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Rapid Procedure for Erythrocyte Packed Cell Volume and Sedimentation Rate Determinations. (22257)

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During laboratory investigations involving large numbers of human blood samples, erythrocyte sedimentation rates and packed red cell hematocrit values were required for correlation with other observations. This need stimulated efforts to develop time saving modifications of the conventional procedures primarily to devise a rapid preliminary screening operation for selection of abnormal samples for further study by standard methods. However, in actual practice the rapid procedure yielded accuracies sufficiently comparable to standard methods that its routine substitution for the more time-consuming technics appeared possible. The procedure was put on a quantitative basis and appropriate comparison studies with accepted methods(1,2) were carried out. Results here presented indicate that the values are satisfactory for routine clinical use and that they may be obtained with substantial time saving and other economies. The method has been used in testing over 10,000 patient blood samples. The essence of the modification consists in determining sedimentation rate and hematocrit values directly in the original blood collection tube without necessity of transfer to a secondary, calibrated hematocrit tube. The recent introduction of rubber stoppered, evacuated, blood collection tubes containing appropriate anti-coagulants contributes to the speed and convenience of this technic. However, any standard test-tube or collection tube may be substituted or adapted to this procedure. A basic problem of this modification, the variable amount of blood sample in each collection tube, has been overcome by use of a proportional volume chart accurately indicating percentage of relative parts of a test tube blood sample irrespective of its total volume (Figs. 1 and 2). The

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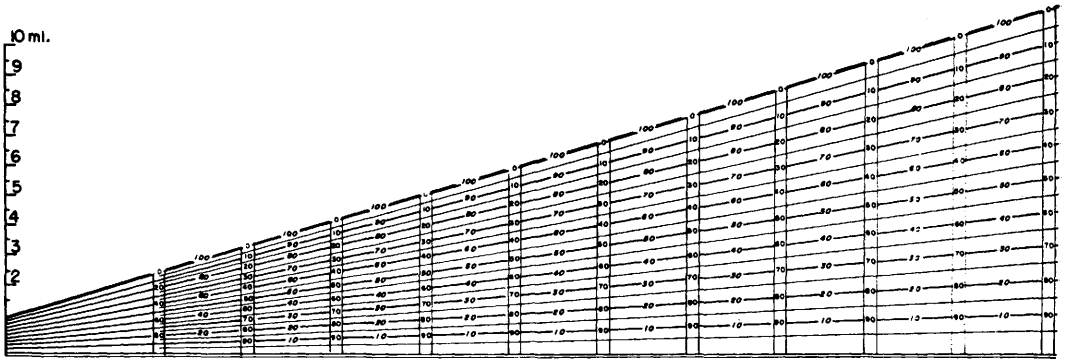

FIG. 1. Proportional volume chart for determining relative percentage of separated substances in an uncalibrated tube irrespective of volume fluctuations.

