

HEMOPOIETIC PROPERTIES OF THE SERA OF HEALTHY AND ANEMIC ANIMALS (CATS)

E. L. Kan

Laboratory of Experimental Pathology and Therapy (Head—G. S. Kan) of the Leningrad Institute of Tuberculosis (Director—Prof. A. D. Semenov), and the Group Directed by Active Member AMN SSSR M. D. Tushinskii

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The humoral mechanisms of regulation of the blood system have been the subject of many hematological investigations in recent years. The role of hemopoietins in the maintenance of the constancy of the composition of the blood and in increasing the cell content in certain anemias has been fully investigated and discussed. This trend in hematology was initiated by the researches of Carnot and Deflandre [9]. These and many other workers [12,14,15] showed that the serum of healthy animals and of animals subjected to bleeding, and also the serum of animals with experimental phenylhydrazine anemia, like the serum of patients with certain forms of anemia, when injected into a recipient animal, causes stimulation of erythropoiesis in the latter.

Although much work has been done on the study of the role of hemopoietins, much remains unclear and unsolved. The presence of hemopoietins in the serum of healthy animals, for instance, has not yet been demonstrated convincingly. The question of the relationships between the hemopoietins and nervous mechanisms has also to be considered.

For a number of years we have investigated the role of the nervous system in the regulation of the blood system [4,5]. In connection with the development of our knowledge of the hemopoietins, it became necessary to ascertain the role and the place of this factor in the changes taking place in the blood system under the influence of certain procedures which we carried out on the nervous system.

In the present communication we describe the results of a study of the hemopoietic properties of the blood of cats during the various phases of development of an experimental hypochromic anemia in these animals after denervation of the spleen.

In our previous investigations [4,5] we showed that division of the splenic nerves leads regularly to the development of a hypochromic anemia with disturbances of erythropoiesis, and this has been confirmed by other authors [1,2,3,6]. In view of the fact that the problem of the hemopoietic properties of the serum of healthy cats, on

which the main experiments were carried out, has not been discussed in the literature, a study was made of this problem also.

METHOD

Blood was taken from experimental donor cats, under anesthesia, from the femoral and splenic veins and the carotid artery. Serum was obtained from the blood by centrifugation, and was injected subcutaneously, in volume of 3-9 ml, into healthy recipient rabbits. The test serum of each experimental donor cat was usually injected into two rabbits. For 2-3 weeks before injection of the serum, the red cell count, the hemoglobin concentration, the reticulocyte count, and the composition of the bone marrow were determined in the rabbits. After injection of the serum, the blood was investigated every day for the first week, on alternate days during the second week, and thereafter once every 5 days for one month. In all, the properties of the sera of 10 healthy cats and 23 cats with experimental anemias were investigated. Test serum was injected into 73 rabbits.

In a proportion of cases a nonprotein extract of the plasma was injected, prepared by Borsuk's method [8]. Blood was obtained from anesthetized cats after intravenous injection of liquid heparin in a dose of 0.2 ml per animal, from the carotid artery, and centrifuged. The pH of the plasma was adjusted to 5.5 with N HCl. The plasma was then boiled for 10-15 minutes, after which it was filtered. The filtrate thus obtained was made up to the original volume with distilled water and injected subcutaneously into recipient rabbits in a dose of 10-15 ml daily for 3-4 days.

Experimental neurogenic anemia was produced by denervation of the spleen, the operative conditions being the same as those previously described [4].

Posthemorrhagic anemia was produced by bleeding from the femoral vein: 60-70 ml of blood was removed through a tied-in cannula. The hemopoietic properties of the serum of these animals were studied 24 hours after this procedure.

RESULTS

In the first series of experiments we studied the hemopoietic properties of the serum of healthy cats. In 12 of 15 cases, this serum caused slight and irregular fluctuations in the recipient rabbits, affecting the red cell count and the hemoglobin concentration, which increased and decreased without transgressing the limits of the "spontaneous" variations in the composition of the blood. The red cell count rose by 380,000-1,350,000/mm³ (8-29%), with an average increase of 550,000/mm³ (11%). The red cell count decreased by 320,000-1,850,000/mm³ (5-25%), with an average fall of 740,000/mm³ (14%). The average increase in the hemoglobin concentration was 7.1 units on the Sahli scale, the average decrease 5.8 units. In all the experiments, as in our previous observations, no parallel trend could be discerned between the changes in the red cells and hemoglobin.

