THE INFLUENCE OF REPEATED INJECTION OF SAPONIN ON THE COLONIZATION PATTERN OF BONE MARROW CELLS IN FOREIGN ORGANS IN RABBITS*

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INTRODUCTION

It is generally believed that repeated administration of various hemotoxins, such as saponin, phenylhydrazin and others, into experimental animals may induce metaplasia of local fixed mesenchymal cells leading to the formation of ectopic foci of hemopoiesis (see the review by Lang, 1938). Since, however, an induction of the state of chronic poisoning with hemotoxins undoubtedly complicates the conditions under which myeloid transformation may make its appearnce, such a procedure is not appropriate when the purpose of the study is to trace the origin of blood cells. It is desirable above all to simplify experimental conditions under which myeloid transformation may emerge.

Nevertheless, experiments along this line have hitherto been seldom reported. So far as the writer is aware, Livadas (1933) is the first who succeeded in producing myeloid transformation of the spleen and liver in rabbits within several hours after a single large dose of saponin. This author interpreted the result as a strong evidence for colonization of bone marrow cells, because autochthonous formation of myeloid cells through metaplasia from local fixed cells in such a short period of time seems hardly conceivable.

Recently Livadas' experiments were greatly extended by Ōmura and Osogoe (1951). They have clearly demonstrated that after intravenous injection of a sublethal dose of saponin in adult rabbits, a liberation of immature blood cells from the bone marrow into the circulation takes place on a large scale, resulting in a striking accumulation of these cells in the spleen, liver, lung and other organs and simultaneously in an almost complete depletion of

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bone marrow parenchyma. The pattern of accumulation of immature blood cells in different organs was the same as observed for the bone marrow cells which were brought in large numbers into the circulating blood of normal rabbits (Osogoe and Ōmura, 1950). Further experiments by Ōmura (1953) on splenectomized rabbits revealed that the rate of liberation of immature blood cells from the bone marrow is considerably accelerated in the absence of spleen, to such an extent that the picture of lung imprints from an animal killed 6 hours after saponin injection resembled closely that of bone marrow imprints.

On the basis of the above mentioned experiments employing a single large dose of saponin, it was concluded that myeloid transformation may occur as a result of colonization of bone marrow cells. This is in sharp contrast to the dominant opinion of previous investigators who believe in an autochthonous formation of myeloid cells through metaplasia from local fixed mesenchymal cells. Since the latter opinion is based chiefly on the experiments in which repeated injections of saponin and other hemotoxins were used, the question which arises is to what extent the repeated use of a hemotoxin, particularly of saponin, may alter the pattern of colonization of bone marrow cells in different organs produced by a single injection of the agent. Incidentally it would be of particular intererest to determine whether or not the repeated administration of saponin and other hemotoxins may induce true myeloid metaplasia of local fixed mesenchymal cells. The present work is an attempt to answer these questions, using sapotoxin as in the previous experiments above referred to.

Material and Method

A total of 19 healthy adult rabbits of both sexes weighing from 2.0 to 3.0 kg were divided into two series: first series consisting of 15 rabbits received intravenously 5 to 8 mg of saponin (E. Merk, Darmstadt) 4 to 9 times at intervals of 7 or 10 days; the second series consisted of 4 rabbits in which intravenous injection of 3 to 6 mg of saponin was repeated as frequently as 31 times at intervals of 3 days. Saponin was dissolved in physiological saline in a concentration of 0.1 % before injection. In both series, approximately 30 ml of blood was withdrawn by heart puncture prior to the initial injection of saponin. Besides, a number of rabbits from the material of the previous experiments (Ōmura and Osogoe, 1951), which had received 5 to 7 mg of saponin in a single dose immediately after withdrawal of about 30 ml of blood, served as controls.

Blood examinations were made at intervals of 24 to 72 hours after the initial injection of saponin. After the last injection, the animals were

sacrificed serially from the 24th hour to 21st day for histological examination of various organs (lung, liver, spleen, bone marrow, mesenteric and popliteal lymph nodes, kidneys and adrenals). The tissues to be examined were fixed in Zenker-formol and stained with Mayer's acid hemalum and eosin or eosin-azur II.

