# THE ERYTHROCYTE SEDIMENTATION TEST: A WIDE-BORE TUBE METHOD USING OXALATED BLOOD AND PERMITTING CORRECTION OF THE RESULT TO A STANDARD RED-CELL VOLUME

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## Introduction

The value of the sedimentation test in the study of patients suffering from rheumatic disease is today well recognised, but the lack of a uniform technique and of a uniform method of expressing the results of the test renders comparison of tests carried out in different clinics well-nigh impossible. The Empire Rheumatism Council, recognising this, have taken action by calling together a Sedimentation Test Sub-Committee of persons interested in the question of the standardisation of the test. The authors of this paper are among the members of that sub-committee and, stimulated by discussions therein, agreed to collaborate in establishing and perfecting a technique for the performance and for the recording of results of the sedimentation test which would enable the four hospitals to attain uniformity in this respect. This has been done, and today in the four largest British hospitals for rheumatic disease, which together admit more than 7,000 patients annually, results of the sedimentation test, which are entirely comparable one with another, are obtained by a uniform technique.

The purpose of this paper is to describe the origin of the method, to detail its technique and to assess its reliability, and also to discuss the various methods which have been tried for correcting the results for anæmia.

The authors wish to make it clear that the work now published does not at present bear the authority of the Empire Rheumatism Council, although it was inspired by the activities of that body. It is independent work which will in future be submitted to the E.R.C. Sub-Committee for their consideration,

and it is fully realised that the method involves certain procedures which other workers may not be prepared to adopt.

It is certain that there can be no single uniform method of doing the sedimentation test, because, for young children and others from whom venous blood cannot be obtained, a simple method, such as that of Payne (1932), capable of giving good results with a few drops of capillary blood, is of great value and cannot be discarded. When more blood can be procured for the test, and where there are adequate laboratory facilities, it seems desirable that a more elaborate method, such as the one now reported, which is sensitive, accurate and capable of correction for anæmia, should be used. The results obtained by this method appear to justify the additional complication of the test.

## Derivation of the Method

A. The Tube.—The basis of the method is the use of a tube similar to that described by Zeckwer and Goodell (1925). The Zeckwer and Goodell tube is a 10 c.c. conical centrifuge tube which has the chief advantage of a very wide bore at its upper part where separation of cells commences. The tapered constriction of the lower end of the tube allows a longer column of blood to be used for a given volume. This tube has now been used with complete satisfaction by one of the authors for some twelve years (Race, 1929). It was concluded by another (Gibson, 1938), that of all the methods used, including most of the standard techniques, this was the most sensitive in detecting abnormal sedimentation and the most consistent in its results.

The first modification comprised the substitution of a 5 c.c. conical tube in place of the 10 c.c. tube in order that a smaller volume of blood should be needed for the test. This tube is of a similar shape to the larger tube and is graduated in 100 parts by volume (Fig. 1). It is supplied by the Scientific Glassblowing Co., Ltd. A comparison of the measurements of the two tubes shows that, in spite of the reduction of volume, there is no important difference in the length of the column of blood or of the bore of the upper part of the tube.

| | *Zeckwer and Goodell* 10 *c.c. Tube.* | *New* 5 *c.c. Tube.* |
|---|---|---|
| Height of blood column .. .. | 74 mm. | 72 mm. |
| Diameter of upper end .. .. | 15 mm. | 12 mm. |

The height of the blood column in the new tubes, as supplied by the manufacturer, is apt to vary from 70 mm. to 74 mm., as a

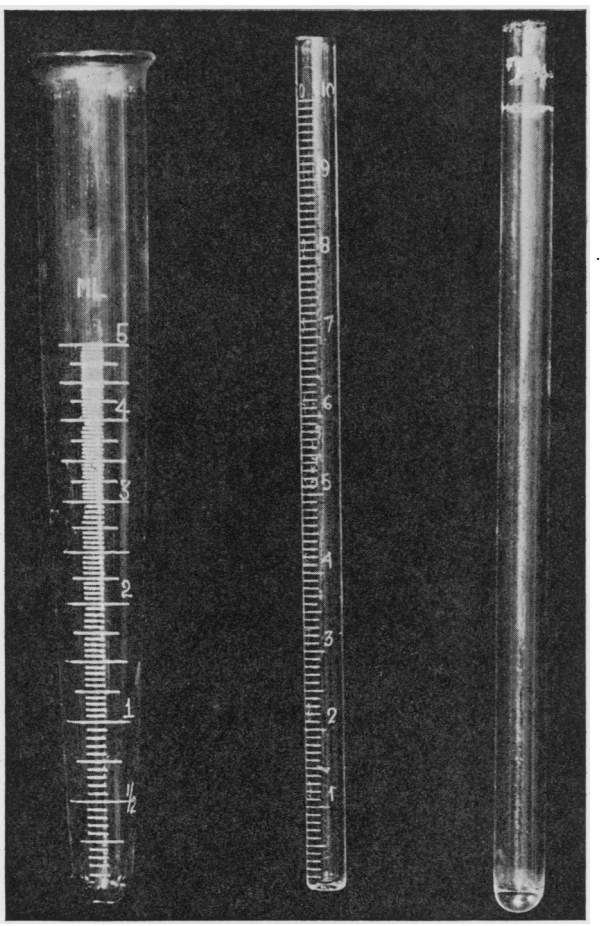


FIGURE 1.

The three tubes shown in the photograph are, from left to right:

(*a*) The 5 c.c. conical tube graduated in 100 parts by volume in which erythrocyte sedimentation is recorded.
(*b*) The Wintrobe hæmatocrit tube.
(*c*) A simple hæmatocrit tube made out of 5 mm. glass tubing with flattened bottom and 100 mm. mark etched on.

result of individual differences in the level at which tapering commences.

Before finally adopting the 5 c.c. tube in place of the 10 c.c.

tube, the reliability of which had long been proved, it was necessary to determine whether the results in the smaller tube were consistent with undiluted oxalated blood and whether the 5 c.c. tube gave results comparable with those given by the larger tube. To test these points the following experiments were carried out:

1. The new 5 c.c. tube was first tested to ascertain the consistency of its results. Duplicate tests were made simultaneously in two 5 c.c. tubes with sixty samples of blood. The following range of differences was observed in the two tubes:

| | | | | |
|---|---|---|---|---|
| No difference in 26 tests. | | | | |
| 1 | per cent. | difference | in | 22 tests. |
| 2 | ,, | ,, | ,, | 6 tests. |
| 3 | ,, | ,, | ,, | 5 tests. |
| 4 | ,, | ,, | ,, | 1 test. |

Thus fifty-four out of sixty (90 per cent.) of the tests showed a difference of 2 per cent. or less, which figure represents approximately 1 mm. linear distance in the upper part of the tube. There was no difference greater than 4 per cent. The mean difference was 0·9 per cent. These figures define the experimental error likely to be associated with the use of the 5 c.c. tubes. The error is very small.

