The marked individual scatter of the initial 17-hydroxyprogesterone concentration in immature male baboons is probably due to the fact that two of the experimental males (Nos. 14465 and 14514) were closer to the period of puberty, and that a hormonal response to stress began to be exhibited in them just as in adult animals as a result of simply being used in the experiment, although in their testosterone concentration they were indistinguishable from the other monkeys of prepubertal age. Considering that 24 h after immobilization no increase in the 17-hydroxyprogesterone level was found in the immature monkeys exposed to stress against the background of a raised 17-hydroxypregnenolone level, it can be postulated that the limiting stage in corticosteroid synthesis during stress is the conversion of 17-hydroxypregnenolone into 17-hydroxyprogesterone. Considering also that in the monkeys under conditions of stress an increase in the blood pregnenolone concentration but not of the progesterone concentration was observed, this suggests that hydrocortisone synthesis in these animals takes place predominantly along the pathway pregnenolone → 17-hydroxypregnenolone → 17-hydroxyprogesterone, but not along the pathway pregnenolone → progesterone → 17-hydroxyprogesterone.

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DETECTION OF ATYPICALLY LIGHT AND IMMUNOLOGICALLY DEFECTIVE ERYTHROCYTES IN PATIENTS WITH PAROXYSMAL NOCTURNAL HEMOGLOBINURIA

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Centrifugation of defibrinated blood on a density gradient at 600g for 30 min revealed the presence of atypically light erythrocytes, located on the boundary between plasma and Ficoll solution with a specific gravity of 1.077 g/cm^3 in 24 of 26 patients with paroxysmal nocturnal hemoglobinuria (PNH). Atypically light erythrocytes are characterized by a reduced content of rhesus-antigen and by a positive direct Coombs' test with anticomplementary serum. The appearance of atypically light erythrocytes in the upper layer was observed in patients with autoimmune hemolytic anemia and with hypoplastic anemia following splenectomy, and starting from the first day after the operation. It is suggested that the presence of atypically light erythrocytes is connected with a deficiency of the immune serum in patients with PNH and, primarily, with deficient ability of the spleen to eliminate defective erythrocytes.

KEY WORDS: paroxysmal nocturnal hemoglobinuria; density gradient.

The pathogenesis of paroxysmal nocturnal hemoglobinuria (PNH) has not yet been explained [3, 5]. The principal current hypothesis is based on the assumption that in this disease two erythrocyte populations – healthy and pathological – exist and are responsible for increased hemolysis in the blood stream [8-10].

The object of this investigation was to fractionate erythrocytes of patients with PNH by centrifugation on a density gradient and to study their immunologic properties.

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TABLE 1. Number of Reticulocytes in Whole Blood from Patients with PNH and in Top Layer

Patient	Reticulocytes, percent		Volume of top layer, percent of volume of blood
	whole blood	top layer	
B.	6,0	44,4	1,0
A.	15,0	80,0	0,5
I.	2,6	85,0	0,25
E.	1,5	5,8	2,0
D.	26,3	50,0	3,0
B-va	10,8	62,7	1,0
M.	8,0	90,0	0,5

TABLE 2. Abundance of Rhesus-Factor and Presence of Complement on Erythrocytes of Patients with PNH

Patients	Blood group and rhesus factor	Presence of rhesus factor in		Result of direct Coombs' test on	
		top layer	bottom layer	top layer	bottom layer
V.	0 (I) Rh+	+	+	+	+
Z.	0 (I) Rh+	−	+	+	+
A-ev	0 (I) rh−	−	−	+	−
S.	0 (I) Rh+	−	+		
K.	0 (I(Rh+	−	+	+	−
E-ov	0 (I) Rh+	+	+	+	−
Sh.	A (II) Rh+	+	+	+	−
N.	0 (I) Rh+	−	+	−	−
K-ov	B (III) Rh+	+	+	+	−
Sh-na	B (III) Rh+	−	+	+	−
D.	0 (I) Rh+	−	+	+	−
A-ich	A (II) Rh+	−	+	+	+
M-ov	AB (IV) Rh+	−	+	+	−
B.	0 (I) Rh+	+	+	+	+

