AN ASSESSMENT OF A NEW MICROMETHOD FOR DETERMINING THE ERYTHROCYTE SEDIMENTATION RATE

STUART SANDERS, M.B., Ch.B., M.R.C.S., L.R.C.P., D.Obst.R.C.O.G., D.C.H.
London

THE ANCIENTS of medicine, Hippocrates and Galen, recognized that when blood was allowed to stand, it separated into four components. These were called ' cholera ', ' sanguis ', ' melancholia ' and ' phlegma ' and upon them, the pathology of disease was based (Fahraeus 1929).

John Hunter (1794) wrote in his *A Treatise on the blood, inflammation and gun-shot wounds* about separation of red cells from the serum. Furthermore, he resuspended the cells in serum and showed that the rate of sedimentation depended on the quality of the serum and not upon the red cells. Babington, working in 1830, collected blood under a layer of olive oil, and observed the rate of sedimentation under various conditions. At the end of the nineteenth century, Biernacki (1894) observed sedimentation of blood using an anticoagulant. Von Limbeck (1901) utilized this sedimentation process to determine the relative volumes of the plasma and corpuscles.

Robin Fahraeus (1918a and 1918b) further developed these basic observations and showed that the rate of separation of red cells was increased during pregnancy. In 1921, he published his monumental work " Suspension-stability of the Blood " in which the physiology and pathology of blood sedimentation were fully explored. This was the birth of the erythrocyte sedimentation rate (ESR) as we know it today.

The earlier methods for estimating the ESR all used blood obtained by venesection. In some patients, however, it is not possible to obtain venous blood (for example, in children, the obese and the elderly). Furthermore, a venesection is aesthetically unpleasant to some patients who would readily accept a finger-prick blood test.

Over the last 40 years, therefore, haematological science has been trying to find a reliable way of estimating the ESR requiring very small

J. COLL. GEN. PRACTIT., 1966, 11, 253

amounts of blood. In the spring of 1964, a new micromethod was placed upon the market by Harshaw Chemicals Limited[1] and advertized to the profession.[2] To date, no properly controlled trial of this method has been published.[3] It was, therefore, considered that there was a good case for a trial of this new method (Harshaw ESR) carried out in parallel with an established technique.

Craddock (1958) states that the ESR estimation is within the scope of the general practitioner. It can be of great assistance to him in diagnosing illnesses and following their progress. He recommends the method first described by Westergren (1921a) and since this is the most commonly used technique in laboratories in Great Britain, it was chosen as the method to be used in comparison with the Harshaw ESR.

Materials

The patients who took part in this trial were all drawn from a 4,000-patient mixed private and national health practice in north-west London. The practice is attended by three partners; all the observations were made by the author who is a principal in the partnership. The patients whose blood was examined were seen either in their own homes or in the surgery; they complained of a wide variety of ailments, some trivial or non-existent, some of a more serious nature. They were asked if they minded having a blood test taken in duplicate (by finger-prick and by venesection) and surprisingly few declined.

While it is appreciated that the Harshaw method is mainly applicable to children because of the small amount of blood required, no patient under the age of ten years was used in this series. It was thought unfair to subject children to unnecessary skin pricks and venepunctures. (The assessment of the Harshaw ESR method with children provides material for a future paper.)

In all, 100 patients took part in this study. While this is not a large number (by comparison with Shannon and Bywaters (1957) who performed 19,000 duplicate ESR readings), the results are such that satisfactory conclusions can be drawn.

Aims

1. Evaluation of the normal range of values for the Harshaw ESR in men and women.
2. Assessment of the Harshaw ESR as a screening test for organic disease.
3. Assessment of the Harshaw ESR for following the progress of an illness.

[1]Harshaw Chemicals Limited, Daventry, Northamptonshire.
[2]*British Medical Journal*, 9 May 1964, p. viii.
[3]Since this was written a paper on the method has been published by J. Phillips in *The Practitioner* (1965, **194**, 672).

