Reprinted from CANCER, Vol. 51, No. 9, May 1, 1983.

Radioactive-Induced Tumors by Phosphorus-32 as Colloidal Compound

A. M. UBIOS, DDS,*† F. S. SILBERMAN, MD,‡ AND R. L. CABRINI, MD*

Chromic colloidal phosphate labeled with ^{32}P, which has been proposed for the treatment of several articular diseases, was injected intra-articularly in the knee joint of adult Wistar rats. After a 270 days minimum latent period, tumors began to appear in the injected zone, to a 70% frequency. Ten lung metastases were detected. In five cases, squamous cell carcinomas were induced in the injected area. The relevance of a sound evaluation of the risk involved in treatments with radioactive isotopes, is discussed.

Cancer 51:1605–1609, 1983.

IONIZING RADIATIONS, besides being a valuable therapeutic medium, are capable of inducing tumor formation. This induction has been reported in human beings as well as in experimental series.[1–4] Among ionizing radiations, those which act as a constant source are highly able to induce tumors.[5] In previous reports, we have reported that intra-articular injections of colloidal chromic phosphate labeled with ^{32}P provoke severe lesions in articular and paraarticular tissues.[6–8] The seriousness of the lesions found and the widespread uses of intraarticular injections of colloidal solutions labeled with ^{32}P and other radioisotopes, such as ^{198}Au, ^{90}Y, and ^{186}Re, for the treatment of articular diseases[9–16] make the exploration of tumoral induction in this experimental model interesting.

Materials and Methods

Fifty-five male Wistar rats of 250 g of body weight at the beginning of the experiment, were used. Forty-five of them received an intra-articular injection of 0.05 ml of radioactive colloidal chromic phosphate in the left knee joint (^{32}P, activity 0.25 mCi, particles 10 to 30 nm[17]), while the right knee was considered as control. The dose was calculated for the total decay of the ^{32}P (half-life 14.5 days, 1.71 MeV, β^-) considering the articular area and the rate levels in hard and soft tissues as described elsewhere.[6,18] Twenty-four animals displaying tumors or ulcerations of discrete size, not smaller than 2 cm in diameter, were killed. Samples of the tumors and of both joints, besides lung and liver, were processed for light microscopy. Samples of tumors were taken for electron microscopy. They were fixed according to Karnowsky's technique,[19] postfixed in osmium tetroxide, thereafter embedded in Epon 812 and stained following Reynold's method.

Ten animals were injected in the left knee joint with equivalent amounts of cold colloidal chromic phosphate. Samples of both knee joints were taken when the animals were killed 56 days later.

Results

In the course of the first 200 days postinjection, eight of 45 injected rats died through causes foreign to the experiment itself. Hard and firm tumorations started to appear from day 270; eight between day 270 and day 300; and another 18 on the following days up to day 730. In all, 26 tumors were detected, yielding an incidence of 70%. Their aspect corresponded to nodular formations, that showed an ulcerated zone in 10 cases. The size of the tumors varied, the largest measuring 12 × 10 × 6 cm.

Microscopic observations of the tumors showed histological images of sarcomatous nature. The tumors were always characteristically aggressive showing evident atypical features, infiltrative activity and a high number of mitosis. The features of highly malignant fibrosarcomas appeared most frequently (Fig. 1), showing remarkable irregular spindle cells in addition to collagen and reticular fibers. It was not exceptional to detect necrotic areas as well as richly vascularizated areas. In nine cases, areas with conspicuous osteogenic activity

Supported in part by Grant 8461 of the National Research Council, Argentina.

* Department of Radiobiology, Comisión Nacional de Energía Atómica, Buenos Aires.
† National Research Council, Argentina.
‡ Dupuytren Foundation, Buenos Aires.
Address for reprints: Dr. A. M. Ubios, Departamento de Radiobiología, Comisión Nacional de Energía Atómica, Avda. del Libertador 8250, 1429 Buenos Aires, Argentina.

Accepted for publication February 5, 1982.

