II. APLASIA OF MARROW AND FATAL INTOXICATION IN DOGS PRODUCED BY ROENTGEN RADIATION OF ALL BONES

By SAMUEL S. SHOUSE, M.D., STAFFORD L. WARREN, M.D., AND GEORGE H. WHIPPLE, M.D.

(From the Departments of Pathology and Radiology of the University of Rochester School of Medicine and Dentistry, Rochester, N. Y.)

(Received for publication, December 12, 1930)

Our main objective in these experiments was the production of *aplasia of the bone marrow.* In this we were successful but at the same time there developed a peculiar and fatal intoxication which terminated the experiments. This peculiar intoxication associated with exposure of the bones to Roentgen radiation has not been previously described and holds some interest for workers in this field.

Many factors may be concerned in the cause of death in these animals. One may believe that the destruction of this mass of cells in the marrow may free split products which are poisonous and cause the fatal intoxication. It is fair to say however that the cell breakdown comes early whereas the serious intoxication comes only the day before death when practically all marrow cells and circulating leucocytes have vanished. The rise in non-protein nitrogen in the blood is also limited to the last day or two of life.

The extraordinary drop in circulating *white blood cells* (agranulocytosis) may be concerned with the fatal intoxication in some experiments where we find massive colonies of bacteria growing in the lung tissue. There is total lack of tissue and white cell reaction to such bacterial invasion.

We recall the drop in *blood platelets* which practically disappear from the circulating blood the day before death. This may be largely responsible for the bleeding into the tissues which takes place during the last 24 hours of life. There is evidence that practically all megakaryocytes of the marrow are destroyed by the radiation and this gives confirmatory evidence to indicate the origin of the platelets from the

megakaryocytes. Furthermore it indicates a short life cycle for the blood platelet in the circulation.

It may be argued that the radiation injures the capillary endothelium which therefore is responsible for the tissue bleeding. It should be recalled that the tissue bleeding occurs in the gastrointestinal muscle coats which are shielded with lead and in addition are known to be resistant to direct radiation (12).

All in all, the sudden bleeding into the tissues in the last day of life seems to be the most important factor in causing death on the 8th or 9th day following radiation of the skeleton. Dogs will survive this massive radiation if a small area of the skeleton escapes the field of radiation. This again suggests the possibility that platelets may be contributed by these uninjured areas in sufficient numbers to prevent the extensive purpura. However it seems wise to admit that other factors may be concerned—some unknown.

Finally we wish to emphasize one conspicuous feature. Death occurs on the 8th or 9th day with great regularity when the standard filtered radiation is given over all bones. It seems as difficult to explain this *latent period* satisfactorily as it is to give a comprehensive explanation for the *delay* in breakdown of the skin ("x-ray" burn) 2 to 3 weeks after radiation. The clinical picture is that of a normal healthy dog—active, good appetite, no loss of weight for 6 or 7 days after radiation. Then suddenly there is loss of appetite and perhaps a little vomiting but no clinical evidence of severe intoxication until a few hours before death.

It should be pointed out that the average of many experiments shows a drop in red cell hematocrit from a normal of about 50 per cent to a level of about 40 per cent within 7 days. One might argue that the exposure to Roentgen radiation caused complete cessation of red cell production and that this loss of circulating red cells represented the normal wear and tear of circulatory function. If this were so then the life cycle of the red cells under such conditions would approximate 5 weeks. There are too many confusing factors to justify a claim that such experiments are conclusive, much as we would like to establish the life cycle period of the normal red cell in the circulation of the dog.

Space will not permit a careful review of the experimental and clinical observations which relate indirectly to the experiments given

below. Those specially interested are referred to papers by Lacassagne (5), Minot and Spurling (7), Krömeke (4), Warren (11), Linser and Helber (2, 6), Heineke (1), Krause and Ziegler (3), Piney (8, 9). The smaller laboratory animals (especially the rabbit) are used by most workers and the dog but rarely.

The acute changes due to radiation in massive doses as recorded in the literature are few in number. The leucopenia with general reduction of the circulating leucocytes and the disappearance of the lymphocytes is well established. There is some disagreement about the immediate leucocytosis occuring a few hours after radiation but the later workers seem to have demonstrated it in rabbits. The leucopenia commences 24 hours or so after the radiation. The single report (Lacassagne) of purpura in new-born rabbits just before death does not seem to have been observed by others. Most reports do not show any disturbance of the platelets or adult red blood cells. Recently Wright and Bulman (1929) (13) have described changes in the platelets in prolonged experiments on the cat. Injury to the lymph nodes and spleen is well known. Many authors state that the marrow injury is extreme yet picture or describe many marrow cells remaining in sections after the radiation. Thorum X given by injection may bring about changes very like those described in our experiments on dogs with massive doses of radiation. We refer to the papers of Valeeff (1927) (10).

