Clinical and Necropsy Findings in Hemoglobin C Disease

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There have been described, to the present time, seven different hemoglobins, in addition to types A and F, which may be identified by their physicochemical characteristics. These hemoglobins have been designated by the alphabet names S, C, D, E, G, H and I.1 The occurrence of clinical syndromes associated with hemoglobins SC, SD, EE, GG and with hemoglobins SC, SD, and SA as well as the combinations of hemoglobin S-thalassemia, hemoglobin S-congenital spherocytosis and hemoglobin C-thalassemia have been described.2 4 With the exceptions of the sickle hemoglobin diseases,9 however, descriptions of the findings at post mortem examination in the hereditary hemoglobinopathies are not available.

It is the purpose of this report to describe the clinical course of a patient with homozygous hemoglobin C disease, together with iron kinetic studies performed before and after a one month period of compound E administration, the findings at post mortem and the results of limited observations of the electrophoretic pattern of myoglobin isolated from skeletal muscle.

Material and Methods

Paper electrophoresis of the prepared hemoglobin samples was performed at pH 8.6 in an 0.05 molar veronal buffer in accord with the technic of Larson and Ranney.11 Plasma iron concentrations were determined by a modification of the method of Barkan and Walker.12 Red cell mass was estimated by the radiochromatim method.13 The rates of plasma iron turnover and incorporation into red cell mass were determined by the method described by Huff.14 Myoglobin was isolated from the frozen samples of skeletal muscle taken at necropsy as follows:15

A 10 gram aliquot of muscle was frozen in dry ice, crushed to a fine powder with a mortar and pestle and then triturated with an equal volume of cold water. After standing overnight the supernatant was separated and carefully adjusted to a pH of 7.0 with N/10 ammonium hydroxide and a one-fourth volume of saturated solution of lead subacetate was added. Time precipitate was discarded and the supernatant dialyzed against several changes of a phosphate buffer (3.0 molar and pH 6.65) during a 12 hour period. After dialysis, the contents of the bag were transferred to a glass tube, centrifuged and the precipitated hemoglobin discarded. The supernate was dialyzed against cold running water for 12 hours. The resultant cherry red solution was concentrated by evaporation and an aliquot of this final solution used for paper electrophoresis.

Fecal and urine urobilinogen were determined by the method of Watson.16 Hematologic determinations were made according to standard methods.17

Case Report

A.D., a 58 year old, male, Negro laborer was admitted to a hospital in April, 1954, with complaints of generalized abdominal discomfort of a few days' duration. He denied having had weight loss, fever, nausea, diarrhea or jaundice. The patient had influenza in 1918, and

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74
one year prior to hospitalization had a right hemiplegia with almost complete motor recovery over a three or four month period. His family had noted minor personality changes and memory defect following this episode. There was no history of arthritis, arthralgia, anemia or jaundice during childhood or adulthood.

Physical examination showed an elderly, oriented male who was thin, but of normal body habitus and without visible skeletal abnormalities. The pulse was 70 per minute, the respirations 18 per minute and the blood pressure 130/70 mm. Hg. The mucous membranes were slightly pallid and there was minimal scleral jaundice present. The retinæ, ears, nose and throat were normal. There was pulmonary emphysema and the heart was normal. The liver was palpable 1 cm. below the right costal margin and the spleen was palpable 7 cm. below the left costal margin. Peripheral arteries were thickened and tortuous with absent pulsations below the popliteal fossae. The neurologic examination was normal except for minimal weakness of the right extremities. Rectal examination was normal.

The urine was normal, VDRL was positive but Kolmer and cardiolipin tests were negative. The hemoglobin was 9.0 Gm. per 100 ml., red cell count 3.55 million per cu.mm., hematocrit 27 per cent, total leukocytes 5,000 per cu.mm. and the differential normal. Sickle cell preparations and examinations for siderocytes were negative. Reticulocyte counts varied from 0.5 to 4.0 per cent and platelets were normal. The blood smears showed target cells which ranged from 20 to 45 per cent at various times during a three month period. There was only moderate anisocytosis, poikilocytosis and polychromatophilia of the erythrocytes and from 0.0 to 3.0 nucleated erythrocytes per 100 leukocytes were seen on various examinations. Bone marrow aspiration revealed normoblastic hyperplasia with M:E ratios of 1:10, on each of three occasions. The total bilirubin level was 0.6 to 4.0 mg. per cent, urinary urobilinogen was less than 2.0 Ehrlich units per 2 hour specimen and fecal urobilinogen in each of four, three day collection periods were 269, 324, 360 and 342 Ehrlich units per day. The Coombs test was negative as were tests for acid hemolysins, cold hemolysins and cold agglutinins. Erythrocyte osmotic fragility was reduced.

