

Erythropoietic Substance in the Serum of Anemic Animals.

NEWTON KRUMDIECK. (Introduced by D. P. Barr.)

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Hemorrhage or blood destruction from whatever cause is reflected in the bone marrow by erythrogenic hyperplasia. Chemical and mechanical factors are thought to be responsible. It is possible, however, that the stimulus to erythrogenic hyperplasia of the bone marrow is some substance in the blood serum of the anemic animal. Carnot and Deflandre¹ reported the presence of such a stimulus but Gordon and Dubin² on repeating their work were unable to demonstrate it. Yu Tin Tei³ in an extensive series of experiments found a hemopoietic substance in the serum of anemic animals and described it as being alcohol, ether, chloroform, acetone

soluble and heat stable at 100°C for 30 minutes. Its activity was reflected by increases in the red blood cells, hemoglobin and reticulocytes in the peripheral blood of recipient animals. The bone marrow was not studied in any of these observations.

The following experiments were designed to determine the effect of injections of serum of animals rendered acutely and chronically anemic by bleeding on the peripheral blood and bone marrow of normal recipient animals.

Healthy white male rabbits weighing between 2500 and 2800 g were used. Donor animals were bled from the marginal ear vein. Blood counts were done at approximately the

TABLE I.
Blood Counts of Rabbits Injected with Serum from Acutely Anemic Animals

	Rabbit No.	Hemoglobin in g/100 cc	Red blood cells in millions/mm ³	Reticulocytes in %
Before injection	4	13.3	5.7	1.2
	5	13.7	6.0	1.8
	6	14.1	6.3	0.6
Day of "	4	13.2	5.4	1.2
	5	13.5	5.9	1.1
	6	13.5	5.8	1.9
1 day after "	4	13.7	5.2	1.6
	5	13.2	5.9	0.8
	6	13.6	6.6	1.2
2 " " "	4	13.1	5.6	1.7
	5	13.0	5.1	4.2
	6	14.1	6.1	3.1
3 " " "	4	13.3	5.7	6.3
	5	13.0	5.2	4.9
	6	13.0	5.9	6.8
4 " " "	4	13.6	5.6	1.3
	5	13.5	5.2	2.1
	6	13.1	5.4	3.2
5 " " "	4	13.2	5.5	2.9
	5	14.5	6.1	2.3
	6	13.7	5.8	3.2

¹ Carnot and Deflandre, *Compt. Rend. de l'Acad.*, 1906, **143**, 384.

² Gordon and Dubin, *Am. J. Physiol.*, 1934, **107**,

704.

³ Yu Tin Tei, *J. Chosen Med. Assn.*, 1938, **28**, 1, 32, 71, 129.

TABLE II.
Blood Counts of Rabbits Injected with Serum from Chronically Anemic Animals.

	Rabbit No.	Hemoglobin in g/100 cc	Red blood cells in millions/mm ³	Reticulocytes in %
Before injection	10	13.6	5.6	2.4
	11	13.4	5.2	3.1
	12	13.5	6.4	0.5
Day of "	10	13.0	5.5	2.8
	11	12.6	4.7	3.7
	12	13.1	5.4	2.8
1 day after "	10	13.5	5.9	4.2
	11	12.7	5.3	4.7
	12	13.1	5.6	5.2
2 " " "	10	13.3	5.5	7.1
	11	13.1	6.0	6.3
	12	12.9	5.3	7.0
3 " " "	10	12.9	5.9	10.5
	11	12.6	6.2	10.1
	12	13.2	5.6	8.9
4 " " "	10	12.9	5.8	9.6
	11	12.9	5.5	9.0
	12	13.2	6.5	7.8
5 " " "	10	12.8	5.9	8.6
	11	14.9	5.3	7.6
	12	13.2	6.8	7.0

TABLE III.
Blood Counts of Rabbits Injected with Serum from Normal Rabbit.

	Rabbit No.	Hemoglobin in g/100 cc	Red blood cells in millions/mm ³	Reticulocytes in %
Before injection	14	13.7	5.6	0.3
	15	14.0	5.1	2.1
	16	14.0	5.9	1.2
Day of "	14	13.4	5.7	0.5
	15	13.9	5.2	1.1
	16	14.1	5.3	1.1
1 day after "	14	13.7	5.2	1.2
	15	13.3	5.4	1.7
	16	13.2	5.7	0.4
2 " " "	14	13.5	5.4	1.4
	15	13.6	5.3	0.7
	16	13.6	5.3	2.1
3 " " "	14	14.0	5.4	2.3
	15	13.8	5.8	1.9
	16	13.6	5.3	2.1
4 " " "	14	13.9	5.3	1.2
	15	13.3	5.0	0.4
	16	13.1	5.7	0.8
5 " " "	14	13.3	5.7	2.5
	15	14.6	5.6	1.7
	16	13.8	5.8	0.4

TABLE IV.
Blood Counts of Rabbits Which Received No Serum.

