-15 percent. It was also found that about 20 percent of the activity could be lost in in the syringe used for injection due to retention on the plastic wall. To minimise this effect the dose was drawn up immediately before injection. (This data was given by Miss G. Hartley, Radiopharmacist, Leicester Royal Infirmary).

(iii) Quality control

Paper chromatography and Instant Thin Layer Chromatography - Silica Gel (ITLC-SG) were adequate for the separation of the "complexed" technetium from reduced technetium and pertechnetate. Two basic chromatographic systems were used at the Radiopharmacy Department, Leicester Royal Infirmary, to check labelling efficiency:-

System I

ITLC-SG with 0.9 percent sodium chloride as solvent.

In this system the complex had Rf of 0.05 and 0.12 and the free pertechnetate had Rf of 1.0.

System II

ITLC-SG with butane-2-one as solvent.

The complex had Rf of 0.9.

Free pertechnetate had Rf of 0.9.

Reduced technetium had Rf of 0.

where Rf = $\frac{\text{Distance travelled by the compound}}{\text{Distance moved by the solvent front}}$

These methods afforded a simple way of estimating complex formation. The labelling efficiency was found to be consistently greater than 90 percent and generally close to 100 percent.

Although the chromatographic separation was always identical initially, it was noted several times that the Rf in system II might move downwards with time. This was not a consistent result and no explanation for it has been found. Additionally, some cloudiness sometimes occurred in the final solution in which case it was then discarded. As this may have been due to old diluted isonitrile - ethanol solution, a fresh isonitrile solution was used in the subsequent studies.

(iv) Uptake by Tc-99m T-BIN in canine heart (Carried out with Dr. Peter Hubner, Dr. David Morton and Mr. Mike Early).

Seven greyhounds (mean weight 26 kg) were studied. Dogs 1 - 4 were normal controls and dogs 5 - 7 underwent coronary artery ligation through a left thoracotomy under general anaesthesia using oxygen, nitrous oxide and fluothane. The left anterior descending coronary artery was ligated in its middle portion, just distal to the edge of the overlying left atrial appendage; this produced a moderately large anteroapical infarct. The chest wound was closed. The animals were allowed to regain consciousness and become haemodynamically stable.

One hour after coronary artery ligation they were given T-BIN.

All dogs (1-7) were imaged under sedation with diazepam and a mixture of etorphine and methotrimeprazine, 0.068 mg/ml and 18 mg/ml respectively (Small Animal Immobilon C-Vet Ltd.). Scintigraphy was performed using a gamma camera (Siemens ZLC) fitted with a low energy general purpose parallel hole collimator.

Administered doses ranged from 16 to 110 MBq (mean=50). From the time of injection serial cardiac scintigrams of 60 or 90 seconds duration were obtained in the anterior (ANT) view for 90-120 minutes in dogs 1 - 4 and in the 45 degrees left anterior oblique (LAO) view for 70 - 90 minutes in dogs 5 - 7. The data obtained were recorded on Scintiview (floppy diskette) and on Nodecrest Medical system using matrix size 64 x 64 x 8 bit. Time activity curves were produced from the regions of interest (lung, background, liver and myocardium). These were drawn on the summed images from early or late frames in the acquisition, either from the scintiview recorded data (using light pen) or the Nodecrest system (using key-board cursor).

Following the dynamic studies, 5 minute static images were performed in the left lateral (LLAT), 45 degrees LAO and ANT views in all dogs. Delayed views were also obtained in dogs 5 and 6 with infarction, at 6 hours and 8 hours respectively. All static images were recorded for post processing on Scintiview using matrix size 256 x 256 x 8 bit.

One minute after injection, 3 ml blood samples were taken into lithium heparinised vials from each animal at regular intervals, over a period of 2 hours. The red blood cells were spun down and the radioactivity in 1 ml plasma was counted in

an automatic well-crystal scintillation counter (Philips PW 4520). Corrections for radioactive decay were made to produce the plasma clearance curves in dogs 5, 6 and 7. At sacrifice tissue samples were taken from the myocardium (viable and infarcted), liver, spleen, kidney and gonads. The samples weighed between 0.6 - 5.3 gm and were counted twice in a well counter. In dog 6 samples from the thyroid, and 5ml each of bile and urine were also counted..

(C) Results

(i) Dynamic images (Fig.2.1)

The lung, liver and spleen were the first organs to appear immediately after injection. The lung uptake was significantly high in the first few minutes after injection and the heart could not be identified. With time activity in the lung gradually cleared and a uniform accumulation of the complex was seen within the ventricular myocardium. At about 30 minutes post injection activity started to accummulate in the kidney which gradually increased with time. At 45 minutes the doughnut appearance of the normal heart and viable parts in dogs with myocardial infarction were clearly seen. By that time activity in the lung had declined significantly which resulted in better definition of the myocardial image (Fig.2.1). Hepatic uptake remained high throughout the study, and in 3 dogs overlapped the inferior surface of the myocardium.

(ii) Time activity curves

Organ activity ratios were derived from the regions of interest drawn on the static AP images. Different background regions of interest were used adjacent to the heart and the

liver regions of interest.

All the curves were normalised and background subtracted to give the net liver and heart activity curves over the regions of interest. In dog 1 as an example (Fig.2.2) the myocardial activity steadily increased to a maximum at about 60 minutes after injection (Fig.2.2,b). At 90 minutes the level of activity in the heart was approximately twice the background (Fig.2.2,a). After correction for background hepatic activity remained relatively high at 2-3 times the myocardial level (Fig.2.2,b).

(iii) Static images

Image processing consisted of altering the grey scale so that the cardiac image just avoided saturation. No background subtraction was used.

In dogs 1-4, the normal myocardium, liver and kidneys were seen and identified in all views (Fig.2.3). In dogs 5-7, the infarction was detected as an area of absent activity at the apex on the LLAT projection (Fig 2.4,c,d) and as reduced activity of the apex on the ANT and LAO projections (Fig.2.5,e,f). The infarcts were still detected in dogs 5 and 6 at 6 - 8 hours after injection. Loops of the intestine could be seen at 3 - 4 hours after injection.

(iv) Plasma clearance curves (Fig.2.6)

As the amount of each dose varied, the number of counts/minute/ml of plasma/MBq injected was plotted against time. There was a rapid drop in the level of activity with only about 10 percent of the initial activity remaining in the plasma at 10 minutes post injection.

(v) Tissue samples (Dogs 5-7)

No correction for blood activity was made but there was a considerable clearance of the radioactivity from plasma by this time. The data obtained (table 2.I, Fig.2.7) showed that the ratio of the number of counts/gm of tissue in the viable and infarcted myocardium varied from 2:1 to 11:1 with a mean ratio of 3:1. High counts were obtained from the liver and bile. Although the counts in the kidney were also high, the urine counts were low.

(D) Discussion

The studies in this chapter have described the development of a new myocardial imaging agent, Tc-99m tertiary butyl isonitrile (T-BIN) complex, which was first introduced by Jones and Davison (Jones et al, 1982). The methods used for its preparation have been shown to be simple, feasible and very reliable. The compound was prepared under strict aseptic conditions using sodium dithionite reduction of pertechnetate. Despite the lack of an HPLC system, quality control using paper chromatography and ITLC-SG indicated that the compound could be synthesized routinely with greater than 90 percent chemical yield.

Previous preliminary biological studies with Tc-99m T-BIN showed that it possessed some remarkable properties in animals. When injected intravenously into rats and dogs, the compound gave good images of the heart after initial clearance of the activity from the lung (Jones et al, 1982). The group also demonstrated in one dog with an experimental myocardial infarction that the remaining viable tissue was well delineated. Rayudu et al (1983), have reported in mice and

dogs that compared with Tl-201, Tc-99m T-BIN showed better myocardial localization but more hepatic interference. They concluded that Tc-99m T-BIN appeared more promising than Tl-201. The studies described in this chapter have shown that Tc-99m T-BIN is taken up by the normal canine myocardium and may be used to scan the heart. The scans also allowed the detection of experimentally induced antero-apical myocardial infarcts.

There was a rapid fall in plasma activity (Fig.2.6), indicating prompt transfer of the compound into the extra vascular space. Although imaging studies (Fig. 2.2,a) showed an immediate uptake by the myocardium it was difficult to visualize the heart until 30 minutes after injection as it was obscured by the lung activity. The reason for visualizing the high lung uptake is uncertain but may be due to sticking of the compound to the endothelial lining of the pulmonary capillary bed during its first pass. At 60 minutes, myocardial activity reached a plateau and background activity from the lung had declined. At that time, the myocardium was clearly seen with a heart to background activity ratio of 2:1. At one to one and a half hours after injection, satisfactory static images were obtained and late scans performed up to 8 hours were also adequate. The hepatic uptake was high, with a liver to heart ratio of 3:1. Despite this, the heart could be seen clearly and separately from the liver. The hepatic uptake might interfere with the detection of inferior infarcts.

Jones et al (1984) studied the biodistribution of the compound in mice. The group showed that the liver uptake was marked with levels above 30 percent of the injected dose being

maintained up to 120 minutes after injection. This was attributed to the lipophilic nature of the compound. Approximately 1.2 to 1.4 percent of the administered material (compared to 4 percent with Tl-201) localized in the myocardial tissue and this appeared to be retained for several hours. In a study by Hamilton (1978), quantitative in vivo tissue distribution of Tl-201 in unanaesthetized dogs demonstrated that myocardial uptake was 50 percent more than that in the liver, lung and skeletal muscles at 30 minutes post injection (Hamilton et al, 1978). It has also been reported that compared with Tl-201, Tc-99m T-BIN cleared more slowly from the whole blood of dogs (Rayudu et al, 1983). These data and the results obtained in this chapter suggest that the pattern of plasma clearance and organ distribution of Tc-99m T-BIN is quite different from that of Tl-201. Also they indicate that the extraction fraction of Tc-99 T-BIN by the myocardium in animals is about 50 percent of that of Tl-201. Despite this, good myocardial images were obtained with T-BIN and showed better localization than Tl-201 (Rayudu et al, 1983). This could be due to the higher photon energy of Tc-99m which gave rise to a better definition of the myocardial image.

Tissue samples obtained in this study showed high concentration of the activity in the liver, bile and kidney but they were low in the urine (table 2.1 and Fig.2.7). The reason why the kidneys were seen could be due to the low molecular weight of the label (620) and its filtration through the glomerulus. The liver also seems to extract Tc-99m T-BIN which may contribute to its excretion in the bile and the colon.

The mechanism of uptake of Tc-99m T-BIN at the cellular

level is different from that of Tl-201 as it is not Na+/K+ ATPase pump dependant. The uptake of the compound by rat myocytes in cultures was not inhibited by either oubain or K, both recognised Na+/K+ ATPase inhibitors (Pendleton et al, 1984). The exact mechanism of uptake has not yet been explained. Jones et al (1984) have suggested that the lipophilic cations of Tc-99m interact with the membranes of certain cells, possibly due to their positive charge.

The principal role of a myocardial imaging agent is in the assessment of coronary artery disease. The cardiac images with Tc-99m T-BIN were of sufficient quality to allow the detection of antero-apical infarcts, 2 hours after production of the infarct and 1 hour after injection of the compound. These experimental studies showed promise, and allowed the next stage of assessment of Tc-99m T-BIN in man to be undertaken.

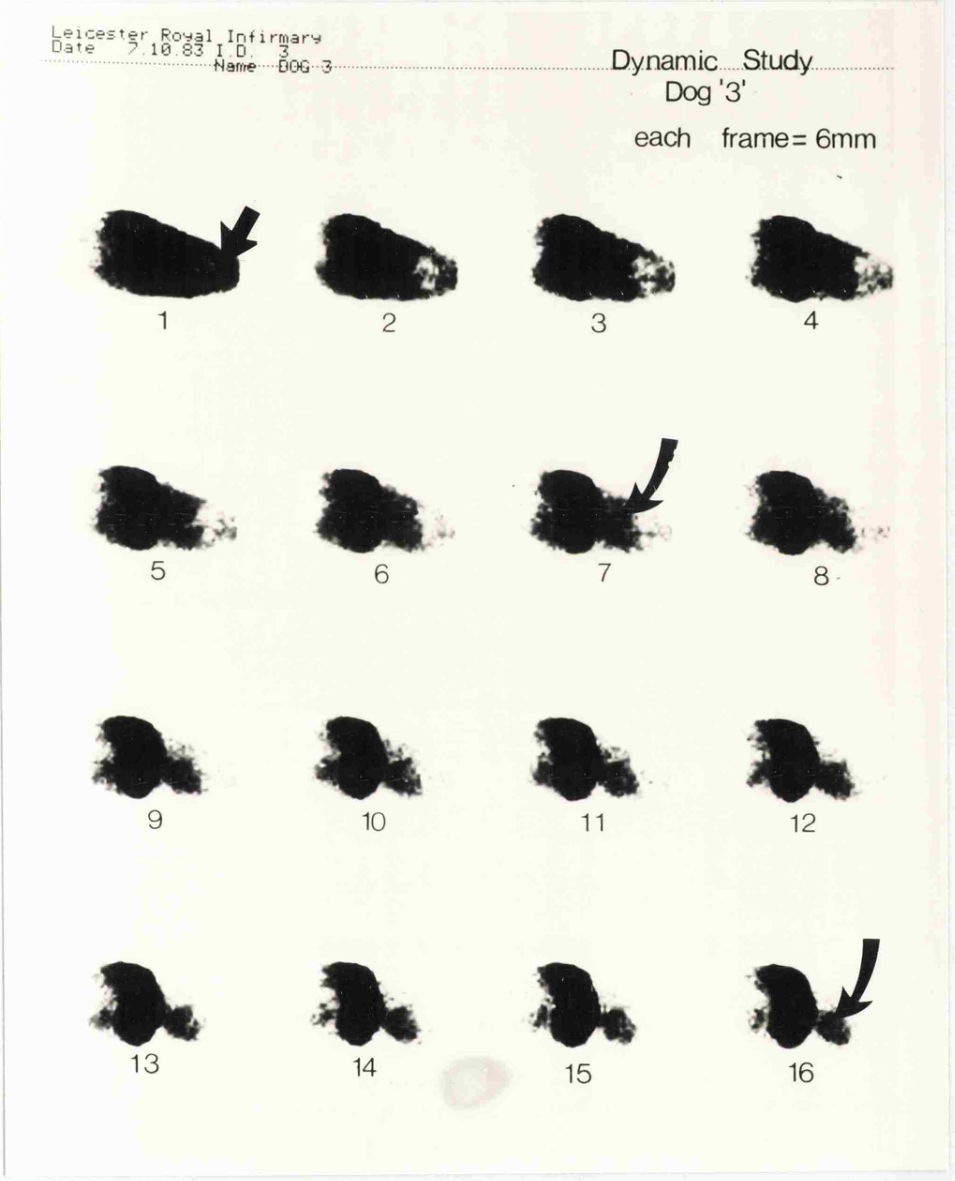


Fig. 2.1

6 minute serial cardiac scintigrams (1-16) in a normal dog showing the initial high uptake of Tc-99m T-BIN by the lung (small arrow) which obscures the heart. A uniform accumulation of the compound is clearly seen within the ventricular myocardium (large arrows) after clearance of the lung activity.

