EXPERIMENTAL BONE MARROW REACTIONS.

I. Anemia Produced by Collargol.

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Plate 19.

(Received for publication, December 14, 1925.)

As a part of an investigation of the processes which underlie the various types of anemia, the attempt is made in the present study to analyze the factors of blood formation and blood destruction in a form of experimental anemia produced by collargol or colloidal silver.

Colloidal silver was employed experimentally by Cohn (1) in 1904. He demonstrated that the silver particles were engulfed in phagocytic cells in the same manner as other substances existing in a finely subdivided state. Since that time numerous experiments have established the fact that many varieties of particulate matter, when introduced in the blood stream, are deposited in phagocytic cells supposedly of reticular and endothelial origin. Because of the functional similarity of these cells, Aschoff (2*a*) introduced the all including term "reticuloendothelial system," but Sabin (3*a*) has recently differentiated the reticulum and endothelium and their respective genetic phagocytic derivatives into "monocytes" and "clasmatocytes." Throughout this paper the terminology of Sabin is adhered to.

Kiyono (4) studied the difference of the deposition of collargol and carmine. He found that collargol passes very slowly through the vessel wall, carmine more quickly. Hence it was much more difficult to make the clasmatocytes of the interstitium of the internal organs take up silver granules even after repeated injections. He concluded, therefore, that collargol has a more selective action than carmine on the endothelium of the so called blood-forming organs. Similar results were obtained by Tschaschin (5). The avidity of Kupffer's cells for colloidal silver in comparison with other colloidal metals has been noted by Brötz (6). A difference of deposition and distribution of colloidal substances has also been observed by Evans (7*a*), Evans and Schulemann (7*b*), Nissen (8), and Drinker, Shaw, and Drinker (9).

Interest in the phagocytosis of particulate matter has been superseded by investigation of other functions of these widely distributed phagocytic cells. Ribbert (10), the first one to demonstrate these cells by use of vital dyes, as early as

1904, suggested that they might be of importance in metabolic activity. More recent efforts have been directed towards two main problems; namely, the site of bile pigment and antibody formation.

Very little attention has been devoted to the effects of particulate matter on the bone marrow from the point of view of blood formation and blood destruction. For this purpose, colloidal silver was employed in the present study. This colloid is not easily eliminated from the body, lodging in the so called hematopoietic organs; *i.e.*, mainly in liver, spleen, bone marrow, and lymph nodes. It is evident that colloids easily dispersed and eliminated from the body may differ in the results produced.

Material and Methods.

The conclusions of this paper are based on observations of 25 rabbits and 46 rats, 20 of the rats being splenectomized. The animals all appeared to be in excellent health.

The collargol or colloidal silver employed is said to contain 78 per cent of metallic silver and a small percentage of egg albumin and its oxidation product (11). It is manufactured by the Heyden Chemical Works, and distributed by Schering and Glatz, New York. The size of the particles has been determined by Bechhold (12) to average 20 millimicra ($\mu\mu$), the individual particle consisting of aggregates of metallic silver and the protective colloid. The concentration of the colloidal suspension, which was made up in small doses in sterile distilled water and filtered immediately before use, varied between 0.1 and 2 per cent. Physiological saline as a solvent was tried, but a white precipitate was formed on standing and the suspension changed color. The same observation was made by Voigt (13*a*).

As a rule injections were given intravenously every 2nd day. Ear veins were used in the rabbits and tail veins and femoral veins in the rats. A small amount of ether was given the rats and their skin was nicked before inserting the needle.

Frequent examinations were made of the peripheral blood. For blood counts the median artery of the ear of the rabbit was pricked with a needle and the tail of the rat snipped with a pair of sharp scissors. The hemoglobin was read in a Duboscq colorimeter against the Newcomer standard. Vital stains with brilliant cresyl blue were made in all instances and the smears counterstained with Wright's stain.

Some of the rabbits were killed with a blow on the head and subsequent bleeding, after one or more injections; others succumbed to the anemia produced. The surviving rats were killed by ether and bleeding. All tissues were fixed in Zenker's fluid, stained with watery eosin 5 per cent, and counterstained with

methylene blue. Ammonium sulfide and potassium ferricyanide were used for iron stain. The latter dissolves out the silver from the cells, thus removing a substance which otherwise would have obscured any iron present in the tissue.

The bone marrow specimens of the rabbits were consistently taken from the right femur. The cross-sections studied were obtained from the upper third at the level of one of the nutrient arteries entering the bone about ½ inch below the head of the femur. Longitudinal sections were taken from the middle and the lower end of the femur. In rats the entire bone marrow from the right femur was fixed.

Normal animals and animals receiving distilled water intravenously were studied as controls.

Experimental Observations on Rabbits.

The most satisfactory results were obtained with rabbits as their femoral bone marrow is normally more fatty and less hyperplastic than that of rats. Of the twenty-five rabbits studied, eleven received colloidal silver in sufficient amounts to produce a fatal anemia. Of these, eight died and three were killed when moribund. The other fourteen animals were killed at various intervals before a fatal anemia had developed.

Three fairly well defined stages followed the injection of colloidal silver into rabbits; namely, an initial stage of erythropoietic hyperplasia, an intermediary stage with myeloid hyperplasia predominating, and a final, comparatively aplastic, stage.

1. The stage of erythropoietic hyperplasia may be illustrated by two groups of experiments.

(*a*) This included three animals killed after one dose of collargol.

Rabbit H-45, a female weighing 1843 gm., received 4 cc. of a 2 per cent suspension, and was killed 24 hours after the injection. The autopsy and microscopical examination gave essentially normal findings except for the deposition of collargol to a small extent in endothelial cells and clasmatocytes.

