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Deposition of Radiogallium (Ga^{72}) in Proliferating Tissues[1]

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It has previously been shown, chemically and autoradiographically, that gallium enters bone at a rapid rate, following injection of the citrate or lactate, concentrating in those areas considered to have marked osteogenic activity (1, 2, 3).

The purpose of the present investigation was to study in experimental animals the deposition of radiogallium (Ga^{72}) in proliferating tissues. The work comprised three parts: (*a*) the quantitative determination of the amount of Ga^{72} deposited in normal and growing bone and in healing fractures, (*b*) a pilot study of the degree of deposition of gallium in certain neoplastic conditions, (*c*) development of technics to provide greater detail in the autoradiographs of tissue containing Ga^{72}.

The isotopes of gallium which appear to have greatest promise for biological studies are Ga^{72} (14.3 hours half-life, 2.5 mev γ, 3.1 mev β^-) and Ga^{67} (78.3 hours half-life, K capture, 0.3 mev γ with conversion e^-). The Ga^{72} can be prepared by neutron bombardment of magnetically separated Ga^{71} to produce an initial specific activity of about 1.0 mc. Ga^{72}/mg. Ga. By deuteron bombardment (*d*, *n*) of a zinc target in a cyclotron, the isotope Ga^{67} can be produced, accompanied by a quantity of Zn^{65} (250-day half-life). This and other contaminants may be eliminated by ether extraction of gallium from a 6 N HCl solution.

The studies described herein were carried out with Ga^{72} supplied by the Atomic Energy Commission. The natural element (Ga^{69} + Ga^{71}) was activated by the (n, γ) reaction, and an initial activity of about 0.4 mc./mg. Ga was obtained. On administration to animals, the activity was reduced by decay to about 0.2 mc./mg. Ga.

METHODS

Preparation of gallium (Ga^{72}) citrate: Quartz ampules were prepared and sufficient purified gallium nitrate was added to provide 75 mg. gallium (5). These ampules were flame-sealed and irradiated in the pile by the Atomic Energy Commission. The initial activity was approximately 30 millicuries (mc.); when shipped by air, the gallium could be administered with an activity of 12–15 mc.

Preparation of the gallium for injection was as follows:

(*a*) Add to the opened ampule 1 c.c. hot HCl (1:1). Heat gently to facilitate solution of the solid. Pour into a graduate cylinder, and rinse ampule with 2-c.c. portions of water.
(*b*) Add 1 c.c. of brom-cresol purple indicator.
(*c*) Add 2 c.c. of 20 per cent citric acid solution. Mix.
(*d*) Add concentrated NaOH solution to a *p*H of 6–7. Color should be red-purple.
(*e*) Make to required volume with water. In our work, 20 c.c. was convenient, since this gave an activity of about 1 mc./c.c.

Observe precautions and provide adequate shielding (5, 6).

The solution prepared as described is suitable for subcutaneous injection if the area is anesthetized, *i.e.*, with procaine.

Intravenous administration may produce acute reactions, often fatal, due to the effect of the citrate on blood calcium. This effect has been overcome by the simultaneous administration of 0.5 gm. calcium chloride for each 10 c.c. of the Ga^{72} citrate solution. The most convenient method of intravenous administration is by means of glucose-saline infusion with the gallium

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1176221

and calcium being introduced into the rubber tube just above the hypodermic needle. The rate of infusion should be moderate, and sufficient of the infusion fluid should be allowed to dilute the gallium so that no localized reaction occurs.

Radiometric Assay: In the determination of Ga^{72} as reported in these studies, the quantitative estimations were carried out on whole fresh tissues, without drying, by making gamma counts at constant geometry. The end-window Geiger tubes were shielded with an aluminum absorber of 1,700 mg./cm.^{2}, which absorbed all beta radiation and back-scatter. By this method, differences due to tissue density and self absorption were eliminated. Because of the high counting rates of samples and the consistently high counting yield, this assay gives results with an error of less than ± 10 per cent.

