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A PRELIMINARY STUDY OF THE IN VIVO AND IN VITRO UPTAKE OF Sr^{90} IN BONE TISSUE AND THE OSSEOUS LOCALIZATION OF RADIOACTIVE FISSION PRODUCTS FROM ATOMIC EXPLOSIONS

By

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INTRODUCTION

It has been shown that the uptake of many radioactive isotopes in bone tissue is concentrated to certain areas. In studies using radioautographic procedure to demonstrate the macroscopic and microscopic localization of isotopes in bone tissue it has been shown that the radioisotopes P^{32}, Ca^{45} and S^{35} (as sulfate) become localized in similar structures. A soft x-ray microradiographic investigation of isotope-labelled bone tissue, revealed that the uptake of the isotopes occurred in areas with a low degree of mineralization i.e. newly-formed bone structures (*Engfeldt, Engström, Zetterström* 1952, *Engfeldt, Engström, Boström* 1954). It has also been demonstrated that bone tissue can be labelled *in vitro* with these isotopes, and that a localization similar to that obtained in the *in vivo* experiments is obtained (*Engfeldt* and *Hjertquist* 1954). These latter findings have been interpreted as an indication that ionic exchange reactions easily take place in newly-formed bone structures with a low degree of mineralization. The characteristic property of the bone tissue, quickly taking up and releasing ions by some surface exchange phenomena or recrystallization, has the consequence that a great number of radioactive isotopes, which are introduced into the body by inhalation or ingestion become localized in specific "hot spots" in the skeleton. These localized areas of high radioactivity may become the cause of radiation sickness and eventually also of malignant transformation of the bone tissue.

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In our time, with the increased risk to the individual of becoming "infected" with bone-seeking radioisotopes such as products from uranium or plutonium fission, it seems extremely important to study bone structure and physiology from the molecular to the anatomical level. Coordinated research may help to find remedies or principles by which a detoxication of the skeleton with respect to radioisotopes can be performed.

The present investigation deals with the deposition of Sr^{90} in bone tissue. This isotope was chosen because it is representative of the bone-seeking isotopes formed by uranium and plutonium fission.

The deposition of Sr^{90} in the skeleton has been studied by several authors. For a general survey see *Fink* (1950) and *Behrens* (1953). A recent contribution to this field was made by *Jowsey, Owen* and *Vaughan* (1953).

The aim of the present investigation is to describe some results of radioautographic studies on the *in vivo* and *in vitro* uptake of Sr^{90} in bone tissue from dogs. The results will be correlated with the pattern of mineral salt distribution studied by microradiography. The present preliminary communication which reports short term experiments is the first in a series which will deal with the localization and metabolism of radioactive fission products in the skeleton. These investigations are undertaken with the intention of discovering how it is possible to remove the radioactivity from the skeleton but also in order to study the effects of prolonged internal radiation.

MATERIAL AND METHODS

For the *in vivo* experiments a normal 2 years old dog weighing about 6 kg was used. The dog was injected intravenously with 1 mC Sr^{90} and was killed 10 days later. The bones were freed from the soft tissues and fixed in absolute alcohol. Thin sections of bone tissue were cut with a rotating saw and these sections were ground on special glass plates to a final thickness of about 50 μ.

Material for the *in vitro* experiments was obtained from a normal 5 months old dog. The sections were prepared in the same way as described above. They were incubated in a solution containing Sr^{90} at a pH of 7 and were then ground another 5 μ on each side.

The ground sections were first used in radioautography. This was performed by sandwiching the sections between sheets of Agfa Printon film and keeping them in a special holder to provide good film-specimen contact. After exposure the films were processed in Kodak