2. Duplicate tests were made on each of 224 blood samples in a 5 c.c. and a 10 c.c. tube. The following range of differences was observed:

| | | | | |
|---|---|---|---|---|
| No difference in 61 tests. | | | | |
| 1 | per cent. | difference | in | 75 tests. |
| 2 | ,, | ,, | ,, | 37 tests. |
| 3 | ,, | ,, | ,, | 31 tests. |
| 4 | ,, | ,, | ,, | 15 tests. |
| 5 | ,, | ,, | ,, | 3 tests. |
| 6 | ,, | ,, | ,, | 1 test. |
| 7 | ,, | ,, | ,, | 1 test. |

Thus 173 out of 224 tests (77 per cent.) showed a difference of 2 per cent. or less, and only five tests (2·2 per cent.) showed a difference greater than 4 per cent. The mean difference was 1·5 per cent. Agreement between the results in the two tubes was therefore close, and there seemed no reason to suppose the 5 c.c. tube to be less accurate than the 10 c.c. tube for recording the erythrocyte sedimentation.

B. The Anticoagulant.—Fahraeus (1921) and others have shown that the rouleau-forming principle of plasma on which the sedimentation reaction depends is extremely susceptible to

dilution. Its concentration appears to be low, and its effects can be readily diminished or annulled by the addition of inert fluid. One effect of adding citrate solution to a sample of blood is, therefore, a retarding of the rate of sedimentation. At the same time, however, the increased dispersion of the cells, amounting to an artificial anæmia, tends to accelerate sedimentation. In some cases these antagonistic effects exactly balance one another, but in the majority of cases the retarding effect of plasma dilution predominates, as has been previously shown (Gibson, 1938). Experiments carried out on duplicate samples of oxalated and citrated blood in the new 5 c.c. tubes confirm this point.

Samples of blood were taken from fifty-two patients. From each patient an oxalated sample and a citrated (one part 3·8 per cent. sod. cit. to four parts blood) sample were obtained, and each was then put up in a 5 c.c. tube. The sedimentation was recorded at the end of one hour.

The following range of differences was observed in the two tubes:

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| No difference in 0 cases. | | | | | 10 per cent. difference in 3 cases. | | | | |
| 1 per cent. difference in 1 case. | | | | | 11 | ,, | ,, | ,, | 2 ,, |
| 2 | ,, | ,, | ,, | 2 cases. | 12 | ,, | ,, | ,, | 2 ,, |
| 3 | ,, | ,, | ,, | 1 case. | 13 | ,, | ,, | ,, | 3 ,, |
| 4 | ,, | ,, | ,, | 3 cases. | 14 | ,, | ,, | ,, | 3 ,, |
| 5 | ,, | ,, | ,, | 6 ,, | 15 | ,, | ,, | ,, | 2 ,, |
| 6 | ,, | ,, | ,, | 3 ,, | 16 | ,, | ,, | ,, | 0 ,, |
| 7 | ,, | ,, | ,, | 8 ,, | 17 | ,, | ,, | ,, | 0 ,, |
| 8 | ,, | ,, | ,, | 7 ,, | 18 | ,, | ,, | ,, | 1 case. |
| 9 | ,, | ,, | ,, | 5 ,, | | | | | |

Only three out of fifty-two (5·8 per cent.) of these tests showed a difference of 2 per cent. or less. A difference greater than 4 per cent. was noted in forty-five (86·3 per cent.) of the tests and the mean difference was 8·3 per cent. In the great majority of instances the rate of sedimentation was slower in the citrated sample than in the oxalated. In only seven tests (13·5 per cent.) was the rate more rapid in the citrated blood.

Gibson (1938), using the Westergren tube, previously found that 15·2 per cent. of duplicate tests proved to be more rapid in the citrated blood, and the majority showed a slowing of the rate with citrate. The mere fact that the same treatment results in acceleration of the rate in some 15 per cent. of blood samples, instead of the usual slowing, indicates the complexity of the test and the complications introduced by citrate dilution.

If the degree of plasma dilution and red-cell dispersion were

constant, the error would always be the same, but both depend upon the relative proportions of plasma and red cells present in any particular sample of blood. For example, if one part of citrate were added to four parts of whole blood from each of two patients, one of whom had a red-cell volume of 25 per cent. and the other a red-cell volume of 50 per cent., the dilution of the plasma in the first case is one part citrate to three parts plasma, and in the second, one part citrate to two parts plasma. In each case the dispersion of the red cells is also significantly different. Dilution of the plasma, therefore, introduces an important and imponderable variable.

Wintrobe and Landsberg (1935) have shown that in tubes of wide bore, oxalate in the proportion of 2 mgm. per 1 c.c. of blood does not materially influence the sedimentation reaction when compared with that obtained in heparinised blood. This concentration of oxalate has been adopted, and pure potassium oxalate has been used in preference to a mixture of potassium and ammonium oxalates, as the latter renders the blood sample useless for urea or plasma protein estimations, both of which are sometimes called for in rheumatic patients.

A further consideration which influenced agreement on the use of oxalate anticoagulant was the practical one of error in dilution when mixing blood and citrate solution in the collecting syringe. Moreover, the mixture so obtained is useless for hæmatological or chemical examinations.

The authors are of opinion that neither convenience nor accuracy is gained from the use of citrate anticoagulant. But at the same time it must be recognised that when the volume of blood obtainable is small, as in children, a narrow-bore tube is required to secure an adequate height of blood column, and under these circumstances dilution of the blood is necessary since undiluted oxalated blood does not fall regularly in such narrow tubes. They therefore agree with Schuster (1939) that in narrow tubes citrated diluted blood gives more regular results than undiluted oxalated blood.

C. Correction for Anæmia or Polycythæmia.—It was felt that a necessary perfection to any sedimentation test should be the ability to correct the results for deficiency or excess of red cells. This seemed especially desirable as the test would be used as a main laboratory index for the diagnosis and control of cases of chronic arthritis in whom there are often found marked

variations of red-cell size and hæmoglobin content and of plasma volume. As a result of preliminary trials of many methods of standardisation it was decided to adopt the principle of Walton (1933), whereby the blood is adjusted experimentally to a standard red-cell content before the test. It has been shown (Collins, 1935) that the anæmia in the chronic rheumatic diseases is most frequently of a hypochromic type with diminished hæmoglobin content but a relatively normal red-cell count, and as the influence of anæmia on sedimentation seems to depend on the relative volume concentrations of red cells and plasma it was decided to standardise the test according to the packed cell (hæmatocrit) volume rather than according to the red-cell count, as described in the original Walton technique. The packed red-cell volume (P.C.V.) is more rapidly obtained than the count, provided that a centrifuge is available for its estimation, and the hæmatocrit tube has a smaller experimental error than the red-cell counting chamber.

