The Mechanisms involved in the scanning of bone using technetium labelled EHDP: its application in the healing of fractures.

By

SEAN PATRICK FRANCIS HUGHES, M.B., B.S., LONDON

F.R.C.S.

A thesis offered for the degree of Master of Surgery in the Faculty of Medicine of the University of London.

1976

TABLE OF CONTENTS

RESULTS

INTRODUCTION

Bone scanning with radioactive isotopes is being increasingly employed in orthopaedics. Technetium labelled ethane -1-hydroxy-1, 1-diphosphonate (^{99m}TcEHDP) is generally used. However it has been difficult to relate the picture obtained from the bone scan, with the clinical and radiographic findings of the patient. In particular, this situation has occurred in patients who have sustained fractures. The problem arises, because the process by which ^{99m}TcEHDP is taken up by bone and the factors that control its clearance from the body, have not yet been elucidated.

This thesis examines in the dog, the disappearance of ^{99m}TcEHDP from the blood, its extraction by bone and its clearance from the kidney. Because ^{99m}TcEHDP is a phosphate compound, the effect of the parathyroid hormone on the renal clearance is also studied.

The extraction of ^{99m}TcEHDP by bone is then examined following a canine tibial fracture; a situation where there is an increase in bone blood flow. ^{99m}TcEHDP bone scans have also been undertaken in patients with fractures, to correlate the experimental findings with the clinical presentation.

HISTORY

Bone scanning with radioactive isotopes has been widely employed in clinical practice (Hughes and Ell 1975). In particular the technique has been used to detect metastases in bone from carcinoma of the breast (Charkes and Sklaroff 1964 and Galasko, 1972), prostate (Roy et al 1971) and lung (Shirazi et al 1973). It has also been helpful in orthopaedics for the diagnosis of infections involving bones or joints (Bauer et al 1973; Kemp et al 1973; Ailsby and Staheli 1974), in studying the rate of union of fractures (Tucker, 1950; Wendeberg, 1961, Johansen, 1973; Bohr, 1973; Muheim, 1973) and in assessing response to treatment of Paget's disease of bone (Rashid et al 1974). Until recently the isotopes that were available included strontium 85 and 87m, and fluorine 18. Each however, has its respective difficulties. Strontium 85 has a long half life of 65 days whereas strontium 87m although having a half life of only 160 minutes, takes over 30 hours to be cleared from the blood. Fluorine 18 has an even shorter half life of 110 minutes, and is a high-energy gamma emitter, but it is usually only produced by a cyclotron reactor and therefore has limited distribution. In 1971, Subramanian and his colleagues labelled a polyphosphate with technetium 99m and so introduced the phosphate compounds as bone scanning agents.

At present there are three phosphate compounds mainly used in bone scanning: pyrophosphate, polyphosphate and EHDP. Of these three, EHDP appears to be the best because it is rapidly cleared from the blood and has a high bone to soft tissue ratio at 4 hours. (Yano et al 1973; Hughes et al 1975).

REVIEW OF THE LITERATURE

A BONE

Bone comprises organic and inorganic constituents. The organic are 35% of the tissue weight and consist of the cells and osseous tissue fluid. The inorganic components consist of minerals and are 65% of the tissue weight (Glimcher et al 1965); their constituents are calcium, phosphate, hydroxyl, carbonate and citrate with traces of magnesium, sodium, potassium, chloride, fluorine, strontium and lead (Vaughan, 1970). The hydroxyapatite is formed from amorphous calcium phosphate (Blumenthal and Posner 1973), its formula being $Ca_{10}PO_{46}(OH)_2$. The crystal has a length of 300-500 Å, with a width of 250 Å and a thickness of 85-100 Å (Robinson, 1952). Neuman and Mulgran (1971) demonstrated that the crystal is surrounded in vitro by a hydrated shell which is 100 Å thick.

To reach the crystal, minerals pass from the capillaries onto the nydroxyapatite. Electron microscopy reveals that there is an extracellular space between the capillary walls and the osteoblasts (Cooper et al 1966; Talmage, 1970). This space has been shown with thiotrast (Seliger, 1970); its ionic content estimated (Triffit et al 1968; Canas et al 1969; Geisler and Neuman, 1969); and its volume calculated by Owen et al (1973) to be 130 μl/g of wet rabbit bone. Total water space of cortical bone has also been measured in dogs as 0.15 ml/g (Kelly et al 1971).

Between the capillaries and the hydroxyapatite, is a family of osteoblasts that may act as a membrane between the latter and the extracellular fluid (Neuman and Ramp, 1971). These osteoblasts do not sit in tight apposition but allow mineral to pass between them. Once on the hydroxyapatite, EHDP has been noted to achieve chemical bonding (chemisorption) (Francis, 1969). EHDP is also believed to exchange with the pyrophosphate that exists on the crystal surface.

Rowland (1966), studying calcium^{45} uptake in bone, introduced the phrase 'short-term' exchange of minerals. This is the exchange that occurs, within one hour of injection, when the isotope is deposited onto those structures "with the greatest exposure to the circulating fluid". These structures are the subperiosteal, endosteal, and trabecular bone; the walls of the resorption cavities and the Haversian canals. Rowland showed all these surfaces to be labelled with Ca^{45} and noted that, within twelve minutes, the periosteal and trabecular bone were heavily labelled. He concluded that the isotope on the surface is not associated with areas of mineralization nor with areas of growth, but with those areas having greatest exposure to the circulating fluid; these are the main sites of rapid exchange between blood and bone.

BLOOD SUPPLY OF BONE

The blood supply of a tubular bone such as the tibia is derived from the nutrient, metaphyseal and periosteal vessels (Kelly and Janes 1968 and Nelson et al 1960).

The nutrient artery traverses the nutrient canal without giving off any collaterals to the bone cortex.

From the medulla, the vessels return to the cortex in a longitudinal direction within the Haversian system (Brookes and Harrison 1957). The terminal branches of the nutrient artery freely communicate with blood vessels in the Haversian canal (LeGros Clark, 1945). The Haversian systems anastomose with one another; they consist of irregular cylindrical structures comprising concentric lamellae of both bone substance and osteocytes. The lamellae are arranged in rings around a central canal (Ham, 1953). Cohen and Harris (1958) reported that the Haversian canals increase in size within the bone itself, the larger vessels being near to the endosteum and the smaller vessels near to the periosteum. Volkmann's canals communicate with the Haversian canals, carrying vessels from the periosteal surface. Capillaries lie within these Haversian canals; the size of capillaries in bone marrow has been estimated as 8μ in diameter (Branemack, 1961). Blood flows from the medulla to the cortex (Johnson, 1927; Brookes et al 1961; Rhinelander, 1972).

B PHOSPHATE COMPOUNDS.

Phosphate compounds have been available to industry as descaling agents for many years. In 1961, Fleisch and Neuman isolated pyrophosphate from human urine and plasma. That compound has been shown in vitro to bind on to the hydroxyapatite crystal, preventing further growth (Krane and Glimcher, 1962).

Pyrophosphates play an important part in calcium homeostasis by retarding the rate of mineral dissolution at the sites of reabsorption (Fleisch et al 1973). Polyphosphates, which are long chain polymers of phosphates, are similar in action to pyrophosphates and both compounds are readily hydrolyzed by the enzymes pyrophosphatase and polyphosphatase (Francis).

Diphosphonates have recently been introduced; they are synthetic compounds possessing a P - C - P bond, are stable in vivo and are able to withstand the chemical and enzymatic degradation processes. One such is ethane-1-hydroxy-1, 1-diphosphonate (EHDP); it is synthesized from acetic acid and phosphorous trichloride (Callahan and Castronovo 1973). Like the pyrophosphates it binds onto the hydroxyapatite and prevents further crystal growth (Jung et al 1973).

This arrest in growth occurs because conversion of calcium and phosphate into hydroxyapatite is prevented and also its dissolution is prevented. Initially, EHDP was used in the treatment of patients with osteoporosis, but it was found to produce osteomalacia, leaving large areas of unmineralized osteoid (Jowsey et al 1971). Currently, EHDP is being used in the treatment of patients with Paget's disease of bone (Russell and Smith, 1973).

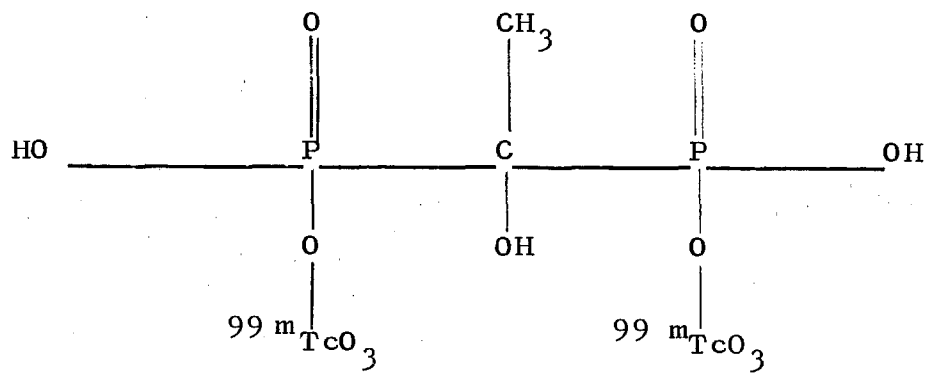
TECHNETIUM

Tecnnetium is a readily available isotope having an energy of 140 Kev which is ideal for scanning. It has satisfactory tissue penetration emitting γ rays but not β rays. It is derived by elution from the parent molybdenum (Wagner, 1968), and has a half life of six hours.

TECHNETIUM LABELLED EHDP

^{99m}TcEHDP is prepared by adding technetium to the stannous salt of EHDP. It is stable in solution and in crystal form. It has been studied in animals and in man (Castronovo et al 1972; Subramanian et al 1972; Silberstein et al 1973) and is shown to have a rapid blood clearance and to give clear bone scans, particularly when the ideal blood to tissue ratio is established.

PROPOSED FORMULA OF TECHNETIUM LABELLED ETHANE-1-HYDROXY-1, 1-DIPHOSPHONATE (M.W.=498)



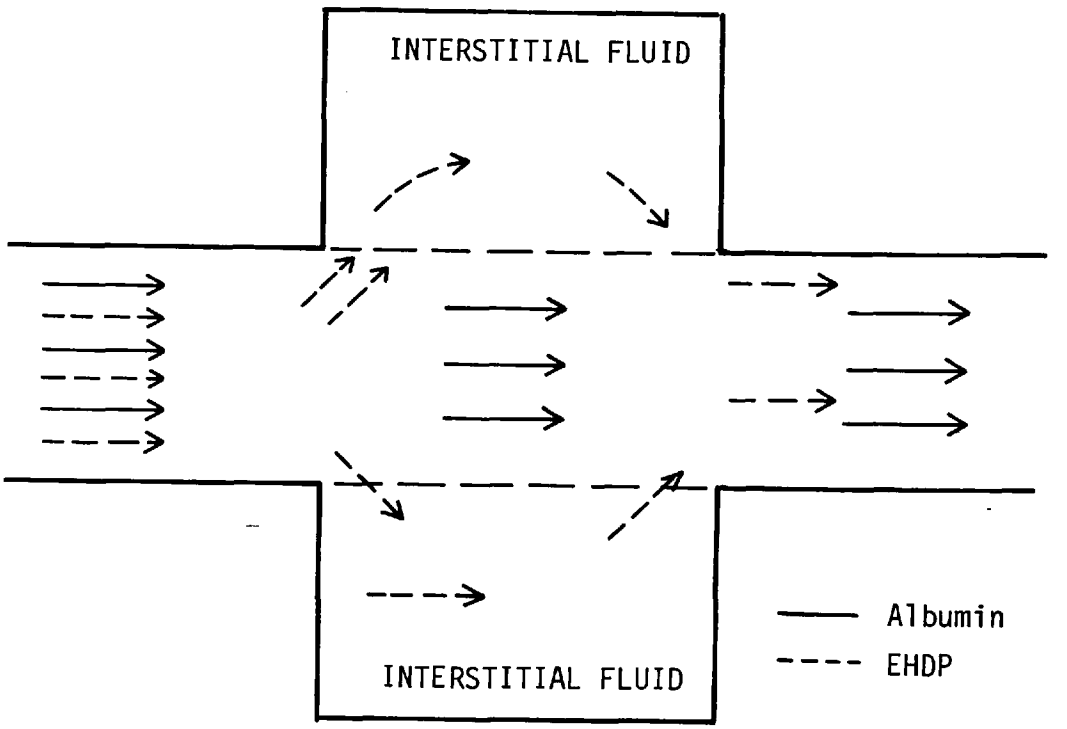
C EXTRACTION OF MINERALS

Transcapillary extraction can be measured by using the outflow dilution technique (Chinard et al 1955). The principle is that two substances are injected simultaneously into an arterial supply of the organ. One substance, the reference marker is unable to escape the intravascular system; the other is the test substance which is permeable and enters the organ under investigation. Observations are made on the blood leaving the tissue; the extraction, that is the fraction of the injected material that leaves the capillary, is calculated.

Figure 1 is a diagram of this principle. The reference tracer is albumin and the test tracer is EHDP. The albumin remains in tne intravascular compartment as the reference tracer and is collected in the venous outflow. EHDP is extracted by bone, however, and the amount extracted is calculated in relation to the reference tracer collected in this venous outflow.

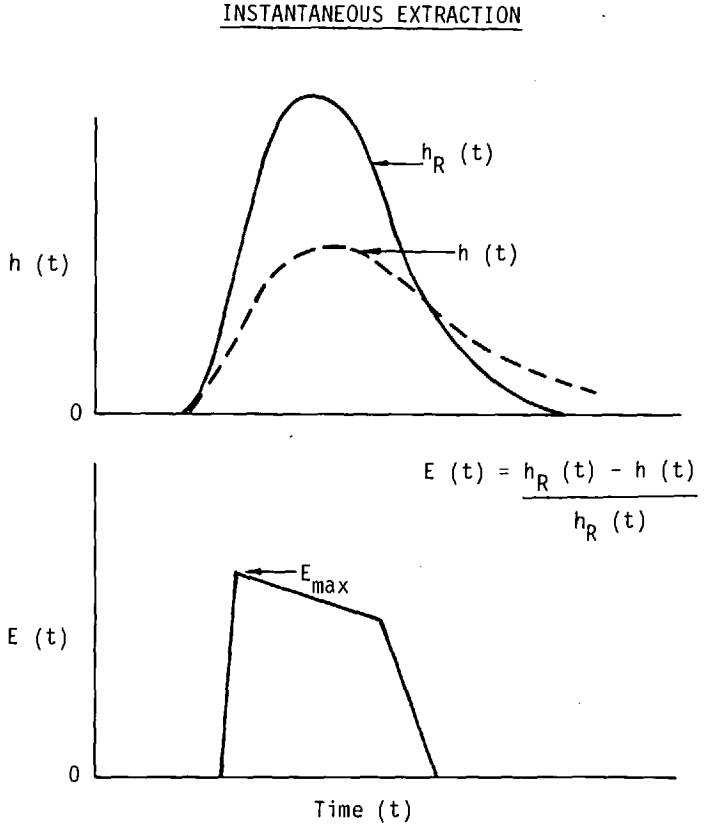
The outflow dilution curve obtained from such a system is shown in Figure 2. The mixture of albumin and EHDP is injected into the arterial side of the organ, and the whole venous end is sampled. The relative concentrations of the two tracers are measured in the venous outflow.

FIGURE 1



A schematic diagram to show the extraction of EHDP in relation to the reference tracer albumin. Both compounds are injected at the arterial end, the albumin remains in the vascular compartment, but the EHDP is extracted. An indicator dilution curve can be constructed from the venous outflow and extraction calculated.

FIGURE 2



Concept of indicator dilution curves for the reference and test tracers from which the instantaneous extraction is calculated.

Calculations of Extractions

The concentration versus time curves observed for each true count, C(t), in the outflow were normalized by dividing by the dose of each tracer injected to provide three functions, h(t); the fraction of the injected dose appearing in the outflow per second.

$$h(t) = \frac{F_v}{I} \cdot C(t)$$

(Zierler 1965)

Eq. 1

Where F_v is the flow in the femoral vein, I is the injected dose and C (t) is the concentration of the tracer in the blood. If the concentration of the reference tracer in the femoral vein at five minutes is zero, then one can assume that all the albumin is out of the bone and that the area under the reference tracer is one. Hence

$$h(t) = \frac{F_v \, C(t) \, / \, I}{\int_0^5 h_R(t).dt}$$

Eq. 2

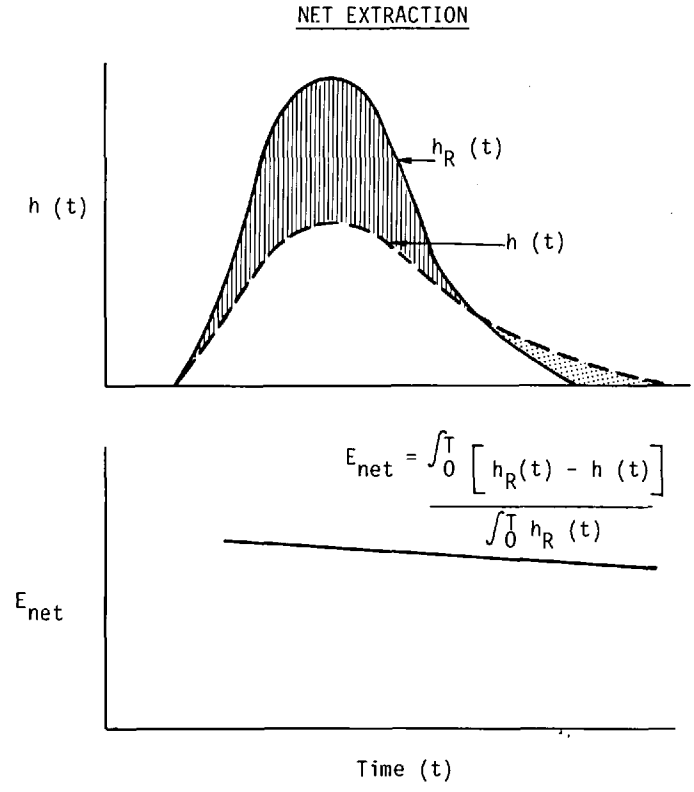
The instantaneous extraction E(t) for ^{99m}TcEHDP was calculated from the formula (Crone 1963)

$$E(t) = \frac{h_R(t) - h(t)}{h_R(t)}$$

Eq. 3

E_{max} is the maximum instantaneous extraction and is taken to be the best indication of extraction. (Bassingthwaighte 1974).

FIGURE 3



Net extraction is the shaded area between the curves divided by the area of the reference curve up to time T. E_{net} is calculated from the area under the curve. The cross area indicates that the back diffusion has become greater than the efflux from the capillaries.

The net extraction E_{net} is the apparent fractional retention by the bone at time T. It is also calculated from the outflow dilution curves by measuring the area under the curve as is shown in Figure 3.

It is the accumulated extraction for the period of sampling.

$$E_{net} = \frac{\int_o^T (h_R(t) - h(t).dt}{\int_o^T h_R(t).dt}$$

Eq. 4

Residuum (R) in the bone can be calculated by counting the whole bone and dividing it by the dose administered.

This is the residuum at time T.

$$R\ (T) = \frac{^{99m}\text{TcEHDP in bone at time T (cpm)}}{\text{dose of } ^{99m}\text{TcEHDP (cpm)}}$$

Eq. 5

The fractional Recovery of the initial ^{99m}TcEHDP was obtained by adding the amount retained in the tibia to the amount collected in the femoral vein during the period of sampling.

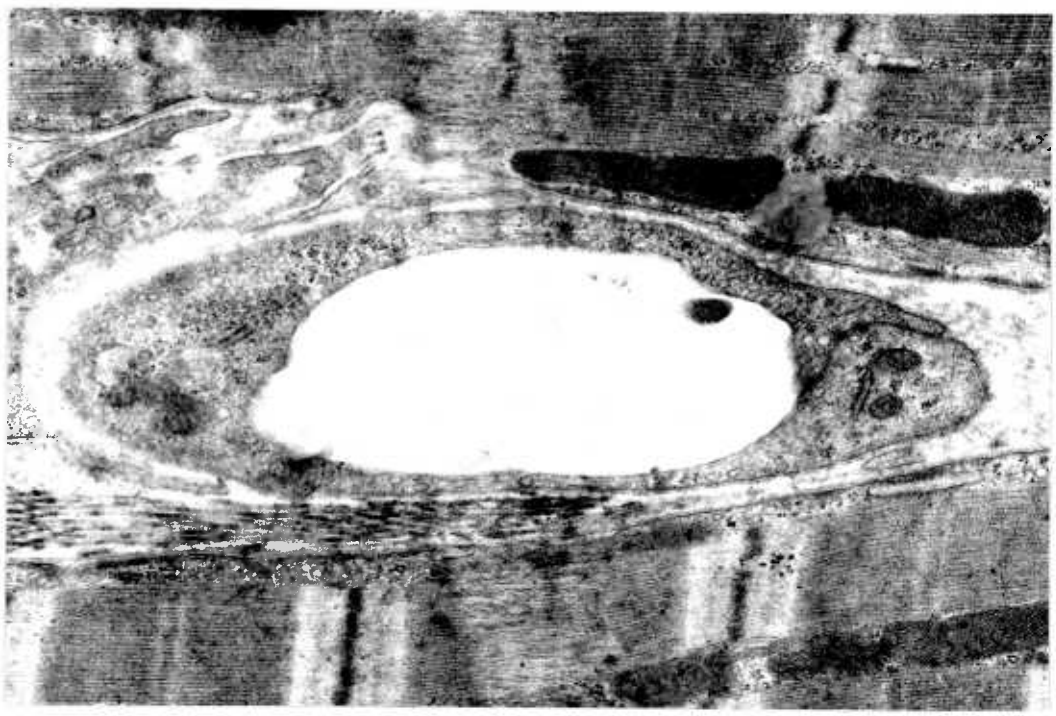
$$\text{Recovery} = \int_o^T h(t).\ dt + R(T)$$

Eq. 6

D DIFFUSION

Minerals pass through the capillaries in muscle by osmosis, filtration and diffusion. Diffusion can be active or passive, diffusion of lipid soluble substances occuring through the cell wall. However Pappenheimer, in 1953, showed that the passage of water and lipid insoluble molecules takes place by diffusion through channels or pores in the capillary walls. He estimated these pores to have a radius of between 30 and 45 Å, and suggested that diffusion rather than filtration was the mechanism by which small molecules could exchange rapidly between blood and interstitial fluid. Electron microscopy has identified these transcapillary clefts in muscle to the 40 Å (Karnovsky, 1968). Figure 4 illustrates this.

FIGURE 4



An electron microscope picture of a capillary in muscle to show the capillary clefts.

The passage of a solute through a membrane is governed by Ficks first Law (1855). This establishes that the amount diffusing through an area A will be given by the product of the diffusion coefficient, D and the concentration gradient. The concentration gradient is the difference in concentration divided by the thickness of the layer being examined:

$$\frac{dQ}{dt} = - DA \frac{C_2 - C_1}{x}$$

or

$$\frac{dQ}{dt} = - DA \frac{dc}{dx} \qquad \text{Eq. 7}$$

However

$$D = \frac{R\,T}{F\,N} \qquad \text{(Nernst 1888)}$$

Where F is the frictional force opposing each molecule, and is given by Stokes Law, when the molecule is spherical;

$$F = 6\,\Pi\,\eta\,a$$

Einstein (1905), used this concept to calculate the diffusion coefficient:

$$D = \frac{R\,T}{6\,\Pi\,a\,\eta\,N} \qquad \text{Eq. 8}$$

Therefore the diffusion coefficient is inversely proportional to the molecular radius and the viscosity of the media.

