ROULEAUX FORMATION INTENSITY OF PLASMA AND E.S.R.

BY

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The term "rouleaux formation intensity" (R.F.I.) is introduced to account for the different degrees of red-cell aggregation due to the effect of plasma factors on the erythrocytes.

It is generally agreed that the erythrocyte sedimentation rate (E.S.R.) corresponds to the R.F.I. of the plasma. This holds true if the Westergren (1921) technique is employed, but not necessarily with the use of the Wintrobe method (Wintrobe and Landsberg, 1935). Both methods may yield discrepant results. Values obtained with Westergren's method are thought to correspond more to the clinical condition of the patient than those secured with the Wintrobe technique. Davis (1946) cited cases with high Wintrobe but normal Westergren E.S.R.s. According to several reports (Gilmour and Sykes, 1951; Goldberg, Glynn, and Bywaters, 1952; Goldberg and Conway, 1952) the very opposite findings of high Westergren and normal Wintrobe E.S.R.s in many seriously ill patients occur even more frequently—for example, in cases in which the Westergren values were high, as anticipated, and the Wintrobe readings were normal or only slightly increased.

The following factors were believed to explain the discrepancy of findings by the use of the two methods: effect of the anticoagulant on the pH (Goldberg, Glynn, and Bywaters, 1952), the existence of the abnormal cryoglobulin in the plasma (Goldberg and Conway, 1952; Milles and Salt, 1952), the influence of diameter of sedimentation tubes (Milles and Salt, 1952), the so-called "reflux phenomenon" (Alexander, 1953), and the inhibition of rouleaux formation in deoxygenated blood (Duxbury, 1953).

This paper is a contribution to the discussion of the reasons for the discrepancy of findings with the Westergren and Wintrobe methods in an attempt to settle the question which method is preferable for clinical use.

Methods

Blood samples were placed into tubes containing heparin (0.02 ml. of 5% pure heparin solution per 10 ml. of blood—that is, 0.1 mg. of heparin per 1 ml. of blood) or the powdered oxalate mixture of Heller and Paul (8 mg. of potassium oxalate and 12 mg. of ammonium oxalate per 10 ml. of blood).

Plasma and cells were then separated after centrifugation at 3,000 r.p.m. for 20 minutes. From each blood sample, eight mixtures or more were prepared, with a packed cell volume (P.C.V.) varying from 12.5 to 55%. Each of these mixtures, non-modified, or altered by the addition of one-fourth of its volume of 3.8% sodium citrate solution (1 ml. of mixture + 0.25 ml. of solution), was transferred into a calibrated Wintrobe haematocrit tube (100 mm. long, 3 mm. diameter) and maintained in a vertical position. For each blood sample, therefore, the sedimentation of at least 16 different combinations of cells and plasma was examined: eight mixtures of plasma and cells, and the same eight mixtures diluted according to the Westergren method. The comparison of the two methods is thus easy to be inter-

preted, since the blood, diluted and undiluted, is allowed to sediment in tubes of the same height and bore.

After E.S.R. readings were taken the tubes were centrifuged 30 minutes at 3,000 r.p.m. for P.C.V. determination. The P.C.V. of the mixture containing citrate solution is uniformly reduced to more than 20% compared with the corresponding undiluted mixture. Thus a very high E.S.R., approaching its maximum in one hour, gives a somewhat greater value in the diluted samples than in the undiluted.

For each sample a microscopical examination of rouleaux formation, the electrophoretic pattern of serum, and the fibrinogen content of plasma was made. The R.F.I. was estimated arbitrarily from observations of its occurrence in different fields, as follows: normal (N), slightly increased (1+), moderately increased (2+), and strongly increased (3+). For the sake of brevity, only the one-hour readings are reported here.

Results

1. Comparison of E.S.R. with Undiluted and Diluted Blood of Varying R.F.I.

Normal Plasma.—When the P.C.V. is normal (45%) the E.S.R. of undiluted blood is very low (Fig. 1 (a)). However, as the P.C.V. is reduced the E.S.R. is strongly accelerated, reaching a high rate when the P.C.V. is very low. The E.S.R. of the same diluted blood is consistently lower, and the difference between the two becomes more pronounced the lower the P.C.V. becomes.