Rabbit H–3, a female weighing 1498 gm., also received one dose of 6 cc. of a 0.4 per cent collargol suspension, but was killed 7 days after the administration. Microscopical examination of the marrow revealed a slight but definite endothelial and erythropoietic stimulation. Not only the endothelium lining the patent sinuses showed this early stimulation, but also the endothelial cells between the fat cells were large and swollen, many of them indicating a potential communication between two widely open sinuses. The picture seen in this early stimulated marrow resembles the description given by Doan, Cunningham, and Sabin

(14*a*) of early stimulated marrow in pigeons and rabbits. It also illustrates beautifully the intersinusoidal capillaries described by Doan (14*b*) and their opening up between the fat cells when the marrow is stimulated. A fair number of clasmatocytes and endothelial cells contained small amounts of collargol, many of them situated in clusters of erythroblasts and normoblasts. A few myelocytes showed mitosis, but erythroblastic stimulation predominated. In Fig. 1 is shown an isolated focus illustrating endothelial hypertrophy and mitosis in close approximation to megaloblasts and clasmatocytes containing small amounts of colloidal silver.

(*b*) Animals receiving more than one injection showed a greater degree of hyperplasia. After a certain number of injections, however, varying in different animals, a hypoplasia was produced.

Rabbit H-13, a large male weighing 2755 gm., was killed when the peripheral blood showed marked erythropoietic activity (22 normoblasts were seen in stained specimen while 100 white blood cells were counted), without appreciable decrease of red cells. The total amount of collargol administered was 174 mg. per kilo. The bone marrow showed a more advanced stage of erythropoietic stimulation than that of Rabbit H-3. This hyperplasia included the endothelial cells, which were definitely swollen in most places, with an increase of the number of foci of megaloblasts, erythroblasts, and normoblasts. The myeloid elements were increased only to a moderate extent. Architecturally, the bone marrow was intact, but many fat cells appeared shrunken with consequent increase of the interstices between the fat cells. The venous sinuses were dilated but there was no apparent extravasation of the blood into the parenchyma. The collargol was deposited mainly in clasmatocytes, and, in very small amounts, in the endothelial cells.

The above experiments show the first stage of the reaction following the intravenous injections of colloidal silver. A considerable amount of the silver was taken up by the phagocytic cells of the liver, spleen, and lymph nodes, and only a small amount reached the bone marrow where it produced a slight stimulation of the endothelial cells with an increase of clasmatocytes and cells of the erythrocytic series. Evidence of this erythropoietic stimulation was found in the outpouring of normoblasts in the peripheral blood. It is interesting to note that an outpouring of young cells occurred at some stage in all the animals receiving moderately large amounts of collargol intravenously, even before any decrease of blood cells had taken place. Stimulation of the myeloid series was less marked.

A similar stimulation of the bone marrow has been observed incidentally after injections of other colloids.

Nissen (8) found a hyperplastic bone marrow after five doses of "elektroferrol" and five doses of carmine, with erythroblastic hyperplasia predominating. Doan, Cunningham, and Sabin (14*a*) describe endothelial and reticular hypertrophy in pigeons after injections of three doses of trypan blue and carmine. A pigeon receiving trypan blue when fasted did not develop a hypoplastic bone marrow as the result of inanition, but the dye had a stimulating effect on cell production in the marrow, and a hyperplastic bone marrow was obtained. The stimulating effect of the dye seemed to be dominant over the influence of inanition, which usually decreases hematopoiesis in pigeons.

2. The second or intermediary stage is represented by Rabbits H-35, H-34, and H-47.

Rabbit H-35, female weighing 1850 gm., received the same amount of collargol per kilo body weight as Rabbit H-13, given, however, in four large doses. The peripheral blood, just before the animal was sacrificed, showed practically no erythropoietic stimulation and no anemia. Microscopically, the marrow showed a hyperplasia, the cellularity being approximately the same as in Rabbit H-13. The interesting feature in this marrow was the definite shifting of the stimulation to the myeloid series. The clasmatocytes were prominent, loaded with collargol, but collargol was also deposited in the endothelium lining the wide open sinuses. The endothelium showed very little activity, and there was practically no evidence of erythroblastic stimulation. The fat cells were shrunken in size and irregular in outline.

Rabbit H-34, of about the same weight and receiving the same treatment as No. H-35, showed marked erythropoietic stimulation, indicated in the peripheral blood by the number of normoblasts present (20 normoblasts seen in stained specimen while 100 white blood cells were counted). Red blood cells were normal (5,100,000). The bone marrow was intensely hyperplastic; this hyperplasia included both the erythrocyte and the myeloid series, the increase of the myeloid elements predominating.

Rabbit H-47, a female, was given 140 mg. per kilo body weight, administered in seven doses. When the animal was killed there were no normoblasts in the peripheral blood and the red blood cells were decreased (3,730,000). The bone marrow picture is practically identical with that of No. H-35 described above.

These animals represent a more advanced phase of the effects of colloidal silver on the bone marrow. In the case of No. H-35, owing to larger dosage and perhaps somewhat to individual susceptibility, the phase of erythrocyte hyperplasia was passed and there was little

or no evidence of hyperplasia of the endothelium. Rabbit H-34 possibly represents a transition between the first and second stage, while Rabbit H-47, as shown by the red blood cell count, was rapidly approaching the final stage.

In all the animals so far described as showing red cell stimulation (Group 1, and No. H-34, Group 2), there was a coincident hyperplasia of endothelial cells, many of which were normal in appearance and without phagocyted particles. In the remaining animals of Group 2, however, many of the endothelial cells contained collargol. Fat absorption and myeloblastic hyperplasia have been noticed by Nissen (8) after five doses of "elektrokollargol." Doan, Cunningham, and Sabin (14*a*) found a mild myeloid hyperplasia with very little red blood cell activity in a rabbit subjected to fifteen intravenous injections of trypan blue. The endothelium in this case contained trypan blue in contrast to that of animals receiving only a few injections of the dye.