Differential counts of the immature myeloid cells occurring in 1 cm^2 of section cut 5 μ thick from the spleen, liver, lung, lymph nodes and other organs were made in the same manner as described previously (Osogoe and Ōmura, 1950; Ōmura and Osogoe, 1951), and the data were compared with those of the control animals from the previous experiments. In most instances, imprint preparations of the organs were made before fixation and stained with Giemsa or May-Giemsa in order to supplement the observations in sections.

Results

Peripheral blood.

It was previously shown by Ōmura and Osogoe (1951) that, shortly after a single injection of about 6 mg (5 to 7 mg) of saponin into adult rabbits numerous erythroblasts appear transiently in the peripheral blood without being accompanied by myelocytes and megakaryocytes. In the rabbits that received 4 to 9 intravenous injections of each 5 to 8 mg of saponin at intervals of 7 or 10 days, a considerable number of erythroblasts also appeared in the peripheral blood, showing many peaks of erythroblast counts. In some instances, the erythroblastic responses were very marked at the beginning, gradually diminishing following repeated injections, whereas in others there was no such a tendency up to the 9th injection. When injection of from 3 to 6 mg of saponin was repeated as frequently as 31 times at intervals of 3 days, all the animals failed to show any marked erythroblastic response to saponin injections at the final stage.

In the total white cell counts, many peaks were also seen in response to saponin injections. In most instances, the height of these peaks was also reduced by repeated injections but to a less well marked degree.

The erythroblasts appearing in the peripheral blood were for the most part normoblasts with a few macroblasts. Myelocytes and metamyelocytes often appeared in small percentages (0.5 to 1.0%), but they were not constantly met with. Megakryocytes were completely absent.

Thus, the alterations of blood picture produced by repeated injections of saponin showed no essential differences from those brought about by a single injection of this agent.

Spleen, liver and lung.

Following a single injections of about 6 mg of saponin in adult rabbits, there occurred, as described previously (Ōmura and Osogoe, 1950), a striking accumulation of bone marrow cells in the red pulp of the spleen, and to a smaller extent also in the small blood vessels of the liver, lung and other organs, in less than 72 hours. In the course of this process an occurrence of numerous emobli of the early large forms, especially the myelocytes, was noticed in the small blood vessels of the lung up to the 48th hour; but later, during the period from the 48th to 72nd hours after injection, the marrow cells in the lung were gradully transported to the spleen and liver, without being detected in the peripheral blood. Thus by the 72nd hour, they were

TABLE I

Differential counts of the bone marrow cells per 1 cm^2 of sections (5μ) from the spleen, liver and lung, following 4 to 9 intravenous injections of each 5-8 mg of saponin at intervals of 7 or 10 days.

Time after the last injection	Rabbit No.	Number of injection	Spleen			Liver			Lung		
			Erythroblasts	Myelocytes	Megakaryocytes	Erythroblasts	Myelocytes	Megakaryocytes	Erythroblasts	Myelocytes	Megakaryocytes
24 hrs.	s 7	1	78,370	106	34	9,980	228	—	8,560	613	—
48 hrs.	s 6	1	75,920	4,510 (246)	82	5,510	2,340	82	7,940	2,065	70
72 hrs.	s 9	1	59,950	31,030 (1,750)	1,230	2,020	4,830 (44)	790	2,140	1,150 (110)	74
24 hrs.	ss 8	5	28,050 (180)	3,100 (10)	1,400	580	180	—	180	180	350
24 hrs.	ss 13	3	115,300 (1,000)	29,800 (100)	100	100	280	10	2,740	240	640
72 hrs.	ss 10	9	51,000 (300)	24,050 (160)	2,150	870	890	400	40	10	200
72 hrs.	ss 12	5	66,000 (250)	11,400 (80)	2,700	1,110	250	40	130	130	250
5days	s 21	1	671,160	109,600 (420)	3,360	16,880	5,160 (53)	232	144	600	192
10days	s 20	1	368,020	36,700	618	1,720	392	245	42	—	42
21days	s 25	1	345	70	—	—	—	—	—	—	—
5days	ss 7	9	77,600 (400)	45,000 (190)	1,500	880	150	50	260	30	290
5days	ss 11	5	38,350 (260)	11,100 (40)	650	1,150	130	440	220	60	320
10days	ss 22	9	39,500 (280)	8,750 (40)	450	20	—	—	20	—	130
10days	ss 3	4	10,700 (80)	9,100 (40)	200	30	410	—	—	30	10
20days	ss 23	9	12,850 (10)	4,950 (20)	50	30	40	10	—	10	20
30days	ss 39	9	750	600	50	—	70	—	—	—	—

All figures in parenthesis indicate the number of mitosis involved.

accumulated in enormous number in the spleen and, though not quite so numerous, yet relatively numerously also in the liver. In the lung these cells remained abundant till the 12th hour.

