THE PRODUCTION OF OSTEOGENIC SARCOMATA AND THE EFFECTS ON LYMPH NODES AND BONE MARROW OF INTRAVENOUS INJECTIONS OF RADIUM CHLORIDE AND MESOTHORIUM IN RABBITS

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Plates 16 to 18

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Early in the use of the X-ray, it became evident that, on account of the repeated exposures, the operators were subject to grave danger. As is well known, they developed cancers from the burns in the skin and signs of damage to the blood-forming organs—anemia and leucopenia. These facts led both to experimental studies of the effects of X-rays and to an analysis of the changes in the blood cells (1–8).

In connection with these early studies on the effects of X-rays, it is of great interest to note the work of Murphy. Following the suggestion of Webb *et al.* (9, 10) that lymphocytes are a measure of resistance in tuberculosis, Murphy and his collaborators (11–26) made extensive studies on the relation of this strain of cells to resistance both to tuberculosis and to cancer. They first found that lymphocytes could be stimulated or depressed by means of X-rays, and determined the appropriate dosage. Nakahara (22) showed that the stimulating effect on the lymph nodes from small doses of X-rays began as early as 48 hours, as shown in his figures (Fig. 2, Plate 8, and Fig. 4, Plate 9). With these data in hand, Murphy and Morton (15) found that mice showing resistance to transplanted mouse cancer had a marked lymphocytosis and that this resistance could be broken down by doses of X-rays which produced no demonstrable changes except a necrosis of lymphoid tissues and the resulting lymphopenia.

When the clinical use of radium became common, it was found again that the danger was to those handling this material in account of the repeated exposures and that the effects were the same as with the X-rays. Mottram *et al.* (28) studied the blood of two groups of workers: those who prepared and measured the emanations and those who gave the radium to the patients. They found a rapid fall in both granulocytes and in lymphocytes and discovered that these

losses were regained only after a considerable interval. There were then several reports of the study of the blood after exposure to radium (29–37) and recently it has become clear that the anemia due to radium may be of the primary type (27, 38–43).

The recent introduction of the use of luminous paint into industry, as well as the sale of radiated waters as tonics, has brought danger to another group of people from exposure to radioactive substances.

In 1929, Martland (44) gave the history of the industrial hazard involved in the painting of dials. Starting in Switzerland, the industry was transferred to this country, and in 1922, 1923, and 1924 there were deaths of dial painters unrecognized as due to radioactive materials. In 1924, Blum reported a case of necrosis of the jaw in one of the dial painters. There followed a series of studies of the people exposed to radium (45–52) in this industry. The early cases in this industrial group died of necrosis of the jaw; now, 8 years after the first reports, some of the cases have died with osteogenic sarcomata and some of those living have this condition, as determined by X-ray photographs. Flinn and his coworkers (49–52) have made special studies of the methods of detecting the presence of radioactive material in the living subject and measuring its amount. They have measured the rate of excretion and have tested methods of increasing this rate and determined the degree of safety of their application.

The development of osteogenic sarcomata in the human cases has increased the interest in the experimental production of tumors by radioactive substances. The first report of the production of osteogenic sarcomata in animals was published in 1910 by Marie *et al.* (53); they attempted to reproduce X-ray burns in animals to see if they would ultimately give rise to the same malignant reaction found in the X-ray operators. They exposed four rats to repeated small doses of X-rays for a period of several weeks. After 6 months one of them developed an ulcer which, 9 months later, suddenly became sarcomatous. The tumor showed active growth and invasion, but did not metastasize. In 1924, Bloch (54) reported the experimental production of epitheliomata of the ear in two rabbits by repeated exposure to X-rays. He succeeded in transplanting one of these tumors into another rabbit. The next year, Goebel and Gérard (55) reported the production of a neoplasm in one guinea pig out of twenty after repeated exposure to X-rays during 15 months. Daels and his coworkers (56–58) have made the most extensive studies of the experimental production of neoplasma with radioactive substances. They have produced sarcomata, some containing bone, and carcinomata of the bile ducts and the mammary glands. In all they have produced 10 sarcomata in 144 treated rats, 5 tumors in 80 treated mice, and 8 tumors in 191 treated guinea pigs. Of the 23 tumors, 19 followed the use of radioactive materials alone; the others developed after the use of a combination of radium and arsenious acid. One of the rat tumors was transplanted into 27 other rats; from 90 to 95

per cent transplants were successful and one tumor was in its tenth passage at the time of writing (1931).

Maisin and Dupuis (59) obtained sarcomata of enormous size in cocks and hens by intravenous injections of ionium, in combination with injection into the pectoral muscles of embryonic extracts. With injections of embryonic extract alone, or with arsenious acid, they could not produce tumors. One of the tumors metastasized; they did not succeed in transplanting them. Schürch and Uehlinger (60) implanted a needle containing 1 microgram of radium under the periosteum of the jaw-bone of a rabbit and allowed it to remain for 20 days. After 1½ years an osteogenic sarcoma developed, limited to the periosteum; it was made up of osteoblasts, and contained newly formed masses of bone and many multinuclear giant cells.

The literature considering the effects of radium on cancer cells in human cases will not be reviewed. It can be followed in the studies of Ewing (61), in the series of reviews published in the *Archives de l'Institut du Radium* (Vol. 1, Paris, 1927, to date), and in the files of the *Strahlentherapie* (Berlin, 1912, to date).

From the references here reported it is clear that radium has a destructive effect on the blood-forming organs; that the lymph nodes are more sensitive than the bone marrow; that there is a period of stimulation during which there is a relative increase in lymphocytes combined with a leucopenia; that the anemias tend to be of the primary type, associated with an increase in immature cells in the marrow; and lastly that radioactive substances produce malignant tumors, both sarcomata and carcinomata, some of which metastasize and can be transmitted in series.

In 1926 we began to study the effects of radioactive substances on the cells of the blood and connective tissues in rabbits. Through the courtesy of Dr. Frederick B. Flinn of Columbia University, radium chloride and mesothorium were obtained from the United States Radium Corporation. These compounds were put up in sealed ampoules of such a strength that 2 cc. of normal salt solution contained about 5 micrograms of the active material. In January, 1928, the strength of the material was tested for us by Dr. Alice H. Armstrong of The Rockefeller Institute and at that time an ampoule of radium chloride contained the equivalent of 5.1 micrograms of radium, and one ampoule of mesothorium, 7.7 micrograms. The dose of the mesothorium was also greater because it is known that it gives off 5 alpha particles to 4 from radium chloride.

