DIPHOSPHONATES
Experimental and Clinical Aspects

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Diphosphonates are simple chemical compounds containing phosphorus-carbon-phosphorus bonds. In recent years there has been considerable interest in compounds of this type because of the profound effects they have on crystal behaviour in vitro and on calcium metabolism in living animals. The story is still far from complete. In this article we will review the experimental and clinical information available at the time of writing (mid-1972).

EXPERIMENTAL ASPECTS

Background—The origins of this work go back to the 1930's, when it was shown that low concentrations of condensed phosphates—compounds which possess chains of P-O-P-O units joined together—were able to prevent the deposition of calcium carbonate from solution. This observation was soon applied to prevent the scaling of industrial and domestic water installations by calcium salts. "Calgon" is a condensed phosphate used in many households today as a water softener.

At the beginning of the 1960's, Neuman and Fleisch recalled this work when trying to explain mechanisms of biological calcification. At that time it was known that the concentrations of calcium and phosphate ions in plasma were insufficient to allow the spontaneous precipitation of calcium phosphate from biological fluids. How then did biological calcification take place? An explanation seemed forthcoming when it was found that collagen, one of the main structural proteins of connective tissues, including bone, promoted the formation of calcium phosphate crystals even from solutions containing physiological concentrations of calcium and phosphate ions. However, this immediately posed a further problem. Why was it that calcification, under normal conditions, was confined to bone and teeth? Many other tissues contain collagens; why did they not all calcify? Fleisch and Neuman (1961) then began to search for substances that might inhibit the nucleating activity of collagen. They found that plasma ultrafiltrates and urine contained substances capable of inhibiting the precipitation of calcium phosphate in vitro, even in the presence of collagen. One of the inhibitors turned out to be inorganic pyrophosphate, the simplest of the condensed phosphates.

During the ensuing years there followed a series of studies to define the role of inorganic pyrophosphate (PPi) in the regulation of calcification and its possible involvement in human diseases. In living animals PPi, given parenterally inhibited various types of experimentally induced calcification. Since PPi is normally present in body fluids it was thought essential that PPi be destroyed before calcification can take place. This destruction could be brought about by pyrophosphatases, one of which is alkaline phosphatase, an enzyme long known to be associated with calcification. When this enzyme is missing, as in the human disease of hypophosphatasia, there is a failure of calcification and increased concentrations of PPi in body fluids (Russell, Bisaz, Donath, Morgan and Fleisch 1971). Low concentrations of PPi in urine may be associated with the production of some renal stones (Russell and Fleisch 1969). This and other work on PPi in calcium metabolism and in diseases of bones and teeth is reviewed in detail elsewhere (Fleisch and Russell 1970, 1972; Fleisch, Bonjour, Morgan, Reynolds, Schenk, Smith and Russell 1972; Fleisch, Russell, Bisaz and Bonjour 1973).
Attempts to utilise the properties of \(PP_1\) and condensed phosphates for therapeutic purposes did not seem to hold much hope of success because these substances were not absorbed intact from the gut and were rapidly destroyed in the body when given by injection. What was needed were compounds related in structure to pyrophosphate with similar effects \textit{in vitro} but which would be more resistant to enzymatic destruction and would be able to produce stronger and longer lasting effects \textit{in vivo}. One analogue of \(PP_1\), imidodiphosphate, in which a P-N-P bond replaces the P-O-P bond of \(PP_1\) was tried and did have effects similar to \(PP_1\) on calcium phosphates \textit{in vitro} (Robertson and Fleisch 1970), but it was labile and ineffective in living systems. However, the diphosphonates which possessed P-C-P bonds did prove more successful. These compounds are much more stable than \(PP_1\) to both chemical and enzymatic degradation. For example, diphosphonates can withstand several hours at 100 degrees Celsius in 1N-hydrochloric acid, whereas \(PP_1\) is completely hydrolysed within a few minutes under these conditions.

![Inorganic pyrophosphoric acid and two diphosphonates](image)

**FIG. 1**
The structure of inorganic pyrophosphoric acid and two diphosphonates. EHDP is the only diphosphonate that has been used clinically.

The two diphosphonates which have been studied in greatest detail are disodium ethane-1-hydroxy-1,1-diphosphonate (EHDP) and disodium dichloromethylene diphosphonate (Cl\(_2\)MDP). Their chemical structure is compared with pyrophosphate (PP) in Figure 1. Longer chain polyphosphates (condensed phosphate) such as are used as water softeners contain more phosphate residues joined together in a similar way to give the structure

\[
\begin{array}{c}
\text{O} \\
\text{H} \\
\text{O} \equiv \text{P} \parallel \text{O} \equiv \text{P} \parallel \text{O} \\
\text{OH} \\
\end{array}
\]

where \(n\) is equal to or greater than 1.

**PHYSICO-CHEMICAL EFFECTS OF DIPHOSPHONATES**

Diphosphonates have effects very similar to those of \(PP_1\) on the behaviour of calcium salts \textit{in vitro}. Thus they inhibit the precipitation of several calcium salts, including calcium phosphate, from solution (Fleisch, Russell, Bisaz, Mühlbauer and Williams 1970). They also block the conversion of amorphous calcium phosphate into crystalline hydroxyapatite (Francis
1969; Francis, Russell and Fleisch 1969). When crystals of hydroxapatite are treated with diphosphonates the rate at which the crystals dissolve, when resuspended in solutions free of calcium and phosphate, is much lower than normal. PP$_1$ has similar effects. The effect on crystal dissolution is important because it may explain why the diphosphonates are able to inhibit bone resorption.

Large amounts of certain diphosphonates partially convert apatite crystals into a colloidal state, a phenomenon called pepitisation (Robertson, Morgan, Fleisch and Francis 1972). EHDP also forms polynuclear complexes in the presence of calcium (Grabensetter and Cilley 1971, Wiers 1971). These two effects may be of biological significance only when large concentrations of diphosphonates are used. Most of the physico-chemical effects of diphosphonates are probably related to the strong affinity which the diphosphonates have for calcium ions and for calcium phosphates. Diphosphonates bind strongly on to the surface of crystals of hydroxapatite, probably to more than one type of binding site, and they progressively displace orthophosphate from the crystals as they become bound (Jung, Bisaz and Fleisch, in press). The total binding capacity follows the order PP$_1$ > EHDP > Cl$_2$MDP, but the affinity of the first binding site follows the sequence EHDP > PP$_1$ > Cl$_2$MDP.