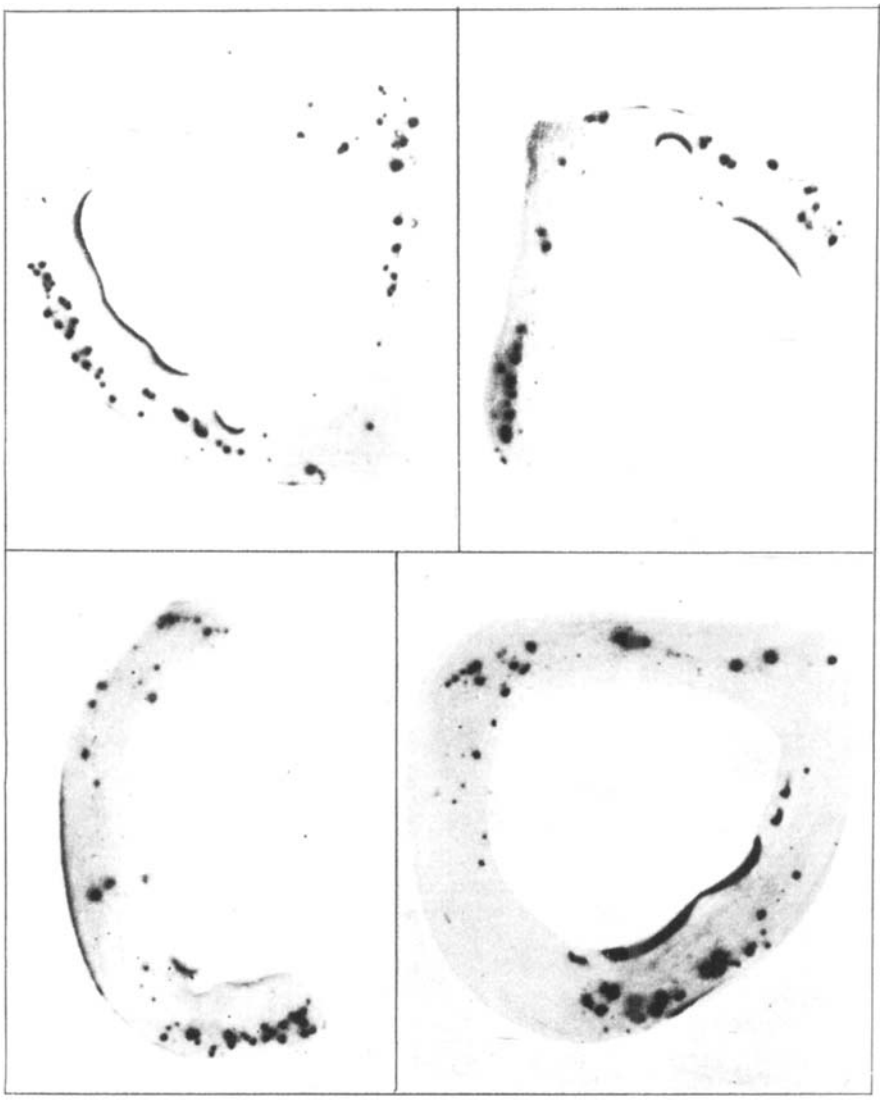

Fig. 1.
Radioautographs of sections from the long bones from a dog which had received Sr^{90}. Note the in-take in the enosteal and periosteal layers and the spotty in-take in certain haversian systems. There is also a general diffuse labelling. All four pictures are cross sections from the diaphyses.

D 19 b developer. The dried films were immediately mounted in canada balsam under a coverslip on a glass slide in order to prevent dust and scratches spoiling the emulsion layer. The radioautographs were enlarged by photomicrography and Kodak B 20 plates were used.

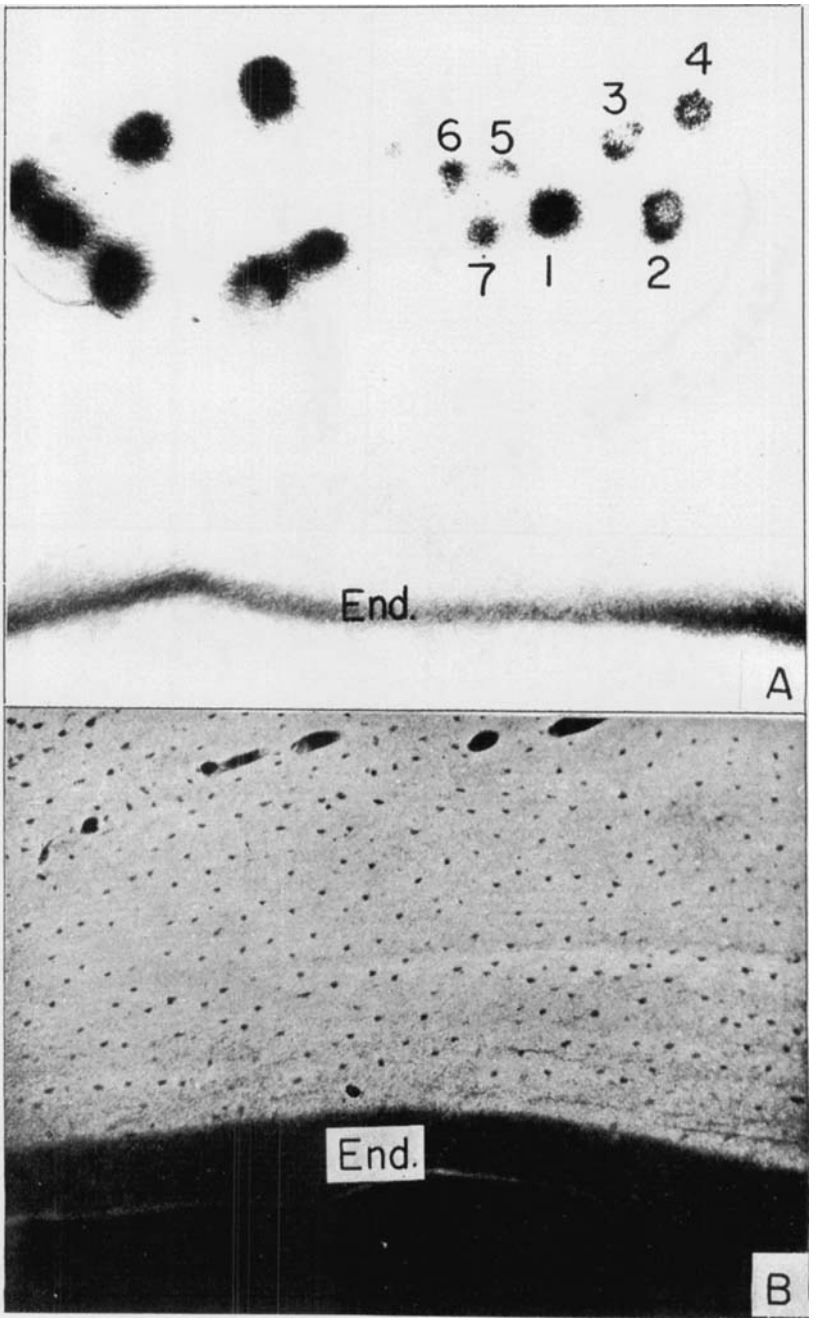

Fig. 2.
Legends see opposite page.

