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Abstract

The author studied the hematopoietic disturbances of rabbit induced by saponin injection and drew the following conclusions: 1) By saponin injection, the structure of bone marrow is disintegrated and hematopoietic cells are released into the circulating blood forming extramedullary hematopietic foci mainly in liver and spleen. The main attacking point of saponin should be RES. Recovery of hematopoietic foci is associated with the recovery of RES. The most marked extramedullary hematopoiesis is found three days after the injection. Thereafter, bone-marrow hematopoiesis proceeds to recovery stage, during which hematopoietic foci in liver and spleen are preserved, especially those in spleen persist fairly for a long time. 2) Daily injections of India ink kept up over a long period of time after the treatment with saponin, prevent the recovery of anemia and bone-marrow hematopoiesis. The lymph nodes, whose RES escaped from the severe damage by India ink, keep the hematopoietic foci for a long time. 3) As far as hematopoiesis is concerned, there seems to be no functional differentiation among RE cells, though they seem to have a special function according to the organs to which they belong, e. g. antibody formation in lymph apparatus, hematopoiesis in bone marrow and red cell destruction in spleen.

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STUDIES ON RETICULOENDOTHELIAL SYSTEM AND HEMATOPOIESIS

I. STUDIES OF EXTRAMEDULLARY HEMATOPOIESIS

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As is well known, reticuloendothelial (RE) cells are widely distributed in the body along with the blood capillaries and lymphoid vessels forming an organized system, reticuloendothelial system (RES). But the function of each cell seems to be differentiated according to the functions of the organ to which it belongs. For example, their function is mainly associated with hematopoiesis in bone marrow, lymphocyte formation in lymph apparatus and red cell destruction in spleen. Besides these, they are closely correlated to antibody formation in spleen and lymph nodes by taking the antigens These functions look to be completely different from each other, but they seem to be something in common in the point that RES phagocytes some materials and transforms them and transfers to other cells, e. g. it takes up red cell and transfers iron to transferrin in serum or directly to erythroblast, or takes up antigen and gives some unknown informations to antibody forming cells. In any event, it is obvious that RES in each organ has a specialized function specific to each organ, and yet it is generally believed that RES is a system forming a functional unit. Consequently, to clarify whether such a different function of RES is fixed or changeable from one to another, the author investigated how the local RES in liver, spleen and lymph nodes is associated with the extramedullary hematopoiesis induced by disintegrating hematopoietic tissues in bone marrow by injecting several agents.

MATERIALS AND METHODS

Normal fifteen male adult rabbits, weighing 2.5—3.0 kg, were used. Each animal received an intravenous injection of saponin, 4—6 mg in 0.1 % solution. After the injection, red cell and white blood-cell counts, hemoglobin contents, reticulocyte counts of peripheral blood were observed quantitatively, and blood cell classifications were done at certain intervals by Giemsa staining. All these rabbits were killed by contusion on the head and cutting off the carotid artery, and two animals each were sacrificed at intervals of fifteen minutes, one hour,

twenty-fours hours, seventy-two hours and thirty days after the saponin injection, and histological observations were made on bone marrow, spleen, liver, kidney, lung and mesenterial lymph nodes by hematoxylin-eosin staining. The remaining three rabbits received the injection of India ink, 3 cc diluted in 20 cc saline solution, daily into ear vein initiating eight hours after the saponin injection. The volume of India ink to be injected was gradually increased day after day and raising the volume to 6 cc on the seventh day, maintained it up to the end. One of them was sacrificed nine days after the saponin injection, the other one after twenty-three days, and the last one after thirty-seven days. The organs of these rabbits were also observed histologically just as with the former.

For histologic observations, the tissues were fixed with 10 % formol, dehydrated with ethanol, embedded in paraffin, sectioned and stained with hematoxylin-eosin in the routine manner.

India ink used was "Fueki-Bokujū", a commercial product. It is composed of 6 % carbon black (400—800 Å in carbon particle diameter), 4 % glucose, 8 % $CaCl_2$, 0.4 % camphor, and some antiseptic and surface activating agents in 80 % water (pH, 5.0—6.0, specific gravity, 1.10).

OBSERVATIONS AND RESULTS

Bone-marrow injury by saponin :

Five minutes after the intravenous injection of saponin in normal rabbits, granulocyte series were diminished in the circulating blood and about one hour after the injection erythroblasts and myeloid cells appeared (Fig 1). Twenty-four hours after the injection, the number of bone-marrow cells in the circulating blood arrived at the maximum level. Erythroblasts occupied at this stage more than 50 % of nucleated cells in the circulating blood. Thereafter, immature

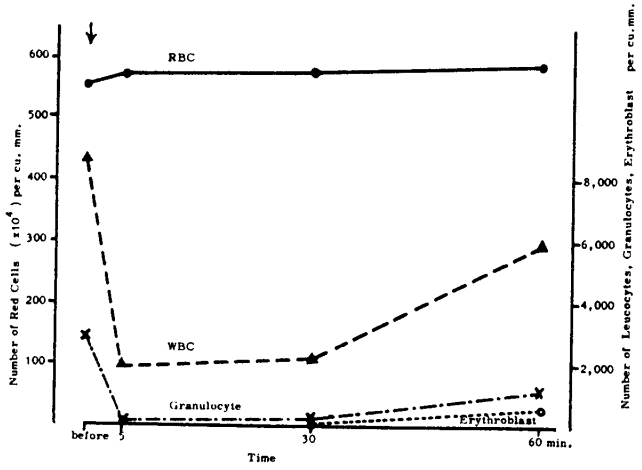


Fig. 1. (a) Changes in number of red blood cells (RBC), white blood cells (WBC), granulocytes and erythroblasts in the circulating blood during 60 minutes after saponin injection (↓).

