

III. THE COMBINED EFFECTS OF COLLOIDAL SILVER AND HIGHLY FILTERED ROENTGEN RADIATION UPON THE HEMATOPOIETIC SYSTEM IN DOGS

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(Received for publication, December 12, 1930)

The production of an anemia in dogs by the repeated intravenous injection of colloidal silver has been described in a preceding paper (Paper I). This caused a severe anemia associated with peripheral blood destruction followed by bone marrow hyperplasia. The intoxication was accompanied by anorexia, lassitude, marked loss of weight, and resulted in death. The number of circulating leucocytes remained essentially normal or became elevated, and there were no signs of a platelet deficiency. Apparently the animals died from the generalized toxic effect of the silver, rather than from the anemia since the hematocrit level was distinctly compatible with life. In Paper II we described the acute changes following exposure of the whole bony skeleton to heavily filtered Roentgen radiation. This is characterized by a leucopenia, an anemia, absence of platelets, bleeding, and destruction of the bone marrow cells. Exitus occurs about 9 days after the radiation.

It was thought that the leucopenia and the failure of the platelets after exposure to radiation were intimately concerned with the death of the animal. Since colloidal silver injections commonly produced a leucocytosis and had no influence upon the platelets, injections of silver might conceivably have some influence in prolonging the life of the animal which had received a fatal amount of radiation over the bone marrow. The reverse was found to be true, however. The acute anemia in the radiated animal comes on at the same time that the hemorrhages occur and seems definitely related to them and not to any disturbance of the supply of red blood cells from the bone marrow. The anemia produced by the silver seems to be accompanied by an

active hyperplastic marrow which is endeavoring to replace the destroyed red blood cells. The effect of a destructive agent such as the heavily filtered radiation upon such a marrow is of great interest since the more actively growing and dividing cells should be more sensitive to radiation than the undisturbed marrow. On the other hand, a hyperplastic marrow by its increased volume alone might be more able to survive the destructive effects of the radiation. Each of the two agents seemed to be equally effective and their combination resulted in a shortening of the period before exitus without an increase in the destructive changes characteristic of each.

Method

Full-grown healthy stock dogs were used throughout. They were kept on a general diet and were observed for 2 weeks previous to being used, to eliminate the possibility of distemper. The colloidal silver preparation used was collargolum which was administered intravenously in a 1 per cent emulsion, as described above (Paper I). The dosage varied in the different animals. In most cases, one or more courses of collargol had been injected before the radiation was used. In one case, the silver was begun 2 days after the exposure to radiation.

The radiation was administered with the animal under amytal anesthesia (intravenous), since complete absence of motion was essential. The fully anesthetized animals were placed upon the deep therapy treatment couch, and the upper half of the body including the head and upper legs down to the costal margin was irradiated. The animal was shifted and the lower half of the body including the tail and lower legs irradiated. It is necessary to do this from the side of the animal so that the intestines can be protected from the radiation while the lumbar spine is exposed. This technique is described more in detail in Paper II in this series. The factors are 200 kilovolts, current 35 milliamperes, target skin distance 58 cm., filtration: copper 0.5 mm., aluminum 1.0 mm. Dosage varied from 350 to 1,050 milliampere minutes.

Blood was obtained from the jugular vein at the proper intervals for the hematocrit, white blood cell count, and blood smears. The latter were stained with Wright's stain. Complete autopsies were performed as soon after death as possible, the tissues being fixed in Zenker's fluid and stained with hematoxylin-eosin.

EXPERIMENTAL OBSERVATIONS

The following experiment illustrates the acute lethal effect from sublethal amounts of silver and heavily filtered radiation, the silver being given first, the hemolysis, anemia, leucocytosis, and loss of weight being characteristic of the silver effect. The precipitate drop

in the white blood cells and the paucity of cells in the bone marrow are typical radiation effects. The combined effects probably overwhelmed the animal so that exitus occurred too quickly for other radiation effects (purpura and hemorrhage) to appear.

Dog 28-158.—A very strong and active male mongrel weighing 21.6 kg. was given daily intravenous doses of 100 mg. to 157 mg. of collargol for 11 days, making a total of 1,467 mg. On the 21st day, he was given 1,050 m.a.m. of heavily filtered radiation over the bony skeleton from the left side. Prior to this the white blood cell count had been 16,000; 2 days after the radiation, it was 14,000, and the general condition was good. On the 4th day after the radiation the leucocyte count was 775; the animal seemed rather quiet, but presented no other abnormal signs. The following morning, he was moribund, with very deep respirations, and a leucocyte count of 250. No signs of hemorrhage were present.