0008-543X/83/0501/1605 $1.05 © American Cancer Society

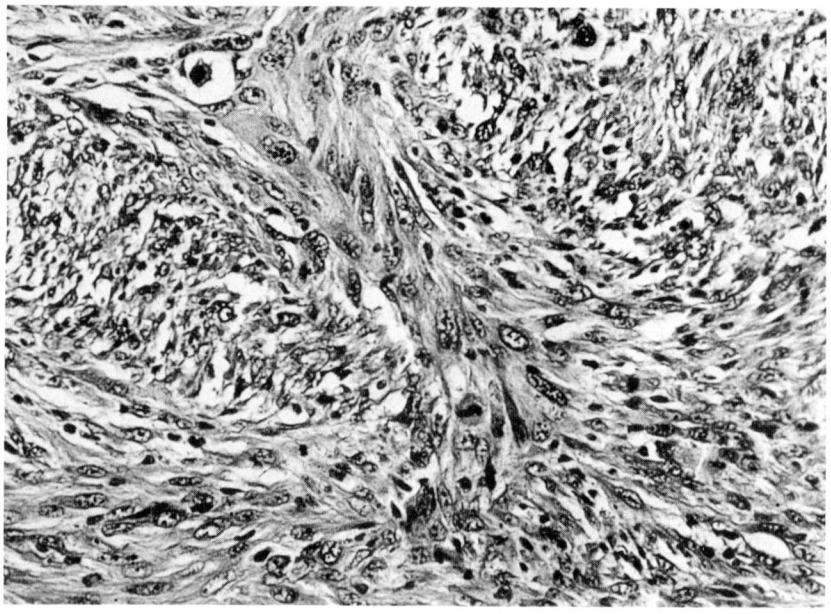


FIG. 1. Sarcomatous area of a tumor. Note irregular spindle cells and mitotic figures (H & E, ×130).

showing clearly atypical trabeculae, were found, an image which corresponds to predominant osteoblastic osteosarcomas (Fig. 2). In five cases, the histologic aspect included extense zones where an endothelial differentiation with atypical cells appeared, sparcely growing on well defined cavities, not associated to vascular structures. Gomori's technique for reticular fibers revealed the lack of endothelium in these cavities. This finding and the existence of PAS-positive areas, directly related to the cavities also proved the presence of glycosaminoglycans, simulating the features of the synoviosarcomas.

No tumors or histologic lesions were found in the right control knee joint. The articular and para-articular tissues of those animals injected intraarticularly with cold colloidal chromic phosphate showed no alterations when studied microscopically.

The ultrastructural observations of tumors showed a

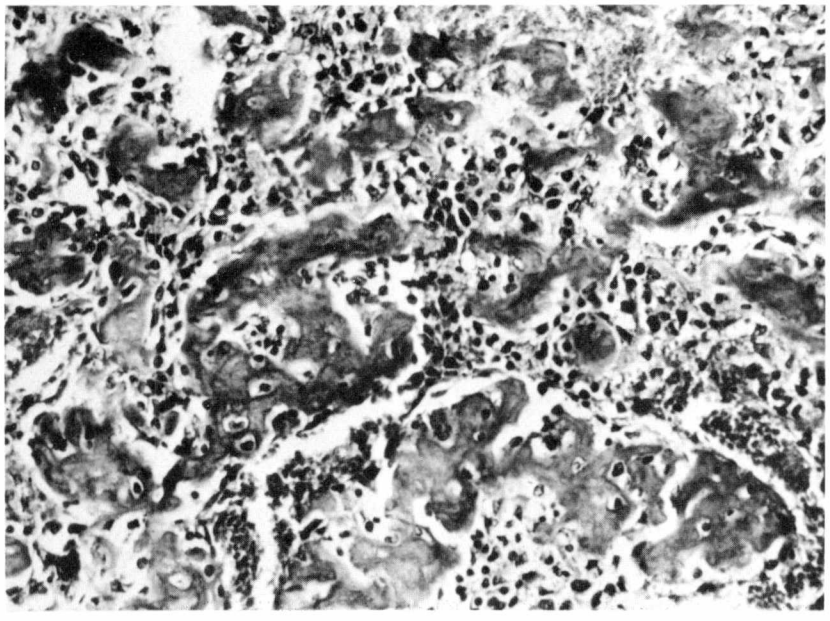


FIG. 2. Characteristic pattern of osteosarcoma with atypical trabeculae (H & E, ×90).

great cellular polymorphism with features of cell damage (Fig. 3). No myofibrils were detected in any of the cases studied with this methodology.

