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Studies on the relationship between the function of reticuloendothelial system and the hematopoiesis. I. Experimental studies on the RES functions using 51Cr-labelled heat-damaged iso-erythrocytes in the induced hematological disorders of mice

Makoto Hasegawa*

*Okayama University,

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Makoto Hasegawa

Abstract

The following conclusions were drawn from the above data concerning the RES function to sequestrate 51Cr-labelled heat-damaged iso-erythrocytes in mice. (l) When the hematological disorders of mice were induced, the RES of the liver and spleen reacted in the same manner. (2) The RES function of the bone marrow and liver were observed to react reversely except in the case of splenectomized mice. (3) Human gamma globulin hypersensitization and chloramphenicol administrations suppressed RES function of the bone marrow and augmented that of the liver and spleen. (4) The RES function of the bone marrow was activated after splenectomy. (5) The massive human gumma globulin administration was followed by the increased RES function of the bone marrow and by the suppressed one of the liver and spleen.

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STUDIES ON THE RELATIONSHIP BETWEEN THE FUNCTION OF RETICULOENDOTHELIAL SYSTEM AND THE HEMATOPOIESIS

I. EXPERIMENTAL STUDIES ON THE RES FUNCTIONS USING ^{51}CR-LABELLED HEAT-DAMAGED ISO-ERYTHROCYTES IN THE INDUCED HEMATOLOGICAL DISORDERS OF MICE

Makoto HASEGAWA

Department of Internal Medicine, Okayama University Medical School, Okayama, Japan (Director: Prof. K. Hiraki)

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Many studies (1, 2) indicate that the spleen has several functions such as: 1) blood formation in embryonic life (3, 4), 2) reservation, sequestration and destruction of erythrocytes, 3) culling of circulation, 4) iron turnover and storage, 5) pooling and destruction of leuocytes and platelets, 6) influence upon hematopoietic function of bone marrow, 7) defence against infection and 8) antibody production.

Since DAMESHEK first used the term hypersplenism in the sense that normal spleen exerts a mild inhibition on the marrow's hematopoietic activity, in hypersplenism the inhibitory effect becomes pathologically severe (5), and many discussions and studies have been made on this point. At present, hypersplenism is used by some as a term meaning augmented sequestration and destruction of erythrocytes in the spleen (6, 7, 8, 9). These functions of the spleen demonstrate its close relationship to the hematopoietic system. In fact splenomegaly can be seen in various blood diseases and splenectomy is helpful in the treatment of some cases of disorders such as congenital hemolytic anemia, idiopathic thrombocytopenic purpura, Banti's syndrome, and hypoplastic anemia. But the influence of splenic functions on hematopoiesis is still obscure in many respects, so that more exact patho-physiological functions of the spleen should be elucidated.

Recently the introduction of radioisotope techniques, especially ^{51}Cr-labelled erythrocyte methods into the field of hematology has made clear the existence of excessive hemolysis which is shown by reduced survival time of erythrocytes in many diseases. And these methods made it possible to examine the site of destruction of erythrocytes and the extent of the role which the spleen plays in this mechanism by body surface counting

of the spleen, liver and bone marrow (10, 11, 12).

The author attempted to show the relationship of RES functions, especially spleen functions to the hematopoiesis by ^{51}Cr-labelled heat-damaged erythrocytes in mice whose hematological disorders were induced by chloramphenicol (CP), human gamma globulin (H. G. G.), the mixture of H. G. G. and Freund's complete adjuvant (H. G. G. & F. C. Adj.) and splenectomy.

MATERIALS AND METHODS

(1) *Amimals:* ICR female mice, weighing 20 to 24 g were used for all the experiments.