TABLE II

Differential counts of the bone marrow cells per 1 cm^2 of sections (5μ) from the spleen, liver and lung, following 31 intravenous injections of each 3–6 mg of saponin at intervals of 3 days.

Time after the last injection	Rabbit No.	Number of injection	Spleen			Liver			Lung		
			Erythroblasts	Myelocytes	Megakaryocytes	Erythroblasts	Myelocytes	Megakaryocytes	Erythroblasts	Myelocytes	Megakaryocytes
24 hrs	ss 21	31	21,000 (160)*	19,050 (60)*	250	100	—	—	30	—	30
48 hrs	ss 15	31	94,130 (720)*	14,550 (60)	700	70	60	—	10	—	50
4 days	ss 19	31	31,900 (210)	45,980 (140)	1,280	140	10	—	—	—	130
9 days	ss 16	31	2,800 (180)	7,950 (35)	100	130	10	10	—	—	10

All figures in parenthesis indicate the number of mitosis involved.

Following the repeated saponin administration, the bone marrow elements were found in varying numbers in the spleen, liver, lung and other organs. Data from differential counts of marrow cells per 1 cm^2 of sections (5μ thick) of these organs, after 4 to 9 injections of 6 mg of saponin at intervals of 7 or 10 days and following 31 injections of 3 to 6 mg of saponin at intervals of 3 days, are represented in Tables I and II, respectively. Table I includes in addition some representative data from the previous experiments with single dose of saponin by Ōmura and Osogoe (1951) for comparison.

As seen in these tables, with respect to the relative amounts of bone marrow cells, the preponderance of the spleen over the liver and lung is very conspicuous in the group that had received repeated administration of saponin, especially in those having received as many as 31 injections, as compared with that in the group that had received a single dose of saponin. Even as early as 24 hours after the last injection, the number of marrow cells in the liver and lung were much smaller than those in the animals given only a single dose of saponin. The occurrence of abundant emboli of marrow cells in the small blood vessels of the lung, as seen after single injection, was not met with in any instances. Thus in the group with repeated saponin injections, there was a prominent shift of marrow cells to the spleen.

With regard to the topographical distribution of the bone marrow cells

accumulated in the spleen, liver and lung, however, there was no essential difference between the two groups. In both, the erythroblasts occurred for the most part intravascularly in the venous sinuses and other small blood vessels, often making large clusters, especially in the splenic sinuses; whereas the myelocytes tended to accumulate outside the vessels. In the spleen, the myelocytes accumulated in the splenic cords outside the venous sinuses to a large extent, sometimes occupying almost the entire cords. In the liver and lung, on the other hand, no myelocytes were found anywhere outside the blood vessels. In the liver, a marked fatty degeneration was often observed in the peripheral zone of the lobules.

The general characteristics of the cellular constitution of the bone marrow cells accumulated in these organs were also entirely the same regardless of whether saponin was injected in a single or repeated doses. Even when the injection was repeated as many as 31 times at intervals of 3 days, neither a particular increase in the large early forms, such as proerythroblasts, basophilic macroblasts and promyelocytes, nor the occurrence of intermediate forms between the local fixed mesenchymal cells, particularly the endothelial cells, and the early myeloid forms could be demonstrated with certainty in any organs and tissues.

After the last saponin injectioon, the bone marrow cells accumulated in the spleen showed a marked tendency toward proliferation with many mitosis up to the 5th day, but to a less extent than following single injection, so that at 5 days the marrow cells in the spleen were less numerous after repeated than single injections (see Table 1). This is probably due to the more pronounced state of poisoning brought about by repeated injections of saponin. Thereafter, they were gradually decreased almost similarly in both groups, but the time of their disappearance was somewhat delayed by repeated injections of saponin (see Table I). In the liver and lung, the accumulated marrow cells were diminished more rapidly than in the spleen in both groups.

