
CASE REPORTS

Nonspherocytic Chronic Hemolytic Anemia with Basophilic Stippling: Report of a Case in a Negro

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THIS REPORT is concerned with a case of chronic hemolytic anemia in a Negro which did not fulfill the criteria for the diagnosis of any of the common forms of chronic hemolytic disease. In 1947, Haden¹ described two white families, one of American and one of Hungarian origin, both of which showed "a new type of hereditary hemolytic jaundice without spherocytosis." In the latter family the most striking feature was the finding of a high percentage of stippled erythrocytes in the blood of the patient, a female of Hungarian extraction, and of four members of the family, representing three generations—the mother, a daughter, and two sisters.

The hematologic findings in our case were strikingly similar to those described by Haden; and, on personal communication, Dr. Haden was also of the opinion that our case had the same type of hemolytic anemia. Because of the rarity of this type of anemia and because of the interesting hematologic studies, the findings will be reported in detail.

Case History

Present illness: R. W., a 27 year old male Negro bank clerk, was admitted to the Beth Moses Division of the Maimonides Hospital on June 28, 1949 for investigation of splenomegaly noted on routine physical examination by his private physician two months previously. The patient was asymptomatic but, on questioning, recalled that his eyes had been yellow and his urine the color of "dark tea" since early childhood. System review was otherwise negative except that he had been rejected by the draft for military service in 1942 because of a heart murmur. He specifically denied any history of joint or abdominal pains, leg ulcerations, fatty food intolerance, or easy fatigability. The patient had been employed in a galvanizing plant from 1939 to 1941 where there was slight exposure to red lead paint. Furthermore, in 1946 and 1947, he had been exposed to benzene fumes in his occupation of clerk in a dry cleaning establishment. Both parents and all four grandparents had been born in the Barbados Islands of the West Indies. Neither they, the patient's eight siblings, nor his two children, had ever had similar symptoms.

Examination disclosed a well developed, healthy looking young Negro male. No gingival lead line was seen. There was moderate pallor of the mucous membranes and the sclerae were icteric. The heart was enlarged to percussion slightly to the left of the mid-clavicular line in the fifth intercostal space. A grade III blowing systolic murmur was heard over the entire precordium. The liver edge was palpated 4 cm. below the right costal margin and the smooth, firm, non-tender edge of the spleen was palpated 10 cm. below the left costal margin. Neurologic examination was negative.

Laboratory data: examination of the blood showed a hemoglobin of 11.1 Gm. per cent, RBC 3.4 M/cu. mm., WBC 7200/cu. mm. and hematocrit 35 per cent. Color index was 1.1. Wintrobe indices showed MCV 104 cu. microns, MCH 32 micromicrograms, and MCHC 32 per

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cent. The reticulocyte count was 17.6 per cent (figure 1). The WBC differential was normal with 70 per cent neutrophils, 24 per cent lymphocytes, 5 per cent monocytes, and 1 per cent eosinophils. One normoblast was seen in counting 100 WBC. Platelets appeared normal on the smear. The most striking feature of the peripheral blood smear was a marked punctate basophilia of the erythrocytes, 10.8 per cent of them being stippled cells (figure 2). In addition, there was considerable polychromatophilia, anisocytosis and poikilocytosis. The red cells appeared slightly macrocytic and normochromic. No spherocytes were present. Sickling preparations were negative, as were two Coombs tests. The icterus index was 24 units. In the osmotic fragility test, hemolysis began at 0.42 per cent NaCl and was complete at 0.30 per cent NaCl. A control gave the same results. After twenty-four hours incubation at 37 C.