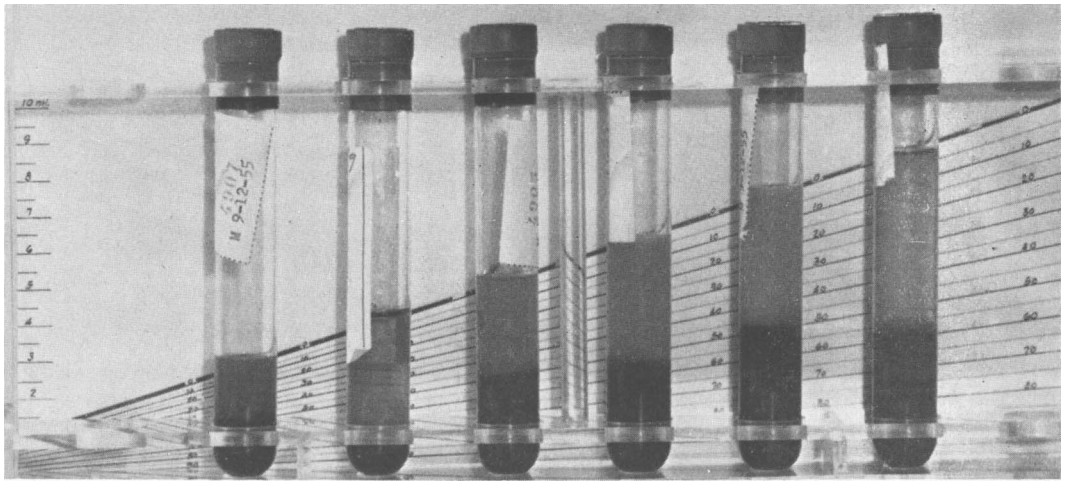

FIG. 2. Blood collection tubes arranged to illustrate use of chart in determining sedimentation rates or hematocrit values irrespective of sample volumes.

design of the chart takes into consideration corrections necessary for the spherical bottom of the tube, the diameter, shape and thickness of glass, and the meniscus. The chart (Fig. 1) was designed for standard blood collection tubes† used in our studies but, as can be readily shown, a reasonable degree of variation in these factors can be tolerated.

Method. Erythrocyte sedimentation rate. Blood is collected by standard venepuncture procedure, employing a syringe and test tube with suitable anticoagulant, or the commercially available rubber-stoppered Vacutainer† tube used with double-ended needle and holder. These studies employed the evacuated tubes containing either heparin or oxalate. The sealed samples are numbered, or otherwise identified, and placed in racks suitable for holding the tubes perfectly perpendicular (Fig. 2).‡ An optional procedure found useful in sharpening the plasma meniscus is the addition of silicone anti-foam emulsion.§ A tiny drop is added to each tube with a No. 24 needle and a one-ml tuberculin syringe. The blood is mixed thoroughly by inverting the entire rack vigorously 10 times, and is then

† The Becton, Dickinson, and Company Vacutainer blood collection tubes containing laboratory grade heparin or mixture of dry ammonium and potassium oxalate.

‡ Chart, racks, and accessory equipment manufactured by Virtis Co., Yonkers, N. Y.

§ Dow Corning antifoam A emulsion, a silicone defoamer.

placed on a level vibrationless surface to sediment for 30 minutes. If more than one rack is run simultaneously, the mixing interval between racks should correspond to the rack reading time of the technician. This interval is approximately one minute with a 10-tube rack for an experienced operator. If a time course curve is desired, appropriate interval readings may be taken, provided tube contents are not unduly disturbed. At termination of the sedimentation period, relative rates are read by placing the rack of tubes in front of the reading chart and sliding the entire rack to the left or right as required to cause the top of the blood sample of a given tube to coincide with the upper heavy meniscus line of the chart. The sedimentation rate of the red cells is then read from the appropriate chart line by noting the position on the chart of the erythrocyte-plasma interface. Either edge of tube may be employed in making the reading but proper eye level should be carefully observed to avoid parallax errors. It will be noted that the chart has 2 reading scales. The numbers reading from top down are for red-cell sedimentation rate, while those reading from bottom up refer to packed red cell volume. If repeat determinations are desired,

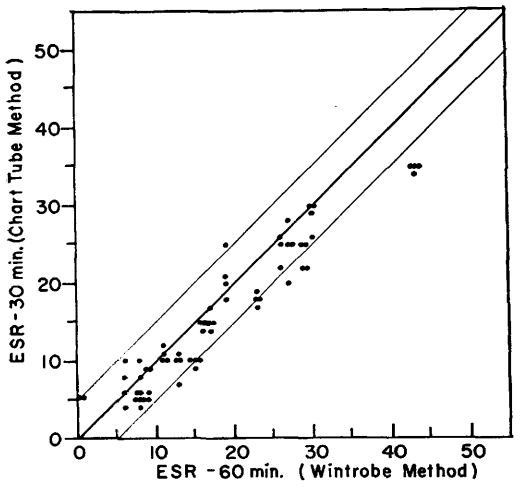

FIG. 3. Values obtained throughout the erythrocyte sedimentation rate range with tube-chart procedure as compared with Wintrobe technic. (Tube values are plotted about the straight line representing Wintrobe values. Scattering reflects combined errors of both methods. Boundary lines equal plus or minus 5 reading points.)

