The development of malignant neoplasms in rats has been observed in the course of studies on the late effects of radioactive phosphorus poisoning. Of thirteen tumors, ten were osteogenic sarcoma and three were squamous-cell carcinoma. Data pertinent to the carcinogenic action of radiophosphorus will be presented.

PROCEDURE AND RESULTS

Neoplasms were observed in two experimental groups of rats, i.e., (a) animals surviving a single LD₅₀ dose of P³² (4.5 μc. per gram of body weight), and (b) those surviving repeated injections of P³² (1.5 μc. per gram of body weight every 3 weeks) for a total dose of 9 or 12 μc. per gram. Adult white male rats of the C. F. Wistar strain, which weighed about 150 gm. each, obtained from Carworth Farms, were used. Spontaneous bone tumors have not been observed among normal animals of this strain (1). The rats were kept in an air-conditioned unit permitting constant temperature and humidity. Radioactive material was injected by the intraperitoneal route.

Complete autopsies were performed on all rats which died or were sacrificed. In almost all animals routine microscopic sections of femur and vertebra were taken. X-rays were taken of the entire skeleton of most animals, usually at the time of death or sacrifice.

For controls, x-rays were made of the skeleton of: (a) twenty normal adult animals of the C. F. Wistar strain, maintained for the same time and under the same conditions as the experimental rats, and (b) eight rats each of which had received, intraperitoneally, a dose of 1 mc. of radioactive iodine¹³¹ 1 year previously. None of these showed evidence of bone neoplasm.

GROUP 1.—In this laboratory 4.5 μc. of P³² per gram of body weight is approximately the LD₅₀ dose. Rats injected with this amount usually died within 3 weeks (20-day LD₅₀) and at autopsy showed marked aplasia of bone marrow and involution of lymphoid tissues. Many of the surviving animals were set aside for extended observation. The object was to determine, in relation to time, the extent of repair in organs severely damaged by a single dose of internal radiation: especially the hemopoietic system, the presence of irreversible tissue lesions; and, finally, any late effects of the P³². To date, study has been completed on nineteen such rats, sixteen of which were sacrificed from 6 months to 1 year later, while three died, i.e., one at 7 months and two at 9 months after administration of radiophosphorus.

Nine rats in this group developed malignant neoplasm. The animals were otherwise in good condition and had gained weight over the experimental period, except in the last few weeks of life when there was progressive anorexia and emaciation.

Rat 56.—This rat died 7 months after injection of the P³². During the last 2 weeks of life, there was progressive swelling of the right side of the face. Autopsy revealed an extensive tumor involving...
right maxillary and premaxillary bones. There was invasion and destruction of the roof of the mouth, also extension into the soft tissues of the face. Microscopically, the neoplasm was an osteogenic sarcoma. No metastasis was demonstrated.

*Rat 59.*—This rat was sacrificed 5 months after receiving an LD$_{50}$ injection of radioactive phosphorus. The anterior and right lateral aspects of the face were expanded, covered by thick, nodular, grayish-red skin, and there was marked exophthalmos of the right eye (Fig. 1). X-ray showed destruction of the right zygoma and posterior portion of the right maxillary bone, and also possible involvement of the right ethmoid and temporal bones (Fig. 2). A section revealed a massive, moderately firm, grayish-white tumor, involving and partly destroying the right nasal, ethmoid, zygomatic, and maxillary bones. The neoplasm infiltrated the soft tissues of the face widely, and involved skin, roof-of-mouth, naso-pharyngeal passage, Harderian gland, ethmoid sinus, and the right olfactory bulb of brain. Superficial and deep cervical lymph nodes were greatly enlarged, measuring up to 1 cm. in diameter. Microscopic study

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**Fig. 1 (Rat 59).**—Face of rat with squamous-cell carcinoma, probably arising from the lining of nasal sinus.

