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BIOLOGY AND MEDICINE QUARTERLY REPORT

January, February, March 1957

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BIOLOGICAL STUDIES OF RADIOACTIVITY AND IRRADIATION

Crocker Laboratory
University of California
Berkeley, California

BIOLOGICAL EFFECTS OF INTERNALLY DEPOSITED RADIOISOTOPES

Patricia W. Durbin and C. Willet Asling in charge

STUDIES OF AN OSTEOGENIC SARCOMA TRANSPLANTABLE IN RATS. II. MORPHOLOGY[1]

Muriel E. Johnston, Nylan Jeung, Marilyn H. Williams, Marshall W. Parrott, George Barr, and Ann Henderson

The growth characteristics and gross morphology of the osteogenic sarcoma maintained in this laboratory were reported in the previous quarter.[2]

Methods

Several specimens of the osteogenic sarcoma were fixed in 80% alcohol, some were decalcified, and all were embedded in nitrocellulose. Sections were cut at 8 to 10 micra, and stained by use of hematoxylin and eosin, Mallory's triple stain, iron hematoxylin and aniline blue, Masson's trichrome stain, Von Kossa (calcium), periodic acid-Schiff (mucopolysaccharide), and toluidine blue (metachromasia).

Results

The undecalcified specimens embedded in nitrocellulose cut at a thickness of 8 μ with a minimum of difficulty. Subsequently it has been found possible to cut the undecalcified tumor, even at 4 μ, in hard paraffin (melting point: 56°–58°C).

The general structure of the tumor was revealed with H and E, Masson's, and IHAB, and best with Mallory's triple stain. In the low-power view shown in Fig. 1 the wide variety of tissue, and the bizarre pattern, are immediately evident. The capsule was a thin layer of dense collagenous fibrous tissue. Immediately exterior to the capsule was loose areolar tissue, and there was striated muscle in some places.

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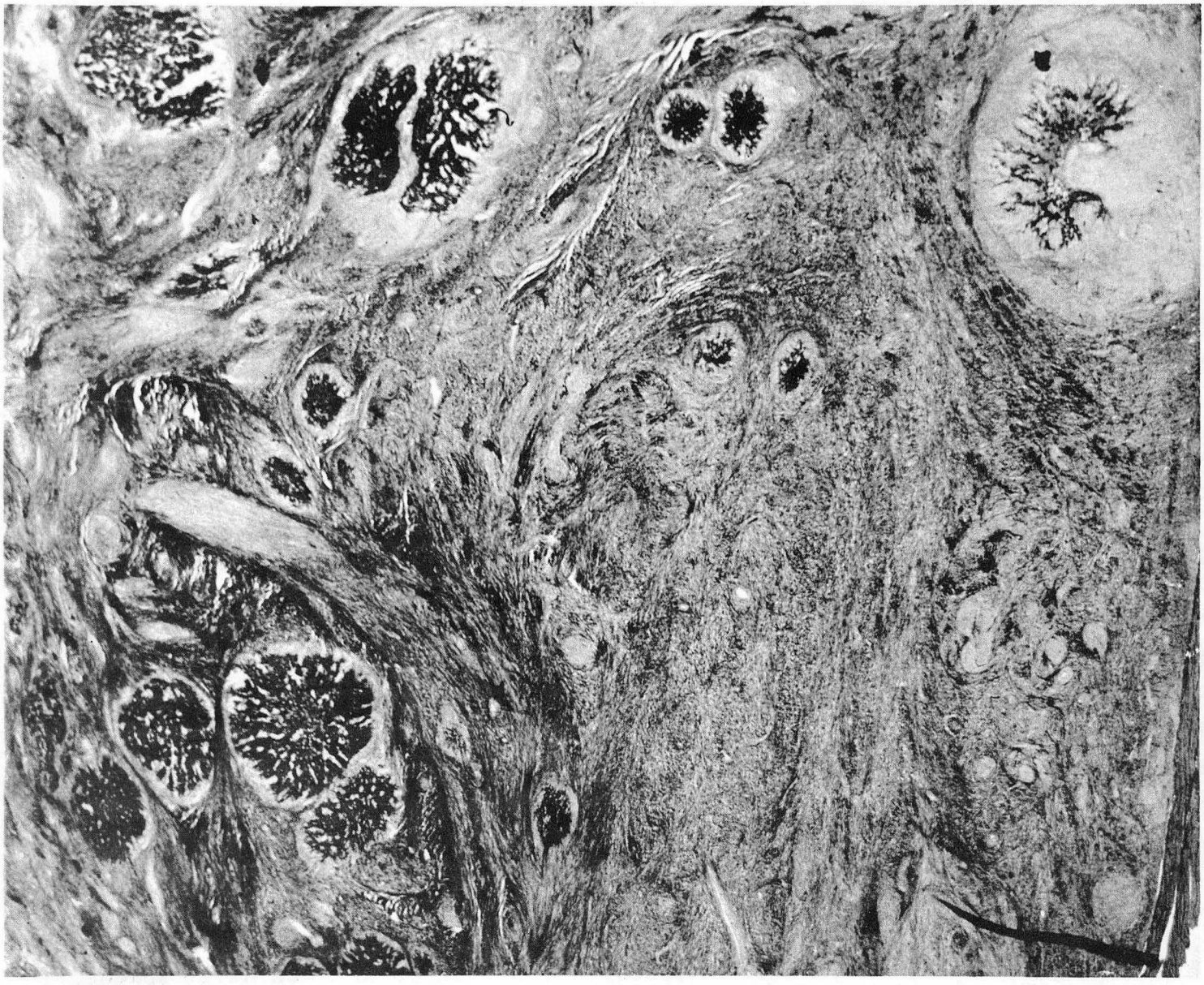
[1] With the assistance of Eugene A. Hessel. A portion of this material was submitted as a special student problem in Anatomy 101.

[2] Medical and Health Physics Quarterly Progress Report, UCRL-3653 Jan. 1957, p. 3.



ZN-1698

Fig. 1. Osteogenic sarcoma, showing numerous nodules with bony spicules (dark), circumscribed by highly variable collagenous fibrous tissue. Undecalcified, 10 μ, Mallory's triple stain. x 13.5

The substance of the tumor itself consisted of two major components (visible to the naked eye in the gross): the bony nodules, and a connective tissue stroma, exclusively collagenous. The nodules, as shown in Fig. 2, varied from 0.1 mm to 1.5 mm in diameter, and consisted of a bony matrix surrounded by a cellular zone. In many cases the matrix appeared to be continuous with the connective tissue of the outlying regions. In some instances only very thin strips of calcified tissue radiated outward from the heavier central portions. In the nodules osteocytelike cells were embedded in the bony matrix. They were smaller than the cells in the periphery of the nodules, and were irregular in shape. Sometimes a single lacuna contained more than one cell; this is not true of normal bone. Large cells surrounding the bony spicules (osteoblasts) penetrated the mass of the nodules and separated the radiating strands of bony matrix. Their cytoplasm was usually basophilic, granular, and vacuolated. The nuclei were large, eccentrically placed, and vesicular, and they possessed a nucleolus. These cells appeared to be a transitional form between the "osteocytes" in the spicules and the fibroblasts of the stroma. Only rarely were osteoclastlike cells seen. In a few instances the matrix of the spicules seemed to be cartilaginous and in close association with the calcified bone; as is illustrated in Fig. 3, no sharp distinction could be made between the two.

The fibrous stroma was extremely variable, and in Fig. 4 two distinct types can be seen in a single high-power field. In some areas the connective tissue fibers were directionally oriented, while in others they were completely disorganized. The stroma was highly cellular, invariably more so than ordinary connective tissue. The connective-tissue cells were also variable, both in size and in shape. The staining reaction of the cytoplasm ranged from light to dark. The nuclei varied in size and shape; some were rounded, some oblong, and others entirely irregular. Some of the connective-tissue-cell nuclei stained heavily, others stained lightly and were vesicular. The fibers of the stroma showed as much variability as the cytoplasm and nuclei of the cells. In some fields the fibers were collected in heavy, deeply staining bundles, either wavy or straight, and in other fields the fibers were lightly stained and thin.