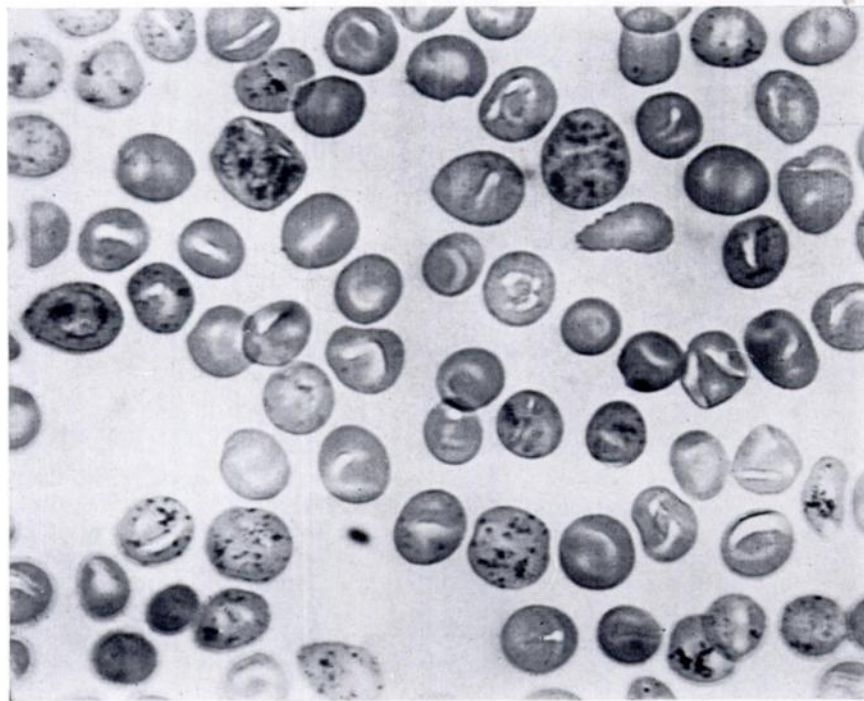


FIG. 1.—Peripheral blood. $\times 1400$. Reticulocytosis. Brilliant cresyl blue stain. A double Cabot ring is present in an erythrocyte at the middle right.

hemolysis began at 0.66 per cent NaCl and was complete at 0.42 per cent NaCl. Again this was the same as the control. The mechanical fragility test was normal. The osmotic fragility method used was that described by Daland and Worthley.² The mechanical test was a modification of the method of Dameshek and Miller.³

The sternal marrow was very cellular with a marked normoblastic response. Differential count disclosed myeloblasts 1 per cent, neutrophilic myelocytes 4 per cent, neutrophilic metamyelocytes 4 per cent, neutrophils 5 per cent, lymphocytes 7 per cent and normoblasts 79 per cent. (Of the normoblasts, 1 per cent were pronormoblasts, 18 per cent were type "A," 16 per cent type "B" and 65 per cent type "C").

A splenic aspiration was performed and failed to reveal any spherocytes. It was noted, however, that the number of stippled cells was markedly reduced as compared with the blood, and that many of the basophilic granules appeared to have been engulfed by the

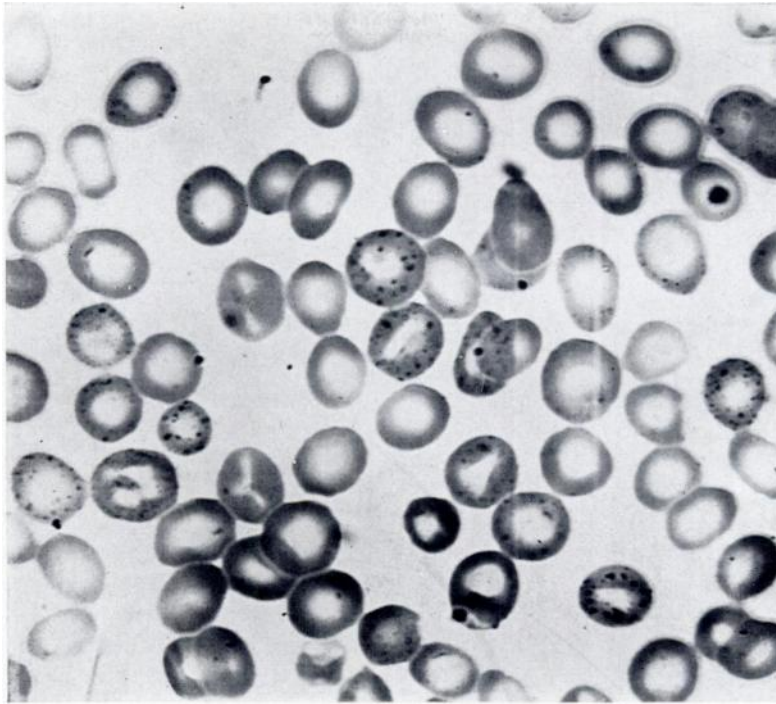


FIG. 2.—Peripheral blood. $\times 1400$. Punctate basophilia. Wright's stain.