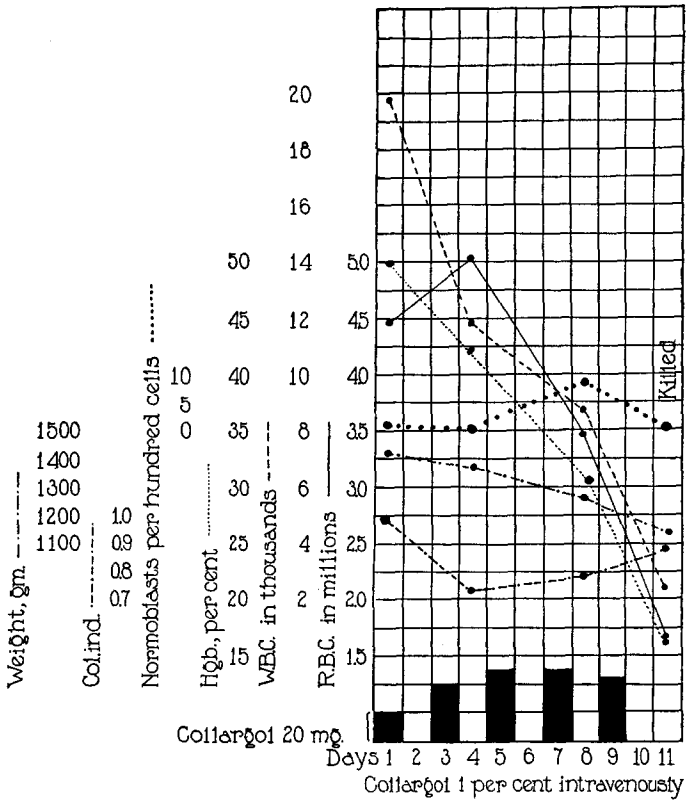
3. The final stage is shown by the animals which were killed when moribund as the result of the collargol injections or which died spontaneously with signs of grave aplastic anemia. Their course is best represented by charts.

Rabbit H-16, Chart 1, a young female, showed an unusually rapid course. On the 2nd day after the last dose the animal was moribund. On microscopical examination of the bone marrow all normal structure was gone, and the fat cells were replaced by an acidophilic fibrin-like ground substance or stroma with few developing cells. The marrow cells were definitely decreased and the few cells remaining were in lines in close relation to the blood vessels. The collargol was deposited in both clasmatocytes and endothelial cells. The endothelial cells lining the patent sinuses and those in between the sinuses were hypertrophied. An occasional megaloblast was seen; most of the red blood cells present, however, were in the erythroblastic stage, while the normoblastic elements were practically absent. The more mature myelocytes had largely disappeared, but a few foci of young myelocytes were seen here and there. The spleen was loaded with iron, which may be due to an early stimulation of the endothelial cells and their derivatives to red blood cell phagocytosis, as has been observed by Motohashi (15) after injection of collargol.

The rapidly fatal course of No. H-16, with the marked diminution of the cellular elements in the peripheral blood, suggests that, in addition to the depression of blood formation, a very active destruction must have taken place, perhaps by means of red blood cell

phagocytosis in the spleen. This, however, was not a prominent feature in the other animals with a fatal course.

Rabbit H-27, Chart 2, a male, had a more protracted course and lived 6 days after the last injection of collargol. The day before exitus the hemoglobin was



In Charts 1 to 4 the number of normoblasts given is the number per 100 *white blood cells*.

CHART 1. Rabbit H-16. Unusually rapid course in young animal. The spleen was filled with iron, which suggests increased blood destruction in addition to depression of blood formation. Striking evidences of blood destruction not found in other animals similarly treated.

12 per cent, red blood cells 1,200,000, and leucocytes 4100. The bone marrow was grossly grayish and firm. Microscopically, the fat cells showed signs of shrinkage, the widened interstices being filled with the same eosin-staining ground substance as in No. H-16. The collargol was aggregated in large masses, consisting, apparently, of several clasmatocytes filled with pigment. Around these masses

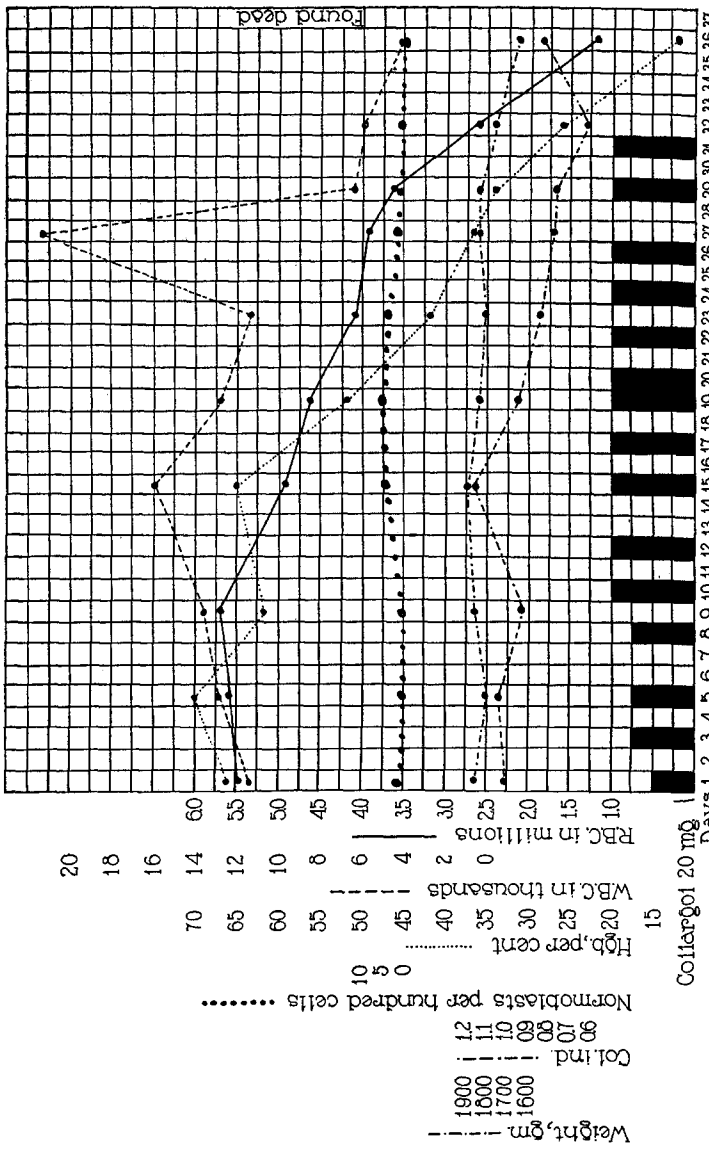


CHART 2. Course of Rabbit H-27, an older animal, which died at a time when the hemoglobin was 12 per cent, red blood cells 1,200,000. Striking hypoplasia of the bone marrow with hypertrophy of the endothelium of the vessels of the mesenteric lymph nodes.

there was no evidence of inflammatory reaction or encapsulation. A moderate amount of collargol was found in the endothelium which, in places, showed hypertrophy. A small number of erythroblasts were present with a moderate number of normoblasts. A moderate number of myelocytes of the later stage were seen. The cells of both the erythrocytic and myeloid series were definitely decreased. This animal showed a decrease of the number of megalocaryocytes. This was noted in two other animals, in one of which death was precipitated by hemorrhages from the intestinal mucosa.