The precise way in which diphosphonates exert their effects on calcium phosphates is not understood. Their effects on crystallisation probably involve an inhibition of heterogenous nucleation and subsequent growth and aggregation of crystal nuclei of hydroxapatite. The diphosphonates probably adsorb to the earliest crystal nuclei, which contain only a few atoms of calcium and phosphate, and so alter the properties of these small particles that subsequent steps in crystal growth and maturation are blocked. The effect of diphosphonates on crystal dissolution is also probably a direct result of their adsorption to the surface layers of the crystal, where they alter the configuration of the sites at which dissolution is actively taking place, and thus decrease the solubility of the crystals.

EFFECTS OF DIPHOSPHONATES ON CALCIFICATION OF SOFT TISSUES

Diphosphonates prevent various types of soft-tissue calcification induced experimentally. Thus they inhibit the aortic and kidney calcification that occurs in rats given large doses of vitamin D (Fleisch, Russell, Bisaz, Mühlbauer and Williams 1970) and the calcium deposition induced by dihydrotachysterol in skin (Casey, Casey, Fleisch and Russell 1972). Diphosphonates differ from PP$_1$, for they are effective whether given parenterally or by mouth whereas PP$_1$ is effective only when given parenterally, probably because it is hydrolysed by the intestinal mucosa. EHDP also prevents the periarticular calcification and the articular changes associated with adjuvant arthritis in the rat (Francis, Flora and King 1972). Furthermore, EHDP given orally to rats inhibits the production of bladder calculi composed of calcium oxalate and calcium phosphate but not those composed of magnesium ammonium phosphate (Fraser, Russell, Pohler, Robertson and Fleisch 1972). This correlates with the ability of EHDP to inhibit the precipitation of calcium phosphate and calcium oxalate in vitro without influencing the precipitation of magnesium ammonium phosphate.

EFFECTS OF DIPHOSPHONATES ON BONE CALCIFICATION

From a theoretical viewpoint, diphosphonates given in large enough doses should be able to inhibit the calcification of bone even though PP$_1$ itself has never been shown to be able to do this, possibly because it is too rapidly destroyed in vivo. In fact it has now been well documented that EHDP, given in high doses to several species, leads to the appearance of osteoid tissue in bone (Fleisch, Bisaz, Care, Mühlbauer and Russell 1970; Jowsey, Holley and Linman 1970; King, Francis and Michael 1971; Russell, Thornton, Casey, Mühlbauer, Kislig, Fleisch, Williams and Schenk 1973) and to a histological appearance in the epiphysial plate which resembles classical vitamin D-deficient rickets (Schenk, Merz, Fleisch,
Mühlbauer and Russell 1973). The defects are reversible when the administration is discontinued (King, Francis and Michael 1971). Studies in rats show that, with careful choice of doses, mineralisation can be selectively inhibited while the laying down of matrix proceeds at normal rates. This suggests that only the mineralisation step is inhibited (Russell, Thornton, Casey, Mühlbauer, Kislig, Fleisch, Williams and Schenk 1973) and that EHDP does not interfere with the synthesis of bone and cartilage matrix at these doses. The dose of EHDP required to produce inhibition of bone mineralisation varies according to the species, the duration of treatment and the route of administration. Roughly, it starts at doses above 1 milligram P/kilogram/day* given parenterally for short periods. This is about ten times higher than the doses currently being given in clinical trials. However, it is obviously difficult to compare effects between species on a body weight basis only, so that caution is needed in man if such effects are to be avoided.

In chicks, in whom bone and cartilage mineralisation was inhibited by EHDP, the lactate production by epiphyseal cartilage was not significantly different from normal when zones of similar histological appearance were compared (Biszaz, Kunin and Fleisch, unpublished). This supports the view that EHDP acts to prevent the mineralisation step per se rather than causing a general alteration of cell metabolism.

Interestingly, C12MDP, unlike EHDP, does not inhibit cartilage and bone mineralisation in rats. This difference between EHDP and C12MDP is puzzling, since C12MDP is as effective as EHDP in preventing soft-tissue calcification and both compounds are potent inhibitors of the precipitation of calcium phosphate in vitro. The difference may be due to differences in the distribution of the two compounds in the body, perhaps related to the fact that EHDP binds more strongly and to a greater extent to crystals than C12MDP. Alternatively, the inhibition of mineralisation may not be due to a simple inhibition of crystal growth but to some unidentified effect on cell metabolism. Whatever the final explanation, the difference between the effects of EHDP and C12MDP may be of practical therapeutic importance.

**EFFECTS OF DIPHOSPHONATES ON BONE RESORPTION**

Since diphosphonates slow the rate at which hydroxyapatite crystals dissolve one might expect them to be able to slow down the rate of bone resorption. Many studies have now shown that diphosphonates can inhibit bone resorption in a variety of experimental systems. In tissue culture, they can prevent the resorption induced by parathyroid hormone (PTH) in mice calvaria, whether this is measured morphologically (Fleisch, Russell and Francis 1969; Russell, Mühlbauer, Bisaz, Williams and Fleisch 1971) or by the release of 44Ca from prelabelled bone (Reynolds, Minkin, Spycher, Morgan and Fleisch 1973). EHDP and C12MDP are effective when added to the medium at concentrations as low as 10^{-6}M, but at equal doses C12MDP is more potent than EHDP. When the effect of C12MDP was compared with that of calcitonin, the recently discovered hormone that produces hypocalcaemia, it was found that both agents inhibited the PTH-induced release of 44Ca and lactate. However, whereas calcitonin had no effect on the PTH-induced increases in various phosphatases and pyrophosphatases, C12MDP blocked the effect of PTH on acid phosphatase and acid pyrophosphatase (Russell, Mühlbauer, Williams, Reynolds, Morgan, Copp and Fleisch 1972). This suggests that the two compounds inhibit bone resorption by different mechanisms. Electron microscopy of bone from rats treated with EHDP reveals a diminution in lysosome content of bone cells (Doty, Jones and Finerman 1972), the lysosomes being the particles in which acid hydrolases such as acid pyrophosphatase are located. Similarly Schenk, Merz, Fleisch, Mühlbauer and Russell (1973) have shown a striking alteration in the histological appearance of osteoclasts from rats treated with C12MDP. The cells are larger and contain more nuclei than usual and their cytoplasm is clearer, suggesting a diminution of intracellular

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* The disodium salt of EHDP contains approximately 25 per cent elemental phosphorus.
particles such as lysosomes. All this does not necessarily mean that diphosphonates act directly on bone cells since alterations in bone mineral solubility and hence in local ion concentrations could lead to changes in cell metabolism (Rasmussen, Kurokawa and deLong 1971).