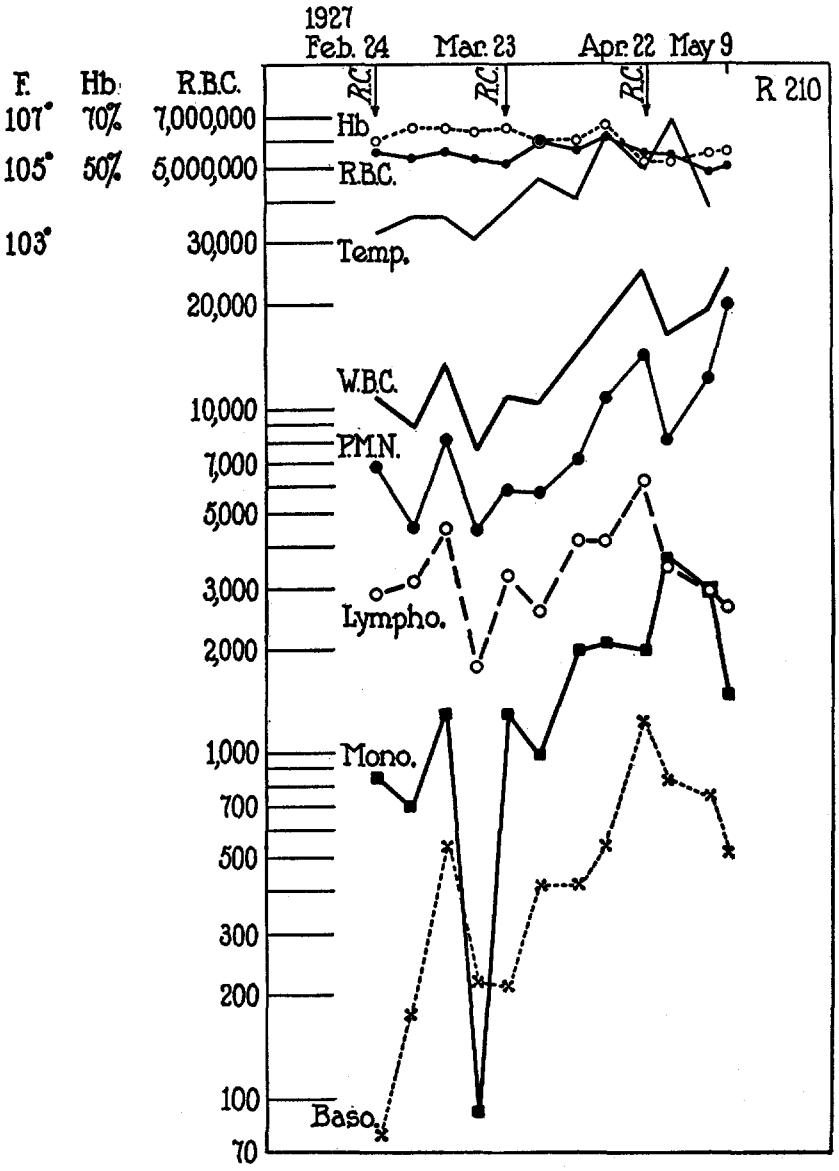
EXPERIMENTAL

Nine rabbits were used for the experiment, five of which received radium chloride and four mesothorium. The injections were given intravenously once a month. The blood cells were counted once a week. The differential counts were made with the supravital technique, except during July and August, when they were made from fixed films. The changes in the lymphocytes are shown in Graph 2, in the red cells in Graph 3. The graphs are all on logarithmic paper. Only the seven animals that lived long enough to show these changes are included. All of the records opposite the numbered months are averages of the 4 counts. The average of the counts before any injection is shown on the upright line at the left. It represents from 5 to 15 counts covering from 1 to 4 months. For Rabbit R 211[1] there was only 1 preliminary count, 2,222 lymphocytes and 5,350,000 red cells, because this animal was substituted for one that died after one injection. From three to five injections were given in the spring, as shown on the graphs; then there was an interval of 3 or 4 months without injections which were resumed in September or October. All of the animals receiving the second series of injections, except R 211, which was killed in December, received an extra injection in January, 1928. One animal died 25 days after one injection; a second was killed after three injections (Graph 1); the other seven were killed at intervals varying from 11 to 19 months after the first injection. Each animal was killed when signs of some damage became evident, such as a fracture or marked weakness. The animals receiving the radium chloride survived the longest and showed fewer signs. The following are the protocols.

Rabbit R 134 was pregnant and 15 days after an injection of radium chloride gave birth to four young. It died 10 days later; the autopsy revealed edema of the lungs and extensive hemorrhages into the bone marrow and into the follicles of the spleen.

Rabbit R 210 received three injections of radium chloride. 6 days after the second injection, the temperature rose; 9 days later, the white blood cells had started to rise and in 2 weeks had reached 25,000 cells per cubic millimeter, without, however, any change in the percentages of any of the different strains (Graph 1).

[1] These are serial numbers of the work of the department covering a term of years.


1927
Feb. 24
Mar. 23
Apr. 22
May 9
R 210
F.
Hb
R.B.C.
107°
70%
7,000,000
105°
50%
5,000,000
103°
30,000
20,000
10,000
7,000
5,000
3,000
2,000
1,000
700
500
300
200
100
70
Hb
R.B.C.
Temp.
W.B.C.
P.M.N.
Lympho.
Mono.
Baso.

GRAPH 1

Then the neutrophilic leucocytes rose to 81 per cent and the lymphocytes fell to 11 per cent. There was no corresponding rise in the red cells nor change in hemoglobin. Except for the one count of 5,326, the lymphocytes were normal. These changes in the blood cells were due to an abscess on the back, on account of which the animal was killed 75 days after the first injection. It was in a good state of nutrition, considering the size of the abscess and the fever; it had gained in weight from 1,840 to 2,240 gm.

The bone marrow and the spleen reflected the presence of the abscess. The erythroid series in the marrow of femur, tibia, and humerus was represented by the usual masses of normoblasts. The fat had not been shifted out of the marrow, but the interstices between the fat cells were hyperplastic, due to an increase in neutrophilic B myelocytes (62). They were in active division and there was some increase in myeloblasts. In the sinuses of the spleen there was marked destruction of leucocytes within clasmatocytes and small masses of plasma cells in their neighborhood. There were also small groups of myelocytes, some of them in division. The lymphoid follicles of the spleen and the mesenteric lymph nodes were normal. The peripheral lymph nodes, on the other hand, were so markedly hyperplastic that almost no distinction could be made between follicles and sinus, as is clear in Fig. 1. The hyperplasia was due to the primitive cells rather than to any increase in mature lymphocytes. This accounts for the predominantly gray tone of the photograph, and is especially well shown in the lower follicle at the right which even lacks the rim of darkly staining small lymphocytes. The centers of all the follicles were filled with primitive cells, many of them in division (Fig. 2).

The protocols of the next five rabbits will be considered together. Three of them, R 157, R 171, and R 175, received the mesothorium, and two, R 133 and R 136, the radium chloride.

Three of these rabbits, R 136, R 171, and R 175, showed a marked shift in the lymph nodes from mature lymphocytes to a predominance of lymphoblasts. For example, in Fig. 5 is shown a small segment of a peripheral follicle from Rabbit R 171, in which the increase in the deeply staining lymphoblasts is clear. This reaction was even more marked in the lymph cords in certain nodes where all of the cells were small lymphoblasts. Wiseman (63) has shown that lymphoblasts may be small or intermediate or large cells. In all of these animals some of the nodes were markedly atrophied, as is shown in Fig. 3, Rabbit R 136. Figs. 1 and 3 are to be contrasted; they are from comparable nodes and are at the same magnification. In Fig. 1 is shown the phase of stimulation after three doses of radium chloride, and in Fig. 3 the aplasia following fourteen doses. A complete absence of lymph cords, such as is shown in Fig. 3, gave to many of these nodes a cystic appearance at autopsy. In the figure it will be noted also that there was a marked reduction in the peripheral follicles. The mesenteric nodes in some instances were represented by tiny nodules, some of them containing follicles but

others only lymphatic sinuses; these sinuses were identified by their endothelial cells which were loaded with the yellow pigment negative for iron, so characteristic a structure of the mesenteric nodes in rabbits. In one rabbit (R 171) some of the peripheral nodes could not be found. Fig. 4 illustrates a condition of great importance, namely, the marked damage of the stem cells; it is from a peripheral node of R 157. The darkly staining cells are not lymphoblasts, but normal lymphocytes which were printed dark in the photograph in order to show the pale damaged cells at all. In the supravital preparations from the nodes of this animal, both mesenteric and peripheral nodes showed a great reduction in all cells; those present were of two types, typical, normal, small lymphocytes and a second described as of the size of small lymphocytes but with an irregular cytoplasmic border and a nucleus in which no structure could be made out, except a dense nuclear membrane. In Fig. 4, these cells are readily identified as the so called pale cells of the follicles, or the stem cell of the lymphocyte. They are marked with arrows in Fig. 4 and are to be compared with the normal cell of the same type, also marked by an arrow, in Fig. 2.

The spleens in these animals showed for the most part a reduction in the lymphoid follicles, except in R 133, in which the follicles were in the state of hyperplasia illustrated for the lymph nodes in Fig. 1. In the sinuses there was a marked increase in iron-containing pigment.

The bone marrows were so mottled in appearance that the change is readily made out in sections with the unaided eye; this appearance is due to areas of aplasia alternating with zones which were either normal or in which there had been some shift toward the immature stages of the erythroid series. There was a change in the megalokaryocytes consisting in a large number of deeply basophilic nuclei almost completely denuded of cytoplasm; in one animal (R 157), 53 per cent of the megalokaryocytes were of this type. They may be considered as representing a shift toward immature stages of these cells. In almost every instance there was a marked change in the fat cells, consisting in a shrinkage due to the loss of fat from the cytoplasm. The bone marrow also showed phagocytic cells filled with pigment containing iron. There were certain special conditions not found in every animal, atrophy of the thymus, multiple abscesses, chronic bronchopneumonia, and certain changes in the liver. The changes in the liver consisted in hemorrhages, in a specific type of damage to the nuclei of the liver cells to be described in detail for Rabbit R 211, and in atrophy. Two of the rabbits showing marked damage of the liver had profound weakness of the muscles or paralyses without signs of damage to the central nervous system.

Rabbit R 157 showed a complete consolidation of the upper lobes of both lungs. There had been a bronchopneumonia and large abscesses alternated with zones in which there had been new growth of bronchi and of the connective tissue septa, so extreme as to suggest a tumor. The cavities of the abscesses contained leucocytes and debris staining like lime salts. The zone showing the most marked changes in bronchial epithelium is illustrated in Fig. 10. Only a small row of typical bronchial epithelial cells (Arrow A) identifies this section as lung. Oppo-

site Arrow B is a solid mass of bronchial epithelium, suggestive of a tumor, but we interpret the condition as due to chronic inflammation.

All of the animals showed a loss in weight ranging from 300 to 680 gm. during the period of the final fall in lymphocytes.