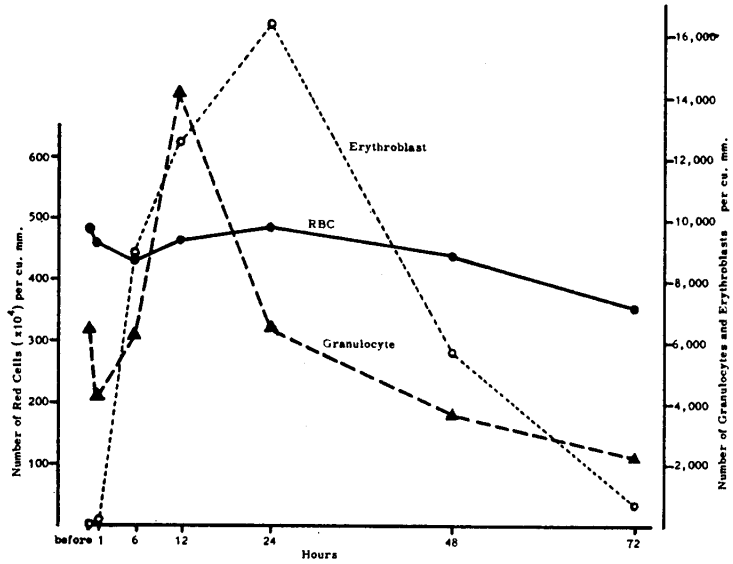


Fig. 1 (b) Changes in number of RBC, granulocytes and eythroblasts in the circualting blood during 72 hours after saponin injection (↓).

bone-marrow cells gradually decreased in number in the circulating blood and at seventy-two hours after the injection the number of erythroblasts was less than 10 % of nucleated cells with the hemogram recovering nearly to the original level. About ten days after the injection, immature cells disappeared in the circulating blood During this period the red cell count decreased very slowly developing a slight anemia reaching about two thirds of original level two weeks after the saponin injection, and there after began to increase and recovered completely thirty days after the injection (Fig. 2).

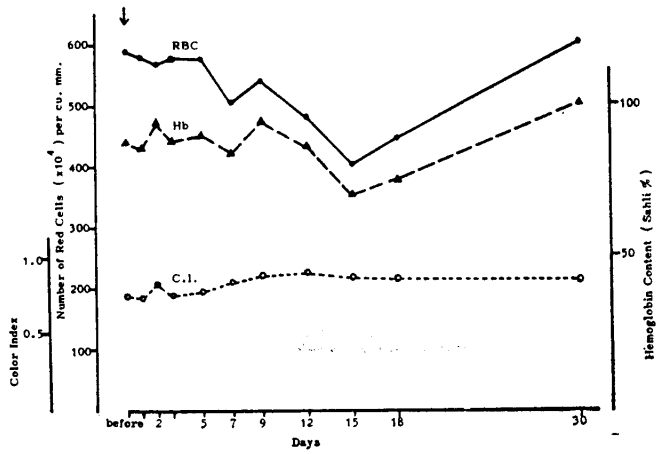


Fig. 2 (a) Changes in number of red blood cells (RBC), in hemoglobin contents (Hb) and color index (C. I.) during 30 days after saponin injection (↓).

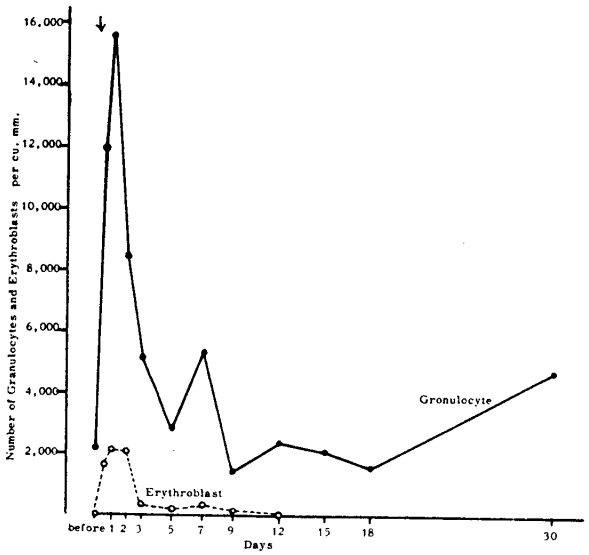


Fig. 2 (b) Changes in number of granulocytes and erythroblasts in the circulating blood during 30 days after saponin injection (↓).

Histologic observation on the bone marrow of those sacrificed fifteen minutes after saponin injection revealed moderate bleeding and congestion, RE cells were swollen but the structural entity was kept nearly normal (Figs. 3, 4). In spleen also, there was no specific change except some accumulation of myelocytes and erythroblasts in the lumen of sinusoids (Figs. 13, 14). In the lung, many myeloid cells were recognized in capillaries. These facts suggest bone-marrow cells are released into the circulating blood just a few minutes after the saponin injection.

One hour after the injection, the structural disintegration of bone marrow became distinct with the distorted sinusoid structure and the swelling of reticulum cells; bone-marrow cells were dislocated by the disintegrated foci (Figs. 5, 6), while in spleen, a number of immature bone-marrow cells were found.

Fig. 3 Section of bone marrow, illustrating focal bleeding. Other fields are almost normal; 15 minutes after saponin injection. × 150