While the silver was being administered and for a few days after it was stopped, he had anorexia, a loss of weight from 21.6 kg. to 17 kg., a leucocyte count of 16,400, definite hemolysis, and a decline of the hematocrit from 55 per cent to 32 per cent on the 21st day, just prior to the radiation. Following the latter, the hematocrit continued between 35 per cent and 32 per cent until the end, and there was no further hemolysis.

Autopsy revealed a few small hemorrhages in one lung, moderate hemorrhages in the mucosa of the large intestine, and considerable hemorrhage in the submucosa of the bladder. The liver showed a moderate degree of central congestion and atrophy with *golden brown material* in some of the endothelial cells. The spleen contained very numerous clumps of similar particles. The pancreas presented a small area of fat necrosis. The bone marrow from the right femur showed a slight increase in the number of marrow cells, among which there were several deposits of *golden brown particles*, (silver), but the marrow from all other regions contained very few cells.

The following experiment shows the acute lethal effect of sublethal doses of radiation and silver with the radiation administered first,—the reverse of the last experiment. The failure of the development of the leucocytosis would indicate that the depletion of the leucocytes by the radiation was generalized and not just restricted to the circulating leucocytes. The leucopenia to below 1,000 cells is usually associated with purpura and bleeding. The sections of some of the organs indicate that this was beginning.

Dog 28-183.—A very active and healthy female mongrel, weighing 17.5 kg. was given 700 m.a.m. of heavily filtered radiation over the skeleton from the left side. After this collargol was injected intravenously between the 3rd and 9th

days inclusive, in daily doses of 75 mg. to 135 mg., making a total of 650 mg. in 7 days. The leucocyte count was 7,850 cells on the 4th day and 275 on the 9th. On the latter date, the animal's general condition was good except for slight inactivity, but the following morning (10th day) she was moribund and exitus occurred before blood specimens could be obtained. No purpura or hemolysis had been observed. The initial hematocrit of 57 per cent had decreased to 53 per cent on the 3rd day, before the collargol was begun, and there was a steady decline to 33.3 per cent on the day before death. The appetite remained good, and there was practically no loss of weight.

Autopsy revealed extensive fresh hemorrhage in the lower ileum, and a few areas of hemorrhage in the bladder wall. Some of the mesenteric lymph nodes had their sinuses filled with erythrocytes, and contained many large phagocytes packed with red blood cells. The spleen showed many large mononuclear cells containing coarsely granular golden brown pigment, and some of them had also ingested red blood cells. The Kupffer cells of the liver contained a similar pigment. The bone marrow from all regions showed only a few marrow cells.

Although the sequence of events differs somewhat, these two experiments show the characteristics of both silver and radiation effects described in the first two papers of this series.

The following rather long experiment shows the effects of a moderate dosage of silver followed by a small dose of radiation. The silver effects (anemia) were maximal and the radiation effects slight and transitory. A transfusion and two more courses (moderate doses) of silver resulted in a marked leucocytosis. The anemia remained stationary for some time and then the hematocrit gradually rose. A large dose of radiation caused the usual leucopenia to below 100 cells with exitus before the development of the usual purpura seen after such a dose of radiation. Each agent thus demonstrated its characteristic influence in turn except that the destructive effects of the radiation upon the marrow cells and leucocytes was overwhelming in the end.

Dog 28-47.—A very strong male mongrel, weighing 27.6 kg., was given 100 mg. to 200 mg. of collargol daily during the first 11 days making a total of 1,875 mg. Conspicuous hemolysis was observed on the 14th day. The initial hematocrit of 53 per cent had declined to 30.4 per cent on the 17th day. That evening, the animal was given 350 m.a.m. of Roentgen radiation over the skeleton. Following that the leucocyte count dropped but did not fall below 7,000. The hematocrit was 25.7 per cent on the 25th day, but had risen to 29.2 per cent on the 29th. A transfusion was done that afternoon which raised the hematocrit to 43.8 per cent. The latter was 37 per cent on the 64th day. Then he was given 100 mg. of collargol

for 3 days, and again from the 70th until the 77th day, making a total of 2,975 mg. As a result of this, the leucocyte count rose as high as 26,000 although the hematocrit was only 25 per cent between the 80th and 90th days; but it gradually rose to 35 per cent on the 103rd day.