Method

In these experiments, normal, strong, healthy stock dogs were used throughout. Care was taken to select animals immune to distemper since the prolonged drop in the temperature following the amytal anesthesia often precipitates an attack of distemper or some acute respiratory infection which usually terminates the experiment. The animals were kept on a general diet and were given as much food and water as they would take.

Preliminary white and red blood cell counts, cover slip blood smear preparations, and hematocrits were made in every case during the 2 or more days of observation before the radiation was administered.

Radiation was at all times given while the animal was under amytal anesthesia. Complete anesthesia was always necessary in order to keep the animal properly located over the portal and properly shielded. At first the amytal was administered intraperitoneally, but often the induction of the anesthesia was prolonged and uncertain. Through the courtesy of Eli Lilly and Company, sodium amytal

was obtained in pure form in ampules. This drug can be given intravenously and is followed promptly by anesthesia with satisfactory experimental results.

The following procedure was finally worked out and used in the complete experiments reported below. ¼ grain of morphine was given subcutaneously, ½ hour before the sodium amytal. Sodium amytal, estimated on the basis of 35 to 40 mg. per kilo of body weight, was then injected at the rate of 10 mg. per minute, until the animal was sound asleep. Enough was injected to reduce the eyelid reflex but not to abolish it. Complete anesthesia lasted usually from 5 to 6 hours. The animal was kept in a warm room, wrapped in cloths, and was fully awake by the next day.

Usually two dogs of equal size and weight were exposed to the radiation at one time, since our equipment consists of a deep therapy couch with two portals equally distant from the target. The maximum portal size is 30 cm. square, so that the exposure had to be divided into two portions in order to cover the whole body of the animal. Both exposures were given at one sitting.

When completely under the anesthetic, the animals were carefully adjusted so that the upper half of the whole body including the head and the paws and forelegs and the trunk down to the costal margin was covered by the boundaries of one portal. After the dose was given over this area, the animal was shifted and placed on its side over the portal. A piece of lead ⅛ inch thick was carefully adjusted to protect completely all of the abdominal viscera in such a manner as not to shield the lumbar spine or the pelvis. This was controlled by fluoroscopic observation. The hind legs and paws, the tail, pelvis, and lumbar spine up to and slightly over the edge of the portion radiated at the previous exposure were carefully included within the field of radiation.

The factors of radiation were: target skin distance, 60 cm.; filter, ½ mm. of copper, 1 mm. of aluminum; 200 kilovolts; 35 milliamperes, watercooled Coolidge tube. The time of exposure varied from 20 to 80 minutes though the usual standard dose was given in 40 minutes, equivalent to 1,050 milliampere minutes. This dosage, which is three times the human epilation dose, always gives an epilation in a dog in 3 weeks with a very mild erythema which is only visible in white or through unpigmented areas of the skin. With repeated trauma such as might occur in the axilla, there is occasionally a slight amount of desquamation of the superficial layers of the skin. The greatest variation in the voltage, as indicated by the sphere gap, in any of the experiments was from 190 to 210 kilovolts. Usually, however, the kilovoltage was maintained throughout the experiment at a constant level as indicated by ionization chamber, electrostatic volt meter, and the sphere gap readings.

On the 2nd day after the radiation, hematocrit and blood counts were again done and continued every other day until death occurred. When the animals became moribund, they were etherized and a complete autopsy done. Sections of all the organs, the bones, and the bone marrow were saved for microscopic study. These were fixed in Zenker's solution and cut and stained with hematoxylin-eosin in the usual manner.

In order to control the peripheral destruction of adult red blood cells by the radiation given, a fragility study was done as follows. Blood was withdrawn from a normal dog and placed in small glass bottles containing a small amount of dry citrate to prevent coagulation. One bottle was kept protected from radiation behind a lead screen in the same room so as to control both the temperature and radiation changes. One bottle was radiated 1 hour, another 1½ hours, and another 2 hours, making a total of 2,100 milliampere minutes, 3,150 milliampere minutes, and 4,200 milliampere minutes respectively; no filter except the glass, 57 cm. target-bottle distance, 200 kilovolts, 35 milliamperes. The filtration of the glass is negligible since these bottles are barely visible upon a fluoroscopic screen and are not as dense as the blood which they contain. These doses are far beyond those given to the dogs treated over the bone marrow and the absence of filters brings into play all of the wave lengths in the unfiltered beam of 200 kilovolts. There was no hemolysis of the cells in any of the bottles. The routine fragility set-up was used and no definite change in the response of the cells to either hypotonic or hypertonic solution was found.