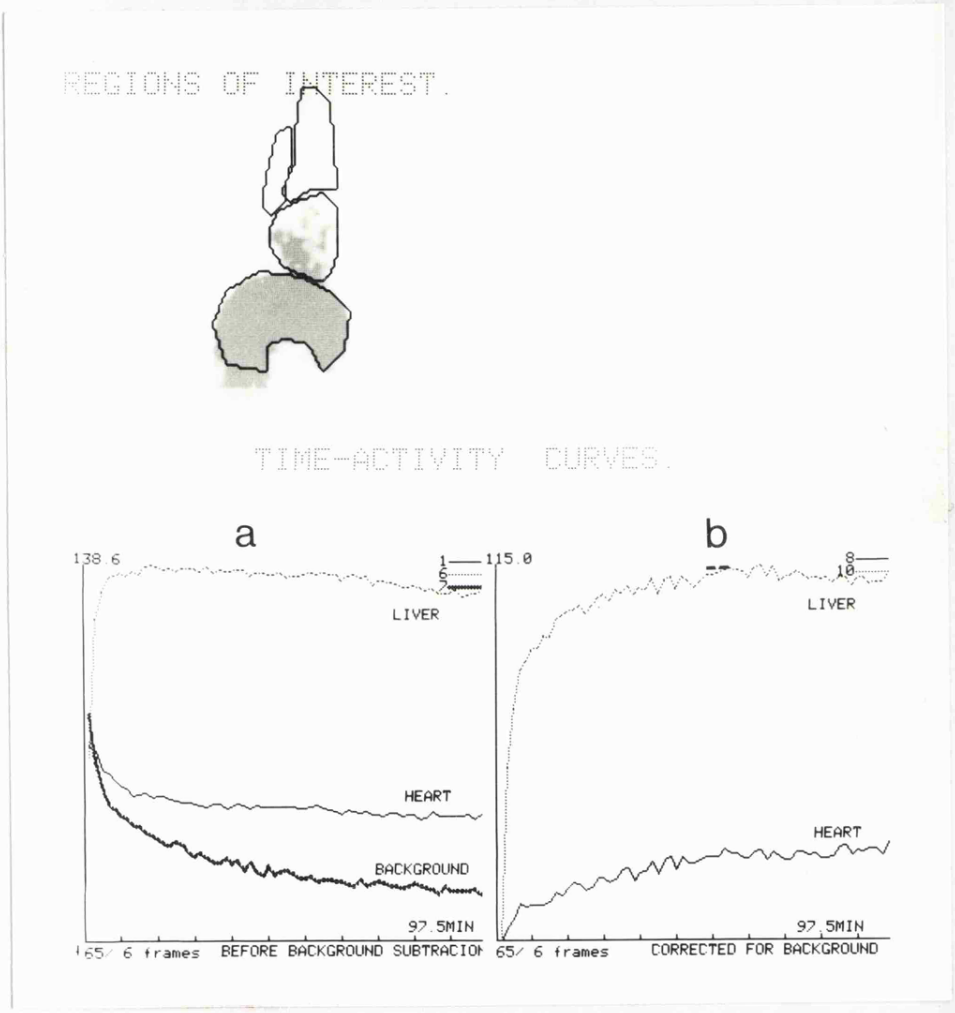


Fig. 2.2

Time activity curves for the regions of interest obtained in a normal dog after the administration of Tc-99m T-BIN. Marks along the horizontal axis denote 10 minute intervals.

(a) Before background correction.
(b) After background correction.

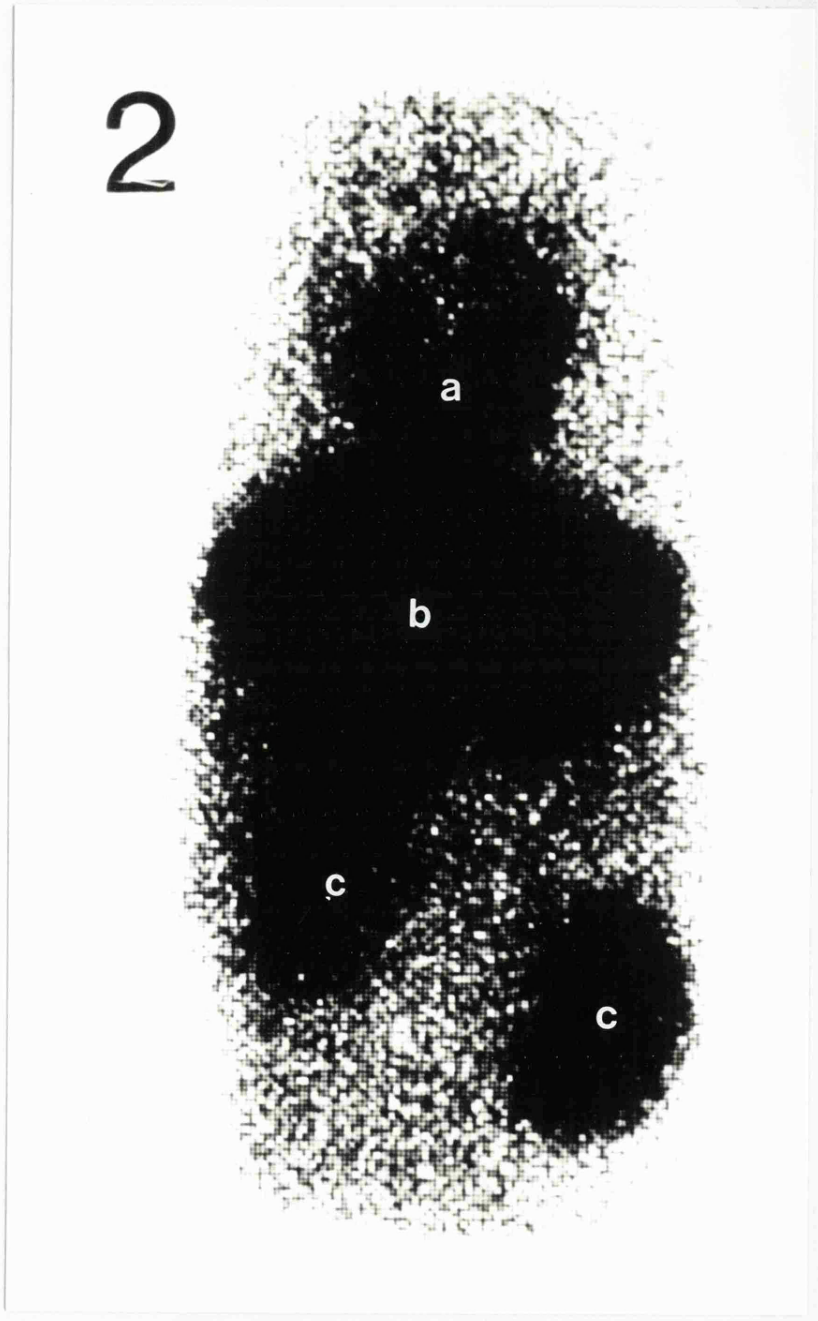


Fig. 2.3

Static Tc-99m T-BIN scan obtained in a normal dog (Number 2) following a dynamic study.
(a) Heart (b) Liver (c) Kidneys

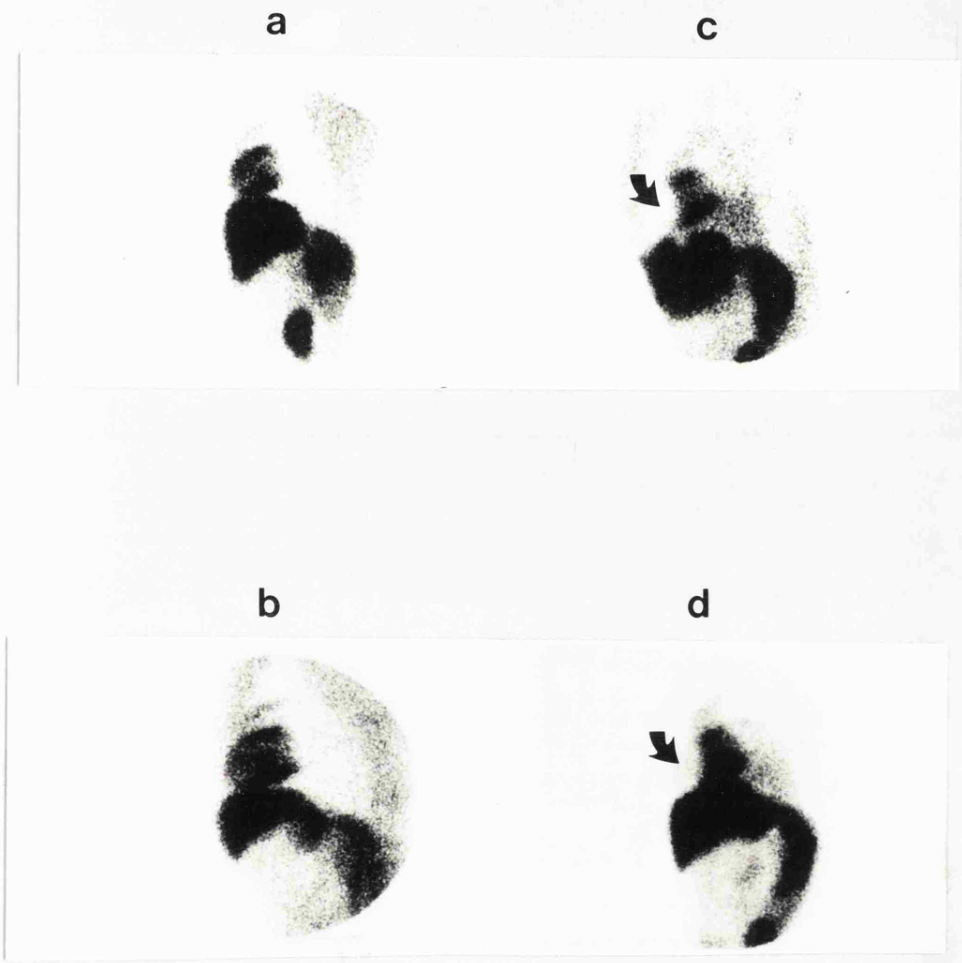


Fig. 2.4

Static imaging with Tc-99m T-BIN in left lateral views.
Normal: (a) dog 1 (b) dog 2
After myocardial infarction (arrows):
(c) dog 5 (d) dog 6

Dog 1 Dog 5

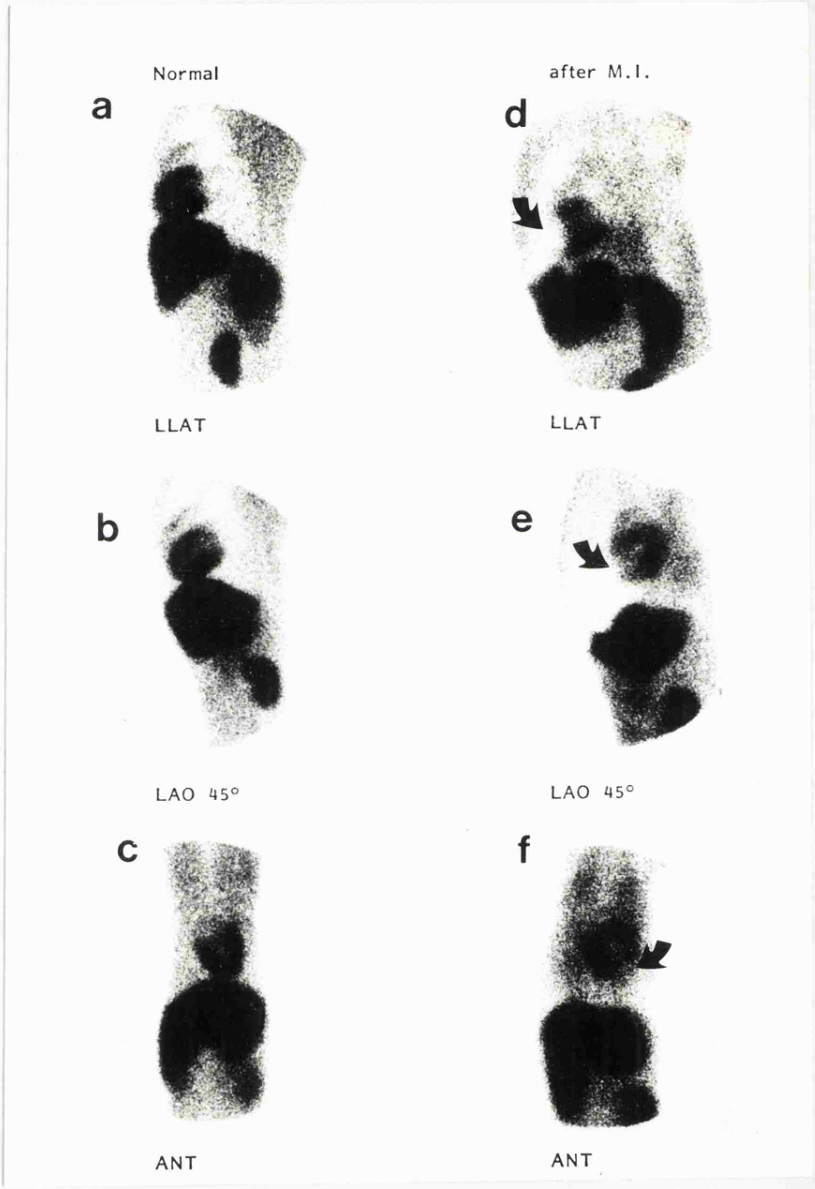


Fig. 2.5

Static imaging with Tc-99m T-BIN.
Normal: [in dog 1] (a) LLAT (b) LAO 45° (c) ANT
After myocardial infarction (arrows):
[in dog 5] (d) LLAT (e) LAO 45° (f) ANT