Methods

Westergren ESR method (Westergren 1921a)

0.4 ml. of a sterile 3.8 per cent sodium citrate solution is drawn into a syringe. Blood is aspirated into the same syringe to make the total up to 2.0 ml. (that is, 1.6 ml. of blood is added). The contents of this syringe are then expelled into a test tube and thoroughly mixed. The blood-citrate mixture is sucked up to the zero mark in a tube, 300 mm. long, which is placed in a stand with a rubber seal at the bottom and a spring clip at the top. The tube is 2.5 mm. in internal diameter and has mm. markings for the lower 200 mm.; the zero mark is at the top of these graduations. The tube is left undisturbed at room temperature for exactly one hour and the upper level of the sedimented red cells is read off on the mm. scale. The ESR is expressed mm./1 hr.

Harshaw ESR method

This method utilizes capillary tubes 1 mm. in diameter and 105 mm. in length. There are two marks on the tube: 15 mm. and 75 mm. from the upper end. This upper end is placed in 3.8 per cent sodium citrate solution and the fluid is allowed to rise by capillary traction to the first (15 mm.) mark. The patient's finger is now pricked and the upper end of the capillary tube is placed in the drop of blood. The blood is allowed to flow into the tube until the fluid level reaches the second (75 mm.) mark. The ratio blood: citrate solution is exactly the same as in the Westergren method (that is, 4:1) and the total volume of blood required is 0.04 ml. The inside of the tube is coated with a mixture of sodium and potassium sequestrene in order to prevent the blood from clotting before it mixes with the sodium citrate solution. The contents of the tube are thoroughly mixed by rotating the tube between finger and thumb and by causing the mixture to flow backwards and forwards in the tube four times. Finally, the blood citrate mixture is arrested at the lower end of the tube which is sealed with, and stuck vertically into, plasticine. The fall in red blood cells after an hour is measured as in the Westergren method against a mm. scale.

(There is in the manufacturer's kit a copper sulphate method for determining the haemoglobin content. After a preliminary trial, this method was found to be too crude and was investigated no further.)

Results

Normal value for the Harshaw ESR

Westergren (1926) stated that the normal values for his ESR method were 1–3 mm./1 hr. in males and 1–7 mm./1 hr. in females; borderline values were 4–7 mm./1 hr. and 8–11 mm./1 hr. respectively. It was decided to take 7 mm./1 hr. and 11 mm./1 hr. as representing the upper limits of normality in men and in women respectively according to Westergren's method.

Men

Out of the 100 patients examined, 31 male cases had normal ESRs according to the above criteria; their ages ranged from 13 years to 85 years (average age 36.4 years) and figure 1 shows their distribution. Figure 2 shows the distribution of the Harshaw ESR values in this group and it can be seen that in 28 out of the 31 cases the Harshaw ESR lies at or below 9 mm./1 hr.; this value was taken as the upper

limit of normal for men. The coefficient of correlation between the Westergren and Harshaw results is +0.953.

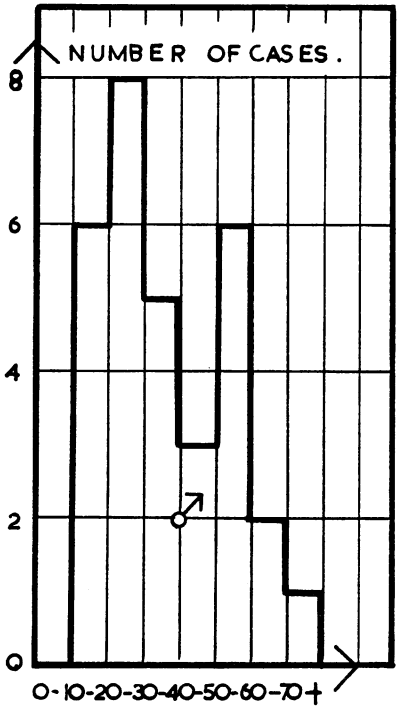

Figure 1.
Age distribution of normal males.

