A Study of Irradiated Bone. III. Scintigraphic and Radiographic Detection of Radiation-Induced Osteosarcomas

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Within 1 yr after localized irradiation of a hind limb with single (1756 rads) or fractionated (4650 rads in 3 wk) x-ray doses, radiation-induced osteosarcomas were observed in four of nine single-dose rabbits and two of 11 fractionated-dose rabbits. Tumors were observed in the proximal tibia in five cases and the distal femur in one case. In terms of production of osteoid or osseous tissue, three tumors were well differentiated, one slightly differentiated, and two (spindle-cell tumors) undifferentiated. This report summarizes the Tc-99m pyrophosphate (TcPPi) imaging and autoradiographic, radiographic, and histologic studies of these osteosarcomas. The four differentiated osteosarcomas were detected 1–2.5 mo earlier by TcPPi imaging than by radiography, whereas the two undifferentiated tumors were suspected 2 wk or 3.5 mo earlier radiographically. Autoradiograms showed TcPPi localization in bone produced by differentiated osteosarcomas, and in regions of reactive bone resorption and formation peripheral to tumors. The results support a recommendation for combined radiographic and scintigraphic techniques for the early detection of osteosarcomas.

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One of the complications following irradiation of bone is the development of osteosarcoma. The first human cases of radiation-induced osteosarcoma in previously normal adjacent bone included in the x-ray beam were described by Beck (*1*) in 1922. Since that time there have been other reports on the induction of osteosarcomas by external-beam irradiation in experimental animals (e.g., *2–6*) and radiotherapy patients (e.g., *7–8*), as well as many reports on radiation induction of osteosarcomas by internally administered bone-seeking radionuclides. In human beings the latent period between localized external-beam irradiation of bone and the appearance of osteosarcoma has been reported to range from about 4 yr to more than 29 yr, after total doses ranging from 1500–23,000 rads (*8*).

This report is not intended to summarize a dose-effect relationship between radiation and osteosarcoma. Rather, it is concerned primarily with techniques of detection of osteosarcoma. This is the third in a series of reports (*9–10*) from an investigation of the pathophysiologic bases for alterations in radionuclide images of bones in rabbits after irradiation. In the course of this investigation, radiation-induced osteosarcomas of varying degrees of differentiation were observed to develop within a year after x-irradiation of a hind leg. These tumors, with their relatively short latency, provide a convenient model to compare methods of clinical detection of developing osteosarcomas. This paper reports the findings of the Tc-99m pyrophosphate† (TcPPi) imaging and autoradiographic, radiographic, and histologic studies of these osteosarcomas.

MATERIALS AND METHODS

These have been reported in greater detail in the

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TABLE 1. OSTEOSARCOMAS OBSERVED

Tumor differentiation (osteoid or bone production)	Location	Months postirradiation for first radiographic indication	Months postirradiation for first imaging indications
		Single-dose rabbits	
Marked	Proximal tibia	6	5
Marked	Distal femur	12	9.5
Little	Proximal tibia	12	9.5
None	Proximal tibia	10.5	11
		Fractionated-dose rabbits	
Marked	Proximal tibia	12	9.5
None	Proximal tibia	6	9.5

previous reports of other aspects of this investigation (*9–10*).

Young adult, male, albino New Zealand rabbits were used in the investigation. The irradiated rabbits received x-ray doses to the left hind leg, with either a single dose of 1756 rads or a dose of 4650 rads fractionated over a period of 3 wk [1756 ret (*11*)]. Nine of the single-dose rabbits and 11 of the fractionated-dose rabbits were allowed to live for about a year after irradiation, when they were killed for postmortem study. The radiation-induced osteosarcomas devleoping in four of the nine single-dose rabbits and in two of the 11 fractionated-dose rabbits were studied by means of the following methods.

