OSTEOSARCOMA INDUCED BY BERYLLIUM OXIDE*

FRANK R. DUTRA, M.D., and EDWARD J. LARGENT, B.A.
(From the Kettering Laboratory of Applied Physiology, College of Medicine, University of Cincinnati, Cincinnati, Ohio)

During the last 10 years, beryllium has become an increasingly important commercial substance. It is now used in the manufacture of x-ray apparatus, fluorescent lamps, certain types of radio tubes, and fatigue-resistant copper alloys. In its natural state, beryllium is a light metal which is not radioactive.

The possibility that beryllium might be capable of inducing neoplasia was noted by Gardner and Heslington.[1] They found that suspensions of zinc beryllium silicate and of beryllium oxide, given intravenously, induced osteosarcomas in rabbits, but not in guinea-pigs or rats.

This observation was highly significant in view of the fact that only one non-radioactive metallic element (arsenic) has been proved to have carcinogenic properties.

As a part of certain studies of the metabolism and toxicology of beryllium being conducted in this laboratory, an attempt has been made to confirm and extend the work of Gardner and Heslington.[1]

METHODS

Young adult white rabbits, male and female, were used. They were kept in individual cages and were fed stock ration throughout the period of the experiment. It is believed that the experiment was adequately controlled by observations made on approximately 50 rabbits obtained from the same source and kept for similar or longer periods while being utilized for other and unrelated experiments. None of these control animals has developed malignant tumors. Moreover, osteosarcomas have not occurred spontaneously among the large numbers of rabbits observed in this laboratory during prolonged toxicologic investigations over the past 2 decades.

The two materials used consisted of (1) a highly purified beryllium oxide, and (2) a calcined phosphor comprised of beryllium oxide, zinc oxide, and silica mixed in the molar ratio of 1:1:1. Neither of these materials was radioactive, as shown by negative tests with the Geiger-Müller counter and by autoradiographs. The mean particle size of each powder was found by electron microscopy to be below 1 μ, and the powders were administered as 1 per cent suspensions in physiologic saline solution. The beryllium oxide was found to be only slightly

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soluble. One gram of beryllium oxide powder was suspended in a liter of distilled water, and 1 week after making the suspension the concentration of beryllium in the solution was 0.7 μg. per 100 cc.

An injection was made into an ear vein of each animal three times per week until the intended amount of material had been administered. The number of doses per animal ranged from 17 to 26, and the amounts of beryllium (calculated as metal) ranged from 0.013 gm. per kg. of body weight to 0.116 gm. per kg. of body weight (Table I).

TABLE I

Osteosarcomas Induced by Beryllium

Rabbit no.	Substance (1% suspension in saline solution)	Dose	Number of doses	Total amount of Be	Date of first dose	Date of last dose	Date tumor found
		cc.		*gm.*			
Be 17	Phosphor	8	21	0.09	8–14–47	10– 3–47	8–16–48
Be 24	BeO	8	23	0.66	8–14–47	10– 6–47	10–16–48
Be 26	BeO	5	20	0.36	9–15–47	11– 3–47	8–27–48
Be 29	BeO	7.5	26	0.70	9–15–47	11–15–47	9–14–48
Be 31	Phosphor	8	25	0.08	9–17–47	11–15–47	9– 2–48
Be 27	BeO	5	20	0.36	9–15–47	11–15–47	10–13–48
Be 4	Phosphor	7	17	0.064	8–27–47	10– 6–47	*
Be 23	BeO	6–7	21	0.50	8–14–47	10– 3–47	†
Be 28	BeO	7.5	24	0.58	9–15–47	11–15–47	†

* No tumors found; observations being continued.

RESULTS

After the animals had been given their final injection, they were examined periodically by palpation of the skeletal structures for the presence of tumors. Osteosarcomas developed in 6 of the 9 animals that lived for 1 year or more after the first injection. The first tumor noted appeared 11½ months after the start of the experiment.

Post-mortem examination was required to reveal the tumors in 3 of the animals, even though progressive loss of weight in the weeks immediately prior to death indicated that tumors might be present. In one of these, the location of the primary tumor in the vertebral column was suspected prior to death when the animal developed signs which were consistent with compression of the spinal cord by tumor (paralysis of the hind limbs and loss of control of anal sphincter). In the other animals, the neoplastic growth was discovered by palpation.