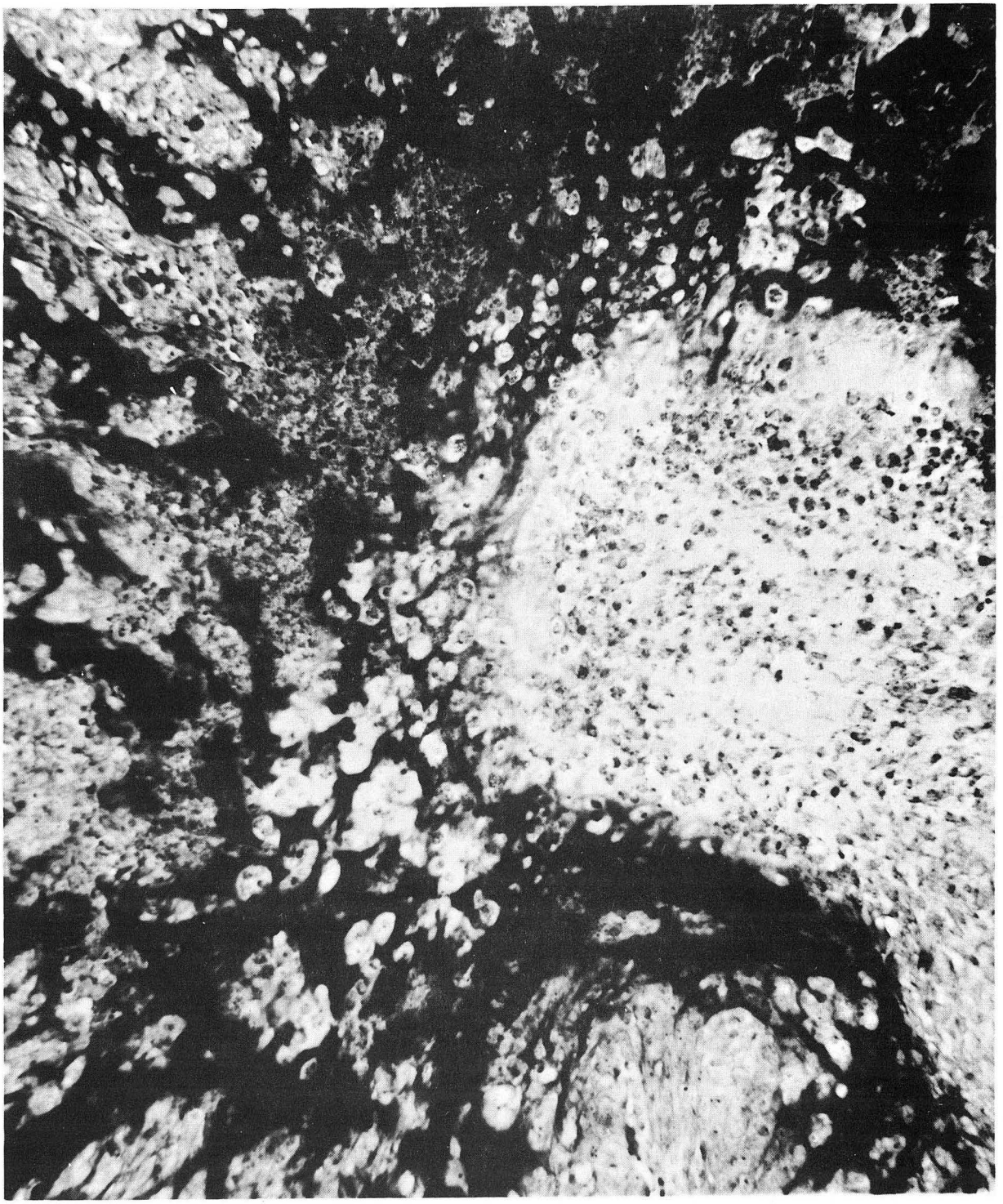
Different specimens of the tumor varied in the proportion between nodular bony masses and a more diffuse osseo-fibrous stroma. Fields were seen that contained almost exclusively osteoblastlike cells without any bony component. Mitoses, contrary to expectations in a rapidly growing tumor like this one, were rather rare. Those that were seen were in the osteoblast-like cells surrounding the nodules.

On the whole the tumors were richly vascular. The larger vessels had thin walls. Some vessels appeared to be only blood-filled tissue spaces. No thick-walled vessels were seen. Frequently vessels, or rather blood sinuses, could be found at the border of a nodule, sending small branches into the center of the nodule. The capsule of the tumor was richly vascular.

The Von Kossa technique revealed the presence of calcium salt, as shown in Fig. 5. Usually the center of the matrix of the nodules was calcified. Some of the smaller nodules showed no evidence of calcification at all. In the larger nodules calcification more commonly reached the periphery of the nodule.

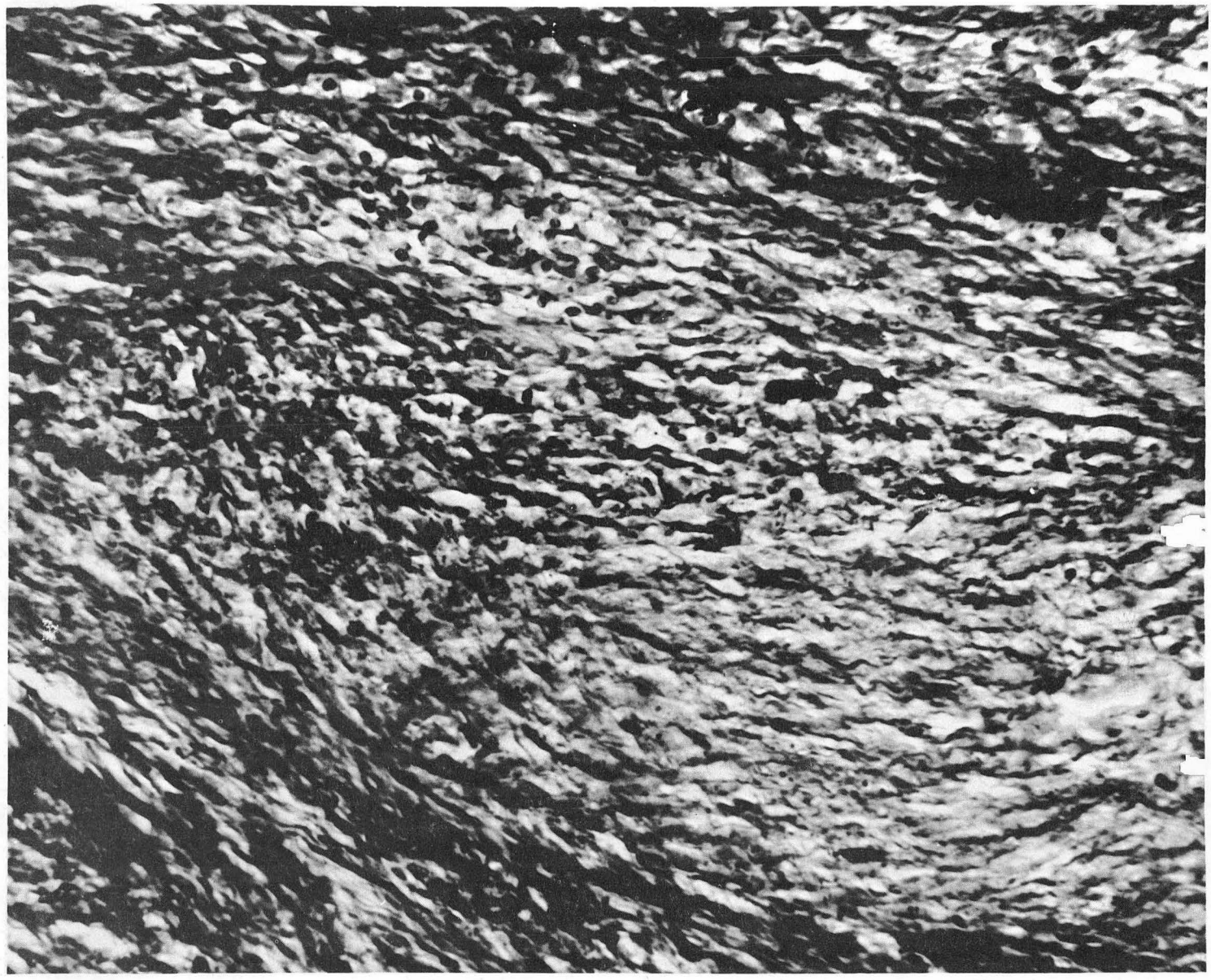
ZN-1699

Fig. 2. The edge of the nodule shown at the lower left of Fig. 1, showing the continuity of the osteoid fibers with those of the stroma, and of the "osteoblasts" with the fibroblasts. Center of nodule is in the lower right corner. Undecalcified, 10 μ, Mallory's triple stain. x 280



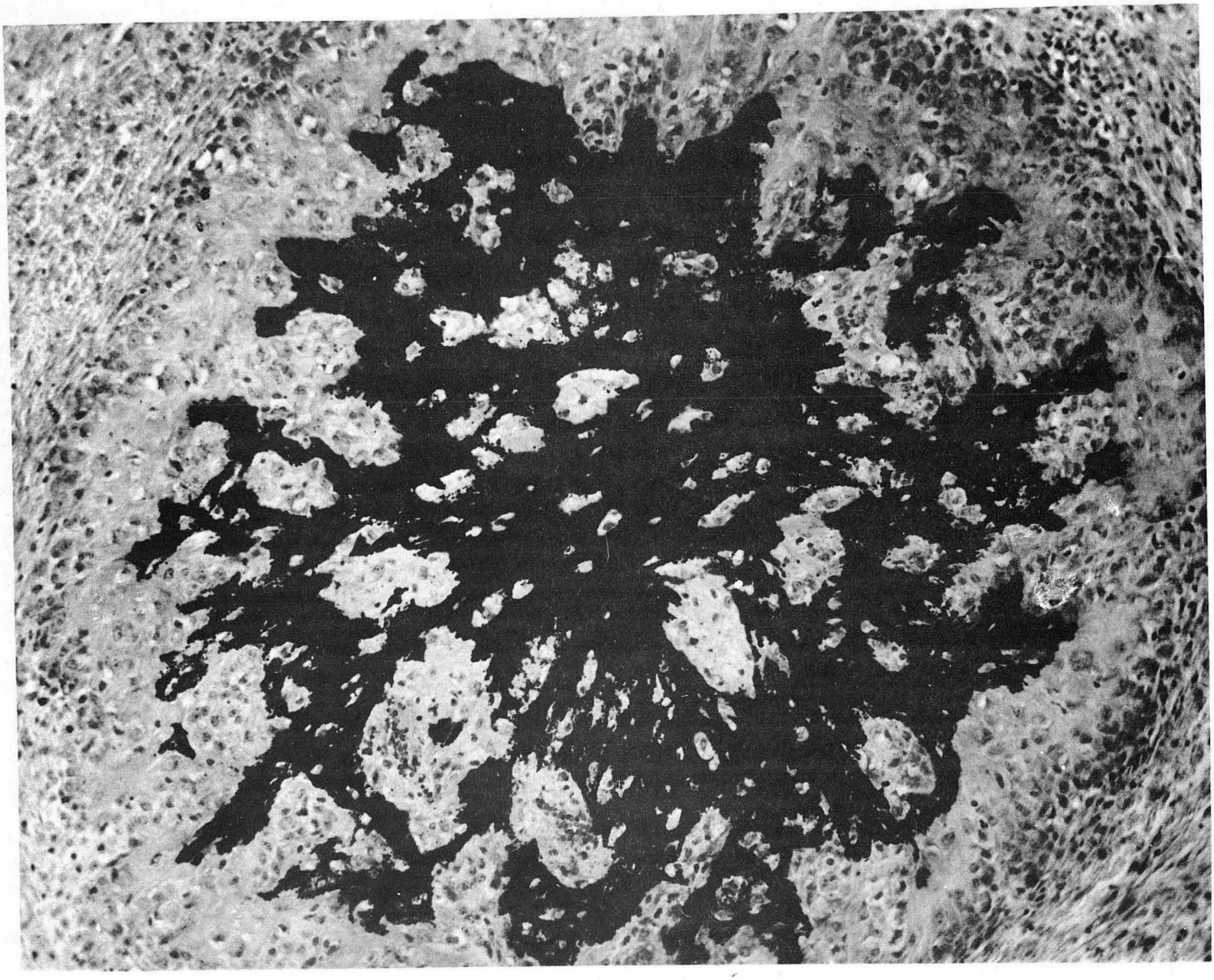
ZN-1700

Fig. 3. The center of the nodule shown at upper right in Fig. 1, showing the central cartilaginous portion continuous with the more peripheral bony matrix. Note fibrous core. Undecalcified, 10 μ, Mallory's triple stain. x 170