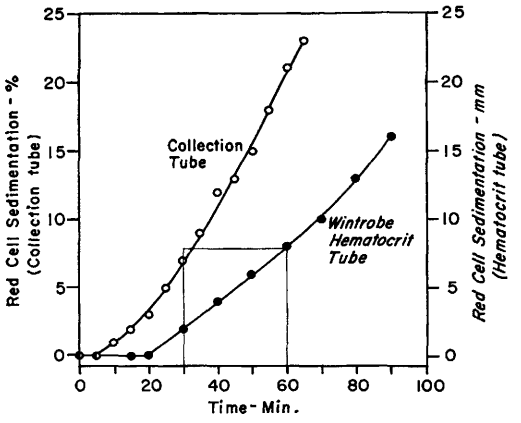

FIG. 4. Comparison of time-course curves of same blood sample determined by tube-chart and Wintrobe procedures.

the entire rack or a given sample may be remixed and reread as many times as required, the only limitation being possible changes in sedimentation rate with time. *Packed red cell volume.* The hematocrit value, or red cell volume, is obtained by transferring the blood collection tubes to a suitable centrifuge and spinning for 10 minutes at 2000 rpm (mean RCF = approximately 700). The large, 4-place horizontal head, No. 284 or 289, of the standard International centrifuge is convenient since each cup will handle from 8 to 12 tubes depending upon diameter of tube used, permitting a total load of 32 to 48 blood samples. Care should be taken in packing the tubes that they are as nearly vertical as possible so that the packed red cell interface will be level in the tube for optimum accuracy in reading. At completion of the run, hematocrit values are read in the same manner as sedimentation rates except that numbers running from bottom of chart upward apply. The percentage of white cells and platelets, or buffy coat, are noted in the usual fashion and their percentage may be read from the chart. Icterus or other abnormalities of the plasma may be recorded as hematocrits are read.

Results. Erythrocyte sedimentation rate. A comparison of results between this procedure and the conventional Wintrobe method is illustrated in Fig. 3 and 4. In Fig. 3, values for a group of blood samples covering a wide range of sedimentation rates are plotted to illustrate normal scattering of points obtained

by this method at all levels of sedimentation rate when referred to Wintrobe values. This was done by employing a standard time factor of 30 minutes in the case of the tube-chart method as compared with 60 minutes with the Wintrobe hematocrit tube. It may be seen that over 80% of the points fall within the plus or minus 5% boundaries established by the standard Wintrobe procedure on the same blood samples. It will be remembered that in this type of graphic plot the combined errors of both methods are reflected in the scattered points about the line. The aberrant points thus may be due to an excessive error by either method or to the smaller combined errors in the same direction by both methods.

Fig. 4 shows the time course plot of the same blood sample when run by the 2 methods. Differences in slope of rate curves illustrate the basis for approximate equivalence of 30- and 60-minute readings for the 2 methods. It is seen that the vertical lines at these 2 time periods intersect the sedimentation curves at approximately the same reading level. This slope increase has the advantage of shortening the time requirement for sedimentation to reach a desirable measuring point, and the disadvantage of increasing the critical importance of the time factor. Thus a lag of 2 minutes in reading the ESR by the tube-chart method would cause a greater error than the same lag period with the Wintrobe hematocrit. Although 30-minute and 60-minute readings are not always equivalent they are statistically close enough to take advantage of the convenience of the 30-minute time period.

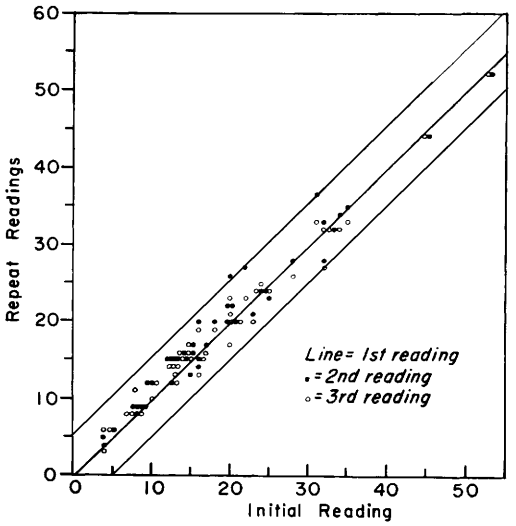

FIG. 5. Reproducibility of repeated ESR determinations over a wide range employing tube-chart technic.