† Since this paper was submitted for publication, these 2 rabbits have died of osteogenic sarcoma with pulmonary metastases.

In 4 rabbits, single primary tumors were found. These tumors were located in the right scapula, the head of the right humerus, the body of the last lumbar vertebra, and the lower end of the right femur. Metastatic tumors were found in the lungs of all 4 of these animals, and in addition there were metastatic nodules in the parietal pericardium, the parietal pleura, and the liver of one of these animals.

There were multiple primary tumors in each of the other 2 rabbits. One of them had an osteogenic sarcoma of the upper end of the right tibia, and a similar primary tumor in the head of the right humerus. In this animal there were masses of metastatic tumor in the lungs and the liver. The second animal had five primary centers of neoplastic growth, and there were metastases in the lungs. The primary tumors were in the lower end of the right humerus, the upper end of the left humerus, the body of one of the anterior lumbar vertebrae, the upper end of the left tibia, and the upper end of the right tibia.

Some primary tumors had broken through the cortical bones and were invading the adjacent muscles (Figs. 1 and 2). These invading tumors were surrounded by pseudo-capsules of fibrous tissue in which degenerating muscle fibers were frequently visible microscopically. The largest primary tumor, arising in the right scapula, was 11 by 9.4 by 6.2 cm. (Fig. 3). In some of the animals the primary tumors were found only after complete dissection of the skeleton with splitting of all bones, and in several instances the tumors had not extended through the cortical bone. In all cases, the fact that tumor was present was confirmed microscopically.

The cut surfaces of the larger tumors were mottled with small yellowish orange necrotic areas and in some places there were foci of recent or partially decolorized hemorrhage. In the 2 cases in which primary tumor was found in the bodies of vertebrae, the tumor had extended through the cortex of the vertebral body and was pressing against, but not invading, the spinal dura mater and the spinal cord.

The metastatic tumors in the lungs consisted grossly of subpleural and intraparenchymal nodules, ranging up to 1.2 cm. in diameter. The larger subpleural nodules tended to be slightly umbilicated. Nearly all of the nodules were firm, and spicules of bone were encountered by the sectioning knife. Some lungs contained only a few small nodules, while in other animals as much as one-half of the total volume of lung tissue was occupied by tumor nodules (Fig. 4). Metastatic nodules in the parietal pleurae, pericardium, and liver were like those in the lungs.

There was focal fibrosis of bone marrow in the ends of some of the long bones in all animals. In one animal the head of the right humerus was occupied by a tumor which was contiguous with dense fibrous

tissue in the neck of the bone, while the body of the humerus was filled with red marrow. In all of the animals, the red marrow contained numerous white flecks which, microscopically, were found to be collections of foreign material (beryllium oxide or phosphor dust) in phagocytic cells. Similar white flecks were seen also in the spleens of the animals, and microscopically the material was found to be within phagocytic cells. The spleen of one animal was grossly fibrotic, and increased fibrous tissue was found in the others by microscopy. In one animal the liver was grossly normal, but in the others there was extensive diffuse fibrosis and cirrhosis, and large areas of liver tissue in some of the animals were completely replaced by dense fibrous tissue.

The microscopic structure of all of the tumors was that of typical osteosarcoma. Several types of tissue could be identified in each tumor (Figs. 5 and 6). There were regions of large, poorly differentiated cells (Fig. 8), and there were other regions where the tissue was made up of atypical, partially differentiated fibroblasts (Fig. 7). Still other areas were comprised of atypical cartilage or of osseous tissue (Figs. 5 and 6).

Large vascular spaces were numerous in the tumors. The endothelial lining was incomplete in some of these spaces so that malignant tumor cells lined the vessels in such regions (Fig. 10). Masses of tumor cells could be seen proliferating within the lumina of vessels in some of the primary tumors (Fig. 9), and in all animals neoplastic emboli were present in the pulmonary arteries (Fig. 11).

None of the tumors contained visible particles of the foreign materials which had been injected, and the quantities of beryllium recovered from the tumors by analysis were small. In an effort to determine if this tumor were capable of existing in a beryllium-free environment, bits of tumor from one rabbit were transplanted to one of the anterior chambers of several guinea-pigs, in the manner described by Greene.[2] It was found that the tumor continued to grow in the eyes of these animals.