ZN-1701

Fig. 4. The fibrous stroma shown in lower right quadrant of Fig. 1, showing some very heavy fibers (lower left) and some thin fibers (lower right). Undecalcified, 10 μ, Mallory's triple stain. x 280



ZN-1702

Fig. 5. Bony nodule comparable to those shown in Fig. 1, stained by the Von Kossa technique to visualize calcification. Note that much of the peripheral, and some of the central, matrix is not calcified. Undecalcified, 8 μ, counterstained with H and E. x 170

The sections stained with periodic acid-Schiff showed PAS positive (presence of mucopolysaccharides) both in the connective tissue stroma and in the nodules, as can be seen in Fig. 6. In the nodules the PAS-positive material was restricted to the matrix, and extended beyond the limits of calcification. In general, the PAS stain was darker in the smaller, more centrally located nodules, and at the edges of the large nodules. Much of the fibrous tissue was also PAS-positive.

Metachromasia, as shown by toluidine blue (Fig. 7), corresponded closely in its distribution to the PAS-positive reaction.

Discussion

From the histological examination of this tumor it is apparent that the bone in the tumor is immature and of abnormal morphology. The inclusion of more than one cell in a single lacuna is not normal, and, according to Greep, a strongly positive PAS reaction is characteristic of immature bone.[3] The bone in this tumor is not like normal lamellar bone; it more nearly resembles immature trabecular bone characteristic of early intramembranous osteogenesis. Since much of the area of the spicules was not calcified, it can be considered to be osteoid. It was this latter portion of the spicules that gave a PAS-positive reaction, stained metachromatically, and did not show evidence of calcification (the Von Kossa test was negative).

Both the PAS stain and the metachromatic stain give positive reactions in the presence of mucopolysaccharides, and are generally found in connective tissue, cartilage, and young bone.[4] In connective tissue the PAS stains usually wash out because the reaction takes place in an aqueous phase.[3] Why the connective tissue of this tumor gave a PAS-positive reaction and yet did not show metachromasia is not fully understood. The PAS method does have a wider range of reaction, and might indicate the presence of glycoprotein or mucoprotein (polysaccharides in these sections were digested with saliva prior to staining). On the other hand this is not normal connective tissue, and some of the polysaccharides may have been polymerized sufficiently to be relatively insoluble in the water phase.

This tumor is believed to be malignant, and therefore has been classified as a sarcoma. Metastases were not observed in this study when the tumor developed from a subcutaneous transplant. In a preliminary study, however, it had been seen to disseminate widely in separate foci throughout the peritoneal cavity when introduced intraperitoneally.

As mentioned earlier, we have used the term "osteogenic" as meaning "giving rise to bone" rather than in the sense of "arising from a bone." The tumor is provisionally being called a "Fibro-chondro-osteo-sarcoma."

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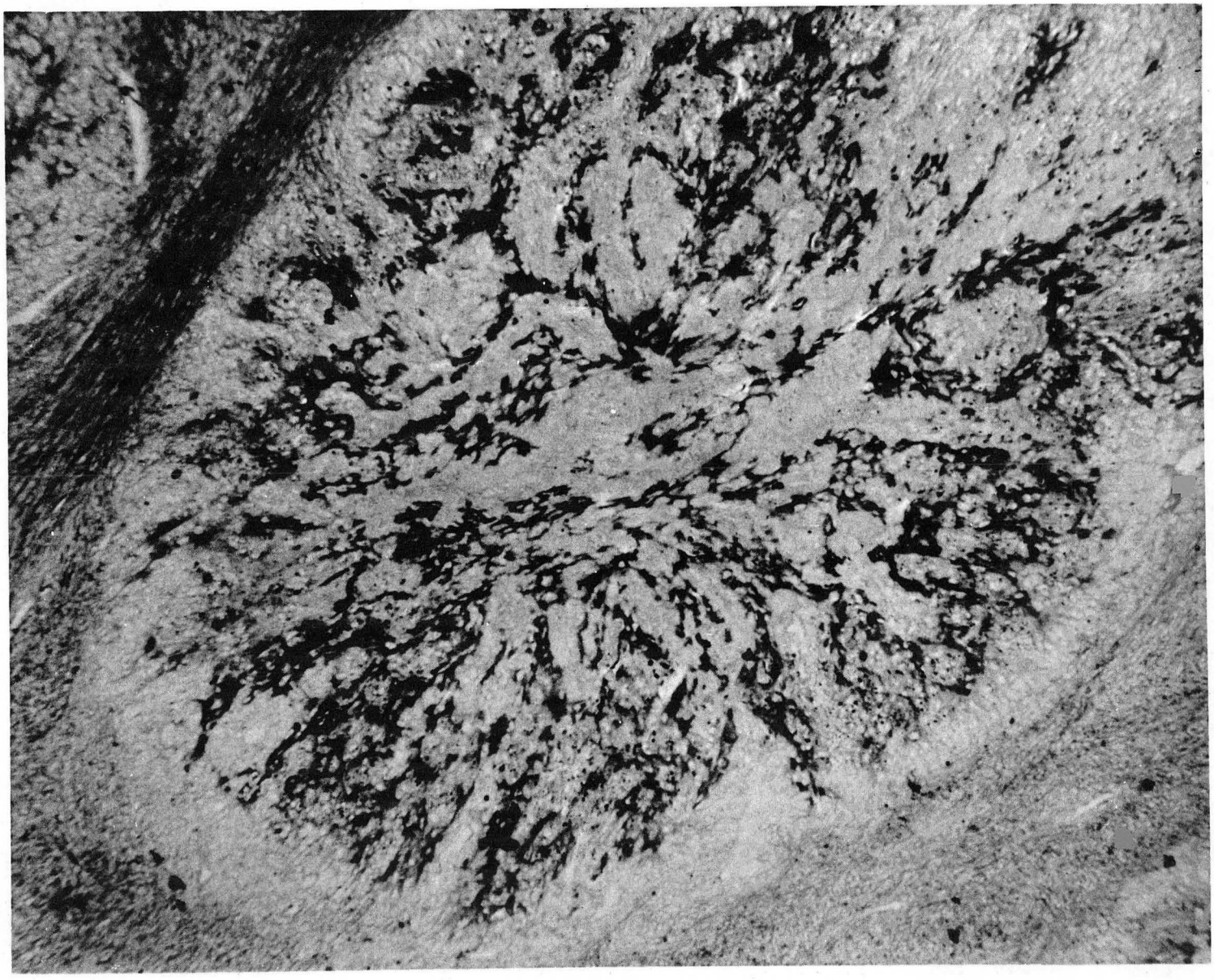
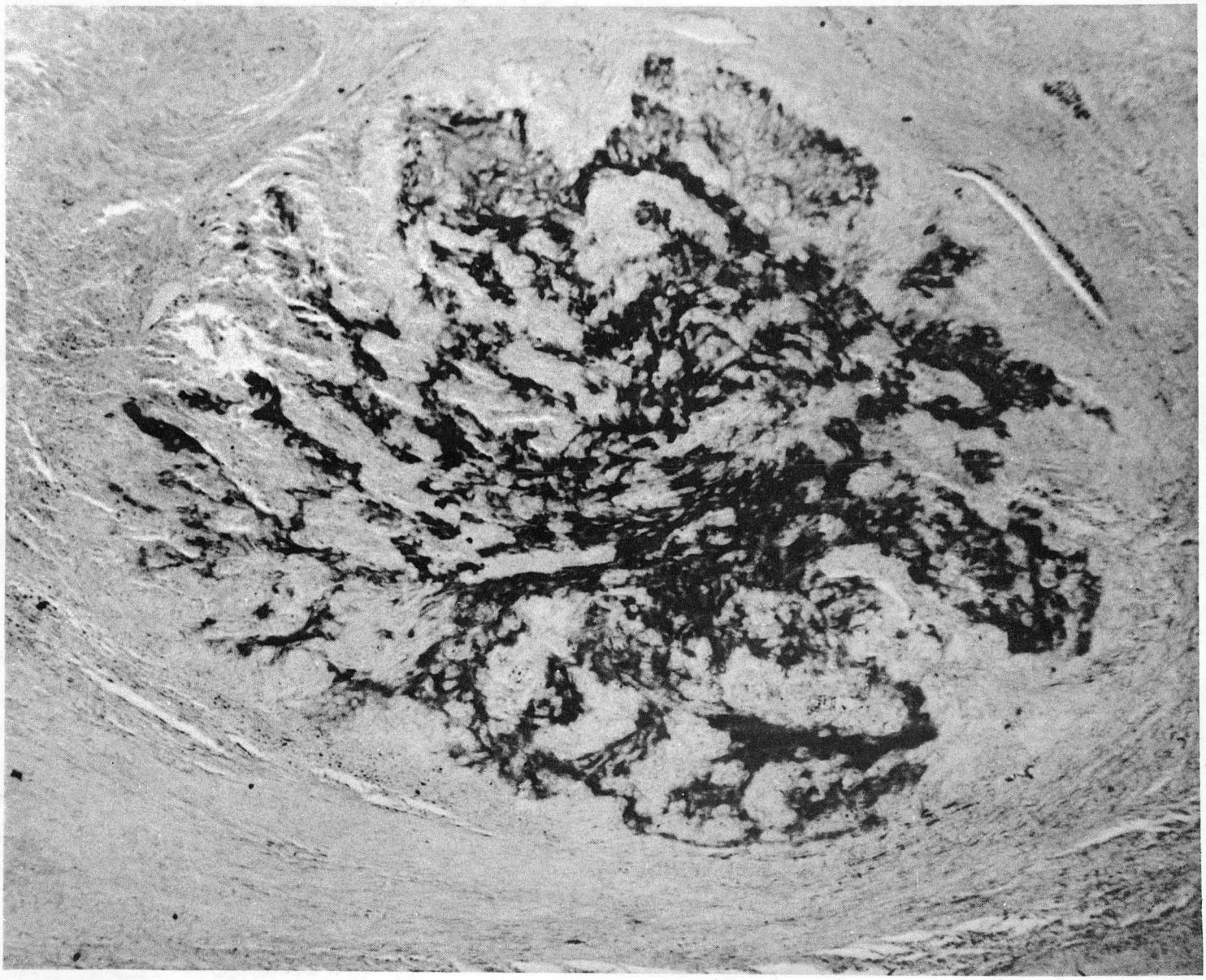
[3] Histology, R. O. Greep, Ed. (Blakiston, New York, 1954).