	Rabbit No.	Hemoglobin in g/100 cc	Red blood cells in millions/mm ³	Reticulocytes in %
Control	17	14.1	5.4	0.1
	18	14.3	5.6	0.7
1 day	17	14.0	5.1	1.2
	18	13.8	5.3	1.0
2 "	17	14.1	5.7	0.2
	18	14.7	5.5	2.4
3 "	17	13.7	5.9	0.3
	18	13.8	5.1	1.9
4 "	17	13.9	5.7	1.3
	18	13.9	5.3	0.5
5 "	17	14.0	5.5	0.7
	18	14.2	5.8	1.3
6 "	17	14.6	5.3	1.0
	18	14.2	5.7	1.4

same time each day on venous blood obtained by pricking the marginal ear vein. Standard red blood cell pipettes and standardized Hellige hemoglobinometers were used. Reticulocytes were stained with brilliant cresyl blue using the usual coverslip technic. Touch preparations of bone marrow from the proximal ends of the femur, humerus and tibia were made at the time each animal was killed. These preparations were stained with Wright-Giemsa stain. Two hundred cells were counted in each preparation.

Experimental. I. Acutely anemic Donor Rabbits. Each of 3 rabbits had 30 cc of blood removed on the first and third days of the experiment. By this means the average original red blood cell count and hemoglobin value were reduced from 5.5 M/mm³ and 13.0 g/100 cc to 3M and 7.9 g respectively. The reticulocytes increased from 2 to 12%. These animals were bled to death from the heart on the fourth day. The blood was allowed to clot and the serum was separated by centrifugation. The serum was pooled and 20 cc were injected intravenously into each of 3 normal rabbits which were killed on the fifth day after injection. The results of the blood counts are shown in Table I.

II. Chronically Anemic Donor Rabbits. Each of 3 rabbits had a total of 240 cc of blood removed from the ear vein by 8 semi-

weekly bleedings of increasing amounts. The average original red blood cell count and hemoglobin fell from 6. M/mm³ and 13.2 g/100 cc to 3.8 M and 6.6 g. The reticulocytes rose from 3 to 25%. This degree of anemia had been maintained for 2 weeks before the animals were bled to death from the heart. The blood was allowed to clot and the serum was separated by centrifugation. Twenty cc of this pooled serum was injected intravenously into each of 3 healthy rabbits. The animals were killed on the fifth day after injection. The blood counts are shown in Table II.

Controls. I. Normal Donor Rabbit. Twenty cc of serum from a healthy rabbit were injected intravenously into each of 3 healthy rabbits which were killed on the fifth day after injection. The peripheral blood counts are in Table III.

II. Normal Rabbits. Two healthy rabbits received no serum but blood counts were done each day. These are listed in Table IV. The bone marrow cell counts for all groups are detailed in Table V.

The bone marrow of the acutely anemic donor rabbits showed a slight increase in the proportion of erythrogenic cells. The bone marrow of rabbits injected with serum from these donor animals showed a normal proportion of erythrogenic and myelogenic cells.

TABLE V.
Average Bone Marrow Cell Counts of All Groups.

Experimental	Rabbit No.	Erythrogenic cells %	Myelogenic cells %
I. Acutely anemic donor rabbits	1	59	41
	2	64	36
	3	63	37
	4	58	42
	5	65	35
	6	47	53
II. Chronically anemic donor rabbits	7	88	12
	8	85	15
	9	78	22
	10	66	34
	11	67	33
	12	66	34
Control	13	55	45
1. Recipients of normal serum	14	58	42
	15	55	45
	16	56	44
II. Normal	17	51	49
	18	52	48

The bone marrow of the chronically anemic rabbits was hyperplastic as demonstrated by the large number (78-88%) of erythrogenic cells in it. Recipients of serum from these donor animals had bone marrows in which the number of erythrogenic cells was increased compared to normal.

Discussion. In neither the recipient nor the control animals was there a significant change in the red blood cell count or hemoglobin. In each of the experimental recipient groups there was an increase in reticulocytes which reached a peak on the third day after the injection of serum from the anemic animals. The reticulocyte count ranged between 4.9 and 10.5%. That this is significant is indicated by the fact that the normal reticulocyte count in rabbits varies usually

between 0.5 and 2.0% and rarely is greater than 3.0%. In contrast to the experimental recipients the control groups showed no increase above the normal range of reticulocytes. The erythrogenic to myelogenic cell ratio in the bone marrow of the group which received serum from the acutely anemic animals showed no conspicuous deviation from that of normal animals. The erythrogenic to myelogenic cell ratio of the group which received serum from the chronically anemic animals was slightly increased above the normal.

Conclusions. These experiments suggest the presence of an erythropoietic substance in the serum of animals which had been rendered anemic by bleeding.