

## A RAPID DIAGNOSTIC TEST FOR SICKLE CELL ANEMIA

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SICKLE cell anemia is a congenital chronic hemolytic type of anemia characterized hematologically by the development of oat-shaped and sickle-shaped erythrocytes. Other cellular abnormalities which are due to excessive blood destruction and active blood formation are also seen in blood smears. Six to 10 per cent of Negroes possess the sickle trait<sup>2, 3</sup>; their red blood cells have the capacity to sickle, but most of these individuals do not develop anemia.

The course of the sickling process as observed under the microscope has been described in detail by several investigators,<sup>4, 5, 6, 10</sup> but little is known about the physical processes involved in sickling. It has been established, however, that the erythrocytes of individuals with sickle cell anemia and sickle cell trait become sickled when the hemoglobin is reduced.<sup>8, 14</sup> When the hemoglobin is combined with oxygen or carbon monoxide, the cells are indistinguishable in form from normal erythrocytes. The term promeniscocyte has been applied to the latter form and meniscocyte to the former.<sup>11</sup> Hahn and Gillespie<sup>8</sup> and Sherman<sup>14</sup> obtained sickling physically by reducing the partial pressure of oxygen over suspensions of promeniscocytes. They were able to reverse the process by passing oxygen or carbon monoxide over meniscocytes. When oxygen is removed from promeniscocytes, their hemoglobin aggregates in one or more foci within the cells, and the cell membrane collapses. When oxygen is added to these cells, they resume their normal contour, and hemoglobin appears to be distributed uniformly throughout their interior. Meniscocytes are strongly birefringent under the polarizing microscope<sup>14</sup> while promeniscocytes are not.

When a drop of blood is sealed between a cover slip and a slide, the decline in oxygen tension due to oxidative processes in the blood cells leads to sickling.<sup>7</sup> This is the common diagnostic test for sickle cell anemia and sickle cell trait used in clinical laboratories. Sherman found that increase in temperature, high leukocyte count, and bacterial contamination, all of which increase the rate of oxygen consumption, accelerated the sickling process. In another method, a saline citrate suspension of blood is allowed to stand in a test tube under a layer of paraffin oil until sickling takes place.<sup>1</sup> In employing any of the common diagnostic tests for sickling it is desirable to obtain blood which has a low fraction of oxyhemoglobin. Thus, the moist stasis method,<sup>13</sup> in which blood is obtained from a patient's finger after its circulation has been occluded for five minutes, gives the most rapid and consistent results. Even with this method it is sometimes necessary to observe the preparation for several hours before the result is conclusive.<sup>5</sup>

In order to find a more convenient and rapid method of producing meniscocytes

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we turned to chemical reducing agents. Sodium dithionite,  $\text{Na}_2\text{S}_2\text{O}_4$ , rapidly reduces oxyhemoglobin to reduced hemoglobin, and this property suggested its use in testing erythrocytes for sickling. When a solution of sodium dithionite was added to promeniscocytes, nearly all of the cells showed sickling or the early changes in the sickling process within a few seconds. Dithionite ion tends to decompose to thiosulfate and sulfite with formation of hydrogen ion<sup>9</sup> so that solutions made up from commercial preparations of sodium dithionite are often strongly acid in reaction; but by adding  $\text{Na}_2\text{HPO}_4$  to the solutions it is possible to increase the pH and at the same time provide a buffering medium. Hahn and Gillespie found that sickling was obtained most consistently if cell suspensions were buffered at a slightly acid pH. We have prepared a satisfactory reagent by adding 0.114 M aqueous  $\text{Na}_2\text{HPO}_4$  to 0.114 M aqueous  $\text{Na}_2\text{S}_2\text{O}_4$  until the final pH was 6.8. The ratio of the volumes of  $\text{Na}_2\text{HPO}_4$  and  $\text{Na}_2\text{S}_2\text{O}_4$  necessary to obtain this pH was about three to two.

The blood used in the following experiments was obtained from 6 different cases of sickle cell anemia, 3 of whom were being treated for exacerbations and 3 of whom were in remission. An excess of the dithionite reagent was added to promeniscocytes on a microscope slide; almost immediately changes were evident in the erythrocytes. Typical crescentic forms did not appear in large numbers, presumably because of the time required for the reduced hemoglobin molecules to become oriented in what Ponder calls the paracrystalline state.<sup>11</sup> However, nearly all of the cells underwent changes in contour, and other changes described by earlier observers took place at an accelerated rate. The forms of many of these cells corresponded to the "holly wreath" cells of Sherman and cells classified as "abnormal" by Reinhard and his co-workers.<sup>12</sup> After about fifteen to thirty minutes the aggregates of hemoglobin in many of the cells became birefringent. The presence of so many holly wreath cells is in accord with Sherman's observation that this form appears in large numbers when the rate of removal of oxygen is rapid. Since dithionite does not react with carbon monoxide, promeniscocytes saturated with carbon monoxide would not be expected to undergo changes in contour upon addition of this reducing agent. This is indeed the case. Although no sickle cell trait blood was available to us for study, there is good reason to believe that such blood would behave in the same manner as sickle cell anemia blood.\*

#### METHOD

The rapidity and simplicity of this test suggests that it would be useful as a clinical laboratory procedure for diagnosing sickle cell anemia and sickle cell trait. No special precautions are necessary in collecting the blood for this test; oxygenated cells may be used since an excess of reducing agent can always be added. The test works equally well with oxalated blood or fingertip puncture specimens and may be applied in several ways. (1) About 0.05 ml. of reagent may be added to a very small drop (about 0.01 ml.) of blood on a slide. A cover slip is then laid over

\* A brief note by da Silva (*Science* 107, 221 (1948)) which appeared since the preparation of this paper indicates that he has successfully identified sickle cell anemia (sickle cell trait) by a procedure similar to method (1) below.

the mixture and cells observed under a microscope. (2) An excess of reagent may be added to a small volume of blood in a test tube and a drop of the mixture observed. (3) A convenient method for studying the entire process of sickling in a short period of time involves the use of a hemocytometer counting chamber. The chamber is half filled with a dilute saline suspension of promeniscocytes; the reagent is then added to fill the rest of the chamber. The erythrocytes may be observed as the reducing agent diffuses into the part of the chamber which they occupy.

Since the dithionite reagent is unstable as mentioned above, its reducing power should be tested frequently by the addition of a test portion to a dilute suspension of oxygenated erythrocytes. If the reagent is satisfactory, a change from the color of oxyhemoglobin to that of reduced hemoglobin should be observed. A large volume of stock  $\text{Na}_2\text{HPO}_4$  solution may be prepared, but it is desirable to make up the  $\text{Na}_2\text{S}_2\text{O}_4$  solution daily.

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