Technical methods

Determination of packed cell volume by centrifugation

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In a recent note Hutchison (1960) has commented on the determination of the packed cell volume and has drawn attention to the importance of the relative centrifugal force as a measure of centrifugal efficiency. Since the relative centrifugal force in g for unit mass is given by the expression,

\[ F = \frac{mg^2r}{w^2} \]

where \( w \) is the angular velocity and \( r \) the radius of gyration, it is clear that since the radius occurs only as a first order quantity and the angular velocity as its square, the latter plays a more important part. This is undoubtedly the reason why 'the figure of 3,000 r.p.m. for 30 min. is too often all that is thought important' rather than 'the force applied by the centrifuge in g' (Hutchison, 1960).

If centrifugation is continued for a sufficiently long time at any speed, the volume of packed cells will reach a constant after a certain time and prolonged spinning after this time will not reduce this volume. The time required will vary from a few minutes to several hours dependant on the relative centrifugal force employed. This does not mean, however, that the cells will be completely packed (Ponder, 1948a) and to regard attainment of constant volume as a criterion of complete packing is quite erroneous.

Millar (1925) used an experimental haematocrit centrifuge in which the rotational speed, and hence the relative centrifugal force, could be varied up to a maximum of approximately 30,000 g and spun samples of oxalated blood at each of several speeds until constant volume was attained. On plotting a graph of cell volume per cent against relative centrifugal force he found that the curve approached an asymptotic line parallel to the relative centrifugal force axis which represented the actual cell volume that would have been attained if the centrifugal force had been indefinitely increased. His results show that at 3,000 r.p.m. with a radius of 10 cm. (relative centrifugal force 1,370 g) the cells are packed to within approximately 1% of their final value. The criticism that very high centrifugal force might cause rupture and also tend to compress the cells to give a low value for the packed cell volume can be rejected (Ponder, 1948b). Indeed, Parpart and Ballentine (1943) have used centrifugal forces of up to 200,000 g and have found that, even after packing under these great forces, the cells are unharmed and can be resuspended and centrifuged many times to give the same result. From these results it would appear likely that true values for packed cell volume, that is, complete packing so ensuring exclusion of all plasma from cell interstices, can only be achieved by prolonged spinning with relative centrifugal forces of several hundred thousand times gravity.

That this is impracticable in the clinical laboratory is obvious and is indeed unnecessary. Ponder uses the original Wintrobe (1942) method in his clinical work which involves spinning at 3,000 r.p.m. for 15 min. originally in a centrifuge with a radius of 15 cm. (relative centrifugal force, approximately 1,500 g). To quote Ponder (1948b): 'It must be borne in mind that the red cell volume in men may range from 82 μ³ to 95 μ³ without its being considered abnormal; small errors in the estimation of volume are accordingly not very important.'

It is interesting to note that Wintrobe (1951) changed his technique in the third edition of his book to spinning at 3,000 r.p.m. for 30 min. but still used a small centrifuge. In the latest edition (1956a) he retains the 3,000 r.p.m. and 30 min., but has now changed his centrifuge to one of greater radius (22.5 cm.) and hence has a higher relative centrifugal force (2,260 g). The normal values of packed cell volume given by Wintrobe remain the same.

The position is further confused by descriptions in various standard textbooks of methods purporting to be Wintrobe's technique but all differing in some detail. The range of normal values given also shows wide variations. Thus Wintrobe gives a normal range in males of 47% ± 7% and technique as above; May and Marrack (1951) a normal range of 42% to 47%, technique 3,000 r.p.m. for 30 min., no radius stated; Wells (1956) spins at 2,500 r.p.m. to 3,500 r.p.m. for 30 min. then for a further 10 min. to ensure complete packing; Whitby and Britton (1957) spin at 3,000 r.p.m. for 55 min.; Darmady and Davenport (1958) spin at 3,000 r.p.m. for 30 min. but give a normal range of 40% to 50% for males; Delaney (1960) is specific with 3,000 r.p.m. for 60 min. at a radius of 15 cm., normal values being 48% to 50%, and Soberad (1958) spins at 3,000 r.p.m. for 10 min. or 'at a high speed until two successive readings are identical'. A number of other variants to the test, all regarded as satisfactory by the respective observers, can be found in the literature, and at least 10 of these have been quoted by Ponder in his book.

Despite these anomalies it is surprising to find that Hutchison considers that the fluctuations in M.C.H.C. of a patient could be attributed to inaccuracies in packed cell volume estimations rather than in Hb estimation. Although it cannot be denied that the Wintrobe technique does not give absolute values of packed cell volume, all authors agree that it is reproducible. Indeed any centrifugal technique in which a standard time and standard relative centrifugal force are employed must be, since such variable parameters as viscosity, temperature, and
so on, are, in this context, negligible. Many haemoglobin methods are by no means so reproducible, the dilution methods being particularly unreliable. An authoritative study of these methods made for the Medical Research Council (Macfarlane, King, Wootton, and Gilchrist, 1948) found that even with a single observer no significance could be attached to differences of less than 10%. In addition haemoglobin pipettes purchased on the open market may carry an error as great as 5% (Lochhead and Purcell, 1952). (An excellent review of errors in Hb estimation can be found in Wintrobe’s book, 1956a.)

With regard to the question of errors in reading haematocrit tubes, it should be noted that Wintrobe (1956b) considers this to be so insignificant that he suggests that certain small angle centrifuges may be used for packed cell volume determination ‘which have the small disadvantage that the cells in the haematocrit are packed at an angle and the true value must therefore be estimated’.

A source of potential error in the determination of packed cell volume is that of sampling. Blood samples, particularly those with a high E.S.R. may sediment very rapidly and appreciable differences in cell content between top and bottom layers can be detected in some cases after only a few minutes.

Finally the question of the M.S.E. haematocrit centrifuge: this machine was designed some 10 years ago to conform with Wintrobe’s specification at that time. The radius of gyration is 137 mm., and a governor may be switched into circuit so that the required speed of 3,000 r.p.m. may be accurately maintained. An automatic time switch is incorporated to facilitate accurate timing. Under these conditions this centrifuge will provide reproducible packed cell volumes which conform to what is generally accepted as Wintrobe’s test. Nevertheless, should the mains voltage be above 230 v then the machine can be operated at 3,800 r.p.m. with the governor switched out of circuit. This will provide a relative centrifugal force of 2,264 g as required by the latest modification of Wintrobe’s test. If required a simple modification can be made so that a governed speed of 3,800 r.p.m. can be maintained. Should the mains voltage be below 230 v, a simple voltage transformer will be required. However, practical tests show that this modification cannot be regarded as worthwhile. It is evident that the greater the true volume of packed cells, the greater will be the difference in the final reading between the two methods. Comparing tests at 2,264 g and 1,372 g respectively with a specimen of high packed cell volume, the results were 49% and 50%. The haemoglobin content was 16.5 g./100 ml. and the M.C.H.C.s were therefore 33% and 33.7%.

In clinical work this is obviously insignificant, especially when one considers that neither method can be regarded as absolute.

CONCLUSIONS

The determination of packed cell volume is a clinical test and absolute values require a relative centrifugal force, much greater, of up to 200,000 g. The clinical test is extremely reproducible and absolute values are of no significance in clinical work because of the wide range of normal values.

Errors in M.C.H.C. determination are more likely to be due to errors in the haemoglobin determinations than in estimates of packed cell volume.

REFERENCES