Supplementary Studies of the Saponin-Induced Colonization of the Bone Marrow Elements in Rabbits

I. A Quantitative Study of the Colonization Pattern by Use of Radioactive Tracer*†

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In a previous study by OMURA and OSOGOE (1951), it was demonstrated that in less than 72 hours after single intravenous injection of a sublethal dose of saponin into rabbits, there occurred a striking accumulation of immature blood cells in the liver and spleen and to a lesser extent also in other organs, with the formation of numerous blood-forming foci. It was also shown that this was accompanied by an extensive depletion of immature blood cells from the bone marrow, without corresponding disintegration of immature cells therein. Furthermore, the pattern of accumulation of immature blood cells in foreign organs after saponin injection was found to be the same as that observed for the transfused marrow described in another paper by OSOGOE and OMURA (1950). On the basis of these observations, it is concluded that the immature blood cells are liberated from the bone marrow on a large scale after a single large dose of saponin.

In order to gain further information concerning the colonization of bone marrow elements in foreign organs, it was attempted in the present work to use radioactive phosphorus for labelling immature blood cells of the bone marrow prior to saponin injection. It was expected that an accumulation of radioactive immature blood cells in an organ might be reflected as an increase in the radioactivity of this organ, and vice versa.

MATERIAL AND METHODS

Adult albino rabbits weighing from 1.8 to 3.5 kg, which had been previously checked and found to be healthy, were used as experimental animals. They were divided into the experimental and control groups.

The animals of the experimental group received a single intravenous injection of

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6 mg of saponin[1], 24 hours after they had beed injected intravenously with 200 μc of inorganic P^{32}. In order to label immature marrow cells alone, the time interval of 24 hours after the injection of P^{32} was adopted in the present study, because longer time intervals were thought to allow labelling of mature cells as well. At 24, 48 and 72 hours after saponin injection (48, 72 and 96 hours after injection of P^{32}), the animals were killed by the injection of air and radioactivity measurements were made of various organs, together with hematological and histological examinations of blood and tissues.

The animals of the control group were also injected intravenously with 200 μc of inorganic P^{32} in the same manner as in the case of the experimental group, but they received no injection of saponin. The animals of this group which were killed 48, 72 and 96 hours after of inorganic P^{32} served as the controls for the animals of the experimental group which were killed 24, 48 and 72 hours after saponin injection, respectively.

The organs to be examined (the lungs, liver, spleen, mesenteric lymph nodes, bone marrow, kidneys and adrenals) were carefully weighed and a portion[2] of the organs were homogenized with a known volume of distilled water. From every tissue 0.5 ml of the homogenate was plated out on a pair of watch glasses. They were then dried by keeping at 60°C. for 5 hours in a thermostadt and used for measurement of radioactivity with a thin mica end-window type of GEIGER-MULLER tube, as described previously by TAMASHIGE (1959). Blood sample was obtained by heart puncture after opening the thorax and prepared for measurement of radioactivity in the same manner as in the case of the other tissues.

All activity measurements were corrected back to the day of administration. The relative specific activity per gram of fresh tissue and that per organ were expressed as percentage of the total specific activity of all the organs examined (lungs + liver + spleen + mesenteric lymph nodes + bone marrow + kidneys + adrenals = 100). The total weight of the bone marrow was taken as 2 per cent of the body weight.

RESULTS

1. *Radioactivity measurements in various organs*

The chief results of radioactivity measurements in various organs from the animals of both the experimental and the control groups are summarized in Tables 1–3.

As seen in Table 1, there occurred numerous erythroblasts in the peripheral blood after saponin injection, whereas these cells never appeared in the circulating blood

[1] Saponin pure white (E. Merk, Darmstadt) was dissolved in physiologic saline in a concentration of 0.1%.

[2] Another portion of the organs were used for histological examination, after fixing in Zenker-formol and embedding in paraffin. The sections, cut 5–7μ in thickness, were stained with Mayer's acid hemalum and eosin.

Table 1. The amounts of radioactivity per gram of blood 24, 48 and 72 hours after saponin injection (48, 72 and 96 hours after injection of radioactive phosphorus).