The bone marrow of No. H-27 gave very little evidence of endothelial hypertrophy or erythropoiesis. This animal, however, showed a proliferation of the endothelium in the small veins of the mesenteric lymph nodes. The endothelium in these places did not show any collargol in contrast to the endothelium of the sinuses, which contained a moderate amount of silver. The phase of division was not caught in this section and there are no evidences in what direction these new cells were differentiating.

Rabbit H-29, male, Chart 3, was killed when moribund 6 days after the last dose. The peripheral blood just before death showed: hemoglobin 18 per cent, red blood cells 2,100,000, white blood cells 3500. Microscopical examination of the bone marrow showed essentially the same as in No. H-27. This animal illustrates beautifully the outpouring of normoblasts (see chart) beginning before any decrease of red blood cells has taken place and stopping when the peripheral blood shows a decrease of red blood cells—a time when one would expect to find normoblasts and other evidences of active blood formation.

Rabbit H-1 is noteworthy from two points of view; namely, the more chronic course with more severe bone marrow aplasia, and the failure to regenerate although life was prolonged for 18 days after the last injection. In all 660 mg. per kilo body weight were administered over a period of 41 days. 8 days before death, the hemoglobin was 22 per cent, red blood cells 2,480,000, leucocytes 10,150 with 1 normoblast in 100 leucocytes counted. The marrow showed the same eosin-staining fibrillar ground substance found in this group of animals and marked cellular aplasia except at the periphery of the cross-section, where there was a cellular area not containing collargol. An enormous number of clasmatocytes contained collargol, in comparison with which the endothelial cells contained but little colloidal silver. The endothelium showed no signs of hypertrophy and most of the endothelial cells present were lining the dilated venous sinuses and capillaries which persisted throughout. The architecture and the striking diminution in number of cells in this bone marrow are well shown in Fig. 2.

In all the animals the number of blood cells remained normal for a considerable length of time after the injection of collargol had begun.

To see whether the animals would recover if the injections were stopped as soon as the red blood cells began to decrease and before a definite anemia had developed, the following experiment was performed. Five rabbits of about the same weight were given eight injections of 4 cc. of 2 per cent collargol each. One animal was killed 6 hours after the last dose, two animals were killed 4 days after the last dose, and two animals succumbed spontaneously.

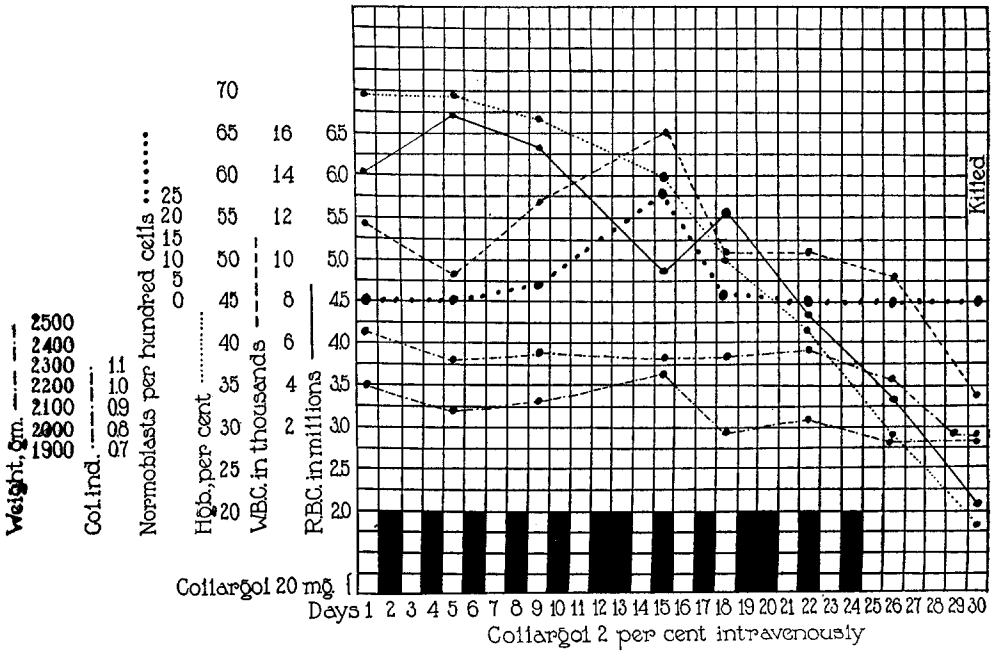


CHART 3. Rabbit H-29, illustrating the outpouring of normoblasts beginning before any decrease of red blood cells has taken place and stopping when the peripheral blood showed a decrease of red blood cells.

The course of three of these experiments is represented graphically in Chart 4, *a*, *b*, and *c*.

Rabbit H-52, female, Chart 4, *a*, was killed 6 hours after the last dose. As may be seen from the chart, the red blood cells showed some decrease in number. The bone marrow showed a moderate hyperplasia of both the erythrocyte and myeloid series, but the latter predominated. One may classify this marrow as belonging to the second or intermediary group. The general architecture and cellularity are represented in Fig. 3.