EXPERIMENTAL OBSERVATIONS

A sufficient number of dogs was used in this study but some experiments were incomplete in one way or another. At times the shielding of the intestinal coils was inadequate and intoxication with diarrhea developed as a result of injury of the intestinal mucosa by radiation. This has been described elsewhere (12) and such experiments were excluded from this series. Other animals received sublethal doses and ultimately recovered. Some animals showed recovery due to the fact that some areas of the skeleton escaped the radiation. These experiments occurred in the early stages of the work when the anesthesia was not deep enough to insure complete immobility. Many animals developed acute respiratory infections or distemper which terminated the experiment before the typical intoxication due to the radiation developed. The leucopenia was probably largely responsible for these infections.

The amount of heavily filtered radiation which was uniformly successful in producing the acute, fatal intoxication was 1,050 milliampere minutes. Less than this or 700 milliampere minutes was occasionally followed by a fatal result. More than 1,050 milliampere minutes caused no appreciable change in the course of events.

We record three typical experiments which are fair examples of a considerable group. We believe these experiments give a fair picture

of the peculiar intoxication and marrow aplasia caused by this standard radiation.

This experiment shows the typical course of events with death 9 days after radiation of the whole skeleton. Radiation 1,050 milliampere minutes given over upper half and again over the lower half of the skeleton with lead shielding of the abdominal cavity. Leucopenia, platelet deficiency, and terminal purpura are conspicuous features.

Dog 28–257.—A young brown female mongrel, well developed, well nourished, and active. Weight 14.1 kg.

6.19.29. Hematocrit 46 per cent; plasma pale lemon colored.

6.20.29. Active and lively. Appetite excellent.

6.21.29. Active and lively. Appetite excellent. Hematocrit 47 per cent, plasma clear straw colored. W. B. C. 15,000; 15,850. Smear of the blood shows the red cells to be of good color. There is some variation in size but no other abnormality. Platelets are numerous. The differential is essentially normal: polymorphonuclears 85 per cent, eosinophiles 6 per cent, large mononuclears and transitionals 6 per cent, small mononuclears 3 per cent. No abnormal cells are noted. 8 p.m. *Deep therapy* under amytal anesthesia, 35 mg. per kg., administered intraperitoneally. 1,050 M.A.M. laterally over the upper half of the right side of the body including the head and extremities to the costal margin. 1,050 M.A.M. laterally over the lower half of the body from the costal margin to and including the tail and extremities. The abdomen is protected by a sheet of $\frac{1}{8}$ inch lead.

6.22.29. Alert but still ataxic from the anesthetic.

6.23.29. Very active and playful. Appetite excellent.

6.24.29. Active—condition and appetite excellent. Hematocrit 41 per cent, plasma somewhat red. W.B.C. 4,850; 4,900.

6.25.29. Active, appetite and condition excellent.

6.26.29. Clinically normal.

6.27.29. Active and alert. Anorexia. Hematocrit 36 per cent, plasma clear straw colored. W.B.C. 0; 25 (checked).

6.28.29. Seems alert and active but nothing eaten.

6.29.29. Condition seems good. Anorexia. Small amount of vomitus in cage (undigested food). No evidence of hemorrhage. Hematocrit 37 per cent, plasma clear straw colored. W.B.C. 25; 50. The layer of W.B.C. in the hematocrit tubes on 6.27 and 6.29 was so thin as to be hardly more than a visible trace. On 6.29 smear only five polymorphonuclear neutrophiles and one disintegrated white blood cell were found on careful search of the two parts of a cover slip preparation. Platelets were nowhere visible. The red blood corpuscles showed pallor but little other abnormality.

6.30.29. 9:45 a.m. Found dead, body warm, slight rigor mortis.

Autopsy at once. The body is well developed and nourished. The skin and hair are apparently normal. There is a small superficial ulceration of the left side of the mouth. Both nasal passages and the accessory nasal sinuses are filled with foul semi-solid grayish material mixed with blood clots. There is no ulceration of the mucous membranes.

The pleural and pericardial surfaces both visceral and parietal show numerous small hemorrhages. There is no free blood in either thoracic cavity or in the pericardial sac. The peritoneal surfaces are smooth and shiny.