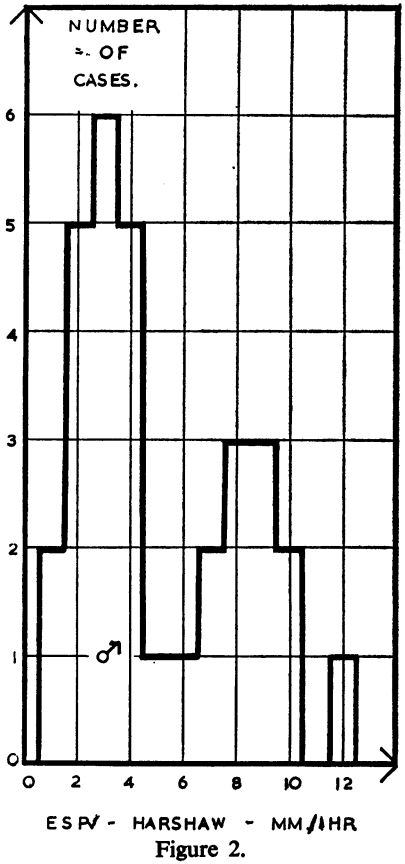

Figure 2.
Distribution of Harshaw ESR results in normal males.

Women

There were 38 females in which the Westergren ESR was 11 mm./1 hr. or less. Their ages ranged from 11 years to 77 years (average age 38.7 years) and their distribution is shown in figure 3. Figure 4 shows the distribution of the Harshaw ESR values in this group and it can be seen that in 35 out of 38 the Harshaw ESR lies at or below 15 mm./1 hr.; this value was taken as the upper limit of normal for women. The coefficient of correlation between the Westergren and Harshaw results is +0.840.

Validity of the Harshaw ESR as a screening test for disease (and its comparison with the Westergren ESR)

There were 17 males and 23 females with abnormal ESRs and their average ages were 49.6 years and 46.0 respectively. The distribution of their ages is shown in figure 5 and their ages range from 18 years to 87 years. Their diagnoses are shown in table I.

Figure 6 shows graphically how the two sets of results comprising 54 duplicate ESR readings compare. The coefficient of correlation between the Harshaw and Westergren ESRs is +0.799. While at normal and low abnormal values, the Harshaw ESR reading is greater than the Westergren reading, in higher values, the position is reversed; at about 20 mm./1 hr. the values are the same. This discrepancy in no way hinders the Harshaw ESR as a test for active disease.

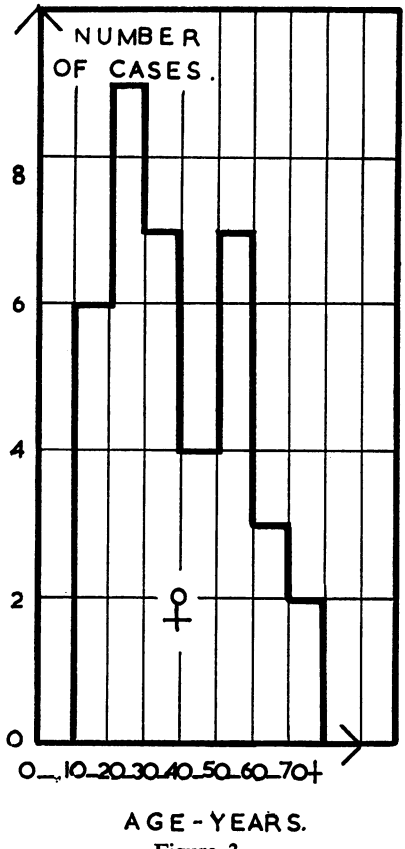

Figure 3.
Age distribution of normal females.

TABLE I
THE DIAGNOSES MADE IN THE ABNORMAL CASES

Disease	*Men*	*Women*
Upper respiratory infection	6	4
Lower respiratory infection	3	2
Skin sepsis	1	2
Anaemia	2	1
Pregnancy	0	2
Toxic hepatitis	0	1
Duodenal ulcer	0	1
Cholecystitis	0	1
Urinary infection	2	1
Diverticulitis	0	1
Orchitis	1	0
Cervical erosion	0	1
Congestive cardiac failure	0	2
Electrolyte imbalance	0	1
No organic disease	2	3
Total	17	23

