1955

Abnormalities associated with hemoglobin C : a review

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ABNORMALITIES ASSOCIATED WITH HEMOGLOBIN C

A REVIEW

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Submitted in Partial Fulfillment for the Degree of
Doctor of Medicine

College of Medicine, University of Nebraska

April 1, 1955

Omaha, Nebraska

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The recognition of more than one type of human hemoglobin and of the existence of abnormal forms has opened up a whole new field for investigation in the rapidly expanding specialty of hematology. The knowledge gained in this investigation has been immediately applicable in today's clinical and laboratory medicine and has in addition, given to us an insight into the pathogenesis of some of the congenital hemolytic diseases. Furthermore, this study has provided proof of the old concept in medicine that a molecular abnormality in a single body protein may cause a sequence of events that characterizes a complex disease.

The purpose of this paper is to present, in a simple form, the basic abnormality associated with these "abnormal hemoglobins," methods used to detect them, and conditions associated with the presence of one of them, namely hemoglobin C.

All human hemoglobins consist of four hemes (Fe-protoporphyrin) which are linked to the protein moiety globin. Globin is composed of polypeptide chains which are arranged in a specific fashion.

The complicated details of the folding or coiling of these chains are incompletely understood. The essential differences of the various hemoglobins are located in the protein moiety, since all have identical hemes.

Although differences in the physical and chemical properties of human adult and fetal hemoglobin have been known for years (1), the recent discovery (2) of an electrophoretically abnormal hemoglobin in sickle cell disease provided the first positive evidence that adult human hemoglobin exists in more than one molecular form.

It was in 1949 that Pauling and his associates (2) examined the physical and chemical properties of the hemoglobins of individuals with sicklemia and sickle cell anemia and compared them with hemoglobin from normal individuals. They found significant differences in the mobilities in the Tiselius apparatus of hemoglobin from sickle cell anemia patients and normal patients. In the sicklemic patients a combination of the two types of hemoglobin was found. They ascribed this difference in mobilities to the globin part of the hemoglobin molecule in that it had a different number or kind of ionizable groups. The exact nature of this difference has not been determined as yet.

In 1950, Itano and Neel (3) began a study of the inheritance of sickle cell anemia encountered in two families in which one or more children had sickling erythrocytes and a hemolytic anemia of a milder form than that usually seen in sickle cell anemia. Erythrocytes of only one parent in each of these families exhibited sickling, contrary to the usual finding of the sickle cell trait in both parents of a child with sickle cell anemia. The electrophoretic analysis of the hemoglobin of the non-sickling parent in each family revealed the presence of a new type of hemoglobin, now called hemoglobin C.

Following the publication of this work it was suspected by many workers that many previously reported cases of "atypical sickle cell anemia" and Mediterranean syndromes were in reality examples of Hb C conditions(3, 4, 5).

Since that time several other types of abnormal hemoglobin (as determined electrophoretically) have been described (6, 7, 8). Most of these have been found in only one family (or person), making their importance extremely limited. However, the importance of the general subject of abnormal hemoglobins lead to a symposium on the subject in 1953, sponsored by the Hematology Study Section of the Division of Research Grants of the National Institutes of Health(9). During this symposium a system of nomenclature for the varieties of human hemoglobin was established. The five

varieties recognized at that time were designated as follows:

1. Normal adult hemoglobin...A. Previously called N or a.
2. Normal fetal hemoglobin...F. Previously called f.
3. Sickle cell hemoglobin...S. Previously called 1.
4. Hemoglobin C. Previously called c, III.
5. Hemoglobin D. Previously called d.

New varieties as discovered were (and are) to be called by letters of the alphabet in order of discovery unless there was some outstanding association which would serve as a convenient mental association.

It might be well to note at this time that fetal hemoglobin (HbF) is somewhat different than the other "abnormal hemoglobins" in that in certain instances it is normal. That is, it is normally present at birth, is usually present up to 6 months (10), and may be present normally up to the age of 2 years, according to Singer and his associates (11). Furthermore, this particular pigment is resistant to alkali denaturation; and since this property sets it apart from all other known hemoglobins, a simple method based on this reaction has been developed by Singer and his co-workers (11) for its quantitative determination. According to the same authors, values obtained by this method which exceed 2% of the total hemoglobin are considered abnormal after the age of 2 years.

The production of fetal hemoglobin may continue from birth throughout the life span of patients with certain hereditary anemias (12). Its production may be resumed in some acquired disorders (12). In addition to its resistance to alkali denaturation, this hemoglobin can be demonstrated by electrophoretic studies as shown by several workers (12, 13, 14), although its separation from Hb A is slight (1).