These, then, were the principles upon which the method under discussion was based: (*a*) the use of a 5 c.c. graduated conical sedimentation tube of wide bore in its upper part to allow of free fall, (*b*) the use of dry oxalate as anticoagulant, and (*c*) the correction of the test to a standard P.C.V. by suitable adjustment of the plasma-cell volume ratio.

Each of the authors commenced the operation of the test in his laboratory on an agreed technique whereby sedimentation was recorded in undiluted blood (crude S.S.), and also in the blood brought to a standard red-cell volume by suitable addition or subtraction of plasma (experimentally corrected S.S.). For reasons to be discussed later, it was thought better to record the results as suspension stability (S.S.) rather than sedimentation rate, the S.S. being the percentage volume of red-cell suspension measured at the end of one hour.

### *Method of Estimating Crude S.S. and Experimentally Corrected S.S.*

Well mixed oxalated blood from the patient was distributed in the following way:

1. Seven c.c. were pipetted into a plain test-tube (Tube 1).
2. Five c.c. were placed direct into a 5 c.c. sedimentation tube (Tube 2) for estimation of crude S.S.

3. A 100 mm. hæmatocrit tube of Wintrobe type was filled to the mark and centrifuged at 3,000 r.p.m. for fifteen minutes. The P.C.V. was then read off ($x$ per cent.).

4. The patient's own plasma was then added to or removed from the 7 c.c. of blood in Tube 1 in such a way that this volume then became $\frac{x}{6}$ c.c. This manipulation brought the packed cell-volume to the chosen standard of 42 per cent.

5. Five c.c. of the adjusted blood were then transferred after thorough shaking to a second 5 c.c. sedimentation tube (Tube 3).

6. Tube 2 was then re-shaken and the sedimentation of the blood in Tubes 2 and 3 were recorded at half-hourly intervals for two hours. It was soon apparent that the one-hour reading gave the most satisfactory index of the erythrocyte sedimentation and all results have been expressed in terms of this one-hour reading.

The standardising P.C.V. of 42 per cent. was chosen for three reasons: (*a*) It was known from previous experience (Gibson, 1938) that oxalated, undiluted blood of this P.C.V. fell with regularity in a tube of wide bore. (*b*) The mean P.C.V. of 400 consecutive rheumatic patients of both sexes was 42·08 per cent. (*c*) It is the mean normal value for women.

By this method three figures were recorded for each test—the "crude S.S." of the unmodified blood, the "experimentally corrected S.S." of the blood adjusted to the standard 42 per cent. P.C.V., and the P.C.V. (hæmatocrit) of the blood sample.

### Calculations for Correction of the S.S.

Theoretically the method detailed above appeared to be excellent, but in hospital practice the adjustment of a large number of blood samples before the tests could be set up proved to be very laborious. Attempts were then made to find a method of calculating the "corrected S.S." from the "crude S.S." and the P.C.V. Each of the authors worked on this problem independently, and each proposed a different correction calculation. These four methods for calculating the "corrected S.S." are described below:

Method I.—The author of this method recorded a number of tests made on different blood samples resulting in identical experimentally corrected S.S. On plotting the "crude S.S."

against the P.C.V. of the blood samples, it was noticed that the curve so formed approximated closely to the basic curve used by Wintrobe and Landsberg (1935) for the compilation of their graphs for correction of the S.R. in their narrow 100 mm. tubes. The Wintrobe and Landsberg chart was accordingly used for the computation of the corrected values. Later experience showed, however, that bloods with P.C.V. widely diverging from the normal failed to behave on experimental correction according to this calculation. This difference from Wintrobe and Landsberg's findings was doubtless due to the considerable difference in bore of the tubes used in the two methods.

METHOD II.—The author of this method felt that even if no curve or factor could be found, it would be of service if a large number of observations were tabulated in such a way that for a wide range of "crude S.S." and hæmatocrit readings a corrected figure could be obtained by reference to a previous sample in which experimental correction had been undertaken.

From observations on 351 blood samples such a table was constructed in which for each hæmatocrit and crude S.S. value the difference between the crude and the experimentally corrected S.S. was recorded. These data are summarised in Table I.

From a study of these figures it was at once seen that the results were somewhat variable, and that modification of the cell-plasma ratio in certain cases introduces complicating factors for which there is at present no explanation. Such anomalous cases were exceptional. The general tendency was for the difference between the crude and the experimentally corrected S.S. figures to increase steadily as the hæmatocrit P.C.V. of the blood sample rose above or fell below 42 per cent. On the other hand, among bloods of the same P.C.V. there was little tendency for the difference to be proportional to the crude S.S. It seemed likely, therefore, that a rough correcting calculation might be possible by taking the crude S.S. and adding or subtracting the mean difference corresponding to the P.C.V. of the blood sample.

A fuller examination of the data showed an interesting relation between the mean-difference figures and the P.C.V. For P.C.V. below 42 per cent. the S.S. showed a difference of approximately 1·5 for each 1 per cent. decrease in P.C.V. Thus the figure to be added to the crude S.S. could be found with fair accuracy by subtracting the patient's hæmatocrit P.C.V. from 42 per cent. and multiplying this difference by 1·5. When the

blood sample was more than 42 per cent. P.C.V. the mean difference was approximately 1·0 for each 1 per cent. excess P.C.V. Thus the figure to be subtracted from the crude S.S. in bloods of P.C.V. greater than 42 per cent. could be calculated simply by subtracting forty-two from the patient's hæmatocrit reading.

