CORRECTION OF E.S.R. IN ANAEMIA

EXPERIMENTAL STUDY BASED ON INTERCHANGE OF CELLS AND PLASMA BETWEEN NORMAL AND ANAEMIC SUBJECTS

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When red cells are mixed with their own plasma in varying proportions the sedimentation rate of the more dilute suspensions is greater than that of the more concentrated ones. This observation was first made by Fåhraeus (1921), and has often been repeated. On the basis of such observations, attempts have been made to allow for the effects of anaemia on the erythrocyte sedimentation rate (E.S.R.) by various correction charts and factors. Among these may be mentioned the correction charts of Gram (1928, 1929) and of Rourke and Ernstene (1930), which were based on experimental dilution of the blood of anaemic patients. The correction chart of Wintrobe and Landsberg (1935) has gained considerable popularity; a slide-rule method for correction based on this chart has even been devised (Best, 1950). The Wintrobe and Landsberg chart is based on dilution experiments with normal blood.

Any such correction chart is valid only if we assume that the E.S.R. is determined by certain properties of the plasma and by the concentration of red cells suspended in it, but not by the shape, size, specific gravity, or any other property of the cells: in other words, it is assumed that any sample of red cells suspended in a given plasma at a given concentration would show the same sedimentation rate. This assumption does not appear justified, and Hynes and Whitby (1938) have shown how Wintrobe's correction chart must be modified to fit in with observed facts. For example, in many cases of anaemia a much slower rate is found than would be expected when normal blood is diluted to a level equivalent to that of the anaemic haematocrit reading. Bönniger and Hermann (1923) noted this effect in chlorosis, and it seems to be particularly marked in sickle-cell anaemia (Bunting, 1939, and other workers). Westergren (1924) states that he has often found the E.S.R. lower than he would have expected from the reduced haemoglobin level. On the other hand, Newham and Martin (1928) maintained that the size and specific gravity of the cells do not affect the E.S.R., and this has been accepted by Wintrobe and Landsberg (1935) and restated by Cutler, Park, and Herr (1938).

Some authors have even gone so far as to deny that the number of red cells present influences the sedimentation rate: Fåhraeus (1929), observing this slowing effect, believed that some compensatory mechanism was at work in anaemia. Bouton (1938), on finding that the red-cell count and the E.S.R. can fluctuate independently in the course of a disease, dismissed the idea that the red cells could have any important effect on the E.S.R. Cutler, Park, and Herr (1938) made a careful study of the formation of red-cell aggregrates, interchanging cells and plasma between normal and anaemic subjects, and concluded that it was only the plasma which influenced the rate: however, they did not use cell–plasma mixtures of equal haematocrit level, and therefore the value of their experimental results is reduced.

Terry (1950) found no significant difference between the sedimentation rates of a group of normal subjects and a group of patients suffering from anaemia "of a benign origin." He does not explain clearly how anaemia of a benign origin is differentiated from other types of anaemia, nor what precautions were taken to avoid bias against including in his benign category any patient with a markedly raised E.S.R. But whatever criteria were used, this is not an unselected series, and a statistical analysis does not improve its significance. It is one thing to say that in many cases of anaemia some compensating factor is at work which masks the potential accelerating effect of dilution—this is undoubtedly true; it is quite another matter to postulate that this compensating factor quantitatively neutralizes the effect of dilution and that therefore a raised E.S.R. has the same significance in an anaemic as in a non-anaemic patient.

In this investigation we have attempted to throw further light on the problem by studying the separate effects on sedimentation of the cell and plasma components of normal and anaemic blood.

Method

Thirty experiments have been performed with the blood of patients suffering from different types of anaemia classified under the four headings of: symptomatic (that is complicating some other disease), post-haemorrhagic, iron-deficiency, and macrocytic anaemias. Blood films were examined with special reference to red-cell size and shape. In each case information was obtained about any abnormality which might influence the E.S.R. The Westergren technique was used in every experiment. Blood was diluted with 3.8% sodium citrate in the proportion of four parts of blood to one part of sodium citrate solution. A column of citrated blood 200 mm. high was set up in a Westergren tube and the E.S.R. read at the end of one hour.

Dilutions were made according to a standardized procedure to cover a haematocrit range of from approximately 40 down to below 10. Haematocrits were always checked in Wintrobe tubes on the blood run out of the E.S.R. tube after reading. When mixtures were made, the cells, after centrifuging and removal of plasma, were washed three times by centrifuging after shaking up thoroughly with normal saline. Five to seven different dilutions were prepared with (1) normal cells in normal plasma; (2) anaemic cells in anaemic plasma; (3) normal cells in anaemic plasma; and (4) anaemic cells in normal plasma. When cells and plasma are interchanged in this way between two samples of normal blood no significant alteration in sedimentation rate is found. This observation rules out a number of possible experimental artifacts: in particular, it is evident that centrifuging and washing cells as we have done does not alter their sedimenting properties. The blood of the normal subject in each case belonged, of course, to the same ABO blood group as the patient.