In the quantitative determination of Ga^{72} (expressed as mc. of Ga^{72}), samples of the initial citrate solution were compared with standard quantities of radium (1 to 2 mg. in 0.5 mm. Pt needles) by means of an ionization chamber meter or a quartz fiber electroscope. The samples were mounted a constant distance from the center of the chamber of the meter or the electroscope, with the Ga^{72} being shielded by means of a 2-mm. brass shield. By this means, the beta radiation was absorbed and the gamma emission of the Ga^{72} was compared with gamma emission of a standard radium source.

Sample Calculations:

1 millicurie (mc.). Amount of radon in equilibrium with 1 mg. of radium.

1 mg. Ra yields at 1 meter 0.84 mr./hr. (milliroentgen/hour).

1 mc. Ga^{72} yields at 1 meter 1.32 mr./hr. (calculated from data of Haynes (9).

Ionization chamber meter (Victoreen 247A)

1.969 mg. Ra at 1 meter. = 1.65 meter reading

7.5 mg. Ga (in 5 c.c.) at 1 meter = 1.98 meter reading

$$\frac{Ga^{72}\text{ reading} \times \text{mg. Ra} \times 0.84}{\text{Ra reading} \times 1.32} = \text{millicuries } Ga^{72}$$

$$\frac{1.98 \times 1.969 \times 0.84}{1.65 \times 1.32} = 1.50 \text{ mc. } Ga^{72}$$

Electroscope (Lauritsen)

1.969 mg. Ra at 50 cm. = 50 division in 2.55 min.

7.5 mg. Ga at 50 cm. = 50 division in 2.14 min.

$$\frac{\text{mg. Ra} \times \text{time of Ra} \times 0.84}{\text{Time of Ga} \times 1.32} = \text{millicuries } Ga^{72}$$

$$\frac{1.969 \times 2.55 \times 0.84}{2.14 \times 1.32} = 1.49 \text{ mc. } Ga^{72}$$

EXPERIMENTAL

Concentration of Ga^{72} in Bone: Young rabbits (2.0–2.5 kg.) were anesthetized and one foreleg was broken. The leg was then placed in a cast and the animals were housed in cages for seven to twenty-one days. At intervals (seven, fourteen, twenty-one days) following the fracture, rabbits were injected subcutaneously with radiogallium (Ga^{72}) citrate (0.5 mc./kg.) and sacrificed sixteen hours after the injection. Samples of both normal bone and the healing callus were examined autoradiographically (3), and the concentrations of gallium in various portions of the fresh bone were determined by photographic densitometric measurements (4), and by means of radiochemical gamma-counting technics.

Immediately after completion of the autoradiographs, the bones were cut into various fractions by means of a thin abrasive wheel. In this way those areas of greatest osteogenic activity (callus, epiphysis) were obtained relatively free of extraneous tissue and adjacent cortical or trabecular bone. The weighed fresh samples were gamma-counted and compared with standard solutions containing Ga^{72}. Results of these findings are summarized in Table I and Figure 1, A and B. Results indicate that in the young rabbit the epiphyseal junction absorbs four to five times the concentration of gallium deposited in the adjacent bone. The callus of the healing fracture concentrates gallium to a degree two to three times that found in the bone adjacent to the fracture.

These findings indicate that gallium enters into the processes of normal bone growth and repair, with particular affinity for those areas where osteogenic activity is greatest. No significant differences