Tissues of the animals which developed tumors were analyzed for beryllium (Table II). The liver and spleen of each of the animals contained relatively large quantities of beryllium, presumably as a consequence of the functional activity of the reticulo-endothelial cells in these organs. The quantities found in the lungs may represent aggregates of particles which were filtered out by the capillary bed of the lungs during and immediately after the intravenous injections.

Entire long bones which were not involved by tumor were analyzed. Microscopic studies of such bones have revealed that most of the beryl-

lium is probably contained in the reticulo-endothelial cells of the marrow. The quantities of particulate material visible in the littoral cells of the marrow were comparable to those seen in the sections of liver and spleen, and presumably, if the marrow had been analyzed alone

TABLE II

Beryllium in Tumors and Other Tissues
Milligrams of Beryllium per 100 Grams of Tissue

Rabbit no.	Primary tumor	Bone without tumor	Lung (including metastatic tumor)	Liver	Spleen	Kidney	Heart	Urine
Be 17	0.0007	0.305	21.8	9.1	64	0.50	0.793	
Be 24	*	4.90	39.5	225		0.41		0.0054
Be 26	0.093	0.308	133	184	235	4.92	1.64	
Be 29	*	0.146	22.2	5.9	75	1.08	0.722	
Be 31	0.035	0.213		8.7	136	0.81		0.004
Be 27	0.038	0.430	43.7	36.3	320	1.99	3.43	0.114

* Tumor too small for analysis.

without cortical bone, the concentrations of beryllium found therein would have been of the order of magnitude found in the spleens and livers. The kidneys and hearts of these animals contained relatively little beryllium, probably because there are few reticulo-endothelial cells in these organs. The amounts found in the primary tumors were minute.

DISCUSSION

The ability of simple beryllium compounds (beryllium oxide, and a calcined mixture of beryllium oxide, zinc oxide, and silica) to stimulate neoplastic growth has been confirmed. The manner in which beryllium is capable of altering normal cells so that they become malignant is unknown. It is recognized that beryllium is capable of inducing proliferation of fibrous stroma in the tissues of man[3] and experimental animals,[4] and it is conceivable that some fundamentally similar process is responsible for the induction of neoplastic proliferation. In the animals which developed malignant tumors, there was, in addition, fibrosis in the livers and spleens, as well as some foci of fibrosis in the bone marrow. In the regions of fibrosis, collections of the irritant dust could be seen in the littoral cells.

That these osteogenic sarcomas could become independent of beryllium after they had begun their development was suggested when only minute amounts of beryllium were found by analyses of them. Further evidence on this score was provided by the successful transplantation

of tumor fragments to the anterior chambers of the eyes of guinea-pigs in which they continued to grow.

At present, there is no reason to believe that the fibrosis which occurs in human berylliosis is likely to lead to the development of tumors. There is no evidence indicating that any tumor has been induced by beryllium compounds except after their intravenous administration to rabbits.

SUMMARY

Malignant bone tumors (osteogenic sarcomas) have developed in 6 of 9 rabbits within the period of 16 months after the beginning of serial intravenous injections of beryllium oxide or of a phosphor containing beryllium oxide. The injections were given three times a week and were continued for from 6 to 8 weeks.

The primary tumors, when subjected to chemical analysis, have been found to contain little beryllium. Tumor fragments from one animal were capable of growth in the anterior chamber of the eyes of guinea-pigs. Thus, the continued growth of the osteogenic sarcomas, once established, appears to be independent of the presence of beryllium.

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DESCRIPTION OF PLATES

PLATE 32

FIG. 1. Rabbit 31. Roentgenogram. Primary osteosarcoma of tibia induced by phosphor containing beryllium oxide.

FIG. 2. Rabbit 26. Primary osteosarcoma of lower end of femur. There is invasion of the skeletal muscle, and hemorrhagic and necrotic areas may be noted in the tumor.

FIG. 3. Rabbit 17. Primary osteosarcoma arising in scapula, with invasion of adjacent muscles. Necrotic areas and hemorrhages are extensive. The glenoid fossa is at the upper right.

FIG. 4. Rabbit 17. Metastatic osteosarcoma of lungs.