Time interval	Procedure	Animal No., body weight and sex	Number of normoblasts per mm^3 of blood	cpm per g of blood $\times 10^3$
24 hours	Saponin injected	No. 3, 2.5 kg, ♂	4,210	38.3
		No. 5, 2.0 kg, ♂	6,048	59.8
		No. 9, 2.5 kg, ♂	24,024	19.6
	Control*	No. 4, 2.7 kg, ♂	0	6.3
		No. 7, 2.3 kg, ♂	0	27.1
		No. 8, 2.4 kg, ♂	0	25.5
48 hours	Saponin injected	No. 11, 2.5 kg, ♂	10,180	10.6
		No. 12, 3.5 kg, ♀	5,040	10.8
		No. 14, 2.0 kg, ♂	3,402	24.8
	Control	No. 15, 2.1 kg, ♂	0	11.2
		No. 16, 2.3 kg, ♂	0	36.0
		No. 17, 2.2 kg, ♂	0	30.4
72 hours	Saponin injected	No. 18, 2.3 kg, ♂	16,170	10.8
		No. 19, 2.8 kg, ♂	8,736	6.8
		No. 22, 2.5 kg, ♂	5,688	9.1
	Control	No. 13, 1.8 kg, ♂	0	37.1
		No. 20, 2.8 kg, ♂	0	7.4
		No. 21, 3.0 kg, ♂	0	8.1

* Control injected with radioactive phosphorus without saponin injection.

of the control group which received no injection of saponin. It should be noticed, however, that despite the abundant occurrence of nucleated red cells in the blood, no significant increase was observed in the values of the specific activity of the blood after saponin injection, as compared with the corresponding values for the control group without saponin injection (control values). This is probably due to the fact that the nucleated red cells occurring in the blood are for the most part orthochromatic normoblasts, which are at the latest stage of maturation among nucleated red cells and therefore have little metabolic capacity of incorporating P^{32} into the cells.

In the organs other than the bone marrow, the values of the specific activity for the experimental group that had received saponin injection tended to be greater than the values for the control group without saponin injection. This tendency was especially evident in the spleen and liver. In the spleen, the values of the relative activity per organ were strikingly increased after saponin injection as compared with the control values (cf. Table 3), and the differences were all statistically significant ($P<0.05$ at 24 and 48 hours; $P<0.01$ at 72 hours after saponin injection). In the liver, lesser differences were found in this respect, however.

As regards the relative activity per gram of fresh tissue, on the other hand, lesser differences were seen between the values for the experimental and those for the

Table 2. The amounts of radioactivity per gram of fresh tissue in various organs 24, 48 and 72 hours after saponin injection (48, 72 and 69 hours after injection of radioactive phosphorus).

Time interval	Procedure	Lungs		Liver		Spleen		Mesenteric lymph nodes		Bone Marrow		Kidneys		Adrenals	
	Radio-activity	cpm per g ($\times 10^3$)	Rel. act.* (%)	cpm per g ($\times 10^3$)	Rel. act. (%)	cpm per g ($\times 10^3$)	Rel. act. (%)	cpm per g ($\times 10^3$)	Rei. act. (%)	cpm per g ($\times 10^3$)	Rel. act. (%)	cpm per g (10^3)	Rel. act. (%)	cpm per g ($\times 10^3$)	Rel. act. (%)
24 hours	Saponin injected	40.9 ±10.43	14.54 ±2.98	52.1 ±5.75	18.92 ±1.95	59.5 ±1.44	21.65 ±1.63	33.0 ±2.93	11.96 ±0.71	21.3 ±5.35	7.75 ±1.95	28.3 ±0.08	10.29† ±0.52	40.6 ±4.03	14.76 ±1.39
	Control**	21.8 ±1.44	9.65 ±0.72	37.1 ±5.69	16.27 ±1.24	36.7 ±2.05	16.26 ±1.23	32.9 ±1.28	14.54 ±0.78	35.9 ±7.71	15.78 ±3.23	29.9 ±2.24	13.22 ±1.13	32.3 ±2.46	14.26 ±1.05
48 hours	Saponin injected	22.0 ±1.42	10.45 ±1.03	33.9 ±6.50	15.96 ±1.35	45.9 ±9.49	20.26 ±0.23	27.5 ±4.64	13.35 ±2.03	18.0 ±3.14	8.67† ±1.06	24.0 ±3.69	11.41 ±0.27	38.2 ±3.20	18.64† ±1.50
	Control	30.0 ±0.06	10.09 ±0.07	56.0 ±0.35	18.80 ±0.08	56.2 ±3.66	18.87 ±1.16	38.4 ±2.14	12.90 ±0.82	39.3 ±1.57	13.20 ±0.51	35.8 ±0.42	13.16 ±1.21	41.9 ±0.93	14.08 ±0.33
72 hours	Saponin injection	18.9 ±3.15	10.17 ±0.67	38.1 ±7.08	20.50 ±1.32	34.7 ±3.99	18.75 ±0.85	24.3 ±2.81	13.30 ±0.44	15.1 ±3.04	8.05 ±0.71	24.7 ±2.13	13.65 ±0.94	27.8 ±2.06	15.72 ±5.99
	Control	30.8 ±4.91	10.29 ±0.81	61.8 ±10.87	20.52 ±1.97	48.5 ±5.56	16.37 ±0.75	36.4 ±3.78	12.34 ±0.44	29.1 ±3.43	9.92 ±1.04	35.3 ±4.33	11.84 ±0.28	56.1 ±8.25	18.68 ±0.76