Radiographs of the hind limbs were made before, and at 3, 6, 7.5, 9, 10.5, and 12 mo after irradiation. Bone images of the rabbits were made at least once every 2 mo until 9 mo after irradiation, and monthly after that. When a region of bone began to show unusual changes, either in the scintigrams or the radiographs, the further development of such changes was closely followed, recorded, and ultimately correlated with histological study. A final retrospective review of the camera images and radiographs was made after histological examination to determine the time of earliest indication of tumor presence. These are the times recorded in Table 1.

Before sacrifice at 12–14.5 mo after irradiation, each animal was injected with tetracycline daily for 3 days to label the sites of new bone formation (*9*). Two hours before sacrifice they were injected with 2 mCi/kg body weight of TcPPi for autoradiographic studies (*10*). Upon death, the tibias and femurs were excised and radiographed. Specimens of bone and suspected tumor were taken to make decalcified and undecalcified histological sections (*9*), and for macroautoradiography (*10*).

RESULTS

Within the 1 yr after irradiation, four of the nine (44%) single-dose rabbits and two of the 11 (18%) fractionated-dose rabbits had developed an osteosarcoma in the irradiated leg. Table 1 summarizes the degree of differentiation of these osteosarcomas in terms of production of osteoid or osseous tissue by the tumor cells, the locations of the tumors, and the postirradiation times of first detection in radiographs and in TcPPi images.

In a retrospective review of periodic radiographs and scintiphotos, the four differentiated osteosarcomas were first indicated 1 mo earlier (one case) to 2.5 mo earlier (three cases) by TcPPi imaging rather than by radiography, whereas the two undifferentiated tumors were indicated 2 wk or 3.5 mo earlier radiographically. Two tumors (one well differentiated and one undifferentiated) were first indicated as early as 5 and 6 mo after irradiation, and the remaining four tumors were first indicated at 9.5 or 10.5 mo after irradiation.

The nontumorous degenerative or atrophic effects of radiation in bone were seen as subtle and diffuse changes

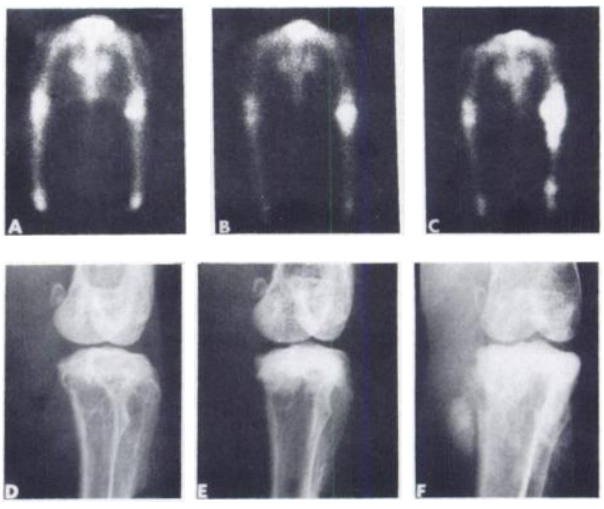


FIG. 1. Well-differentiated osteosarcoma developing in left hind leg after irradiation with 4650 rad fractionated over 3 wk. Supine TcPPi images at 9.5 (A), 12 (B), and 14.5 (C) mo, and radiographs at 9 (D), 12 (E), and 14.5 (F) mo after irradiation. Tumor first seen on images at 9.5 mo (A) by focal increase of TcPPi in proximal tibia and subsequently on radiographs at 12 mo (E) as osteoblastic lesion in same location. Progression of tumor growth is evident on later images and radiographs.