The heart shows several ecchymoses in the epicardial fat and endocardium. The muscle is in good condition.

Lungs: There are several small solid hemorrhagic areas in each cut section of every lobe. The middle lobe of the right lung is entirely filled by dark red blood.

The spleen appears congested but shows no gross hemorrhage.

The oesophageal mucosa shows many tiny ecchymoses. The stomach contains about 200 cc. of a dark brown thin liquid and the mucosa shows a few ecchymoses. The duodenum appears to be normal. The jejunum and ileum show a few ecchymoses scattered along in the mucosa. There is a section of the lower ileum which shows extensive hemorrhage into the muscle and intestinal wall. In the central portion of this segment the intestine is dilated and thin walled, and the mucosa is desquamated from its surface for a length of about 6 to 8 cm. The lumen contains a small amount of blood-tinged mucus.

The colon shows many ecchymoses and contains a moderate amount of blood-tinged mucus. There is a section about 8 to 10 cm. long in the lower third where the wall of the colon shows extensive hemorrhagic infiltration, causing the wall to be doubled in thickness.

The lymph nodes in the mesentery are enlarged (3 x 2 cm.) and show considerable hemorrhage within their structure.

The bladder shows a few ecchymoses in the mucosa, otherwise it is normal.

The kidneys aside from a moderate number of hemorrhages in the cortex are normal in appearance.

The bone marrow of the long bones is fatty and the marrow contains liquid blood in considerable quantity. That of the ribs is quite red and is mixed with much liquid quickly-clotting blood. The vertebrae are grayish red and show some little cellular material but mostly free blood.

The brain, thyroid, pancreas, liver, adrenals, ovaries, uterus, and other structures show no hemorrhages or other abnormalities.

Microscopical sections were made from all tissues. Organs presenting no features of interest are not mentioned.

The *lungs* in some places present a normal aspect, in others a slight amount of atelectasis, but in most sections red cells are numerous in the alveoli. There are frequently solid *masses of bacteria* scattered in among the larger hemorrhagic areas usually fairly well in the center of the densely packed red cells. There is usually considerable destruction and autolysis of the alveolar cell remnants and the red cells adjacent to these bacteria but there is a notable absence of any poly-

morphonuclear cells or phagocytes here or elsewhere in the lung sections. In the portions of the lung free of hemorrhage the capillaries are engorged but the alveoli are intact, and the bronchi and larger vessels seem normal.

The *liver* shows a rather marked congestion of the central veins. No hemorrhages noted.

The *kidneys* show an occasional small hemorrhage into the tubules, otherwise they are normal.

In the *adrenal* in some areas there is a rather diffuse invasion of all of the spaces between the cell columns by red cells. Many small areas show focal autolysis of moderate degree.

The *stomach* aside from a massive hemorrhage in the submucosa is not abnormal.

In the *jejunum*, a cross section shows hemorrhage into the villi, into the muscularis mucosae, and occasionally into the muscle coats. The mucosa is seen to be lifted from the muscularis mucosae by the hemorrhage in many places. There is edema of the areolar tissue.

In the ileum the cells of the mucosa show occasional pyknosis and frequent mitotic figures, probably the result of moderate injury by the radiation. Extensive submucosal and intra-mucosal hemorrhage is present and in several places the mucosa has disappeared probably due to the loosening of its attachments by hemorrhage. In the section of ileum which was much dilated and thinned out, the mucosa has been stript off over a large area. A few of the mucosal cells show pyknosis and slight injury from the radiation. There is some hemorrhage into the villi remaining in this section. The lead shielding of the intestine was not complete in this small area.

The *spleen* appears to have lost much of its substance so that the trabeculae seem more prominent and compact than usual. The germinal centers are reduced in size and contain paler cells more widely separated than usual. The pulp cells are pale and show occasional pyknosis and frequently mitoses. An occasional eosinophile is present. Red cells have infiltrated many areas extensively, involving both the pulp and germinal centers indiscriminately. Occasional phagocytes are seen containing what appears to be shrunken red cells or fragments of red cells. Apparently this has occurred quite recently.

A mesenteric lymph node contains many hemorrhagic areas in the edematous areolar tissue and among the pulp cells. There is occasional necrosis of the germinal centers where they can be identified. There are large numbers of very large giant cells and large phagocytes at the sites of the germinal centers which have been infiltrated by red blood cells. The phagocytes are often distended with 1 to 3 full sized red blood cells.