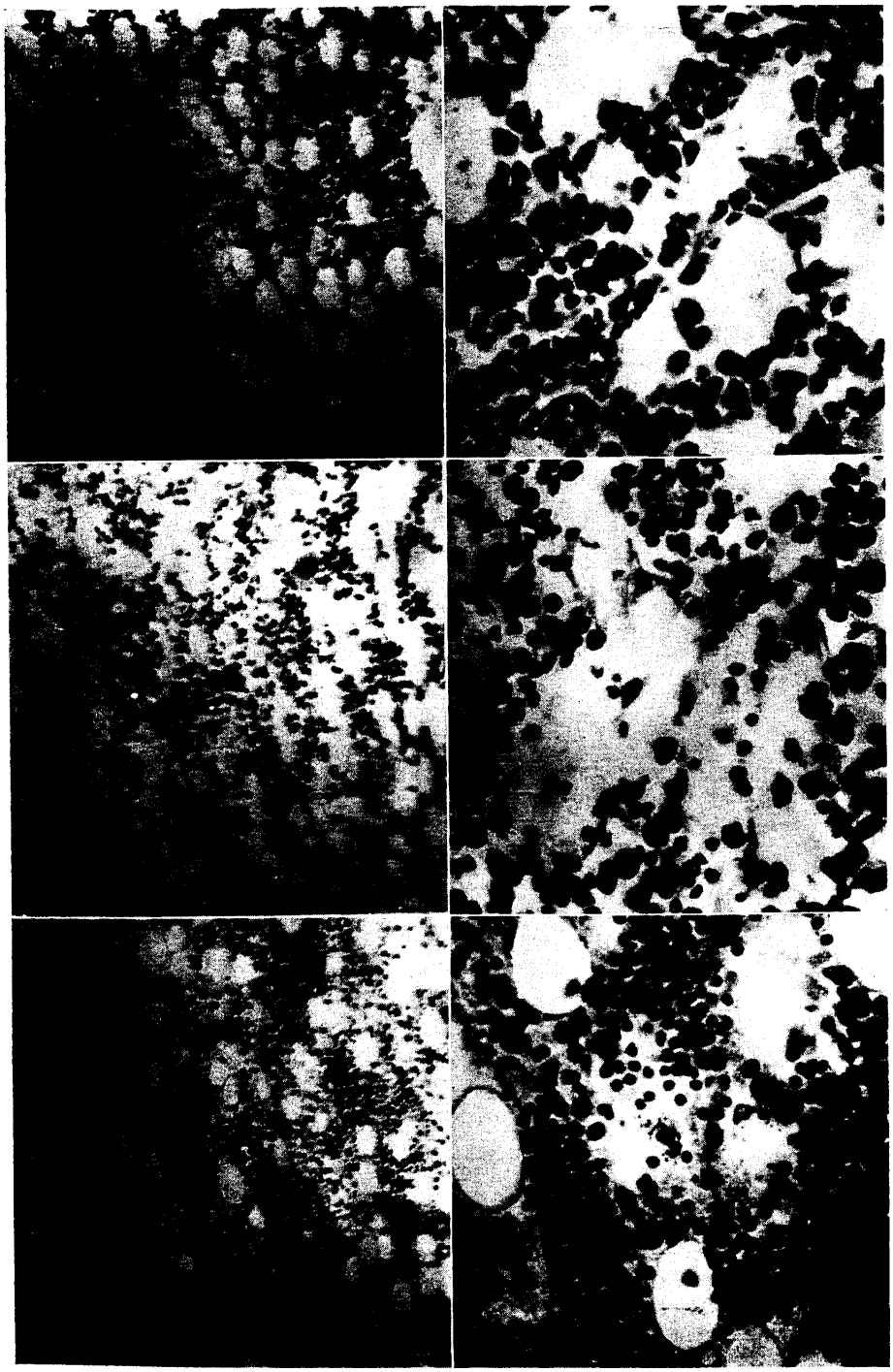
Fig. 4 High magnification of the above. Almost normal hematopoietic picture is seen. × 470

Fig. 5 One hour after injection, structural disintegration of bone marrow becomes visible. × 150

Fig. 6 High magnification of Fig. 5. Hematopoietic foci are dissociated. Reticulum cells are considerably swollen and opening of sinusoids is observed. × 470

Fig. 7 Section of bone marrow 24 hours after injection, showing marked structural destruction. × 150

Fig. 8 High magnification of Fig. 7. Bleeding and edema are observed. Hematopoietic foci are completely destroyed, showing depletion of bone-marrow cells and marked degenerative swelling of reticulum cells. × 470



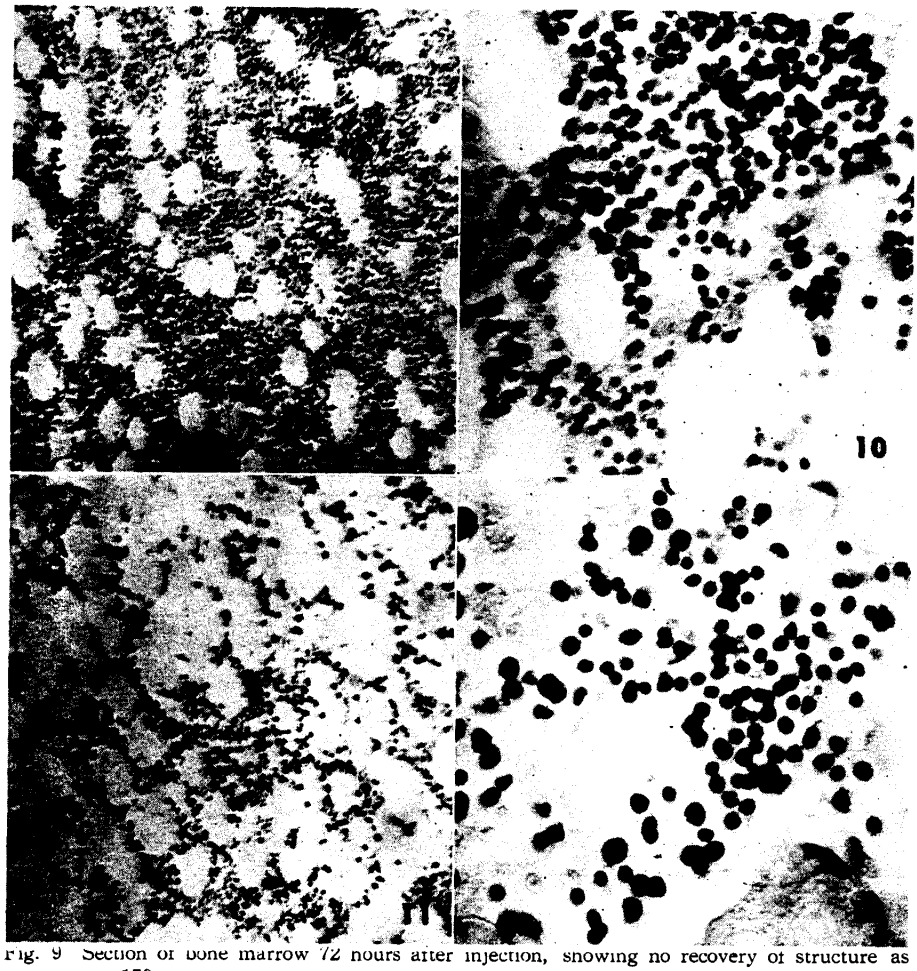


Fig. 9 Section of bone marrow 72 hours after injection, showing no recovery of structure as yet. × 150

