SUMMARY  Spherocytic deer mice provide a model of human spherocytosis. Their erythrocytes are abnormal in shape and are more rigid than normal red blood cells (RBC). Like their human counterparts, spherocytic mice are anemic. Measurements of RBC velocity in microvessels on the cerebral surface failed to reveal a difference between the velocity of cells in spherocytic as compared to normal deer mice. However, plasma transit, as measured by fluorescein microangiography, was faster than normal. Both decreased plasma transit time and increased RBC velocity are expected in nonspherocytic, anemic mice. Since the former, but not the latter, was found in the spherocytic, anemic mice, it appears that increased RBC rigidity has a greater effect on RBC movement than on plasma movement within the cerebral microcirculation. Thus it would seem that this increased RBC rigidity prevents the increased RBC velocity that otherwise would be observed in anemia but does not prevent the decrease in plasma transit time.

APPROXIMATELY 20 years ago hereditary spherocytosis was recognized in the deer mouse. The hematological, pathological, and physiological features of this syndrome were shown to be similar to those of spherocytosis in man. In spite of the interest one might expect in this model of a disease with abnormal red blood cells (RBC), little use appears to have been made of the affected animals for studies of blood rheology or of the microcirculation.

Our interest in the animal model of spherocytosis was prompted by our previous studies of the cerebral microcirculation in other models of rheological disorders. In these earlier investigations on mice we demonstrated that two hypersensitive, macroglobulinemia and polycythemia, exerted a greater effect on plasma flow than on RBC velocity. Thus plasma flow was retarded more than RBC velocity, a phenomenon that appeared to accentuate the normal difference between the velocity of cells and plasma in RBC. We ascribed this accentuation to an increase of plasma skimming in the cerebral microcirculation. On the other hand, we also investigated acutely anemic mice and found that both plasma velocity and RBC velocity were accelerated, without a demonstrable difference in the degree to which the plasma was accelerated in relation to the RBC. The studies just referred to were investigations of conditions in which blood viscosity was altered either by increased plasma viscosity, increased hematocrit (Hct), or decreased Hct. The availability of deer mice with spherocytosis provided us with an opportunity to study the effects of abnormal RBC shape and rigidity in a small rodent resembling the mouse of our earlier investigations. The new study employed the same techniques and measured the same parameters as the old. Unfortunately, the availability of deer mice was severely limited by the loss of one of two colonies maintained in this country, and by the small size of the other. Nevertheless, the data presented below appear to provide a basis for valid conclusions which, in retrospect, fit a hypothesis that could have been constructed from our data concerning the other models of rheological abnormality.

Methods

Deer mice (Peromyscus maniculatus) of both sexes were used. Spherocytosis was established on the basis of splenomegaly, increased RBC fragility, and examination of a blood smear. Studies of RBC velocity and plasma transit time were made by observing vessels on the cerebral surface. These vessels were exposed by craniotomy in anesthetized mice, prepared as previously described. Measurements of RBC velocity were made with ultra high speed microcinematography which permits RBC tracking from frame to frame. Plasma transit time from arteriole to venule was determined by analyzing movie film exposed at lower framing rates (40/sec) during passage of fluoresceinated plasma after intravenous injection of sodium fluorescein.

Microhematocrits were determined by routine techniques using a Clay-Adams centrifuge, and blood gases were analyzed by an Instrumentation Laboratories Ultramicro blood gas apparatus. Blood pressures were measured by employing a tail cuff and pulse transducer.
Results

ERYTHROCYTE VELOCITY IN CEREBRAL MICROVESSELS

Fifteen spherocytic mice were examined. In some, RBC velocity was measured in arterioles; in others, in venules; and in a few, measurements could be made in both types of vessel. In some vessels RBC could be seen with sufficient clarity on the film, to be tracked as they moved in the center of the blood stream. In a greater proportion of vessels, clarity was sufficient for tracking only along the vessel wall. In some vessels a velocity pulse could be observed and measurements were obtained at the peak and nadir of a cardiac cycle. In many more vessels the cardiac cycle was not readily manifest in the microcirculation, and therefore measurements were made at random. The data are summarized in Figure 1, which compares normal and spherocytic mice with respect to nadir, peak, or random velocities of RBC. All data points are entered in the figure, which clearly shows no difference between RBC velocities in spherocytic and control mice. The venules in this portion of the study were 14-55 μm in diameter and the arterioles were 13-26 μm in diameter. The control and experimental mice did not differ with respect to the size of the vessels examined.