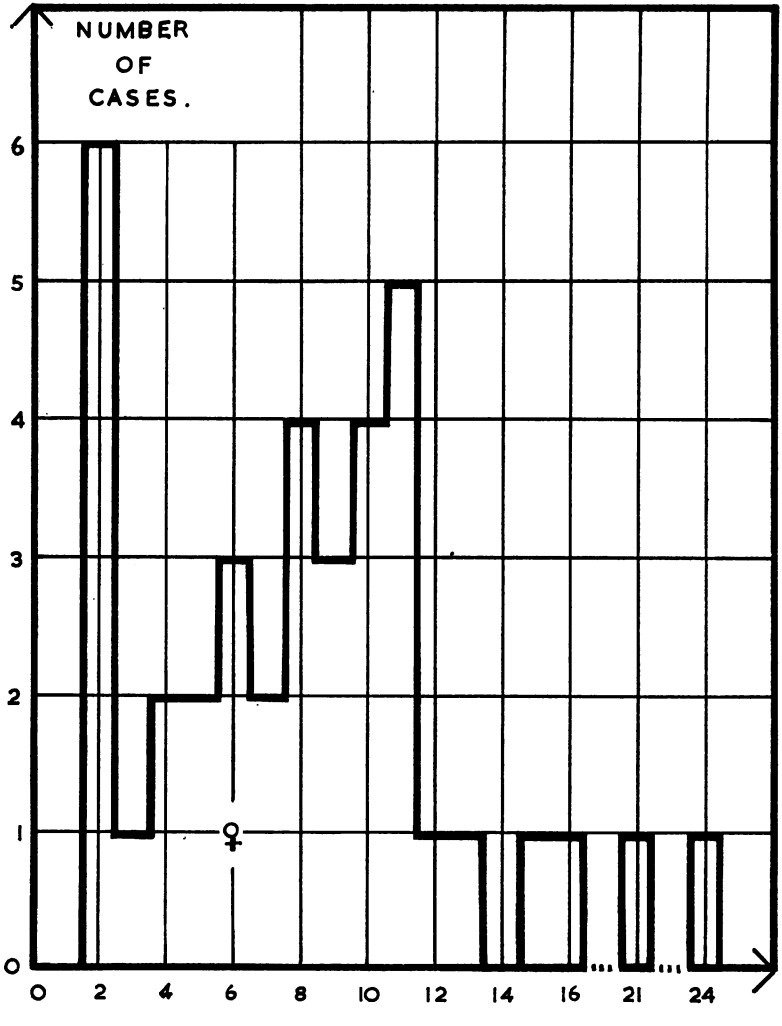

Figure 4.
Distribution of Harshaw ESR results in normal females,

The Harshaw ESR method as a means of following the activity of a disease process

This is best discussed by taking examples of cases in which repeated determinations of the ESR were taken; figures 7–13 are self-explanatory.

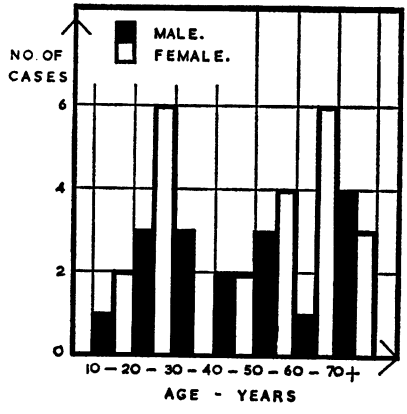

Figure 5.
Age distribution of abnormal cases (male and female).

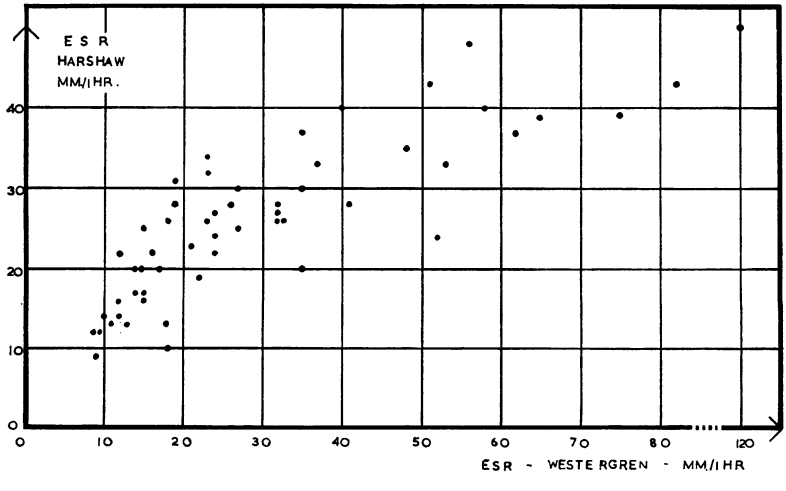

Figure 6.
Relationship of the Harshaw ESR result to the Westergren ESR result in abnormal cases (male and female).