Fig. 10 High magnification of Fig. 9. Compared with Fig. 8, bleeding and edema are decreased, but hematopoietic foci have not as yet recovered. × 470

Fig. 11 Section of bone marrow 30 days after injection, showing a considerable but not quite complete recovery of hematopoietic foci. × 150

Fig. 12 High magnification of Fig. 11. Hematopoietic foci are clearly recognized, while swollen mesenchymal cells containing lipids are observed. × 470

Fig. 13 Section of spleen 15 minutes after saponin injection, showing scarcely any changes in its structure but a little bone-marrow cells in sinusoids. × 150

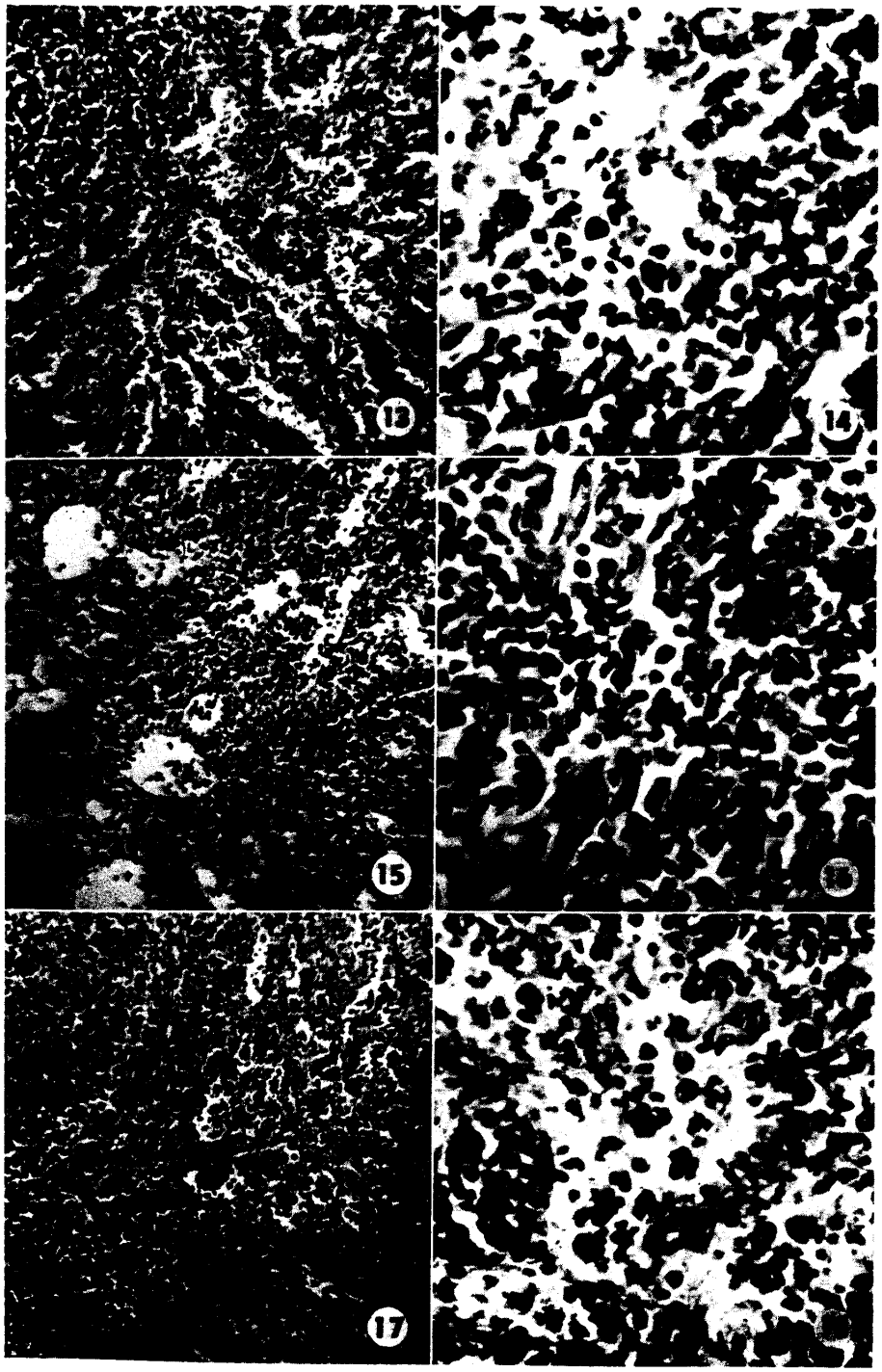
Fig. 14 High magnification of the above. × 470

Fig. 15 Section of spleen one hour after injection, illustrating more bone-marrow cells than the above and moderate dilatation of sinusoidal lumen. × 150

Fig. 16 High magnification of Fig. 15. × 470

Fig. 17 Section of spleen 24 hours after injection, showing marked opening of sinusoids not so as in bone marrow and appearance of many bone-marrow cells in them. × 150