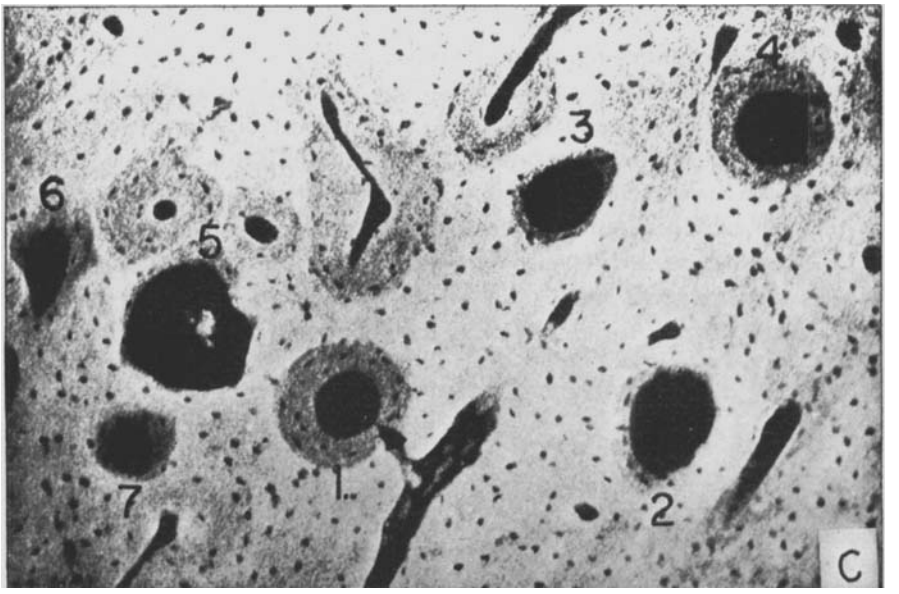

Fig. 2.
Part of Fig. 1 but further enlarged is shown in A, where "End" stands for the endosteal layer. B and C are microradiograms from the same section but the magnification is higher than that of A. The numbers refer to identical structures. Areas with a low mineralization take high amounts of isotope.

After the radioautographic exposure the sections were microradiographed and a Philips x-ray diffraction unit acted as radiation source. The x-ray tube had a copper target. The tube was run with 30 kV peak voltage and 12 mA. The distance from the focus to the film was 25 cm. The microradiographs were registered on Eastman Kodak Spectroscopic Plate no. 649, which has a resolving power of more than 1000 lines per mm. The microradiograms were enlarged by photomicrography and Kodak O 250 plates were used. It can be shown both theoretically and experimentally that a microradiogram of a section of bone registered under the conditions just mentioned show the true distribution of mineral salts. Full details of the microradiographic technique are found in the paper by *Engfeldt* and *Engström* (1954). In order to study the molecular structure of endosteal bone with a very low degree of mineralization pieces of this bone were dissected and mounted for x-ray diffraction in a Chesley microdiffraction camera adapted to a Siemens crystalloflex II x-ray unit. Nickel filtered CuK-radiation was used and the diffractograms were registered on Ilfex x-ray film. For details of micro x-ray diffraction see *Carlström* (1954).

RESULTS

The radioautograms of bone tissue from the animals that had received Sr^{90} disclosed two types of isotope localization in the bone tissue. It could be seen (Fig. 1) that there was a faint diffuse uptake of isotope throughout the mineralized tissue. Within this diffuse labelling there were spots with extremely high activity. These localized areas with high isotope content were especially abundant in the periosteal and endosteal areas. Certain haversian systems of the compact bone tissue also showed a high isotope uptake. An examination of the microradiographs from these sections demonstrated that the areas which

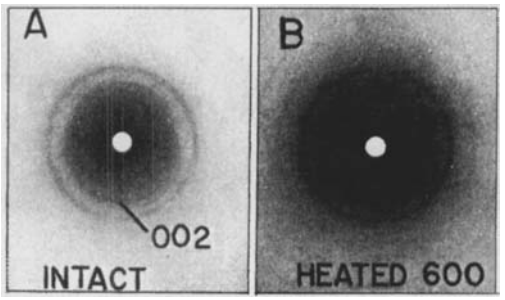

Fig. 3.
X-ray diffraction patterns (flat film) from the endosteal layer marked "End" in Fig. 2. It is seen that the intact bone gives very diffuse lines indicating a small particle size. After heating the crystallites grow and the orientation is also better brought out. The pattern is that of hydroxyapatite.

showed a localized high uptake of Sr^{90} were mineralized to a low degree. Fig. 2 A shows a part of a radioautograph at higher magnification. The endosteal zone marked with "End" in the radioautograph exhibits a low x-ray absorption, which is well demonstrated in the microradiogram of the same bone section (Fig. 2 B). The haversian systems marked 1, 2, 3 and 4 etc. in the radioautograph also have a low mineral content which can be seen from the microradiogram in Fig. 2 C. Resorption cavities, in which new formation of bone tissue has just started in a small area, show labelling in this area of bone deposition.