The diffusion of lipid insoluble molecules is through the capillary clefts and is dependent on the radius of the molecule. The larger the molecule, the lower the diffusion coefficient and the slower the flux. Apart from the molecular size, there is restriction to diffusion, either from friction between the pore walls or from hindrance when the molecule strikes the edge of the cleft. Restriction to diffusion in theoretical membranes was proposed by Renkin (1959) and its existance has been confirmed by Beck and Schultz (1970) using a microporous membrane.

PERMEABILITY

The indicator dilution technique has been developed in muscle by Crone (a) (1963) and by Martin and Yudelevich (1964), in order to estimate capillary permeability. Extraction is calculated from the outflow dilution curves and providing there is a large enough fluid space in the tissue under study to minimise early return to the blood, it is possible to measure permeability of the capillary membrane by the formula

$$PS = - F_s \log_e (1-E)$$

Eq. 9

Crone (b) (1963) went onto show that if three tracers are injected simultaneously into the artery, a ratio of permeabilities can be derived. The three substances would be two test substances and a reference substance.

The extraction is calculated as before.

Thus:

$$\frac{P_a S}{P_b S} = \frac{-F_s \log_e (1-E_a)}{-F_s \log_e (1-E_b)}$$

Eq. 10

Because the injection of the three substances is simultaneous, the surface area and the flow cancel out and a permeability ratio can be calculated.

The advantage of this equation is that it contains only values determined in the experiment whereas a calculation of permeability coefficients alone involves assumption about capillary surface area and rate of perfusion.

RELATIONSHIP BETWEEN FREE DIFFUSION AND CAPILLARY PERMEABILITY

Crone (b) calculated the permeability ratio for inulin to sucrose in muscle and found that they were approximately proportional to the ratio of their free diffusion coefficients in water. He postulated therefore, that for these molecules there was probably no restriction to free diffusion through the capillary cleft and that it was possible to relate the diffusion coefficient ratio of the substances, based on their molecular weight, and the permeability ratio based upon measuring extraction.

E TISSUE UPTAKE AND DISTRIBUTION

The blood clearance and tissue uptake of the phosphate compounds has been examined in animals and man. (Subramanian et al 1972; Kaplan et al, 1973; Tofe and Francis 1974; Krishnamurthy et al 1974). In rabbits at 4 hours there was 24% tripolyphosphate, 8% pyrophosphate, but less than 2.5% EHDP still present in the blood showing that the blood clearance is rapid for EHDP. The average radioactivity present in 1g of tissue to 1ml of blood also confirms that EHDP has the highest uptake in the bone then in either of the other two substances at 4 hours (Hughes et al 1975).

Calculations

The observed tracer concentrations in tissue and plasma were standardised with respect to the injected dose to give the concentrations C_t in tissue and C_p in plasma.

$$C_t \text{ or } C_p = \frac{\text{cpm/g of tissue or cpm/ml of plasma}}{\text{cpm of injected dose.}}$$

Eq. 11

The fractional uptake f of the whole tissue is obtained by multiplying by the tissue weight W_t.

$$f = C_t \cdot W_t$$

Eq. 12

The tissue concentration of each time; t, relative to that in the plasma at the same time is C_t/C_p.

Eq. 13

F RENAL CLEARANCE

^{99m}TcEHDP is excreted by the kidney and phosphate excretion is largely controlled by the kidneys. Albright and Reifenstein (1948) demonstrated that parathyroid hormone (PTH) by increasing the glomerular filtration rate, acts upon the renal tubules to increase the amount of phosphate in the urine. Recker et al (1973) examined the effect of EHDP on patients and noted a significant increase in phosphate in the blood and a reduced renal phosphate clearance. However he was unable to show any effect of PTH on the tubular reabsorption of phosphate by EHDP.

Calculations

$$\text{Clearance} = \frac{C_u \cdot F_u \text{ ml/min}}{C_p} \qquad \text{Eq. 14}$$

A clearance ratio of one substance to another can be calculated, as a measure of reabsorption.

$$\left[C_u \cdot F_u / C_p \right]_a \Big/ \left[C_u \cdot F_u / C_p \right]_b \qquad \text{Eq. 15}$$

G THE BLOOD SUPPLY OF FRACTURES

The vascular changes that follow a fracture have been studied by microangiography (Gotham 1961, Rhinelander 1972) and by measuring bone/blood flow with chromium labelled microspheres (Brookes et al 1970); electromagnetic probes (Wray 1964); clearance techniques (Laurnen and Kelly 1969) and recently using iodoantipyrine washout techniques (Paradis and Kelly 1975). All these methods have shown that vascularity increases following a fracture.

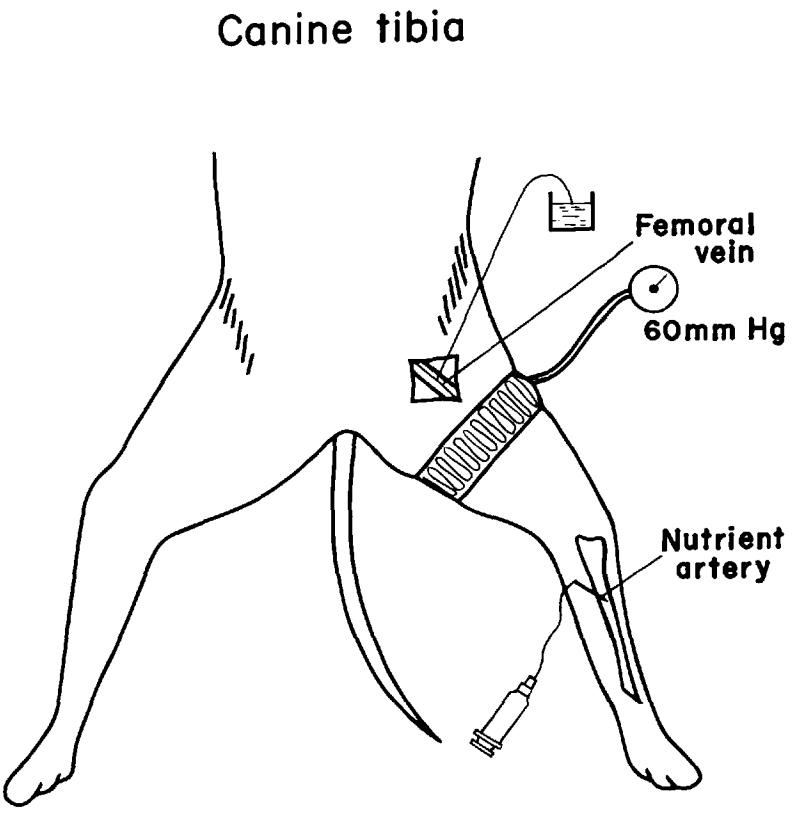
If the flow through a capillary is increased, the transcapillary extraction may be diminished (Guller et al 1975). However, in a recent study of the transcapillary extraction of strontium following the increase in bone/blood flow associated with a fracture, the instantaneous extraction of the mineral remained unaltered by the increase in flow (Hughes et al 1976). The fact that extraction is undiminished by changes in flow following a fracture is probably due to enlargement of the surface area from recruitment and dilatation of the capillaries in the cortex. This would confirm the work of Rhinelander and Wray and Lynch (1959) who showed by microangiography that the number of blood vessels in bone increased following a fracture.

MATERIALS AND METHODS

A. EXTRACTION OF ^{99m}TcEHDP AND THE MEASUREMENT OF PERMEABILITY

Ten adult dogs were anaesthetized and the nutrient artery of the tibia approached by the trans-fibular route. The artery was cannulated with fine PE 50 (diameter 0.58 mm) polyethene tubing. Previous studies have shown that such a cannulation does not decrease total tibial strontium clearance but does decrease diaphyseal clearance by one-third (Cofield et al 1975). The ipsilateral femoral vein was isolated in the femoral triangle and cannulated with PE 360 tubing (diameter 5.3 mm). Tributaries of the femoral vein proximal to the cannula were ligated and a pneumatic cuff, inflated to 60 mm Hg, was applied to the thigh to prevent recirculation (Bosch, 1969). A 1ml mixture of 30μCi 51 chromium albumin (Squibb), 10μC1 14 carbon sucrose (New England) and 20μCi ^{99m}TcEHDP (Diagnostic Laboratories) was injected into the nutrient artery over a 47 second period so as not to affect the blood flow through the artery. Twenty sequential samples of venous blood were collected at fifteen second intervals from the ipsilateral femoral vein after which the dog was killed. These samples were the total outflow of the femoral vein during that 5 minute period. Figure 5.

FIGURE 5



A diagram of the animal preparation. The isotopes are injected into the nutrient artery of the tibia and the blood collected from the femoral vein.

After centrifuging, 1ml samples of plasma were counted along with a standard for two minutes in a Nuclear Chicago Model C120-1. These specimens were recounted at 48 hours to allow for the decay of technetium. 0.2 ml aliquots of the original plasma, in 0.3ml of distilled water and 10ml of Instagel, were counted in a liquid scintillating counter (Nuclear Chicago Model Mark II), and the results analysed on a CDC 3500 and CDC 3600.

From four of the ten dogs, the amount of isotope in the bone was estimated by counting the whole tibia, cleaned of all soft tissue, in a small animal whole-body counting chamber (Lomayoco).

In a further three dogs the instantaneous extraction and permeability ratio of ^{99m}TcEHDP was compared with ^{85}Sr by the same technique as has been described, using chromium labelled albumin, but this time excluding ^{14}C sucrose and substituting ^{85}Sr.

In another eight dogs the instantaneous extraction and permeability ratio of ^{99m}TcEHDP to sucrose was compared with that of ^{99m}Tc pyrophosphate using the same technique.

MEASUREMENT OF ^{99m}TcEHDP IN BONE

At the end of 5 minutes each dog was killed, the tibia disarticulated and cleaned of soft tissues. Then the whole bone was counted for ^{99m}TcEHDP in the small animal counting chamber, to give the fractional residuum (R) at five minutes

B TISSUE UPTAKE AND BLOOD CLEARANCE

Eleven adult dogs were anaesthetized, the left jugular vein and the right carotid artery cannulated a urinary catheter was inserted and the bladder emptied. Thirty μCi ^{99m}TcEHDP was injected into the cannulated jugular vein. Blood samples were then withdrawn from the carotid artery at one minute intervals for ten minutes, and at ten minute intervals for between one and four hours. At one, two, three and four hours the animals were killed and samples of tissue taken from their skin, muscle, liver, kidney, lung, gut and the whole of the left tibia excluding the marrow.

These samples were weighed and counted, along with a pilot, in the small animal-counter. The blood and urine samples were centrifuged and 1ml aliquots of plasma and urine were counted, along with a pilot, for two minutes in a Nuclear Chicago C 120-1.

C RENAL CLEARANCE

In four adult dogs used as controls the clearance of ^{99m}TcEHDP was measured and compared with the clearance rate of inulin labelled witn ^{14}C. The dogs were anaesthetized and the left jugular vein and right carotid artery cannulated. The bladder was opened and a wide bore polyetnene tube sutured into place. A urethral tube was also inserted and sutured, tnus producing a closed circuit system.

Thirty μCi ^{99m}TcEHDP and 10 μCi ^{14}C inulin were injected into the jugular vein and 1 ml blood samples were withdrawn from the carotid artery at ten minute intervals for four nours. The bladder was wasned out with saline at ten minute intervals and the volume measured. The blood and urine samples were centrifuged and 1 ml aliquots of serum and urine were counted for technetium along with a pilot in the Nuclear Chicago C 120-1. The ^{14}C was counted in an automatic liquid scintillation system, Nuclear Chicago Mark II.

Parathyroid Hormone Control

The effect of parathyroid hormone on tubular reabsorption was studied in seven dogs. In four dogs, four days after a total thyroparathyroidectomy, the clearance rate of ^{14}C inulin was compared with that of ^{99m}TcEHDP using the method just described.

In a further three dogs, the response of phosphate excretion to parathyroid hormone was compared with the ^{99m}TcEHDP and ^{14}C inulin response to parathyroid hormone. In these animals, after total thyroparathyroidectomy, the ureters were isolated and cannulated; two hours later an infusion of ^{99m}TcEHDP and ^{14}C inulin was started. One hour later a pharmacological priming dose (3.3 units/kg) of parathyroid hormone (Lilly) was given intravenously, followed by a sustained dose of 0.1 units/kg/min. The parathyroid hormone was infused for two hours and samples of blood and urine were obtained at fifteen minute intervals for phosphate analysis, ^{99m}TcEHDP and ^{14}C inulin. These isotopes were counted in a similar manner to that described for renal clearance and the concentration phosphate was estimated by colorimetric method (Young, 1966).

D BLOOD FLOW AND EXTRACTION FOLLOWING A FRACTURE

The instantaneous extraction of ^{99m}TcEHDP was measured following a fracture. The right tibias of four adult dogs were divided under anaesthesia at the junction of the middle and distal thirds, using an oscillating Stryker saw. The fracture was displaced, then reduced and a 4 - hole Vitallium plate applied to the medial surface with 0.9cm screws. The dogs were able to weight bear after three days without difficulty. At two weeks the extraction of ^{99m}TcEHDP was calculated using the outflow dilution techniques.

E BONE SCAN

Bone scanning was undertaken on a patient who had sustained a subcapital femoral fracture, to assess the presence of avascular necrosis; and on another who exhibited non union of his tibial fracture, to assess the rate of union.

The procedure used was as follows 15 mCi of oxidant free ^{99m}Tc pertechnate ($^{99m}TcO_4$) is added to a vial containing 1 - 2 ml of a sterile pyrogenic aqueous solution of a sodium salt of EHDP (0.5mg/ml) with stannous chloride (0.14 mg/ml) and sodium carbonate, to yield a pH of between 5.0 and 8.0. After thorough mixing, the solution was ready for injection. It was given intravenously by slow injection into an antecubital vein. Three hours later,

the patient had a bone scan with a rectilinear scanner or a gamma camera. Because ^{99m}TcEHDP is excreted in the urine, the bladder concentration is high, and, to reduce the background, the patient was encouraged to drink copiously and to empty his bladder as frequently as possible.

RESULTS

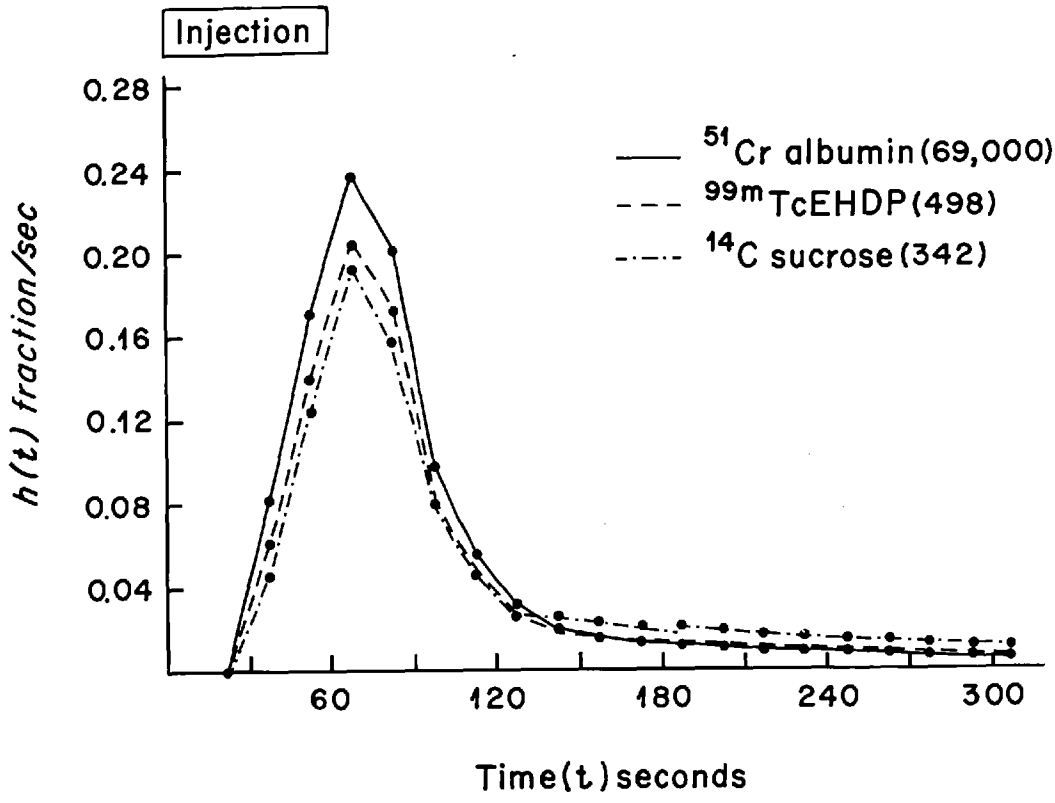
A EXTRACTION OF ^{99m}TcEHDP BY INSTANTANEOUS EXTRACTION, ACCUMULATIVE EXTRACTION AND RESIDUUM.

Figure 6 (a) shows typical indicator dilution curves for the three substances ^{99}TcEHDP, ^{14}C sucrose and ^{51}Cr albumin. The fraction of the injectate is plotted against time in seconds. The ^{99m}TcEHDP curve is separate from the sucrose curve. At about 2 minutes the sucrose curve crosses the albumin curve whereas the ^{99m}TcEHDP curve does not. The corresponding extraction curves calculated from equation 3 are shown in Figure 6 (b). Thepeak of the instantaneous extraction curve is E_{max}, (mean $\pm$ S.D.), for ^{14}C sucrose it was 0.37 $\pm$ 0.08 (N=10) compared with that for ^{99m}TcEHDP which was 0.27 $\pm$ 0.05 (N=10). See Table I.

The net extraction E_{net} is the accumulated extraction at 5 minutes and is calculated from equation 4. E_{net} for ^{14}C sucrose (mean $\pm$ S.D.) was 0.14 $\pm$ 0.09 (N=10) and for ^{99m}TcEHDP was 0.18 $\pm$ 0.05 (N=10).

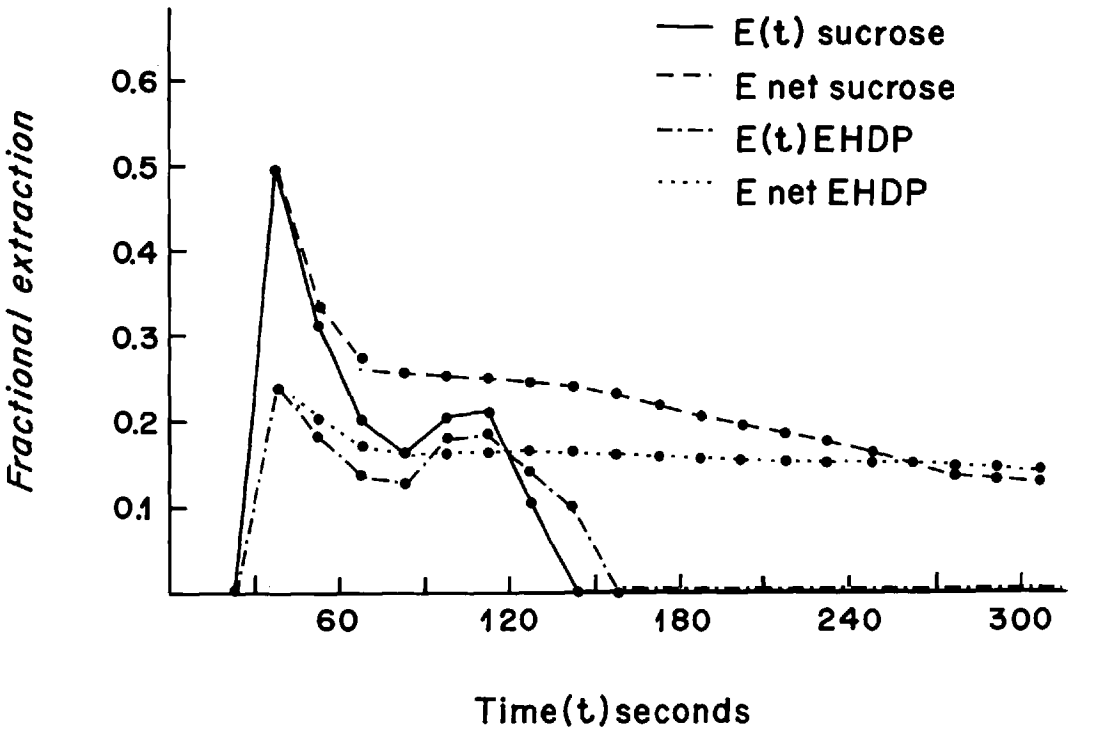
The amount of ^{99m}TcEHDP in the bone at 5 minutes was 21%. The fractional residuum of ^{99m}TcEHDP in the bone

FIGURE 6 (a)



Indicator dilution curves for ^{99m}TcEHDP, ^{51}Cr albumin and ^{14}C sucrose.

FIGURE 6 (b)



The extraction curves for ^{99m}TcEHDP and ^{14}C sucrose.

at 5 minutes derived from equation 5 was 0.215. The fractional recovery of ^{99m}TcEHDP at 5 minutes derived from equation 6 was 99%.

Comparative Extractions

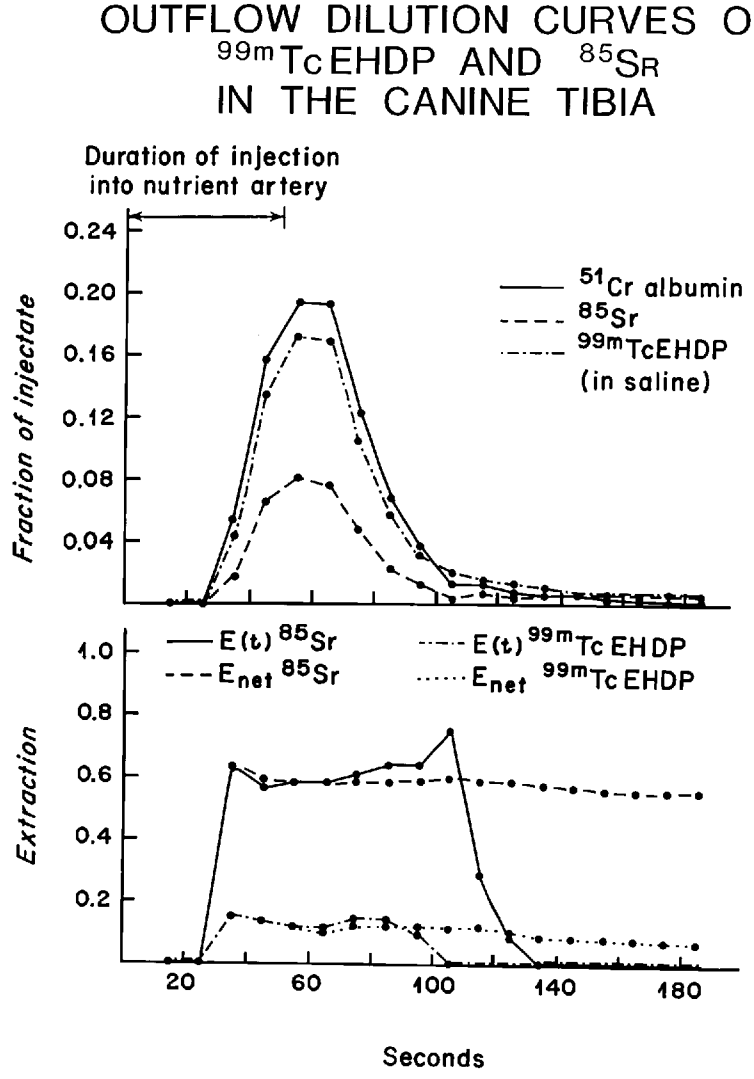
(i) Pyrophosphate:

The extraction of ^{99m}TcEHDP was compared with that of ^{99m}Tc pyrophosphate in 8 dogs. The E_{max} for ^{99m}Tc pyrophosphate was 0.43 $\pm$ 0.08 (N=4) and E_{max} for ^{99m}TcEHDP was 0.20 $\pm$ 0.1 (N=4). The net extraction at 2 minutes E_{net} for ^{99m}Tc pyrophosphate was 0.36 $\pm$ 0.1, N=4 and for ^{99m}TcEHDP was 0.16 $\pm$ 0.04, N=4. Statistically there was no difference between the extraction of the two substances.