Fig. 18 High magnification of Fig. 17. Many erythroblasts, myeloid cells are found, but arrangement of these series are irregular. × 470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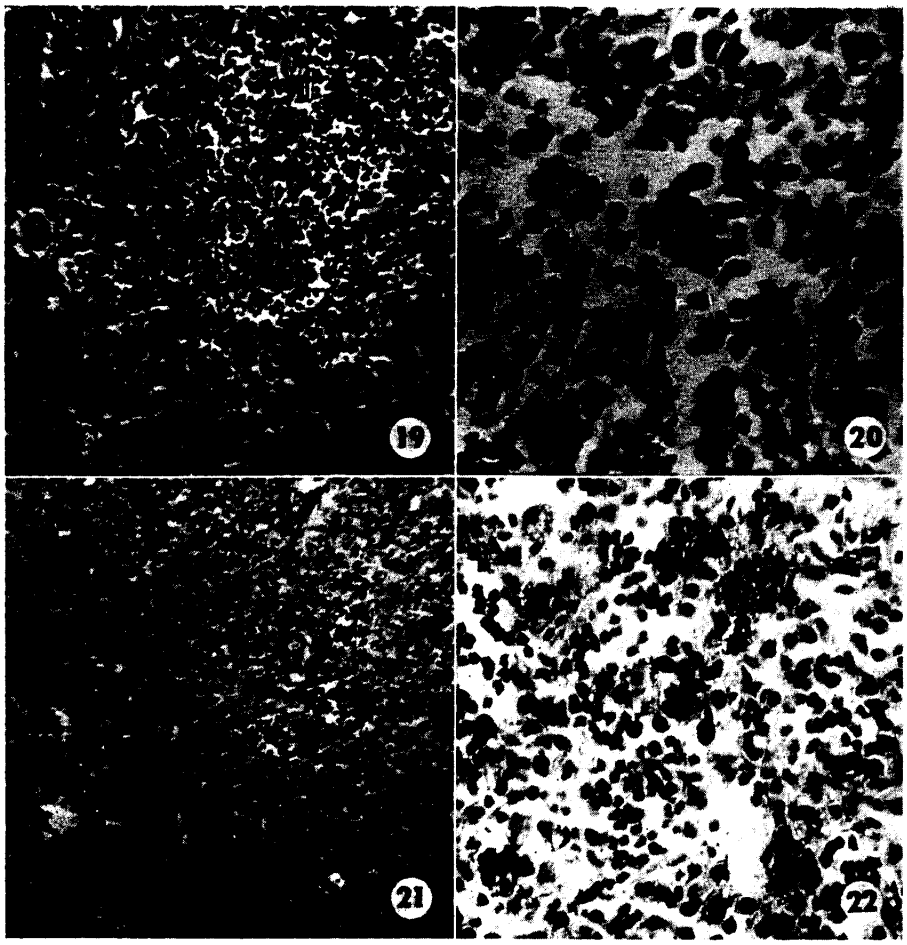


Fig. 19 Section of spleen 72 hours after injection, demonstrating appearances of erythroblasts, myeloid cells and megakaryocytes in the lumen of sinusoids forming groups of the same series, resembling hematopoietic foci in bone marrow. × 150

Fig. 20 High magnification of Fig. 19. × 470

Fig. 21 Section of spleen 30 days after injection, showing almost complete recovery to normal structure. × 150

Fig. 22 High magnification of Fig. 21. In several sinusoids, a little of groups of erythroblasts remain. × 470

Fig. 23 Section of liver 15 minutes after saponin injection, illustrating almost normal picture. Effect of saponin are scarcely visible. × 150

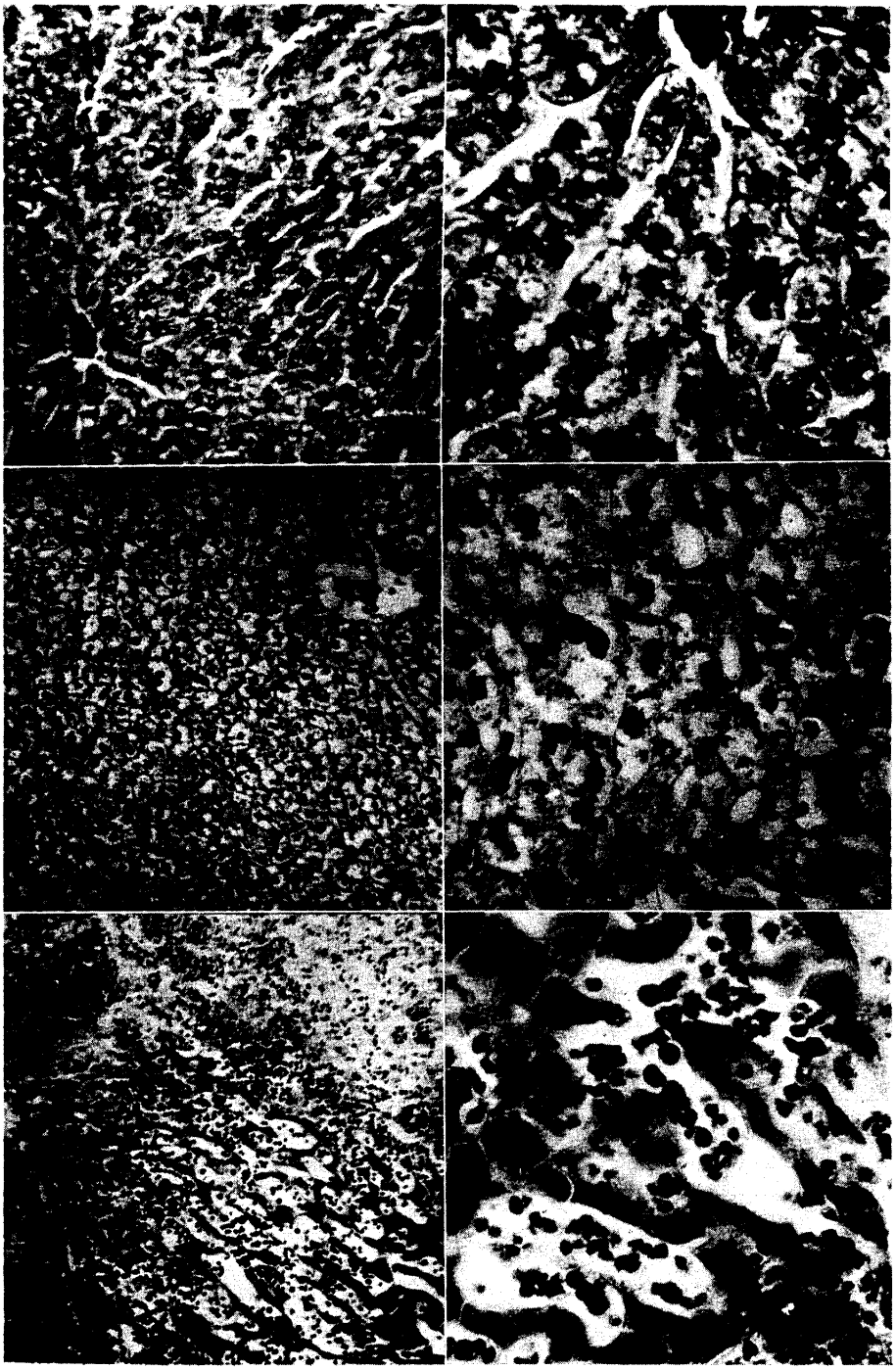
Fig. 24 High magnification of the above. × 470