EXPERIMENTAL METHOD

Blood was taken from a vein of the patients and healthy donors in a volume of 10-15 ml into an Erlenmeyer flask with beads and defibrinated by rotary movements. The blood was then poured as a layer above 5 ml of a solution forming a density gradient of 1.077 g/cm^3, and consisting of a mixture of ten parts of 33.9% Verografin solution and 24 parts of 9% Ficoll solution [1, 6]. The tubes were centrifuged at 600g for 30 min. The result was assessed depending on the location of the erythrocytes – at the bottom of the tube or on the boundary with the density gradient also. Erythrocytes from the top layer did not pass through the solution of the density gradient but were located at the boundary between the plasma and the Ficoll solution, and formed a clearly demarcated ring. Erythrocytes of the bottom and top layers were drawn off into separate tubes, washed three times with isotonic NaCl solution, and subjected to immunologic investigation.

The blood group was determined with the aid of standard isohemagglutinating sera. The rhesus factor was determined on a tile by the express method using sera with albumin [4]. The direct Coombs' test was carried out by the usual method with antiglobulin serum [4]. This test also was performed with the patient's erythrocytes in relation to anticomplementary serum after incubation of the cells for 1 h at 37° C with fresh human group AB (IV) serum. The serum for the Coombs' test was obtained by immunizing rabbits with allogeneic reticulocytes, sensitized with fresh healthy human serum [2].

EXPERIMENTAL RESULTS

During centrifugation on a density gradient under the conditions specified above, erythrocytes of the defibrinated blood of 60 healthy subjects behaved similarly. They settled completely on the bottom of the tube.

It was a different matter with blood from patients with PNH. In 24 of the 26 patients tested, separation of the erythrocytes into two layers was observed: A normal bottom layer and an atypically light, top layer. The top layer accounted for between 0.01 and 10.8% of the volume of blood. Morphologically it consisted of erythrocytes, microspherocytes, and reticulocytes ranging in number from 5.8 to 90% (Table 1).

It is important to note that blood from healthy donors contained an increased number of reticulocytes after blood donation (about 1.4%). However, during its centrifugation on a gradient with specific gravity of 1.077 g/cm^3, no top layer was formed. This indicates a significant decrease in the density of the reticulocytes in patients with PNH.

The immunologic study showed a decrease in agglutinability of the top layer with antirhesus serum in eight of the 13 RH-positive patients tested. In 6 cases no rhesus factor whatever could be detected. Meanwhile, it was clearly present in the erythrocytes of the bottom layer. In two of six cases the agglutinability of group factor B in the top layer was reduced. The top layer of blood from 14 of the 15 patients tested also reacted with anti-complementary serum in the direct Coombs' test (Table 2). In four cases the positive result was also obtained with the erythrocytes of the bottom layer.

The appearance of a top layer also has been observed in other hematologic diseases. In particular, the formation of atypically light erythrocytes was established in six of 16 patients with autoimmune hemolytic anemia, five of 12 patients with hypoplastic anemia, and six of 10 patients with dyshematopoiesis.

It is important to note the appearance of atypically light erythrocytes in eight of 13 patients with hemolytic and hypoplastic anemia after splenectomy in the course of treatment. Pathologically light erythrocytes were not formed in any of these patients before splenectomy. An atypically light top layer began to appear on the first day after the operation, and it persisted for 2-3 weeks.

These investigations suggest that the presence of atypically light erythrocytes in patients with PNH is a characteristic feature of this disease. The formation of the atypical top layer in patients with PNH is evidently the result of liberation of defective erythrocytes into the blood stream. Imperfection of erythrocytes is largely associated with an inadequate hemoglobin content [7] and also with defects of the membrane. The latter are manifested as increased ability of the erythrocytes to adsorb complement and a deficiency of rhesus antigen (D), firmly bound with the erythrocyte stroma, on them. The presence of atypically light erythrocytes in patients with PNH is evidently connected with unsoundness of the immunologic system of the patient and, in particular, of the spleen, which is unable to eliminate pathologically changed erythrocytes. Such defective erythrocytes accumulate in the patient's body and evidently give rise to intravascular hemolysis.

This hypothesis on the role of the spleen in the pathogenesis of PNH explains the appearance of atypically light erythrocytes in patients with other hematologic diseases (autoimmune hemolytic anemia, hypoplastic anemia) as early as on the first day after splenectomy.

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