1176222

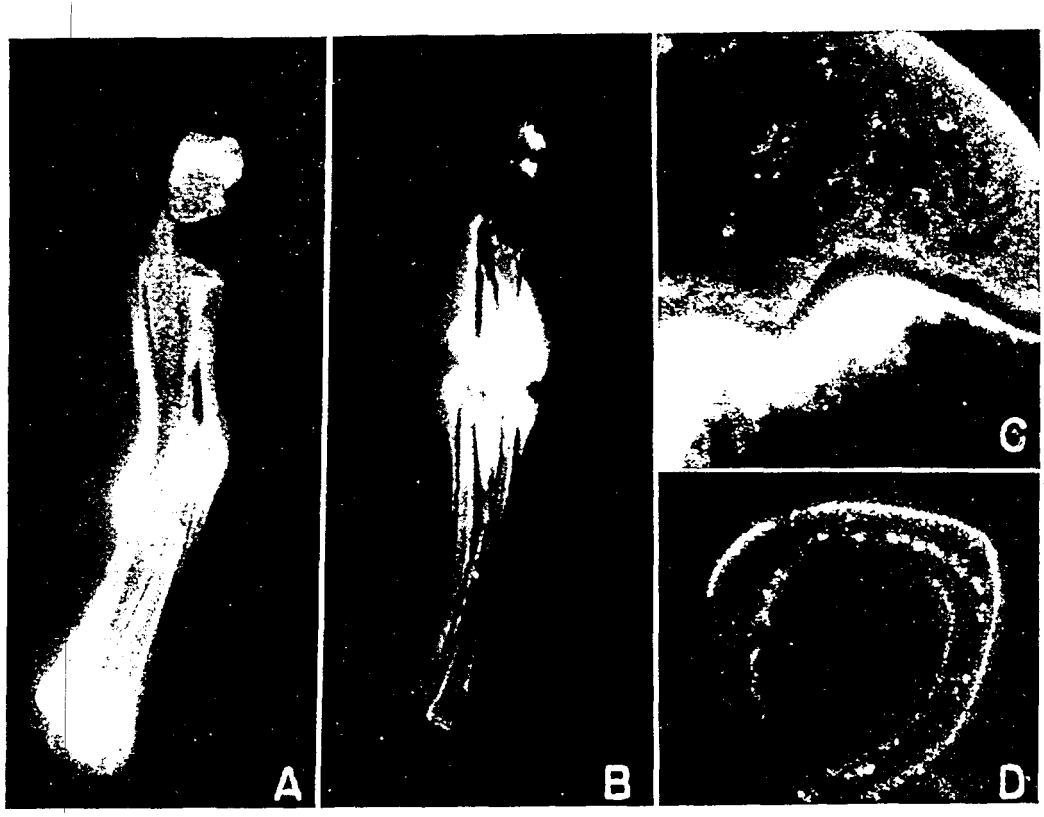

Fig. 1. Prints of autoradiographs of rabbits receiving 0.5 mc. Ga^{72}/kg. A and B. Healing fractures of foreleg fourteen days after break, showing deposition of Ga^{72} in callus. ×1. C. Head of femur, showing area at epiphyseal junction. ×7. D. Cross section of shaft of femur showing localization of Ga^{72}. ×7.

TABLE I: CONCENTRATION OF GALLIUM (Ga^{72}) IN GROWING AND HEALING BONE OF RABBITS

	—Microcuries per Gram of Fresh Bone—	
Sample	Determined Autoradiographically	Determined by Gamma Counting
Normal Bone		
Epiphyseal junction	4.0	3.9
Trabecular	1.1	1.6
Cortical	0.8	0.9
Healing Bone		
Callus (14 days)	3.1	2.2

Note: Values here are the mean of samples from four animals. Reference standards of Ga^{72} were prepared and used to estimate the concentrations at time of death.

were noted in the deposition of Ga^{72} in the fourteen to twenty-one days following the fracture. At seven days there was a diffuse, less well defined deposition in the areas surrounding the fracture.

Deposition of Ga^{72} in Neoplastic Tissues: In order to determine the affinity of gallium for various neoplastic growths, a group of animals having spontaneous non-osteogenic neoplasms were injected subcutaneously with 0.5 mc. Ga^{72}/kg., as the citrate, and sacrificed fourteen to sixteen hours thereafter. The tissues were removed and gamma counts made by the methods previously described.

In Table II is given a résumé of the results of these studies with one strain of rat, one strain of mouse, and one dog. It will be seen that no significant localization of Ga^{72} occurred in the fibroadenoma or the fibrocarcinoma of the rat and mouse or in the mixed mammary tumor of a dog. While localization in the tumor was always greater than that seen in the muscle of the corresponding animal, this deposition did not approach the concentrations found in the osteoid structures.