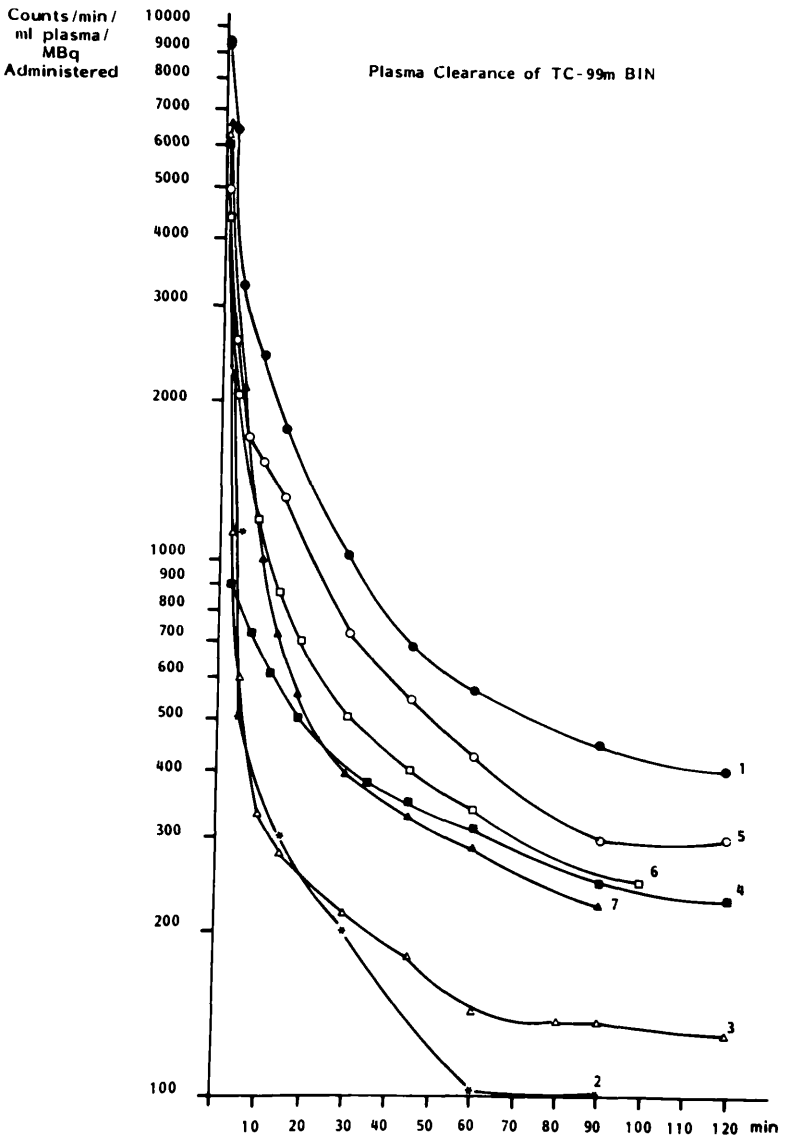


Fig. 2.6

Plasma clearance curves of Tc-99m T-BIN in dogs (1-7) showing a rapid drop in the level of activity to approximately 10% of the initial activity at 10 minutes post injection.

Number of counts/gm of tissue

Dog	Time*	Heart		Liver	Spleen	Kidney	Gonads	Thyroid	Bile	Urine
		Normal	Infarcted							
5f	8 hr	7794	2773	6568	-	11717	614	-	-	-
6f	6 hr	6107	3016	9773	2244	12821	569	3392	12328	290
7m	2 hr	4696	402	7424	3731	4774	7248	-	-	-

*. Time after injection of Tc-99m T-BIN

m = male
F = female

Table 2.1

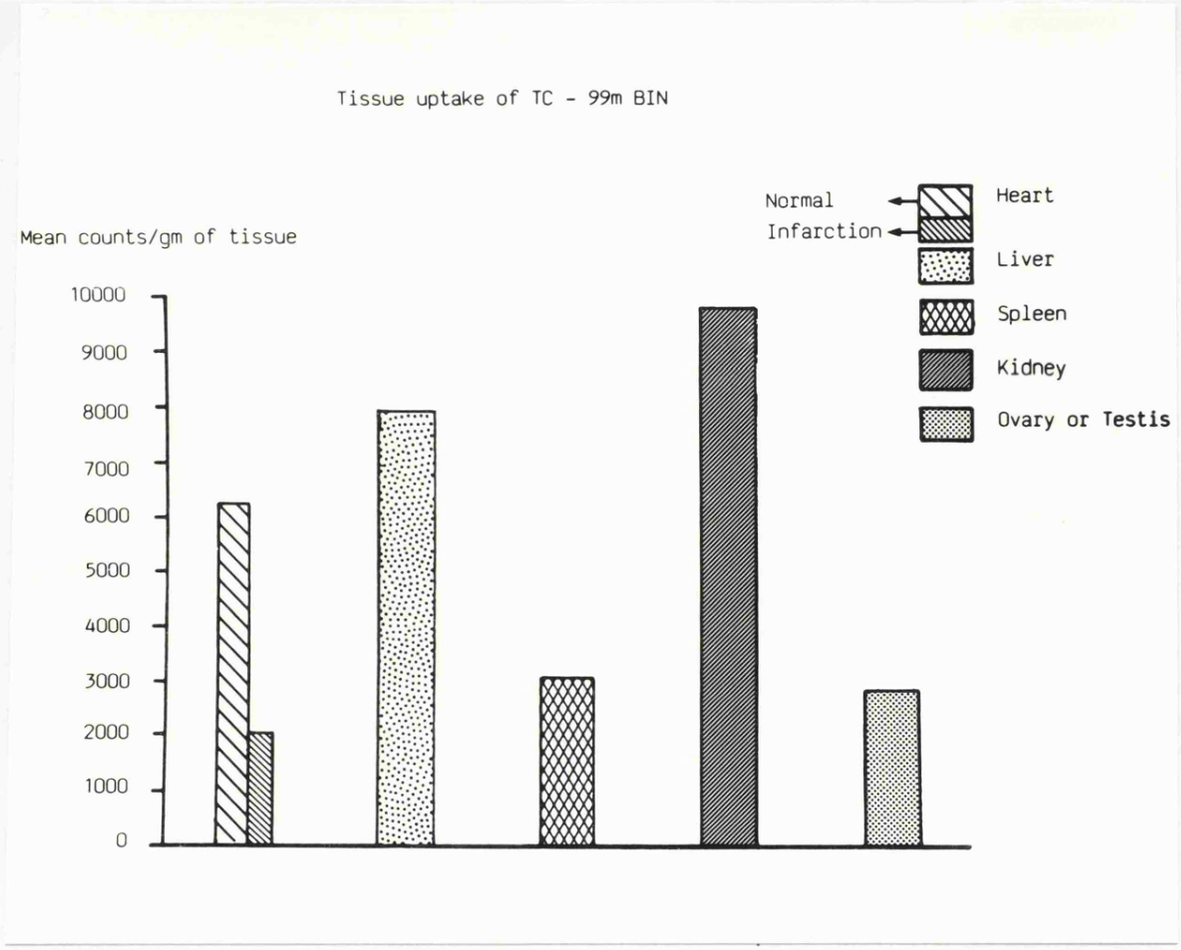

Tissue uptake of TC - 99m BIN
Mean counts/gm of tissue
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Normal
Infarction
Heart
Liver
Spleen
Kidney
Ovary or Testis

Fig. 2.7

CHAPTER 3 ASSESSMENT OF Tc-99m T-BIN IN NORMAL HUMANS

(A) Introduction

The biological characteristics of Tc-99m T-BIN in dogs recorded in the previous chapter suggested that the compound must be suitable for use as a cardiac scanning agent in man. Approval from the Leicester Area Health Authority Ethical Committee was sought and granted before each stage of the work in human volunteers and patients. The work in this chapter assessed the following parameters in normal human subjects:-

(1) Imaging time and projections.

(2) Analysis of scans.

(3) The potential effects of the compound on the functions of the vital organs, as information about toxicity was not available.

(B) Materials and methods

(i) Subjects

Six males and four females, aged 23-59 years (mean 39) were studied. Five were normal volunteers with normal clinical examination, normal electrocardiogram (except one with right bundle branch block) and negative exercise test. There were five patients presenting with recurrent persistent atypical chest pain. Each patient had a normal exercise test to a maximal level. Three had normal coronary arteriograms, one of the two without an arteriogram, had also a normal exercise thallium scan. All the five patients had a normal resting electrocardiogram and they did not show any clinical evidence of cardiopulmonary disease.

The details of the test were explained to each subject before carrying out the study and informed consent was obtained. Each of the 5 volunteers had two separate studies, at rest and after a multigrade treadmill exercise using the

Sheffield protocol. In the five patients studies were performed after exercise. All imaging acquisition and computer processing were done by the attending staff of radioisotope service at Groby Road Hospital and Leicester Royal Infirmary.

(ii) Imaging protocol

A gamma camera fitted with a low-energy all-purpose parallel or converging collimator was used. A No 18 butterfly needle (Abbot laboratories)was inserted into an arm vein. 100-150 MBq of Tc-99m T-BIN were administered to each subject per study. Five minute serial scintigrams beginning 5-10 minutes post injection were obtained for 60-90 minutes in the 45 degree left anterior oblique (LAO) modified with 20 degree cranial tilt. Time activity curves were produced for heart, lung, liver and background by taking the average activity per pixel in each region normalised to the largest area. In 4 volunteers, 5 mls venous blood samples were collected at 1, 3, 5, 10, 15, 30, 60, 90 and 120 minutes after injection. The plasma was separated and activity was counted by a gamma counter to determine plasma clearance curves (as described in Chapter 2). At the end of the dynamic study, 7 minute static views were obtained in the same modified LAO projection and the LAO 60 degree, anterior (ANT) and the left lateral (LLAT) views. These were repeated at 3-5 hours after exercise. All images were stored on a computer using matrix size 128 x 128 x 8 bit. Imaging of the arms in 2 subjects was also done immediately and at 4 hours after injection. In two cases images also of neck, lower abdomen and thighs were performed to aid in dosimetry calculation.

(iii) Biochemical and biological measurements

These were undertaken on venous blood drawn in 3 normal volunteers to study the immediate and short term effects of the complex on the vital organs. Blood samples were collected immediately before and after injection and were repeated 3-4 times over a period of 7 days. They were analysed by the laboratories at Groby Road Hospital or Leicester Royal Infirmary for:-

-Full blood count, total and differential.

-Cardiac enzymes (alanine amino transfrase, aspartate amino transfrase, lactate dehydrogenase, alpha hydroxy acid butyrate, creatine phosphokinase and isoenzyme MB of creatine phosphokinase).

-Liver function tests (serum bilirubin, alkaline phosphatase and gamma glutamyl transferase).

-Kidney function tests (Urea, electrolytes and serum creatinine).

-Serum calcium, phosphate, urates, total protein and albumin.

-Thyroid function (in one subject).

(C) Results

(i)Dynamic images

The lung, liver and spleen were the organs to appear immediately after injection. In all subjects, initial uptake in the lung was high and obscured the myocardium (Fig.3.1, 3.2). There was a gradual clearance of the activity from the lungs, and a gradual appearance of the left ventricular myocardium. At rest, the myocardium was clearly seen at 45-60 minutes after injection. On exercise, particularly at maximal level, more rapid clearance of the activity from the lungs

allowed the visualisation of the heart at 20-30 minutes (Fig.3.2). A uniform uptake of the activity was seen throughout in the left ventricular wall and in some subjects the right ventricle could also be identified.

Time activity curves for the regions of interest showed that myocardial uptake increased slightly with time (Fig.3.3, 3.4). The liver uptake gradually increased and reached a plateau at approximately 30 minutes after injection. At 60 minutes the liver to heart ratio was about 4:1 at rest (Fig.3.3) and 2:1 after exercise (Fig.3.4). The lung uptake was high after injection but it cleared gradually with time.

(ii) Static images

The course of the vein at the site of injection was seen up to the axilla (Fig.3.5,a). Serial scans of the same arm at 1 and 2 hours showed gradual clearance of activity with time (Fig.3.5,b,c). The vein on the contralateral side could not be seen.

The liver uptake was high but it did not interfere with the myocardium in the ANT view and the 45 degree LAO projection modified with the 20 degree cranial tilt (Fig.3.6,a,b). The latter view allowed the maximum separation between the myocardium and the liver in most of the cases. The LLAT view was found to be of no value due to superimposition of the liver (Fig.3.6,c). As an alternative the 60 degree LAO projection was used, in which myocardial borders were usually defined (Fig.3.6,d).

Delayed images obtained at 2-4 hours showed high levels of activity still remaining in the myocardium, liver and spleen (Fig.3.6). Also both kidneys and loops of the small intestine

were seen in some cases.

(iii) Plasma clearance curves

The number of counts/minute/ml of plasma was plotted against time. No correction for variations in administered activity was made as the range of doses was smaller than that used for the dogs (Chapter2). There was a rapid drop in the level of activity with only about 10 percent of the initial activity remaining in the plasma at 15 minutes post injection (Fig.3.7).

(iv) Side effects

A mildly unpleasant taste (bitter) and a smell (metallic) were experienced by all subjects immediately after injection. It usually lasted up to 1 minute. Only 2 subjects felt slight pain in the arm during injection. Sometimes the smell of the free ligand (T-BIN) could be detected by the attending persons. No other side effects were reported.

Laboratory investigations carried out on the 3 normal volunteers over one week were very satisfactory. They showed no adverse effects on the functions of the vital organs. The results obtained after the test did not show any significant changes from those obtained prior to each study.

(D) Discussion

The work described in this chapter confirms and extends previous experimental work and shows that there is a good uptake by Tc-99m T-BIN into the myocardium including that of man. With subjects who were scanned at rest and after exercise, the post exercise scans were best. This could be due to two factors, (1) increased myocardial blood flow associated with exercise which can lead to an increase in cardiac uptake of T-BIN, (2) a concomitant faster washout of the activity from the

lung and wash-in into the myocardium. This was particularly noticed at maximal levels of exercise which resulted in better quality myocardial images than those obtained at rest or after mild exercise (Fig.3.1, 3.2).

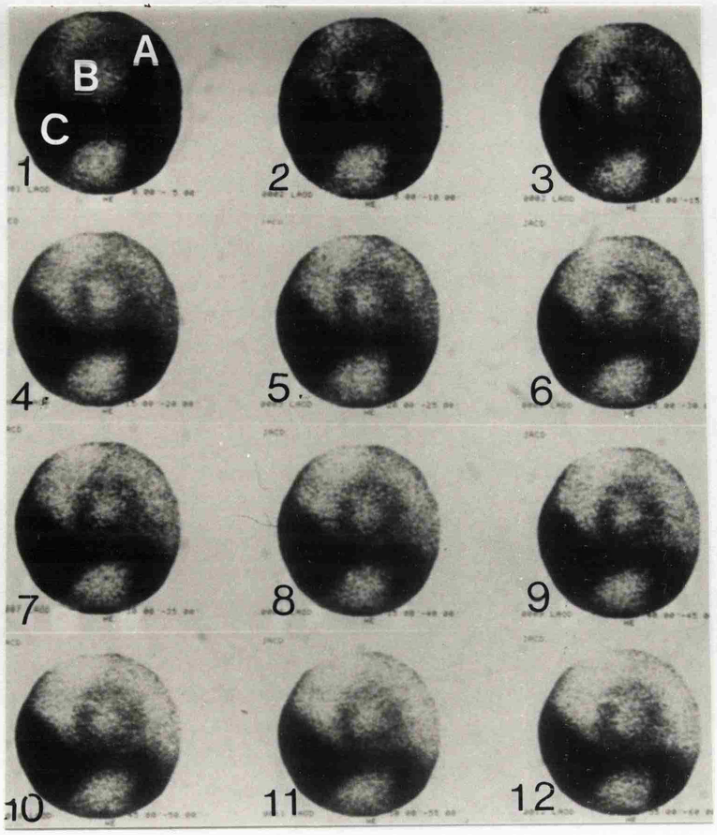
As with the canine studies there was high initial lung uptake which obscured the myocardium in all studies (Fig.3.1, 3.2). Time activity curves showed that the cardiac uptake occurred early after injection and remained constant both at rest and after exercise (Fig.3.3, 3.4). The clearance of the lung activity was the essential factor to obtain optimal myocardial images. Dynamic studies have shown that from the time of injection, satisfactory myocardial scans can be obtained after 60 minutes at rest and 30 minutes after exercise, since by then the lung activity has significantly declined (Fig.3.1, 3.2). Delayed myocardial images of the same or better quality could also be obtained up to 4-5 hours after injection, as by that time clearance of the lung was completed.