8th rib on the left and right shows hemorrhage into the fat. The connective tissue framework and the blood vessels represent practically all of the visible cells with the exception of an occasional normoblast.

The *cervical, thoracic, and lumbar vertebrae* have a similar paucity of bone marrow elements, the normoblasts being seen occasionally and undifferentiated

cells rarely. When present they stain poorly and are pyknotic. Hemorrhage is diffuse.

In the *femur marrow* of each side, the fat and the framework are practically all that are visible. Only a few atypical darkly staining normoblasts remain.

The *end of the femur* shows a few more marrow cells but they stain darkly and frequently show chromatin granulation and pyknosis of the nuclei or they are very pale and partially autolyzed. Normoblasts are slightly more numerous. Hemorrhage is a prominent feature.

The following experiment shows the relative ineffectiveness of doubling the amount of radiation. The animal died on the 8th day instead of the 9th. Transfusion did not modify the intoxication nor the hemorrhagic tendency.

Dog 28–196.—A black and white adult female, well nourished, strong, and active. Weight 17.8 kg.

5.14.29. Hematocrit 53 per cent; plasma pale lemon color.

5.15.29. Condition and appetite excellent. Hematocrit 52 per cent; plasma pale lemon color. 8 p.m. Sodium amytal administered intraperitoneally. 1,080 M.A.M. with the usual factors were given over the upper and lower halves of the body as described except that both sides were treated with this amount making the total double the usual treatment or 2,160 M.A.M.

5.16.29. Condition good, able to walk but slightly ataxic.

5.17.29. Condition excellent. Appetite good. Hematocrit 50 per cent; plasma cloudy and very slightly pink. W.B.C. 10,900; 9,850.

5.18.29. Condition and appetite excellent. No diarrhea. Hematocrit 49 per cent; plasma lemon colored. W.B.C. 7,000; 6,700.

5.19.29. Condition good but some anorexia.

5.20.29. Condition good, though slightly inactive; anorexia. Hematocrit 44 per cent; plasma lemon colored. W.B.C. 600; 700.

5.21.29. Rather quiet; about $\frac{2}{3}$ of food eaten. Hematocrit 43 per cent; plasma pale lemon color. W.B.C. 200; 125. Weight 16.4 kg.

5.22.29. Weak and stuporous. No food eaten. Hematocrit 44 per cent, plasma lemon colored. W.B.C. 100; 100. Apparently somewhat dehydrated. Because of low W.B.C. the animal was *transfused* with 225 cc. of whole normal blood. Blood stained material was observed to pass from the rectum.

5.23.29. Moribund at 8:30 a.m. Death shortly after 10 a.m.

Autopsy, 2 p.m. The body is cold and rigor mortis present. The skin and mucous membranes are normal in appearance. The subcutaneous fat is somewhat reduced in amount. The musculature is good. The serous surfaces are all smooth, shiny, showing no hemorrhages. In the duodenum about 20 cm. below the pylorus is a segment about 10 cm. long, which is dark bluish red in color involving the mucosal, serosal, and muscular layers. The terminal ileum has a similar segment

about 6 cm. long just above the ileocaecal valve. The mucosa has not been injured by the radiation but the changes are due to hemorrhagic infiltration. The remainder of the gastrointestinal tract and the oesophagus appear entirely normal.

The *bone marrow* of the long bones, ribs, and vertebrae shows abundant fat but aside from a slight pinkish tinge there is no evidence of free blood or hemorrhage.

The heart, lungs, liver, pancreas, spleen, kidneys, adrenals, bladder, uterus, and ovaries are free from gross hemorrhages and apparently normal.

Microscopical sections made from all tissues.

The *lungs* show many alveoli containing free red blood cells. The epithelium of many of the smaller bronchi is also covered with a layer of red blood cells. There is no massive hemorrhage. Some atelectasis is present here and there and in these areas the capillaries are engorged. There is no evidence of a pneumonic process.

The *liver* shows some congestion, with engorgement of the central veins. There is a small amount of focal central necrosis in many of the liver lobules. Around the hyalinized cells the liver cells are pale and show frequent mitoses. Invasion by phagocytes has not occurred and the rest of the liver cells seem normal.

In the *kidneys* the glomeruli seem congested. Many of the tubules contain a large number of red blood cells. Aside from a moderate amount of postmortem autolysis there is no other deviation from the normal.

Sections from the small *intestines* are somewhat autolyzed and show occasional small areas in which the mucosa has been injured by the radiation. There is a considerable amount of hemorrhage into the smooth muscle bundles, into the areolar tissues, the submucosa, and under the peritoneum. This does not seem associated with the mild injury to the mucosa which is strictly localized to a small area. The capillaries are dilated and congested everywhere.