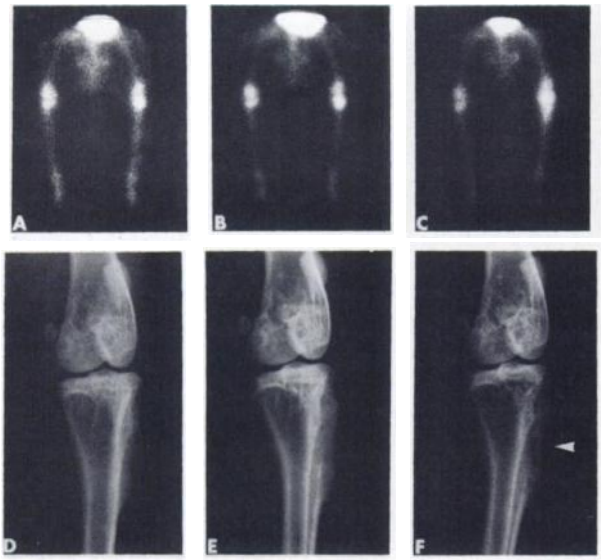


FIG. 2. Poorly differentiated osteosarcoma developing in left hind leg after irradiation with 1756-rad single dose. Supine TcPPi images at 9.5 (A), 10.5 (B), and 12 (C) mo, and radiographs at 9 (D), 10.5 (E), and 12 (F) mo after irradiation. Tumor first seen on images at 9.5 mo (A) by a focal increase of TcPPi in proximal tibia and subsequently on radiographs at 12 mo (F) in anterolateral aspect of proximal tibia as a subtle radiolucent lesion (arrow). Progression of tumor growth evident on later images.

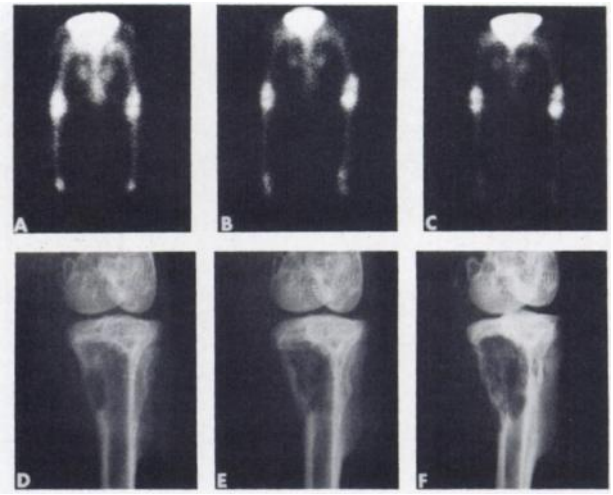


FIG. 3. Undifferentiated osteosarcoma (fibrous spindle-cell type) developing in left hind leg after irradiation with 4650 rad fractionated over 3 wk. TcPPi images at 6 (A), 9.5 (B), and 12 (C) mo after irradiation and radiographs at 6 (D), 9 (E), and 12 (F) mo. Tumor first seen on radiographs at 6 mo (D) in posteromedial aspect of proximal tibia as radiolucent lesion with minimally sclerotic margin, and subsequently on scintigrams at 9.5 mo (B) as moderately elevated activity in proximal tibia. Progression of tumor growth evident on later radiographs; little change seen on later TcPPi images.

in radiographs and in TcPPi camera images (*10*). A well circumscribed increase in TcPPi activity or a decrease in bone density seen radiographically (lytic change) was observed initially at those sites that eventually developed tumors (Figs. 1–3). In general, the more differentiated the tumor, the greater was the TcPPi uptake differential between tumor and contralateral control regions. "Photon-deficient" lesions were not observed in the rabbits that developed the "lytic" tumors (Fig. 3). This may have been because the generalized decrease in uptake of TcPPi in the rabbits' irradiated knee joints at these times after irradiation (*10*) masked the small changes that occurred in conjunction with the "lytic" tumor.