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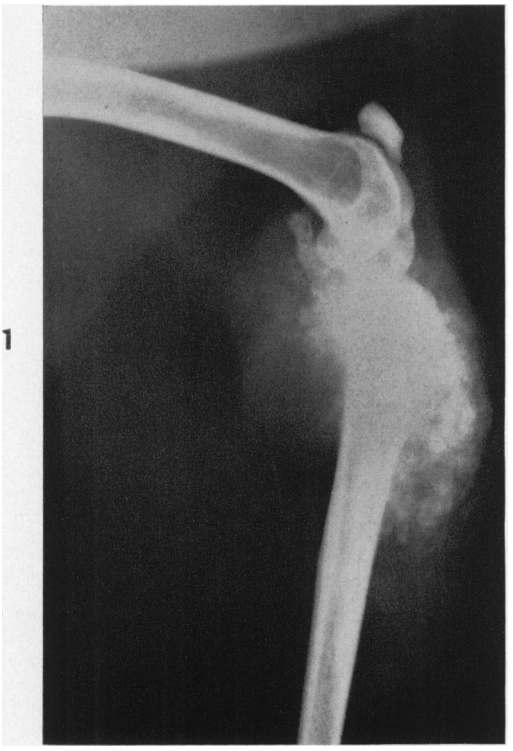

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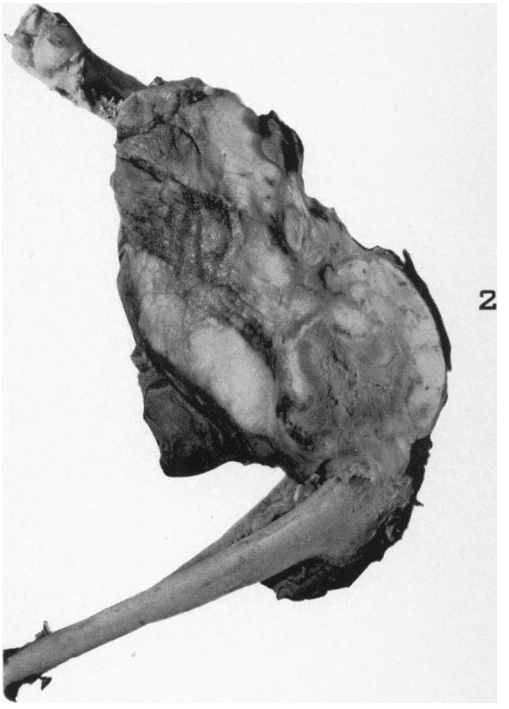

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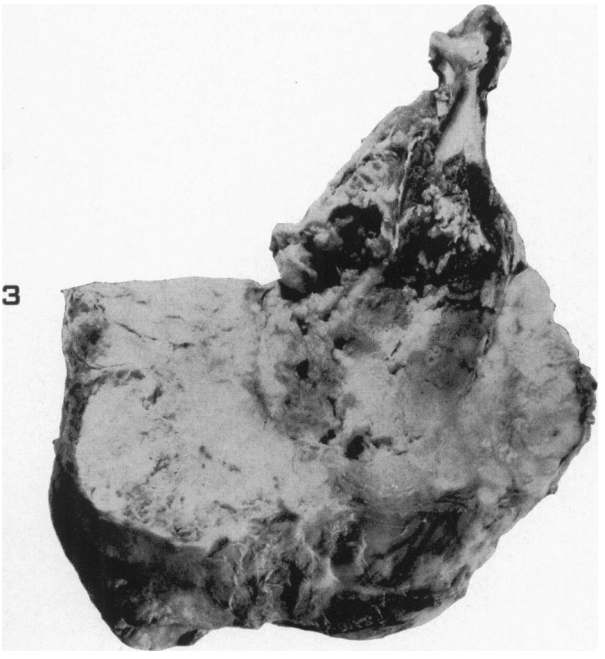

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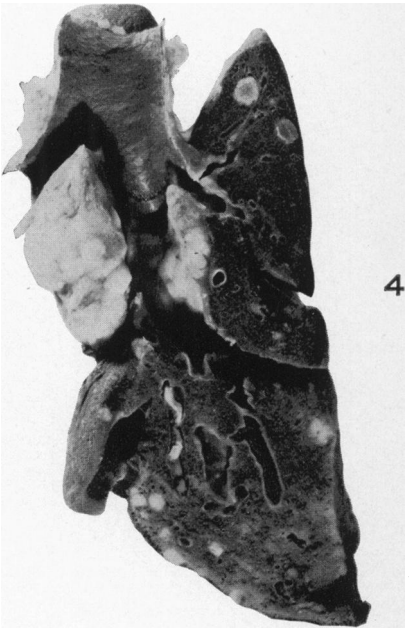

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PLATE 33

FIG. 5. Rabbit 31. Tissue taken for biopsy of a primary osteosarcoma of the tibia, showing heterogeneous nature of the structure of the tumor. Abnormal cartilage, with focal calcification, and poorly differentiated tumor cells are seen. A fibrous pseudo-capsule in which there is a degenerating skeletal muscle fiber is included in the lower right corner. × 160.

