PATHOGENESIS OF HEMOLYTIC ANEMIA CAUSED BY PHENYLHYDRAZINE

(Experimental data)

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The application of modern methods of physicochemical and immunological analysis to hematology has led to considerable progress in the study of the causes of the hemolytic anemias [8]. Problems in the pathogenesis of this group of blood diseases are less clear [8,10,12].

The author has previously studied the activity of the adrenal glands during the development and compensation of the hemolytic process [5] and obtained data indicating the role of the central nervous system in the pathogenesis of experimental hemolytic anemia [3].

During the present investigation, the importance of the RES in the destruction of abnormal (damaged by phenylhydrazine) erythrocytes and the pathogenetic mechanisms accounting for the development of the macrocytosis in hemolytic anemia caused by phenylhydrazine were investigated. Too little attention has been paid to the study and discussion of these problems in the literature [8,9,13].

EXPERIMENTAL METHOD

Two series of experiments were carried out on 124 sexually mature healthy rabbits and rats.

In the experiments of series I, the importance of the RES in the development of the pathological hemolysis was studied in animals after receiving phenylhydrazine. It was assumed that, if the RES plays a dominant role in the destruction of the pathologically changed erythrocytes, the changes in the functional state of the RES would be reflected in the severity of this process.

The experiments of series I consisted of four groups of observations. In the animals of group 1 (7 rabbits), the functional state of the RES was modified by splenectomy. Phenylhydrazine was given on the second day after the operation. The control animals (5 rabbits) received simply the hemolytic poison. The dose of phenylhydrazine in the experimental and control series was 50 mg/kg.

Subsequent experiments were carried out on rats. In the animals of group 2 of this series, the activity of the RES was modified by application of an electric current. This stimulus depresses the activity of the reticuloendothelium [7]. For a period of one week before administration of the hemolytic poison and during the same period thereafter, an alternating electric current of voltage 40 V was applied daily to the rats for 10-15 min. Observations were made on 10 animals. Control rats received only phenylhydrazine. The dose of the hemolytic poison was 20 mg/kg.

The functional state of the RES of the animals of the third group of this series was modified by the parenteral administration of bacterial polysaccharides in a dose of 100 mg/kg (10 rats). This treatment is known to stimulate the reticuloendothelium [1]. The five control animals received only the hemolytic poisons, the dose of which in both the experimental and control animals was 40 mg/kg.

The functional state of the RES of the animals of the 4th group (20 rats) was modified by whole-body x-ray irradiation in a dose of 200 R. According to data in the literature [13], this dose of radiation does not affect the composition of the red blood but modifies the activity of the reticuloendothelium. Irradiation was given with the

Composition of Red Blood and Certain Physicochemical Properties of the Erythrocytes and Serum of Healthy and Anemic Rabbits

Index	Experimental conditions	Number of observations	M ± m	P
Erythrocytes (in millions)	Normal	10	5.65 ± 0.2	< 0.05
	Anemia	10	2.26 ± 0.15	
Hemoglobin (in %)	Normal	10	73.7 ± 2.2	< 0.05
	Anemia	10	33.8 ± 2.3	
Color index	Normal	10	1.02 ± 0.02	< 0.05
	Anemia	10	1.4 ± 0.1	
Mean diameter of erythrocytes (in μ)	Normal	600	6.05 ± 0.03	< 0.05
	Anemia	1000	9.04 ± 0.05	
Minimal osmotic resistance of erythrocytes	Normal	10	0.44 ± 0.008	
	Anemia	10	0.42 ± 0.006	0.05
Maximal osmotic resistance of erythrocytes	Normal	10	0.33 ± 0.005	
	Anemia	10	0.30 ± 0.005	< 0.05
Osmotic depression of serum (in deg)	Normal	10	0.57 ± 0.004	
	Anemia	10	0.59 ± 0.02	> 0.05

RUM-3 apparatus working in the following conditions: currents 10 mA, voltage 180 kV; filters of copper (thickness 0.5 mm) and aluminum (1 mm); total distance 30 cm; duration of irradiation 5 min. Phenylhydrazine (40 mg/kg) began to be given 4 days after irradiation, when the effect of the radiation was most marked. The changes in the blood composition of the irradiated animals were compared with those in 10 intact rats receiving only the hemolytic poison.

The experiments of series II were carried out to examine the mechanism of development of the macrocytosis in hemolytic anemia caused by phenylhydrazine. The pathogenetic mechanisms responsible for the increase in the diameter of the erythrocytes in the hemolytic anemias and other diseases have received little study. The appearance of this phenomenon has been attributed to a disturbance of the metabolism of the antianemia factor (vitamin B_{12}) or to changes in the osmotic equilibrium between the erythrocytes and the surrounding medium [8,12]. There is indirect evidence that many of these mechanisms may be concerned in the increase in the diameter of the erythrocytes in animals with hemolytic phenylhydrazine anemia [6]. To verify these hypotheses experimentally, two groups of observations were made in the experiments of series II.

