I. EFFECTS OF THE INTRAVENOUS INJECTION OF COLLOIDAL SILVER UPON THE HEMATOPOIETIC SYSTEM IN DOGS

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(Received for publication, December 12, 1930)

In this laboratory during several years much experimental work has been done to study the effect of diet upon blood regeneration in dogs with anemia caused by bleeding (6). The anemia is continued at a uniform level of about one-third normal by repeated blood withdrawal from the jugular vein. Under such conditions the *bone marrow* of all bones shows *extreme hyperplasia* and presumably is functioning at maximal capacity due to a continued maximal demand associated with severe anemia.

If an *aplastic anemia* with great depletion of bone marrow cells could be produced experimentally, a rare opportunity would be offered to study the life cycle of the red cell under carefully controlled conditions. This ideal condition has never been produced experimentally in the dog although Muller (3) reports suggestive observations in the rabbit. We had hopes that by means of colloidal silver or Roentgen radiation or both it might be possible to cause a type of aplastic anemia which would yield much valuable information.

As sometimes happens in such experiments we were able to take a step toward our goal of experimental aplastic anemia but so many complicating factors developed simultaneously that we were unable to get any clear-cut evidence as to the life cycle of the red blood cell in the circulation of the dog.

As is evident from the protocols given below the *colloidal silver* injections in dogs cause no aplasia of the marrow but rather a distinct *marrow hyperplasia.* Moreover there is some hemolysis which in some way must be related to the silver injections. This hemolysis may be in

part responsible for the marrow hyperplasia although the phagocytosis of silver by marrow cells may also be concerned.

It is possible, although very difficult, to produce a condition of aplastic anemia in dogs by means of Roentgen radiation. At the same time however there is almost complete disappearance of white cells and platelets from the blood. Multiple hemorrhages then develop due to platelet deficiency or to endothelial injury or both. Terminal infections (agranulocytosis) are all too frequent. It is obvious that this type of experimental aplastic anemia with the hemorrhagic complications is not suitable for the proposed study of the circulating red cells.

Method

Our studies were made on healthy and well nourished full-grown dogs. All of them were under observation at least 2 weeks before the experiments were begun. A diet of mixed kitchen scraps was used generally, but a few of the animals were fed small amounts of raw beef after they had become inactive and refused to eat the usual food.

All the blood studies were made on blood obtained by hypodermic puncture of the jugular vein. The red blood cell hematocrit was determined before the silver injections were begun and at 1 or 2 day intervals thereafter. The same hematocrit tube was used for each dog throughout. Approximately 10 cc. of blood were added to 2 cc. of 1.6 per cent sodium oxalate solution, and centrifugalized at 2,400 revolutions per minute for ½ hour.

In these experiments we employed only colloidal silver. The preparation used was collargolum, a crystalline material supplied by the Heyden Chemical Company. It is said to contain 85.87 per cent of metallic silver and a small percentage of albumen. A 1 per cent emulsion of this was prepared with distilled water, filtered, and kept in a brown bottle in a refrigerator. As an additional precaution against its disintegration, the stock bottle of the crystals was also kept in a refrigerator. Just prior to each injection the emulsion was carefully warmed in the syringe to approximately body temperature. It was slowly introduced into a jugular vein. The dosage varied, as described in the following protocols.

EXPERIMENTAL OBSERVATIONS

A single intravenous injection of 500 mg. of collargol is followed as a rule by death in less than 12 hours without convulsions or clinical evidences of shock. This single large dose produces moderate congestion and marked edema of the lungs.

Doses of 1,300 to 1,500 mg. of collargol are tolerated if divided and given at the proper intervals over a period of 3 to 7 days. There is no conspicuous tolerance afforded by repeated doses nor is the effect cumulative in the strict sense. Single doses of 200 to 300 mg. are well tolerated. Death usually follows a single large dose even after several preliminary smaller doses. Evidence of hemolysis is always present.

The following brief protocol gives the outline of a typical short experiment:

Dog 27–249.—A male Dalmatian coach, weighing 23 kg., was given 400 mg. of collargol intravenously in divided doses, on the 1st day of the experiment. The next day he received two doses of 200 mg. and 300 mg. respectively. The 3rd day he was given one dose of 400 mg. making in all 1,300 mg. No reaction occurred during the next 40 minutes after the injection, but 4 hours after the last injection he was found dead. Necropsy revealed marked edema and moderate congestion of both lungs. The spleen and liver had a dark slaty color, and their reticulo-endothelial cells contained coarsely granular golden brown pigment (silver). The bone marrow showed slight hyperplasia and many large mononuclear cells filled with brown pigment.

In these short experiments the hematocrit drops 10 to 14 per cent and on examination the bone marrow may present a normal appearance or a hyperplasia. This is illustrated by the following protocol in which a large total dose was given over a somewhat longer period.