Autoradiograms of the well-differentiated tumors showed a marked increase in TcPPi deposition in the osteoid or osseous tumor tissue (Fig. 4). There was no increase in TcPPi uptake in the purely cellular regions of these or other forms of osteosarcoma. Only very minimal TcPPi uptake was found autoradiographically in the central necrotic calcified regions of the most advanced well-differentiated osteosarcomas. In general, for all the types of osteosarcoma observed in this study, increased TcPPi localization as seen autoradiographically appeared to be at the sites of tumor-related lightly calcified new bone, which was observed on radiographs of the bone sections (Figs. 4–6). In the poorly differentiated and the two undifferentiated osteosarcomas, the increased TcPPi localization was predominantly in reactive new osseous tissue, and on the surface of areas of bone resorption surrounding tumor tissue. Tetracycline was also found deposited in the areas of reactive bone formation (Fig. 5). A focus of osseous-tissue formation in the center of one of the undifferentiated osteosarcomas was found to be a major site of increased TcPPi uptake (Fig. 6).

DISCUSSION

This paper is concerned primarily with detection of osteosarcoma and is not intended to represent a dose-effect relationship study of radiation induction of osteosarcoma. However, as data related to the induction of osteosarcoma are implicit in the information given and are of some importance, a brief discussion of certain aspects is warranted for both information and cautionary purposes.

Naturally occurring osteosarcomas in the rabbit are rare, and the few cases that have been reported in the literature have occurred in rabbits more than 5 yr of age (*6*). In our study, nonirradiated legs of rabbits showed no bone tumors. Therefore, it appears that the incidence of six osteosarcomas in 20 of the young rabbits irradiated in the present study represents a significant increase due to irradiation. Despite an apparent large percentage difference, the frequency of osteosarcoma in fractionated-dose rabbits is not significantly lower statistically ($P = 0.44$) than that in the single-dose rabbits by the chi-squared test corrected for continuity (*12*). The postirradiation observation time was too short to permit a complete assessment of the tumorigenic effects of the

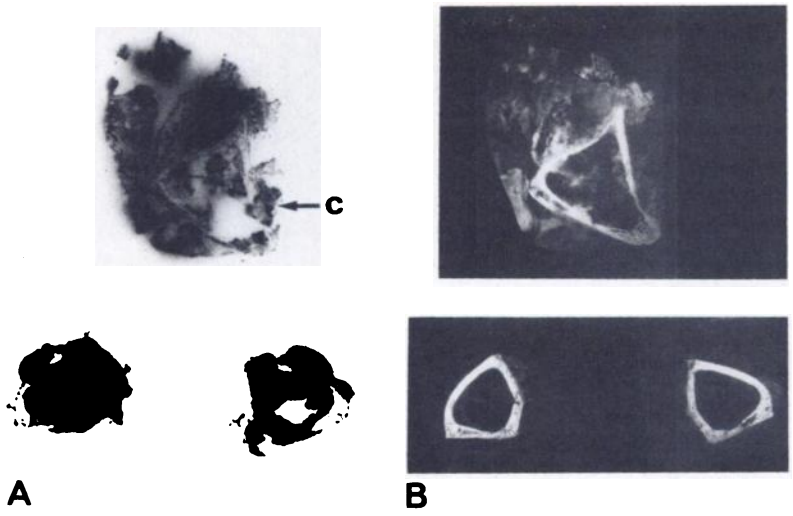
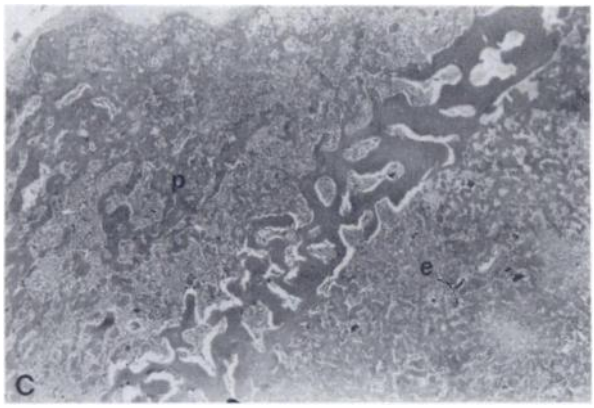