The endosteal zone has a very low x-ray absorption (End in Fig. 2) which means a low content of mineral salts. Micro x-ray diffraction studies (Fig. 3) of this zone showed that it contained mineral salts giving a pattern characteristic of hydroxyapatite. The diffraction lines were diffuse, indicating a small particle size, a result always found in osseous tissues. After heating to 600° for one hour the pattern became sharp due to the growth of the crystallites and an unequivocal

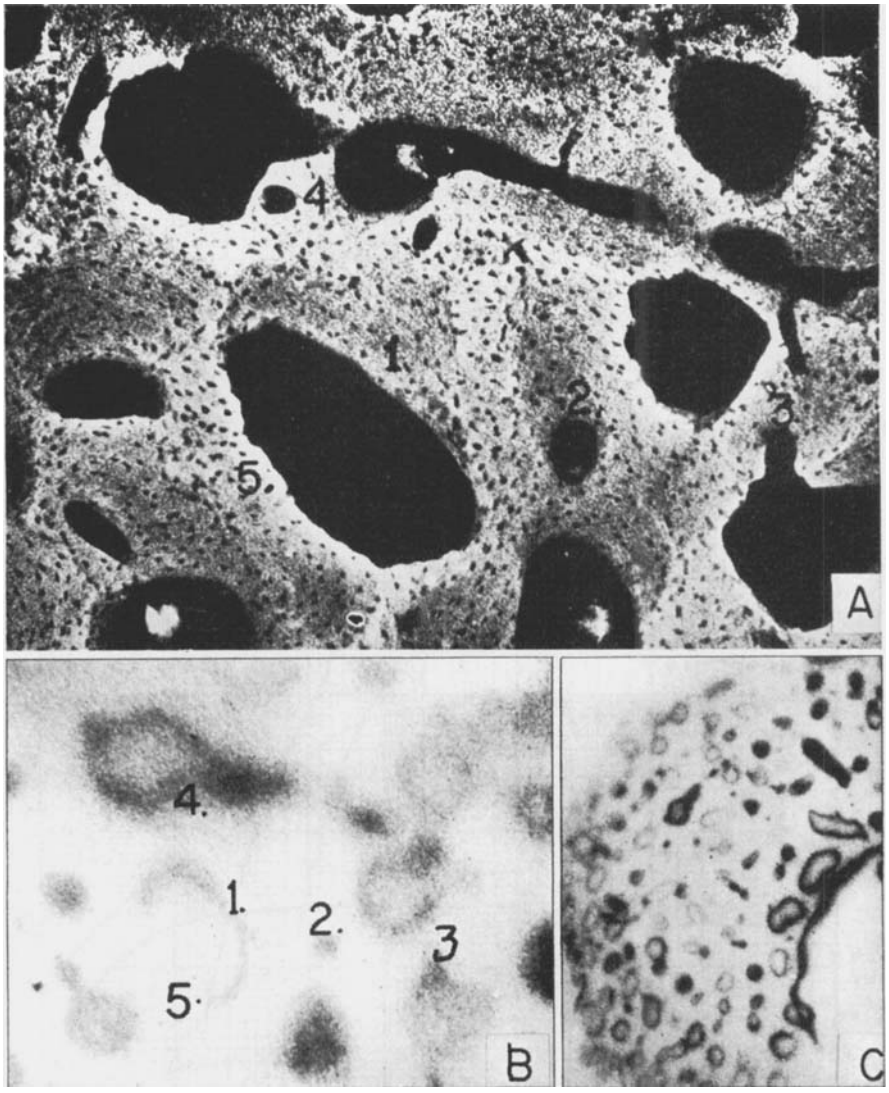

Fig. 4.

In vitro labelling of sections of bone tissue. A, is the microradiogram taken at high magnification, B and C are the radioautographs of the same section which was incubated in a solution containing Sr^{90}. The figures indicate identical structures.

indexing is possible. The diffraction pattern showed the unexpected result that the hydroxyapatite crystallites have a preferential radial orientation. Fig. 4 C shows the results of the *in vitro* experiments with Sr^{90} and in general the localization of the isotope is the same as in the *in vivo* labelling. The comparison between the microradiogram and the