(ii) Strontium:

Figure 7 shows the indicator dilution curves for ^{85}Sr and ^{99m}TcEHDP. In this situation there is an appreciable difference in the extraction rate of ^{85}Sr when compared to that of ^{99m}TcEHDP. E_{max} for ^{99m}TcEHDP was 0.33 $\pm$ 0.16 (N=3) and for ^{85}Sr was 0.75 $\pm$ 0.09 (N=3). E_{net} at 3 minutes for ^{99m}TcEHDP was 0.25 $\pm$ 0.2 (N=3) and for ^{85}Sr was 0.67 $\pm$ 0.1 (N=3), see also Table II.

FIGURE 7



(a) Outflow dilution curves for ^{51}Cr albumin, $^{99m}TcEHDP$ and ^{85}Sr.

(b) The extraction curves of $^{99m}TcEHDP$ and ^{85}Sr.

B TRANSCAPILLARY MOVEMENT OF ^{99m}TcEHDP

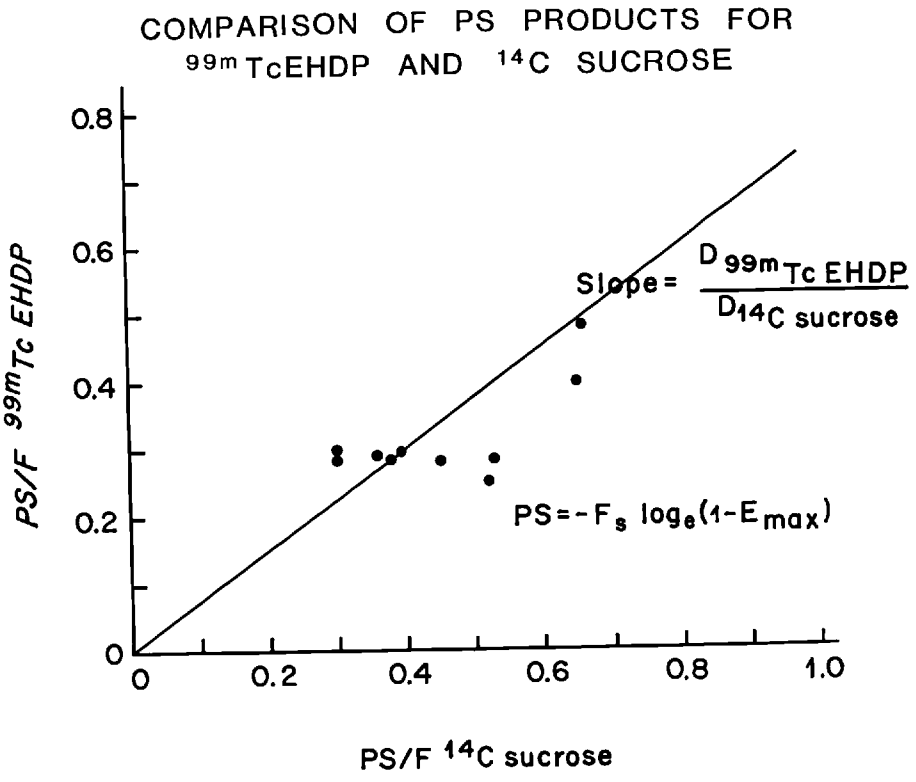
The permeability ratio of ^{99m}TcEHDP to ^{14}C sucrose calculated from equation 10 was 0.71 ± 0.16 (N=10). (mean ± S.D.). The diffusion coefficient of ^{99m}TcEHDP, (molecular weight 498) is 0.398 x 10^{-5} cm^2/sec. at 20°C and zero concentration and for ^{14}C sucrose (molecular weight 342) is 0.5226 x 10^{-5} cm^2/sec at 20°C and zero concentration (based upon figure 3.2 p. 68 of Stein 1967). The ratio of diffusion coefficients of ^{99m}TcEHDP to ^{14}C sucrose is 0.76.

The product of PS/F_s for ^{99m}TcEHDP is plotted against that of ^{14}C sucrose and is shown in Figure 8, the continuous line is the ratio of free diffusion coefficients. The data is distributed along the theoretical line for the diffusion coefficients, suggesting that there is no restriction to diffusion.

C BLOOD DISAPPEARANCE AND TISSUE UPTAKE

There was a rapid blood clearance of ^{99m}TcEHDP, particularly within the first twenty minutes, when 80 per cent of the injected dose disappears from the blood. By four hours, 97 per cent of the original injected dose was removed from the blood. In Figure 9 the percentage of injectate per ml is plotted against time on semi-logarithmic scale and shows the rapid clearance of the isotope from the circulation. There are essentially two components; the time constant of the first component is nine minutes and that of the second is 160 minutes.

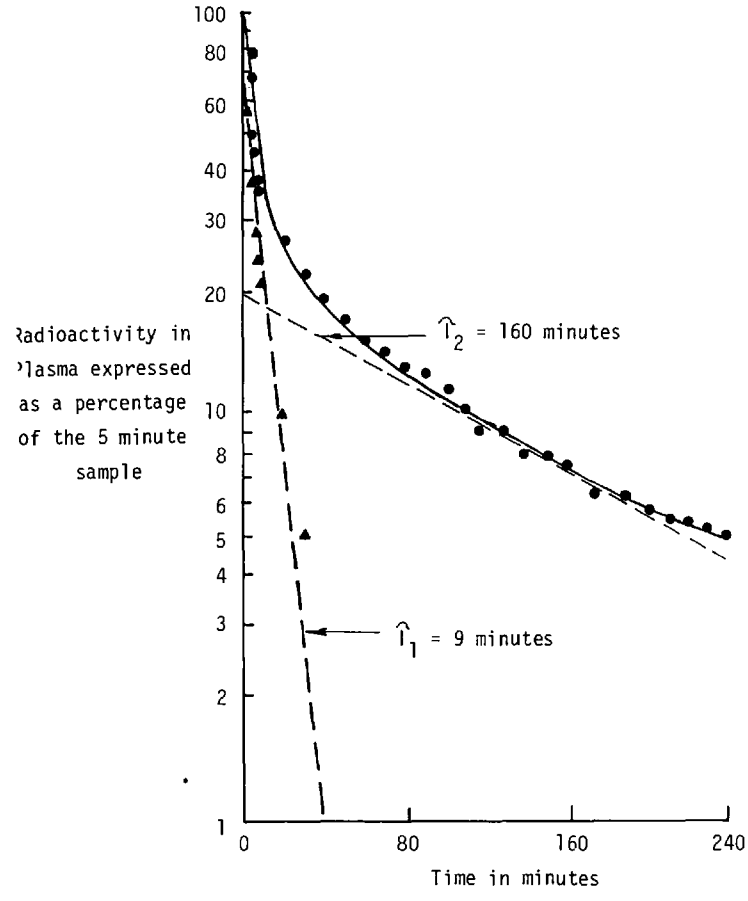
FIGURE 8



The relationship between PS/F_s ^{99m}TcEHDP and PS/F_s ^{14}C sucrose to tne ratio of their diffusion coefficients.

FIGURE 9

CAROTID ARTERY PLASMA DISAPPEARANCE CURVE FOR
^{99m}Tc EHDP IN 15.4 Kg DOG AFTER JUGULAR INJECTION

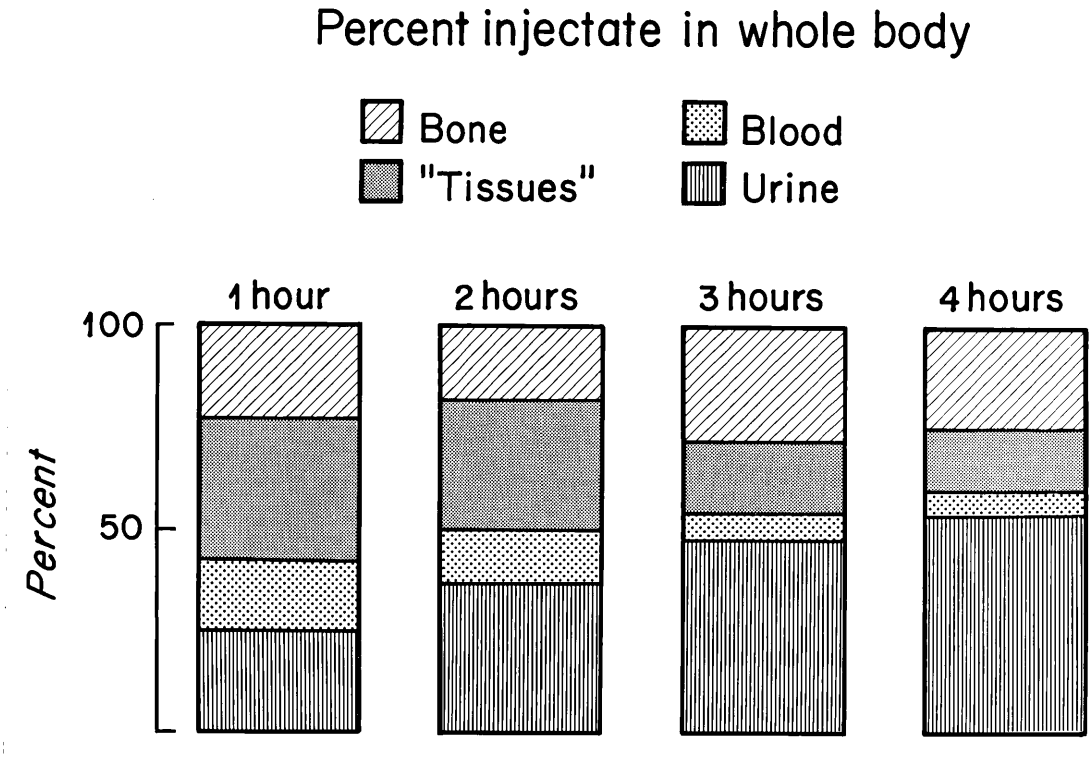


Plasma clearance curves for ^{99m}TcEHDP over 4 hours, to show the rapid plasma clearance and the 2 components of the curve.

The uptake in the tissues varies with time and is a reflection of the activity in the blood, Table III.

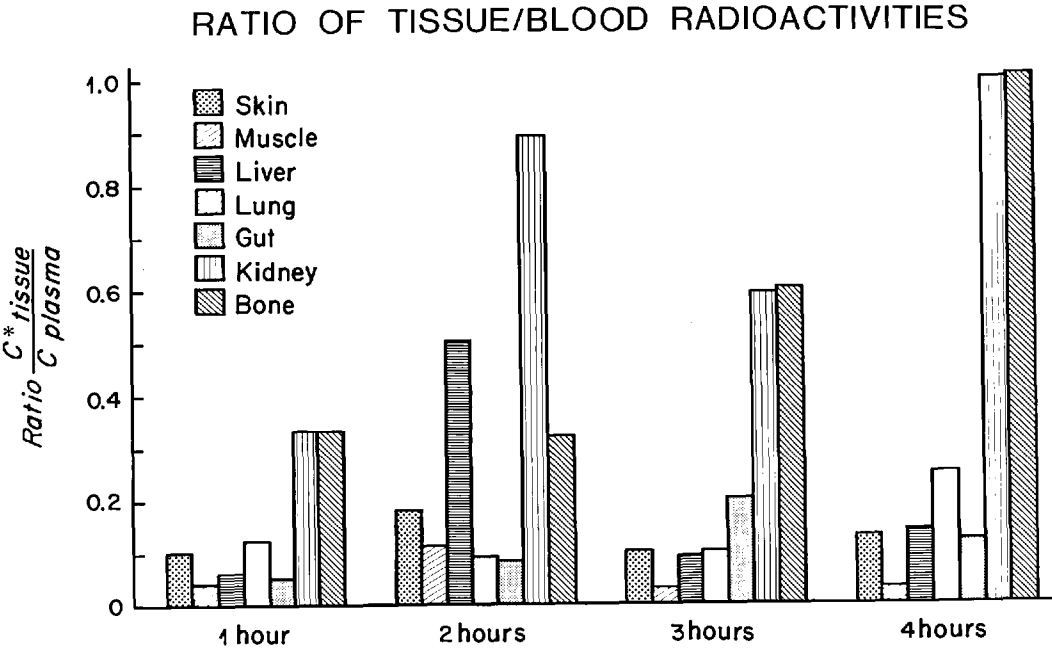
For an expression of the percentage of injectate in the body as a whole, five uninjected dogs were killed and dissected completely. The results of the mean percentageof tissue per body weight are shown in Table IV. From these figures were calculated the results given in Table V. The radioactivity of skin and muscle dropped 69% and 64% respectively between the first and the fourth hour. In contrast the liver, kidney, lung and gut show but a small drop in activity over the same period, ranging from 8 to 27%. The bone has a relatively consistent uptake over the 4 hour period with a mean of 23 $\pm$ 3% (N=11). The amount of ^{99m}TcEHDP in the urine increases from 25% to 54% by four nours, as snown in Figure 10. The ratio of radiactivity is expressed in Table VII and depicted in Figure 11. This shows that, at four hours, the ratio of radioactivity of bone to blood is four times that at one hour. This ratio reflects the background activity seen at tne time of bone scanning and although the kidney activity was high, that of the other tissues was relatively low and therefore the bone scanning picture will be clearer.

FIGURE 10



Percentage of injectate in various tissues of the body at 1,2,3 and 4 nours, showing the decreasing blood, increasing urine, but constant bone uptake.

FIGURE 11



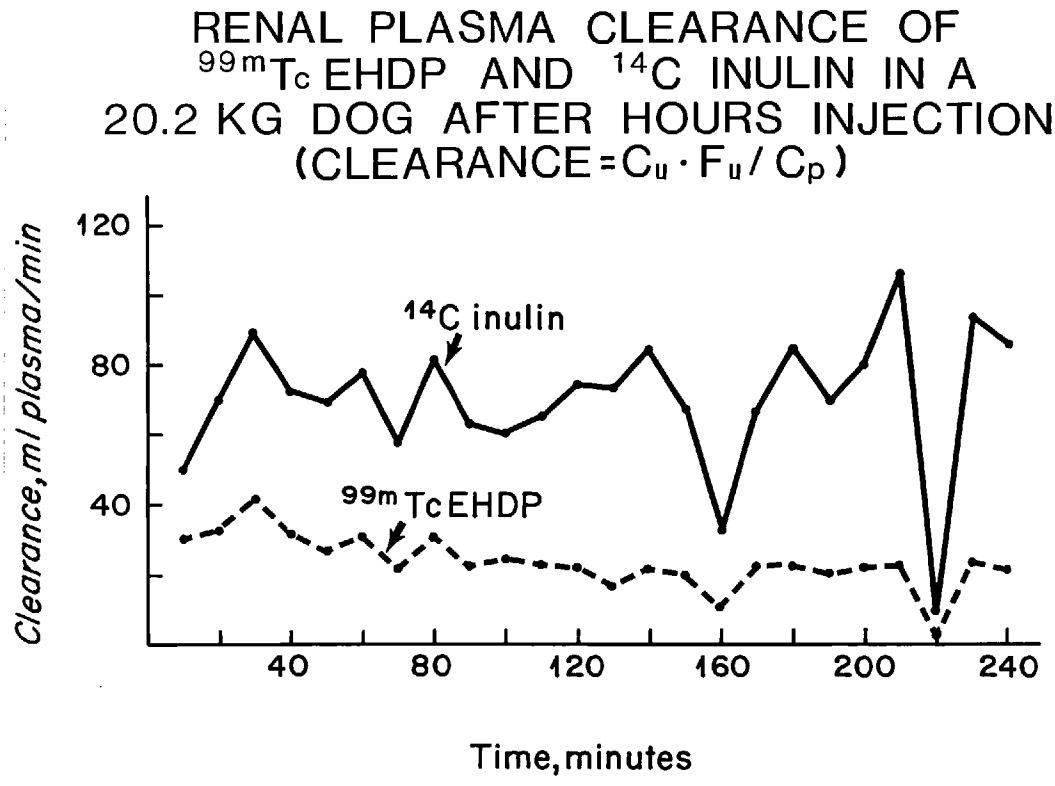
Ratios of radioactivity of tissue to that of plasma and 1,2,3 and 4 hours.

D RENAL CLEARANCE

The clearance for inulin in the control dogs, was 84,94,142 and 41ml/min, with a mean ± S.D. of 90.4 ± 42 ml/min; that for EHDP was 25,24,35 and 12ml/min with a mean of 24.1 ± 9.7ml/min. An example of the clearance is shown in Figure 12. The clearance in 3 dogs after total thyroparathyroidectomy did not differ from that in the control dogs, being for ^{99m}TcEHDP 30,30,21ml/min with a mean of 27 ± 5ml/min and for inulin 128,97,97ml/min with a mean of 107 ± 10ml/min. The serum calcium in this group fell from 10 to 5mg/100ml. In a third group of 3 dogs the effect of parathyroid hormone on phosphate clearance was compared with ^{99m}TcEHDP. The clearance for ^{99m}TcEHDP was similar to that in the previous experiments. The response of phosphate to parathyroid hormone is summarised in Table VI in which the fractional excretion of ^{99m}TcEHDP, that is the clearance of ^{99m}TcEHDP divided by the clearance of ^{14}C inulin is compared to the fractional excretion of phosphate, that is the clearance of phosphate divided by the clearance

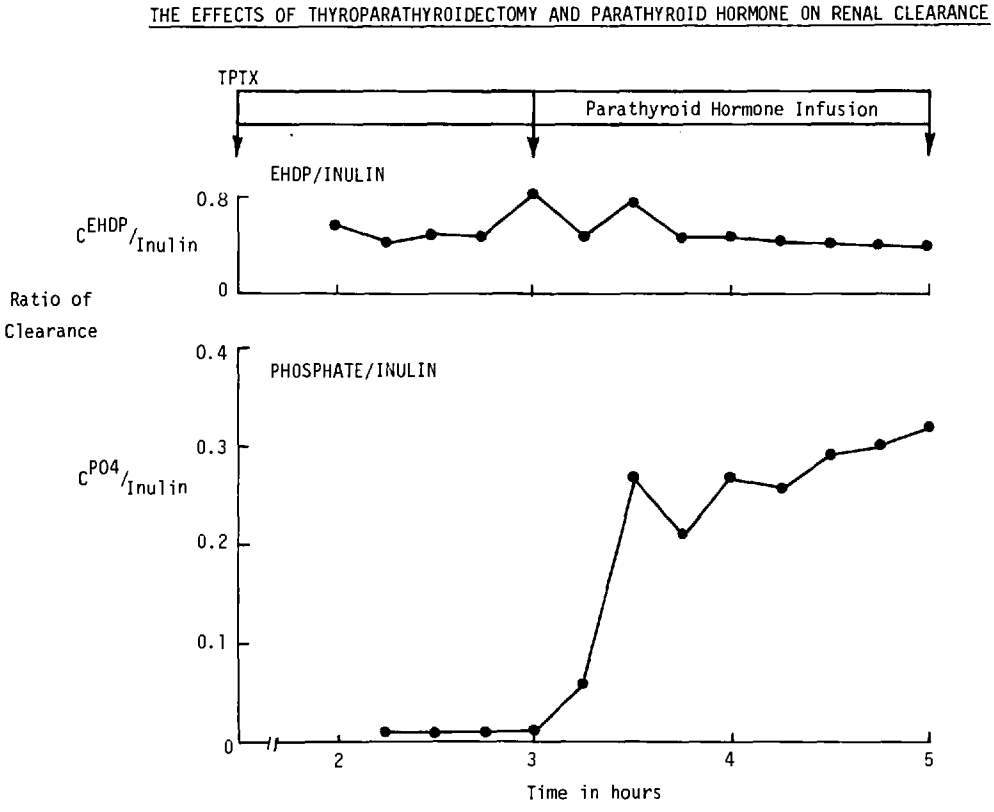
of ^{14}C inulin. In the thyroparathyroidectomised dog there was a marked increase in fractional excretion of phosphate, but there was no real difference in fractional excretion of ^{99m}TcEHDP following the administration of parathyroid hormone. Figure 13 and Table VII illustrate this.

FIGURE 12



Renal clearance of EHDP and inulin over 4 hours.

FIGURE 13



Renal clearance of phosphate, EHDP and inulin and the effect of PTH.

E FRACTURES

(i) Extraction following a canine tibial fracture.

At two weeks following the fracture E_{max} for ^{99m}TcEHDP was (mean ± S.D.) 0.32 ± 0.18 (N=4) and that for sucrose was 0.37 ± 0.2 (N=4). The net extraction at 5 minutes for ^{99m}TcEHDP was 0.28 ±0.16 (N=4) and sucrose was 0.17 ± 0.1 (N=4). The permeability ratio was 0.78 ± 0.2. Figure 14 shows a radiograph of the fracture at two weeks. Table VIII shows there is no statistical difference between controls and fractures even though the blood flow is known to increase at two weeks after fractures (Paradis et al 1975). (Hughes et al 1976).

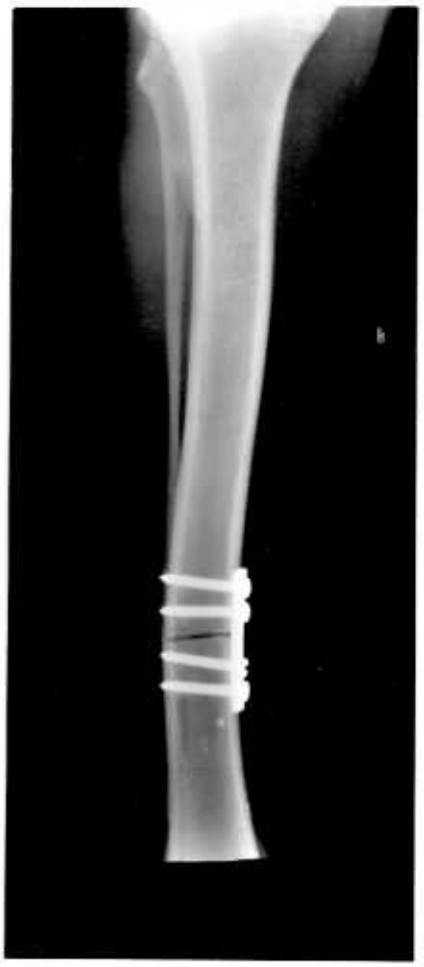
If the flow increases and the extraction remains the same the PS product must be raised (equation 9). That the capillaries become more permeable is unlikely, more probably there is an increase in surface area from recruitment and dilatation of the capillaries within the cortex of the bone, thus enlarging the surface area that is available for exchange.

(ii) ^{99m}Tc EHDP Bone Scans in patients

a. Subcapital fracture of the neck of femur.

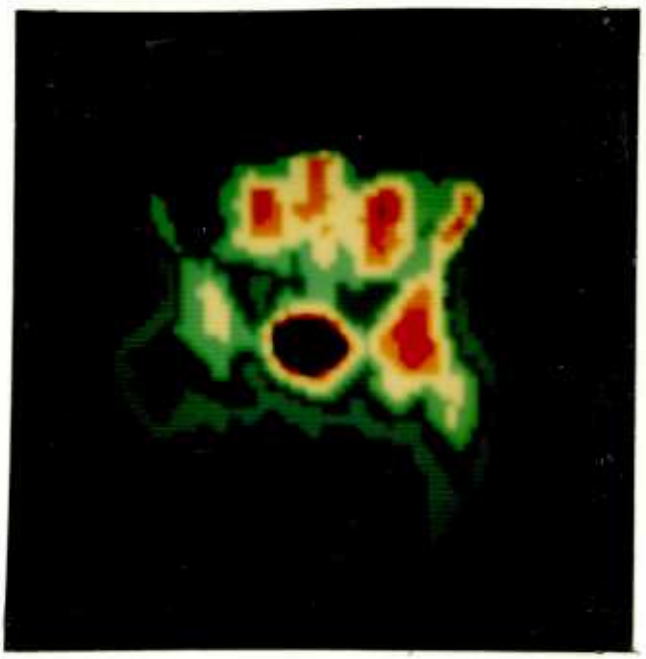
A 50 year old woman with a subcapital fracture of the neck of the left femur which was displaced, was treated by closed reduction and internal fixation with a Watson Jones nail. At two months she was painfree and walking with one stick.