Diphosphonates also inhibit bone resorption in living animals. Thus, bones explanted from new-born mice previously treated with EHDP or CI₂MDP exhibit a reduction of bone resorption rate in tissue culture (Reynolds, Minkin, Spycher, Morgan and Fleisch 1973). Furthermore, both EHDP and CI₂MDP partly prevent the increase in blood calcium induced by parathyroid hormone in thyroparathyroidectomised rats on a low calcium diet (Fleisch, Russell and Francis 1969; Russell, Mühlbauer, Bisaz, Williams and Fleisch 1971). In young mice, CI₂MDP given at a dose of 10 milligrams P/kilogram/day subcutaneously causes a severe impairment of normal bone remodelling resulting in a skeleton which resembles that of the “grey lethal” strain of congenitally osteopetrotic mice (Reynolds, Morgan, Mühlbauer and Fleisch, unpublished). In young growing rats, 10 and 30 milligrams P/kilogram/day of CI₂MDP retard the remodelling of the metaphysis to such a degree that, as the bone grows in length, the metaphysis becomes club-shaped and radiologically more dense than normal (Schenk, Merz, Fleisch, Mühlbauer and Russell 1973). All these effects on bone resorption and remodelling are more pronounced with CI₂MDP than with EHDP given at an equal dose.

Diphosphonates have also been tested in rats in which osteoporosis was induced by immobilising a limb by nerve section (Michael, King and Francis 1971; Mühlbauer, Russell, Williams and Fleisch 1971). This type of bone loss may resemble that seen in fractured limbs and in patients subjected to prolonged bed rest. Both EHDP and CI₂MDP, at doses as low as 0·01 milligram P/kilogram/day subcutaneously, reduced the difference between immobilised and non-immobilised limbs. Again, at equal doses, CI₂MDP was generally more effective than EHDP. Polyphosphates and calcitonin are unable to prevent this type of bone change. It is significant, from the point of view of clinical applications, that in animals there is about a hundredfold difference between doses of EHDP that are effective in this system and the minimum doses required to induce inhibition of bone and cartilage mineralisation.

EFFECTS OF DIPHOSPHONATES ON CALCIUM METABOLISM

In intact animals, diphosphonates have significant effects on bone turnover as measured by ⁴⁰Ca kinetic techniques (Gasser, Morgan, Fleisch and Richelle 1972). Thus, increasing doses of both EHDP and CI₂MDP progressively prevent the increase in the rate of bone resorption which occurs when rats are switched from a high to a low calcium diet. The effect of CI₂MDP is again greater than that of EHDP at equal doses. Furthermore, effective doses— that is, down to 0·01 milligram P/kilogram/day subcutaneously—are again lower than those necessary to inhibit soft-tissue calcification. Except with high doses of EHDP, the net calcium balance of the animal and the mineral content of bone increase only slightly. This is explained by the fact that bone mineralisation rate is decreased to nearly the same extent as bone resorption rate, so that the net result is a reduction in bone turnover rate without a large change in the chemical composition of the bones. It may seem paradoxical that studies on ⁴⁰Ca kinetics reveal this reduction in bone mineralisation rate with a wide range of doses of CI₂MDP and EHDP while much higher doses seem to be required to inhibit mineralisation of soft tissues, and moreover frankly rachitic bones are only produced with the highest doses of EHDP. One explanation is that the type of reduction of bone mineralisation rate that occurs with low doses of CI₂MDP is different from that which occurs with the highest doses of EHDP. With CI₂MDP there is no accumulation of unmineralised matrix as osteoid and cartilage, so that presumably there is a depression of matrix synthesis which precisely matches the reduction in mineralisation rate. One can speculate that this reduction in matrix synthesis is secondary to the inhibition of bone resorption and is brought about by some signal which links rates of bone formation to rates of bone destruction. This type of linking mechanism has been
proposed before (Harris and Heaney 1969) to explain why, in man, the rates of bone mineral accretion and bone mineral resorption always seem to be very closely correlated in a variety of normal and disease states. The linking seems disturbed only in very few situations, of which thyroid disease and the early stages of immobilisation osteoporosis may be examples. In contrast, the inhibition of mineralisation that occurs with high doses of EHDP probably involves a direct action at the site of newly formed matrix so that unmineralised matrix accumulates. In this case in the growing rat the calcium balance becomes markedly less positive, in contrast to an unchanged or slightly increased balance when the same dose of \( \text{Cl}_2\text{MDP} \) is administered (Gasser, Morgan, Fleisch and Richelle 1972).

This change in calcium balance with large doses of EHDP is associated with a reduction in intestinal absorption of calcium, the size of which corresponds roughly to the decreased net entry of calcium into bone (Morgan, Bonjour, Gasser, O'Brien and Fleisch 1971). This change suggests that there is some mechanism in the body for adjusting the rate of calcium absorption from the gut to the calcium requirement of the organism. Many years ago Nicolaysen, Eeg-Larsen and Malm (1953) proposed a similar mechanism to account for calcium adaptation in humans. In the case of EHDP-treated rats, when calcium can no longer be deposited in bone because it is blocked by EHDP, the proposed signal would inform the gut to absorb less calcium. If this did not happen calcium absorption would continue and the animal would become hypercalcaemic with its accompanying dangers. In studies of this phenomenon in EHDP-treated rats it has been established that there is a close relationship in time between the appearance of changes in the epiphysial cartilage and the decrease in intestinal absorption of calcium. The change in the absorption rate seems to be specific for calcium and is associated with a reduction in the content of calcium-binding protein and Ca-stimulated ATP-ase in the intestinal mucosa, but not in other brush border enzymes (Bonjour, Russell, Morgan and Fleisch, unpublished). Both the Ca-binding protein and Ca-stimulated ATP-ase are thought to be involved in calcium absorption (Wasserman and Taylor 1966, Melancon and DeLuca 1970) and are depressed in vitamin D-deficiency when calcium absorption is impaired.