In the experiments of group 1, the effect of vitamin B_{12} on the severity of the microcytosis was studied in rats with hemolytic phenylhydrazine anemia (15 animals). Vitamin B_{12} in a dose of 15 μg was given by subcutaneous injection starting immediately after the injection of phenylhydrazine (40 mg/kg) and continuing daily until the end of regeneration of the blood. The control animals (12 rats) received only the hemolytic poison. In the experiments of group 2 in this series, the importance of osmotic forces in the increase in the diameter of the erythrocytes was investigated in animals with hemolytic anemia caused by phenylhydrazine. Starting 4-5 days after injection of the hemolytic poison (50 mg/kg), when the signs of macrocytosis were most marked, the changes in the diameter of the erythrocytes and the osmotic concentration in the cells and the blood serum were studied in 10 rabbits.

The changes in the osmotic concentration in the erythrocytes were judged indirectly by determining their osmotic resistance to hypotonic sodium chloride solution. The osmotic concentration in the plasma was determined from the degree of osmotic depression of the serum as measured by means of Beckmann's cryometer [11]. The hematological indices were studied by the usual methods. The diameter of the erythrocytes was measured by the method suggested earlier by the author [4].

EXPERIMENTAL RESULTS

The control observations showed that the factors used to influence the RES of the experimental animals themselves produced no statistically significant changes in the red blood picture of the animals throughout the experiment.

The study of the development of hemolytic process in the animals with modified reactivity of their RES in every case gave consistent results. After the injection of phenylhydrazine, the process of hemolysis followed the same lines in the splenectomized and healthy rabbits. No differences could be detected between the rats subjected to stimulation by the electric current and the control animals in relation to the development of hemolytic anemia. In the rats receiving the bacterial polysaccharides, as in the control animals, after the injection of phenylhydrazine the difference between the erythrocyte count and the hemoglobin concentration throughout the period of observation was not statistically significant ($P > 0.05$). Four days after injection of identical doses of phenylhydrazine, the hemolytic anemia was equally severe in the healthy and irradiated animal.

Hence, the results of these observations showed that a change in the functional state of the RES brought about in the experimental animals by various procedures had no significant effect on the development of the process of hemolysis caused by injection of phenylhydrazine. This conclusion is confirmed by the work of Rothberg and co-workers [14], who showed that erythrocytes, when damaged by phenylhydrazine, are destroyed at the same rate in splenectomized and healthy rabbits. During his study of the course of hemolytic anemia in guinea pigs irradiated in lethal doses, E. D. Gol'dberg [2] found no differences between the development of hemolysis in these animals and in unirradiated guinea pigs.

Evidently, the increase in the pathological hemolysis in the animals with hemolytic anemia caused by phenylhydrazine was due primarily to the direct action of this chemical compound on the erythrocytes, as a result of which their resistance was lowered. The role of the RES in this phenomenon probably consisted of the ingestion and subsequent conversion of the disintegration products of the red blood cells. Hyperplasia of the RES developed secondarily.

The results of the study of the mechanisms of the increase in erythrocyte diameter in the animals with hemolytic phenylhydrazine anemia showed that vitamin B_{12} had no effect on the severity of this phenomenon. For instance, in the rats receiving vitamin B_{12}, the number of macrocytes in the blood at the height of the anemia was 16.5%, while in the control animals receiving the hemolytic poison alone, it was 17.6%. These observations show that the increase in the diameter of the erythrocytes in hemolytic anemia caused by phenylhydrazine is not connected with a deficiency of the anti-anemic factor, vitamin B_{12}.

The results of the second group of experiments of this series are given in the table. They show that the height of the macrocytosis in the rabbits with hemolytic anemia (mean diameter of the erythrocytes 9.3 μ compared with 6 μ), the minimal and maximal osmotic resistance of the erythrocytes,was increased. These changes reflect changes in the osmotic concentration in the erythrocytes.

The results of the study of the osmotic concentration of the blood plasma of these animals showed that this may either rise or fall slightly. The mean value of the osmotic depression of the serum in the anemic animals was found to be similar to that in the healthy rabbits. Most of the macrocytes, when stained, showed polychromatophilia. The increase in the diameter of the young erythrocytes in the animals with experimental hemolytic anemia was evidently due to a disturbance of the osmotic equilibrium between the erythrocytes and the surrounding medium, and undoubtedly the change in the osmotic concentration in the red blood cells played a leading role in the development of this phenomenon.

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All abbreviations of periodicals in the above bibliography are letter-by-letter transliterations of the abbreviations as given in the original Russian journal. *Some or all of this periodical literature may well be available in English translation.* A complete list of the cover-to-cover English translations appears at the back of this issue.