As noted above, the original discovery of these abnormal hemoglobins was made with the use of the Tiselius apparatus for electrophoresis (2). Since then many tests for physical and chemical properties of these hemoglobins have been run, including solubility studies, ultra-violet absorption spectra, ultra-centrifugation, and alkali denaturation (15). Of all the studies done, the only one which will reveal the specific abnormality is electrophoresis with the use of solubility studies for final differentiation in some cases. Notable of these cases is Hb F (11) as discussed above, and Hb D which has the same mobility in electrophoretic studies as Hb S, but which differs greatly in its solubility (1, 6, 12). Of course, sickling studies will usually reveal the presence of Hb S, but even this is not infallible (16). Hb C, with which we are mainly concerned in this paper, can be determined only by electrophoretic studies (12, 15).

The Tiselius apparatus offers nearly exact separations and provides precise quantitative analysis of the hemoglobin solutions. But the high cost of the apparatus, need for skilled personnel, and the fact that but one sample can be analysed over a period of six to twenty hours, lead to the search for a simpler method of electrophoresis that could be applied in general clinical use. In 1951, Kunkel and Tiselius (17) presented a method for filter paper electrophoresis which was applicable to hemoglobin separations. Since then various methods and modifications have been presented along with clinical results (18, 19, 20, 21), but the most practical and applicable remains that of Kunkel and Tiselius (17) or a modification of it as used by Larson (22) or Smith and Conley (23). With this method, a buffer solution at pH 8.6 is used which results in Hb A having the fastest mobility, then Hb F, Hb S, and Hb C in that order. As noted before, Hb S and Hb D have the same mobility. This is the reverse of the mobilities as seen in the Tiselius apparatus, where a buffer at pH 6.5 is used. This, of course, is due to the change of buffer pH to the acid side of the isoelectric points of the hemoglobin molecules instead of the relatively basic buffer as used in the filter paper technique (1). Motulsky (24) described a simple method of paper electrophoresis which allows accurate quantitation of various hemoglobin mixtures by inspection alone.

For more exact quantitative results he described technics of elution and photoelectric scanning. (This technique can also be used for red cell life span determinations by serially following the disappearance of a certain hemoglobin type transfused into a patient with a different hemoglobin variety.)

As has been previously alluded to, the presence of these abnormal hemoglobins is determined by hereditary mechanisms, with the exception of Hb F.

In 1949, Neel (25) postulated the genetics of sicklemia and sickle cell anemia as heterozygosity and homozygosity respectively for the Hb S gene. Pauling (2), with his discovery of the molecular abnormality, agreed with this concept. Somewhat later Neel (26) made three hypotheses to explain the various gradations between the "anemia" and "trait" as follows: 1) That there are associated modifying genes which, although incapable alone of producing sickling, can in the presence of a sickle cell gene influence the production of the abnormal hemoglobin. 2) Hb S associated with the thalassemia gene. 3) Hb S associated with other abnormal hemoglobins. Since then all cases which have been adequately studied have confirmed this genetic aspect (16).

Following the discovery of Hb C, Kaplan, Zuelzer, and Neel (4) postulated a similar hypothesis. They stated that the presence of

Hb C is determined by a single gene which is transmitted as a simple, though incomplete, Mendelian dominant. In 1953 these same writers (27) presented further evidence in support of this hypothesis and also evidence that the gene was not sex linked. During that same year Ranney (15) published a case of a Negro woman with pure Hb C whose husband had only Hb A. Each of their four children had Hb C and Hb A. This provided strong support for this hypothesis. The genetic studies of Singer and his co-workers (28) and Schneider (29) were also in accord. The method of quantitative inheritance of Hb S and Hb C is at present poorly understood, but Singer (30) noted that "the expressivity of the gene for Hb C is frequently greater than for Hb S." Thus, the presence of the abnormal gene for Hb S "enhances the activity" of the gene for Hb C when both are present, resulting in a larger percentage of Hb C than Hb S. They also stated that the genes for the abnormal hemoglobins and the genes for thalassemia are not allelomorphs; that is, the segregation of these genes takes place independently. Whether or not the genes for Hb S and Hb C are allelomorphs has not yet been established for certain (28), but Schneider (29) feels his work indicates that they are not.