* Rel. act. =relative activity is expressed as percentage of the total activity of all the organs examined (lungs+liver+spleen+mesenteric lymph nodes+bone marrow+kidneys+adrenals=100).

** Control injected with radioactivity phosphorus without saponin injection.

† Significant at 5% level ($0.05 \geqq P > 0.01$)

Table 3 The amounts of radioactivity per organ in various organs 24, 48 and 72 hours after saponin injection (48, 72 and 96 hours after injection of radioactive phosphorus).

Time interval	Procedure	Lungs		Liver		Spleen		Mesenteric lymph nodes		Bone Marrow		Kidneys		Adrenals	
		Weight of organ (g)	Rel. act.* (%)	Weight of organ (g)	Rel. act. (%)	Weight of organ (g)	Rel. act. (%)	Weight of organ (g)	Rel. act. (%)	Weight of organ (g)	Rel. act. (%)	Weight of organ (g)	Rel. act. (%)	Weight of organ (g)	Rel. act. (%)
24 hours	Saponin injected	9.13 ±0.91	6.27 ±1.51	76.45 ±8.36	66.08 ±2.50	1.56 ±0.27	1.38† ±0.07	2.14 ±0.25	1.22 ±0.32	42.53 ±0.53	14.59 ±1.05	20.18 ±0.30	9.92 ±1.76	0.53 ±0.10	0.36 ±0.06
	Control**	8.67 ±1.02	4.27 ±0.56	68.33 ±7.02	54.97 ±5.97	1.33 ±0.32	0.65 ±0.22	1.75 ±0.30	1.13 ±0.23	41.44 ±2.01	29.82 ±6.20	15.85 ±2.13	9.35 ±1.89	0.34 ±0.01	0.23 ±0.02
48 hours	Saponin injected	8.94 ±0.98	4.26 ±0.32	88.28 ±4.41	63.55 ±0.99	2.07 ±0.54	1.96† ±0.34	3.88 ±0.98	2.46 ±0.78	48.13 ±5.35	19.04 ±1.78	17.67 ±1.69	8.23 ±1.16	0.58 ±0.10	0.49 ±0.07
	Control	7.41 ±0.43	3.44 ±0.19	66.97 ±5.65	61.45 ±1.08	0.56 ±0.17	0.50 ±0.09	1.63 ±0.14	1.03 ±0.10	36.96 ±0.97	24.07 ±1.36	15.15 ±0.04	8.98 ±1.10	0.55 ±0.03	0.38 ±0.03
72 hours	Saponin injected	12.10 ±0.53	4.51 ±0.22	92.58 ±12.43	67.87 ±2.22	2.38 ±0.48	1.63†† ±0.07	2.59 ±0.38	1.28 ±0.17	51.56 ±15.08	14.76† ±2.59	19.43 ±0.95	9.78† ±0.91	0.32 ±0.03	0.18 ±0.02
	Control	9.92 ±2.13	5.08 ±0.63	58.57 ±4.41	61.61 ±1.29	0.74 ±0.18	0.63 ±0.18	1.75 ±0.30	1.09 ±0.13	43.23 ±5.58	23.06 ±1.07	13.58 ±0.50	8.26 ±0.16	0.32 ±0.06	0.34 ±0.07

* Rel. act.=relative activity is expressed as percentage of the total activity of all the organs examined (lungs+liver+spleen+mesenteric lymph nodes+bone marrow+kieneys+adrenals=100).