Hepatic uptake was high at rest but less marked after exercise. There was adequate separation of the heart from the liver using a modified 45 degree LAO projection with 20 degree cranial tilt (Fig.3.6,b). Hepatic activity could interfere with the detection of postero-inferior defects if the LLAT view was used (Fig.3.6,c). The ANT and the 60 degree LAO were also found to be useful views to evaluate different myocardial regions on the T-BIN scans (Fig.3.6,a,d).

Activity was seen in the kidneys and the small intestine at 3-4 hours after injection. These results agree with the animal studies (Chapter 2) and those obtained by Holman et al (1984). The appearance of the activity in these two organs

later on suggests excretion of the compound through the hepatobiliary system into the small bowel and by the kidney.

The unpleasant taste and the smell immediately after injection are probably caused by the passage of the injected material into the lung, with reaction and release of the free tertiary butyl isonitrile. The pain during injection in some patients could be due to the relatively high concentration of T-BIN in ethanol (6 percent) used as a solvent or the solvent itself. Later in this project, the method of preparation was altered as a lower T-BIN concentration in ethanol was used (6 percent to 1 percent) which resulted in a lower frequency of these side effects. There have been no other adverse effects reported during this study. Haematological and biological profiles have shown no change for one week after the administration of Tc-99m T-BIN.



5 minute serial scintigrams obtained in normal subjects. 3 main biological characteristics of Tc-99m T-BIN are demonstrated.

- Initial high lung uptake (A) which clears gradually with time.
- Good myocardial uptake (B) all through the study but appears higher on exercise.
- Relatively high hepatic uptake (C) especially at rest.

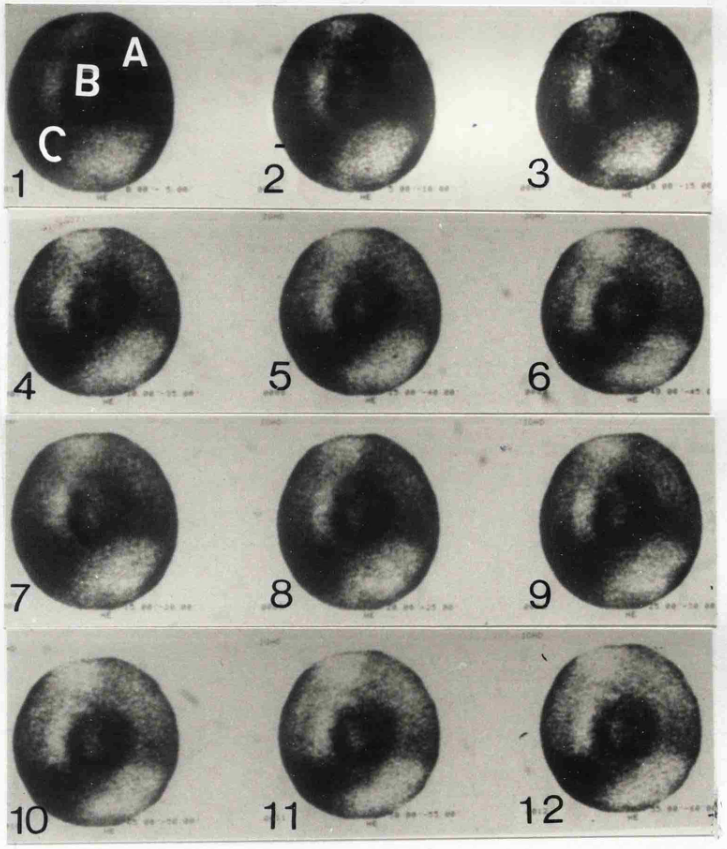
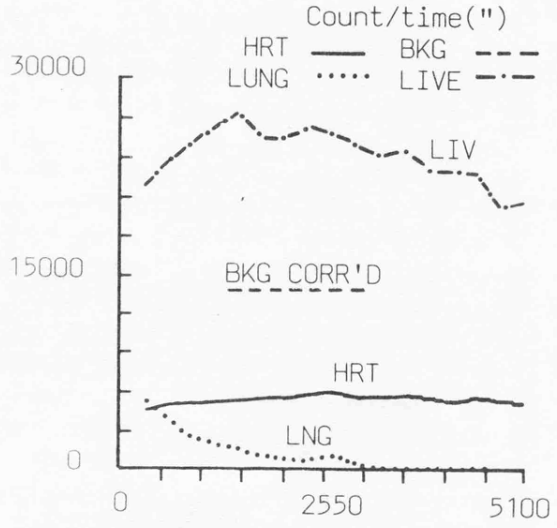


Fig. 3.1
At rest

Fig. 3.2
After exercise



Time activity curves for the regions of interest obtained after the administration of Tc-99m T-BIN.

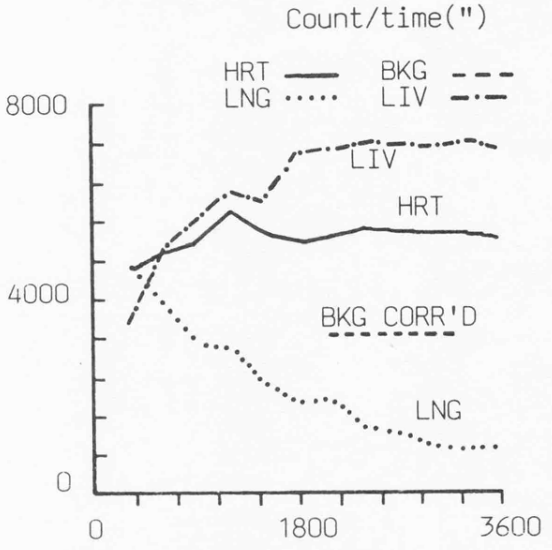
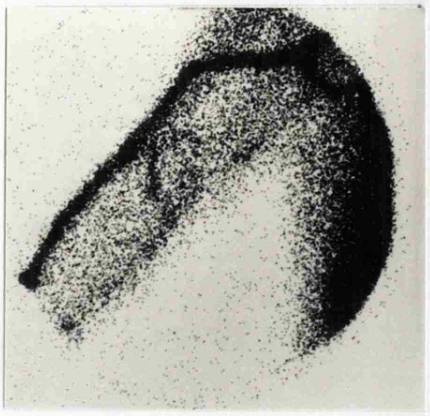


Fig. 3.3

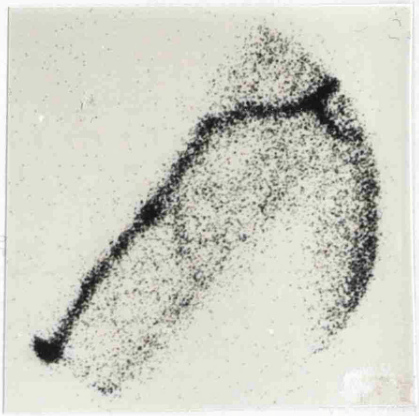
At rest.
The mean hepatic to myocardial activity ratio is approximately 4 : 1.
Early and constant cardiac uptake is demonstrated.

Fig. 3.4

After exercise.
The mean hepatic to myocardial activity ratio is approximately 2 : 1.
Early and constant cardiac uptake is also demonstrated.



(a) Immediately after injection



(b) At 60 minutes

(c) At 120 minutes

Fig. 3.5

Serial scans of an arm at the site of injection of Tc-99m T-BIN. The course of the vein is seen up to the axilla (a). Clearance of the activity with time is also demonstrated at 60 minutes (b) and 120 minutes (c) after injection.

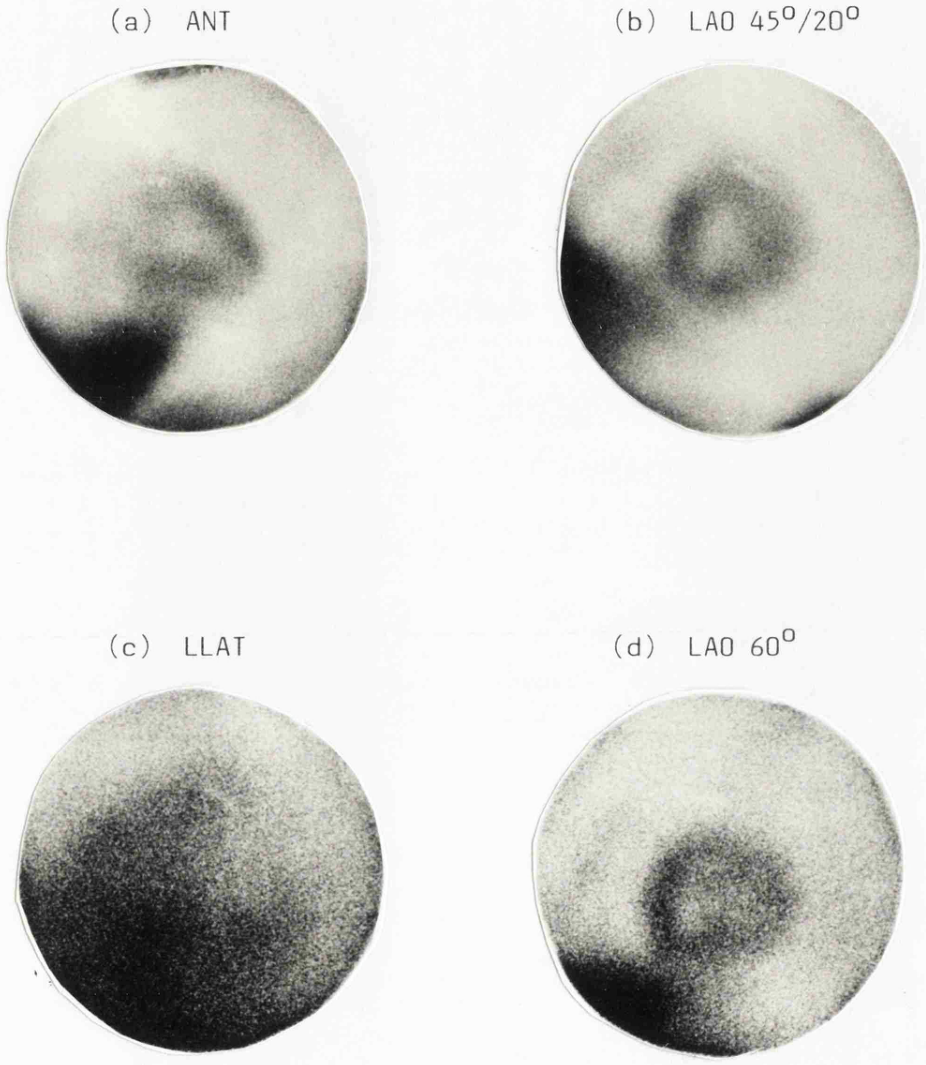


Fig. 3.6

Normal Tc-99m T-BIN scan obtained in 4 views at 4 hours after exercise. Hepatic uptake is seen high but it does not interfere with the myocardial image in the ANT (a), LAO $45^{o}/20^{o}$ (b) and LAO 60^{o} (d) projections. The superimposition of the liver on the myocardium in the LLAT view (c) is demonstrated.

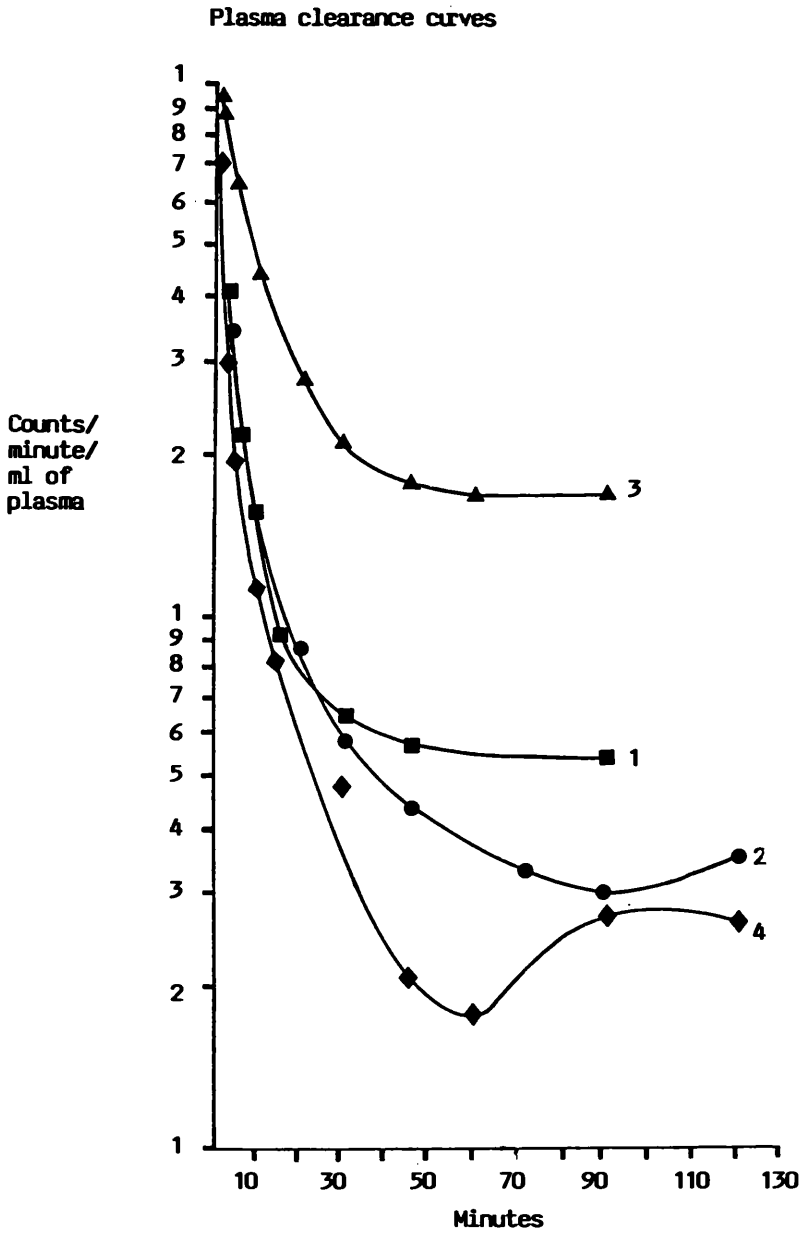


Fig. 3.7

Plasma clearance curves obtained in 4 normal humans.