Rabbit R 117 was one of the two animals which developed an osteogenic sarcoma. The animal had a litter of five young after the third injection and another litter after the sixth. During the period of the lymphopenia (Graph 2), the basophils fell and there was a loss of 680 gm. in weight. All of the bones, including the skull, were radioactive. The bones were tested for us by Dr. Armstrong, both in the fresh state and after being dried and ground.

The lymph nodes were all smaller than usual and looked cystic. The liver was small and the thymus markedly atrophic. The spleen was slightly larger than normal and weighed 1.6 gm. The most interesting changes were in the bones. All of them were extremely brittle. In the marrow of the left humerus there was a small white placque near the epiphysis which proved to be a sarcoma (Fig. 7). There were many vessels between bone and marrow. The right humerus showed more profound changes; this marrow was more markedly adherent to the bone, but was cut out and represented a calcified cast of the marrow cavity. It showed no blood formation, no supravital preparations could be made, and the mass was put aside for decalcification. The marrows of the radii and ulnae were gelatinous and showed more blood formation than is usual in these bones. The femoral and tibial marrows were likewise gelatinous; the distal end of the tibia being most markedly so and as usual almost aplastic. The active marrow in these bones was deep red and showed the same increase in blood vessels between marrow and bone as in the humeri. Counts of marrow cells of the active areas were normal in the proportion of erythroid to myeloid cells (64, 65), and there had been no shift to immature forms in either myeloid or erythroid series.

Figs. 7 to 9 show the tumor of the humoral bone marrow. In Fig. 7 is the nodule and its border at a magnification of 120 diameters. The tumor, as shown in the left half of the photograph, was relatively uniform, made up predominantly of the sarcoma cells, but there were remnants of the sinusoids marked by perivascular clasmatocytes filled with iron-containing pigment; there were also a few myelocytes, some of them in division. The nuclei of tumor cells varied in size but not in type (Fig. 8); their chromatin was in fine particles along the linin framework; they had conspicuous basophilic nucleoli. Many of the nuclei were in division, as is shown in Fig. 8. The border of the tumor shown on the right side of Fig. 7, and at higher magnification in Fig. 9 showed the invasion of the tumor cells into the marrow. In this zone the marrow was hyperplastic due to a marked increase in neutrophilic leucocytes. This is clear in Fig. 9, where the characteristic tumor cells marked by arrows are shown between the marrow cells which are predominantly leucocytes.

There was also a tumor in the left axilla, about $2\frac{1}{2}$ x 1 x 1 cm., which was made up of cells like those of the sarcoma of the marrow. In supravital preparations the cytoplasm showed no reaction to neutral red, as is characteristic of tumor cells.

The nucleoli were conspicuous. There were considerable numbers of multinucleated giant cells with nuclei of the same type. At one end there was a small necrotic area. There were three similar nodules in the lungs, two small ones in the left and a larger one in the right lung. Unfortunately these tissues were lost, but the supravital studies of the cells indicate quite clearly that there were metastases of the sarcoma. The study of the tissues of the next animal leads to the suggestion that the calcified marrow of the right side was an older stage of the tumor.

Rabbit R 211 was the other animal that developed a sarcoma. The sections are shown on Figs. 11 to 14. This rabbit received six intravenous injections of mesothorium during a period of 11 months, as is shown in Graph 2. In December the animal had a spontaneous fracture of the lower end of the right femur and was killed. Thus the experiment was the shortest of the series of seven which received the second series of injections. No anemia developed; the red cells which had averaged 5,400,000 for the 1st month were 6 million the last month. The final fall in the white cells during November and December was due mainly to lymphocytes; in small part to basophils. During this period the loss in weight was 300 gm. Almost the entire marrow cavity above the fracture was occupied by a tumor. The sarcoma replaced almost the entire marrow of the right femur. The contours of the tumor were sharp, as is shown in Fig. 11. At the upper end of the tumor there was a zone of aplasia (Fig. 11), beyond which there was a remnant of normal bone marrow. The fracture of the bone was opposite the lower end of the tumor. The sarcomatous nature of the new growth is clear in Fig. 11. The type of cell is shown in Fig. 12; the nuclei are of the same type as in the tumor of R 117. In many places there were small masses of newly formed bone in the tumor, some of them in small placques, such as the one shown opposite Arrow A in Fig. 12, others in irregular networks. Near these small masses of bone were giant cells, such as the one marked by Arrow B, Fig. 12, which is of the type of the osteoclast. The nuclei in these giant cells were like those of the tumor cells, which is in agreement with the theory of Arey (66) that the osteoclast is formed by the fusion of osteoblasts.

That the tumor itself was derived from osteoblasts is indicated in Fig. 13, which is a small mass of bone against the tumor; it is denuded of osteoblasts, but three typical tumor cells nearby probably represent them. Fig. 14 is a small piece of damaged bone near the fracture. It was cut without decalcification; the bone corpuscles are shown with their processes entirely free from the canaliculi of the bone and in the upper part of the section the mineral matter of the bone is shown much fragmented.

The bone marrow in this animal does not appear mottled with the development of the aplastic zones seen in other animals. The cells, however, are difficult to discriminate because of a damage to the nuclei which makes them all look alike. Mottram (67) has seen the same change in the bone marrow of rats exposed to the gamma radiation from radium. The fat cells and the matrix were normal. A count of marrow cells with the supravital technique showed an increase in the

proportion of erythroid elements, 1,023 erythroid to 990 myeloid. Of the erythroid cells, there was an increased proportion of the early erythroblasts (18 per cent), with late erythroblasts 29 per cent and normoblasts 52 per cent. The early erythroblasts were described as very large cells which were entirely typical. 31 per cent of the megalokaryocytes were of the type already described, represented by deeply staining nuclei, apparently denuded of cytoplasm. Some of the less darkly stained nuclei showed marked signs of damage to the chromatin.

All of the lymphoid tissues including the follicles of the spleen, the mesenteric, and the peripheral nodes were small but in sections were hyperplastic, owing to the same marked stimulation of the primitive cells seen in Rabbit R 210. None of these cells, however, were in division, and both in the spleen and in the lymph nodes there were large masses of them, showing a peculiar type of necrosis. This process involved only the pale stem cells. The reaction is illustrated in Fig. 6; in the photograph the nuclei are so pale that they are difficult to differentiate and in the section stained with hematoxylin and eosin the entire area had a slate blue color. The cells look as if there had been a complete solution and diffusion into the tissues of the stainable matter of the nuclei. This is the only animal in which such extensive necrosis of lymphoid tissues was found. In the mesenteric nodes there were large areas covering several low power fields showing this necrosis; some of the small peripheral nodes were completely involved and the entire thymus showed the reaction. Besides this reaction in the lymphoid tissues, there was a similar extensive damage to the liver cells. Most of the cells of this organ showed the same slate blue color of the cytoplasm with signs of damage to the chromatin of the nuclei; but the nuclei were much less depleted of chromatin than those of the primitive cells of the lymphoid tissues.

DISCUSSION

In this series of experiments a number of phenomena have been observed: marked changes in the lymphoid tissues eventually registered by a lymphopenia; a slight anemia associated with a destruction of red blood cells and, within the limits of this experiment, with only minimal changes in the bone marrow; atrophy of the thymus; a damage to the liver cells and a reduction in size of this organ; the frequent development of abscesses; one spontaneous fracture; and in two instances the development of osteogenic sarcomata. Throughout this discussion it is to be borne in mind that the number of animals used was small.

All studies on the biological effects of radioactive substances show that the dose is an important factor; as shown in the literature, depending on the dose, the effects have ranged from conditions in which only lymphoid tissues have been altered (44) to death in a short time