The determining factor for the presence of Hb F is not known. Singer's group (11) advanced the theory that "the resistant fraction

(Hb F) in the hereditary hemolytic syndromes may represent a continual production of fetal pigment beyond the physiologic age limit and the appearance of the abnormal hemoglobin (Hb F) in the acquired disorders may indicate a reactivation of such a mechanism." However, this is no real explanation even at best. It may even be that the Hb F present at birth and that associated with various hematologic disorders of later life are not the same. This was alluded to by Singer, Chernoff and Singer (31). But even this is not a final answer.

Since the discovery of Hb C four specific hematologic and clinical entities have been described in which it is associated. They are as follows: 1) Hemoglobin C trait, 2) Sickle cell-hemoglobin C disease, 3) Hemoglobin C-thalassemia disease, and 4) Pure hemoglobin C disease. Combinations of Hb C with other abnormal hemoglobins (example Hb D) are possible, but none have been reported as yet.

With the exception of one case report by Diggs (32), all reported cases of Hb C have been in Negros or in patients with some Negro blood. The incidence of hemoglobin C trait in American Negros was reported by Schneider (29) as 3.0% and by Smith and Conley (23) as 2.2%. There have been only scattered reports of the other

three conditions and no accurate statement of their occurrence can by made from the literature now available.

Each of the four subtypes of Hb C disorders will be discussed.

1. Hb C Trait.

This anomaly is the heterozygous condition for Hb C and has the hemoglobin formula of A-C. This condition according to Kaplan, Zuelzer, and Neel (4) is expressed as an asymptomatic carrier state. The only abnormalities found are a high incidence of target cells and a correspondingly increased resistance to hypotonic saline. There is no evidence of hemolysis in the patient and no anemia. The survival times of erythrocytes transfused into normal recipients revealed normal results according to these same authors (33) and Motulsky (24). The amount of Hb C present in the hemolysates varied from 25-40% (3, 12, 24, 29) with no significant amounts of Hb F (12, 24). Reports of this condition by other workers (15, 23, 27, 34) were consistent with the above description.

2. Sickle Cell-Hb C Disease.

It was in this disorder that Itano and Neel (3) made the original discovery of Hb C. This anomaly is the heterozygous condition for both Hb C and Hb S and has the hemoglobin formula of C-S or C-S-F (12); that is, Hb F is inconstantly found in abnormal amounts (greater than 2%).

Although the clinical picture of this disease may be variable, most patients present a hemolytic syndrome intermediate between sickle cell trait and sickle cell anemia (4, 15, 23). Concerning this feature of the disease, it is noteworthy to point out that many cases now known to be sickle cell-Hb C disease were formerly diagnosed as "mild sickle cell anemia" or "atypical sickle cell anemia" (3, 5, 23). There is usually a mild chronic anemia with symptoms directly referable to the anemia, or a compensated hemolytic process (4, 5, 23, 33). There are, of course, the associated findings of the hemolytic process such as increased reticulocytosis, erythroid hyperplasia of the bone marrow, increased serum bilirubin and fecal urobilinogen, etc. Jaundice may or may not be present (4, 23). The erythrocyte survival time in normal recipients is decreased greatly (4, 33). The resistance to hypotonic saline is increased with no increase in mechanical fragility (28, 33). Mild hemolytic crises similar to those seen in classical sickle cell anemia were noted by several authors (15, 34, 35, 36). These occurred more frequently and were more severe with associated pregnancies (5, 23), especially during the last trimester.

In nearly all cases of this disorder a slowly progressive splenomegaly was noted (4, 5, 15, 23, 35). Most cases in which there was no splenomegaly were in children (5, 23, 28, 34). Singer and

his group (28) reported hepatomegaly with no splenomegaly in a Negro boy with this disease. Splenectomy done in one patient and reported by Ranney (15) did not correct the mild anemia.

Only rarely will sickled cells be seen on the slide, but the cells will sickle rapidly and completely in sealed preparations with sodium bisulfite (33). The peripheral blood smear also reveals a marked increase in the number of target cells present (12, 15, 23, 33, 35). Concerning the peripheral blood smear, it is worthwhile to emphasize that the findings of a low MCV and MCH with a normal MCHC in this disease, as noted by Singer and his associates (28) as well as by Kaplan and his associates (33), do not, in these instances, suggest the expression of a thalassemia gene.

Smith and Conley (5) as well as Ranney and his group (15) noted the frequent occurrence of radiologic evidence of aseptic bone necrosis. Ranney (15) also noted a high incidence of associated arthritis. In another report by Smith and Conley (23) they noted gross unilateral hematuria in two of their six reported cases.

In this condition the percentage of Hb C present in the hemolysates varies from 40-67% (3, 12, 24, 29). Most commonly it is 50% of each Hb C and Hb S. Occasionally there is an associated abnormal amount of Hb F also present (12, 23).