FIG. 4. Necropsy studies of well-differentiated osteosarcoma shown in Fig. 1, at 14.5 mo after irradiation. (A) TcPPi contact autoradiograms of transverse bone sections. (B) Radiographs of bone sections used for autoradiograms. (C) Photomicrograph of decalcified transverse bone section. (H & E, ×50 before reproduction). Intense TcPPi uptake in marrow and periosteal regions (arrow c in A) corresponds to areas of calcification on radiographs in B and to tumor formation in endosteal (e) and periosteal (p) regions in C.



two doses and the degree to which latency may have been different as compared with induction effectiveness (*13*). In spite of these limitations, the data still may be compatible with the view that fractionation of dose may decrease the sarcomagenic effectiveness of dose, provided the dose is not too large (*4,13*).

Because of the limitations of the present data, it would be unwarranted and misleading to attempt to compare them closely and extensively with other data relating to experimental animals or human beings under other than reasonably similar conditions and limitations. A fairly reasonable comparison can be made with a recent pilot study (*6*) in which three of four young adult rabbits (75%), irradiated to the head twice weekly for 6 wk with fast neutrons to a total dose of 1680 rads, developed osteosarcoma in the irradiated field within 1 yr after irradiation. These data for 1 yr of observation, in comparison with the 1-yr data for fractionated x-irradiation (4650 rads) to a hind leg in the present study, though involving different bones and bone masses and subject to the same problems concerning latency, are at least compatible with the well-known greater relative biological effectiveness of the neutrons (*6*).

As shown in Table 1, the four cases of differentiated osteosarcoma were detected by TcPPi scintigraphy before radiographic changes became apparent. The indications of development of the undifferentiated osteosarcomas were detected in one case at about the same time by the two techniques, and in the other case considerably earlier by radiography. The intervals between studies were large, however, and it is possible that malignant changes could have been observed earlier had there been more frequent applications of the techniques.

This detection pattern is in agreement with earlier clinical studies where bone imaging was found to be a sensitive technique for the study of both primary and secondary skeletal neoplasms (*15–18*). The pattern is also in agreement with the differences in the underlying pathophysiological changes assessed by the two techniques (*18–19*). The false-negative bone images that do occur are reported as being due to highly anaplastic tumors where little reactive bone is formed (see Figs. 3 and 6), or imaging when the phase of active bone remodeling is over (*17,19*).

In following the time course of the differentiated osteosarcomas, the extension of the tumor along the shaft of the bone, as determined histologically, was seen sooner, and could be assessed better, in the gamma camera images than in the radiographs (Fig. 1). This contradicts one recent clinical study of osteogenic sarcoma (*20*) in which no difference was noted, but is in agreement with another study (*17*). In the four rabbits with differentiated osteosarcomas, a slight increase in the ipsilateral femur or tibia was seen. This has also been observed in clinical studies (*21*) and may be a manifestation of the "recruitment phenomenon". In two of our animals, however, histological evidence of tumor spread across the joint was found.

The autoradiographic findings that TcPPi is highly localized in new tumor bone and osteoid tissue—and not in the highly cellular (with no bone or osteoid produc-