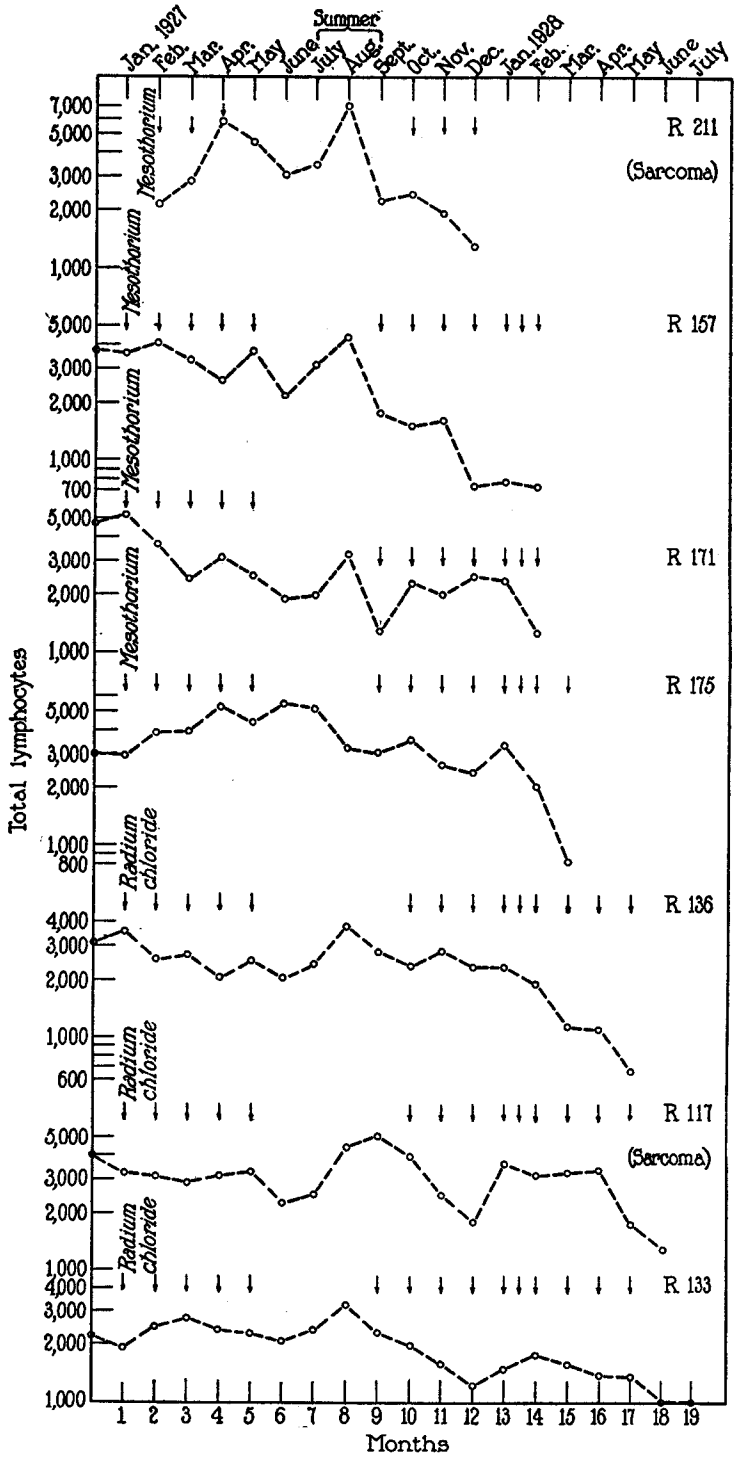
(1). In these experiments the dose of mesothorium was higher than of radium chloride in the proportion of 7.7 to 5.1 which in terms of alpha particles means a ratio of 38 to 20. In considering the biological effect of radium it is necessary to know the amount stored in the tissues, since a considerable amount of the radium given is excreted (49). Methods for measuring radioactive materials in the living person have been developed by Flinn *et al.* (49–52).

Effects on Lymph Nodes and Lymphocytes

The effects on the lymph nodes in our series can be followed in Figs. 1 to 6, and they are to be compared with the changes in the lymphocytes shown in Graph 2. In general it will be noted that the lymphocytes fell faster after mesothorium than after radium chloride. After three injections of 5.1 micrograms of radium chloride (R 210), only the peripheral lymph nodes were affected, indicating that they are more sensitive to radioactive materials than the mesenteric nodes and spleen. They showed a uniform hyperplasia of the primitive stem cells (Fig. 1) with extremely active cell division.

By the end of 11 months after six injections of 7.7 micrograms of mesothorium (R 211) all of the lymphoid tissues were involved. The animal still showed the increase in the primitive cells, but, in contrast to the previous experiment, there was no cell division. Instead, large areas of the primitive cells showed a peculiar type of degeneration in which the changes that could be detected were in the chromatin of the nuclei (Fig. 6). The sections look as if the chromatin had dissolved and diffused through the nuclear membrane into the cytoplasm. The same type of cellular damage involved all of the cells of the thymus. This was the only animal in which we found damage to the stem cells in the stage of their hyperplasia; in the other animals a similar damage was seen to the stem cells scattered in their normal proportions in the follicles. This difference will be clear by comparing Figs. 2 and 4.

The six remaining animals were allowed to live from 14 to 19 months after the first injection. The study of their tissues shows that after the period of stimulation to the primitive stem cell, there was a marked development of immature lymphocytes, the type of cells with deeply basophilic cytoplasm, known as the lymphoblast. In the follicles


Jan. 1927
Feb.
Mar.
Apr.
May
June
Summer
July
Aug.
Sept.
Oct.
Nov.
Dec.
Jan. 1928
Feb.
Mar.
Apr.
May
June
July
Total lymphocytes
Mesothorium
Radium chloride
R 211
(Sarcoma)
R 157
R 171
R 175
R 136
R 117
(Sarcoma)
R 133
Months

GRAPH 2

this was shown by a marked increase in the proportion of the large lymphoblasts to the mature small lymphocytes as seen in Fig. 5, while in many of the lymph cords there was a complete replacement of mature lymphocytes by lymphoblasts.

This condition was both accompanied and followed by signs of damage to the stem cell of the lymphocytes, illustrated by the cells with arrows in Figs. 4 and 6. This damage was a shrinkage of the nuclei, as can be seen by comparing with the corresponding normal nuclei in Fig. 2, and with a gradual depletion of the chromatin until nothing stainable was left in the nuclei but an inner rim to the nuclear membrane. No such signs of damage could be made out either in the lymphoblasts or in the mature lymphocytes.

The next stage in the changes of the lymph nodes was depletion, starting with the cells of the lymph cords and gradually involving the follicles as well. The contrast between the period of atrophy and the early stimulation is shown in Figs. 1 and 3. A node in which all of the lymph cords had disappeared looked cystic in the gross specimen; the process went on to the complete atrophy of some nodes. These processes were not all in the same stage in the different nodes of an animal. For example, in R 133 the follicles of the spleen showed a hyperplasia of the stem cell while the lymph nodes showed atrophy. In general the depletion was more extreme in the peripheral nodes than in the mesenteric nodes and in the spleen; in some instances the peripheral nodes had disappeared entirely. The effects on the lymphoid tissues may thus be summed up in three stages: first, a period of stimulation to the stem cell; second, a shift to the immature stage known as the lymphoblast; and third, a depletion of the nodes due to such a damage of the stem cell that the losses in lymphocytes could no longer be made up.

With these processes in mind it is interesting to follow the changes in the lymphocytes in the blood. The blood cells of the animal which developed the abscess and was killed after three injections are shown in Graph 1. We do not know the reason for the rise of all of the strains of white blood cells, unaccompanied by a rise in red cells, which is shown for April 21; the curve of the red cells indicated that it was not a process of dehydration. However, the subsequent curve of the lymphocytes shows that there was no sustained rise in circulating

lymphocytes in this animal to reflect the hyperplasia of the primitive stem cells shown in Fig. 1.

In Graph 2 it will be seen that during the initial 5 months in which the primitive cells of the lymph nodes were stimulated, three animals show a rise in lymphocytes, three a fall, and one no change. Thus there was no constant early reaction of the lymphocytes in the blood which may have been due to variations in the condition of the different lymph nodes.

All of the graphs show a rise in lymphocytes during the summer months. There are two factors to be considered in relation to the rise in lymphocytes in summer; for July and August the differential counts were made on fixed films and it is our experience that lymphocytes run on an average 4 per cent higher in counts made with fixed films than in counts of the living cells (68). In every instance, the first counts of lymphocytes, taken before the second series of injections was started, were lower than the counts in August. These points make the rise during the summer months of less significance as an effect of the injections of radioactive material.

Every graph shows that there was a final, significant, and steady fall in lymphocytes covering in general the period of the second series of injections. The study of the tissues for each animal has shown that the lymph nodes finally exhibited a marked shift to the lymphoblast and a depletion of the nodes due to a failure of the stem cells to replace lymphocytes.

The final period of the more precipitous fall in lymphocytes, covering the 3 or 4 months before each animal was killed, has been in each instance correlated with a loss in weight in the animals. They all showed a gain in weight during the early months of the experiment, probably due to the better feeding of the animals which were all bought from dealers and not bred in the laboratory. Four were females and of them three gave birth to young during the experiment. The loss in weight which is associated with the period of depletion of the lymph nodes is of great significance in connection with the studies of the relation of lymphocytes to resistance to cancer and to tuberculosis (9–26, 69, 70). Thomas (71) has found in a study of a series of tuberculous rabbits that in the final period of broken resistance there is a loss in weight which is proportional to the fall in lymphocytes.