Lymph nodes, kidneys and adrenals.

After repeated administration of saponin, the bone marrow cells also occurred in these organs but in much smaller numbers than after single injection, except in the lymph nodes in which more numerous marrow cells, particularly the myelocytes, appeared in the former case. Here it is of particular interest that, in the former case, foci of myelocytes occurred not only in the medullary cords but also in the secondary nodules more frequently than in the latter case. The detailed description of this phenomenon will be made elsewhere. The mode of occurrence of bone marrow cells in the kidneys and adrenals after repetition of injection was entirely the same

as described in the previous paper dealing with single injection of saponin (Ōmura and Osogoe, 1951).

Bone marrow.

The characteristic features of bone marrow in acute poisoning with single large dose of saponin are the almost complete depletion of morrow parenchyma and extensive hemorrhage occurring within 24 hours after administration, as described previously (Ōmura and Osogoe, 1951). After the repetition of saponin injection, the picture was entirely the same as seen after single injection. However, regeneration of the marrow parenchyma was somewhat delayed by repeated injections, probably due to the more pronounced state of intoxication with saponin.

Discussion

As mentioned above, the repeated administration of saponin does not significantly alter the pattern of colonization of bone marrow cells produced by a single injection of this agent. Even when injection was repeated as many as 31 times at interval of 3 days, neither a particular increase in the early myeloid forms such as proerythroblasts and promyelocytes, nor the occurrence of transition forms between the local fixed mesenchymal cells and the early myeloid forms could be demonstrated with certainty in any organs and tissues. Thus, so far as the present experiments are concerned, there was no evidence indicative of true myeloid metaplasia.

It should not be overlooked, however, that with increase in the number of injections the relative amounts of bone marrow cells in the spleen were increased while those in the liver and lung, especially the latter, considerably decreased. Interpretation of this phenomenon may be summarized as follows: Since a single large dose of saponin produces a liberation of bone marrow cells into the circulation on a large scale as described previously (Ōmura and Osogoe, 1951), it is conceivable that the repeated use of similar dose of saponin may also exert the same effect in dislodging the bone marrow cells from the liver, lung and other organs where they have once accumulated, and these dislodged cells may eventually be transfered to the spleen by the blood stream. This inference is supported by the fact that the spleen is the most favorable organ for accumulation of the bone marrow cells which are brought in large numbers into the circulating blood of normal rabbits (Osogoe and Ōmura, 1950).

It must be emphasized in this connection that a marked shift of marrow cells to the spleen after repetition of saponin injection by no means indicates the spleen as being the sole organ in which myeloid metaplasia may emerge,

because Ōmura (1953) has demonstrated that myeloid transformation of the lung, liver and other organs may be produced by a single large dose of saponin to a greater extent in the absence of the spleen than in its presence.

It is also noteworthy that after repetition of injection, the bone marrow cells accumulated in the spleen showed less tendency toward proliferation but these cells persisted for a somewhat longer period, than after single injection. This is ascribable to the more pronounced state of intoxication produced by repeated injections of saponin.

The interesting finding, that in the secondary nodules of lymph nodes, foci of myelocytes were frequently produced by repeated injections of saponin, will be discussed in another paper.

Summary

A large dose of saponin was repeatedly injected into adult rabbits in an attempt to determine to what extent the repeated use of saponin may alter the pattern of colonization of bone marrow cells in different organs produced by a single injection of this agent.

It was revealed that no significant alterations in the pattern of colonization of bone marrow elements in different organs resulted from repetition of saponin injection, except for a marked shift of these cells to the spleen. Even when the injection had been repeated as many as 31 times at intervals of 3 days, neither a particular increase of the early myeloid forms such as proerythroblasts and promyelocytes, nor the occurrence of transition forms between the local fixed mesenchymal cells and the early myeloid forms, which may be regarded as indicative of an autochthonous formation of myeloid cells, could be demonstrated with certainty in any organs and tissues.

A marked shift of bone marrow cells to the spleen observed after repeated injections of saponin is interpreted as an effect of this agent causing dislodgment of these cells from the liver, lung and other organs and their transference to the spleen by way of the blood stream. It is to be emphasized that such shift by no means signifies that the spleen is the sole organ in which myeloid metaplasia may emerge.

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