FIGURE 14



Radiograph of the canine tibia, 2 weeks after fracture and fixation with 4 hole plate.

FIGURE 15



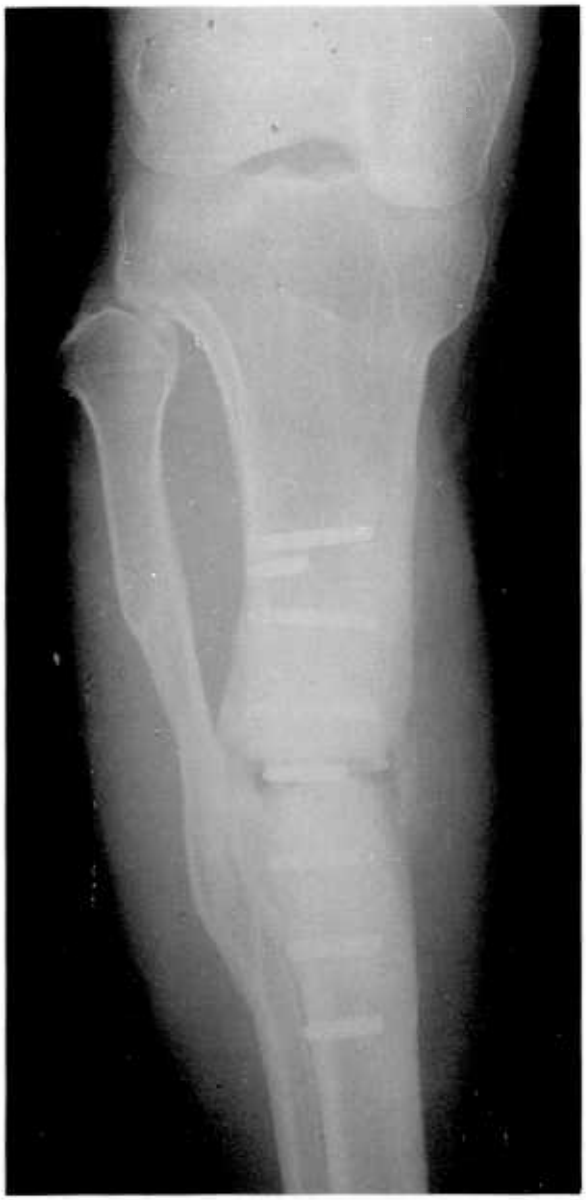
A ^{99m}TcEHDP bone scan of a patient with a 2 month old subcapital fracture which had been treated by internal fixation with a Watson Jones nail.

A bone scan at 8 weeks is shown in Figure 15 and demonstrates the vascularity associated with a healing fracture.

b. Tibial shaft fracture.

A 21 year old man was seen 3 years after fracture of the left tibia from a road traffic accident. His fracture had been treated by internal fixation and by bone graft, and had failed to unite. The x-ray is shown in Figure 16 there is non union. A bone scan was performed, there is activity around the ends but no activity at the fracture site Figure 17. Non union was confirmed at operation and the fracture was treated by a sliding bone graft and cancellous bone chips. The histology at the fracture site is shown in Figure 18. The bone is acellular, but there are a number of capillaries in the bone structure. At the actual fracture ends there was fibrous union. Further away from the fracture, where the bone scan was normal, the bone itself appeared normal.

FIGURE 16

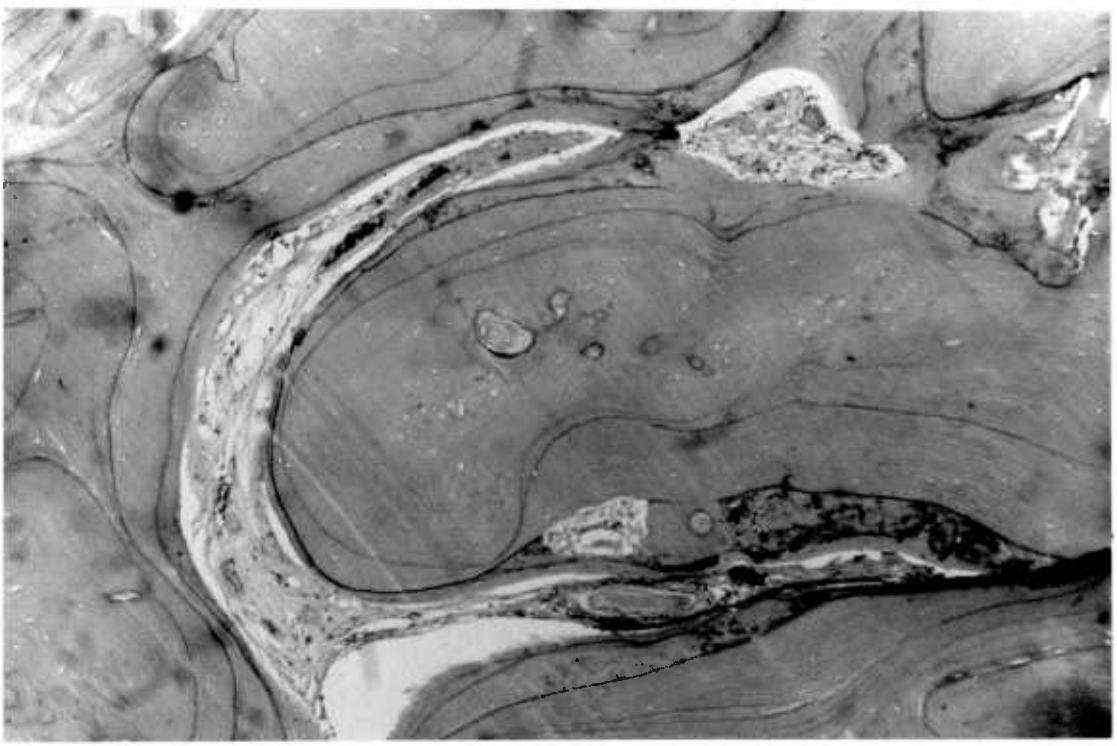


X-ray of NON UNION of a tibia in a patient 18 months after injury.

FIGURE 17

^{99m}TcEHDP bone scan of this non union of the tibia, showing diminished uptake at the fracture site, with increased activity around the fracture.

FIGURE 18



Histology of the corresponding area of increased uptake, showing capillaries within the bone.

DISCUSSION

I have studied the movement of ^{99m}TcEHDP across the capillaries within the Haversian canals, using the technique of simultaneous arterial injection of diffusible and nondiffusible tracers, which was introduced by Chinard et al (1955) Martin and Yudilevich (1964) and Crone (1963). The technique of adding a further isotope to the injection to produce a mixture of diffusible, and non diffusible tracers has been developed by Crone (1963). Davies et al (1976) have applied this method to bone to estimate the transcapillary exchange of strontium. A third tracer, sucrose, was used as the diffusible tracer because it is inert, penetrates the capillary wall, but does not penetrate cells and has a hydrated radius of 5.4 Å. It is therefore small enough to pass through the capillary clefts.

One of the main assumptions of the single-injection technique is that there is no back diffusion of the diffusible tracer into the flowing blood during the initial phase of the passage of the bolus. The effect of back diffusion in the present investigation is small because of the low extraction of ^{99m}TcEHDP and the relatively slow circulation of the blood through diaphyseal canine bone of 1.47 ml/100g. min. (Cofield et al 1975). The other main assumption is that the extravascular space is large enough for diffusion of the sucrose to occur. The presence of an extravascular space has been demonstrated by electron microscopy and by other techniques which I have previously discussed.

Recently this space has been measured in the canine tibia, using a triple isotope technique. Total water space was defined by ^{125}I antipyrine; vascular space by ^{99m}Tc albumin and extracellular space by ^{51}Cr EDTA. The findings were, that in the cortex, the cellular space was 14ml/100ml, the vascular space was 3ml/100ml and the interstitial space was 11ml/100ml of bone, Lopez-Curto et al (1976). The space in bone therefore appears to be large enough to allow the instantaneous extraction technique to be applied.

The extraction of $^{99m}TcEHDP$ by the canine tibia was low, as measured by the maximum instantaneous extraction (E_{max}), the accumulative extraction (E_{net}), and by the residuum at 5 minutes, 1,2,3 or 4 hours. This was particularly so in comparison with that of ^{85}Sr, which was 0.69, and ^{18}F which was 0.70 $\pm$ 0.08 N=8, Davies et al (1976).

The permeability ratio of $^{99m}TcEHDP$ to the freely diffusible sucrose was 0.71, which is similar to the ratio of free diffusion coefficients of $^{99m}TcEHDP$ to sucrose which is 0.76. The similarity between these two ratios suggests that the mechanism for transcapillary exchange of $^{99m}TcEHDP$ is through the capillaries in bone by unrestricted free passive diffusion across the capillary pore. $^{99m}TcEHDP$ is a much larger molecule than the others that have been studied and its low extraction is related to the effect of size on its passage through the transcapillary cleft. $^{99m}TcEHDP$ is rapidly cleared from the blood particularly within the first 20 minutes. In an earlier experiment investigating phosphate compounds in rabbit I demonstrated the rapid blood clearance of EHDP compared with that of pyrophosphate or tripolyphosphate. In this experiment there is also a rapid blood clearance of EHDP.

The analysis of the mathematical components of the blood disappearance curve shows two compartments in parallel. This may well be an over simplification of the situation, however, it gives information of the relative speeds of movement of ^{99m}TcEHDP from the blood into the main tissues of distribution - namely the bone and the urine.

The percentage uptake in the organs varies, and whereas the bone uptake is constant at between twenty and thirty % of the initial dose over the 4-hour period, the tissue and blood levels decrease and the urine excretion increases. The total bone uptake occurs in the first few minutes and probably by the process of "short term exchange", and then the blood level falls with time, while the urine level rises.
The percentage radioactivity in the tissue varies, being highest in the first hour and lowest by 4 nours. The ratio of radioactivity of tissue to that of plasma is highest for bone at 4 hours, this confirms that this time is the most favourable one for a bone scan.

The renal clearance of ^{99m}TcEHDP was found to be less than that of ^{14}C inulin in all three groups studied; controls thyroparathyroidectomized dogs and thyroparathyroidectomized animals given parathyroid hormones. This indicates that EHDP is not secreted by the dog kidney. Troehler et al (1975) have shown that diphosphonates are secreted by the rat nephron, but they also indicated that phosphate is secreted by the tubule of the rat. This suggests a difference in renal handling of phosphate and diphosphonates

by the rat as compared with the dog. I did not find any difference in the clearance of ^{99m}TcEHDP compared with that of inulin after total thyroparathyroidectomy or after parathyroid infusion, although in the same experiment the phosphate excretion was influenced by the parathyroid hormone. Recker et al (1973) after giving EHDP without technetium to male volunteers could not find any influence of parathyroid hormone control on that compound.

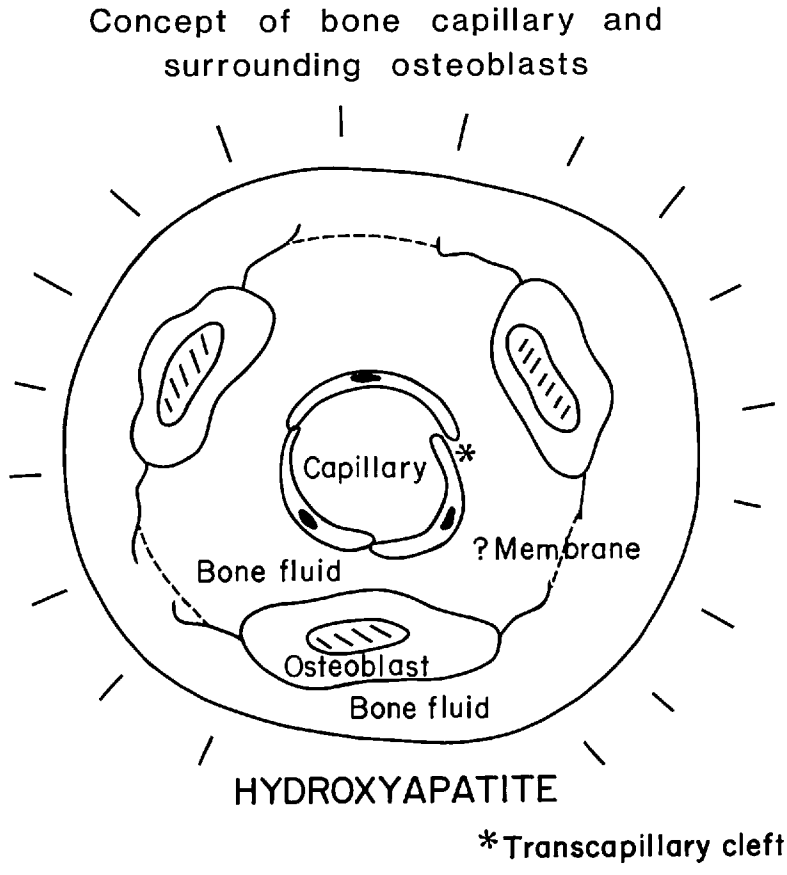
Bone blood flow increases following a fracture, reaching a maximum level at two weeks, but the extraction of ^{99m}TcEHDP does not change. This is because there is an increase in the surface area made available for exchange of minerals, from recruitment and dilatation of capillaries already in the Haversian system. Previously I had studied the clearance of ^{99m}TcEHDP and the microangiographic changes in a rabbit's tibial fracture and found that the number of blood vessels increased as did the bone clearance of ^{99m}TcEHDP returning to normal when the fracture had healed (Hughes 1976). This characteristic of fracture healing has been demonstrated using other techniques by Rhinelander and by Gotham.

Therefore the isotopic activity is proportional to the surface area available for exchange in the bone. This relationship was clearly seen in the patient who had non-union of the tibia; there was increased isotopic activity in the bone where there were numerous capillaries, and no activity in the area of fibrous union.

In a study of 14 patients who had osteosarcoma involving their femurs or tibias, ^{99m}TcEHDP bone scans were performed at various time intervals during the patient's treatment. The bone scans showed an increase in the uptake of ^{99m}TcEHDP well away from the tumour site, which appeared to be more extensive than the radiographic and pathological appearances of the tumour (Hughes et al 1976). In infected hips following total replacement the ^{99m}TcEHDP bone scan again showed areas of increased activity out of proportion to the extent of cellular turnover and formation (Hughes and Benson 1976). Therefore based upon the experimental findings it is now possible to relate the amount of activity seen on the bone scan with the state of the blood flow through the cortical bone.

It is my conclusion that ^{99m}TcEHDP, a bone scanning agent which is being used increasingly frequently, enters bone by the process of free passive diffusion. It leaves through the capillary cleft, having a low extraction due to the relatively large size of the molecule. It passes from the capillary to the hydroxyapatite through the family of osteoblasts that surround the capillary, as is shown in Figure 19. The increased activity seen on a bone scan is a blood flow phenomenon and is dependent on the increase in the surface area that is available for the exchange of minerals, from recruitment, enhancement and dilatation of capillaries.

FIGURE 19



Concept of a capillary in bone surrounded by fluid and a family of osteoblasts and separated from the hydroxyapatite crystal.

SUMMARY

^{99m}TcEHDP is a compound which is now routinely used in bone scanning. In this thesis it has been studied in the experimental animal and in patients. Following intravenous injection the isotope passes to the bone and out in the urine. In the bone ^{99m}TcEHDP leaves the capillaries within the Haversian System, through the capillary clefts and then passes to the hydroxyapatite crystal. It has a low extraction, whether measured by instantaneous extraction, accumulated extraction or by residuum at 5 minutes, 1,2,3 or 4 hours. This is because it is a large molecule and therefore moves relatively slowly through the capillary clefts by the process of free passive diffusion. In the kidney the clearance is lower than that of inulin and is unaffected by parathyroid hormone.

Following a fracture there is an increase in the bone blood flow, particularly at 2 weeks. However the extraction of ^{99m}TcEHDP by the canine tibia is not affected and that therefore an increase in flow in the capillary produces an

increase in surface area from recruitment and dilatation of the capillaries.

Hence ^{99m}TcEHDP behaves in a manner similar to that of other minerals in its transport through bone, obeying biophysical principles.

^{99m}TcEHDP is now generally available for bone scanning and can be used repeatedly without major radiation hazards to the patient. This can be helpful to patients with fractures of bones who can now be followed up regularly and their response or resistance to treatment analysed. Further studies are under way to examine the uptake of ^{99m}TcEHDP in patients who have had subcapital fractures and those who have had infections in bone.

Many factors influence the movement of the bone seeking isotopes onto the hydroxyapatite crystal. These include the rate of blood clearance, the tissue distribution and the renal control.

Equally important is the transcapillary exchange, which depends on surface area, blood flow and capillary permeability. But in the end it is the vasculature that is important for the changes which occur in bone as "they bring the supplies to the bone for its increase" (John Hunter 1798).

---oOo---

GLOSSARY

Q	=	Flux
D	=	Diffusion coefficient cm^2/sec
A	=	Cross sectional area cm^2
C	=	Concentration mg/cm^3
t	=	Time in seconds
x	=	Distance in cm
R	=	Gas constant (8.3143×10^7 erg/k/mol)
T	=	Time constant
π	=	3.1416
η	=	Viscosity cm/g/sec
a	=	Particle radius, cm
N	=	Avagadro's number (6.022×10^{23} mol.)
P	=	Permeability of the capillary cm/sec
S	=	Capillary surface area cm^2/g
F_s	=	Flow of the solute carrying fraction of the perfusate (plasma) ml/g/sec
E	=	Extraction (dimensionless)
$E(t)$	=	Instantaneous fractional extraction
E_{max}	=	Maximum instantaneous extraction
$h(t)$	=	Fraction of injected dose/sec
$h_R(t)$	=	Fraction of injectate of nondiffusible tracer, albumin
C_t	=	Concentration in tissues in cpm
C_p	=	Concentration in plasma in cpm
τ_1	=	Time constant for fast component
τ_2	=	Time constant for slow component

C_u = Concentration of isotope in urine cpm/ml

F_u = Flow in urine ml/min

C_p = Concentration of isotope in plasma cpm/ml

R(T) = Residuum at time (T)

F_v = Flow in the femoral vein ml/g/sec

C(t) = Concentration of the tracer in the blood

F = Frictional force opposing each molecule

f = fractional uptake

W_t = tissue weight gm

ACKNOWLEDGEMENTS

I should like to express my gratitude to Dr. Patrick Kelly, Mr. Selwyn Taylor and Mr. Rodney Sweetnam, for their continuous encouragement and guidance with this study.

I should also like to thank Mr. Rob Davies and Dr. Jim Bassingthwaighte for the extremely helpful advice.

I am indebted to Mr. Glen Christensen for instructing me in laboratory techniques. I should like to thank Mrs Rose Garmers for typing the original manuscript and also Miss Elaine Etgert for typing the final manuscript.

The laboratory work for this thesis was carried out while I was a Research Assistant to Dr. Kelly at the Mayo Clinic, Rochester. The patients were under the care of Mr. Sweetnam at the Middlesex and I am grateful for the facilities made available to me by Dr.Keith Britton of the department of Nuclear Medicine at The Middlesex Hospital.

---oOo---

TABLE I

EXTRACTION

	^{14}C sucrose		^{99m}TcEHDP		P ratio
	E_{max}	E_{net}	E_{max}	E_{net}	
	0.49	0.13	0.32	0.17	0.57
	0.30	0.003	0.25	0.12	0.81
	0.28	0.01	0.26	0.15	0.92
	0.36	0.18	0.24	0.14	0.61
	0.41	0.17	0.22	0.18	0.47
	0.41	0.10	0.24	0.15	0.52
	0.33	0.15	0.25	0.19	0.72
	0.26	0.09	0.25	0.17	0.96
	0.48	0.23	0.37	0.24	0.71
	0.31	0.32	0.25	0.27	0.78
Mean	0.36	0.14	0.27	0.18	0.71
S.D.	0.08	0.09	0.05	0.05	0.18
N	10	10	10	10	10

TABLE II

COMPARISON OF EXTRACTIONS OF PYROPHOSPHATE, ^{85}Sr AND EHDP

	^{99m}Tc Pyrophosphate		^{99m}TcEHDP	
	E_{max}	E_{net}*	E_{max}	E_{net}*
	0.32	0.24	0.36	0.20
	0.40	0.37	0.16	0.14
	0.44	0.30	0.14	0.17
	0.53	0.50	0.06	0.11
Mean	0.43	0.36	0.20	0.16
S.D.	±0.08	±0.1	±0.1	±0.04
N	4	4	4	4

	Strontium 85		^{99m}TcEHDP	
	E_{max}	E_{net}**	E_{max}	E_{net}**
	0.66	0.56	0.17	0.07
	0.79	0.67	0.30	0.24
	0.84	0.78	0.50	0.42
Mean	0.75	0.67	0.33	0.25
S.D.	0.09	0.1	0.17	0.16
N	3	3	3	3

*E_{net} = 2 minutes

**E_{net} = 3 minutes

TABLE III

MEAN PERCENTAGE INJECTATE PER 100 g TISSUE

	1 hr*	2 hr*	3 hr*	4 hr ± S.D.**
Skin	0.42	0.30	0.13	0.09 ± 0.05
Muscle	0.13	0.08	0.04	0.03 ± 0.01
Liver	0.22	0.88	0.44	0.14 ± 0.05
Kidney	1.4	1.7	0.79	0.86 ± 0.5
Lung	0.15	0.19	0.13	0.18 ± 0.18
Gut	0.37	0.13	0.10	0.11 ± 0.02
Bone	1.07	0.56	0.85	0.57 ± 0.08

* N = 2

**N = 5

TABLE IV

STANDARD % WEIGHT OF 5 DOGS

Skin	=	13.0 %
Muscle	=	50.0 %
Liver	=	3.4 %
Kidney	=	1.1 %
Lung	=	1.7 %
Gut	=	7.3 %
Bone	=	13.5 %
	=	90.0 %

TABLE V

PERCENTAGE OF INJECTATE IN WHOLE BODY

	1 hr	2 hr	3 hr	4 hr	% drop
Skin	9	9	4	3	69
Muscle	10	10	4	3	64
Liver	1	7	3	1	8
Kidney	2	4	2	2	16
Lung	1	1	1	1	27
Gut	2	2	2	2	5
Bone	23	18	28	27	-8
Blood	17	13	9	6	67
Urine	25	37	48	54	-16

TABLE VI

MEAN RATIO OF RADIOACTIVITY 1 g TISSUE TO 1ml BLOOD

	1 hr	2 hr	3 hr	4 hr
Skin	0.10	0.18	0.10	0.13
Muscle	0.04	0.06	0.03	0.03
Liver	0.06	0.50	0.09	0.14
Kidney	0.33	0.89	0.59	1.0
Lung	0.12	0.09	0.10	0.25
Gut	0.05	0.08	0.20	0.12
Bone	0.33	0.32	0.60	1.03

TABLE VII

RESPONSE OF PHOSPHATE AND EHDP TO PARATHYROID HORMONE (MEAN ± S.D)

Exp.	No. Clearance Periods	C_{EHDP} (ml/min)	C_{PO_4} (ml/min)	C_{In} (ml/min)	C_{EHDP}/C_{In}	C_{PO_4}/C_{In}
1.	Thyroparathyroidectomy (3)	30.6 ± 1.1	0.20 ± 0.06	77.8 ± 4.4	0.39 ± 0.03	0.003 ± 0.0008
	Parathyroid Infusion (3)	21.2 ± 6.3	3.90 ± 1.20	46.1 ± 3.2	0.46 ± 0.11	0.090 ± 0.03
2.	Thyroparathyroidectomy (3)	26.8 ± 0.43	2.90 ± 0.2	58.3 ± 3.9	0.46 ± 0.03	0.049 ± 0.0002
	Parathyroid Infusion (10)	32.8 ± 2.2	13.0 ± 0.9	64.7 ± 2.3	0.51 ± 0.04	0.200 ± 0.02
3.	Thyroparathyroidectomy (2)	33.5 ±12.5	0.45 ± 0.11	47.0 ± 7.0	0.71 ± 0.16	0.009 ± 0.001
	Parathyroid Infusion (8)	28.6 ± 3.8	13.5 ± 1.5	57.2 ± 4.9	0.49 ± 0.04	0.250 ± 0.03

TABLE VIII

TWO WEEK FRACTURES AND EXTRACTION

	^{99m}TcEHDP		Sucrose		P EHDP/ Sucrose
	E_{max}	E_{net}	E_{max}	E_{net}	
	0.31	0.36	0.42	0.15	0.68
	0.07	0.07	0.11	0.09	0.59
	0.51	0.43	0.58	0.35	0.86
	0.39	0.29	0.38	0.10	1.03
Mean	0.32	0.28	0.37	0.17	0.78
S.D.	± 0.18	± 0.16	±0.2	± 0.12	± 0.2

E_{net} = 5 minutes.