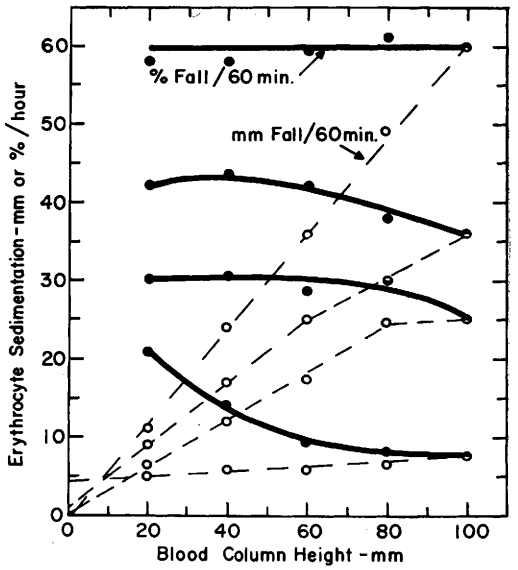

FIG. 6. Comparison of mm and percentage sedimentation rate readings employing identical blood samples with different column heights. Four levels of sedimentation rate are illustrated. Solid lines = percentage sedimentation rate (% of total height /60 min). Broken lines = mm of red cell sedimentation/60 min.

Reproducibility of this procedure with repeat determinations on the same blood samples over a wide ESR range is illustrated in Fig. 5. The original readings are plotted as the diagonal reference line and subsequent second and third readings are plotted as the closed and open circles. The moderate degree of scattering throughout the entire range indicates that reproducibility is not biased by magnitude of the sedimentation rate.

The published procedures for measuring erythrocyte sedimentation rate employ "millimeters" of fall of the top boundary of the settling red cell population. The several technics in current clinical use employ hematocrit tubes of varying lengths fluctuating from 40 to 200 mm(2-5). Since the procedure reported here employs "percentage" of fall rather than absolute distance in mm it was necessary to investigate the relationship between abso-

lute and percentage measurements within the anticipated extremes of sample volume fluctuation and thus of blood column height. Under conditions of our testing operations the extremes of whole blood sample volume gave column heights of 20 to 80 mm with the preponderance of samples from 50 to 70 mm in height. Fig. 6 illustrates the relationship between absolute mm of fall and percentage, or relative fall, when testing identical blood samples of different height. The data also show the relationship between blood samples with widely differing sedimentation rates.

For all sedimentation rate levels except the lowest one, the blood column height has little influence on percentage fall, whereas there are substantial differences in the reading of various height tubes when mm are the criteria of measurement. This accounts for some of the numerical confusion in attempting to establish normal and abnormal standards of sedimentation rate between several methods utilizing different column heights(2-7). With the percentage technic described here the sample column height is not critical between the range of 20 to 100 mm unless the ESR is low, in which case samples less than 40 mm in height should be avoided, corrected, or transferred to a smaller bore tube which will increase the sample height to 50 or 60 mm.

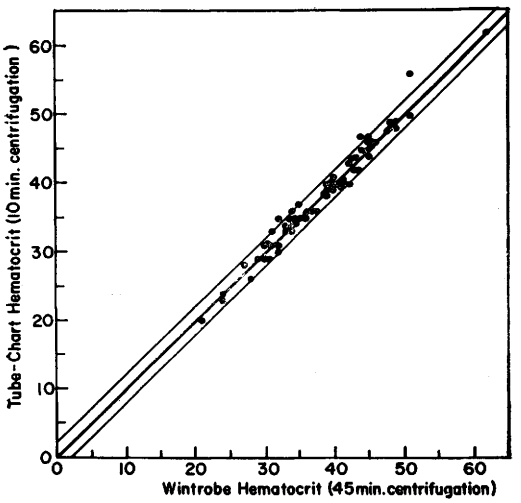

FIG. 7. Comparison of hematocrit values on 68 blood samples determined by both tube-chart and Wintrobe procedure employing 10 and 45 min. centrifugation respectively. (rcf = 700, rpm = 2,000.)

