To Norma and Melanie.

THE USE OF RADIOACTIVE NUCLIDES IN THE INVESTIGATION OF MALIGNANT DISEASE.

(A Laboratory and Clinical Evaluation of the ^{99m}Tc phosphates as Bone Scanning Agents in the detection of skeletal metastases)

by

Dennis Lionel Citrin M.B. Ch.B., M.R.C.P. (U.K.)

Senior Registrar in Nuclear Medicine,

University Department of Medicine,

Royal Infirmary, Glasgow.

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SUMMARY

The relative insensitivity of standard radiological methods in the diagnosis of early metastatic disease of the skeleton is well recognised. The development in the 1960's of isotope bone scanning using ^{85}Sr, ^{87m}Sr and ^{18}F permitted the earlier and more accurate diagnosis of malignant involvement of bone, but as none of these radionuclides was ideal the technique of bone scanning has not been optimally applied in clinical medicine and surgery. In 1972 and 1973, new radiopharmaceuticals were developed which have made available to all hospitals with even limited facilities the capacity to obtain high quality bone scans. These new radiopharmaceuticals are the ^{99m}Tc labelled phosphates. This thesis describes the laboratory and clinical evaluation of the available ^{99m}Tc phosphate compounds.

The introductory section describes the principle of the bone scan and how it differs from the radiograph. There follows a description of the conventional bone scanning agents ^{85}Sr, ^{87m}Sr and ^{18}F, and a brief discussion of the disadvantages of each of these nuclides. The development of the ^{99m}Tc phosphates is then described and the relevant physical and chemical properties of these agents is summarised.

The choice of which of the available ^{99m}Tc phosphates, ethane hydroxy diphosphonate, pyrophosphate, polyphosphate and monofluorophosphate, is the most suitable agent depends on a comparison of their pharmacological properties, as they are all very similar chemically and all utilise the same radionuclide, ^{99m}Tc. The second section of the thesis describes a detailed quantitative comparison of the pharmacological properties of each compound. Several new investigative techniques were developed for this study and these techniques are described in detail. On the basis of the comparative studies, it was concluded that ethane hydroxy diphosphonate is the most favourable agent, because of the higher target:background ratios it provides, and because of its more rapid blood clearance, greater urinary excretion and lower whole body retention.

A detailed evaluation of the in vitro and in vivo properties of ethane hydroxy diphosphonate is then described. The in vitro properties studied are those which are of importance to the use of the compound in routine clinical use. It was concluded that the compound is very flexible and that high quality images can be obtained despite the variation in presentation, labelling and injection procedures which may occur in a busy Department of Nuclear Medicine.

The in vivo experiments performed in volunteer subjects and in patients with known bony metastases demonstrate that E.H.D.P.

clearly satisfies the important biological criteria necessary for a satisfactory bone scanning agent, namely

(1) rapid skeletal uptake of a significant proportion of the administered activity.

(2) skeletal binding which is irreversible, in the short term at least.

(3) higher uptake in tumour-involved compared with normal bone.

(4) no significant retention in soft tissue.

(5) rapid urinary excretion of the diphosphonate not taken up by bone.

(6) lack of toxicity.

The clinical sections of the thesis are introduced with a discussion of the problems of technique and scan interpretation which are particularly relevant to the ^{99m}Tc phosphates. The study performed to assess the sensitivity and clinical value of the ^{99m}Tc phosphate bone scan in patients with malignant disease is then described: three hundred and seventy two patients and 75 control subjects were studied and the results support the conclusion that the ^{99m}Tc phosphate bone scan allows earlier and more accurate identification of bone metastases than the radiograph. If the bone scan is read with all clinical details and the results of complementary radiographs available, the incidence of false positive results is acceptably low.

The application of the ^{99m}Tc phosphate bone scan to the preoperative staging and clinical follow up of patients with primary breast cancer is described in the next section. The high incidence of bony metastases in patients with breast cancer is well recognised and the value of a technique which permits the identification of metastases in asymptomatic patients with normal radiographs is potentially great. Previous studies with strontium and fluorine have suggested that about 20% of patients with apparently curable breast cancer (Stages I and II) have bone metastases at the time of first presentation, and a similar incidence of occult metastases was observed in our study using ^{99m}Tc phosphates. The clinical importance of this study is discussed.

The thesis concludes with a brief discussion of future developments in the use of isotope methods in the study of malignant disease of the skeleton. Technical improvements in scanning equipment and available radiopharmaceuticals have been significant, and the need now is for critical clinical studies which utilise the isotope bone scan to improve the diagnosis and management of patients with malignant disease of the skeleton.

PREFACE

All of the studies described in this thesis were designed personally, and carried out in the Department of Nuclear Medicine, Glasgow Royal Infirmary, from November 1972 until the present date.

Selected aspects of the work described have been (or are to be) published as original papers in:

(1) British Journal of Surgery, 61:73 (1974).

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(5) Journal of Nuclear Medicine, 15:1110 (1974).

(6) Proceedings of the 11th Annual Meeting of Gesellschaft fur Nuclear Medizin (Society of Nuclear Medicine). Published by F.K. Schattauer Verlag, Stuttgart (1974).

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SECTION I. INTRODUCTION

A. GENERAL

"Nuclear medicine procedures are very useful in certain types of cancer. The procedures are simple and not hazardous to the patient; they provide both structural and physiologic information that can be quantified readily, and they aid in determining therapy and assessing its value. Better imaging devices, improved radiopharmaceutical preparations, and new diagnostic techniques will bring expansion of this field" (James and Wagner, 1970).

The above statement was remarkably prophetic, predicting as it did the general expansion of nuclear medicine techniques in the investigation of malignant disease. During the past 3 years there has been a great increase in the use of one particular nuclear medicine technique, radionuclide bone scanning, in the investigation of patients with cancer. The reasons for the increased use of the bone scan are firstly, the development of high resolution total body imaging devices (rectilinear scanners and gamma cameras) which permit the detailed study of a large area of the patient within a relatively short period of time. Of greater and more specific importance, however, has been the introduction of a new group of radiopharmaceuticals, the ^{99m}Tc labelled phosphates, which are excellent bone scanning agents. This thesis describes the

laboratory and clinical evaluation of the ^{99m}Tc phosphates.

In this introductory section are described the principles of the bone scan and radiograph. The properties and disadvantages of the conventional bone scanning agents ^{85}Sr, ^{87m}Sr and ^{18}F, are discussed and the development of alternative agents is then described. A brief summary of the available information regarding the physical and chemical properties of the most promising of the alternative bone scanning agents, the ^{99m}Tc phosphates, is then given.

Following the introduction of bone scanning with ^{85}Sr (Fleming et al, 1961) there have been many reports of the clinical use of isotope bone scanning in the study of patients with suspected bone metastases. Extensive experience has now been obtained with ^{85}Sr (Charkes and Sklaroff, 1964; Sklaroff and Charkes, 1964; Erjavic, 1965; Simpson and Orange, 1965; De Nardo, 1966; De Nardo and Volpe, 1966; Charkes et al, 1966; Briggs, 1967; Parsons et al, 1969; Gnekow et al, 1972), with ^{87m}Sr (Charkes et al, 1964; Meckelnburg, 1964; Spencer et al, 1967; Samuels, 1971; Sauer, 1971; Scott and Adams, 1974), and with ^{18}F (Blau et al, 1962; French and McReady, 1967; Galasko et al, 1968; Harmer et al, 1969; Hopkins et al, 1972).

On the basis of these studies the bone scan has been accepted as the most sensitive indicator of early metastatic involvement of the skeleton, and is the investigation of first choice in the

investigation of suspected bone metastases in patients with primary cancer (De Nardo, 1968; Charkes, 1970; Verdon et al, 1971; De Nardo et al, 1972). This statement is particularly true of those patients with cancers which have a known predilection to metastasize to bone, particularly carcinoma of the breast (Sklaroff and Charkes, 1968; Galasko, 1969; Rubin and Ciccio, 1969), carcinoma of the prostate (Faber et al, 1967; Williams and Blahd, 1967; Morgan and Mills, 1968; Roy et al, 1971) and lymphoma (Weber et al, 1968; Harbert and Ashburn, 1968).

B. PRINCIPLE OF THE RADIOGRAPH AND SCAN

The bone scan is more accurate and sensitive than the skeletal radiograph because of the different principles on which each is based. Overall the radiograph indicates the net result of bone destruction and repair while the scan indicates the dynamic response of bone to tumour invasion (Charkes et al, 1966; Charkes et al, 1968).

When tumour cells invade bone they produce two basic effects: bone destruction, and an osteoblastic reaction which represents attempts by the surrounding bone to repair the destructive effects (Milch and Changus, 1956). The radiograph demonstrates both processes - bone destruction is seen as radiolucencies (osteolytic areas) and bone repair as radiodensities (osteosclerotic areas). Bone destruction must be advanced before any abnormality is seen on the radiograph: the trabecular bone of the axial skeleton is the commonest site for bone metastases (Jaffe, 1958). A destructive lesion in trabecular bone must be greater than 1-1.5 cm in diameter, and there must be a loss of approximately 50% of bone mineral before radiolucencies will be apparent on a conventional radiograph (Borak, 1942; Shackman and Harrison, 1948; Edelstyn et al, 1967). Early in bone repair, insufficient numbers of calcium atoms have been laid down to be visualised

radiographically as radiodensities. For these reasons the skeletal radiograph is normal during the early phase of tumour involvement.

The bone scan is based on an entirely different principle to the radiograph. The scan is independent of bone destruction, but depends on the reaction of the bone to tumour invasion. The local increase in bone blood flow and increased production of hydroxy-apatite crystals following tumour invasion is demonstrated by the locally increased concentration of a gamma-emitting bone seeking nuclide or radiopharmaceutical administered to the patient. The increased concentration of bone scanning agent is recorded as a "hot spot" by an appropriate detecting system, such as a recti-linear scanner or gamma camera (Figure I.1). It is important to note that the increased concentration of tracer is not due to or directly dependent on the metabolism of the tumour cells themselves, but is directly related to the local changes in bone metabolism consequent upon tumour invasion.

Early in bone invasion by tumour, bone destruction is not marked enough to be visible radiographically. At this time early bone repair can be detected by bone scanning although radiodensities are not visible radiographically. A positive bone scan may, there-fore, be associated with normal radiographs. As the tumour pro-gresses, the bone destruction it causes will become visible on the radiograph as radiolucencies (osteolytic lesions). In these circumstances bone reaction is considerable, and the bone scan is

also strongly positive. If the tumour does not progress calcium will be laid down during the healing process in such quantities that radiodensities (sclerotic areas) will be visible radiographically. At this stage both investigations are positive. Eventually, if the lesion heals completely, there is extensive calcification producing a dense appearance on the radiograph. At this stage the bone scan may be normal.

As the bone scan depends on the metabolic reaction of the bone, it is clear that in the absence of bone reaction to tumour invasion, the scan may be normal despite radiographic evidence of bone destruction. This occurs infrequently (in less than 5% of cases) but is particularly likely in some cases of myeloma, in cases of rapidly growing anaplastic carcinoma or conversely in cases of indolent tumours such as thyroid cancer (Charkes, 1970). If bone destruction is extensive (Goergen et al, 1974), or if bone metabolism is modified by radiotherapy (Cox, 1974b) a "cold" area may be visible, corresponding to the area of diminished bone activity.

It is clear that the techniques of bone scanning and skeletal radiology are in many instances complementary and maximum diagnostic information can be obtained by performing both studies.

C. BONE SEEKING RADIONUCLIDES

(1) Historical

The first clinical demonstration of the localisation of radionuclides in bone was the development of bone necrosis, osteomyelitis and bone tumours in radium dial workers. It was recognised that these diseases of the skeleton resulted from chronic ingestion of radium which was deposited in bone and produced intense local irradiation (Blum, 1924; Looney, 1954).

The first radionuclide specifically used in the investigation of the skeleton was ^{32}P (Chiewitz and Hevesy, 1935). The ideal bone seeking agent would be a radionuclide of calcium. There is, however, no suitable radionuclide of calcium for use as a bone scanning agent: ^{47}Ca is the only gamma emitting nuclide of calcium, and the 1.31 MeV gamma rays it produces are of too high an energy to be detected efficiently with available scanning systems (Verdon et al, 1971), although external counting techniques have been used successfully with ^{47}Ca in the study of patients with bone tumours (Bauer and Wendeberg, 1959; Greenberg et al, 1961). The gamma emissions of Scandium-47, the daughter product of ^{47}Ca (Taylor, 1966) have been used for bone scintigraphy using the gamma camera (Basse-Cathalinat et al, 1968). In general, however, ^{47}Ca is considered unsuitable as a bone scanning agent, and alternative agents have been sought.

Elements which behave biologically in a manner similar to calcium were investigated, and the bone seeking properties of Strontium have been extensively studied. The deposition of ^{89}Sr, a pure β-emitter, in active osteogenic areas in normal bone and osteogenic sarcoma was demonstrated in 1942 (Treadwell et al, 1942). Clinical application of this fact was delayed until a suitable radionuclide of Strontium became available. Bauer and Ray (1958) showed that ^{85}Sr behaved biologically in a similar manner to calcium, and that it would exchange with calcium in the calcium hydroxy-apatite crystal of bone. ^{85}Sr was subsequently used to obtain point counting quantitative data in patients with bone metastases (Bauer and Wendeberg, 1959; Gynning et al, 1961). Following these studies, radionuclide visualisation of bone (bone scanning) was introduced using ^{85}Sr (Fleming et al, 1961) and another nuclide of strontium, ^{87m}Sr (Charkes et al, 1964).

Another radionuclide which localises in the hydroxy-apatite crystal is ^{18}F: fluoride ions exchange not with calcium but with the hydroxyl ions in hydroxy-apatite, and the clinical usefulness of ^{18}F-fluoride was first demonstrated in 1962 (Blau et al, 1962). During the past 10 years ^{85}Sr, ^{87m}Sr and ^{18}F have been extensively used as bone scanning agents despite certain disadvantages and limitations associated with their use.

(2) Strontium-85

^{85}Sr is a pure gamma emitter and its 513 KeV photon may be detected by rectilinear scanner and gamma camera systems. The important studies which established the principles and pathological basis of the bone scan were performed with ^{85}Sr (Charkes et al, 1966; Charkes et al, 1968) despite the significant limitations on its use due to relatively unfavourable physical characteristics. The long physical half life of ^{85}Sr (64 days) and the long biological half life of minerals in the skeleton result in a relatively high dose to the skeleton and the U.S. Atomic Energy Commission restricts the use of the nuclide to adult patients with known malignant disease (Verdon et al, 1971). The maximum permissible injected activity of 100 uCi gives a low photon flux resulting in low information density on the scintiscan and a long scanning time is required. Gastrointestinal excretion of the nuclide may be confused with lesions of the lumbar spine or pelvis, and cleansing of the bowel with laxatives or enemata is usually required prior to scanning.

(3) Strontium-87m

Because of the long physical half life of ^{85}Sr, alternative nuclides of Strontium were considered, and ^{87m}Sr was introduced (Charkes et al, 1964). Its biological behaviour is identical to that of ^{85}Sr but the physical half life of ^{87m}Sr is only 2.8

hours compared with 64 days for ^{85}Sr. ^{87m}Sr is produced from a Yttrium-strontium generator, and emits a 388 KeV gamma ray which is readily collimated and therefore very suitable for both rectilinear scanners and gamma cameras (Bell, 1972). The short physical half life of ^{87m}Sr means that the restrictions on the use of ^{85}Sr do not apply to ^{87m}Sr, and the latter has been used in children (Samuels, 1972). Large activities of ^{87m}Sr (1-3 mCi) can be injected and scans with a high information density can therefore be obtained with a relatively short patient scanning time (Volpe, 1971).

The main disadvantage of ^{87m}Sr remains the slow blood clearance of the nuclide. High background activities in the blood and soft tissues results in low target/background ratios and relatively poor visualisation of normal and pathological bone until many hours after injection (Weber et al, 1969). The high blood and extra-cellular fluid activities shortly after injection of ^{87m}Sr may lead to erroneous false-positive diagnoses, or the visualisation of non-bony tumours (Charkes, 1969). The slow blood and soft tissue clearance of ^{87m}Sr limits its clinical usefulness, as the short physical half life precludes waiting until background levels are low, when optimum target:background ratios would be obtained.

(4) Fluorine-18

Of the three most widely used bone scanning agents, ^{85}Sr, ^{87m}Sr and ^{18}F, ^{18}F is in many respects the most suitable. It

is reactor produced, by the neutron irradiation of ^{6}Li-enriched Lithium carbonate (Thomas et al, 1966). Where available, cyclotrons provide a more convenient and effective method for the production of large quantities of pure ^{18}F-fluoride. The usual cyclotron method is the bombardment of pyrogen free water with 30 MeV α-particles (Clark and Silvester, 1966). The resulting nuclide is obtained carrier free. ^{18}F has a very short physical half life (1.83 hours), decaying by positron (β^+) emission. In most centres, either a rectilinear scanner (Dunson et al, 1973) or a gamma camera with additional collimation (Galasko et al, 1968) is used to detect the 511 KeV annihilation photons resulting from the positron emission. Those nuclear medicine centres with positron cameras may find ^{18}F particularly suitable (Blau et al, 1972).

The superiority of ^{18}F when compared with ^{85}Sr and ^{87m}Sr is due to its more favourable biological properties, in particular the rapid clearance from the body of the fluoride ion not taken up by bone. Because of the rapid urinary excretion of ^{18}F, blood and soft tissue levels are low, and target:background ratios obtained with ^{18}F are higher than those obtained with the nuclides of Strontium (Weber et al, 1969). ^{18}F is administered intravenously as sodium fluoride in saline solution and the activity routinely injected is 2 mCi (Blau et al, 1972). The administered activity is limited more by expense and availability than by

radiation dose. The main disadvantage with ^{18}F is its short physical half life which together with its cyclotron production, severely limits its use, due to expense and difficulty with distribution. In the United Kingdom its use is restricted to the Greater London Area.

(5) Development of Alternative Agents for Skeletal Imaging

As described above, the conventional agents used for bone imaging, ^{85}Sr, ^{87m}Sr and ^{18}F have certain problems associated with their use, and none is ideal (O'Mara and Subramanian, 1972). Because of the undoubted superiority of the bone scan when compared with the radiograph in the early detection of metastatic disease, many alternative nuclides and radiopharmaceuticals have been considered. These include ^{68}Ga (Hayes et al, 1965; Edwards et al, 1966) and ^{99m}Tc pertechnetate (Tow and Wagner, 1967). Because of their chemical similarity to calcium, several nuclides of the alkaline earth element Barium have been evaluated. Both ^{131}Ba and ^{135m}Ba were found to have excellent physical characteristics and rapid blood clearance (Spencer et al, 1970; Lange et al, 1970; Subramanian, 1970).

Another promising group of agents are chelates of the rare earth elements (the lanthanides) (O'Mara et al, 1969). Of these compounds the HEDTA (N-hydroxy ethylenediamine triacetic acid) chelate of $^{157}Dysprosium$ appears to have the most suitable properties (Subramanian et al, 1971; Yano et al, 1971).

Unfortunately the production of ^{157}Dy in a typical medical cyclotron is not possible because high energy protons are required, and the more readily available ^{171}Er H.E.D.T.A. is probably a reasonable second choice (O'Mara and Subramanian, 1972).

D. INTRODUCTION OF THE ^{99m}Tc PHOSPHATES

(1.) Development

The potential importance of the agents just described has been overshadowed (and their further development probably curtailed) by the recent introduction of an entirely new group of compounds which are by far the most promising of all the available bone scanning radiopharmaceuticals. These are phosphate and phosphonate compounds labelled with ^{99m}Technetium.

The excellent physical characteristics of ^{99m}Tc are well documented. The short physical half life of 6.2 hours is ideal for many studies. The monoenergetic gamma emission of 140 KeV is easily collimated and the absence of biologically hazardous beta decay further reduces radiation absorbed dose. These properties, together with the relatively low cost and convenience of generator production, as the daughter product of ^{99}Mo, make ^{99m}Tc the radionuclide of choice in radioisotope imaging procedures for nearly every major organ system in man (Wagner, 1968).

The introduction of techniques for reducing the oxidation state of ^{99m}Tc with stannous chloride (Eckelman and Richards, 1970) have made it possible to label with ^{99m}Tc various phosphate and phosphonate compounds, which have a high skeletal affinity, thus producing potentially very valuable bone scanning agents.

Polyphosphates, also known as condensed phosphates, are compounds which possess chains of -P-O-P-O- units joined together. The ability of these linear compounds to prevent the deposition of calcium carbonate from solution is well known (Fleisch and Russell, 1970). It was predicted, therefore, that polyphosphates would have a strong affinity for the hydroxy-apatite crystal in the mineral phase of bone, particularly in actively growing areas of the skeleton, and this affinity has been demonstrated using ^{32}P labelled polyphosphates (Fels et al, 1959). Subramanian and McAfee (1971) first described the production and tissue distribution of a ^{99m}Tc labelled tripolyphosphate compound. These early studies in rabbits demonstrated that the bone concentration of ^{99m}Tc tripolyphosphate from 3 to 24 hours after injection was 65-70% of that of ^{85}Sr injected simultaneously. Blood levels of the tripolyphosphate compound were higher than those of ^{85}Sr, however. Subsequent studies in adult rabbits (Subramanian et al, 1972a) with a longer chain polyphosphate compound, with a chain length of 46 and a molecular weight of 4660, showed similar skeletal uptake to ^{85}Sr, but blood levels of this polyphosphate compound were only slightly higher than ^{85}Sr and three times lower than tripolyphosphate. With the long chain polyphosphate compound, higher bone:background activity ratios were obtained than with tripolyphosphate, and skeletal visualisation was better. Using this compound skeletal metastases were identified in patients with

both a rectilinear scanner and a gamma camera (Subramanian et al, 1972a).

Following the initial development of ^{99m}Tc polyphosphates, attention turned to other phosphates which would be suitable as bone scanning agents. A particularly promising compound was ^{99m}Tc pyrophosphate. Pyrophosphate is a simple chemical, an anhydro-dimer of orthophosphate, and is normally present in body fluids. It is known that pyrophosphate is important in the regulation of the calcification of bone, although its precise role in calcium metabolism and in diseases of bones and teeth is not well defined (Fleisch and Russell, 1970; 1972). When labelled with ^{99m}Tc, pyrophosphate provided high bone/background activity ratios, and satisfactory skeletal scintigrams were obtained in experimental animals and early patient studies (Perez et al, 1972; Cohen et al, 1972; Hosain, 1973; Fletcher et al, 1973; Huberty et al, 1974).

The third compound proposed as a bone scanning agent was ^{99m}Tc diphosphonate. The possibility of using polyphosphate or pyrophosphate as a therapeutic agent in the treatment of bone diseases has been considered (Russell and Smith, 1973), but the rapid destruction of these compounds by tissue phosphatases made their therapeutic use impossible. The development of the diphosphonates stemmed from the search for a compound which combined the biological properties of pyrophosphate and polyphos-

phates, together with resistance to enzymatic destruction in vivo (Fleisch et al, 1969; Francis et al, 1969). Diphosphonates possess P-C-P instead of P-O-P bonds and are consequently much more stable to both chemical and enzymatic degradation than pyrophosphate and polyphosphate (Russell and Smith, 1973). Several groups of workers independently proposed and evaluated ethane-1-hydroxy-1 1-diphosphonate, also known as ethylene hydroxy diphosphonate or hydroxy ethylidene disodium phosphonate (E.H.D.P. or H.E.D.S.P.A.) as a bone scanning agent (Tofe and Francis, 1972; Castronovo and Callahan, 1972; Subramanian et al, 1972b; Yano et al, 1973a). Satisfactory concentration of ^{99m}Tc-E.H.D.P. in rat and rabbit skeleton was demonstrated by these studies, and high quality gamma camera scintigrams obtained. Recently a different diphosphonate compound ^{99m}Tc labelled methylene diphosphonate has also been suggested (Subramanian et al, 1974).

Following the initial successful evaluation of polyphosphate, pyrophosphate and ethane hydroxy diphosphonate, a fluorophosphate compound was also introduced. The initial clinical evaluation of this compound, ^{99m}Tc labelled monofluorophosphate which utilises stannous fluoride as reducing agent, is described in this thesis. This compound also permits satisfactory visualisation of both normal and tumour-involved bone in clinical studies.

(2) In Vitro Properties of the ^{99m}Tc Phosphates (Table I.1)

These have been extensively studied and summarised by Dunson et al (1973). All of the compounds are compounded as sodium salts, and are available as white lyophilised powders, mixed with stannous chloride. The pyrophosphate compound used in all the experiments described in this thesis was in aqueous solution, but it is now available in a lyophilised form. An oxygen free atmosphere improves the in vitro stability of all compounds. Diphosphonates and pyrophosphates are more stable in vitro than the polyphosphates which tend to hydrolyze spontaneously. Accurate estimation of the chain length of commercially available polyphosphates, using end-point titration and nuclear magnetic resonance, has shown a significantly lower chain length than that claimed by the manufacturer. It appears that any one polyphosphate compound is usually a mixture of compounds of varying chain length (King et al, 1973).

All ^{99m}Tc phosphate compounds are available in a "single step" kit form, and are easily labelled by the addition of reduced ^{99m}Technetium (IV) as sodium pertechnetate solution, obtained from a commercial ^{99}Molybdenum-^{99m}Technetium generator. A range of phosphate:stannous chloride weight ratios (5:1-50:1) permits satisfactory labelling of the phosphate with ^{99m}Tc (Tofe and Francis, 1974). For satisfactory labelling it is essential to maintain the technetium in the reduced state, and this is

accomplished by the presence of stannous chloride, and by the avoidance of oxidising agents in the generator eluate. Because of instability of the labelled compound in the chromatography solvent, instant thin layer chromatography may not give an accurate estimate of labelling efficiency and starch gel chromatography is necessary if an accurate measure of labelling efficiency is desired (Eckelman and Richards, 1972). In practice labelling efficiencies of greater than 95% are consistently obtained, and routine measurement of labelling prior to injection is not required.

(3) Mechanism of Skeletal Uptake of the ^{99m}Tc Phosphates

The actual mechanism of skeletal uptake of these compounds is incompletely understood but is thought to be related to their chemistry. Neuman and Neuman (1953) described the localisation of anionic metal complexes in bone. The technetium-tin-phosphate complex is anionic and it is thought that bone uptake of the complex is a physicochemical reaction leading to the "chemisorption" of a mono-molecular layer onto the hydroxy-apatite fraction of bone (Francis et al, 1969). The reaction is probably between the phosphate, and the hydroxyl groups of the hydroxy-apatite molecule (Tofe and Francis, 1972). This suggested mechanism of chemisorption of phosphate or phosphonate to the hydroxy-apatite crystal, though conceptually attractive, has not yet been established. There are several problems regarding the

bone uptake of these compounds, and these are discussed below.

The ^{99m}Tc-tin-polyphosphate and ^{99m}Tc-tin-pyrophosphate complexes are stable in vivo in the short term at least (thus permitting skeletal scintigrams to be obtained). The uncomplexed compounds, however, are rapidly destroyed in vivo by tissue polyphosphatases (Harold, 1966) and pyrophosphatases (Russell and Smith, 1973). There is some evidence to suggest that long chain ^{99m}Tc-tin-polyphosphate (chain length 40-50) is readily hydrolysed in vitro by the amounts of alkaline phosphatase normally found in blood, and it has been shown that following intravenous injection, long chain polyphosphate is hydrolysed at least in part to ^{99m}Tc-Sn-pyrophosphate (Bowen and Garnett, 1974). It is likely that the presence of tin and/or technetium prevents the further hydrolysis of ^{99m}Tc-Sn-pyrophosphate to orthophosphate, and it may be that a cyclical molecule of pyrophosphate is the stable unit that localises in bone after injection of both long chain polyphosphates and pyrophosphate (Bowen and Garnett, 1974). A comparison of the relative bone deposition of polyphosphates of different chain lengths shows that the shorter chain lengths of polyphosphate yield the highest bone concentration, while the highest bone uptake of all is found with degraded long chain polyphosphates (King et al, 1973). There is a possibility that different mechanisms may operate with polyphosphate and diphosphonate uptake. Enzyme activity apparently plays no significant part in polyphosphate uptake (Cox, 1974a) while tissue binding of

diphosphonate by acid and alkaline phosphatase has been postulated as the mechanism of its uptake and retention by bone (Zimmer et al, 1975).

It has even been suggested that the role of phosphates is a secondary one and not directly concerned in the mechanism of technetium uptake in bone (Cox, 1974a). The high bone affinity for tin chelates is well documented (Yano et al, 1973b). Following the intravenous injection of a ^{99m}Tc-tin-^{32}P pyrophosphate compound, the ^{99m}Tc radioactivity remains associated with the skeleton after the ^{32}P pyrophosphate has been degraded into orthophosphate, and much of the ^{32}P radioactivity has been redistributed to the blood, liver and other soft tissues (Dunson, Personal Communication, 1974). These observations lend support to Cox's suggestion that the bone seeking properties of the ^{99m}Tc-tin-phosphates are primarily related to either the technetium (IV) or tin, and that the role of the phosphates lies in their ability to prevent oxidation of the technetium tin complex to bone marrow seeking colloids (Cox, 1974a).

Whatever the precise mechanism of bone uptake of the ^{99m}Tc phosphates, there is no doubt that there is enhanced uptake of labelled radiopharmaceutical in pathological areas, due either to increased local blood flow, or increased osteogenesis, resulting in increased active transport of calcium and phosphate. Autoradiographs of human femoral heads obtained at operation show

deposition of previously administered ^{99m}Tc polyphosphate in proximity to bone marrow, to osteocytes and in linear patterns at the junction of bone of different maturities (Tilden et al, 1973). It is likely that of the two factors, bone blood flow and osteoblastic activity, blood flow is more important (Genant et al, 1974).

(4) Other Technetium Labelled Bone Scanning Agents

Following the introduction of polyphosphates, pyrophosphate and ethane hydroxy diphosphonate, several other ^{99m}Tc labelled bone scanning agents have been suggested. In view of the affinity of fluoride for the hydroxy apatite crystal, a ^{99m}Tc stannous fluoride complex was evaluated in experimental animals with promising results which have not yet been confirmed in man (Chervu et al, 1973). ^{99m}Tc-Sodium monofluorophosphate has been briefly referred to, and recently two interesting new compounds, technetium titanium citrate and a technetium titanium hexametaphosphate complex, have been suggested as potentially valuable bone scanning agents (Cox, 1974a). These compounds have not yet been clinically evaluated.

(5) Comparison of the ^{99m}Tc Phosphates with ^{85}Sr, ^{87m}Sr and ^{18}F

A comparison of the relevant physical properties of ^{99m}Tc with the conventional agents (Table I.2) emphasizes the advantages of the physical properties of ^{99m}Tc, which include high yield of

easily collimated 140 KeV photons, short physical half life, and convenient availability from a 67 hour half life parent, ^{99}Mo. It is clear that, providing the ^{99m}Tc phosphates have suitable biological and pharmacological properties, they will be very suitable bone scanning radiopharmaceuticals, and that they constitute a real advance over the existing agents.

Animal studies have demonstrated similar in vivo distribution of tripolyphosphate, long chain polyphosphate and ^{85}Sr (Subramanian and McAfee, 1971; Subramanian et al, 1972a). Similar studies with diphosphonate by the same workers demonstrated better characteristics for this compound when compared with ^{85}Sr (Subramanian et al, 1972b). The overall superiority of ^{99m}Tc phosphates when compared with ^{85}Sr and ^{87m}Sr has been confirmed in preliminary clinical studies (Hosain et al, 1973; Marty and Denney, 1973; Oxley et al, 1973). The biological properties of ^{18}F are superior to those of the ^{99m}Tc phosphates (Yano et al, 1973a; Ackerhalt et al, 1973; Krishnamurthy et al, 1974a;) but clinical comparison of ^{18}F with ^{99m}Tc polyphosphate (Weber et al, 1974; Barrett and Smith, 1974; Krishnamurthy et al, 1974b) and ^{99m}Tc diphosphonate (Silberstein et al, 1973), showed better results in terms of image quality and detection rate of lesions with the ^{99m}Tc phosphates.

There is a similar pattern of distribution of both the ^{99m}Tc phosphates and ^{18}F, and the greater detail of the normal and abnormal skeleton seen with the phosphates is due in part to the

increased photon flux due to the higher injected activity (Silberstein et al, 1973; Pendergrass et al, 1973). Another factor of importance in determining image quality is the better resolution obtainable with the 140 KeV photon of ^{99m}Tc when compared with the high energy emission of ^{18}F (Charkes et al, 1973).

The early clinical results reported above, together with the more favourable physical properties, lower cost and easy availability of ^{99m}Tc make it likely that in virtually all bone scanning studies, the ^{99m}Tc phosphates will become the agents of first choice.

(6) Background to Studies described in Thesis

In late 1972 there were made available to us for study several different ^{99m}Tc labelled phosphate preparations:

(1) Ethane Hydroxy Diphosphonate manufactured by Diagnostic Isotopes Incorporated, New Jersey, U.S.A. (E.H.D.P.).

(2) Pyrophosphate manufactured by Departement des Radio-elements, Centre d'Etude Nucleaires de Saclay, Gif-Sur-Yvette, France (PYRO).

(3) A Polyphosphate compound manufactured by Diagnostic Isotopes Incorporated, New Jersey, U.S.A. (D.I.I. POLY).

(4) A Polyphosphate compound manufactured by New England Nuclear Incorporated, Massachusetts, U.S.A. (N.E.N. POLY).

Shortly thereafter a fifth compound became available for study:

(5) Monofluorophosphate manufactured by The Radiochemical Centre, Amersham, Bucks, England (M.F.P.).

All of the compounds were made available in very convenient and easy to use "single step" kit forms, requiring only the addition of sodium pertechnetate (^{99m}Tc) solution for instant labelling prior to injection. As previously discussed, there is no highly significant difference between the in vitro properties of the different compounds although overall E.H.D.P. is chemically the most stable and reproducible (Dunson et al, 1973). The same radio-nuclide (^{99m}Tc) is used with all compounds and so there is no advantage in this respect in choosing any particular phosphate. The choice of which of these compounds is the most suitable depends, therefore, on a comparison of their pharmacological properties. In 1972 and 1973 no such comparison had been performed and the choice of which agent to use remained arbitrary. A detailed quantitative comparison of the relevant pharmacological properties of the five compounds detailed above is a major part of the work which we have carried out in the past few years and these comparative experiments are described in detail in the next section of this thesis.

SECTION II. COMPARISON OF THE PHARMACOLOGICAL PROPERTIES OF THE ^{99m}Tc PHOSPHATES

A. INTRODUCTION

In this section, are described the experiments carried out to compare the in vivo pharmacological properties of the ^{99m}Tc phosphates. Five different compounds were studied:

(1) Ethane hydroxy diphosphonate (E.H.D.P.).

(2) Pyrophosphate (PYRO).

(3) A Polyphosphate (D.I.I. POLY).

(4) A Polyphosphate (N.E.N. POLY).

(5) Monofluorophosphate (M.F.P.).

Throughout this thesis, these abbreviations are used to simplify the text.

Two different polyphosphate compounds were studied because of the known chemical instability (King et al, 1973) and occasional radiopharmaceutical inconsistency (Dunson et al, 1973) of this compound. As will be seen later, small but consistent differences were found between the properties of the two polyphosphate compounds. Chronologically, M.F.P. was made available to us after the 4 other compounds had been studied. By this time we had concluded that E.H.D.P. was superior to PYRO and POLY, and our

evaluation of M.F.P. was, therefore, basically a direct comparison with E.H.D.P. only.

Although animal experiments are essential in the pre-clinical evaluation of any new diagnostic or therapeutic agent, results in animal experiments may not always be directly extrapolated to the human subject. In particular, measurement of isotope concentration in tumour-involved bone is very difficult in animals, as there are available very few satisfactory animal models with bone tumours. As the main clinical importance of bone scanning at present is in the study of malignant disease, it seemed important to consider the localisation of these compounds in tumour-involved bone. For these reasons we restricted all our pharmacological experiments to humans, patients with known or suspected bone tumours and volunteer subjects with no evidence of bone disease.

All experiments described in this and subsequent chapters were performed with the permission of the Isotope Advisory Panel of the Medical Research Council; the Medicines Commission of the Department of Health and Social Security; the Ethical Committee of Glasgow Royal Infirmary and Associated Hospitals, and where appropriate, the informed verbal or written consent of the individual patients and volunteer subjects concerned.

The following pharmacological properties are relevent to a discussion of bone seeking radiopharmaceuticals and were measured

in the studies described in this section:

(1) Uptake by tumour-involved bone.

(2) Uptake by normal bone.

(3) Soft tissue uptake.

(4) Blood clearance.

(5) Urinary excretion.

(6) Whole body retention.

(7) Toxicity.

B. VALIDATION OF EXPERIMENTAL TECHNIQUES DEVELOPED TO COMPARE THE PHARMACOLOGICAL PROPERTIES OF THE ^{99m}Tc PHOSPHATES

New experimental methods were developed to investigate 3 different pharmacological properties of the ^{99m}Tc phosphates:

(1) Uptake by tumour-involved bone.

(2) Uptake by normal bone.

(3) Serial measurement of blood levels, urinary excretion and whole body retention of microcurie activities of each compound administered to young healthy male volunteers.

(1) Radionuclide Uptake by Tumour-involved Bone

Description of Technique

The visualisation on a scan of any lesion labelled with a positive label depends on a high target:background activity ratio. Clearly the higher the target to background ratio, the "hotter" is the hot spot and the easier its visual identification. As previously described, a bone tumour is seen on the bone scan as a hot spot, because of the focally increased concentration of bone seeking radiopharmaceutical due to increased blood flow and increased metabolic activity at the site of tumour involvement.

It is clear that in any comparison of bone scanning radiopharmaceuticals used to identify bone tumours, a comparison of the target to background ratios obtained with each compound is of great importance. Unfortunately the appearance of the lesion seen on the image recorded from the gamma camera on polaroid or x-ray film is highly variable, as it depends on the variable exposure of the detecting system, which varies with the total number of counts and oscilloscope intensity. No quantitative data is obtainable from the display, and it is not possible to directly compare the different hot spots unless an identical exposure has been used in obtaining the scintigram.

To permit statistically valid comparisons in our studies, a relatively simple electronic technique was devised by Dr. R.G. Bessent, to provide quantitative data from the gamma camera display, independent of exposure. Such facilities are available if the gamma camera is on line to a computer or hard-wired processor. The system developed in our department provides the same quantitative facility without a computer, using simply a single-channel analogue pulse height analyser (P.H.A.), a Nuclear Data multi-channel analyser (M.C.A.) and some ancillary circuitry interfaced to a Nuclear Enterprises Scinticamera IV gamma camera (Figure II.1).

The X co-ordinate pulses from the gamma camera are taken to the analogue P.H.A. whose output pulses feed the coincidence in-

put of the M.C.A. The Y co-ordinate pulses are taken directly to the analogue-to-digital converter input of the M.C.A., but only Y pulses whose associated X pulses fall within the window of the P.H.A. are accepted. The M.C.A., therefore, generates an activity profile in the Y direction which is arranged to occupy about 50 channels. The "ΔE" control of the P.H.A. is used to select the width of a narrow vertical strip on the gamma camera field and the "E" control is used to move the strip to the anatomical region through which the profile is required. Because the P.H.A. output pulses feed the intensity control unit, the strip appears as a band of diminished intensity on the gamma camera display (Figure II.2). This allows accurate direct adjustment of the profile position. After a suitable acquire-time the profile appears on the screen of the M.C.A. and is then typed out in digital form. A profile in the X direction can be simply obtained by interchanging the X and Y co-ordinate signals to the circuit.

Definition of Tumour:Bone Ratio

Using the quantitative profiling facility it is possible to quantitate the activity in any visibly tumour-involved area of bone. The tumour:bone ratio is defined as the gross activity from an area of tumour-involved bone (A) divided by the gross activity from an equal area of corresponding normal bone (B).

In the case of spinal metastases the "normal bone" used is an area of apparently healthy bone in the same part of the spine (Figure II.3). In the case of metastases in areas other than the spine, for example the ribs, the contralateral area of the skeleton is used as the normal area for the calculation of the tumour:bone ratio (Figure II.4). Calculation of the tumour: bone ratio from the digital print out of the gamma camera M.C.A. system is shown (Figure II.5).

Validation of the System

We have described this gamma camera/multi-channel analyser system in some detail as it is the basic system used in all the quantitative gamma camera studies described in this thesis. To validate the system and establish its reproducibility, ten patients with known, x-ray positive bone metastases were studied.

Each patient was scanned twice, using an identical technique and the same radiopharmaceutical (^{99m}Tc-E.H.D.P.). Tumour: bone ratios were measured four hours after the intravenous injection of 10 mCi of E.H.D.P. The ratios obtained from the same tumour areas were compared by paired "t" test and also by correlation of the duplicate results from the individual pathological areas.

Results and Discussion

In the ten patients studied, there were 33 areas of tumour-involved bone in which measurement of isotope activity was measured. Thus 33 tumour:bone ratios were measured on two occasions. The mean and standard error of the mean for each set of observations was 1.79 ± 0.09 and 1.75 ± 0.09. Using the paired Student's 't' test, there was no significant difference between the results. Correlation between the duplicate results was good ($r = 0.95$, $n = 33$, $y = 0.19 + 0.87\,x$, $p < 0.001$) (Figure II.6).

