THE EFFECT OF STASIS OF BLOOD IN VARICOSE VEINS ON ERYTHROCYTE FRAGILITY, WITH ACCOMPANYING STUDIES COMPARING RED CELLS AND OTHER BLOOD ELEMENTS WITH CUBITAL VEIN BLOOD

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IT HAS been demonstrated by Fahraeus\(^4\) that normal red blood cells have a tendency to become spheroidal in shape after standing in vitro at body temperature. Gänsslen,\(^6\) Haden\(^6\) and Castle and Daland\(^7\) have shown that spheroidal red cells are more susceptible to osmotic hemolysis than are normal corpuscles. Haden found that normal red cells when suspended in graded hypotonic salt solutions become progressively more globular as the solution becomes more hypotonic, and that there is a direct relationship between the volume thickness index and fragility of the red blood cells. Ham and Castle\(^8\) and Tsai, Lee and Wu\(^9\) have further stated that red cell fragility is increased by stasis of the blood in vitro at body temperature. These investigators found that between one-half and two and one-half hours of stasis was necessary before the first manifestation of increased fragility appeared. Spontaneous hemolysis appeared after approximately twelve hours of stasis.

During stasis of blood at body temperature there is also an increase in packed cell volume due to the development of spheroidal cells.\(^8\) There is also evidence that under some conditions, in vivo stasis produces increased red cell fragility. Waller\(^12\) and Cormick\(^13\) found an increase in red cell fragility in capillary blood following tourniquet stasis, but Waller found no increased fragility in blood removed from the cubital vein under conditions of stasis except after expressing capillary blood into the veins.

There is evidence that concentration and stasis of red blood cells occur normally in the spleen.\(^11\) Red cells obtained from the splenic vein were found to be more fragile when suspended in hypotonic salt solutions than red cells from blood in other veins.\(^8\) As further proof that in vivo stasis may cause red cells to become more fragile, Tsai and co-workers\(^8\) found that osmotic fragility of red cells removed from both the splenic and renal veins increased progressively following stasis produced by occlusion of the veins and arteries of the spleen and kidney.

Ham and Castle\(^8\) have attached great importance to the effects of stasis on red cells and consider this factor to be the common denominator in many of the anemias due to hemolysis. They believe that erythrostasis in the spleen is probably the mechanism producing increased blood destruction in the hemolytic anemias with increased red cell fragility, and also, that an unusual degree of erythrostasis might account for some hemolytic anemias in which there is normal or secondarily increased red cell fragility. A number of investigators\(^8\) have shown that the red cells usually become less fragile to hypotonic salt solutions.

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following splenectomy. Ham and Castle interpret the beneficial effect of splenectomy as being due to the removal of the organ in which a large degree of red cell stasis (and thus increased fragility) occurs. They explain certain anemias associated with splenomegaly on the basis of a probable increase in normal splenic function with respect to erythrocytic stasis.

In an attempt to evaluate further the effect of in vivo stasis as a possible mechanism for increasing red cell fragility, the present investigation was undertaken to measure the osmotic fragility of red blood cells from veins in 20 patients without any known hemolytic tendency or other blood dyscrasia. Although the degree of stasis in varicose veins is not known it seems well established that the movement of blood in varicosities is very sluggish. Ochsner and Mahorner visualize the leg with varicose veins as having a circulation of its own. They consider the possibility of a given blood cell remaining in the venous system of the leg indefinitely ‘coming up each time perhaps to the opening of the saphenous where it again becomes one of the unhappy ones to fall through the opened sluices peripheral in the superficial venous system.’