TABLE I.—DATA FROM 351 COMPLETE TESTS UPON WHICH CALCULATION METHOD II. WAS BASED.

| *P.C.V. of Blood Samples. (Per Cent.)* | *Number of Tests.* | *Aggregate Differences between Crude and Exp. S.S.* | *Mean Difference.* | *Mean Difference ÷ (42 – P.C.V.)* |
|---|---|---|---|---|
| 29 | 4 | 74 | 18·5 | 1·4 |
| 30 | 7 | 129 | 18·4 | 1·5 |
| 31 | 3 | 38 | 12·7 | 1·2 |
| 32 | 5 | 76 | 15·2 | 1·5 |
| 33 | 7 | 91 | 13·0 | 1·4 |
| 34 | 7 | 81 | 11·6 | 1·5 |
| 35 | 10 | 110 | 11·0 | 1·6 |
| 36 | 7 | 75 | 10·7 | 1·8 |
| 37 | 21 | 151 | 7·2 | 1·4 |
| 38 | 22 | 130 | 5·9 | 1·5 |
| 39 | 33 | 159 | 4·8 | 1·6 |
| 40 | 37 | 116 | 3·1 | 1·6 |
| 41 | 30 | 61 | 2·0 | 2·0 |
| | | | | *Mean Difference ÷ (P.C.V. – 42).* |
| 43 | 24 | 25 | 1·0 | 1·0 |
| 44 | 27 | 64 | 2·4 | 1·2 |
| 45 | 32 | 111 | 3·5 | 1·2 |
| 46 | 16 | 65 | 4·1 | 1·0 |
| 47 | 23 | 107 | 4·6 | 0·9 |
| 48 | 11 | 67 | 6·1 | 1·0 |
| 49 | 11 | 82 | 7·5 | 1·1 |
| 50 | 7 | 58 | 8·3 | 1·0 |
| 51 | 5 | 42 | 8·4 | 0·9 |
| 52 | 2 | 27 | 13·5 | 1·4 |

Table II. shows a recalculation of these factors from a total of 500 tests (351 tests by the author of this method, shown in Table I., and a further 149 tests made independently by the other authors). It will be seen that for hæmatocrit readings below 42 per cent. the factors vary little about the approximate mean of 1·5, and for hæmatocrit readings above 42 per cent. the factors are all approximately 1·0.

TABLE II.—DATA FROM 500 COMPLETE TESTS CONFIRMING THE VALIDITY OF THE FACTORS ARRIVED AT IN METHOD II.

| *P.C.V. of Blood Samples. (Per Cent.)* | *Number of Tests.* | *Aggregate Differences between Crude and Exp. S.S.* | *Mean Difference.* | *Mean Difference ÷ (42 – P.C.V.)* |
|---|---|---|---|---|
| 26 | 2 | 47 | 23·5 | 1·5 |
| 27 | 1 | 26 | 26·0 | 1·7 |
| 28 | 2 | 49 | 24·5 | 1·8 |
| 29 | 5 | 94 | 18·8 | 1·4 |
| 30 | 9 | 162 | 18·0 | 1·5 |
| 31 | 5 | 75 | 15·0 | 1·4 |
| 32 | 12 | 177 | 14·8 | 1·5 |
| 33 | 10 | 141 | 14·1 | 1·6 |
| 34 | 14 | 172 | 12·3 | 1·5 |
| 35 | 19 | 209 | 11·0 | 1·6 |
| 36 | 17 | 170 | 10·0 | 1·7 |
| 37 | 33 | 243 | 7·4 | 1·5 |
| 38 | 36 | 229 | 6·4 | 1·6 |
| 39 | 50 | 237 | 4·7 | 1·6 |
| 40 | 48 | 154 | 3·2 | 1·6 |
| 41 | 31 | 62 | 2·0 | 2·0 |
| | | | | *Mean Difference ÷ (P.C.V. – 42).* |
| 43 | 30 | 33 | 1·1 | 1·1 |
| 44 | 35 | 85 | 2·4 | 1·2 |
| 45 | 40 | 140 | 3·5 | 1·2 |
| 46 | 22 | 93 | 4·2 | 1·1 |
| 47 | 30 | 148 | 4·9 | 1·0 |
| 48 | 16 | 95 | 5·9 | 1·0 |
| 49 | 14 | 102 | 7·3 | 1·0 |
| 50 | 9 | 80 | 8·9 | 1·1 |
| 51 | 6 | 57 | 9·5 | 1·1 |
| 52 | 4 | 45 | 11·3 | 1·1 |

On theoretical grounds, assuming that the rate of rouleau formation depends upon a simple property of plasma only, the graph of correction ought to be a curve of the type used by Rourke and Ernstene (1930), Wintrobe and Landsberg (1935), Hambleton and Christianson (1937-38) and others; but as the sedimentation of blood is no simple event which obeys simple mathematical laws, there is no reason to suppose that a smooth curve is a more accurate correction graph than the refracted straight line based on the two factors. Furthermore, any attempt to round off a curve might suggest an accuracy greater than our data warrant. The method is a practical one involving a very simple calculation. It may be summarised thus:

1. Determination of the " crude S.S." of undiluted oxalated blood.

2. Estimation of P.C.V. of the blood sample by centrifuging in a hæmatocrit tube for fifteen minutes at 3,000 r.p.m. (It is as well to test the performance of any centrifuge by spinning a test batch of filled hæmatocrit tubes until a constant P.C.V. is reached and noting the time required by the machine to achieve this.)

3. Correction of " crude S.S." by:

(*a*) If P.C.V. of blood is less than 42 per cent., adding 1·5 (42 – P.C.V.).

(*b*) If P.C.V. of blood is more than 42 per cent., subtracting 1 (P.C.V. – 42).

METHOD III.—The correction curves in this method were obtained as follows: A comparatively large sample of blood was collected and divided into at least three parts, which parts were then, by addition or subtraction of plasma, manipulated to three or more different P.C.V.s, and the S.S. was determined at each P.C.V. The results were plotted and the points joined by a smooth curve. From a large series of such experiments a chart of multiple curves was constructed and the crude stabilities were corrected by noting the point of intersection of vertical and horizontal lines drawn from the P.C.V. and crude S.S. values respectively, and following the nearest curve to its intersection with the vertical line at the standard C.V. of 42 per cent.

METHOD IV.—This method depended on the use of correction factors by which the P.C.V. difference from 42 per cent. was to be multiplied, but it differed from Method II. in that multiple factors were established and that these factors were grouped according to the range of corrected S.S. Like Method II., different factors were found to be necessary for bloods above 42 per cent. and for those below 42 per cent. The factors were the average slopes of correction lines for bloods whose corrected S.S. fell within groups of ten integers. The correction lines were obtained from 158 blood samples in the following way:

For every sample, the crude S.S. and the experimentally corrected S.S. were plotted on a graph whose abscissa represented P.C.V. per cent. and whose ordinate represented S.S. values. Each pair of points was joined by a straight line. The experimentally corrected S.S. points, of course, always lay on the vertical line representing 42 per cent. P.C.V. The slope of each line was

expressed as the tan of the angle—*i.e.*, the difference between crude S.S. and corrected S.S. divided by the difference between P.C.V. of the blood and 42 per cent.

