ALLOXAN ANEMIA

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It is known that the injection of animals with alloxan evokes a characteristic picture of diabetes: hyperglycemia, increased appetite, thirst, decline in weight, etc. [3, 5, 8, 9, 10, 13, 16, 17, 18]. I. M. Azbukina [1] showed that in alloxan diabetes hypochromic anemia develops, accompanied by progressive reticulocytosis, an increase in the average diameter of the erythrocytes, and neutrophilosis with the appearance of immature forms. The author connects the anemia with the development of diabetes, and not with the toxic action of alloxan indicated by F. A. Zilberman[4], L. I. Fandeev [16], N. A. Naumova [11]. Rose and Gyorgy [20] and also Maske and Wolff have reported on the hemolytic action of alloxan.

In one series of our experiments devoted to study of the regulating role of the pancreas in blood-formation we studied the problem of shifts in the blood system with alloxan diabetes.

EXPERIMENTAL METHODS

The experiments were performed on 20 adult rabbits, in the border ear veins of which a 10% solution of alloxan (monohydrate) was injected in a dose of 180 mg per 1 kg of weight. The injections were made after examination of the constitution of the blood; the hemoglobin content, the number of erythrocytes, and leucocytes, the leucocytal formula, the number of reticulocytes, and the amount of sugar were determined. These indicators were investigated 1-2 days and 7-8 days after the injection of alloxan. The animals were weighed regularly.

On the second day after the alloxan injection the amount of sugar in the rabbit's blood increased sharply – to 200-260 mg % and it remained at high levels (Fig. 1), sometimes reaching 300-400-500 mg % and more. In some cases the sugar-content curve oscillated: a rise to high figures (200-300 mg %), a drop to the original level, and a considerable rise again. The weight also began to drop on the second day, and in many cases it was 50-60% of the original figures at the time of the rabbits' death. In animals with a sharp drop in weight and a high content of sugar in the blood, increased appetite and thirst were observed.

In study of the blood picture it was noted with in the 2nd-3rd day anemia began to develop, which remained to the 18th-48th day of the experiment with a 7-40% reduction of hemoglobin, of erythrocytes by 450,000 to 2,300,000. In animals surviving to 2-4 months the blood indicators were restored by the 37th-47th day, although the picture of diabetes remained for a prolonged period, and in rabbits Nos. 10 and 11 anemia developed in the absence of symptoms of diabetes (Figs. 1 and 2).

In the leucocyte count the regular shifts were not detected. In all cases in calculation of the leucocytal formula a shift was observed in the direction of increase of pseudoeosinophils with a decrease in the percentage of lymphocytes.

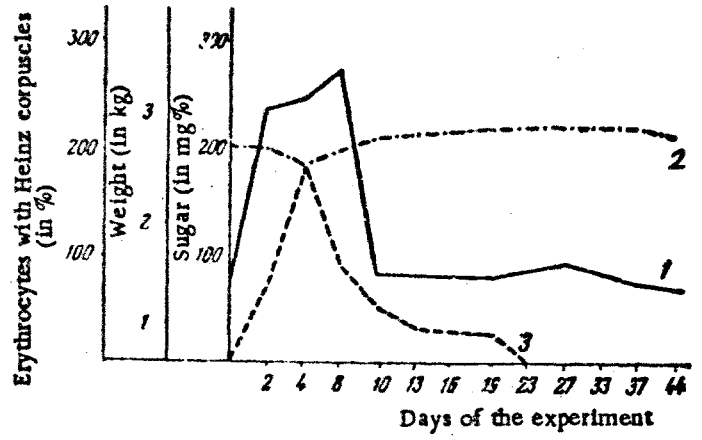

Fig. 1. Change of the sugar content in the blood (1), of weight (2), and of erythrocytes with Heinz bodies (3) in alloxan diabetes in rabbit No. 3.

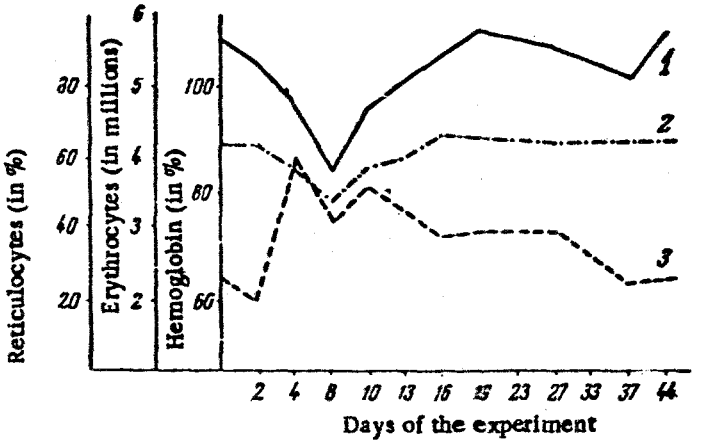

Fig. 2. Change of the number of erythrocytes (1), of hemoglobin (2), and of reticulocytes (3) in rabbit No. 3 in alloxan diabetes.