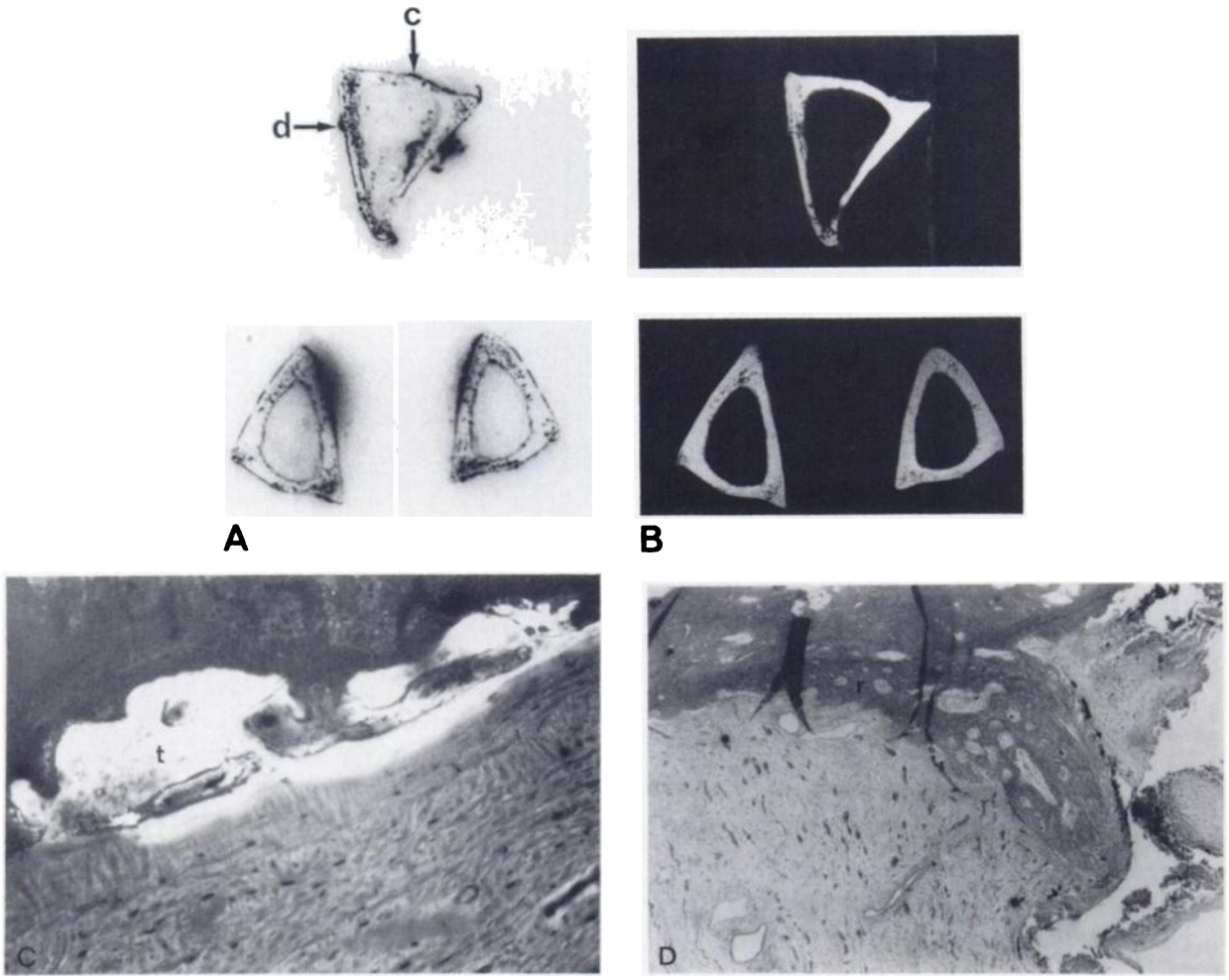


FIG. 5. Necropsy studies of poorly differentiated osteosarcoma shown in Fig. 2, at 12 mo after irradiation. (A) TcPPi contact autoradiograms of transverse bone sections. (B) Radiographs of bone sections used for autoradiograms. (C) Ultraviolet photomicrograph of undecalcified transverse bone section (×400 before reproduction). (D) Photomicrograph of decalcified transverse bone section (H & E, ×200 before reproduction). TcPPi uptake in A corresponds with cortical cavities and lightly mineralized areas extending from bone surface on radiographs in B. Note: Sites of intense periosteal uptake (arrows c and d in A) correspond to sites of tetracycline localization (t in C) and reactive bone formation (r in D).

tion) or necrotic, mineralized, tumor tissue—are similar to those reported by other investigators in studies of osteosarcomas in patients and experimental animals using Sr-85 (*22,23*), F-18 (*24*), or Tc-99m polyphosphate (*25*). The finding, in the poorly differentiated and undifferentiated osteosarcoma cases in the present study, that TcPPi concentrated in areas of reactive bone formation is in agreement with reports of studies of metastatic carcinoma in bone using Ca-45, F-18, or Tc-99m polyphosphate (*26*). Note, however, that although the imaging, radiographic, and histologic findings in rabbit osteosarcomas in the present study may be qualitatively similar to those in human osteosarcomas in principle and in general terms, there are undoubtedly quantitative and temporal differences.