The validation study shows that the technique of quantitative bone scanning, using the system described, provides highly reproducible data. It follows that if a series of patients were scanned using the same technique but different radiopharmaceuticals, then any significant difference demonstrated between the tumour: bone ratios obtained from the same tumour area, would be due to a difference between the properties of the compounds under study.

(2) Radionuclide Uptake by Normal Bone

Description of Technique

The standard method employed to measure the uptake of gamma emitting nuclides and radiopharmaceuticals involves the administration of small activities to experimental animals, usually rat or rabbit, with normal skeletons. At successive intervals after intravenous injection the animals are killed and samples of bone,

bone marrow, blood, muscle and internal organs are counted in a well scintillation counter. A standard sample obtained from the injected material is also counted. In this way the absolute uptake of the agent under study by bone and other internal organs is obtained. Bone:soft tissue activity ratios are then calculated (Subramanian and McAfee, 1971; Subramanian et al, 1972a). In this way an indication of the skeletal uptake of the compound in the human subject is obtained.

No satisfactory method is available to directly study normal bone uptake in man. An estimate of bone uptake may be made by measuring the activity excreted after administration of a known activity to a human subject. The excreted activity is subtracted from the total activity injected. In the case of ^{99m}Tc phosphates where all the excreted activity is in the urine, and faecal excretion is negligible, this approximation is valid (Krishnamurthy et al, 1974c; 1974d). The method is indirect, however, and no account is made of unexcreted non-osseous activity in blood, muscle and other soft tissues. We have devised a non-invasive method which provides a direct quantitative index of normal bone uptake. This method permits repeated measurements to be made at any time interval after injection, thus facilitating dynamic uptake measurements.

Definition of Spine:Background Ratio

If a region of interest is drawn transversely across the lower dorsal spine just above the kidneys using the gamma camera/multi-channel analyser system previously described, quantitative measurement of activity can be obtained from the spine and surrounding non-spine background (Figure II.7). The spine: background ratio is defined as the total counts from the spine area divided by the total counts from an equal area of the non-spine background.

A more detailed discussion of the spine:background ratio will be found in a subsequent chapter. The present discussion relates to the use of the ratio as an index of normal bone uptake in the study to compare the relative merits of the different ^{99m}Tc phosphates under study. For this purpose spine:background ratios were measured 2, 4 and 6 hours after the intravenous administration of the labelled pharmaceutical in 35 control subjects.

(3) Measurement of Blood, Urine and Whole Body Levels of Microcurie Activities

Description of Technique

Serial measurement of the levels of gamma emitting radionuclides in blood, urine and whole body, using a well scintillation detector, bulk sample scintillation detector and shadow shield whole body monitor, respectively, are standard laboratory

techniques. Serial blood levels of the ^{99m}Tc phosphates were measured during 95 routine scanning studies of patients with suspected metastatic disease after the intravenous injection of 10 mCi of the compound. To measure the kinetics of these compounds in greater detail would require repeated measurements and it would have been unpleasant for patients with skeletal cancer, many of whom feel unwell and are in pain, to take part in tests that involve multiple venepunctures, timed urine collections and repeated whole body monitor studies. For this reason we decided to study the kinetics of microcurie activities of each compound injected into healthy young male volunteers. The standard technique when scanning patients is to add 30 mCi of ^{99m}Tc-pertechnetate solution to a vial of unlabelled phosphate, and inject 2 ml of the resulting 7 ml solution into the patient. In the case of Pyro which is a liquid (volume 2 ml), 3 ml of the resulting 9 ml solution is given to the patient. Accordingly for the microcurie study, 100 uCi in 7 ml was added to the vials of compounds under study and either 2 ml or 3 ml of the resulting solution, in the case of Pyro, injected.

The ratio of the volume of pertechnetate solution to radiopharmaceutical was the same in the controls and scanning studies, although the molar concentration of the pertechnetate solution was 3×10^2 times higher in the patient studies. Previous work

had shown that an eighty fold dilution of a tripolyphosphate solution did not alter tissue localisation to any significant extent (Subramanian and McAfee, 1971). It seemed, likely, therefore, that a technique using uCi activities would be a valid method of assessing the distribution and kinetics of the compounds under study. To confirm this validity an experiment was performed.

Validation of Technique: Materials and Methods

The principle of the experiment was to measure and compare serial blood and urine levels of activity in 10 volunteer subjects on 2 different days, once after the injection of 5 mCi of E.H.D.P. (1.5 mg in 1 ml solution), and on the second occasion after the injection of 20 uCi of the same compound (2 mg of E.H.D.P. in 2 ml solution).

Five ml venous blood samples were obtained at 5 min, 15, 30, 45, 60, 120, 180, 240, 300 and 360 minutes after injection: the subjects emptied their bladders 15, 30, 45, 60, 120, 180, 240, 300 and 360 minutes after injection. All urine was stored until after the completion of the study. Blood and urine samples were subsequently counted in a well scintillation counter and bulk sample scintillation counter respectively. In each case standard samples obtained from the injected material were also counted. Blood results were expressed as % injected activity/litre of

whole blood and cumulative urine excretion was expressed as % injected activity excreted.

The duplicate results from each subject were compared by correlation.

Results

The blood and urine levels of the ten subjects are shown in Table II.1. When the duplicate results for each individual subject were compared, a high degree of correlation was obtained between the results obtained in the paired studies for blood levels ($r = 0.88$, $n = 100$, $p < 0.001$) and urine excretion ($r = 0.86$, $n = 82$, $p < 0.001$) (Figures II.8; II.9).

It is clear, therefore, that the microcurie technique provides a satisfactory method to study the blood and urinary levels of the compounds in human subjects. Accurate and consistent measurement of serial blood and urinary levels of the radiopharmaceutical were obtained with a 3×10^2 reduction in injected radioactivity and consequently a comparable reduction in radiation absorbed dose. It is possible to extrapolate the results obtained in such a study to similar studies involving larger activities (e.g. 10 mCi), and the results of such studies involving the repeated injection of microcurie activities in healthy young volunteers, are presented later in this section. A further advantage in studying healthy young volunteers whose co-operation

is assured, is the ease of performance of these experiments and the resulting high degree of reproducibility is illustrated by the low standard error of the mean in the results.

C. COMPARISON OF THE PHARMACOLOGICAL PROPERTIES OF THE ^{99m}Tc PHOSPHATES

In the preceding part of this section several experimental methods are described and validated. These methods were developed to permit a detailed quantitative comparison of the important pharmacological properties of bone scanning agents. In this part of the section is presented a comparison of the pharmacological properties of the five ^{99m}Tc phosphate compounds studied.

(1) Uptake by tumour-involved bone

Principle

The tumour:bone ratio has already been defined and its importance briefly discussed. In any comparison of compounds used to positively identify bone tumour, it is clearly important to compare the tumour:bone ratios which each compound can provide. Tumour:bone ratios have been measured in a large number of patients with bone metastases and the values obtained with each compound compared.

The principle of the comparison of the ^{99m}Tc phosphates, with respect to tumour:bone ratios, was to study patients with known x-ray positive metastases on 2 or 3 different occasions using an identical technique but a different phosphate compound on each

occasion. Treatment remained unchanged during the period of study, and the only variable, therefore, was the compound under study. As the initial validation study had shown the tumour:bone ratio to be a highly reproducible entity, it followed that if a significant difference were found between the tumour:bone ratios obtained with any one radiopharmaceutical when compared with the ratios obtained with a different compound from the same tumour areas, then this would represent a significant difference between the compounds under study.

Materials and Methods

Thirty eight patients with known x-ray positive bone metastases were studied (Table II.2). A total of 92 studies were performed in these patients (Table II.3). Sixteen patients had three studies (one study with one of the Poly compounds and further studies with E.H.D.P. and Pyro) and 22 patients were scanned on two occasions (with two of the different compounds under study). 144 tumours were studied in the 38 patients. The total number of tumours visualised with each compound is shown in Table II.3.

All studies were conducted in the following way: 30 mCi of ^{99m}Tc pertechnetate solution was added to the vial containing the stannous phosphate. Immediately after thorough mixing, 2 ml of the resulting solution was injected intravenously. As previously discussed the pyrophosphate compound studied was not lyophilised

but was available in aqueous solution. Accordingly 3 ml of the labelled pyrophosphate was injected. Thus 10 mCi of ^{99m}Tc PYRO was administered to the patients, while 8.6 mCi of all other compounds was used. Four hours after injection, the known tumour-bearing areas were visualised using the gamma camera. At the same time total activity in tumour-involved and normal bone were measured during a 100 second acquire time, using the gamma camera-M.C.A. system previously described. Tumour:bone ratios were thereafter calculated from the digital printout of the M.C.A. The different values obtained with the different compounds for the tumour:bone ratios obtained under similar conditions from the same tumour areas in the same patients were compared using a paired Students' 't' test.

Results

The tumour:bone ratios obtained in this study with E.H.D.P. were significantly higher than those obtained with all other compounds (Table II.4). PYRO gave higher ratios than D.I.I. POLY, but no higher than the N.E.N. POLY. M.F.P. was only compared directly with E.H.D.P. and gave lower tumour:bone ratios than E.H.D.P. Overall the tumour:bone ratios obtained with M.F.P. were similar to those obtained with D.I.I. POLY.

(2) Uptake by normal bone

Principle

As previously described, the transverse spine:background ratio was devised as an index of normal bone uptake which could be simply measured using non-invasive methods in human subjects. To compare the relative merits of the ^{99m}Tc phosphates, serial spine:background ratios obtained with the different compounds in a number of similar patients were studied. This study was performed before M.F.P. became available, and as the study in patients with bone metastases confirmed that M.F.P. was inferior to E.H.D.P. and was quite similar to D.I.I. POLY, experiments to measure normal bone uptake were not carried out with M.F.P.

Materials and Methods

Thirty five patients with no evidence nor history of bone disease nor cancer were studied. There were 17 male patients (aged 34-71, Mean = 50.8) and 18 post menopausal females (aged 35-67, Mean = 53.7). All had been admitted to Glasgow Royal Infirmary with a variety of medical and surgical conditions (e.g. haemorrhoids, inguinal hernia, myocardial ischaemia, diabetes mellitus) and were in a convalescent phase. Each patient was studied on one occasion only, and received either 8.57 mCi of a POLY compound or E.H.D.P., or 10 mCi of PYRO by intravenous injection (Table II.5). Two, 4 and 6 hours after injection

the total counts from spine and background areas were measured for a 200 second period. From the total counts recorded, spine: background ratios at 2, 4 and 6 hours were calculated for each patient and the Mean and Standard Error of the mean for each compound calculated.

Results

The results are summarised in the Table (II.6). The spine: background ratios obtained with E.H.D.P. at 4 and 6 hours were higher than the ratios obtained with all other compounds. The difference between E.H.D.P. and PYRO at 4 hours just failed to achieve statistical significance using an unpaired 't' test. PYRO gave higher spine:background ratios than both POLY compounds.

When the absolute number of counts from the spine and soft tissue background, corrected for the physical decay of the nuclide, were compared a similar pattern was found with all compounds: a fall in activity in both areas was noted from 2 till 6 hours (Table II.7). If a uniform soft tissue background across the region of interest is assumed, subtraction of the background activity from the spine activity gives a measure of net bone uptake. There was no significant difference between the net bone uptake of the four compounds studied in this experiment.

(3) Soft Tissue Uptake and Blood Clearance

Principle

Animal studies have shown no significant concentration of ^{99m}Tc phosphates in any non-osseous tissue apart from the urinary tract. Scanning studies in man confirm the absence of concentration of ^{99m}Tc phosphates in normal non-osseous tissues other than kidney and bladder with occasional exceptions which are discussed in a subsequent section. For these reasons, and because of the technical difficulties involved, detailed quantitative measurement of activity in muscle, connective tissue and internal organs in humans were not carried out. In common with other workers, to estimate the levels of activity in non-skeletal tissue, we have simply measured the whole blood radioactivity levels obtained with the different compounds. As we discuss in a subsequent chapter, whole blood clearance is one of the most important pharmacological properties of bone seeking compounds, and the "soft tissue" activity seen on the scintiscan largely reflects blood levels.

Material and Methods

In 19 patients with known bony metastases who had repeated studies with different compounds, and in a further 41 patients routinely scanned, blood activity levels were measured at 5 minutes and 2, 4 and 6 hours after intravenous injection. A

total of 95 studies were carried out in 60 patients. A 5 ml sample of venous blood was counted in a well scintillation counter and the activity compared in each case with the activity of a standard sample obtained from the injected material. Blood levels were then expressed as a percentage of the injected activity/litre of whole blood. The different values for whole blood activity of the different compounds at 2, 4 and 6 hours were compared by unpaired 't' test. M.F.P. was not studied in this experiment. Blood levels of this compound were measured using the microcurie technique described below.

Results

Blood levels of E.H.D.P. were significantly lower than those of all the other compounds studied in this experiment. Blood levels of PYRO were significantly lower than the blood levels of the two POLY compounds (Table II.8).

(4) Blood Clearance, Urinary Excretion and Whole Body Retention of Microcurie Activities

Earlier in this section is described the development and validation of a method which allowed accurate measurement of serial blood, urinary and whole body levels of 30 uCi activities of the compounds under study in healthy young subjects.

Materials and Methods

A detailed study of serial blood clearance, urinary excretion and whole body levels was performed in 20 control subjects, healthy male medical students and doctors (age 20-26). The 5 compounds were studied in 2 related experiments which employed identical methods. In the first experiment, 10 of the control subjects were each studied with 3 compounds (E.H.D.P., PYRO and one POLY). Each subject received 30 uCi of labelled phosphate by intravenous injection on 3 different occasions at weekly intervals. Some months after the first experiment a further ten subjects were studied with M.F.P. when this compound became available. A total of 40 studies were performed in the two experiments (Table II.9).

Immediately prior to injection, the subject emptied his bladder. One hour after injection and hourly thereafter, blood radioactivity and whole body retention were measured until 6 hours after injection. 5 ml samples of venous blood were counted in a well scintillation counter and the activity expressed as a percentage of injected activity/litre of whole blood. Whole body levels were measured in a Shadow Shield Whole Body Monitor, and results were expressed as a percentage of the whole body radioactivity measured immediately after injection (100%). In every instance whole body levels were measured after the subject had completely emptied his bladder. The results of blood and

whole body activity obtained at the same time with each different compound were compared by paired 't' test.

In 17 studies (6 E.H.D.P., 6 PYRO and 5 D.I.I. POLY) cumulative hourly urine excretion was measured. The urine voided at the end of each hour, prior to the whole body count, was collected and later counted using a bulk scintillation counter. The excreted activity was compared with that of a standard sample obtained from the injected material. Results were expressed as a percentage of injected material excreted and were compared by unpaired 't' test.

In the second experiment serial levels of M.F.P. in blood, urine and the unexcreted activity retained in the whole body, were measured in ten of the control subjects. Equipment and techniques used were identical to those used in the first study. The results obtained were compared by unpaired 't' test with those previously obtained with E.H.D.P. in the first group of 10 controls.

Results

Blood clearance of E.H.D.P. was significantly faster than that of PYRO, POLY and M.F.P. PYRO was removed from the blood more rapidly than both POLY compounds and M.F.P. (Table II.10). Cumulative urinary excretion of E.H.D.P. was significantly higher than that of PYRO, D.I.I. POLY, and M.F.P. Urine

samples were not collected in the 5 studies with N.E.N. POLY. Urinary excretion of PYRO was significantly higher than that of M.F.P. but using the unpaired 't' test the difference between urinary excretion of PYRO and D.I.I. POLY just failed to reach statistical significance (Table II.11).

Whole body retention of E.H.D.P. was significantly lower than that of all other compounds. PYRO again occupied an intermediate position: whole body retention of PYRO was greater than E.H.D.P., but less than that of M.F.P. and the two POLY compounds (Table II.12).

The results for whole body retention directly measured in our human subjects were used to calculate the whole body radiation absorbed dose for each compound. The results are shown in Table II.13.

(5) Toxicity

In addition to considering the radiation dose from the ^{99m}Tc phosphates it is also important to consider possible toxicity of the pharmaceutical. In the experiments described in this section, 134 patients received an injection of labelled phosphate. Many patients and control subjects had multiple studies performed. On no occasion was any toxic effect noted. In all our experience with these compounds which now totals more than 1,200 patient studies, the only untoward effect recorded is occasional local pain produced at the site of an accidental perivenous injection.

D. DISCUSSION OF THE COMPARISON OF THE PHARMACOLOGICAL PROPERTIES OF THE ^{99m}Tc PHOSPHATES

The most important property of a bone scanning agent is that it should provide high target:background ratios, that is high contrast between the concentration of activity in normal and pathological areas of the skeleton. The most relevant way, therefore, to compare these compounds is to compare the tumour: bone ratios obtained from the same tumour areas. The significant differences in the recorded tumour:bone ratios in this controlled study of a large number of patients with unequivocal metastases, demonstrates that by this criterion E.H.D.P. is the most favourable compound. We also measured normal bone:background ratios in the spine of volunteer subjects, and found that the spine:background ratios were higher with E.H.D.P. than with all other compounds studied. Our results from the direct measurement of activity in normal and tumour-involved bone in humans are confirmed by animal experiments where bone/blood and bone/soft tissue ratios were higher with E.H.D.P. than PYRO and POLY (Subramanian et al, 1972a; 1972b; Hughes et al, 1973; Yamamoto et al, 1974). From indirect measurements of blood and urinary levels of ^{99m}Tc phosphates administered to a small number of patients, bone:blood ratios were calculated and found

to be higher with E.H.D.P. than PYRO and POLY (Krishnamurthy et al, 1974c; 1974d). In a paired patient study similar to that described above, higher target:background ratios in normal and abnormal bone were obtained with diphosphonate than with a polyphosphate compound (Serafini et al, 1974).

The calculation of net bone uptake from the recorded gross activity in spine and background areas shows that the net bone uptake of E.H.D.P. is no higher than that of the other phosphates, and this observation confirms early animal experiments with these compounds (Subramanian and McAfee, 1971; Subramanian et al, 1972a). The higher target:background ratios obtained with E.H.D.P. are due not to higher bone uptake but, as discussed in detail in the next section, are due to lower background activity consequent upon more rapid clearance of unfixed diphosphonate from blood and soft tissues.

In addition to comparing the tumour:bone and the spine: background ratios obtained with each compound, we thought it important to compare blood and whole body activities for two reasons: firstly the quality of the skeletal image is improved by lowered blood and soft tissue activity on the scintiscan, and secondly, the whole body radiation absorbed dose depends on the effective half life of the compound under study, which in turn depends on the biological half life.

Having shown that blood levels of E.H.D.P. at 2, 4 and 6 hours were lower than blood levels of the other compounds under study in 60 patients, we then compared the detailed blood clearance of these compounds in healthy young volunteers. The results of this study confirm our earlier observations, and those of other workers who have demonstrated a more rapid blood clearance of E.H.D.P. (Krishnamurthy et al, 1974c; 1974d).

The more rapid urinary excretion of E.H.D.P. is also confirmed by Krishnamurthy et al (1974c; 1974d). The higher values for urinary excretion of all compounds in our studies compared with the published results in the literature, is probably due to the fact that we measured urinary excretion in healthy young male subjects with normal renal function who were encouraged to void urine frequently. Whole body levels of E.H.D.P. were lower than those of all other compounds studied. There was good correlation between the blood and whole body levels of each compound and it is clear that measurement of blood activity gives an accurate estimate of whole body activity at all times (Figure II.10).

From our measurement of whole body activity levels, the whole body radiation absorbed dose was calculated for each ^{99m}Tc phosphate. These calculated values are of a similar order to those obtained from extrapolation of animal data (Castronovo and Callahan, 1972). The calculated whole body

radiation dose for E.H.D.P. is 15 mRad/mCi, and this compares favourably with 20 mRad/mCi for ^{87m}Sr and 50 mRad/mCi for ^{18}F (Castronovo and Callahan, 1972). The whole body radiation dose from a ^{99m}Tc phosphate bone scan is very low and is similar to that from other standard Nuclear Medicine procedures which utilise ^{99m}Tc (Table II.14). Critical organ dose is also acceptably low. When compared overall with some standard radiological procedures, it can be seen that the radiation dose from a bone scan is acceptable and is low enough to permit repeated studies (Table II.14). To reduce radiation exposure, especially to the bladder which is the critical organ, frequent voiding of urine should be encouraged.

Many thousands of bone scans have now been obtained with these compounds, including repeated studies in many patients, and no toxic effects have been reported (Castronovo and Callahan, 1973; Subramanian et al, 1974). There is no evidence that any of the ^{99m}Tc phosphates studied is potentially more toxic than any other compound (Castronovo, 1973). The potential risk of acute intravenous toxicity has been examined in detail with E.H.D.P. and this experiment is described in detail in the next section. Overall we have no doubt that these compounds are safe and suitable for routine clinical use.

All of the ^{99m}Tc phosphate compounds described in this section provided reasonable visualisation of both normal and

tumour-involved bone. The variation in image quality experienced when the gamma camera is used to take multiple views in discontinuous sections makes it difficult to compare different scans. No formal subjective comparison was made, therefore, of the images obtained with each compound, although overall E.H.D.P. was preferred it provided scans with lower background activity and greater contrast between normal and tumour-involved bone.

It is perhaps a happy coincidence that E.H.D.P., which is chemically the most stable and reproducible of the ^{99m}Tc phosphates studied, should also possess the most suitable pharmacological properties. Because of the significantly higher tumour:bone and spine:background ratios, more rapid blood clearance, greater urinary excretion and lower whole body retention of E.H.D.P., this compound is the phosphate bone scanning agent of choice at present. This view is confirmed by several workers who have extensively studied the available compounds, E.H.D.P., PYRO and POLY, both in terms of their in vitro properties (Dunson et al, 1973) and in vivo properties (Yamamoto et al, 1974; Subramanian et al, 1974). In the next section of the thesis is described a series of experiments carried out to evaluate in detail the properties of ^{99m}Tc-E.H.D.P.

SECTION III. EVALUATION OF ^{99m}Tc LABELLED ETHANE HYDROXY DIPHOSPHONATE (E.H.D.P.)

In the previous section are described in detail the experiments performed to compare E.H.D.P. with the alternative ^{99m}Tc phosphate compounds available. On the basis of these experiments we have concluded that E.H.D.P. is the agent of choice at the present time. In this chapter we describe a series of experiments carried out to evaluate in detail those pharmacological properties of E.H.D.P. which are of relevance to its clinical use as a bone scanning agent. The characteristics of E.H.D.P. which we have investigated are considered in 2 main groups. The first series of experiments describe the evaluation of certain *in vitro* aspects of diphosphonate pharmacology, and the second series of experiments investigate the *in vivo* kinetics of the compound in human subjects.

A. IN VITRO PROPERTIES OF E.H.D.P.

(1) Introduction

A detailed examination of the chemistry of ethane hydroxy diphosphonate is beyond the scope of this thesis. Several detailed studies of this compound have been made and are reported in the literature (Michael et al, 1972; Castronovo and Callahan, 1972; Subramanian et al, 1972b; Russell and Smith, 1973; Callahan and Castronovo, 1973; Dunson et al, 1973; Tofe and Francis, 1974; Castronovo, 1974). The most important chemical property of E.H.D.P. is its stability both in vitro and in vivo, and this by itself represents a major advantage over the polyphosphates, quite apart from any consideration of biological properties (Dunson et al, 1973). The in vitro properties of E.H.D.P. which we have investigated and discuss here are those which are relevant to the use of the compound in the clinical environment.

It is of some importance in the routine clinical use of a bone scanning agent that there should be available to the medical, technical and nursing staff, a degree of flexibility in terms of the quantity of radiopharmaceutical, and the volume and activity of radioactive label injected into the patient. Because of the size of the technetium generators routinely used in nuclear medicine departments, towards the end of the generator's life a

larger eluate volume may be necessary to supply the relatively large nuclide activities needed for rapid imaging of the whole skeleton. If the use of a larger eluate volume resulted in a poor scan image then this would clearly be disadvantageous. Other important considerations include variation in scan image with the quantity of radiopharmaceutical and/or activity of ^{99m}Tc injected, as these factors can vary from day to day depending on the number of patients to be scanned. It may also be necessary on occasions to store the labelled product for some hours prior to injection. It is clearly most desirable to inject the labelled compound immediately after labelling, but in the clinical situation, delays in patient arrival and other scheduling problems sometimes make it necessary to wait for some time after labelling before injection.

For these reasons a series of experiments was conducted to investigate the flexibility of use which a lyophilised stannous diphosphonate kit provides. Studies were carried out to examine four different but related problems; these are the possibly deleterious effect on scan quality of:

(1) varying eluate volume.

(2) varying the quantity of E.H.D.P. injected.

(3) varying the amount of radioactivity (mCi) injected.

(4) prolonged storage of the labelled compound prior to injection.

All the studies described in this section were performed using a lyophilised sterile preparation of E.H.D.P., manufactured and kindly supplied by Philips-Duphar B.V., Petten, Holland. Each vial contained 3 mg. of E.H.D.P. and 0.06 mg. of stannous chloride. The diphosphonate was labelled using ^{99m}Tc obtained as sodium pertechnetate solution from a standard ^{99}Mo-^{99m}Tc generator manufactured and supplied by Philips-Duphar B.V. In all studies, except those where the labelled material was stored for some hours, the labelled diphosphonate was injected intravenously immediately after labelling.

(2) Variation in Eluate Volume

Materials and Methods

To assess the possibly deleterious effect on scan quality of increasing the volume of pertechnetate solution added to the diphosphonate vial, a paired study was performed. Six patients, five with bone metastases and one with Paget's disease, were each studied on 2 occasions. In the first of the 2 studies, 2 ml. of sodium pertechnetate was added to the diphosphonate, and in the second study, 8 ml. of sodium pertechnetate was used. In both studies the same activity of ^{99m}Tc (10 mCi) and the same quantity of E.H.D.P. (3 mg) was injected. The only variable, therefore, was the volume of labelled pertechnetate solution added to the diphosphonate vial, and subsequently injected into the patient

(2 ml in one study; 8 ml in the second).

Using the previously described gamma camera system, Polaroid scans and tumour:bone ratios (or Paget's:bone ratios) were recorded in each study four hours after the intravenous injection of labelled diphosphonate. The two sets of Polaroid scintigrams of the same abnormal areas were visually compared. The pairs of target:background ratios obtained from the same pathological areas were compared by paired Students 't' test, the method previously used to compare the relative performance of all the ^{99m}Tc phosphates.

Results

There was no difference on visual inspection of the Polaroid scans from the paired studies. There was no significant difference in the ratios obtained from the same pathological areas in the paired studies (Mean $\pm$ S.E.M. = 2.68 $\pm$ 0.48; Mean $\pm$ S.E.M. = 2.69 $\pm$ 0.47; $0.90 < p < 0.95$) (Table III.1).

(3) Variation in Quantity and Activity of labelled Diphosphonate

Materials and Methods

A wide range of quantities of labelled E.H.D.P. (0.25 - 3 mg) have been used in our patient studies. Satisfactory visualisation of normal and pathological bone has been obtained with all the different quantities of E.H.D.P. employed. To more critically

assess the effect on scan quality of varying the quantity of E.H.D.P. and of varying the activity of ^{99m}Tc injected a paired study was performed.

Five patients with known bone metastases were each studied on two occasions. The same technique was used throughout, the only variables being the volume, quantity and activity of labelled pharmaceutical injected (Table III.2). As far as possible the ratios of ^{99m}Tc to E.H.D.P., and E.H.D.P. to eluate volume, were kept constant in the paired studies. Polaroid scans and quantitative tumour:bone ratios were measured four hours after injection. The tumour:bone ratios recorded from the same tumour areas were compared by paired 't' test.

Results

There was no difference on visual inspection of the Polaroid scans from the paired studies. There was no significant difference between the tumour:bone ratios obtained from the same tumour-involved areas in the paired studies (Mean $\pm$ S.E.M. = 1.90 $\pm$ 0.14; Mean $\pm$ S.E.M. = 1.91 $\pm$ 0.13; $0.95 < p < 0.975$) (Table III.2).

(4) Storage of Labelled Product

Materials and Methods

In the majority of studies performed, the radiopharmaceutical was injected immediately after labelling. This is clearly the optimum method. In many departments it may be more convenient

to inject the material some hours after labelling, if for example several patients are to be scanned. To ensure that storage at room temperature did not adversely effect the scan image, eight patients were studied. Seven patients had a known primary cancer (3 breast, 3 lung, 1 prostate) and one patient was a control subject with no evidence of cancer. In these patients the E.H.D.P. was labelled in the usual way, but the labelled compound was not injected until 3-5½ hours after labelling. During this time the solution was kept at room temperature. The activity of the solution was 10 or 15 mCi at the time of injection. Polaroid scans and, where appropriate, tumour:bone ratios were recorded in the standard way 2 hours after injection. Each patient was studied on one occasion only.

Results

Satisfactory images were recorded in all cases. Three of the cancer patients had radiologically proven bone metastases. These were clearly seen on the bone scans. The remaining four patients and one control subject had normal bone scans. In the 3 patients with positive bone scans, tumour:bone ratios were measured. The results clearly demonstrate that satisfactory visualisation of focal bone lesions can be anticipated even in patients where injection has been delayed until several hours after labelling (Table III.3).

(5) Discussion of In Vitro Experiments

The results of these experiments clearly confirm that the lyophilised stannous diphosphonate studied provides in a very convenient single step kit a highly flexible bone scanning agent which can be used with the technetium supplied throughout the effective life of a standard technetium generator. No significant deterioration in scan image should be anticipated despite the variation in technetium presentation, labelling and injection procedures which may occur in any busy Department of Nuclear Medicine.

In the first study, a fourfold increase in eluate volume had no deleterious effect on scan image, nor a significant effect on the quantitative tumour:bone ratios obtained. The absence of harmful effect due to dilution in these clinical studies is in accord with the absence of any change in pharmacological properties due to dilution of the labelled phosphate observed in the earliest work with polyphosphate in rabbits (Subramanian and McAfee, 1971).

We observed and described a similar lack of effect on the pharmacological properties of the ^{99m}Tc phosphates with a 300-fold dilution in molar concentration of the pertechnetate solution used for labelling in the "microcurie" method described earlier. Dunson et al (1973) have reported a slight deterioration in image quality when large quantities of E.H.D.P. are compounded, presumably due to less favourable phosphate:tin ratios. This should

not be a problem with single vials in commercially available kits, where the ratio of E.H.D.P. to tin is standardised.

In the second experiment a twofold reduction in the quantity of E.H.D.P. and the activity of ^{99m}Tc injected produced no significant change in scan image or tumour:bone ratios. A wide range of activities of ^{99m}Tc and quantities of E.H.D.P. may be used without any significant change in the scan image produced.

There are clear advantages in being able to label the diphosphonate some hours before injection: at present most centres wait several hours after injection before starting to scan, to obtain satisfactory target:background ratios. It may, therefore, be necessary to inject several patients at different times depending on their time of scanning. Because of the flexibility of use of diphosphonate it is possible to label the vial at say, 10 a.m., inject the patients between 10 a.m. and 11.30 a.m. and begin scanning at 1.30 - 2.00 p.m. In this way patient inconvenience may be minimised, and the most economic and rational use of the pharmaceutical achieved. The general policy suggested for a vial containing 3 mg. of E.H.D.P. would be:

Number of patients : 3 per vial

Activity per patient : 10-15 mCi

Eluate volume to be added : 2-8 ml

Inject within 6 hours of labelling.

Start scan within 2-4 hours after IV injection.

In an earlier discussion of the relative merits of the ^{99m}Tc phosphates the reliability and reproducibility of E.H.D.P., especially when compared with ^{99m}Tc POLY, was stressed. We have studied three samples of E.H.D.P. from different commercial sources*. In general the three E.H.D.P. products are very similar and provide highly reproducible and reliable results.

* Diagnostic Isotopes Incorporated, New Jersey, U.S.A.
Procter and Gamble Incorporated, Cincinnati, Ohio, U.S.A.
Philips-Duphar B.V., Petten, Holland.

B. IN VIVO PROPERTIES OF E.H.D.P.

(1) Introduction

In the first part of this section are described experiments which indicate the favourable in vitro properties which make E.H.D.P. a flexible and reliable bone scanning agent. In a previous section are described the series of experiments in which the important pharmacological properties of the ^{99m}Tc phosphates were compared. On the basis of these studies it was concluded that E.H.D.P. was the ^{99m}Tc phosphate of choice at present. Studies are now described in which the in vivo pharmacological properties of E.H.D.P. are examined in detail. The following properties of E.H.D.P. were measured:

(1) Blood clearance.

(2) Urinary excretion.

(3) Uptake by normal bone.

(4) Uptake by tumour-involved bone.

(5) Acute toxicity.

(6) Cumulative toxicity.

(2) Measurement of Blood Clearance, Urinary Excretion and Uptake by Normal Bone

Materials and Methods

Blood clearance, urinary excretion and normal bone uptake of ^{99m}Tc labelled E.H.D.P. were measured in 10 healthy young

male volunteer subjects with no evidence of bone disease (aged 22-25). Each subject was given 5 mCi of ^{99m}Tc labelled E.H.D.P. by intravenous injection. Whole blood radioactivity levels were measured at 15 minute intervals for one hour, and hourly thereafter until 6 hours after injection: 5 ml blood samples were collected by venepuncture, and whole blood radioactivity measured in a well scintillation counter. A standard sample obtained from the injected material was also counted. Results were expressed as a percentage of injected activity/litre of whole blood.

Prior to injection the subject emptied his bladder. The voided urine was discarded. Urine was collected at regular intervals during the 6 hour study period. The radioactivity in each sample was later counted in a bulk sample scintillation counter. The activity of a standard sample obtained from the injected material was also counted. In this way the cumulative urinary excretion from 0 to 6 hours after injection was derived and expressed as a percentage of the injected activity.

Serial uptake of diphosphonate in the normal bone of the lower dorsal spine was measured using the quantitative spine: background profile described in the previous section. Using this system a profile was drawn transversely across the gamma camera field of view just above the kidneys to include the lower dorsal spine and surrounding area. The gross counts from the

region of interest corresponding to the spine and the gross counts from the same number of channels in the surrounding soft tissue background were calculated from the teletype printout of the Multi-Channel Analyser. Counts from the two areas were recorded serially from 15 minutes until 6 hours after injection and their ratio (spine:background ratio) was calculated for each time of study.

Results

Blood Clearance

Initial clearance of ^{99m}Tc-E.H.D.P. from the blood is extremely fast, whole blood levels falling to 7.22 $\pm$ 0.64% of the injected activity/litre of whole blood at 5 minutes after injection (Table III.4). From 15 minutes until the end of the 6 hour period of study the blood clearance is well described by a biexponential function, A (t) (percentage of injected activity per litre) = $4.36e^{-0.032t} + 2.19e^{-0.0045t}$, where the decay constants are in minutes^{-1} and correspond to half lives of 21.8 and 155 minutes respectively (Figure III.1). The parameters were obtained from an iterative least squares fit to the bi-exponential expression itself and the correlation coefficient between observed results and those calculated from the bi-exponential expression is 0.9996 with a regression co-efficient of 0.9995.

Urinary Excretion

Cumulative urinary excretion is shown in the table (III.4). Six hours after injection a total of 70.6 ± 1.8% (Mean ± S.E.M.) of the injected activity has been excreted in the urine.

Normal Bone Uptake

In the control subjects, when the total number of counts from the regions of the profile corresponding to the spine and to the soft tissue background corrected for the physical decay of the nuclide, were plotted separately against time, a regular fall in activity was observed in both areas. The gross activity in the spine area fell more slowly than that in the non-spine background area (Figure III.2). Because of this, the spine: background activity ratio rose continuously throughout the 6 hours of the study (Figure III.3).

The gross activity recorded from the spine includes activity in the non-osseous tissue in the area under study. If it is assumed that the soft tissue background across the profile is uniform then subtraction of this background activity from the gross counts measured in the spine area gives a measure of net uptake by the spine. Net spine uptake rose rapidly and by 60 minutes had attained 80% of its value at 6 hours, when the study was terminated (Figure III.4). This dynamic uptake can be well described by a biexponential function ($T_{\frac{1}{2}}$ of Exponent I = 11.8 minutes, $T_{\frac{1}{2}}$ of Exponent II = 53.0 minutes).

(3) Measurement of Uptake by Tumour-Involved Bone

Materials and Methods

In a second related study serial uptake of ^{99m}Tc diphosphonate in tumour-involved bone was recorded in thirteen patients with known bone tumours. There were 11 female and 2 male patients (ages 34-72). Ten patients had bone metastases (9 breast, 1 prostate, 1 lung and 1 occult primary) and the other patient had a Ewing's sarcoma of pelvis. In all but one patient the tumour-involved areas studied were radiologically visible. In the remaining patient (carcinoma of breast) a previous scan had demonstrated a lesion in the lumbar spine and post-mortem confirmation was obtained within 2 months of the study described here.

Gamma camera scintigrams were obtained serially from 30 minutes until 4 hours after the intravenous injection of 15 mCi of ^{99m}Tc labelled E.H.D.P. By means of the gamma camera multi-channel analyser system previously described, serial ^{99m}Tc diphosphonate activity was recorded from 30 minutes until 4 hours after injection in 24 pathological areas of the skeleton and 24 corresponding areas of normal bone in the 13 patients studied. The ratio between the gross counts from the tumour-involved bone and those from the corresponding normal bone areas (tumour:bone ratios) were calculated for all times of study.

Results

When the gross counts from the gamma camera image of the tumour-involved bone were plotted against time, after correcting for the physical decay of the nuclide, a rapid rise was noted for the first hour of the study, after which a constant level was maintained throughout the remainder of the 4 hour study. In the corresponding normal bone there was a regular decrease in gross activity, similar to that observed in the spine of the control subjects (Figure III.5). The gross counts recorded from the tumour-involved bone and corresponding normal bone were obtained from equal areas of the skeleton. Thus, a measure of the increased uptake of diphosphonate in tumour-involved areas due to the tumour involvement can be obtained by subtracting the two sets of data (i.e. net increase in diphosphonate uptake in tumour-involved area = gross recorded counts in tumour-involved area minus gross recorded counts in area of corresponding normal bone). To simplify the nomenclature, we have called this increase in activity due to tumour involvement "net tumour uptake". Net tumour uptake rose throughout the 4 hours of the study (Figure III.6) in contrast to the net normal bone uptake in the control subjects, which reached a maximum value within 120 minutes of injection.

Because of the continued increase in activity in the tumour-involved bone and the regular decrease in gross activity in the

normal bone, the tumour:bone ratio rose steadily throughout the 4 hours of the study (Figure III.7).

(4) Acute Toxicity Studies

Principle

There is a theoretical risk of acute hypocalcaemia following the rapid intravenous injection of a phosphate or phosphonate compound due to its chelating action on serum calcium (Gosselin et al, 1953). Accordingly a study was performed to investigate the possibility of acute hypocalcaemia following the intravenous injection of a quantity of E.H.D.P. equivalent to that used in patient scanning studies.

Materials and Methods

Twenty five subjects (young healthy male volunteers) received an intravenous injection of either 2.5 mg or 3 mg of E.H.D.P. (labelled either with 5 mCi, 20 uCi or 30 uCi of ^{99m}Tc) during the pharmacological studies previously described. During these studies venous blood samples were taken at regular intervals after injection. The opportunity was taken, therefore, to measure venous blood calcium and phosphate levels immediately prior to injection of diphosphonate, and at 5, 15, 30, 45, 60, 120, 180, 240, 300 and 360 minutes after injection.

Ten ml of venous blood, obtained without the use of a tourniquet if possible, was collected in plain glass tubes. Serum

calcium and phosphate were measured using a Standard Technicon Autoanalyser method. Results were expressed as mg/100ml. Fifteen subjects were studied on one occasion and 10 subjects were studied twice. A total of 35 studies were, therefore, obtained.

Results

There was no clinical evidence of acute hypocalcaemia in any subject. Blood calcium and phosphate levels remained absolutely normal throughout the period of study apart from a post-prandial rise in serum phosphate (Table III.5).

(5) Cumulative Toxicity Studies

Principle

The experiment described above showed that no significant acute changes in serum calcium or phosphate should be anticipated after intravenous injection of the quantity of diphosphonate routinely used for bone scanning. The possibility of a cumulative toxic effect was investigated in six healthy young male subjects.

Materials and Methods

Six young male volunteers (age 22-30) each received 5 mg of unlabelled E.H.D.P. by intravenous injection daily for a total of seven days. The diphosphonate was dissolved in

non-radioactive sterile isotonic saline. During the twenty four hours immediately before the week of the study, and during the twenty four hours immediately after its completion, collections were made of all urine passed. Twenty four hour urinary excretion of calcium and phosphate were measured using the standard biochemical methods used in the Department of Biochemistry, Glasgow Royal Infirmary. In this way twenty four hour urinary calcium and phosphate excretion were measured immediately before and after the daily injection of 5 mg. of E.H.D.P. During the week of study serum calcium, phosphorus, alkaline phosphatase, albumin and globulin were measured daily. On 3 non-consecutive days of the study SGOT, SGPT, bilirubin, full blood count, blood film and platelet counts were estimated using standard biochemical and haematological techniques. The overall design of the study is shown in the figure (Figure III.8).