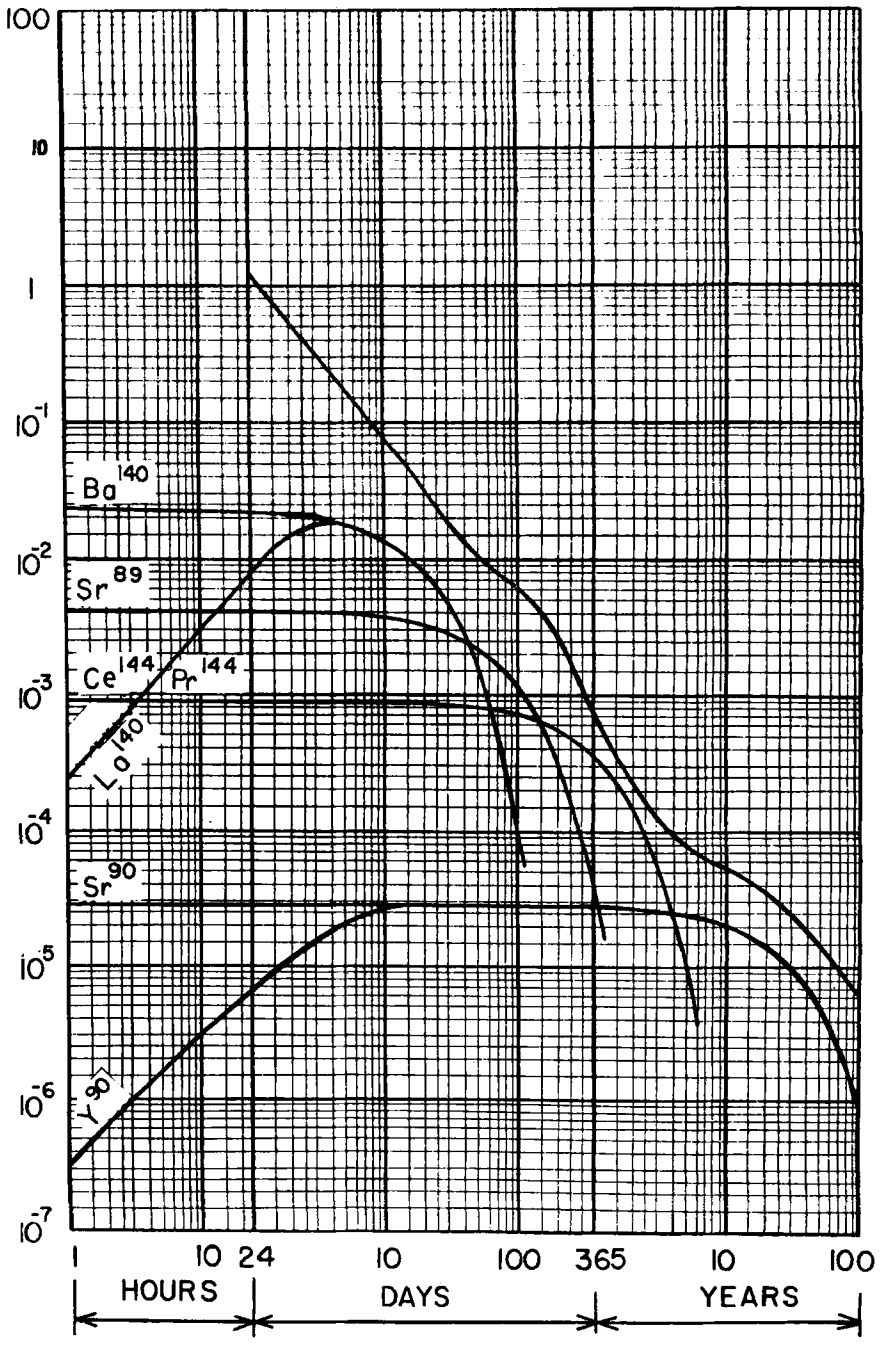

Fig. 5.

Number of radioactive disintegrations resulting from 10000 fissions. The unlabelled curve is the total amount. All curves calculated per minute.

in vitro labelling with Sr^{90} of the same section is shown in Fig. 4 A and B. It is clearly seen that bone structure with a low degree of mineralization has a high *in vitro* exchange of its mineral component.

DISCUSSION

It is known that the greatest part of strontium administered to the body is fixed in the skeleton (*Pecher* 1941, *Kidman, Tutt* and *Vaughan* 1950). The localization of Sr^{90} in rabbit bone has been studied by *Jowsey, Rayner, Tutt* and *Vaughan* 1953 and they were able to demonstrate that areas of active bone growth concentrated the isotope. In a later communication *Jowsey, Owen* and *Vaughan* 1953 reported the distribution of Sr^{90} in bone tissue of monkeys, which died after injection of Sr^{90}. In these animals they could demonstrate a high isotope labelling of such bone structures which had a low content of mineral salts. Our results which are obtained in experiments with dogs are in good agreement with their findings. These areas with a low mineralization and high isotope labelling have been shown in earlier experiments to be newly formed. The localization of Sr^{90} after intravenous injection of Sr^{90} thus follows the same general pattern as that of radiophosphorus, radiocalcium and radiosulfate (*Engfeldt, Engström* and *Zetterström* 1952, *Engfeldt, Engström* and *Boström* 1954).

The *in vitro* uptake of Sr^{90} in bone tissue also takes place in the same structures where radiophosphorus, radiocalcium and radiosulfate are fixed *in vitro* (*Engfeldt, Hjertquist* 1954). These findings seem to indicate that the mechanism involved in the labelling of bone tissue with Sr^{90} is essentially similar to that of the above-mentioned isotopes. In the case of Sr^{90}, however, there seems to be a slight difference in the type of fixation which is demonstrated by the diffuse faint labelling of the whole bone tissue in the diaphyses. This diffuse labelling thus occurs in highly mineralized bone tissue which was actually formed long before the injection of Sr^{90}. Such an intake has not been shown to occur in experiments over a short period with the other isotopes mentioned above. However, in these earlier experiments the animals were killed 3 hours to 4 days after the injection of the isotope while in this case the animal was killed 10 days after isotope administration. The uptake in the old, highly mineralized bone tissue can probably be interpreted as a slow ionic exchange reaction.