Slightly Increased R.F.I. of Plasma.—The E.S.R. of diluted blood is slower when compared with undiluted blood. Only when the P.C.V.s are very low or very high are the E.S.R.s equal (Fig. 1 (b)).

Moderately Increased R.F.I. of Plasma.—The E.S.R. of undiluted blood is very rapid and exhibits a new phenomenon, as shown in Fig. 1 (c). When the P.C.V. is very high (above 48%) the E.S.R. is suddenly lowered, as illustrated by the sharp bend of the curve. The E.S.R. of diluted blood is slightly lower than that of the undiluted when the P.C.V. ranges from 28 to 50%, and, on the contrary, slightly higher when the P.C.V. is either very low (less than 28%) or very high (about 50%).

Strongly Increased R.F.I. of Plasma.—The abrupt downward inflection of the curve shows that the sedimentation of undiluted blood is slowed down suddenly when the P.C.V. is within physiological limits (Fig. 1 (d)). When the P.C.V. is above 40% the E.S.R. values are low. On the contrary, the E.S.R. is very high when the P.C.V. is below 40%. This particular feature is not observed in the sedimentation of diluted blood: then the E.S.R. remains high and is always greater than in undiluted blood. The sudden downward bend of the sedimentation curve of undiluted blood stresses the important fact that the difference between the E.S.R.s of diluted and undiluted blood is particularly high when the P.C.V. is above 40%.

The above findings show that the E.S.R. is not necessarily proportional to the R.F.I. of the plasma. This is demonstrated with undiluted blood, especially in two conditions: (1) rapid sedimentation when the R.F.I. is normal and the P.C.V. is very low (Fig. 1 (a)), and (2) a very slow sedimentation when the R.F.I. is very strong and the P.C.V. exceeds 40% (Fig. 1 (d)).

2. Effect of Fibrinogen Content of Suspension Medium of Erythrocytes on the S.R. with Varying P.C.V.

It is well established that a correlation exists between the fibrinogen content and R.F.I. of plasma. The higher the fibrinogen content of a given sample the greater is the R.F.I. (Fahraeus, 1929; Zarday and Farkas, 1931; Kylin, 1935; Gray and Mitchell, 1942; Frimberger, 1942).

We examined the sedimentation rate of red cells suspended in plasma exhibiting strong R.F.I., in the serum from the same blood sample, and in mixtures of plasma and serum calculated to give fibrinogen contents of 20, 40, 60, and 80% of the total fibrinogen of the native plasma. Thus R.F.I. increases with increasing fibrinogen contents—

that is, the lowest R.F.I. is found in serum and the highest in native plasma. Fig. 2 shows the findings of E.S.R. plotted against fibrinogen content of the suspension medium with P.C.V.s of 45% and of 30%. The blood for this experiment was secured from a patient suffering from rheumatoid arthritis. The fibrinogen content of his native plasma amounted to 10 g. per litre, and upon electrophoretic analysis a marked increase of alpha and beta globulins was found.

Fig. 2 (a) shows that the sedimentation is very slow in the undiluted sample containing native plasma. Paradoxically, it is increased when the fibrinogen content is reduced. The curve has two maxima, and falls off at the lowest

increase the E.S.R., and this is particularly important when the R.F.I. of the plasma is very strong (Fig. 1 (d)). For example, when the P.C.V. is reduced from 45 to 36%, a decrease of 20%, the E.S.R. is increased from 18 to 57 mm.

Dilution of Plasma.—The plasma is diluted to a great extent when citrate solution is added in the customary proportion of one-fourth of the volume of the blood sample. The plasma dilution is approximately one-third when the P.C.V. of the blood sample is 45%, and only one-fourth when the P.C.V. is 20%. The effect of this dilution on the E.S.R. differs according to the case. When the R.F.I. of plasma is normal, the dilution of plasma reduces the R.F.I. considerably—nearly to the point of abolishing it

FIG. 1.—E.S.R. (one-hour readings) plotted against the P.C.V. of undiluted blood samples. The E.S.R. of diluted samples is plotted on the same vertical line as that of the corresponding undiluted sample. (a) Normal plasma. (b) Plasma with slightly increased R.F.I. (c) Plasma with moderately increased R.F.I. (d) Plasma with strongly increased R.F.I.