Results

All results of all the investigations described above were within normal limits. No significant change in any of the recorded parameters were observed. To simplify the presentation none of the detailed results are shown, as all of the investigations are standard tests and their ranges of normality well established.

(6) Discussion of In Vivo Experiments

The blood kinetic data reported here are very similar to those obtained by other workers using E.H.D.P. obtained from different sources (Krishnamurthy et al, 1974c; Berg et al, 1974). Diphosphonate clearly provides reliable, highly reproducible results whatever its source and this confirms in humans the biological consistency noted in experimental animals (Dunson et al, 1973). The bi-exponential blood clearance is thought to represent early rapid clearance into bone followed by a slower renal excretion (Krishnamurthy et al, 1974c). Although this statement is generally true, part of the rapid clearance of diphosphonate from the blood is also due to renal excretion; within 30 minutes of intravenous injection over 20% of the administered dose was excreted in the urine. In our control subjects, healthy young men with normal renal function, 70% of the administered activity was excreted within six hours, without the use of diuretics or a water load.

The rise in the observed spine:background ratio with time confirms what is apparent visually: scan quality improves the longer the delay between injection and scanning. The increase in spine:background ratio with time is due to two factors: a continuous rise in bone uptake of diphosphonate and a continuous decrease in soft tissue background activity due to renal excretion of the diphosphonate not taken up by bone. The

second of these factors is by far the most important and is in accord with our observation in a previous section that E.H.D.P., which has the fastest blood clearance of the ^{99m}Tc phosphates, provides the highest spine:background ratios, although its net bone uptake is no higher than that of the alternative phosphate compounds.

A similar normal bone uptake pattern to that described above has been observed in rabbits, where there was no significant difference between bone levels of ^{99m}Tc-E.H.D.P. at one hour and four hours after injection (Subramanian et al, 1972b). In the same study, absolute skeletal uptake of E.H.D.P. was actually lower than that of ^{85}Sr, the higher bone:background ratios observed with E.H.D.P. being entirely due to its more rapid excretion resulting in lower blood and soft tissue levels.

The gross activity recorded from tumour-involved bone includes a proportion of activity in the blood and soft tissues within the image of the "hot spot". Gross recorded activity remains constant because the activity lost due to decreasing blood levels is compensated by the continuous uptake in the tumour: involved bone. In both normal and abnormal bone the rapid rise in activity represents rapid clearance from blood, and the absence of any short term fall in net activity as the blood activity drops rapidly is in accord with the concept of chemisorption of a stable, non-hydrolysable compound (Castronovo and Callahan, 1972). In

normal bone net activity increases little after 120 minutes, by which time only 6% of the injected activity remains in the blood. This observation is in accord with the recent suggestion that, in normal bone, blood flow may be more important than metabolic activity in determining diphosphonate uptake (Genant et al, 1974). By contrast, the net uptake in tumour-involved bone continues to increase despite falling blood levels, and this is presumably a consequence of the increased metabolic activity of the tumour-involved bone.

The tumour:bone ratio as defined is of practical value as a direct quantitative expression of the contrast between the tumour-involved and normal bone in the gamma camera image. Clearly the higher the tumour:bone ratio the easier is the detection of the tumour on visual inspection of the image. Tumour:bone ratios were higher four hours after injection than at any earlier time during the study although ratios obtained within 120 minutes of injection were generally high enough to allow visualisation of most tumours (Figure III.9).

Acute hypocalcaemia due to chelation of ionised calcium is well recognised following the intravenous injection of phosphates (Gosselin et al, 1953). Diphosphonate is not a true phosphate and in the form used in scanning it is complexed with tin and technetium. Acute hypocalcaemia is, therefore, unlikely unless extremely large doses are employed.

Electrocardiographic changes have been observed in dogs following the rapid bolus intravenous injection of E.H.D.P. at a dosage level of only 20 mg/kg body weight (Stevenson and Dunson, 1973). Calcium or phosphate levels in the blood are not quoted in this report, but the electrocardiographic changes were apparently consistent with hypocalcaemia and were reversed by the intravenous infusion of calcium chloride. The same authors observed acute toxic symptoms of tachycardia, hyperpnoea andtetany at a dose level of 30 mg/kg. The LD_{50} following acute injection of diphosphonate in several animal species is reported as being below 45 mg/Kg (Stevenson and Dunson, 1973), although a more reasonable figure for the LD_{50} for diphosphonate is probably of the order of 100-200 mg/Kg body weight (Castronovo and Callahan, 1973). Rapid deaths are associated with lung haemorrhage andtetany.

As previously discussed, satisfactory visualisation of the skeleton is obtained after injection of 0.5 mg of diphosphonate which is equivalent in a "standard" 70 Kg patient to 0.007 mg/Kg. In our acute toxicity experiment the injection of 3 mg of E.H.D.P., equivalent to a dose of 0.04 mg/Kg, produced no observable clinical effect, and no acute change in the recorded serum calcium or phosphate. There is clearly a very large margin of safety between the lowest dose associated with clinical symptoms (30 mg/Kg) and the dose required to permit visualisation of the skeleton (0.007 mg/Kg) (Castronovo and Callahan, 1973).

The available evidence suggests that the diphosphonate and tin do not dissociate in vivo (Castronovo and Callahan, 1973) and the potential danger of acute tin toxicity is, therefore, less likely. In any event, the quantity of stannous chloride injected is of the order of 0.06 mg (0.0009 mg/Kg body weight). This is trivial when compared with the reported lethal dose of stannous chloride in dogs after intravenous administration (20-50 mg/Kg) (Spector, 1956). There is little danger from cumulative tin toxicity, as tin is already present at relatively high concentration in bone (0.5 ppm Sn) (Kehoe et al, 1940) and the additional burden from one bone scanning dose of stannous diphosphonate is 0.0014 ppm for a 70 kilogram man (Tofe and Francis, 1974).

It is clear that acute alterations in calcium metabolism following intravenous injection of a bone scanning dose of E.H.D.P. are extremely unlikely, and it has been our experience that patients with pre-existing hypocalcaemia due to renal disease suffer no untoward effect following acute injection of E.H.D.P. Of greater importance, however, is our observation that there was no change in blood and urinary levels of calcium and phosphate, and serum alkaline phosphatase after the daily intravenous injection for 7 days of 5 mg of E.H.D.P., equivalent to 0.07 mg/Kg body weight. This is equivalent to approximately 0.02 mg of elemental phosphorus/Kg body weight/day, as the disodium salt of E.H.D.P. contains approximately 25% elemental phosphorus.

The absence of any discernible change in calcium metabolism despite daily intravenous injection of a relatively large amount of E.H.D.P. (larger by a factor of 5 than the average scanning dose) is particularly gratifying in view of the reported effects on immobilisation osteoporosis in rats of the daily subcutaneous injection of E.H.D.P. at doses as low as 0.01 mg P/Kg/day (Michael et al, 1971; Muhlbauer et al, 1971).

E.H.D.P. given at a daily oral dose of 20 mg/Kg body weight to patients with Paget's disease reduced the initially elevated levels of plasma alkaline phosphatase (Russell and Smith, 1973). Clinical experience with the oral administration of large doses of E.H.D.P. for the treatment of Paget's disease and other bone diseases may not be directly relevant to intravenous administration, however, because as little as 0.5% of an oral dose may be absorbed in man (range 0.5 - 15%, mean 3%) (Russell and Smith, 1973).

The limited experience available in man suggests that histological abnormality in bone and cartilage matrix may be anticipated after the daily parenteral administration of 1 mg of elemental phosphorus per kilogram body weight given for short periods of time. The dosage of E.H.D.P. employed by us was smaller by a factor of 50 and it is, therefore, unlikely that any significant alteration in calcium or phosphate metabolism would be anticipated, even in patients having bone scans repeated at frequent intervals.

The measurement of other simple parameters of haematological and biochemical function during prolonged administration of the unlabelled pharmaceutical failed to reveal any evidence of toxocity.

Our own personal experience now consists of over 800 patient doses of E.H.D.P. including many repeated doses in individual patients. There has been no evidence of toxic effects and this experience is in accord with that of other workers (Castronovo and Callahan, 1973; Subramanian et al, 1974; Tofe and Francis, 1975). Many thousands of patient doses have now been given without any untoward effect being reported in the literature.

In summary, ^{99m}Tc labelled E.H.D.P. clearly satisfies the following biological criteria which are necessary for a satisfactory bone scanning agent:

(1) Rapid skeletal uptake of a significant proportion of administered activity.

(2) Skeletal binding which is irreversible, in the short term at least.

(3) Quantitatively higher uptake in tumour-involved bone compared with normal bone.

(4) No significant retention in soft tissue.

(5) Rapid urinary excretion of the administered diphosphonate which is not taken up by bone.

(6) Lack of toxicity.

We now have a clear understanding of the in vivo distribution of the compound, although the precise mechanism of its localisation and uptake in the skeleton remain unclear.

There are several important practical considerations which follow from the work described in this and the previous section. Rapid blood clearance of that proportion of the administered radiopharmaceutical not taken up by the skeleton is more important in attaining high target to background ratios than absolute bone uptake. The studies in normal and pathological bone described above clearly demonstrate why diphosphonate is superior to the other ^{99m}Tc phosphates and the nuclides of Strontium, despite the fact that in absolute terms skeletal uptake of diphosphonate is no higher than the alternative agents. The realisation that absolute bone uptake is not the most important pharmacological property is of importance in considering new bone scanning techniques and radiopharmaceuticals. There seems little justification for the potentially hazardous pre-treatment of patients with metastatic lesions with testosterone and oestrogens (Hertz, 1950) or parathyroid and/or thyroid hormones (Charkes, 1973) in attempts to increase the uptake of bone seeking isotopes. Measures designed to promote the renal excretion of the radiopharmaceutical not bound to bone appear more rational, although there is no published evidence to confirm that the administration of a diuretic, as advocated by Yeates et al (1972) will significantly

increase the urinary excretion of the ^{99m}Tc phosphates or that a diuretic will improve the quality of scan image obtained. Patients are routinely requested to drink large quantities of fluids during the interval between injection and scanning, as frequent voiding reduces the radiation dose to the bladder and may also increase the rate of excretion.

In the development of new bone scanning radiopharmaceuticals, rapid blood clearance of non-osseous activity should be sought and in this context it is possible that methylene diphosphonate (M.D.P.) which apparently has a marginally more rapid blood clearance than E.H.D.P. will prove to be a superior agent (Subramanian et al, 1974).

Another potentially important practical point to emerge from the studies described above depends on the demonstration of a quantitative difference between the dynamic uptake of diphosphonate by normal and tumour-involved bone. It is possible that the measurement of dynamic uptake of diphosphonate by a metastatic lesion when compared with uptake by normal bone may provide information regarding the biological activity of the lesion in study. Serial measurements of diphosphonate uptake by a known malignant lesion in bone may be used as a method of assessing response to various treatment regimes.

SECTION IV. CLINICAL ASPECTS OF BONE SCANNING WITH THE ^{99m}Tc PHOSPHATES

In the introductory section we referred to many clinical studies where the overall accuracy of strontium and fluorine bone scans in the early detection of bone metastases was compared with the radiological skeletal survey. The increased sensitivity of the radionuclide technique was established by these studies. The consequent recognition of the earlier and more accurate diagnosis of skeletal metastases provided by bone scanning stimulated the intensive investigations which culminated in the development of the ^{99m}Tc phosphates.

In this section is described our experience of some clinical aspects of bone scanning with the ^{99m}Tc phosphates in patients with malignant disease. The standard method used to scan the skeleton is first described. Some of the problems of technique and scan interpretation which are particularly relevant to the ^{99m}Tc phosphates are then discussed. This section is concluded by a description of the study carried out to evaluate the sensitivity and clinical value of the ^{99m}Tc phosphate bone scan in patients with malignant disease.

A. METHOD OF BONE SCANNING

The method used in our department utilises a gamma camera to obtain scans of the skull, axial skeleton, thorax, pelvis and proximal ends of the humerus and femur, using a modification of Galasko's method (Galasko et al, 1968). In all studies, patients are scanned three to four hours after the intravenous injection of 10-20 mCi of a ^{99m}Tc-labelled phosphate. We now routinely administer 17.5 mCi of E.H.D.P. in 2-3 ml of sterile aqueous solution. Patients are encouraged to drink large quantities of water during the interval between injection and scanning. Frequent voiding of urine is encouraged. Immediately before the scan, the patient empties his bladder to improve the visualisation of the pelvis.

Scans of the skeleton are recorded on Polaroid film using either a Nuclear Enterprises Scinticamera IV or Ohio Nuclear Series 100 gamma camera. Multiple overlapping views of the skeleton are obtained with the patient seated. Scans of the pelvis are obtained with the patient standing. In all, twelve views are obtained: right and left lateral skull (2 views), posterior views of cervical, dorsal and lumbar spine (3 views) and posterior views of right and left shoulders and proximal humeri (2 views). An anterior view of sternum and ribs (1 view), and right and left anterior and posterior pelvis with

proximal femora (4 views), complete the gamma camera skeleton scan obtained (Figure IV.1). Spinal views are taken with 300,000 counts each and all other views with 200,000 counts. Using this method satisfactory scintigrams of the skeleton are obtained with one hour of patient scanning time.

In general, the ^{99m}Tc phosphates reliably provide scans of high diagnostic quality. In elderly patients where urinary clearance of the compound is reduced, high blood and soft tissue background levels may seriously affect scan quality. Due to the relatively low gamma ray energy of ^{99m}Tc, attenuation by subcutaneous fat may be a problem and in the obese patient, scan quality is generally poorer. As previously discussed, the longer the interval between injection and scanning, the better the scan image produced due to higher target:background ratios. With E.H.D.P., good quality scans will generally be obtained after a 2-3 hour delay between injection and starting the scan. Patient positioning is important and patient rotation should be minimised. When using a whole body scanner or scanning gamma camera where the patient lies down, patient rotation is less marked when the patient lies supine rather than in the prone position (Charkes et al, 1973).

Because of urinary excretion of the phosphates, contamination of skin with voided urine can cause difficulties of interpretation especially in infants and children (Gilday and Paul, 1974).

Attenuation of the 140 KeV gamma photon by jewellery and other metallic objects carried by the patient, breast prostheses and other items will produce artefacts which are seen as cold areas (Figure IV.2). Such articles should be removed or identified prior to the scan (Pistenma et al, 1975).

B. INTERPRETATION OF THE ^{99m}Tc PHOSPHATE BONE SCAN

(1) The Normal Bone Scan

The criteria of normality of the bone scan have been described as bilateral distribution of radioactivity symmetrical about the midline, and a uniform or smooth gradation of radioactivity along the spine (Merrick, 1975). This statement is, however, an oversimplification: it is clear that before the presence of an abnormality can be recognised, the variability of appearance of the normal skeleton must be known, and every Nuclear Medicine Department should, therefore, develop its own experience of the normal bone scan. The detailed appearance of the normal skeleton on the ^{99m}Tc phosphate scan has been described (Charkes et al, 1973; Thrall et al, 1974). It should be stressed that much greater detail of the normal skeleton is seen that on scans obtained with Strontium or Fluorine (Charkes et al, 1973; Krishnamurthy et al, 1974b) and we agree that the ^{99m}Tc phosphate bone scan is not easy to read. The decision as to whether a certain area is normal or abnormal may be difficult and errors in interpretation are bound to be experienced during the initial use of the technique (Charkes et al, 1973).

(2) Problems of Interpretation

The more favourable physical properties of ^{99m}Tc compared with ^{85}Sr, ^{87m}Sr and ^{18}F, permit the injection of much higher activities. The increased information density resulting from the higher injected activity provides better counting statistics and theoretically improved resolution (Charkes et al., 1973). Overall, the quality of scans obtained with the ^{99m}Tc phosphates is superior to those obtained with all other agents, including ^{18}F. The increased information density on the ^{99m}Tc phosphate scan results in the visualisation of many details of skeletal, and in some instances non-skeletal, structure (Figure IV.3). As previously discussed any bone disease will be seen on the scan as an area of increased uptake, irrespective of histology. The combination of high sensitivity and low specificity makes it necessary to consider the possibility of false positive scans. It is, therefore, important to consider the many different conditions which may lead to positive focal accumulation of ^{99m}Tc phosphate on a bone scan, before accepting a diagnosis of metastatic disease.

Normal Extra-Skeletal Concentration

As previously discussed, the rapid blood clearance of ^{99m}Tc phosphates results in a very low soft tissue background activity. Due to the high urinary excretion of the compounds there is significant visualisation of the kidneys and urinary bladder on the scan. The renal images are generally of diagnostic quality

and asymmetry of the renal images may indicate unsuspected unilateral renal disease. In many large series having bone scans several such patients have been noted (Park et al, 1973).

The renal images are absent in renal failure (Figure IV.4). Bladder activity may obscure or mimic a bone lesion in the pubic or ischial bones and whenever possible the patient should empty the bladder prior to scanning the pelvis.

In some patients concentration of ^{99m}Tc phosphate in the normal breast has been observed (McDougall and Pistenma, 1974). This is apparently irrespective of the age of patient, although in our experience this is most common in younger patients (Figure IV.5). In patients with high blood background levels due to poor renal excretion or because of scanning too early after injection, a "blood pool" image of heart and liver may be obtained. Visualisation of thyroid, salivary glands or stomach is only seen where the labelling of the compound has been inefficient, and usually indicates the presence of oxidants in the ^{99m}Tc generator eluate.

Accumulation in Pathological Conditions Affecting Soft Tissues

A large number of diseases affecting non skeletal tissues may be associated with focal accumulation of ^{99m}Tc phosphate. The diseases in which this phenomenon may be seen include cerebral infarction (Grames and Jansen, 1973; Wenzel and Heasty, 1974); soft tissue tumours, both primary (Berg et al, 1973;

Chaudhuri et al, 1974) and metastatic (Thrall et al, 1974); myocardial infarction (Murray, 1974); abcesses (Chaudhuri et al, 1974); haematomata, surgical and traumatic wounds (Fordham and Ramachandran, 1974); thrombophlebitis and Monckeberg's medial sclerosis (Murray, 1974); pleural effusion and ascites (Thrall et al, 1974) and metastatic calcification in soft tissue (Richards, 1974). This list is not comprehensive and in many of these conditions the mechanism of uptake of the radiopharmaceutical is incompletely understood. Increased activity may be due to disease-mediated increased blood flow, micro-calcification in areas of haemorrhage or infarction, or binding of the phosphate by enzymes released in response to tissue damage (Zimmer et al, 1975). Different mechanisms may operate with the different ^{99m}Tc phosphate compounds.

The importance of these conditions is in their recognition as potentially misleading causes of a "positive" bone scan. A full clinical history and physical examination, together with other appropriate investigations, should allow the correct diagnosis to be made in many of these cases (Figure IV.6).

Benign Bone Disease Associated with Positive Bone Scan

Virtually every focal bone disease will produce an area of focally increased uptake on the scan. In the differential diagnosis of metastatic disease, the most important of these

conditions to consider are Paget's disease; arthritis, especially osteoarthritis of hips and shoulders; degenerative disease of the spine; osteomyelitis and fractures. Metabolic bone disease may also cause focal abnormalities on the bone scan, for example pseudofractures due to osteomalacia, and fractures or vertebral collapse in patients with severe osteoporosis. As previously discussed the bone scan is non-specific in that any disease affecting bone will result in increased accumulation of radio-pharmaceutical. The scan abnormality produced by certain disease processes may, however, be sufficiently characteristic to permit a specific diagnosis to be suggested.

Of all the benign bone diseases causing diagnostic confusion, Paget's disease is the most important. This condition may be anticipated in about 3-4% of all hospital patients over the age of 40 (Collins, 1956) and diagnostic difficulty is particularly likely in elderly patients. The position may be further complicated by the presence of a pagetoid response of bone to metastatic involvement which may be confusing both radiologically and histologically (Milch and Changus, 1956). We have observed several patients with apparently co-existent metastatic cancer and Paget's disease. Histological confirmation of the Paget's disease was not obtained, however.

In true Paget's disease there is often characteristically involvement of a large area of bone, e.g. involvement of the

hemi-pelvis (Figure IV.7) or diffuse involvement of the skull (Figure IV.8). Another means of differentiating between metastases and Paget's disease is the intensity of the scan abnormality. In general higher target:background ratios are seen with Paget's disease than metastases. When a vertebral body is uniformly involved by a lesion which produces high ^{99m}Tc phosphate uptake with no loss of vertebral height, Paget's disease is likely. The typical appearance of Paget's involvement of the spine is compared with that of metastatic disease and osteoporotic collapse (Figure IV.11).

Focal accumulation of isotope around a large joint, especially the shoulders or hips, indicates the likelihood of osteoarthritis (Figure IV.9). In patients with degenerative disease of the spine, especially where osteophyte formation is marked, focal hot spots may be seen. In general, a solitary abnormality in the cervical or lumbosacral spine of an elderly patient is usually due to degenerative disease. The bone scan of such patients should not be interpreted without reference to the corresponding radiograph.

Trauma to bone will produce lesions on the bone scan, even if there is no radiographic abnormality. Fractures of bone, whether complete or incomplete, produce lesions with very high levels of nuclide concentration (Fordham and Ramachandran, 1974). In many cases the distribution of the lesions gives a

clue to diagnosis, for example a row of lesions in the ribs is almost invariably the result of trauma (Figure IV.10). Collapse of a vertebral body due to benign disease such as osteoporosis may be difficult to differentiate from malignant collapse, although in malignant disease the pedicles are often involved whereas in osteoporotic collapse the whole vertebral body is uniformly affected (Figure IV.11). In cases of marked osteoporotic collapse, reduction of height in the vertebra is seen, giving a characteristic appearance.

Bone Involvement by Tumour Associated With a Normal Bone Scan

There are several clinical situations in which the bone scan may be normal, or considered normal despite radiological evidence of tumour involvement of the skeleton. As discussed in the introductory section, the uptake of a bone seeking nuclide or radiopharmaceutical depends on the presence of reactive bone. When there is little or no reactive bone, no abnormality is seen on the scan. Absence of reactive bone may be due to the biological nature of the primary lesion: multiple myeloma and indolent tumours such as well differentiated thyroid cancer may provide minimal bone reaction and occasionally there may be little bone reaction to rapidly progressive cancers. Another important clinical situation where the bone scan may be modified is after radiation therapy. Incidental irradiation

of normal bone during treatment of a primary cancer of lung or breast results in either increased or decreased uptake of ^{99m}Tc polyphosphate (Cox, 1974b). The late effect of irradiating normal bone is invariably reduced uptake of tracer, corresponding closely to the radiation field (Bell et al, 1969). Treatment of bone metastases by radiotherapy will also alter the uptake of bone seeking nuclides. After such treatment there is generally an initial increase in uptake followed several months later by a fall, and this fall is associated with radiographic evidence of recalcification (Greenberg et al, 1972). In a patient studied with ^{99m}Tc diphosphonate there was no initial rise in nuclide uptake, but a measurable decrease was noted after 18 days irradiation (Castronovo et al, 1973). After successful radiotherapy a minimally abnormal scan is associated with radiographic evidence of sclerotic metastases, aptly described as the "scars of previous battles" (Charkes et al, 1968). If bone destruction is particularly extensive an area of reduced activity may be seen on the scintigram (Goergen et al, 1974). In these cases the radiograph is unequivocal.

Another rather uncommon situation in which metastatic disease of the skeleton may not be easily recognised on the bone scan, is where there is diffuse skeletal involvement. When the skeleton is totally involved by tumour, although there is marked bone reaction and generally increased nuclide uptake, there is no contrast between areas of the skeleton, and no focal "hot spot"

is seen (Frankel et al, 1974; Thrupkaew et al, 1974). The possibility of diffuse metastatic disease should be considered where there is generally increased uptake throughout the skeleton. We have seen such a picture in a patient with chronic myeloid leukaemia, presumably due to increased bone blood flow associated with extensive marrow involvement.

If all the clinical circumstances described in this chapter are kept in mind, and if full clinical and radiological details are available at the time of scan interpretation, the incidence of false positive bone scans will be kept to an absolute minimum.

As previously discussed in the introduction, it is clear that bone scanning and radiology are in many instances complementary and maximum diagnostic information can be obtained from a combination of the two studies (Charkes et al, 1966).

C. EVALUATION OF THE SENSITIVITY AND CLINICAL VALUE OF THE ^{99m}Tc PHOSPHATE BONE SCAN

(1) Principle of the Study

To critically assess the sensitivity and clinical value of the ^{99m}Tc phosphate bone scan a large number of patients with known or suspected malignant involvement of the skeleton were studied. In every patient a gamma camera bone scan was obtained using the standard technique previously described and a full radiological skeletal survey was also obtained. Each investigation was reported and the results of each test compared with one another, and compared also with the clinical status of the patient at the time of scanning and during subsequent follow-up. A control group, consisting of 75 patients with no history nor clinical evidence of cancer, were also studied.

(2) Materials and Methods

Patients

372 patients with a positive histological diagnosis of malignant disease were studied (Table IV.1). They were referred to the Department of Nuclear Medicine in Glasgow Royal Infirmary during the period November 1972 to March 1975 for bone scanning to confirm or exclude the presence of malignant disease of the skeleton. They represent a consecutive patient population with

the exception of another 16 patients with known malignant disease who were scanned during this period but were considered unsuitable for the study because of either inadequate clinical information, a poor quality bone scan, or because radiographs were not available for comparison.

There were 64 male and 308 female patients, the preponderance of females being due to the large number of patients with breast cancer. Seven patients had primary bone tumours, the remainder having known or suspected bone metastases from primary tumours elsewhere. Especially common were patients whose primary tumour had a known predilection to metastasize to the skeleton (breast, lung or prostate) (Table IV.1).

Controls

Seventy five control patients with no history nor clinical evidence of malignant disease were also scanned using an identical technique to that employed in the cancer patients. There were 55 female patients and 20 male patients. Fifteen female patients with a lump in the breast were scanned as part of their initial preoperative assessment. In all cases histology of the lesion was later shown to be benign. A further 60 patients, post-menopausal females and males over the age of 35, admitted to Glasgow Royal Infirmary for treatment of a variety of conditions such as myocardial ischaemia, asthma, diabetes, gall bladder

disease, inguinal and femoral herniae, were also scanned. Informed verbal or written consent was obtained from all these patients.

Bone Scan

Each patient and control was scanned three to four hours after the intravenous injection of 10-20 mCi of a ^{99m}Tc-labelled phosphate compound (Table IV.2). Details of the technique employed are described in the earlier part of this section. In patients whose clinical examination precluded a full isotope skeletal study, a more limited examination, restricted to known tumour-bearing areas, was obtained.

Radiological Skeletal Survey

In all cancer patients skeletal radiographs in 2 planes were obtained, generally within two weeks of the scan. The radiographic skeletal survey comprised antero-posterior and lateral views of skull; antero-posterior and lateral views of the cervical dorsal and lumbar spine, a postero-anterior radiograph of chest, rib views and postero-anterior view of pelvis.

A radiological skeletal survey was not performed in the control subjects, unless the bone scan showed abnormalities, in which case corresponding radiographs were obtained.

Comparison of Results and Follow-Up

The scan and radiograph were read independently, with knowledge of the primary diagnosis and any other relevant clinical information available at the time of study. As the criterion of normality of the ^{99m}Tc bone scans had not been established at the start of this study, all the scans were re-reported during the months of January to March 1975. In only a few cases did the retrospective interpretation of the scan differ from the original report. In all cases the radiograph and scan were recorded as positive, negative or suspicious of metastatic involvement. Any focal accumulation of radioactivity was reported as abnormal and held to be due to tumour involvement unless the corresponding radiograph clearly showed a benign bone lesion. In such cases the scan was reported as negative. In several patients the appearances of the abnormality on the bone scan allowed a confident diagnosis of non-malignant disease to be made. The radiographs were accepted as positive when the abnormalities were reported as "due to metastatic disease", "consistent with metastases" or some similar expression.

The results of the two investigations were compared and related to the clinical status of the patient at the time of study and during the subsequent follow up period which ranged from a month to over two years. Where available, the results of pathological studies (biopsy or post-mortem findings) were

correlated with the scans and radiographs. In many patients the details of follow up were obtained retrospectively from the clinical notes. In a large number of patients with breast cancer, however, a detailed follow up involving repeated bone scans and radiographs was obtained. In this thesis, for the purposes of the comparison of the diagnostic accuracy of the scan and radiograph, the result of only one bone scan in each patient is recorded.

(3) Results

The overall comparison of the scan and radiograph is shown in the table (IV.3). The detailed results are presented and discussed in the following way. We have accepted the radiograph as the standard investigation for two reasons: firstly, it is the standard, universally available technique against which any new development must be judged, and secondly, the radiograph generally allows a specific diagnosis of bone tumour to be made, due to the anatomical detail displayed. In contrast, the bone scan represents a non-specific abnormality, namely increased concentration of isotope which is seen with many bone diseases. All patients studies have, therefore, been classified as X-Ray Positive, X-Ray suspicious or X-Ray negative. Where the radiograph shows unequivocal evidence of bone tumours, no further details regarding clinical or pathological follow-up were sought.

In all other patients, especially where there was a difference between the radiographic and scan results, clinical and where available pathological details during follow-up were obtained.

X-Ray Positive

In 102 patients, the radiographic skeletal survey showed evidence of bony involvement by malignant disease. Seven patients had primary bone tumours (4 osteogenic sarcoma and 3 Ewing's sarcoma) and the remaining 95 patients had bony metastases from primary tumours elsewhere. In all but 2 of these 102 patients the bone scan independently confirmed the presence of focal malignant disease of bone. The two patients with normal bone scans but abnormal radiographs are of interest in that both had known bony metastases from a breast cancer for at least five years prior to the study. Each had been treated with radiotherapy and systemic hormone therapy and the radiographs revealed diffuse sclerotic metastases. The absence of abnormal uptake of ^{99m}Tc phosphate on the bone scan is probably a reflection of the previous radiotherapy (Cox, 1974b) and represents inactive disease at the time of study (Charkes et al, 1968).

Of the 100 patients with abnormal scans and radiographs, a single area of abnormality was noted in each study in 11 patients. In the remaining 89 patients multiple metastases were present. All areas of abnormality visible on the radiograph were also

seen on the scan but in 45 patients (45%), the scan showed additional areas of abnormality not noted on the corresponding radiograph. Several examples of this phenomenon are shown (Figures IV.12; 13).

X-Ray Suspicious

In 10 patients (9 breast and 1 colonic cancer) the appearance of the radiograph was suspicious but not diagnostic of tumour involvement. In seven of these patients the scan was positive. In each case the abnormality on the scintigram was in the same area of the skeleton which was suspicious on the radiograph. In 3 patients the bone scan was normal (Table IV.4).

To date, follow up of the seven scan positive patients has revealed subsequent radiological evidence of bone metastases at the sites indicated by the bone scan in three (Figure IV.14). Another patient had multiple liver metastases with malignant ascites at the time of study and the clinical suspicion of bone metastases was very high. Two further patients have evidence of progressive disease on repeated bone scans and one of these patients died eight months after the initial study. The seventh X-Ray suspicious, scan positive patient is alive twelve months after the study but has developed pain in the areas shown to be tumour-involved on the scan. The three patients with negative scans are alive and asymptomatic after follow up periods of 2, 6 and 16 months respectively.

X-Ray Negative, Scan Negative

In 260 patients, the radiographs showed no evidence of bone metastases. In 189 of these patients the bone scan was also normal. Of these 189 patients with normal radiographs and bone scan, 116 have been followed up for at least six months, or until their death if this occurred sooner (Table IV.5). 98 of these patients remain clinically well with no radiological nor scan evidence of bony metastases (mean follow up of this group = 12.8 months, range 6-24). Twelve patients have died during follow up: in none was there clinical or pathological evidence of bone metastases. The available information regarding the cause of death in these patients is summarised in Table IV.6. To date, of the 116 patients with negative scans and radiographs followed up for more than six months, six (5.2%) have developed radiological or pathological evidence of bone metastases (Table IV.7). The mean interval between the initial negative studies and the confirmation of metastases was 9.1 months (range 6-15 months). No patient developed clinical evidence of bone metastases within six months of a negative scan. Five patients developed symptoms suggestive of bony metastases confirmed by scan and radiograph. The six patient's symptoms were associated with a positive bone scan but negative radiograph. Metastases were confirmed at post mortem two months later.

Ten of the 372 patients (3.8%) had a definite focal

abnormality on the scan which would have been wrongly diagnosed as bone metastases if corresponding radiographs had not been obtained. In all cases the radiographs showed evidence of a benign bone disease which was responsible for the abnormal bone scan (Table IV.8). These ten patients were considerably older than the average patient studied (Mean = 69.4 years, range 58-82).

X-Ray Negative, Scan Positive

In 63 patients with a negative radiological skeletal survey, the bone scan showed abnormalities consistent with metastatic involvement of the skeleton. Follow up of these patients has so far revealed clinical evidence of metastatic malignant disease in all but 8 (Table IV.9). To date, absolute confirmation of metastatic tumour in the skeleton has been confirmed by pathological examination in 6 (Table IV.10), (Figures IV.15; IV.16) and the development of radiographic evidence of metastases in 15 patients (Table IV.11) (Figures IV.17; IV.18; IV.19). The mean interval between the abnormal scan and subsequent development of radiological abnormality was 6 months (range 1-10 months).

In 42 patients the presence of metastatic malignancy in the skeleton has not yet been confirmed. In all but 8 of these patients, however, the clinical features at the time of study

or during follow up have been highly suggestive of bone involvement. In eleven patients who died, post mortem examinations were not obtained. In all of these patients there was a high clinical suspicion of metastatic disease in the skeleton, and several patients had metastases in non-skeletal sites (Table IV.12). A further 19 patients with positive bone scans had clinical evidence of recurrent or metastatic disease in tissues other than the skeleton. All but two patients have been followed up. Mean follow up of the remaining 17 patients is only 5.1 months (range 2-21). To date, none has developed radiological evidence of bone metastases, but in many there is a strong clinical suspicion of bone metastases (e.g. bone pain, relief of bone pain following radiotherapy, elevated acid phosphatase in patients with carcinoma of prostate) (Table IV.13). Four patients who were thought to have localised disease only, were scan positive. They have all subsequently developed symptoms during follow up (Table IV.14).

Eight further patients, asymptomatic at the time of their positive bone scan, remain well during follow up. Mean follow up of this group is 6.9 months (range 2-16). All have breast cancer and 6 were scanned at the time of their initial presentation.

X-Ray Negative, Scan Suspicious

In 8 patients with normal radiographs the bone scan was suspicious of metastatic involvement. In 7 cases there was focal accumulation of isotope but this was not considered to be unequivocally outwith normal limits. In the eighth patient there was generalised increased uptake of radiopharmaceutical throughout the skeleton, and the suspicion of generalised metastatic disease was considered. Details of the eight scan suspicious patients are shown in Table IV.15. Follow up of these patients has confirmed the presence of metastases in four, while further follow up is required in the other patients.

Control Subjects

In 4 of the 75 (5.3%) control subjects, abnormalities were noted on the scan which could be interpreted as metastatic deposits. In 2 of these subjects the radiograph was normal and in the other 2 demonstrated a localised concentration of bone in the area of abnormality seen on the scan (pelvis). Follow up of these 4 controls has shown no evidence of metastatic disease and the scan abnormalities have been considered as false positive results.

Summary of Results

Our results to date may be summarised as follows:

(1) 102 patients had radiological evidence of metastases. In all but two the bone scan showed corresponding abnormalities, and in 45 (45%) the scan showed additional lesions which the radiograph had failed to demonstrate.

(2) Ten patients had a suspicious radiograph. In 7 of these patients the scan was positive. Three of the scan positive patients subsequently developed positive radiographs. In the remaining 4 scan positive patients there was a high clinical suspicion of bone metastases. The three X-Ray suspicious scan negative patients remain well during follow up.

(3) 260 patients had a normal radiographic skeletal survey. 189 of these patients had a normal bone scan. Of these patients 116 have been followed up for at least 6 months, or until their death if this occurred sooner than six months. 98 of the 116 are alive and well (mean follow up 12.8 months, range 6-24 months). Twelve have died without evidence of bone metastases. Six (5.2%) patients developed bone metastases after a negative bone scan.

(4) 63 patients had a positive bone scan and negative skeletal survey. In 21 of these patients the presence of metastatic disease in the skeleton has been confirmed by histological or subsequent radiographic study. Only 8 of the 63 X-Ray

negative scan positive patients remain clinically well and symptom free during follow up. 8 patients had a suspicious bone scan and negative skeletal survey. The presence of metastatic disease has been subsequently confirmed in 4 of these patients to date.

(5) The incidence of false positive scans in the control group was 4 of 75 patients (5.3%).

(4) Discussion

Follow up of the patients in this study is not yet complete: radiographic confirmation of the presence of bone metastases indicated by a positive bone scan may not be anticipated for a period ranging up to 18 months after study or even longer (Galasko, 1969). Our results to date, however, have confirmed the greater overall sensitivity of the bone scan when compared with the skeletal radiograph, in the detection of focal metastatic disease of the skeleton.

In virtually every patient with radiological evidence of metastases the bone scan showed corresponding abnormalities and in 45%, the scan showed additional lesions which the radiographs had failed to demonstrate. In several cases these additional lesions have been of profound clinical significance.

The most important group of patients to consider are those with a normal or suspicious radiograph, as it is in these patients that a positive bone scan provides information unavailable

from conventional radiological techniques. All the available clinical data in these patients has been presented.

In seventy patients where the radiograph failed to show definite evidence of metastatic disease (either X-Ray suspicious or negative) the scan was positive, and confirmation of bone metastases by biopsy or the subsequent development of radiologically evident lesions has now been obtained in 24 of these patients. Conversely, 98 of 116 scan negative patients (85%) followed up for at least six months remain clinically and radiologically free of bone metastases. The incidence of subsequent bone metastases in scan negative patients is clearly much lower than in scan positive patients but as these patients were not strictly comparable clinically, no formal statistical test has been employed. It should be stressed that a negative bone scan does not entirely exclude the possibility of bone metastases, as six of 116 patients (5.2%) with a negative bone scan followed up for more than six months developed definite bone metastases.

The question of false positives is clearly the most important one to consider in the clinical application of bone scanning. The denial of radical treatment to a patient with early cancer because of the false presumption of metastatic disease would be a disaster, and it is this very possibility that makes it mandatory to consider all other conditions before ascribing a positive diagnosis of metastatic disease to any

focal area of increased uptake seen on the bone scan. The various physiological and pathological conditions which may be associated with a "positive" bone scan have been discussed. If the bone scan is reported with knowledge of the clinical details and with the corresponding radiographs available then the incidence of false positive results is acceptably low. Four of the 75 (5.3%) control subjects had a significant abnormality on the bone scan. Of greater relevance, however, is the incidence of non-malignant bone disease causing focal abnormalities in the large series of unselected cancer patients we have studied. Ten of the 372 patients (3.8%) had definite focal abnormalities thought to be due to benign disease. In general, these patients were elderly, and there is no doubt that particularly in the elderly the bone scan should be interpreted with caution, and with full clinical details and corresponding radiographs available.

Overall we have concluded that the ^{99m}Tc phosphate bone scan is the procedure of first choice in the investigation of suspected metastatic involvement of the skeleton because of its greater sensitivity when compared with conventional radiography. Our experience in this respect is similar to that of other workers using polyphosphate (Yeates et al, 1972; Barrett and Smith, 1974; Krishnamurthy et al, 1974b; Fletcher et al, 1975); pyrophosphate (Murray, 1974; Fletcher et al, 1975) and diphos-

phonate (Silberstein et al, 1973; Pendergrass et al, 1973; Papadimitrou et al, 1974; Pistenma et al, 1975). We agree that to rely on the bone scan alone is unwise (Yeates et al, 1972). Unlike Yeates, who suggests a radiological skeletal survey as the initial investigation, followed by a bone scan to reduce the number of false negative results from the radiological survey, we would suggest the following protocol in the investigation of patients with suspected bone metastases:

(1) Isotope bone scan of axial skeleton, including proximal humeri and femori.

(2) Radiographs of any area of increased uptake on bone scan to reduce danger of false positives due to non malignant bone disease. If radiographs are negative then metastases are likely and biopsy confirmation should be sought.

(3) If the bone scan is negative it is extremely unlikely that a radiographic skeletal survey will be positive except in the unusual clinical circumstances described in the first part of this section.

(4) If the bone scan is negative and the clinical suspicion of metastatic disease is high, the scan should be repeated approximately six months after the initial negative study.

There is no absolute contraindication to bone scanning and in general the scan is well tolerated by the patient as there

is less physical manipulation than with conventional radiology. Using E.H.D.P. and either a gamma camera or whole body scanner, high quality bone scans are readily obtained within one hour. For these reasons and because of the greater sensitivity of the isotope technique it is likely that there will be a great increase in the number of isotope bone scans requested in patients with cancer, and this is already the experience of many Nuclear Medicine Centres. The situation might become similar to that of radioisotope brain scanning, where a recent study suggested that even in a highly specialised neurological hospital, no fewer than 25% of brain scans requested were inappropriate and unjustifiable on clinical grounds (Claveria et al, 1973).