Fig. 25 Section of liver one hour after injection, showing degeneration of parenchymal cells and appearance of a little of bone-marrow cells in sinusoids × 150

Fig. 26 High magnification of Fig. 25. Degeneration of liver cells is markedly and a little of erythroblasts and myelocytes are recognized in sinusoids. × 470

Fig. 27 Section of liver 24 hours after injection, demonstrating predominant destruction of structure with central necrosis and infiltration of many bone-marrow cells. × 150

Fig. 28 High magnification of Fig. 27. The lumens of sinusoids are open and in them bone-marrow cells are scattered. × 470



The disintegration of sinusoids was also recognized in this organ (Figs. 15, 16). In liver, parenchymal cells were found to be degenerated and in the lumen of sinusoids a small number of bone-marrow cells were observed (Figs. 25, 26). In lymph nodes, sinusoids were dilated and there were observed many reticulum cells phagocyting red cells.

Twenty-four hours after saponin injection, injury of bone marrow became

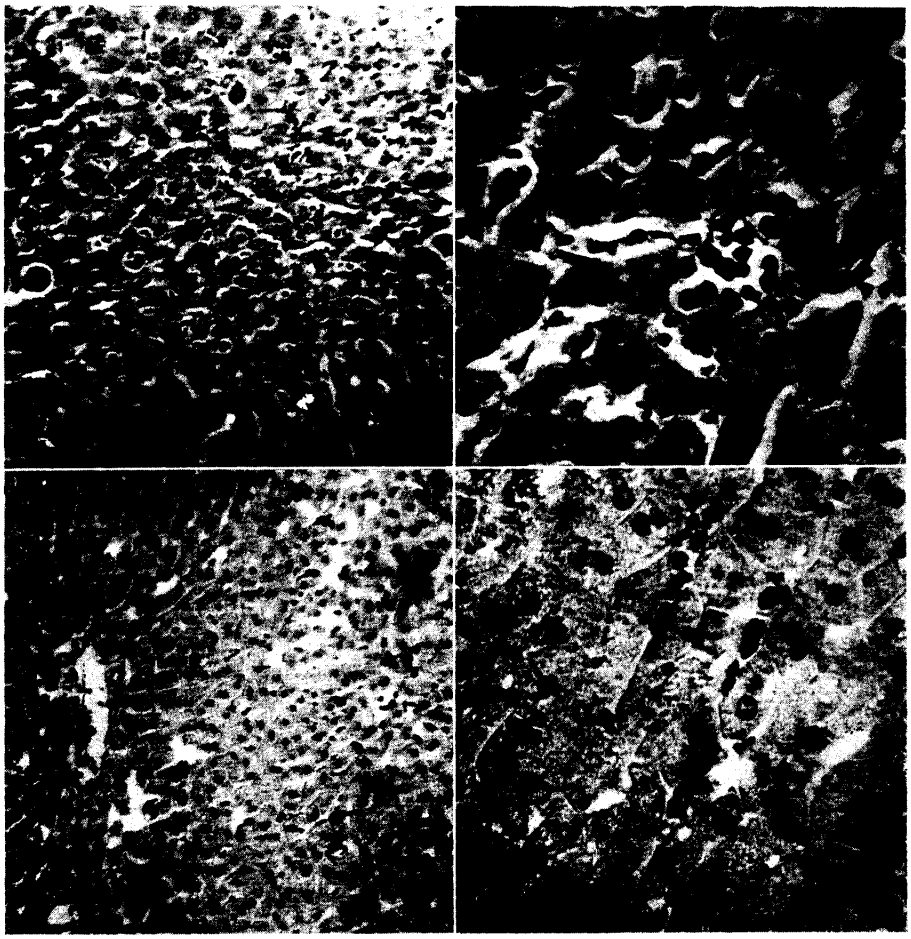


Fig. 29 Section of liver 72 hours after injection, illustrating recovery of degenerated parenchymal cells and grouping of bone-marrow cells and appearance of megakaryocytes. × 150

Fig. 30 High magnification of Fig. 29. In the lumens of sinusoids, erythroblasts and myelocytes are grouping in each of series. Endothelial cells and Kupffer cells are clearly recognized. × 470

Fig. 31 Section of liver 30 days after injection, showing almost complete recovery without appearance of bone-marrow cells. × 150

Fig. 32 High magnification of Fig. 31. × 470

marked, making the original structure hardly distinguishable, and with a severe congestion and hemorrhage, most hematopoietic tissues were obliterated. Reticulum cells were swollen and degenerated (Figs. 7, 8). Comparing to such a severe damage of bone marrow, the structural change of spleen was not so marked, though the sinusoids were dilated and impregnated with many immature bone-marrow cells. However, among these cells there was not any structural arrangement or formation of hematopoietic focus (Figs. 17, 18). In liver, central necrosis was marked and sinusoids were dilated by congestion, and in consequence endothelial cells or Kupffer cells located near the central vein grew indistinct. Irregular infiltration of bone-marrow cells were observed similarly as in spleen (Figs. 27, 28). In lymph nodes, sinusoids were extremely dilated being filled with red cells. Lymph follicles were found in the blood meer.