In the *spleen* the trabeculae seem more prominent than usual due to a generalized reduction of the pulp substance. The germinal centers are greatly reduced in size and number of cells. The sinuses are filled with blood and the pulp and germinal centers are pretty generally infiltrated by red blood corpuscles. There is no localized collection of red cells suggesting hemorrhage but rather a generalized diapedesis of these cells. Large phagocytes containing brown granules and cell fragments resembling shrunken red cells are seen in great numbers. They are also spread diffusely throughout the spleen substance. The stroma seems to be very loosely placed and more open than usual suggesting moderate edema.

A large mesenteric *lymph node* shows marked hemorrhage into the node substance with separation of the node into small islands of cells. These are no longer compact but seem separated by edema. There are a few phagocytes containing brown granules but they are much less evident than in the spleen. The good condition of the red cells and the few phagocytes suggest that the hemorrhage is of recent occurrence.

A *rib* shows much hemorrhage in among the fat. In addition to the connective tissue framework are found occasional large round cells with vacuolated round nuclei. The cytoplasm stains faintly pink and the nucleus is not very dark and

shows generally a nucleolus. A normoblast is rarely seen. An occasional phagocyte containing brown granules is present. There is no evidence of any blood formation.

A thoracic *vertebra* shows much the same picture—there is a great deal of blood present between marrow fat cells. Aside from the connective tissue framework and a few phagocytes containing granules there are very few of the normal marrow cells to be found. There are a fair number of rather shrunken, abnormal looking normoblasts in each high power field, a few large round cells with pale cytoplasm and large round nucleus, and a few shrunken undifferentiated cells apparently of the myeloid series. The endosteum covering the bony trabeculae is normal in appearance.

The *femur marrow* shows marked hemorrhage, and a similar paucity of marrow cells. Cells resembling plasma cells and normoblasts without the pink cytoplasm are seen here and there in groups of two or three in the connective tissue framework.

Phagocytes with brown granules are about half as numerous as the marrow cells. Many areas of the section show only congested blood vessels and the connective tissue framework of the marrow.

The third experiment shows the characteristic course of events, unaffected by two transfusions, and death on the 9th day.

Dog 28-268.—A small black and white female mongrel. Somewhat thin, weight 8.6 kg.

6.5.29. Active. Hematocrit 46 per cent; plasma clear and normal.

6.6.29. Appetite good.

6.7.29. Lively, has gained weight—weight 9.1 kg. Hematocrit 46 per cent; plasma clear and normal. 8 p.m. Intravenous amytal—40 mg. per kg. Animal asleep in deep anesthesia before termination of injection. Deep therapy 1,050 M.A.M. were administered over the upper and lower halves of the body in two treatments, as described under Method.

6.8.29. Active—all food eaten, condition excellent.

6.9.29. Very active—appetite excellent. Hematocrit 43 per cent; plasma faint lemon color. W.B.C. 5,150; 5,000.

6.10.29. Active and playful—appetite good. Hematocrit 43 per cent; plasma faint pink color. W.B.C. 3,750; 4,450.

6.11.29. Active and playful—appetite good. W.B.C. 2,150; 1,900.

6.12.29. Condition good. Active and playful—appetite excellent. Hematocrit 39 per cent; plasma pale lemon color. W.B.C. 1,125; 1,125.

6.13.29. Most of food eaten—vomitus in cage. Somewhat less active than before. Mouth in good condition. No bleeding. Hematocrit 37 per cent. Plasma slightly pink. Weight 9.1 kg. W.B.C. 425; 350. 2 p.m. Transfused 100 cc. citrated whole normal blood from donor with hematocrit of 51 per cent—no reaction noted.

6.14.29. Inactive—nothing eaten, dog quiet and seems intoxicated. Hematocrit 41 per cent. Plasma lemon color. W.B.C. 75; 150.

6.15.29. Nothing eaten—inactive—no changes noted. Mouth in good condition. W.B.C. 50; 50. Smear shows only 2 definite platelets in careful search of both cover slips. There are a very few white blood cells only 10 being found in the two cover slips, 6 being polymorphonuclear neutrophiles, 1 disintegrated basophile, and 3 large mononuclears with kidney shaped or oval nuclei. There is considerable variation in size of the red cells, but no poikylocytosis. There is some achromia but this is variable since many of the cells have the usual amount of hemoglobin content. There is no stippling or basophilia. 1:30 p.m. Transfused with 200 cc. of citrated blood from same donor. 6:00 p.m. 200 cc. of Klim and Karo syrup given by stomach tube and immediately vomited. 11:00 p.m. No change—inactive and lethargic—not cold—does not look unusually sick.