That night (103rd), the animal was radiated with 1,050 m.a.m. over the skeleton. The leucocyte count remained as high as 13,000 for 2 days (until the 105th day), but the next morning it was 6,150. On the 108th day, there were only 425; on the 109th, 225; and on the 110th day, 100 leucocytes per c. mm. Previously his general condition had remained fairly good, but on that day (110th), he was definitely weak and lethargic; however, no antemortem hemorrhages were observed. He died that night, 7 days after the last radiation. The hematocrit was 31 per cent on the day of death. A postmortem blood examination showed no leucocytes in two counting chambers. The initial weight of 27.6 kg. had declined to 22 kg. prior to the last "x-ray" exposure, and terminally, it dropped to 19.5 kg.

Autopsy revealed numerous small hemorrhages in the parietal and visceral peritoneum. The intestinal lumen contained very abundant clotted blood, and in several loops, the entire thickness of the wall was filled with hemorrhage. The spleen had a dark slaty color, and microscopical study disclosed numerous deposits of coarsely granular golden brown particles. The liver presented a slight excess of connective tissue radiating from the portal spaces, and many large deposits of coarsely granular golden brown particles. The pancreas had a slaty gray color, and contained numerous small deposits of granules similar to those in the liver and spleen. The bone marrow in the shaft of each femur and each humerus had a very dark bluish red color and a very soft consistency. Microscopic study revealed engorged sinuses, abundant fat, and very few marrow cells. There were many deposits of coarsely *granular golden brown particles*, both intra- and extracellular, and also many large phagocytes containing red blood cells. The marrow from the cervical, thoracic, and lumbar vertebrae contained abundant fat and dilated congested blood vessels. Only a very few marrow cells were present. Here, there were also a few intracellular deposits of coarsely granular brown particles. Likewise, the marrow from the second and the eighth ribs on each side contained only a few marrow cells and was congested.

The following long experiment shows the characteristic acute and chronic effects of the silver and radiation, the anemia being especially marked. Repeated transfusion near the end of the experiment did not change the course of the experiment. The second and third doses of radiation should have caused exitus on the 9th or 10th day. The survival of this animal after the second radiation and the long period after the third radiation can be explained only by the failure to include some of the bone marrow in the field of radiation each time. The presence of silver in the tissues does not seem to change the effect of the radiation any, *i.e.* by intensive local radiation.

Dog 28-200.—A vigorous male Airedale, weighing 20 kg., was given daily doses of 75 mg. to 150 mg. of collargol for 11 days, making a total of 1,375 mg. Near the end of this course of injections, the buffy layer in the hematocrit tube became about three times its original thickness. Hemolysis was observed between the 10th and 20th days and occasionally thereafter. The initial hematocrit of 46.8 per cent red blood cells had dropped to 27.1 per cent on the 17th day. Then it remained between 30 per cent and 35 per cent until the 121st day. On the 43rd day, he was given 700 m.a.m. heavily filtered over the bony skeleton from the left side. There was practically no change in the hematocrit but the leucocyte count declined from 16,000 to 3,750 on the 51st day (8 days after the irradiation). It was 3,900 on the 52nd day, but had risen to 6,000 on the 55th. It remained between 6,500 and 11,000 until the 71st day. That night, he was again radiated, this time with 1,050 m.a.m. over the right side. Following this, there was a gradual decrease in the number of white blood cells to 3,000 on the 82nd day (11 days after the irradiation), and then a slow rise to 12,000 on the 99th day. On the 102nd day, when the count was 10,200, he was again given 1,050 m.a.m. of heavily filtered radiation over the skeleton from the left side. The leucocytes declined to 1,100 on the 109th day (7 days after the irradiation), but gradually returned to 8,000 on the 128th day, and were 7,000 on the day of exitus. Although the hematocrit had not been affected by the previous exposures to Roentgen radiation, having maintained a value of 30 per cent to 35 per cent since before the first radiation, yet on the 121st day, it dropped to 29 per cent, and gradually declined to 19 per cent on the 129th day. That afternoon, he was transfused with the calculated amount of blood to raise his hematocrit to 30 per cent. The following day, it was down to 22 per cent, and he was transfused again. He was found dead the next morning, the 131st day of the study, and the 29th since the last exposure to the radiation. Prior to the transfusions, the blood smears showed very marked achromia and anisocytosis, a few normoblasts and basophilic erythrocytes, a very few myelocytes, but no lymphocytes. Although only a very few platelets were seen in the smears, no hemorrhages were observed during life.

The initial weight of 20.2 kg. fell to 18.3 kg. after the course of collargol and before the radiation was employed, and down to 16.4 kg. near the end. He showed lethargy and anorexia during the last 13 days, and was gavaged with milk and glucose. Pitting edema of the legs was present the last 10 days.