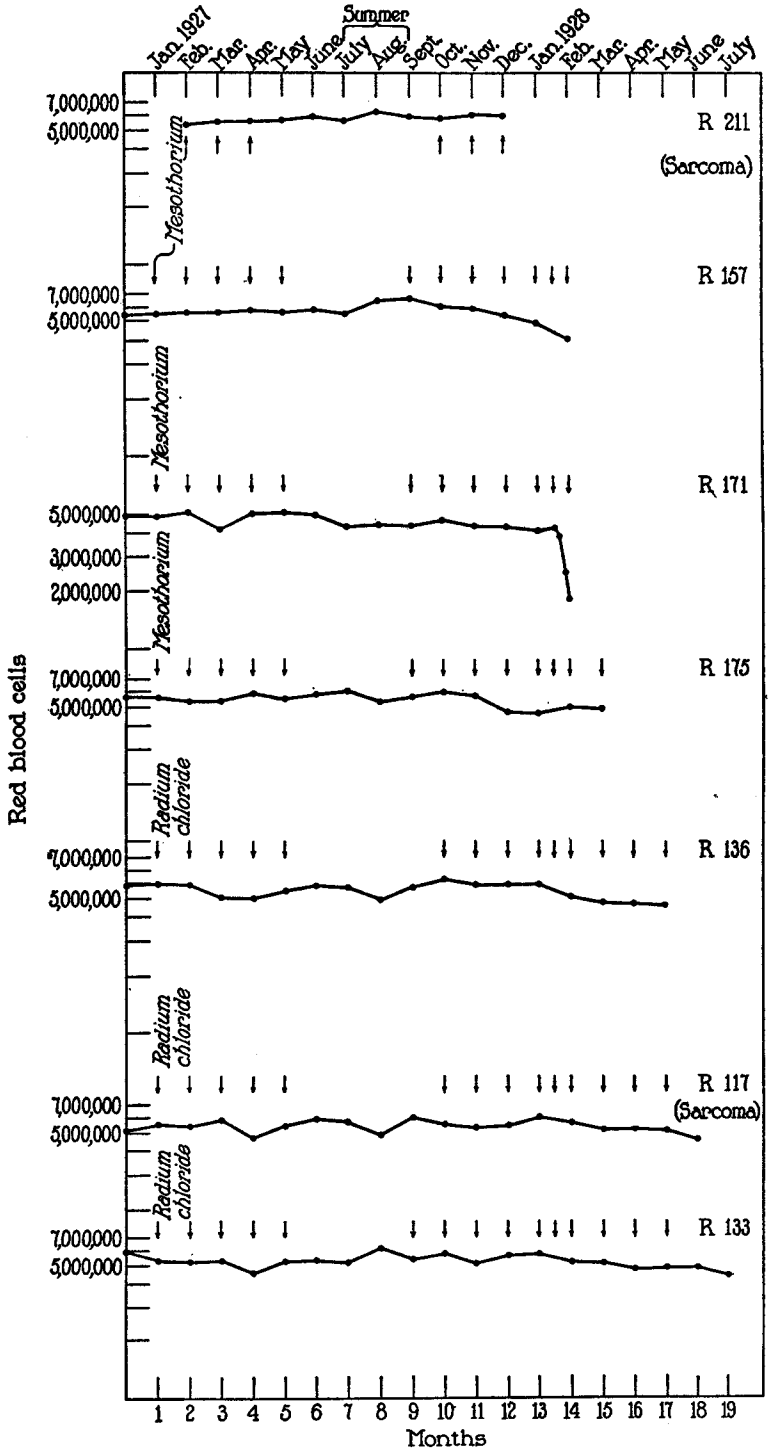
This series of radiated animals demonstrates the same phenomenon,—an atrophy of the lymph nodes, a lymphopenia, and at the same time a loss in weight. This correlation was constant and significant.

Effects on the Bone Marrow and the Red Cells

There were no changes in the neutrophilic leucocytes in this series other than those associated with the development of the abscesses. There were also no changes in eosinophilic leucocytes and in monocytes, but in every instance there was a fall in basophils during the period of the final fall in lymphocytes.

The fact that the bone marrow as a hematopoietic organ is less sensitive than the lymph nodes is shown not only by the lack of change in granulocytes but also by the slight changes in the red cells. This will be clear by comparing the graphs of the red cells (Graph 3) with those of the lymphocytes in Graph 2. There was no change whatever in the color index throughout the experiments, even in R 171, at the time of the precipitous fall in red cells. Rabbit R 211, which was killed in 11 months, showed no anemia whatever; the original count of red cells was 5,200,000 and the final count was 6,000,000.

It will be noted on Graph 3 that all of the animals except the first two showed a slight fall in red blood cells by March or April, but all except R 171 showed an excellent recovery to the level of 6,000,000 cells by August or the early months of the fall. All of the animals which received the extra injection in January showed then a progressive fall in red cells. The graph of R 171 shows that this animal was a special case in regard to the red blood cells; the original level of the red cells in this animal was lower than in the others and, from July on, there was a slight loss of red cells which became extreme after the extra injection in January. All of the figures on this chart are averages except the records shown for February for R 171, in which the 4 counts for the first half of the month are plotted separately in order to bring out better the rapidity and severity of the fall in red cells from 4,380,000 to 1,800,000. In the preceding months the counts of the red cells did not vary much from the average. The signs were of a peripheral destruction of red cells: marked fragmentation of these cells, as seen in the supravital preparations, and the extreme increase in iron-containing pigment in spleen and bone marrow seen


Jan. 1927
Feb.
Mar.
Apr.
May
June
Summer
July
Aug.
Sept.
Oct.
Nov.
Dec.
Jan. 1928
Feb.
Mar.
Apr.
May
June
July
Red blood cells
7,000,000
5,000,000
Mesothorium
R 211
(Sarcoma)
R 157
R 171
3,000,000
2,000,000
R 175
Radium chloride
R 136
R 117
(Sarcoma)
R 133
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19
Months

GRAPH 3

in sections. However, the bone marrow also showed greater damage than any of the other animals in the series, for it not only revealed the change in the fat cells and the marked mottling of active areas and zones of complete aplasia, but in the active areas there were some large zones of immature erythroblastic cells comparable to the great increase in lymphoblasts described in the lymph nodes. This is interesting in connection with the reports of the occurrence of a primary anemia due to exposure to radioactive substances in human cases (27, 38–43).

All of the other animals in the series, except the three that were killed too early (R 134, R 210, and R 211), showed changes in the fat cells and the mottling of the marrow but not the shift to early erythroblastic or megaloblastic marrow. The mottling of the marrow is due to a change in the normal distribution of active and inactive marrow; in the normal rabbit there are large areas, often at the periphery, of active marrow and smaller, centrally placed, inactive zones. The mottling is a change in this pattern. It is easily seen with the unaided eye and may indicate a local damage to the stem cells of the marrow, due to an uneven distribution of the radioactive material in the bone. The great reserve in the tissues of the marrow may account for the fact that so much change in the marrow was not reflected to a greater extent in the blood. From the results on Graph 3, it is likely that the first series of injections of the radioactive material caused enough peripheral destruction of the red cells to give rise to a slight anemia but that there was not enough damage to the bone marrow to be detected in the blood (R 211). With the second series, however, the crowding of the two injections into the 1 month caused not only an increased peripheral destruction but also instigated changes in the bone marrow which were progressive. In one animal in the series (R 171) both the red cells and the bone marrow were more susceptible to radioactive material than in the other animals, and in this instance there occurred both extreme destruction of mature red cells and the early signs of the changes in the marrow of the increased proportion of immature cells of the erythroid series which eventually results in an anemia of the primary type. Thus, the effects on the red cells were similar in type but less in degree than those on the lymph nodes. They are, increased destruction of red cells, a shift to the immature phases in the marrow, and finally aplasia.

Effects on Thymus and Liver

The observations on the thymus in our series are meager, but they suggest that the cells of the thymus (R 211) are affected in the same manner as the primitive stem cells of the lymph nodes and that they are not as resistant to radioactive material as mature lymphocytes. The end-result of this effect is a complete atrophy of the organ. A damage to the liver has been noted in four animals; with mesothorium (three animals) there were changes in the nuclei, similar in kind but less in amount than those of the stem cell of the lymph nodes; namely, a loss of chromatin from the nucleus into the cytoplasm. The damage was reflected in a marked reduction in the size of this organ. Both of the animals which showed paralysis—one of them certainly without damage of the central nervous system—exhibited the reduction in size of the liver. These symptoms are possibly explained by the demonstration by Mann (72) of weakness and paralysis in dogs after removal of the liver and of the same phenomena in rabbits after partial liver insufficiency by McMaster and Drury (73, 74) and after total removal of the liver by Drury (75).

Production of Osteogenic Sarcomata through Storage of Radioactive Material in the Bones

Certain results in our series of animals are interesting in relation to the industrial hazard in the use of radioactive materials: first, the number of abscesses that developed; second, the spontaneous fracture; and third, the development of osteogenic sarcomata in two animals. Our percentage of tumor formation, two out of seven animals, was high in comparison with the figures of Daels *et al.* It has been demonstrated that the bones store the radioactive material. The sarcomata were clearly derived from osteoblasts; in one instance there was newly formed bone associated with giant cells of the type of the osteoclast. In one case there were metastases. We have lacked one element in the proof of malignancy; namely, successful transplantation of the tumors. No attempts in this direction were made.