FIG. 6. Rabbit 31. Another region in the excised tissue used for Figure 5, in which fragments of osseous tissue are numerous. × 160.

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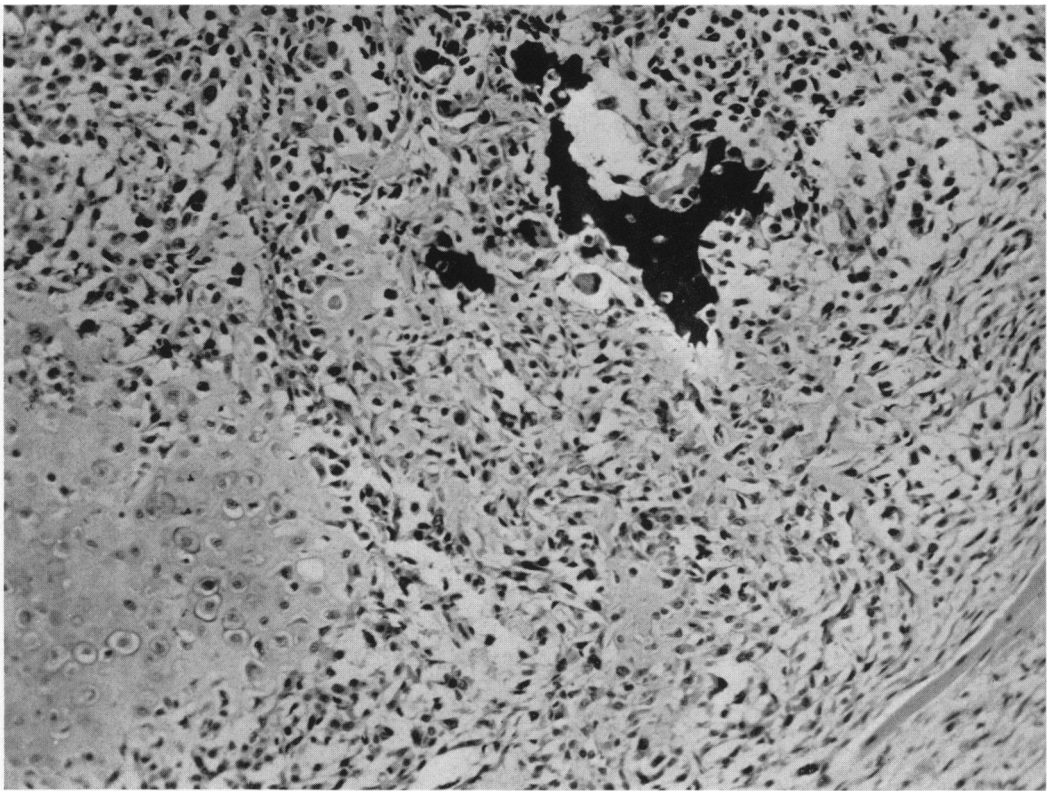