Lung metastasis, forming nodes or miliar dissemination, were found in ten cases. Histologically they corresponded either to fibrosarcomas or osteosarcomas (Figs. 4 and 5).

In five animals, persistent vegetating ulcers with indurated base were observed, which microscopically corresponded to squamous cell carcinomas of a high mitotic index, with atypical cells, horn pearl formation and infiltrative activity (Fig. 6).

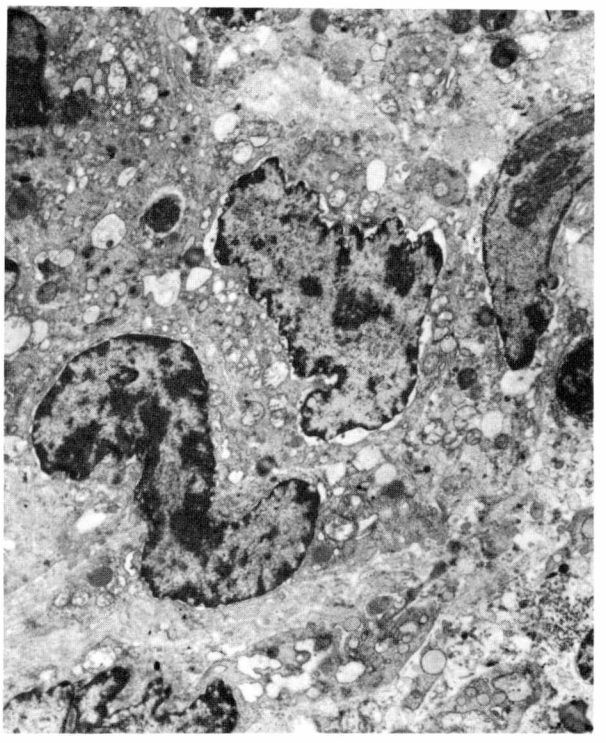


FIG. 3. Ultrastructural image of a tumor showing the irregular features of the cell components (H & E, ×4500).

Discussion

The oncogenic potentiality of ^{32}P has been demonstrated in experimental models[20,21] as well as in humans. It is well known that leukemia is induced in patients treated for polycithemia vera with ^{32}P.[22,23] Our results indicate a higher potentiality when ^{32}P is injected as colloidal solution. The high tumoral incidence yielded by this experiment (70%) could be attributed to the adsorption on cavity walls of the colloidal particles and the fact that there is practically no leakage out of the cavity.[24–26] In this way, the radioisotope acts as a constant source of irradiation until its total decay. The minimum latent period of 270 days is similar to that reported for osteosarcomas induced by other types and conditions of irradiation in experimental models,[20,21,27,28] as well as in humans.[28–33]

We have observed 30% of metastases in the tumor-bearing animals, but this number would probably be higher if all the animals had been kept alive for a longer period.

The localization of the radioisotope accounted for the sarcomatous type of the tumors. The histologic study has demonstrated indifferentiated forms, presumably of synovial origin, and osteosarcomas, which as observed in humans[34] display very polimorphic patterns.

The induction of malignant epithelial tumors would be due to injections applied through a false pathway. This finding indicates that this risk must be not underestimated.

It is very interesting to note that no tumors appeared in control noninjected knee joints nor in those injected with cold colloidal chromic phosphate. In a previous report, we have demonstrated that radioactive colloidal chromic phosphate (^{32}P) provokes severe lesions in articular and growth cartilage, and bone marrow, 56 days after its injection.[6] In the same period, cold colloidal chromic phosphate produced no histologic alterations in articular and para-articular tissues. The lack of lesions in this period enables us to assume that no change will occur later due to the colloidal nature of the chromic solution.