CHAPTER 4 MYOCARDIAL PERFUSION IMAGING IN PATIENTS USING Tc-99m T-BIN

(A) Introduction

The work reported in the last chapter showed that Tc-99m T-BIN given intravenously localized in the myocardium of normal humans and gave good scintigrams of the normal heart. The next step was to assess the value of the agent in patients with angina and myocardial infarction (M.I.) due to coronary artery disease (CAD). The studies in this chapter were designed to find the optimal time and views to scan patients. A comparison of Tc-99m T-BIN with Tl-201 was also performed. The proposed study was approved by the Leicester Area Health Authority Ethical Committee.

(B) Materials and methods

(i) Study groups

Thirty patients (26 males and 4 non pregnant females) aged 35-73 years (mean = 57) were divided into two groups. Informed consent was obtained from each patient prior to the test after explaining the nature of the procedure.

Group I consisted of 10 patients with M.I. documented on the resting electrocardiogram (ECG). Nine of them had old (6 weeks-8 years duration; mean = 2.2 year) transmural MI (5 anterior and 4 inferior) and one had an acute anterior subendocardial MI. The 10 patients were scanned with Tc-99m T-BIN at rest. Out of the 10, 3 patients also had resting Tl-201 scans and one of the three had ECG gated cardiac T-BIN scintigraphy.

In group II, 20 patients with chronic stable angina due to angiographically proven CAD (>70 percent luminal narrowing of at least one coronary artery), were selected for the

exercise studies. There were 11 with single vessel CAD, 4 with 2 vessel CAD and 5 with 3 vessel CAD (table 4.1). Coronary angiography was done 8 weeks from the study as a part of their routine investigation. No patient in this group had any evidence of acute myocardial ischaemia at rest, co-existent valvular or congenital heart disease and/or cardiomyopathy. The stress was performed by exercising the patient on a treadmill using the Sheffield protocol. The test was terminated when a limiting symptom (eg chest pain, extreme dyspnoea, fatigue) developed. Three weeks after exercise one patient had a second injection at rest. Ten patients in this group also had a separate exercise Tl-201 scan. Both T-BIN and Tl-201 were injected at the same level of exercise and the patient continued exercising for a further30-60 seconds. The two tests were separated by at least 10 days and not more than 8 weeks. Out of the 20 patients, 5 also had exercise ECG gated T-BIN cardiac scintigraphy.

(ii) Imaging protocol

A gamma camera fitted with a low energy general purpose converging collimator was used in all patients. Prior to the test a butterfly needle was placed into an arm vein. Each patient was given 100-200 MBq of Tc-99m T-BIN intravenously. When ECG gated scintigraphy was required, the dose was increased to 400 MBq. Two patients in group I and 5 in group II had dynamic studies immediately after injection using the same imaging protocol performed on the normal subjects (chapter 2). This was followed by 7 minute static views obtained in the modified LAO 45 degree, ANT and LAO 60 degree projections. The rest of the patients in the two groups were scanned in the same

four static views at 20-30 minutes after injection for studies during exercise and 1-2 hours for studies at rest. In group II these views were repeated at 3-4 hours to study redistribution of the compound.

For Tl-201 studies, each patient was given 70-80 MBq of thallous chloride intravenously. 7 minute myocardial images were performed in the 45 degree LAO, ANT and LLAT views at 15 minutes in Group I and within 10 minutes and at 3 hours after exercise in group II.

All images were stored on a computer using matrix size 128X128 X8 bit.

ECG gated studies were acquired in the modified 45 degree LAO and the ANT projections. The imaging time was 15 minutes for each view. During data acquisition the R wave of the ECG was used to signal the beginning of each cycle which was divided into 16 equal segments. The data obtained were stored on a computer using matrix size 64 X 64 X 8 bit.

(C) Results

(i) Patients with myocardial infarction (Group I)

On T-BIN scans, 9 patients showed defects in myocardial uptake corresponding to the infarct sites. In the tenth patient, who had an inferior M.I., the inferior surface of the myocardium could not be seen due to hepatic interference. Antero septal defects were easily detected in the 5 patients with anterior transmural M.I. (Fig.4.1). The patient who had acute anterior subendocardial M.I. showed a marked decrease in tracer concentration in the regions of the upper septum and anterior wall (Fig.4.2). Despite the high hepatic uptake, defects in the postero-inferior areas of the myocardium could

be identified in 3/4 patients with inferior M.I. (Fig.4.3). This was best achieved on the modified 45 degree LAO. In the 3 patients who also had Tl-201 scintigraphy, both scans equally detected the infarction but, the defects had better definition on T-BIN scans. However, liver uptake was high with T-BIN which made the detection of inferior infarcts difficult (Fig.4.4).

(ii) Patients with angina pectoris (Group II)

After exercise satisfactory myocardial scans could be obtained at 20-30 minutes after injection. 16 patients had areas of decreased radioactivity on the exercise T-BIN scans and 4 did not show any abnormality (Table 4.1). These areas corresponded to the site of coronary artery disease shown on the coronary angiogram in 9/11 patients with single vessel CAD (Fig.4.5, 4.6). In the case of two and three vessel CAD, a defect in myocardial uptake was seen in at least one area (Fig.4.7) in 3/4 and 4/5 patients respectively (Table 4.1).

From the 16 patients with perfusion defects on exercise, 15 showed a gradual partial or complete filling-in of the activity in these defects within 4 hours after injection (Fig.4.5, 4.6, 4.7). In the sixteenth patient the situation was different as the defect in myocardial uptake was still seen at 4 hours after termination of exercise. When the study was repeated at rest three weeks later, the myocardial image appeared normal (Fig.4.8).

In the 10 patients who also had a Tl-201 scan, 8 had a +ve T-BIN scan compared to 6 patients with Tl-201 (table 4.2). The defects in myocardial uptake on T-BIN scans had greater definition and were more easily identified than on Tl-201 (Fig.4.9, 4.10).

By gating the acquisition in the 2 groups a better resolution of the myocardial image was obtained. The gated images showed a larger ventricular cavity and an easier detection of areas of reduced uptake on the diastolic frame (Fig.4.11). Systolic myocardial thickening and wall motion of the left ventricle and in some cases the right ventricle could be seen and assessed on the cine display even when there was a high background contribution from the lung activity.

The same taste and the smell after injection were experienced in 12 patients, and 3 had a mild burning sensation in the arm during the administration of T-BIN. These symptoms lasted approximately for 30 seconds and eased off within two minutes after injection.

(D) Discussion

The studies in this chapter showed that Tc-99m T-BIN scanning can be used to detect areas of reduced myocardial uptake in patients with M.I. and angina due to CAD. These areas were better identified on the T-BIN scans and the images were generally superior to those obtained with Tl-201 despite the higher lung and liver uptake found with Tc-99m T-BIN. This could be due to the higher photon energy of Tc-99m which gave rise to a better resolution and definition of the myocardial image.

In patients with myocardial infarction optimal T-BIN myocardial scans were obtained at 1-2 hours after injection. This was the time required for the clearance of the activity from the lung. Resting perfusion scans are mainly performed on

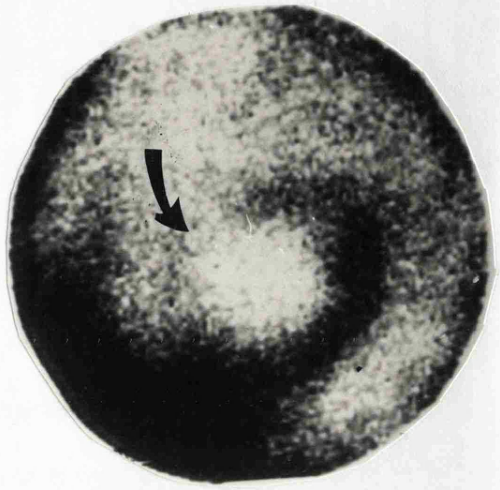
patients with suspected acute MI in whom imaging time is not as critical as in the exercise studies. For this reason, it may be preferable, unless early intervention is required, to delay myocardial imaging until clearance of the lung activity is complete. Imaging studies at rest suggest that the later the scanning the better is the contrast of the myocardial image and the delineation of the infarct.

Hepatic uptake is relatively high particularly in the resting studies. It can be a potential problem in the detection of infero-apical defects. The modification of the standard views which are currently used, e.g. A 20 degree cranial tilt of the camera in 45 degree LAO projection could separate the liver from the inferior surface. Additional views such as the 60 degree LAO and the anterior projections have been found to have additional value in exploring different myocardial regions without significant superimposition of the liver.

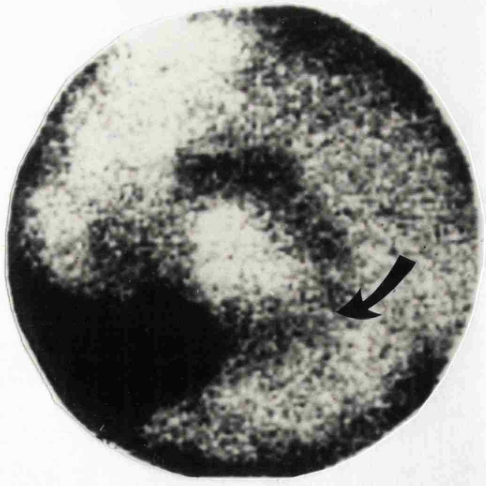
The study has also shown stress induced myocardial defects on T-BIN scintigraphy in patients with angina (Table 4.1). After exercise satisfactory myocardial scans could be obtained at 20-30 minutes from the time of injection. This was the minimum time required for defects to be seen without significant changes in the distribution (redistribution) of T-BIN having occurred. The data suggest that redistribution of T-BIN takes place within 4 hours after injection. It may be due to washout from the lungs or the adjacent normal myocardium. Holman et al (1984) studied two patients with angina due to CAD and they concluded that there was no or little evidence of the redistribution with T-BIN. They also suggested that 2

injections would be necessary to distinguish transient exercise induced ischaemia and irreversible myocardial damage. The studies in this chapter suggest that this may be true for patients where redistribution is very slow and takes longer than 4 hours. It has been shown in this study that 4 hours was the best time for the post exercise scan to assess reversible perfusion defects.

ECG gating studies using Tc-99m T-BIN look very promising. The use of one technique to study both left ventricular myocardial perfusion and function by one single injection would be very valuable. The utility of this technique needs further assessment, particularly in terms of calculating the ejection fraction and studying regional wall movements either at rest or during stress.



LAO45°



ANT

Fig. 4.1

Resting Tc-99m T-BIN cardiac scan obtained at 60 minutes after injection.
A defect in myocardial uptake is seen at the site of an old anterior transmural M.I. (arrows).

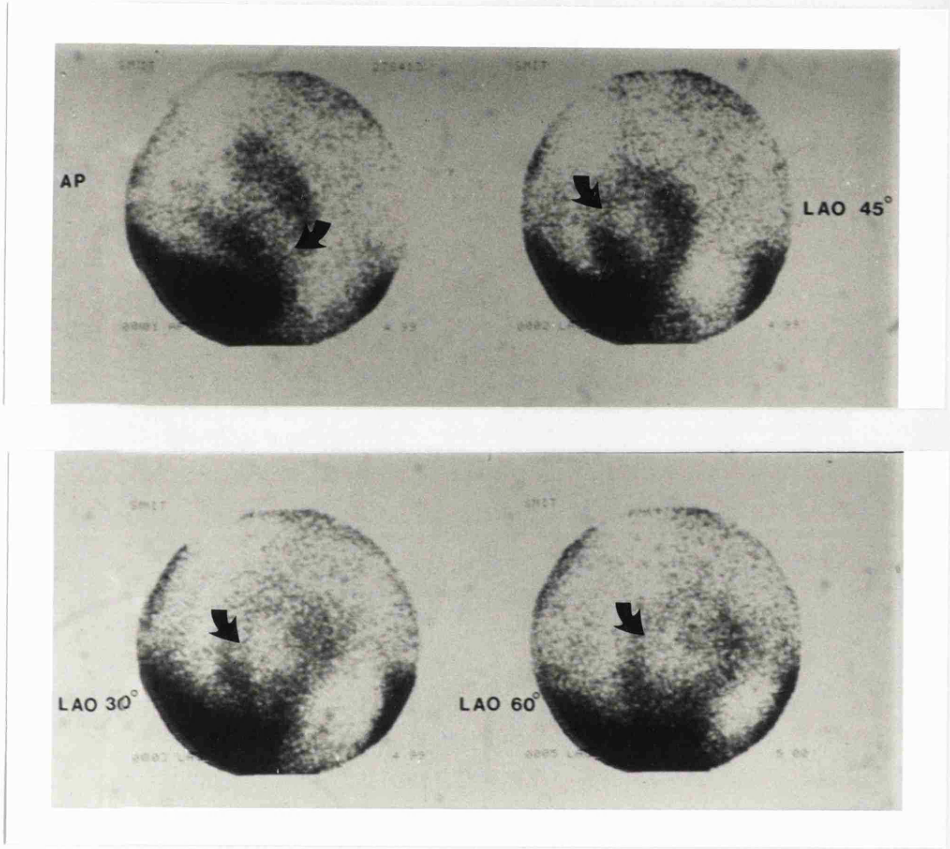


Fig. 4.2

Resting Tc-99m T-BIN cardiac scan obtained at 2 hours after injection in a patient with acute anterior subendocardial M.I. An area of diminished activity (arrows) is seen in the upper septum on the LAO views and at the apex on the AP view.