Recording of Results

Graphs were drawn of E.S.R. against haematocrit for each experiment, and typical examples of the results obtained in the four principal categories of anaemia are given in Figs. 1, 2, 3, and 4. In all the graphs reproduced the haematocrit figure is the figure given by the diluted blood. The results do not seem to vary very widely from a similar dilution series made with dry oxalate anticoagulant (Hambleton and Christianson, 1939), and closely comparable series of figures

can be obtained with dry sodium citrate and with 3.8% sodium citrate solution, the sedimentation rates always being slower with the citrate solution to about the same extent at each haematocrit level (Westergren, 1924). Nevertheless, these results should be applied only to E.S.R.s determined by the Westergren method.

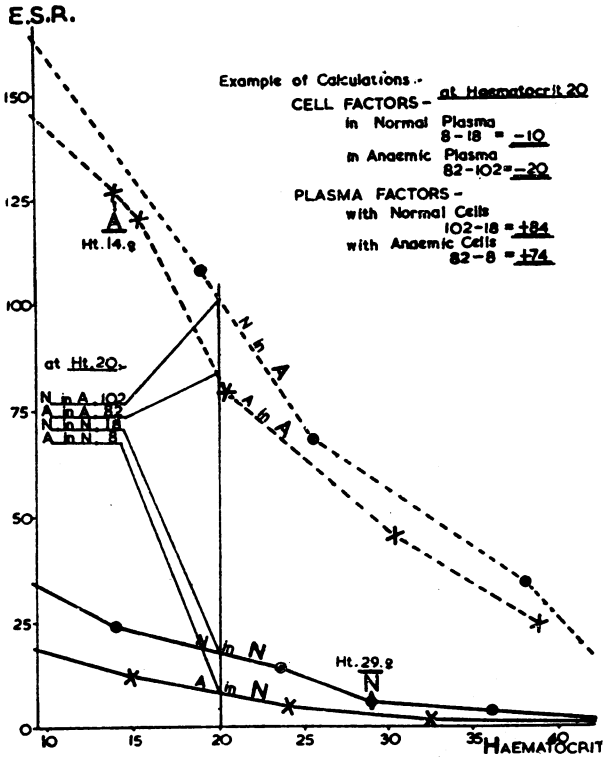

FIG. 1.—Symptomatic anaemia (carcinoma). E.S.R. plotted against haematocrit for: normal cells in normal plasma (N in N); anaemic cells in anaemic plasma (A in A); normal cells in anaemic plasma (N in A); anaemic cells in normal plasma (A in N).

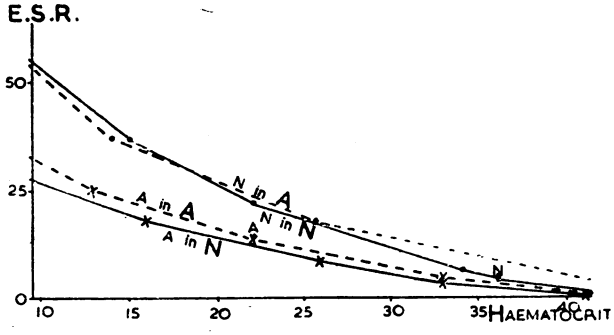

FIG. 2.—Post-haemorrhagic anaemia (haematemesis) See legend to Fig. 1.

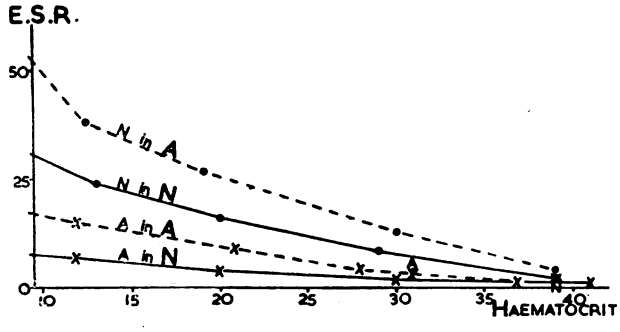

FIG. 3.—Iron-deficiency anaemia (menorrhagia). See legend to Fig. 1.