The knowledge of the behaviour of Sr^{90} in the body is of the greatest importance as this isotope is one of the biologically most important fission products from uranium and plutonium. Strontium 90 has a half life of 25 years and therefore it will have considerable opportunity to

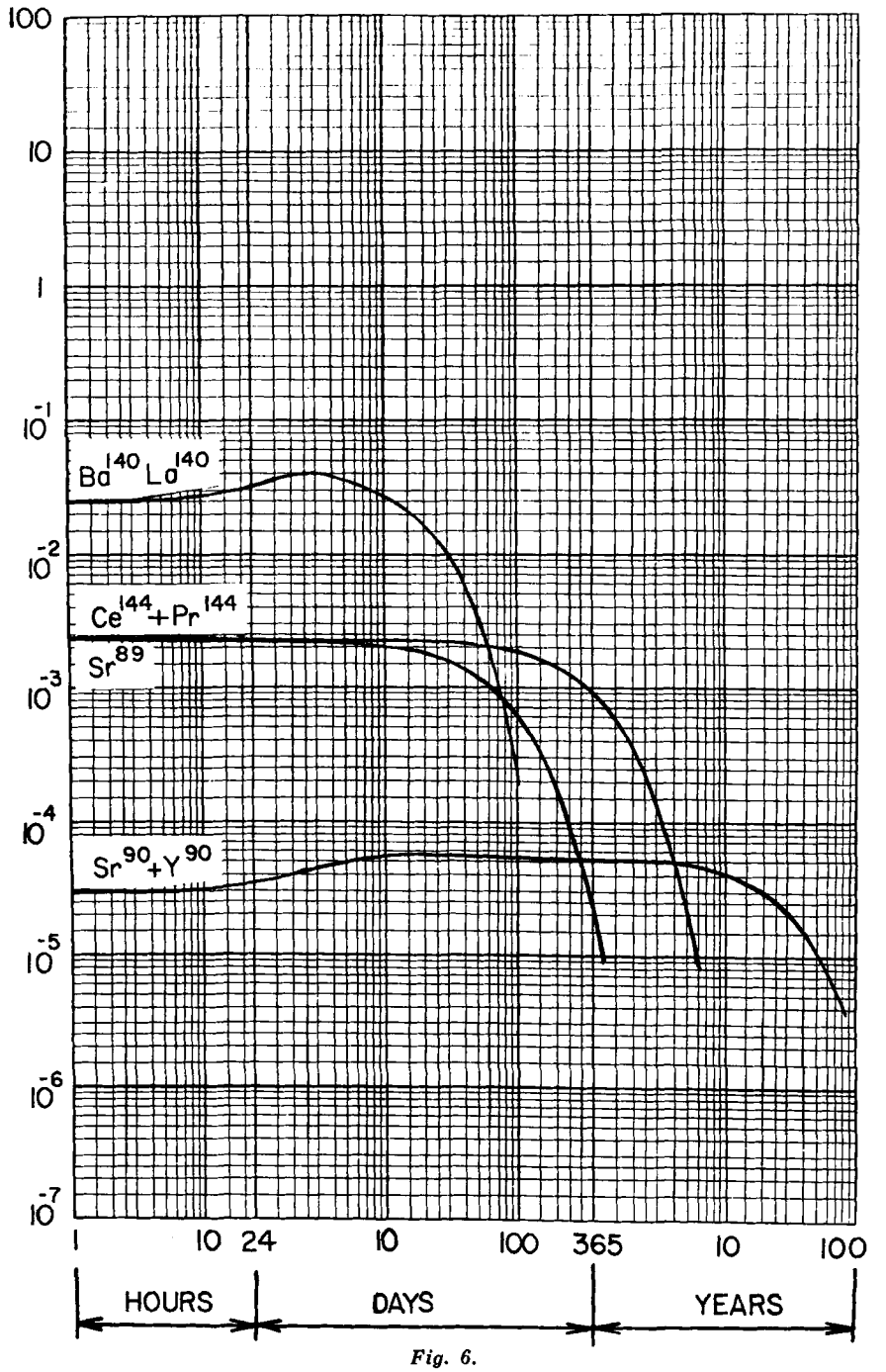

Fig. 6.

The curves in Fig. 5 but multiplied with the effective energy of the various isotopes.