Recently there has been a series of fascinating advances in our understanding of the metabolism of vitamin D. It is now thought that vitamin \( \text{D}_3 \) (cholecalciferol) is transformed in the liver to 25-hydroxycholecalciferol. This metabolite is transported to the kidney where it is further hydroxylated to produce 1,25 dihydroxycholecalciferol. Other hydroxylated metabolites are also produced from 25-hydroxycholecalciferol but it is now thought that the 1,25 dihydroxy derivative is the agent responsible for the well known stimulatory effect of vitamin D on the intestinal absorption of calcium. Studies of EHDP-treated rats have helped to shed light on the way that calcium metabolism is controlled by vitamin D. Thus the defective absorption of calcium in EHDP-treated rats, as measured in tied gut loops in situ, can be specifically reversed by low doses of the vitamin D metabolite, 1,25-dihydroxycholecalciferol (Bonjour and colleagues, unpublished). This suggests that the effect of EHDP on gut absorption of calcium may be due to a depression of synthesis of 1,25-dihydroxycholecalciferol by the kidney. Indeed, Stanbury and his colleagues (Hill, Mawer, Lumb and Stanbury 1972; Stanbury 1973) have demonstrated an inhibition of production of 1,25-dihydroxycholecalciferol in rats treated with high doses of EHDP. Since the synthesis of 1,25-dihydroxycholecalciferol seems to be controlled by plasma calcium (Boyle, Gray and DeLuca 1971), perhaps through its effect on secretion of parathyroid hormone, it is possible that the mild hypercalcaemia (Gasser, Morgan, Fleisch and Richelle 1972) which occurs soon after injection of EHDP is the signal for reduction in renal synthesis of 1,25-dihydroxycholecalciferol. These results support the suggestion (Boyle, Gray and DeLuca 1971; Boyle, Gray, Omdahl and DeLuca 1971) that 1,25-dihydroxycholecalciferol may be the agent responsible for adjusting the rate of calcium absorption to the calcium requirements of the organism.
The mechanism of the hypercalcaemia observed in the experiments mentioned above is not yet elucidated. It is probably different from the large increases in plasma calcium produced by infusing large amounts of EHDP into dogs and pigs (Fleisch, Bisaz, Care, Mühlbauer and Russell 1970; Gitelman 1970); these large rises are probably due to non-ultrafiltrable components and may represent the formation of polynuclear complexes between calcium and EHDP (Grabenstetter and Cilley 1971, Wiers 1971). The mechanism by which EHDP can bring the plasma calcium up to normal after parathyroidectomy (Russell, Mühlbauer, Bisaz, Williams and Fleisch 1971) is also not understood.

METABOLISM OF DIPHOSPHONATES

Diphosphonates are extremely resistant to chemical breakdown and no enzyme has yet been found that can catalyse their hydrolysis. This probably explains why they are more potent than PP$_3$. However, phosphonatases capable of catalysing the cleavage of certain types of C-P bonds are present in some micro-organisms (LaNauze, Rosenberg and Shaw 1970).

EHDP is absorbed in the gastro-intestinal tract, probably mainly in the stomach, to an extent which varies in different species—between 1 and 10 per cent in rats, rabbits, monkeys and man, but more in dogs. The absorption can vary more than tenfold from one individual to another within one species. Generally it is higher in younger animals than in the old (Michael, King and Wakim 1972). Approximately half of the absorbed compound goes into the bone and the rest is excreted in the urine. Only minimal amounts go into soft tissues (Michael, King and Wakim 1972). There is no significant breakdown in the body. In the rat, the half time for bone retention of EHDP has been calculated to be about four weeks after subcutaneous administration (King, Francis and Michael 1971; Michael, King and Wakim 1972). We do not yet know the extent of differences in metabolism that exist between EHDP and CI$_2$MDP.

MODE OF ACTION OF DIPHOSPHONATES

Most of the effects of diphosphonates on calcification and on bone can be accounted for in terms of what we know of their physico-chemical effects on calcium phosphate in vitro. However, there are some discrepancies which may point to other factors of importance in their mode of action. For instance, why does CI$_2$MDP not inhibit cartilage and bone mineralisation when it is able to inhibit crystal formation in vitro and soft-tissue calcification in vivo? Also, why is CI$_2$MDP more effective than EHDP on bone resorption in vivo in spite of having a weaker effect on crystal dissolution in vitro?

There is not much information available about the influence of diphosphonates on various biochemical processes. There have been some studies of the effects on phosphatases and pyrophosphatases, enzymes on which one might expect to see effects because diphosphonates structurally resemble pyrophosphate, the natural substrate for pyrophosphatases. In vitro the diphosphonates can be shown to have either no effect, to inactivate or inhibit, or even to activate various of these enzymes, depending on the conditions used (Wöltgens, Bonting and Bijvoet 1971; Russell, Preston and Fleisch, unpublished). The significance of these enzyme effects in living animals is difficult to judge because the concentrations of substrate and inhibitors are unknown. However, it might be of relevance that pyrophosphate concentrations in plasma of patients are not altered during treatment with EHDP. Diphosphonates have also been shown to inhibit renal adenyl cyclase in vitro (Pilczyk, Sutcliffe and Martin, personal communication). This is the enzyme that produces that ubiquitous intracellular chemical messenger, cyclic AMP, which in the renal cortex seems to be involved in the phosphaturic action of parathyroid hormone. However, Recker, Hassing, Lau and Saville (in press) have shown that oral administration of EHDP to men had no effect on basal or parathyroid hormone-stimulated rates of renal production of cyclic AMP. EHDP and CI$_2$MDP also slow down the rate of release of accumulated calcium from mitochondria in vitro (Guilland, Sallis...
and Fleisch, unpublished), but do not influence the uptake of aminoacids by isolated cells (Touabi, unpublished). Finally, EHDP was found to inhibit growth and acid production by Streptococcus salivarius (Guggenheim 1970).

CLINICAL ASPECTS

In the previous pages we have outlined the effects of the diphosphonates in vitro and in animals. It is on the basis of this work that their effects in man are now being studied.