CONCLUSION

We believe that both bone imaging and skeletal radiographs should be used for the early detection of radiation-induced osteosarcoma and its differentiation from radiation atrophy of bone. Autoradiograms of bone and tumor specimens indicate that the abnormal scintigrams reflect the enhanced deposition of TcPPi in newly formed bone and osteoid tissue produced by, and in reaction to, the tumor.

FOOTNOTE

† Pyrophosphate was obtained from Phosphatec kits supplied by E. R. Squibb and Sons, Inc., New Brunswick, NJ.

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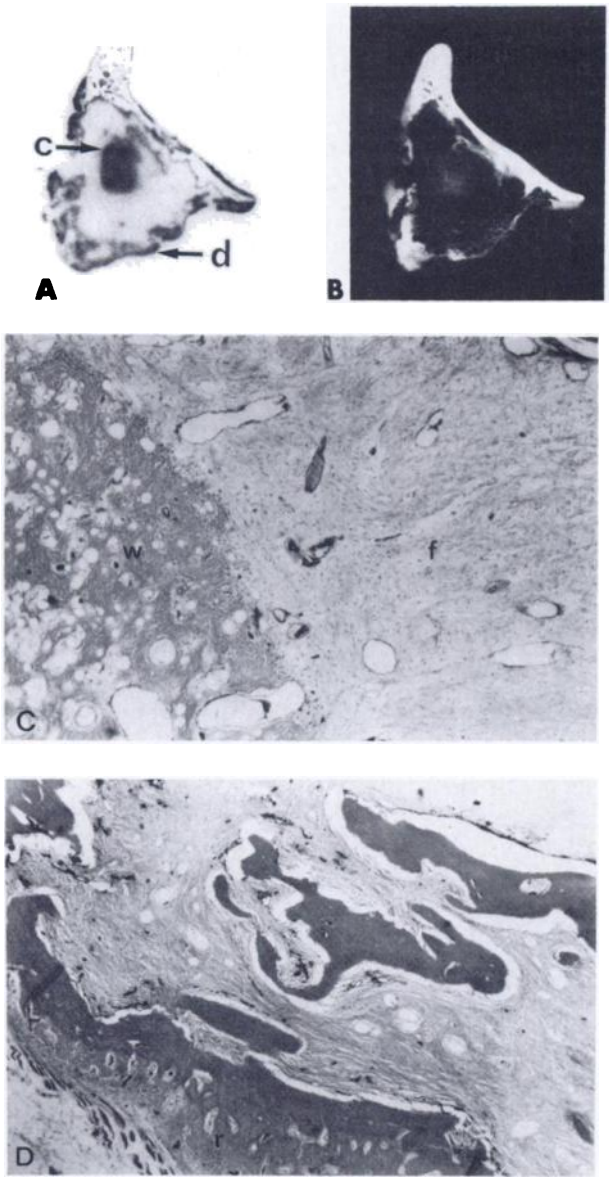

FIG. 6. Necropy studies of undifferentiated osteosarcoma shown in Fig. 3, at 12 mo after irradiation. (A) TcPPi contact autoradiograms of transverse bone section. (B) Radiograph of bone section used for autoradiograph. (C and D) Photomicrographs of undecalcified bone section (H & E, ×200 before reproduction). Intense TcPPi uptake (arrow c in A) corresponds to site of light mineralization in B and location of woven bone (w) as opposed to fibrous tumor (f in C). Other areas of intense TcPPi uptake (arrow d in A) correspond to regions of light bone mineralization on remains of cortex in B and region of reactive bone formation (r in D).

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