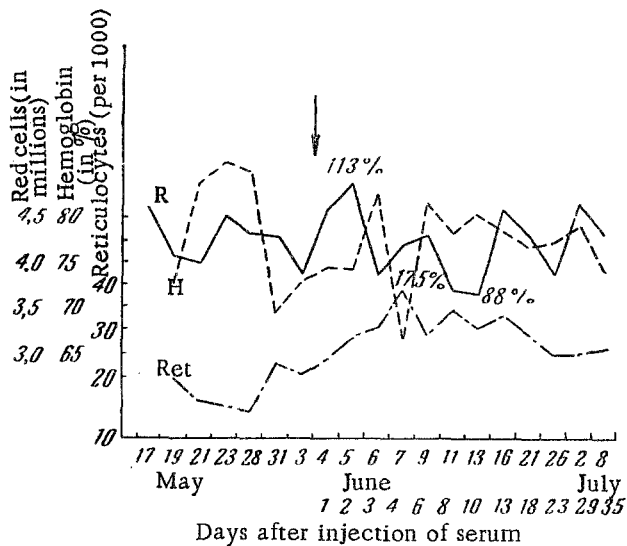


Fig. 1. Changes in the red cell count (R), hemoglobin concentration (H), and reticulocyte count (Ret) in a rabbit in response to the injection of 3 ml of serum from a healthy cat. The arrow (\downarrow) indicates the day on which the serum was injected.

In 14 of 15 experiments, the serum of healthy cats caused an increase in the reticulocyte count regularly in the recipient rabbits. The average increase was 16 per 1000 (92% in relation to the original level). An increase in the reticulocyte count was observed starting with the 3rd-4th day after injection of the serum, and the maximum reticulocytosis was found on the average on the 8th-10th day (Fig. 1).

Apart from a moderate reticulocytosis, no other marked changes of erythropoiesis were present in the bone marrow.

Injection of physiological saline into 8 control rabbits caused no changes in the composition of the blood or bone marrow in any experiment that exceeded the limits of "spontaneous" variations.

In the second series of experiments we investigated the hemopoietic properties of the blood of experimental

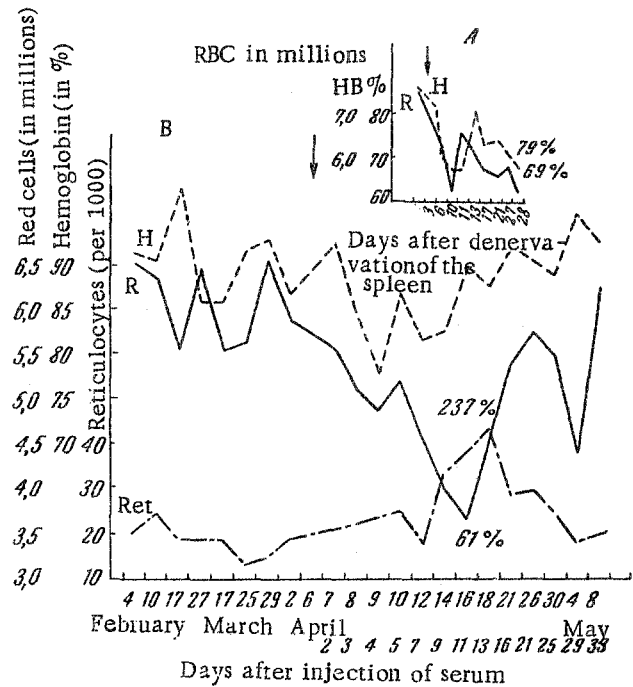


Fig. 2. A) Changes in the red cell count (R), and hemoglobin concentration (H) in a cat after denervation of the spleen just before the time of taking the blood. The arrow (\downarrow) denotes the time of denervation. B) Changes in the red cell count (R), hemoglobin (H), and reticulocyte count (Ret) in a rabbit in response to the injection of 3 ml of serum from a cat at the height of development of anemia, on the 28th day after denervation of the spleen (the red cell count fell to 2,880,000 or 69% of the initial value—A). The arrow (\downarrow) denotes the day the serum was injected.

cats from 4 to 66 days after the operation of division of the splenic nerves, when anemia had developed, and also at later periods, when the initial composition of the blood had been restored. In all 36 experiments, within a few days of the injection of "anemic" donors' serum, the recipient rabbits developed a regular and pronounced fall in the red cell count (by 270,000-3,320,000/mm³). On the average, the red cell count fell by 1,250,000/mm³, or by 22% in relation to the original level. The increase in the red cell count was irregular and did not exceed 9%, i.e. the percentage of error.