(2) *Measurements of RES functions by ^{51}Cr-labelled heat-damaged iso-erythrocytes:* Heparinized whole blood was withdrawn from decapitated ICR mice, and was labelled with ^{51}Cr (the form of sodium chromate, Dinabot Laboratory) added in the ratio of 100 μc of ^{51}Cr to 3 ml of whole blood, through the incubation giving gentle agitation for 20. minutes at 37°C. After the addition of 10 mg of ascorbic acid to 3 ml of ^{51}Cr-labelled whole blood, it was heated at 49.5 ± 0.5°C for 20 minutes in the water bath with gentle agitation. These heated erythrocytes were washed three times and resuspended in sterilized physiological saline to the same volume as that initially withdrawn. 0.3 ml of ^{51}Cr-labelled heat-damaged erythrocytes was injected into the mouse tail vein. Twenty-five μl of mouse blood was obtained from retroorbital venous plexus using the capillary tubes at 5, 20, 40 and 60 minutes after injection. The ^{51}Cr radioactivity in the capillary tubes were calculated in the well-type scintillation counter (TEN scaler SA-250). The count per minutes (cpm) in each specimen was plotted against time on semilogarithmic paper and ^{51}Cr clearance (T 1/2) was determined. The mice were sacrificed by decapitation 120 minutes after injection of ^{51}Cr-labelled erythrocytes. The liver, spleen, kidney, lungs, femur, tibia and fibula were excised and weighed. The radioactivity of each whole organ was determined in a well-type scintillation counter. Percent uptake of each organ was determined by dividing the cpm in each organ by the cpm of standard ^{51}Cr-labelled erythrocytes prepared at the time of injection. The specific ^{51}Cr activity (cpm per mg) in each organ was calculated. The uptake of whole bone marrow was assumed to be 13 times the sum of cpm of femur, tibia and fibula. The specific ^{51}Cr activity in the bone marrow was of course not taken. Normal mice were used for control in each experiment, in order to avoid the erroneous results in clearance and organ uptake due to the denaturation during the procedures.

(3) *Induction of the hematological disorders:* For the purpose of inducing hematological disorders, the following approaches were made; administration of CP, splenectomy, and administration of H. G. G. alone and with combination of Freund's complete adjuvant or CP. Five mg of chloramphenicol succinate, which corresponds to 250 mg per kg of body weight, was administered intraperitoneally twice daily for 3 days (CP 3-day group), and in the

other group 5 mg of CP was administered once a day for 30 days (CP 30-day group). The splenectomy was performed aseptically under pentobarbital anesthesia 10 days (Spx. 10-day group) and 20 days (Spx. 20-day group) before the examination of RES function. Intraperitoneal administrations of 5 mg of CP twice daily for 3 days was started 10 days (Spx. 10-day plus CP group) and 20 days (Spx. 20-day plus CP group) after splenectomy.

Two and half mg of human gamma globulin was administered intraperitoneally three times every other day (H. G. G. group). The mixture of H. G. G. and Freund's complete adjuvant (H. G. G. & F. C. Adj. group) was injected intraperitoneally into the mice three times every other week, then a splenomegaly and ascitic fluid were obtained. To examine the combined effect of CP and H. G. G., 1.75 mg of Chlorabulin (Midori Juji Co. Ltd), which contained 0.5 mg of CP and 1.25 mg of H. G. G., was administered three times intraperitoneally every other day (Chlorabulin 3-day group). ^{51}Cr-labelled heat-damaged erythrocytes were administered at 12 hours after the last injection of CP, and 4 days after H. G. G. and Chlorabulin injections.

(4) *Others:* The peripheral blood pictures and the ferrokinetics were observed as shown in the accompanying paper (13).

RESULTS

(1) *Normal mice :* The changes of each organ uptake were small 30, 60 and 120 minutes after ^{51}Cr-labelled heat-damaged erythrocytes injection. The highest percent uptake was in the liver, the next was in the spleen and the next was in the bone marrow (Figure 1). The specific ^{51}Cr activity in the spleen was about seven times higher than that in the liver

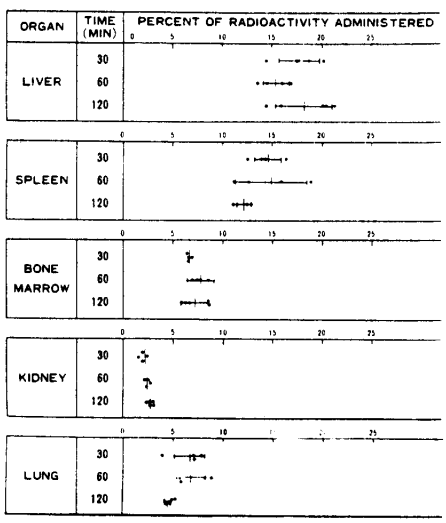

Fig. 1 Organ distribution of ^{51}Cr-labelled heat-damaged iso-erythrocytes in normal mice

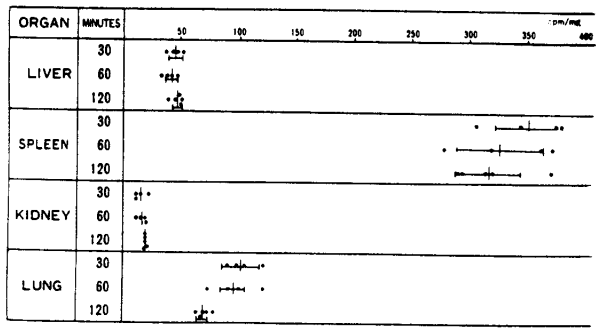

Fig. 2 Specific ^{51}Cr activity (cpm/mg) in organs of normal mice

(Figure 2).