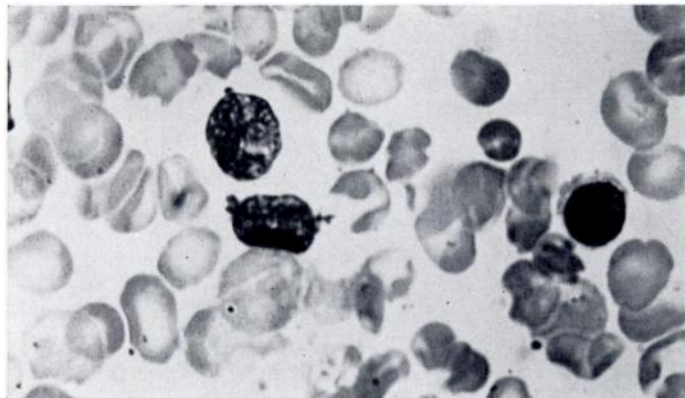


FIG. 3.—Spleen aspirate. $\times 1400$. Wright's stain. Note the two reticulo-endothelial cells containing basophilic aggregates. Note the absence of stippling in the erythrocytes. A normal lymphocyte is shown at the right.

reticulo-endothelial cells of the spleen (figure 3). This point will be considered later at greater length. Differential count of the splenic aspirate revealed neutrophils 14 per cent, lymphocytes 74 per cent, monocytes 1 per cent, eosinophils 1 per cent, normoblasts 3 per cent, and reticulo-endothelial cells 7 per cent.

The urine contained no bilirubin. The two hour urine urobilinogen excretion was 8.7

Ehrlich units (normal up to 0.8 E.U.). The twenty-four hour urine excretion of lead was 91 micrograms (normal up to 100 μg). The twenty-four hour fecal urobilinogen excretion was 600 mg./100 Gm. (normal up to 200 mg./100 Gm.); method that of C. J. Watson.

X-rays of the gall bladder and of the long bones were negative. A teleroentgenogram of the heart was interpreted as showing slight left ventricular enlargement. X-ray of the skull (figure 4) revealed localized zones of fine longitudinal striations in the parietal and occipital regions, giving the typical "hair on end" appearance of certain cases of hereditary hemolytic anemias.

Family studies: examination of four of the eight siblings and of both of the patient's children failed to reveal any hematologic abnormalities.

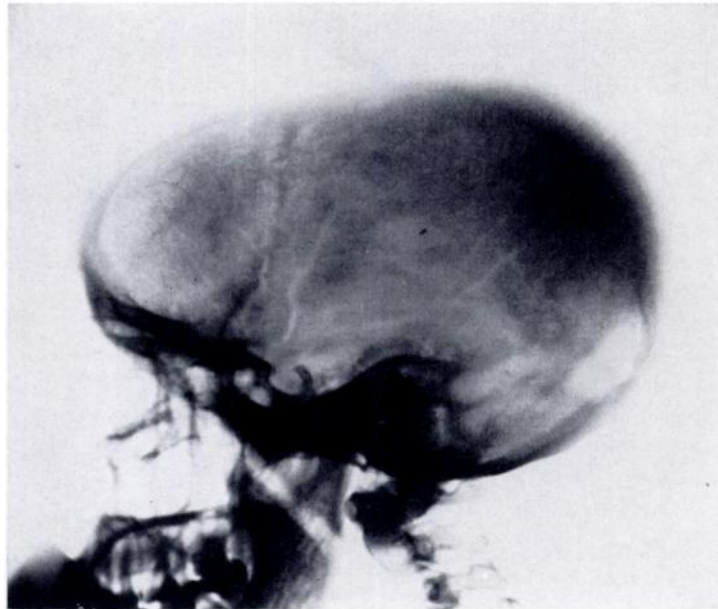


FIG. 4.—Skull x-ray. Note the "hair on end" appearance of the posterior margin and the increased granulation of the entire skull.