In order to determine the degree of localization of Ga^{72} in osteogenic lesions, a transplanted osteogenic sarcoma of the mouse was introduced subcutaneously on the abdominal wall of several mice (C_3H

1176223

TABLE II: COMPARATIVE CONCENTRATIONS OF Ga^{72} IN NORMAL AND NEOPLASTIC TISSUES

Species	No. of Animals	Gamma Counts per Minute per Gram of Fresh Tissue						Type Tumor
		Composite Bone	Kidney	Liver	Spleen	Muscle	Tumor	
Albany rat	4	7,990	6,081	1,115	693	140	313	Fibroadenoma
Mouse (C_3H)	2	620	373	136	106	59	219	Fibrosarcoma
Dog	1	2,273	2,853	325	111	17	245	Mixed mammary tumor

Note: The results shown above are the mean of at least duplicate samples taken from the indicated number of animals. Counts are corrected for background and are calculated to time of death.

strain). One of these mice was injected subcutaneously with 0.5 mc. Ga^{72}/kg. as the citrate, and the tumor was removed after sixteen hours. An autoradiograph was prepared of the dense tumor tissue, which indicated localization of Ga^{72} in this mass equivalent to that found in the epiphyseal junction or healing fractures of rabbits, namely 3–5 μc./gm. (microcuries/gram) of tissue (Fig. 2).

Additional serial transplants of this tumor produced a fibrous mass different in character from the original tumor. When autoradiographs of these soft masses were prepared, it was found that in only a few small discrete areas was there any localization of Ga^{72}. This indicates that, while the mass of the tumor was fibrous in nature, there were centers of osteogenic activity still present.

It is recognized that the number of animals having spontaneous tumors presented in this report is small. However, the difficulties of obtaining spontaneous tumors in experimental animals on a laboratory basis has not permitted further extension of these studies. The data presented must be considered as a pilot study. The results are given in order to provide the best available basis for further investigation.

Autoradiographic Technics: The degree of definition obtainable by autoradiographic technics applied to Ga^{72} is determined by the penetration of the beta spectrum of this radioactive isotope. Since the maximum beta energy is 3.1 mev, there appears in the autoradiograph a somewhat indistinct outline caused by the scattering of the high-energy particles, not only by the tissue specimen but also by the emulsion, the film, and the slide backing. The sharpness of the image is also reduced due to *Bremstrahlung* produced by the accompanying gamma spectrum (max. 2.5 mev).

Technics have been improved over those previously reported (3) and include a method which permits the production of a more detailed survey type autoradiograph from undecalcified bone specimens using the short half-life 14.3-hr. isotope, Ga^{72}. The animals were injected subcutaneously with 1 mc. Ga^{72}/kg. as the citrate and killed four hours after injection, which permitted about 75 per cent of the maximum absorption of Ga^{72} to take place in the bone. The animal was then sacrificed and the bone specimens were removed and immediately sectioned to a thickness of 0.10–0.12 mm. by means of a self-feeding circular saw (7). These sections were mounted on microscope slides with a minimum amount of warm (40° C.) photographer's gelatin. As the gelatin hardened, the slides were immersed in 90 per cent and then absolute ethanol for fixation and dehydration. Fixing time did not exceed fifteen minutes. After drying, the bone specimens were placed in direct contact with the emulsion of panchromatic fine grain film (Weston speed, daylight, 50). The film and specimens were held in close contact by mounting between two aluminum plates, on each of which were pasted two layers of blotting paper, covered with black paper. The whole was held tightly together by means of two screw clamps (C type). The specimens were left in contact with the film for sixteen hours, after which the film was slightly overdeveloped in ultra-fine grain developer (Von-L No S-35; 7 minutes at 20° C.) to bring out

1176224

greater detail with minimum grain size. In Figure 1, C and D, are shown enlarged (×7) prints of the autoradiograph of sections of the head and shaft of the femur of a young rabbit (1.5 kg.).

A modification of a technic previously described (8) has proved useful in producing autoradiographs having good definition. A special emulsion (Ansco, autoradiographic "A") was obtained in the fresh semisolid state, melted at 40° C., and diluted with two volumes of water plus a few drops of a wetting agent. This diluted emulsion was used to impregnate fresh undecalcified bone sections (0.1 mm.) cut as described above. The impregnated sections were dried at 38° C. for thirty minutes, and were then placed in a light-tight container for sixteen to twenty-four hours. They were then slightly overdeveloped in the fine grain developer. After fixing in 30 per cent hypo followed by acid hardener, the sections were cleared in xylol and mounted in balsam on microscope slides. The unstained silver-imbedded sections are used for photographic reproduction, but microscopic examination is best done after staining lightly with hematoxylin-eosin. The autoradiographs in Figure 3 (×100) were made as described, from a bone taken from a mature rabbit four hours after intravenous injection of 1 mc. Ga^{72}/kg., as the citrate.