(2) *CP 3-day group :* The clearance of this group was within the normal range. The marrow uptake of ^{51}Cr-labelled heat-damaged erythrocytes was low but both the liver and spleen uptake was high ($p<0.01$) (Figure 3).

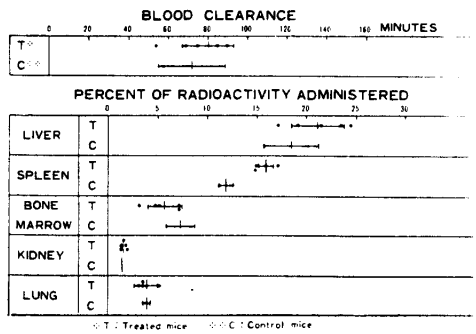

Fig. 3 Blood clearance ($T\frac{1}{2}$) and organ distribution of ^{51}Cr-labelled heat-damaged iso-erythrocytes in mice treated with chloramphenicol for 3 days

(3) *CP 30-day group :* The clearance of this group was also within the normal range. The uptake of liver and spleen, which was increased in CP 3-day group, returned to the normal range in this group (Figure 4).

(4) *Spx. 10-day group :* This group showed an extremely prolonged clearance (normal group 47.1 ± 7.0 minutes, this group 157.0 ± 36.0) ($p<0.01$) and increased uptake of the liver (normal group 16.3 ± 1.8 %, this group 23.1 ± 1.3 %) ($p<0.01$) and bone marrow (normal group 4.9 ± 1.5 %, this group 6.6 ± 0.8 %) (Figure 5).

(5) *Spx. 20-day group :* The clearance of this group was significantly prolonged beyond that of the normal group (normal group 73.4 ± 17.2,

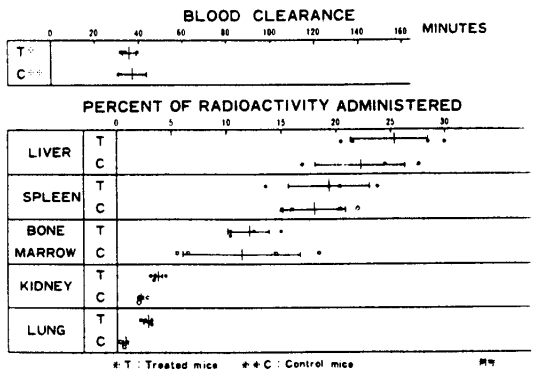

Fig. 4 Blood clearance ($T\frac{1}{2}$) and organ distribution ^{51}Cr-labelled heat-damaged iso-erythrocytes in mice treated with chloramphenicol for 30 days

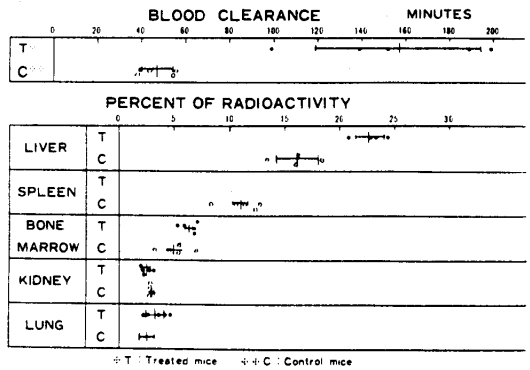

Fig. 5 Blood clearance ($T\frac{1}{2}$) and organ distribution of ^{51}Cr-labelled heat damaged iso-erythrocytes in mice 10 days after splenectomy

this group 120.0 ± 4.1 minutes) ($p < 0.01$). However, it was shortened markedly compared with that of Spx. 10-day group. The uptake of the liver was slightly higher and that of the bone marrow was significantly higher than in the normal group ($p < 0.01$) (Figure 6).

(6) *Spx. 10-day plus CP group :* Two mice showed prolonged clearance (85 and 215 minutes), but the other 2 showed the normal range of clearance (68.0 ± 8.6 minutes). And all the 4 showed the uptake of normal range in the liver and bone marrow (Figure 7).