DISCUSSION

The prominent hematologic features of our case were the combination of hemolytic anemia with a marked degree of basophilic stippling. The moderate erythrocytic macrocytosis was probably a reflection of the increase in the number of reticulocytes. The presence of hemolytic anemia was established by the findings of splenomegaly, reticulocytosis, and increased excretion of urinary and fecal urobilinogen. The long standing history of jaundice and dark urine indicated that the disease had started in early childhood. Furthermore, the skull x-ray changes were typical of those found in the chronic hemolytic anemias of childhood⁴ and were so marked as to suggest early inception of the process.⁵

We were confronted then with the differential diagnosis of hemolytic anemia present since infancy in a young Negro male. Despite the absence of history of

crises, the obvious thought was that of sickle cell anemia but this was discarded because of repeatedly negative sickling preparations.

The occurrence of thalassemia in 22 Negroes has now been described, the most recent review being by Schwartz and Mason.⁶ However, the diagnosis of thalassemia could not be established because of the absence of increased resistance of the erythrocytes to hypotonic saline, the lack of target cells and the marked degree of basophilic stippling and reticulocytosis.

The rarity of congenital hemolytic jaundice in the Negro race has been commented upon by many observers. Isolated reports have appeared in the literature from time to time, the most recent being that of Churg and Rosenbaum⁷ who described three well documented cases in one Negro family. Again, this diagnosis could not be supported in our case in the absence of spherocytes and with a normal erythrocyte fragility. In rare cases, an increase in fragility compared with the control can be demonstrated only by incubation at 37 C. for twenty-four hours.⁸ This test was found to be negative in our patient.

Because of the high degree of basophilic stippling, lead poisoning (which can also cause a hemolytic anemia) was considered. However, the anemia of lead poisoning is usually hypochromic and has a lesser degree of reticulocytosis. The twenty-four hour urinary lead excretion was within normal limits. Furthermore, the slight exposure to lead was incurred ten years previously, whereas the jaundice had antedated this exposure by several years. We feel, therefore, that the possibility of lead poisoning as an etiologic factor has been adequately excluded.

Another possibility was an atypical form of acquired hemolytic anemia, but the negative Coombs test^{9, 10} provided further evidence against this—at least of the type associated with an antibody adsorbed to the red blood cell surface. Recently Crosby¹¹ reported a white family, nine members of which presented an hereditary nonspherocytic type of hemolytic anemia. However, basophilic stippling was not present in these cases.

Under a similar title, "A new type of hereditary hemolytic jaundice without spherocytosis," Haden¹ described two families, one of which presented hematologic data identical to those found in our case. This is the only report we have been able to find which appears identical with the findings in our case. Haden's family had five members, aged 4 to 48 years, representing three generations, all of whom had a chronic hemolytic anemia associated with a high degree of basophilic stippling. The author postulated that the defect might lie within the stroma of the red blood cell, but had no explanation for the underlying etiology. Since studies of various members of our patient's family were completely negative, the factor of heredity must be discounted—unless, of course, the patient was an illegitimate child.

"Siderocytic" hemolytic anemia, reviewed recently by Estren and Dameshek,¹² is a hemolytic anemia characterized by the presence of erythrocytes containing inclusion bodies. These bodies are usually coarser than those seen in punctate basophilia and tend to occur in aggregates. They stain blue or purplish with Romanovsky stains, and some of them give a positive reaction with iron stains.

In Pappenheimer's three cases¹³ these inclusion bodies were noted only after splenectomy. In McFadzean and Davis' seven cases¹⁴ they were present in a high proportion of the red cell precursors in the bone marrow, but became abundant in the peripheral blood only after splenectomy. These authors¹⁵ obtained similar blood pictures when experimental lead poisoning was produced in guinea pigs by feeding lead carbonate. The animals developed a progressive anemia while taking lead, with the appearance in the peripheral blood of a small number of basophilic stippled cells. After splenectomy, not only was there a sharp increase in the number of stippled cells but the anemia was corrected. The bone marrow always showed more stippled cells than the peripheral blood, and many of the granules, both in blood and marrow, gave a positive reaction for iron. Splenic aspirations were not performed, but histologic examination of splenic material showed abundant iron staining particles.