There are several clinical situations in which these compounds might be effective. These can be divided into disorders of ectopic mineralisation and disorders of excessive bone resorption. Among the disorders of ectopic mineralisation one should also include the successful use of EHDP against dental calculus in man (Mühlemann, Bowles, Schait and Bernimoulin 1970; Sturzenberger, Swancar and Reiter 1971) and its possible future use against renal stone disease. In describing clinical experience with EHDP we will deal mainly with our own results. However, there are several other clinical studies in progress and results from these should soon become available. So far EHDP is the only diphosphate studied in man.

ECTOPIC MINERALISATION

There are many disorders of ectopic mineralisation. In soft tissues calcification may occur either in the presence of true ectopic bone tissue, as in myositis ossificans progressiva or in the myositis ossificans that occurs after paraplegia; or in its absence, as in scleroderma or dermatomyositis. The mechanisms underlying the abnormal calcification are completely unknown although the present rapid advances being made in studies of normal calcification may soon provide an answer. None of these conditions is common, and in most the course is unpredictable. The factors which lead to myositis ossificans in one patient and not in another are not understood. In these disorders of soft-tissue calcification it is recognised that spontaneous improvement may occur. This makes it difficult to evaluate procedures aimed at the prevention or treatment of ectopic mineralisation. Our studies have concentrated on one of the better characterised disorders of ectopic mineralisation, namely myositis ossificans progressiva, but we have also studied the less predictable generalised calcinosis which may occur in dermatomyositis.

Myositis ossificans progressiva—This is a rare inherited disorder of connective tissue in which various phalangeal abnormalities, particularly of the big toes, are associated with a tendency to ossification of the muscles (Lutwak 1964, McKusick 1966). The disorder is most active in childhood, but recurrent ossification in adolescence or early adult life may contribute to the final disability. Episodes of "myositis" with intense inflammation, swelling and pain in the muscles, are followed by the formation of ectopic bone matrix and mineralised bone. This process eventually fixes many joints, leading to complete immobility. Myositis ossificans progressiva is distinct from myositis ossificans due to other causes, such as paraplegia or after total hip replacement. It was chosen for a trial of EHDP because of its severity and crippling consequences, and because no other accepted form of treatment exists. EHDP has been used in two main ways: firstly, to prevent calcification after "myositis" in the active phase, particularly in children, and secondly, to prevent or to slow down recalcification after surgical removal of existing ectopic bone. Few people have personal experience of more than one or two cases of myositis ossificans progressiva, and its natural history is not completely defined. It seems, however, that some, and possibly most, of the areas of "myositis" in the active phase eventually ossify; and it seems to be the common experience of surgeons that removal of ectopic bone is usually, if not inevitably, followed by recalcification. Recalcification is probably more likely to occur if a patient is operated upon during the acute phase of the disease than when the disease is quiescent. Evidence for ectopic mineralisation is usually radiological and the process is probably best referred to as calcification. The term ossification
is best reserved to describe histological evidence of bone formation. The first report (Bassett, Donath, Macagno, Freisig, Fleisch and Francis 1969) of the use of EHDP in myositis ossificans progressiva was based on the results in three children, two of whom were in the active phase. These two showed regression of newly formed soft-tissue swellings, without radiological evidence of calcification; joint movement was said to be increased, and exacerbation of the disorder followed cessation of treatment.

EHDP has since been widely used in active myositis ossificans progressiva; detailed results are not yet published, but a recent report of fifty-two cases collected from many centres showed that about half were improved (Geho and Whiteside 1973). Regression of the lesions with EHDP was said to occur occasionally. Children treated with EHDP appear to grow normally and there is no evidence of rickets at the dose used (10–20 milligrams EHDP/kilogram/day orally).

**TABLE I**

**MYOSITIS OSSIFICANS PROGRESSIVA. CLINICAL DETAILS OF SEVEN PATIENTS TREATED WITH EHDP**

<table>
<thead>
<tr>
<th>Case number</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Age of onset (years)</th>
<th>Disability</th>
<th>Activity of disease</th>
<th>Ectopic bone removed</th>
<th>Date</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>33</td>
<td>Female</td>
<td>5</td>
<td>Confined to bed</td>
<td>Active</td>
<td>Elbow</td>
<td>1970</td>
<td>Delayed calcification</td>
</tr>
<tr>
<td>2</td>
<td>22</td>
<td>Female</td>
<td>3</td>
<td>Wheelchair</td>
<td>Active</td>
<td>Foot Hip</td>
<td>1970/1971</td>
<td>Delayed calcification/Recalcification</td>
</tr>
<tr>
<td>3</td>
<td>17</td>
<td>Male</td>
<td>2</td>
<td>Confined to bed</td>
<td>No new lesions seen</td>
<td>Hip</td>
<td>1971</td>
<td>Delayed calcification</td>
</tr>
<tr>
<td>4</td>
<td>16</td>
<td>Female</td>
<td>2</td>
<td>Able to walk</td>
<td>Active</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>Female</td>
<td>2</td>
<td>Wheelchair</td>
<td>Active</td>
<td>Quadriceps</td>
<td>1972</td>
<td>Recalcification</td>
</tr>
<tr>
<td>6</td>
<td>25</td>
<td>Female</td>
<td>2</td>
<td>Able to walk</td>
<td>Inactive</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>22</td>
<td>Female</td>
<td>4</td>
<td>Able to stand</td>
<td>Inactive</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* See Russell, Smith, Bishop, Price and Squire (1972).

In adults, as in children, an increase in mobility of the patient has been claimed (Weiss, Fisher and Phang 1971) but the significance of this is difficult to assess. Metabolic studies by these authors in one such patient showed a reduction in both bone resorption and bone mineral accretion, without a change in external calcium balance.

We have recorded our preliminary experience in removal of established ectopic bone in adults (Russell, Smith, Bishop, Price and Squire 1972). In two women aged twenty-three and thirty-four, ectopic bone was removed surgically while the patients were receiving EHDP, 20 milligrams/kilogram daily by mouth. In the first patient (Case 1, Table I) the improved mobility of the left elbow produced by operation was maintained for six months until death occurred from pneumonia. During this time there was no radiological evidence of recalcification, but tissue excised at necropsy from the area of the operation showed microscopic islands of partly mineralised bone matrix. The bone of the normal skeleton was osteoporotic but showed no evidence of a mineralisation disorder.