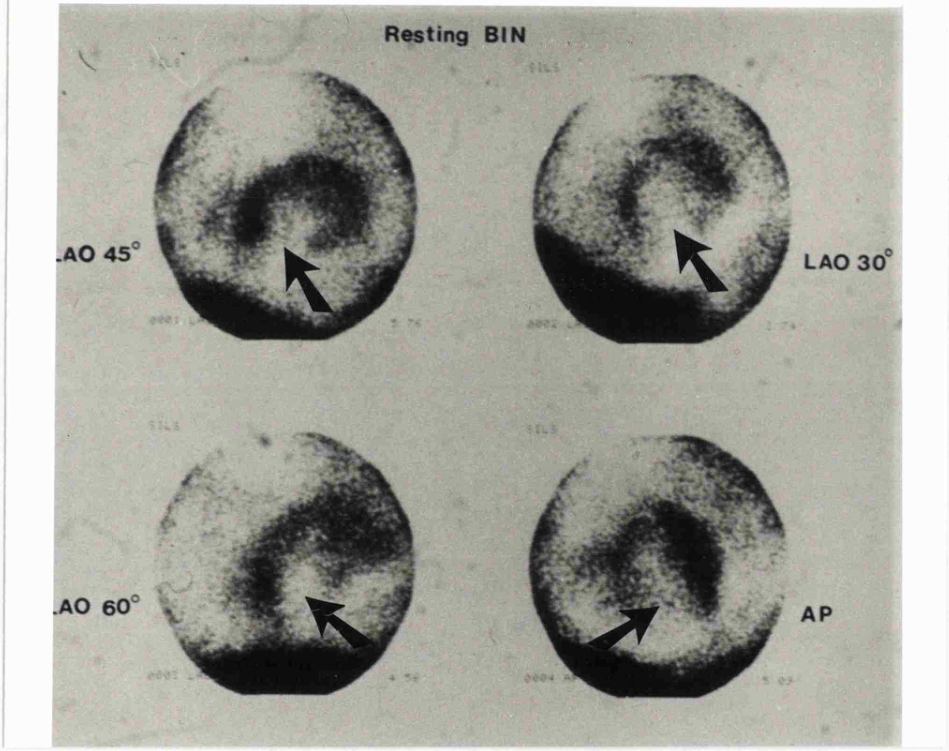


Fig. 4.3

Resting Tc-99m T-BIN cardiac scan obtained at 90 minutes after injection. A large defect in myocardial uptake is seen at the site of an old inferior transmural M.I. (arrows). In this example no interference from the hepatic uptake is demonstrated.

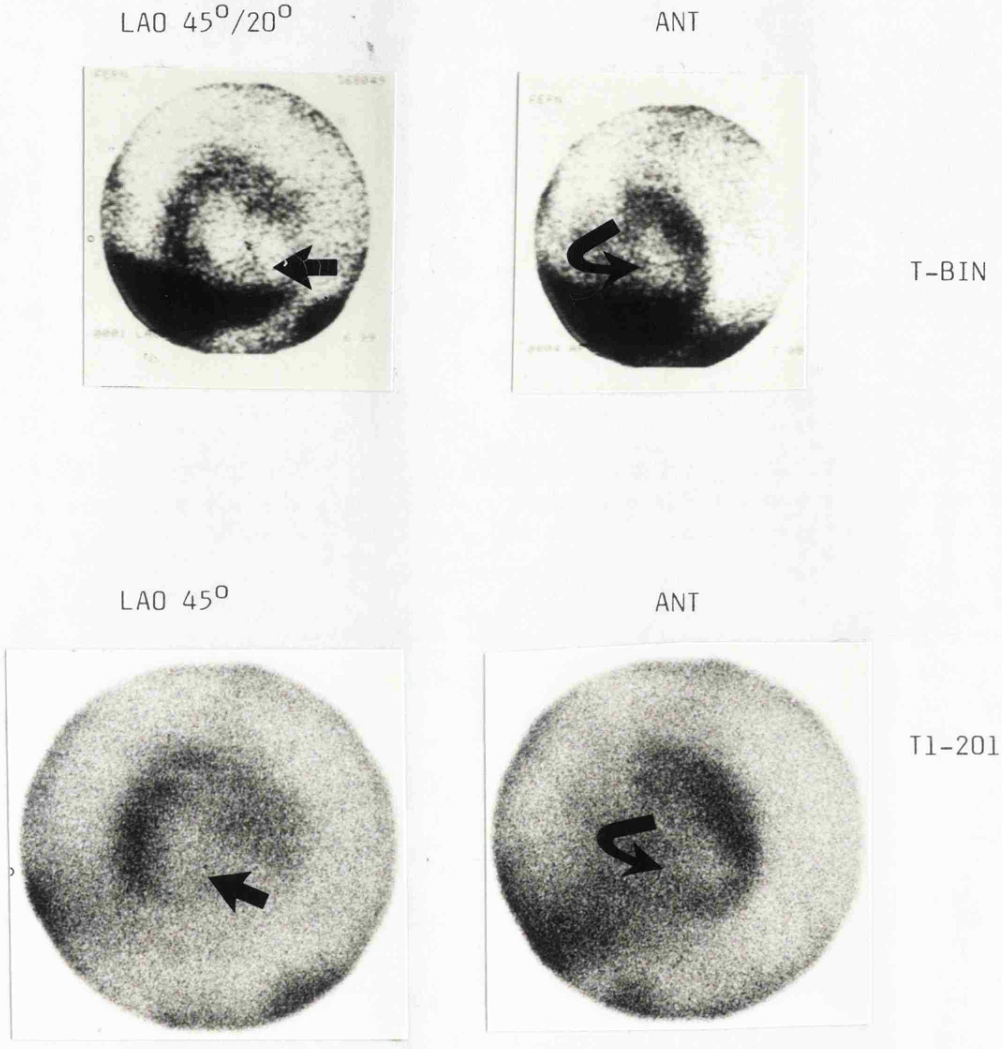


Fig. 4.4

Comparison between resting Tc-99m T-BIN (top panel) and Tl-201 (lower panel) cardiac scintigraphy in a patient with an old inferior transmural M.I. The infarct (arrows) has a better definition on T-BIN than on Tl-201 scan but the high hepatic uptake with T-BIN makes its detection difficult.

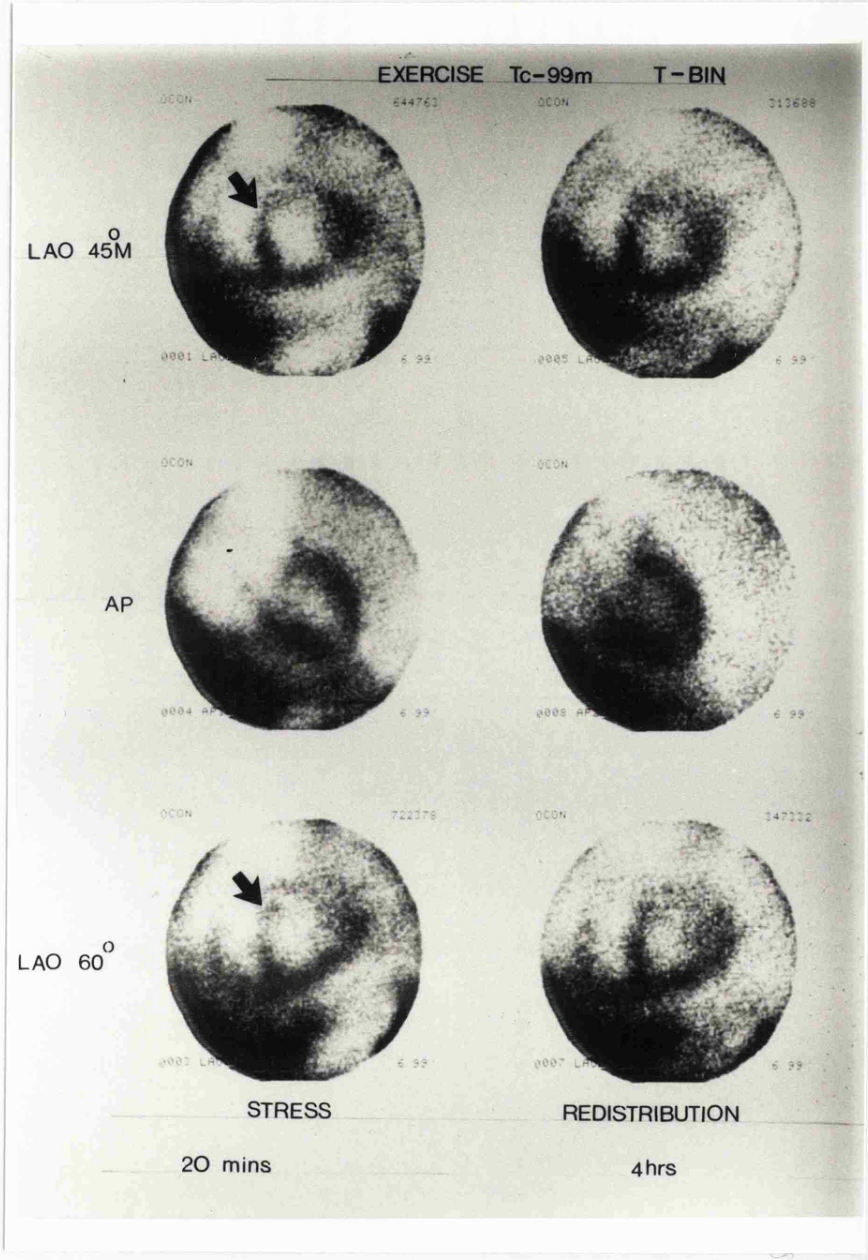


Fig. 4.5

Exercise (left panel) and redistribution (right panel) Tc-99m T-BIN cardiac scintigraphy in a patient with angina due to LAD coronary artery stenosis. Reversible ischaemia is demonstrated in the septal region (arrows).

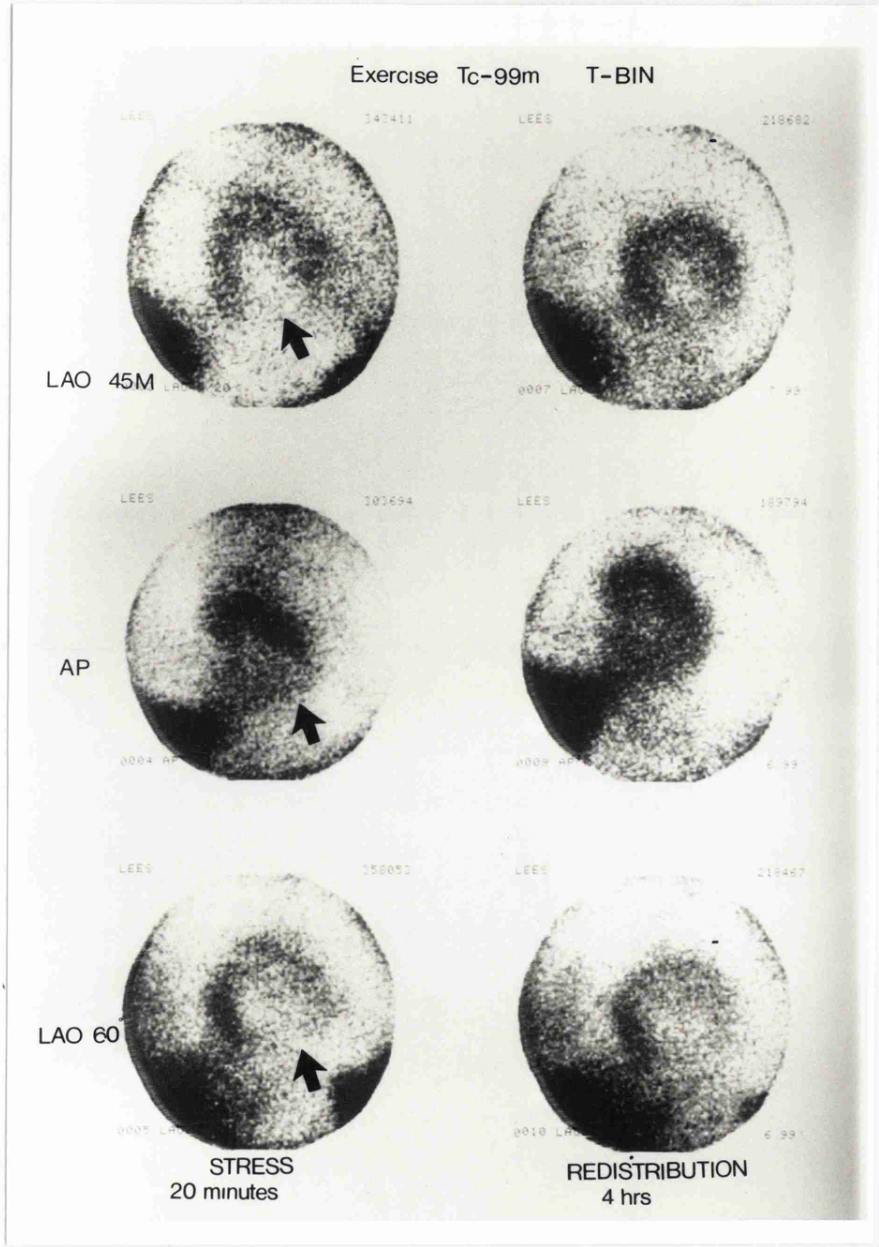


Fig. 4.6

Exercise (left panel) and redistribution (right panel) Tc-99m T-BIN cardiac scintigraphy in a patient with angina due to RCA stenosis. Reversible ischaemia is demonstrated in the postero-inferior region (arrows).

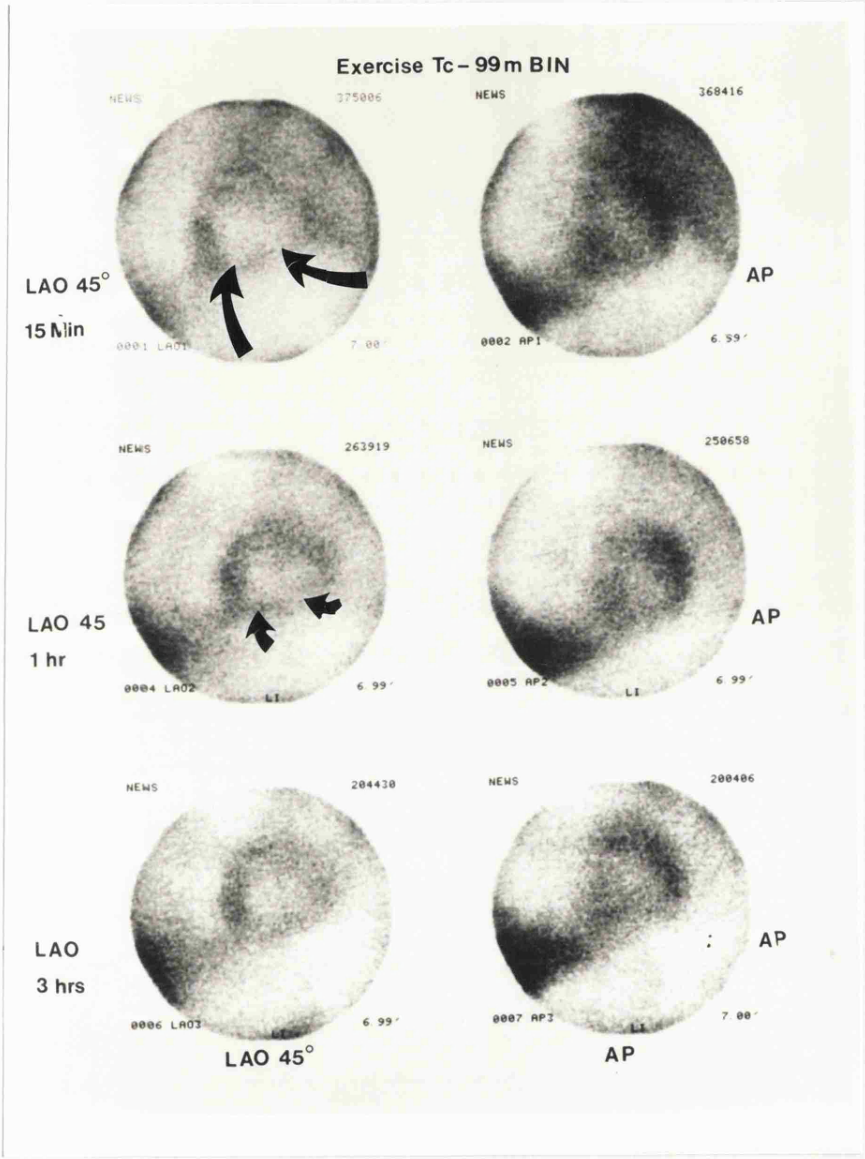


Fig. 4.7

Exercise Tc-99m T-BIN cardiac scintigraphy in a patient with 2 vesel CAD. LAO 45 M view (left panel) and AP view (right panel). A perfusion defect is seen in the postero-lateral region (large arrow) at 15 minutes after exercise (upper panel) which shows partial redistribution (small arrow) at 1 hour (middle panel) and complete normalization at 3 hours (lower panel).