It was found that these slopes could be grouped with fair accuracy according to the range of corrected S.S. in the following way:

(*a*) Where observed P.C.V. was below 42 per cent.:

| *No. of Cases.* | *Corrected S.S. Range.* | *Slope of Average Correction Line.* |
|---|---|---|
| 13 | 100 to 90 | 1·61 |
| 24 | 89 to 80 | 1·82 |
| 24 | 79 to 70 | 2·00 |
| 22 | 69 to 60 | 1·68 |
| 16 | 59 to 50 | 1·45 |

(*b*) Where observed P.C.V. was above 42 per cent.:

| | | |
|---|---|---|
| 11 | 100 to 90 | 0·83 |
| 22 | 89 to 80 | 1·17 |
| 11 | 79 to 70 | 1·53 |
| 7 | 69 to 60 | 1·64 |
| 8 | 59 to 50 | 1·88 |

The average slope figures in the last column are the factors by which the P.C.V. difference from 42 per cent. was multiplied to obtain the difference between crude and corrected S.S.

In order to determine which correction factor to apply for any test, it was necessary to find the point representing crude S.S. and original P.C.V. on a large scale graph on which the average correction lines were drawn. The graph was divided by these lines into areas to which the different correction factors applied, and it was then simply necessary to use the factor for the appropriate area.

This method has been described in some detail because it was the only correction method which employed multiple factors, and it is extremely interesting to compare the accuracy of these multiple factors with the simple factors employed in Method II.

### Comparison of "Corrected S.S." obtained by the Four Calculation Methods with "Corrected S.S." obtained by Experiment

In order to test the accuracy of our four correction calculations when applied to tests wherein the experimentally corrected S.S. had been determined, a system of "blind" corrections was

evolved. Each of the authors performed fifty complete tests, recording the crude and experimentally corrected S.S. and the P.C.V. The crude S.S. and the P.C.V. figures were then circulated to each of the other authors, who calculated the corrected S.S. according to his own method and returned the figures to be checked against the corrected S.S. which had been determined by experiment. In this way each author performed fifty complete tests and calculated corrections for a total of 200 tests. Except for the fifty tests which he himself had carried out, he was ignorant of the experimentally corrected results at the time of his calculations.

The four sets of calculated corrections have since been correlated with the experimental corrections and the deviations of the former from the latter in the 200 tests are recorded in Tables III. and IV.

It is obvious from the statistical analysis of the results of our different methods of calculating the corrections for different hæmatocrit readings that all the methods gave reasonably accurate determinations. Method I. is statistically inferior to the other three methods, but any of the latter would meet all practical requirements.

It remained to decide which method should be adopted and recommended for general use. The choice fell unanimously on Method II. because of the extreme simplicity of the calculation. From Table IV. it will be seen that the difference between the experimentally observed corrected S.S. and that calculated by this method was 4 per cent. or less in over 90 per cent. of cases. It may be noted that 4 per cent. represents a linear fall of about 2 mm.

This method has been described in detail above (p. 341). It may be useful to construct a simple table of the necessary amounts to be added to or subtracted from the "crude S.S." at each hæmatocrit volume to obtain a result corrected to the standard P.C.V. of 42 per cent.

It is of no material value to report the corrected S.S. as a figure containing a 0·5 value, and to maintain all our results as integers we have adopted the practice of raising any corrected S.S. containing this fraction to the nearest whole number above. Thus a crude S.S. of 64 in a blood of 35 per cent. P.C.V. when corrected by Method II. yields the figure of 74·5. This is reported as 75.

TABLE III.—FREQUENCY DISTRIBUTION OF DEVIATION OF CALCULATED CORRECTED S.S. FROM OBSERVED S.S. IN BLOOD EXPERIMENTALLY CORRECTED TO 42 PER CENT. P.C.V.

| *Relation of Calculated to Observed Correction.* | *Corrections Calculated by :* | | | |
|---|---|---|---|---|
| | *Method I.* | *Method II.* | *Method III.* | *Method IV.* |
| −22 | — | — | 1 | — |
| −21 | — | — | — | — |
| −20 | — | — | — | — |
| −19 | — | — | — | — |
| −18 | — | 1 | — | — |
| −17 | — | — | — | — |
| −16 | — | — | — | — |
| −15 | — | — | — | — |
| −14 | — | — | — | — |
| −13 | — | 1 | — | — |
| −12 | — | — | 1 | — |
| −11 | — | — | — | 1 |
| −10 | — | — | — | — |
| − 9 | — | — | — | — |
| − 8 | — | 1 | — | — |
| − 7 | 3 | — | — | 4 |
| − 6 | 4 | 2 | 3 | 1 |
| − 5 | 2 | 5 | 3 | 4 |
| − 4 | 7 | 4 | 3 | 8 |
| − 3 | 15 | 10 | 10 | 6 |
| − 2 | 15 | 20 | 18 | 15 |
| − 1 | 25 | 27 | 28 | 29 |
| 0 | 43 | 57 | 51 | 40 |
| + 1 | 22 | 34 | 38 | 37 |
| + 2 | 23 | 15 | 13 | 20 |
| + 3 | 12 | 12 | 10 | 12 |
| + 4 | 6 | 4 | 13 | 10 |
| + 5 | 8 | 3 | 3 | 8 |
| + 6 | 4 | 3 | 3 | 1 |
| + 7 | 1 | — | — | 1 |
| + 8 | 4 | — | 1 | 1 |
| + 9 | 1 | 1 | 1 | 1 |
| +10 | 1 | — | — | 1 |
| +11 | 2 | — | — | — |
| +12 | 2 | — | — | — |
| +13 | — | — | — | — |
| Total cases .. | 200 | 200 | 200 | 200 |
| Standard deviation | 3·4 | 2·8 | 3·0 | 2·9 |

TABLE IV.—DEVIATIONS OF (*a*) 2 PER CENT. OR LESS; (*b*) 4 PER CENT. OR LESS OF CALCULATED CORRECTED S.S. FROM EXPERIMENTALLY CORRECTED S.S.

(Each group contained 50 cases.)

| *Tests carried out by:* | *Corrected S.S. Calculated by:* | | | |
|---|---|---|---|---|
| | *Method I.* | *Method II.* | *Method III.* | *Method IV.* |
| (*a*) *Differences of 2 per cent. or less:* | | | | |
| The author of I. | 28 | 28 | 31 | 27 |
| The author of II. | 25 | 42 | 30 | 27 |
| The author of III. | 36 | 40 | 49 | 41 |
| The author of IV. | 39 | 43 | 38 | 46 |
| Totals (200 tests) | 128 | 153 | 148 | 141 |
| (*b*) *Differences of 4 per cent. or less:* | | | | |
| The author of I. | 42 | 43 | 41 | 41 |
| The author of II. | 35 | 48 | 47 | 38 |
| The author of III. | 42 | 44 | 47 | 49 |
| The author of IV. | 49 | 48 | 49 | 49 |
| Totals (200 tests) | 168 | 183 | 184 | 177 |

## ANOMALOUS RESULTS

1. INCONSISTENT SEDIMENTATION.—Two groups of anomalies have been encountered in the course of this study. The first, which is described by the term "inconsistent sedimentation," is a failure of the red cells to separate from the plasma after being set up in the sedimentation tube; but separation takes place after re-shaking the blood, or it may occur normally in a duplicate test with the same blood sample. This anomaly has been described by Shackle (1938). It is not to be explained by clotting, inadequate tube-bore, high red-cell volume, low temperature, or any of the usual causes of retarded fall. Nor does it occur twice in blood samples from the same patient. It is an inconsistent defect unlike the anomalous sedimentation in anæmia described below.