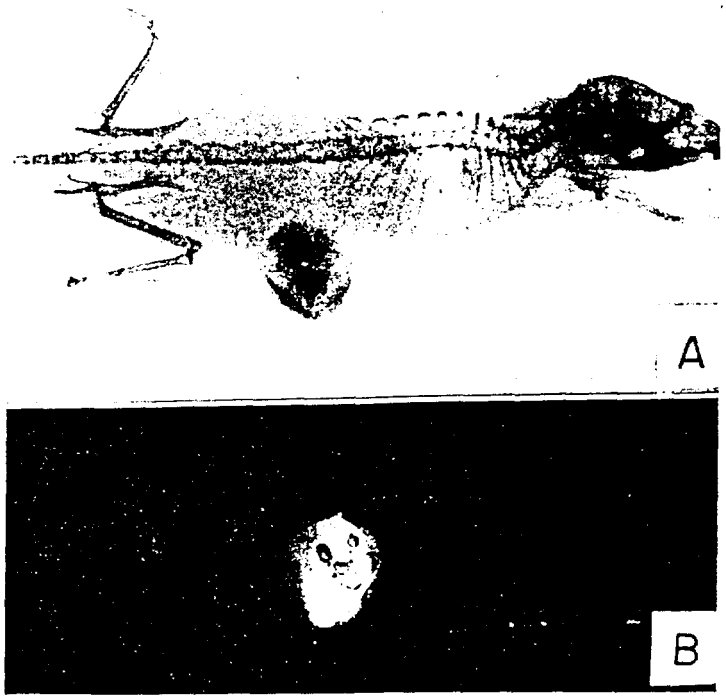

Fig. 2. A. Roentgenogram showing transplanted osteogenic sarcoma in mouse. ×1. B. Autoradiograph of tumor sixteen hours after subcutaneous injection of 0.5 mc. of Ga^{72}/kg. ×1.

These autoradiographic studies extend earlier ones (3). The findings indicate that Ga^{72} is deposited in the periosteum, the endosteum, in the lining of the blood vessels, in the haversian canals, and in the calcifying cartilage at the ends of the diaphysis. The trabeculae of cancellous bone are outlined by the Ga^{72}, since it is presumed to be laid down in the endosteum. The epiphyseal cartilage plate appears to contain little or no Ga^{72}, while the fine structure of the adjacent epiphysis is clearly outlined. The apparent deposition of Ga^{72} throughout cortical bone, as suggested by our earlier work, now appears

1176225

to be due to the deposition of Ga^{72} on the periphery of blood vessels and in the haversian canal system. The scattering of the energetic beta radiation by the dense bone gives an appearance in the gross autoradiographs (3) of a more or less homogeneous distribution of Ga^{72} in the shafts of the long bones.

The difficulties encountered with the autoradiographic study of Ga^{72}, particularly on the cellular level, are those due to its short half-life (14.3 hr.). The bone sections must be cut while fresh and placed in contact with some photographic emulsion within a few hours after the death of the animal. Since 70 per cent of the activity of the Ga^{72} deposited in the bones decays in twenty-four hours, it can be seen that the usual histologic methods of imbedding and cutting are not applicable. Other difficulties were encountered in using many types of photographic emulsions, since most are relatively insensitive to the gamma rays and beta particles given off by Ga^{72}. Also, in enlarging the contact autoradiographs, the size of the developed silver granules makes extensive magnification impractical. These difficulties have in part been overcome by the use of the slower fine grain panchromatic films and slight over-development in an ultra-fine grain developer. In cases of low intensity radiations, the use of special developers (Von-L, No. S-35) or intensifiers ("Hydram") added to the usual fine grain developers, has made possible the bringing out of the latent image more strongly. In general, the slower panchromatic emulsions rather than x-ray emulsions have given more satisfactory results when finer detail was desired.