PLASMA TRANSIT TIME

The passage of fluoresceinated plasma was measured as it moved from surface arteriole to surface venule in 14 spherocytic and 14 control mice. Transit time was 0.67 ± 0.21 seconds in the controls, and 0.48 ± 0.25 seconds in the spherocytics. This difference is significant (t = 2.1, P < 0.05). The venules observed in this part of the study were 15-70 μm in diameter, and the arterioles were 19-76 μm in diameter. The control and experimental mice did not differ with respect to the size of the vessels observed.

HEMATOCRIT, BLOOD GASES, AND BLOOD PRESSURE

The Hct of spherocytic mice was 29 ± 5, and that of nonspherocytic animals was 34 ± 5. Thus the spherocytics were significantly anemic, or significantly more anemic than the controls (P < 0.02).

Arterial Pco₂ was obtained at the end of the experiment in 13 control and 15 spherocytic mice. It was 36 ± 8 mm Hg in the former and 42 ± 10 mm Hg in the latter, an insignificant difference. Arterial Po₂ was 82 ± 13 mm Hg in the former and 93 ± 10 mm in the latter. This difference was significant at the 5% level.

The blood pressure was successfully measured for only six spherocytic animals and six normal mice. It was 98 ± 29 mm Hg in the former and 87 ± 12 mm Hg in the latter, an insignificant difference.

Discussion

We expected that in spherocytosis, RBC velocity would be retarded because of decreased cell flexibility or deformability. Moreover, the decreased deformability would be expected to lead to an increased blood viscosity and would place spherocytosis among the hyperviscosity syndromes. In which our previous investigations showed a reduction of plasma velocity even greater than the reduction in RBC velocity. Thus we expected not only that RBC velocity might be reduced, but that plasma transit time might be even more strikingly prolonged. Instead, our data supplied two surprises. First, RBC velocity in spherocytics was in the same range as that in normal deer mice. Second, rather than a prolonged transit time, plasma showed a significantly reduced transit time.

One possible explanation for these data comes from a consideration of the effects of anemia observed in the spherocytic mice. It is well known that viscosity falls as the Hct falls. We have shown in an earlier study of cerebral microcirculation that anemia is accompanied by an increased RBC and plasma velocity. Since in the spherocytic mice Hct was significantly lower than the controls, we can explain our data by suggesting that the anemia was reflected in an actual reduction in plasma transit time, an acceleration of plasma transit like that observed in our previous study of acutely anemic but otherwise normal mice. We can suggest also that anemia counteracted the effects of RBC rigidity on cell velocity, so that reduced RBC velocity was not observed. We have demonstrated a similar "protective" effect of anemia in macroglobulinemia. However, although RBC's were not slowed in spherocytics, it is also of interest that an increased RBC rigidity did have an effect in the spherocytic mice; an effect sufficient to prevent the RBC acceleration that otherwise would have appeared in the presence of anemia.

It is of interest to compare the present data with that of our earlier studies of hyperviscosity states. In the latter we found a differential effect of hyperviscosity on RBC and
plasma movement. Thus, if viscosity is elevated by increased numbers of RBC, by RBC aggregation, or by increased plasma viscosity, the effect is greater on plasma than on cells, and thus retards the former more than the latter. In contrast, if our inferences from the present study are correct, increased cell rigidity exerts a greater effect on cell velocity than on the velocity of the surrounding plasma; it thus prevents an absolute increase in RBC velocity in anemia, but does not prevent an increase in the velocity of plasma, as shown by the decreased plasma transit time.

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