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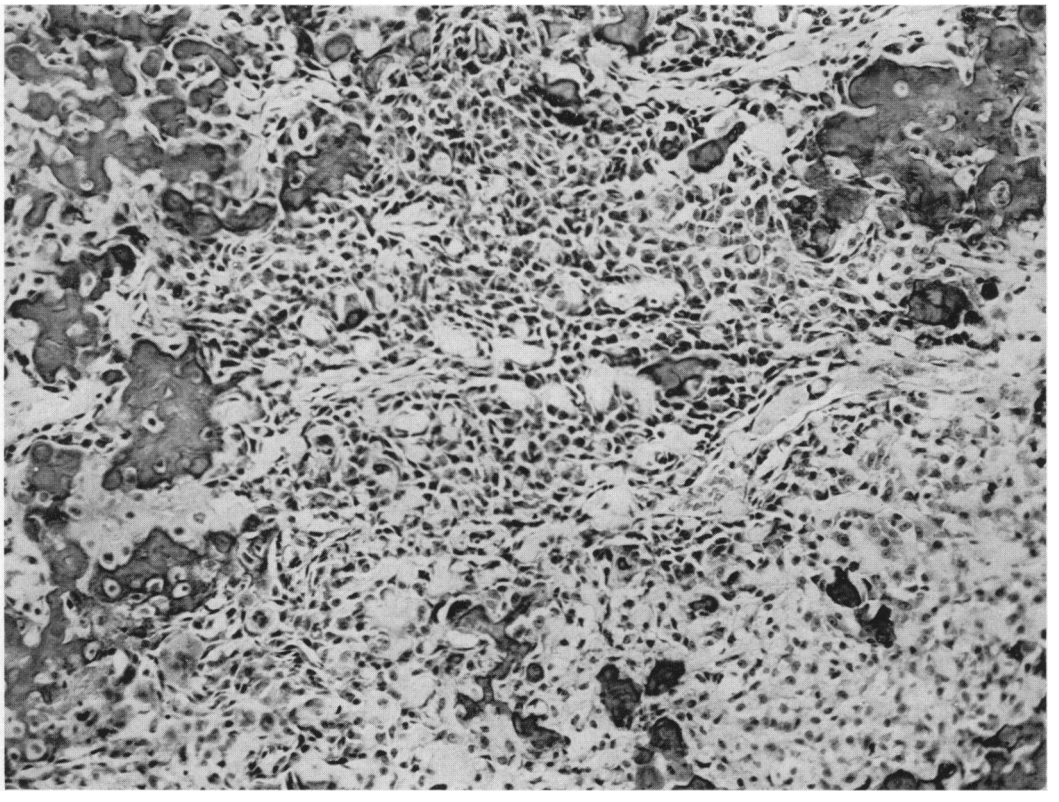
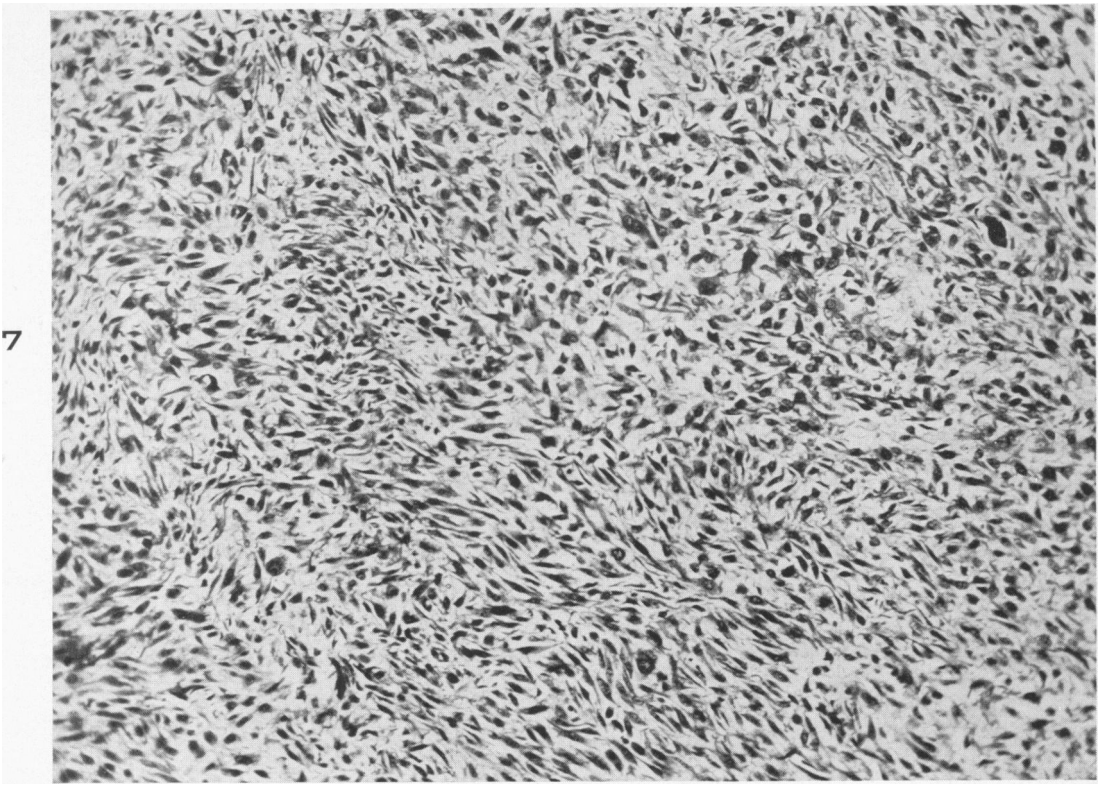


PLATE 34

FIG. 7. Rabbit 29. Region of poorly differentiated spindle and polygonal cells, some multinucleated, from a primary osteosarcoma. × 160.