DISCUSSION

Deposition of Ga^{72} in bone appears in many respects similar to that of other bone seekers (Ra, Pu, P^{32}, Sr^{89}). However, gallium is deposited more rapidly than many others and the short half-life does not offer such chance for the production of neoplastic growths. Localization of Ga^{72} in the endothelial cells of the skeletal structures, and particularly the deposition of gallium in that zone of osteogenesis between the cartilaginous epiphyseal plate and the metaphysis (*i.e.*, the zone of calcifying cartilage), indicates that gallium enters into the calcification process in a dynamic manner. It is these tissues which are active in the development of new bone and the regeneration of changes of the haversian canal system, and it is in these areas that the phosphatases are believed to be most actively concentrated. Therefore, as a working hypothesis, it is suggested that the injection of small amounts of Ga^{72} citrate, followed by preparation of autoradiographs by the emulsion impregnation technic as described above, may provide a means of differential staining and localization of structures containing large amounts of the phosphatase enzymes.

The localization of gallium in osteogenic centers makes possible a more or less localized irradiation of these sites by means of Ga^{72}. This suggests that radiogallium may be useful in the therapy of osteogenic neoplasms. Also because of this localization, the *in situ* irradiation of osteoid structure may be useful in certain conditions in which whole body roentgen irradiation is now an accepted practice. By reason of its short half-life (14.3 hr.), repeated injections of this isotope would seem to offer a means of applying ionizing radiation of high intensity, in divided doses, with no danger of the residual radiation inherent in the use of the longer-life bone seekers.

In considering the therapeutic trial of Ga^{72}, the possible toxicity of the carrier gallium must be anticipated. In moderate doses this element produces nephritis followed by albuminuria and glycosuria. With an increase of specific activity of Ga^{72}, through the magnetic separation of Ga^{71}, resulting in a reduction of the carrier Ga, it is believed that experimental clinical trials will be warranted. This is in addition to the clinical studies of osteoid lesions and metastases with tracer (500 μc.) quantities of Ga^{72} now being carried out.