The hemoglobin concentration rose on the average by 7.1 and fell by 8.1 Sahli units. The fall in the hemoglobin concentration was 1.5 times greater than the fall in the control experiments (Fig. 2).

Injection of serum from "anemic" donor cats into rabbits, in 35 of the 36 experiments caused an increase in the reticulocyte count (by 7-39 per 1000). The reticulocyte count rose on the average by 13.2 per 1000 or by 89% by comparison with the original level. The maximum reticulocytosis in these experiments took place on the 8th day from the moment of injection of the serum. The increase observed in the reticulocyte count following injection of "anemic" serum was slightly smaller than that after injection of the serum of healthy cats.

TABLE 1. Correlation between the Degree of Anemia in the Donor (Cat) and the Degree of the Changes in the Composition of the Recipient's (Rabbit) Blood

Cats		Rabbits				
Day after denervation	Mean fall in the red cell count (in millions)	Day of maximum fall in the red cell count	Mean fall in the red cell count (in millions)	Day of maximum increase in the red cell count	Mean increase in the red cell count (in millions)	Mean increase in the reticulocyte count (per 1000)
4-10th	2.18	20th	0.63	3rd	0.51	17.1
11-20th	1.86	13th	0.98	8th	0.42	18
21-30th	2.13	10th	1.65	8th	0.43	15
31-40th	1.81	16th	1.27	2nd	0.54	16

TABLE 2. Mean Results of Experiments to Study the Hemopoietic Properties of the Blood of Healthy and Anemic Cats

Indices of the composition of the recipients blood Characteristics of donor's serum	Red cells				Hemoglobin		Reticulocytes	
	increase		decrease		increase	decrease	increase	
	in mil- lions	as % of origi- nal value	in mil- lions	as % of origi- nal value	Sahli units		per 1000	as % of original value
Serum in splenogenic anemia	0.48	9	1.25	23	7.1	8.1	13.2	89
Nonprotein extract of plasma in splenogenic anemia	0.56	8	1.06	22	7.3	8.0	23.1	140
Serum in period of compensation of splenogenic anemia	0.82	13	0.71	13	11.5	5.3	12.2	74
Serum of healthy cats	0.55	11	0.74	14	7.2	5.8	16.1	92
Serum in posthemorrhagic anemia . . .	0.76	12	0.27	5	8.3	3.2	19	103

The fall in the red cell count in the peripheral blood of the rabbits which resulted from injection of the test serum was to some degree due to disturbance of erythropoiesis. This occurred in 11 of the 12 experiments in which the changes in hemopoiesis in the bone marrow were studied. In the first period after injection of the serum there were signs of stimulation of erythroblastic maturation, associated with an increase in the reticulocyte count; subsequently, in the 2nd-3rd week, stimulation of erythropoiesis was replaced by depression, with a fall in the number of reticulocytes. The total number of erythroblasts fell by 4-20.2%.

Analysis of the results obtained also showed that a correlation was present between the depth of anemia in the donors and the severity of the changes in the composition of the blood and in hemopoiesis in the bone marrow in the recipient. Furthermore, it was found that the serum did not affect the red cell composition of the blood of the rabbits to an equal extent at all periods after denervation (Table 1). In the period of maximum anemia in the experimental cats, observed on the 21st-30th day after denervation of the spleen, the serum of these animals

evoked the most pronounced fall in the red cell count and hemoglobin concentration and the smallest rise in the reticulocyte count. Another important fact was that the changes listed above in the blood system of the rabbits took place soonest after the injection of the "anemic" serum.

In the period of restoration of the composition of the blood in the cats suffering from anemia after denervation of the spleen, their serum caused changes in the composition of the blood of the recipient rabbits similar to those observed in control experiments.

From the results obtained, the question arose as to the nature of the humoral factor present in the blood at the height of development of anemia after denervation of the spleen. Some authors [11,15] associate the hemopoietins with the protein fractions of the blood; others [8,10] deny that they are blood proteins.

In order to study this problem, and to exclude the possibly toxic influence of the foreign serum, a third series of experiments was performed in which a nonprotein extract of plasma was injected. In a volume of 23-45 ml, nonprotein plasma extract, taken at the height

of theanemia, caused the same reaction in principle in the rabbits as did the whole serum or plasma under the same experimental conditions. The degree of severity of the reaction, however, was different: In all 8 experiments, a less pronounced fall in the red cell count and a more marked reticulocytosis were observed that after the injection of whole serum (Table 2). On the average, the red cell count in these experiments fell by 1,060,000 (by 22%) and the reticulocyte count rose by 23.1 per 1000 (by 140% compared with the initial level). Just as after the injection of serum, the bone marrow showed signs of disturbance of erythropoiesis. It was thus established that a nonprotein extract of the plasma also possesses the property of producing anemia. At the same time it must be emphasized that the last series of experiments showed that the factor found in the serum in neurogenic anemia was to some extent associated with the proteins of the blood.