This experiment defines quite clearly a model for tumor induction that has a high degree of reproducibility. In other experimental models with ^{32}P, osteosarcomas were induced in 15% of the cases,[20] whereas in the same period, we found a tumoral incidence of 43%.

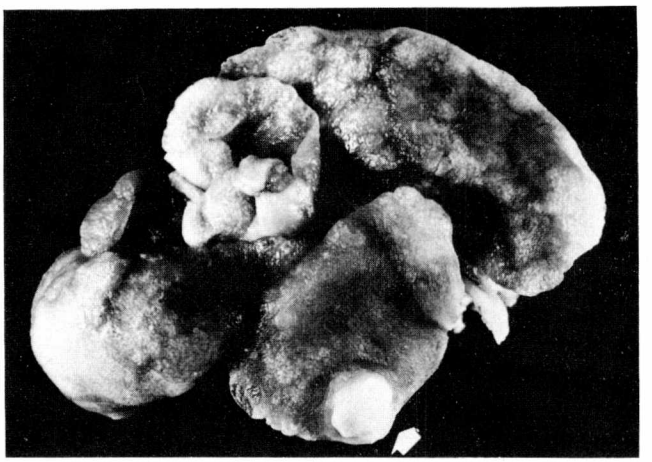
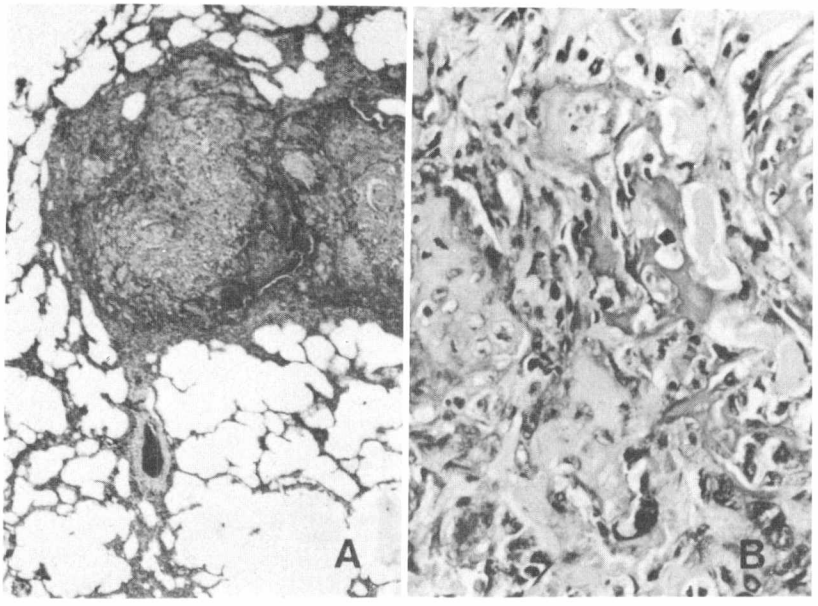


FIG. 4. Lung with metastatic nodule (arrow).



FIGS. 5A AND 5B. Metastatic nodule in lung, histologically osteogenic sarcoma (H & E: A, ×30; B, ×200).

The dose used in our experiment[6] is equivalent to that employed for the therapy of rheumatoid arthritis in the second interphalangeal joint.[16] Considering that the use of colloidal forms of β-emitter radioactive isotopes have been proposed for the treatment of chronic synovial effusions,[9,10] diffuse villonodular synovitis[11,14] and rheumatoid arthritis[12–16] it is clear that our results deserve serious attention. The therapeutic employment of radioactive isotopes must be carefully programmed, taking into account the severity of the lesion under treatment, as much as the risk entailed by the use of radioactive isotopes and the safeguard of a high security level.