It is important, therefore, to discuss the clinical situations in which a bone scan may provide valuable clinical information. In general terms the clinical indication for a bone scan is the clinical suspicion of bone involvement by tumour. Such a general statement clearly includes many different clinical situations in which the index of suspicion may be high or low. A detailed study of the clinical indications for bone scan and, the relative merits of bone scanning versus biochemical, haematological and other studies in the investigation of metastatic bone disease is not presented in this thesis. At the present time with our present state of knowledge and experience, it is justifiable to recommend a bone scan in all patients in whom

there is a reasonable clinical suspicion of metastatic disease, for example in patients with a known primary tumour who develop bone pain, in patients with local or nodal recurrence of cancer following primary treatment and in cancer patients with unexplained anaemia or hypercalcaemia. The bone scan is also valuable in assessing the extent of disease of patients with known metastatic disease and may be of value in assessing response to therapy, in the accurate delineation of radiotherapy portals, and in indicating a site or sites suitable for biopsy in patients with a strong clinical suspicion of cancer but no tissue diagnosis. It should be stressed that the bone scan does not replace the need for skeletal radiology, and indeed in many patients with known bone metastases the two investigations are complementary, providing information regarding the extent of bone destruction and an indication of the dynamic response of bone to tumour involvement, as previously discussed.

It is likely that the scan will be of great value in the pre-operative assessment of patients presenting with primary cancer. At the present time it is probably unjustified to withhold surgical treatment from a patient with a clinically resectable cancer because of a positive bone scan, in the absence of other evidence of metastatic disease. There is an urgent need to accurately define the clinical significance of a positive bone scan in patients with apparently early cancer.

A study designed to answer this question in patients with breast cancer is described in the next section of the thesis.

SECTION V. APPLICATION OF THE ^{99m}Tc PHOSPHATE BONE SCAN TO THE STUDY OF PRIMARY BREAST CANCER

A. INTRODUCTION

The ability of the bone scan to positively identify the presence of skeletal metastases in an asymptomatic patient with apparently circumscribed primary cancer at a time when conventional radiology is normal, is clearly applicable to many different clinical situations in routine practice. In this section of the thesis is described a study which demonstrates the research potential of the technique in the clinical study of patients with malignant disease. We have specifically attempted to assess the prognostic significance of a positive bone scan in the study of patients with primary breast cancer.

Breast cancer is the commonest malignancy in women in the United Kingdom. One in 17 women will develop breast cancer, and every year over 10,000 patients die because of the disease (Baum, 1974). The incidence of bone metastases in patients with advanced breast cancer is extremely high - a figure of 85% is quoted from post mortem studies (Jaffe, 1958), and a similar incidence has been reported from a study with ^{18}F of patients

known to have advanced disease (Galasko, 1969). It is clear, therefore, that bone scanning is highly relevant to the study of patients with breast cancer (Rubin and Ciccio, 1969; Hopkins and Kristensen, 1973), especially as the majority of bony metastases in this condition contain reactive new bone (Milch and Changus, 1956) and will therefore be readily visible on the scan.

It has previously been suggested that the bone scan may be of use in evaluating patients with primary breast cancer at the time of their initial presentation: the incidence of clinically occult metastases demonstrable by the technique in such patients has been variously reported as 16% (Sklaroff and Charkes, 1968), 24% (Galasko, 1969) and 19% (Hoffman and Marty, 1972). The introduction of the ^{99m}Tc phosphates and the consequent ready availability of high quality bone scans in all Nuclear Medicine Departments makes it essential to know precisely the prognostic significance of a positive and negative ^{99m}Tc phosphate bone scan in patients with apparently "early" breast cancer.

B. MATERIALS AND METHODS

105 female patients with breast cancer were studied. To avoid patient selection in our population, all patients with breast cancer presenting to the participating surgeons were referred for study, irrespective of their clinical stage or symptoms. Each patient had a full clinical examination, the clinical staging being assessed by the T.N.M. method (U.I.C.C., 1960). A full radiological skeletal survey and bone scan were performed in every patient using the methods described in the previous section of the thesis. Both the initial radiological and scanning study were performed before or within two weeks of the patient's primary treatment. The result of the bone scan was not considered when the patient's primary treatment was determined.

During follow up whenever possible the patient was re-examined clinically every 3 months. A repeat bone scan was performed every 5-6 months in all patients, or sooner if the patient developed any symptoms suggestive of bony disease. If, during follow up, any area on a repeat bone scan was suspicious or positive for tumour involvement or if the patient developed symptoms, corresponding radiographs in 2 planes were obtained. The entire protocol for the study may be summarised:

(1) Preoperative: Clinical Staging (T.N.M.).

(2) Bone Scan and X-Ray before or within 2 weeks of mastectomy.

(3) Repeat bone scan every 5-6 months or sooner if symptoms indicate.

(4) X-Ray any area of suspicion on repeat scan, or if symptoms develop.

C. RESULTS

(1) Clinical Details

105 female patients whose ages ranged from 28 to 79 years (mean 54.9 years) were studied. Their clinical staging expressed using the accepted International summary scheme used in end results reporting, (Cutler, 1974), is shown:

Stage I	59.
Stage II	29.
Stage III	12.
Stage IV	5.

Two of the 105 patients (one clinically Stage I and one clinically Stage IV) complained of bone pain at the interview prior to the bone scan. In no other patient was there a clinical suspicion of bony metastases prior to study. The evidence of metastatic disease in the 5 Stage IV patients was skin nodules on the chest wall in 2 patients, and radiologically demonstrated lung metastases in another 2 patients. The fifth patient, who had radiologically positive bone metastases, was the only one of the five patients Stage IV who complained of bone pain.

The patients have now been followed up for a period of time ranging from 2 to 24 months (mean 10.4 months). During this time each patient has had 1-6 bone scans (mean 2.5). A total of 261 bone scans have been performed. During the follow up

period, 12 patients (11.4%) have died, and 15 (14.3%) have developed definite evidence of bone metastases as shown by positive histology or radiographic evidence of bone destruction.

(2) Results of Radiological Survey and Bone Scan at the time of first presentation

Of the 105 patients studied, only one had a positive radiological skeletal survey at the time of presentation. Bone scan confirmed extensive metastatic disease of the skeleton in this patient. Twenty four patients (22.9%) had a positive bone scan at the time of presentation (Table V.1). In two patients the scan was considered suspicious but not absolutely diagnostic of bone metastases. In the remaining 79 patients the initial bone scan was reported as negative. Of the 88 patients with Stage I and Stage II breast cancer, 16 (18.2%) had scan evidence of bone metastases. Three of the twelve patients (25%) with locally advanced (Stage III) disease had a positive bone scan at the time of presentation and in a fourth patient the scan was suspicious. All five of the Stage IV patients (100%) had positive bone scans at the time of first presentation.

(3) Results of Follow Up Scans

During follow up, all the initially scan positive patients have remained positive and in most cases the scan has shown progression of disease, demonstrated by the appearance of new

lesions (Figure V.1). One of the 2 scan suspicious patients became scan and X-Ray positive seven months after the initial study. The other scan suspicious patient's repeat bone scan shows no change after an 8 month interval. Nine of the seventy nine patients who were initially scan negative have become scan positive during follow up ("scan converters"). In these 9 patients, the mean interval between the initial negative scan and the positive study was 10.4 months (range 5-17 months). In 70 patients repeated bone scans have remained normal.

(4) Clinical Follow Up

In this study, the basic reason for detailed follow up was to obtain information regarding the subsequent presence or absence of bony metastases and to correlate this information with the results of bone scan and radiographic skeletal survey. For this purpose the patients are classified and discussed on the basis of the results of the bone scan (Table V.2).

INITIAL SCAN POSITIVE GROUP

Twenty four patients had a positive bone scan on initial study. To date, 10 of these patients (4 Stage I, 2 Stage II and 4 Stage IV) have evidence of bone destruction on subsequent radiographs. Included in this group is the one patient with positive radiographs at the time of presentation. Nine patients,

therefore, who initially had positive bone scans and normal radiographs have developed radiological evidence of metastatic disease at the sites indicated on the scan (Figures V.2; V.3; V.4). The mean interval between the initial negative and the positive radiographs was 6.4 months (range 2-12 months). Three of these patients have since died and post mortem confirmation of metastases obtained. At present, therefore, seven patients are alive but with radiographic evidence of bone metastases.

Four other patients have died, without radiological evidence of bone metastases. In one of these patients (initially Stage III), post mortem examination confirmed metastases in the skull and spine as indicated by the scan (Figure IV.16). No post mortem examination was performed on the other 3 patients who died: one patient (clinically Stage II) whose bone scan showed rib involvement died with pulmonary metastases 4 months after mastectomy. Another patient (clinically Stage II) died "of carcinomatosis" six months after mastectomy. Her death occurred in a distant hospital while she was on holiday and no further details are available. The third patient who died presented with a large tumour (T4), lung metastases, a positive bone scan and negative skeletal survey. She died 10 months later.

Four patients have developed bone pain suspicious of bone metastases but serial radiographs remain negative. In one

patient (initially Stage I) a trephine biopsy of the iliac crest has confirmed the presence of metastatic tumour. The remaining three patients have evidence of bone metastases clinically although there is as yet no radiographic or pathological confirmation: one patient (initially Stage I) is bed ridden at home with severe generalised bone pain 16 months after the initial study. Bone scans have shown extensive abnormalities despite negative X-Rays. Another patient (initially Stage II) has developed lower lumbar pain 9 months post mastectomy. Radiographs remain normal although a repeat scan is positive. A third patient presented with locally advanced disease (Stage III), bone pain and negative radiographs. The bone scan was grossly abnormal. Follow up interval is only 3 months and at present her bone pain has been controlled by androgen therapy.

Six patients (1 Stage I, 4 Stage II and 1 Stage III) remain alive with no evidence of bone metastases 3-16 months (mean = 7.7 months) after the initial study.

SCAN SUSPICIOUS GROUP

Two patients had a "suspicious" bone scan at the time of initial presentation. One patient (Stage III) became scan and x-ray positive 7 months later while the other (Stage I) remains clinically well eight months after the initial study.

SCAN NEGATIVE GROUP

In seventy nine patients the initial bone scan was negative. In all patients the initial radiographs were also negative. During follow up 9 of these patients have developed positive bone scans. The patients with an initially negative scan may be considered in 2 main groups - those whose initial scan was negative but a subsequent scan became positive ("scan converters"), and those patients in whom repeated bone scans are persistently negative.

Nine patients whose initial bone scan was negative developed abnormalities on subsequent scans. The mean interval between the initial, negative scan and the positive scan was 10.4 months (range 5-17 months). Three of the 9 patients (2 Stage I and 1 Stage II) have developed radiological evidence of metastases, two at the time of the positive scan, and one patient 7 months later. One of these patients died 8 months after the positive radiographs were obtained. A further patient (Stage II) developed a positive scan six months after her mastectomy. Repeat radiographs were negative (Figure V.5) but the presence of metastatic tumour was confirmed by post mortem examination two months later. Two patients (one Stage I and one Stage III) have developed clinical symptoms of bone metastases despite persistently negative radiographs. In one patient metastases were shown in the dorsal spine 11 months after mastectomy for

Stage I disease. At the time she had back pain radiating to the arms. A further 13 months later she developed severe generalised bone pain. Radiographs remain normal.

The second patient is of great interest in that the conversion from a negative to a positive scan preceded the development of symptoms of metastatic bone disease by 6 months, and occurred at a time when the primary tumour had responded favourably to systemic treatment. She presented with a large $T_3N_0M_0$ tumour. Because of an axillary vein thrombosis she did not have a mastectomy but was treated with oestrogens. Initial bone scan and radiographs were negative. With oestrogen therapy the primary tumour regressed to a great extent and by 1 year was clinically impalpable. Fourteen months after her initial presentation a bone scan showed evidence of disease in the right clavicle and right 1st rib. This abnormality clearly progressed in subsequent scans and further lesions developed (Figure V.6). Twenty months after her initial presentation she returned with ulceration of the breast, and bone pain and tenderness localised to the scan positive areas.

Three patients (all Stage I initially) in whom the scan has "converted" remain well at the time of follow up 3, 7 and 3 months respectively after the positive bone scan.

In 70 patients repeated bone scans have remained normal. These 70 scan negative patients have been followed up from 3-24

months (mean 10.9 months). No patient has developed clinically obvious bone metastases. Two patients have died: one patient (Stage II) died of pulmonary embolism and congestive cardiac failure 16 months after mastectomy: post mortem revealed no bone metastases. The second patient (also Stage II) died of cerebral secondaries 11 months after mastectomy. No post mortem was obtained.

Summary

The clinical course and confirmation of bone metastases by positive histology or subsequent radiological evidence of bone destruction in 105 unselected patients presenting with breast cancer after mean follow up of 10.4 months, is summarised in Table V.2.

(5) Prognostic Significance of a Positive Bone Scan in "Early" Breast Cancer

It can be seen from Table V.1. that a simple clinical comparison of the scan positive and scan negative groups is invalid, as the clinical stage of the disease is different in each group: in particular, in the scan positive group there are four patients with known soft tissue metastases. To accurately define the clinical significance of a positive bone scan in primary breast cancer it is necessary to study clinically similar patients in whom the only major variable

is the status of the bone scan. For these reasons we have examined the results of the bone scans and radiographs performed in the 88 patients who presented with Stage I and Stage II breast cancer, to specifically answer the question: "What is the clinical significance of a positive bone scan in patients with "early" (Stage I and II) primary breast cancer?".

Eighty eight of the 105 patients in the study had Stage I (59) or Stage II (29) disease. The detailed results of the bone scans in clinical follow up in these patients is shown in Table V.3. Sixteen of the 88 patients had a positive bone scan at the time of first presentation. One patient had a suspicious area on the bone scan, and the remaining 71 patients had a normal bone scan. In every patient the radiographic skeletal survey was negative. Repeated scans in the initially scan positive patients have shown progression of the disease, demonstrated by the appearance of new lesions, in many. In no patient has an initially positive bone scan reverted to normal. There has been no progression of the suspicious areas in the "scan suspicious" patient. Of the 71 patients with an initially negative bone scan 8 have converted from scan negative to scan positive during the repeated studies while 63 have remained persistently scan negative.

The mean follow up is 11.2 months (range 2-24 months) since initial study. The initially scan positive group have been

followed up for 8.2 months (range 3-16 months). The "scan converter" group have been followed up for 5-24 months (mean 14.4 months) and the persistently scan negative group from 3-24 months (mean 11.6 months). The mean age of patients in the scan negative group (53.08) was no different from that of patients in the scan positive (53.56 years) and "scan converter" groups (53.00 years).

The eight patients whose bone scan was initially negative but became positive during follow up are of interest. Five of these eight patients were either pre-menopausal or within 2 years of the menopause (age 27-46, mean 38.6 years), and in these patients the mean interval between negative and positive scan was 7.6 months (range 5-13 months). Four of these 5 patients have since developed significant symptoms and radiological evidence of metastases within 12 months of the positive bone scan. The remaining 3 "scan converter" patients were elderly (aged 75, 76 and 79 respectively) and the intervals between the negative and positive scans were 11, 17 and 14 months respectively. Radiographs remain normal at 24, 24 and 14 months respectively although one of these patients has subsequently developed severe bone pain.

The relevant clinical details in the individual patients have already been described. In Tables V.3. and V.4. are shown the clinical results recorded in these patients during

follow up to date. The two most important clinical parameters are clearly subsequent mortality and significant morbidity, which in this context refers to the occurrence of histologically proven or radiologically evident bone metastases. The difference in mortality and morbidity between the 3 main groups: scan positive patients, scan "converters", and patients with persistently negative scans, was found to be significant using a standard chi squared test:

$$\text{mortality } \chi^2 = 7.63,\ p < 0.025;$$

$$\text{bone metastases } \chi^2 = 30.46,\ p < 0.005.$$

D. DISCUSSION

Follow up of the patients in this study is not yet complete. Radiological or pathological confirmation may not be anticipated for several years. A previous study has shown that during follow up of 12 X-Ray negative scan positive patients with "early" breast cancer, only 67% were confirmed as having bony metastases at 12 months although subsequently all 12 developed evidence of bony disease (Galasko, 1969). It is also well known that there may be a delay of many months between the onset of symptoms due to bony metastases and their subsequent radiological confirmation (Sharpe and McDonald, 1942). Our results to date, however, demonstrate that in a large number of patients presenting with breast cancer, a positive bone scan does indicate the presence of clinically occult skeletal metastases - to date 9 of 24 patients with an initially positive bone scan have developed radiologically evident bone metastases at the areas indicated by the scan as being tumour-involved and in another 2 patients definite histological proof of metastases has been obtained. Conversely not one of the 70 patients with persistently negative scans has yet developed evident bone metastases. Not unexpectedly, the incidence of occult bone metastases is higher in these patients with known metastatic (Stage IV) or locally advanced disease (Stage III) than in those patients with apparently "early" (Stage I or II) tumours.

The incidence of positive bone scans in the 88 patients in our series who presented with Stage I or Stage II tumours was 18.2%, and this figure is similar to that reported by other workers who studied similar numbers of patients using the less suitable bone scanning agents ^{85}Sr (Sklaroff and Charkes, 1968), ^{87m}Sr (Hoffman and Marty, 1972) and ^{18}F (Galasko, 1969). The results presented here suggest that a positive bone scan at the time of presentation in a patient with Stage I or Stage II disease is of serious prognostic significance, both in terms of morbidity (i.e. subsequent development of bone metastases) and mortality. The significantly greater morbidity and mortality in the scan positive groups is not due to longer follow up, as the mean follow up of the scan negative group (11.6 months) was longer than that of the scan positive group (8.2 months) while the follow up in the scan "converter" group was 14.4 months.

The ability of the isotope bone scan to identify the presence of occult metastases in patients with apparently early breast cancer is clearly of great prognostic significance in the individual case. It also may be of great importance in the design of prospective clinical trials which compare different forms of surgical treatment of primary breast cancer. The selection of patients for such trials requires accurate staging of disease and an isotope bone scan is an essential part of preoperative assessment.

Another important application of the bone scan is in the follow up of patients after treatment of primary breast cancer. Because of the very suitable properties of the ^{99m}Tc phosphates repeated bone scans are readily performed and such serial studies, which have never been previously reported, provide a unique opportunity to study the development and progression of malignant disease in patients in whom symptoms are entirely absent and radiographs are normal (Figure V.7). In this context the conversion of a previously negative scan to positive is a highly significant event: eight patients who were initially scan negative became scan positive during follow up and 4 (50%) of these patients have been subsequently shown to have bone metastases.

It is clear that a negative bone scan does not exclude the presence of bony metastases, but in general terms, the likelihood of a patient developing clinical or radiological evidence of bone metastases within 12 months of a negative bone scan is low. An exception to this rule may be younger patients or patients with unfavourable histology. It is suggested that in post-menopausal patients a yearly bone scan should be part of the routine follow up, while in younger patients, and possibly in patients with less favourable histology and/or clinical staging, a bone scan should be repeated at six monthly intervals during the first 2 years of follow up, whether or not the patient has symptoms.

SECTION VI. FUTURE DEVELOPMENT IN ISOTOPE BONE STUDIES IN MALIGNANT DISEASE

A. INTRODUCTION

This thesis describes the evaluation and comparison of the new ^{99m}Tc labelled phosphate bone scanning agents. Those pharmacological properties of importance in obtaining reliable high quality bone scans have been discussed. It is clear that a high target to background ratio, good counting statistics, easy collimation and therefore high resolution, and low radiation absorbed dose are all provided by ^{99m}Tc-E.H.D.P. The results of the clinical studies described, although to some extent preliminary, demonstrate the value of the technique in the investigation of patients with suspected tumour involvement of bone. In this concluding section possible future advances in the use of bone scanning in the study of malignant disease are discussed.

Future developments in the use of bone scanning may be anticipated in two main areas, firstly further improvements in the technical aspects of the technique, and secondly in clinical studies designed to critically utilise the bone scan to improve the diagnosis and treatment of patients with metastatic disease.

B. TECHNICAL IMPROVEMENTS IN THE BONE SCAN

The quality of the visual image of the skeleton obtained from the rectilinear scanner or gamma camera (scintiscan) depends on many factors, the most important of which are

(1) adequate information density,

(2) adequate target:background ratio,

(3) adequate resolution of the physical system

and

(4) satisfactory display of the recorded data.

Technical improvements in the bone scan can be achieved by improving the radiopharmaceuticals used and also by improvements in the physical detecting and display systems.

(1) Improvements in the Available Radiopharmaceuticals

As discussed in detail in this thesis, the ^{99m}Tc phosphates, particularly ^{99m}Tc-E.H.D.P., represent a major improvement in the available bone scanning agents. The physical properties of ^{99m}Tc are nearly ideal, and ^{99m}Tc-E.H.D.P. provides high information density scans with higher target to background ratios and better resolution than conventional bone scanning agents. Higher target:background ratios than those now available may be achieved either by increasing the uptake of radiopharmaceutical

in tumour-involved bone or reducing the levels of activity in surrounding normal bone. In this respect methylene diphosphonate (M.D.P.), which has a marginally faster blood clearance, may be superior to E.H.D.P. (Subramanian et al, 1974).

It is clear that the limiting factor which determines the target:background ratio, and therefore whether a tumour is visualised or not, is biological. There must be clearly a minimum degree of bone reaction below which the focal concentration of radiopharmaceutical will be too low to permit visualisation of a lesion. All of the bone scanning agents described depend on the principle of increased blood flow or metabolic activity due to tumour involvement causing increased focal concentration of radiopharmaceutical. The ^{99m}Tc diphosphonates are certainly the best of the available agents and one cannot envisage any major improvement in terms of physical or pharmacological properties in this class of compounds. Measures aimed at manipulating the biological system to increase the uptake of ^{99m}Tc-E.H.D.P. might improve the scan quality but these measures are potentially harmful to the patient, and further research should probably be directed towards developing new agents whose mechanism of localisation in tumour-involved bone is entirely different, based perhaps on the metabolism of the tumour cells. Such a compound would be a true positive tumour label. It is likely that the use of such an agent would permit

the identification and visualisation of tumour cells in bone at a time when bone reaction is minimal, and a ^{99m}Tc-E.H.D.P. scan is negative.

(2) Improvements in the Available Physical Detection and Display Systems

A detailed discussion of the physics of detection and display systems is outwith the scope of this thesis. At its simplest level, however, the most important property of the system used to detect focal hot spots (whether gamma camera, rectilinear or hybrid scanner) is spatial resolution. The introduction of the ^{99m}Tc phosphates has itself acted as a great stimulus to the development and marketing of a new generation of whole body scanners and gamma cameras with improved resolution which are capable of providing high quality images of the total skeleton with ½-1 hour of patient scanning time. Most of the clinical studies described in this thesis were carried out with an older instrument with relatively poor resolution, and an example is shown of the improvement in image quality which the latest generation of commercially available gamma cameras provides (Figure VI.1). Differing exposure of the scintigram in different "spot" views of the skeleton is not a problem with newer instruments which provides a complete image of the whole skeleton on one 35 mm, 70 mm or radiographic film. These images can be viewed through transmitted light, as with a conventional radiograph.

Another area where improvements may occur is in the area of image manipulation and display. The whole field of computer analysis of scan data has a great potential. Improvements in scan image can be obtained by a variety of computer techniques (Figure VI.2) (Barber and Mallard, 1971; Waxman et al, 1971). Whether or not there is a consequent improvement in terms of diagnostic accuracy has not yet been established (Kuhl et al, 1972; Galasko, 1973). Of greater importance is the ability to convert the analogue gamma camera scintigram into digital form and so provide quantitative data which may be objectively assessed. There are now commercially available hard wired systems and digital computers which can be operated on-line to the gamma camera, and provide reliable quantitative data. These advances are particularly relevant to the ^{99m}Tc phosphates as the higher injected activities of these compounds provide much better counting statistics for quantitative studies.

C. USE OF THE BONE SCAN TO IMPROVE THE DIAGNOSIS AND TREATMENT OF MALIGNANT DISEASE

In general the technical developments in bone scanning over the past few years have made available a reliable high quality investigative technique for the study of malignant disease. The potential value of a technique which can positively identify the presence and possibly the biological activity of bone metastases in asymptomatic patients with normal radiographs is great. What is now more important than further technical advances is that clinical studies be undertaken to critically determine those clinical situations in which the bone scan is of value in improving our understanding and treatment of patients with metastatic cancer (Table VI.1). Although these techniques are applicable to many different malignancies they are discussed here mainly in the context of breast cancer.

In the previous chapter the use of the bone scan in the study of patients with primary breast cancer was described. The results in that study, although not yet complete, indicate how the clinical staging of patients with primary breast cancer can be significantly altered by the bone scan. It may well be necessary to re-design existing surgical trials for

the treatment of early breast cancer, in the light of the significant new developments in preoperative staging which bone scanning provides.

By permitting serial bone scans to be performed, ^{99m}Tc-E.H.D.P. now makes it possible for the first time to document accurately and non-invasively the progression of occult bony metastases in asymptomatic patients with no clinical nor radiographic evidence of local recurrence nor metastatic disease. This has enormous implications in the definition of the disease-free interval, the length of which is considered at present to be of considerable prognostic significance (Kennedy, 1973).

The bone scan is also of great potential benefit in clinical research aimed at improving the treatment of metastatic disease, an area where present therapeutic efforts are generally unsuccessful. The conclusion that conventional forms of treatment of metastatic cancer are unsuccessful is drawn from experience of treatment of metastases identified by conventional methods of diagnosis. The earlier use of systemic therapy, initiated because of a positive bone scan, may well improve the results of treatment in patients with metastatic disease. Of relevance also is the use of the bone scan as a quantitative, objective index of response or failure of response of metastases

to systemic treatment. In this context the opportunity to obtain repeated bone scans with ^{99m}Tc-E.H.D.P. is particularly important, and serial studies may be used to rationalize the treatment of patients with established metastatic disease (Figure VI.3). The only problem with such a scheme is the fact that the phosphate bone scan does not reflect tumour activity directly, but reflects the response in surrounding bone. This technique may, therefore, be more useful in the study of radiologically invisible metastases where bone destruction is less marked.

Further useful research applications of the bone scan may include the study of haematological malignancies, where the combination of bone scanning and bone marrow scanning techniques may be used as an index of successful therapy.

Another possible application is the development of radiotherapeutic compounds based on diphosphonate, where ^{99m}Tc is replaced by a suitable β-emitting nuclide such as ^{32}P. Local radiotherapy using conventional radium or x-rays is often of great value in treating local manifestations of cancer. Such treatment is simply palliative, however, and it is clear that metastatic cancer requires systemic treatment if any hope of cure is to be anticipated. "Radio-chemotherapy" with ^{32}P-diphosphonate or similar agent would result in irradiation of all metastatic foci in the skeleton, with the added advantage

that those areas which are most active metabolically would receive a proportionally greater radiation dose. Prior to such treatment an uptake study with ^{99m}Tc diphosphonate could be performed and the therapeutic activity to be injected calculated from this, much in the way radioactive iodine uptake is measured prior to treatment in thyrotoxicosis.

A summary of the thesis

THE USE OF RADIOACTIVE NUCLIDES IN THE INVESTIGATION OF MALIGNANT DISEASE.

(A laboratory and clinical evaluation of the ^{99m}Tc phosphates as bone scanning agents in the detection of skeletal metastases)

by

Dennis Lionel Citrin M.B. Ch.B., M.R.C.P. (U.K.)

Presented for the degree of Doctor of Philosophy in the Faculty of Medicine, University of Glasgow.

July 1975.

In Two Volumes.

The relative insensitivity of standard radiological methods in the diagnosis of early metastatic disease of the skeleton is well recognised. The development in the 1960's of isotope bone scanning using ^{85}Sr, ^{87m}Sr and ^{18}F permitted the earlier and more accurate diagnosis of malignant involvement of bone, but as none of these radionuclides was ideal the technique of bone scanning has not been optimally applied in clinical medicine and surgery. In 1972 and 1973, new radiopharmaceuticals were developed which have made available to all hospitals with even limited facilities the capacity to obtain high quality bone scans. These new radiopharmaceuticals are the ^{99m}Tc labelled phosphates. This thesis describes the laboratory and clinical evaluation of the available ^{99m}Tc phosphate compounds.

The introductory section describes the principle of the bone scan and how it differs from the radiograph. There follows a description of the conventional bone scanning agents ^{85}Sr, ^{87m}Sr and ^{18}F, and a brief discussion of the disadvantages of each of these nuclides. The development of the ^{99m}Tc phosphates is then described and the relevant physical and chemical properties of these agents is summarised.

The choice of which of the available ^{99m}Tc phosphates, ethane hydroxy diphosphonate, pyrophosphate, polyphosphate and monofluorophosphate, is the most suitable agent depends on a comparison of their pharmacological properties, as they are all very similar chemically and all utilise the same radionuclide,

^{99m}Tc. The second section of the thesis describes a detailed quantitative comparison of the pharmacological properties of each compound. Several new investigative techniques were developed for this study and these techniques are described in detail. On the basis of the comparative studies, it was concluded that ethane hydroxy diphosphonate is the most favourable agent, because of the higher target:background ratios it provides, and because of its more rapid blood clearance, greater urinary excretion and lower whole body retention.

A detailed evaluation of the in vitro and in vivo properties of ethane hydroxy diphosphonate is then described. The in vitro properties studied are those which are of importance to the use of the compound in routine clinical use. It was concluded that the compound is very flexible and that high quality images can be obtained despite the variation in presentation, labelling and injection procedures which may occur in a busy Department of Nuclear Medicine.

The in vivo experiments performed in volunteer subjects and in patients with known bony metastases demonstrate that E.H.D.P. clearly satisfies the important biological criteria necessary for a satisfactory bone scanning agent, namely

(1) rapid skeletal uptake of a significant proportion of the administered activity.

(2) skeletal binding which is irreversible, in the short term at least.

(3) higher uptake in tumour-involved compared with normal bone.

(4) no significant retention in soft tissue.

(5) rapid urinary excretion of the diphosphonate not taken up by bone.

(6) lack of toxicity.

The clinical sections of the thesis are introduced with a discussion of the problems of technique and scan interpretation which are particularly relevant to the ^{99m}Tc phosphates. The study performed to assess the sensitivity and clinical value of the ^{99m}Tc phosphate bone scan in patients with malignant disease is then described: three hundred and seventy two patients and 75 control subjects were studied and the results support the conclusion that the ^{99m}Tc phosphate bone scan allows earlier and more accurate identification of bone metastases than the radiograph. If the bone scan is read with all clinical details and the results of complementary radiographs available, the incidence of false positive results is acceptably low.

The application of the ^{99m}Tc phosphate bone scan to the preoperative staging and clinical follow up of patients with primary breast cancer is described in the next section. The high incidence of bony metastases in patients with breast cancer is well recognised and the value of a technique which permits the identification of metastases in asymptomatic patients with normal radiographs is potentially great. Previous studies

with strontium and fluorine have suggested that about 20% of patients with apparently curable breast cancer (Stages I and II) have bone metastases at the time of first presentation, and a similar incidence of occult metastases was observed in our study using ^{99m}Tc phosphates. The clinical importance of this study is discussed.

The thesis concludes with a brief discussion of future developments in the use of isotope methods in the study of malignant disease of the skeleton. Technical improvements in scanning equipment and available radiopharmaceuticals have been significant, and the need now is for critical clinical studies which utilise the isotope bone scan to improve the diagnosis and management of patients with malignant disease of the skeleton.

THE USE OF RADIOACTIVE NUCLIDES IN THE INVESTIGATION OF MALIGNANT DISEASE.

(A Laboratory and Clinical Evaluation of the ^{99m}Tc phosphates as Bone Scanning Agents in the detection of skeletal metastases)

by

Dennis Lionel Citrin M.B. Ch.B., M.R.C.P. (U.K.)

Senior Registrar in Nuclear Medicine,
University Department of Medicine,
Royal Infirmary, Glasgow.

A thesis presented for the degree of Doctor of Philosophy in the Faculty of Medicine, University of Glasgow.

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Table I.1. In Vitro Properties of ^{99m}Tc Phosphates

	Polyphosphate	E.H.D.P.	Pyrophosphate	M.F.P.
Physical Properties	All compounds are presented as white lyophilised powders (non-radioactive) *			
Active Constituents	(a) 100mg DII Polyphosphate 2 mg Stannous Chloride	5mg E.H.D.P. 0.5mg Stannous Chloride	100mg Pyrophosphate 1.3mg Stannous Chloride	100mg M.F.P. 2mg Stannous Fluoride
	(b) 40mg NEN Polyphosphate 1 mg Stannous Chloride			
Solubility	All compounds are soluble in aqueous solution			
In Vitro Stability	Stability of all compounds is improved by storage in oxygen-free (nitrogen) atmosphere. E.H.D.P. is chemically the most stable. All compounds have a shelf life of at least several months.			
Labelling	All compounds are easily and rapidly labelled with ^{99m}Tc (pertechnetate solution). A low concentration of oxidising agents in ^{99m}Tc generator eluate is necessary. Labelling yield may be rapidly checked by paper chromatography or more accurately by Sephadex gel chromatography.			
Sterility	All compounds are presented in sterile and pyrogen-free condition. Millipore filtration is not required.			

* Pyrophosphate is now available lyophilised. The preparation studied in the experiments described in this thesis was in aqueous solution.

Table I.2. Comparison of ^{85}Sr, ^{87m}Sr and ^{18}F with the ^{99m}Tc Phosphates

Nuclide	Physical Half life	Principal γ Ray Energy	Injected Activity	Estimated Radiation Dose (Rads/mCi)		Main Disadvantages
				Whole Body	Bone	
^{85}Sr	65 days	514 KeV	100 uCi	13	55	(1) High radiation dose (2) Injected activity restricted to 100 uCi (3) Use restricted to adults with known cancer
^{87m}Sr	2.8 hours	388 KeV	1-3 mCi	0.02	0.16	(1) Low target:background ratio (2) Faecal excretion may obscure or mimic lesion in lumbar spine or pelvis
^{18}F	110 min	510 KeV	2-10 mCi	0.05	0.29	(1) Cyclotron production (expense, availability) (2) Needs additional collimation for gamma camera, or positron camera
^{99m}Tc-phosphates	6.2 hours	140 KeV	10-20 mCi	0.02	0.05	(1) Attenuation of photon by subcutaneous fat, items of clothing (2) Activity in bladder or kidney may mimic or obscure pelvic lesions (3) Occasional radiopharmaceutical unreliability (polyphosphates)

Table II.1. Blood and Cumulative Urinary Activity Levels of E.H.D.P. in 10 volunteer subjects

(Mean ± S.E.M.)

	Blood Activity		Cumulative Urinary Activity	
	mCi	uCi	mCi	uCi
5	7.22 ± 0.64	8.00 ± 0.76	-	-
15	4.74 ± 0.26	5.07 ± 0.59	12.98 ± 1.13	14.02 ± 1.37
30	3.66 ± 0.20	3.50 ± 0.30	21.89 ± 1.42	24.40 ± 2.07
45	2.74 ± 0.24	2.88 ± 0.27	29.20 ± 1.80	31.57 ± 2.40
60	2.36 ± 0.20	2.52 ± 0.31	34.50 ± 1.98	37.22 ± 2.65
120	1.38 ± 0.12	1.54 ± 0.12	48.10 ± 2.58	51.06 ± 3.43
180	0.99 ± 0.10	1.10 ± 0.13	55.80 ± 2.96	60.03 ± 3.88
240	0.73 ± 0.06	0.86 ± 0.09	60.10 ± 3.25	66.16 ± 4.07
300	0.59 ± 0.04	0.60 ± 0.08	68.40 ± 1.65	69.89 ± 4.12
360	0.43 ± 0.03	0.52 ± 0.08	70.60 ± 1.80	72.19 ± 4.20

Each subject studied on 2 occasions following intravenous injection of 5 mCi and 20 uCi of ^{99m}Tc-E.H.D.P.

Table II.2. Comparison of ^{99m}Tc Phosphates: Site of Primary Tumour in Patients with Bone Metastases

Breast	25
Lung	6
Prostate	4
Occult	1
Colon	1
Bladder	1
TOTAL	38

Table II.3. Number of Patients and Tumours Studied with each ^{99m}Tc phosphate

	No. of Patients *	No. of tumours
E.H.D.P.	35	128
PYRO	19	70
D.I.I. POLY	17	47
N.E.N. POLY	9	42
M.F.P.	12	55
TOTAL	92	144

* All patients had at least two separate studies

Table II.4. Comparison of Tumour:Bone Ratios.

Results of Paired 't' Tests

		n	Mean ± S.E.M.	p
1.	E.H.D.P.	54	1.90 ± 0.11	< 0.02
	PYRO		1.80 ± 0.09	
2.	E.H.D.P.	39	1.84 ± 0.12	< 0.001
	D.I.I. POLY		1.59 ± 0.07	
3.	E.H.D.P.	34	1.97 ± 0.14	< 0.005
	N.E.N. POLY		1.82 ± 0.12	
4.	E.H.D.P.	55	1.82 ± 0.07	< 0.001
	M.F.P.		1.58 ± 0.06	
5.	PYRO	33	1.79 ± 0.11	< 0.02
	D.I.I. POLY		1.68 ± 0.11	
6.	PYRO	37	1.85 ± 0.12	< 0.40
	N.E.N. POLY		1.80 ± 0.12	

n = number of tumours in each paired study.

$p < 0.05$ considered significant.

Table II.5. Spine:Background Study.

Number of Patients

	Male	Female	Total
E.H.D.P.	6	3	9
PYRO	4	5	9
D.I.I. POLY	5	5	10
N.E.N. POLY	2	5	7
TOTAL	17	18	35

Table II.6. Spine:Background Ratios

(Mean ± S.E.M.)

	n	2 hr	4 hr	6 hr
E.H.D.P.	9	2.12 ± 0.08	2.70 ± 0.12	3.01 ± 0.10
PYRO	9	2.19 ± 0.07	2.67 ± 0.09	2.69 ± 0.10
D.I.I. POLY	10	1.83 ± 0.11	1.93 ± 0.11	2.04 ± 0.11
N.E.N. POLY	7	2.11 ± 0.08	2.19 ± 0.06	2.32 ± 0.05

n = number of subjects studied with each compound

Table II.7. Total Counts from Spine and Background Areas in Control Subjects: *

Counts/mCi of injected activity

(Mean ± S.E.M.)

	2 hr			4 hr			6 hr		
	Spine	Background	Net Bone	Spine	Background	Net Bone	Spine	Background	Net Bone
E.H.D.P.	3179 ± 216	1525 ± 107	1654	2820 ± 162	1060 ± 73	1760	2438 ± 116	818 ± 116	1620
PYRO	3082 ± 266	1363 ± 103	1719	3203 ± 195	1209 ± 83	1994	3042 ± 273	1121 ± 82	1921
D.I.I. POLY	4189 ± 579	2170 ± 236	2019	3850 ± 428	1950 ± 173	1900	3828 ± 357	1848 ± 106	1980
N.E.N. POLY	3457 ± 361	1650 ± 180	1807	3289 ± 331	1509 ± 152	1780	3280 ± 347	1419 ± 157	1861

* Corrected for physical decay of nuclide

Table II.8. Blood Levels of mCi Activities: % activity/litre whole blood

	E.H.D.P.		PYRO		D.I.I. POLY		N.E.N. POLY	
	n	Mean ± S.E.M.	n	Mean ± S.E.M.	n	Mean ± S.E.M.	n	Mean ± S.E.M.
5 min	26	5.88 ± 0.37	26	6.26 ± 0.42	16	7.99 ± 0.88	21	8.42 ± 0.73
2 hr	23	1.42 ± 0.18	24	1.71 ± 0.16	23	3.08 ± 0.38	21	2.59 ± 0.24
4 hr	25	0.90 ± 0.11	25	1.44 ± 0.13	23	2.53 ± 0.29	22	2.15 ± 0.20
6 hr	25	0.59 ± 0.07	25	1.24 ± 0.12	23	2.21 ± 0.27	22	1.97 ± 0.21

n = number of patients

Results of unpaired 't' tests

			2 hr	4 hr	6 hr
E.H.D.P.	vs	PYRO	p < 0.30	< 0.005	< 0.001
E.H.D.P.	vs	D.I.I. POLY	p < 0.001	< 0.001	< 0.001
E.H.D.P.	vs	N.E.N. POLY	p < 0.001	< 0.001	< 0.001
PYRO	vs	D.I.I. POLY	p < 0.005	< 0.005	< 0.005
PYRO	vs	N.E.N. POLY	p < 0.005	< 0.005	< 0.005

Table II.9. Measurement of Kinetics of Microcurie Activities of ^{99m}Tc Phosphates in Control Subjects

	No. of Subjects *	No. of Studies
E.H.D.P.	10	10
PYRO	10	10
D.I.I. POLY	5	5
N.E.N. POLY	5	5
M.F.P.	10	10
TOTAL	20	40

* Each subject had 3 studies in this experiment

Table II.10. Blood Levels of Injected Microcurie Activities: % activity/litre of blood

(Mean ± S.E.M.)

	n	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
E.H.D.P.	10	2.48 ± .17	1.68 ± .13	1.29 ± .09	1.03 ± .07	.90 ± .06	.77 ± .06
PYRO	10	2.35 ± .07	1.69 ± .08	1.55 ± .10	1.39 ± .09	1.27 ± .10	1.21 ± .08
D.I.I. POLY	5	3.69 ± .27	3.10 ± .18	2.93 ± .11	2.69 ± .17	2.55 ± .14	2.41 ± .12
N.E.N. POLY	5	3.07 ± .21	2.18 ± .10	1.91 ± .12	1.75 ± .06	1.62 ± .05	1.59 ± .05
M.F.P.	10	2.23 ± .26	1.68 ± .21	1.51 ± .19	1.32 ± .18	1.34 ± .16	1.21 ± .16

n = number of subjects

Table II.11. Cumulative Hourly Urinary Excretion of 30 Microcurie Activities: % of Administered Activity

(Mean ± S.E.M.)

	n	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
E.H.D.P.	6	42.11 ± 1.15	57.95 ± 1.40	65.52 ± 2.52	71.91 ± 2.66	76.52 ± 2.8	79.88 ± 2.87
PYRO	6	32.09 ± 1.42	42.97 ± 1.47	47.99 ± 1.59	51.05 ± 1.64	52.87 ± 1.74	54.43 ± 1.77
D.I.I. POLY	5	33.65 ± 3.39	43.52 ± 4.19	49.16 ± 4.73	53.38 ± 5.23	56.38 ± 5.46	58.63 ± 5.60
M.F.P.	10	29.79 ± 1.72	38.91 ± 1.91	43.47 ± 1.86	46.78 ± 1.92	49.11 ± 2.01	51.21 ± 2.21

n = number of subjects studied

Table II.12. Whole body retention levels: % of administered activity

(Mean ± S.E.M.)

	n	1 hr	2 hr	3 hr	4 hr	5 hr	6 hr
E.H.D.P.	10	70.75 ± 1.50	56.66 ± 1.59	48.50 ± 1.65	42.82 ± 1.62	39.16 ± 1.44	35.96 ± 1.47
PYRO	10	71.41 ± 1.33	61.97 ± 1.31	56.69 ± 1.42	54.48 ± 1.45	52.28 ± 1.49	50.29 ± 1.41
D.I.I. POLY	5	76.94 ± 1.73	70.10 ± 1.81	65.41 ± 2.00	62.26 ± 1.92	59.92 ± 2.11	58.77 ± 1.75
N.E.N. POLY	5	75.92 ± 1.49	67.06 ± 1.15	62.67 ± 1.26	57.99 ± 1.31	56.96 ± 1.47	54.97 ± 2.22
M.F.P.	10	78.00 ± 1.23	68.91 ± 1.28	63.91 ± 1.21	60.40 ± 1.21	58.22 ± 1.13	56.48 ± 1.07

n = number of subjects

Table II.13. Calculated Whole Body Radiation Absorbed Dose for ^{99m}Tc Phosphates*

E.H.D.P.	15 mRad/mCi
PYRO	20 mRad/mCi
D.I.I. POLY	22 mRad/mCi
N.E.N. POLY	21 mRad/mCi
M.F.P.	21 mRad/mCi

* Assumes:

(1) All the energy of the decaying nuclide is absorbed in tissue.