Autopsy showed emaciation, extensive pitting edema of the legs, and 500 cc. of thin yellowish fluid in each pleural cavity. The lungs presented extreme edema and congestion, and microscopic study revealed some regions with many red blood cells in the alveoli. There was moderate congestion of the gastrointestinal tract. The liver showed gross scarring along the margin of some of the lobes. Slides from these regions showed extensive fibrosis, in which occurred remnants of the liver columns and numerous large mononuclear cells filled with coarsely granular golden brown pigment. In other sections, the Kupffer cells were also filled with similar pigment which, however, had a greenish blue color when stained for iron. The spleen contained many normoblasts and a few small scattered deposits of coarsely

granular golden yellow pigment, both intra- and extracellular, which was colored greenish blue with the iron stain. The retroperitoneal lymph nodes showed extensive clumps of similar pigment in the stroma, the sinuses filled with erythrocytes, and many large mononuclear cells with ingested red blood cells. Some of the germinal centers showed islands of activity, but the most of them seemed to be destroyed. The bone marrow was congested and showed a few areas of hemorrhage. There was a marked loss of the normal elements; but several islands of densely packed cells remained which consisted chiefly of normoblasts and some myeloid cells.

DISCUSSION

The combined effects of silver and radiation did not differ much from what would be expected from the effects of each alone. Those animals which were given a course of silver injections several days prior to the irradiation showed toxic signs such as anorexia, loss of weight, leucocytosis, definite hemolysis, and a severe anemia. All of these symptoms had improved considerably before the Roentgen radiation was given. Abundant silver particles were found in the reticulo-endothelial cells. The less acute experiments showed scarring in the liver in which there were numerous deposits of silver. Thus the action of the silver here was similar to its effect when used alone.

Following the exposures to moderate amounts of heavily filtered Roentgen radiation, there was a marked decrease in the number of leucocytes, which almost completely disappeared within 5 to 10 days. Exitus occurred soon after this. It is remarkable that most of the animals remained in apparently good condition, becoming lethargic only a few hours before death. The blood smears showed only a few platelets, yet none of the dogs presented any signs of purpura. Autopsy revealed rather definite depletion of the bone marrow in all of the animals, and also prominent hemorrhages in the viscera. No terminal infections were observed.

It is interesting that one experimental animal showed a definite leucopenia after each of his three exposures to radiation, but had a leucocyte count of 7,000 when death occurred, 29 days after the last radiation.

Edema which occurred in an occasional animal in the longer experiments, was thought to be the result of malnutrition and a consequent lowering of the plasma proteins, since it was improved slightly by a

transfusion, but unfortunately no protein determinations were done. The serum non-protein nitrogen was taken in one of the animals. It remained normal until a very few days before death, and then became moderately elevated.

In the shorter experiments, the anemia resulting from the silver injections was not further affected by the radiation but remained the same until death occurred. In the last experiment described above, the hematocrit was not affected by any of the radiation until 10 days before death. Then it declined very rapidly, and could not be maintained by repeated transfusions. Since this animal withstood the first two exposures to the radiation and survived an unusually long time after the third, it seemed probable that not all of the bones had been completely radiated. This was borne out by the finding of a few islands of hyperplasia in one femur.

In most of the experiments, the autopsy revealed either brown granular pigment or phagocytosed red blood cells in the various organs. We can not say positively whether the hemophage activity was stimulated by the colloidal silver or whether this represented a sieving out process of injured erythrocytes. The amount present did not seem to be increased over that found after the use of silver or radiation alone. Along with these signs of peripheral blood destruction, there was an extensive aplasia of the bone marrow. Blood smears showed marked achromia and many young forms of red blood cells. These young red blood cells probably represent a desperate attempt of the remaining islands of bone marrow cells to regenerate the blood. Mortland reported similar changes in several human cases of thorium poisoning.

Even though all of these animals had a severe anemia and depletion of the bone marrow, death was not apparently the result of an insufficient oxygen carrying capacity of the blood but in some way was caused by a deficiency of thrombocytes and leucocytes. There might have been some unknown irreparable injury to the fixed tissues, especially the platelet forming mechanism, since life could not be saved by repeated transfusions. The actual effect of this on the body is not clear, since the hemorrhages *per se* were entirely too small to be fatal and no infection was superimposed on the agranulocytic condition. The toxic effect of the phagocytized silver is not to be for-

gotten as a direct cause of death with the animal's general condition so much depressed.

CONCLUSION

1. The individual destructive effects of colloidal silver and heavily filtered radiation are still evident when the two are used together.
2. The combined effects are cumulative in that small doses are more destructive than when either is used alone.
3. The leucocytosis resulting from the injection of the colloidal silver affords no protection against the terminal leucopenia following the radiation.