McPheeters and Rice studied the direction of blood flow in leg varicosities and discussed in detail the movement of lipiodol injected into the varicosities of two patients with a positive Trendelenburg test. One subject was recumbent and the other sitting with legs horizontal. In both cases the injected lipiodol remained stationary until the patient tensed the abdominal muscles or moved the feet, following which the lipiodol was seen to move distally. In their experience the dye never moved centrally. However, Schmier and Heller observed that injected radio opaque material moved in a central direction. Heller determined the specific gravity of blood removed from the varicose vein under observation and then injected radio opaque dye of the same specific gravity. He found in patients having varicose veins and competent valves that the circulation was directed centrally but at a slower rate than in the normal control. In patients with incompetent valves the flow was nearly stationary but after the patient remained standing for some time a very slow upward flow developed. Coughing or straining rapidly reversed the flow and forced the opaque substance distally. He also noted that when the patient first stands after being in a supine position there is a surge of blood down the varicose vein. While there is no definite proof that a significant quantity of blood stagnates in the varicose vein for hours it is evident that abnormal erythrocytic stasis does occur.

**Method**

The patients used in this study had all been previously examined in the Varicose Vein Clinic of the University of California Medical Center. Each patient was requested to stand quietly for a period of at least fifteen minutes. Blood was then drawn from a tortuous dilated superficial vein, usually on the calf, and immediately afterward a similar sample was obtained from the cubital vein without the aid of a tourniquet.

The following studies were made on the two specimens of blood: (1) The osmotic fragility of the red cells. (2) The packed cell volume. (3) Hemoglobin, red blood count, white blood count and platelet count (Rees and Ecker method). (4) Plasma protein (Falling drop method).

All of the laboratory determinations were performed by one of us.
The valves of the long saphenous veins were incompetent in each of the patients tested (Sp., Di., and Whi. were not tested). The clinical degree of tortuosity and dilatation of each patient is indicated in table 1. One patient (Di) had a varicose ulcer. There was neither evidence of congestive heart failure nor obvious blood dyscrasia in any of the patients studied.

Results

1. Hypotonic fragility. The resistance of the red blood cells to hypotonic solutions of saline was determined on blood from the cubital and varicose veins of 19 otherwise healthy patients. The fragility of the red blood cells from the varicose veins was not significantly different from the fragility of red cells obtained from the cubital veins (table 1). In each patient the red blood cell fragility fell within the normal range in both the varicose vein and the cubital vein specimens.

2. Red cell count, packed cell volume, serum protein, hemoglobin, white cell count and platelet count. The increased pressure in varicose veins should cause fluid transudation into the tissues which would be expected to produce hemoconcentration of the varicose vein blood. However, Erb and Tickense found no increase in red cells, red cell fragility, white cells or platelets in blood from varicose veins as compared with cubital vein blood.

(a) Our results show a small but significant increase in red blood cell count in blood from varicose veins as compared with the cubital vein blood. Statistical calculations show P < 0.05, the mean difference being 108 million red cells. This is indicative of a minor degree of hemoconcentration.

(b) Surprisingly enough the packed cell volume of varicose vein blood was only suggestively higher

*Statistical analyses of our data were made by Dr. John C. Talbot of the University of California Medical Center.
than cubital blood (P slightly < 0.05). If, as result of stasis, some red cell swelling had occurred some increase in packed cell volume would result, and added to a certain amount of hemoconcentration it would be expected that the packed cell volume would increase out of proportion to the red cell increase.

(c) There was a suggestive increase in total serum protein in varicose veins compared with cubital veins (P somewhat > 0.05). A more accurate technic or a larger series would be necessary to establish the significance of this apparent increase.

(d) There was no significant difference in hemoglobin, platelets, or white cells in the varicose and cubital vein samples.