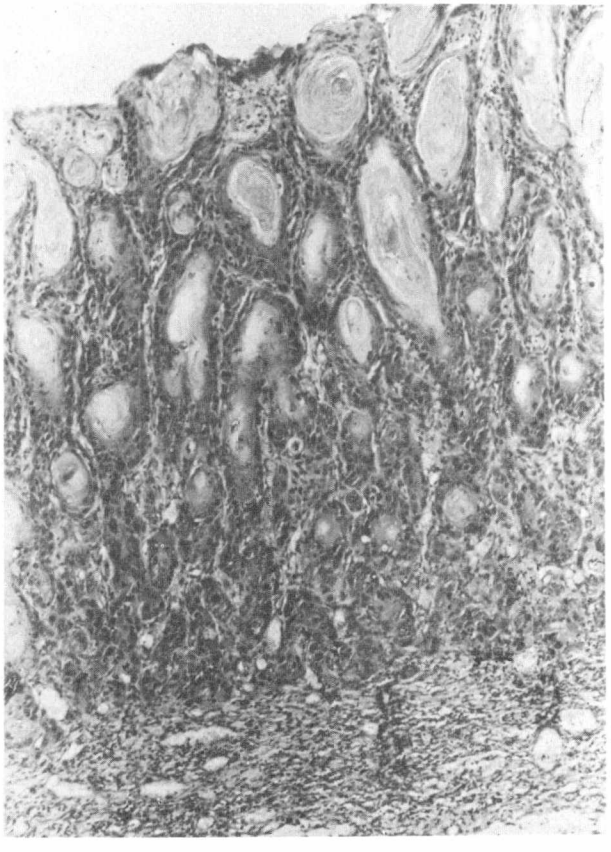


FIG. 6. Squamous cell carcinoma developed in the injected area showing abundant horn pearl formation (H & E, ×90).

REFERENCES

1. Mole RH. Ionizing radiation as a carcinogen: Practical questions and academic pursuits. *Br J Radiol* 1975; 48:157–169.

2. Bustad LK. Inferences on radiation carcinogenesis revealed by selected studies in animals. In: Yuhas JM, Tennant RW, Regan JD, eds. Biology of radiation carcinogenesis. New York: Raven Press, 1976; 13–29.

3. Feo F. Current knowledge concerning the carcinogenic effects of ionizing radiations. *Minerva Med* 1977; 68:2611–2613.

4. Hazel JJ. Radiation and the induction of malignancy. *Clin Nucl Med* 1979; 4:84–85.

5. Rosenblatt LS, Hetherington NH, Goldman M, Bustad LK. Evaluation of tumor incidence following exposure to internal emitters by application of the logistic dose–response surface. *Health Phys* 1971; 21:869–875.

6. Ubios AM, Cabrini RL, Silberman FS, Miranda FF. Radiation effects produced by the intraarticular injection of ^{32}P. *Clin Orthop* 1978; 136:299–303.

7. Ubios AM, Silberman FS, Cabrini RL. Daño por radiación en articulaciones sometidas a diferentes dosis de ^{32}P. *Acta Ortop Latinoam* 1978; 5:149–154.

8. Ubios AM, Silberman FS, Cabrini RL. Ultrastructural aspects of radiation injury by ^{3};2P in articulations (Abstr). *Eur J Cell Biol* 1980; 22:562.

9. Ahlberg A, Mikuloski P, Odolberg-Johnson O. Intraarticular injection of radioactive gold in treatment of chronic synovial effusion in the knee. *Acta Rheumatol Scand* 1969; 15:81–89.

10. Prichard HL, Bridgman JF, Blechen NM. An investigation of radioactive yttrium (^{90}Y) for the treatment of chronic knee effusions. *Br J Radiol* 1970; 43:466–470.

11. Gumpel JM. Use of yttrium-90 in persistent synovitis of the knee. *Am Rheum Dis* 1973; 32:223–227.

12. Wiston MA, Bluestone R, Cracchiolo A, Blahd WH. Radioisotope synovectomy with ;3²P-chromic phosphate: Kinetic studies. *J Nucl Med* 1973; 14:886–889.