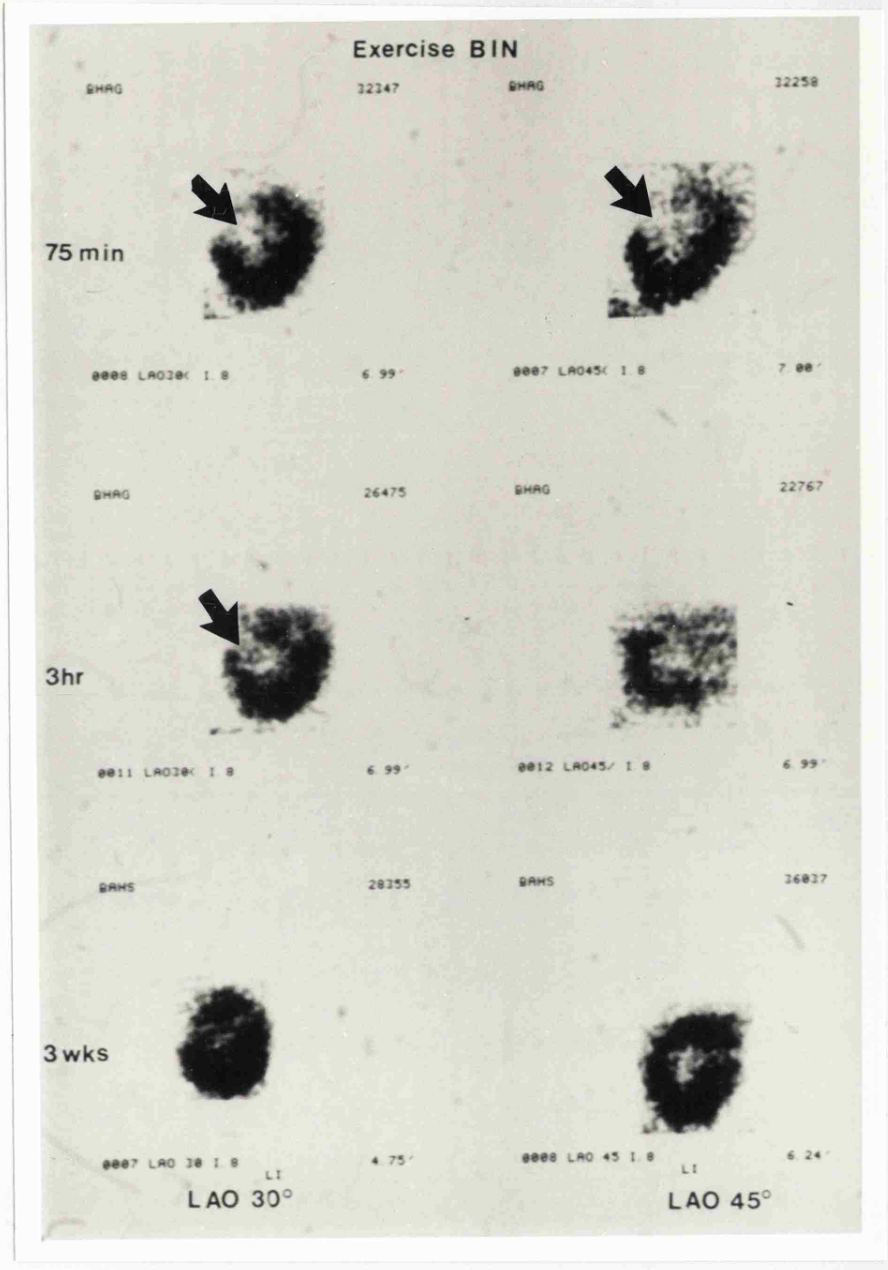


Fig. 4.8

Exercise Tc-99m T-BIN cardiac scintigraphy in a patient with LAD coronary artery stenosis. A perfusion defect is seen in the upper septal region (arrow) at 75 minutes after exercise (upper panel). No significant redistribution is demonstrated at 3 hours (middle panel). Near normalization of the scan is seen after 3 weeks following a second injection at rest (lower panel).

	Patient	Age in Years	Sex	History	Coronary arteriogram (% of stenosis)			Site of perfusion defect (S) on exercise T-BIN scan	Number of +ve scans
					LAD	LCX	RCA		
Single Vessel C.A.D.	1	68	M	Angina	90	-	-	Septum	9/11
	2	63	M	Angina	90	-	-	-ve	
	3	35	M	Angina	100	-	-	Septum	
	4	58	M	Angina	-	-	95	Postero-inferior	
	5	54	M	Angina	90	-	-	Septum	
	6	64	M	Angina	-	95	-	Inferior	
	7	49	M	Angina	-	-	90	-ve	
	8	66	M	Angina	-	-	90	Postero-inferior	
	9	36	M	Angina	90	-	-	Septum	
	10	57	M	Atypical Angina	95	-	-	Septum	
	11	54	M	Angina	-	-	90	Inferior	
2 Vessel C.A.D.	12	45	M	Angina	-	90	50	Postero-lateral	3/4
	13	55	M	Atypical Angina	90	-	60	Septum	
	14	48	M	Angina	90	-	90	Inferior	
	15	57	M	Angina	100	100	-	-ve	
3 vessel C.A.D.	16	43	M	Angina	50	90	100	Septum & postero-lateral	4/5
	17	60	F	Angina	100	100	70	Postero-lateral & apical	
	18	59	F	Angina	80	80	100	Postero-lateral	
	19	63	M	Angina	100	80	100	-ve	
	20	54	M	Angina	95	70	50	Postero-lateral & septum	
		Mean = 54.4						Total:	16/20

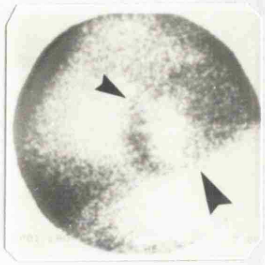
CAD = Coronary artery disease
LCX = Left circumflex

Table 4.1

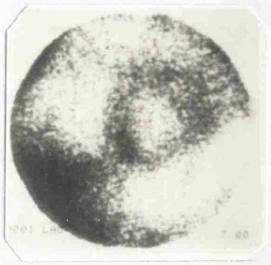
RCA = Right coronary artery
LAD = Left anterior descending

Stress

Redistribution



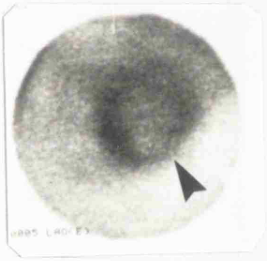
LAO $45^{o}/20^{o}$



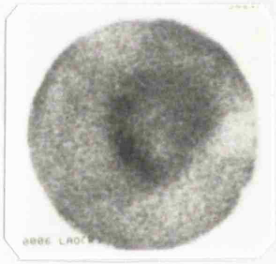
T-BIN

(20 mins)

(4 hrs)



LAO 45^{o}



Tl-201

(10 mins)

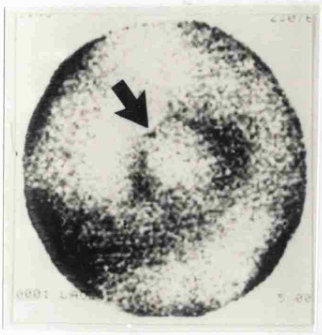
(3 hrs)

Fig. 4.9

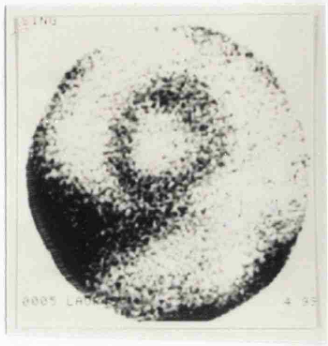
Comparison between exercise Tc-99m T-BIN (upper panel) and Tl-201 (lower panel) cardiac scans in a patient with angina due to triple vessel CAD. Reversible ischaemia is demonstrated on both scans in the postero-lateral region (large arrows) but is seen better on T-BIN. There is also a reversible perfusion defect in the upper part of the septum on T-BIN scan (small arrow) which is not seen on Tl-201.

Stress

Redistribution



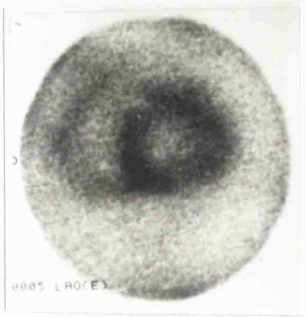
45^{o} LAO/20^{o}



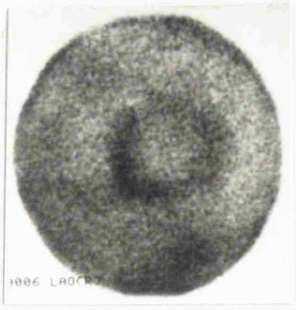
T-BIN

(30 mins)

($3\frac{1}{2}$ hrs)



45^{o} LAO



Tl-201

(10 mins)

(3 hrs)

Fig. 4.10

Comparison between exercise Tc-99m T-BIN (upper panel) and Tl-201 (lower panel) cardiac scans in a patient with angina due to LAD coronary artery stenosis. Reversible ischaemia is demonstrated in the upper septal region (arrow) on T-BIN while Tl-201 scan looks normal.

Comparison between Tl-201 and Tc-99m T-BIN in patients with angina pectoris

Patient	Age in Years	Sex	History	Beta Blocker	Exercise Level						Reasons for stopping the exercise	Days between Tl-201 & T-BIN Stress test	Arteriogram (% of stenosis)			Site of perfusion defects(s) on exercise scan	
					Tl-201			Tc-99m T-BIN									
					Max HR	Max BP(S)	RPP (10^3)	Max HR	Max BP(S)	RPP (10^3)			LAD	LCX	RCA	Tl-201	Tc-99m T-BIN
1	45	M	Angina	Yes	130	200	26	140	180	25.2	Dyspnoea	41	-	90	50	Postero-lateral	Postero-lateral
2	43	M	Angina	Yes	104	160	16.6	106	160	17	Angina	27	50	90	100	Postero-lateral	Septum + postero-lateral
3	68	M	Angina	No	130	150	19.5	123	150	18.5	Angina, dyspnoea, fatigue	12	90	-	-	-ve	Septum
4	63	M	Angina	Yes	125	180	22.5	136	170	23.1	Angina & leg pain (Tl-201)	12	90	-	-	-ve	-ve
5	49	M	Angina	Yes	130	160	20.8	120	160	19.2	Angina & dyspnoea	13	-	-	90	-ve	-ve
6	35	M	Mild Angina	No	163	160	26.1	175	170	29.8	End of protocol	12	100	-	-	Septum	Septum
7	58	M	Angina	Yes	110	170	18.7	100	170	17	Angina	29	-	-	95	Postero-inferior	Postero-inferior
8	64	M	Angina	No	140	160	22.4	145	170	24.6	Angina & dyspnoea	57	-	90	-	Inferior	Inferior
9	57	M	Angina	Yes	104	160	16.6	108	170	18.4	Angina	44	-	80	100	-ve	Postero-lateral
10	59	F	Angina	Yes	105	170	17.9	98	170	16.7	Angina & leg pain (T-BIN) Angina & dyspnoea (Tl-201)	28	80	80	100	Postero-lateral	Postero-lateral
	Mean = 54.1				Mean = 124.1	Mean = 167	Mean = 20.72	Mean = 125.1	Mean = 167	Mean = 20.89		Mean = 27.5				Total +ve $\frac{6}{10}$	Total +ve $\frac{8}{10}$

BP(S) = Systolic blood pressure
RPP = Rate pressure product

Table 4.2

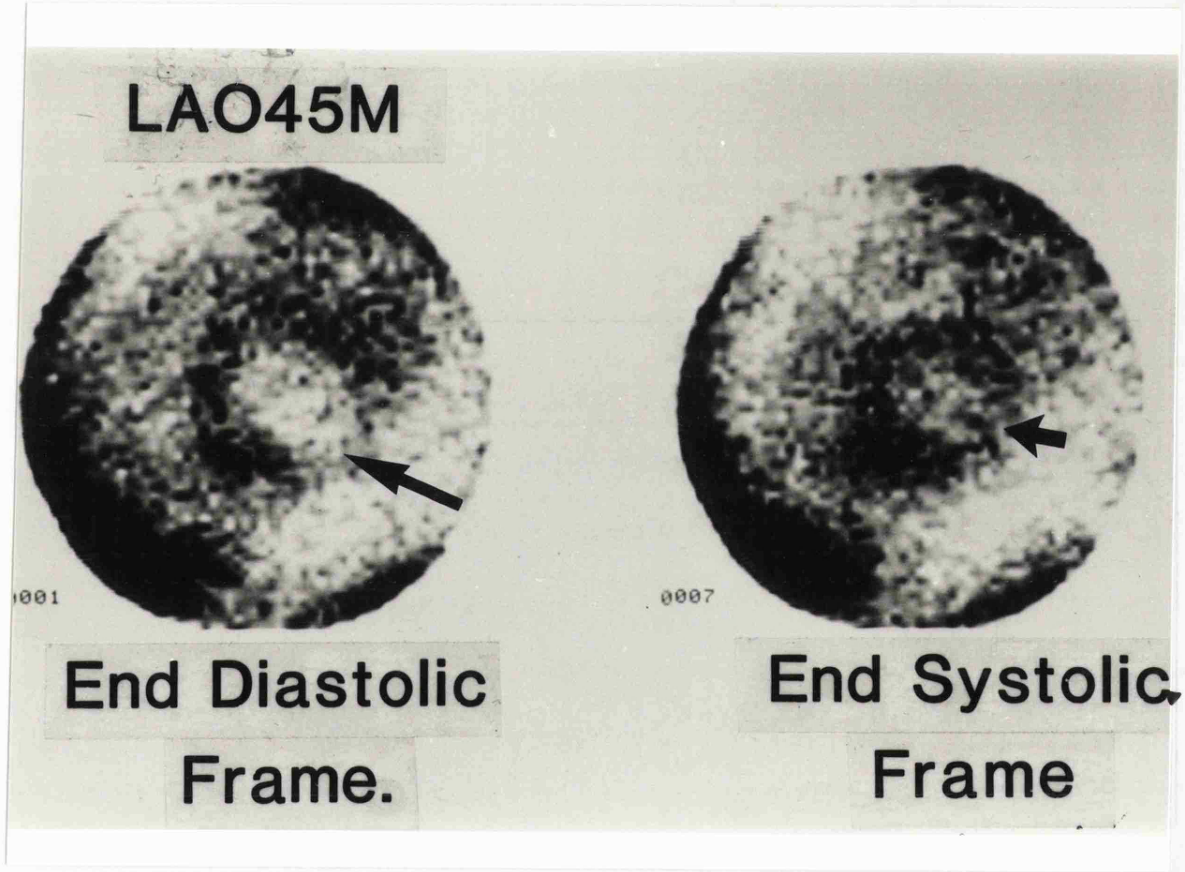


Fig. 4.11

ECG gated Tc-99m T-BIN cardiac scintigraphy. A perfusion defect is seen on both the end-diastolic (large arrow) and end-systolic (small arrow) frames but is better demonstrated in diastole.