FIG. 8. Rabbit 29. Another area of poorly differentiated tumor, in which there are numerous giant cells. × 160.



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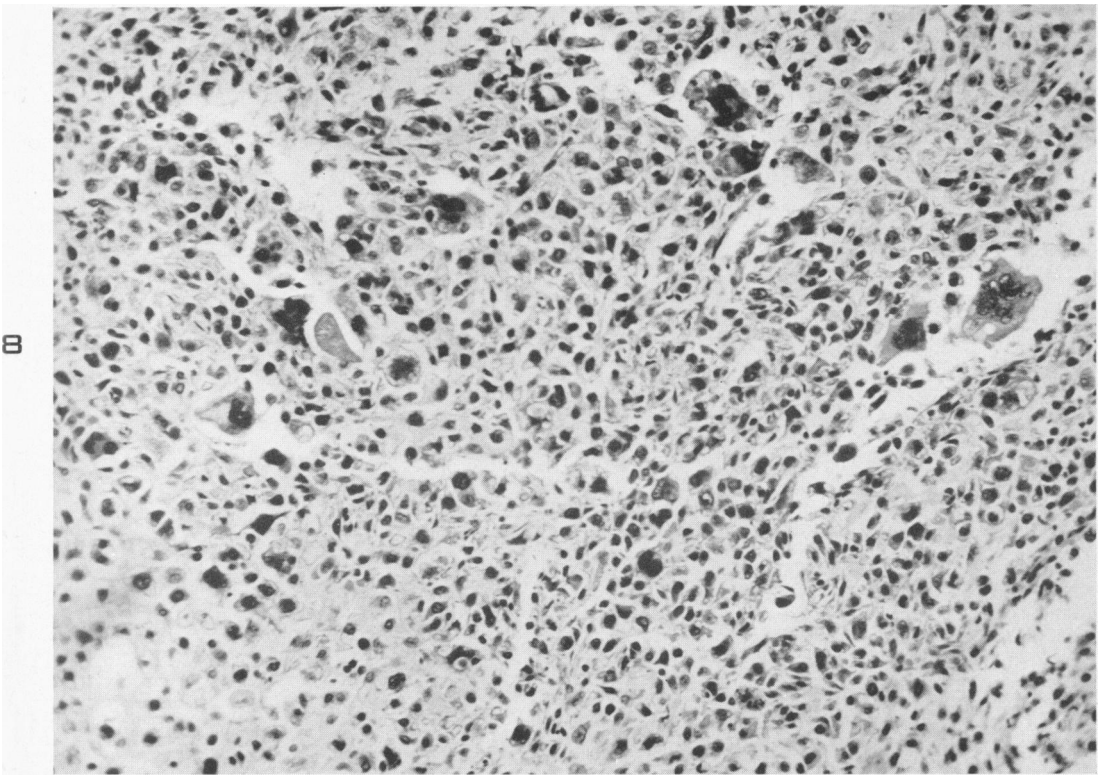

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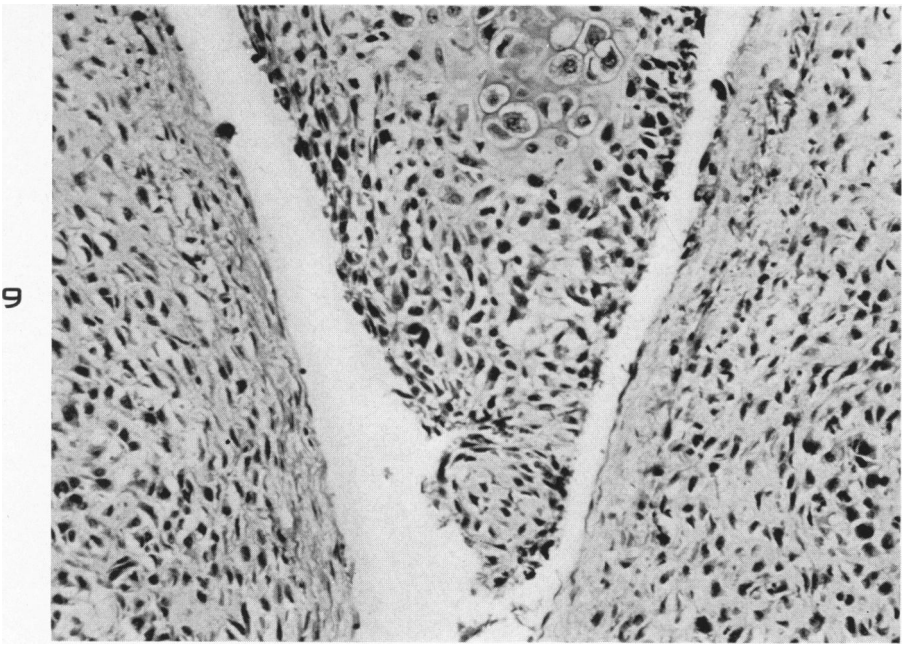
PLATE 35