** Control injected with radioactive phosphorus without saponin injection.

† Significant at 5% level ($0.05 \geqq p > 0.01$).

†† Significant at 1% level ($p \leqq 0.01$).

control groups (cf. Table 2). In certain organs such as the lungs, liver, mesenteric lymph nodes, kidneys and adrenals, the values of the relative activity for the experimental group were sometimes smaller than the corresponding values for the control group. It should be added here that in the lungs, kidneys and adrenals, the values of the relative activity per organ for the experimental group were also sometimes smaller than the corresponding values for the control group.

The findings in the bone marrow are in sharp contrast with those in the spleen and liver. Namely, in this tissue a more or less remarkable decrease was constantly observed both in the values of the relative activity per gram of fresh tissue and in those per organ after saponin injection, as compared with the control values (cf. Tables 2 and 3).

2. *Histological findings in various organs*

As briefly mentioned in the introduction, the previous study by OSOGOE and OMURA (1951) demonstrated: (1) that within 72 hours after a large dose of saponin, a striking accumulation of immature blood cells took place in the sinusoids of the liver and the red pulp of the spleen and to a lesser extent also in the small blood vessels of lungs, kidneys, adrenals and lymph nodes, with the formation of numerous blood forming foci; and (2) that the bone marrow parenchyma was depleted almost completely and flooded with blood by the 24th or 48th hour, without corresponding disintegration of immature blood cells therein.

In the present study, the mode of accumulation of immature blood cells in the liver, spleen and other organs generally followed the pattern outlined above. It was revealed that the immature blood cells accumulated to the greatest extent in the spleen and to a somewhat lesser degree in the liver. The degree of accumulation of these cells was greatly reduced in other organs such as the lungs, mesenteric lymph nodes, kidneys and adrenals. Thus, the histological findings of the organs examined fairly well agree with the results of radioactivity measurements in these organs. It should be noticed, however, that in the lungs, kidneys and adrenals, the findings of both methods did not always agree, as already stated.

DISCUSSION

The chief results of the present experiments may be summarized as follows: After saponin injection into adult rabbits which had been injected with 200μc of inorganic P^{32} 24 hours previously, there occurred a striking increase in the amount of radioactivity in the spleen and to a lesser extent also in the liver in less than 72 hours; whereas the amount of radioactivity in the bone marrow was invariably reduced to a large extent, as compared with the values for the control group without saponin injection. In view of the fact the immature blood cells are capable of rapidly incorporating radioactive phosphorus, it is expected that an increase in the radi-

oactivity of an organ would indicate an accumulation of these cells in this organ and vice versa. It is therefore of interest to know to what extent the results of radioactivity measure ments in an organ might be correlated with the histological findings of this organ.

The results of the present experiments have shown that the histological findings of the organs examined fairly well agree with the results of radioactivity measurements in these organs. Although in some organs such as the lungs, kidneys and adrenals the findings did not always agree, it can be stated that the results of radioactivity measurements may reflect the histological findings to a certain extent.

It should be emphasized here that the histological findings in the present experiments are quite similar to those in the previous experiments by OMURA and OSOGOE (1951), in which saponin alone was injected in a large dose into adult rabbits subsequent to bleeding of 30 cm^3 of blood. Moreover, as already stated, the pattern of accumulation of immature blood cells in foreign organs after saponin injection was found to be the same as that observed for the transfused marrow cells reported in another paper by OSOGOE and OMURA (1950). It is therefore evident that a large dose of saponin causes a liberation of immature blood cells from the bone marrow on a large scale.