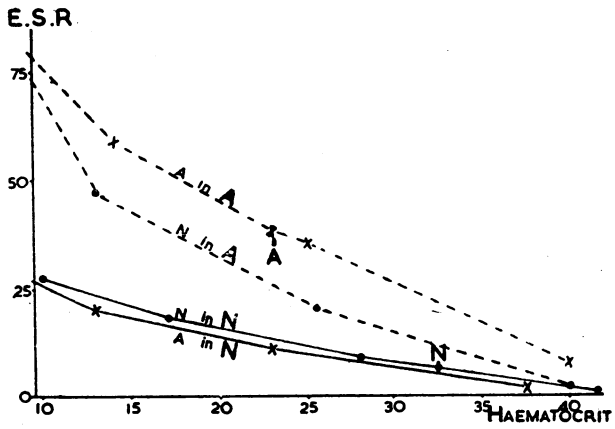

FIG. 4.—Macrocytic anaemia (pernicious anaemia). See legend to Fig. 1.

Fig. 5 is a composite graph showing the E.S.R. at different haematocrit levels for 34 normal blood samples. This graph therefore shows the approximate range to be expected of normal cells in normal plasma at the haematocrits given by the different dilutions, and it can be used for rough computation of the expected degree to which the E.S.R. should be

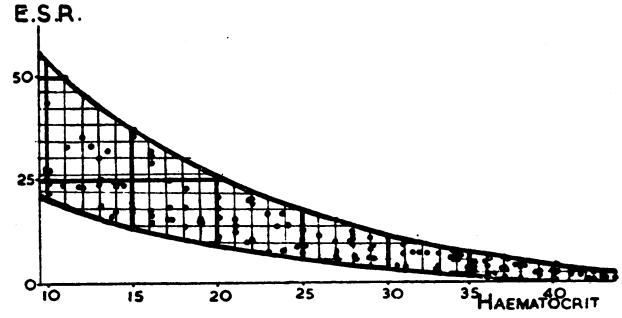

FIG. 5.—Composite graph showing E.S.R. at different haematocrit levels for 34 normal blood samples.

raised by anaemia. In order to show the approximate degree to which the different factors in the cells and plasma affect one another in the interchanges used in the experiments, the E.S.R.s were read off the graphs at the arbitrary haematocrit level of 20. The figures so obtained from the four haematocrit/E.S.R. curves drawn in each experiment indicate the effect of the plasma and of the cells upon the sedimentation rate.

Results

In describing the results of these experiments it is convenient to speak of the cells of a particular patient as having a "cell retarding factor" if they sediment more slowly in a given plasma than do normal cells, or a "cell accelerating factor" if they sediment more rapidly. Similarly, plasma may be said to have a "plasma retarding factor" if cells suspended in it sediment more slowly than they do in normal plasma, or a "plasma accelerating factor" if they sediment more rapidly. It is emphasized that these factors are postulated for convenience only and that no attempt was made to demonstrate their physical basis: indeed, this conception of accelerating or retarding factors breaks down when we consider the behaviour of blood in the macrocytic anaemias.

The accompanying Table shows cell and plasma accelerating and retarding factors for 30 cases of anaemia classified into the four groups shown. The mean values for these factors are given for each group, and, in parentheses, the lowest and highest figures obtained in each group. Accelerating factors are indicated by a plus sign, and retarding factors by a minus sign. The figures are obtained from

	Symptomatic Anaemias (14 Cases)	Post-haemorrhagic Anaemias (5 Cases)	Iron-deficiency Anaemias (6 Cases)	Macrocytic Anaemias (5 Cases)
Cell accelerating or retarding factors:				
In anaemic plasma	−17 (−86 to +5)	−6 (−14 to +3)	−11 (−31 to +2)	+27 (0 to +95)
In normal plasma	−7 (−15 to +5)	−8 (−14 to +8)	−7 (−12 to −3)	+7 (−2 to +24)
Plasma accelerating or retarding factors:				
With anaemic cells	+57 (+17 to +138)	−1 (−13 to +7)	+8 (+4 to +13)	+20 (−8 to +69)
With normal cells	+67 (+25 to +134)	−2 (−8 to +10)	+12 (+4 to +24)	0 (−13 to +17)

the graphs at the arbitrary haematocrit level of 20. For example, the cell accelerating or cell retarding factor in anaemic plasma is found by subtracting the figure for the sedimentation rate of normal cells in anaemic plasma from that for anaemic cells in anaemic plasma at the 20% haematocrit level (see Fig. 1).