[4] A. G. E. Pearse, Histochemistry: Theoretical and Applied (Churchill, London, 1954).



ZN-1703

Fig. 6. Nodule comparable to that shown in Fig. 5, treated with periodic acid-Schiff to visualize mucopoly-saccharides. Note positive reaction (dark color) extending beyond the region of calcification (see Fig. 5), and lack of reaction in central core. Note also the positive reaction of the fibrous tissue in upper left corner. Undecalcified, 10 μ. x 56



ZN-1704

Fig. 7. Nodule comparable to those shown in Figs. 5 and 6. Note metachromasia distributed in a manner similar to the PAS-positive regions (see Fig. 6), and extending beyond the areas of calcification. Decalcified, 10 μ, toluidine blue. x 56

STUDIES OF AN OSTEOGENIC SARCOMA TRANSPLANTABLE IN RATS. III. METABOLISM OF CALCIUM-45

The presence of abnormal rapidly growing bone in the osteogenic sarcoma described in the preceding section suggested the need to learn something of the metabolism of calcium in this tumor. Experiments conducted with Ca^{45} are summarized as follows.

Methods

The animals, the procedures used for transplanting the tumors, and the method of estimating growth rate have been reported.[2]

Sixteen rats from our fourth transplant generation, F_4, were used for this study. Each rat received an intravenous injection of 0.2 ml of an isotonic sodium citrate solution containing 2 μC of high-specific-activity Ca^{45} which was obtained from Oak Ridge. Eight rats were sacrificed 4 hours after the injection, and the remainder were sacrificed 24 hours after the injection.

Serial blood samples (about 0.1 ml) were obtained from the tail veins of five animals in the 4-hour group. These were spread on aluminum discs, weighed immediately, and air-dried for radioactive assay. Just prior to sacrifice each rat was placed under deep ether anaesthesia, and 5 ml of blood was drawn from the inferior vena cava. Microhematocrits were determined. A portion of each whole-blood sample was centrifuged to separate cells and plasma.

The rats were sacrificed by decapitation. The tumors and right femurs were dissected and weighed. The terminal whole-blood and plasma samples, femurs, and tumors were oven-dried and ashed in a muffle furnace for 24 hours. The ashed tumor samples were weighed and dissolved in dilute HCl. The femurs and blood samples were dissolved in dilute HNO_3. Aliquots of the dissolved ash were prepared for radioactive assay by methods described elsewhere.[5] The stable calcium in the tumors was determined gravimetrically by precipitation as the oxalate. The calcium content of the femurs was calculated, with calcium assumed to constitute 16% of the wet weight.

Results and Discussion

Growth rates were determined for all the tumors used. The average doubling time for the F_4 generation was found to be 4.9 days. This agrees fairly well with 3.75 days determined for the F_2 and F_3 generations.[2]

The time rate of disappearance of intravenously administered Ca^{45} from the blood of tumor-bearing rats is shown in Fig. 8. The raw data, with standard errors, are shown in Table I. The data in Fig. 8 have been corrected for the 24-hour Ca^{45} concentration of the blood that was assumed to be an equilibrium value. A two-component curve was obtained on a semilog plot with half-time values of 6 and 117 min. The initial very rapid component due to mixing was not determined.

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[5] Medical and Health Physics Quarterly Progress Report, UCRL-2881, Dec. 1954, p. 15.

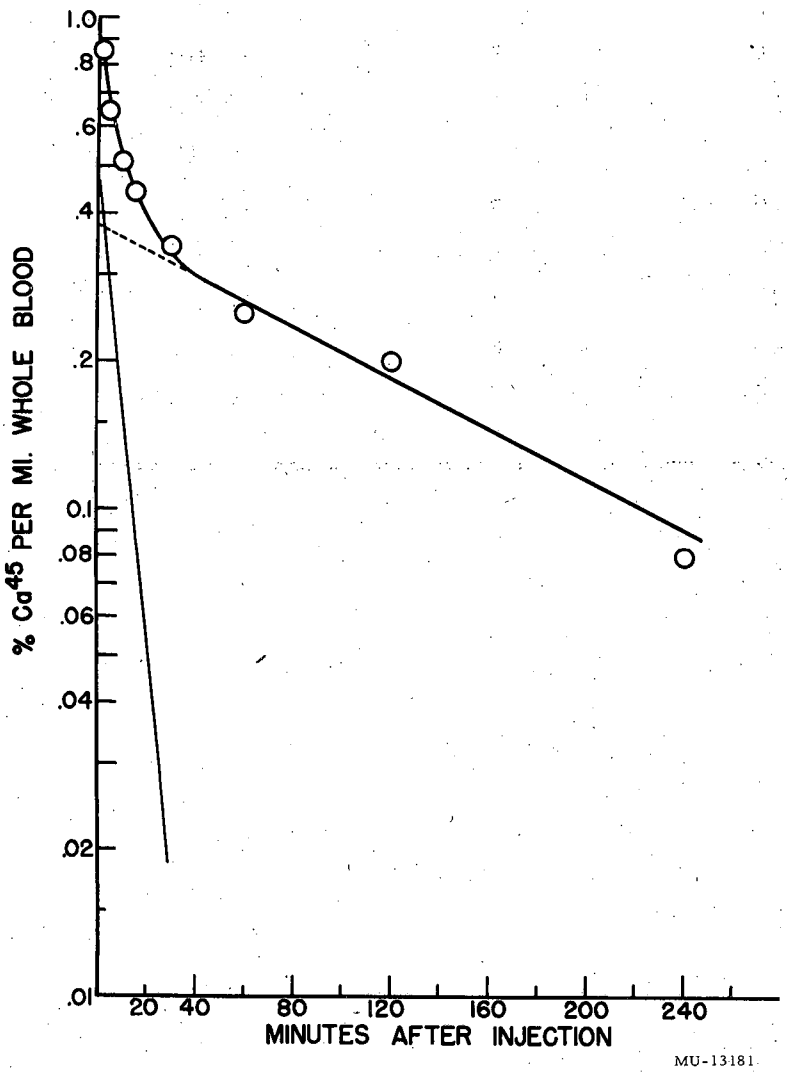

Fig. 8. Disappearance of intravenously administered Ca^{45} from the blood of rats bearing osteogenic sarcomas. Each point represents five rats.

Table I

Rate of disappearance of intravenously administered Ca^{45} from the blood of rats bearing osteogenic sarcomas.

Time interval (min)	No. of rats	% Ca^{45} per ml whole blood	Standard error
2	5	0.89	±0.03
5	5	0.69	±0.07
10	5	0.55	±0.03
15	5	0.49	±0.03
30	5	0.38	±0.04
60	5	0.29	±0.03
120	8	0.24	±0.03
240	8	0.12	±0.02
2400	8	0.04	±0.007

Table II summarizes the measurements made of stable calcium and Ca^{45} in the tumors and femurs. The mean Ca^{45} uptakes in the tumors and femurs were the same at 4 hours as at 24 hours, and the data for both groups have been combined. The variability of calcium content and Ca^{45} uptake in the tumors was in agreement with the often extreme differences seen in the quantity of bone in the individual tumors. It was therefore not surprising that there was no correlation between tumor size and Ca^{45} uptake or stable calcium content. There appeared to be a linear relationship between the percent Ca^{45} uptake in the tumor and the logarithm of the stable calcium content. For tumors containing from 30 to 500 mg of calcium the Ca^{45} uptake followed the exponential curve shown in Fig. 9. A least-squares fit yielded the equation

$$Ca^{45} = 62 \log Ca - 92,$$

where Ca^{45} is the percent of Ca^{45} uptake in the tumor at 24 hours, and Ca is the stable calcium content of the tumor.

The rapidity of bone formation in the tumors is strikingly demonstrated by the mean specific activities (% Ca^{45}/g stable calcium) of the tumors and femurs, 360 and 21.9, respectively.

The effect of the tumor mass on the composition of the blood with respect to cells and plasma is shown in Fig. 10. The regression line obtained by a least-squares fit shows an inverse linear relationship between red blood cell volume and tumor size. This has been interpreted as an indication of failure of the bone marrow to produce red cells at a sufficient rate to keep up with the rapid increase in the total body weight of the hosts.

