ESTIMATION OF THE ERYTHROCYTE SEDIMENTATION RATE OF CAPILLARY BLOOD

DESCRIPTION OF A NEW METHOD

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Venous blood is necessary for the estimation of the erythrocyte sedimentation rate (E.S.R.) by the methods of Westergren (1921) and Wintrobe and Landsberg (1935), but venepuncture may occasionally prove difficult or impossible, owing to such factors as inadequate veins, obesity, and flexion deformities. A search was therefore undertaken for a reliable method of estimating the E.S.R. using capillary blood. The consensus of opinion (Nichols, 1942) is that under similar conditions the erythrocyte sedimentation rates of capillary and venous blood are comparable.

Review of Micromethods

Many micromethods are to be found in the literature, and unpublished methods undoubtedly exist. Table I summarizes those methods which seem to be the most widely known; details are also given of two unpublished micromethods and of the Westergren macromethod. Westergren (1957) warns against micromethods, and several other writers believe that the narrow tubes used in certain micromethods cause slowing and irregularity of the sedimenting column of cells (Ham and Curtis, 1938). Thygesen (1942) states that occasionally the E.S.R. is accelerated by narrow tubes. The recommended internal diameter has been variously stated as: over 1.5 mm. (Thygesen), over 2 mm. (Ham and Curtis, 1938), over 2.2 mm. (Westergren, 1957), and 2.5 mm. (Mårtensson and Hansen, 1953). Many authors, however, claim reliable results from methods utilizing tubes of an internal diameter of 1 mm. or even less (Table I). In part these discrepancies may be accounted for by the anticoagulant, as Mårtensson and Hansen and other workers have shown that diluted blood is less likely to be affected by the bore of the tube than blood to which a solid anticoagulant

has been added. Table I (opposite) shows that five micromethods employ tubes of an internal diameter of 2 mm. or over. It is certain that these methods will distinguish normal from abnormal sedimentation rates, but since a micromethod should not need more than 0.3 ml. blood, it is obvious that the wider the bore of the tube the shorter the column must be. It was considered that the results from any micromethod chosen should be capable of accurate conversion at all rates of sedimentation into the corresponding Westergren values, the Westergren method being probably the commonest venous method in use in Great Britain (Dacie, 1956a) and the most reliable (Lawrence, 1953; Duxbury, 1957; and others). In effect, this means that the sedimenting column used in the micromethod must be of the same length as that used in the Westergren method, if Westergren values in the range of 70-130 mm. at 1 hour are to be differentiated; for it is obvious that the maximum possible E.S.R. in mm. at 1 hour will vary directly with the length of the column used. As an example of the undesirability of using a short tube, Nelson and Whyte (1955), using Method 8 (sedimenting column only 38 mm. long), found that "any reading by the micromethod of more than 10 mm. in half an hour or 15 mm. in one hour, may correspond to a Westergren value of anything from 40-140 mm. in one hour". They state that the maximum rate of fall would give a much closer correlation between the methods, but, since multiple readings are inconvenient, it seemed advisable to test the claims of Peters (1945) for the only method (No. 7, Table I) employing a sedimenting column 200 mm. long. Good agreement with the Westergren method is apparent in the values obtained by Peters in thirty patients, though it is not clear from his paper whether he used capillary or venous blood in the smaller tubes.

