On the Optical Properties of the Hemoglobin in Microdrepanocytic Disease

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MICRODREPANOCYTIC disease was first described by Silvestroni and Bianco in Italy in 1945, and later studied in detail by the same authors. It is a familial affection which arises on the constitutional substratum of two hereditary abnormalities, microcythemia (or thalassemia) and sickle cell trait, simultaneously present in the patients. It is characterized by a chronic hemolytic anemia, splenomegaly, recurrent crises of osteoarticular and abdominal pains, sickling, and marked morphologic abnormalities of erythrocytes.

To the present, Silvestroni and Bianco have found, in Italy, eleven families of white race and of Italian extraction, in which one or more children affected by microdrepanocytic disease were discovered. Some cases of this disease, quite identical, were subsequently described by others in the United States and in Italy. A genetic study, recently published in this journal, provided evidence that both the microcythemic and sicklemic genes are present in patients with microdrepanocytic disease.

In the drepanocytic (sickle cell) anemia of Negroes, various authors have shown that a percentage of the hemoglobin of the patients and carriers of the sickle cell trait is structurally abnormal. It is called type S as opposed to type N, or normal hemoglobin.

Lately, Singer and Chernoff have found that sickling is due to type S hemoglobin only, and not to fetal hemoglobin (type F), which is also present in the erythrocytes of drepanocytic anemia patients. This last observation is in agreement with the studies of Liquori and Liquori and Bertinotti, who showed that fetal hemoglobin is present both in patients with Cooley's disease and in the carriers of thalassemia (or microcythemia), though the erythrocytes do not exhibit any change of the shape when kept at reduced oxygen pressure.

Sickling of the red cells in drepanocytic anemia occurs only in conditions of reduced oxygen pressure and it was postulated that this change is caused by such an abnormally high viscosity of reduced hemoglobin S as to form pseudocrystalline aggregates or tactoids. These can also be obtained with isolated hemoglobin S (see Harris).

The presence of reduced hemoglobin S can also be revealed by its characteristic optical properties (birefringence) either in the erythrocyte or in hemoglobin ob-
optical properties of hemoglobin

OPTiAL PROPERTIES OF HEMOGLOBIN

1062

Optical properties of hemoglobin

Optical properties of hemoglobin obtained from patients with microdrampanocytic disease and from carriers of the sickle cell trait. All subjects were of the white race.

The chief aim of our study was to establish whether there would be an identity or analogy between the hemoglobins of Negro subjects with sickle cell anemia or carriers of the sickle cell trait and the hemoglobin of white patients with microdrampanocytic disease.

Erythrocytes and hemoglobin from the blood of nine subjects belonging to four families were used. Six subjects out of nine were affected with microdrampanocytic disease, while the remaining three were carriers of the sickle cell trait.

Our observations were mainly qualitative and partly quantitative.

MATERIALS AND METHODS

Preparations of erythrocytes were so made as to realize conditions of sickling with the following technic. A drop of blood was spread on a slide and protected with a cover slip rimmed with paraffin. The slides were then kept in the incubator at 37 C. for the minimum time required to obtain complete deformation of the erythrocytes.

Tactoids of isolated reduced hemoglobin were obtained following the method reported by Singer and Chernoff.

For the study of the birefringence of red cells and tactoids, the technical devices suggested by Swann and Mitchison (see also Perutz and Mitchison) were, on the whole, adopted.*

The essential points of our technic were the following: (1) use of the arc lamp of a Leitz XI C. microprojector, in order to have a light of high intensity; (2) minimum opening of the iris diaphragms; (3) insertion of the additional condenser lens for the observation in convergent light; (4) choice of small numerical aperture objectives and high magnification eyepieces.

With these simple devices we were able to make qualitative observations and, by using a Berek compensator, to measure the retardations.

In order to make photographs, it was found essential to further increase the sensitivity of the apparatus. This was obtained by removing the analyser Nicol prism and by using a Wright eyepiece fitted with a diaphragm and a special Nicol prism inserted above it. This device made it possible to exclude practically all secondary rays and to obtain a central beam in the analyser.

RESULTS AND DISCUSSION

1. When red blood cells of carriers of the sickle cell trait or of patients with microdrampanocytic disease are observed at the polarizing microscope, under conditions of reduced oxygen pressure, only the sickled erythrocytes are birefringent (fig. 1). The birefringence is shown not only by the true sickled cells, but also by other erythrocytes corresponding to the granular form described by Sherman. The other erythrocytes are altogether isotropic. The birefringent cells exhibit a white interference color with a purplish blue nuance. This is probably due to an anomalous optical behavior caused by the small retardation of the single elements. It is

* The apparatus was set up by one of us (Ascenzi) in the Institute of Morbid Anatomy, University of Rome, where studies on the erythrocytes in Cooley's disease had formerly been made (see Liquori and Bertinotti).
known to mineralogists that minerals with a low birefringence power, e.g. chlorites, show anomalous interference colors, namely those not corresponding to the Newton color scale (see Winchell\textsuperscript{23}).