FIG. 2.—E.S.R. (one-hour readings) plotted against the fibrinogen content of the erythrocytes' suspension medium (see text). (a) The P.C.V. of undiluted samples is 45%. (b) The P.C.V. of undiluted samples is 30%.

fibrinogen content. However, when the same samples are diluted with the citrate solution the E.S.R. corresponds to the fibrinogen content of the preparation, reaching the highest value for the native plasma, while the undiluted samples show the lowest E.S.R. value. Thus the curve of the E.S.R. of undiluted samples demonstrates that in certain cases the lowering of the R.F.I. can accelerate the E.S.R. This paradoxical phenomenon, which may be compared to the "zone phenomenon" of immunology, does not occur under the following conditions: (1) when the samples are diluted in accordance with Westergren's method; and (2) when the P.C.V. is lowered to 30%, as shown in Fig. 2 (b), or less.

Discussion

1. Causes of Discrepancy Between the E.S.R. of Undiluted and Diluted Blood

Three factors of unequal importance contribute to this discrepancy—that is, the variation of the P.C.V., the presence of plasma dilution, and the action of sodium citrate.

Variation of P.C.V.—We have shown that the P.C.V. of diluted blood is more than 20% lower than that of undiluted blood. Such a reduction in the P.C.V. tends to

completely. However, when the R.F.I. of plasma is strongly increased it still remains rather high after dilution. Moreover, such a weakening of the R.F.I. can accelerate the E.S.R., as we have shown. It is understandable that under certain conditions—for example, high P.C.V. and high R.F.I. of a given blood sample—the dilution of blood has the cumulative effect of increasing the E.S.R., both in lowering the P.C.V. and the R.F.I.

Action of Citrate Salt.—It has been claimed that this salt inhibits rouleaux formation. We found that in the amounts employed in this study the action of this salt is insignificant compared with the two factors discussed above. These factors are sufficient to explain the differences observed between the E.S.R. of undiluted and diluted blood. A blood with a strong R.F.I. and a normal P.C.V. shows marked discrepancy in its E.S.R. according to the manner in which it is collected. Such blood, made incoagulable by dried heparin or the powdered oxalate mixture of Heller and Paul, has a low E.S.R. The same blood mixed with one-fourth its volume of 3.8% sodium citrate solution or with 7.6 mg. of powdered sodium citrate per ml.—the amount of salt used in the Westergren's method of dilution—gives a very high E.S.R. Thus the single fact of blood

dilution or its absence cannot explain the discrepancy of E.S.R. findings. Therefore the explanation must lie in variations of the P.C.V. The heparinized and the oxalated blood samples have their P.C.V. unchanged and their plasma undiluted. Conversely, the blood in citrate solution has a reduced P.C.V. and a diluted plasma. The blood mixed with powdered sodium citrate, although apparently undiluted, behaves like diluted blood: its P.C.V. is lowered because of cell shrinkage, and consequently the plasma is diluted by the intracellular fluid given off. Thus it is not the citrate salt itself which is responsible for the changes in the E.S.R., but rather the changes in the cell volume and plasma dilution.

The difference, therefore, in sedimentation between diluted and undiluted blood is essentially due to the simultaneous action of two factors: P.C.V. lowering and plasma dilution. When the R.F.I. of plasma is normal or slightly increased these two factors act in an inverse way. The increase of the E.S.R. due to the lowering of the P.C.V. is less than the lowering of the E.S.R. due to plasma dilution. Consequently, the E.S.R. of diluted blood is slower than that of undiluted blood. When the R.F.I. of plasma is strongly increased the accelerating effect of the P.C.V. decrease is marked, while the dilution of plasma has only a slightly decreasing effect or, sometimes, an accelerating action. Therefore sedimentation is much quicker with diluted than with undiluted blood.

The discrepancy between the E.S.R. of diluted and undiluted blood occurs under the following conditions: (1) slow E.S.R. of diluted blood with rapid E.S.R. of undiluted blood signifies a normal R.F.I. of plasma and a very low P.C.V. (Fig. 1 (a)); (2) slow E.S.R. of undiluted blood with very rapid E.S.R. of diluted blood signifies strong R.F.I. of plasma with a high P.C.V. (Fig. 1 (d)); (3) moderately increased E.S.R. of undiluted blood with very rapid E.S.R. of diluted blood signifies a strong R.F.I. of plasma with a P.C.V. between 30 and 45% (Fig. 1 (d)).