From the table it will be seen that in the symptomatic anaemias a marked plasma accelerating factor and a much smaller, inconstant, cell retarding factor are found. In the post-haemorrhagic anaemias the differences are less than in the other groups, although a cell retarding factor is usually found: in fact, the blood of a patient with a post-haemorrhagic anaemia behaves much more like a dilution of normal cells in normal plasma than does any other type of anaemic blood. The iron-deficiency anaemias are characterized by a cell retarding factor and a plasma accelerating factor of approximately equal degree. In some cases the cell retarding factor predominated, giving an E.S.R. lower than would be expected from a dilution of normal cells in normal plasma to an equivalent haematocrit level.

In pernicious anaemia the position is more complicated. In all other types of anaemia it seems that if, for example, cells possess a cell retarding factor they will exhibit this property both in the normal and in the anaemic plasma; equally, if plasma is found to possess a plasma accelerating factor it will usually exert this effect both on normal and on anaemic cells. Such uniformity is not found in pernicious anaemia. Generally speaking, the cells seem to possess an accelerating factor, but this property is always more marked in the anaemic plasma than in normal plasma, and in some cases the effect is reversed—that is to say, the anaemic cells sediment more rapidly than normal cells in anaemic plasma, but more slowly in normal plasma.

Discussion

Attempts to correct the E.S.R. for the effects of anaemia are designed to assist in solving a common practical problem: an anaemic patient is found to have a raised E.S.R. —is this due solely to the anaemia, or does it suggest underlying organic disease? The authors of correction charts believe that they can answer this question, at least in the majority of cases. All correction charts are based on the assumption that abnormalities of the cells exert at most a negligible influence on the sedimentation rate. Previous workers have cast doubts on the validity of this assumption, and we believe that our experiments show conclusively that it cannot be maintained. In fact, cell factors operate in most cases of anaemia, and are often of the same order of magnitude as the plasma factors. This being so, the "corrected" E.S.R. may often be misleading to the clinician.

The nature of the cell retarding factor found in most cases of anaemia is not clearly brought out by our experimental results. It is easy to imagine that anisocytosis might interfere with rouleau formation. In all cases, apart from the macrocytic anaemias, the extent of anisocytosis as judged from the appearance of the film did seem to bear at any rate a rough relation to the magnitude of the cell retarding factor. However, this is by no means true of the macrocytic anaemias, in which marked anisocytosis may be accompanied by a cell accelerating factor. It may be that some complicating factor masks the potential effect of anisocytosis in these cases. The alternative suggestion that the relative specific gravity of the cells is the important factor cannot be ruled out: this possibility was raised by Bendien, Neuberg, and Snapper (1932) and by Ham and Curtis (1938) on the basis of a few experiments. From our comparatively small number of observations it would be unwise to infer that any one property of the erythrocytes is responsible for the accelerating or retarding factors we describe.

A limited amount of information may be obtained from consideration of the E.S.R. and the haematocrit in conjunction with a chart like that given by Hynes and Whitby (1938) showing the sedimentation rates obtained at varying dilutions of normal cells in their own plasma, with curves lying above these limits representing degrees of elevation of the E.S.R. The basis of such a chart is given in Fig. 5. If the value found in a particular case lies well above the normal range the patient probably has some organic disease apart from his anaemia. If the reading is within the normal range or below it no definite conclusions can be drawn. While useful information could no doubt often be obtained by carrying out exchange experiments of the type we describe, the method seems altogether too time-consuming in relation to the value of the results.

Summary

The problem of the erythrocyte sedimentation rate in anaemia has been studied experimentally by interchanging erythrocytes and plasma of normal and anaemic subjects: the E.S.R. was determined at varying haematocrit levels for both types of blood, each type of erythrocyte being suspended in both types of plasma. The results of each experiment were recorded graphically.

In the symptomatic anaemias the anaemic cells sedimented rather more slowly than did normal cells both in normal and in anaemic plasma. Both types of cells sedimented much more rapidly in the anaemic plasma than in the normal plasma. Thus the anaemic cells have a slight retarding influence on sedimentation and the anaemic plasma a marked accelerating influence.

Post-haemorrhagic anaemias behaved more like dilutions of normal cells in normal plasma than did other types of anaemia, but some retarding effect of the cells was usually present.

Iron-deficiency anaemias showed a retarding influence due to the cells and an accelerating effect of the plasma of approximately the same magnitude; in some cases the retarding effect of the cells predominated, giving an E.S.R. lower than would be expected for a dilution of normal cells in normal plasma to the same haematocrit level.

In macrocytic anaemias the sedimenting properties of the cells showed marked differences according to the type of plasma. If uncomplicated, such anaemias did not usually give an E.S.R. greatly above that expected for an equivalent dilution of normal cells in normal plasma.

It may be tentatively inferred that the cell retarding effect is associated with anisocytosis.