1176226

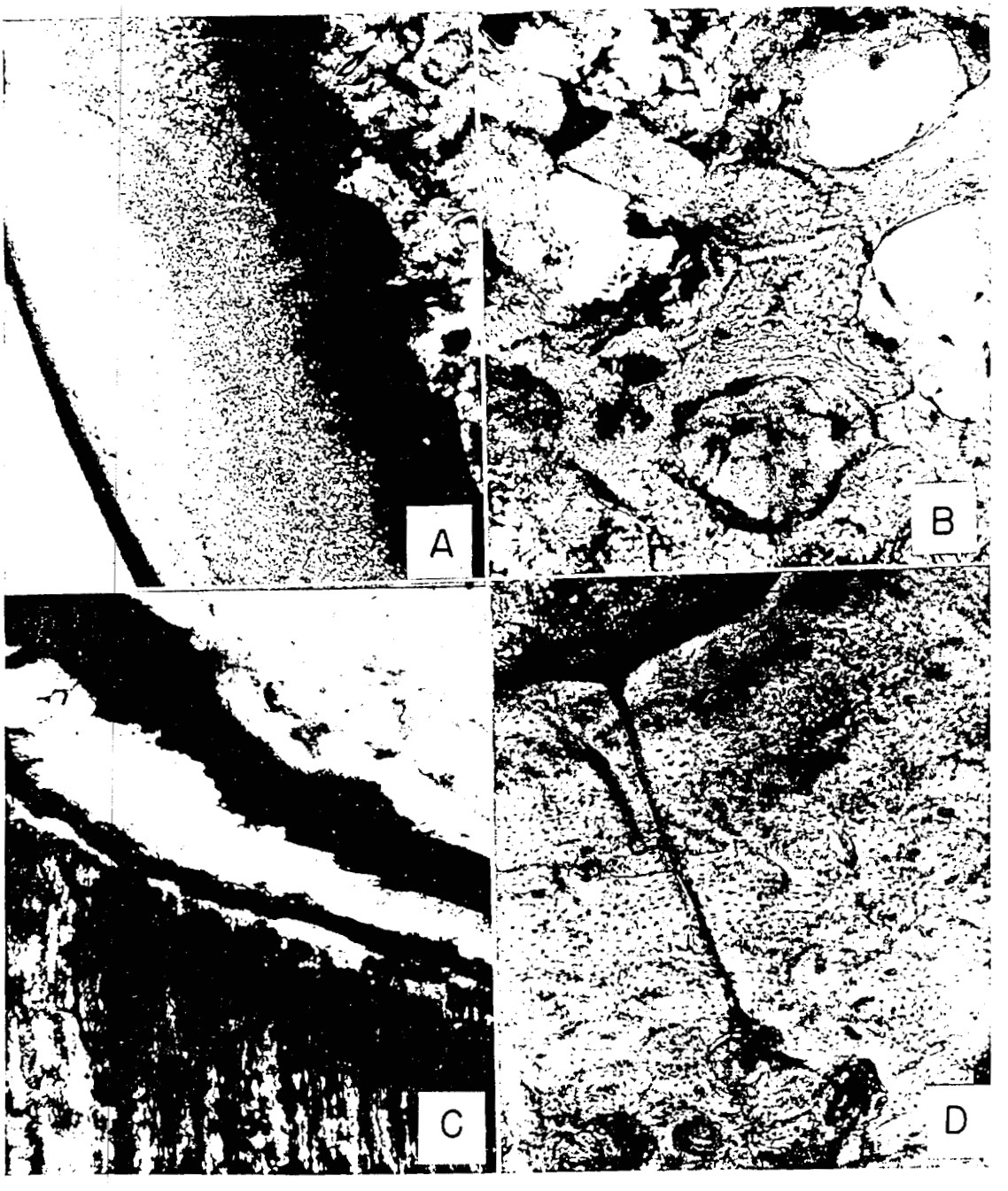

Fig. 3. Photographs (×100) of 0.1-mm. bone sections of femur from 3.5-kg. rabbit, four hours after injection of 4 mc. of radiogallium (Ga^{72}) citrate. Dense black areas are silver granules deposited from a photographic emulsion, affected by the radioactivity, and developed in the usual way:

A. Head of condyle, showing outer layer of high Ga^{72} content, with underlying zone of cartilaginous material, followed by a zone of high Ga^{72} content, and finally by the trabeculae of the epiphysis.

B. Details of Ga^{72} in the trabeculae of the epiphysis.

C. Epiphyseal junction. Lower columnar area shows high content of Ga^{72}, increasing as the cartilaginous plate is approached. The white area in this plate is devoid of Ga^{72}, while above is an area of the epiphysis which is very rich in Ga^{72}.

D. Nutrient vessel connecting the periosteal layer with an haversian canal. Note dense localization adjacent to periosteum.

1176227

SUMMARY AND CONCLUSIONS

Radiogallium (Ga^{72}) has been shown to concentrate at areas of osteogenic activity, *i.e.*, the epiphyseal junction and in the callus of healing fractures in rabbits, and in osteogenic sarcoma of the mouse.

In certain fibroadenomas, fibrosarcomas, and mixed mammary tumor of animals, no significant localization of Ga^{72} occurred.

Autoradiographic studies of the deposition of Ga^{72} in bone, by impregnation of bone sections with liquid photographic emulsion, has made possible more detailed localization of this element.

It is suggested that radiogallium citrate (Ga^{72}) is worthy of clinical trials in tracer amounts for diagnostic purposes and at higher levels for possible therapeutic effect.

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SUMARIO

Depósito de Radiogalio (Ga^{72}) en los Tejidos Proliferantes

Según se observó, el radiogalio (Ga^{72}) se concentra en las zonas de actividad osteógena, por ej., la unión epifisaria y el callo de las fracturas en vías de cicatrización en los conejos y el sarcoma osteógeno del ratón. En ciertos fibroadenomas, fibrosarcomas y tumores mamarios mixtos de los animales, no hubo localización significativa de Ga^{72}.

Los estudios autorradiográficos del depósito de Ga^{72} en el hueso, por medio de la impregnación de cortes óseos con emulsión fotográfica líquida, ha permitido localizar con mayor precisión el elemento mencionado.

Sugiérese que el citrato de radiogalio (Ga^{72}) es acreedor a pruebas clínicas, en cantidades indicativas para fines de diagnóstico y a dosis mayores por su posible efecto terapeútico.

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