2. Clinical Significance of the E.S.R. of Diluted and Undiluted Blood

The E.S.R. test is employed in the clinic in order to detect approximately modifications in the protein composition of plasma. The degree of abnormality of plasma composition, shown by the increase of globulin and fibrinogen, corresponds to the degree of the R.F.I. increase of plasma. A method of measuring the E.S.R. cannot be useful to the clinician unless the E.S.R. values correspond to the R.F.I. of plasma. In the following it will be shown which of two techniques generally employed—blood undiluted (Wintrobe) and blood diluted (Westergren)—is more apt to give adequate information about plasma alterations.

First, let us suppose that the blood to be examined has a normal haematocrit value, averaging 45%. In normal plasma the sedimentation of undiluted blood is slow, but is rapidly accelerated with increasing R.F.I. of plasma, reaching the maximum when the R.F.I. is moderately increased. If the plasma change becomes still more pronounced, the E.S.R., far from becoming increased, is progressively decreased. As illustrated in Fig. 2 (a) the E.S.R. becomes very slow in plasma with the highest alteration, and therefore is misleading to the clinician. This is not the case when the blood is diluted by one-fourth its volume with sodium citrate solution. Here the E.S.R. increases progressively according to the degree of plasma alteration. It is slower in comparison with undiluted blood when the plasma is normal, and reaches a very high value when the plasma is greatly altered.

Let us suppose that the blood to be examined is anaemic, with a haematocrit reading of 30%. In the case of normal plasma this anaemic undiluted blood already has a relatively rapid E.S.R. A very small change in the protein composition of the plasma will bring the E.S.R. to its maximum, as shown in Fig. 2 (b). Further accentuation of the plasma change will not further modify it. With blood diluted one-fourth its volume by sodium citrate solution, the results are

quite different. Dilution decreases the E.S.R. in the case of normal plasma, increases it in highly altered plasma, and shows that whenever the plasma is more abnormal the sedimentation is more rapid.

Conclusion

In conclusion, the E.S.R. of undiluted blood is not a true mirror of plasmatic changes. If the haematocrit reading is low, the sedimentation rate of undiluted blood is high with a normal or slightly modified plasma. If the haematocrit is normal, the sedimentation of undiluted blood is slow in a highly altered plasma. The dilution of blood, independent of the haematocrit value, decreases the E.S.R. in the case of normal or slightly modified plasma, and increases the E.S.R. in the case of highly altered plasma. Therefore the E.S.R. findings of diluted blood give adequate information about the degree of plasma alteration. The Westergren method is more useful than the Wintrobe test, not because the tube of 200 mm. is employed, but because the blood under investigation is diluted.

Summary

The apparent anomaly of a low sedimentation rate with the Wintrobe method and a very high E.S.R. with Westergren's method, as described by many authors, occurs consistently when the following conditions are present: a very strong rouleaux formation intensity (R.F.I.) of the plasma, and a packed red blood cell volume with an average normal value of 45% or higher.

The reason for this discrepancy between the two methods of measuring the E.S.R. lies in the fact that the sedimentation rate does not necessarily correspond to the R.F.I. of the plasma, but, on the contrary, becomes very slow under the two conditions mentioned above.

The addition of sodium citrate solution according to Westergren's technique masks this phenomenon in lowering the packed cell volume and in diluting the plasma.

The determination of the E.S.R. of diluted blood gives a more adequate account of the plasma abnormalities than does the determination with undiluted blood.

For clinical purposes, therefore, it is preferable to use Westergren's dilution method in tubes of 100 or 200 mm. length.

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A few weeks ago Miss M. P. HORNSBY-SMITH, Parliamentary Secretary to the Minister of Health, opened the newly built twin-operating-theatre suite at St. Mary's Hospital, Portsmouth. She said it was the largest capital scheme in the western area of the hospital service for the current year. The building cost was £40,997, and a further £10,000 was spent on equipment. The new theatre, together with the hospital's old main theatre, will serve some 224 hospital beds.