Dog 28–114.—A male, weighing 19.4 kg., received rather small divided doses of collargol during the first 6 days, but the next 3 days he was given single daily doses of 300 mg., making a total of 1,900 mg. He was inactive and had not eaten when the last dose was given; his condition was unchanged 8 hours later but the following morning he was found dead. Necropsy revealed considerable edema and congestion of the lungs. The spleen and liver had a dark brown color; sections showed abundant coarsely granular golden brown material (silver) in the reticulo-endothelial cells, and fine brown particles in the parenchyma cells of the liver. The femur marrow showed hyperplasia and contained numerous deposits of coarsely granular golden brown pigment. The initial hematocrit of 54 per cent had dropped to 47 per cent on the 8th day.

Two dogs receiving smaller individual and smaller total injections over 2 to 3 weeks developed distemper and death was due to this intercurrent infection. The hematocrits dropped 20 per cent and a marked loss of weight occurred. The bone marrow was either normal or slightly hyperplastic.

The following experiment shows the fluctuation of the hematocrit and white blood cells under *prolonged administration* of silver in moderate amount.

Dog 27–90.—A female, weighing 14 kg. During the first 28 days she received small doses of 20 to 50 mg. of collargol at intervals, making a total of 150 mg. During that time there was moderate leucocytosis and an increase in the red cell hematocrit from 46 per cent to 52 per cent. Between the 28th and 36th days she was given 900 mg. collargol in doses of 100 to 300 mg. each. The silver injections were stopped then because of anorexia and loss of weight. By the 49th day she had lost 4 kg. in weight, the hematocrit was down to 39 per cent, and a nasal discharge was observed. Her general condition and appetite improved after cessation of the silver injections. On the 62nd day the red cell hematocrit was 36 per cent and the leucocyte count was 13,600. 400 mg. of collargol were given that day. On the 70th day the hematocrit was 29 per cent and the leucocyte count was 13,400; nevertheless, she was given another 400 mg. dose of collargol. 5 days later the hematocrit was 24 per cent, the leucocyte count 9,500; the white count continued between 9,000 and 15,000 and she remained very lethargic until the end. The hematocrit was 18 per cent from the 101st until the 115th day. On that day she was given 300 mg. collargol, although her condition was bad at the time. 3 days later the hematocrit had risen to 27 per cent and it was 22 per cent on the 122nd day. At that time she was given 600 mg. of collargol, making a total of 2,600 mg. in 4 months. She was found dead the next morning. The day before death she weighed 9.8 kg., whereas the initial weight had been 14 kg. Hemolysis was present during and for several days after each course of collargol.

Necropsy showed an extreme emaciation; slimy exudate in the nose. The spleen had a dark slate color, and microscopic examination showed very numerous clumps of yellowish brown granular particles (silver), chiefly intracellular. The latter were not colored blue when stained for iron. A few megakaryocytes were seen. The liver was chocolate brown, and showed numerous yellowish brown granular particles in the sinusoids, most of which were in large phagocytes. There were extensive central necroses in which there were moderate numbers of mononuclears and very prominent deposits of the granular material. The pancreas had a gray color but microscopic examination showed many yellowish brown granular particles (silver) scattered in the stroma. The kidney sections revealed a small amount of similar particles in the glomerular tufts, which did not turn blue with the iron stain. A lymph node showed numerous clumps of these particles. The femur marrow was very hyperplastic, and all the fat had disappeared; there were abundant parent marrow cells and several mitotic figures were noted. The vertebral marrow was similar to that of the femur, but in addition it contained a moderate amount of the yellowish brown granular particles.

The following protocol shows the results of 3 courses of silver, followed each time by an hemolysis, an anemia, and later by recovery.

After the second course of silver the hematocrit was so low (20 per cent) that a transfusion was given. This apparently had only a temporary effect. The peripheral destruction of the blood and the hyperplasia of the marrow are the outstanding findings.

Dog 28–178.—A male, weighing 15.5 kg., received between 50 mg. and 125 mg. of collargol daily for 13 days, making a total of 1,150 mg. The original hematocrit of 54 per cent had dropped to 25 per cent on the 22nd day, and the weight had fallen to 12.9 kg. on the 25th day. By the 57th day the hematocrit had risen to 32 per cent. He was then given 70 mg. of collargol daily for 10 days. On the 71st day the hematocrit was 20 per cent; that day a transfusion was done which brought the red cell hematocrit up to 30 per cent. On the 86th day it had declined to 21 per cent and another transfusion was done which increased the red cell hematocrit to 26 per cent. On the 97th day the red cell hematocrit was 28 per cent and his general condition seemed very good. Because of a rising hematocrit a third course of silver was given, consisting of 150 mg. for 4 days, on the 98th to 101st days. On the 99th day the hematocrit was 34 per cent and on the 101st it was 33 per cent—that day he was inactive and did not eat. This condition continued, he gradually became weaker, and died on the 105th day. The hematocrit was 28 per cent the day before death.