Singer and Singer (37) noted that the presence of Hb C with Hb S definitely decreased the quantity of Hb S necessary for gel formation to occur. Hb A also interacts the same way but to a lesser degree. These demonstrable differences in the "lowest gelling points of hemolysates" of various types of sickling cells formed the basis of a diagnostic gelling test to differentiate sickle cell trait, sickle cell anemia, and sickle cell-Hb C disease.

3. Hb C-Thalassemia Disease.

This Hb C disorder represents a heterozygous condition for Hb C and the gene for thalassemia. The hemoglobin formula is C-A-F, for thalassemia, although a disease involving the erythrocytes, does not reveal an "abnormal hemoglobin" that has so far been distinguished from the normal Hb A (12). Only three cases of this disorder have been reported so far.

In the latter part of 1954, Singer and his co-workers (28) presented a paper which described a Negro family in which several members had either thalassemia or Hb C trait. In two members of this family the combined occurrence of both anomalies was demonstrable. The condition manifested itself in these two as a microcytic erythrocytosis. The erythrocytes revealed a low MCV and MCH, but a normal MCHC. About 45% of the red cells appeared as target cells in the peripheral blood films. The amount of Hb C

in the hemolysates was about 75%, the rest composed of Hb A and in one case also a small amount of Hb F. This is analogous to the results of hemoglobin analysis in sickle cell-thalassemia disease where 60-80% Hb S is found. The hypothesis was advanced by these authors (28) that "the thalassemia gene enhances the expressivity of the gene for the pathologic pigment."

These same authors (28) found during their work with the above described family that a second component was seen in the electrophoretic studies of the hemolysates of almost all the members of that family who did not reveal Hb C. This component had a mobility faster than Hb A in the Tiselius apparatus and constituted 5.2 to 13.5% of the total hemolysates. This same component has also been reported by Itano's group (38, 39) who felt it represented Hb F. Singer (28) does not agree with this but offers no further suggestion. Further investigations are indicated, particularly in thalassemia syndromes.

At the same time the above cases were reported, Zuelzer and Kaplan (40) reported a case of severe hypochromic, microcytic anemia in a Negro boy which they attributed to the interaction of the Hb C gene with the thalassemia gene. The boy's father had asymptomatic Hb C trait and his mother had thalassemia minor as did several of his siblings. The patient's blood film exhibited extreme pleo-

morphism with the coexistance of hypochromic cells and deeply stained microcytes and many (up to 60%) target cells. There was no sickling. His hemoglobin level was 6.1 gm. and all cell indices were low. There was no hepatosplenomegaly. Bone marrow was hyperplastic but there was only 3.6% reticulocytosis. Total serum bilirubin was 0.4 mg.%. Red cell survival times were not done. The patient's hemoglobin consisted of 71% Hb A and 29% Hb C with only a trace of Hb F. The diagnosis of thalassemia rests, of course, on essentially morphological grounds.

There is little similarity between the two cases of Singer's and the one of Zuelzer and Kaplan's, but with only three cases reported no conclusions can be drawn at this time. These cases are presented only for completeness. In all probability, both cases represent true cases of Hb C-thalassemia disease. Perhaps the difference in the percentages of Hb C present in the hemolysates will explain the differences in the symptoms and findings.

4. Pure Hb C Disease.

This Hb C disorder represents the homozygous condition of Hb C. The erythrocytes contain only Hb C. The disease is characterized by a hemolytic process with or without anemia (15, 30, 34). Crosby and Ackeroyd (41) have pointed out that anemia may be absent in a hemolytic disorder if the mean cell life (42, 43, 44) exceeds 15-20 days, and that in such instances the marrow compensates for

the increased rate of erythrocyte disintegration by producing 6-8 times the normal output of erythrocytes. The hemolytic process reveals itself in the usual clinical laboratory findings. The anemia, when present, is usually normochromic and normocytic or microcytic in type (24, 30, 45).

All cases of pure Hb C disease reported have had a conspicious number of target cells in the film (15, 30, 35). In spite of the fact that disorders associated with Hb C are frequently also associated with target cells, this shape anomaly is by no means characteristic for the presence of Hb C since it is also a common finding, according to Crosby (46), in sickle cell anemia, thalassemia, liver disease, following hemorrhage or dehydration, and after splenectomy. Also, since in Hb C disease all the erythrocytes contain only Hb C, it is not clear why all the cells are not target cells. Singer (30) suggested that the shape anomaly might be due to some factor located in the erythrocyte stroma.