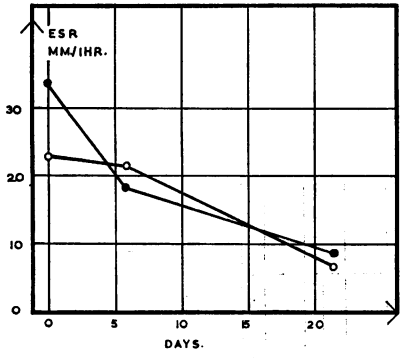

Figure 7.
Female. *Age:* 64 years.
Diagnosis: Bronchopneumonia (bilateral).

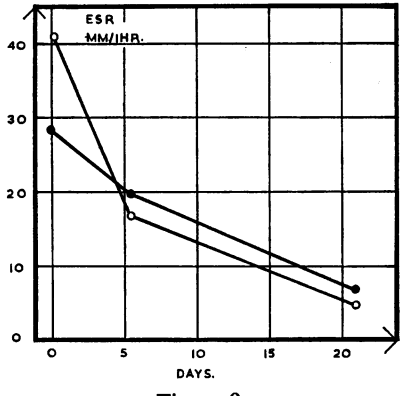

Figure 9.
Male. *Age:* 30 years.
Diagnosis: Pharyngitis.

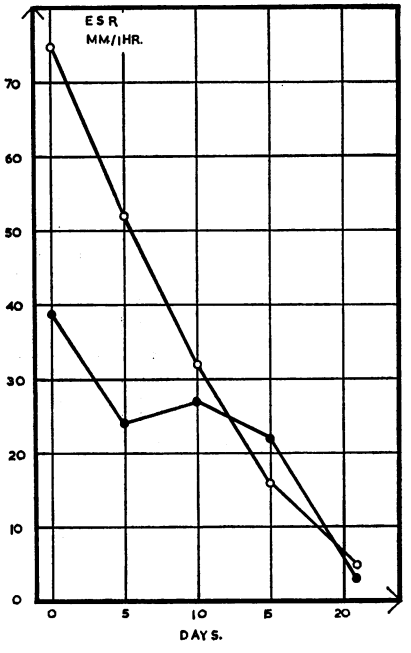

Figure 8.
Male. *Age:* 25 years.
Diagnosis: Lobar pneumonia (left lower lobe).

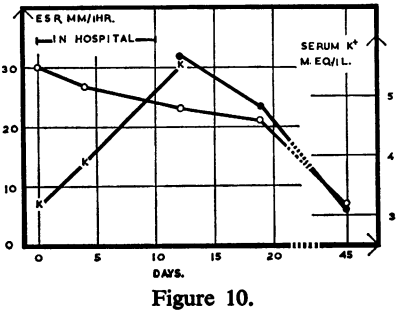

Figure 10.
Female. *Age:* 67 years.
Diagnosis:
1. Chronic congestive cardiac failure.
2. Hypokalaemia (due to diuretic overdosage).

Key to figures 7–13
- ● — Harshaw ESR. (mm./1 hr.)
- O — Westergren ESR. (mm./1 hr.)
- K — Serum potassium (mEq./L.)
- S — Serum glutamic oxalascetic transaminase (units/ml.)
- H — Haemoglobin (G/100 ml.)