**Fig. 2 (Rat 59).**—X-ray of skull showing destruction by tumor of right zygomatic arch and maxillary bone.

**Fig. 3 (Rat 59).**—Cervical lymph node, H & E x 80. Metastatic well differentiated squamous-cell carcinoma.
revealed a well-differentiated squamous-cell carcinoma with the mouth, naso-pharynx, or epithelial lining of the ethmoid sinus as possible sites of origin. There were metastases to the right cervical lymph node chain (Fig. 3).

Rat 53.—This rat died 9 months after administration of the P\(^{32}\). Terminally, there was anorexia and weight loss. An extensive lesion, probably an osteogenic sarcoma, was found, which was evidently neoplastic and involved the right maxillary bone and roof of the mouth. The latter was necrotic, ulcerated, and largely destroyed. This animal was discarded by accident, and microscopic confirmation of the tumor could not be made.

Rat 187.—This rat was sacrificed 8 months after receiving an LD\(_{50}\) dose of P\(^{32}\). The animal was otherwise in good condition and showed no clinical evidence of tumor. X-ray at the time of sacrifice showed irregular sclerosis of the proximal diaphysis of the right tibia, with bone formation and extension into the surrounding soft tissues (Fig. 4). Microscopic study revealed an invasive osteogenic sarcoma, involving the periosteum, cortex, and cancellous part of the bone. There were several metastatic nodules in the lungs and within the lumens of pulmonary blood vessels (Fig. 5).

Rat 187.—This rat was sacrificed when in poor condition 1 year after receiving radiophosphorus in LD\(_{50}\) dose. Several weeks before death, the animal showed atrophy and paralysis of the left leg, which was inwardly rotated and fixed. X-ray of the pelvis at the time of death disclosed a large well-defined mass, arising from the wing of the left ilium. There were numerous calcified areas at the center. The ilium was partially destroyed (Fig. 6). At autopsy a hard mass, 4 cm. in diameter, was found.

Fig. 4 (Rat 187).—A-P and lateral films showing a destructive and invasive tumor of the proximal diaphysis of the right femur.

Fig. 5 (Rat 187).—Lung, H & E \(\times\) 86. Nodule of osteogenic sarcoma within lumen of pulmonary blood vessel.
observed, which was attached along the entire pelvic aspect of the left ilium and which protruded into the abdominal cavity, filling most of the entire left lower quadrant (Fig. 7). The sigmoid colon plasm was an osteogenic sarcoma. Microscopic sections revealed metastasis to the lungs.

Rat 72.—This rat was sacrificed 1 year after injection of the P32. The animal was in fairly

![X-ray of pelvis. Large tumor mass attached to left ilium](image)

was displaced to the right by the neoplasm, which extended up to the inferior pole of the left kidney, compressed the left iliac vessels and nerves, and was attached to the oblique abdominal muscles. The outer and cut surfaces were hard, bony in consistency, grayish-white or pink, nodular, and richly vascularized. Microscopically, the tumor was a typical osteogenic sarcoma, and there were multiple metastatic nodules in the lungs (Fig. 8).

Rat 188.—This rat was sacrificed 10 months after receiving radiophosphorus. For several weeks prior to death there was progressive swelling and protrusion of the anterior and lateral aspects of face. Films of the head, at the time of sacrifice, showed a soft tissue swelling of the face containing small areas of calcific density. The tumor extended to the right premaxillary bone (Fig. 9). At autopsy a large, hard, grayish-white, bony tumor was observed attached to the periosteal surface of the right nasal and premaxillary bones, compressing the right nasal cavity, widely infiltrating the soft tissues of face, and extending to the skin. The neo-

![The neoplasm of left ilium extends into the abdominal cavity.](image)
good condition, despite moderate weight loss over the last 2 months. Exophthalmos of the right eye was noted a few weeks before sacrifice. X-ray findings of the head and the rest of the skeleton were negative. Autopsy revealed an infiltrating well-differentiated squamous-cell carcinoma of the soft tissues beneath the right eye, with involvement of the Harderian gland and extension to the ethmoid sinus. The precise site of origin was not evident. No metastasis was found.