(7) *Spx. 20-day plus CP group :* The clearance was normal in this group. The uptake of the liver in this group increased significantly over that of the normal group as well as that of the Spx. 20-day group ($p < 0.01$). The uptake of the bone marrow in this group decreased slightly as compared with that of the normal group as well as that of the Spx. 20-day group (Figure 8).

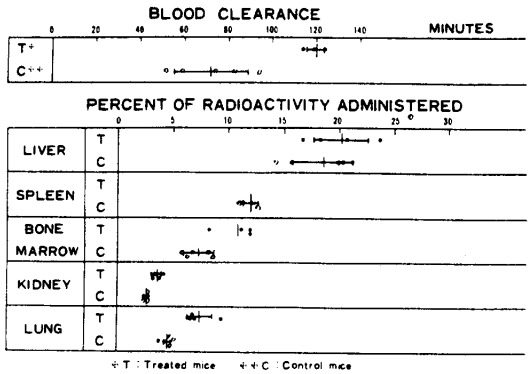

Fig. 6 Blood clearance ($T\frac{1}{2}$) and organ distribution of ^{51}Cr-labelled heat-damaged iso-erythrocytes in mice 20 days after splenectomy

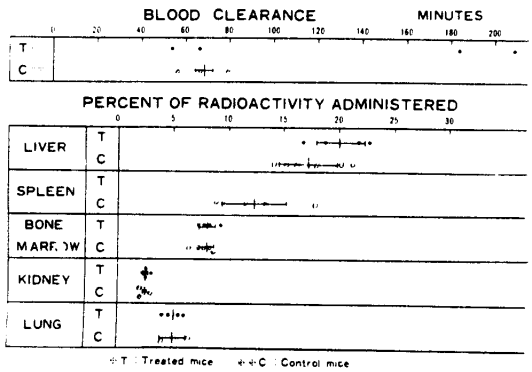

Fig. 7 Blood clearance ($T\frac{1}{2}$) and organ distribution of ^{51}Cr-labelled heat-splenectomy iso-erythrocytes in mice treated with chloramphenicol 10 days after splenectomy

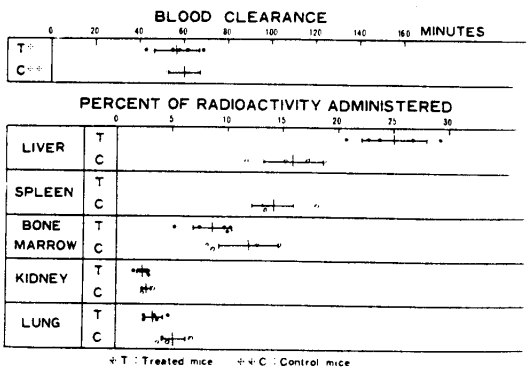

Fig. 8 Blood clearance ($T\frac{1}{2}$) and organ distribution of ^{51}Cr-labelled heat-damaged iso-erythrocytes in mice treated with chloramphenicol 20 days after splenectomy

(8) *H. G. G. group :* This group showed a slightly prolonged clearance. The uptake of liver and spleen was slightly decreased and that of the bone marrow increased (Figure 9).

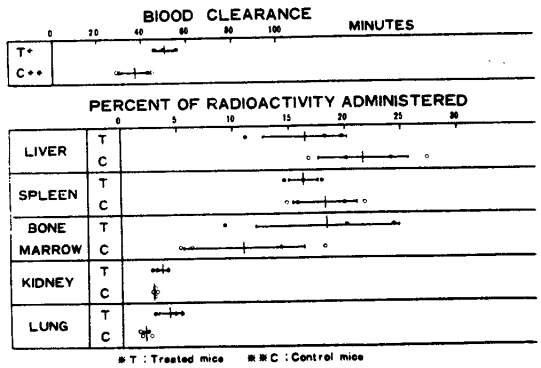

Fig. 9 Blood clearance ($T\frac{1}{2}$) and organ distribution of ^{51}Cr-labelled heat damaged iso-erythrocytes in mice treated with H. G. G. for 3 days

(9) *H. G. G. & F. C. Adj. group :* This group showed splenomegaly weighing 481.0 ± 56.5 mg in contrast to the normal mouse spleen of 131.0 ± 2.1 mg in weight. This group had a significantly shortened clearance (normal group 94.0 ± 13.0, this group 43.3 ± 15.3 minutes) ($p < 0.01$). The uptake of the liver was slightly increased, and that of the spleen significantly increased in this group (normal group $11.2 \pm 2.1\%$, as against $20.0 \pm 3.5\%$ of this group) ($p < 0.01$). On the contrary, the uptake of the bone marrow was significantly decreased (normal group $6.6 \pm 1.0\%$, this group $2.3 \pm 0.2\%$) ($p < 0.01$) (Figure 10). The specific