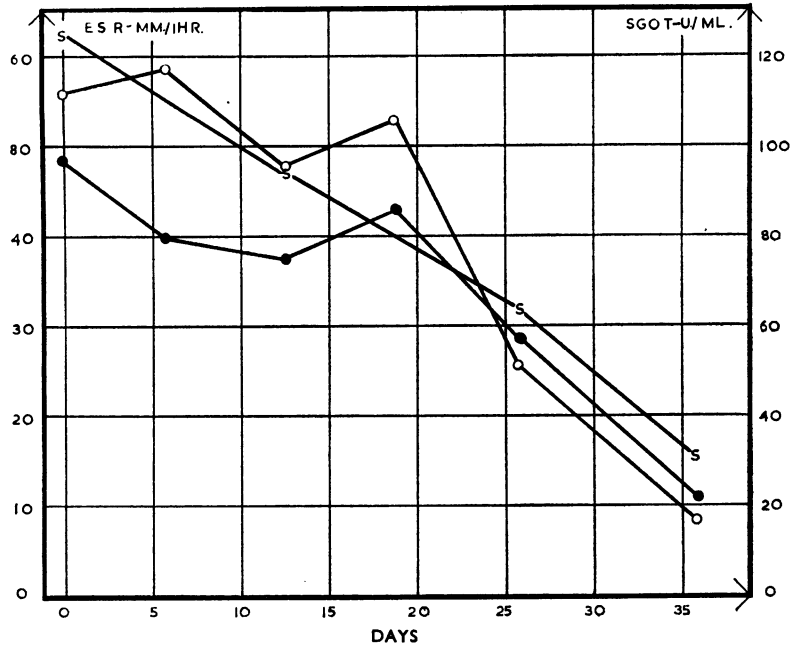

Figure 11.
Female. *Age:* 26 years.
Diagnosis: Toxic hepatitis secondary to a self-induced chemical abortion.

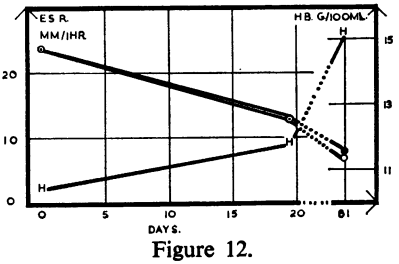

Figure 12.
Male. *Age:* 53 years.
Diagnosis:
1. Iron-deficiency anaemia (treated with oral iron).
2. Varicose eczema.

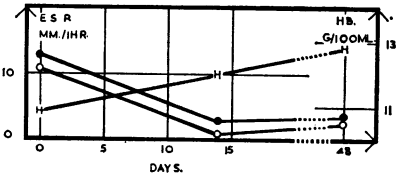

Figure 13.
Male. *Age:* 85 years.
Diagnosis:
1. Iron-deficiency anaemia (treated with oral iron).
2. Chronic hypertensive heart failure.

Discussion

Table II was constructed after an extensive and exhaustive search of the literature and summarizes the various methods which have been described for determining the ESR. In the hands of an experienced technician, 0.5 ml. is the most blood that can be obtained by

finger (or heel) prick (Cecil 1964); obviously the smaller the quantity of blood that can be used, the easier the method will be. The so-called micromethods of Brinckman and Wastl (1921), Cutler (1927), Kato (1938), Obermer (1943), Payne (1932), Peters (1945), Rappaport (1943), Smith (1936) and Whitby and Britton (1963) all require 0.2 ml. of blood or more and this is a volume which I find difficult to obtain by skin prick.

I consider the methods described by Balachowsky (1925), Langer and Schmidt (1926) and Linzenmeier (1929) to be the only techniques which are truly micromethods which can be performed accurately and easily on very small quantities of blood; however, all these latter methods suffer from the same disadvantage—they all require complex and costly apparatus.

The advantage of the Harshaw ESR method is that it uses a disposable capillary tube which is easy and inexpensive to obtain. The volume of blood required is very small (0.04 ml.) and from inspection of the results, it can be seen that they stand good comparison with the standard Westergren method.

As shown by Westergren (1926), the lower limit for the normal ESR is greater in women than in men. The same observations have been made on Harshaw ESRs. There has been no valid explanation made for this fact. The manufacturers of the Harshaw method state in their literature that the normal ESR value is 10 mm./1 hr. for both men and women. My results do not substantiate this: their value would be slightly high for men and definitely low for women.

Table II shows the wide variations in the anticoagulent used by different workers. Some use a chemical in solution and some use it dry. (Chandler (1960) collects blood very carefully and uses no anticoagulent.) Goldberg and Conway (1952) utilized dry potassium oxalate to prevent clotting and then diluted the blood with 3.8 per cent sodium citrate; their results are directly comparable with Westergren's (1921a). Later, Dawson (1960) did the same, but he used sequestrene as the anticoagulant. The Harshaw method works on the same principle: the sequestrene coating of the capillary tube prevents the blood from clotting before it has a chance of mixing with the sodium citrate solution.