Instances of this phenomenon are rare. They do not appear more than once or twice in some thousand tests. They may usually be recognised by observing the sedimentation in two tubes of two portions of the same blood sample. For this reason one of the authors invariably records the sedimentation in the hæmatocrit tube before centrifuging as a check on the sedi-

mentation in the 5 c.c. tube, but in one observed case retardation occurred in both tubes. A repeat test, however, will not give the same false result.

Instances of the phenomenon are given below:

| Date. | 1 *Hour S.S.* | | *P.C.V. per Cent.* | *Corrected S.S.* |
|---|---|---|---|---|
| | *5 c.c. Tube.* | *Hæmatocrit Tube.* | | |
| CASE 2,026: Rheumatoid arthritis; female, aged sixty. | | | | |
| 23/12/38 .. .. | 37 | 34 | 26 | 61 |
| 27/2/39 .. .. | *92* | *92* | 29 | *112* |
| 27/2/39 (re-shaken) | 42 | — | 29 | 62 |
| 27/5/39 .. .. | 43 | 43 | 30 | 61 |
| CASE 1,438: Rheumatoid arthritis; male, aged sixty. | | | | |
| 12/4/38 .. .. | 49 | 47 | 36 | 58 |
| 31/5/38 .. .. | 50 | 48 | 36 | 59 |
| 8/9/38 .. .. | 52 | 47 | 37 | 60 |
| 28/2/39 .. .. | *92* | 36 | 34 | *104* |
| 28/2/39 (re-shaken) | 46 | — | 34 | 58 |
| CASE V.Y.: Rheumatoid arthritis; female, aged seventy-one. | | | | |
| 9/5/39 .. .. | *94* | — | 30 | *112* |
| 9/5/39 (re-shaken) | 45 | — | 30 | 63 |
| CASE 3,417: Old rheumatoid arthritis; female, aged seventy-two. | | | | |
| 22/4/39 .. .. | *98* | 52 | 35 | *109* |
| 22/4/39 (re-shaken) | 47 | — | 35 | 58 |

In case 2,026 an anomalous result was obtained for the second test in both the 5 c.c. tube and the hæmatocrit tube. The fact that this was an inconsistent sedimentation and not a deficiency of the sedimentation factors in the plasma associated with anæmia (see below) was recognised by comparison with the test performed two months earlier. Re-shaking of the sample resulted in proper sedimentation.

In the other cases the rapid sedimentation in the hæmatocrit tube or of the blood remaining in the collecting tube indicated the anomalous result in the other tube. The calculation of corrected S.S. to over 100 also showed something to be amiss with the test.

2. ANÆMIA IN THE ABSENCE OF DIMINISHED SUSPENSION STABILITY.—Anæmia may undoubtedly occur without an increase in the rate of sedimentation. In certain cases of anæmia not only the cells may be deficient, but the plasma may also

lack the constituents which make up the rouleau-forming principle. Recovery from the anæmia may mean not only a return of the red cells to normal number and hæmoglobin content, but also a recovery of the plasma deficiency. As an example the following case of pernicious anæmia may be quoted: At the time of the first sedimentation test the patient had 1,420,000 R.B.C.s and a colour index of 1·4. The P.C.V. at this time was only 17 per cent., a very abnormal blood in which to record sedimentation. As a check on this test, therefore, the same blood was manipulated by the addition of red cells to a P.C.V. of 32 per cent. and re-tested.

| *Date.* | *Crude S.S.* | *P.C.V. per Cent.* | *Corrected S.S.* |
|---|---|---|---|
| 9/12/38 .. .. .. | 54 | 17 | 92 |
| 9/12/38 (adjusted) .. | 80 | 32 | 95 |
| 17/1/39 .. .. .. | 84 | 38 | 90 |
| 13/4/39 .. .. .. | 87 | 42 | 87 |

It is to be noted that recovery from anæmia was accompanied by a diminishing, not increasing, corrected S.S. Hence, the phenomenon described by Gregg (1936-37) in rabbits, and by Schuster (1938) in man, may occur—viz., anæmia masking an increased rate of sedimentation with the latter becoming apparent as the red-cell count and volume improve. Such a sequence would of course upset any method of correction. This state of affairs is somewhat rare in rheumatic patients, and can be recognised in bloods by the corrected suspension stability approaching or even exceeding 100. The sedimentation test is fundamentally an attempt to assess the presence or absence of certain conditions of the plasma, and this is one of the conditions which may be identified by the test. Without correction for P.C.V. such an abnormality would be missed and the blood might be reported as normal. When this condition is recognised it should be reported to the physician that the patient's blood is abnormally deficient in sedimenting factors and that the sedimentation test should be disregarded in arriving at a diagnosis.

In a total of 2,012 patients observed at Centre II. only eight such cases have been noted. Generally these cases had a crude S.S. of eighty or more, with a P.C.V. of 35 per cent. or less, and they were women. In this series the condition occurred in about

0·8 per cent. of all female rheumatic patients, but a more accurate impression of its incidence is conveyed by the fact that it occurred in 2·6 per cent. of all women in this hospital with a normal (more than eighty) S.S. Its frequency in uncomplicated hypochromic anæmia is not known. Cutler *et al.* (1938) have also remarked on this condition. The eight cases from Centre II., together with five cases from other centres, are tabulated below as examples of their identification (Table V.).

