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Hemolytic Mechanism in Sickle Cell-Hgb C Disease.* (22095)

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Splenic overactivity has been implicated in the hemolysis observed in many of the acquired hemolytic syndromes. The role of the spleen in the red cell destruction characteristic of the hereditary hemolytic anemias, however, has not been clearly defined. The available data suggest basically different mechanisms for the pathogenesis of the increased hemolysis in 2 of the hereditary hemolytic syndromes. In sickle cell anemia (homozygous Hgb S disease) the frequent development of an atrophic spleen, the so-called autosplenectomy, implies that the spleen cannot play an active role in red cell destruction. Furthermore, splenectomy in cases of uncomplicated sickle cell anemia neither leads to hematologic improvement nor results in a prolongation of the lifespan of the erythrocyte. Thus, sickle cell anemia erythrocytes transfused into a splenectomized sickle cell anemia patient still showed a markedly reduced lifespan(1). In hereditary spherocytosis, however, the evidence suggests that the increased destruction of the red cells may be mediated through the presence of a normal spleen, since hereditary spherocytosis erythrocytes transfused into a normal individual lacking a spleen have a normal survival time whereas the same cells given to a normal individual with a spleen demonstrate a sharply reduced lifespan(2). This situation exists irrespective of the pre- or post-splenectomy state of the donor with hereditary spherocytosis, or his hematologic status at the time of study. In view of these considerations, it seemed of some interest to perform similar studies on patients with sickle cell-Hgb C disease. Sickle cell-Hgb C disease is characterized by splenomegaly and signs of hemolysis which appear to vary in degree intermittently during the course of the disease. Because splenomegaly often becomes evident at times of hemolytic crises in sickle cell-Hgb C disease, there is reason to suspect that enlargement of the spleen may lead to accelerated rates of red cell destruction. By defining the part played by the spleen in the hemolysis of this disease, it was hoped that an evaluation of the effects of splenectomy and the possible use of this operation as a therapeutic procedure might thereby be made.

Material and methods. Three patients with the diagnosis of sickle cell-Hgb C disease, confirmed by electrophoretic analysis of their hemoglobin types, were used as donors. All were in a relatively asymptomatic state. The spleens of 2 of the donors were palpable just below the costal margin. From one patient, A. F., two 500 ml aliquots of blood were obtained and transfused into a normal individual with a spleen and into a hematologically normal splenectomized recipient who had

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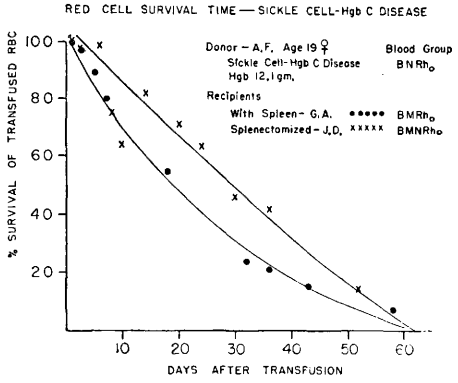

FIG. 1.

been subjected to surgery some years earlier for idiopathic thrombocytopenic purpura. From the second patient, I. F., the younger sister of A. F., 500 ml of blood were removed and transfused into a second splenectomized recipient. The survival time of the erythrocytes was followed by the Ashby technic of differential agglutination in the standard way using dried anti-M serum which gave inagglutinable counts of less than 10,000 cells per cu mm(1). The third donor with sickle cell-Hgb C disease, L. M. (blood type O N Rh_0) was utilized for a combined Ashby and Cr^{51} tagged red cell survival time study. Approximately 500 ml of blood were removed by venesection and three 20 ml aliquots of blood were tagged with 75 microcuries of Cr^{51} by a modification of the method of Weinstein, *et al.*(3). One aliquot of the Cr^{51} tagged cells was auto-transfused into the donor, a second given to a compatible normal recipient (blood type B M Rh_0), the third to a splenectomized normal individual of blood type AB MN Rh_0. The remainder of the blood was transfused into the normal recipient with a spleen who had been given the tagged cells. The disappearance of the Cr^{51} from the peripheral blood, determined at intervals of one or 2 days, was carried out in a well-type scintillation counter and the half-time disappearance ($t_{1/2}$) calculated(3). The Ashby technic was used to follow the survival of the transfused red cells in the normal recipient.