McFadzean and Davis postulated that in these cases of acquired hemolytic anemia associated with punctate basophilia, and in their cases of experimental lead poisoning, there was a defect in the hemoglobinization of the red cell due to a

TABLE 1.—Comparative Reticulocyte and Stipple Cell Counts in Blood, Marrow and Spleen

Source	Stippled cells per 1000 RBC	Reticulocytes per 1000 RBC
Blood	102	345
Marrow	144	428
Spleen *1	53	342
2	2	—

* Study made on 7/13/49. All other values obtained on 1/21/50. In Spleen *1, differential showed 45 per cent neutrophils; in *2, there were 14 per cent neutrophils.

partial failure to incorporate iron into the protoporphyrin nucleus. According to their theory, cells derived from these precursors, after their entry into the general circulation, are rapidly taken up by the spleen, and probably by the rest of the reticulo-endothelial system, and destroyed. This would explain the increase in red cell count and the rise in percentage of stippled cells after splenectomy.

It is of interest that in lead poisoning in humans, stippled cells have also been found in greater numbers in the marrow than in the blood.¹⁶

Iron stains of our material yielded negative results. However, in an attempt to study the hemolytic mechanism in our patient, he was re-admitted to the hospital six months later for further study. At this time a somewhat greater degree of reticulocytosis of the peripheral blood was present. In order to investigate the marked punctate basophilia, simultaneous blood, marrow and splenic aspirations were performed, and both reticulocyte preparations and ordinary smears were obtained. Reticulocyte and stipple cell counts were made and compared (table 1). The highest percentage of stippled cells was in the marrow, with the peripheral blood next, the spleen showing the lowest number of these cells. The splenic puncture done six months previously (*2 in table 1) disclosed only 2 stippled cells per 1000 red blood cells. Spleen sample *1 was more diluted

with peripheral blood than #2 because of the necessity of withdrawing a larger amount of material for the reticulocyte preparations. The greater dilution is shown by the presence of 45 per cent neutrophils in sample #1 compared with only 14 per cent neutrophils in sample #2. This probably explains the greater number of stippled cells in sample #1 as being due to contamination with peripheral blood (which had a much larger number of stippled cells).

In both splenic punctures a large number of basophilic granules were seen outside of the red blood cells, and many granules appeared to have been engulfed and aggregated by the reticulo-endothelial cells of the spleen (figure 3). The marked paucity of stippled cells in the spleen combined with the well-known phagocytic activity of the reticulo-endothelial system incriminates the spleen as being a likely organ either for stippled cell destruction, or for "sifting out" the inclusions within the stippled red cells.

In the bone marrow, only a rare reticulum cell showed similar phagocytic activity. No granules were seen engulfed by the monocytes of the marrow, blood or spleen. The fact that more stippled cells were seen in the marrow than in the blood would suggest the marrow as a possible site for their formation; however, the greater number may not be significant.

The relationship of reticulocytes (diffuse basophilia) to stippled cells (punctate basophilia) is still somewhat obscure, although it is thought that they may be different forms of the same general abnormality,¹⁷ the former requiring supravital staining for demonstration of their existence. Both are associated with polychromasia. In the brilliant cresyl blue stains of our patient's blood (figure 1) the stippled cells were no longer discernible as such, the granules possibly having become enmeshed in the precipitated reticulum and no longer distinguishable from it. The reticulocyte counts of the spleen and blood were almost identical, in contrast to the great difference noted in the stippled cell counts. We have no explanation for this, except that this point favors a difference in the significance of stippled cells and reticulocytes. The greater number of reticulocytes in the marrow was expected, since that is the usual finding both in normal and in anemic subjects.

It would appear, then, that the hypothesis offered by McFadzean and Davis may well explain the hemolytic process in our patient. The hemolysis would appear to be related to the abnormality of the erythrocytes, manifested by basophilic stippling, with the spleen a probable site of red blood cell destruction. If this view is correct, splenectomy would seem to be indicated. However, this was not advised because our patient was completely asymptomatic.