Rat 159.—This rat was sacrificed 1 year after receiving an LD₅₀ dose of P³². Condition of the animal was fair, although marked left exophthalmos had been observed about 2 weeks before sacrifice. X-ray findings of the head were negative. Autopsy showed an extensive ulcerating tumor of the roof of the mouth extending laterally to the left mandible and also invading the Harderian gland. The neoplasm was a well-differentiated squamous-cell carcinoma. There was no demonstrable metastasis.

Rat 197.—This rat was sacrificed 11 months after receiving P³². One week before death, paralysis of the right fore limb was noticed, and also weakness of the left fore limb and of both hind extremities. X-ray showed a sclerosing neoplasm of the second cervical vertebra (Fig. 10). At autopsy a hard white neoplasm was noted, 1 cm. in diameter, involving C₂, invading the spinal canal, and compressing the cord (Fig. 11). The neoplasm was an osteogenic sarcoma. No metastasis was found.

GROUP 2.—In order to observe the tolerance of rats to large, repeated doses of radiophosphorus, a preliminary experiment was carried out, using fifteen rats. The schedule called for eight successive intraperitoneal injections, each of 1.5 µc. per gram body weight of the radioactive material, at 3-week intervals. This was a total of 12 µc. per gram, almost three times the single LD₅₀ dose.

One rat was dead 10 days after injection of the first dose of radiophosphorus; one died after two doses, one after four doses, one after five doses, two after six doses, and one after seven doses.
Fig. 10 (Rat 197).—Film of spine showing sclerosing neoplasm of the second cervical vertebra.

Fig. 11 (Rat 197).—Tumor mass attached to the right side of the second cervical vertebra.

Fig. 12 (Rat 225).—Osteogenic sarcoma of jaw. H & E $\times$ 186. There is pleomorphism of tumor cells and abundant formation of bone.
Eight of the fifteen rats survived all eight doses; of these, three died at intervals of a few to several weeks after injection of the eighth dose, while the remaining five were sacrificed from 1 to 2 months after receiving the eighth dose.

Complete autopsies were performed on eleven of the fifteen rats. Four animals were either discarded accidentally after death or showed too advanced autolysis for study.

Three rats in this group developed a malignant tumor of bone which was evident in the gross, while a fourth showed a probable early osteogenic sarcoma of the femur, diagnosed microscopically. All four animals showed progressive weakness, anorexia, and weight loss over periods from 2 to 6 weeks before death or sacrifice. Prior to this terminal phase, the weights had increased or were maintained.

Rat 225.—This rat died 2 weeks after receiving the sixth successive dose of P32, i.e., 4 months after the onset of the experimental period. Total dosage was 9 µc. per gram. There was an extensive, destructive, and expanding neoplasm of the right maxillary bone involving the roof of the mouth. Time showed involvement of the proximal one-fourth of the right tibia by a destructive and invasive lesion. The latter formed a large mass with irregular calcific density (Fig. 13). Autopsy revealed

![Fig. 13 (Rat 225).—Film showing destructive neoplasm of the proximal fourth of the right tibia with invasion of soft tissues.](image)

Rat 226.—This rat was sacrificed when in poor condition at 6 months, 1 month after injection of the eighth successive dose of P32. The total amount received was 12 microcuries per gram. Films at this time showed involvement of the proximal one-fourth of the right tibia by a destructive and invasive lesion. The latter formed a large mass with irregular calcific density (Fig. 13). Autopsy revealed

![Fig. 14 (Rat 226).—Right tibia, H & E X 134. Osteogenic sarcoma with prominent intercellular bony matrix.](image)

The tumor infiltrated the adjacent soft tissues widely, forming a large, hard, grayish-white mass. Microscopic examination showed an osteogenic sarcoma (Fig. 12). No metastasis was found.