Results and discussion. In the first experiment, performed by the Ashby technic alone, no appreciable difference was noted between the survival times of red cells from the patient with sickle cell-Hgb C disease transfused into the normal and splenectomized individuals. In each instance the red cells disappeared from the recipient's circulation in approximately 50 to 60 days (Fig. 1). Similar data were obtained in the second experiment in which sickle cell-Hgb C disease erythrocytes were transfused into a splenectomized recipient. The red cell lifespan was again approximately 60 days (Fig. 2). Since no normal control was run at the same time, it is impossible to say whether this result represents a prolongation of the erythrocyte lifespan in the splenectomized subject. However, the similarity of the findings in each of

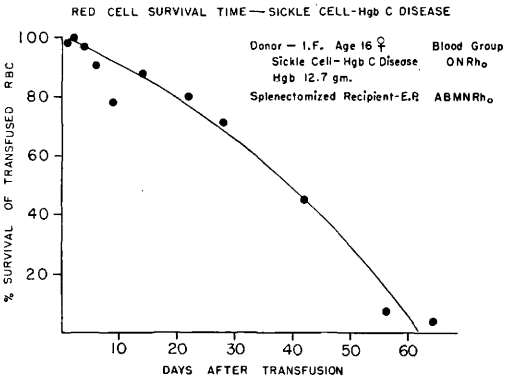

FIG. 2.

the first 2 experiments suggests that any increase in red cell survival time in the splenectomized recipient must have been slight, if at all present. In the third experiment, the half-time of disappearance of Cr^{51} tagged

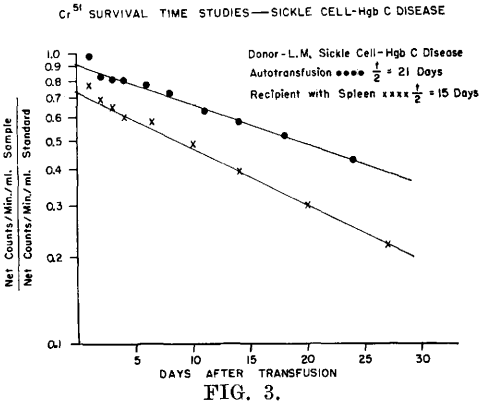

FIG. 3.

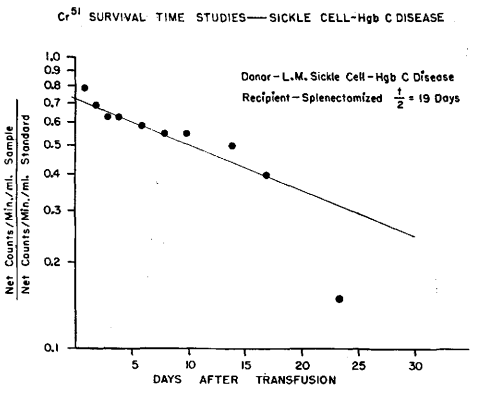

FIG. 4.

cells transfused back into the donor was 21 days, while in a normal individual these cells had a t/2 of 15 days (Fig. 3). The aliquot of labelled sickle cell-Hgb C disease erythrocytes followed in the splenectomized recipient appeared to have a t/2 of approximately 19 days, although a single satisfactory line of disappearance for the Cr^{51} could not be drawn (Fig. 4). The configuration of the curve suggested that 2 or more populations of red cells were present or that the Cr^{51} was being eluted from the pathologic cells at an inconstant rate. Nevertheless, the major proportion of the Cr^{51} disappeared at approximately the same rate in the splenectomized and non-splenectomized recipients. The erythrocyte lifespan by the Ashby technic was, as in the instance of the first 2 donors, approximately 50 days in the normal recipient (Fig. 5).