13. Gumpel HM. Irradiation treatment of rheumatoid arthritis. *Br Med J* 1973; 3:633.

14. Koptan MT, El-Zornaky K. Intaarticular radioactive colloidal gold in the treatment of diffuse villo-nodular synovitis of the knee. *Strahlentherapie* 1974; 148:295–297.

15. Mueller W, Friedrich R, Pavelka K. Synoviortesis with yttrium 90. *Deutsch Med Wochenschr* 1974; 99:996–1000.

16. Onetti CM, Gutiérrez E, de Baretta EO, Daziano MR. Sinovectomía radioisotópica con ^{32}P-coloidal en artritis reumatoidea. I. Resultados clínicos (Abstr). *Rev Biol Med Nucl* 1975; 7:167–168.

17. Anghileri LJ, Marqués R. New colloidal chromic radiophosphate (^{3};2P) for local irradiation of the central nervous system. *Int J Appl Radiat Isotope* 1967; 18:97–100.

18. Silberman FS, Lanari EM, Miranda FF, Ubios AM, De Grossi OJ, Cabrini RL. Transformaciones patológicas relacionadas a inyección intra-articular de ^{32}P coloidal. *Rev Biol Med Nucl* 1980; 12:7–12.

19. Karnowsky MJ. A formaldehyde–glutaraldehyde of high osmolarity for use in electron microscopy. *J Cell Biol* 1965; 27:137A–138A.

20. Cobb LM. Radiation induced osteosarcoma in the rats as a model for osteosarcoma in man. *Br J Cancer* 1970; 24:294–299.

21. Furth J, Tullis JL. Carcinogenesis by radioactive substances. *Cancer Res* 1956; 16:5–21.

22. Modan B, Lillienfeld AM. Polycythemia vera and leukemia: The role of irradiation treatment: A study of 1222 patients. *Medicine* 1965; 44:305–344.

23. Modan B, Lillienfeld AM. Carcinogenic effect of ionizin-irradiation treatment in polycytaemia. *Lancet* 1964; 2:439–441.

24. Root SW, Tylor MD, Andrews GA. Distribution of colloidal radioactive chromic phosphate after intracavitary administration. *Radiology* 1954; 63:251–257.

25. Anghileri LJ. *In vivo* distribution of radioactive chromic phosphate: influence of the particle size and route of injection. *Int J Appl Radiat Isotope* 1965; 16:623–630.

26. Silberman FS, Ubios AM, Miranda F, DeGrossi O, Cabrini RL. Distribución del fosfato crómico (^{32}P-CROP) en cavidad articular. *Acta Ortop Latinoam* 1976; 3:186–190.

27. Arlen M, Higinbotham NL, Huvos AG, Marcove RC, Miller T, Shah IC. Radiation-induced sarcoma of bone. *Cancer* 1971; 28:1078–1099.

28. Solheim OP. Development of osteosarcoma in rats after irradiation. *Acta Radiol (Ther) Stockh* 1977; 16:433–446.

29. Kim JH, Chu FC, Woodard HO, Melamed MR, Huvos A, Cantini J. Radiation induced soft-tissue and bone-sarcoma. *Radiology* 1978; 129:501–508.

30. Tountas AA, Fornasier VL, Harwood AR, Leung PMK. Post-irradiation sarcoma of the bone: A perspective. *Cancer* 1979; 43:182–187.

31. Rushforth GF. Osteosarcoma of the pelvis following radiotherapy for carcinoma of the cervix. *Br J Radiol* 1974; 47:149–151.

32. Brady LW. Radiation-induced sarcomas of bone. *Skeletal Radiol* 1979; 4:72–78.

33. Boice JD. Cancer following medical irradiation. *Cancer* 1981; 47:1081–1090.

34. Schajowicz F. Bone-forming tumors. In: Schajowicz F, ed. Tumors and tumor-like lesions of bone and joints. New York: Springer-Verlag, 1981; 25–108.