In Rabbit H-33, female, Chart 4, *b*, killed 4 days after the last dose of collargol, the marrow showed a comparatively normal structure as far as fat cells,

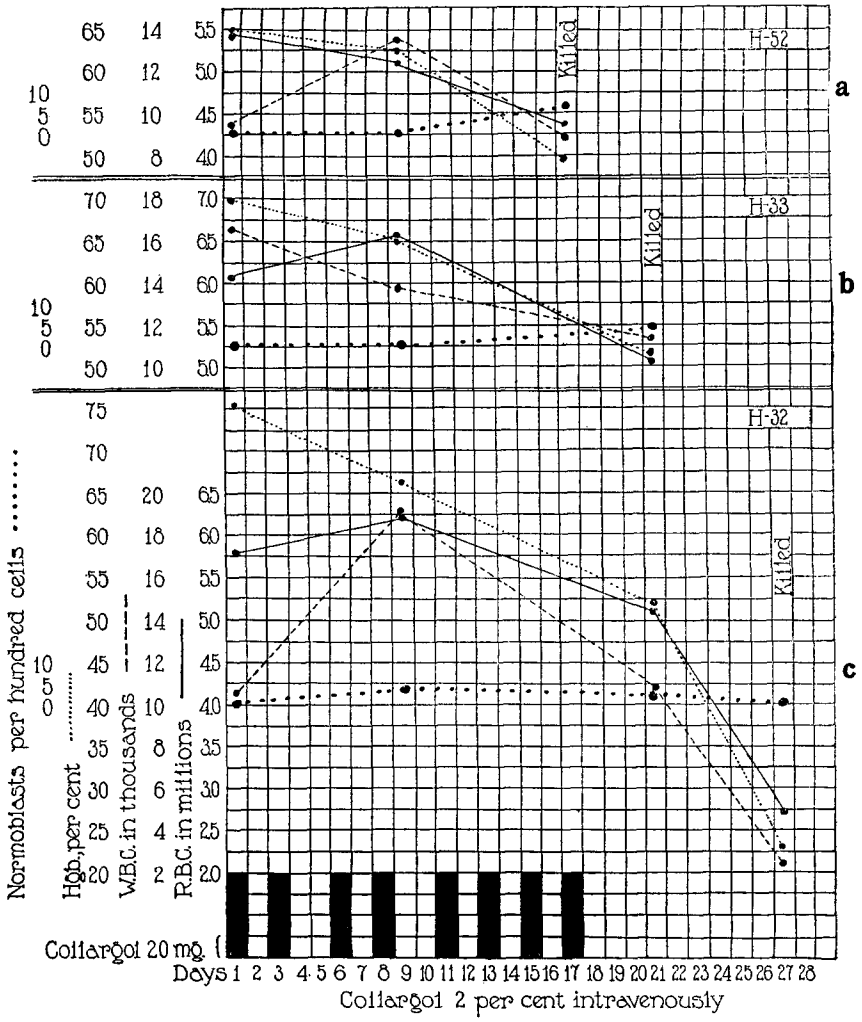


CHART 4, *a* to *c*. (*a*) Rabbit H-52, killed 6 hours after the last dose. For architecture and cellularity of bone marrow see Fig. 3. (*b*) Rabbit H-33 killed 4 days after the last dose at a time when blood was beginning to show some decrease in erythrocytes and hemoglobin. The marked decrease in marrow cells and deposition of collargol in the endothelium is illustrated in Fig. 4. (*c*) Rabbit H-32 killed when moribund. Bone marrow similar to that in Fig. 4.

blood vessels, and megalocaryocytes were concerned. There was, however, a marked decrease in cells of both the myeloid and erythroid series. The collargol was deposited in considerable amounts in the endothelium, which did not seem hypertrophied and was present as well in large amounts in clasmatocytes.

The bone marrow of Rabbit H-32, female, Chart 4, *c*, killed when moribund, showed very few marrow cells and fat cells decreased in number and shrunken in size. The blood capillaries and sinuses were well maintained, practically all of them lined with a hypertrophied endothelium containing collargol. An occasional focus of red blood cells was present, mainly in the erythroblastic stage with a rare megaloblast. A few groups of normoblasts also remained. The myelocytes were scant, most of them being immature forms.

Fig. 4 illustrates the deposition of collargol in the endothelium as well as the general structure and cellularity of the marrow of No. H-33. In several of the animals receiving 2 per cent collargol, the fat cells did not disappear to the same extent as in those animals receiving less concentrated solutions. This seems to indicate that the stage of stimulation was less protracted in those animals receiving the higher concentration of the silver suspension. Ribadeau-Dumas and Debré (16) have also noticed that the bone marrow reaction in rabbits was modified towards hyperplasia by weak and moderate doses of collargol, but that the reaction was less intense after concentrated doses.

The marrow of the other two of the five animals similarly treated was practically the same as that of those described above.

The animals just described (Nos. H-52, H-33, and H-32) present several interesting points. The administration of collargol was stopped after eight injections and before the animals had begun to show any appreciable anemia. The bone marrow of the rabbit (No. H-52) killed 6 hours after the last dose showed considerable signs of activity and stimulation, and was in marked contrast to that of the animals allowed to live without further administration of collargol. This suggests that the action of the colloidal silver continues after the administration has been stopped, probably by the mechanism of disintegration of the clasmatocytes containing collargol and the engulfing of the particles thus set free by new clasmatocytes and endothelial cells. The bone marrow of the animals killed 4 days after the last dose showed a greater depression of the erythropoietic function than that of the animals allowed to live until moribund. The striking coincidence of a bone marrow aplasia without any anemia in the peripheral blood indicates that collargol injures the bone marrow without affecting the formed red cells. After the injury is accomplished, the animal continues to live until the circulating formed blood elements

decrease in the course of normal blood destruction to a point incompatible with life. There is, however, some slight effort on the part of the bone marrow to produce blood cells, evidenced by the occasional foci of erythroblasts, although this effort is wholly inadequate and the animal dies with signs of grave anemia.