In a fourth series of experiments we showed that the serum of cats taken 24 hours after acute loss of blood caused a reaction in recipient rabbits which, in principle, was of quite a different type from that due to the serum of cats with neurogenic anemia: An increase in the red cell count (on the average by 760,000/mm³, or 12%) and an insignificant fall in this count (on the average by 270,000/mm³, or 5%), and also a more marked reticulocytosis, which was observed sooner—on the 5th day after injection of the serum (Fig. 3 and Table 2). The reticulocyte count rose on the average by 19 per 1000 (by 103%). The results of these last experiments thus showed that

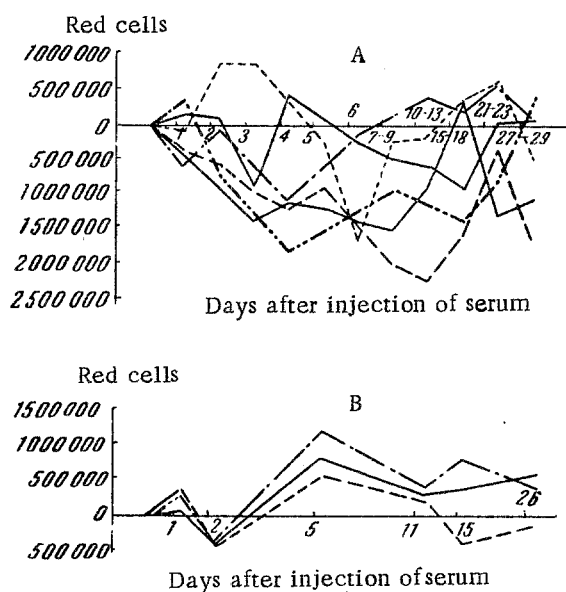


Fig. 3. A) Changes in the red cell count in 6 rabbits in response to the injection of 3 ml of serum from cats in the period of maximum development of anemia caused by denervation of the spleen. B) Changes in the red cell count in 3 rabbits in response to the injection of 3 ml of serum taken from cats 24 hours after acute loss of blood.

the serum of cats in certain anemic states could have a stimulating, hemopoietic effect on erythropoiesis.

We must now consider the cause of the fall in the red cell count of the recipient rabbits in response to the injection into these animals of the serum of cats in the phase of development of anemia due to denervation of the spleen. It is possible that the foreign cats' serum causes hemolysis in rabbits, and anemia develops as a result of this process. While we cannot altogether dismiss this mechanism, we cannot regard it as the most important, for there is indirect evidence against the occurrence of hemolysis. The increase in the reticulocyte count, for instance, was 1.5 times greater after injection of the nonprotein plasma extract than after injection of serum (see Table 2). Furthermore, the increase in the reticulocyte count itself in response to injection of "anemic" serum was smaller than that after injection of serum from healthy animals. It could be assumed that cats' serum, whatever the form of anemia, would possess the property of causing anemia when injected into other animals. The results obtained, however weigh against this hypothesis: The serum in posthemorrhagic anemia possesses hemopoietic properties and causes stimulation of erythropoiesis in recipients. We must therefore consider that after denervation of the spleen, substances are formed which possess the power to interfere with erythropoiesis. The nature of the factor thus found is not yet known. It may be a factor of the erythrodieterin type [7], or it may possibly be the factor described in the literature under the name of "Anemie-stoff" [13].

SUMMARY

It was demonstrated experimentally that the subcutaneous injection of sera (3-9 ml) obtained from healthy cats into recipient rabbits causes moderately marked fluctuations in the red cell count and hemoglobin level and reticulocyte count. At the height of anemias after splenic denervation both the serum and plasma contain an anemic factor which decreases the red cell count and hemoglobin level when injected into other animals and disturbs erythroblast maturation with subsequent inhibition of erythropoiesis. In similar experiments the nonprotein plasma extract causes analogous, although less pronounced changes. The serum in posthemorrhagic anemia possesses hemopoietic activity and causes moderate polyglobulopenia and pronounced reticulocytosis in recipient rabbits.

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