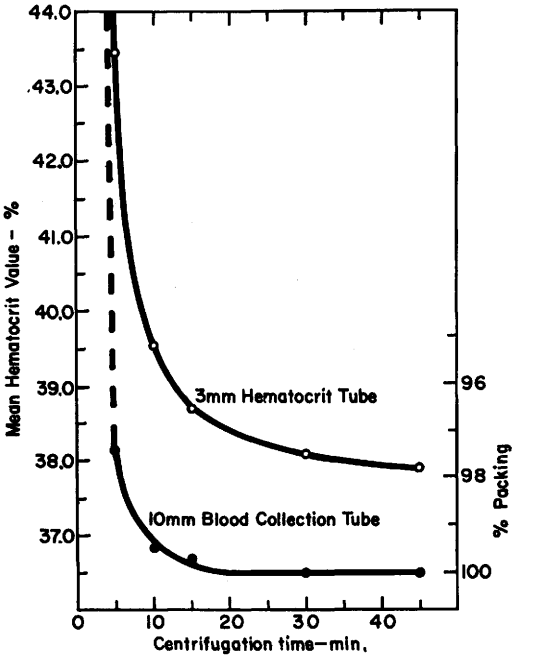

FIG. 8. Comparison of sedimentation curves in centrifugal packing of red cells when employing a 13 mm blood collection tube and a 3 mm hematocrit tube. These curves are mean values of 20 samples run simultaneously from the same group of standard hospital blood samples.

The foregoing data illustrate that the expression of erythrocyte sedimentation by percentage fall is simpler and more uniform than arbitrary sedimentation rate in mm for columns of varying heights which will yield strikingly different values for the same blood sample depending upon the style of tube employed. None of the ESR values reported here are corrected for differences in packed red cell volume although this correction does not significantly alter the relationships illustrated. The standard Wintrobe chart can be used for this purpose with this method in the same way as employed for the Wintrobe values.

Red cell packed volume. A comparison of this procedure and the Wintrobe in hematocrit determinations on 68 samples of blood covering a wide range of values is illustrated in Fig. 7. It is seen that the 2 methods agree very closely and that 95% of the points lie within the plus or minus 2% boundaries. The

difference in required centrifugation time between the 2 methods is shown in Fig. 8. It may be noted that the stationary packing point for a given relative centrifugal force is reached much more rapidly in the larger diameter collection tube and that 5 minutes of centrifugation at 2000 rpm (rcf = 700) is equivalent to the packing obtained in about 30 minutes with the narrow bore hematocrit tube. After 10 minutes of centrifugation the packing value is about 1 or 2% greater than the Wintrobe tube after 45 minutes of centrifugation.

Discussion. The advantages of this method are principally convenience, speed, and economy. Relative accuracy is indicated by Fig. 3, 5, 6, and 7 which illustrate that the obtained values are comparable with a standard current method and distinguish the normal and abnormal categories in the same quantitative manner.

A beneficial result derived from the use of relatively large diameter collection tubes of 10 to 15 mm as compared with 3 mm bore Wintrobe tubes is the substantial increase in speed of the red cell sedimentation. The erythrocyte sedimentation rate is approximately doubled, making it possible to reduce the sedimentation period by one-half, *i.e.*, from 60 to 30 minutes (Fig. 3, 4). The same advantage may be exploited in the packed red cell determination (Fig. 8) where the hematocrit value in the collection tube after 5 minutes of centrifugation is approximately equivalent to the standard Wintrobe procedure at 30 minutes. As a consequence of the various economies, a single technician can run sedimentation rate or hematocrit determinations on several hundred patient blood samples per day. In addition to modification of technical procedure, this method introduces and utilizes the concept of presenting sedimentation rates, as well as hematocrits, as percentages rather than absolute millimeters of fall as commonly expressed (Fig. 6). In the case of the 100 mm Wintrobe hematocrit tube, both mm and percentage criteria are the same, of course.