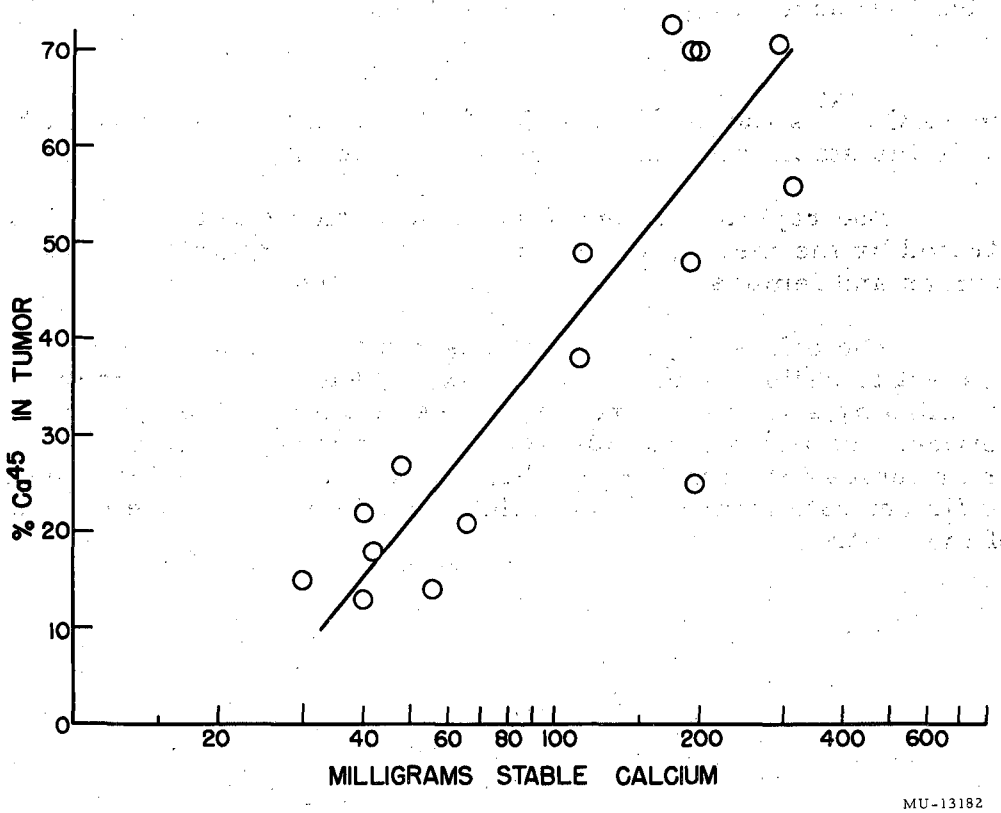

Fig. 9. Percent of administered Ca^{45} taken up by an osteogenic sarcoma as a function of the stable calcium content of the tumor. Each point represents an individual rat, curve fitted by least squares.

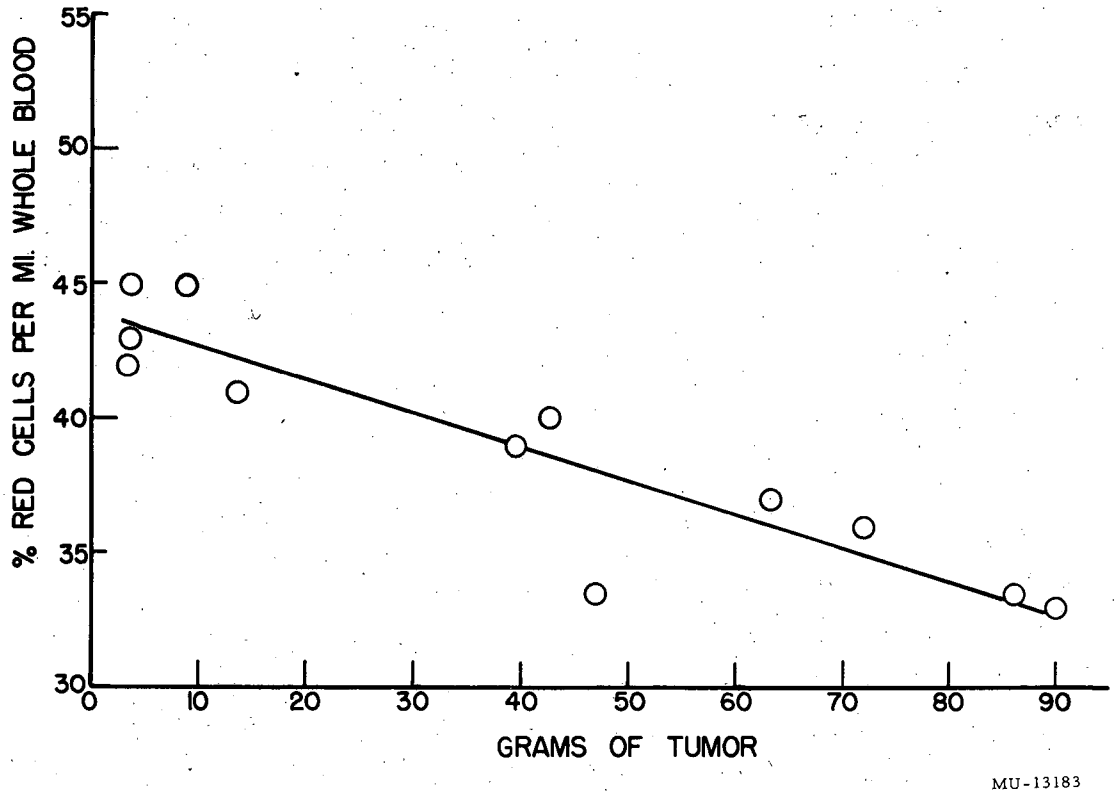

Fig. 10. The effect of size of an osteogenic sarcoma on the hematocrit of the host rat. Each point represents an individual rat, curve fitted by least squares.

Table II

Summary of Ca^{45} in osteogenic sarcoma

Tumors	Mean	One standard deviation	Range
Time to double in volume, days	4.9	± 1.5	3.0 - 8.4
Weight at sacrifice, g	39.7		3.6 - 90.0
Stable calcium			
(mg/tumor)	132		40 - 312
(mg/g tumor)	5.7	± 4.4	0.66 - 15.0
Uptake Ca^{45} (4-hr same as 24-hr)			
% dose/tumor	40		13 - 73
% dose/g tumor	1.9	± 1.7	0.30 - 7.4
% dose/g stable Ca in tumor	360	± 135	130 - 670
Bone			
Uptake Ca^{45} (4-hr same as 24-hr)			
% dose/g bone	3.0	± 0.6	
% dose/g stable Ca in bone	21.9	± 4.6	
Blood			
Microhematocrit	37	± 4	
Uptake Ca^{45}			
24-hr whole blood, % dose/ml	0.040	± 0.007	
4-hr plasma, % dose/ml	0.18	± 0.06	
24-hr plasma, % dose/ml	0.045	± 0.007	

INDUCTION OF MAMMARY TUMORS BY ASTATINE-211

The increased incidence of mammary tumors in female Sprague-Dawley rats following the administration of sublethal amounts of At^{211} (0.5 μC/g) was first reported by Hamilton et al.[6] To test the validity of this original observation, experiments have been conducted in this laboratory with the following aims: (a) determination of the number of animals developing tumors after a single injection of At^{211}, and the age at which they appear; (b) histological classification of the tumors produced; (c) assessment of the relationship between tumor production and endocrine function (At^{211} administered at a level of 0.5 μC/g produces partial thyroid deficiency[7]); and (d) determination whether the causative agent is endocrine imbalance or direct action of alpha-particle irradiation of immature mammary tissue.

Methods

The animals employed were female rats of the Sprague-Dawley strain that were obtained from the original colony. At 55 days of age 120 rats were each given an intravenous injection of At^{211} (prepared by methods previously described[8]) at a level of 0.5 μC/g body weight. For 8 days prior to the At^{211} injection, 40 of these rats received a daily subcutaneous injection of 230 μg of l-thyroxine to reduce the thyroidal uptake of At^{211}.[9] A second group of 20 rats was given l-thyroxine in their drinking water at a level of 1 μg/day, starting the day after the At^{211} injection. There were, in all, three groups of At^{211}-treated rats, (a) 60 that received only the At^{211} injection, (b) 40 that were pretreated with l-thyroxine--TH pretreated--and (c) 20 that received l-thyroxine in the drinking water--TH therapy. Sixty uninjected rats served as controls.

All animals were housed in groups of five in stock cages on wood shavings and were fed Purina Lab Chow and given tap water ad lib, with the exception of the TH-therapy group. All animals were weighed and examined for tumors every 2 weeks for the first 6 months after the At^{211} injection, and every month for the remaining 6 months.

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[6] Hamilton, Durbin, and Parrott, The Accumulation and Destructive Action of Astatine-211 (eka-iodine) in the Thyroid Gland of Rats and Monkeys, J. Clin. Endocrinology Metabolism 14, 1161-1178 (1954).

[7] Hamilton, Durbin, Asling, and Johnston, Physiological and Biochemical Studies of Astatine-211 (eka-iodine), Proc. International Conference, Peaceful Uses of Atomic Energy, Geneva, August 155, Vol. 10, Radioactive Isotopes and Nuclear Radiations in Medicine, 175-181.

[8] Parrott, Garrison, Durbin, Johnston, Powell, and Hamilton, The Production and Isolation of Astatine-211 for Biological Studies, UCRL-3065, July 1955.

[9] Shellabarger, Durbin, Parrott, and Hamilton, Effects of Thyroxine and KSCN on Capacity of Rat Thyroid to Accumulate Astatine-211, Proc. Soc. Exptl. Biol. Med. 87, 626-629, (1954).

