bution of spinal anesthesia in rabbits (Fig. 2c). This subject is being investigated by Bieter and Ridges.[4]

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Variability Due to Technique of the Sedimentation Index.

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The striking variation in sedimentation rate as between individuals has led us to devote considerable attention to an analysis of this phenomenon. Studies of the influence of the technique employed and the interrelationships of the results by those techniques are proving fruitful in revealing important biological measures. Discussion of these results is being reserved until more extensive data can be accumulated, but it seems advisable at this stage to publish a note on the trustworthiness of a single determination of the sedimentation index.

Of fundamental importance at all times in the interpretation of the data of observation or experimentation is a knowledge of the variability to be expected in replicate measures of the same type. Not infrequently the worker in the fields of the so-called "exact sciences" is able to estimate *a priori* and from theoretical premises the error attaching to any method. Such estimates cannot, of course, be accepted as valid until verified in practical work, a fact which is all too often neglected. The biological worker can rarely follow such a procedure, if for no other reason than that the organism itself contributes an amount to the variability which can only be determined by the critical mathematical analysis of actual results.

Many techniques of making the sedimentation test have been in use during the past 10 years, but since the Cutler,[1] the Linzenmeier,[2] and Westergren[3] methods have had the greatest number of followers, the present discussion will be limited to these methods.

The extent to which any single measure, or average of replicate measures of blood sedimentation is reliable as a basis of generaliza-

[4] Bieter, R. N., and Ridges, A. H., unpublished experiments.

[1] Cutler, J., *Am. J. Med. Sci.*, 1926, **171**, 882; *Am. Rev. Tuberc.*, 1929, **19**, 544.

[2] Linzenmeier, G., *München med. Wchnschr.*, 1923, **120**, 1243.

[3] Westergren, A., *Am. Rev. Tuberc.*, 1926, **14**, 94.

tions must be dependent basically upon a precise determination of the variability of a large number of replicate determinations.

To obtain information regarding the accuracy of the Cutler (5 cc.) technique, which we have favored because of its convenience in certain respects, 2 relatively large series of determinations were carried out on samples of blood as nearly homogeneous as possible and drawn at one time. The first, Series I, made use of horse blood. The very rapid sedimentation of the equine erythrocytes necessitated the filling of the tubes from an open vessel in which the fluid was constantly stirred. This led to an error of a range of ± 2 mm. in filling the tubes to the zero mark. Also, it was exceedingly difficult to make precise readings rapidly at the beginning of the experiment and the observations at the 5 and 10 minute periods were rejected on this account.

Since some of the sources of error were inherent in the nature of the sedimentation of the blood used, the experiment was repeated upon a sample of human blood drawn from a healthy individual. These results comprise Series II. Using a pipette, the sedimentation tubes were filled exactly to the zero mark. The readings were all made by one observer. The sedimentation was read to the nearest mm. and the authors believe that the records are precise within that range of error.

In establishing the variability of any one sedimentation technique for general purposes, it would seem desirable to include in the study results from a number of subjects of a wide range of sedimentation indices. Accordingly, for a comparative analysis of the accuracy of the determinations by each of the 3 methods, sedimentation rates were determined *in duplicate* for each of 45 young women attending the preliminary nursing course at the University of Minnesota, all apparently in good health. For this group, comprising Series III, the Cutler 1 cc. technique was employed, the Linzenmeier and Westergren methods being followed according to the original specifications.

As a measure of dispersal or "error" in magnitudes quantitatively measurable, the standard deviation, σ, enjoys an unchallenged popularity after the many decades of vigorous analysis in which it has been employed. Accordingly, the error inherent in the sedimentation techniques being studied herein will be measured by that statistic as defined originally by Karl Pearson. For Series I and II of the present data, the application of the well-known formula for the standard deviation is straightforward and presents no difficulty. In Series III, however, where determinations are available in dupli-

cate only, σ may be evaluated most directly by employing another technique. The differences between duplicate measures provide a series of ranges for samples of 2, and the mean range may readily be computed. The equation

$$\sigma = \frac{\text{mean range}}{1.12838},$$

where the denominator is drawn from tables by Tippett,[4] may then be employed for evaluating the required standard deviation.

The form of distribution of error has not been investigated for the data under consideration because of the inadequacy of the number of cases for such purposes. Accordingly the hypothesis of a "normal" distribution of error will be made. This hypothesis has been tested by one of us[5] on extensive data of a physico-chemical nature, and therein has been found justifiable. Theoretically, the normal curve is of infinite range. For practical purposes it is necessary to consider its range as limited by ordinates bounding, say, the central 98% of the total area. Such ordinates occur at $\pm 2.3263\ \sigma$ from the mean, and in the following discussion "range of error" will be defined accordingly.

Results. Series I. The series employing equine blood provided information that is, from certain points of view, merely incidental to the main task, which must center about blood from human subjects. Nevertheless it is of interest to note the statistics of sedimentation obtained for this type of vascular fluid.