TABLE I
PARTICULARS OF TWELVE METHODS

Method No.	Authors	Date	Internal Diameter of Tube (mm.)	Length of Sedimenting Column (mm.)	Approx. Volume of Blood Required (ml.)	Anticoagulant	Normal Values	Comment
1	Cutler	1927	2.5	50	0.3	3 per cent. Na citrate; that amount adhering to walls of tube	A nearly horizontal line	Serial readings must be taken
2	Landau	1933	1	50	0.04	5 per cent. Na citrate to 12 mm. mark	1-6 mm. ♂ } at 1-8 mm. ♀ } 1 hr	Modification of the Linzenmeier-Raunert method, see also Rogatz (1943)
3	McSweeney ..	1934	1	100	0.08	3.8 per cent. Na citrate; 1 vol. to 4 vols blood	10 mm. at 1 hr (max.)	
4	Smith	1936	2.5	50	0.3	5 per cent. Na citrate; 1 vol. to 7.5 vols blood	4 mm. at 30 min. (max.) 9 mm. at 1 hr	Modification of Cutler's method
5	Kato	1938 1940	0.79-1	64-102	0.05	Various dry anticoagulants	0-20 per cent. of length of column at 1 hr	
6	Obermer	1943	2	100	0.3	Saturated Na oxalate to 4 mm. mark	4 mm. at 15 min. 10 mm. at 30 min. 25 mm. at 1 hr (max.)	Modification of Balachowsky's method
7	Peters	1945	1.2	200	0.25	3.8 per cent. Na citrate; 1 vol. to 4 vols blood	As for standard Westergren method	Blood taken directly into tube
8	Nelson and Whyte	1955	2.5	38	0.16	3.8 per cent. Na citrate; 1 vol. to 4 vols blood	10 mm. at 1 hr (max.)	Crista apparatus (Messrs. Hawksley, London, W.I.). See also Goldberger (1940).
9	Bodian	Unpublished	2	100	0.3	3.8 per cent. Na citrate; 1 vol. to 4 vols blood	3-10 mm. at 1 hr	Apparatus obtainable from Messrs Hawksley.
10	Masters	Unpublished	1.5	100	0.2	3.8 per cent. Na citrate; 1 vol. to 4 vols blood	3-10 mm. at 1 hr	Method similar to that used at Hospital for Sick Children, Gt. Ormond Street, London
11	Present Method ..		1.25 ±0.01	200	0.25	5 per cent. Na citrate; 1 vol. to 5 vols blood	2-15 mm. at 1 hr	Modification of Peters' method. Results corrected to correspond to Westergren values
12	Westergren ..	1921 1957	2.5	200	1.6	3.8 per cent. Na citrate; 1 vol. to 4 vols blood	3-5 mm. ♂ } at 4-10 mm. ♀ } 1 hr	

Results using Peters' Method

Peters used tubes 300 mm. in length; accordingly "Veridia"* tubes of this length and of 1.25 mm. internal diameter (tolerance ± 0.01 mm.) were obtained. These tubes have stout walls, and may be put in Westergren stands. A single mark was etched on each capillary tube at 200 mm.

(a) *Venous Blood.*—75 samples of venous blood diluted with 3.8 per cent. sodium citrate in the proportion 4 vols blood to 1 vol. citrate (*i.e.* the Westergren mixture) were put up in Westergren tubes and in Veridia microtubes. Analysis of Table II (overleaf) shows that equivalent readings were obtained, except in the cases marked with an asterisk, throughout the scale of E.S.Rs. The microtubes were no more difficult to read than the macro tubes, and slowing and irregularity of the sedimenting column of cells did not present problems.

* Obtainable from Messrs. Chance Bros., Ltd., Smethwick Birmingham, 40.

TABLE II
75 SAMPLES OF VENOUS BLOOD

Tube	Westergren	Micro.
Blood (4 vols.)	Venous (4 vols.)	Venous (4 vols.)
Citrate (1 vol.)	3·8 per cent.	3·8 per cent.
1	2	2
2	3	3
3	4	4
4	4	5
5	5	4
6	5	5
7	5	6
8	6	6
9	6	7
10	7	5
11	7	6
12	7	6
13	7	8
14	7	8
15	8	6
16	8	6
17	8	7
18	8	7
19	8	8
20	8	9
21	8	9
22	8	9
23	9	8
24	9	10
25	10	11
26	10	12
27	10	12
28	11	9
29	11	14
30	12	10
31	12	12
32	12	14
33	12	14
34	13	11
35	13	12
36	13	14
37	15	13
38	16	15
39	18	12*
40	18	14
41	18	17
42	19	14
43	20	12*
44	20	15
45	22	23
46	23	21
47	23	28
48	27	17*
49	27	22
50	28	26
51	29	24
52	29	33
53	30	27
54	30	33
55	32	39
56	36	39
57	37	30
58	37	42
59	40	45
60	41	38
61	41	43
62	48	55
63	48	59
64	48	65*
65	49	56
66	50	47
67	53	70*
68	54	54
69	55	62
70	57	65
71	58	68
72	70	74
73	78	72
74	93	90
75	123	132