The peripheral blood of all the animals in which a fatal anemia was produced showed a marked anisocytosis towards the end, with pronounced central pallor and a tendency to microcytosis. Platelets, as noted in the smears, were abundant. Normally, the blood of rabbits shows some polychromatophilia and reticulated cells. Discounting this, all the animals receiving several doses of collargol showed certain constant characteristics; namely, the progressive increase of young cells in the peripheral circulation followed by decrease, until finally neither polychromatophilia, reticulated cells, nor normoblasts were present. This later stage was seen only in animals running a fatal course. The color index in most instances was decreased.

The autopsy findings, other than those already discussed, were uniform in the eleven animals which died from anemia. As was to be expected, large accumulations of collargol were found in phagocytic cells in the liver, spleen, and lymph nodes. In the kidneys the collargol was deposited in the endothelium of the capillaries, but very little was found in the glomeruli or in the epithelium of the tubules.

Voigt (13*b*), studying the fate of collargol in rabbits, found that kidneys and intestines contained silver. He examined urine and feces but could not demonstrate the presence of silver.

That the distilled water used as solvent for the collargol played no part in the results produced was conclusively proven by findings in control animals.

Experimental Observations on Rats.

A. Normal Rats.

As indicated above, rats did not prove to be as satisfactory experimental animals as rabbits. Many factors probably contributed to this, the most important being the very active hematopoietic function in rats, and the comparatively large spleen and liver which seem to

form an efficient protective barrier to substances injected in the blood stream. Nevertheless, in spite of this, anemia, secondary in type, was produced in all animals receiving a moderate amount of collargol, and a few animals died as a result of this anemia.

While the red blood cells and hemoglobin of the rabbits gradually decreased if the injections were stopped when the initial decrease had begun, the rats recovered promptly as soon as the injections were discontinued, unless hemoglobin and red blood cells had reached a dangerously low point. Therefore, in order to produce an anemia, injections had to be continued. In the later stages the administration became increasingly precarious, and the experiments were interrupted in many cases by the animals dying under the ether or shortly after.

Chart 5 illustrates the course of Rat R-26, which died 6 days after the last injection with signs of grave anemia. The bone marrow in this, as well as in many other animals, could not be analyzed as no definite structure could be made out, but there was a relative decrease of cellular elements and most of the cells present were young forms. The megalocaryocytes were definitely decreased.

Six rats were injected with a 0.1 per cent suspension, the lowest concentration employed in these experiments. The course of all these animals seems to point to a direct relationship between the amount of collargol injected and the anemia produced.

That the general experimental procedure and the distilled water used for solvent of the collargol played little or no part in the production of the anemia was shown by the five controls.

In comparing rats and rabbits certain differences stand out. The bone marrow of the rabbit contained more collargol after the administration of comparatively smaller amounts, and the degree of depression of the hematopoietic function was more marked in rabbits than in rats. Leucopenia, a comparatively constant finding toward the end in rabbits, was not obtained in rats. Iron was always found in the spleen of rabbits dying from anemia, but in rats it was practically absent after the administration of a small number of medium sized injections in spite of the fact that the spleen of the adult rat is normally loaded with iron.

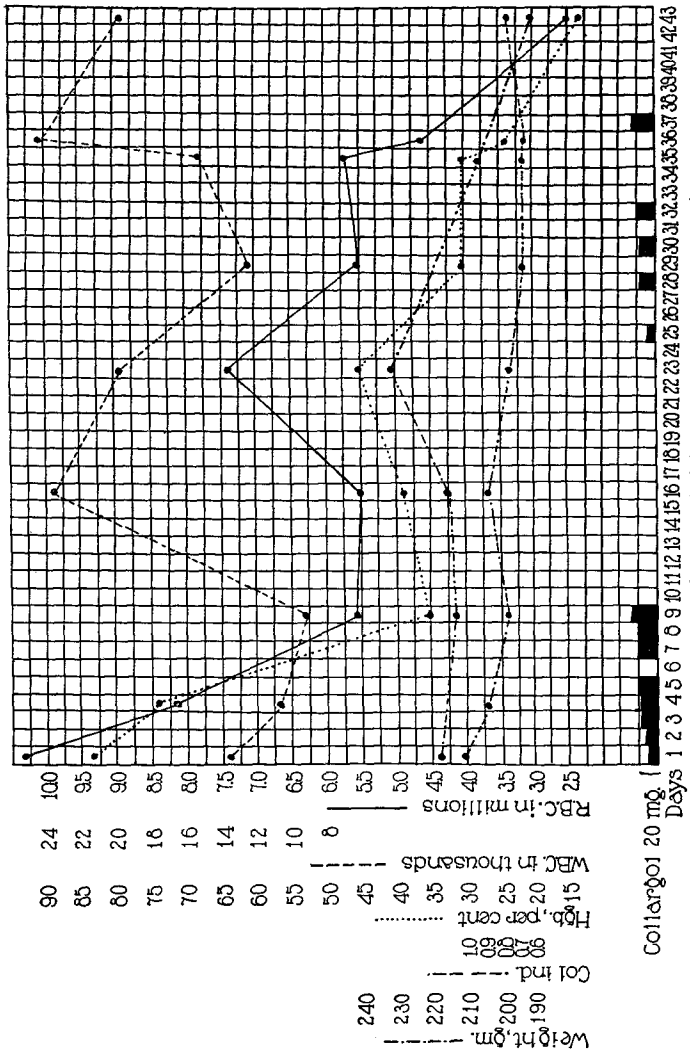


CHART 5. Course of Rat R-26. Death occurred 6 days after the last injection. Signs of grave anemia.

B. Splenectomized Rats.

To see whether it would be possible to cause collargol to be deposited in larger amounts in the bone marrow, a series of rats was splenectomized.

The general course of the splenectomized rats did not differ from that of the non-splenectomized ones. As in the non-splenectomized series, animals were killed at different stages. As far as could be determined, there was no appreciable difference in the degree of anemia produced in the same time interval with the same amount of collargol, nor did there seem to be any marked increase in the amount of collargol deposited in the bone marrow.

The findings described above seem to indicate that the chief site of clasmatocyte formation differs in animals of different species. In the rabbit, the bone marrow participates to a considerable extent, while in rats, after the removal of the large spleen, most of the compensatory function is shifted to the liver. This probably holds true for non-splenectomized animals also, after overloading the spleen with particulate matter.