TABLE IX

COMPARISON OF EXTRACTION
(Mean ± S.D.)

Present Study	E_{max}	E_{net} (3 min)
1. ^{99m}TcEHDP	0.27 ± 0.05 (N = 10)	0.18 ± 0.05* (N = 10)
2. ^{99m}Tc Pyrophospnate	0.43 ± 0.08 (N = 4)	0.36 ± 0.1** (N = 4)
3. ^{85}Sr	0.75 ± 0.1 (N = 3)	0.67 ± 0.1 (N = 3)
Earlier Studies		
^{85}Sr (Copp and Shim 1965)	---	0.76 ± 0.06 (N = 10)
^{85}Sr (Cofield et al 1975)	0.53 ± 0.08 (N = 12)	0.41 ± 0.06 (N = 12)
^{85}Sr (Davies et al 1975)	0.69 ± 0.1 (N = 14)	0.56 ± 0.1 (N = 14)

* at 5 minutes

**at 2 minutes

REFERENCES

1. Ailsby, R.L. and Staheli, L.T.: Pyogenic infections of the sacro-iliac joint in children. Radioisotope bone scanning as a diagnostic tool. Clin. Orth & Related Res. 100:96-100, 1974.

2. Albright, F. and Reifenstein, E.C.: The parathyroid gland and metabolic bone diseases. Williams and Wilkins, Baltimore, 1948.

3. Bassingthwaighte, J.B.: A concurrent flow model for extraction during transcapillary passage. Circ. Res. 35:483-503, 1974.

4. Bauer, G.C.H., Lindberg, L., Naversten, Y. and Sjöstrand L.O.: ^{85}Sr radionuclide scintimetry in infected total hip arthroplasty. Acta. Orth. Scand. 44:439-450, 1973.

5. Beck, R.E. and Schultz, J.S.: Hindered diffusion in microporous membranes with known pore geometry. Science. 170:1302-1305, 1970.

6. Becker, R.R. Hassing, G.S., Lar, J.R. and Saville, P.D.: The hyperphosphatemic effect of disodium ethane-1-hydroxy-1, 1-diphosphate (EHDP ^{TM}): renal handling of phosphorus and the renal response to parathyroid hormone. J. Lab. Clin. Med. 81. 258-266, 1973.

7. Blumenthal, N.C. and Posner, A.S.: Hydroxyapatite: Mechanism of Formation and Properties. Calc. Tissue Res. 13:235-243, 1973.

8. Bohr, H.: Densitometry and ^{18}F scintigraphy in the study of the revascularization of the femoral head in coxa plana (C-P). Acta Orth. Scand. 44:417-425, 1973.

9. Bosch, W.J.: Plasma ^{45}Ca clearance by the tibia in the immature dog. Am. J. Physiol. 216:1150-1157, 1969.

10. Branemark, Per-Ingrar: Experimental investigation of microcirculation in bone marrow. Angiology, 293-305, 1961.

11. Brookes, M. and Harrison, R.G.: The vascularization of the rabbit femur and tibiofibula. J. Anat. 91:61-72, 1957.

12. Brookes, M., Elkin, A.C., Harrison, R.C. and Heald, C.B.: A new concept of capillary circulation in bone cortex. Lancet. 1:1078-1081, 1961.

13. Brookes, M., Richard, D.J., and Singh, M.: Vascular sequelae of experimental osteotomy. Angiology 21: 355-367, 1970.

14. Callahan, R.J. and Castronovo, F.P.: Development of technetium-99m labelled 1-hydroxy-ethylidene-1,1-disodium phosphonate for skeletal imaging. Am. J. Hosp. Pharm. 30:614-617, 1973.

15. Canas, F., Terepka, A.R. and Neuman, W.F.: Potassium and milieu interieur of bone. Am. J. Physiol. 217:117, 1973.

16. Castronovo, F.P., Jr., and Callahan, R.J.: New bone scanning agent: ^{99m}Tc-labelled 1-hydroxy-ethylidene-1, 1-disodium pnosphonate. J. Nuclear Med. 13:823-827, 1972.

17. Charkes, N.D. and Sklaroff, D.M.: Early diagnosis of metastatic bone cancer by photoscanning with strontium-85. J. Nuclear Med. 5:168-179, 1964.

18. Chinard, F.P., Vosborough, G.J. and Enns, T.: Transcapillary exchange of water and of other substances in certain organs of the dog. Am. J. Physiol. 183:221-234, 1955.

19. Cofield, R.H., Bassingthwaighte, J.B. and Kelly, P.J.: Strontium-85 extraction during transcapillary passage in tibial bone. J. Appl. Physiol. 39: 596-602, 1975.

20. Cohen, J. and Harris, W.H.: The three dimensional anatomy of Haversian systems. J.B.J.S. 40(A):419-434, 1958.

21. Cooper, R.R., Migram, J.W., and Robinson, R.A.: Morphology of the osteon. J.B.J.S. 48(A)1239-1271, 1966.

22. Copp, D.H. and Shim, S.S.: Extraction ratio and bone clearance of Sr^{85} as a measure of effective bone blood flow. Circ. Res. 16:461-467, 1965.

23. Crone, C.: The permeability of capillaries in various organs as determined by use of the 'indicator diffusion' method. Acta. Physiol. Scand. 58:292-305, 1963.

24. Crone, C.: Does "restricted diffusion" occur in muscle capillaries? Proc. Soc. Exper. Biol.and Med. 112:453-455, 1963.

25. Davies, D.R., Bassingthwaighte, J.B., and Kelly, P.J.: Transcapillary exchange of strontium and sucrose in canine tibia. J. Appl. Physiol. In press.

26. Davies, D.R., Hughes, S.P., Bassingthwaighte, J.B. and Kelly, P.J.: Transcapillary exchange of ^{18}F, ^{14}C sucrose and ^{14}C-antipyrine in bone. Submitted to J. App. Physiol.

27. Einstein, A.: Über die von der molekularkinetischen theorin der wärme geforderee bewegung von in ruhenden flussigkeisen suspendierten teilchen. Annalen der Physik. 17:549-560, 1905.

28. Fick, A.: Ueber Diffusion: Annalen der Physik. 94: 59-86, 1855.

29. Fleisch, H. and Neuman, W.F.: Mechanisms of calcification: Role of collagen polyphosphate and phosphatase. Am. J. Physiol. 200:1296-1300, 1961.

30. Fleisch, H., Russell, R.G.G., Bisaz, S. and Bonjour, J.P: The effects of pyrophosphate and diphosphonates on calcium metabolism. in: Hard Tissue Growth Repair and Remineralization. Ciba Foundation. pp 331-358, 1973.

31. Francis, M.D.: The innibition of calcium hydroxyapatite crystal growth by polyphosphonates and polyphosphates. Cal. Tissue Res. 3:151-162, 1969.

32. Galasko, C.S.B.: Skeletal metastases and mammary cancer. Annals of the Roy College of Surg. Eng. 50:3-28, 1972.

33. Geisler, J.Z. and Neuman, W.F.: The membrane control of bone potassium. Proc. Soc. Exp. Biol. Med. 130:608-611, 1969.

34. Glimcher, M.J., Andrikides, A. and Kossiva, J.: Studies of the mechanisms of calcification. In: Structure and Function of Connective and Skeletal Tissue. pp 234-255, Butterwortn, London, 1965.

35. Gotham, Lar Vascular reactions in experimental fractures, microangiographic and radioisotopic studies. Acta Chir. Scan. Supp 284:1-34, 1961.

36. Guller, B., Yipintsoi, T., Orvis, A.L. and Bassingthwaighte J.B.: Myocardial sodium extraction at varied coronary flows: Estimation of capillary permeability by residue and outflow detection. Circ. Res. (In press).

37. Ham, A.W.: Histology. 2nd Ed. pp 250-252, Lippincott, Philadelphia. 1953.

38. Hughes, S.P.F. and Ell, P.J.: An appraisal of bone scanning in clinical practice. J. Irish College of Physicians and Surg. April, 4 154-157 1975.

39. Hughes, S.P.F., Jeyasingh, K. and Lavender, P.J.: Phosphate compounds in bone scanning. J.B.J.S. 57(B) 214-216, 1975.

40. Hughes, S.P.F., Davies, D.R., Bassingthwaighte, J.B. and Kelly, P.J.: Extractions of minerals following fractures of the canine tibia. Submitted J.B.J.S. 1976.

41. Hughes, S.P.F.: Clearance of ^{99m}TcEHDP following a fractur of the rabbits tibia. Submitted Acta Orthop. Scand. 1975.

42. Hughes, S.P.F., Benson, M.K.D., Ell, P.J. and Britton, K.D. Bone scanning with technetium labelled EHDP in the detection of metastases from osteosarcoma. Submitted Brit. J. Radiol. 1976.

43. Hughes, S.P.F., and Benson, M.K.D.: Infection following total hip replacement in a general hospital without separate orthopaedic facilities. Presented BOA April, 1976.

44. Hunter,J.: Experiments and observations of the growth

of bones from the papers of the late Mr. Hunter. E. Home, 1798. Works of John Hunter, Volume IV, Animal Economy, London. Longman, 1837.

45. Johansen, A.: Fracture healing controlled by ^{87m}Sr uptake. Acta Ortho. Scand. 44:628-639, 1973.

46. Johnson, R.W.: A physiological study of the blood supply of the diaphysis. J.B.J.S. 9:153-184, 1927.

47. Jowsey, J., Riggs, B.L. Kelly, P.J., Hoffman, D.L. and Bordier, P.: Treatment of osteoporosis with disodium ethane-1-hydroxy-1, 1-diphosphonate. J. Lab & Clin. Med. 78:574-584, 1971.

48. Jung, A., Bisaz, S., and Fleisch, H.: The binding of pyrophosphate and two diphosphonates by hydroxyapatite crystals. Cal. Tissue Res. 11:269-280, 1973.

49. Kaplan, W.D., Holman, F.L., Liebow, P.A. and Davis, M.A.: Enhanced detection of a skeletal lesion with delayed ^{99m}Tc polyphosphate bone scanning. J. Nuclear Med. 15:47-49, 1973.

50. Karnovsky, M.J.: The ultrastructure basis of transcapilla exchanges. J. Gen. Physiol. 645-955, 1968. Supplement.

51. Kelly, P.J. and Janes, J.M.: Microangiographic and histologic studies of vascular anatomy of the femur and tibia distal to femoral and iliac arteriovenous fistulas in dogs. Anat. Record. 162:255-268, 1968.

52. Kelly, P.J., Yipintsoi, T. and Bassingthwaighte, J.B.: Blood flow in canine tibial diaphysis estimated by iodoantipyrine ^{125}I-washout. J. Appl. Physiol. 31: 38-47, 1971.

53. Kemp, H.B.S., Johns, D.L., McAlister, J. and Godlee, J.N.: The role of fluorine-18 and strontium-87m scintigraphy in the management of infected spondylitis. J.B.J.S. 55(B): 301-311, 1973.

54. Krane, S.M. and Glimcher, M.J.: Transphosphorylation from nucleoside di and triphosphate by apatite crystal. J. Biol. Chem. 237-2991-2998, 1962.

55. Krishnamurthy, G.T., Thomas, P.B., Tubis, M., Endow, J.S., Prichard, J.H. and Blahd, W.H.: Comparison of ^{99m}Tc polyphosphate and ^{18}F. I. Kinetics. J. Nuclear Med. 15:832-836, 1974.

56. Laurnen, E.L. and Kelly, P.J.: Blood flow: Oxygen consumption; carbon dioxide production and blood calcium and pH changes in tibial fractures in dogs. J.B.J.S. 51(A): 298-308, 1969.

57. LeGros, Clark: Tissues of the body. pp 104, 1945. 2nd Edition. Oxford. Clarendon Press.

58. Lopez-Curto, J., Hughes, S.P.F., Kelly, P.J. and Bassingthwaighte, J.B.: Insterstial fluid space in bone. Abstract F.A.S.E.B., April 1976.

59. Martin, P. and Yudilevich, D.: A theory for quantification of transcapillary exchange by tracer dilution curves. Am. J. Physiol. 207:162-168, 1964.

60. Muheim, G.: Assessment of fracture healing in man by serial ^{87m}strontium scintimetry. Acta Ortho. Scand., 44:621-627, 1973.

61. Nelson, G.E., Kelly, P.J., Peterson, L.F.A. and Janes, J.M.: The blood supply of the human tibia. J.B.J.S. 42(A)625-636 1960.

62. Nernest, W.: Zur kinetik der in lösung befindlichen körper. Zeitschrift fur Physikalische Chemie. 2: 613-637, 1888.

63. Neuman, W.F. and Mulgran, B.J.: Synthetic hydroxyapatite crystals. Cal. Tissue Res. 7:133-138, 1971.

64. Neuman, W.F. and Ramp, W.K.: The concept of a bone membrane. Some implications. In: Cellular mechanisms for calcium transfer and homeostasis. Edited by Nichols, G. and Wasserman, R.H. pp 197-209, Academic Press. New York/London, 1971.

65. Owen, M., Triffit, J.T. and Melick, R.P.: Albumin in bone. In: Hard Tissue Growth, Repair and Mineralization. pp. 263-287, Ciba, 1973.

66. Pappenheimer, J.R.: Passage of molecules through capillary walls. J. Gen Physiol 33:387, 1953.

67. Paradis, G.R. and Kelly, P.J.: Blood flow and mineral deposition in canine tibial fractures. J.B.J.S. 57(A) 220-226, 1975.

68. Rashid, M., Khairi, A., Johnston, C.C. Airman, R.D., Wellman, H.N., Serafini, A.N., and Sankey, R.R.: Treatment of Paget's disease of bone. J.A.M.A. 230: 562-567, 1974.

69. Recker, R.R. Hassing, G.S., Lau, J.R. and Saville, P.D.: The hyperphosphatemic effect of disodium ethane-1-hydroxy 1-diphosphonate (EHDP ^{Tm}): Renal handling of phosphorus and the renal response to parathyroid hormone. J. Lab. Clin. Med. 81: 258-265, 1973.

70. Renkin, E.M.: Transport of potassium-42 from blood to tissu in isolated mammalian skeletal muscle. Am. J. Physiol. 197: 1205-1210, 1959.

71. Rhinelander, F.W. Circulation in Bone. In: Biochemistry and Physiology of bone. Vol. II; 2nd Ed. Academic Press., New York, 1972.

72. Robinson, R.A.: An electron microscope. Study of tne crystalline inorganic component of bone analysis relationship to the organic matrix. J.B.J.S. 34(A): 389-935, 1952.

73. Roy, R.R., Nathan, B.L., Beales, J.S.M. and Chisholm, G.D. 18 Fluorine total body scans in patients with carcinoma of the prostate. Brit. J. of Urology. 43:58-64, 1971.

74. Rowland, R.E.: Exchangeable bone calcium. Clin. Orthop. and Related Res. 49:223-248, 1966.

75. Russell, R.G.G. and Smith, R.: Diphosphonates. J.B.J.S. 55(B): 66-86, 1973.

76. Seliger, W.G.: Tissue fluid movement in compact bone. Anat. Rec. 166:247-256, 1970.

77. Shirzai, P.H., Rayudu, G.V.S., Rigan, W.G. and Fordham, F.W.: Bone scanning in the staging and management of bronchogenic carcinoma. A review of 205 cases. J. Nuclear Med. 19:450-451, 1973.

78. Silberstein, E.B., Saenger, E.L., Tofe, A.J., Alexander, G.W., Jr., and Park, H.M.: Imaging of bone metastasis with ^{99m}Tc-Sn-EHDP, ^{18}F and skeletal roentgenography: A comparison of sensitivity. J. Nuclear Med. 14:454, 1973.

79. Stein, W.D.: The movement of molecules across cell membranes. (Figure 3.2, page 68). Academic Press, New York, 1967.

80. Subramanian, G., McAfee, J.G., O'Mara, R.E., Rosentreich, M. and Mehler, A.: ^{99m}Tc polyphosphate. A new radiopharmaceutical for skeletal imaging. J. Nuclear Med. 12:399-400, 1971.

81. Subramanian, G., McAfee, J.G., Blair, R.J., Mehler, A. and Connor, T.: ^{99m}TcEHDP: A potential radiopharmaceutical for skeletal imaging. J. Nuclear Med., 13:947-950, 1972.

82. Talmage, R.V.: Morphological and physiological considerations in a new concept of calcium transport in bone. Am. J. Anat. 129:467-476, 1970.

83. Tofe, A.J. and Francis, M.D.: Optimization of the ratio of stannous tin: Ethane-1-hydroxy-1,1-diphosphonate for bone scanning with ^{99m}Tc-Pertechnetate. J. Nuclear Med. 15:69-74, 1974.

84. Triffit, J.T., Terapka, A.R. and Neuman, W.F.: A comparative study of the exchange in vivo of major constituents of bone mineral. Calc. Tissue Res. 2: 165-176, 1968.

85. Troehler, Ulrich, Bonjour, J.P. and Fleisch, H.: Renal secretion of diphosphonates in rats. Kidney International 8:6-13, 1975.

86. Tucker, F.R.: The use of radioactive phosphorus in the diagnosis of avascular necrosis of the femoral head. J.B.J.S. 32(B): 100-107, 1950.

87. Vaughan, J.M.: The Physiology of Bone. Clarendon Press. Oxford. 1970.

88. Wagner, H.N.: Principles of nuclear medicine. pp 282-284, Saunders, Philadelphia, 1968.

89. Wendeberg, B.: Mineral metabolism of fractures of the tibia in man studied with external counting of Sr^{85}. Acta Orthop. Scand. Supp. 52:1961.

90. Wray, J.B. and Lynch, C.J.: The vascular response to fracture of the tibia in the rat. J.B.J.S. 41(A): 1143-1148, 1959.

91. Wray, J.B.: Acute changes in femoral arterial blood flow after closed tibial fractures in dogs. J.B.J.S. 46(A): 1262-1268, 1964.

92. Yano, Y., McRae, J., Van Dyke, D.C. and Anger, H.O.: Technetium ^{99m}-labelled stannous ethane-1-hydroxy 1-1 diphosphonate. A new bone scanning agent. J. Nuclear Med. 14:73-78, 1973.

93. Young, D.S.: Improved method for automatic determination of serum inorganic phosphate. J. Clin. Path. 19:397-399, 1966.

94. Zierler, K.L.: Equations for measuring blood flow by external monitoring of radioisotopes. Circ Res. 16:309-321, 1965.

---oOo---

1. An Appraisal of Bone Scanning in Clinical Practice.
Journal of South Colleges of Physicians and Surgeons. Vol 4. 1975.

2. Phosphate Compounds in Bone Scanning.
Journal of Bone and Joint Surgery
57B 1975

3. Infection following total hip Replacement
Acta Orthopaedica Scandinavica.
46 1975.

4. Extraction of Bone of Technetium 99m - labelled Ethane 1- hydroxy-1-1- diphosphon
Federation of American Societies
of Experimental Biology. 1975

5. Extraction of Minerals following fractures of the tibia.
Orthopaedic Research Society 1976.

An Appraisal of Bone Scanning in Clinical Practice

S.P.F. HUGHES, FRCSI., FRCS., FRCS.Ed.
Senior Registrar, Department of Orthopaedics.
and
P. J. ELL, MD., MSc.
Registrar, Department of Nuclear Medicine.
The Middlesex Hospital, London, WIN 8AA.

Summary

Bone scanning is now available for the detection of metastases in bone, for the assessment of a fracture and for investigating bone infections and vascularity. It is safe, sensitive and inexpensive.

Increased bone blood flow and local metabolic activity remain the main causes for abnormal concentration of these radiopharmaceuticals in the bone. Of the substances used, the technetium 99m labelled phosphate compounds appear to be the best, because they are so readily available. Bone scanning may now be performed without major risks to the patient and thus allows for adequate follow-up particularly with regard to their response or resistance to treatment.

Bone scanning with radioactive isotopes has been available for many years. Recently, however, both the radioisotopes and the instruments for their detection have improved considerably. This has made bone scanning a routine clinical procedure.

Isotopes

The isotopes that are used include strontium 85 and 87m, fluorine and the technetium labelled phosphate compounds which have been introduced lately as bone scanning agents. Strontium 85 has a long half life of 65 days, and although strontium 87m has a half life of only two hours and forty minutes, it takes over 30 hours to be cleared from the blood. Fluorine 18 has a very short half life of 110 minutes, and is a high energy gamma emitter, but is usually only produced by a cyclotron reactor and therefore it has a limited distribution. In 1971, Subramanian and McAfee labelled a polyphosphate with technetium 99m. Technetium 99m is readily available, has a short half life of six hours and has an ideal energy for scanning (Table I). At present, there are several phosphate compounds used in bone scanning; pyrophosphate, triolyphosphate and ethylhydroxydiphosphonate (EHDP). Of these three, EHDP appears to be the best, because it is rapidly cleared from the blood and has a high bone to soft tissue ratio at 4 hours. (Yano et al., 1973; Hughes, Jayasingh and Lavender, 1975).

The bone seeking isotopes act on the hydroxyapatite crystal; strontium exchanges with calcium; fluorine exchanges with the hydroxyl group and Jung, Bisaz and Fleisch (1973) have shown that the pyrophosphates and diphosphonates bind directly on to the hydroxyapatite crystal. Galasko (1974) has demonstrated that fluorine 18 is incorporated into the actively dividing cells of a metastases and that following radiotherapy, the amount of uptake by the metastases decreases. Tilden et al (1973) have shown that technetium 99m labelled polyphosphates are concentrated in the developing osteocytes of a formal head removed for total replacement of the hip.

Technique

Recent advances in instrumentation consist mainly in the the use of whole body rectilinear

Present address: The Mayo Clinic, Rochester, Minnesota 55901.

	85 Sr	87m Sr	18 E	99m Tc
Half life	65 days	2.7 hours	1.85 hours	6 hours
Main energy (Kev)	514	390	511	140
Dose to skeleton (in rads/mCi)	30.0	0.13	0.18	0.05

Table 1. Main Bone seeking Isotopes relevant to Clinical Skeletal Scanning

scanners and large field gamma cameras attached to "scanning beds". With these devices images of the entire skeleton can be easily obtained in less than 45 minutes. Anterior and posterior views of the skeleton are preferred and almost always are sufficient to provide the answer to the clinical problem. The display of these images is a matter of continuous improvement. Standard X-ray plates can be used or more sophisticated hard copy printouts can be obtained with a grey or indeed a colour scale. The images are generally reduced in size by a 5 to 1 minification factor but regions of particular interest can be produced on a 1 to 1 basis.