Micro = Veridia microtubes, internal diameter 1·25 mm.

* Results considered unsatisfactory.

(b) *Capillary Blood.*—For these reasons capillary and venous samples from 22 volunteer patients were compared (Table IIIA). In view of the clotting that occurred in two samples of capillary blood (Nos. 19 and 22) 5 per cent. citrate was substituted, the dilution being kept constant, as Ham and Curtis have shown that such a substitution is without effect on the sedimentation rate of venous blood. The results from fifteen further patients are given in Table IIIB.

TABLE III (A AND B)
CAPILLARY AND VENOUS SEDIMENTATION RATES
COMPARED IN 37 PATIENTS

Patients Group	A		B	
	Wester- gren	Micro.	Wester- gren	Micro.
Blood	Venous 4 vols.	Capillary 4 vols.	Venous 4 vols.	Capillary 4 vols.
Citrate	3·8 per cent. 1 vol.	3·8 per cent. 1 vol.	3·8 per cent. 1 vol.	5 per cent. 1 vol.
1	2	2	1	5
2	5	9	2	12
3	6	5	3	15
4	7	11	4	25
5	8	6	5	24
6	8	8	6	19
7	9	9	7	31
8	9	11	8	45
9	12	10	9	32
10	12	12	10	40
11	13	18	11	38
12	16	19	12	40
13	20	14	13	51
14	28	36	14	48
15	30	28	15	69
16	30	29		
17	32	31		
18	37	clotted		
19	41	clotted		
20	48	65		
21	55	79		
22	58	clotted		

The tendency for an accelerated E.S.R. to occur in the microtubes is readily seen, though this effect is not constant. As such a factor would, if constant, aid recognition of slightly increased sedimentation rates, an effort was made to find a citrate concentration and dilution at which this occurred. A number of experiments were performed with venous blood using varying proportions of 3·8 and 5 per cent. sodium citrate. The effect of the addition of the correct quantity of Wintrobe's solid anticoagulant mixture of oxalates (Wintrobe and Landsberg, 1935) was also determined. These experiments led to the following observations:

- (1) A dilution of 5 vols blood with 1 vol. 5 per cent. sodium citrate gave the most reproducible results when compared with the Westergren method. This was therefore adopted as the definite modification of Peters' method.

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(2) Using a solid anticoagulant, the results with these microtubes were invariably slower than the corresponding Westergren values (sometimes by as much as 45 mm./hr).