CHAPTER 5 SUMMARY AND FUTURE WORK

(A) Summary

The thesis has described the first successful imaging of the myocardium using a Tc-99m labelled compound; T-BIN in the U.K. This new agent is taken up well by the canine and the human myocardium.

In chapter 1, the radionuclide methods that have been used to assess myocardial perfusion were described. The advantages and limitations of each technique were discussed with a special reference to the current methods.

In chapter 2, the formulation of Tc-99m T-BIN and initial studies in dogs were described. Paper chromatography was sufficient to test quality control. Satisfactory uptake by the normal canine heart was found. Experimentally induced antero apical myocardial infarcts were detected. The biodistribution of the compound was assessed by plasma clearance curves, time activity curves for the regions of interest and tissue sampling.

Chapter 3 assessed the success of T-BIN in scanning the human myocardium. The data obtained paralleled that obtained with dogs (Chapter 2). Plasma clearance was fast after I.V. administration (approximately 90 percent of the initial activity cleared within 15 minutes). The cardiac uptake was rapid which was then maintained for 4 to 6 hours. The initial lung uptake made early visualization of the myocardium difficult. The best myocardial scans were obtained at 60 minutes at rest or 30 minutes after exercise, when the lung activity had declined. Hepatic uptake was high and could interfere with visualization of the inferior surface of the heart. However, the myocardium could be scanned satisfactorily using a modified 45 degree LAO

projection. The thyroid, spleen, skeletal muscles, kidney and intestine also accumulate T-BIN. Excretion of the compound appeared to be mainly through the hepatobiliary system into the small intestine, and partly by the kidney. Toxicity was not assessed but no adverse reactions occurred. Biochemical and haematological tests showed no changes over one week.

Chapter 4 dealt with the clinical application of Tc-99m T-BIN in patients with CAD. At rest patients with M.I. showed defects corresponding to the infarct sites. In patients with angina, reversible ischaemia was demonstrated. A comparison with Tl-201 scans was made on a small number of patients. Visual analysis of the scans was easier with T-BIN than with Tl-201. The detection rate of M.I. and CAD in patients with angina was similar with both techniques, but slightly in favour of T-BIN. Very impressive ECG gated T-BIN scans were obtained. Regional wall motion could be seen on the cine display and defects in myocardial uptake were best detected in diastole.

The work reported in this thesis has shown that Tc-99m T-BIN is a good myocardial perfusion scanning agent and is at least as successful in the detection of coronary artery disease as Tl-201.

(B) Future work with Tc-99m isonitrile compounds

(i) Mechanism of cellular uptake

The mechanism of Tc-99m T-BIN cellular extraction is still unknown. The data suggest that the uptake is not dependent on the membrane Na+/K+ ATPase but it may be related to its lipophilicity (Sands et al, 1986). However, it is unclear whether the high lipophilicity of the Tc-99m isonitriles is the reason for the relative lack of species specificity seen with these agents (Pendleton et al, 1984). Also, if lipophilicity is an important characteristic for a human cardiac imaging agent, then by what mechanism(s) can the Tc-99m T-BIN accumulate in the myocardium rather than accumulate non specifically in the blood cells and vessels between the injection site and the heart (Sands et al, 1986). Further studies at the cellular level are needed to determine the mechanism(s) of T-BIN and any Tc-99m analogues that become available.

(ii) Quantitation

In order to measure washout/washin of Tc-99m T-BIN by the use of quantitative myocardial profiles, further examination of the background subtraction method is required. According to an initial report from the medical physics department at Leicester Royal Infirmary, interpolative background subtraction to remove lung activity was not appropriate. The lung activity was quite high especially on the exercise views and the liver activity adjacent to the myocardium would cause anomalies on the subtracted image. Also background subtraction using percentage of maximum and regions of interest methods were both unreliable

as the distribution of activity in the image was so variable. The selection of a background area around the myocardium was difficult since the four regions around the left ventricle (liver, diaphragm, lung and right ventricle) have their own different amounts of normal uptake.

Further work on quantifying washout/washin of T-BIN in the myocardium needs to be done. The technique has its limitation due to the change in amount of overlying lung activity over the first 2-3 hours after injection. Probably the introduction of other derivatives of the isonitrile group in the future with less or no lung uptake may make quantitation easier.

(iii) Clinical studies

Tc-99m T-BIN does have the potential to be better than Tl-201 in common use despite the high lung and liver uptake with T-BIN. Further assessment of specificity and sensitivity are required to compare directly with Tl-201 in a large number of patients. This comparison should be made in patients with M.I. and angina pectoris due to CAD documented on coronary arteriogram.

One attribute of a Tc-99m labelled myocardial agent is the possibility to measure both ventricular function and myocardial perfusion with a single injection. In comparison with Tl-201, a substantially higher photon yield is possible, because of the higher injected dose permitted by the superior dosimetry of Tc-99m (Holman et al, 1984). So improved count rate with Tc-99m T-BIN should permit ECG studies with less imaging time. The author's experience with a relatively small number of patients suggests that ECG gated T-BIN scintigraphy

is well worth further investigation. Very impressive scans showing both infarction and ischaemic areas and regional wall movement were obtained. The method also lends itself to calculation of left ventricular ejection fraction, either at rest or after exercise, in a fashion similar to cine angiography. However, difficulties may arise in defining the outline of the left ventricular cavity particularly in systole. The successful use of phase and amplitude analysis to study regional wall motion objectively and edge detection to assess ejection fraction would be very valuable.

Another alternative that has been recently reported (Perez-Balino et al, 1986) is the use of first pass ejection fraction and myocardial perfusion with a single injection of a Tc-99m isonitrile; carbomethoxy isopropyl isonitrile (Tc-CPI). This is a newly introduced derivative of the isonitrile group that is currently under investigation. The results with this agent support the concept of dual ventricular function and perfusion studies using a single Tc-99m labelled myocardial agent, and suggest that this could become the standard radionuclide stress test in the future.

The most recent advance in myocardial imaging is the development of quantitative approaches using SPECT. In case of Tl-201 scintigraphy, the technique can localize disease somewhat better by avoiding problems of overlap, but its exact value is still uncertain. As discussed in Chapter I, a major limitation of SPECT with Tl-201 is the lack of adequate photon attenuation correction. The availability of Tc-99m T-BIN, and probably its analogues in the near future, should expand the applicability of SPECT for myocardial imaging. SPECT with T-

BIN may provide a better myocardial to background ratio in the thorax allowing early scanning within 30 minutes after injection (Rigo et al, 1986). Also the improved energy range and image contrast with Tc-99m T-BIN should decide the most serious controversy regarding the application of transaxial SPECT to Tl-201 myocardial imaging, which is the choice between 360 degrees compared with 180 degrees data sampling techniques.

(iv)Tc-99m isonitrile analogues

Tc-99m T-BIN is only one of several alkyl isonitrile complexes of technetium that have been shown to exhibit myocardial uptake in animals. It has been suggested recently that the sustituent group in the isonitrile ligand can be readily altered and not merely with simple alkyl or aryl groups (Holman et al, 1984). It is thus possible that among the analogues of this class there may be other complexes with biological properties better suited to the intended use. For example, lower uptake and faster clearance from the lung and liver and perhaps also more rapid washout from the myocardium itself to facilitate a dual injection protocol.

The most promising derivatives that have been recently suggested are the aliphatic (C-4 to C-5) ethers (Williams et al, 1986 and Mousa et al, 1986). Two of these were found to have the best overall characteristics; Tc-99m-hexakis-2-methoxy-2-methylpropyl-1-isonitrile (MMI) and Tc-99m-hexakis-1-methoxypropyl-2-isonitrile (CPI) (Williams et al, 1986). These newer compounds will need to be evaluated against Tc-99m T-BIN and Tl-201.

A comparison between the two agents and T-BIN was made in

patients with CAD to select the Tc-isonitrile with the best properties for myocardial imaging (McKusick et al, 1986).The 3 isonitrile agents were injected at peak exercise (the same level of Tl-201) and imaging was repeated after a second injection at rest. The authors showed that the liver uptake obscured the heart in 6/40 with T-BIN but none of CPI or MMI patients. CAD was detected by all 3 agents, but because of good heart uptake and lung and liver parameters, MMI displayed the highest initial contrast and had the best potential using dual injection protocol. This agent is currently under intense clinical investigation to assess its value in place of Tl-201.

Appendix

ORGAN DOSIMETRY OF Tc-99m T-BIN (Given by Mr M Y Early)

The quantity of radioactive material administered to the subject is called the administered dose, and is measured in units of Becquerels (Bq) or Megabecquerels (MBq). A Becquerel represents one disintegration per second.

The energy absorbed by matter exposed to radiation is called the radiation Absorbed Dose (A.D.), and is measured in units of Grays (Gy). One Gray is defined as 1 Joule kg^{-1}. An older unit, the rad, is still used and represents 100 ergs gm^{-1}. 1 Gray = 100 rads.

Different biological effects may result from the same absorbed dose delivered by different types of radiation. The Dose Equivalent (D.E.), measured in Sieverts (Sv), takes the Relative Biological Effectiveness (R.B.E.) of the radiation into account when measuring the absorbed dose in humans.

Thus D.E. (Sv) = A.D. (absorbed dose) (Gy) x R.B.E.

Again, an older unit is still in use, the rem. which is defined by

D.E. (rem) = A.D. (rad) x R.B.E.

For Beta, Gamma and X radiation, the R.B.E. is in fact equal to 1, and so absorbed dose and dose equivalent are numerically equal for the agents considered in this paper.

To compute the absorbed dose from the administration of a radiopharmaceutical, the following factors must be known:

1) Distribution of the agent anatomically.

2) Rate of uptake into different tissues.

3) Rate of elimination from different tissues.

It can be seen that organs will receive widely different absorbed doses from a single administration. Furthermore, tissues themselves have different degrees of sensitivity to radiation.

In order to simplify and compare radiation doses, an Effective Dose Equivalent (E.D.E.) has been defined, which represents the total radiation dose to a number of organs weighted according to a risk estimate for each organ involved. Thus the E.D.E. is the whole body dose which would produce the same risk as a non-uniformly distributed absorbed dose.

Although originally conceived to help in assessing risks to people working with radiation, the E.D.E. has been widely used in the context of radiopharmaceuticals.

Estimation of the absorbed dose from a Tc-99m T-BIN study proceeded through the following stages:

1. Distribution pattern determined from gamma-camera studies.
2. Assumptions about and approximations to T-BIN metabolism.
3. Calculations of organ doses.
4. Estimation of whole body E.D.E.

1. The distribution of Tc-99m T-BIN was assessed by looking at the acquired images of five patients who were scanned using a large field of view gamma camera. Forty to sixty dynamic frames were acquired following on IV administration of T-BIN. The frames were then summed and the distribution was observed on the resulting image.

Organ	Fraction of administered dose (f)
Liver	.25
Lung	.13
Heart	.06
Spleen	.15
Rest	.41

Cumulated Activity, $A = \frac{A_o}{\lambda} = \frac{A_o . T_{\frac{1}{2}}}{\ln(2)}$

where A_o = administered activity

λ = decay constant (hr^{-1})

$T_{\frac{1}{2}}$ = radioactive decay half-life (hr)

Now, 100 MBq = 2702 μCi and for Tc-99m, $T_{\frac{1}{2}}$ = 6.03 hr.

Therefore, $\underline{A = 23506\ \mu Ci\text{-}hr}$

2. Assume that no gamma photons are allowed to escape the body (worst case) and that they are all absorbed in the source organ.
Assume no biological elimination.

3. Therefore, the cumulated activity within the source organ is:

$$A\ (source) = A * f \quad \mu Ci\text{-}h$$

Therefore, dose to target organ:

$$D_{(T \leftarrow S)} = A * f * S_{(T \leftarrow S)}$$

where $S_{(T \leftarrow S)}$ = conversion factor for μCi-h to rad.

These values are listed in the Medical Internal Radiation Dose (MIRD) pamphlet under Tc-99m.

Source Organ	f	Target	$S_{(T \leftarrow S)}$ ($\times 10^{-5}$)	w	Organ Dose (mrad)	Whole Body (mrad)
Liver	.25	Liver	4.6	.06	270	16
Lungs	.13	Lungs	5.2	.12	159	19
Spleen	.15	Spleen	33	.06	1160	70
Other (heart etc)	.47	Other	.19	1.0	21	21
					TOTAL WHOLE BODY DOSE (mrad)	126

w = a weighting factor relating to the risk estimate of each organ.

4. Therefore, the Whole Body effective equivalent dose from a 100 MBq administration of Tc-99m T-BIN is 126 mrad. This is less than the radiation dose delivered to the patient per 37 MBq (1 mCi) of Tl-201 which has been estimated to be 349 mrad to the heart and 240 mrad to the total body (Atkins et al, 1977). The usual amount of injected radioactivity for Tl-201 myocardial imaging in clinical practice is approximately 75 MBq.

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