The NPN was 34 mg. per cent and serum uric acid 7.5 mg. per cent. Fasting blood sugar, total protein, albumin and globulin, serum electrolytes, thymol turbidity, bromsulphthalein excretion and alkaline phosphatase were normal. Needle biopsy of the liver was normal, multiple tests for occult fecal blood were negative and the cerebrospinal fluid was normal.

Roentgenographic studies which were normal included a complete bone survey, gastrointestinal series, barium enema, cholecystograms, intravenous pyelograms and examinations of the chest. The electrocardiogram on admission showed nonspecific T wave changes in chest leads 3, 4, 5 and 6.

The patient's course in the hospital was uneventful and the above data were obtained to

Fig. 1.—Electrophoretic pattern of the patients hemoglobin (Veronal buffer, pH 8.6, 0.05 M).
HEMOGLOBIN C DISEASE

Fig. 2.—Patients course while under observation

determine the cause of the anemia. He received oral iron, pteroylglutamic acid and intra-
muscular vitamin B₁₂ without improvement. Paper electrophoresis of the patient's hemo-
globin showed 100 per cent hemoglobin C (fig. 1); alkali denaturation showed less than 1.5
per cent resistant hemoglobin.

The patient was transferred to another hospital where, during a three month period of
observation, no change occurred in the clinical course and iron kinetic studies were per-
formed prior to and during the 5th week of a 5 week course of 150 mg. of compound E per
day. The hematologic data obtained during this period of time are shown in figure 2. Approx-
imately one month following the completion of the course of compound E, the patient
noted anorexia and midepigastric discomfort. On physical examination the patient had a
diastolic gallop, sinus tachycardia and a to-and-fro precordial friction rub. Electrocardiograms
revealed changes over a 48 hour period of time which were consistent with either myocardial
infarction or pericarditis. Despite the usual management for myocardial infarction the pa-
tient expired 48 hours after the onset of these new symptoms.

PATHOLOGY FINDINGS

Autopsy was performed 8 hours after death. There was no jaundice, edema nor skeletal
abnormality. Approximately 100 ml. of yellow tinged fluid was present in the abdominal
cavity; there were adhesions about the spleen and gallbladder. The spleen weighed 575 Gm.,
the capsule was wrinkled, the cut surface was dark red, smooth and firm. The liver, gall-
bladder and biliary ducts were normal and calculi were not present. The gastrointestinal
tract was normal. The kidneys were normal in size, but there was diffuse atrophy of the
right and focal atrophy of the left renal cortices. The brain showed an area of cortical de-
generation with yellow discoloration lateral anterior to the anterior pole of the left
lateral ventricle.

Bone marrow of the ribs, sternum and vertebrae appeared red and that of the femora
yellow.

The heart weighed 330 Gm. and appeared normal. There was neither excess pericardial
fluid nor adhesions. The coronary vessels were carefully examined and found to be patent
throughout their grossly visible portions. The myocardium of the left ventricle was 13 mm.
and that of the right ventricle 3 to 5 mm. in thickness, there was no evidence of either recent
or old myocardial infarction present. There was extensive atheromatous involvement with calcareous plaques in the thoracic and abdominal aorta.

On microscopic examination, the marrow taken from ribs, sternum and vertebrae showed marked erythroid hyperplasia. There were normal amounts of iron demonstrable by iron stain in the marrow. The spleen showed marked congestion with erythrocytes of the malpighian corpuscles and in some sections the erythrocytic congestion involved the splenic pulp (fig. 3). Only small amounts of hemosiderin were seen in the spleen. The liver showed moderate congestion of the portal areas by erythrocytes, the hepatic parenchymal and Kupffer cells contained large amounts of fine and coarse brown pigment which gave a positive iron staining reaction. Other than the moderately severe degree of hemosiderosis, the liver appeared normal.

