PHOSPHATE COMPOUNDS IN BONE SCANNING


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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 ethylenediaminetetraacetate (EDTA)—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 Each substance was injected into ten rabbits and blood was withdrawn at regular intervals.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 EDTA 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 EDTA 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, tripolyphosphate and ethylenediaminetetraacetate (EDTA). 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 ethylenediaminetetraacetate 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.

Ethylenediaminetetraacetate was prepared by adding 0·5 millilitre of stannous chloride to 0·5 millilitre of ethylenediaminetetraacetate 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.
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 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>
<thead>
<tr>
<th>Tissue</th>
<th>EHDP</th>
<th>Tripolyphosphate</th>
<th>Pyrophosphate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Skin</td>
<td>0.039</td>
<td>0.117</td>
<td>0.035</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.010</td>
<td>0.043</td>
<td>0.011</td>
</tr>
<tr>
<td>Liver</td>
<td>0.030</td>
<td>0.464</td>
<td>0.434</td>
</tr>
<tr>
<td>Kidney</td>
<td>0.532</td>
<td>2.018</td>
<td>0.715</td>
</tr>
<tr>
<td>Bone</td>
<td>1.673</td>
<td>1.716</td>
<td>2.722</td>
</tr>
</tbody>
</table>

*(N=10)*
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, Panneiciere 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 $^{32}$P in subcapital fractures of the femoral neck (McNeur 1970), in the study of fracture healing (Wendelberg 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 (Charles 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.

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**REFERENCES**


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