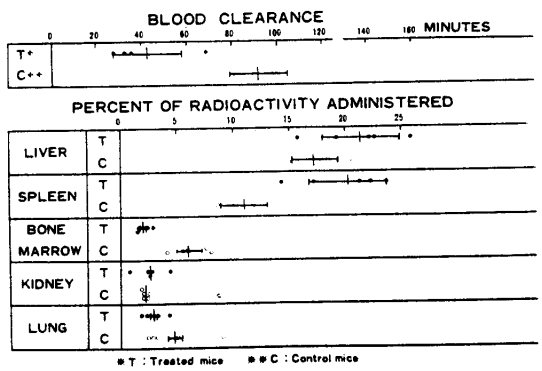

Fig. 10 Blood clearance ($T\frac{1}{2}$) and organ distribution of ^{51}Cr-labelled heat-damaged iso-erythrocytes in mice sensitized with H. G. G. & F. C. Adj.

^{51}Cr-radioactivity in the spleen of this group was lower than that of the normal group (normal group 338.5 ± 49.9 cpm, this group 170.4 ± 38.2 cpm) (Figure 11).

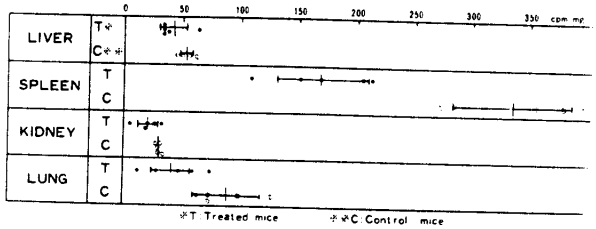

Fig. 11 Specific ^{51}Cr-activity (cpm/mg) in mice sensitized with H. G. G. & F. C. Adj.

(10) *Chlorabulin 3-day group :* The clearance was almost within the normal range in this group. The uptake of the liver was significantly increased (normal group 14.8 ± 1.9, this group 21.9 ± 0.5 %) ($p < 0.01$). The bone marrow uptake showed a decreasing tendency (Figure 12).

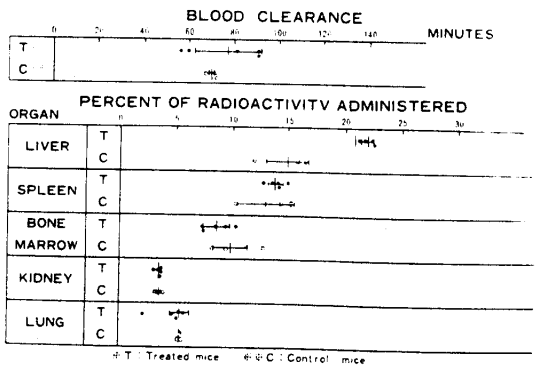

Fig. 12 Blood clearance ($T\frac{1}{2}$) and organ distribution of ^{51}Cr-labelled heat-damaged iso-erythrocytes in mice treated with chlorabulin for 3 days

DISCUSSION

The precursor of the hematopoiectic organ is known as the reticulo-endothelial cells from the studies of developmental anatomy (14, 15, 16). The reticuloendothelial cells have been observed to have two kinds of functional potentials; one is phagocytic activity, the other is proliferative activity to supply blood cells (14, 15, 16). From these observations, it has been suggested that there should exist some relationship between the reticuloendothelial system in the spleen and the other hematopoietic organs throughout natal and post-natal stage. However, the observations

which proved such relationship are scanty in the aspect of cellular functions, especially regarding the post-natal hematopoiesis. The study presented here was carried out with the purpose to make clear the role of the reticuloendothelial functions, especially of the spleen in the hematopoietic disorders which had been induced by administration of CP, H. G. G., H. G. G. and F. C. Adj., and Chlorabulin, splenectomy and splenectomy plus CP administration.

