Confirmation of a Structural Abnormality in the Stroma of Erythrocytes from Paroxysmal Nocturnal Hemoglobinuria (PNH) After Hemolysis in Distilled Water

By H. Braunsteiner, E. Gisinger and F. Pakesch

The results of many investigations on the pathogenesis of paroxysmal nocturnal hemoglobinuria (PNH) point in the direction of a stromal abnormality of the erythrocytes. In 1951 Matthes et al. described the patchy appearance of electron microscopic preparations of hemolyzed erythrocytes from one case of PNH. Recently, however, Douglas and Eaton were unable to find similar changes. The present report fully confirms the result of Matthes et al.

In view of the theoretic and practical importance of this finding more available cases should be tested.

Case history: A 30 year old female was admitted to the medical department of the University Hospital on April 25, 1955. She had no special complaints except for a slight general weakness. Since 1950 she had been treated for anemia with iron, liver extracts and vitamin B12. She had never had blood transfusions. For short periods she had been very slightly jaundiced. The family history was negative.

At admission the patient was pale but not markedly jaundiced. The physical examination was completely negative except for a questionable enlargement of the spleen and a cystic tumor of the left ovary. Laboratory findings were as follows: erythrocytes—2,600,000; hemoglobin—7.8 Gm; reticulocytes—7%; hematocrit—25; mean diameter of erythrocytes—7.4 μ; resistance of erythrocytes—0.44 to 0.32% saline; leukocytes—7900 with normal differential count; thrombocytes—104,000; sedimentation rate—17 mm in 1 hour; serum iron—17 γ%; total iron-binding capacity—267 γ%; blood group—A₁, Rh neg.; Coombs test, blocking test and Donath-Landsteiner test were negative; cold agglutinins—1:16; serum bilirubin—0.9 mg%. The sternal marrow showed 55% of erythropoietic cells.

Total protein of the serum—7.1 Gm; albumin—4.9 Gm; NPN—23.3 mg%; fasting blood sugar—109 mg%; cholesterol—134 mg%; thymol turbidity—0.8 units; serodiagnosis for lues—negative; concentration and dilution of the urine were normal; a creatinine clearance was also normal. Urinalysis—protein, sugar and bilirubin were negative, urobilinogen slightly augmented. At microscopic examination an amorphous sediment was found.

A provisional diagnosis of hemolytic anemia of unknown origin was made. At this point PNH was suspected, although no hemoglobin was found in the urine. An acid serum test, however, was negative. Retrospectively this was proved to be due to a technical error. The serum had been acidified to a pH of approximately 6.0. Since several cases of acquired hemolytic anemia combined with a dermoid cyst have been described, the tumor of the left ovary was removed. It proved to be a simple cyst; the clinical state of the patient remained unchanged.

Cortisone (300 mg daily) was then administered to the patient for 7 days without result. Immediately afterwards she received 2 injections of 50 mg iron saccharate to follow the variations of her serum iron and iron-binding capacity.

After these injections a very dark, reddish urine was found in the morning, and spectroscopic determination revealed hemoglobin. Immediately the tests for PNH were rechecked and gave strongly positive results. The acid-serum test was highly positive at pH 6.8; hemolysis was still augmented by addition of 30 to 50 units thrombin. At 37°C. spontaneous hemolysis was strong after 4 hours, this was inhibited by addition of sodium citrate. Spon-
general, spontaneous hemolysis was stronger in serum of blood groups O and B; rhesus groups showed no effect.

Morning urine remained positive for hemoglobin. At the same time the erythrocyte count dropped to 2,300,000 and the reticulocytes rose to 20%. The patient became slightly jaundiced with a serum bilirubin of 2.0 mg%. She now was started on Dicoumarol and the prothrombin level kept at approximately 30%. Under this treatment she improved very slowly. After 2 months the erythrocyte count reached 2,900,000 with 6% reticulocytes, serum bilirubin was back at 0.8 mg%, the urine was hemoglobin free but still contained hemosiderin. At present the patient is working as a secretary and feels rather well. All tests in vitro for PNH have remained positive.

Electron microscopy: Electron microscopic investigations were performed at the time of hemoglobinuria and in relative remission.

Methods: Blood was drawn from the cubital vein and one part hemolyzed in 30 parts of distilled water for 3 minutes. In some experiments HCl or NaOH was added and the pH varied from 6.0 to 8.5. A droplet of the fluid containing hemolyzed erythrocytes was then transferred directly on formvar membranes for electron microscopic observation and allowed to dry. In some instances fixation was first carried out by adding one part of a 1% solution of osmium tetroxide to three parts of fluid. A Philips microscope EM 100 A was used.

Results

In figure 1 and 2 stromas of a hemolyzed normal erythrocyte and of an erythrocyte from the patient with PNH are depicted. The difference of structure is obvious. The normal stroma is smooth or slightly granular, whereas the stroma from PNH is patchy, as if a coarse precipitation of the matrix had taken place. This abnormality of structure was found in approximately 60 to 70 per cent of stromas from PNH. Variation of pH or fixation with osmium tetroxide had no fundamental influence. However, when the erythrocytes from PNH were previously centrifugated and washed in saline and then hemolyzed, differences in the structure of stromas were less marked.
Discussion

These investigations fully confirm the work of Matthes et al. Erythrocytes from PNH show a definitive structural abnormality after water hemolysis. No similar abnormality was found in normal erythrocytes nor in erythrocytes from iron-deficiency anemia, pernicious anemia and acquired hemolytic anemia. Electron microscopy of hemolyzed erythrocytes from PNH seems therefore to present diagnostic value. We have no explanation for the negative finding of Douglas and Eaton, but since we have seen in our case that differential centrifugation and washing of PNH erythrocytes diminished the structural abnormality, it is conceivable that differences in technic might influence the result. Perhaps a small amount of plasma should be present during hemolysis. This question needs further consideration. It will be important to have the test performed on a larger group of patients.

The pathophysiologic significance of this defect is not clear. In our opinion it is most likely that the visible abnormality in the stroma is only a secondary phenomenon under nonphysiologic conditions. The fundamental defect might rather lie in the lack of an enzyme or the presence of an abnormal compound of the stroma, which then leads to pathologic precipitation of the matrix in distilled water.

Summary

The presence of a structural abnormality in the stroma of erythrocytes from PNH after hemolysis in distilled water is confirmed.

Summary in Interlingua

Esseva confirmate le presentia de un anormalitate structural in le stroma de erythrocytos in nocturne hemoglobinuria paroxysmal post hemolyse in aqua distillate.
REFERENCES


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