Description of the Modified Peters' Method

A 0.2 ml. pipette should be calibrated, preferably with mercury, and marks etched at 0.05 ml. and 0.15 ml. (marks "A" and "B"). The pipette is slightly overfilled to mark "A" with 5 per cent. citrate, the end wiped with cotton-wool, and the citrate level adjusted to mark "A". Capillary blood is obtained by ear-lobe puncture with a straight No. 2 Haegedorn needle, after the lobe has been cleaned with spirit and briskly rubbed with dry cotton-wool. Freely-flowing capillary blood is essential, and only the gentlest squeezing is permissible (a second operator to steady the lobe and encourage the flow of blood while the first fills the pipette is a great advantage). The pipette is filled to the 0.2 ml. mark; 0.15 ml. of the blood-citrate mixture is expelled on to a waxed watch glass, and the pipette is then refilled with capillary blood from Mark A to Mark B. In this way, the citrate solution is in contact with the maximum amount of blood. Blood and citrate are then gently but thoroughly mixed and left to stand for 3 to 5 minutes (not longer) in the watch glass, another watch glass of equal diameter being put over it (total volume 0.3 ml.: 0.25 ml. blood, 0.05 ml. 5 per cent. citrate). The microtubes are most conveniently filled to the 200 mm. mark by means of a small teat. An unbroken column free from air bubbles is essential, and this is easily achieved with practice. The tubes are put up in a Westergren stand and read after 1 hour with a millimetre ruler. (When possible, independent readings were made during this trial by two observers; when this was impracticable, the microtubes were read first.) At the completion of the test, the blood-citrate mixture should be run into blotting-paper (as suggested by Montgomery, 1943), as the presence of small clots invalidates the test. The pipette should be cleaned with distilled water, alcohol, and acetone between each sample. The microtubes are conveniently cleaned in the same way and at the same time as the Westergren tubes; that is, with cold tap water and distilled water. Excess water should be shaken out before the tubes are dried in an oven.

(a) *Results with Venous Blood.*—Venous blood from thirty patients was collected in plain venules* and diluted immediately with the appropriate

quantity of 5 per cent. citrate solution in the 0.2 ml. pipette as described for capillary blood. The results were compared with those obtained by the Westergren method (Table IVA).

(b) *Results with Capillary Blood.*—Capillary and venous samples from a further fifty patients were compared (Table IVB). No patient who appeared

TABLE IV (A AND B)
(A) VENOUS BLOOD DILUTED AS FOR CAPILLARY BLOOD IN THIRTY PATIENTS
(B) CAPILLARY AND VENOUS SEDIMENTATION RATES (mm.) COMPARED IN FIFTY PATIENTS

Patients Group	A		B	
	Westergren	Micro.	Westergren	Micro.
Blood	Venous 4 vols.	Venous 5 vols.	Venous 4 vols.	Capillary 5 vols.
Citrate	3.8 per cent. 1 vol.	5 per cent. 1 vol.	3.8 per cent. 1 vol.	5 per cent. 1 vol.
1	6	9	1	2
2	8	12	2	3
3	8	16	3	5
4	9	14	4	3
5	14	21	5	4
6	14	24	6	4
7	15	30	7	5
8	17	22	8	7
9	17	41	9	10
10	22	38	10	8
11	29	44	11	13
12	29	53	12	7
13	35	62	13	11
14	39	70	14	13
15	40	53	15	11
16	45	62	16	13
17	45	62	17	14
18	45	65	18	13
19	47	74	19	16
20	51	65	20	18
21	53	77	21	15
22	54	82	22	21
23	55	79	23	24
24	57	92	24	30
25	63	88	25	20
26	70	92	26	21
27	77	95	27	31
28	85	105	28	7*
29	95	125	29	23
30	130	134	30	26
			31	23
			32	15*
			33	32
			34	28
			35	33
			36	37
			37	30
			38	36
			39	41
			40	51
			41	52
			42	53
			43	48
			44	51
			45	54
			46	76
			47	67
			48	90
			49	107
			50	123

* Results unsatisfactory. Blood difficult to obtain: small clots present.

* Obtainable from Bayer Products, Ltd., London.

ill was asked to co-operate in this trial, and this accounts for the number of normal values obtained. However, except in the two cases marked with an asterisk, where small clots occurred and results were unreliable, it was observed that:

- (1) The acceleration in the microtubes is constant within narrow limits (Figure) when the Westergren E.S.R. is raised;
- (2) Capillary and venous samples give results that are strictly comparable.

Figure.—Sedimentation rates by Westergren and modified Peters' methods.