These observations suggest that the intrinsic defect of sickle cell-Hgb C erythrocytes, like that of sickle cell anemia red cells, is primarily responsible for the shortened survival time of the red cells. As in sickle cell anemia, hemolysis proceeds at approximately the same rate whether a spleen is or is not present. The pathogenesis of the hemolytic mechanism is, therefore, somewhat different from that seen in hereditary spherocytosis where the presence of a normal spleen is required for red cell destruction to take place at an increased rate. These studies do not throw any light on the site of red cell destruction in sickle cell-Hgb C disease. It has been postulated that in sickle cell anemia, by virtue of the abnormal shape of the red cells, plugging of small vessels with resultant stasis and hemolysis takes place. Since this phenomenon may occur in any small vessel suitably situated, the spleen does not selectively trap such cells. In hereditary spherocytosis, the spherical erythrocytes are believed to be selectively trapped in the sinusoids of that organ. It would seem, therefore, that the sickle-shaped erythrocytes in sickle cell-Hgb C disease behave much like the red cells in the related homozygous Hgb S condition.

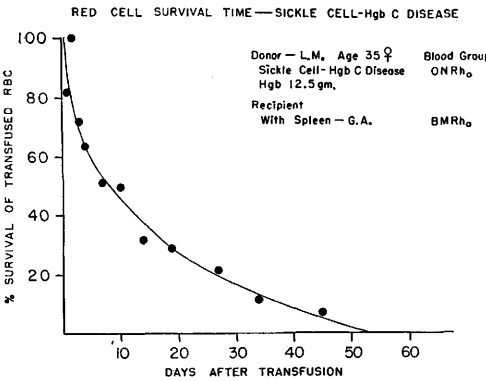

FIG. 5.

In vivo sickling can be correlated with the percentage of the total hemoglobin in the form of Hgb S(4,5). Since the majority of patients with sickle cell anemia have approximately 90% Hgb S(6,7) while those with sickle cell-Hgb C disease have from 45-50% Hgb S(8), it would be expected that under similar conditions hemolysis as a consequence of the mechanical factors mentioned above would take place more readily in sickle cell anemia. Furthermore, since some patients with homozygous Hgb S disease have as little as 60% Hgb S(9) while some with sickle cell-Hgb C disease have over 63% Hgb S(10), some overlap in the severity of the anemia and degree of hemolysis should be encountered if red cell destruction in these sickling diseases is to be explained on the basis of mechanical trapping, stagnation and lysis. Clinical and hematologic data in the 2 sickle cell diseases are consistent with these tenets and support this concept. It would be of extreme interest to try to localize if possible

major sites of stasis and destruction in these sickling disorders. Unfortunately, body scanning following the injection of Cr^{51} tagged cells was not possible, but such studies would undoubtedly provide important relevant information.

Another problem left unanswered by the present investigation is the role of the spleen during periods of clinical crisis. Characteristic of the crisis of sickle cell-Hgb C disease is the progressive development of marked anemia and a rapidly enlarging spleen. The clinical findings are, therefore, in marked contrast to those observed in the usual variety of crisis seen in sickle cell anemia, where the abdominal, muscle and joint pains are not accompanied by any specific hematologic changes nor any increase in the degree of hemolysis(11). Abdominal pain in sickle cell-Hgb C disease is usually localized in the left upper quadrant and can be correlated with the remarkable change in the size of the spleen. It seems more than likely that splenic overactivity, either by virtue of its increasing mass, or as a result of a more specific role in sequestering the abnormally shaped red cells, leads to the development of the severe anemia seen during periods of crisis in sickle cell-Hgb C disease. Furthermore, the number of patients with sickle cell-Hgb C disease who appear to develop the first episode of anemia following trauma to the left upper quadrant area strongly suggests that splenic enlargement may actually be the precipitating factor in the pathogenesis of the crisis, while the anemia is a secondary phenomenon. Unfortunately none of our patients was suffering from this complication at the time of observation, but repeat Cr^{51} auto-survival time studies of the erythrocytes during periods of quiescence and crisis will make it possible to evaluate this problem.

Summary. Red cells from 3 patients with sickle cell-Hgb C disease transfused into normal individuals with and without a spleen, were found to have a survival time of approximately 50 days. The spleen, therefore, does not appear to have a critical role in the hemolysis occurring in this disease. Possible mechanisms for the red cell destruction, based on the per cent of Hgb S present, as well as the role of the spleen during periods of crisis, are discussed.

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