SUMMARY

The observations in this work suggest that with certain doses of radioactive material, the fundamental damage in the lymphoid tissues

is to the stem cell and that the damage is to the chromatin of the nuclei of these cells. The erythroid tissues are apparently less susceptible to radioactive material than the lymphoid tissues but an original anemia of secondary type from peripheral destruction may eventually be changed to one of primary type through decreased maturation of primitive cells in the marrow. The damage of lymph nodes and bone marrow leads to atrophy of these organs. The cells of the liver and thymus suffer nuclear damage of the same general character as is seen in the lymph nodes, and there is an atrophy of these organs.

The storage of the radioactive material in the bones gave rise to osteogenic sarcomata in two out of seven rabbits surviving from 11 to 19 months. A repetition of the experiment has been undertaken with more intensive studies to test the validity of the findings.

BIBLIOGRAPHY

1. London, E. S., *Berl. klin. Woch.*, 1903, **40**, 523.
2. Heineke, H., *Münch. med. Woch.*, 1904, **51**, 785; 1903, **50**, 2090.
3. Milchner, R., and Mosse, M., *Berl. klin. Woch.*, 1904, **41**, 1267.
4. Helber, E., and Linser, P., *Münch med. Woch.*, 1905, **52**, 689.
5. Aubertin, C., *Arch. électr. méd.*, 1912, **20**, 150, reviewed in *Arch. mal. coeur.*, 1912, **5**, 550; *Compt. rend. Soc. biol.*, 1912, **72**, 84.
6. Aubertin, C., and Bordet, E., *Arch. mal. coeur.*, 1909, **2**, 321.
7. von Jagic, N., Schwarz, G., and von Siebenrock, L., *Berl. klin. Woch.*, 1911, **48**, 1220.
8. Gudzent, F., and Halberstaedter, L., *Deutsch. med. Woch.*, 1914, **40**, 633.
9. Webb, G. B., and Williams, W. W., *Nat. Assn. Stud. and Prev. Tuberc., Tr. 5th Ann. Meeting*, 1909, 231.
10. Webb, G. B., Williams, W. W., and Basinger, A. F., *Nat. Assn. Stud. and Prev. Tuberc., Tr. 6th Ann. Meeting*, 1910, 279.
11. Murphy, Jas. B., and Ellis, A. W. M., *J. Exp. Med.*, 1914, **20**, 397.
12. Taylor, H. D., and Murphy, Jas. B., *J. Exp. Med.*, 1917, **25**, 609.
13. Murphy, Jas. B., and Sturm, E., *J. Exp. Med.*, 1919, **29**, 35.
14. Hussey, R. G., *J. Exp. Med.*, 1921, **33**, 337.
15. Murphy, Jas. B., and Morton, J. J., *Science*, 1915, **42**, 842; *Proc. Nat. Acad. Sc.*, 1915, **1**, 435; *J. Exp. Med.*, 1915, **22**, 204, 800.
16. Murphy, Jas. B., and Nakahara, W., *J. Exp. Med.*, 1920, **31**, 1.
17. Nakahara, W., and Murphy, Jas. B., *J. Exp. Med.*, 1921, **33**, 327.
18. Hussey, R. G., *J. Gen. Physiol.*, 1921–22, **4**, 511.
19. Murphy, Jas. B., and Taylor, H. D., *J. Exp. Med.*, 1918, **28**, 1.
20. Taylor, H. D., Witherbee, W. D., and Murphy, Jas. B., *J. Exp. Med.*, 1919, **29**, 53.

21. Thomas, M. M., Taylor, H. D., and Witherbee, W. D., *J. Exp. Med.*, 1919, **29,** 75.
22. Nakahara, W., *J. Exp. Med.*, 1919, **29,** 83.
23. Murphy, Jas. B., and Sturm, E., *J. Exp. Med.*, 1925, **41,** 245.
24. Nakahara, W., and Murphy, Jas. B., *J. Exp. Med.*, 1920, **31,** 13; 1921, **33,** 429; 1922, **35,** 475; 1921, **33,** 433.
25. Murphy, Jas. B., Liu, J. H., and Sturm, E., *J. Exp. Med.*, 1922, **35,** 373.
26. Murphy, Jas. B., The lymphocyte in resistance to tissue grafting, malignant disease, and tuberculous infection. An experimental study. Monograph of The Rockefeller Institute for Medical Research, No. 21, New York, 1926.
27. Mottram, J. C., *Arch. Radiol. and Electrother.*, 1920–21, **25,** 194; *Brit. Med. J.*, 1921, **2,** 269; *Proc. Roy. Soc. Med.*, 1922–23, **16,** path., 9.
28. Mottram, J. C., and Clarke, J. R., *Proc. Roy. Soc. Med.*, 1920, **13,** electrother., 25; *Arch. Radiol. and Electrother.*, 1919–20, **24,** 345; *Am. J. Roentgenol.*, 1920, **7,** 355.
29. Pfahler, G. E., *Am. J. Roentgenol.*, 1922, **9,** 647.
30. Caffaratti, M., *Radiol. Med.*, 1922, **9,** 317.
31. Lazarus-Barlow, W. S., *Great Britain Med. Research Council, Special Rep. Series, No. 62*, 1922, 33.
32. Williams, R. C., *Pub. Health Rep., U. S. P. H. S.*, 1923, **38,** 3007.
33. Amundsen, P., *Acta Radiol.*, 1924, **3,** 1.
34. Jaulin, *J. Radiol. et Electr.*, 1927, **11,** 193.
35. De Laet, M., *Ann. Med. Legale*, 1928, **8,** 443.
36. Lavedan, J., *Radiophys. et Radiothér.*, 1928–29, **1,** 477.
37. Rosenthal, N., *Am. J. Clin. Path.*, 1931, **1,** 7.
38. Brulé, M., and Boulin, R., *Bull. et mém. Soc. méd. hôp. Paris*, 1925, series 3, **49,** 529.
39. Emile-Weil, P., and Lacassagne, A., *Bull. Acad. méd.*, Paris, 1925, series 3, **93,** 237.
40. Laignel-Lavastine, and George, P., *Bull. et mém. Soc. méd. hôp. Paris*, 1926–27, series 3, **50,** 1617.
41. Emile-Weil, P., *Bull. et mém. Soc. méd. hôp. Paris*, 1926–27, series 3, **50,** 1627.
42. Martland, H. S., *Arch. Path.*, 1926, **2,** 465.
43. Reitter, G. S., and Martland, H. S., *Am. J. Roentgenol.*, 1926, **16,** 161.
44. Martland, H. S., *J. Am. Med. Assn.*, 1929, **92,** 466, 552; *Am. J. Cancer*, 1931, **15,** 2435.
45. Hoffman, F. L., *J. Am. Med. Assn.*, 1925, **85,** 961.
46. Castle, W. B., Drinker, K. R., and Drinker, C. K., *J. Ind. Hyg.*, 1925, **7,** 371.
47. Martland, H. S., Conlon, P., and Knef, J. P., *J. Am. Med. Assn.*, 1925, **85,** 1769.
48. Martland, H. S., and Humphries, R. E., *Arch. Path.*, 1929, **7,** 406.
49. Flinn, F. B., *Boston Med. and Surg. J.*, 1928, **197,** 1309; *Am. J. Roentgenol.*, 1929, **22,** 554; *J. Am. Med. Assn.*, 1926, **87,** 2078; 1931, **96,** 1763.
50. Flinn, F. B., and Seidlin, S. M., *Bull. Johns Hopkins Hosp.*, 1929, **45,** 269.