That the site of compensatory activity varies in different animals has been shown by several observers.

Pearce and Austin (17) found that in splenectomized dogs a compensatory activity took place in lymph nodes and liver. In splenectomized rats, Lepehne (18) found compensatory activity in Kupffer's cells in the liver. Motohashi (15), in a series of splenectomized rabbits, observed that hemophagic activity of the spleen in rabbits had been transferred to the bone marrow and, to a lesser extent, to the liver. This compensatory function has been emphasized by Krumbhaar (19).

DISCUSSION.

The above experiments indicate that the administration of comparatively small amounts of collargol produced, first, a stimulation of the endothelium, of the erythrocyte series, and, to some extent, of the myeloid series; and, second, the accumulation of the collargol mainly in the clasmatocytes and, to a much less extent, in the endothelial cells. The animal's health remained unimpaired and the blood counts were normal.

If larger amounts of collargol were administered over a longer period of time, the bone marrow changed from a hyperplastic to a compara-

tively aplastic one, and, in addition to the clasmatocytes, the endothelial cells took up a considerable amount of collargol. At this stage, there was a very marked anemia and scarcely any evidence of red cell formation in the bone marrow. The myeloid series showed more activity and the leucocytes in the peripheral blood decreased in number only towards the end.

That collargol produces similar results in man has been shown by accidental fatalities from therapeutic use.

Two cases were reported by Herzog and Roscher (20*a*), in 1922, in which, after large doses of collargol, constitutional symptoms appeared, both cases ending fatally, one in 9 days and the other in 16 days. Leucocytosis and outpouring of normoblasts occurred in these cases just as in the rabbits, before anemia had developed. In the chronic case leucocytosis was replaced by leucopenia before the end. The prominent feature, however, was the purpura and the bleeding from the mucous membranes.

Bleeding was not seen in my series of animals except in Rabbit H-29, which showed extensive hemorrhages from the intestinal mucosa, with a marked diminution of the megalocaryocytes in the bone marrow. The authors considered the human cases similar to those of benzene and radium poisoning, interpreting their findings as an initial stimulation of the bone marrow followed by depression.

As the above cases had received, in addition to collargol, two doses of salvarsan, Herzog and Roscher (20*b*), in an attempt to find out the cause of death, injected one rabbit with collargol, administering 912 mg. in twelve doses, distributed over a period of 27 days. The animal died with signs of severe anemia and their results are similar to those of the experiments described above, with this difference, that no leucopenia was obtained and myeloid metaplasia was observed in the spleen.

The explanation of the anemia produced by the injections of collargol must, in the light of the experiments described in this article, be sought in the bone marrow. The coincidence of a stimulated endothelium, with erythropoietic activity, and of a quiescent endothelium, with decrease or lack of erythropoiesis, is, to say the least, striking, while myeloid activity seems to be independent of the endothelial changes. The suggestion, therefore, presents itself that a close relation must exist between the erythrocytes and the endothelium and its derivatives.

As the result of recent research, the theory that the endothelium is the progenitor of the clasmatocytes and the red blood cells is gaining ground. The

most recent investigation and the most convincing proof of the endothelial origin of the clasmatocytes, first suggested by Mallory (21) in 1898, are offered by Sabin (3*b*).

Due to the researches of Sabin *et al.*, new evidence has been brought forth also in support of the theory of the endothelial origin of the erythrocytes. Sabin (3*c*), in 1920, demonstrated this genetic relationship in the chick embryo, while Doan (14*c*) in 1922, analyzing the bone marrow of the adult pigeon, and Cunningham and Doan (22*a*), studying the mammalian bone marrow, drew the same conclusions. From this analysis the conclusion was also drawn that white blood cells arise from the reticular cells extravascularly in the intervascular parenchyma (Cunningham, Sabin, and Doan (22*b*)).

Accepting the evidences offered of the endothelial origin of the clasmatocytes and the red blood cells as a working hypothesis, it ought to be theoretically possible to influence functionally this widespread system of endothelial cells and indirectly the production of both red blood cells and clasmatocytes.

As seen in the experiments described in this paper, the first action of collargol is a stimulation of the bone marrow with outpouring of young erythrocytes and normoblasts. This stimulation is observed after the injection of many colloidal substances. The increased activity is, however, not confined to the erythrocytic series of cells, but also includes the clasmatocytes.

Experimentally, large phagocytic cells were produced by Evans and Winternitz (7*c*), in 1911, Aschoff and Kiyono, in 1913 (2*b*), and Kiyono, in 1914 (4), who found these cells in the circulation after repeated injections of carmine, isamine blue, trypan blue, and collargol. Simpson (23) confirmed these observations, employing various colloidal suspensions.

It is of significance to note that the stimulation caused by the colloidal substances includes both red blood cells and clasmatocytes. Theoretically, one may conceive that if the rate of excretion of the colloid injected is in any way commensurable with the rate of introduction, a continued stimulation of the endothelium could be obtained with new formation of erythrocytes and clasmatocytes. If, however, colloidal substances with a low rate of diffusibility, such as collargol, are injected continuously, the development of the clasmatocytes from the endothelium becomes paramount at the expense of the red blood cell formation and finally the endothelium is filled with

particulate matter and becomes genetically inactive; the animal lives only until the already formed blood elements have been destroyed in the daily wear and tear of the red blood cells. The reticulum, which is less phagocytic than the endothelium, is moderately stimulated throughout and not until the end is a leucopenia observed in the peripheral blood.

CONCLUSIONS.

1. The effect produced by the intravenous administration of collargol on the bone marrow of rabbits varies directly with the amount of collargol injected, and three fairly well defined stages could be recognized.

(*a*) An initial stage after comparatively few and small doses, with erythrocytic and endothelial hyperplasia in the bone marrow and with evidences of this stimulation in the peripheral blood in the form of young erythrocytes and normoblasts.

(*b*) An intermediary stage which followed the injection of larger amounts of collargol, and which was characterized by a predominant myeloid hyperplasia.