In the second patient (Case 2, Table I) receiving EHDP, ectopic bone removed from the right foot had not recurred a year and a half after operation, whereas, without EHDP, previous excision of bone from the same area had been followed by radiological evidence of recalcification within two months.

We now have further experience of the effect of EHDP in myositis ossificans progressiva. Six patients are currently receiving EHDP (Table I). Bone has been removed from four of
them. A summary of our results is given in the Table, and detailed findings will be published elsewhere.

In Case 3 there was no recalcification six months after removal of bone from the right hip. The ectopic bone which was removed showed an excessive thickness of osteoid. The patient had been receiving EHDP at an oral dose of 20 milligrams per kilogram body weight for six months previously. This experience with the delay in recalcification is the same as in Case 1 and after the first operation in Case 2.

In contrast, removal of ectopic bone from the quadriceps muscle in Case 5 was followed by recalcification within a month, rather in the way one would expect in an untreated patient. In this patient active myositis in other muscles continues to occur, with redness and swelling, but so far without radiological evidence of calcification. Similarly, a second operation in Case 2, in an attempt to mobilise the left hip, has also been followed by recalcification during EHDP treatment. This contrasts with the lack of calcification in the operation area in the foot of the same patient. Clearly there are factors which influence the response in different patients, and in the same patient at different times, to treatment with EHDP.

Except for short periods, our patients with myositis ossificans progressiva had a single oral daily dose of EHDP at 20 milligrams/kilogram body weight before, throughout and after operation. Factors most likely to cause a difference in response when the oral dose is constant are the activity of the disorder and the amount of the dose which is absorbed. It is well known that the activity of the disease is greatest in childhood and tends to be less in adult life, when active “myositis” lesions become uncommon. Perhaps recurrent mineralisation of ectopic bone matrix also occurs more readily in younger patients treated with EHDP. The absorption of EHDP is variable, and is affected by the presence of other substances in the stomach, such as calcium. Absorption in man varies from 0.5 to 15 per cent (mean 3 per cent) and this variation alone could account for the different response. The contribution of variation in absorption needs to be studied, but the work is hampered at present by lack of a convenient method for measuring EHDP in body fluids.

We have not observed in our patients either regression of established ectopic bone, or the increased mobility of joints not operated upon which has been recorded by others.

In summary it appears that EHDP can slow the mineralisation of ectopic bone matrix in myositis ossificans progressiva under certain circumstances which remain to be defined. The evidence for this effect of EHDP is largely radiological, but our histological findings, and those of Shaw (1972), show that although bone matrix re-forms at the operation site, EHDP delays its mineralisation. There is no evidence that EHDP inhibits the formation of ectopic bone matrix itself, which, after all, is probably the basic abnormality in the disease. It may therefore be effective only against the complications due to calcification of this matrix. It is possible that unmineralised ectopic bone matrix may eventually fix a joint as effectively as if it were mineralised. Thus even when mobility of a joint is increased by operation this increase may not be maintained. These considerations should not weigh unduly against using EHDP in myositis ossificans progressiva because there is no other known effective treatment for this crippling disease.

**Calcinois after dermatomyositis**—Other types of soft-tissue calcification, mainly due to dermatomyositis, have also been treated with EHDP, apparently with good result. These disorders are very different from myositis ossificans progressiva. Thus, although there is radiological calcification in the soft tissues, ectopic bone is not formed and there is no evidence that the condition is inherited. Furthermore, and importantly, the calcification may improve spontaneously. Calcification after dermatomyositis nevertheless presents a fairly constant clinical picture. There are masses of calcium within the soft tissues and these deposits may ulcerate through the skin. In a nine-year-old girl with this condition EHDP at a daily dose of 10 milligrams/kilogram was associated with striking improvement (Cram, Barmada, Geho and Ray 1971). In this patient, as in others, calcinois had previously failed to respond to a
variety of measures. Improvement began in a month and continued for a year, except when the dose of EHDP was halved. Metabolic studies including $^{85}\text{Sr}$ uptake studies suggested a reduction in the rate of calcium deposition in the body, together with a fall in calcium absorption.

A recent survey (Geho and Whiteside 1973) of eighteen patients with calcinosis mainly due to dermatomyositis suggested that twelve were improved. Our own experience with four patients does not allow any firm conclusion to be drawn.

There are other conditions with ectopic mineralisation in which EHDP may be useful. However, because of their variable natural history, carefully controlled studies will be needed. Thus the myositis ossificans which follows total hip replacement may be uncommon, but for the individual it can be a disastrous complication, particularly since it tends to recur in the same patient after any subsequent operation. Effective prevention, particularly during second operations, would be a considerable achievement. Ectopic calcification in blood vessels may be an unwelcome complication in other disorders, such as renal failure where it may preclude effective transplantation. In this situation treatment aimed at prevention would be justified.

EXCESSIVE BONE RESORPTION

There are many conditions in which the resorption of bone is excessive. These include Paget's disease (ostitis deformans), immobilisation osteoporosis, parathyroid bone disease, some forms of renal bone disease, and neoplastic bone disease.

**Paget's disease of bone**—This is a relatively common disorder (Barry 1969). When it is active and generalised, there are gross biochemical changes with an increase in the plasma alkaline phosphatase and urinary total hydroxyproline (THP). The raised plasma alkaline phosphatase in Paget's disease is probably due to the increased osteoblastic activity in this disorder. The total hydroxyproline excretion is a measure of the rate of collagen "turnover" (both resorption and formation), and in Paget's disease it is increased due to the excessive "turnover" of the collagen of bone matrix (Kivirikko 1970). The total hydroxyproline excretion is closely correlated with the non-protein-bound hydroxyproline in the plasma (referred to hereafter as plasma hydroxyproline). The hydroxyproline measurements should be made while the patients are on a low gelatin diet, which excludes ice cream, mousse and other obvious sources of gelatin. This exclusion is necessary because ingestion of gelatin, which is thermally denatured collagen, will considerably increase the amount of hydroxyproline in the blood and urine. In Paget's disease the effects of a compound which acts on bone, such as EHDP, can easily be detected biochemically by its effect on alkaline phosphatase and hydroxyproline.