(2) The fraction of the activity remaining in the body decreases exponentially with time due to excretion.

(3) Mass of the patient is 70 Kg.

(4) The radiant energy is absorbed uniformly throughout body.

Table II.14. Radiation Dose from Radionuclide and Radiological Procedures

Radioisotope Procedure	Radiopharmaceutical	Whole Body Radiation Dose (mRad/mCi)
Liver Scan	^{99m}Tc Sulphur Colloid	20
Brain Scan	^{99m}Tc as Pertechnetate	12
Pancreas Scan	^{75}Se methionine	8100
Blood Pool Scan	^{131}I labelled H.S.A.	2000
	^{99m}Tc albumin	5
Renal Scan	^{203}Hg Chlormerodrin	200
Thyroid Studies	^{131}I	400

Radiological Procedure	Skin Dose in m Rad per film	Mid plane Dose
Lumbar spine	480 - 17000	600 - 1500
Dorsal spine	400 - 10000	550 - 1000
Pelvis A.P.	350 - 4700	50 - 600
I.V.P.	500 - 1750	50 - 600
Pelvimetry	1500 - 120000	200 - 3300

Table III.1. Effect of Varying Volume of Eluate

Patient	Primary Disease	Target:Background Ratio	
		2 ml Study	8 ml Study
1	Breast Cancer	2.07	2.05
2	Prostatic Cancer	2.18	1.98
3	Breast Cancer	2.12	2.32
		3.12	2.92
		1.29	1.44
		1.30	1.52
		3.34	3.58
		3.13	4.54
		2.30	2.14
4	Breast Cancer	1.39	1.10
		1.89	1.73
		2.14	2.25
		3.43	2.82
5	Paget's Disease	8.97	8.47
6	Occult Primary	1.61	1.54
(Mean ± S.E.M.)		2.68 ± 0.48	2.69 ± 0.47

$(0.90 < p < 0.95)$

Table III.2. Effect of Varying Quantity and Activity of Labelled Radiopharmaceutical

Patient	Primary Disease	^{99m}Tc	Volume	Quantity of E.H.D.P.	Tumour:Bone Ratio	
					a	b
1	Breast Cancer	a. 10 mCi	8 ml	3 mg	1.60	1.60
		b. 5 mCi	4 ml	1.5 mg	1.56	1.67
					1.81	1.81
					2.21	2.21
					1.60	1.47
					1.89	1.90
					1.73	1.98
					1.37	1.77
					2.56	2.45
					1.81	1.76
2	Lung Cancer	a. 10 mCi	2 ml	1 mg	1.36	1.36
		b. 5 mCi	1 ml	0.5 mg		

Table III.2. con't

Patient	Primary Disease	^{99m}Tc	Volume	Quantity of E.H.D.P.	Tumour:Bone Ratio	
					a	b
3	Lung Cancer	a. 5 mCi	1 ml	0.5 mg	3.36	3.32
		b. 2.5 mCi	0.5 ml	0.25 mg		
4	Lung Cancer	a. 10 mCi	2 ml	3 mg	1.91	1.75
		b. 10 mCi	2 ml	1 mg		
5	Occult Primary	a. 10 mCi	2 ml	3 mg	1.86	1.77
		b. 5 mCi	1 ml	0.5 mg		
				Mean ± S.E.M.	1.90 ± 0.14	1.91 ± 0.13
					(0.975 < p < 0.95)	

* a and b paired studies. Quantity of E.H.D.P. and ^{99m}Tc activity in a > b.

Table III.3. Storage of Labelled Product

Patient	Primary Disease	Delay between labelling and injection	Scan Image	Tumour:Bone Ratios	
1	Breast Cancer	3 hours	Satisfactory quality	-	
2	Lung Cancer	3 hours	Satisfactory quality	-	
3	Control (Benign)	3 hours	Satisfactory quality	-	
4	Breast Cancer	5 hours	Satisfactory quality	-	
5	Breast Cancer	5 hours	Satisfactory quality	-	
6	Lung Cancer	5 hours	Satisfactory quality Positive	Right shoulder Left rib Left scapula Left clavicle Right rib Lumbar Dorsal	2.56 2.77 4.50 2.61 1.57 1.46 1.39
7	Lung Cancer	5 hours	Satisfactory quality Positive	Lumbar	1.79
8	Prostatic Cancer	5½ hours	Satisfactory quality Positive	Lumbar Lumbar Dorsal Dorsal Dorsal Right pelvis Left pelvis	1.37 1.46 1.37 1.35 1.50 1.43 1.53

Table III.4. Whole Blood and Urinary Levels of ^{99m}Tc E.H.D.P.

(Mean ± S.E.M.)

Time after injection	Blood Activity (% of injected activity/ litre of whole blood)	Cumulative Urinary Excretion (% of injected activity)
5 min	7.22 ± 0.64	-
15 min	4.74 ± 0.26	13.0 ± 1.13
30 min	3.66 ± 0.20	21.9 ± 1.42
45 min	2.74 ± 0.24	29.2 ± 1.80
1 hr	2.36 ± 0.20	34.5 ± 1.98
2 hr	1.38 ± 0.12	48.1 ± 2.58
3 hr	0.99 ± 0.10	55.8 ± 2.96
4 hr	0.73 ± 0.06	60.1 ± 3.25
5 hr	0.59 ± 0.04	68.4 ± 1.65
6 hr	0.43 ± 0.03	70.6 ± 1.80

(n = 10)

Table III.5. Serial Blood Calcium and Phosphate Levels after intravenous diphosphonate

(Mean ± S.E.M.)

		Calcium	Phosphate
Pre injection		9.58 ± 0.06	3.20 ± 0.10
Post injection	5 min	9.51 ± 0.07	3.17 ± 0.09
	15 min	9.48 ± 0.06	3.05 ± 0.12
	30 min	9.54 ± 0.08	3.00 ± 0.11
	45 min	9.51 ± 0.06	3.16 ± 0.11
	60 min	9.50 ± 0.08	3.24 ± 0.10
	120 min	9.51 ± 0.07	3.33 ± 0.10
	180 min	9.43 ± 0.08	3.47 ± 0.10
	240 min	9.41 ± 0.09	3.62 ± 0.10
	300 min	9.40 ± 0.07	3.71 ± 0.11
	360 min	9.34 ± 0.10	3.80 ± 0.09

All results expressed as mg/100ml.

(n = 35)

Table IV.1. Primary Diagnosis in Cancer Patients Scanned with ^{99m}Tc Phosphates

Breast Cancer	283
Lung Cancer	33
Prostatic Cancer	14
Primary bone tumour	7
Kidney cancer	6
Colon	6
Lymphoma	5
Miscellaneous (including oesophagus, bladder, thyroid, uterus, ovary)	18
TOTAL	372

Table IV.2. Agents used in Evaluation of ^{99m}Tc Phosphate Bone Scan

Agent	Patients	Control Subjects	Total
E.H.D.P.	276	15	291
PYRO	35	16	51
POLY	47	44	91
M.F.P.	14	0	14
TOTAL	372	75	447

Table IV.3. Overall Comparison of Bone Scans and Radiographs

	Total	X-Ray +ve Scan +ve	X-Ray +ve Scan -ve	X-Ray ? Scan +ve	X-Ray ? Scan -ve	X-Ray -ve Scan -ve	X-Ray -ve Scan +ve	X-Ray -ve Scan ?
Breast	283	63	2	6	3	159	46	4
Lung	33	14	-	-	-	7	9	3
Prostate	14	7	-	-	-	3	3	1
Primary bone tumour	7	7	-	-	-	-	-	-
Kidney	6	2	-	-	-	4	-	-
Colon	6	1	-	1	-	3	1	-
Lymphoma	5	1	-	-	-	4	-	-
Miscellaneous	18	5	-	-	-	9	4	-
TOTAL	372	100	2	7	3	189	63	8

Table IV.4. Clinical Details and Follow Up of X-Ray Suspicious Patients

	Clinical Details	X-Ray Suspicious	Bone Scan	Clinical Follow Up
(1)	Mastectomy 3 yrs before. Complaint of pain in neck.	Cervical spine	Cervical Spine	Radiotherapy to neck relieved pain. X-Ray +ve 6/12.
(2)	Stage I breast cancer. Time of presentation.	Skull	Extensive	X-Ray +ve 4/12.
(3)	Cancer of colon. Complaint of hip pain.	Right sacro iliac region	Right sacro iliac region	X-Ray +ve 8/12.
(4)	Mastectomy 2 yrs before. Presented with ascites and liver metastases	Right ribs	Extensive	Started Stilboestrol. "Very ill" 3/12.
(5)	Fungating Cancer of breast.	Lumbar spine	Lumbar spine Right shoulder	Repeat bone scan showed progression. Died 8/12.

Table IV.4. con't

	Clinical Details	X-Ray Suspicious	Bone Scan	Clinical Follow Up
(6)	Mastectomy 15/12 before.	Right ribs	Right ribs	Pain right ribs 12 months.
(7)	Mastectomy 10/12 before. Complaint of pain in pelvis.	Left pelvis	Pelvis Lumbar spine	Repeat bone scan positive.
(8)	Stage II breast cancer. Time of presentation.	Left pelvis Right shoulder	Negative	Repeat bone scan -ve 12/12. Clinically well 16/12.
(9)	Stage I breast cancer. Time of presentation.	Left pelvis	Negative	Clinically well 6/12.
(10)	Stage I breast cancer. Time of presentation.	Skull	Negative	Clinically well 2/12.

Table IV.5. X-Ray -ve Scan -ve Patients followed up for at least 6 months

No evidence of bone metastases during follow up*	98
Died	12
Definite bone metastases	6
TOTAL	116

* Mean duration of follow up = 12.8 months (range 6-24 months)

Table IV.6. Clinical Details and Follow Up of X-Ray -ve Scan -ve

Patients Dying During Follow Up

	Clinical Details	Interval between Study and Death	P/M	Cause of Death	Bone Metastases
(1)	Mastectomy 4/12 before study: anaemia, P.U.O., backache.	1 month	Yes	Stomach cancer, liver metastases, P.T.E., C.C.F.	No
(2)	Mastectomy 4 yrs before study: lymph node recurrence.	18/12	No	Liver metastases	None clinically obvious
(3)	Stage II breast cancer. Studied at time of mastectomy.	16/12	Yes	P.T.E., C.C.F., no metastases	No
(4)	Stage II breast cancer. Studied at time of mastectomy.	11/12	No	Surgically proven brain metastasis	None clinically obvious
(5)	Stage I breast cancer. Age 75. Studied at time of mastectomy.	6/12	No	Died suddenly at home ? cause	None clinically obvious

Table IV.6. con't

	Clinical Details	Interval between Study and Death	P/M	Cause of Death	Bone Metastases
(6)	Previous mastectomy. Hepatomegaly.	5/12	No	Liver metastases	None clinically obvious
(7)	Kidney Cancer. Age 75.	1/12	Yes	Died postoperatively after nephrectomy	No
(8)	Previous Cancer of Cervix. Previous simple fracture neck of right femur. Fracture neck of left femur, biopsy - no tumour cells.	2/12	Yes	Simple fracture, occult bronchial carcinoma	No
(9)	Lymphoma. Back pain. Lymphangiogram positive. Repeat bone scan 6/12 later -ve.	8/12	No	Unknown	None clinically obvious
(10)	Lung cancer treated with radiotherapy.	3/12	No	Unknown	None clinically obvious
(11)	Lung Cancer. Dysphagia, weight loss.	2/12	Yes	Local extension into oesophagus	No
(12)	Lung cancer treated with radiotherapy.	4/12	Yes	Massive haemoptysis from local recurrence	No

Table IV.7. Clinical Details and Follow Up of Patients with "False Negative" Bone Scans

Site of Tumour	Clinical Details
(1) Breast	Repeat scan and x-ray +ve. 9/12 after initial study which was at time of mastectomy. Developed back pain 3/12 after 2nd study.
(2) Breast	Developed nodal recurrence and back pain 15/12 after initial study which was at time of mastectomy. Repeat bone scan +ve, x-ray suspicious only.
(3) Breast	Developed widespread bone metastases 10/12 after negative scan and x-ray.
(4) Breast	Scan and x-ray at time of mastectomy normal. 6/12 later scan +ve, x-ray -ve. Backache. Died 2/12 later, P.M. +ve.
(5) Breast	Initial scan and x-ray at time of mastectomy -ve. Young woman. Unfavourable histology. 6/12 later clinical deterioration. Scan and x-ray +ve.
(6) Occult Adenocarcinoma	Presented with axillary lymph node metastasis: occult primary ? breast. Initial scan and x-ray -ve. Repeat 10/12 later, both +ve.

Table IV.8. Clinical Details of Patients with "False Positive" Bone Scans

Age	Site of Primary Tumour	Radiographic diagnosis
67	Breast	Paget's disease of right humerus.
64	Breast	Ununited fracture right humerus. Degenerative disease of spine.
71	Breast	Osteoarthritis left hip.
74	Breast	Osteoarthritis left shoulder.
58	Breast	Degenerative disease of lumbar spine with osteophyte.
74	Breast	Paget's disease of skull.
82	Prostate	Paget's disease of pelvis.
70	Prostate	Paget's disease of pelvis.
69	Lung	Paget's disease of pelvis.
65	Lung	Bilateral simple fracture neck of femur.

Table IV.9. Summary of Results of Follow Up of X-Ray -ve Scan +ve Patients

Pathological Confirmation	6
Subsequent X-Rays positive	15
Died without post mortem confirmation	11
No evidence of bone metastases but clinical evidence of recurrent or metastatic disease at time of study	19
Well at time of study but subsequent clinical evidence of recurrent metastatic disease	4
Well at time of study and follow up	8
TOTAL	63

Table IV.10. Pathological Confirmation in X-Ray -ve Scan +ve Patients

Clinical Details	Site of Metastases	Clinical Follow Up
(1) Previous mastectomy Back pain.	Lumbar spine	Died two months. P.M. +ve.
(2) Presented with Stage III breast Cancer. No back pain.	Skull Thoracic spine	Died 2/12 after study. P.M. +ve.
(3) Presented with Stage II breast cancer. Back pain.	Extensive	Leukoerythroblastic anaemia. Trephine biopsy of iliac crest +ve.
(4) Lung Cancer. Back pain.	Lumbar spine	Died 1/12 after study. P.M. +ve.
(5) Lung Cancer. No symptoms of bone metastases.	Right ribs	Rib involvement confirmed at operation.
(6) Lung Cancer. Local rib pain.	Right ribs	Rib involvement confirmed at operation.

Table IV.11. Clinical Details of X-Ray -ve Scan +ve Patients where X-Rays Subsequently Confirmed Metastases

	Clinical Details	Site of Metastases	Follow Up
(1)	Mastectomy 12 yrs before. Local recurrence back pain.	Thoracic spine Lumbar spine	X-Rays +ve L.V. one month later.
(2)	Stage I breast cancer at time of study. No symptoms of bone metastases.	Skull	Lytic metastases skull 5/12 later.
(3)	Bilateral primary breast cancer (Stage III) at time of study. No symptoms of bone metastases.	Extensive metastases in spine	Lumbar spine positive 5/12.
(4)	Primary breast cancer with skin nodules (Stage IV) at time of presentation. No symptoms of bone metastases.	Multiple in spine, ribs, and pelvis.	+ve pelvis 4/12. Rest of skeleton still negative.
(5)	Mastectomy 3 years before study. Local recurrence. No symptoms of metastases.	Multiple metastases in spine	+ve X-Ray 8/12.

Table IV.11. con't

	Clinical Details	Site of Metastases	Follow Up
(6)	Stage II breast cancer at time of study. No symptoms of bone metastases.	Left shoulder girdle	Clinically well one year. Metastases left ribs 2/12.
(7)	Mastectomy 6 yrs before. Back pain.	Lower Thoracic Spine	10/12 later x-ray +ve. Pain relieved by DXT and oophorectomy. Recurrent node and tumour in 2nd breast 10/12.
(8)	Stage II breast cancer at time of study. No symptoms of bone metastases.	Right ribs	X-Ray +ve 8/12. Severe generalised bone pain 10/12.
(9)	Stage I breast cancer. No symptoms of bone metastases.	Ribs. Thoracic, lumbar spine.	X-Ray +ve left rib, T12, L3, 4/12. Back pain 6/12.
(10)	Node recurrence and back pain 7 yrs after mastectomy and oophorectomy.	Extensive	+ve spine x-ray 6/12.
(11)	Mastectomy 10/12 before study. Complaint of low back pain.	Multiple	X-Ray +ve lumbar spine 4/12.

Table IV.11. con't

	Clinical Details	Site of Metastases	Follow Up
(12)	Cervical and axillary lymph node metastases and back pain, 3 yrs after mastectomy.	Multiple	X-Ray +ve 5/12.
(13)	Previous radiotherapy for cancer of bladder. Complaint of pain in right shoulder.	Right scapula	X-Ray +ve 2/12.
(14)	Back pain 6/12 after oesophagectomy for cancer of oesophagus.	Lumbar spine, right 3rd rib, left first rib	Radiotherapy to lumbar spine relieved pain. X-Ray +ve left 1st rib 4/12.
(15)	Scanned at time of presentation. Stage IV breast cancer (multiple pulmonary metastases)	Multiple, especially cervical spine	Mastectomy and oophorectomy. Clinically well 6/12 but +ve x-ray cervical spine.

Table IV.12. Clinical Details of X-Ray -ve Scan +ve Patients Who Died Without Post Mortem Confirmation of Bone Metastases

	Clinical Details	Site of Metastases	Clinical Follow Up
(1)	Scanned at time of presentation with gross ulcerated breast cancer. Chronic lymphatic leukaemia. Low back pain.	Pelvis	Died at home 5/12 after scan.
(2)	Scanned 10/12 after local excision cancer left breast. No symptoms.	Skull, Pelvis	Died 14/12. Leukoerythroblastic anaemia.
(3)	Scanned at time of presentation with Stage II breast cancer. Rheumatic heart disease.	Left ribs	Died lung metastases 4/12.
(4)	Scanned at time of presentation with Stage II breast cancer. No bone symptoms.	Skull, left ribs, thoracic spine	Died 6/12 later on holiday in distant hospital. "Carcinomatosis". No further information.
(5)	Scanned at time of presentation with Stage IV breast cancer (malignant pleural effusion). No bone symptoms.	Extensive in spine	Died 11/12.

Table IV.12. con't

	Clinical Details	Site of Metastases	Clinical Follow Up
(6)	Previous resection of cancer of rectum. Severe low back pain.	Lumbar spine, Pelvis	Pain relieved by local DXT. Died 4/12. No P.M.
(7)	Lung cancer on chest x-ray. Tender left ribs. Presented with pain.	Left ribs	Pain relieved by local DXT. Died 3/12. No P.M.
(8)	Presented with uraemia, confusion and back pain. Prostatic biopsy +ve. Both acid and alkaline phosphatase elevated.	Extensive	Died 5/12.
(9)	Previous removal of ovarian tumour. Presented with metastases in cervical lymph node and severe pain in cervical spine.	Cervical Spine	Developed jaundice and died 16 days after study.
(10)	Lung Cancer. Presented with soft tissue metastases in thigh and back pain.	Cervical, lumbar spine	Died 5/12.
(11)	Lung Cancer. Severe back pain.	Extensive	Pain relieved by DXT. Died 2/12.

Table IV.13. Clinical Details of X-Ray -ve Scan +ve Patients with Clinical Evidence of Recurrent or Metastatic Disease at Time of Study

	Clinical Details	Site of Metastases	Clinical Follow Up
(1)	Mastectomy 18/12 before scan. Pain left ribs.	Left clavicle and left upper ribs	Oophorectomy. Pain relief. Repeat scan shows progression 5/12.
(2)	Mastectomy 5/12 before study. On androgens because of lymph node metastases.	Left ribs	Scan abnormality disappeared after 6/12 of systemic radiotherapy. Well at 21/12.
(3)	Mastectomy 22/12 before study. Supraclavicular metastases at time of study.	Extensive skull, sternum, spine, ribs	Follow up 2/12 only.
(4)	Mastectomy one year before study. Complaint of back pain, paraesthesia arm.	Thoracic spine	Started on oestrogens. Alive one year later but generalised bone pain.
(5)	Mastectomy 30/12 before study. Pain left ribs.	Left ribs	Pain relieved by DXT 2/12.
(6)	Mastectomy 6 years before. Multiple skin nodules. Liver metastases confirmed on laparotomy 4 yrs before. On androgens for 6 yrs with resulting regression of skin nodules.	Ribs, shoulder, thoracic spine	Remains in remission one year later.

Table IV.13. con't

	Clinical Details	Site of Metastases	Clinical Follow Up
(7)	Breast Cancer. Mastectomy 2 yrs before. Back pain.	Extensive	Lost to follow up. No information available.
(8)	Breast Cancer. Mastectomy 6/12 before. Pain back and right shoulder.	Thoracic spine	Pain persists, follow up 4/12.
(9)	Local recurrence 26/12 after mastectomy.	Left ribs	No evidence of bone metastases. Follow up 3/12.
(10)	Local excision breast cancer. Recurrence three years later.	Lumbar spine	2/12 follow up.
(11)	Mastectomy 2 yrs before. Malignant pleural effusion.	Skull, thoracic, lumbar spine	3/12 follow up.
(12)	Radiotherapy and androgens for locally advanced breast cancer 2 yrs before study. Malignant pleural effusion.	Extensive	3/12 follow up.

Table IV.13. con't

	Clinical Details	Site of Metastases	Clinical Follow Up
(13)	Mastectomy 10/12 before study. Low back pain.	Extensive	Pain relieved by DXT. 5/12 follow up.
(14)	Previous mastectomy. Recurrent nodules in chest wall.	Right and left ribs, pelvis, lumbar spine	2/12 follow up.
(15)	Prostatic cancer. Generalised bone pain.	Extensive metastases	Elevated acid phosphatase 6/12.
(16)	Prostatic cancer. Back pain.	Lower dorsal	Deterioration. Alive but bed ridden 8/12. Acid phosphatase elevated.
(17)	Lung Cancer. Back pain.	Pelvis	Lost to follow up.
(18)	Cancer of bladder. Back pain.	Dorsal spine	For radiotherapy. 2/12 follow up.
(19)	Lung cancer. Back pain.	Dorsal spine	For radiotherapy. 2/12 follow up.

Table IV.14. Clinical Details of X-Ray -ve Scan +ve Patients Well at Time of Study who Subsequently Developed Symptoms

	Clinical Details	Site of Metastases	Clinical Follow Up
(1)	On oestrogens because of Stage III breast cancer with axillary vein thrombosis. Clinically well at time of study.	Right and left ribs	16/12 after positive scan developed pain and ulceration of primary tumour.
(2)	Stage II breast cancer at time of study.	Extensive	Clinically well 10/12. Terminal condition with generalised bone pain 18/12.
(3)	Stage I breast cancer at time of study.	Extensive	Severe leukcerythroblastic anaemia 2/12.
(4)	Lung cancer. Scanned before pneumonectomy.	Left ribs	Painful local recurrence in left chest wall 8/12.

Table IV.15. Clinical Details of X-Ray -ve, Scan Suspicious Patients

	Clinical Details	Site of Metastases	Clinical Follow Up
(1)	Stage III Primary Breast Cancer. No symptoms of bone disease.	Suspicious skull	Scan and X-Ray +ve 7/12.
(2)	Scanned 5/12 after mastectomy. Clinically well.	Multiple suspicious areas spine	Local recurrence 4/12. Generalised bone pain. X-Ray +ve 7/12 after scan.
(3)	Mastectomy 1 yr before. Abdominal pain. Probable liver metastases.	Suspicious skull and spine	Chemotherapy. Abnormalities not seen on scan 12/12 later. Clinically well at that time.
(4)	Time of mastectomy, Stage II breast cancer.	Suspicious cervical and thoracic spine	3/12 follow up well.
(5)	Lung Cancer. Back pain.	Suspicious cervical and lumbar spine	Lytic area 8/12 later on X-Ray cervical spine.
(6)	Prostatic Cancer. No symptoms of bone metastases. On oestrogens.	Generalised high uptake. ? significance	Well 3/12 follow up.
(7)	Lung Cancer. Backache. Previous right middle and lower lobectomy.	Suspicious lumbar spine	Died 1/52, P.M. +ve L.V.2, 3, 5.
(8)	Lung Cancer. Backahce. Previous lobectomy.	Suspicious thoracic spine	Follow up 3/12, backache persists.

Table V.1. Primary Breast Cancer Study.

Results of Bone Scans

		Clinical Stage			
	Total	I	II	III	IV
Scan Positive	24	6	10	3	5
Scan Suspicious	2	1	0	1	0
Scan initially Negative → Positive	9	6	2	1	0
Scan Persistently Negative	70	46	17	7	0
TOTAL	105	59	29	12	5

Table V.2. Primary Breast Cancer Study.

Follow Up of Patients

(All Clinical Stages)

	Total	Dead	Alive X-Ray Positive Metastases	Alive Clinical Evidence Bone Metastases but X-Ray Negative	Alive No Clinical Evidence of Bone Metastases
Scan Positive	24	7 (3*, 1+)	7	4 (1+)	6
Scan Suspicious	2	1*	0	0	1
Scan Negative initially but later Scan Positive	9	2 (1*, 1+)	2	2	3
Scan Persistently Negative	70	2	0	0	68
TOTAL	105	12	9	6	78

* X-Ray positive before death. + X-Ray negative but biopsy positive.

Table V.3. Primary Breast Cancer Study.

Follow Up of Patients Presenting

with Stage I and II Disease

	Total	Dead	Alive X-Ray Positive Metastases	Alive Clinical Evidence Bone Metastases but X-Ray Negative	Alive No Clinical Evidence of Bone Metastases
Scan Positive	16	3 (1*)	5	3 (1+)	5
Scan Suspicicus	1	0	0	0	1
Scan Negative initially but later Scan Positive	8	2 (1*, 1+)	2	1+	3
Scan Persistently Negative	63	2	0	0	61
TOTAL	88	7	7	4	70

* X-Ray positive before death. + X-Ray negative but biopsy positive.

Table V.4. Primary Breast Cancer Study.
Summary of Clinical Follow Up
To Date in Patients Presenting
with Stage I and Stage II
Disease

	Total	Dead	Total With Proven Bone Metastases	Mean Follow Up (months)
Scan Positive	16	3	7	8.2
Scan Suspicious	1	0	0	11.0
Scan Negative initially but later Scan Positive	8	2	4	14.4
Scan persistently Negative	63	2	0	11.6
TOTAL	88	7	11	

Table VI.1. Some Examples of the Research Applications of Bone Scanning in Malignant Disease

(1) Assessment and selection of patients for clinical trials of treatment of primary cancer.

(2) Accurate, non-invasive method for the study of the progression of occult malignant disease.

(3) Earlier identification of patients with metastases for clinical trials of systemic treatment.

(4) Rationalisation of systemic therapy by providing a quantitative index of response.

(5) Combination of bone and bone marrow scanning in haematological and other malignancies.

(6) Development of therapeutic agents based on the available diagnostic agents.

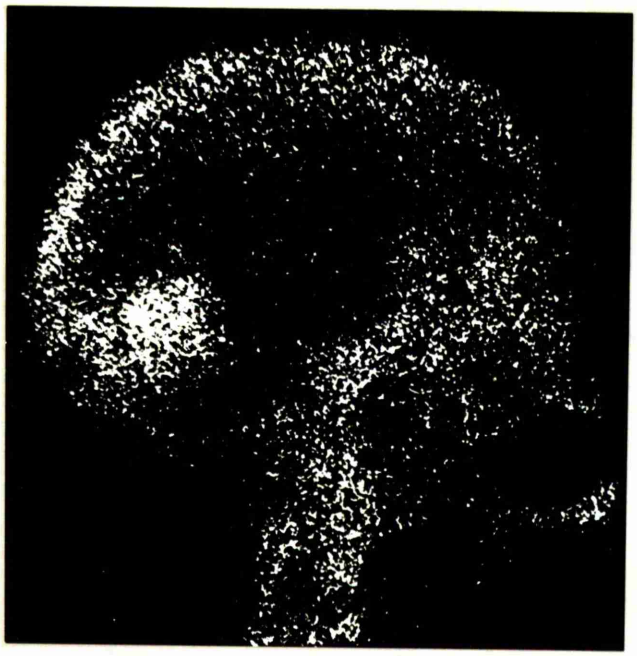


Figure I.1. **Positive Bone Scan**

Gamma camera right lateral skull view in a 58 year old woman with a T_3N_1 primary breast cancer. Metastasis in occipital bone is seen as an area of increased activity ("hot spot").

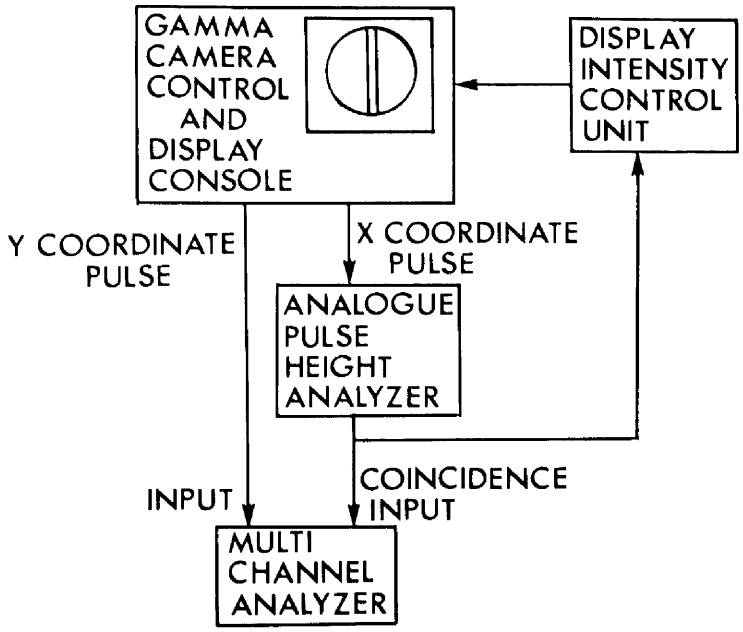


Figure II.1.

Circuit used to quantitate bone scans.

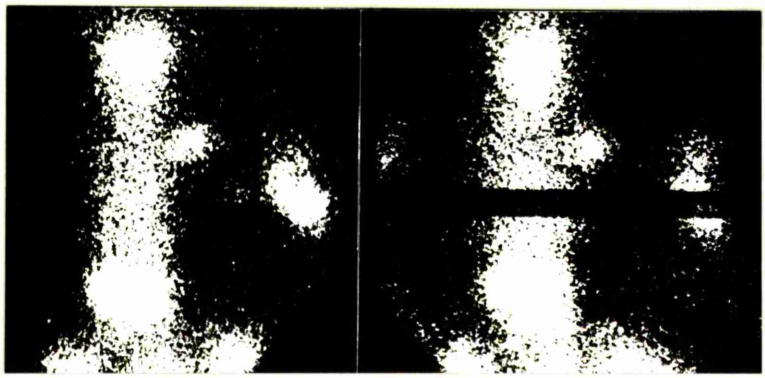
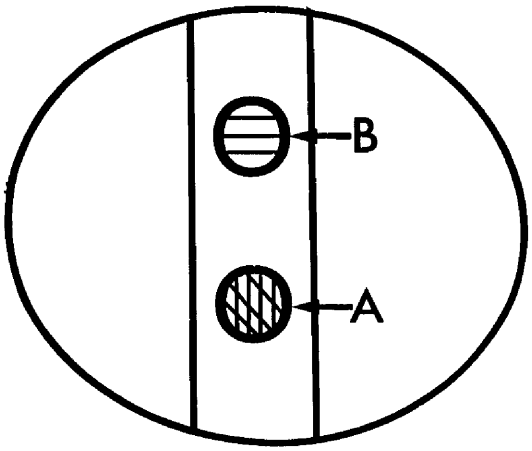


Figure II.2. Region of Interest Profile

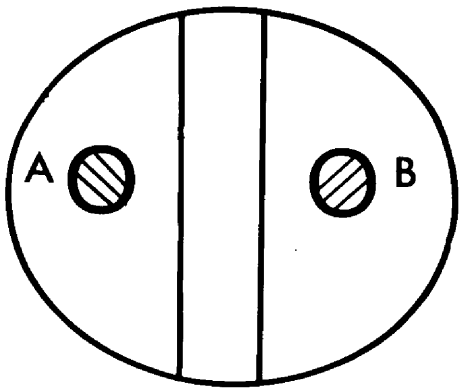
Gamma camera scan of thoracic spine in a patient with multiple bone metastases. Region of interest profile is seen as band of diminished intensity. Area under study here is right rib metastases.



$$\frac{\text{Activity in A}}{\text{Activity in B}} = \text{Tumour : Bone ratio}$$

Figure II.3. Tumour:Bone Ratio for Spinal Tumours

Large Circle represents the field of view of the gamma camera. The central area enclosed by the two vertical lines represents the spine. A and B represent areas of tumour-involved and corresponding normal bone, respectively.



$$\frac{\text{Activity in A}}{\text{Activity in B}} = \text{Tumour: Bone ratio}$$

Figure II.4. Tumour:Bone Ratio for Non-spinal Tumours

Large Circle represents the field of view of the gamma camera. The central area enclosed by the two vertical lines represents the spine. A and B represent areas of tumour-involved and corresponding normal bone, respectively.

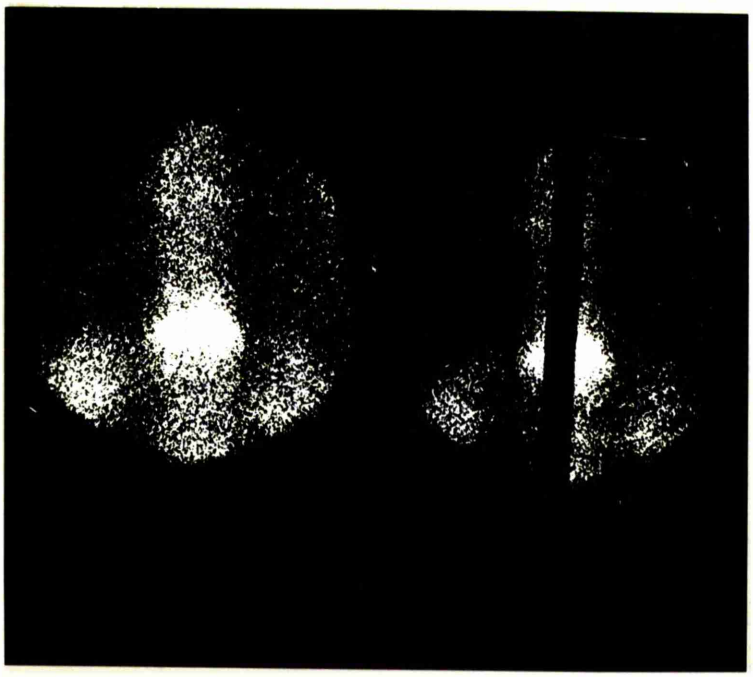
Figure II.5. Calculation of Tumour:Bone Ratio

(a) Lower thoracic and lumbar spine of a 45 year old woman who had a right mastectomy 5 years before study. Obvious metastasis T12. Region of interest Profile is seen.

(b) Digital print out from Multi Channel Analyser. Counts corresponding to tumour underlined in black. Counts from normal bone area underlined in red.

$$\text{Calculated tumour:bone ratio} = \frac{4423}{2432}$$

$$= 1.82$$



(a)

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(b)

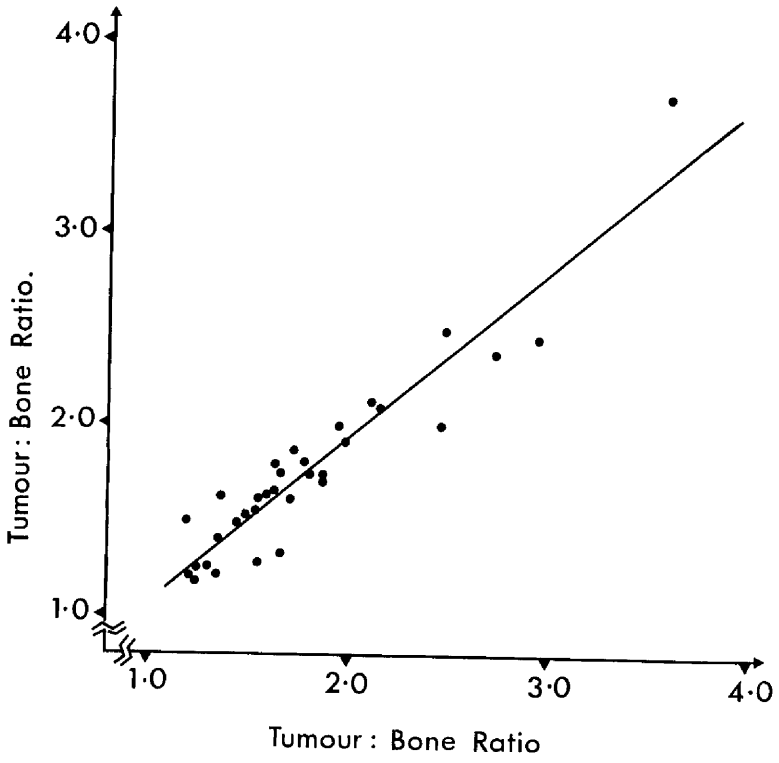


Figure II.6.

Correlation between duplicate results of tumour:bone ratios.

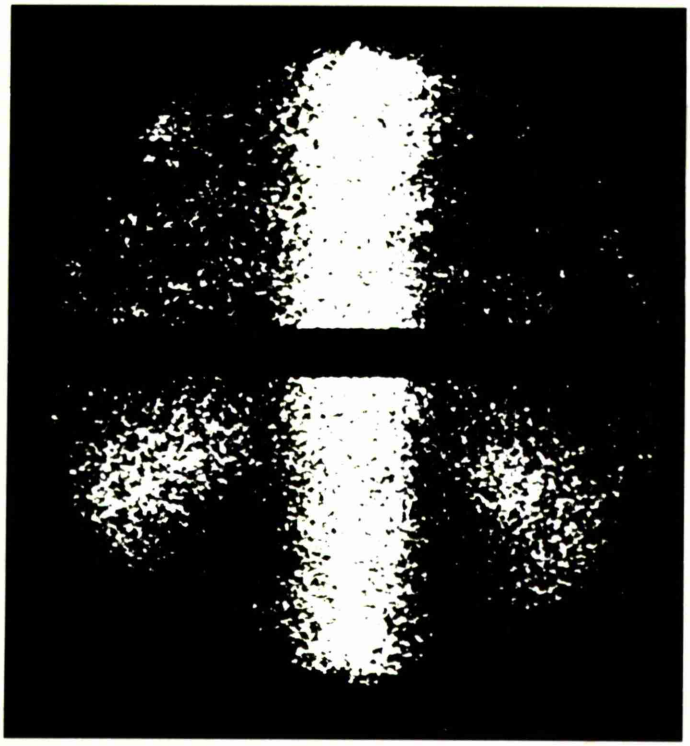


Figure II.7. **Region of Interest for Spine:background Ratio**

Horizontal region of interest across lower dorsal spine and surrounding tissues.