The experiment was terminated at 1 year postinjection in an attempt to avoid confusion with the normal tumor incidence of this strain, which begins to be significant at about 14 months of age.[10] During the year tumor-bearing animals were sacrificed in batches of five or more, regardless of the sizes of the tumors or the time elapsed since their appearance. Five controls were sacrificed each time tumor rats were autopsied. The procedures were the same for the sacrifice of rats that developed tumors during the course of the year and for the 1-year survivors. During the year six At^{211}-injected and three control rats died of pneumonia and were not autopsied. Twenty-four hours prior to autopsy each rat received a tracer dose of I^{131} (5 μC to 10 μC intraperitoneally). The animals were sacrificed with chloroform and weighed, and the gross appearance of the internal organs was recorded. Thyroid remnants, liver, spleen, kidneys, ovaries, adrenals, pituitary, and tumors were dissected and weighed. Samples were also taken of lung, lymph nodes, and uterus. For histological examination thyroid, ovary, and uterus were fixed in Bouin's fluid, the pituitary fixed in Zenker-formol, and the remainder of the tissues fixed in 10% neutral formalin. The thyroid glands (in the fixative) were assayed for I^{131} gamma activity with a well-type scintillation counter.

Basal metabolic rates were determined for 14 rats that received only At^{211}, and for 7 TH-pretreated rats at 13.5 months of age, according to the method described by Watts.[11] At 7 months of age (5 months after the At^{211} injection), white blood cell counts, red blood cell counts, hemoglobin, and hematocrit levels were determined for 17 rats that received At^{211} only, 18 TH-pretreated rats, and 45 normal controls.

Results

Histological examination of the tissues is still in progress, and only the tumor incidence, organ weights, I^{131} uptakes, BMR measurements, and blood data are discussed in this report.

The comparison of the incidence of mammary tumors in the three groups of At^{211} rats with that in normal controls is shown in Fig. 11. The tumor incidences for the three At^{211} groups were comparable, and a composite curve has been drawn. The first tumors appeared in the experimental animals just prior to the 150th day of life; no tumors were found in the normals prior to the 210th day. By the end of one year after the At^{211} injection there were four times as many tumor bearers (often multiple) among the At^{211}-treated rats as in the control group.

The data on thyroidal uptake of I^{131} and the body and organ weights are shown in Table III. It can be seen that within each treatment group there were few, if any, differences between the tumor-bearing animals and those that had not developed mammary tumors. Pretreatment with l-thyroxine provided partial protection of the thyroid gland from the alpha-particle radiation of At^{211}. The glands were larger and retained a more

[10] Davis, Stevenson, and Busch, Tumor Incidence in Normal Sprague-Dawley Female Rats, Cancer Res. 16, 194-197 (1956).

[11] R. W. E. Watts, Metabolic Rate Changes Following Thyroid Destruction by At^{211} and I^{131} in the Rat, Proc. Soc. Exptl. Biol. Med. 89, 220-222 (1955).

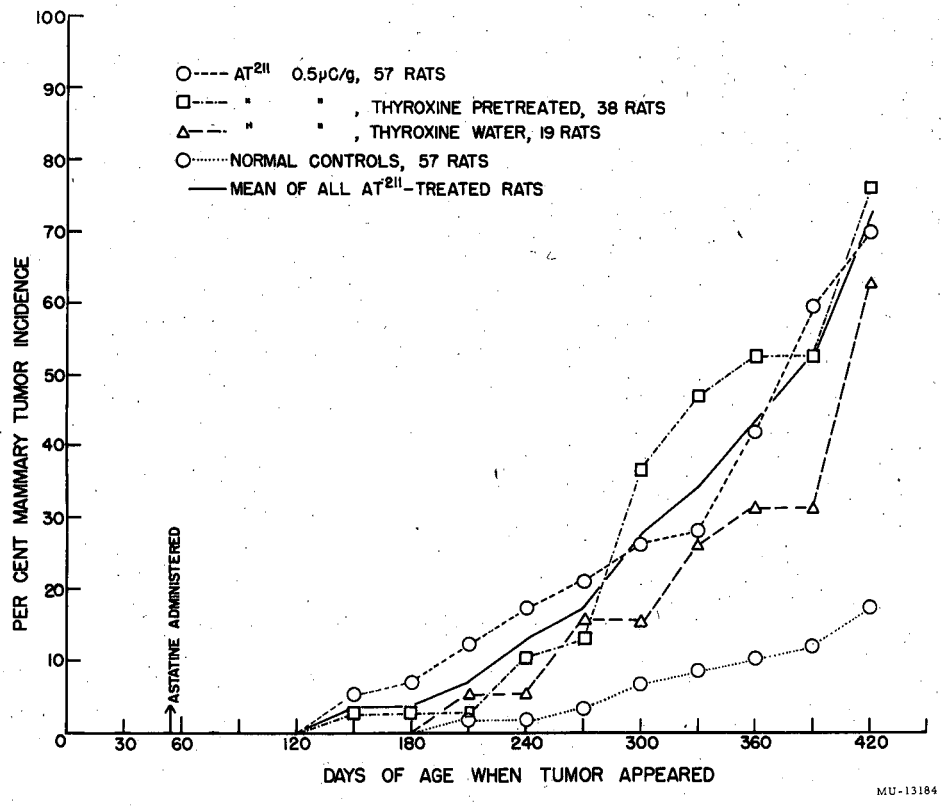

Fig. 11. The incidence of mammary tumors in female Sprague-Dawley rats following a single intravenous injection of 0.5 μC/g body weight of At^{211}, and in normal controls. Solid line is the mean incidence for 114 At^{211}-treated rats.

Table III

Comparison of body weights, organ weights, and thyroidal uptake of I^{131} by mammary-tumor-bearing and non-tumor-bearing female rats 3 to 12 months following the administration of 0.5 μC/g of At^{211}.

Experiment	No. rats autopsied	Body wt (g)	Thyroid analysis: wt (mg)	Thyroid analysis: %I^{131}	Thyroid analysis: %I^{131}/g	Weight of organ (mg): Spleen	Weight of organ (mg): Adrenal (1 only)	Weight of organ (mg): Pituitary	No. rats bearing uterine fibroids
At only--tumorous[a]	36	261	8.5	3.41	377	390	27.4	13.9	24/36
At only--nontumorous	21	257	8.4	2.71	305	370	26.1	12.9	18/21
At + thyroxine pretreatment--tumorous[a]	28	282	11.2	5.39	469	514	29.3	13.0	14/28
At + thyroxine pretreatment-nontumorous	8	270	13.4	7.64	534	408	30.9	13.2	3/8
At + thyroxine therapy	13 tumorous[a] 3 non-tumorous	253	8.0	3.72	447	383	26.8	13.8	11/16
Normals	5 tumorous 41 non-tumorous	283	23.2[b]	10.97[b]	487[b]	544	32.6	13.6	2/46

[a] Some of these animals had more than one mammary tumor.

[b] Data available for 9 additional rats for a total of 55 individual measurements.

nearly normal ability to concentrate I^{131}. Body weights and the weights of the three organs listed were within normal limits for the TH-pretreated rats.

The number of rats having uterine fibroids was greater than 50% in the At^{211}-treated groups; uterine fibroids were observed in only 4.5% of the normal controls.

For the At^{211}-treated rats the basal metabolic rate was 37.9 cal/m^2/hr, for the TH-pretreated rats it was 44.1 cal/m^2/hr, and for normal controls of this age the mean BMR was 40 cal/m^2/hr. The BMR's of rats receiving 0.5 μC/g At^{211} were therefore within the normal range.

White cell count, red cell count, hemoglobin, and hematocrit are shown in Table IV. The red count and hemoglobin were very nearly normal. White cell count and hematocrit were beyond one standard deviation from normal.

Table IV

Formed elements of the blood 5 months after the administration of At^{211} at a level of 0.5 μC/g body weight. Mean values are shown with standard errors.

	Normal Controls (45)	At^{211}-injected (17)	At^{211}-injected thyroxine pretreated (18)
White cell count thousands/mm^3	13.3 ± 0.6	8.43 ± 0.54	10.2 ± 0.6
Red cell count millions/mm^3	7.15 ± 0.08	7.04 ± 0.2	7.17 ± 0.14
Hemoglobin g/100 cc	14.3 ± 0.1	13.8 ± 0.1	14 ± 0.1
Hematocrit cc RBC/100 cc whole blood	56 ± 0.7	46 ± 0.5	48 ± 0.5

Discussion

These studies substantiate the earlier report of an increased incidence of mammary tumors in rats injected with At^{211}. Body weights, organ weights, and I^{131} uptakes in the residual thyroid tissue were not different for tumorous and non-tumor-bearing rats. The foregoing measurements, when added to data on BMR and on the formed elements of the blood, suggest that the functional state of the thyroid gland is not a primary factor in the development of these tumors.

RADIATION CHEMISTRY

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Boyd Weeks, Michael Jayko, Winifred Bennett,
Sibyl Cole, and Margery A. Andrews

The radiation chemistry group has continued to direct its work towards elucidation of the detailed mechanism of radiation-induced chemical change in aquo-organic systems, keeping in mind the ultimate application in radiation biology. We are concerned with the two types of radiation interaction: (a) indirect action of H and OH on dissolved organic substances, (b) direct action resulting from radiation absorption in the organic component. Our approach has been to study the radiation chemistry of particular chemical groups and bonds in the simpler organic derivatives, and to apply the information so obtained to parallel studies of the corresponding processes as they occur in more complex biological systems. A correlated activity is the development of irradiation facilities making use of the Crocker Laboratory 60-inch cyclotron as a principal radiation source.