We have investigated the effect of EHDP in about forty patients with Paget's disease. The results which follow are based on our detailed results on the first ten of these patients, given EHDP at a daily oral dose of 20 milligrams per kilogram body weight. A preliminary study of four of these patients (Smith, Russell and Bishop 1971) showed that EHDP could reduce the initially elevated values of plasma alkaline phosphatase, of plasma hydroxyproline and of the urinary excretion of hydroxyproline. This implied that there was a reduction by EHDP of both the excessive formation of bone and its excessive resorption.

In our first ten patients this response to EHDP was consistent in nine (Smith, Russell, Bishop, Woods and Bishop 1973). In one patient absorption may have been poor because of diarrhoea, and there were no convincing biochemical changes. In the nine patients who responded, the alkaline phosphatase and hydroxyproline measurements began to fall at about one month and reached normal or near normal levels during periods of treatment of three to seven months of EHDP.

The biochemical significance of these results needs to be judged against the well known natural variation in Paget's disease (Nagant de Deuxchaisnes and Krane 1964). Thus these biochemical measurements and, by implication, the activity of the Paget's disease, can be varied by analgesics (Henneman, Dull, Avioli, Bastomsky and Lynch 1963), by steroids, and
The biochemical changes after admission to hospital (February 14) in a man age 50 with severe Paget's disease. There is a temporary increase in plasma hydroxyproline and in urine THP excretion, and in plasma and urine calcium. This response is uncommon and is similar to that seen in total immobilisation. It is probably due to increased bone resorption. For normal limits of the biochemical data see Figure 5.
by change in physical activity. Complete immobilisation of a patient with severe Paget’s disease may increase plasma and urine calcium and urinary hydroxyproline, and occasionally admission to hospital in a patient with severe Paget’s disease will do the same (Fig. 2), presumably because of decreased physical activity. It is therefore necessary in such studies to keep patients as active as possible when they enter hospital and not to alter any drugs that they may be receiving. Patients taking analgesics should continue on the same dose; those not on analgesics should not be given them.

The long-term changes in the activity of Paget’s disease and the level of alkaline phosphatase have been well documented by Woodard (1959). However there are few measurements on the short-term variation in plasma alkaline phosphatase or plasma and urine hydroxyproline. In our study these were obtained from a group of patients with Paget’s disease who attended
the hospital at monthly intervals without treatment. In these patients (as in all the others in
the Paget's study) the twenty-four-hour urine collections have been obtained on a low gelatin
diet. The consecutive monthly measurements have been expressed in relation to the initial
value as 100 per cent. The monthly percentages on each patient have been grouped together
and are expressed in terms of a mean ± 1 S.E.M. (Fig. 3). It is clear from this figure that the
plasma alkaline phosphatase and total hydroxyproline excretion in untreated patients remain
quite constant for periods up to six months. By comparison, the biochemical changes with
EHDP are highly significant. Similar changes occur in the plasma hydroxyproline, which
is closely correlated with alkaline phosphatase and urinary hydroxyproline (Preston
and Smith, unpublished). Measurement of the plasma hydroxyproline which, within the
normal range, is subject to wide error, becomes more accurate when the values are above
normal. Thus the changes in plasma hydroxyproline provide useful additional evidence of
the effect of EHDP in patients with severe Paget's disease and are also useful where twenty-
four-hour urine collections are inaccurate or impossible.

![Graph showing changes in plasma calcium and phosphate](image)

Fig. 4

The changes in plasma calcium and phosphate in the same groups as Figure 3.

Figure 4 shows the changes in plasma calcium and phosphate which occur in these groups.
The changes in calcium are probably not significant; the variable increase in plasma phosphate
is a feature of EHDP therapy most marked in the least severely affected patients. The mechanism
of the rise in plasma phosphate, which has also been observed by others, is not fully worked
out. There is an increased tubular reabsorption of phosphate which appears unrelated to
changes in parathyroid activity. The effect is not seen in animals.

In Paget's disease EHDP produced changes in the histological appearance of iliac crest
biopsies. The trabecular bone in five out of six such bone biopsies, taken after treatment with
EHDP for three to seven months, consistently showed a considerable increase in osteoid
thickness and in the number of birefringent lamellae seen under polarised light in sections of
undecalcified bone (Paterson, Woods and Morgan 1968). There was also a reduced number
of bone cells present and these cells appeared histologically inactive. We did not have bone biopsies from all our patients before treatment was started. In those in whom we have had biopsies, there is either the appearance of normal bone or of active Paget's disease with irregular trabeculae, excessive numbers of active-looking cells, and vascular fibrosis of the marrow. The trabecular bone from the iliac crest may appear to be normal when there is clear radiological and biochemical evidence of Paget's disease elsewhere. Even bone from such cases has shown an excess of osteoid after EHDP.

EHDP appears to have several effects in these patients. The dose of 20 milligrams/kilogram body weight apparently reduces excess resorption of bone but also reduces mineralisation of bone matrix. The appearance of increased amounts of osteoid under EHDP may be due to direct inhibition of mineralisation of new matrix or to demineralisation of existing matrix. Whether it is associated with any change in vitamin D metabolism and the formation of 1,25 dihydroxycholecalciferol (Hill, Mawer, Lumb and Stanbury 1972) as in the animal experiments is not known. The effects of smaller doses of EHDP are now being tested to see whether this defective mineralisation is invariably present in patients who respond biochemically to EHDP. Animal experiments discussed previously suggest that there is a significant difference between the minimum dose which will reduce bone resorption rates and the higher doses which also interfere with mineralisation. There is a suggestion that a daily oral dose of 10 milligrams/kilogram body weight (Bijvoet, Froeling and van der Sluys Veer 1972) can reverse the biochemical changes of Paget's disease without the appearance of excess osteoid. However, variation in intestinal absorption may make it difficult to be very precise about the exact optimal oral dose.

Although our results have shown clearly that EHDP may reduce the activity of bone in Paget's disease, the clinician will particularly want to know its effect on the symptoms and complications of Paget's disease. It is possible at this stage only to give a partial answer to these questions. One reason for this is the difficulty in deciding whether pain of which the patient complains is due to Paget's disease. Thus, although Paget's disease may sometimes cause a bone, such as the tibia, to become painful and deformed, more often the patient complains of pain in the hip or knee, adjacent to a bone radiologically affected with Paget's disease. In the latter case it can then be impossible to decide whether the pain is due to associated joint disease, or to the Paget's disease itself. Further, patients with extensive Paget's disease with considerable deformity often have no pain in the bones; in these cases clinical assessment may be based on the effects on complications, such as deafness and spinal cord compression, in which improvement can be fairly accurately measured.