In this connection, it is of interest to compare the results of the present experiments with the results of transfusion of radiophosphorus-labelled bone marrow elements, leukocytes and lymphocytes. OSOGOOE (1958) and TAMASHIGE (1959) reported that the amounts of radioactivity recovered per organ 24 hours after administration of radioactive marrow cells into X-irradiated guinea-pigs indicated that chief accumulation of the transfused cells occurred in the spleen and bone marrow. After transfusion of P^{32}-labelled leukocytes in rabbits, LEATHY et al. (1954) found the greatest amount of radioactivity in the lungs within a few minutes, but they also demonstrated that the organ which shows the highest level of radioactivity was shifted from the lung to the liver after 5 hours. The initial accumulation of the transfused, P^{32}-labelled lymphotcytes in the lungs has been demonstraed by WEISBERGER et al. (1951), FICHTELIUS (1953) and MONDEN et al. (1955, 1959). MONDEN et al. further observed a gradual shift of radioactivity from the lungs to the liver and spleen. It has been repeatedly shown that the transfused cellular elements of the hematopoietic tissues accumulate in the liver and spleen to the greatest extent (OSOGOE, 1950; and OMURA, 1950; FICHTELIUS, 1953; OSOGOE, 1958).

As mentioned above, the characteristic feature of the the transfused leukocytes and cellular elements of the hematopoietic tissues is that they accumulate in the liver and spleen to the greatest extent. This is in good agreement with the pattern of distribution of radioactivity observed in the present study.

SUMMARY

1. A large dose (6 mg) of saponin (E. MERCK) was administered in a single, intravenous injection into adult albino rabbits, which had been intravenously injected with 200μc of inorganic P^{32} in a single dose 24 hours previously.

2. In less then 72 hours after saponin, there occurred a striking increase in the amount of radioactivity in the spleen and to a lesser extent also in the liver, whereas the amount of radioactivity in the bone marrow was invariably reduced to a large extent, as compared with the values for the control group without saponin injection.

3. The histological examination of various organs reavealed a striking accumulation of immature blood cells in the spleen and liver, with a simultaneous depletion of these cells in the bone marrow.

4. The above-mentioned pattern of accumulation of immature blood cells in foreign organs after saponin injection was the same as that observed for the transfused marrow cells.

REFERENCES

FICHTELIUS, K. E. 1953. The fact of the lymphocyte. *Acta Anat.*, **19**, Suppl. 1 ad 19: 1–78.

LEATHY, W. V. C., T. F. MCNICKLE and P. K. SMITH 1954. Fate of injected radiophosphorus-labelled leucocytes. *Am. J. Physiol.*, **179**: 570–576.

MONDEN, Y., A. KANESADA, H. IMAMURA and K. FUKUTANI 1956. Fate of the transfused lymphocytes labelled with radioactive phosphorus. An experiment with splenectomized albino rats. *Acta Haem. Jap.*, **19**: 644–650.

OMURA, K. and B. OSOGOE 1951. Saponin induced colonization of the bone marrow elements in foreign organs in rabbits. *Anat. Rec.*, **110**: 289–312.

OSOGOE, B. 1950. Transplantation of hematopoietic tissues into the circulating blood. I. Experiments with lymph nodes in normal rabbits. *Anat. Rec.*, **107**: 193–220.

———— 1958. Behavior of transfused cellular elements of hematopoietic tissues. Proceedings of the Sixth International Congress of the International Society of Hematology (Boston, 1956), pp. 139–143. Grune and Stratton, New York.

OSOGOE, B. and K. OMURA 1950. Transplantation of hematopoietic tissues into the circulating blood. II. Injection of bone marrow into normal rabbits, with special reference to the histogenesis of extramedullary foci of hematopoiesis. *Anat. Rec.*, **108**: 663–686.

TAMASHIGE, T. 1959. On the behavior of the transfused cellular elements of bone marrow in X-irradiated guinea-pigs. *Bull. Yamaguchi Med. Sch.*, **7**: 71–90.

WEISBERGER, A. S., R. A. Cuyton, R. W. Heinle, J. P. Storaashi 1951. The role of the lungs in the removal transfused lymphocytes. *Blood*, **6**: and 916–925.