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domen (cord bladder) and complete paralysis of the hind extremities was noted. Antero-posterior and lateral x-rays showed a decrease in density of the body and neural arch of the second lumbar vertebra. The appearance suggested destruction of the vertebra by neoplasm. No accompanying soft-tissue mass was visualized (Fig. 15). At autopsy an extensive osteogenic sarcoma was observed, principally periosteal, but also involving marrow which was the seat of diffuse hemorrhage. There was invasion of the soft tissues, and also encroachment on the spinal canal with compression of the spinal cord. No metastasis was found.

*Rat 236.*—This rat was dead at 5 months, 1 week after the eighth dose of radiophosphorus was injected. There was a lesion, detected microscopically, 2 mm. in diameter, situated in the distal metaphysis of right femur just above the knee, which probably represented an early osteogenic sarcoma. This showed marked proliferation of atypical, irregular or spindle-shaped cells with many mitoses, also pleomorphic and hyperchromatic forms including bizarre giant cells and formation of spicules of atypical bone (Fig. 16).

Morphologically, all the osteogenic sarcomas were essentially similar. In the gross, the tumors varied from firm to hard and were white or grayish-white, solid, and resistant to section. The cut surface usually showed tiny, gritty nodules, evidently of bone.

Microscopically, the tumors consisted of com-

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*Fig. 15 (Rat 298).*—A-P and lateral views of spine showing destruction of the second lumbar vertebra.

*Fig. 16 (Rat 236).*—Early osteogenic sarcoma of right femur, H & E X 298. Marked proliferation of atypical cells with pleomorphic and hyperchromatic nuclei, also matrix of atypical bone.
pact groups of highly pleomorphic oval, irregular or spindle-shaped cells with hyperchromatic or vesicular nuclei and poorly defined cytoplasm. There were numerous mitoses. Giant cells were common. An abundant intercellular matrix was present, either of fibrous tissue or bone, and the latter showed an abundance of well-formed trabeculae. Occasionally osteoid or cartilage were found.

SUMMARY OF DATA ON MALIGNANT NEOPLASMS (Table 1)

There were thirteen neoplasms, all in adult, white male rats. Three were squamous-cell carcinomas involving the face and were associated with exophthalmos. Ten were osteogenic sarcomas distributed as follows: four in the jaw, two in the spine, two in the tibia, and one each in the femur and pelvis. Four of these tumors metastasized to the lungs.

Incidence.—Malignant tumors occurred in nine out of nineteen rats surviving a single dose of P32, an incidence of 47 per cent. This figure is probably low, since most of the rats without neoplasm were sacrificed or died prior to the average latent time for the development of tumor. There were four tumors among the fifteen rats which received repeated injections of radiophosphorus, an incidence of 27 per cent.

Latent period.—With a single dose of P32, the latent period varied from 210 to 360 days and averaged 290 days. With repeated doses of radiophosphorus, the neoplasms occurred from 120 to 200 days and the average was 185 days. These periods indicate detection of the neoplasm, not the exact time of origin. The data suggest that the time of tumor development may be shortened by larger total doses of the radioactive material.

Origin.—The site of origin of the osteogenic sarcomas was difficult to determine. The three tumors of the lower extremities were located in the metaphysis. Of these, two involved both cancellous portion and cortex, including endosteal and periosteal surfaces, while the third was situated mainly within marrow and also involved the endosteum. The neoplasms of ilium and spine appeared to be essentially periosteal.

There were three squamous-cell carcinomas involving the soft tissues of the face. These may have originated from the roof of mouth, nasopharynx, accessory nasal sinuses, or Harderian gland. In these structures the epithelium is closely approximated to bone and was presumably irradiated from this source.

DISCUSSION

This study shows that malignant tumors of bone can be produced experimentally in rats by radioactive phosphorus. Hence, P32 is similar in this respect to other radioactive elements such as radium (2, 3), plutonium, cerium144, yttrium91, and strontium89 (4).