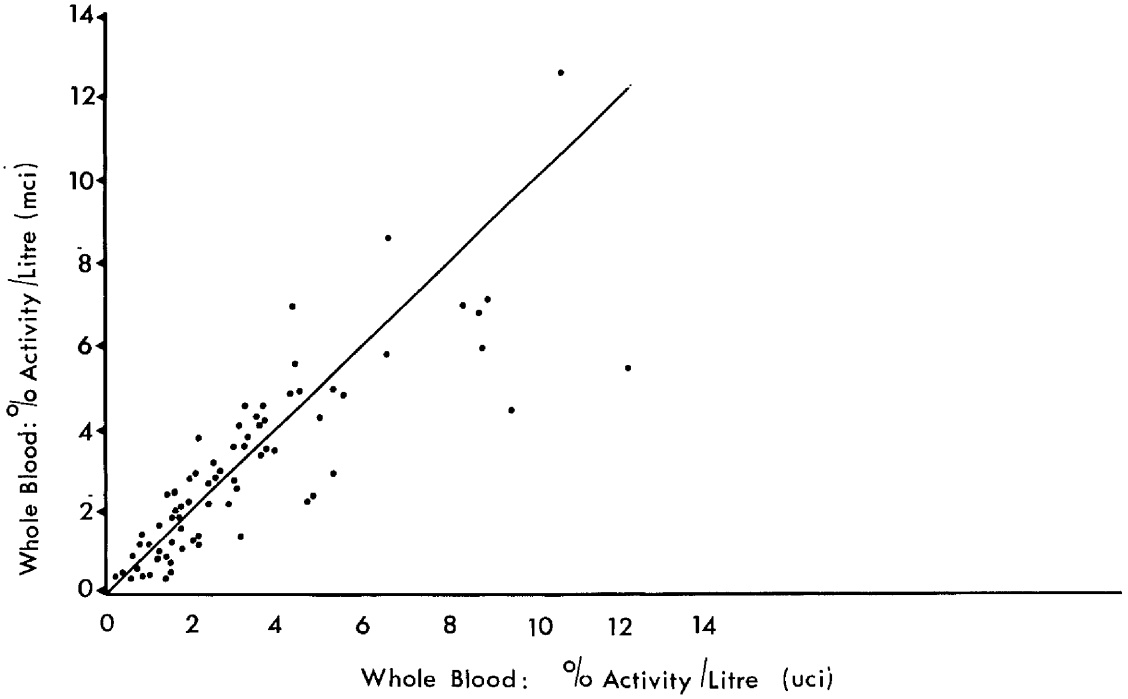


Figure II.8.

Correlation of whole blood levels of mCi and uCi activities.

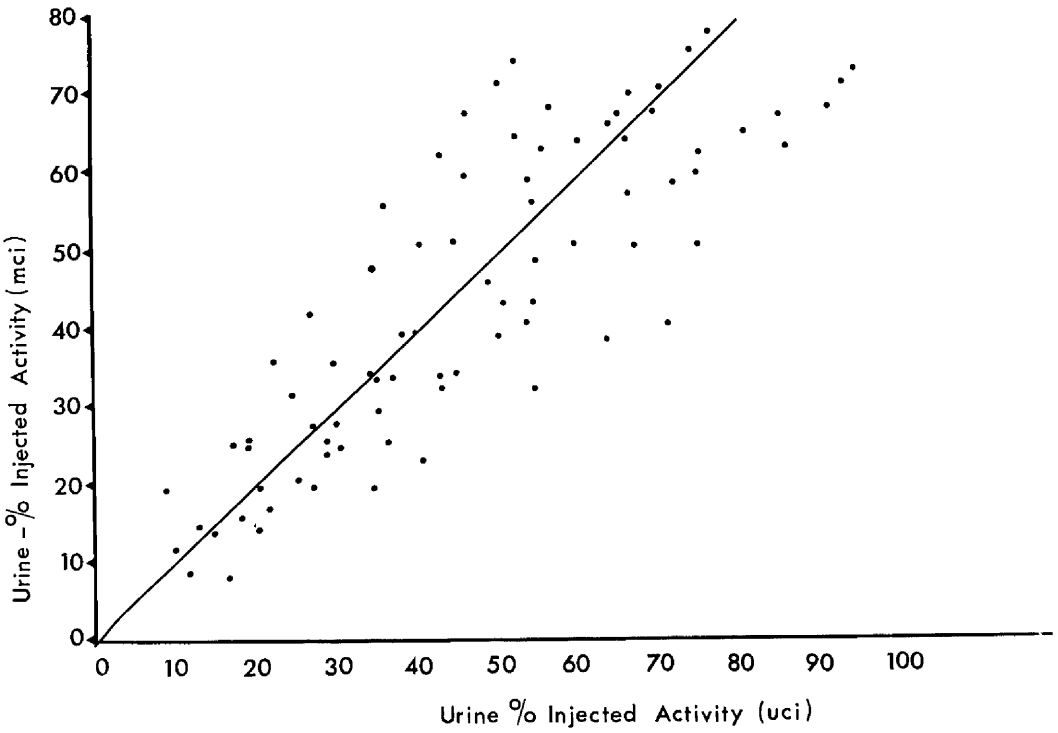


Figure II.9.

Correlation of urinary levels of mCi and uCi activities.

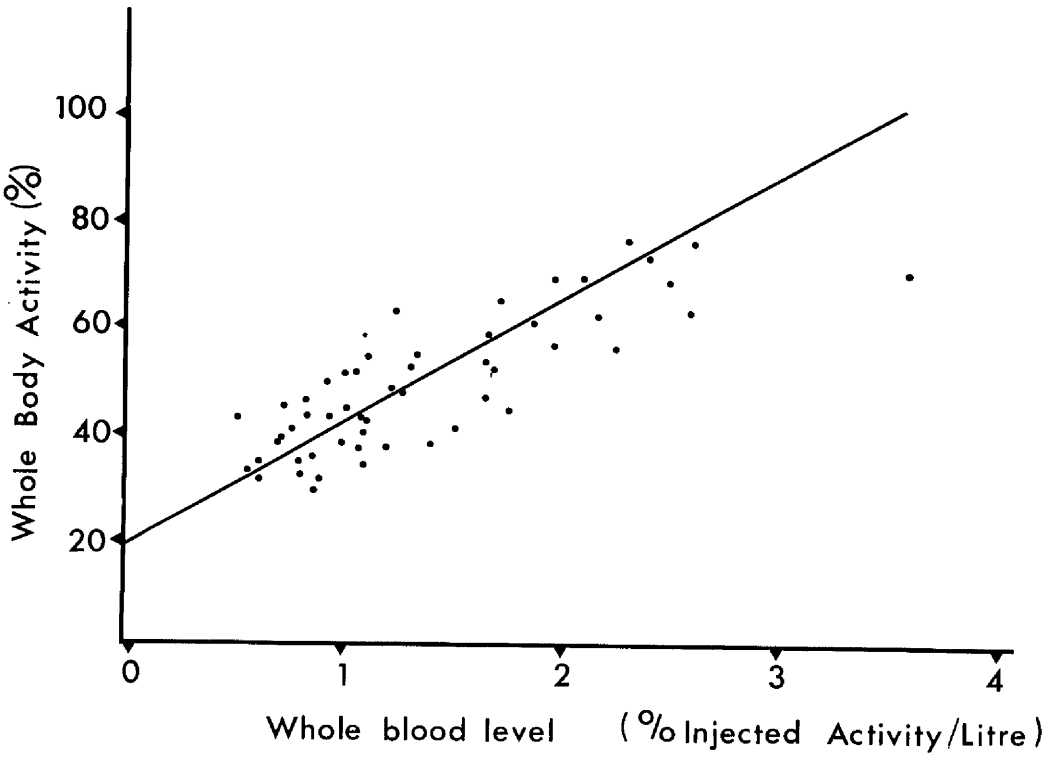


Figure II.10.

Correlation of whole body activity and blood levels of E.H.D.P.

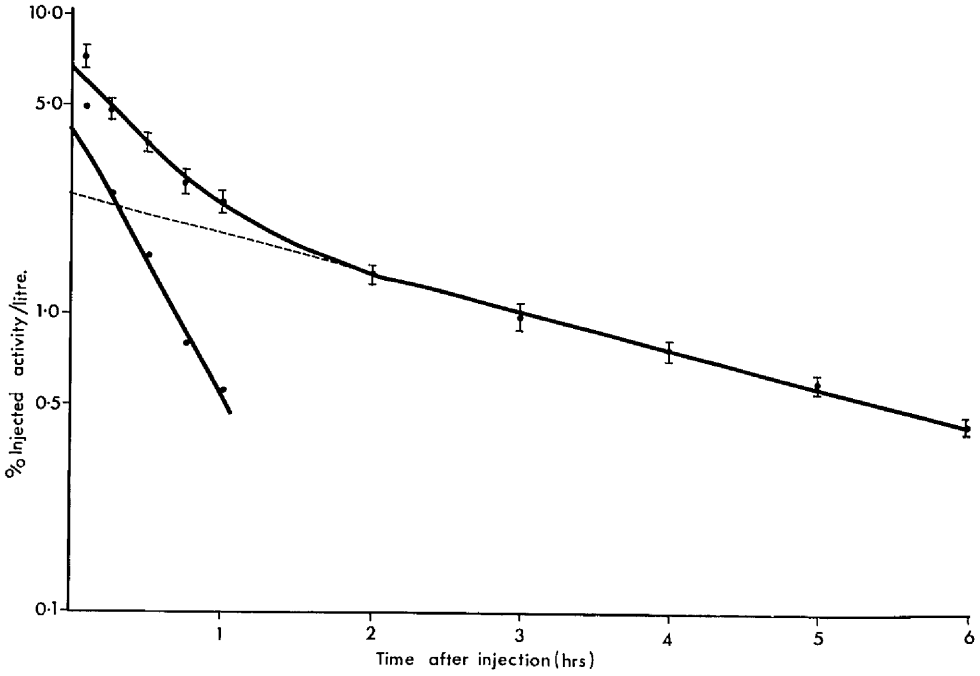


Figure III.1.

Blood clearance of ^{99m}Tc-E.H.D.P.

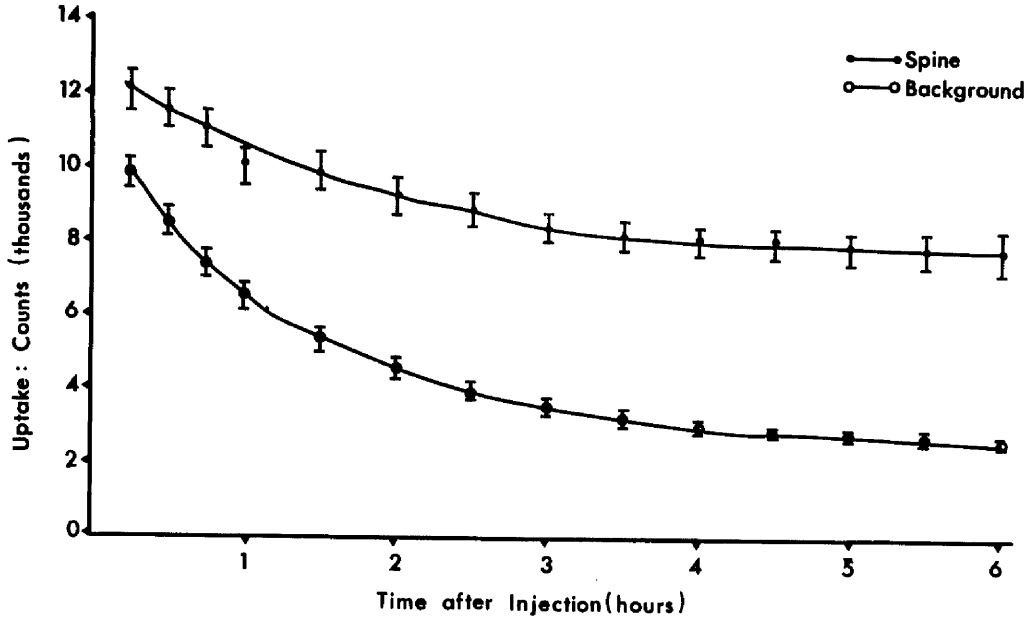


Figure III.2.

Change in gross activity in spine and background area with time.

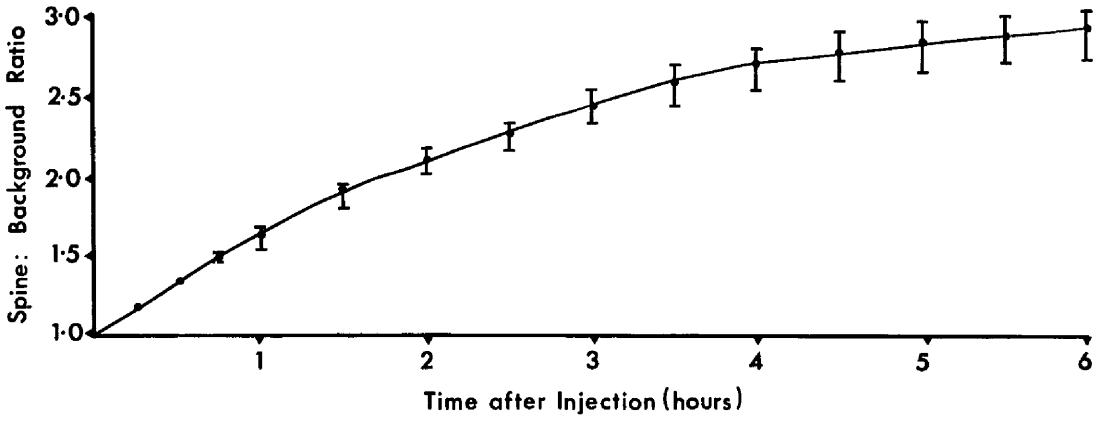


Figure III.3.

Rise in spine:background ratios with time.

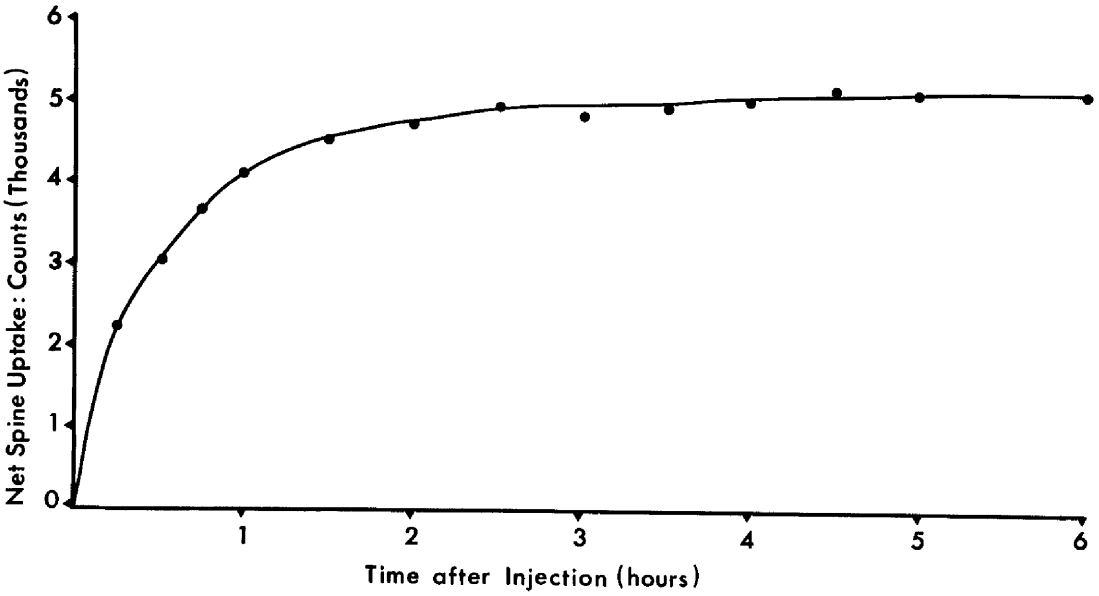


Figure III.4.

Serial net spine uptake of ^{99m}Tc-E.H.D.P.

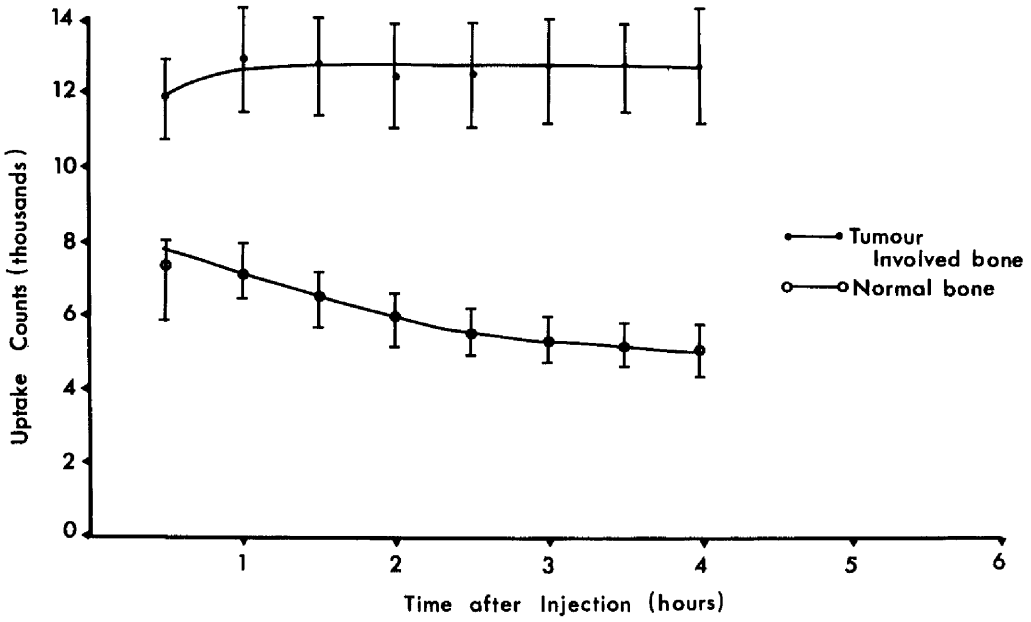


Figure III.5.

Change in gross activity in tumour-involved and normal bone with time.

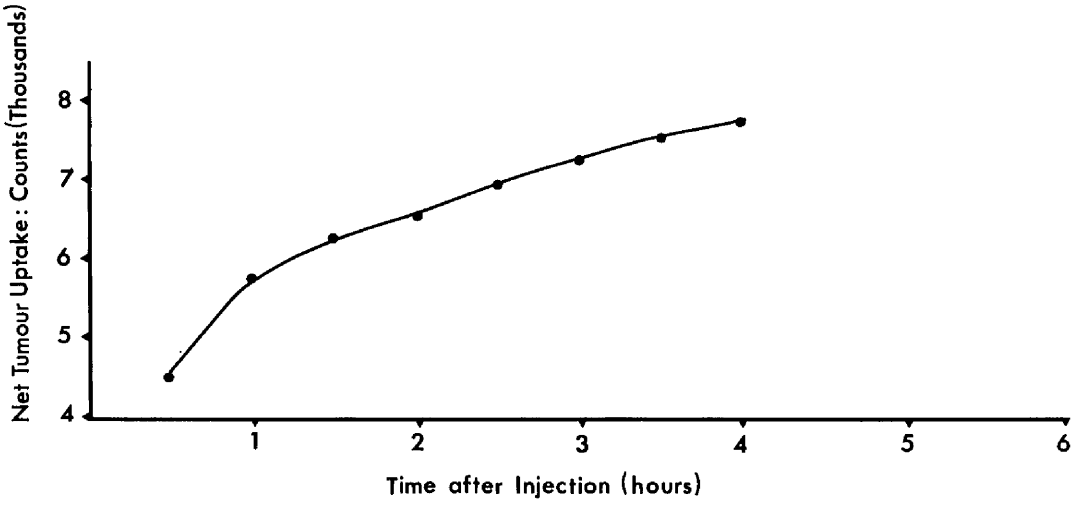


Figure III.6.

Serial net tumour uptake of ^{99m}Tc-E.H.D.P.

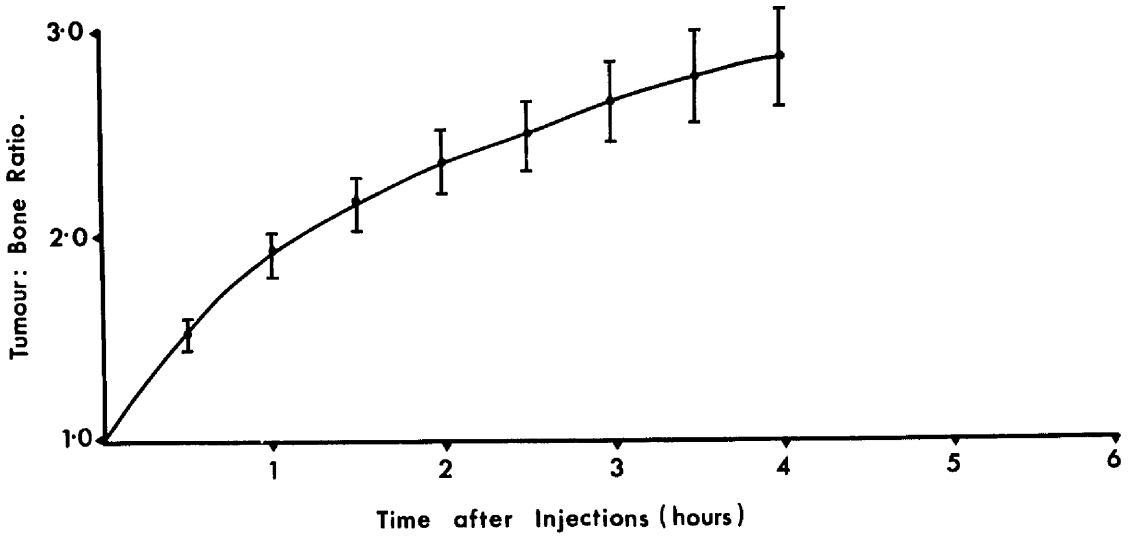


Figure III.7.

Rise in tumour:bone ratios with time.

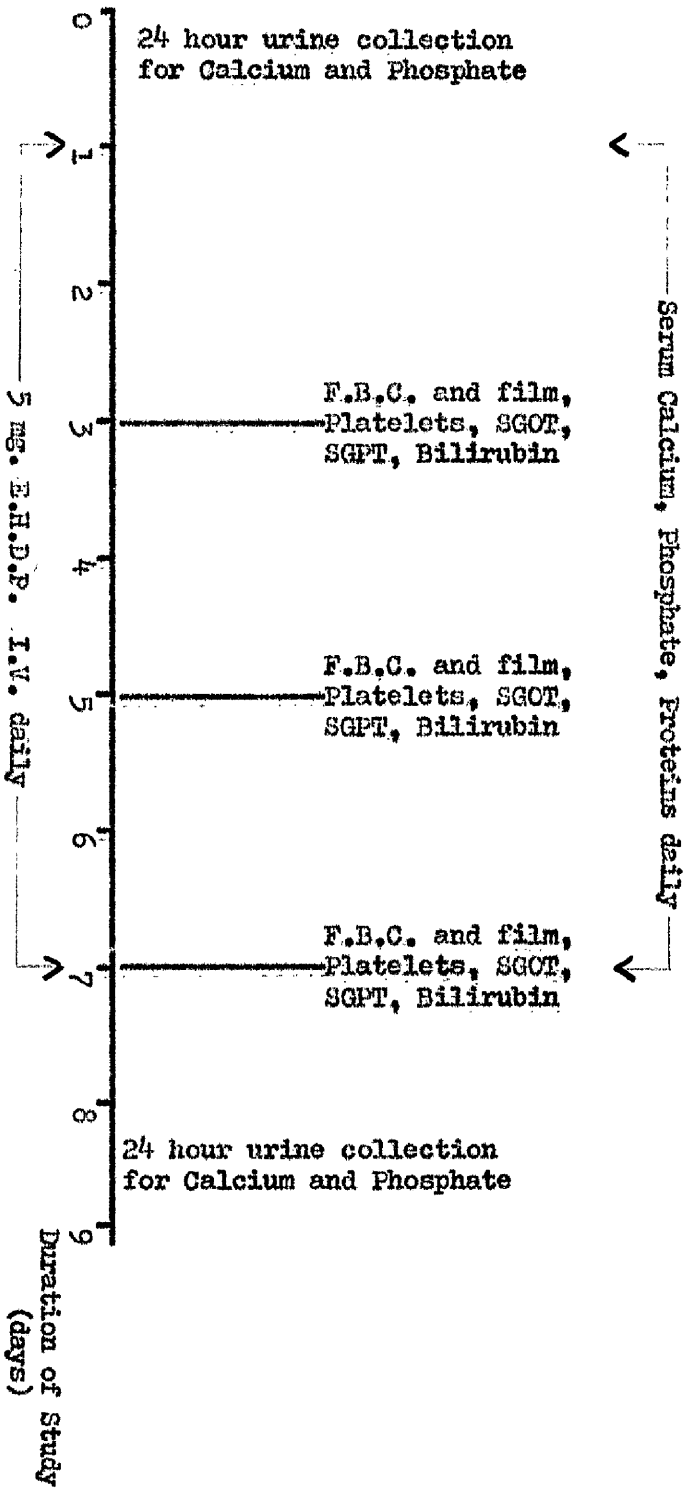
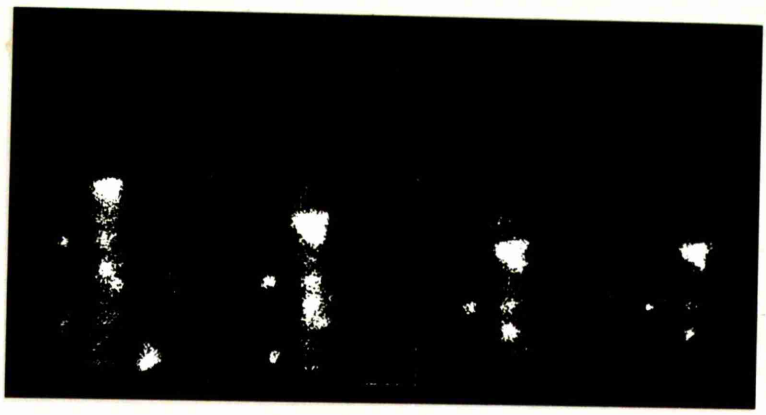


Figure III.8. Design of Study of Cumulative Toxicity of ^{99m}Tc E.H.D.P.



60 90 120 210

Figure III.9. **Serial Visualisation of Bone Tumours with ^{99m}Tc-E.H.D.P.**

Serial images at 60, 90, 120 and 210 minutes of the thoracic spine of a 45 year old woman with metastatic breast cancer. Note improving contrast between tumour-involved and surrounding bone.

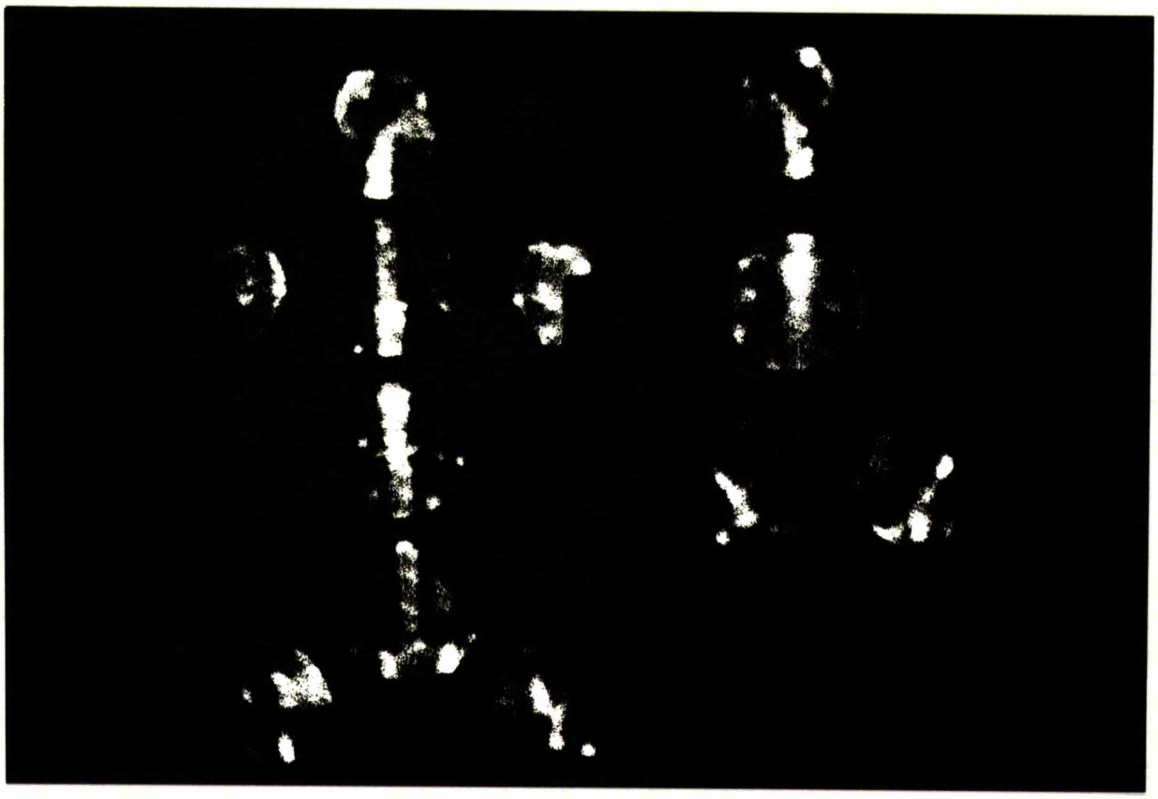
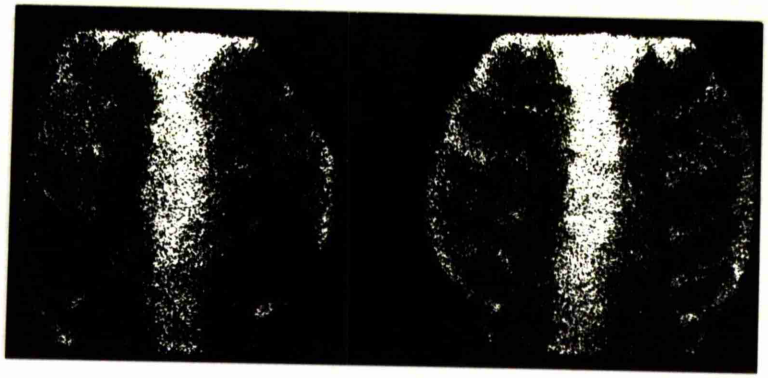


Figure IV.1. **Gamma Camera Bone Scan**

Anterior and posterior overlapping views of the proximal skeleton of a 45 year old woman who had a mastectomy 18 years previously. Widespread bony metastases are evident.



(a) (b)

Figure IV.2. Artefact due to breast prosthesis

Anterior views of sternum and ribs in a woman who had previously undergone bilateral mastectomy, and wore a special padded brassiere. Details of lower ribs are obscured (a). After removal of the prostheses a normal scan is obtained (b).

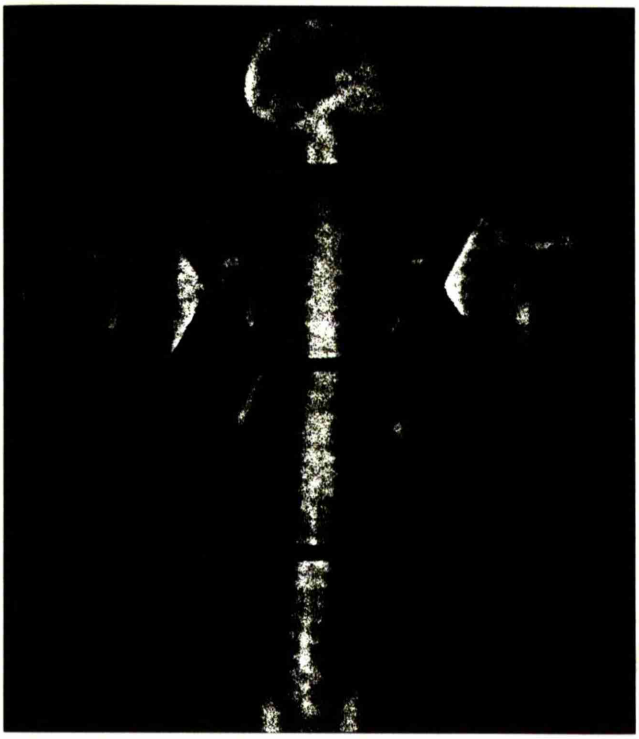


Figure IV.3. Normal Posterior Bone Scan

Multiple overlapping views on gamma camera. Especially prominent are the spines and angles of the scapula, the spinous processes of the lumbar vertebrae and the sacro-iliac joints.

In the vault of the skull the region of the frontal suture is often clearly seen. The transverse sinus is also visible.

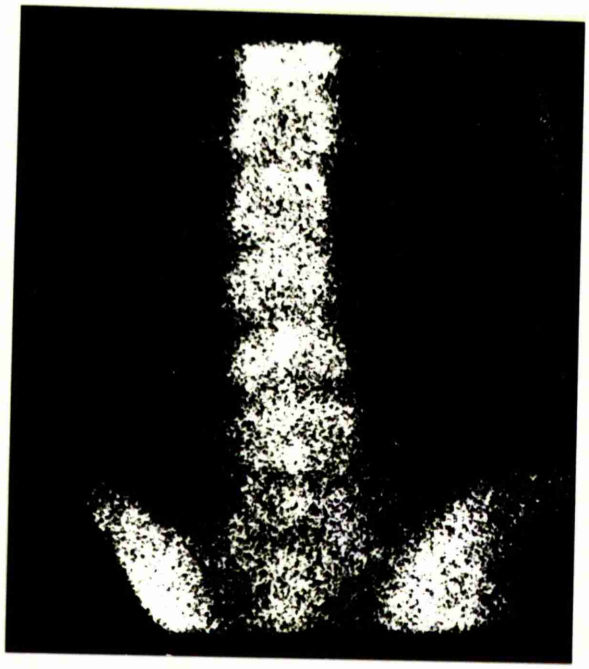


Figure IV.4. Bone Scan of Lumbar Area in Renal Failure

There is high uptake of radiopharmaceutical in bone, because of renal osteodystrophy. Renal images are not seen.

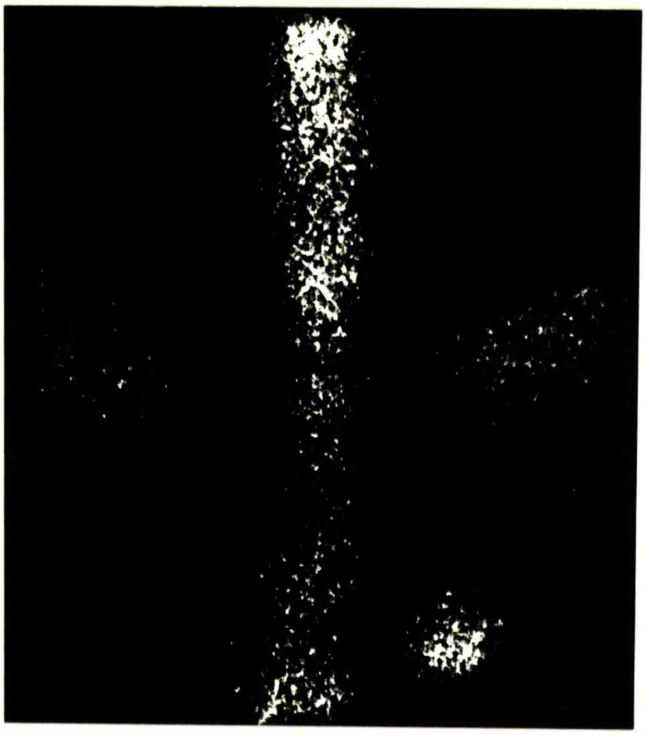
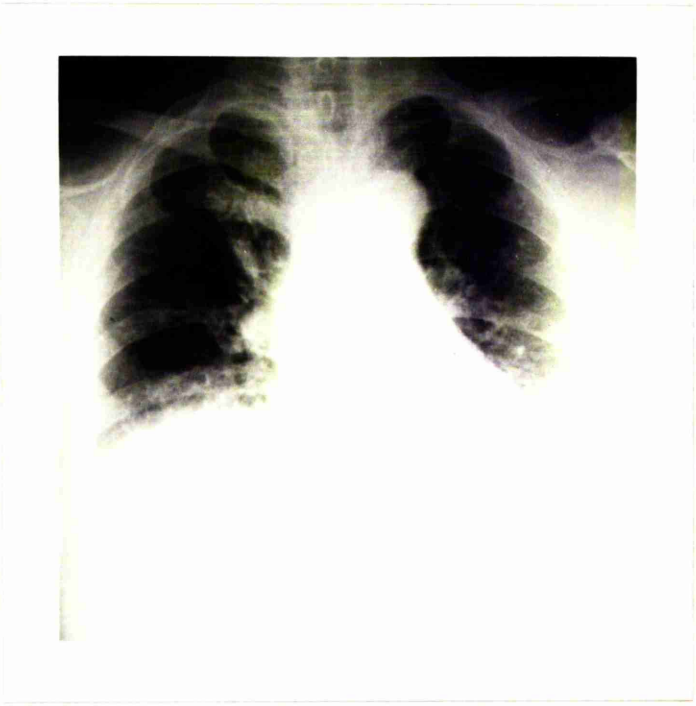


Figure IV.5. Concentration of ^{99m}Tc E.H.D.P. in the Breast

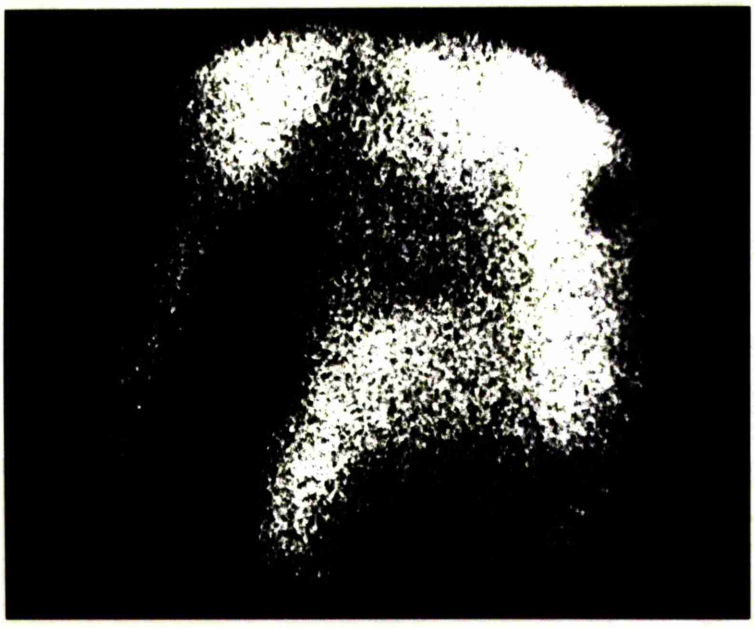
Anterior view of sternum and ribs in a 31 year old woman. Marked symmetrical accumulation of E.H.D.P. is clearly seen in the breasts. Activity in the lower part of the image is in the lumbar spine and upper pole of the left kidney.

Figure IV.6. Concentration of ^{99m}Tc E.H.D.P. in a pleural effusion

(a) Chest radiograph and (b) anterior gamma camera view of the right side of chest in a 61 year old woman with right pleural effusion due to metastatic carcinoma of breast. Activity in right pleural effusion is clearly seen.