PROTEIN

Pepsin

The study of chemical changes involved in the irradiation of oxygen-saturated pepsin solutions has continued. This work developed out of previously reported studies in which it was shown that glycine, diethylamine, and certain other organic molecules containing the monovalent nitrogen function undergo radiation-induced oxidation in dilute aqueous solution to yield the carbonyl compound that would be expected on the assumption that an imino derivative of the parent molecule is formed as an intermediate.[1, 2, 3] As noted in the preceding quarterly report (UCRL-3653), the presence of carbonyl groups in high-molecular-weight (nondialyzable) fractions, isolated from irradiated pepsin solutions, has been demonstrated by their reaction with 2, 4-dinitrophenylhydrazine.

Since the monocarbonyl and 1, 2-dicarbonyl hydrazones formed have different and characteristic absorption patterns in methanol-KOH,[4] it has been possible to follow the formation of each of these products separately. The procedure found to be most suitable has been to heat-precipitate the protein immediately after irradiation, separate the fraction by centrifugation, add 2, 4-dinitrophenylhydrazine to each fraction, and dialyze separately. The procedure has the advantage of keeping to a minimum any self-digestion by

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[1] Boyd M. Weeks, Indirect and Direct Action of Heavy-Particle Radiation on Glycine in Aqueous Solution (Thesis), UCRL-3071, July 1955.

[2] W. M. Garrison and B. M. Weeks, Mechanism in the Radiolysis of Aqueous Glycine Solutions, J. Chem. Phys. 24, 616 (1956).

[3] M. Jayko and W. M. Garrison, Indirect Action of Radiation on the -NH-CH_2- Linkage in Diethylamine (A Mechanism for the Radiation-Induced Decomposition of the Peptide Chain), J. Chem. Phys. 25, 1084 (1956).

[4] G. R. Lappin and L. C. Clark, Anal. Chem. 23, 541 (1951).

the protein. It has been possible in this manner to accurately reproduce results and to investigate yields at low dosages. When the above procedure is used, the dicarbonyl function is found only in the supernatant or "heat-soluble" material. As determined by the absorption spectra the separation of the two classes of carbonyls is complete, and since the concentration of dicarbonyls can be determined accurately at a much lower concentration than the monocarbonyls, the former were chosen as a means of studying yields at very low doses. The sensitivity of this test is such that radiation doses equivalent to approximately 10^4 r can easily be followed with the techniques now employed. It is expected that further development will permit studies at doses in the 100-r range. As indicated in previous quarterlies, the dicarbonyl compounds are most likely to be the oxidation products of serine, threonine, and (or) the aromatic amino acids, phenylalanine and tyrosine. Several attempts have been made to isolate individual carbonyl-containing residues from the protein hydrolyzate. Thus far, these attempts have not been successful, owing to the labile nature of the 2, 4-dinitrophenylhydrazones under the conditions required for protein hydrolysis.

Tissue Homogenates

The possibility exists that the radiation-induced formation of 1, 2-dicarbonyls may provide a sensitive direct chemical method for the study of radiation effects in biological systems. A preliminary study of neutron-irradiated liver homogenate has been made. At a neutron dose equivalent to approximately 2×10^3 r (see the following section neutron irradiation procedures), a positive test for dicarbonyl formation is readily observed. This work is being continued.

GLYCINE

Chemical reaction induced in aqueous glycine by radiation decomposition of water,

$$H_2O \rightsquigarrow H + OH, \quad (1)$$

$$H_2O \rightsquigarrow \tfrac{1}{2}H_2 + \tfrac{1}{2}H_2O_2, \quad (2)$$

yields hydrogen, ammonia, acetic acid, and glyoxylic acid as principal products in oxygen-free systems. Hydrogen peroxide formed in the primary radiation processes (2), however, is not observed. It has been proposed in a previously presented mechanism[1,2] that the hydrogen peroxide steady-state concentration is kept at low value by the chain sequence

$$NH_2CH_2COOH + OH = NH_2CHCOOH + H_2O, \quad (3)$$

$$NH_2CHCOOH + H_2O_2 = NHCHCOOH + OH + H_2O. \quad (4)$$

The imino acetic acid NHCHCOOH then hydrolyzes to form ammonia and glyoxylic acid:

$$NHCHCOOH + H_2O = NH_3 + CHOCOOH.$$

The hypothesis regarding Reactions (3) and (4) is now being tested (a) by radiation studies of oxygen-free glycine solutions containing added hydrogen peroxide, and (b) by Fenton's-reagent studies of oxygen-free glycine solutions in which OH radicals are formed chemically by the Haber-Weiss reaction,

$$Fe^{+2} + H_2O_2 = Fe^{+3} + OH^- + OH. \qquad (5)$$

Results of preliminary experiments employing Fenton's reagent are reported here; radiation studies of oxygen-free glycine solutions with added hydrogen peroxide are currently in progress and will be reported later.

Experimental Procedures and Results

The glycine was prepared by multiple crystallization from water according to a previously reported procedure.[1] Water for the solutions was doubly distilled, first in a Barnstead still, and second in an all-Pyrex system from alkaline permanganate. The glycine solution was made 3 M and adjusted to pH 1 with sulfuric acid. Reagent-grade hydrogen peroxide (Baker and Adamson 30%) was added without additional purification. Reagent-grade crystalline ferrous sulfate (General Chemical Co.) was used as supplied, and the solution was adjusted to pH 1 with sulfuric acid. The reaction vessel, an inverted U-tube arrangement, was suitably constructed so that appropriate volumes of each of the solutions could be evacuated in separate arms prior to mixing. The vessel was opened about 1/2 hour after the solutions were mixed, and hydrogen peroxide remaining in the mixture was titrated with 0.01 N ceric sulfate to the ferrous ortho-phenanthroline end point. After the entire sample was titrated with ceric sulfate the solution was made alkaline and was distilled to dryness in vacuo at slightly above room temperature. The distillate was titrated with 0.1 N hydrochloric acid for ammonia content.

The data summarized in Table V indicate that the chain reaction (3), (4) occurs under the experimental conditions employed. Ammonia yield can be calculated on the basis of the above stoichiometry, in which it is assumed that all the ferrous ion reacts according to Eq. (5), and all the hydrogen peroxide consumed reacts according to Eqs. (4) and (5). The calculated ammonia yield for the second experiment is 0.220 mmole; the observed yield was 0.178 mmole. The difference between the observed and calculated values in the latter case (higher peroxide concentration) is attributed to the competing reaction

$$OH + H_2O_2 = HO_2 + H_2O.$$ [5]

[5] N. Uri, Inorganic Free Radicals in Solution, Chem. Revs. 50, 375 (1952).

Table V

Action of Fenton's reagent on glycine in oxygen-free solution

	Experiment I	Experiment II
Initial volume of mixture	29 ml	24 ml
Initial H_2O_2 in mixture	0.226 mmole (0.0078 M)	0.297 mmole (0.0124 M)
Initial $FeSO_4$ in mixture	0.0428 mmole (0.00148 M)	0.0428 mmole (0.00177 M)
Final H_2O_2 in mixture	0.0024 mmole	0.055 mmole
Observed NH_3 yield	0.198 mmole	0.178 mmole
Calculated NH_3 yield	0.203 mmole	0.220 mmole

ORGANIC ACIDS

Papers on the mechanism of indirect action of radiation in (a) oxygen-free formic acid solutions and (b) oxygen-aerated acetic acid solutions are being prepared. Initial reports of this work have appeared.[6,7]

CHEMICAL ACTINOMETRY OF CYCLOTRON RADIATIONS

In the preceding quarterly report (UCRL-3653) we presented data on water-decomposition yields for 10-Mev protons and for neutrons produced by bombardment of beryllium with 24-Mev deuterons. Here, summarized briefly, are the experimental procedures used in obtaining the aforementioned data. We employed the aqueous formic acid–oxygen dosimeter, the radiation chemistry of which at pH values of 2.7 and above

[6] Garrison, Bennett, and Jayko, Mechanism in the Radiolysis of Aqueous Formic Acid Solutions, J. Chem. Phys. 24, 631 (1956).

[7] Garrison, Haymond, Bennett, and Cole, Radiation-Induced Oxidation of Acetic Acid-Oxygen Solutions, J. Chem. Phys. 25, 1282 (1956).

has been quantitatively interpreted[8b] in terms of the two primary processes

$$H_2O = H + OH, \quad (1)$$

$$H_2O = \frac{1}{2} H_2 + \frac{1}{2} H_2O_2. \quad (2)$$

The absolute yields of Reactions (1) and (2) are essentially independent of pH over the corresponding range, and are related to the yields of observed products by the Eq. (i) $G(1) = G(CO_2) = -G(O_2)$, (ii) $G(2) = G(H_2)$ (iii) $G(1) + 0.5\, G(2) = G(H_2O_2)$. Hart[8a] employed the formic acid-oxygen system at pH 2.7 in obtaining the values $G(1) = 3.00$, $G(2) = 0.88$ for Co^{60} γ-rays and the values $G(1) = 0.26$, $G(2) = 2.94$ for the $B^{10}(n, \alpha)\, Li^7$ reaction. The data of Table IV (UCRL-3653) gave the initial results of our studies with cyclotron-produced radiations. The values G(1) and G(2) were calculated on the basis of Eq. (i) and (iii) from hydrogen peroxide and carbon dioxide yields. Addition of $HC^{14}OOH$ in tracer amounts to the target solution permitted the radiometric determination of carbon dioxide as $BaC^{14}O_3$. Hydrogen peroxide was determined by titration with ceric sulfate.

The all-glass target cells used in the proton irradiations have been described.[9] Oxygen aeration was maintained during exposure. Additional mixing was obtained with an air-driven glass stirrer which was rotated at approximately 2000 rpm, a value 4 to 5 times as great as required to give yields independent of stirring speed. Preliminary studies also showed that the yields were independent of beam current and total dose within the range of values employed in obtaining the reported data. The supporting target assembly and the beam-monitoring circuit were similar to those described earlier.[10] The deflected cyclotron beam is passed in sequence through (a) a water-cooled collimator (which was covered with a 1-mil Al foil to bring about the stripping reaction $H_2^+ = 2H^+ + e$), (b) the Al foil cyclotron window, (c) the air space required for the shutter arrangement, (d) the glass target window. The energy of the particles absorbed in the solution is calculated from range-energy data.[11]

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[8] E. J. Hart, (a) Radiation Research 1, 53 (1954);
(b) J. Am. Chem. Soc. 76, 4198 (1954).