COMMENT

As mentioned earlier, the preponderance of evidence indicates a slow, steady progression of blood centrally in varicose veins with reflexes of blood following straining and coughing and other activities which increase the intra-abdominal pressure. It seems likely, then, that the slow movement of blood through varicose veins does not produce stagnation comparable to that which has been shown necessary to produce red cell swelling and increased fragility in the test tube. In an attempt to establish roughly the duration that a measurable quantity of blood remains in the varicose vein, we, in collaboration with Doctors J. Hopper, Jr., and C. J. Mudrick, performed the following experiment:

Evans blue dye (Ti814) was injected into a varicose vein of a patient with marked varicosities. No dye appeared in the cubital vein blood until two minutes after the injection. The dye concentration gradually increased and finally leveled off fifteen minutes after the injection. Dye did not appear in the varicose veins of the opposite leg until four minutes following injection and failed to reach the dye concentration of the arm in a period of thirty minutes. It has been shown that dye injected in an arm vein of normal subjects appears in samples of blood taken from the other arm within thirty seconds and levels off within three to four minutes.34 Obviously our experiment in one patient and without controls has no comparative value but it does indicate that in this patient a certain amount of stasis occurred in the varicosity for about fifteen minutes. Further experiments on this problem are in progress.

Our data permit no final conclusions regarding the importance of the factor of stasis in hemolytic diseases. However, the lack of increased red cell osmotic fragility under the conditions of stasis that occur in varicose veins suggests that erythrostasis of a moderate degree does not play a major part in most hemolytic diseases. We are inclined to agree with the viewpoint of Dameshek and Miller32 that the hemolytic states are due to a number of different causes such as hemolysins, agglutinins and inherited red cell abnormalities with such supplementary factors as stasis, trauma, and possibly, chemical and hormonal changes augmenting the occurrence of hemolysis. It seems likely that several factors are operating at once. For example, in the presence of hemolytic disease, increased stasis and increased trauma to the red cells might be expected to produce some increase in the degree of hemolysis. It also seems likely that in order for stasis appreciably to augment hemolysis in any given hemolytic syndrome it is necessary for stagnation to occur over a prolonged period of time. Tsai9 showed that increased red blood cell fragility did not appear until stasis had been present for one-half hour to two and one-half hours and that hemolysis did not begin until about twelve hours of stasis. Also in
our experiment and in Waller's\textsuperscript{12} a degree of stasis beyond that normally existing did not produce a significant increase in fragility in blood from veins. Therefore, the amount of stasis present in congestive failure or produced by increased blood viscosity caused by the increase in globulin in infections as suggested by Castle\textsuperscript{8, 17} would hardly seem sufficient to produce hemolysis. It is probable that the spleen is the only organ in the body in which stasis, sufficient to cause a significant increase in hemolysis, might occur.

The absence of a greater degree of hemoconcentration than we found in varicose vein blood is difficult to understand. Beecher\textsuperscript{33} found a gross filtration pressure of 50 cm. of water in excess of the colloid pressure of the blood in varicose veins and concluded that normal resorption of tissue fluid at the venous end of the capillary was impossible and all tissue fluid must be carried off by the lymphatics. This should result in marked hemoconcentration but our experiments showed evidence of only mild hemoconcentration. Obviously, factors are involved which have not been adequately explored.

Our results confirm the findings of Erb and Tiefensee\textsuperscript{39} that there is no significant increase in white cells, platelets, and red cell fragility in blood from varicose veins as compared with cubital vein blood. However, our finding of a significant increase in red cells in the varicose vein is at variance with their conclusion that the red cells were not significantly higher than in the cubital vein.

**SUMMARY AND CONCLUSIONS**

1. Blood from varicose veins was compared with cubital vein blood in 20 patients in order to determine whether or not the degree of stasis present in varicose veins would increase red cell fragility. Corollary studies consisted of comparative determinations of red cells, hemoglobin, packed cell volume, white blood cells, platelets and serum proteins.

2. There was no increase in red cell fragility in the varicose vein specimen, indicating that the theory that minor degrees of intravascular erythrostasis contribute substantially to some of the hemolytic anemias is untenable.

3. There was a small but statistically significant elevation in red cells per cu. mm. in varicose vein blood as compared with blood from cubital veins. There was a suggestive, but not significant, increase in packed cell volume and serum protein in the varicose vein samples. The evidence indicates a mild degree of hemoconcentration.

4. White cells, platelets and hemoglobin determinations were found to have the same values in varicose vein blood as in blood from the cubital vein.

**REFERENCES**


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