With progress dealing mainly with the crystal-photomultiplier ratio, the resolution of these modern imaging devices has progressively improved and considerable detail can now be recognised on the skeletal images. The individual vertebra can be clearly seen, and the ribs well outlined. However, in some patients, mostly the obese ones, even the best radiopharmaceutical cannot produce this amount of detail. Moreover, the size of a lesion alone is not the only important factor to be seen on the scan. The metabolic activity is of paramount importance and sometimes small, but very active lesions are more clearly seen on the scan than larger ones.

Clinical Application

At present bone scanning is being used in clinical practice mainly for the early detection of metastatic disease. However, there are non-malignant conditions where bone scanning may be useful (Table II).

Orthopaedics

In Orthopaedic surgery, there are instances when the ability to detect infection is vitally important. The patient may not have all the clinical signs of an infection and haematological investigations may be unhelpful. This is particularly true in the diagnosis of late infection after prosthetic replacement (Bauer, et al., 1973. Kemp et al., 1973), found scintigraphy, or serial scanning, valuable in assessing reactivation of chronic spinal infections. A recent paper by Ailsby and Staheli (1974) has shown that scanning can help in the diagnosis of pyogenic infections involving the sacroiliac joints in children.

Radioisotopes have also been used to assess the rate of fracture healing. Johannsen (1973)

NON-MALIGNANT DISEASE

Infective
- Osteomyelitis
- Infection following total hip replacement
- Infective spondylitis

Traumatic
- To assess the rate of union of fractures
- Subcapital fractures of the femur

Vascular
- Perthes' disease

Metabolic
- Paget's disease

MALIGNANT DISEASE

Primary
- Osteosarcoma
- Ewing's tumour

Secondary
- Breast
- Prostate
- Lungs
- Thyroid
- Kidney

Table II. Indications for Bone Scanning

studied the ratio of uptake of strontium 87m in a fractured tibia compared with a normal tibia and noted an increase in activity over the fracture as union occurred. Muheim (1973) used serial bone scanning to compare the methods of treating fractures. He found that in a small group of patients, compression plating produced a fairly constant bone scan over 28 weeks, but that conservative treatment produced a progressive uptake of the radioisotope at the fracture site which increased until union had occurred. For some time one of the worse fractures to give a prognosis for has been the subcapital fracture of the neck of the femur. To a large extent, clinical classification has not been very helpful and a common practice is to treat all fractures over a certain age with a replacement prothesis. However, closed reduction and internal fixation is preferable in young people; thus the capacity to predict which fracture needs replacement would be invaluable. Riggins, Denardo D'Ambrosia and Goldman (1973) used fluorine 18 to detect vascularity after a subcapital fracture in dogs. They found that a positive fluorine 18 bone scan was indicative of vascularity, whereas a negative scan indicated an absent circulation at the femoral head. Technetium 99m polyphosphate has also been used to study subcapital fractures with very similar results (Korvald and Sundsford, 1974).

In Perthes' disease there is impairment of the blood supply to the femoral head, producing a transient or absolute anoxia (Kemp, 1973). Bohr (1973) using fluorine 18 has shown that revascularisation of the epiphysis of the femoral head has usually taken place by the radiographic stage of condensation, and concluded that scintigraphy was very effective in assessing the progress of the disease.

Metastatic Malignant Disease

The role of bone scanning in the management of malignant disease is now generally accepted. Malignant disease often spreads to the skeleton particularly from the breast (Fig. 1), lungs and prostate and, to a lesser extent from the thyroid and kidney. However, the ability to detect metastatic deposits in bone on routine radiographs is not always satisfactory. Much of the vertebral bone can be destroyed before the lesion becomes apparent on radiographs (Jaffe, 1958) and therefore negative radiographs are not all that helpful. Biochemical investigations such as serum calcium, phosphorus, alkaline phosphase, lactic acid dehydrogenase and urinary calcium are on the whole non-specific for malignant disease and are relatively insensitive indices of malignant spread.

Galasko (1972) has studied metastatic disease from carcinoma of the breast and he found that in 50 patients with advanced disease — that is, some form of spread of the tumour — radiographs were positive in only 50% of the

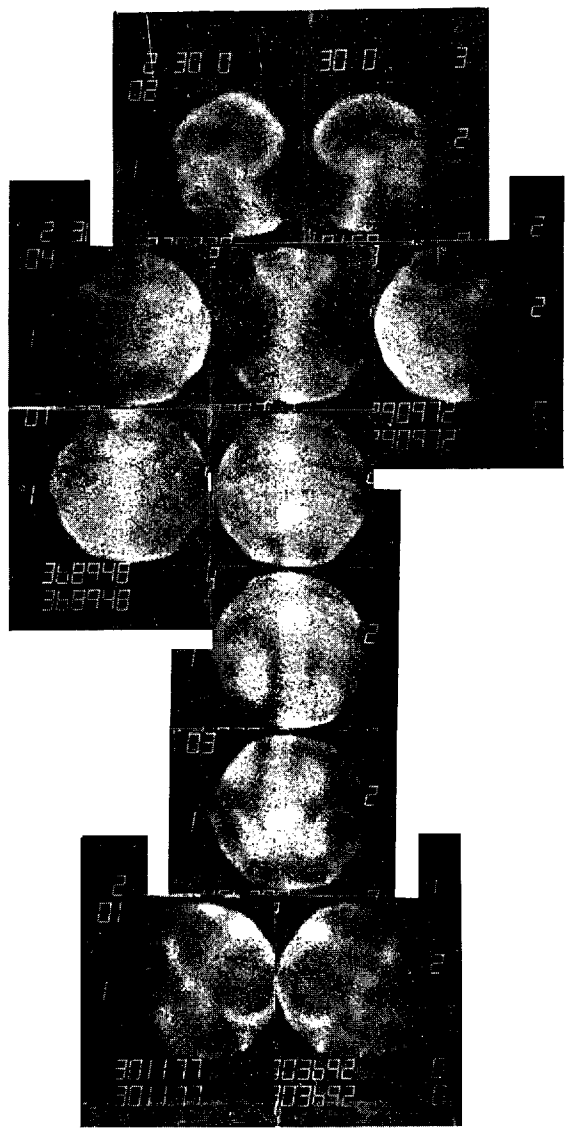


Fig. 1 Patient with carcinoma of the breast. Metastases are clearly seen in the dorsal and lumbar spine at a time when the skeletal X-ray survey was normal. Multiple views obtained with a gamma camera.

cases, but a fluorine 18 bone scan was positive in 84%. In 50 patients with early cancer — that is, without any detectable metastatic

disease — 12 patients had positive bone scans which were later confirmed to be due to metastases. In this situation, bone scanning is invaluable in order to accurately stage the disease and to prevent extensive surgery. In carcinoma of the bronchus, 30% out of 114 patients with early disease had positive bone

Fig. 2 Patient with primary osteosarcoma in the lower third of the left femur. Patient was scanned after radiotherapy. Tracer uptake is therefore suppressed in the tumour area but abnormal concentration of the tracer is seen in the chest indicating left lung metastasis at a time where the chest X-ray was clear.

scans (Shirazi et al. 1973), an incidence which rose to 50% at later stages of the malignancy. Patients with carcinoma of the prostate were recently reviewed (Shearer et al, 1974) and bone scanning with technetium-99m-EHDP was found to be useful in detecting the lesions and assessing the response of the metastases to treatment.

Primary malignant disease

The role of phosphate compounds in bone scanning in primary malignant bone disease was recently studied by us (to be published). It was helpful in defining the site and extent of the osteosarcoma and it did appear to be [redacted] useful in revealing soft tissue pulmonary metastases before they were apparent on radiography (Fig. 2). In the case of Ewing's sarcoma, phosphate scanning has been helpful in staging the tumour and in deciding the appropriate therapy. Failure of suppression of the technetium 99m labelled phosphate after a course of radiotherapy is being used to diagnose a recurrence of the tumour, an infection of the bone or a fracture.

References

AILSBY, R. L. and STAHELI, L. T. (1974). *Clinical Orthopaedics and Related Research*, **100**, 96.

BAUER, G. C. H., LINDBERG, L., NAVERSTEN, Y. and SJÖSTRAND L. O. (1973). *Acta Orthopaedica Scandinavia*, **44**, 439.

BOHR, H. (1973). *Acta Orthopaedica Scandinavia*, 44, 417.

GALASKO, C. S. B. (1972). *Annals of the Royal College of Surgeons of England*, **50**, 3.

GALASKO, C. S. B. (1974). *Journal of Bone and Joint Surgery*, **56B**, 207.

HUGHES, S. P. F., JEYASINGH, K. and LAVENDER, P. J. (1975). *Journal of Bone and Joint Surgery, (in press)*.

JAFFE, H. L. (1958). *Tumours and Tumerous Conditions of the Bones and Joints*. Lea and Febiger, p. 604.

JOHANNSEN, A. (1973). *Acta Orthopaedica Scandinavia*, 44, 628.

JUNG, A., BISAZ, S. and FLEISCH, H. (1973). *Calcified Tissue Research*, **11**, 269.

KORVALD, E. and SUNDSFORD, A. J. (1974). *Acta Orthopaedica Scandinavia*, **45**, 572.

KEMP, H. B. S. (1973). *Annals of the Royal College of Surgeons of England*, **52**, 18.

KEMP, H. B. S., JOHNS, D. L., MCALISTER, J. and GODLEE, J. N. (1973). *Journal of Bone and Joint Surgery*, **55B**, 301.

MUHEIM, G. (1973). *Acta Orthopaedica Scandinavia*, 44, 621.

RIGGINS, R. S., DENARDO, G. L., D'AMBROSIA, R. and GOLDMAN, M. (1973). *Journal of Nuclear Medicine*, **15**, 183.

SHEARER, R. J., CONSTABLE, A. R., GIRLING, M., HENDRY, W. F. and FERGUSON, J. D. (1974). *British Medical Journal*, **18**, 362.

SHIRAZI, P. H., RAYUDU, G. V. S., RIGAN, W. G., FORDHAM, F. W. (1973). *Journal of Nuclear Medicine*, **19**, 450.

SUBRAMANIAN, G. and MCAFEE, J. G. (1971). *Radiology*, 99, 192.

TILDEN, R. L., JACKSON, J., ENNEKING, W. F., DELAND, F. H. and MCVEY, J. T. (1973). *Journal of Nuclear Medicine*, **14**, 576.

YANO, Y., MCRAE, J., VAN DYKE, D. C. and ANGER, H. O. (1973). *Journal of Nuclear Medicine*, **14**, 73.

PHOSPHATE COMPOUNDS IN BONE SCANNING

S. P. F. HUGHES, F.R.C.S.
Senior Registrar Orthopaedics
The Middlesex Hospital
London, England

K. JEYASINGH, B.Sc.
Physicist
and
P. J. LAVENDER, F.F.R., F.R.C.P.
Consultant Radiologist
The Hammersmith Hospital
London, England

REPRINTED FROM
THE JOURNAL OF BONE AND JOINT SURGERY
BRITISH NUMBER
MAY 1975
British Editor: R. C. F. Catterall

PHOSPHATE COMPOUNDS IN BONE SCANNING

S. P. F. HUGHES, F.R.C.S.
Senior Registrar Orthopaedics
The Middlesex Hospital
London, England

K. JEYASINGH, B.Sc.
Physicist
and
P. J. LAVENDER, F.F.R., F.R.C.P.
Consultant Radiologist
The Hammersmith Hospital
London, England

British Editorial Offices:
8 HALLAM STREET
LONDON, W1N 6NY

PHOSPHATE COMPOUNDS IN BONE SCANNING

S. P. F. HUGHES, K. JEYASINGH and P. J. LAVENDER, LONDON, ENGLAND

From the Departments of Surgery, Medical Physics and Radiodiagnosis, the Hammersmith Hospital, London

Bone scanning with radioactive isotopes has been used to study a wide variety of disorders. Recently certain phosphate compounds, labelled with technetium, have been used as bone scanning agents. The comparative merits of three technetium-labelled phosphate compounds currently available for bone scanning—pyrophosphate, tripolyphosphate and ethylhydroxydiphosphonate (EHDP) have been compared in rabbits. Each substance was injected into ten rabbits and blood was withdrawn at regular intervals. The animals were killed at four hours and the blood and tissue samples were assayed for radioactivity. The results show that EHDP has a more rapid blood clearance than the other two agents, with a resultant improvement in the bone to soft-tissue ratio. Of the three substances investigated technetium-labelled EHDP was the best and might allow the technique of scanning to be used on a wide scale for the general study of bone and its pathology.

Bone scanning with radioactive isotopes has been used to study a wide variety of disorders, including the healing of fractures, infective lesions of the spine and hip and neoplastic deposits in bone before they are visible in radiographs.

Bone consists of the minerals calcium, phosphorus, sodium and magnesium, with traces of strontium, barium, fluorine and chlorine. No radioactive isotope of calcium suitable for bone scanning purposes is available. Strontium 85 and 87m have both been used extensively, but the count rate from these isotopes is rather low and therefore skeletal detail is not well shown. Fluorine 18 is a good bone scanning agent with a short half life of 110 minutes; unfortunately its use is limited to centres within the range of a cyclotron. Recently phosphate compounds labelled with technetium have been introduced as bone scanning agents.

Polyphosphates, that is long-chain polymers of phosphate, have been used for many years as detergents and scale removers. It has recently been shown that these phosphate compounds bind on to the hydroxyapatite crystal of bone (Jung, Bisaz and Fleisch 1973) and prevent further crystal growth (Francis 1969). Use of these properties has been made in the treatment of Paget's disease, metastatic calcification and osteoporosis (Russell and Smith 1973; Michael, King and Francis 1971).

In 1971 Subramanian and McAfee labelled a stannous chelate of sodium tripolyphosphate with technetium 99m and showed that it was taken up by the skeleton. Since then, further reports have described the use of different technetium-labelled phosphate compounds (Subramanian, McAfee, Blair, Mehter and Connor 1972; Castronovo and Callahan 1972; Yano, McRae, Van Dyke and Anger 1973).

We have investigated in rabbits the comparative merits of three technetium-labelled phosphate compounds currently available for bone scanning—pyrophosphate, tri polyphosphate and ethylhydroxydiphosphonate (EHDP). Polyphosphates were not investigated, because their properties and molecular weight vary with different chain lengths (Subramanian, McAfee, Bell, Blair, O'Mara and Ralston 1972).

METHOD

Sorens pyrophosphate was supplied ready labelled with technetium by Micro Bio Laboratories. Sodium tripolyphosphate and ethylhydroxydiphosphonate were labelled by one of us (K. J.).

Five hundred milligrams of tripolyphosphate were dissolved in 10 millilitres of sterile oxygen-free water and allowed to stand for thirty minutes. The solution was then added to 200 milligrams of stannous chloride in 10 millilitres of sterile oxygen-free water. The mixture was left until a clear solution was formed and then filtered through a 0·22 μ millipore filter into a sterile vial containing nitrogen. One millilitre of this solution was mixed with about 20 milligrams of technetium and autoclaved at 132 degrees Celsius for six minutes.

Ethylhydroxydiphosphonate was prepared by adding 0·5 millilitre of stannous chloride to 0·5 millilitre of ethylhydroxydiphosphonate in a sterile sealed evacuated vial: 4 millilitres of technetium were then added to the solution and the pH adjusted to 6·0. The final solution was sterilised by filtering through a 0·22 μ filter into a sterile multidose vial.

Thirty New Zealand white female adult rabbits of similar weight and age were then divided into three experimental groups. There were ten animals in each group, which were given a different technetium-labelled phosphate compound. Approximately 500 μc of technetium was given to each rabbit. The solution was injected into the peripheral vein of one ear and after five minutes 1-millilitre samples of blood were withdrawn at regular intervals from the central artery of the other ear. At four hours, the animals were killed and samples of tissue were taken from the skin, muscle, liver, right kidney and the whole of the right femur including the marrow. The blood and tissue samples were assayed for radioactivity together with a radioactive standard, and the radioactivity in the tissue sample was expressed as a percentage of the original dose for 10 grams of tissue.

S. P. F. Hughes, F.R.C.S., Senior Registrar Orthopaedics, the Middlesex Hospital, London W.1, England.
K. Jeyasingh, B.Sc., Physicist, the Hammersmith Hospital, London W.12, England.
Dr P. J. Lavender, F.F.R., F.R.C.P., Consultant Radiologist, the Hammersmith Hospital, London W.12, England.

RESULTS

The blood clearance of the substances is shown in Figure 1. The radioactivity in the blood is expressed as a percentage of the five-minute sample and is plotted on a semi-logarithmic scale against time in minutes. The clearance

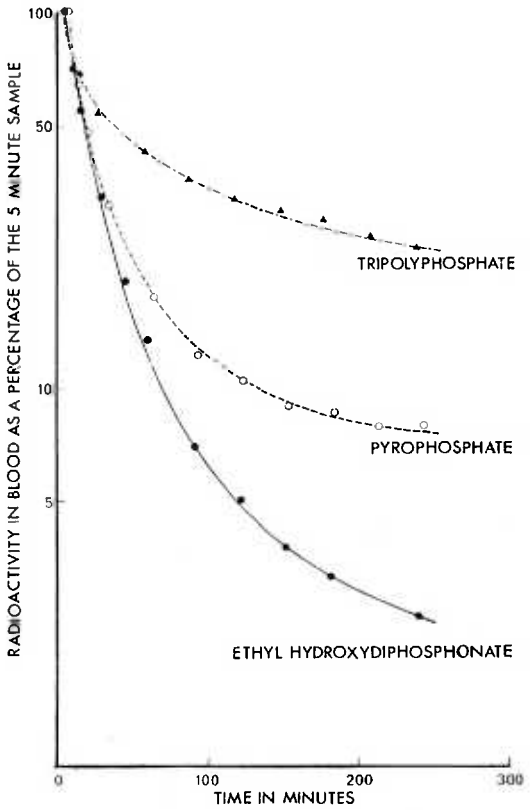


FIG. 1

Graph of the blood clearance of each phosphate compound.

of tripolyphosphate was slower than that of the other two substances; at four hours there was more than 24 per cent tripolyphosphate, 8 per cent pyrophosphate, but less than 2·5 per cent ethylhydroxydiphosphonate still present in the blood. The patient is scanned at four hours, and therefore the lower the blood activity at this time the higher the contrast between the bone and the surrounding tissues.

The average radioactivity present in 10 grams of tissue expressed as a percentage of the injected dose is shown in Table I. It can be seen that tripolyphosphate has the highest percentage radioactivity in all the tissues except in bone, and that ethylhydroxydiphosphonate has the lowest percentage radioactivity of the three. However, when the ratio of radioactivity present in 1 gram of tissue to 1 millilitre of blood at four hours is shown (Table II) different values are apparent. The ratio of radioactivity is equally low for all three substances in skin and muscle. In the liver, pyrophosphate has the highest ratio and in the kidney ethylhydroxydiphosphonate has the highest ratio of radioactivity. But it is clear that the ratio of radioactivity in bone is highest by far with ethylhydroxydiphosphonate, and it is the contrast between the bone and the surrounding tissue which gives the favourable definition of the scan.

TABLE I

MEAN PERCENTAGE RADIOACTIVITY PRESENT IN 10 GRAMS OF DIFFERENT TISSUE

	EHDP	Tripolyphosphate	Pyrophosphate
Skin	0·039	0·117	0·035
Muscle	0·010	0·043	0·011
Liver	0·030	0·464	0·434
Kidney	0·532	2·018	0·715
Bone	1·673	1·716	2·722

TABLE II

MEAN RATIO OF RADIOACTIVITY 1 GRAM OF TISSUE TO 1 MILLILITRE OF BLOOD

	EHDP	Tripolyphosphate*	Pyrophosphate
Skin	0·900	0·399	0·501
Muscle	0·276	0·143	0·171
Liver	0·746	1·600	6·32
Kidney	23·68	6·99	10·21
Bone	47·60	5·99	39·80

*(N=10)

FIG. 2

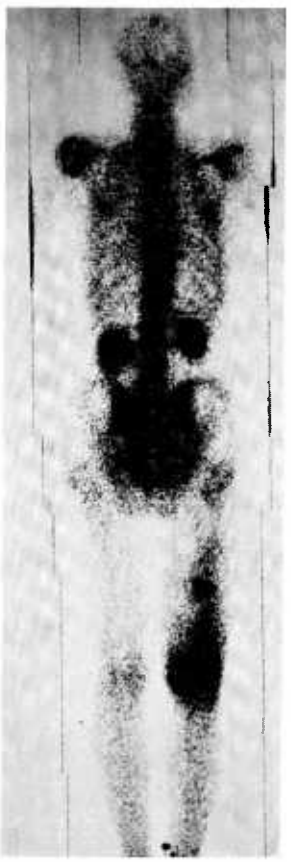


FIG. 3

Figure 2—Radiograph of a chondrosarcoma of the left femur.
Figure 3—A bone scan of the same patient.

A patient with a chondrosarcoma (Fig. 2) gave a clear outline of the tumour in the left femur as well as excellent definition of the skeleton, the kidney and the bladder (Figs. 2 and 3).

DISCUSSION

We believe that ethylhydroxydiphosphonate has better properties for scanning bone than pyrophosphate or tripolyphosphate, because it is rapidly cleared from the blood and at four hours shows a higher bone to blood ratio than the other two substances. Direct comparisons between fluorine 18 and technetium-labelled ethylhydroxydiphosphonate indicate that the fluorine achieves a higher bone to blood ratio at comparable times after injection (Bok, Perez, Panneciere and Di Paola 1973). However, its high energy gamma emission is not suited to present scanners or cameras, and clinical comparisons show no advantage over technetium-labelled compounds (Marty and Denney 1973; Silberstein, Saenger, Tofe, Alexander and Park 1973). The reason for the better properties exhibited by ethylhydroxydiphosphonate may be that it is not hydrolysed so rapidly *in vivo* because it is resistant to the action of the naturally occurring enzymes, phosphatase and phosphonatase.

The use of bone scanning will no doubt increase in orthopaedics. That it has a place is seen in the detection of avascular necrosis using P^{32} in subcapital fractures of the femoral neck (McNeur 1970), in the study of fracture healing (Wendeberg 1961) and in the comparison of osteonecrosis and osteoarthritis in the knee (Muheim and Bohne 1970). Scintigraphy or serial bone scanning is used in tumour diagnosis, in the early detection of metastases in malignant disease of the breast (Charkes and Sklaroff 1964; Galasko 1972) and prostate (Roy, Nathan, Beales and Chisholm 1971), and assessment of osteosarcoma (Gerson, Dorfman, Norman and Mankin 1972). Recently, scanning has been used to study the changes of bone in Perthes' disease (Bohr 1973), in infective spondylitis (Kemp, Johns, McAlister and Godlee 1973) and in infections after total replacement of the hip (Bauer, Lindberg, Nauversdten and Sjöstrand 1973).

With the introduction of phosphate compounds labelled with technetium, bone scanning can become generally available in orthopaedics. Of the three substances investigated, ethylhydroxydiphosphonate appears to be the best because it gives a high bone to soft-tissue ratio, a rapid blood clearance and, probably most important of all, it is bound to the isotope technetium which is readily available and is in general use for other medical isotopic investigations.

We should like to express our gratitude to Mr Selwyn Taylor and Dr H. Glass for their help and advice on this project, and to the Cancer Research Fund for financial assistance.

REFERENCES

Bauer, G. C. H., Lindberg, L., Nauversdten, Y., and Sjöstrand, L.-O. (1973) ^{85}Sr radionuclide scintimetry in infected total hip arthroplasty. *Acta orthopaedica Scandinavica*, **44**, 439–450.

Bohr, H. (1973) Densitometry and ^{18}F scintigraphy in the study of the revascularization of the femoral head in coxa plana. *Acta orthopaedica Scandinavica*, **44**, 417–425.