51. Schlundt, H., Barker, H. H., and Flinn, F. B., *Am. J. Roentgenol.*, 1929, **21,** 345.
52. Barker, H. H., and Schlundt, H., *Am. J. Roentgenol.*, 1930, **24,** 418.
53. Marie, P., Clunet, J., and Raulot-Lapointe, G., *Bull. Assn. franç. étude cancer*, 1910, **3,** 404.
54. Bloch, B., *Schweiz. med. Woch.*, 1924, **5,** 857.
55. Goebel, O., and Gérard, P., *Compt. rend. Soc. biol.*, 1925, **93,** 1537.
56. Daels, F., *Brit. J. Radiol.*, 1925, **30,** 474; *Bull. Assn. franç. étude cancer*, 1926, **15,** 103.
57. Daels, F., and Baeten, G., *Bull. Assn. franç. étude cancer*, 1926, **15,** 162; *Lancet*, 1926, **2,** 666.
58. Daels, F., and Biltris, R., *Bull. Assn. franç. étude cancer*, 1931, **20,** 32; 1927, **16,** 772.
59. Maisin, J., and Dupuis, P., *Rev. Belge Sc. Med.*, 1929, **1,** 409.
60. Schürch, O., and Uehlinger, E., *Z. Krebsforsch.*, 1931, **33,** 476.
61. Ewing, J., Neoplastic diseases, Philadelphia, W. B. Saunders and Co., 3rd edition, 1928.
62. Sabin, F. R., Austrian, C. R., Cunningham, R. S., and Doan, C. A., *J. Exp. Med.*, 1924, **40,** 845.
63. Wiseman, B. K., *J. Exp. Med.*, 1931, **53,** 499; **54,** 271; *Folia haematol.*, 1932, **46,** 346.
64. Sabin, F. R., and Doan, C. A., *Proc. Soc. Exp. Biol. and Med.*, 1927, **25,** 121.
65. Sabin, F. R., *Physiol. Rev.*, 1928, **8,** 191.
66. Arey, L. B., *Am. J. Anat.*, 1919–20, **26,** 315.
67. Mottram, J. C., *Arch. Radiol. and Electrother.*, 1920–21, **25,** 197.
68. Sabin, F. R., Cunningham, R. S., Doan, C. A., and Kindwall, J. A., *Bull. Johns Hopkins Hosp.*, 1925, **37,** 14.
69. Cunningham, R. S., Sabin, F. R., Sugiyama, S., and Kindwall, J. A., *Bull. Johns Hopkins Hosp.*, 1925, **37,** 231.
70. Sabin, F. R., Doan, C. A., and Forkner, C. E., *J. Exp. Med.*, 1930, **52,** suppl. 3, 1.
71. Thomas, R. M., *J. Exp. Med.*, 1932, **56,** 185.
72. Mann, F. C., *Medicine*, 1927, **6,** 419.
73. McMaster, P. D., and Drury, D. R., *J. Exp. Med.*, 1929, **49,** 745.
74. Drury, D. R., and McMaster, P. D., *Proc. Soc. Exp. Biol. and Med.*, 1929, **27,** 48.
75. Drury, D. R., *J. Exp. Med.*, 1929, **49,** 759.

EXPLANATION OF PLATES

PLATE 16

FIG. 1. Peripheral lymph node of Rabbit R 210, killed 17 days after the third intravenous injection of 5.1 micrograms of radium chloride, to show the degree of hyperplasia of the pale cells of the follicles. Stained in hematoxylin and eosin. ×50.

FIG. 2. Center of one of the peripheral follicles of the same node as in Fig. 1, to show the predominance of large pale cells and the number of them in division. Stained in hematoxylin and eosin. ×1,000.

FIG. 3. Cervical lymph node of Rabbit R 136, killed 22 days after the fourteenth intravenous injection of 5.1 micrograms of radium chloride, to show the stage of depletion of the nodes. Stained in hematoxylin and eosin. ×50.

FIG. 4. Mesenteric lymph node from Rabbit R 157, killed 5 days after the twelfth intravenous injection of 7.7 micrograms of mesothorium, to show the damage to the stem cells, which are indicated by arrow. The normal small lymphocytes are printed very dark in order to bring out the pale nuclei of the stem cells. Stained in hematoxylin and eosin. ×1,000.

FIG. 5. Peripheral lymph node of Rabbit R 171, killed 15 days after the twelfth injection of 7.7 micrograms of mesothorium to show the decrease in mature lymphocytes and the increase in lymphoblasts in a peripheral follicle. Stained in Giemsa. ×270.

FIG. 6. Mesenteric lymph node of Rabbit R 211, killed 22 days after the sixth injection of 7.7 micrograms of mesothorium, to show the damage to masses of the stem cells in a peripheral follicle. The small dark nuclei are of normal small lymphocytes. Stained in hematoxylin and eosin. ×1,000.

PLATE 17

FIG. 7. Sarcoma in the bone marrow of the left humerus of Rabbit R 117, which was killed 41 days after the fourteenth injection of 5.1 micrograms of radium chloride. The left half of the figure is the sarcoma; the right half shows the zone of invasion of the bone marrow by the tumor cells, with more normal bone marrow at the extreme right. Stained in hematoxylin and eosin. ×120.

FIG. 8. Section from the center of the sarcoma seen in Fig. 7, to show the character of the tumor cells, one of which is in division. Stained in hematoxylin and eosin. ×1,000.

FIG. 9. Section through the edge of the sarcoma seen in Fig. 7, to show the tumor cells, marked by arrows, invading the marrow between masses of myelocytes, leucocytes, and megalokaryocytes. Stained in hematoxylin and eosin. ×1,000.

FIG. 10. Section of the lung of Rabbit R 157, killed after the twelfth intravenous injection of mesothorium, to show the complete absence of air sacs and the proliferation of bronchial epithelium due to a chronic inflammatory process. Arrow A—bronchus; Arrow B—membrane of bronchial epithelium. Stained in hematoxylin and eosin. ×200.

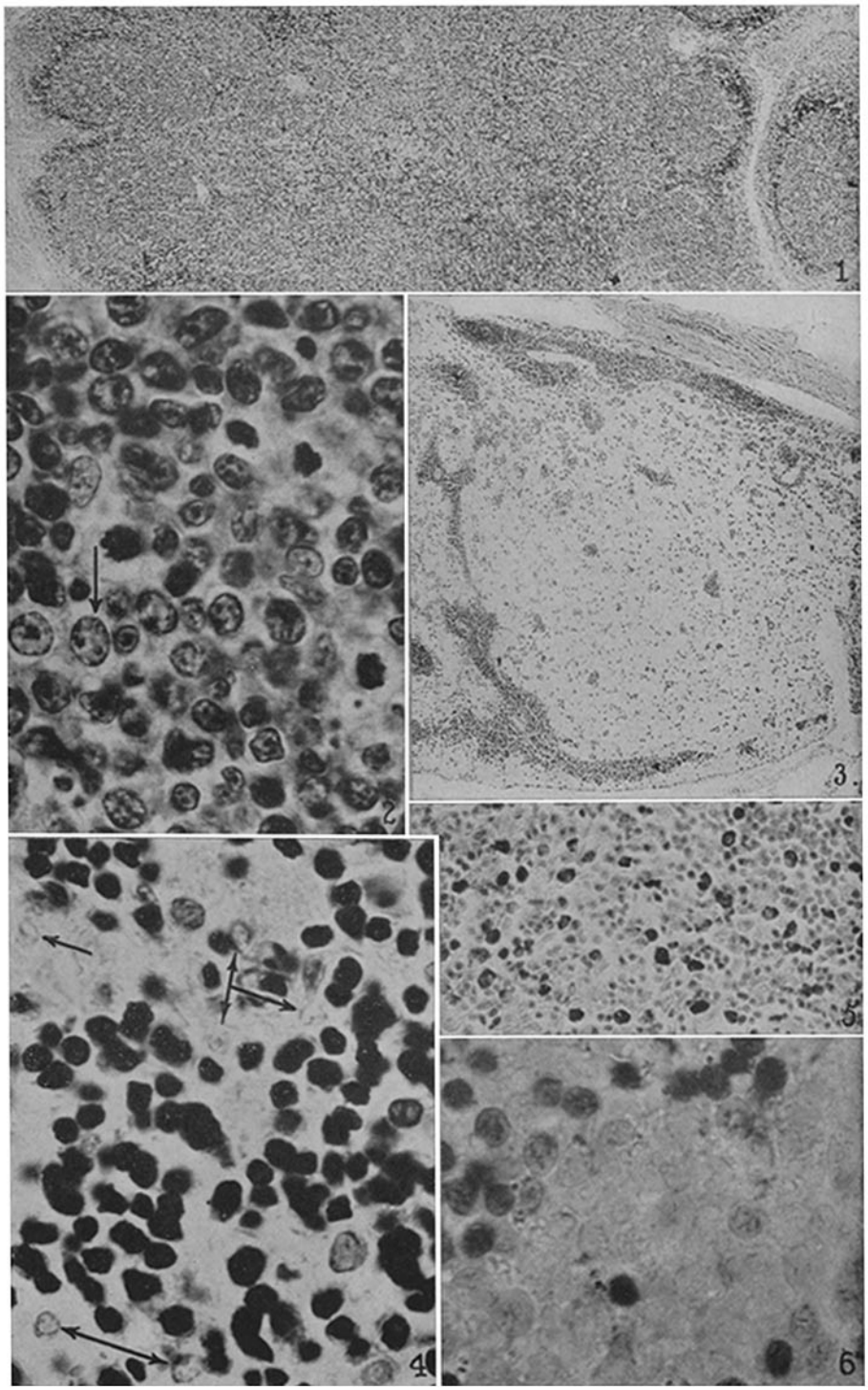
PLATE 18

FIG. 11. Section of a sarcoma which almost completely replaced the bone marrow of the right femur of Rabbit R 211, killed 22 days after the sixth injection of 5.1 micrograms of radium chloride. Stained in hematoxylin and eosin. ×120.