Even seventy-two hours after saponin injection, bone marrow showed recovery tendency (Figs. 9, 10), while in spleen bone-marrow cells were assuming some arrangement in the hematopoietic tissue, i. e. forming some erythroblastic islets and myeloid foci were suggesting the initiation of extramedullary hematopoiesis (Figs. 19, 20). In liver, the parenchymal cells showed the recovery from degeneration, megakaryocytes were often encountered, and erythroblasts and myelocytes formed separate groups in the lumen of sinusoids similarly as in spleen (Figs. 29, 30). Lymph nodes appeared to be in the recovery phase.

Thirty days after saponin injection, the bone marrow presented a fairly good state of recovery, with the appearance of hematopoietic foci. However, the tissue was still slack when compared with that of normal bone marrow (Figs. 11, 12). Spleen, liver and lymph nodes recovered their normal structure, but some sinusoids had a few groupings of erythroblasts showing residual hematopoietic foci (Figs. 21, 22, 31, 32).

Effect of RES-block by India ink after saponin injection :

Intravenous injection of India ink, repeated daily for a long period of time starting eight hours after saponin injection, brought about some changes in hemogram of the circulating blood, erythroblasts did not disappeare from the circulating blood (Fig. 33), and anemia persisted progressively without showing any tendency of recovery so long as India ink injection was continued.

Histologic observation of bone marrow from the animal killed thirty-seven days after saponin injection revealed an increase of enormously swollen RE cells phagocyting a mass of carbon black, located among strikingly faded hematopoietic foci. Liver and spleen likewise had swollen RE cells filled with carbon black, included among them were a few myelocytes and erythroblasts. Carbon-black phagocyting cells were less in lymph node than in bone marrow, liver and spleen, but surprisingly there developed marked myelopoietic foci (Figs. 34, 35).

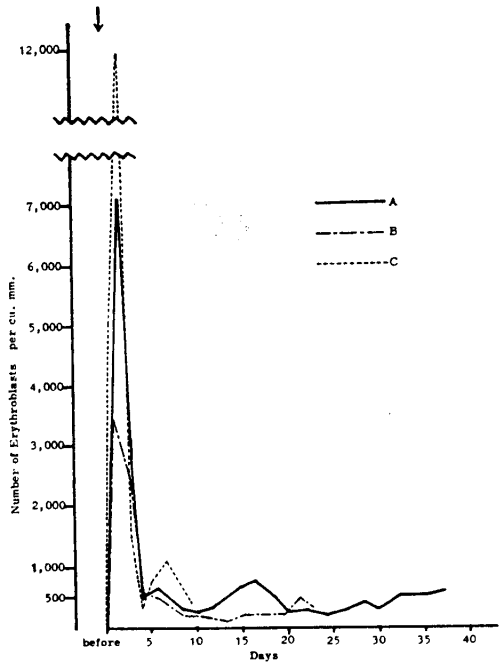


Fig. 33 (a) Appearance of erythroblasts in the circulating blood after saponin injection (↓), following India ink injection continuously for a long time. A: during 37 days, total volume of India ink is 150 cc, B: during 23 days, 120 cc, C: during 9 days, 70 cc.

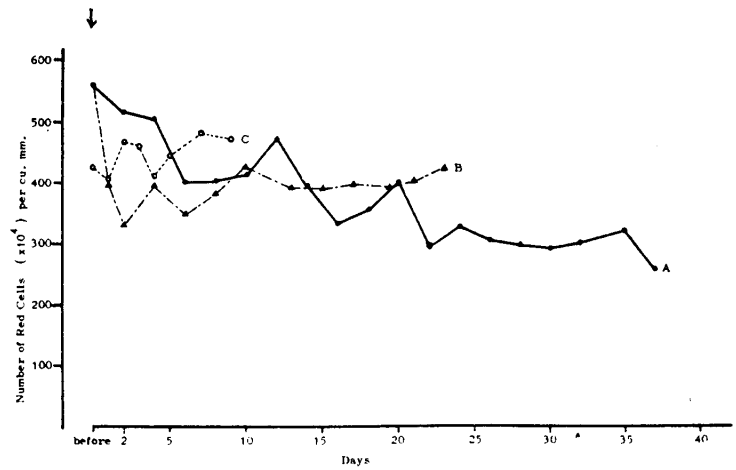


Fig. 33 (b) Change in number of red blood cells in the same case as Fig. 33 (a).