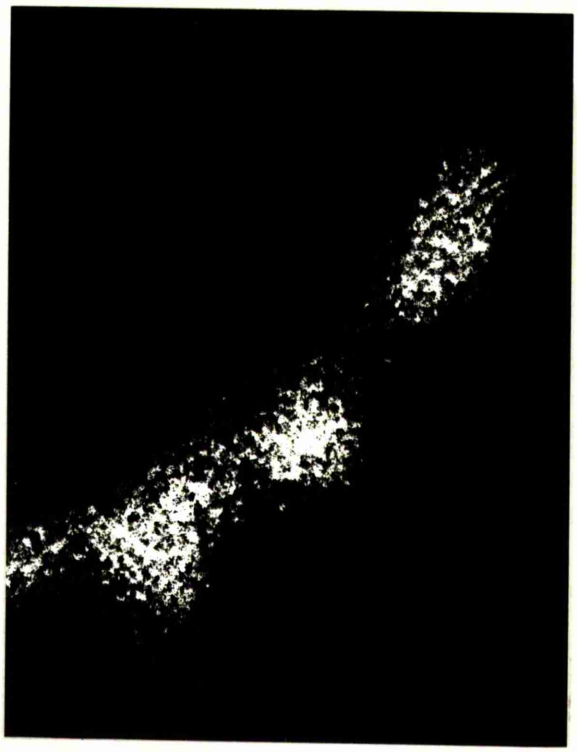
(a)



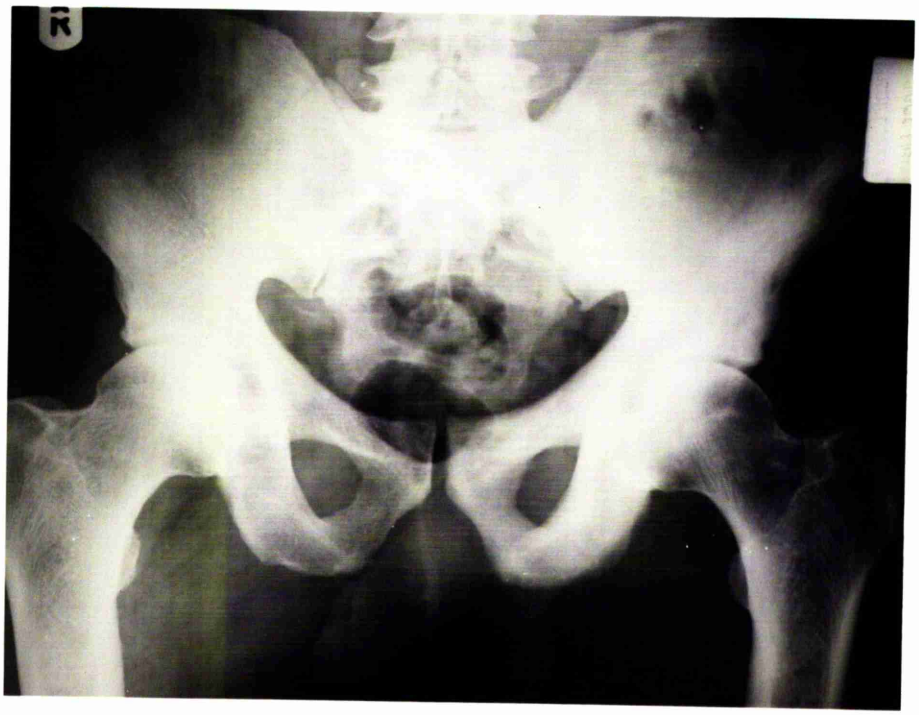
(b)

Figure IV.7. Paget's disease of the pelvis

Diffuse involvement of the left side of the pelvis is seen on the gamma camera scan (a) and the radiograph (b).



(a)

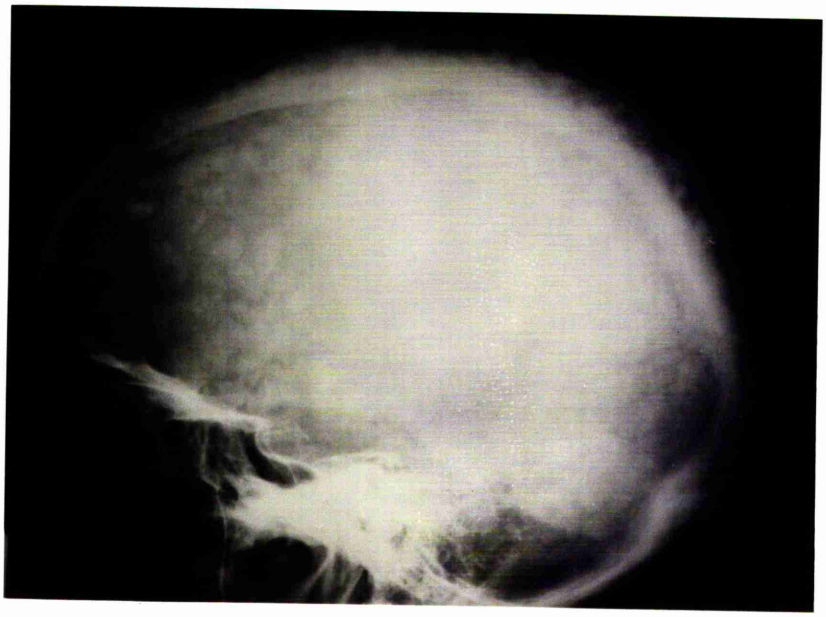


(b)

Figure IV.8. Paget's disease of the skull

Diffuse involvement of the skull vault is seen on the scan (a) and the radiograph (b).

(a)

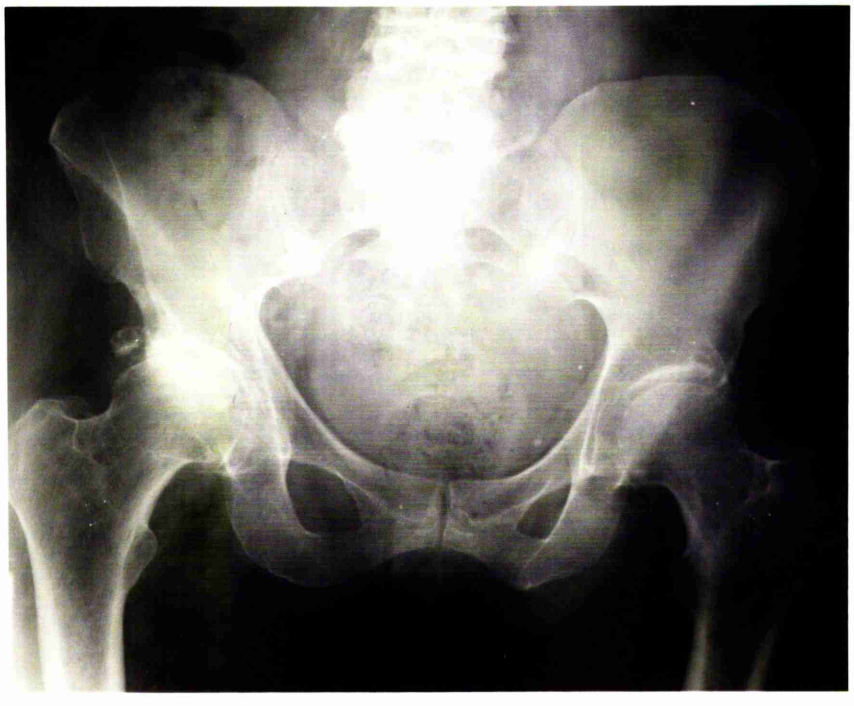


(b)

Figure IV.9. Osteoarthritis of the hips

Gamma camera anterior composite view of pelvis (a). Increased activity is seen in the area of the right hip joint. Radiograph shows osteoarthritis with new bone formation (b).

(a)



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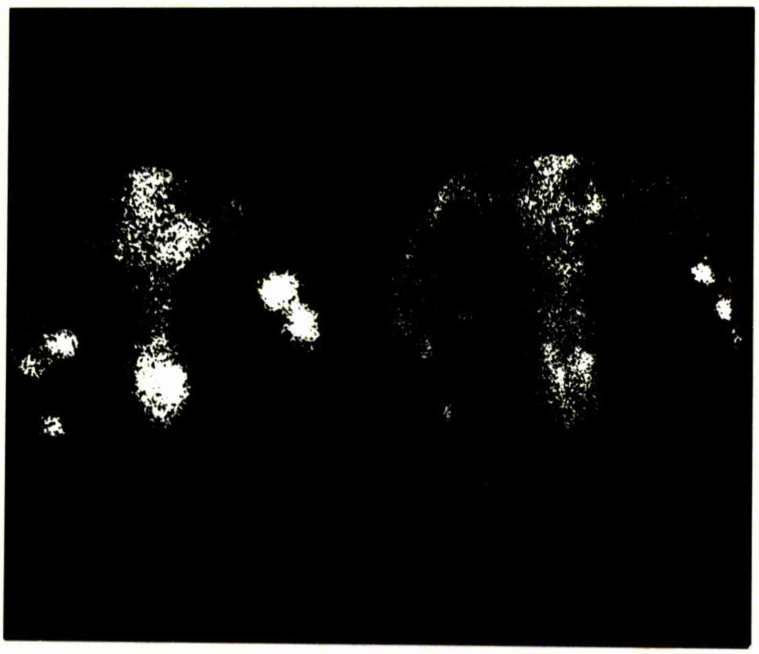
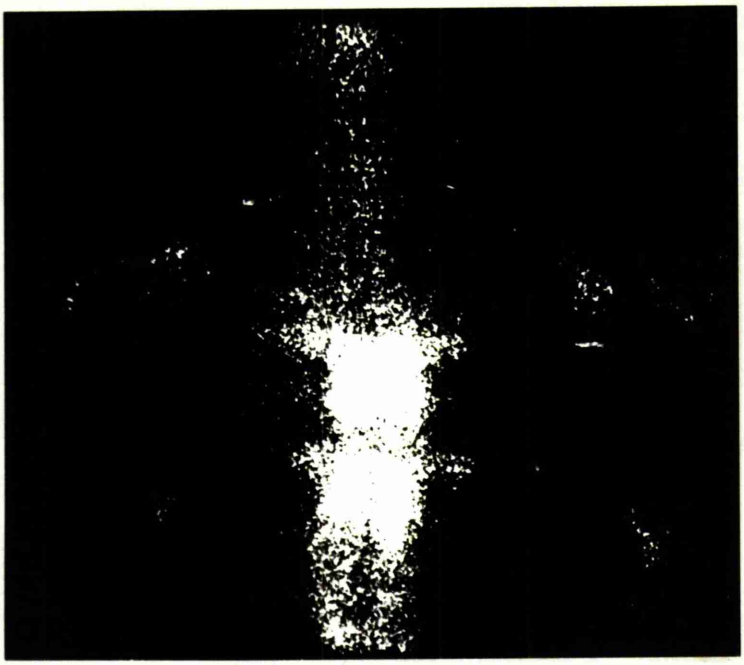


(a) (b)

Figure IV.10. Trauma to bone

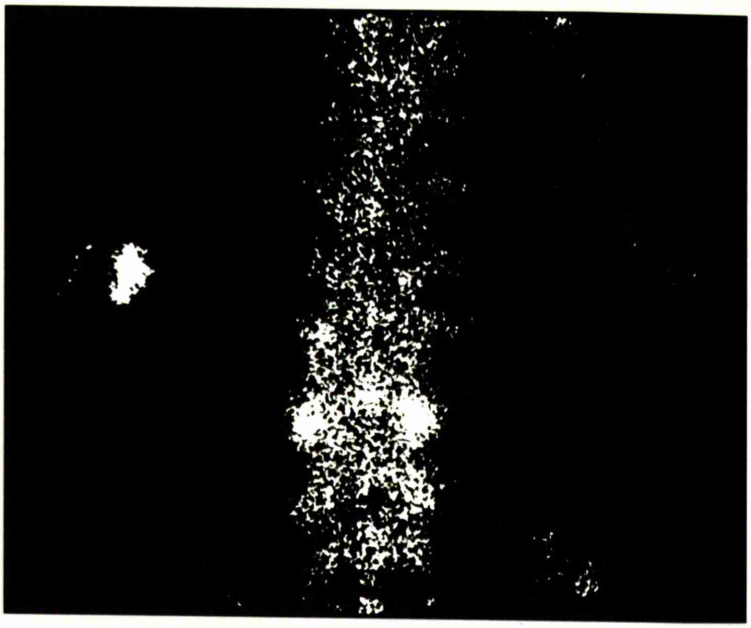
Serial bone scans in a 64 year old woman who was resuscitated with external cardiac massage after an anaphylactic reaction to penicillin. (a) Multiple lesions in ribs and sternum. (b) Repeat bone scan 3 months later shows reduction in activity consistent with healing. Radiographs obtained at time of second scan showed evidence of healing fractures.



(a)

Figure IV.11. Comparison of Paget's disease, Metastases and Osteoporotic Collapse of the spine

(a) Paget's disease: uniform involvement of the vertebral bodies without loss of height. (b) Metastases: involvement of the pedicles. There is also a metastasis in left rib. (c) Osteoporotic collapse: uniform involvement of vertebral bodies with marked loss of height (bone scan equivalent of "codfish vertebrae").



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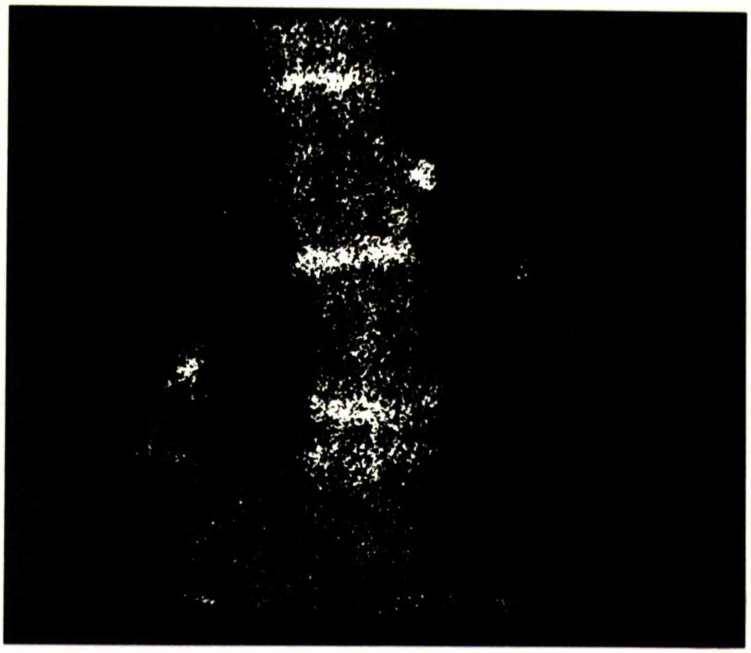
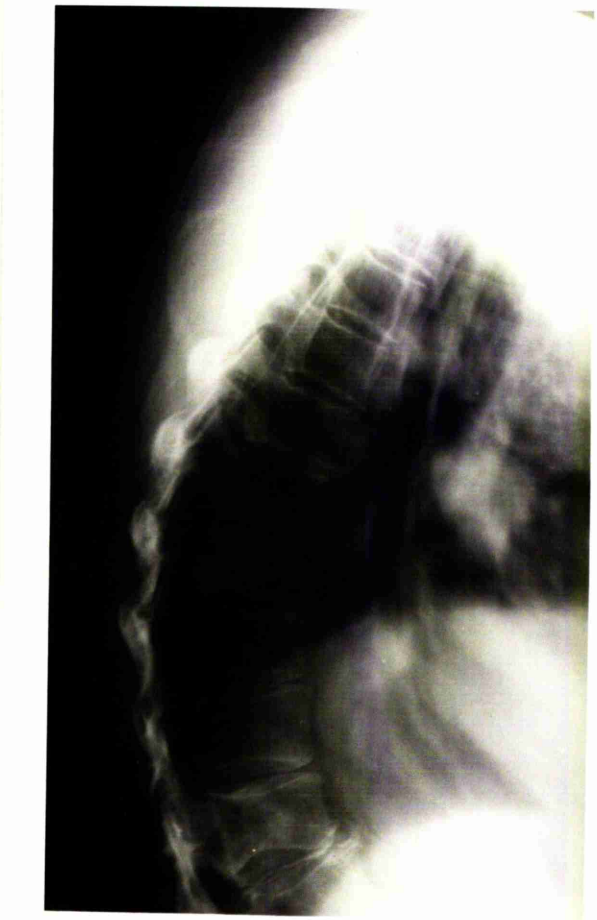


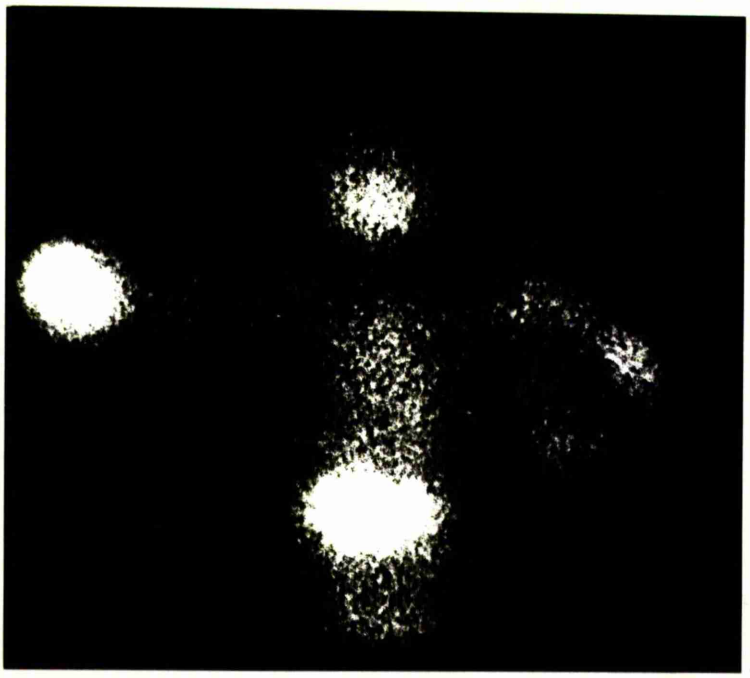
(c)

Figure IV.12. X-Ray Positive Metastases: Comparison of X-Ray and Scan

A 77 year old woman presented 6 years after mastectomy with abdominal pain and hepatomegaly (liver metastases).
(a) Radiograph of dorsal spine showed partial vertebral collapse due to metastases which were confirmed by bone scan (b). Radiograph of the skull (c) was normal but right lateral gamma camera scan showed 3 distinct metastatic deposits in the vault of the skull (d). Uptake by the tumour-involved bone is so great that activity has been diverted away from normal skull. Lesions at lower part of scintiscan are in the cervical spine and shoulder.



(a)



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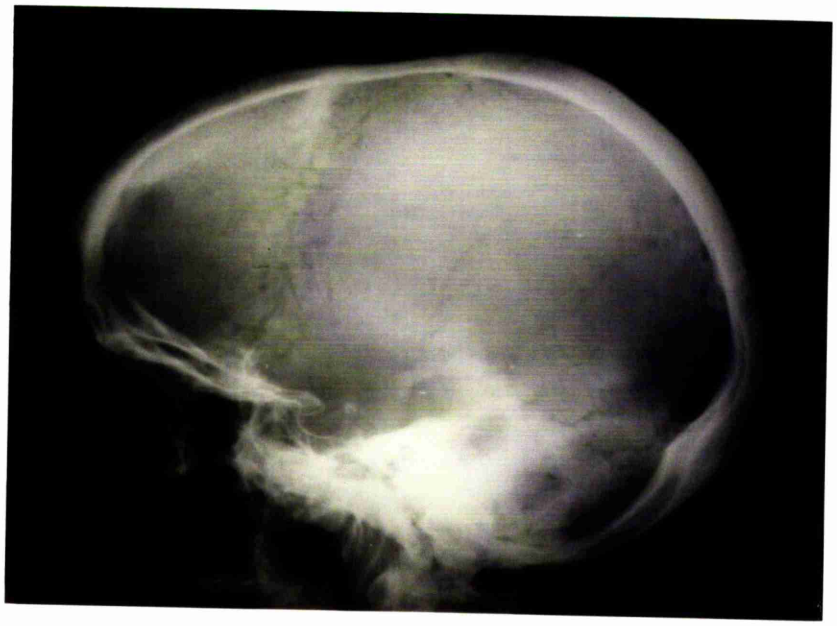
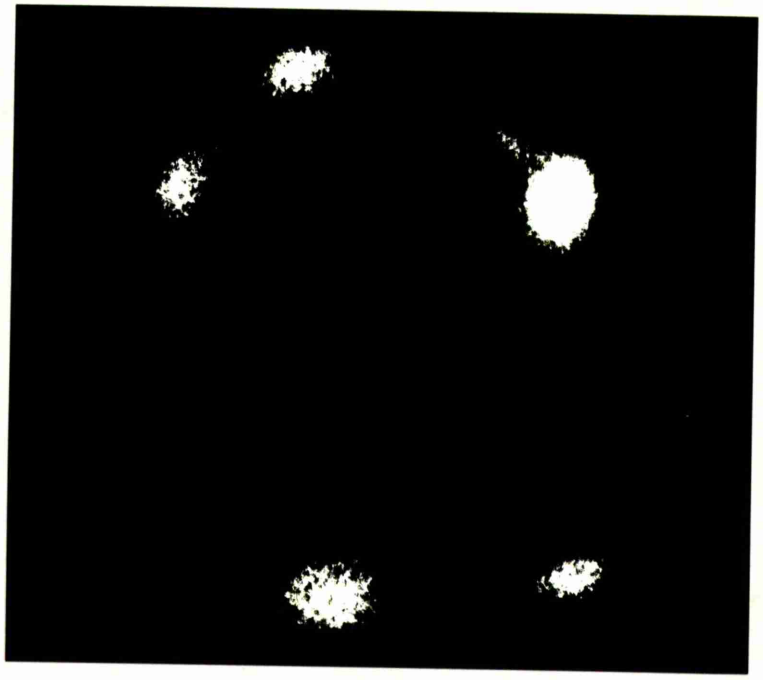


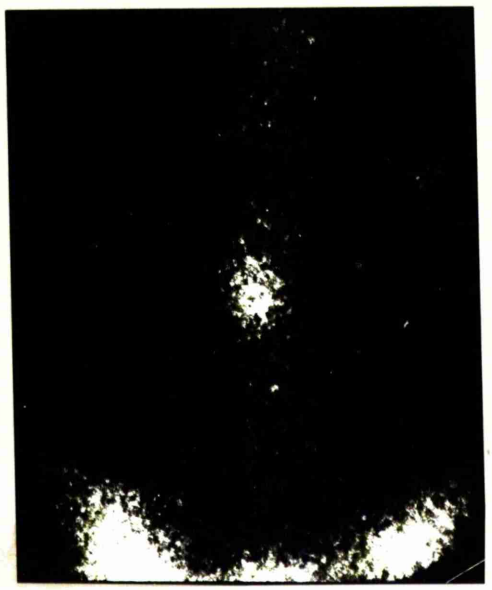
(c)



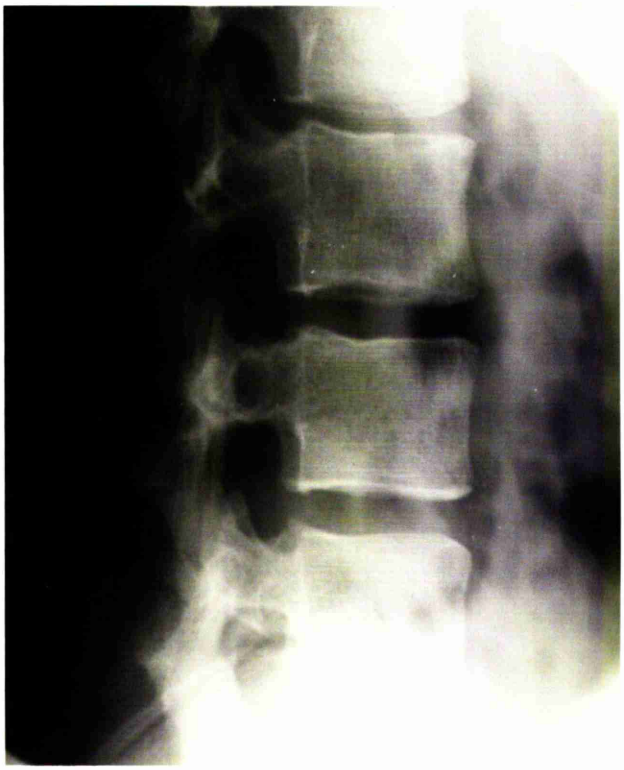
(d)

Figure IV.13. Detection of Occult Metastases from Osteogenic Sarcoma

A 16 year old girl presented with osteogenic sarcoma of the right tibia. Bone scan showed a lesion in the mid lumbar spine (a). There were no symptoms from this deposit and radiograph was normal (b). Six months later she developed back pain, weakness of the legs, and urinary retention. Repeat radiographs at this time showed collapse of L4 (c). Residues of a myelogram are also seen. Because of the lumbar lesion seen on the initial bone scan an amputation of the right leg was avoided.



(a)



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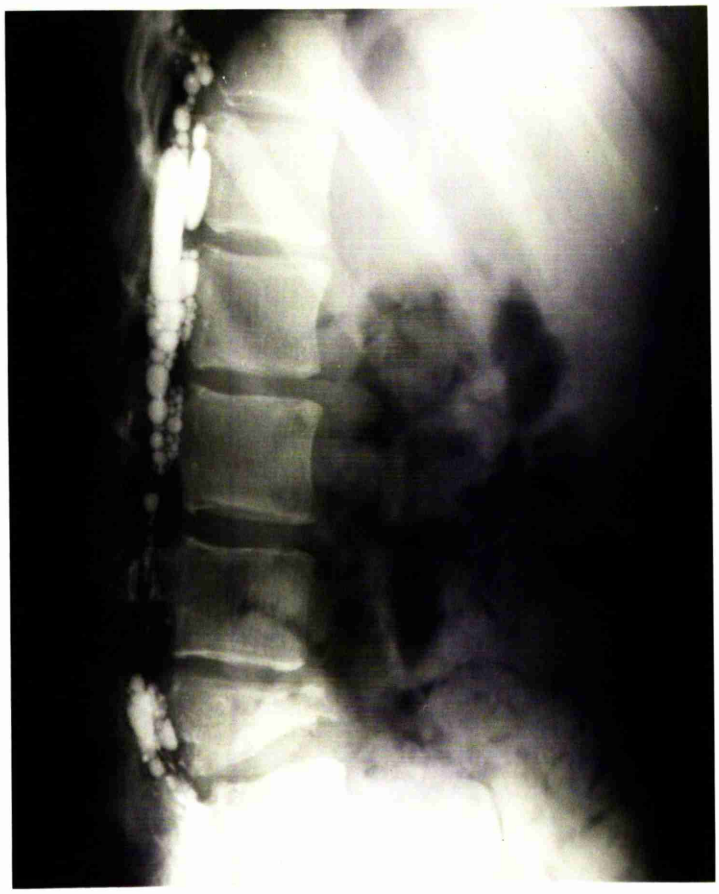

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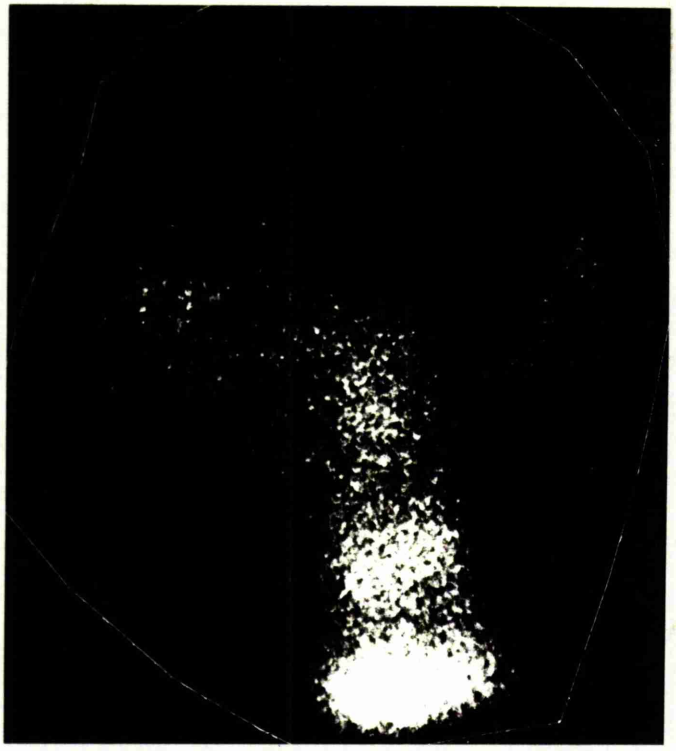
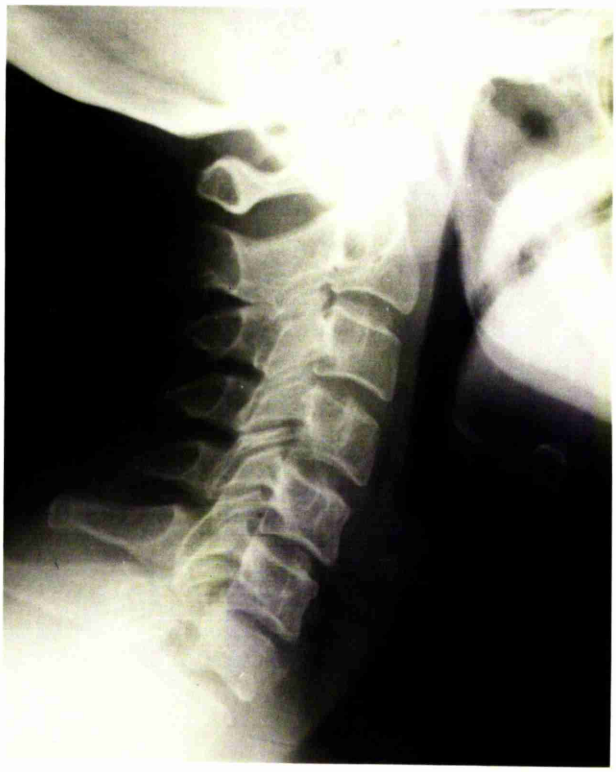


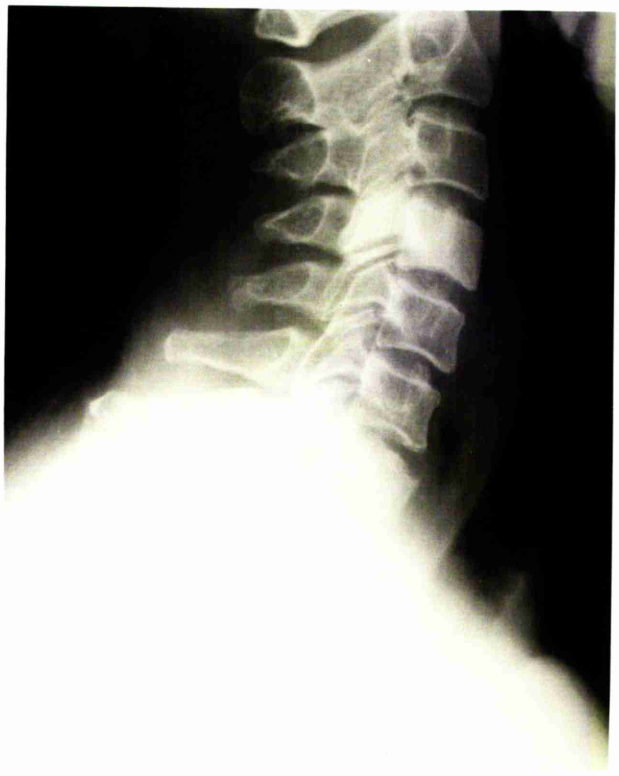
(a)

Figure IV.14. X-Ray Suspicious Patient: Development of Metastases

A 62 year old patient developed cervical pain 3 years after mastectomy. (a) Bone scan showed 2 definite lesions mid and lower cervical spine. At that time the only radiographic abnormality was a suspicion of metastatic involvement of C7 (b). She received radiotherapy to the cervical spine with prompt pain relief. (c) Radiograph 6 months later confirms osteosclerotic metastases C7 and also in C4, which had been previously radiologically normal.



(b)



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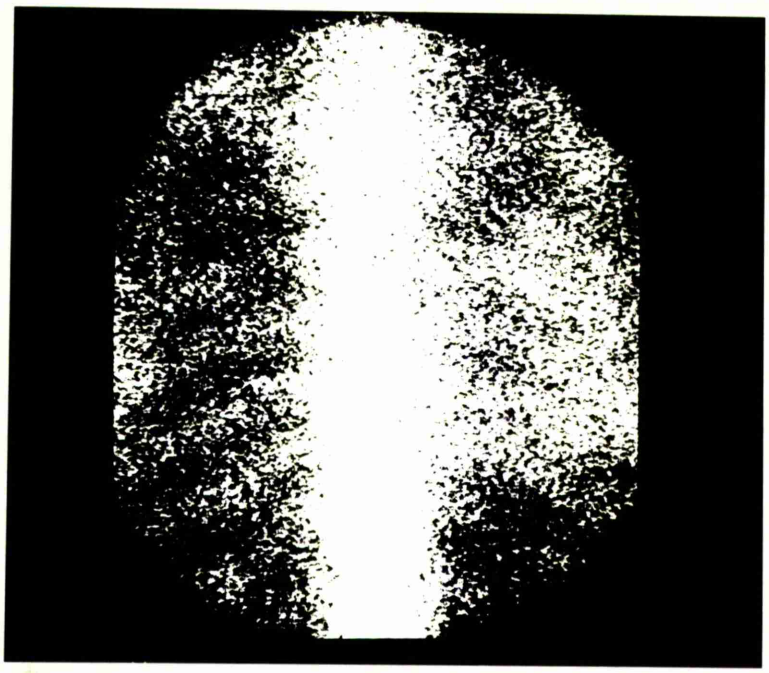
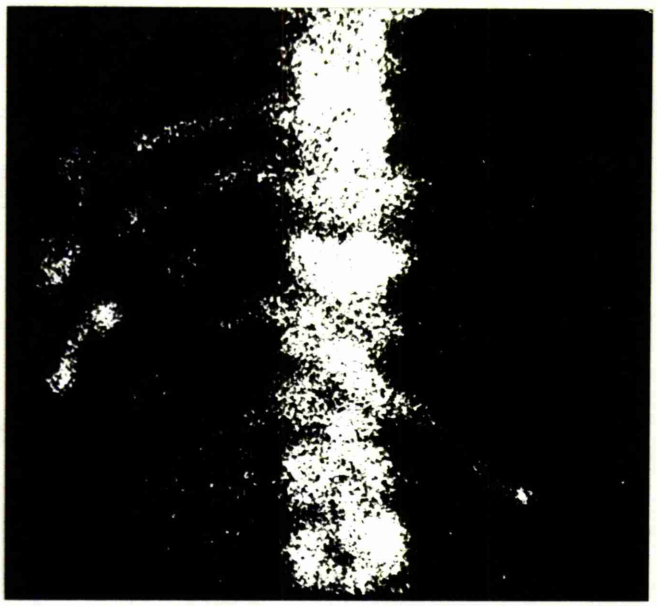


Figure IV.15. Pathological Confirmation of Metastases in an X-Ray Negative Patient

A 62 year old man presented with right rib pain. Chest X-Ray showed a large carcinoma of right lung. Specific x-rays of ribs were normal. In view of bone scan which showed diffuse involvement of right ribs, a formal thoracotomy was not carried out. A limited resection under local anaesthesia confirmed extensive tumour involvement of the ribs.

Figure IV.16. Pathological Confirmation of Metastases In X-Ray Negative Patient

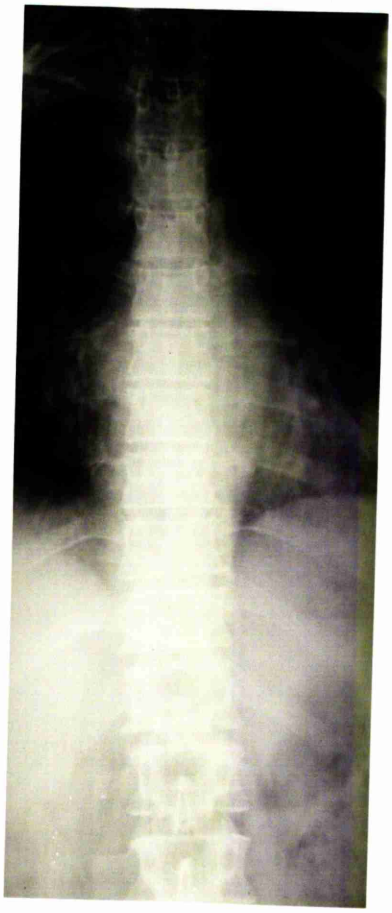
A 78 year old woman presented with a T_3N_1 breast cancer. Bone scan showed a distinct lesion in the vault of the skull. There was also uniformly high uptake in the cervical spine. Oestrogen therapy was started and she died of pulmonary embolism two months later. Metastatic involvement of the skull and spine were confirmed at post-mortem examination.



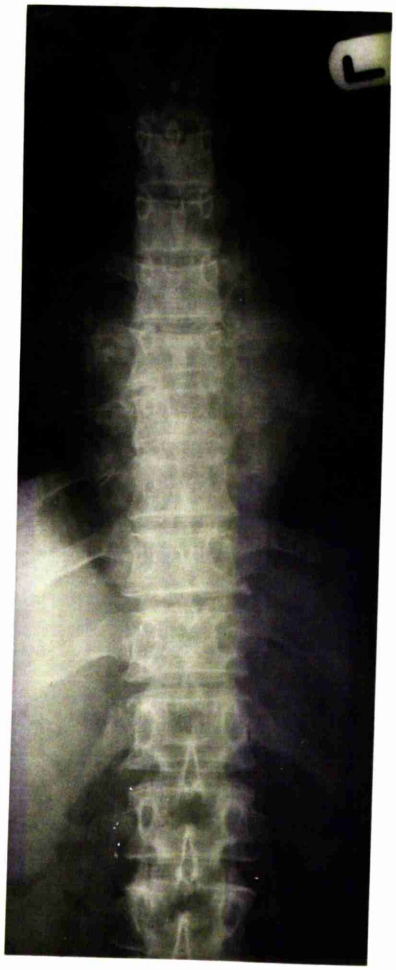
(a)

Figure IV.17. Subsequent Radiological Confirmation of Metastases Diagnosed by Bone Scanning

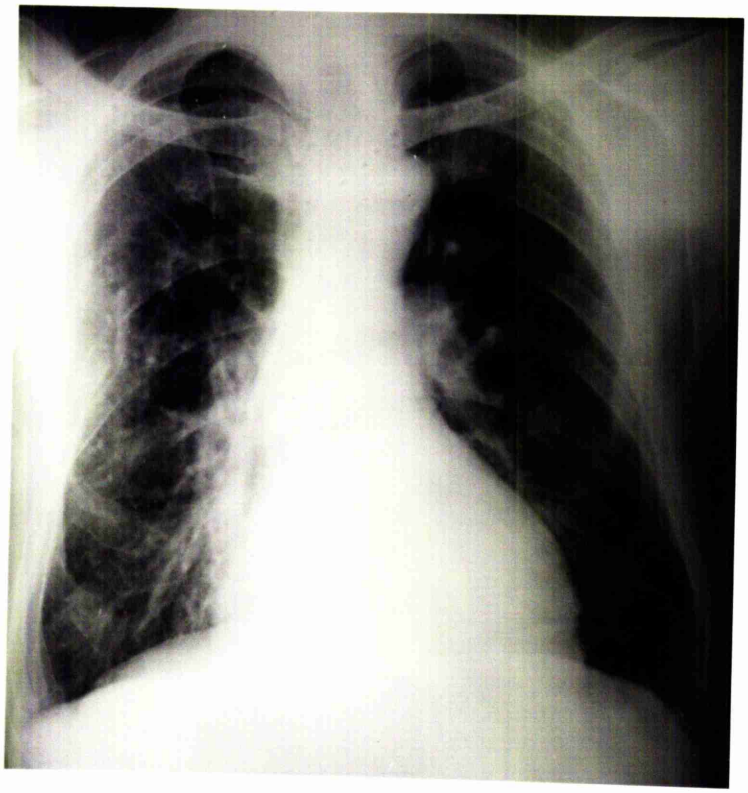
A 60 year old woman presented with lymph node recurrence and back pain 7 years after a mastectomy and oophorectomy. A bone scan (a) demonstrated extensive metastases in thoracic and upper lumbar spine (note especially lesion of right pedicle L2). Radiograph at this time was normal (b). (c) Radiograph 6 months later showed partial collapse T7 and loss of definition left pedicle T8. Right pedicle of L2 is also involved.