The very fast settling of the red corpuscles will be noted from the summary of results presented in Table I. The standard deviations, measuring the error inherent in the method, do not give any significant evidence of real differentiation at the various time intervals. Accordingly, the average "variance" (σ^2) may be accepted as the most reliable basis for the calculation of the standard devia-

TABLE I.

Statistics for 49 replicate determinations of sedimentation for equine blood (Cutler 5 cc. method).

Time Interval	Mean mm.	σ mm.	Time Interval	Mean mm.	σ mm.
min.			hr.		
15	23.45	1.09	1	27.59	1.01
20	24.96	0.90	2	28.51	0.95
25	25.84	0.93	6	29.18	1.04
30	26.10	0.95	18	29.43	1.01

[4] Tippett, L. H. C., *Biometrika*, 1925, **17**, 364.

[5] Treloar, Alan E., *Cereal Chem.*, 1932, **9**, 449.

tion of replicate determinations, giving $\sigma = 0.99$ mm. Correcting for the error of filling the tubes (range = ±2 mm.), we find a new value for σ of 0.49 mm. It is of interest to compare this value with those arising from Series II and III.

Series II. Passing to the series of 50 determinations on the one sample of human blood (Table II), a sharp contrast will be noted in the mean rate of sedimentation. The sedimenting corpuscles take 6 hours to reach a level attained by the red cells of the horse blood in less than 15 minutes. However, the standard deviations of the replicates at each interval of time approximate to the value of 0.49 mm. secured for Series I. Securing the average value as indicated above, we find the standard deviation of replicated determinations for human blood to be 0.48 mm.

TABLE II.
Statistics for 50 replicate determinations of sedimentation for human blood (Cutler 5 cc. method).

Time Interval	Mean mm.	σ mm.	Time Interval	Mean mm.	σ mm.
min.					
10	2.16	0.46	60 min.	10.54	0.50
15	2.94	0.51	90 "	14.32	0.47
20	3.64	0.52	2 hr.	16.36	0.48
25	4.48	0.48	3 "	18.78	0.41
30	5.22	0.50	4 "	20.56	0.49
35	6.16	0.54	6 "	23.02	0.37
40	7.02	0.47	18 "	26.20	0.40
45	7.96	0.49			

Series III. The standard deviations arising from application of the method of ranges, as derived from the duplicate analyses by 3 techniques on the 45 women subjects, are given in Table III. The progressive increase in σ for the successive time intervals in the Linzenmeier tubes is without question statistically significant. Although not so orderly, the same trend is apparent for the Westergren

TABLE III.
Standard deviations of the errors of the Cutler (C), Westergren (W), and Linzenmeier (L) techniques, based on 45 duplicate tests.

Time Interval	Standard Deviation* C	W	L
hr.			
½	0.53±.06	0.26±.03	0.47±.05
1	0.61±.07	0.35±.04	0.59±.07
2	0.41±.05	0.32±.04	0.71±.08
6	0.55±.06	0.87±.10	0.97±.11
16-18	0.45±.05	1.22±.14	1.08±.12
Average	0.51±.03		

* ± standard error in each case.

technique. In the Cutler series, however, there is no such trend, the individual standard deviations deviating by random errors only from the average value of 0.51 mm. Thus the results by the Cutler (1 cc.) method on the heterogeneous blood samples are in excellent agreement with those arising in Series I and II from the homogeneous samples, and the weighted average σ of 0.50 must surely give a highly dependable estimate of the variation due to technique in blood sedimentation readings by the Cutler methods.

Considering now the Westergren and Linzenmeier* values, it would appear to be a reasonable deduction that, for the one hour readings, the Westergren method is somewhat more consistent than the Cutler, and the Linzenmeier less accurate in replication. The latter is clearly more open to question than the former. For the half-hour readings, the Westergren method is without doubt more consistent in its results than either of the other 2, while after several hours the Cutler readings are unquestionably more accurate than those arising from either the Westergren or Linzenmeier techniques.

We reserve for later consideration the relative merits of the half-hour or one hour readings for the measurement of the sedimentation characteristics of human blood.

Conclusions. The Cutler methods of blood sedimentation tests show consistent accuracy at all periods of time so far as errors of measurement are concerned. Replicated tests on the same sample of blood may be expected to vary over a range of 2.3 mm. in 98% of such tests, provided the error is of the type described by the "normal law of errors".

The Westergren method shows higher accuracy than the Cutler method at the half-hour period of sedimentation, the error increasing steadily with increase in the time interval until it is approximately double that of the Cutler at 16 hours.

The Linzenmeier technique appears to have an error equal to or greater than the Cutler according as the time of sedimentation is short or long, so far as absolute fall of the red-cell column is concerned. The method of accumulation of the data considered herein did not permit of a study of sedimentation time as ordinarily recorded for the Linzenmeier method. Such a study, if made, would have had little comparative value in the present connection.

* We are considering the Linzenmeier method here in terms of distance of fall as for the other techniques, and not in terms of time taken to reach the 18 mm. mark.