6.16.29. Found dead though still warm this morning. No vomitus or stool in cage—no bleeding noted.

Autopsy, 11 a.m. The *body* is that of a poorly nourished female mongrel showing no gross lesions of the skin or mucous membranes. There is no evidence of bleeding in the mouth or rectum. There is no hemorrhage in the muscles nor is there any gross damage to the vascular system.

There are many ecchymotic areas in the parietal pleura especially in the left side of the chest, but no fluid or adhesions. There are several epicardial hemorrhages but none in the pericardial sac proper; no fluid or pericardial adhesions. The peritoneal surfaces are negative. The *heart* is essentially normal in size and shape and musculature shows no hemorrhages in its substance.

The *lungs* show very extensive rather solid hemorrhagic areas 1 to 2 cm. in diameter in sufficient number to occupy one-third of the total volume of the lung substance. The pulmonary vessels are free of thrombi.

There are two small and one fairly large sharply circumscribed bluish red areas in the mucosa of the lower ileum. The mucosa is thickened apparently by the infiltrating blood. The gastrointestinal tract except for postmortem autolysis is entirely normal. There is no gross evidence of injury from the radiation.

The *spleen* is normal in size—the architecture is undisturbed and shows no hemorrhage or congestion. Two of the *mesenteric lymph nodes* measure 1 x 1 x 1.5 cm. and are filled with blood. All other nodes here and elsewhere are small and pinkish in color, without gross hemorrhage.

The *bone marrow* of each femur and humerus contains abundant fat mixed with free blood suggesting hemorrhage. The rib and vertebral marrow are also hemorrhagic so that cellular details are obscured. The liver and other organs appear free of gross hemorrhage and are normal.

Microscopical sections are made from all tissues.

The *heart* shows some extravasation of blood into the epicardial fat and occasionally between the muscle bundles. There are no other abnormalities, the muscle cells being normal in appearance.

The bronchi of major lobules of the lungs are filled with blood. The alveoli are filled with blood cells, fibrin, and masses of bacteria—there are very few

wandering cells. Some alveolar walls are destroyed and cells disintegrated probably due to bacterial growth during the last day of life.

The *pancreas, adrenals, and kidney* aside from a moderate amount of capillary hemorrhage seem undisturbed in any way.

A section of *small intestine* through the abnormal area noted in gross shows extensive infiltration of the muscle, areolar tissue, and mucosa by red cells. Much of the epithelium of the villi has disappeared and that noted in the occasional villus is pyknotic and darkly stained. Hemorrhage into the submucosa has infiltrated and elevated the mucosa with the result that it is destroyed down to the muscularis mucosa. The pyknosis without destruction of the crypt nuclei indicates a moderate effect upon these cells directly by the radiation but the injury is insufficient to cause extensive destruction. In the *large intestine* a few localized hemorrhages in the mucosa are the only abnormal findings.

In the *spleen* the germinal centers are reduced in size and number. Their cells are darkly stained except for the numerous mitotic figures. The pulp cells are reduced in number but the pulp volume is increased by the red cells which are everywhere in large numbers. The red cells are well preserved and there are very few phagocytes present containing brown granules. There is no necrosis nor are pyknotic cells visible.

In the *mesenteric lymph node* the pulp is diffusely infiltrated by red cells, so much so as to separate the individual cells rather widely. The germinal centers are divided up into little islets of a few cells by the hemorrhage. The cells of the germinal center are not abnormal except perhaps that mitotic figures are quite numerous. A large number of huge phagocytic cells are everywhere and contain brown granules and whole red cells.

The central shaft marrow from the *humerus and femur* shows a few normoblasts here and there in the connective tissue framework supporting the fat and blood vessels. There are occasional phagocytes, containing brown granules. Small localized hemorrhages are everywhere. The usual number of marrow cells is greatly decreased. The diaphysis of the femur shows similar hemorrhage though not limited by the connective tissue framework. The marrow cells are greatly reduced in number yet are still quite numerous. Many "shadows" of marrow cells are visible. Those cells remaining are very densely stained with hematoxylin and show shrunken coalescing chromatin material in the nuclei. The normoblasts and the cells of the same size and appearance except for the unstained cytoplasm show less disturbance than the more undifferentiated cells. There are a few phagocytes containing brown granules.