Bok, B., Perez, R., Panneciere, C., and Di Paola, R. (1973) Bone scanning radiopharmaceuticals: a comparison of three products. *Journal of Nuclear Medicine*, **14**, 380–381.

Castronovo, F. P., Jun., and Callahan, R. J. (1972) New bone scanning agent: ^{99m}Tc-labeled 1-hydroxy-ethylidene-1, 1-disodium phosphonate. *Journal of Nuclear Medicine*, **13**, 823–827.

Charkes, N. D., and Sklaroff, D. M. (1964) Early diagnosis of metastatic bone cancer by photoscanning with Strontium-85. *Journal of Nuclear Medicine*, **5**, 168–179.

Francis, M. D. (1969) The inhibition of calcium hydroxyapatite crystal growth by polyphosphonates and polyphosphates. *Calcified Tissue Research*, **3**, 151–162.

Galasko, C. S. B. (1972) Skeletal metastases and mammary cancer. *Annals of the Royal College of Surgeons of England*, **50**, 3–28.

Gerson, B. D., Dorfman, H. D., Norman, A., and Mankin, H. J. (1972) Patterns of localization of ^{85}Strontium in osteosarcoma. *Journal of Bone and Joint Surgery*, **54–A**, 817–827.

Kemp, H. B. S., Johns, D. L., McAlister, J., and Godlee, J. N. (1973) The role of Fluorine-18 and Strontium-87m scintigraphy in the management of infective spondylitis. *Journal of Bone and Joint Surgery*, **55–B**, 301–311.

Jung, A., Bisaz, S., and Fleisch, H. (1973) The binding of pyrophosphate and two diphosphonates by hydroxyapatite crystals. *Calcified Tissue Research*, **11**, 269–280.

McNeur, J. C. (1970) Observation on radioactive phosphorus uptake in high fractures of the femoral neck. *Journal of Bone and Joint Surgery*, **52–B**, 393.

Marty, R., and Denney, J. D. (1973) Bone scanning agents: clinical comparison of ^{99m}Tc-Diphosphonate, ^{87m}Sr and ^{18}F. *Journal of Nuclear Medicine*, **14**, 423–424.

Michael, W. R., King, W. R., and Francis, M. D. (1971) Effectiveness of diphosphonates in preventing "osteoporosis" of disuse in the rat. *Clinical Orthopaedics and Related Research*, **78**, 271–276.

Muheim, G., and Bohne, W. H. (1970) Prognosis in spontaneous osteonecrosis of the knee. *Journal of Bone and Joint Surgery*, **52–B**, 605–612.

Roy, R. R., Nathan, B. E., Beales, J. S. M., and Chisholm, G. D. (1971) 18 Fluorine total body scans in patients with carcinoma of the prostate. *British Journal of Urology*, **43**, 58–64.

Russell, R. G. G., and Smith, R. (1973) Diphosphonates. *Journal of Bone and Joint Surgery*, **55–B**, 66–86.

Silberstein, E. B., Saenger, E. L., Tofe, A. J., Alexander, G. W., Jun., and Park, H. M. (1973) Imaging of bone metastasis with ^{99m}Tc-Sn-EHDP, ^{18}F and skeletal roentgenography: a comparison of sensitivity. *Journal of Nuclear Medicine*, **14**, 454.

Subramanian, G., and McAfee, J. G. (1971) A new complex of ^{99m}Tc for skeletal imaging. *Radiology*, **99**, 192–196.

Subramanian, G., McAfee, J. G., Bell, E. G., Blair, R. J., O'Mara, R. E., and Ralston, P. H. (1972) ^{99m}Tc-labeled polyphosphate as a skeletal imaging agent. *Radiology*, **102**, 701–704.

Subramanian, G., McAfee, J. G., Blair, R. J., Mehter, A., and Connor, T. (1972) ^{99m}Tc-EHDP: a potential radiopharmaceutical for skeletal imaging. *Journal of Nuclear Medicine*, **13**, 947–950.

Wendeberg, B. (1961) Mineral metabolism of fractures of the tibia in man studied with external counting of Sr^{85}. *Acta orthopaedica Scandinavica*, Supplementum 52.

Yano, Y., McRae, J., Van Dyke, D. C., and Anger, H. O. (1973) Technetium-^{99m}-labeled stannous ethane-1-hydroxy-1-diphosphonate: a new bone scanning agent. *Journal of Nuclear Medicine*, **14**, 73–78.

Acta orthop. scand. 46, 968–978, 1975

The Middlesex Hospital, London, W1N 8AA, England.

INFECTION FOLLOWING TOTAL HIP REPLACEMENT IN A GENERAL HOSPITAL WITHOUT SPECIAL ORTHOPAEDIC FACILITIES

M. K. D. Benson & S. P. F. Hughes

Accepted 28.iv.75

Total prosthetic replacement of the hip has become a standard orthopaedic procedure. A serious complication is for infection to occur as it results not only in pain in the hip, but it may lead to further disability and eventual removal of the whole prosthesis.

Charnley & Eftekhar (1969) investigated this problem and showed that the incidence of infection could be reduced by using a specially designed operating theatre incorporating a system of nearly sterile air with laminar flow. However, not all hospitals have these facilities and we have reviewed the incidence of infection among patients who have had a total hip replacement undertaken in a general hospital without special orthopaedic theatres.

METHOD

All total hip replacements performed at The Middlesex Hospital from 1968 to 1972 inclusive have been reviewed. There were 274 patients who had 321 arthroplasties; 202 women and 72 men. The mean age was 63 years (Figure 1) and the average follow-up period was 2 years (Figure 2).

The operations were performed in general theatres, which were built in 1936; two further theatres were added in 1968. The plan of the operating suite is shown in Figure 3. The theatres are situated on the top floor of the six-storey hospital. The clean zone includes the changing and recovery rooms; dirty material is dispersed through a clean corridor to a sluice and holding area. The scrub room is incorporated in the operating theatre, but the sterilising and anaesthetic rooms are separate. The ventilation system is designed to give a minimum of 15 air changes each hour in the theatre. Air enters from outside through high level wall ducts and is extracted through low level vents. With this system, the air flow is turbulent rather than laminar. Air filters remove particulate matter greater than 5 μm in diameter. This is an effective barrier to dust particles but not to isolated bacteria.

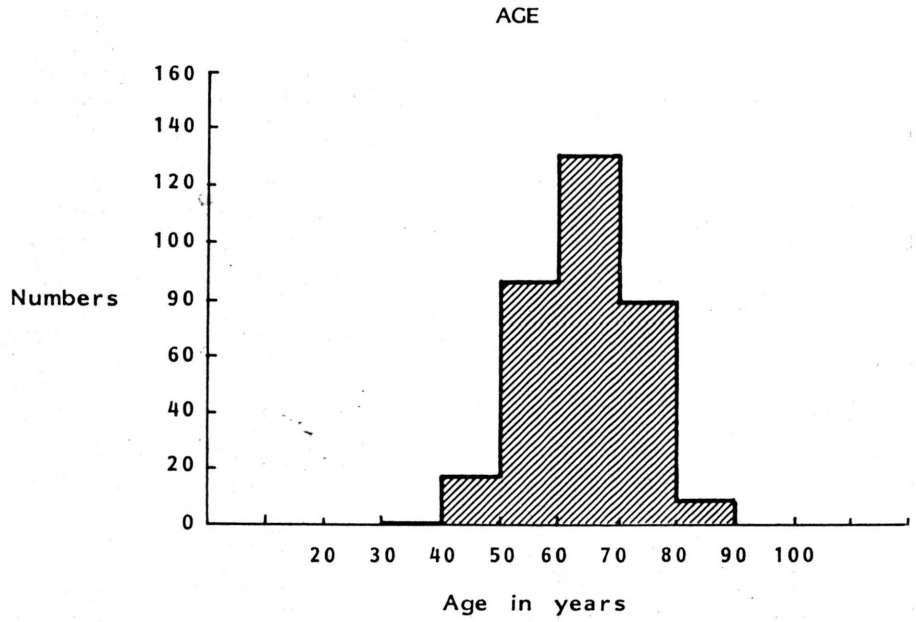


Figure 1.

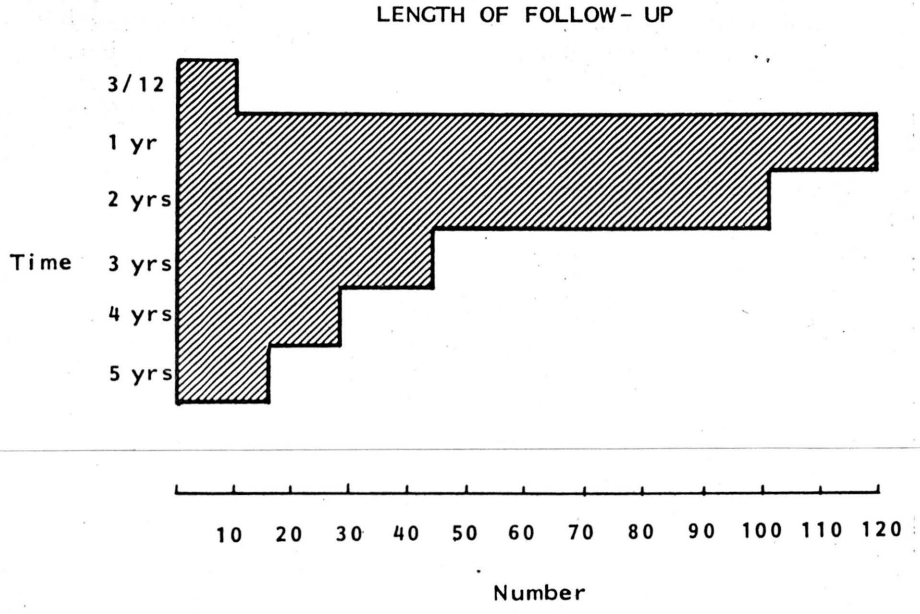


Figure 2.

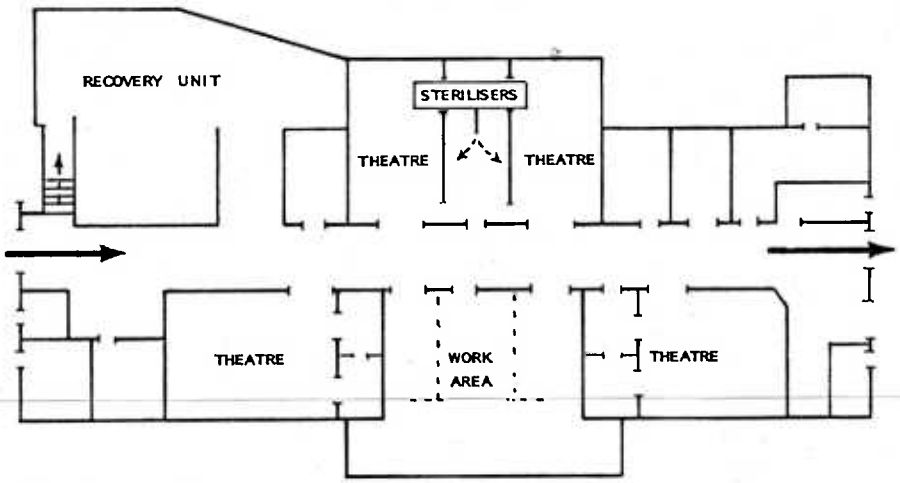


Figure 3.

However, bacteria are rarely completely unattached to particulate matter and particles which carry pathogenic bacteria have a medium diameter of 12 μm (Noble et al. 1963). In practice, therefore, a 5 μm filter is highly efficient (Blowers & Crew 1960).

No theatre is reserved entirely for orthopaedic use; general surgeons, urologists, gynaecologists, cardiothoracic and neurosurgeons all operate in these theatres. No theatre is used for 2 hours after an operation on a patient who is potentially or actually infected. In addition to the normal attendants at a major operation, there are always some medical and nursing students in the theatre itself. The spectators are encouraged to remain as still as possible, as activity has been shown to increase the content of bacteria in the air (Brøckner & Jessen 1963).

Patients are admitted to the orthopaedic ward 24 to 48 hours before operation. In the theatre, the skin is cleaned with 0.5 per cent chlorhexidene in 70 per cent alcohol. No patient received prophylactic antibiotics, but one third had chloramphenicol powder inserted into the wound at the end of the operation. A closed system of drainage was used in all patients and the types of replacement included McKee-Farrar, Charnley and Stanmore; one patient had a Howse prosthesis.

As infection following hip replacement can become apparent at any stage after the operation, we arbitrarily divided infections into those occurring early, within 3 months of the operation, and those occurring late, more than three months after the operation. The diagnosis of early infection rarely presents a problem; the patient has continuous severe pain, is obviously unwell, usually has a fever and frequently has a discharge from his wound. The white cell count is elevated and pathogenic organisms can almost always be cultured. By contrast, late infections may prove difficult to distinguish from mechanical disorders, or from immune reactions based on metal sensitivity. The patient has pain, but is generally well, frequently afebrile and often has a normal white cell count. We considered late infection to be present when two or more of the following criteria were present.

a) pain in the hip at rest and on movement.
b) a persistent sinus in communication with the hip joint.
c) pathogenic organisms isolated from the hip joint, either by discharge, aspiration or at operation.
d) an E.S.R. that is 30 mm/hour above the pre-operative level, in the absence of other causes for the elevation.
e) radiological evidence of infection; that is, periosteal reaction, bone resorption and progressive erosion of the calcar femorale.

RESULTS

Of the 321 hip arthroplasties performed, 17 became infected, an incidence of 5.3 per cent. Nine of these infections were early (2.8 per cent) and eight were late (2.5 per cent). Six of the 103 patients who had chloramphenicol powder inserted into the wound at the end of the operation developed an infection, compared with 11 out of 218 who did not. There was, therefore, no statistically significant difference in the infection rates of those patients with local antibiotic powder applied to the wound and those without.

The organism isolated from the infected hips showed a wide variety (Table 1). There was no obvious difference in the type of organism cultured from the early infections and that cultured from the late infections. It was notable that several of the organisms cultured were of the supposedly less virulent type, and are usually regarded as skin commensals only. Whether *staphylococcus albus* may be considered pathogenic and be responsible for deep infections in the abnormal environment created by a hip arthroplasty remains uncertain.

In all 17 infections, the patient complained of severe pain in the hip; although not abolished by rest, the pain was aggravated by movement. None of the patients with late infection showed signs of

Table 1. Organisms isolated.

Organism	Early	Late
Staphylococcus aureus	2	1
Escherichia coli	3	1
Proteus species	1	1
Pseudomonas aeruginosa	2	–
Streptococcus faecalis	2	1
Staphylococcus albus	2	2
Micrococci or diphtheroids	1	2

Table 2. X-ray changes in 17 patients.

Change	Minimal	Marked
Periosteal reaction	4	7
Resorption of the calcar femorale	6	5
Bone destruction	4	3

systemic upset, and none complained of general ill health or of fever. The patients who developed what was regarded as late infection were initially pain-free following their arthroplasty. Similarly, the white cell count was not raised. The E.S.R., however, was elevated, ranging from 44 to 120 mm/hour (mean 65 mm/hour). The E.S.R. varies with surgery and usually increases by about 30 mm/hour, returning to its original level at 8 weeks (Hughes 1966).

Radiographic evidence of infection about the prosthesis is shown in Table 2. The most constant findings were periosteal reaction around the shaft of the femur and resorption of the calcar. Periosteal reaction about the acetabulum was not seen even in the presence of gross acetabular loosening and migration resulting from infection. Charnley (1967) believes that resorption of the calcar is a physiological effect and that

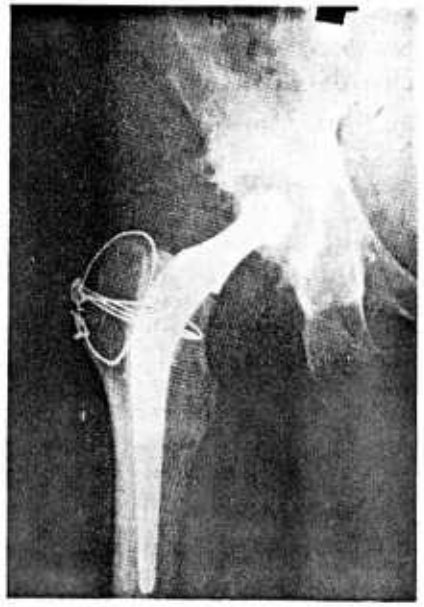


Figure 4.

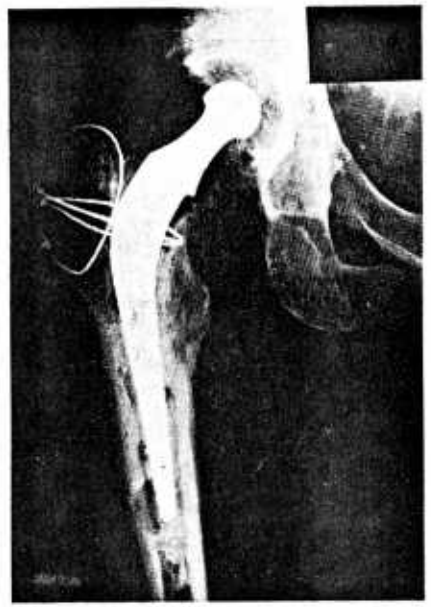


Figure 5.

the resorption confirms the efficacy of weight transmission by cement low down in the medullary cavity. In this physiological resorption, however, there is a smooth loss of the full thickness of the calcar, while in the infective process, the calcar is irregularly eroded. Bone resorption was also seen in the greater trochanter but infrequently in the femoral shaft (Figures 4 and 5).

In 10 of the 17 patients with infection, sinograms were performed and these confirmed a direct communication with one or both of the components of the hip prosthesis. Bone scans, using ethylhydroxydiphosphonate, were performed on three patients, and each demonstrated increased isotope uptake about the infected prosthesis (Figure 6).

The incidence of infection was no different, whether Consultants or Senior Registrars performed the operation. Out of 321 hip replacements, there were 118 McKee-Farrar, 161 Charnley, 41 Stanmore and one Howse. The rate of infection was 7.6 per cent, 4.8 per cent and 0 per cent; the single Howse replacement became infected. The length of follow-up for the McKee-Farrar, Charnley and Stanmore was 5, 4 and 2 years respectively, and for this reason the relative rates of infection are not statistically significant.

Of the eight patients with late infection, three gave a clear history of a recent intercurrent infection. One patient had a dental abscess and

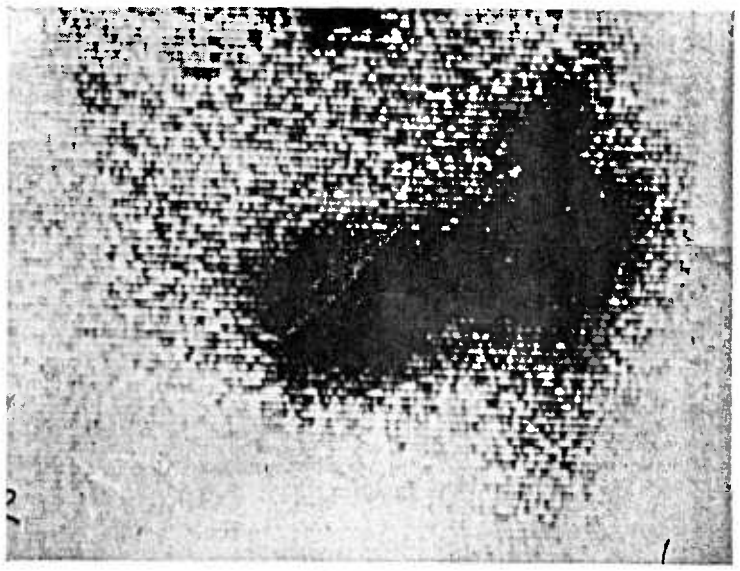


Figure 6.

one an infected ulcer; however, both these were treated before their hip infection developed and there is unfortunately no record of the pathogenic organism. The third patient had had a urinary tract infection caused by a coliform organism of exactly similar sensitivity as that subsequently isolated from the infected hip prosthesis. Three other patients had episodes of trauma to their hip prior to the onset of infection. In each case, the initial pain of their fall was completely relieved before the persistent deep pain of infection developed. No patient who had bilateral arthroplasties developed infection in both hips.

Eleven of the 17 patients with infection have had Girdlestone arthroplasties. Of the remaining six, one has died and five are either unwilling or not sufficiently incapacitated for the prosthesis to be removed.

DISCUSSION

We have found that the incidence of infection following total hip replacement in this retrospective series compares favourably with that reported by others (Table 3). Most authors (Patterson & Selby Brown 1972, Charnley 1972, Harris et al. 1972, Todd et al. 1972, Sillar & Connor 1971, Ericson et al. 1973, and Arnett 1973) agree that infection

Table 3. Comparative rates of infection.

Name	Year	Number	Early %	Late %	Total %	Type of theatre, prosthesis an prophylaxis
Charnley	1965	582	1.6	2.2	3.8	Special orthopaedic theatre. 130 air changes/hour. Charnley. No prophylaxis.
Charnley	1969	708	1.0	0.3	1.3	Special orthopaedic theatre. 300 air changes/hour. "Laminar flow". Charnley. No prophylaxis.
Roles	1971	471	–	–	5.9	General theatre. Knees and hips replaced. Mixed prophylactic antibiotics.
Sillar	1971	60	1.6	1.6	3.2	Orthopaedic theatre. Charnley. Gentamycin. Cloxacillin and Ampicillin.
Harris	1972	73	7.0	3.8	10.8	General theatre. Charnley. Rheumatoid arthritis, Ampicillin and Cloxacillin.
Todd	1972	320	2.5	2.8	5.3	General theatre. Charnley. Ampicillin and Cloxacillin.
Patterson	1972	368	2.99	5.16	8.15	Orthopaedic theatre. McKee. No prophylaxis.
Ericson	1973	83	0	–	0	Orthopaedic theatre.
		60	–	0	0	Charnley. Probenecid and Cloxacillin.
Coventry	1974	2,012	–	–	0.6	Orthopaedic theatre. Charnley. Methicillin.
The Middlesex	1974	321	2.8	2.5	5.3	General theatre. McKee, Charnley, Stanmore and Howse No prophylaxis.

following prosthetic replacement may occur either early or late. There is disagreement, however, as to why late infections occur. Late infections may arise because bacteria introduced at the time of the operation become activated, or because the prosthesis may be the site for bacteria to settle during a bacteraemia. A significant bacteraemia may be produced by trauma, burns or intercurrent infections (Lerner &

Weinstein 1966 a) and the possible effects of a bacteraemia on patients who have damaged heart valves are well established. Patients with abnormal heart valves are advised to avoid these procedures, including dental or urological manipulations, which might provoke bacteraemia, unless they are adequately protected by prophylactic antibiotics (Lerner & Weinstein 1966 b). It may be that patients with hip prostheses should be similarly protected.

Certainly, intercurrent infections, no matter how trivial, should be treated with the appropriate antibiotics for a period sufficient to cover any bacteraemia which may arise. Similarly, all injuries to the hip joint should be taken seriously and the patient given a prophylactic antibiotic, as a local haematoma formation following such an accident may act as a suitable culture medium for bacteria to grow and for infection to take place.

In order to reduce the per-operative rate of infection, Charnley (1968) has paid particular attention to the air in the theatre. He operates in an isolated chamber in which the air is filtered to 1 μm and delivered at 4,500 cf/min. A system of aluminium diffusion vanes attempts to distribute the air flow in a linear fashion. Air enters through the ceiling and is dispersed at ground level; it is changed 300 times per hour. The air inside the operating enclosure is, therefore, of high sterility and the ventilation approximates as closely as possible to the ideal of laminar flow. To prevent bacterial contamination by the surgeon and his assistants, each wears a totally investing helmet and gown with individual exhaust systems to remove convection currents, skin scales and exhaled breath.