From the observed micro-E.S.R., the standard Westergren value may be calculated by taking the mid-point through the curves drawn in the Figure (these limits are curved because of the effect of erythrocyte packing at the higher sedimentation rates). If, for example, the micro-E.S.R. reading is 50 mm./hr, then the Westergren value in over 90 per cent. of cases will be 33 ± 6 mm./hr. It can be seen that though capillary results of 20 and 31 mm. have been found to correspond to Westergren values of 15 mm., these results would have been reported as equivalent to the following Westergren values, 13 ± 3 mm., and 19 ± 4 mm.; indeed, analysis of the most divergent points shows that in no case would the clinician have been significantly misled if the Westergren values as calculated from the micro-E.S.R. values had been the only data available.

In certain cases, serial readings were taken of the cases compared in Table IV at 10-minute intervals. These observations showed that though sedimen-

tation at 10 minutes might be slower in the microtubes, presumably because of a slight prolongation of the phase of aggregation, this relationship was reversed after a further 10 minutes and for the remaining period.

Discussion

In the 5 months during which this study was in progress, six patients were seen (out of approximately 1,200) in whom venepuncture had been unsuccessfully attempted on at least one occasion in the past. In two, venepuncture was not attempted. In all six patients capillary blood was easily obtainable and their preference for this procedure was emphatic. In these patients, results were obtained that were consistent with the clinical picture, and in no case were differences noted between the behaviour of these micro-E.S.R.s and those which were compared with the Westergren method.

All the patients who volunteered to take part in this study were adults, but this modification of Peters' method could be adapted for paediatric use. Tubes of an internal diameter of 1.25 mm. have given reliable results, and this holds true for samples from non-anaemic patients (Hb 13 to 15 g./100 ml.). This method was designed for estimating the E.S.R. of patients attending a Department of Physical Medicine, and has proved of value in the six cases mentioned.

Puncture of the ear-lobe was used in preference to that of the finger pulp in every patient except one (who had very small lobes). Ear-lobe puncture is less painful and provides blood more readily (Dacie, 1956b). A satisfactory result was obtained from finger-prick blood in the one patient in whom this method was used.

The acceleration that occurred in the microtubes was reproducible when 5 vols blood were diluted with 1 vol. 5 per cent. citrate, and the results obtained were capable of conversion at all sedimentation rates into the corresponding Westergren values with more accuracy than those obtained by the technique described by Peters.

Summary

An account is given of some common methods employed for determining the erythrocyte sedimentation rate of capillary blood. The claims of Peters (1945) are investigated and a modification of his method is described, that requires 0.25 ml. blood and 0.05 ml. 5 per cent. Na citrate.

From data obtained when results by this method, using capillary blood (fifty patients) and venous blood (thirty patients), were compared with results

by the Westergren method, it is shown that accurate prediction of the Westergren values is possible at all rates of sedimentation with the use of this micro-method.

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Détermination de la vitesse de sédimentation érythrocytaire du sang capillaire par un nouveau procédé

RÉSUMÉ

On décrit quelques procédés habituels pour déterminer la vitesse de sédimentation érythrocytaire du sang capillaire. On étudie les résultats de Peters (1945) et on décrit une modification de son procédé, qui demandait 0,25 c.c. de sang et 0,05 c.c. de citrate de soude.

La comparaison des résultats de ce procédé, employant du sang capillaire (50 malades) et veineux (30 malades), à ceux qu'on obtient avec la méthode de Westergren, montre que la micro-méthode permet de prévoir précisément, à toutes les vitesses de sédimentation, les chiffres donnés par la méthode de Westergren.

Determinación de la velocidad de sedimentación eritrocitaria de la sangre capilar por un nuevo método

SUMARIO

Se describen algunos procedimientos habituales para determinar la velocidad de sedimentación eritrocitaria de la sangre capilar. Se estudian los resultados de Peters (1945) y se describe una modificación de su método, que requería 0,25 c.c. de sangre y 0,05 c.c. de citrato de sodio.

Al comparar los resultados con este método, empleando la sangre capilar (50 enfermos) y venosa (30 enfermos) a los obtenidos con el método de Westergren, se ve que el micro-método permite anticipar con precisión, a todas las velocidades de sedimentación, las cifras que se obtiene con el método de Westergren.