FIG. 9. Rabbit 26. A large vein surrounded by tumor cells in a primary osteosarcoma in lower end of femur. In the lumen of the vessel is a mass of viable neoplastic tissue, an example of tumor spreading within vascular channels, and a ready source of embolic metastasis. × 160.

FIG. 10. Rabbit 26. Two large vascular channels in a primary osteosarcoma of the femur. The endothelial lining of each is fragmentary and tumor cells project into the lumina. × 150.

FIG. 11. Rabbit 26. A mass of metastatic osteosarcoma lodged in a pulmonary artery. The tumor is comprised of abnormal cartilage in which there is calcification. Invasion of the arterial wall is visible at the margin of the embolus toward the right. × 150.



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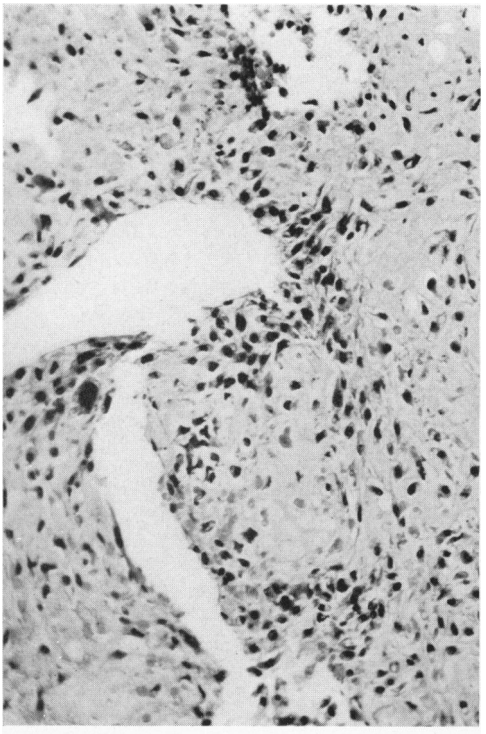

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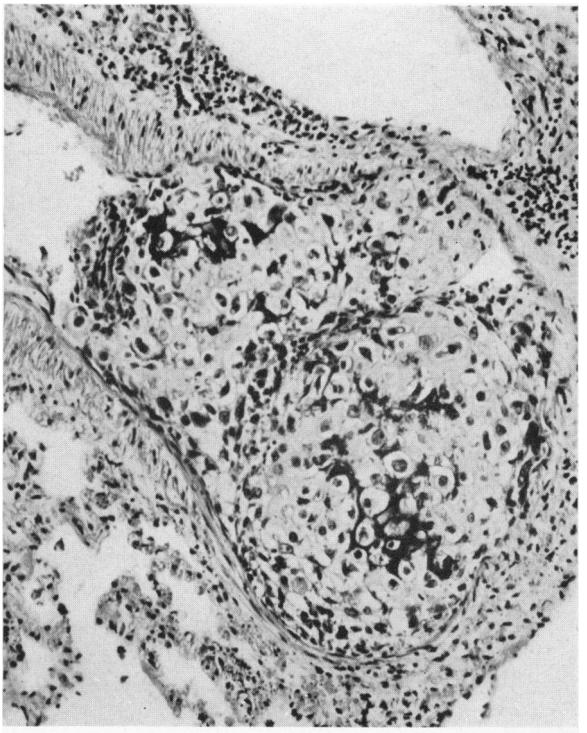

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