The *8th rib* on the right shows no marrow cells whatever, merely hemorrhage and the connective tissue and blood vessel framework. This side of the thorax was the side radiated. The 8th rib on the left shows extensive hemorrhage, a rare normoblast, the framework cells. No other marrow cells are to be found.

The cervical, thoracic, and lumbar *vertebrae* all show similar depletion of the marrow cells with only an occasional normoblast present. Hemorrhage is everywhere, filling the spaces among the bony trabeculae.

DISCUSSION

The large *phagocytes* of the marrow, lymph glands, and spleen in these experiments present features of unusual interest relating especially to the origin of these phagocytes. We record above that these large phagocytes are numerous and contain red cells and other cell fragments. The lymphocytic cell chain is especially sensitive to radiation and all marrow cells are destroyed in satisfactory experiments. Yet these phagocytes persist and retain their usual capacity of engulfing much cell debris. It would follow from this observation that these particular phagocytes take their origin from the capillary endothelium which is obviously resistant to the radiation. This method may be a profitable one to study various types of phagocytes about whose origin there is some diversity of opinion.

The behavior of the blood platelets in this type of experiment calls for much more study but some points stand out in an analysis of these experiments. Platelets are present in the circulating blood up to the last 24 hours before death when they vanish. This platelet lack probably is in part responsible for the diffuse purpura found at autopsy. If the platelets were sensitive to radiation, we might expect an earlier decrease in the blood stream although not necessarily so. Assuming the probability that the megakaryocyte of the bone marrow produces the platelets, this observation suggests a life cycle of 7 to 8 days for the platelets in the circulation, as all megakaryocytes are destroyed by the marrow radiation.

SUMMARY

Constant findings were obtained in the acute reaction to the specified amount of heavily filtered radiation over the bony skeleton.

1. There develops without warning a short and fatal intoxication on the 8th or 9th day after the exposure to the radiation.

2. A profound leucopenia appears after 5 to 6 days and is maintained in the peripheral blood (200 white blood cells or less per c. mm.) for the 2 to 3 days before death.

3. The platelets suddenly disappear from the blood smears the day before death. This has some bearing on the life cycle of the platelet.

4. All of the organs and body structures present extensive and generalized capillary hemorrhage of recent origin.

5. The substance of the spleen and lymph nodes is greatly reduced and the germinal centers are visible only as remnants.

6. The red cell hematocrit reading drops from about 50 per cent or normal to approximately 40 per cent.

7. The bone marrow is depleted of all its cells except the connective tissue and fat cells, blood vessel endothelium, phagocytes filled with brown granules, and occasional normoblasts.

BIBLIOGRAPHY

1. Heineke, H., *Mitt. Grenzgeb. Med. u. Chir.*, 1904–5, **14,** 21.
   Heineke, H., *Deutsch. Z. Chir.*, 1905, **78,** 196.
2. Helber, E., and Linser, P., *Münch. med. Woch.*, 1905, **52,** 689.
3. Krause, P., and Ziegler, K., *Fortschr. Geb. Röntgenstrahlen*, 1906, **10,** 126.
4. Krömeke, F., *Strahlentherapie*, 1926, **22,** 608.
5. Lacassagne, A., Lavedan, J., and de Leobardy, J., *Compt. rend. Soc. biol.*, 1922, **86,** 668.
   Lacassagne, A., and Lavedan, J., *Compt. rend. Soc. biol.*, 1922, **86,** 713.
   Lacassagne, A., and Lavedan, J., *Paris Med.*, 1924, **1,** 97.
   Lacassagne, A., and Lavedan, J., *Medecine*, 1924, **5,** 683.
6. Linser, P., and Helber, E., *Deutsch. Arch. Klin. Med.*, 1905, **83,** 479.
7. Minot, G. R., and Spurling, R. G., *Am. J. Med. Sc.*, 1924, **168,** 215.
8. Piney, A., *Brit. Med. J.*, 1925, **2,** 343.
9. Piney, A., and Mayneord, W. V., *Brit. J. Radiol.*, 1928, N. S. **1,** 263.
10. Valeeff, I., *Strahlentherapie*, 1927, **26,** 363.
11. Warren, S. L., *Physiol. Rev.*, 1928, **8,** 92.
12. Whipple, G. H., and Warren, S. L., *J. Exp. Med.*, 1922, **35,** 203 and 213.
13. Wright, S., and Bulman, H. A., *Lancet*, 1929, **2,** 217.