Another common feature of this disease is the splenomegaly (15, 24, 30, 34, 36, 45). The only reported case which did not reveal a splenomegaly was that of Watson's (35). A splenectomy was performed on one of Singer's (30) patients but it did not ameliorate the hemolytic process. In spite of the fact that a splenectomy will not, in all probability, relieve the hemolytic process, Singer (30)

feels such a procedure may become indicated due to the large size that organ may attain or because of developing hypersplenism.

No hemolytic crisis as such have been reported, even during pregnancies (15), but there is frequently transient arthritis and vague abdominal pains (15, 24, 30, 35, 36).

No significant quantities of Hb F have been reported in this disease (12, 24, 30).

Until late in 1954, all reported cases associated with Hb C were in Negros. At that time Diggs and his associates (32) reported a case of pure Hb C disease in a white male of Italian parentage. The clinical and hematological features described were similar to those described above. This patient had had a splenectomy a year and a half before the diagnosis of Hb C disease had been made, because of an attack of acute abdominal pain which he had had at that time.

How does the presence of this abnormal hemoglobin make the erythrocytes susceptible to hemolysis? The pathologic physiology of these disorders is another of the unsolved problems concerning the subject of "abnormal hemoglobins" (29, 30, 35, 40, 45). Watson (35) states that target cells are mechanically fragil but presents no proof. Spaet (45) suggests that in spite of a normal mechanical fragility test in vitro, these cells may have a greater liability to

mechanical fragmentation in vivo. The possibility that these cells have a different type of stroma has not been ruled out, but Schneider (29) is attempting to do this by trying to produce antibodies against the abnormal erythrocytes. Singer and his associates (30) also noted that even in pure Hb C disease there is a distinct heterogeneity of the red cell population which cannot be explained by the presence of the pathologic pigment alone.

Review:

Abnormal hemoglobins differ from the normal hemoglobin molecule in that the former have a different number or kind of ionizable groups in the globin part of the molecule. The exact difference is not known as yet, but the abnormal hemoglobins can easily be determined by electrophoretic studies of the hemolysates.

The presence of one of these abnormal pigments in the patient's erythrocytes either by itself or in combination with any other abnormal hemoglobin, leads to a hemolytic disease.

The presence of these abnormal hemoglobins is determined by genetic factors which are transmitted as Mendelian dominant genes.

The abnormal Hb C is found in about 2% of American Negros. Four different subtypes of Hb C disease have been described.

1.) Hb C trait. This disorder is present as an asymptomatic

carrier state. The only abnormality which is consistently found is the presence of increased numbers of target cells. No evidence of hemolysis.

2.) Sickle cell-Hb C disease. This disorder is characterized by a hemolytic syndrome intermediate in severity between the sickle cell trait and sickle cell anemia. There is a slowly progressive splenomegaly and there may be hemolytic crises. The erythrocytes will sickle in vitro.

3.) Hb C-thalassemia disease. This condition presents itself as a microcytic disorder with or without anemia. The MCH and MCV are low and the MCHC is usually normal. There are numerous target cells. No hepatosplenomegaly.

4.) Pure Hb C disease. Basically this disorder is characterized by a hemolytic process with or without an anemia, a congestive splenomegaly, and numerous target cells.

The pathogenesis of the disease produced by the presence of Hb C is not known.

Summary:

1. The basic concept and associated defect of abnormal hemoglobins are presented.

2. Methods of determining the presence of these abnormal hemoglobins are given.

3. The genetics involved in determining the presence of these abnormal pigments are explained inasmuch as they are known.

4. A complete review of the literature concerning hemoglobin C is organized into the four subtypes of disorders associated with that abnormal pigment.

5. The pathogenesis of these diseases is alluded to, but it remains enigmatic.

Conclusions:

1. A differential hemoglobin analysis has become indispensable for the proper classification and differential diagnosis of a variety of hematologic disorders.

2. Electrophoretic studies of hemolysates are indicated:

   a. In most hemolytic disorders whether an anemia is present or not.

   b. In cases where sickling erythrocytes are present.

   c. In cases where significant numbers of target cells are found.

   d. In cases of splenomegaly from unknown cause.

   e. In selected genetic studies.

3. A molecular abnormality in a single protein may cause a sequence of events that characterizes a complex disease.

4. Study of the pathologic pigments has created many problems concerning the biochemical, pathophysiologic, and genetic features of the diseases in which they are found. Some of these problems have been solved, some are still controversial, and some remain ambiguous.

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