In a busy general theatre such as that of The Middlesex, used by a variety of surgical specialities, such a system would be difficult to emulate. Therefore, in addition to standard precautions against infection, the use of prophylactic antibiotics may be considered. Scales et al. (1972) reviewed 1,623 patients who had metal implanted during various orthopaedic operations and found that the incidence of wound infection could be reduced from 8.8 per cent to 5.3 per cent by introducing antibiotics into the wound during the operation. In our patients, however, the use of chloramphenicol powder made no difference to the infection rate, early or late. Coventry et al. (1974) reported a 0.6 per cent infection rate, following total hip replacement. They gave Methicillin systemically as a prophylactic and operated in theatres reserved entirely for orthopaedic use. Ericson et al. (1973) showed that prophylactic Cloxacillin can reduce the infection rate from

13 per cent to 0 per cent in early infection and from 14 per cent to 0 per cent in late infections. However, the total follow-up was only 2½ years; their series included fractured necks of femurs and the operations were performed entirely in orthopaedic theatres. Because of the wide variety of organisms cultured from infected hip prostheses, we believe that a single anti-staphylococcal antibiotic may not be adequate protection and that a broader spectrum antibiotic given systemically at the time of the operation may be more effective.

SUMMARY

Infection following total hip replacement is a serious complication for it is frequently impossible to resolve without removal of the prosthesis.

We have reviewed 321 total hip replacements undertaken in a general hospital without special orthopaedic theatres. There were 17 deep infections, nine early and eight late. Although the diagnosis of early infection is usually not difficult, the differentiation between late infections, mechanical failure and metal sensitivity may be a problem. This paper discusses the use of ESR, radiographs, isolation of pathogenic organisms and bone scanning in reaching the diagnosis of infection of the hip.

There is possibly a parallel between prosthetic infection and subacute bacterial endocarditis. Therefore all intercurrent infections and episodes of trauma should be given an adequate course of a broad spectrum antibiotic. Sterile air and laminar flow systems are discussed and compared with prophylactic antibiotics, both systemic and local, in attempting to reduce the overall rate of infection following total replacement of the hip.

ACKNOWLEDGEMENTS

We should like to express our thanks to Mr. P. H. Newman and Mr. D. R. Sweetman for allowing us to study their patients, and to Mr. Sweetman and Dr. R. E. M. Thompson for their help and advice in preparing this paper.

REFERENCES

Arnett, J. H. (1973) Delayed infection after total hip replacement. *J. Amer. med. Ass.* **223**, 1042.

Blowers, R. & Crew, B. (1960) Ventilation of operating theatres. *J. Hygiene* **58**, 427.

Brøckner, J. & Jessen, C. (1963) Risk of airborne infection at operation. *Acta chir. scand.* **125**, 8.

Charnley, J., Follacci, F. M. & Hammond, B. T. (1967) The long-term reaction of bone to self-curing acrylic cement. Centre for Hip Surgery, Wrightington Hospital. Internal Publication No. 9.

Charnley, J. (1968) Clean air operating room enclosure. Centre for Hip Surgery, Wrightington Hospital. Internal Publication No. 13.

Charnley, J. & Eftekhar, N. (1969) Post-operative infection in total prosthetic replacement arthroplasty of the hip joint. *Brit. J. Surg.* **56**, 641.

Charnley, J. (1972) The long-term results of low friction arthroplasty of the hip performed as primary intervention. *J. Bone Jt Surg.* **54**-B, 61.

Coventry, M. B., Beckenbaugh, R. D., Nolan, D. R. & Ilstrup, D. M. (1974) 2,012 total hip arthroplasties; a study of post-operative course and early complications. *J. Bone Jt Surg.* **56**-A, 273.

Ericson, C., Lidgren, L. & Lindberg, L. (1973) Cloxacillin in the prophylaxis of post-operative infections of the hip. *J. Bone Jt Surg.* **55**-A, 808.

Harris, Jacqueline, Lightowler, C. D. R. & Todd, R. C. (1972) Total hip replacement in inflammatory hip disease using the Charnley prosthesis. *Brit. med. J.* **2**, 750.

Hughes, S. P. F. (1966) The change in the ESR following surgery. A report to the St. Mary's Hospital Orthopaedic Club.

Lerner, P. I. & Weinstein, L. (1966 a) Infective endocarditis in the antibiotic era. *New Engl. J. Med.* **274**, 199.

Lerner, P. I. & Weinstein, L. (1966 b) Infective endocarditis in the antibiotic era. *New Engl. J. Med.* **274**, 388.

Noble, W. C., Lidwell, O. M. & Kingston, D. (1963) The size distribution of airborne particles carrying micro-organisms. *J. Hygiene* **61**, 385.

Patterson, F. P. & Selby Brown, C. (1972) The McKee-Farrar total hip replacement. *J. Bone Jt Surg.* **54**-A, 257.

Roles, N. C. (1971) Infection in total prosthetic replacement of the hip and knee joints. *Proc. roy. Soc. Med.* **64**, 636.

Scales, J. T., Towers, A. G. & Roantree, B. M. (1972) The influence of antibiotic therapy on wound inflammation and sepsis associated with orthopaedic implants. *Acta orthop. scand.* **43**, 85.

Sillar, W. & Conner, A. N. (1971) Total hip replacement. Initial experience with the Charnley low friction arthroplasty. *Scottish med. J.* **16**, 228.

Todd, R. C., Lightowler, C. D. R. & Harris, Jacqueline (1972) Total replacement in osteoarthrosis using the Charnley prosthesis. *Brit. med. J.* **2**, 752.

Key words: infection; total hip replacement; general hospital

Correspondence to:

S. P. F. Hughes
The Middlesex Hospital
London W1N FAA
England

EXTRACTION OF MINERALS FOLLOWING FRACTURES OF THE TIBIA

S.P.F. Hughes, D.R. Davies, J.B. Bassingthwaighte and P.J. Kelly,
Department of Orthopedics, Mayo Clinic and Mayo Foundation,
Rochester, Minnesota 55901

Diaphyseal blood flow increases after a fracture. However, because the clearance techniques for measuring blood flow depend on a knowledge of extraction, we have examined the relationship between extraction of the bone-seeking isotope ^{85}Sr and bone blood flow in the fractured canine tibia.

The right tibias of 19 adult dogs were divided at the juncture of the middle and distal thirds. The fracture was reduced and fixed with a four-hole plate. The dogs were able to bear weight after 3 days. At 2, 6, and 12 weeks, groups of dogs were anesthetized and the tibial nutrient artery was cannulated via the transfibular route. Diaphyseal blood flow was measured by the iodoantipyrine (I-Ap) washout technique (J Appl Physiol 31:38-47, 1971). Extraction of ^{85}Sr was measured by an indicator-dilution technique wherein a 1-ml sample of 5 μCi ^{85}Sr, 5 μCi [^{14}C]sucrose, and 15 μCi [^{51}Cr]albumin was infused in the nutrient artery over 47 seconds. Strontium is a small molecule that exchanges with calcium on the hydroxyapatite crystal; sucrose is freely diffusible, penetrates capillaries, and permeates the extravascular space but does not enter cells. Chromium-labeled albumin is a non-diffusible reference tracer. Indicator-dilution curves are constructed from the venous outflow samples collected from the ipsilateral femoral vein over 10-second periods for 3 minutes. Samples of plasma (0.2 ml) are counted in a liquid scintillation system.

Instantaneous extraction of the strontium and sucrose, E(t), can be estimated from the formula

$$E(t) = \frac{h_R(t) - h(t)}{h_R(t)}$$

where h(t) is the fraction of the injected dose appearing in the outflow per second, for sucrose or strontium, and h_R is the fraction of the reference tracer, ^{51}Cr. The maximal value of each E(t), E_{max}, is taken to be the best indication of extraction (Circ Res 35:483-503, 1974). By knowing the extraction, it is possible to estimate the capillary permeability-surface area product, PS, from the formula $PS = -F_B \log_e (1 - E_{max})$ (Am J Physiol 197:1205, 1959).

At 2 weeks after the fracture, the blood flow was (mean ± SE) 4.29 ± 0.5 ml/min per 100 g (N = 3) as estimated from I-Ap washout; when the fracture was healed, at 12 weeks, the estimated flow was 1.49 ± 0.1 (N = 4), which was closer to the normal value of 1.47 ± 0.63 (N = 27).

E_{max} of Strontium and Sucrose (SE)

	2 weeks	6 weeks	12 weeks
^{85}Sr*	0.77(N=9) ±0.05	0.67(N=5) ±0.08	0.74(N=5) ±0.04
Sucrose†	0.69(N=9) ±0.05	0.55(N=5) ±0.06	0.68(N=5) ±0.06

Control: *0.77±0.03(N=10), †0.56±0.03(N=10)

PS Product, Strontium (ml/min per 100 g)

2-week fracture	3.30 ± 0.5 (N=3)
12-week fracture	1.29 ± 0.2 (N=4)
Control	0.78 (N=12)

These results show that while blood flow increases after a fracture, the fractional extraction of minerals remains unchanged. This might occur in spite of the increased blood flow if there was an increase in surface area from recruitment of capillaries.

It is our conclusion that the clearance technique in the experimental animal is a reliable means of defining bone blood flow and that, in the fractured bone, increase in clearance is proportional to the increase in blood flow, for the extraction of the isotopes does not change, although the amount of ^{85}Sr extracted increases because of recruitment of capillaries at the fracture site.

Supported in part by NIH Research Grants AM-15980 and HL-9719.

Department of Orthopedics, Mayo Clinic and Mayo Foundation, Rochester, Minnesota 55901

MARCH 1, 1975 • VOLUME 34, NUMBER 3

Federation Proceedings

Abstracts

59th Annual Meeting
Atlantic City, New Jersey
April 13–18, 1975

FEDERATION OF AMERICAN SOCIETIES FOR EXPERIMENTAL BIOLOGY

AMERICAN PHYSIOLOGICAL SOCIETY
AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS
AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY
AMERICAN INSTITUTE OF NUTRITION
AMERICAN ASSOCIATION OF IMMUNOLOGISTS

Capillary Blood Flow

PHYSIOLOGY

1019

SLOW COMPONENTS OF TISSUE WASHOUT: FLOW OR DIFFUSION LIMITED? R.D. Varn*, W.N. Durán* and E.M. Renkin. F.G. Hall Laboratory and Dept. of Physiology and Pharmacology, Duke Univ. Med. Ctr., Durham, N.C. 27710.

Experiments were done to determine whether slow phases of multi-exponential washout of highly diffusible solutes are due to uneven distribution of blood flow, or to diffusion gradients around capillaries. Blood-perfused dog gracilis muscles were loaded with tritiated water (THO) and ^{14}C-antipyrine (AP) or ^{14}C-urea by 60 min. i.a. infusion. Simultaneous washout of the two tracers was then studied by measuring their concentrations in samples of venous effluent. Washout curves at constant blood flow were multi-exponential. Early washout rates of THO and AP were rapid and closely similar, indicating flow-limited transport. After a brief rapid phase, urea washout became slower than that of THO and AP, indicating diffusion-limitation. During subsequent slow phase washout of all three substances, two experimental maneuvers were done: 1) step reduction of blood flow, 2) momentary interruption of blood flow. After both procedures, venous concentration of urea increased substantially, while concentrations of THO and AP increased only slightly. We conclude that slow component washout is at least partly diffusion-limited for urea, but almost entirely flow-limited for THO and AP. (Supported by USPHS Grant HP 12749)

PHYSIOLOGY

1020

LYMPH FLOW TRANSIENTS FOLLOWING ELEVATION OF VENOUS PRESSURE IN THE DOG'S HIND PAW. H. I. Chen*, H. J. Granger, and A. E. Taylor. Univ. of Miss. Sch. Med., Jackson, Miss. 39216

In 8 pentobarbital-anesthetized dogs, the mean arterial pressure was kept constant at 100 mm Hg, and venous pressure (VP) changed by altering the level of an external venous reservoir. Two lymphatic vessels on each side of the lateral saphenous vein near the ankle region were cannulated and lymph flow (LF) was measured each minute in order to observe LF transients following sudden elevation of VP by either 20 or 30 mm Hg. At a VP of 5 mm Hg the baseline LF was 3.3 X 10^{-5} ml/min. The LF transient during 30 min. of venous hypertension consisted of a rapid 10 minute peak phase followed by a 20 minute steady-state plateau phase for VP's of 25 and 35 mm Hg. The peak and steady-state plateau flow for 20 mm Hg VP elevation were 32.5 X 10^{-5} ml/min (10.4-fold) and 18.6 X 10^{-5} ml/min (5.5-fold), respectively; and 63.9 X 10^{-5} ml/min (18.1-fold and 35.1 X 10^{-5} ml/min (9.8-fold) for 30 mm Hg venous pressure elevation. Upon returning VP to control levels, LF rapidly decreased to one-half the plateau level in 2 minutes, and gradually approached the baseline flow, requiring 26 and 46.5 minutes for VP elevations of 20 and 30 mm Hg, respectively. The data demonstrate that LF increases in proportion to the extent of venous pressure. Regardless of the underlying mechanism that causes the changes in LF, both the on and off transients consist of fast and slow components.

(Supported by NIH grants HL 11678, HL 11477, and HL 15680.)

PHYSIOLOGY

1021

INTERSTITIAL SPACE STRUCTURE: AN EXPLANATION OF CAPILLARY-LYMPH SOLUTE TRANSPORT DYNAMICS. Philip D. Watson* and Fred S. Grodins. USC, Los Angeles, California 90007

The dynamics of capillary-lymph transport for sucrose and various dextrans are shown to be inconsistent with the current, well-mixed single compartment models of the interstitial space (IS), even when the model is modified with variable excluded volumes and transport delays. The simplest IS structure found consistent with the published dynamic data and with the steady-state volumes of distribution, is a two-phase (gel and free-fluid), distributed system with restricted diffusion in the gel phase.

This proposed structure produces a flat, steady-state, capillary-lymph concentration profile for all solutes. It predicts that the largest dextrans, > 250,000 mol. wt., are not in steady-state after 6 hours, and could appear in the lymph faster than some smaller dextrans.

When the same structure is used as the extra-vascular space in an "arterial disappearance curve" simulation, the arterial concentration curve obtained is well fitted by two exponentials. Hence the proposed IS structure may be an alternative to the nutritional-shunt flow hypothesis.
(Supported by NIH Grants GM-01724 and GM-53110.)

PHYSIOLOGY

1022

ISOGRAVIMETRIC CAPILLARY PRESSURE (PCI) AND NEGATIVE INTERSTITIAL FLUID PRESSURE (PISF) IN THE ISOLATED HINDLEG. Robert A. Brace*, Aubrey E. Taylor, and Arthur C. Guyton. Univ. of Miss. Med. Ctr., Jackson, MS 39216

We have measured isogravimetric pressure after very rapid removal of the hindleg of the dog. The method involved clamping all except the femoral artery and vein just distal to the hip joint and then severing at the joint. During the initial perfusion period, arterial and venous pressures were adjusted so that no slow weight change occurred. Upon reaching a steady state, isogravimetric capillary pressure averaged (n = 9) 8.1 ± 0.4 (SE) mm Hg while plasma colloid osmotic pressure (πC) was 16.4 ± 1.0 mm Hg. If it is assumed that the colloid osmotic pressure of the interstitial fluid was 4 mm Hg, interstitial fluid pressure averaged −4.3 ± 0.8 mm Hg. Increasing tissue weight by filtration into the tissues produced the following:

% CHANGE WEIGHT	PCI (mm Hg)	πC (mm Hg)	PISF (calculated) (mm Hg)
0	8.1	16.4	-4.3
1.2	11.9	16.6	-0.7
2.9	14.0	16.9	+1.1
5.8	17.9	17.8	+4.1
9.2	19.1	18.7	+4.4

This data indicate interstitial fluid pressure is normally negative in the hindleg. However, very small fluid shifts cause this pressure to become positive and the isogravimetric pressure to increase greatly. Supported by NIH grant HL 05184

PHYSIOLOGY

1023

CALCULATION OF THE EDEMA SAFETY FACTOR IN ISOLATED DOG LUNG. Robert E. Drake* and Aubrey E. Taylor. Univ. of Mis. Med. Ctr. Jackson, Miss. 39216

The increase in perimicrovascular pressure, decrease in lymph colloid osmotic pressure, and increase in lymph flow were either measured or calculated at different pulmonary venous pressures in a perfused dog lung. A pressure volume curve for the lung tissue was generated by increasing pulmonary venous pressures by small increments. Perimicrovascular pressure increased from the lowest value of −4.8 mm Hg to +2 mm Hg before large fluid accumulation occurred in the lung. The compliance increased from 3 to 20 ml/mm Hg/100 gm. As fluid collected in the lung, the perimicrovascular osmotic pressure decreased in a range of 2.1 to 12.1 mm Hg. The filtration coefficient of the pulmonary capillaries was 0.21 ml/min/mm Hg/100 gm for all lungs studied. The lymph flow was small in our preparation and does not represent total lung lymph flow. To calculate the maximum pressure drop we have used a value of .06 ml/min/100 gm for total lung lymph flow. The pressure drop is .3 mm Hg at low pulmonary venous pressures and increases to 2 mm Hg at the high pulmonary venous pressures, since lymph flow increased an average of 6 fold. The total safety factor calculates to be 10.9 to 20.9 mm Hg and agrees with the safety factor of 18-20 mm Hg as calculated for intact dog lung (Guyton and Lindsey, Circ. Res., 7: 649, 1959).

Supported by NIH Grant HL 11477

PHYSIOLOGY

1024

EXCHANGE BETWEEN BLOOD, TISSUE AND PERIPHERAL LYMPH IN MESENTERY. Alan R. Hargens* and Benjamin W. Zweifach. Dept. of Bioengineering, Univ. of Calif., San Diego, La Jolla, CA. 92037.

An attempt was made to measure directly the forces responsible for fluid transfer between blood and tissue. The four parameters of the Starling equation thus determined are blood capillary hydrostatic pressure (P_b), blood colloid osmotic pressure (π_b), tissue fluid hydrostatic pressure (P_t), and tissue fluid colloid osmotic pressure (π_t). Representative values in normal cat mesentery are 30 cm H_2O for P_b, 25 cm H_2O for π_b, zero cm H_2O for P_t, and 5 cm H_2O for π_t. Perturbations in the blood variables elicit immediate and compensatory changes in the tissue variables so that the Starling equilibrium is maintained. However, compensation also occurs by abrupt adjustments in lymph flow. Substantial protein is transferred from blood, across tissue, and into peripheral lymph, a further indication that the lymphatic system is important in maintaining the equilibrium. In addition, the lymphatic system itself functions in a Starling-type framework by concentrating protein by ultrafiltration throughout its network of collecting vessels.
(Supported by USPHS NIH Grant HL 10881 and by the San Diego County Heart Association.)

1025 PHYSIOLOGY

RELATIVE WASHOUT KINETICS OF PAIRED SUBSTANCES INJECTED INTO RED BONE MARROW. E.L. Dobson, H.G. Parker,* D.C. Van Dyke,* M.L. Mohr* and J.J. Lynch.* Donner Laboratory, University of California, Berkeley, CA 94720

Following a direct intramedullary injection into the bone marrow, we compared the rate of washout of pairs of radioactive tracers administered mixed in the same solution. The detection system used was an Anger scintillation camera with fast digital recording. With this system, we could follow the washout from the instant of injection because the injection syringe and the injection site are easily separable with the Anger Camera. The initial portion of the washout pattern from the distal femoral marrow of the dog's leg was very rapid, with half times of a few seconds. Iodide ion, pertechnetate ion, sodium ion, and labeled albumin in various pairs followed essentially identical multiexponential washout patterns. Iodoantipyrine, however, washed out somewhat more slowly. Retention of a sizeable fraction of injected colloidal particles (microaggregated albumin and Tc-sulphur colloid), as well as fluoride ion, verified that these were not simply intravenous injections. The similarity in washout pattern between iodide ion and albumin suggests that there is no extracellular compartment separate from the plasma space in the marrow. (Supported in part by the U.S. Atomic Energy Commission)

1026 PHYSIOLOGY

EXTRACTION BY BONE OF TECHNETIUM-99m-LABELED ETHANE-1-HYDROXY-1,1-DIPHOSPHONATE. Sean P. F. Hughes*, David R. Davies*, Patrick J. Kelly and James B. Bassingthwaighte. Mayo Graduate School of Medicine, Rochester, MN 55901

The transcapillary extraction of technetium-99m-labeled ethane-1-hydroxy-1,1-diphosphonate (EHDP), a substance used in bone scanning, was measured in 10 dogs by injecting 20 μCi of EHDP, 30 μCi of ^{51}Cr-labeled albumin, and 10 μCi of [^{14}C]sucrose into the tibial nutrient artery (injection duration, 1 minute) and sampling at 15-second intervals from the ipsilateral femoral vein for construction of outflow concentration-time curves. A pneumatic cuff inflated to 60 mm Hg applied to the thigh prevented recirculation. At 5 minutes the animal was killed, and radioactivity in the tibia was counted. The mean (±SD) net 5-minute extraction of EHDP calculated from the venous outflow curve was 0.18 ± 0.05 (N = 10). The maximal instantaneous extraction of EHDP was 0.27 ± 0.05 (N = 10). By direct counting of tibial samples, the fraction of the EHDP remaining in the bone was 0.21 ± 0.06 (N = 4); 0.07 ± 0.03 (N = 4) of the albumin was also retained. The average ratio of permeabilities of EHDP to sucrose, using $PS = -F \log_e(1 - E_{max})$ for each tracer, was 0.72 ± 0.18 (N = 10) which is not statistically different from the ratio of free diffusion coefficients, 0.86. These results suggest that the mechanism for transcapillary exchange of sucrose and EHDP is free diffusion through the clefts in the capillary endothelium. (Supported in part by USPHS NIH Grants AM-15980 and HL-9719)

1027 PHYSIOLOGY

MECHANISMS OF TRANSENDOTHELIAL EXCHANGE. Emil Aschheim, 6803 Clyde Str., Forest Hills, New York, N.Y. 11375

Evidence discussed elsewhere (Microvasc. Research 8:64-69, 1974) suggests that at the level of the terminal vascular bed there exist two separate and functionally distinct modes of transport that appear to be largely independent of each other. The first, the Starling-Folkow mechanism is concerned with shifts of water between the extra and intravascular compartments and serves the regulation of blood volume. The locus of passage are the interendothelial clefts, the forces are those originally described by Starling and the controlling mechanism the modulation of pre and postcapillary resistances by the autonomic nervous system.

The second, or Krogh-Palade mechanism is nutritive in nature, since it deals with the passage of small molecular weight solutes. It is based first on the transcellular peregrinations of Palade's vesicles, a mode of transport in which the overall magnitude is under cellular regulation, and second, on the combined effects of diffusion and convection that act on molecules outside the endothelial tubes. The convective component of this transport is due to water flows that are generated between closed and open capillaries by the out of phase contraction and relaxation of precapillary sphincters.

1028 PHYSIOLOGY

VASCULAR DAMAGE FROM INTRA-ARTERIAL INJECTIONS OF BARBITURATES. Mary P. Wiedeman and Ronald F. Tuma*. Temple Medical School, Philadelphia, Pa. 19140.

In vivo microscopic observations of intra-arterial injections of secobarbital in the wing of the bat revealed that platelet aggregates at arteriolar branch sites rather than vasospasm were the cause of subsequent ischemia. Further studies were undertaken to determine what component of the oral preparation was responsible for the initiation of the platelet aggregations. Comparison with the effects of an intravenous preparation of sodium pentothal showed no differences. Attempts to reinstitute flow with hydrocortisone injected intra-arterially immediately after the barbiturate injection have been moderately successful. Evidence for an interaction with the vessel wall will be presented and the responses will be shown by color cinephotomicrography.

Supported by the National Institutes of Health, S.C.O.R. Grant # HL 14217-05.