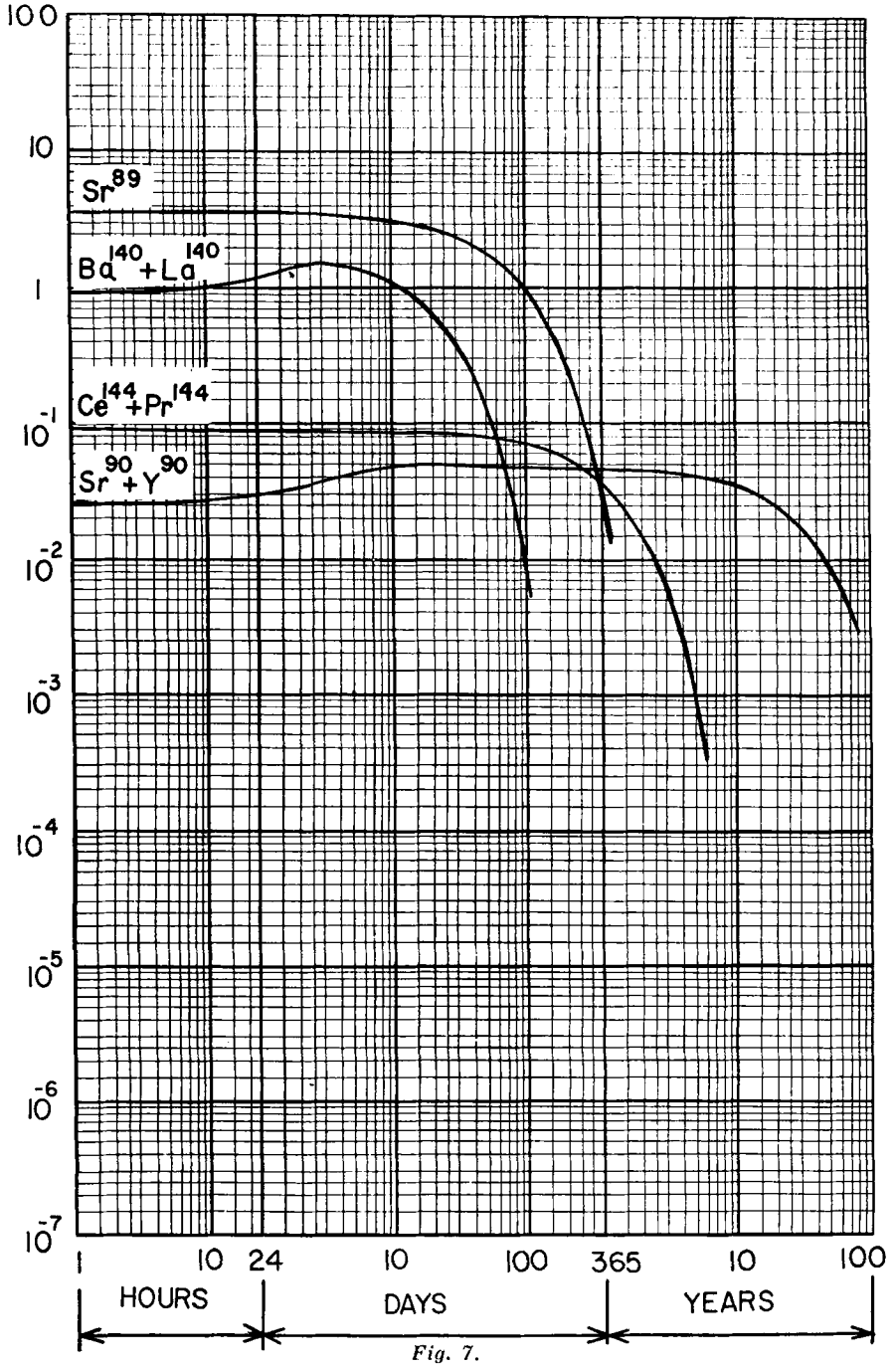

Fig. 7.

The curves in Fig. 6 multiplied with $T_b \cdot f_a$ where T_b is the biological half life and f_a the fraction of isotope retained in the critical organ (bone), after being introduced into the organism by inhalation.

damage by radiation the tissue where it is fixed. It is an β-emitter with an energy of 0.61 MEV. The fact that the isotope is fixed in high concentrations in areas of bone which is being newly formed must be considered unfavorable since these areas are situated near the blood-forming tissues (cf. the endosteal uptake). It is also evident that in the case of infection with radiostrontium a young individual will concentrate more isotopes in the skeleton than an old one. On the other hand the rebuilding processes may run faster in a young individual and therefore the isotope may be disposed of more quickly. However, even a relatively short period of internal radiation may be hazardous.

In uranium and plutonium fissions there appear a great number of radioactive products. Several of those which occur in great quantities can be classified as bone-seeking. Fig. 5 shows the relative abundance of such fission products which are known to, or from chemical relationships can be expected to become localized in the bone tissue. The diagram is constructed in such a way that each curve represents the number of radioactive disintegrations for each isotope as a function of time. It is assumed that there is an initial fission of 10,000 atoms. The biological action of the isotope depends also on the energy of the emitted radiation. In Fig. 6 therefore the values in Fig. 5 are multiplied with the effective energy of the radiation.

In order to get an approximate estimate of how these curves may be interpreted the values in Fig. 6 are multiplied by a factor equalling the intake into the body by inhalation and ingestion, results of which are presented in Fig. 7 and 8 respectively. These two latter curves also take the "biological half life" of the isotope into account.

It is clear from the diagrams that almost immediately after an atomic explosion the bone-seeking isotopes occur in great quantities and remain as a health hazard at least as long as a human life time.

SUMMARY

The in vivo and in vitro uptake of Sr^{90} in bone tissue of dogs has been studied using radioautographic and microradiographic techniques. The labelling with Sr^{90} has been shown to occur in certain bone structures which have a low content of mineral salts and which are newly formed. Both the in vivo and in vitro distribution of Sr^{90} in the bone tissue follow the general patterns which characterise phosphorus, calcium and sulfate. The importance of this localized intake of Sr^{90} in bone tissue is discussed from the point of view that it is an important fission product of an atomic explosion.