The most striking changes were found in the lungs (fig. 4). The large arteries from all lobes of the lungs contained thrombi. Some of the smaller vessels contained old recanalized thrombi with hemosiderin deposits, while others were occluded by more recent thrombi with hyalinized clots and fibroblastic proliferation. Sections from ten different areas of the lungs were examined and all contained vascular thrombi of various ages. Infarction of the pul-

Fig. 3. Spleen from patient (A) shows loss of follicular substance around the artery and congestion by erythrocytes of the sinuses as compared to a normal spleen (B).
Fig. 4. (A) Recanalized thrombus in a medium sized pulmonary artery. Hemosiderin appears as black granules. Hematoxylin and Eosin (X31). (B) Recent thrombus in a medium sized pulmonary artery. Fibroblastic proliferation can be seen at the top of the thrombus. Hematoxylin and Eosin (X53).
monary parenchyma was not found. A search for evidence of myocardial infarction and pericarditis in seventeen different areas of the heart failed to reveal abnormalities. The right kidney revealed severe fibrosis consistent with healed pyelonephritis and the left kidney contained occasional cortical scars. Hyalination and intimal proliferation of the arterioles were frequently seen in both kidneys. There were microscopic changes of focal encephalomalacia present in the brain.

Results

Genetic Data

Examination of the few available family members (fig. 5) showed that the daughter and one half-sibling had numerous target cells and approximately 35 per cent and 30 per cent, respectively, hemoglobin C. Routine hematologic examination on the members of the family failed to show other abnormalities.

Fig. 5. Genetic chart of patient A.D.

Table 1

<table>
<thead>
<tr>
<th>Author and Case</th>
<th>Hct</th>
<th>RBC Mass</th>
<th>Plasma iron</th>
<th>T 1/2 Pl-Fe</th>
<th>Pl-Fe-T-R</th>
<th>FE-T-R</th>
<th>RBC-Fe-T-R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Subjects</td>
<td>46.4</td>
<td>1746</td>
<td>96</td>
<td>1.27</td>
<td>0.37</td>
<td>69</td>
<td>0.25</td>
</tr>
<tr>
<td>A.D. before Compound E</td>
<td>27.5</td>
<td>1022</td>
<td>89</td>
<td>1.17</td>
<td>0.59</td>
<td>50</td>
<td>0.29</td>
</tr>
<tr>
<td>A.D. after Compound E</td>
<td>33.0</td>
<td>1436</td>
<td>112</td>
<td>0.63</td>
<td>1.44</td>
<td>44</td>
<td>0.62</td>
</tr>
<tr>
<td>Terry et al. before Compound E</td>
<td>31.0</td>
<td>1340</td>
<td>85</td>
<td>1.25</td>
<td>0.59</td>
<td>60</td>
<td>0.36</td>
</tr>
<tr>
<td>Terry et al. after Compound E</td>
<td>35</td>
<td>174</td>
<td>1.42</td>
<td>0.81</td>
<td>80</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>Thomas et al. No treatment</td>
<td>24</td>
<td>35</td>
<td>87</td>
<td>1.75</td>
<td>Normal</td>
<td>79</td>
<td></td>
</tr>
</tbody>
</table>

T 1/2 Pl-Fe = Time required for 1/2 of Fe59 to be cleared from plasma.
Pl-Fe-T-R = Calculated plasma iron turnover rate.
RBC-Fe-T-R = Calculated red cell iron turnover rate.
Radio Iron Studies

The results of iron kinetic measurements prior to and after the 5 weeks of compound E treatment are presented in table 1. Plasma iron levels were within the normal range on all determinations, the disappearance rate of the plasma iron was approximately normal prior to the administration of compound E, and was increased to approximately twice the pretreatment level during therapy.

The percentage uptake of the labeled iron was in the normal range prior to compound E treatment (68%) and decreased to 44% during therapy. There was no difference observed in plasma volume but the red cell mass showed an increase from 1022 ml. to 1436 ml. during the time compound E was given. The hematocrit increased from 27.3 to 34.7 per cent during this same period.