Our first patients were chosen because they had biochemically active Paget's disease and in none of these was pain severe. In six who had pain this was improved in three and strikingly so in one of these. We have now begun to study the effect of EHDP in patients with spinal cord compression; no definite conclusions can yet be drawn, but rapid improvement does not occur. Indeed it is difficult to see how any of the known medical treatments for Paget's disease might bring about rapid improvement in cord compression, because it would necessitate a change in the total configuration of the affected bone. Further observations which might help in the clinical assessment of EHDP could include measurement of hearing, of cardiac output, and of temperature over the affected bones.

One of the most important complications of Paget's disease is a sarcoma of bone. We have treated a patient with generalised Paget's disease with EHDP, who was subsequently shown to have a sarcoma of the vertebral body. Despite suppression of the biochemical parameters (Fig. 5), there was no evidence at necropsy to suggest that the phosphonate altered the course of the malignant process.

In conclusion, our studies in Paget's disease have shown that EHDP is capable of suppressing the activity of rapidly metabolising bone. This biochemical suppression is associated with the accumulation of osteoid tissue and a disorder of mineralisation. These
Biochemical changes in a man of 62 with extensive Paget's disease and sarcoma of a vertebral body. The patient was at first completely immobilised in hospital and the temporary hypercalcaemia may be due to this. The THP excretion fell to near normal while EHDP was being given, but the plasma alkaline phosphatase increased. Radiotherapy was given between June 10 and July 8. Death occurred on October 11. The normal limits of the biochemical values are shown by the shaded areas.
effects occur with a daily dosage of 20 milligrams/kilogram body weight, by mouth. The next stage of investigation will be to test whether lower doses can produce suppression of the biochemical activities without impairing mineralisation, and whether other phosphonates such as CI, MDP might be better than EHDP. The effect on symptoms and complications needs further study.

EHDP should be compared with other agents capable of reversing the biochemical abnormalities of Paget's disease (Lancet 1971a and b). In the past, large doses of aspirin and of corticosteroids have been shown to lower the plasma alkaline phosphatase, but they have not proved effective in long-term therapy. The agents currently used, apart from the diphosphonates, are mithramycin (Condon, Reith, Nassim, Millard, Hilb and Stainthorpe 1971) and calcitonin (Haddad, Birge and Avioli 1970). Glucagon has also had a limited trial (Condon 1971). Mithramycin is a cytotoxic agent, active against some forms of neoplastic disease. It is toxic, has to be given by intravenous infusion, and appears to have a direct effect on the bone cells. In high doses renal and hepatic side effects are reported. Mithramycin (and the related actinomycin) rapidly reduces the plasma alkaline phosphatase and urinary hydroxyproline and is said to reduce the bone pain in Paget's disease. Although it may be used in repeated short courses it is not a suitable agent for long-term therapy. Calcitonin, the main effect of which is to reduce excessive bone resorption, is effective in Paget's disease. The most convincing results have been produced with human calcitonin (Woodhouse, Reiner, Bordier, Kalu, Fisher, Foster, Joplin and MacIntyre 1971); the results from porcine and salmon calcitonin are less consistent and biochemical recurrence may occur together with the production of antibodies (De Rose, Singer, Afiramides, Baker and Wallach 1973). As with all calcitonins, human calcitonin has to be given by subcutaneous injection. Human calcitonin produces biochemical changes which are very similar to those of EHDP. Limited published histological studies suggest that normal bone is formed under the influence of human calcitonin, instead of the disorganised bone of Paget's disease. The osteoclast count is reduced and by implication resorption is decreased. Glucagon, which can stimulate the release of calcitonin from the thyroid C cells, seems to reverse the biochemical abnormalities of Paget's disease. In large doses it may not be entirely without side effects (Shedden 1971).

Other bone disorders—There are a number of bone disorders, apart from Paget's disease, in which the use of EHDP has been considered. Of these the commonest is senile osteoporosis, in which the aim is to prevent the progressive bone loss which occurs with age. Animal studies with immobilisation osteoporosis showed the effectiveness of EHDP and the greater effectiveness of CI, MDP. In elderly osteoporotics bone turnover and bone loss are far less rapid than in the young (idiopathic juvenile) osteoporotic or the immobilised. The only published findings so far are those of Jowsey, Riggs, Kelly, Hoffman and Bordier (1971) in four elderly patients given EHDP in daily doses of 10–20 milligrams/kilogram, who showed an increase in unmineralised osteoid after three months and no consistent changes in bone resorption, assessed by quantitative microradiography. There are no published results on the rapidly progressive forms of osteoporosis due to immobilisation, or in the young. In the bone disease of hyperparathyroidism (osteitis fibrosa cystica) there is no indication for a trial of EHDP where excessive resorption can be cured by removal of the parathyroid tumour. But it might be considered when, for instance, there is a parathyroid carcinoma, or when the parathyroid bone resorption is associated with metastatic calcification, as in renal failure. The excessive bone resorption, sometimes associated with hypercalcaemia, which may occur with multiple bone metastases or myeloma, may provide another indication for a trial of EHDP.

Conclusion—The use of the diphosphonates in man is based on considerable research on these and related compounds in animals, which continues to suggest their use against soft-tissue mineralisation and in excessive bone resorption. In man EHDP is clearly biologically active. Nevertheless it is only partly successful in the treatment of ectopic mineralisation, and the marked effects which it has in Paget's disease are produced by a dose (20 milligrams/kilogram)
DIPHOSPHONATES

which is probably too high for routine use. Further work should establish the best conditions for the effective clinical use of EHDP and related compounds in man.

SUMMARY

1. The phosphonates are simple chemical compounds containing P-C-P bonds which are resistant to the action of naturally occurring phosphatases and pyrophosphatases. They inhibit the formation and dissolution of apatite crystals in vitro and prevent ectopic mineralisation and bone resorption in animals.

2. In man one diphosphonate (EHDP) has been shown to reduce the excessive turnover of bone in Paget’s disease and also appears to slow the mineralisation of ectopic bone matrix in myositis ossificans progressiva.

3. The possible uses of the diphosphonates in bone disorders with excessive resorption and in ectopic mineralisation are being further investigated.

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DIPHOSPHONATES