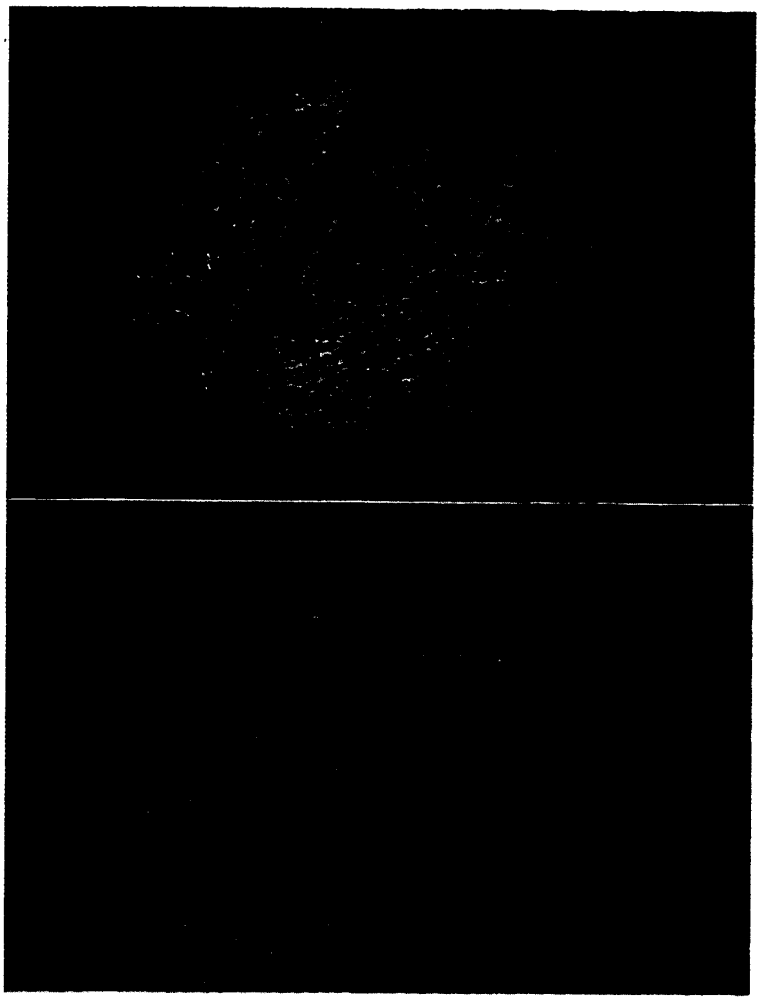


Fig. 34 Section of lymph node after saponin injection, following India ink injection continued for 37 days. RE cells phagocyte many carbon black and swell, while myelocytes are grouping, suggesting myelopoietic focus. × 100

Fig. 35 High magnification of the above, illustrating myelopoietic focus. × 400

DISCCUSION

For a long time hemolytic action of saponin has been known. In 1906 BUNTING[1] used saponin in his experiment for the purpose to produce anemic rabbits. He noticed that nucleated red cells appear in the circulating blood rapidly after the intravenous injection of saponin, 2—4 mg accompanied with the appearance of extramedullary hematopoiesis in spleen sinusoid. He was of the opinion that saponin injures the delicate blood capillaries of bone marrow, and consequently bone-marrow cells are released into the circulating blood.

Since then, ISAAC[2], DRINKER[3], LANG[4], LIVADAS[5], CUSTER[6], OMURA and OSOGOE[7], FRESEN and LIERENFELD[8], and others used saponin for the studies of blood circulation in bone marrow and histogenesis of myeloid metaplasia, reconfirming that intravenous injection of saponin induces the release of bone-marrow cells into the circulating blood with the formation of hematopoietic foci in liver and spleen. OSOGOE and his associates investigated changes in the circulating blood and organs of rabbits received the intravenous injection of saponin after withdrawing some amounts of blood, and found hematopoietic foci in liver and spleen seventy-two hours after saponin injection.

Similar results were also obtained on the animals received no pretreatment of withdrawing of blood. They also observed the complete recovery of bone marrow thirty days after the saponin injection. But, by the author's observation thirty days seemed to be not long enough for the complete recovery of bone marrow. The bone marrow showed some groups of swollen reticulum cells containing some lipids, though the recovery of hematopoietic foci, especially especially erythropoietic one, were recognized to a certain extent. From the findings of this experiment, it is suggested that the main acting points of saponin lie in the damage of reticulum cells which support the normal structure of bone marrow and erythropoietic islets. The damage of RE cells will result in the disintegration of normal structure and islets of bone marrow. The appearance of the myeloid and the erythroid cells from bone marrow is accompanied with the degeneration of RE cells and their recovery initiates the recovery of the RES structure or the formation of erythropoietic foci. Gradual decrease in red cell counts in the circulating blood after saponin injection is not due to the direct hemolytic action of saponin, because saponin does not show hemolytic action in the concentration under 0.03 %[9]. The anemia resulting from the saponin injection will be mainly due to the disintegration of erythropoietic islet. The progress of anemia can be interpreted as a retarded erythropoiesis with the normal destruction process of faded red cells. Taking the life-span of red cells of rabbit as sixty days, the decrease in the red cell count to about two thirds of normal count within two weeks after saponin injection is reasonably understood from

the supposition mentioned. The fact that the complete recovery of hemogram of the circulating blood preceeds to the recovery of histologic structure of bone marrow is probably due to the active extramedullary hematopoiesis, which can be seen even thirty days after the saponin injection.

The author's hypothesis that the effect of saponin is represented as the attack on RES can be supported by the observations on the rabbit receiving India-ink injection following the saponin injection. The blockade of RES by India ink injection should result in the retarded recovery of the function of RES. By carbon-black blockade RES in the convalescent stage will be attacked in bone marrow as well as in spleen and liver. Thus persistent anemia is induced. This proves that the recovery of the function of RES is essential for the recovery of hematopoiesis.

Besides these, the experiments have proven that there seems to be no functional differentiation of RES in various organs, as far as the hematopoiesis is concerned. RES in liver, spleen, lymph nodes and others can form erythroblastic islets with the erythroblast metastasized from bone marrow. Therefore, the translocation of hematopietic foci from liver and spleen to bone marrow after birth is not due to the functional differentiation of RES, but due to some environmental factors, e. g. oxygen tension and others.

SUMMARY

The author studied the hematopoietic disturbances of rabbit induced by saponin injection and drew the following conclusions:

1) By saponin injection, the structure of bone marrow is disintegrated and hematopoietic cells are released into the circulating blood forming extramedullary hematopietic foci mainly in liver and spleen. The main attacking point of saponin should be RES. Recovery of hematopoietic foci is associated with the recovery of RES. The most marked extramedullary hematopoiesis is found three days after the injection. Thereafter, bone-marrow hematopoiesis proceeds to recovery stage, during which hematopoietic foci in liver and spleen are preserved, especially those in spleen persist fairly for a long time.

2) Daily injections of India ink kept up over a long period of time after the treatment with saponin, prevent the recovery of anemia and bone-marrow hematopoiesis. The lymph nodes, whose RES escaped from the severe damage by India ink, keep the hematopoietic foci for a long time.

3) As far as hematopoiesis is concerned, there seems to be no functional differentiation among RE cells, though they seem to have a special function according to the organs to which they belong, e. g. antibody formation in lymph apparatus, hematopoiesis in bone marrow and red cell destruction in spleen.

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