In our experiments, the neoplasms occurred in animals which received very high doses of the P32. Whether the carcinogenic property is retained with smaller amounts, and in what proportion, is not known. It is quite probable that the incidence decreases with lower doses in much the same manner as for strontium89. Brues, Lisco, and Finkel (5) reported a high incidence of osteogenic sarcoma in mice following single doses of 5 and 25 μc. of this isotope per gram of body weight (LD50 at 30 days for strontium89 was 8 μc. per gram body weight) and a considerably smaller incidence with doses of 1, 0.5, and 0.25 μc. per gram. Repeated monthly injections with doses of 1 and 0.5 μc. per gram gave a high incidence for neoplasm, while 0.2 and 0.1 μc. per gram resulted in a much lower incidence.

Radioactive elements such as radium, plutonium, strontium89, and phosphorus32 are deposited...
heavily in the skeleton, producing radiation damage to bone. The changes have been described under the term "radiation osteitis" (2, 6–8) and consist essentially of degeneration and necrosis of old bone, accompanied by reactive formation of new bone which is usually atypical in arrangement and staining, and often fibrous. Frequently, the new bone also becomes devitalized. The lesions occur in epiphysis and metaphysis, also in the shaft where both endosteal and periosteal surfaces may be involved. The processes of devitalization and formation of atypical bone are similar for various types of radiation (9) (alpha, beta, and gamma ray), apparently with only quantitative differences in the extent of the bone damage which appears due to variation in character and number of the ionizing particles.

In our studies with P³², such bone changes, although varying in degree, were observed almost constantly in femur and vertebrae of rats with or without sarcoma. One lesion, situated at the distal epiphysis of the femur, was sufficiently marked to cause expansion of the bone and was associated with pathological fracture (demonstrated by x-ray). There is strongly presumptive evidence that the lesions serve as a precursor to malignancy, although the precise steps in the metamorphosis are not known (2). If this concept is correct, the neoplasm occurs as part of, or as the end result of, atypical bony growth representing attempted regeneration in regions of radiation necrosis.

The amount of radiation delivered to the skeleton of the rat by an LD₅₀ dose of 4.5 μc. of P³² per gram was calculated to be about 12,500 rps. This calculation was made by assuming the biological half-life of P³² to be 10.8 days. The average skeletal weight of a 200-gm. rat as determined in this laboratory was 18 gm.

The production of malignant neoplasms by P³² is of interest in relation to the therapeutic use of this isotope. The doses used in our study are much larger proportionately than those employed clinically. An LD₅₀ dose of 4.5 μc. per gram in the rat corresponds to 300 mc. for a 70-kg. human, i.e., about twenty times the usual therapeutic dose. Whether smaller amounts of P³² will prove carcinogenic for animals is not known at present. However, very large therapeutic doses of radioactive phosphorus—and, in fact, of all radioactive isotopes which deposit heavily in bone—would seem to be contra-indicated where long term survival is expected.

SUMMARY

One of the late effects observed in rats injected with large doses of radioactive phosphorus was the development of malignant neoplasms. Of thirteen tumors, ten were osteogenic sarcomas, situated most frequently in the jaw and also occurring in the spine, tibia, femur, or ilium. Four of the neoplasms metastasized to the lungs. There were three squamous-cell carcinomas involving the face and associated with exophthalmos.

The neoplasms occurred in animals which received (a) a single LD₅₀ dose of P³² (average latent period of 290 days) and (b) repeated doses of 1.5 μc. per gram of P³² (average latent period of 165 days). The incidence of tumor development was about 4% per cent.

Atypical proliferation of bone was usually found in these rats, and the lesions may have served as a precursor to malignant change.

REFERENCES

Production of Malignant Tumors in Rats with Radioactive Phosphorus

Simon Koletsky, Frederick J. Bonte and Hymer L. Friedell


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