TABLE V.—CASES WITH ANÆMIA AND NORMAL SEDIMENTATION TEST.

| *Clinical Features.* | *Sex.* | *Age.* | *Crude S.S.* | *P.C.V. per Cent.* | *Corrected S.S.* |
|---|---|---|---|---|---|
| Severe rheumatoid arthritis .. | F. | 25 | 78 | 28 | 99 |
| Subacute rheumatic infection .. | F. | 28 | 84 | 31 | 101 |
| Old Still's disease .. .. | F. | 29 | 82 | 32 | 97 |
| Rheumatoid arthritis .. .. | F. | 45 | 84 | 32 | 99 |
| Rheumatoid arthritis .. .. | F. | 26 | 80 | 33 | 94 |
| Early osteo-arthritis .. .. | F. | 49 | 88 | 33 | 102 |
| Subacute rheumatic infection .. | F. | 16 | 90 | 35 | 101 |
| Fibrositis .. .. .. | F. | 28 | 91 | 35 | 102 |
| Fibrositis .. .. .. | F. | 42 | 95 | 36 | 104 |
| Fibrositis .. .. .. | F. | 34 | 84 | 23 | 113 |
| Rheumatoid arthritis .. .. | F. | 65 | 88 | 31 | 105 |
| Old Still's disease .. .. | M. | 16 | 93 | 35 | 104 |
| Non-rheumatic .. .. .. | M. | 27 | 95 | 34 | 107 |

The following repeated tests on the third of these patients may be quoted to show that this anomalous result is consistently repeated in different blood samples from the same patient. The point has been mentioned before in discussing the nature of the inconsistent sedimentation in the previous section:

| *Date.* | *Crude S.S.* | *P.C.V. per Cent.* | *Corrected S.S.* |
|---|---|---|---|
| 31/3/37 .. .. .. | 79 | 32 | 94 |
| 11/5/37 .. .. .. | 82 | 32 | 97 |
| 12/5/37 .. .. .. | 87 | 32 | 102 |
| 7/6/37 .. .. .. | 84 | 33 | 98 |
| 9/11/37 .. .. .. | 86 | 34 | 98 |

### The Value of Corrected S.S. in Rheumatism

Repeated sedimentation tests are frequently performed to control or to assess the value of treatment in rheumatism. In chronic rheumatism particularly it is the practice to administer hæmatinics in addition to therapy directed at the rheumatic process, and improvement in the anæmia may well account in a large measure for the improvement in the sedimentation rate observed in a crude (uncorrected) test. Alternatively the use of drugs (*e.g.*, gold preparations) which are toxic to the hæmatopoietic system may induce an anæmia and accelerate the rate of sedimentation in the crude test. Such errors in the follow-up of cases can be largely discounted if a corrected sedimentation test result is also available. Two cases, from Centre I., illustrating these points are abstracted below:

Case 1.—Mrs. M. B., aged twenty-seven. Typical rheumatoid arthritis for three years. Sedimentation tests performed on admission (17/2/39) and five and a half weeks later (26/3/39). Meanwhile the patient had received hæmatinics as well as other treatment. The apparent improvement in the S.S. is seen from the corrected figures to be mainly due to recovery of the anæmia:

| *Date.* | *Crude S.S.* | *P.C.V. per Cent.* | *Corrected S.S.* |
|---|---|---|---|
| 17/2/39 .. .. .. | 55 | 32 | 70 |
| 28/3/39 .. .. .. | 66 | 40 | 69 |

Case 2.—Mr. A. S., aged thirty-nine. Rheumatoid arthritis for nine months, rapidly advancing. Irregular pyrexia. Sedimentation tests were made shortly after admission (20/3/39) and again three and a half weeks later. Small doses of gold salts had been administered meanwhile, but the patient had responded badly and had become anæmic. The apparent fall in the S.S. is seen from the corrected figures to be attributable to the increasing anæmia:

| *Date.* | *Crude S.S.* | *P.C.V. per Cent.* | *Corrected S.S.* |
|---|---|---|---|
| 20/3/39 .. .. .. | 57 | 43 | 56 |
| 12/4/39 .. .. .. | 46 | 35 | 57 |

In addition to the value of the corrected S.S. in the follow-up of cases, it is of obvious value in estimating the true activity of a disease by its effect on the sedimentation factors in the plasma dissociated from its effects on the red-cell number and volume.

## Value of the Hæmatocrit as an Independent Index of Abnormality in Rheumatism

The hæmatocrit tube described by Wintrobe (1933) and obtainable from most instrument makers may be used. This tube holds a 100 mm. column of blood and is about 2·5 mm. in diameter. Cheaper and equally satisfactory, however, is a 5 mm. bore tube which can be made in any laboratory from 5 mm. glass quill tubing. One end is sealed off and the bottom flattened by means of a heated metal rod of the appropriate diameter, and the 100 mm. filling-mark is etched on (Fig. 1). This tube has the additional advantage that it may be filled and set up for a duplicate observation of the S.S. before centrifuging. Its bore of 5 mm. allows a freer fall of oxalated blood than does the narrower tube, and the duplicate reading, though not reported, may prove a useful check on the 5 c.c. tube in cases of inconsistent sedimentation. Reading of the S.S. and of the P.C.V. after centrifuging is effected by holding the tube vertically against a millimetre scale. This tube holds 2·0 c.c. of blood.

It has been the experience of the authors that the additional labour involved is more than repaid by the additional information afforded by the hæmatocrit reading. The various uses of the hæmatocrit tube have been fully described by Wintrobe (1933). Apart from its importance in the interpretation of the sedimentation test it is itself an independent means of estimating anæmia or abnormal plasma volume. Both of these are of particular importance in rheumatism. Collins (1935) has described the anæmia commonly co-existing with chronic rheumatism, and Gibson and Kersley (1938) have described it in terms of cell-plasma volume ratio. In fibrositic conditions the packed cell volume is substantially normal, whereas in rheumatoid arthritis and ankylosing spondylitis a normal value is uncommon. After a short experience with the hæmatocrit it becomes a habit to think of anæmia in terms of red-cell volume, and in a busy laboratory this method of assessing and controlling the progress of anæmia saves many hours of painstaking cell counting. The readiness with which toxic failure of the blood-forming organs in the course of gold therapy can be identified is exemplified in Case 2 quoted above.

The depth of the leucocyte cream observed in the hæmatocrit tube is also an indication of any gross departure from normal of

the white cell count. In one case the first indication of a lymphatic leukæmia in a case of " osteoarthritis " was the observation of a leucocyte volume of 6 per cent. When the white cell count was subsequently made in this case it was 120,000 per c.mm. Finally, the plasma colour may be of assistance in recognising icterus in its sub-clinical stages, and the use of a hæmatocrit tube 100 by 5 mm. gives a volume of plasma sufficient for the performance of the Van den Bergh test. The application of this in rheumatism and gout is in association with possible liver damage from gold or atophan treatment.

In short, the hæmatocrit tube provides, with the minimum of labour, a brief hæmatological review and indicates at a glance those cases in which more detailed investigations would be profitable.

The hæmatocrit volume is not to be identified with the hæmoglobin content of the blood except in a very general way. Variations in red cell size, number, and hæmoglobin content of individual cells may combine to produce packed cell volume and total hæmoglobin figures which are not necessarily correlated. Twelve bloods which had a P.C.V. of exactly 42 per cent. had hæmoglobin contents, estimated by the Hellige Neoplan method, ranging from 87 to 98 per cent., with a mean of 92·6 per cent. (12·4 to 13·8 gm. per cent., mean 13·09 gm. per cent.). The hæmatocrit tube has also been used by Wintrobe (1933) and Wintrobe and Landsberg (1935) as a sedimentation test-tube, but its bore is considered to be too narrow for accurate results with bloods of high red-cell volume. The hæmatocrit tube, therefore, is used only as an auxiliary part of the test which is made in the large 5 c.c. tube.