In all the animals reticulocytosis developed beginning on the 2nd day, reaching a maximum on 5th-7th day, when the number of reticulocytes was 1.5-5 times the original number (Fig. 2).

On supravitally stained smears (Nile blue) beginning on the 2nd day we detected erythrocytes with Heinz corpuscles – from 200 to 970‰. In the determination of an acute reaction to the injection of alloxan it was established that 20-30 minutes after the injection erythrocytes with Heinz corpuscles began to appear in the form of weakly noticeable blue granules, sometimes singly, sometimes 2-3 to one erythrocyte; such erythrocytes numbered 100-200‰. Then their number increased, and after 2-3 hours it had reached 800-900‰. As the number of erythrocytes with these corpuscles increased the morphology of these inclusions also changed. They were gradually transformed from barely visible blue granules into irregular crumblike or flaky larger formations, 1-3 per erythrocyte, arranged in a close accumulation or scattered around the periphery in the form of a chain, or, finally, at the poles of the cell. In single erythrocytes the bodies were at the center, in the others they were drawn in the direction of the center. The dimensions of the granules varied from 0.8 to 1.9 μ (Fig. 3).

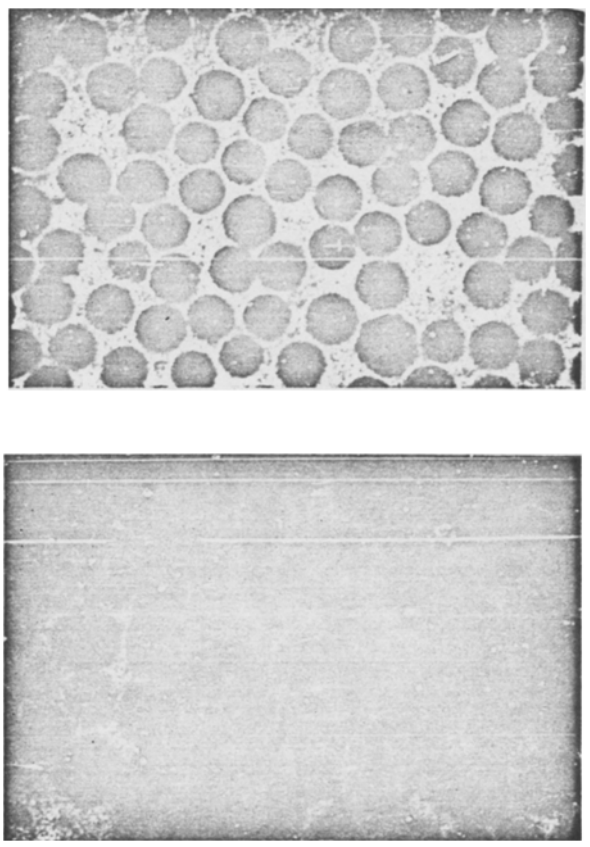

Fig. 3. Erythrocytes with Heinz corpuscles and reticulocytes in rabbit No. 17, on the 2nd day (above) and 9th day (below) after the injection of alloxan. Objective Reichert's Hom. Imm. 1/12, eyepiece 6 compens.

The nature of the inclusions in hemolytic anemia has been a matter of discussion. Some authors have considered these formations as changed normal structures (Shilling), others have considered them of a nuclear nature (E. O. Freyfeld, E. A. Tatarinov, and others), while still others have seen them as necrotically altered hemoglobin (Pappengeym and Suzuki).

In 1938 in our laboratory D. I. Goldberg described a granular degeneration of hemoglobin with supravital staining of smears in animals poisoned with phenylhydrazine. In studying the action of turpentine, V. A. Sorokina [15] also detected internal inclusions in erythrocytes, which she called "turpentine bodies". Analogous data were obtained by A. S. Saratikov and E. I. Kleitman [11, 12] in the parenteral administration of phytoncides, and by E. I. Kleitman [6, 7] in poisoning with picric acid and methylene blue.

In our laboratory it was shown that Heinz corpuscles and multiple granularity are artificial structures which appear as a result of a change in the colloidal hemoglobin phase. Similar formations are not encountered in diseased erythrocytes circulating in the blood system; they arise only in preparation of a smear as a result of a change in the dispersedness of the hemoglobin phase. This physicochemically changed hemoglobin assumes an affinity to basic stains and easily coagulates in the form of granules [2, 14].

We did not find any indications in the literature that alloxan causes the formation of pathological internal structures in erythrocytes, so that our observation is the first. The appearance in alloxan diabetes of hemoglobinemic degenerative structures indicates the community of the action of alloxan and a number of hemolytic poisons known to us. Hence, we are entitled to draw the conclusion that alloxan anemia cannot be fully linked

with affection of the insular apparatus of the pancreas, but depends also on the hemolytic action of the alloxan itself. In this connection it is not uninteresting to note that in a series of our experiments with surgically induced diabetes anemia developed in dogs only at later periods, at a time when deep disturbances of the metabolic processes developed.

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* In Russian.