FIG. 12. Section of the tumor, shown in Fig. 11, to show the newly formed bone, Arrow A, and the giant cell, Arrow B, of the type of osteoclast near the bone. Stained in hematoxylin and eosin. ×1,000.

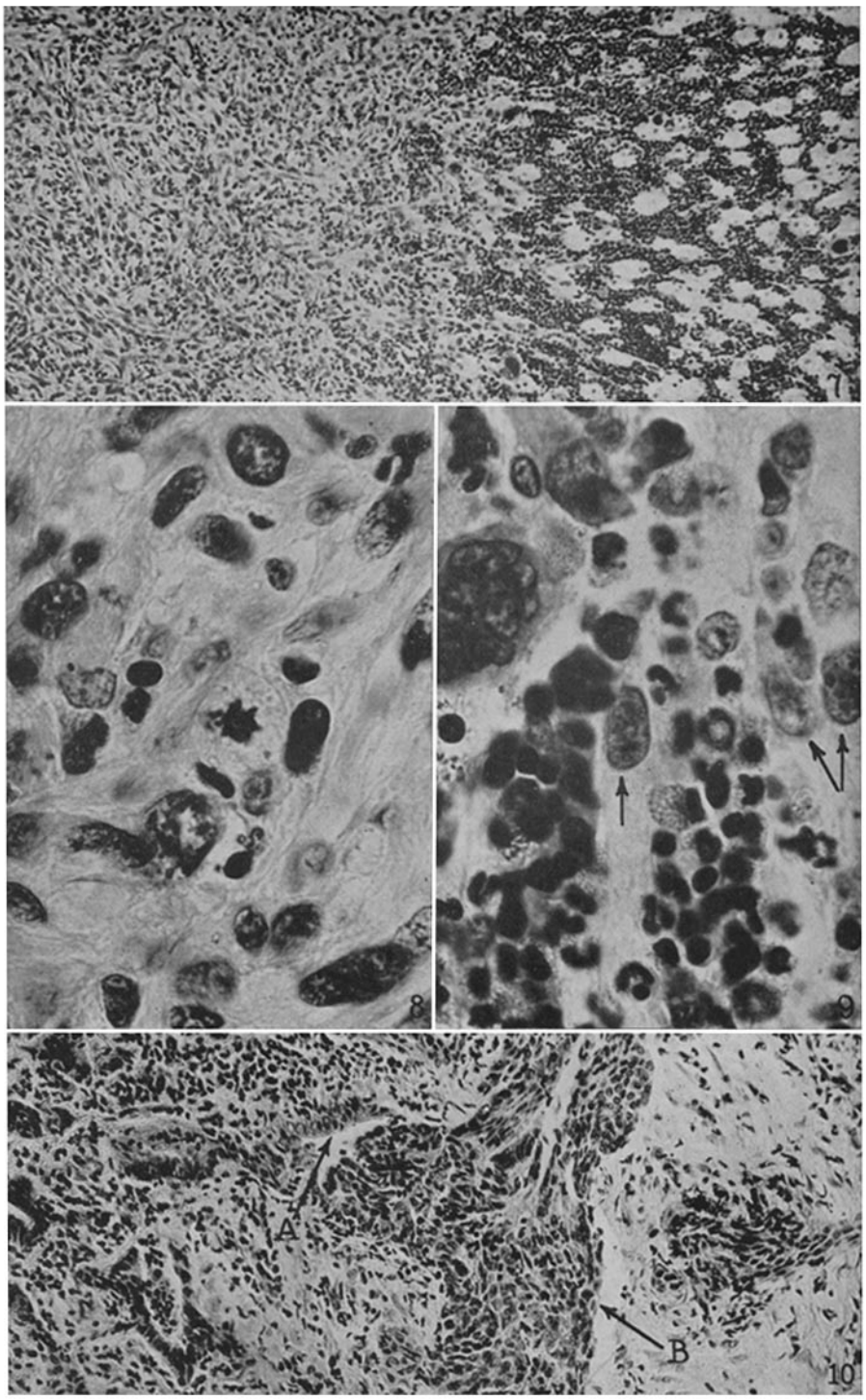
FIG. 13. Section of bone from Rabbit R 211, near the edge of a sarcoma of the bone marrow. The edge of the bone is denuded of osteoblasts, but they may be represented by the three large tumor cells nearby. Stained in hematoxylin and eosin. ×1,000.

FIG. 14. Damaged bone near the place of fracture from the right femur of Rabbit R 211 to show the complete freeing of the bone corpuscles from their canaliculi and the fragmentation of the bony matrix. Stained in hematoxylin and eosin. ×270.



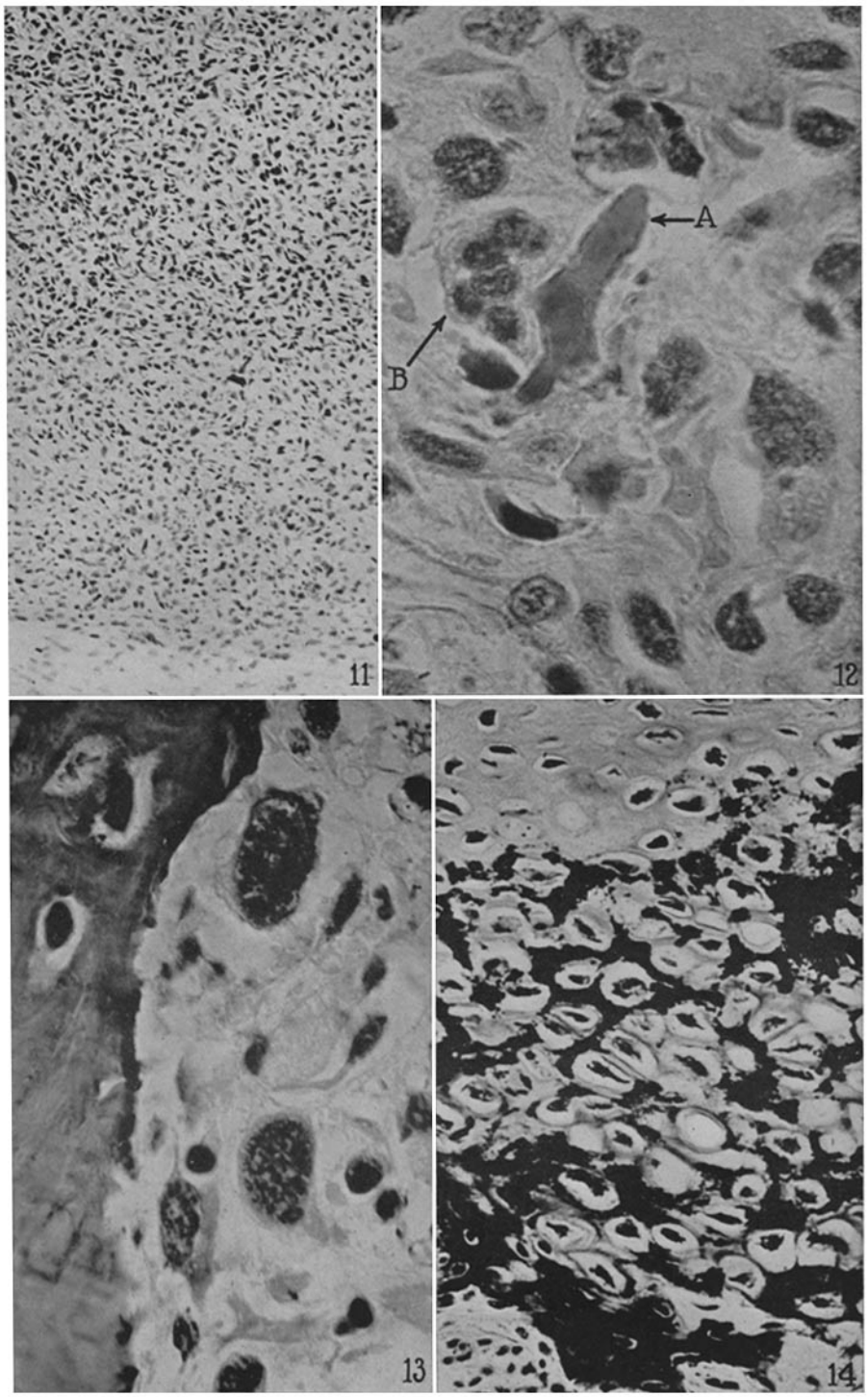
Photographed by Louis Schmidt

(Sabin *et al.*: Radium chloride and mesothorium injections)



Photographed by Louis Schmidt

(Sabin *et al.*: Radium chloride and mesothorium injections)



Photographed by Louis Schmidt

(Sabin *et al.*: Radium chloride and mesothorium injections)