### Suspension Stability or Sedimentation Rate ?

In the method of the sedimentation test which has been described the actual measurement which is read off the sedimentation tube at the end of one hour is a measurement of volume—the volume occupied by the suspension of red cells, or, alternatively, the volume of supernatant plasma which has been cleared of red cells. If the latter figure is read and recorded as the sedimentation rate (S.R.), it must be clearly understood that it deals with a volume rate per hour and not with a linear distance fall per hour, which is usually implied in other methods in which the S.R. is the recorded result. In parallel-sided tubes

this linear fall per hour is also a volume fall per hour, but a complication is introduced in the tube that is used in this work by its tapering lower part, which interferes with packing of the cells so that in rapidly sedimenting bloods, which approach packing at the end of one hour, comparison of volume rate in the conical tube and linear rate in the straight tube may be fallacious. For these reasons all the results have been recorded as suspension stability—*i.e.*, the volume of red-cell suspension still standing at the end of one hour. The figure 100 – S.S. may, however, generally be considered the sedimentation rate, provided that the reservations mentioned above be held in mind.

### Comparison with Other Techniques

It is not proposed to discuss this matter fully here. The particular advantages of certain of the measures employed in the new test have already been indicated. Suffice it to say that each of the authors has been so favourably impressed with the reliability and uniformity of the results of the new method that it has been established as the routine method in the laboratories and institutions represented, and that it has been thought fit to replace other methods which were in use at the time of its introduction (viz., the 10 c.c. tube method, the Westergren tube and the Walton techniques). The decisions, which were individually arrived at, were reached after consideration had been given to parallel tests by two methods and to clinical applications.

### Limits of Normality and Manner of Recording Results

It has not been found possible to make sedimentation tests on a large series of healthy persons such as were available to Wintrobe and Landsberg (1935). Each of the authors, however, tested a number of presumably healthy persons, colleagues, assistants, and blood donors, but they do not feel in a position to state a definite numerical limit of normality for the test. No such hard-and-fast limit exists, for reduced stabilities are to be found on occasion in apparently healthy persons and high stabilities are sometimes encountered in obvious disease. The test is a test for presence or absence of an excess of rouleau-forming principles in plasma. It is not a direct test for the presence or absence of disease, but in certain diseases, including rheumatism

and tuberculosis, the results of the test are well known to bear a close correlation to the clinical activity and prognosis of the disease. Experience with this new method of conducting the sedimentation test only permits the statement that a corrected S.S. below eighty to eighty-five is of clinical significance. Results higher than eighty to eighty-five are likely to be normal.

Throughout this paper the aim has been to give greater precision to the numerical expression of the results of the sedimentation test. It is recognised that a numerical record of the S.S. is merely an index of a complicated phenomenon and not an absolute number, as is the figure expressing, for example, P.C.V. percentage or concentration of uric acid in blood.

The only alternatives in reporting the sedimentation test are: firstly, in general terms, such as normal, slightly or markedly increased or diminished; secondly, by a graphed curve either recorded photographically or plotted from multiple observations. The first method gives little more information than that which was available to the old phlebotomists, and limits comparison of one test with another. The graphical record is in routine use in some laboratories and allows easy comparison of repeated tests on an individual, though comparison with tests on other persons is made rather difficult. Numerical records permit easier comparison and have been considered more practicable for this paper. A graphed curve of suspension stability usually records the crude S.S. In order to obtain the full benefit of the test performed in the method we have described, both the P.C.V. of the blood and the corrected S.S. of the one-hour reading should be recorded alongside the graph.

## Summary

1. The practical method elaborated and now adopted in four British spa hospitals is as follows:

(*a*) Collect 8 to 10 c.c. venous blood oxalated with dry potassium oxalate to a concentration of 2 mgm. per 1 c.c. of blood.

(*b*) Fill to the 5 c.c. mark a 5 c.c. graduated (in 100 parts by volume) conical centrifuge tube. At the end of one hour read the volume of red-cell suspension still standing. This is the "crude S.S." (suspension stability). If a curve is desired, half-hourly readings may be taken over two hours.

(*c*) Fill a 100 by 5 mm. hæmatocrit tube and centrifuge this at 3,000 r.p.m. for fifteen minutes. Read off the packed red-

cell volume (P.C.V. per cent.). Alternatively, the P.C.V. may be obtained by centrifuging the 5 c.c. sedimentation tube after the sedimentation readings have been completed.

(*d*) The crude S.S. is viewed in the light of the presence or absence of anæmia, as shown by the P.C.V., and the S.S. is corrected to a standard P.C.V. of 42 per cent. by means of the following simple calculation:

If P.C.V. $<$ 42 per cent., add $1{\cdot}5 \times (42 - \text{P.C.V.})$ to crude S.S.

If P.C.V. $>$ 42 per cent., subtract $1{\cdot}0 \times (\text{P.C.V.} - 42)$ from crude S.S. (Correction Method II., p. 334.)

2. This correction method was chosen from four different mathematical methods propounded by the authors and statistically tested against corrections obtained by experimental manipulation of the cell-plasma volume ratio. It does not pretend to a high degree of mathematical accuracy, but it is designed as a simple device for the interpretation of the test in the light of variations in packed cell volume. Without assistance of this kind the mental adjustments necessary to assess the significance of fluctuations in S.S. when the cell volume percentage is also changing would be extremely difficult to make.

3. The standard P.C.V. at which corrected readings are given is 42 per cent. This is the mean of 400 unselected rheumatic cases of both sexes, and has been chosen as the standard figure for other reasons discussed in the text.

4. The sedimentation tube is of such dimensions that free fall of undiluted blood takes place, thus giving a true or natural reading and not one which is modified by the dilution of plasma by a citrate solution.

5. The hæmatocrit tube requires the use of a laboratory centrifuge, but possesses many advantages in addition to giving the P.C.V. upon which correction calculations may be based. Information concerning the red cells, the white cells, and the plasma may be gathered from it. It may also be used after filling but before centrifuging for a parallel sedimentation test, whereby the rare cases of inconsistent sedimentation may be detected.

6. The sedimentation test, as here described, is in the opinion of the authors the most accurate and informative method

where suitable conditions exist for its performance. In the absence of proper laboratory facilities, and in children where venous blood cannot be easily collected, its application may be limited.

7. Anomalies of sedimentation due to (*a*) inconsistent sedimentation, and (*b*) anæmia without diminished S.S., have been discussed in some detail and the manner in which such abnormalities may be detected has been described.

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