[9] Garrison, Haymond, and Weeks, Radiation Research 1, 97 (1954).

[10] Garrison, Haymond, Corum, and Hamilton, Rev. Sci. Instr. 24, 462 (1953).

[11] Aron, Hoffman, and Williams, Range-Energy Curves AECU-663, 1949.

The sections 2, 3, 4 are in electrical contact and act as a Faraday cage for the monitored beam. The charge input is amplified by a feedback electrometer and fed to a capacitor-charging integrator, the accuracy of which is maintained to within 0.5%. Calorimetric measurements have shown that this targetry measures charge input to within ± 1%.[12] The assembly has been used both at the cyclotron port within the fringing field and at the end of a target snout extension which provides a magnetically focused beam outside the cyclotron water-wall shielding.[13] Most of the proton data were obtained under the latter condition, in which case the effects of secondary electron emission are reduced to a negligible value by an imposed magnetic field or by a pair of Faraday cup apertures located between the collimator and the Al window that delimits the cyclotron vacuum.

The neutron data are not based on an absolute dosimetry measurement, and hence provide only a direct measurement of the ratio G(1)/G(2) for the particular geometry employed. Standard volumes of the formic acid solution were irradiated under 1 atmosphere of oxygen in cylindrical target cells mounted in a motor-driven reel situated 15 cm from the beryllium target along the forward axis of the neutron spectrum. Under these conditions the background γ-ray dose is equivalent to less than 2% of the energy transferred to the solution via recoil nuclei.[14]

We gratefully acknowledge the cooperation of Mr. William B. Jones, Mr. Kenneth Jenkins, and the staff of the Crocker Laboratory 60-inch cyclotron.

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[12] Warren M. Garrison and Boyd M. Weeks in Medical and Health Physics Quarterly Report, UCRL 1826, 1952, p. 27.

[13] R. E. Ellis and L. Schecter, Phys. Rev. 101, 636 (1956).

[14] Tochilin, Ross, Shumway, Kohler, and Golden, Radiation Research 4, 158 (1956).

BIOLOGICAL STUDIES OF RADIATION EFFECTS

John H. Lawrence, M. D., in charge

Donner Laboratory of Biophysics and Medical Physics
University of California, Berkeley, California

Material for this report had not been received at the time of publication, but may be expected in a subsequent report.

RADIATION DETECTION AND PROTECTION

Radiation Laboratory
University of California
Berkeley, California

HEALTH CHEMISTRY

Nelson B. Garden in charge

Reported by Rosemary J. Barrett

The ever-increasing radiation intensity levels, both for accelerator targets and for radioactive isotopes, and the growing number of graduate students, postdoctoral personnel, and visiting scientists, plus the imminent plan to reduce tolerance levels as recommended by the Bureau of Standards, have necessitated closer planning for increase of equipment for handling radioactive materials, more efficient techniques where possible, and a greater number of trained Health Chemists. The forthcoming start-up of the Heavy Ion Accelerator and the remodeled and improved 184-in. cyclotron and Bevatron specifically point to increased load in Health Chemistry functions.

More and more it becomes apparent, after ten years, that adequate protection against radioactivity requires the specialized services of groups in the fields of technical assistance to researchers, monitoring, decontamination functions, transportation, storage and disposal of radioactive materials, airborne activity control, and equipment development, and calls for the closest cooperation possible among these groups. Some of the specific items receiving attention are noted below.

Building, Remodeling, and Planning

Plans for the enlargement of Bldg. 70 are being formulated, with Health Chemistry personnel in consultation for insurance that basic protective principles will be incorporated in these plans--and that our space recently relinquished to a research group will be replaced.

The creation of decontamination facilities in Room 105, Bldg. 5, to replace the decontamination space relinquished to research projects in Bldg. 70, is nearing completion.

The renovation of the ducting and ventilation facilities of Donner Laboratory continued, with the goal of making this building entirely suitable for housing radioactive work. Health Chemistry personnel continued checking and advising on this project.

Room 106, Old Radiation Laboratory, available for general use for radioactivity processing, is being revamped through improvements in the manifold system. The ducts and exhauster were removed and found alpha-contaminated, the former being then disposed of and the latter decontaminated and reinstalled.

Bevatron revamping was assisted by provision of personnel, techniques, and equipment for effecting complete capture of metallic dust and chips arising from grinding and drilling near the magnet and beam tank.

Containment and Decontamination

Techniques have been developed whereby a large part of liquid active waste is solidified "on the spot" in Bldg. 70. This is carried out by immersing the 500-ml bottles in cement immediately; this parcel then is handled as dry waste rather than being added to the bulk liquid waste, which requires special gelling treatment described previously.

Technical assistance was given in the handling and storing (including diluting and canning) of tritiated water being used by a medical group in Donner.

High-activity waste from the last plutonium napkin-ring processing in the 6-in. lead caves of Bldg. 70 has been prepared for sea disposal and the cave made ready for the next runs.

A closed dissolver system for use in the Bldg. 70 6-in. lead caves has been assembled and installed, and will be used in the next re-irradiated napkin-ring processing.

The cleanup of the 6-in. cave in Bldg. 5, subsequent to the loading of the 2300-curie Co^{60} source, has been completed.

Routine inspection for presence of radioactivity continues on oil samples from house and individual vacuum systems, water samples from the target-handling cart for targets from the 60-in. cyclotron, and incinerator ash samples. A study of the applicability of membrane filtration to target-cart water decontamination indicates that a rigid specification for feed water must be established. Present demineralized water is too high in filter-plugging solids.

Routine air sampling now involves more than 1000 samples per month.

Stack gas sampling by use of membrane filters was initiated in areas other than cave operations.

Development of a sealed-sock or bagging assembly for attachment to Berkeley boxes or other enclosures was completed, the assembly was satisfactorily field-tested, and a stock was ordered.

Continued studies confirm that the CWS-6 filter-passing aerosols arise from multicurie radiochemical operations in enclosures having "normal" throughput ventilation, whereas little or no such contaminants appear from so-called "low leak" systems.

Garden

A double scrubber system has been created for Dr. Calvin's group for use in their experiments with material containing both C^{14} and cyanide; the system controls both hazardous aspects of the materials. Field evaluation continues.

Methods for quantitative assessment of I^{131} in air continued under study.

Apparatus and Equipment

A plaque covered with a proper amount of Cs^{137} for use in intracavity insertion in the treatment of cancer in humans was created by Health Chemistry personnel; this specifically chosen type of source material, in proper quantity, affords means by which desired treatment may be administered; formerly the desired treatment had to be compromised to fit the characteristics of materials usually used, such as radium.

A failure of a specially fabricated Sr^{90} source (in service for five years) has resulted in the recall of all Sr^{90} sources for thorough examination (these sources have been fabricated on request by researchers desiring a source with a specified half life, energy, and quantity). Evidence has been obtained that the failure resulted from premature corrosion of the aluminum foil within the source; this corrosion was probably due to the presence of chlorine, released through the bombardment of SrCl by beta radiation. One is thus cautioned that the longevity of parts of sources is less than that normally expected and that therefore an even tighter system for source inspection and control--checking, replacing parts, remounting--is mandatory. An evaluation of all sources with respect to their body-burden content will give criteria on which to base the tightness of the necessary control.

The rupture of a centrifuge head, within a shielded lead box, has led to the construction of a new type, fabricated from a single piece. The escaping radioactive solution was adequately captured in the box tray.

HEALTH PHYSICS

Burton J. Moyer in charge

STATISTICAL SUMMARY OF MONITORING PROGRAM

Survey Instruments Maintained

Beta-gamma meters	22
I D L meters	21
Juno ion chamber	20
Abacus logarithmic ion chamber	30
Recording-intensity meters	8
Victoreen proteximeter	3
Slow-neutron proportional counters	15
Fast-neutron proportional counter (portable)	11
Slow-neutron portable unit	4
Balanced chamber--fast neutron--portable	3
Special tissue wall survey instrument	1

Survey Instruments in Storage

Beta-gamma meters	7

Personnel Meters in Use - Berkeley Only

Total personnel covered with film badges	.1800
Total man-days' coverage with electroscopes	.5870
Total man-days' coverage with dosimeters	.3150
Total man-days' coverage with slow-neutron chambers	.3150

	Man-days	Number per day (approximate) for 3 months
Bevatron		
Electroscopes	1360	15
Bldgs. 10, 53, and 80		
Slow-neutron chambers	2250	25
Dosimeters	2250	25
Electroscopes	2250	25
Crocker		
Slow-neutron chambers	900	10
Dosimeters	900	10
Electroscopes	900	10
Bldg. 70		
Electroscopes	1360	15

Cases of Weekly Exposure Above 0.3r*

Weekly Film Expos. Above	184-inch Area	60-inch Area	Linac	Chem	Other	Total
0.3	1	8	44	5	**2	60
0.5	0	2	2	0	1	5
1.0	0	1	0	0	1	2
1.5	0	0	0	0	0	0
2.0	0	0	0	0	0	0
2.5	0	0	0	0	0	0
3.0	0	0	0	0	0	0
4.0	0	0	0	0	0	0
6.0	0	0	0	0	0	0
6.5	0	0	0	0	0	0

*excluding Livermore

**Bldg. 46. Reading 1.09: Elma Williams. This reading was apparently received by someone other than Elma Williams, who has not been wearing her badge.
Reading 0.374: Carl Svoboda. (He uses this badge elsewhere, also.)

Information Division
5-7-57 sa

Moyer