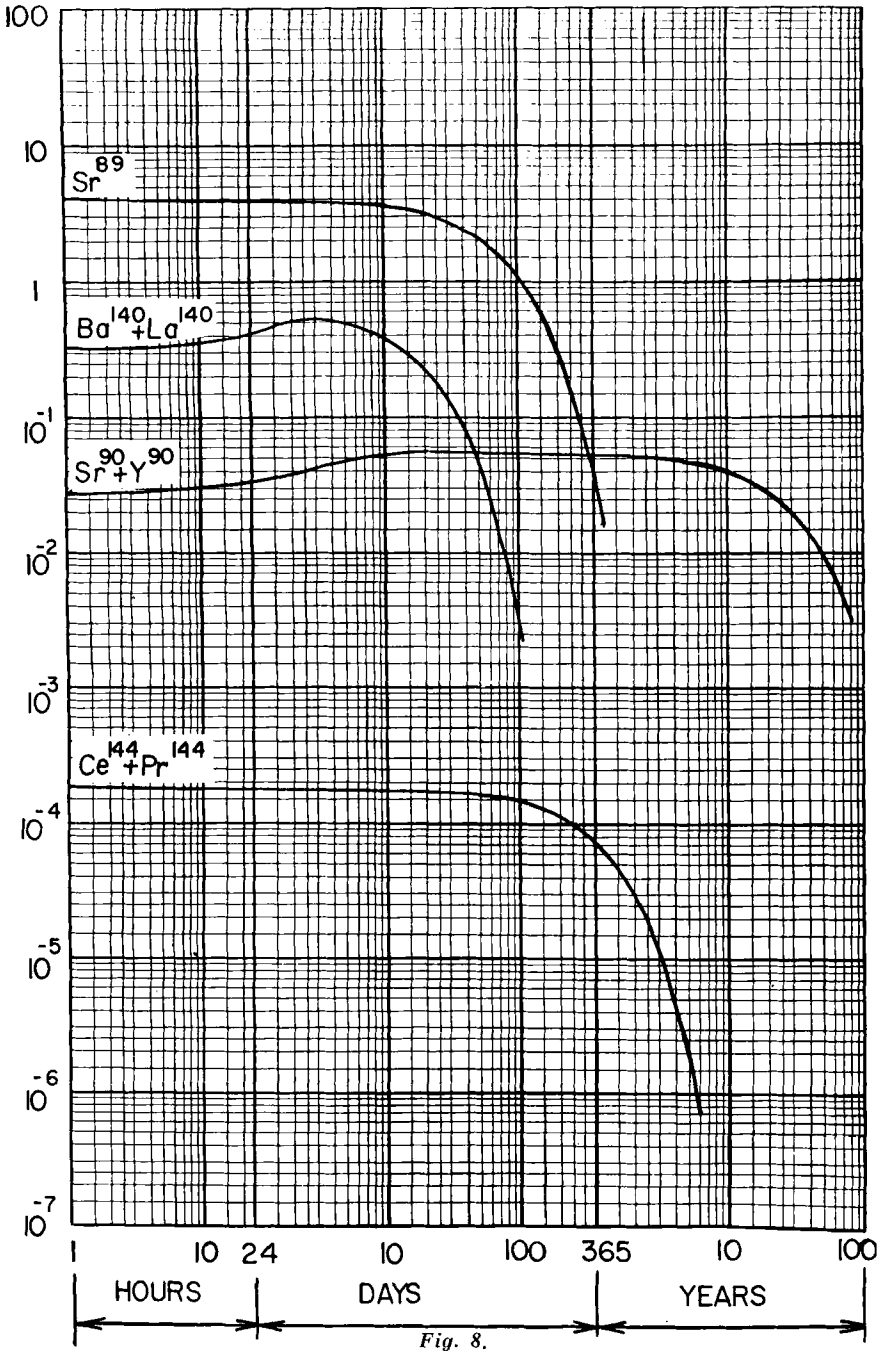

Fig. 8.

The same as in Fig. 7 but instead of being introduced by inhalation the isotope is taken in by food and water. The factor f_a in Fig. 7 therefore is replaced by another, f_w which characterizes the isotope retained in the skeleton after oral administration.

RESUME

Le prélèvement in vivo et in vitro de Sr^{90} dans le tissu osseux des chiens a été étudié en utilisant la radiographie et la technique microradiographique. La rubrification Sr^{90} s'est manifestée dans certaines structures osseuses ayant une faible contenance en sels minéraux et qui étaient nouvellement formées. La distribution in vivo et in vitro de Sr^{90} dans le tissu osseux correspond aux données ordinaires en ce qui concerne la teneur en phosphore, calcaire et sulfate.

L'importance de ce prélèvement de Sr^{90} localisé dans le tissu est discutée en partant du point de vue de savoir si c'est un produit important en cas d'explosion atomique.

ZUSAMMENFASSUNG

Die in vivo und in vitro Aufnahme von Sr^{90} in das Knochengewebe von Hunden wurde mittels radioautographischer und mikroradiographischer Methoden untersucht. Die Kennzeichnung mit Sr^{90} trat in gewissen Knochenstrukturen auf, die einen geringen Mineralsalzgehalt haben und die erst kurz zuvor geformt wurden. Die in vivo und in vitro Verteilung von Sr^{90} folgt der allgemeinen Regel, die für die Verteilung von Phosphor, Calcium und Sulfat gilt. Die Wichtigkeit dieser örtlichen Aufnahme von Sr^{90} in den Knochen wird unter Hinweis darauf besprochen, dass es ein wichtiges Produkt einer Atomexplosion ist.

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