(*c*) A final stage with marked bone marrow aplasia and with colloidal silver deposited in endothelial cells, as well as in clasmatocytes. This was associated with a high grade anemia with low color index, resembling aplastic anemia in its main features. This stage terminated fatally.

2. There was no evidence of injury to blood cells in the peripheral circulation. The erythrocytic bone marrow aplasia was present before any appreciable decrease of red blood cells was found in the peripheral blood.

3. The results were less clear-cut in a series of rats, but anemia of a similar type was produced in all animals when sufficiently large doses were injected.

4. Splenectomy did not alter the course in rats materially.

5. It is fair to conclude that the cause of the anemia produced may be sought in the deviation of the parental endothelial cell toward clasmatocyte formation at the expense of the development of erythrocytes.

6. It is suggested that the results may be offered in support of the

theory of the endothelial origin of both clasmatocytes and red blood cells.

I am deeply indebted to Dr. Francis W. Peabody for valuable suggestions in the course of the experiments and it gives me great pleasure to acknowledge his and his staff's constant assistance.

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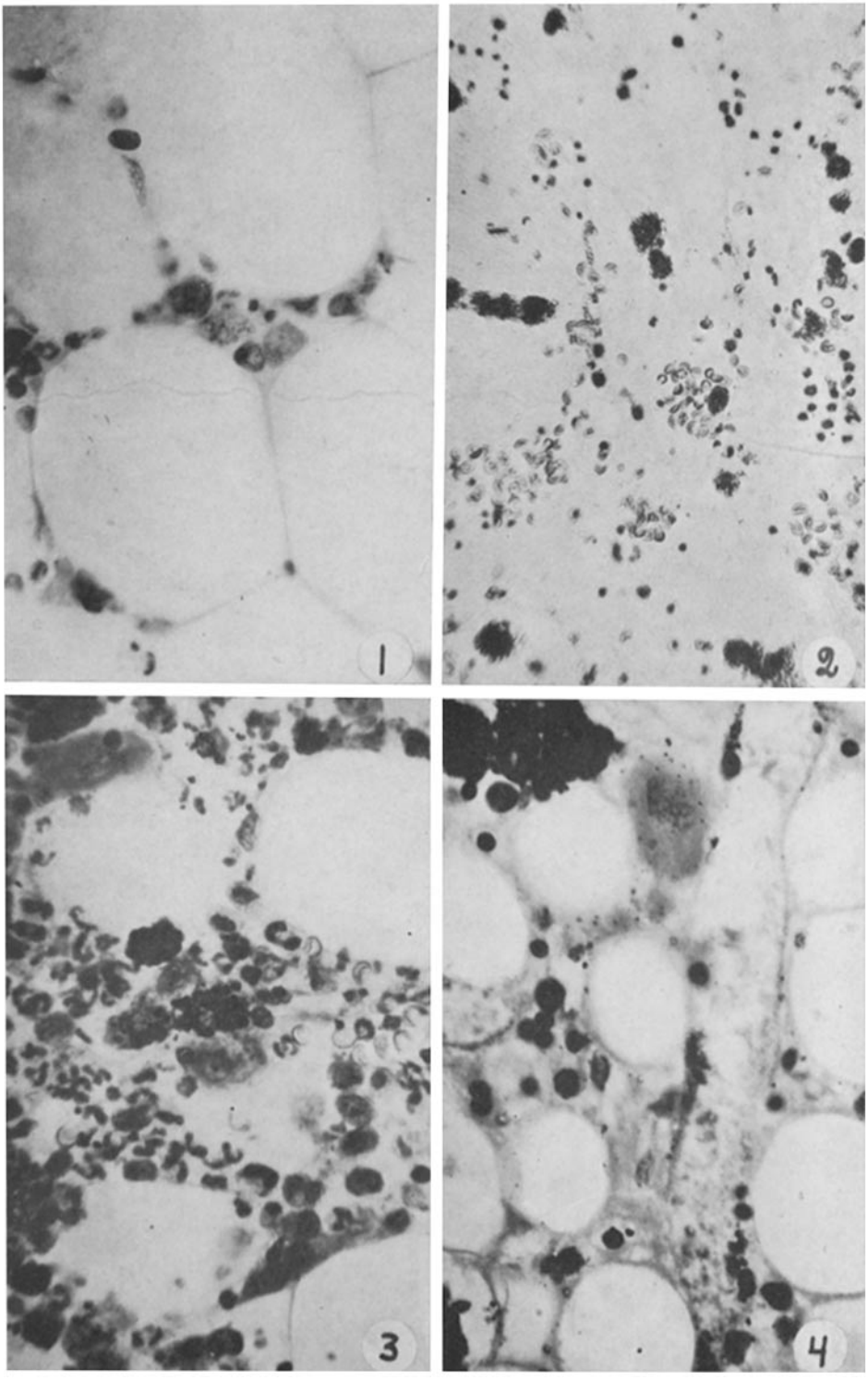
EXPLANATION OF PLATE 19.

FIG. 1. Rabbit H-3. Isolated focus between the fat cells in early stimulated marrow showing hypertrophied endothelium and mitosis adjacent to megaloblast and clasmatocytes containing silver granules. × about 800.

FIG. 2. Rabbit H-1. Bone marrow of animal succumbing 18 days after the last injection of collargol. The normal architecture is gone, being replaced by an eosin-staining ground substance containing many clasmatocytes filled with collargol, but very few cells of the erythromyeloid series. Blood vessels are intact, lined with apparently inactive endothelium. × 370.

FIG. 3. Bone marrow of Rabbit H-52 given eight doses of 2 per cent collargol and killed 6 hours after the last dose. Moderate hyperplasia of both the erythrocyte and myeloid series, the latter predominating. × about 800.

FIG. 4. Bone marrow of Rabbit H-33 subjected to the same treatment as No. H-52, but killed 4 days after the last dose. The peripheral blood showed some decrease in erythrocytes and hemoglobin. Fat cells are intact, but very few cells of either myeloid or erythrocyte series remain. The collargol is deposited in clasmatocytes and in the endothelium lining blood vessels. × about 800.



(Muller: Bone marrow reactions. I.)