The usefulness of methods of "correcting" the E.S.R. for the effects of anaemia is criticized in the light of

these experimental results. Some information can be gained by using a chart of the Hynes–Whitby type.

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CORTISONE IN IDIOPATHIC STEATORRHOEA

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Taylor, Comfort, Wollaeger, and Power (1951) have reported favourable results in the treatment of idiopathic steatorrhoea with cortisone in doses of 25–100 mg. daily. Following the administration of the drug, there was subjective improvement, with increased appetite, a rise in the level of plasma proteins and prothrombin, and a significant decrease in the amount of fat and nitrogen excreted in the faeces. There is little evidence of impaired adrenal function in the sprue syndrome, but it is well known that cortisone in this dosage may not be acting as adrenal replacement therapy, and that improvement may occur in a number of conditions in the absence of adrenal insufficiency. We therefore decided to attempt to confirm the results of the Mayo Clinic group.

Remissions are common in idiopathic steatorrhoea, and only patients in whom the clinical condition appeared stable were chosen for trial with cortisone. We selected two untreated patients who had been under observation in hospital, and two others in whom considerable improvement had already occurred after a prolonged period of treatment with diet and vitamins, including folic acid. We felt that if the cortisone had any specific effect on the metabolic defect in steatorrhoea it should produce further improvement in the treated cases and perhaps abolish the steatorrhoea. With established methods of treatment the absorption of fat remains impaired in the great majority of patients.

Method

Each patient was given a diet containing 70 g. of protein and 70 g. of fat daily. The total amount of fat excreted in the stools during three consecutive periods of four days, separated by carmine markers, was estimated by the method of van de Kamer, ten Bokkel Huinink, and Weyers (1949). One patient who had also been the subject of a calcium balance had a control period of 36 days. At the end of the control period each patient was given 100 mg. of cortisone daily by intramuscular injection for 24 to 28 days, followed by a smaller dose for two to three days before the drug was discontinued.

During the last two weeks of treatment with cortisone a second fat balance was carried out, and the results were compared with those obtained during the control period. In each case the excretion of nitrogen in the faeces was estimated during the period of control, and again during the second fat balance while the patient was receiving cortisone. A radiograph of the chest was taken before treatment was started, and changes in weight, blood pressure, and serum electrolytes were recorded during the trial. Three of the patients, Nos. 1, 2, and 3, were allowed to go home between the two balances, but all were admitted to hospital during the periods of collection of faeces.

Results

Both the patients who had had previous treatment felt better, and in one the bowels were opened less frequently and the stools became more formed. In one of the untreated cases (No. 4) there was a great improvement in appetite and morale, although little change occurred in the action of the bowels. In the other (No. 3) there was no subjective improvement, but the diarrhoea decreased. One patient (No. 2) gained 2.4 kg. in weight during the period of treatment, owing to the retention of fluid.

Excretion of Fat and Nitrogen.—The Chart shows the percentage of dietary fat excreted in the faeces and the amount of faecal nitrogen in each period of four days, before and during treatment with cortisone. The great variation in the results of consecutive estimations in Case 4 was due to irregular bowel action, which made separation of the periods difficult. The Table gives the difference between

Excretion of Fat and Nitrogen in the Faeces Before and During Treatment with Cortisone

	Case No.	Mean % Dietary Fat Excreted During Consecutive Periods of 4 Days. (Number of Periods in Parentheses)		Mean 4-day Excretion of Nitrogen in Faeces (g.)		Length of Treatment (Days)	Total Dosage of Cortisone (g.)
		Before Treatment With Cortisone	During Treatment With Cortisone	Before Treatment With Cortisone	During Treatment With Cortisone		
Previously Treated	1	16·7 (3)	12·0 (3)	7·4	5·2	28	2·80
	2	16·5 (3)	11·1 (3)	6·9	6·9	33	2·88
Previously Untreated	3	43·6 (3)	29·5 (2)	11·1	11·9	31	2·89
	4	25·0 (9)	23·0 (3)	5·6	9·9	28	2·55

For fat excretion: $t = 2{\cdot}49$; $p > 0{\cdot}05$.

the mean four-day excretion of dietary fat before and during treatment with cortisone. The difference between the amounts of fat excreted is not significant. The effect on the excretion of nitrogen was variable.

Discussion

Hypotension, pigmentation of the skin, and asthenia are common in patients suffering from steatorrhoea, and several observers, noting the close resemblance to Addison's disease, have suggested that adrenal insufficiency may play some part in the pathogenesis of the sprue syndrome (Gloor, 1930; Thaysen, 1932). However, in spite of the fact that the hormones of the adrenal cortex exert an influence on the