(b)



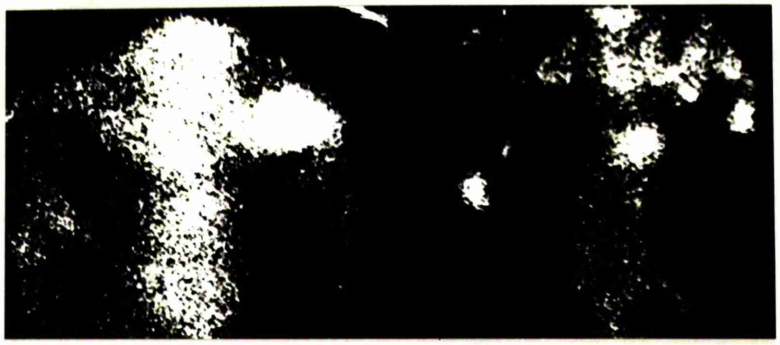
(c)



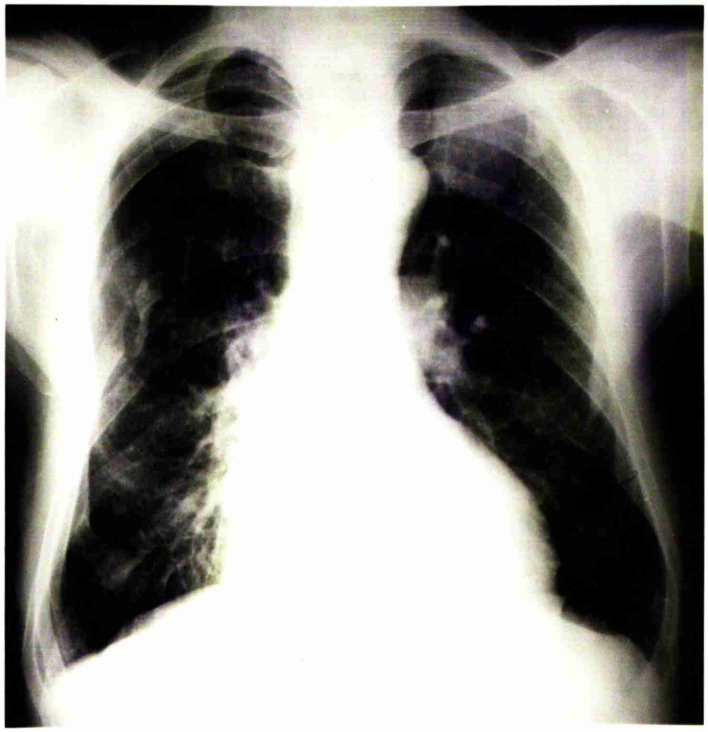
(a)

Figure IV.18. Subsequent Radiological Confirmation of Metastases Diagnosed by Bone Scanning

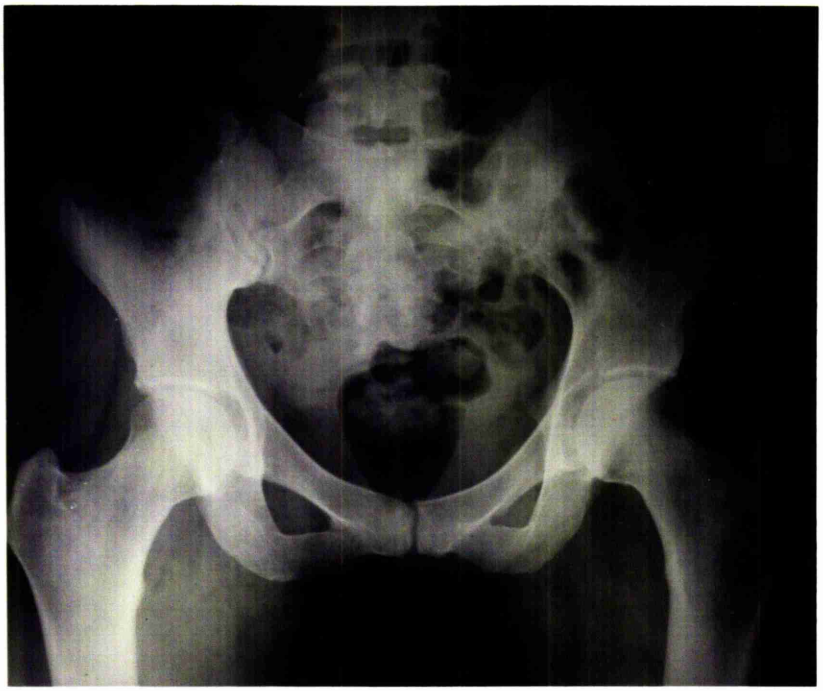
A 65 year old man presented with back pain six months after resection of an oesophageal cancer. (a) Chest radiograph at this time showed no abnormality apart from the rib resection due to the right thoracotomy. Bone scan showed metastases in the anterior left ribs (b). Repeat scan five months later showed more definitive lesions in the sternum and ribs (c). Chest radiograph at this time showed an opacity at the apex of the left lung due to metastatic involvement with associated destruction of the left first rib (d).



(b) (c)



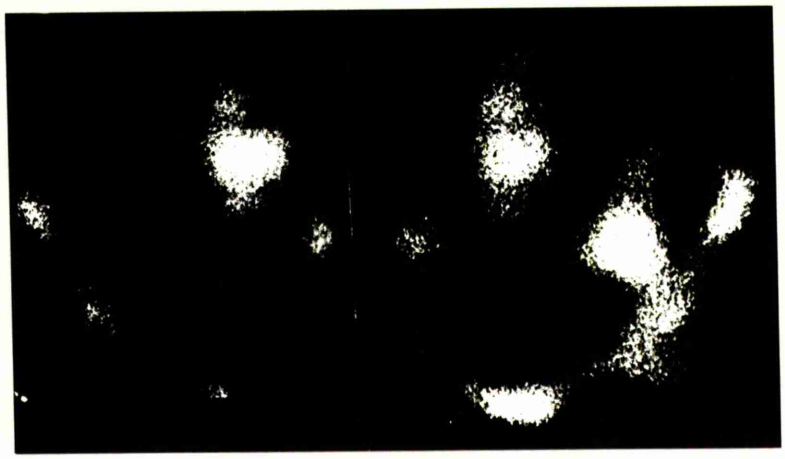
(d)



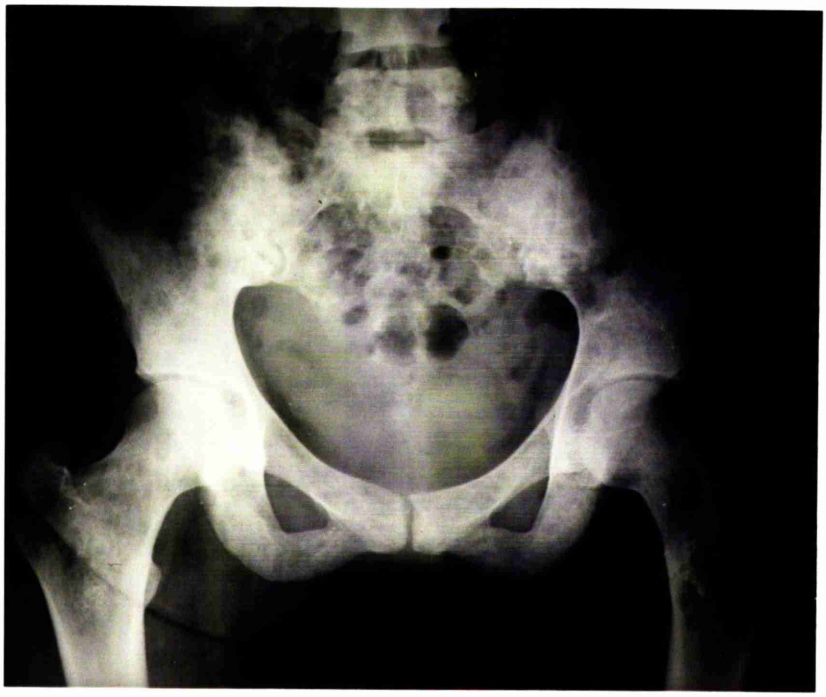
(a)

Figure IV.19. Subsequent Radiological Confirmation of Metastases Diagnosed by Bone Scanning

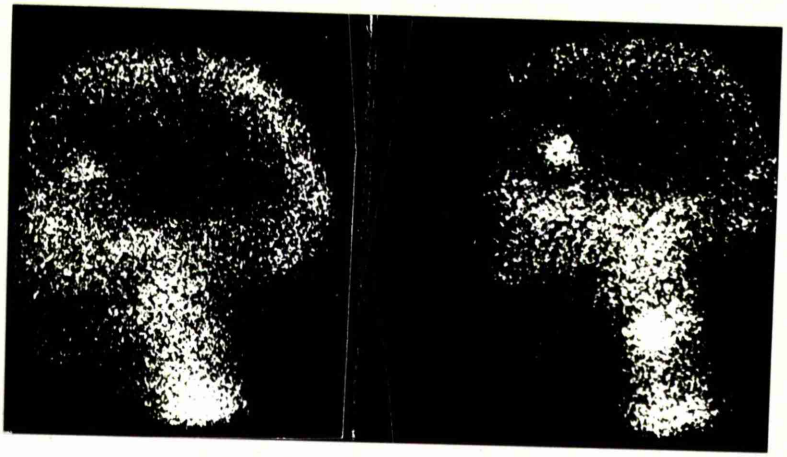
A 36 year old woman developed low back pain and cervical and axillary lymph node enlargement 3 years after a mastectomy. Radiograph of pelvis was normal (a). (b) The bone scan (a composite of right and left anterior pelvic views) shows involvement of L5 and extensive disease of the left half of the pelvis and the upper end of the left femur. Six months later radiograph confirms extensive bony involvement of the pelvis. (c).



(b)



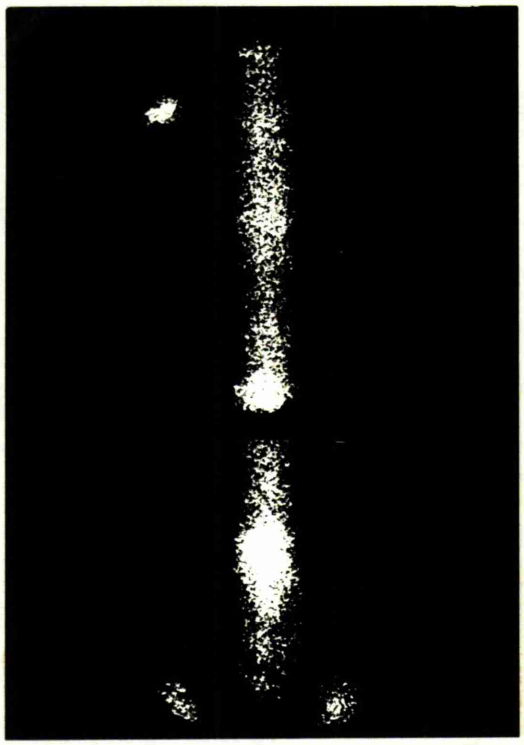
(c)



(a) (b)

Figure V.1. Primary Breast Cancer: Progression of Occult Metastases

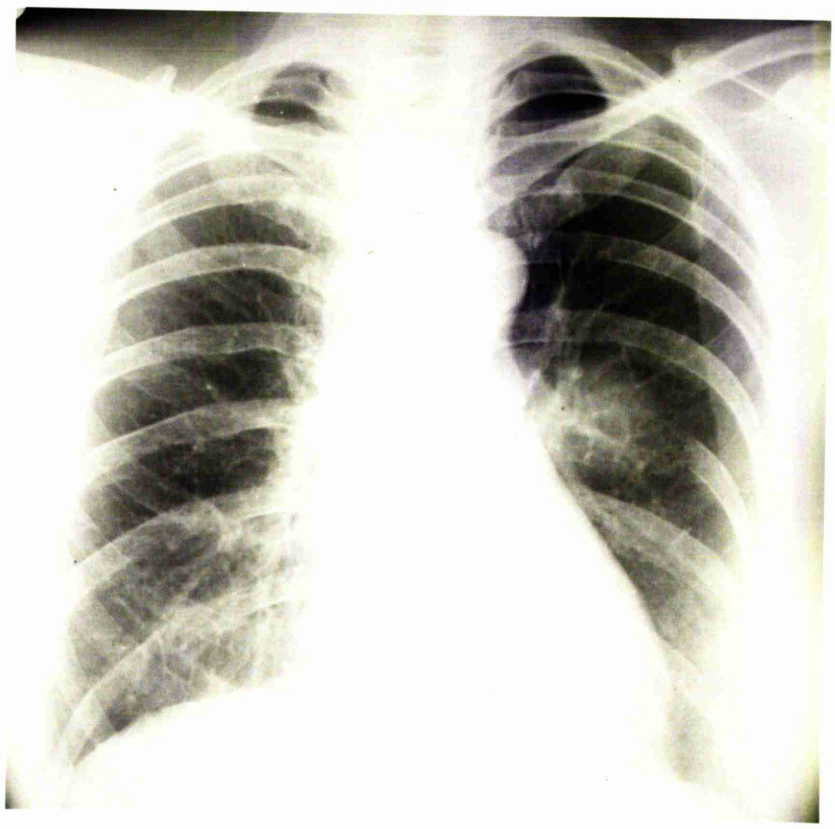
A 49 year old woman presented with T_2N_0 carcinoma of the breast. Radiographs were normal. Bone scan showed a metastasis in the left frontal bone (a). A repeat bone scan five months later showed progression of the lesion and an additional metastasis was also visible in the cervical spine (b). Subsequent radiological confirmation of both metastases was obtained.



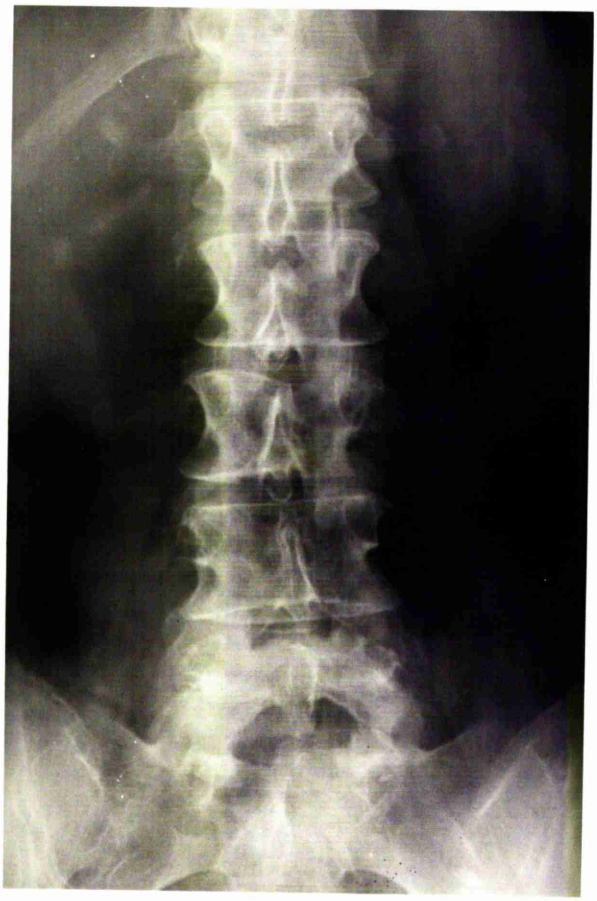
(a)

Figure V.2. **Primary Breast Cancer: Scan Positive Patient who Developed Radiological Metastases**

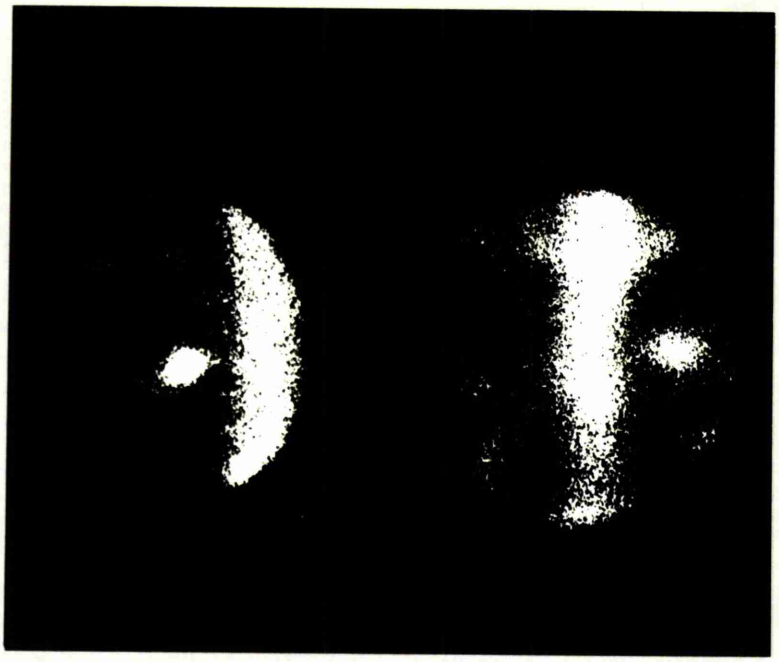
A 58 year old woman with T_2N_0 carcinoma of the breast had a normal radiological skeletal survey. Bone scan showed lesions in left ribs, lower thoracic spine and mid lumbar spine (a). Four months later radiographs confirmed metastases in left ribs (b) and T12 and L3 (c). At this time she was asymptomatic but two months later developed back pain.



(b)



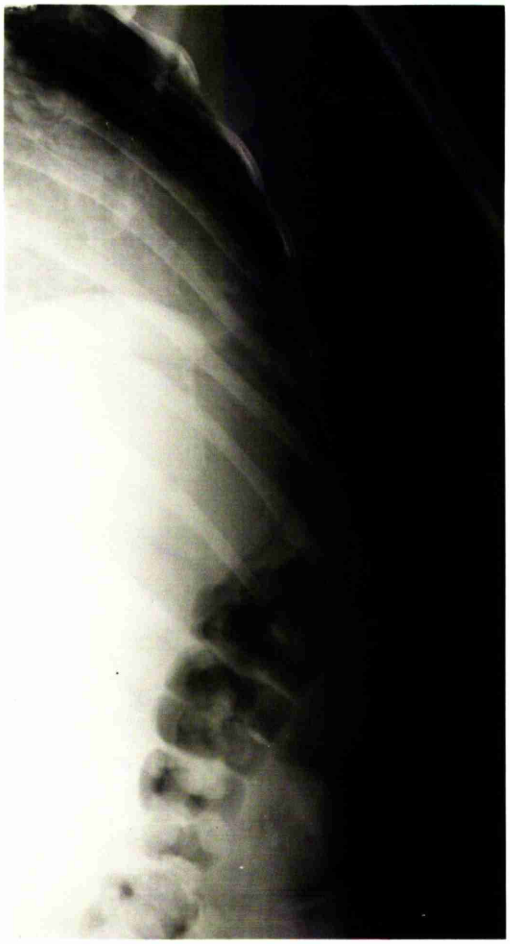
(c)



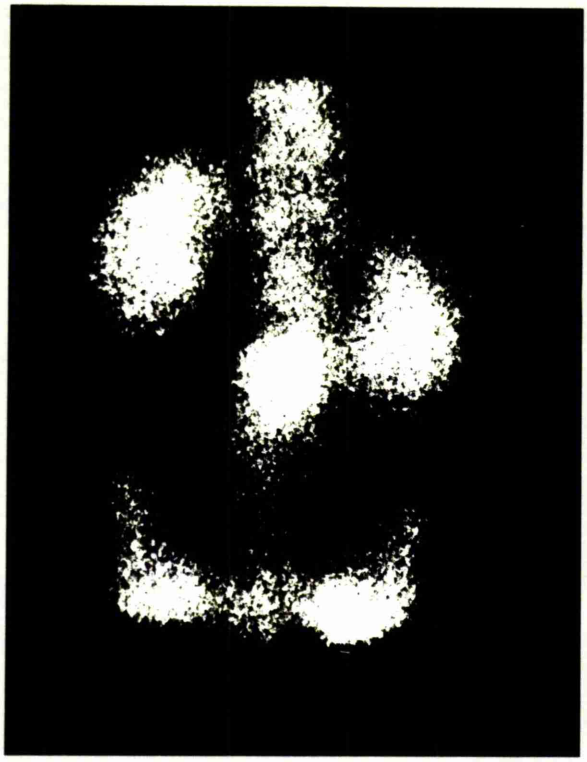
(a) (b)

Figure V.3. Primary Breast Cancer: Scan Positive Patient who Developed Radiological Metastases

59 year old woman with T_2N_1 primary breast cancer. Asymptomatic. Normal radiographs. Bone scan showed metastases left ribs seen in (a) posterior view; (b) anterior view. Confirmed radiologically eight months later (c).



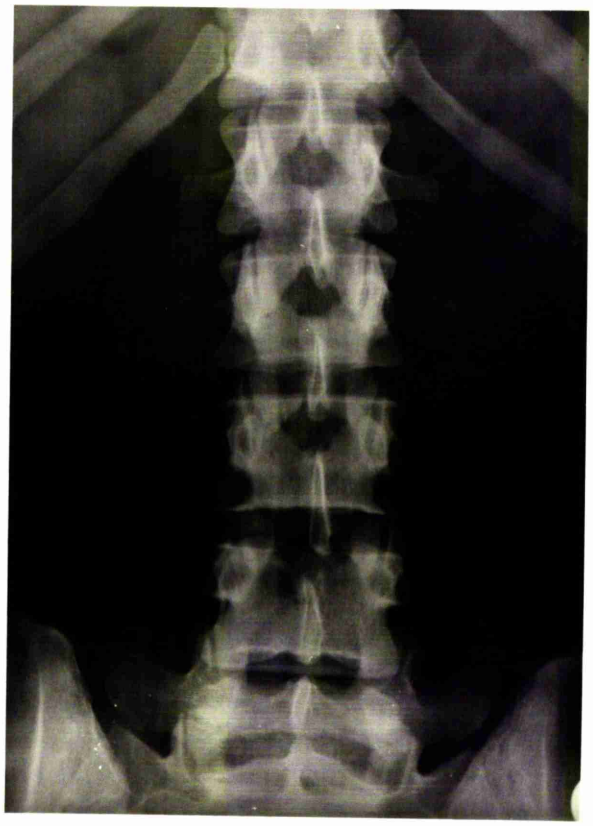
(c)



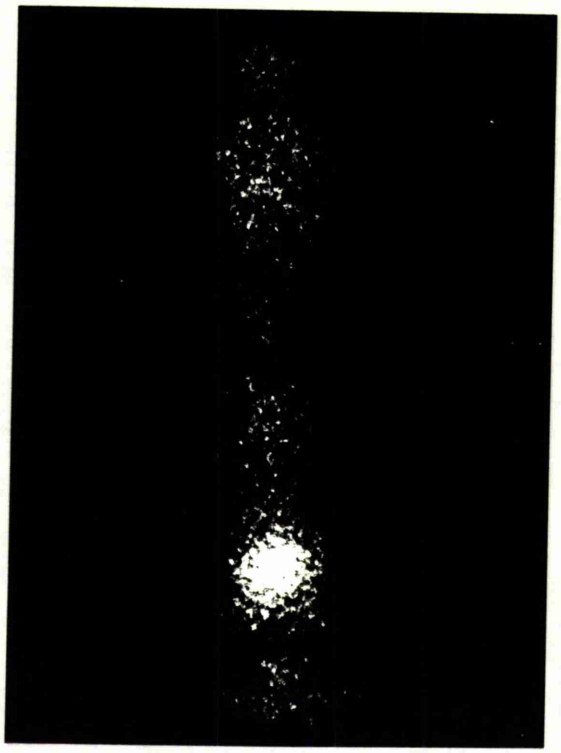
(a)

Figure V.4. Primary Breast Cancer: Scan Positive Patient who Developed Leukoerythroblastic Anaemia and Radiological Metastases

35 year old woman with T_1N_0 breast cancer. (a) Bone scan is positive lumbar spine (there were also extensive metastases in thoracic spine). (b) Radiograph is normal. Two months after study she developed leukoerythroblastic anaemia. She responded to oophorectomy and twelve months after original bone scan she has radiological metastases in lower thoracic spine. Lumbar spine remains normal.



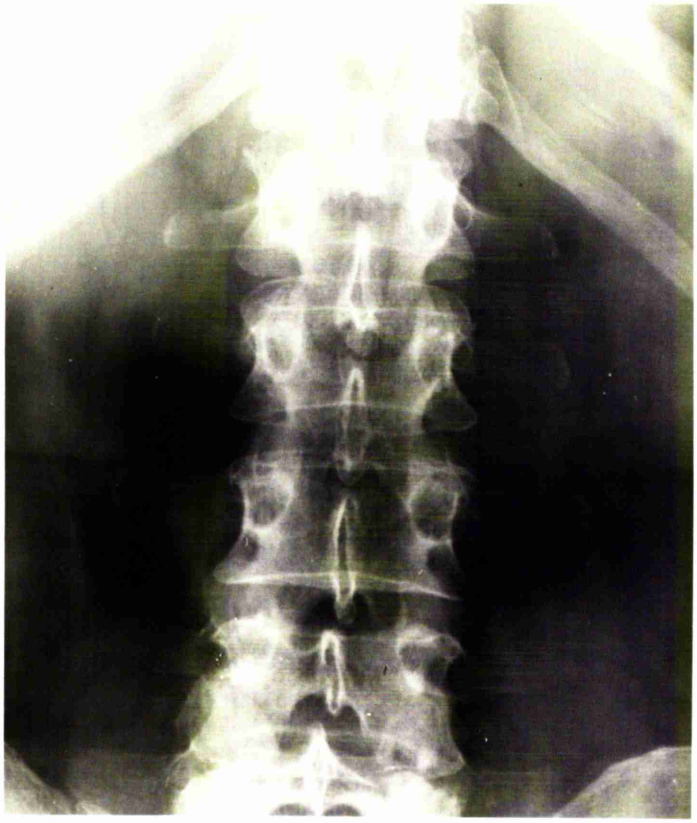
(b)



(a)

Figure V.5. Primary Breast Cancer: Scan Converter

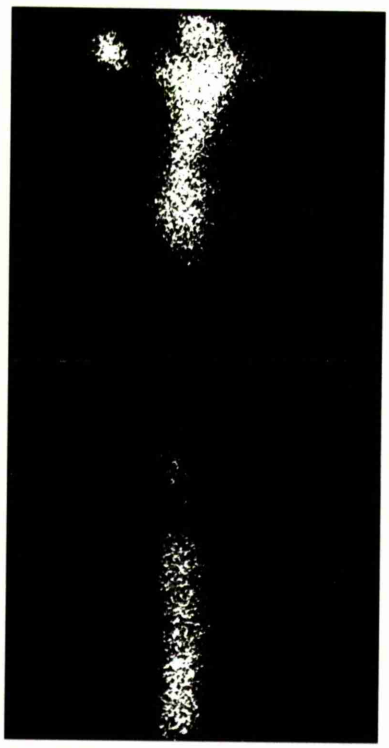
46 year old woman with T_2N_1 breast cancer. Six months after normal bone scan and radiographs at the time of mastectomy she developed lumbar pain and at this time bone scan showed a lesion in the lumbar spine (a). Radiographs were normal (b). Treated by oophorectomy. Post-mortem confirmation of metastases in lumbar spine was obtained two months later.



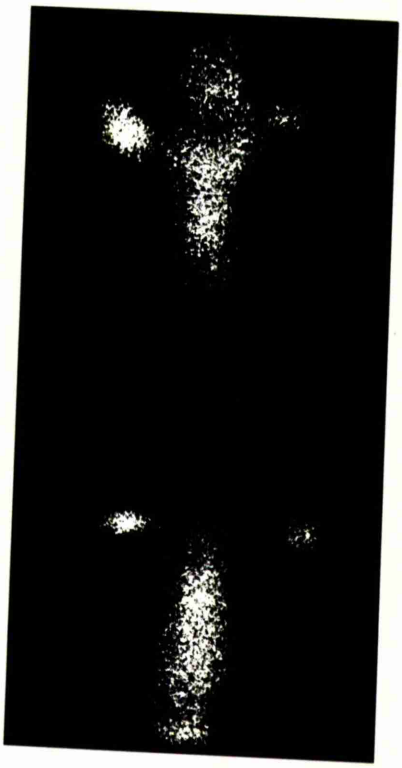
(b)

Figure V.6. Primary Breast Cancer: Scan Converter

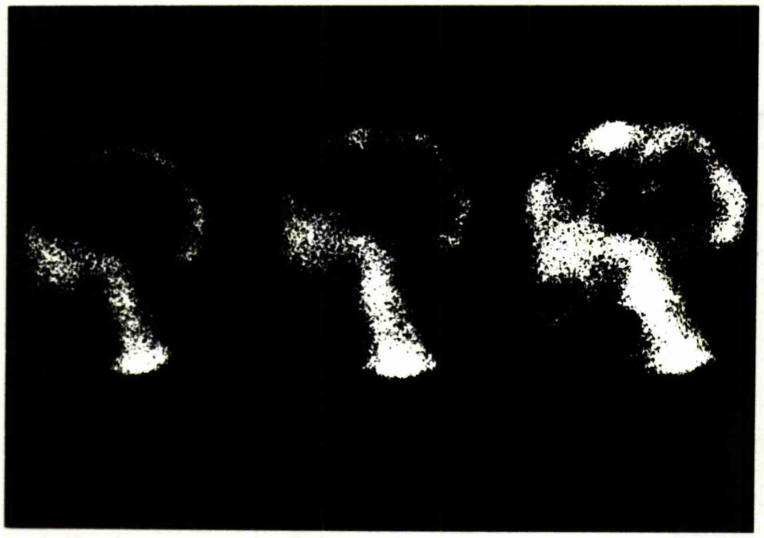
72 year old woman. Negative bone scan at the time of presentation with T_3N_0 tumour and axillary vein thrombosis. Primary tumour regressed with oestrogen therapy. Fourteen months later the bone scan showed a lesion of right shoulder girdle. (a) Composite view; upper: anterior view of sternum and upper ribs. Lower: posterior view of thoracic spine. (b) Repeat scan five months later shows progression of lesion in right ribs and a new lesion in left ribs, best seen on the posterior view. Radiographs still normal. Shortly thereafter she developed pain in the ribs and ulceration of the breast.



(a)



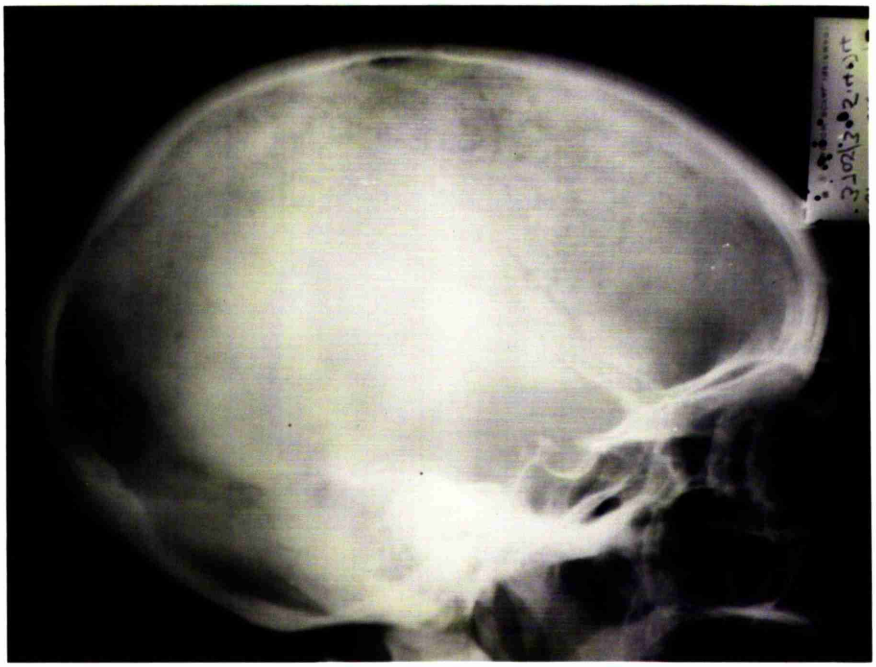
(b)



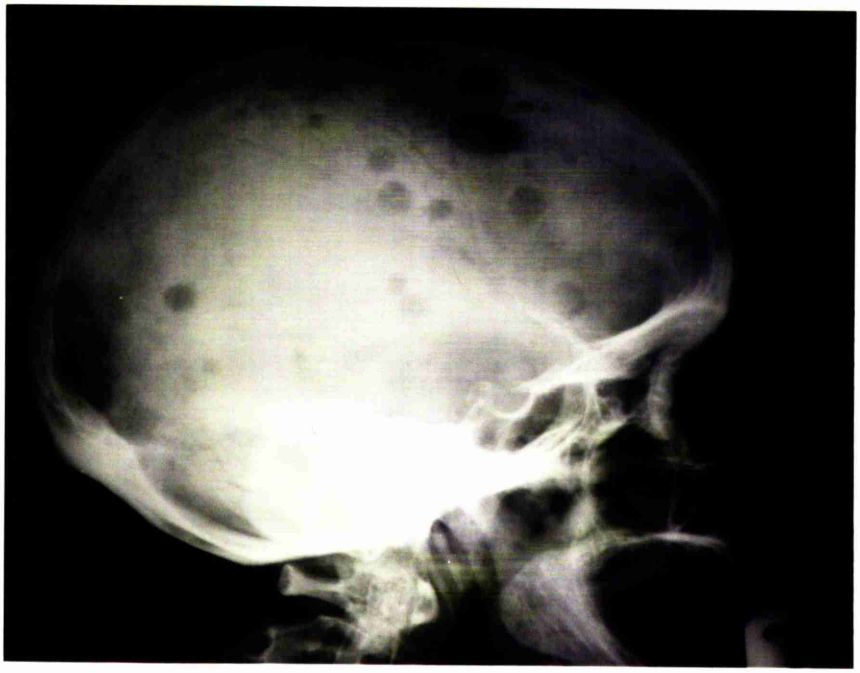
(a) (b) (c)

Figure V.7. Primary Breast Cancer: Scan Evidence of Progressive Disease

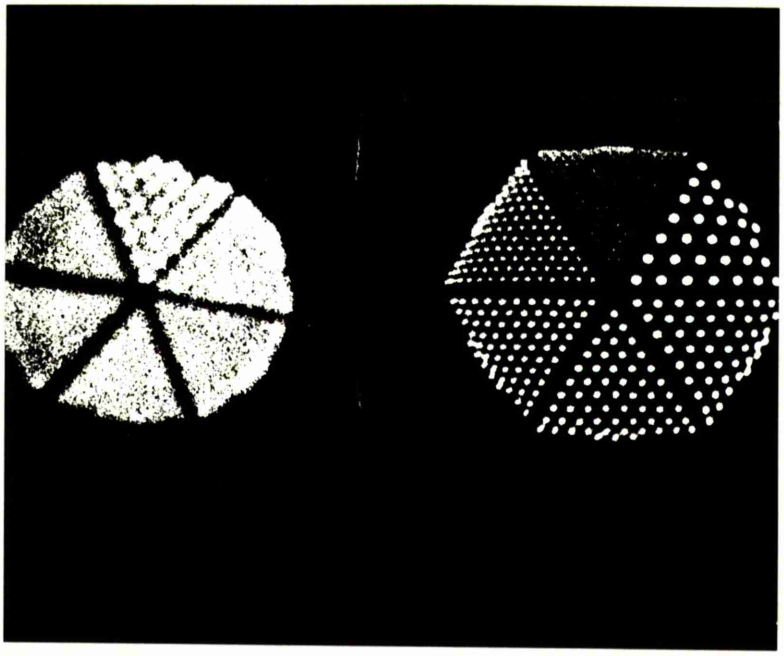
Serial bone scans of skull in a patient with scan evidence of metastases in ribs at the time of presentation with T_2N_1 cancer. Same patient as illustrated in Figure V.3.
(a) Normal skull. (b) Five months later early metastases in vault of skull and cervical spine. (c) Further five months later gross disease of skull and cervical spine.
(d) Normal skull radiograph at time of scan (b).
(e) Skull radiograph at time of scan (c) confirms extensive metastases.



(d)



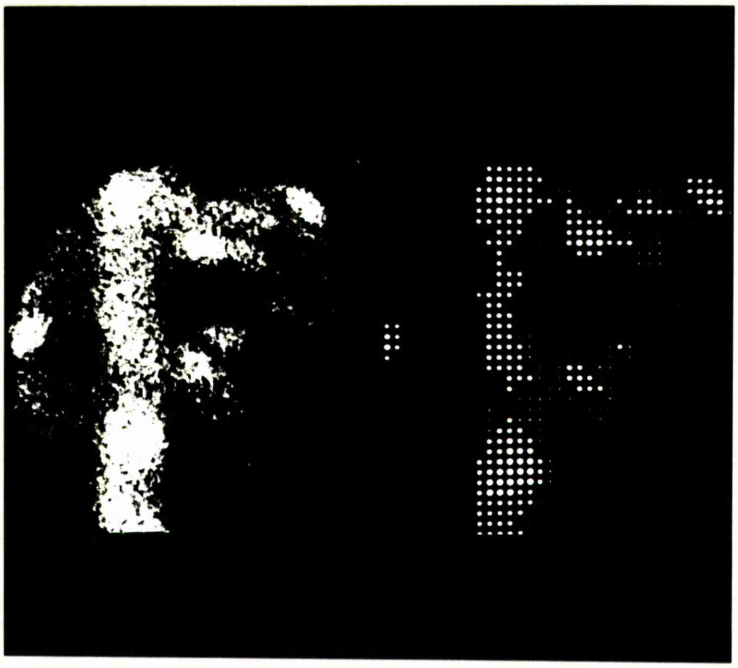
(e)



(a) (b)

Figure VI.1. Comparison of Gamma Cameras

Gamma camera images of a Standard Anger Phantom filled with ^{99m}Tc. Each image obtained with 10^6 counts, under identical conditions. (a) Image obtained with Nuclear Enterprises Scinticamera IV Gamma Camera. (b) Image obtained with Ohio Nuclear Series 100 Gamma Camera. Marked improvement in uniformity and resolution with the newer instrument is clearly seen.



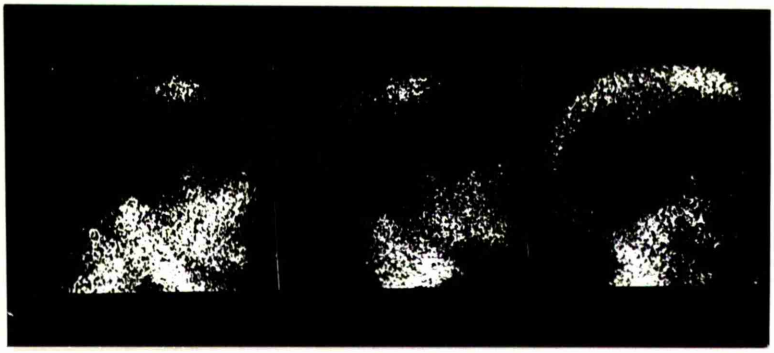
(a) (b)

Figure VI.2. Improvement in Bone Scan Due to Computer Smoothing

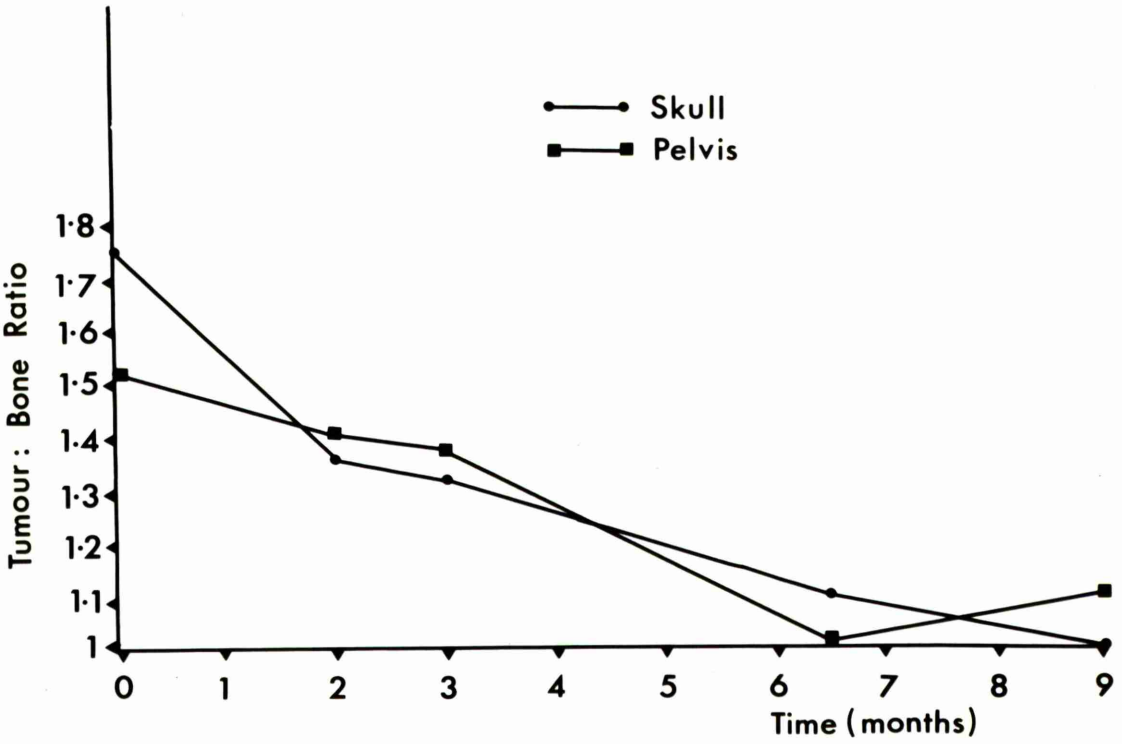
Patient with extensive bony metastases from carcinoma of prostate. Gamma camera view of thoracic spine and ribs. (a) Analogue image from gamma camera. (b) Digitized image following computer smoothing.

Figure VI.3. Serial Bone Scans as an Index of Therapeutic Response

A 23 year old woman was scanned ten months after a partial mastectomy for carcinoma of the breast. She was asymptomatic at the time of study. (a) Definite lesion in the vault of the skull. A further metastasis was seen in the left side of pelvis. Because of the scan appearance she was treated with systemic chemotherapy. (b) Three months later, no significant change in scan image. (c) Six and a half months after original scan the lesion in the skull is virtually invisible. Because of different camera exposure the images are not entirely comparable. (d) Serial quantitative tumour:bone ratios recorded from skull and pelvic metastases show a significant reduction in measured activity following chemotherapy. Chemotherapy was stopped nine months after original scan. Patient was clinically very well at that time. Five months later she developed severe leukoerythroblastic anaemia, and died.



(a) (b) (c)


Skull
Pelvis
Tumour : Bone Ratio
1·8
1·7
1·6
1·5
1·4
1·3
1·2
1·1
1
0
1
2
3
4
5
6
7
8
9
Time (months)

(d)

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