SUMMARY

1. A case of chronic hemolytic anemia, present since infancy, in a young Negro male has been reported. There were no hematologic abnormalities in the six family members who were examined.

2. The unusual features in this patient were normal red blood cell fragility, the absence of spherocytosis and marked punctate basophilia. These findings appear to conform to the syndrome of hereditary hemolytic anemia without

spherocytosis described by Haden. This is the first reported case of this syndrome in a Negro.

3. Examination of material obtained by splenic puncture revealed that:

a. Stippled cells were much fewer in the spleen aspirate than in the peripheral blood.

b. Basophilic granules were found engulfed by the reticulo-endothelial cells of the spleen.

These data suggest as a possible explanation for the hemolytic mechanism that the spleen selectively destroys the abnormal stippled erythrocytes. The mechanism of formation of these erythrocytes remains obscure.

REFERENCES

- ¹ HADEN, R. L.: A new type of hereditary hemolytic jaundice without spherocytosis. *Am. J. M. Sc.* 214: 255, 1947.
- ² DALAND, G. A. AND WORTHLEY, K.: The resistance of red blood cells to hemolysis in hypotonic solutions of sodium chloride. *J. Lab. & Clin. Med.* 20: 1122, 1935.
- ³ DAMESHEK, W. AND MILLER, E. B.: Pathogenetic mechanisms in hemolytic anemias. *Arch. Int. Med.* 72: 1, 1943.
- ⁴ CAFFEY, J.: The skeletal changes in the chronic hemolytic anemias (erythroblastic anemia, sickle cell anemia, and chronic hemolytic icterus). *Am. J. Roentgenol.* 37: 293, 1937.
- ⁵ SMITH, C. H.: Familial blood studies in cases of Mediterranean (Cooley's) anemia. *Am. J. Dis. Child.* 65: 681, 1943.
- ⁶ SCHWARTZ, S. O. AND MASON, J.: Mediterranean anemia in the Negro. *Blood* 4: 706, 1949.
- ⁷ CHURG, J. AND ROSENBAUM, M.: Congenital hemolytic jaundice in a Negro family. *Am. J. M. Sc.* 217: 383, 1949.
- ⁸ EMERSON, C. P., SHEN, S. C., HAM, T. H. AND CASTLE, W. B.: The mechanism of blood destruction in congenital hemolytic jaundice. *J. Clin. Investigation* 26: 1190, 1947.
- ⁹ COOMBS, R. R. A., MOURANT, A. E. AND RACE, R. R.: A new test for the detection of weak and "incomplete" Rh agglutinins. *Brit. J. Exper. Path.* 26: 255, 1945.
- ¹⁰ SINGER, K. AND MOTULSKY, A. G.: The developing (Coombs) test in spherocytic hemolytic anemias. *J. Lab. & Clin. Med.* 34: 768, 1949.
- ¹¹ CROSBY, W. H.: Hereditary nonspherocytic hemolytic anemia. *Blood* 5: 233, 1950.
- ¹² ESTREN, S. AND DAMESHEK, W.: Current concepts of hemolytic anemias, in *Advances in Internal Medicine*, Vol. 3. New York, Interscience, 1949. P. 45.
- ¹³ PAPPENHEIMER, A. H., THOMPSON, W. P., PARKER, D. P. AND SMITH, K. E.: Anaemia associated with unidentified erythrocytic inclusions, after splenectomy. *Quart. J. Med.* 14: 75, 1945.
- ¹⁴ MCFADZEAN, A. J. S. AND DAVIS, L. J.: Iron staining erythrocytic inclusions with special reference to acquired hemolytic anemias. *Glasgow M. J.* 28: 237, 1947.
- ¹⁵ —AND—: On the nature and significance of stippling in lead poisoning, with reference to the effect of splenectomy. *Quart. J. Med.* 18: 57, 1949.
- ¹⁶ MOVITT, E. R.: Plumbism: Punctate basophilia in bone marrow. *Am. J. Clin. Path.* 15: 557, 1945.
- ¹⁷ WINTROBE, M. M.: *Clinical Hematology*, Philadelphia, Lea & Febiger, 1946.