The observations with the aid of the Berek compensator make it possible to establish, in accordance with results reported by Perutz and Mitchison, that the optical axis of the true sickled cells is perpendicular to their length.

The average retardation obtained from the measurement of one hundred red cells was $7.5 \pm 0.15 \ \mu $. If one assumes a value of approximately $2\mu$ as the average maximum thickness of a sickled cell, the resulting birefringence is of the order of 0.0037. This value is within the range of those found in other biologic structures, e.g. muscle, bone, etc. (see Høncke\textsuperscript{24}, Ascenzi\textsuperscript{25, 26}).

Fig. 1.—With the polarizing microscope, the sickled cells show illumination (X 350).

As already stated, the nonsickled red cells are not birefringent. This fact is remarkable, since in microdrepanocytic disease many of these red cells are morphologically abnormal, i.e. they show micropoikilocytosis. These features are typical of thalassemia, which is itself a component of microdrepanocytic disease. Since variable amounts of $F$ hemoglobin have been found in thalassemia, our observations confirm indirectly the findings of Singer and Chernoff that $F$ hemoglobin is not responsible for the sickling.

Our results indicate that nonsickled cells do not contain $S$ hemoglobin; however they do not allow one to determine the distribution of $S$ and $F$ hemoglobin within the sickle cells (Singer and Fisher\textsuperscript{27}).

2. Hemoglobin solutions obtained either from patients with microdrepanocytic disease or from Italian carriers of sickle cell trait, rapidly undergo an increase
of viscosity and form pseudocrystalline aggregates or tactoids. Using the phase microscope, the tactoids exhibit shapes corresponding perfectly to those shown by sickled cells (fig. 2).

With the polarizing microscope the tactoids appear birefringent and their optical axis is perpendicular to their length. Therefore they show properties similar to those of sickled cells. In this instance also the interference color is white with a purplish blue nuance. These findings support the suggestion that in patients with microdrepanocytic disease, as well as in the white carriers of sickle cell trait, sickling is primarily due to a pseudocrystalline aggregation of reduced $S$ hemoglobin.

![Fig. 2.—Tactoids of reduced hemoglobin from a patient with microdrepanocytic disease with the phase microscope. Their shape is similar to that of the sickled red cells ($X$ 1200).](image)

**Conclusions**

1. In patients with microdrepanocytic disease and in the white carriers of the sickle cell trait, sickling is associated with optical anisotropy of erythrocytes. Birefringence is negative in respect to the major axis of the cell. The mean retardation of each cell is $7.5 \pm 0.15 \text{ m}\mu$ and the resulting calculated birefringence is approximately 0.0037.

2. Reduced hemoglobin, either from patients with microdrepanocytic disease or from white carriers of sickle cell trait, forms tactoids which optically behave like sickled cells.

3. According to Singer and Chernoff, the above mentioned facts lead to the suggestion that in these two pathologic conditions, $S$ hemoglobin is present. The possibility remains open that the type III (C) hemoglobin, described by Itano and Neele,\textsuperscript{28} is also able to form tactoids; however, to our knowledge, this has not yet been proved.

4. The presence in microdrepanocytic anemia of isotropic red cells similar to those found in thalassemia, which are known to contain $F$ hemoglobin, confirms indirectly that sickling is not due to $F$ hemoglobin.
5. The fact that morphologic properties of the sickle cell from Italian carriers of the trait and from patients with microdrepanocytic anemia are identical with those from Negros with drepanocytic disease or carrier of the trait, is a strong indication that the chemical bases of these conditions are likely identical.

SUMMARY

In analogy with sickle cell anemia of the Negro, white carriers of the sickle cell trait and patients with microdrepanocytic disease show sickling which is associated with the presence of type S hemoglobin. The sickled cells are birefringent and the reduced hemoglobin forms tactoids which reveal a striking resemblance to the sickled erythrocytes.

The mean retardation of each sickled red cell is 7.5 ± 0.15 μ and the birefringence is approximately 0.0037.

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1066

OPTICAL PROPERTIES OF HEMOGLOBIN

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On the Optical Properties of the Hemoglobin in Microdrepanocytic Disease

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