The CP administrations have demonstrated to cause suppressed erythropoiesis, including anemia, decreased reticulocytes, prolonged ^{59}Fe plasma iron disappearance time, suppressed percent of ^{59}Fe reappearance, and decreased uptake of ^{59}Fe into the bone marrow and/or the spleen, as will be described in the accompanying paper (13). These mice showed normal range of the clearance, which was consisted of a lower uptake of the bone marrow and a higher one of the liver and spleen after administration of ^{51}Cr-labelled heat-damaged erythrocytes in CP-3 day group. In CP 30-day group all the organs showed normal uptake. Though these experiments suggested that RES functions might be restored with longer term of CP administration, there was no conclusive proof. It was assumed that at first the toxicity of CP suppressed hematopoiesis and stimulated RES functions of the liver and spleen, and after prolonged CP administrations, RES cells became tolerant to CP and restored RES functions.

The splenectomy is one of the methods to estimate the spleen function. But the splenectomy has direct influences upon the RES functions and hematopoiesis by the stress of the operation and bleeding. Prolonged ^{51}Cr-clearance and higher uptake of ^{51}Cr-labelled heat-damaged erythrocytes into the RES of liver and bone marrow were observed after splenectomy. It might be said that the total RES functions were suppressed by splenectomy, but liver and bone marrow compensated for the depleted spleen RES function. Compared with Spx. 10-day group, Spx. 20-day group showed a highly compensated state of the RES function.

The CP administration in splenectomized mice made the RES function in the liver further accentuated, and made the clearance normal or nearly normal. This accentuated RES function in the liver was also observed in the CP 3-day group, while the RES function of the bone marrow in this group was suppressed by CP.

It is well known that the hypersplenism can be produced by sensitization of heterologous or homologous immunoglobulin, and that the hypersplensim usually accompanies hypofunction of the hematopoietic organs. But the relationship between the hypersplenism and the RES function of bone marrow, liver and spleen should be elucidated. After administrations

of H. G. G., the RES function was suppressed in liver and spleen and was activated in bone marrow, and as a whole the clearance was prolonged. The hypersensitizations with H. G. G. & F. C. Adj. made this function reverse, as shown by the shortened clearance, accentuated RES function of liver and spleen, and depressed function of bone marrow. It could be postulated about these reverse facts that the massive dose of H. G. G. suppressed RES except in bone marrow, although H. G. G. & E. C. Adj. induced hyperfunction of RES of liver and spleen with depressed bone marrow function by the hypersensitivity.

Of course, in the present experiments there were many problems about the technique of the estimation of RES function. One of them was the difficulty of getting constant damage of erythrocytes by heating. Secondly, circulating blood in the organ contributed to modify the uptake of ^{51}Cr radioactivity. Furthermore, exsanguination from the organs by decapitation was not always complete. Thirdly, there were technical problems to inject equally in all mice ^{51}Cr-labelled heat-damaged erythrocytes and to obtain a constant blood volume for counting the radioactivity. Fourthly, the measurement of the uptake of the whole bone marrow was so difficult that femur, fibula and tibia were measured as the representative. The final problem of individual difference was solved by using the inbred mice. These problems in this method were solved in the following ways: (1) The temperature to get heat-damaged erythrocytes was strictly controlled in a range of 49.5 ± 0.5°C, and the time was kept constant at 20 minutes. The fragility of the erythrocytes was relatively constant because of inbred mice. (2) Excess circulating blood pool in the organ was washed out with saline after excision. (3) ^{51}Cr-labelled heat-damaged erythrocytes were injected carefully by tuberculin syringe with a 25G needle. The blood was taken from retroorbital venous plexus into a heparinized capillary hematocrit tube marked at 25 μl. (4) At first all the bones of the mouse were removed. The ratio of ^{51}Cr uptake into the whole bone marrow to the sum of uptake into femur, tibia and fibula was 13 to 1. Thereafter the bone marrow uptake was obtained by multiplying the sum of cpm of femur, tibia and fibula by the index of 13. In all the mice there was a close correlation between the results of the Congo red test and those of the sequestration of ^{51}Cr-labelled heat-damaged erythrocytes.

CONCLUSION

The following conclusions were drawn from the above data concerning the RES function to sequestrate ^{51}Cr-labelled heat-damaged iso-erythro-

cytes in mice.

(1) When the hematological disorders of mice were induced, the RES of the liver and spleen reacted in the same manner.

(2) The RES function of the bone marrow and liver were observed to react reversely except in the case of splenectomized mice.

(3) Human gamma globulin hypersensitization and chloramphenicol administrations suppressed RES function of the bone marrow and augmented that of the liver and spleen.

(4) The RES function of the bone marrow was activated after splenectomy.

(5) The massive human gumma globulin administration was followed by the increased RES function of the bone marrow and by the suppressed one of the liver and spleen.

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