The plasma showed hemolysis from the 4th to the 15th day, but had regained its normal color by the 17th day. It had a lemon color after the second course of collargol, even before the transfusion. It was very red during the last 3 days of life, following the third course of silver. The leucocyte count dropped to 4,600 on the 23rd and 24th days, but thereafter it remained between 8,000 and 13,000.

Necropsy revealed a dark brown spleen in which microscopic examination showed very numerous clumps of coarsely granular golden brown particles (silver). The liver showed many focal necroses with an infiltration of mononuclears and the Kupffer cells were filled with coarsely granular golden brown particles. Bone marrow from the femur, vertebrae, and ribs showed marked hyperplasia, with no death of the parent cells.

DISCUSSION

Five of the acute experiments were characterized by edema and congestion of the lungs, obviously due to the acute toxic action of the massive doses of silver. There is nothing specific about this reaction which is typical of the heavy metals.

Patein and Roblin (4) reported a human death which occurred within 2 hours after an intravenous injection of 50 mg. of collargol. The autopsy showed acute

pulmonary edema. Chemical analysis of the tissues of their patient showed no silver in the lungs, a definite trace in the kidneys, and an abundance in the spleen and liver. Those results are in harmony with the microscopic findings in our animals, in which the endothelial cells of the liver and spleen contained large deposits of the silver. The spleen in our dogs usually presented a dark slaty color. An iron stain was done on several cases, which indicated that these deposits did not contain any hemosiderin. Voigt (5) mentions a rabbit weighing 2 kg. which was given an intravenous injection of 120 mg. of collargol. There was no immediate reaction, but the next morning it was found dead. Autopsy showed definite edema of the lungs; the liver and spleen had a black color, and contained abundant accumulations of dark particles; the kidney contained none but the reticulo-endothelial cells of the bone marrow were laden with silver particles.

Herzog and Roscher (1) injected collargol into rabbits and obtained a rapidly developing anemia. More recently Muller (3) has found that colloidal silver, administered intravenously to rabbits, produced first an erythrocytic hyperplasia, followed by a condition which resembled aplastic anemia. Aplasia of the erythroblastic bone marrow seemed to be present before there was an appreciable decrease in the number of circulating red blood cells. She concluded that the anemia was caused by the endothelial cells of the bone marrow forming clasmatocytes at the expense of the development of the erythrocytes.

It is noted that three of our dogs contracted distemper soon after the silver injections were begun. We feel justified in attributing the onset of this disease to the lowered vitality of the animals which resulted from the silver intoxication. It is possible that these were latent cases of distemper and that an exacerbation occurred when the resistance was lowered. In this connection it may be noted that 30 years ago collargol was used as an internal antiseptic under various conditions.

During the first 5 to 8 days of the injection of collargol the red cell hematocrits drop 7 to 14 per cent, although the bone marrow may appear normal or moderately hyperplastic at autopsy. Although the animals which contracted distemper have shown a decline in the hematocrit of 20 to 23 per cent, the bone marrow seemed normal or slightly hyperplastic. In animals which were studied almost 4 months a marked anemia was observed, with a terminal hematocrit of 22 per cent to 28 per cent, but this was accompanied by a hyperplastic bone marrow. Thus, in none of the animals did we find the slightest sign of bone marrow *aplasia*; indeed quite the opposite picture was presented, for there were numerous parent cells which seemed perfectly capable

of producing mature blood cells. Likewise the human cases which died after repeated injections of collargol presented moderate anemia and hyperplasia of the bone marrow.

This anemia observed in our experiments is probably in large part due to an increased destruction of the circulating red blood cells, since we observed hemolysis in the plasma of several of the animals. Muller (3) stated that she obtained no evidence of injury to the red blood cells in the peripheral circulation, but she notes a rapid decrease in the number of erythrocytes in one rabbit, in which the spleen was found loaded with pigment. Motohashi (2) found marked phagocytosis of the red blood cells in rabbits following the injection of collargol. Voigt (5) states that after being injected intravenously, the colloidal silver is gradually changed into the ionized form, and he attributes collargol poisoning to this transformation.

CONCLUSIONS

1. Colloidal silver has no specific action on the bone marrow in dogs but is a systemic poison which may cause anorexia, weakness, loss of weight, anemia, and death.

2. Hemolysis can be demonstrated after large doses of colloidal silver and the anemia presumably is due in part at least to a destruction of red blood cells in the peripheral circulation.

3. The colloidal silver, injected intravenously, is deposited as granules almost exclusively in the cells of the reticulo-endothelial system after the manner of particulate substances.

4. Repeated injections of non-lethal amounts of this substance are invariably followed by hyperplasia of the bone marrow. In no case was aplasia found.

5. Large single doses of this material cause rapid death in 12 hours or less characterized by pulmonary edema and congestion.

6. An initial increase in the number of erythrocytes and leucocytes may occur following smaller amounts of silver, but repeated injections cause a considerable anemia, without a definite increase in the leucocytes and with no signs of blood platelet deficiency.

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