Myoglobin

The electrophoretic mobility (veronal buffer at pH 8.6) of myoglobin obtained from the patient's lumbar muscle was entirely similar to that of individuals without hematologic disease (fig. 6). Both normal and the patients myoglobin exhibited a mobility greater than that of hemoglobin C and less than that of hemoglobin S at pH 8.6.

Discussion

Approximately 28 cases of homozygous hemoglobin C disease have been reported.15-20 These suffice to define the clinical picture as one of mild intermittent abdominal discomfort, occasional arthralgia and intermittent mild jaundice which usually occurs in a Negro and is compatible with advanced age. In most of the reported patients, the complaints for which medical attention was sought were unrelated to the hemoglobin C disease and the symptoms were rarely referable to the mild to moderate anemia present. Only two of twenty-eight re-
ported patients failed to exhibit splenomegaly and all but two, who were of Italian and of German-Dutch ancestry respectively were Negroes.

The laboratory findings are a mild to moderate, normocytic, normochromic or hypochromic anemia, a large number of target cells present in the peripheral blood smear, a decreased osmotic fragility, a modest reticulocytosis and a normoblastic marrow. In all of the patients reported, the diagnosis has been established by the characteristic electrophoretic pattern of the abnormal hemoglobin.

The calculated rate of iron turnover and rate of renewal of red cells is approximately twice the value observed in the normal subject. This increased rate of red cell production was not, however, adequate to compensate for the rate of red cell destruction and to provide a normal concentration of erythrocytes in the peripheral blood. The substantial increase in plasma iron turnover rate and red cell iron turnover which was observed concurrently with the administration of compound E could represent variations inherent to the disease. That this is unlikely, however, is suggested by the long base line obtained prior to treatment, during which time, hemoglobin values did not reach the level obtained during compound E administration. Observations entirely similar to these were observed in the patient studied by Terry. Upon discontinuing the compound E, there was a prompt return to the pretreatment levels, but quantitative data for rates of red cell production and destruction were not obtained during this period.

Although it has been suggested that myoglobin abnormalities may exist in those diseases characterized by the presence of abnormal hemoglobins, it was not found in patients with sickle cell anemia. The inability to demonstrate evidence of myocardial infarction in the pathologic studies on this patient suggested the possibility that an abnormality of cardiac muscle protein might be a possible explanation for the cause of death. From the clinical course of this patient and the relatively benign nature of the disease in other patients, this does not seem likely. Because of this possibility, the myoglobin studies were carried out. Paper electrophoresis failed to show a difference between the myoglobin of the patient and that from subjects with type A hemoglobin.

The pathologic findings which were of greatest interest were the pulmonary arterial thrombi. Vascular thrombi are known to occur in acquired hemolytic anemia, and in sickle cell anemia and its clinical variants, but has not been found in other types of hereditary intracorporeal hemolytic anemia. Diggs and others have postulated that the thrombi present in sickle cell anemia result from the intravascular sickling of the cells and subsequent mechanical blockage of small vessels and resultant local anoxia. At present, there is no similar known phenomenon in patients with hemoglobin C disease. The red cells in the tissues of this patient were indistinguishable from those in tissues of patients with type A hemoglobin. It may be suggested that the intracellular crystals or the folded red cell membrane described by others represent morphologic abnormalities which could initiate vascular damage. This seems unlikely, however, since these phenomena were not seen in this patient and there is no adequate explanation for the thrombi seen.

SUMMARY

A patient with homozygous hemoglobin C disease was observed during a five month period of time. Estimates of the rates of red cell synthesis were made prior
HEMOGLOBIN C DISEASE

to and during the administration of compound E. The post mortem findings and results of paper electrophoresis of myoglobin are presented.

SUMMARIO IN INTERLINGUA
Un patiente con homozygotic morbo a hemoglobin C esseva observate durante un periodo de cinque menses. Estimatos del nivello del synthese de cellulas rubie esseva executate ante e durante le administration de composito E. Es presentate le constataiones post morte e le resultatos del examine de myoglobin per electrophoresa a papiro.

REFERENCES
15 Perkoff, G. AND Tyler, F. H.: Personal communication.


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