Since there is no necessity for the transfer of an accurate representative sample, as there is in the usual determination, the danger of sampling error due to improper mixing or measuring is avoided. The additional safety factor of handling a sealed container when testing bloods which may contain infectious agents is a distinct advantage and protection for laboratory personnel. With the use of disposable test tubes there is, of course, no glass washing or hematocrit tube cleaning problem.

Finally, since the blood sample is not used up or contaminated it is available for all other routine determinations which can utilize plasma or whole blood. Bacteriological studies, cellular counts, and many of the blood chemistry determinations may be made from the one blood sample tube(1,6).

Under some circumstances a rapid screening of blood samples for abnormalities is useful. A high degree of accuracy in spotting abnormal specimens may be obtained by taking a 15-minute reading. Values exceeding 10 should be considered abnormal. Final readings should, of course, be made at 30 minutes for confirmation and to obtain standard comparative values.

The proportional volume chart and procedure may also be used for other purposes such as measuring the relative percent of cells in ascitic fluid, the various percentile components of a centrifuged homogenate, sedimented bacteria, chlorella, etc., with no concern for a precise sample volume or calibrated tubes.

Summary. The conventional erythrocyte sedimentation rate and the packed red cell volume procedures have been modified to effect more than a 50% reduction in the operational time required. The essence of the modification consists in determining the sedimentation rates and hematocrit values directly in the original blood collection tube without the necessity of the usual transfer to a secondary, calibrated hematocrit tube. This is accomplished by employing a proportional volume chart which gives the sedimentation rate or hematocrit value for the red cells irrespective of the sample volume fluctuations. All values are expressed as percentages rather than the absolute measurements commonly employed. Erythrocyte sedimentation time is reduced from the standard of 60 to 30 minutes, and hematocrit centrifugation time is re-

duced from 30 to 10 minutes to yield approximately equivalent values. The method has been successfully tested with over 10,000 patient blood samples. The procedure may also be used for similar proportional measurements with ascitic fluid, tissue homogenates, bacterial suspensions, etc., with no need for exact sample volume or calibrated tubes.

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Autoradiography of Carbon-14 Labeled Isoniazid in Brain.* (22258)

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The widespread interest in drugs affecting the central nervous system, together with the increasing numbers of isotopically labeled drugs available in research, has stimulated the search for methods of producing autoradiograms of nervous tissue containing compounds labeled with such soft beta emitters as C-14. To date no suitable method has been developed for preparing brain autoradiograms for C-14 containing specimens. Previous methods have been described showing the localization of certain other isotopes not only in individual cells but even in inclusion bodies(1,2). These methods have not, however, been applied to such soft tissues as brain.

The autoradiograms described in this paper, together with supporting differential counting data, show the localization of C-14 labeled isonicotinic acid hydrazide (Isoniazid) and/or its metabolites in gross anatomical structures of brains. C-14 labeled Isoniazid was used as a prototype for these studies because of its known central nervous system effects and because it has previously been shown to localize in the nervous system of mice, rats, and guinea pigs(3). These observations on the localization of C-14 labeled Isoniazid have been confirmed by us in rats and cats, which were used in the autoradiograph studies.

Materials and methods. The Isoniazid used in these studies was synthesized by Murray and Langham of the Health Division, Los Alamos Scientific Laboratories(4). The compound was labeled in the carboxyl position and had a specific activity of 0.031 mc/mg. The labeled drug was diluted with normal Isoniazid in saline to a concentration of 2.5 mg/ml with a resultant activity of 34 x 10^6 dpm/ml. Adult cats were given an intraperitoneal dose of 10 mg/kg of Isoniazid dissolved in normal saline one hour before being anesthetized with pentobarbital sodium. During anesthesia the chest was opened and perfusion with normal saline begun in order to remove the vascular contents from the capillary bed of the brain. The left ventricle of the heart was cannulated and the descending

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