From figure 6 it can be seen that at low readings, the Harshaw ESR is higher than the Westergren, whilst at higher readings (more than 20 mm./1 hr.) the position is reversed. I cannot explain the former difference in the readings, but the latter difference is that the red cells have much further to fall due to a higher column of blood.

The verticality of the column of blood has been indicated as an important factor in reliability and reproducibility of results (a column which is not standing at 90° will sediment faster than one

TABLE II

METHODS OF ESTIMATING ERYTHROCYTE SEDIMENTATION RATE

Author	*Year*	*Volume of blood (ml.)*	*Height of column (mm.)*	*Anticoagulant*
Bach and Gray Hill	1932	1.00	200	Sodium Citrate Soln.
Balachowsky	1925	0.07	100	Potassium Oxalate Soln.
Bannick *et al.*	1937	4.50	200	Sodium Citrate Soln.
Beaumont and Maycock	1935	(8 drops)	100	Sodium Citrate Soln.
Brinckman and Wastl	1921	0.25	60	Potassium Oxalate Soln.
Chandler	1960	0.66	60	Nil
Collins *et al.*	1939	5.00	72	Dry Potassium Oxalate
Cutler	1926	4.50	50	Sodium Citrate Soln.
Cutler	1927	0.50	50	,, ,, ,,
Cutler	1940	0.90	50	,, ,, ,,
Dawson	1960	1.60	200	,, ,, ,, +Dry Sequestrene
Della Vida	1942	5.00	200	Dry Potassium Ammonium Oxalate
Fahraeus	1921	8.00	170	Sodium Citrate Soln.
Fahraeus	1929	0.80	200	,, ,, ,,
Gaskell	1945	4.00	100	,, ,, ,,
Goldberg and Conway	1952	4.00	200	Dry Potassium Oxalate +Sodium Citrate Soln.
Haskins *et al.*	1930	0.88	200	Dry Potassium Oxalate
Kato	1938	0.20	100	Dry Potassium Ammonium Oxalate
Langer and Schmidt	1926	0.12	45	Sodium Citrate Soln.
Linzenmeier	1923	0.80	50	,, ,, ,,
Linzenmeier	1925	0.04	62.5	,, ,, ,,
McSweeney	1934	1.00	100	,, ,, ,,
Obermer	1943	0.30	100	Sodium Oxalate Soln.
Payne	1932	0.40	100	Sodium Citrate Soln.
Peters	1945	0.23	200	,, ,, ,,
Rappaport	1943	0.21	120	,, ,, ,,
Smith	1936	0.30	50	,, ,, ,,
Walton	1933	1.00	60	,, ,, ,,
Westergren	1921a	1.60	200	,, ,, ,,
Whitby and Britton	1963	0.50	100	Dry Potassium Oxalate
Wintrobe and Lansberg	1935	5.00	100	,, ,, ,,
Zeckwer and Goodell	1925	8.00	74	Sodium Citrate Soln.

which is completely vertical) (Nichols 1942). The Westergren tube is held vertically by a spring clip and a frame. The Harshaw tube stands free in plasticine; however, it is in close proximity to its scale which stands upright and hence reliable results can be obtained.

Fahraeus, in his original observations made in 1918, thought that the ESR was always raised in pregnancy; he went further and suggested that the ESR should be used as a pregnancy test. This has since been disproved and my figures for blood examined during pregnancy bear this out (*see* table III).

TABLE III

ESR RATES IN PREGNANCY

Age (years)	*Maturity (weeks)*	*Westergren ESR (mm./1 hr.)*	*Harshaw ESR (mm./1 hr.)*
30	12	3	10
23	14	51	43
25	38	2	2
24	24	15	25
25	37	10	11

The effect of anaemia on the ESR has produced material for innumerable papers (*see* Terry 1950) in the medical literature; complex correction methods have been described to adjust the ESR when raised due to anaemia. While it is accepted that a reduction in the red cell volume will accelerate the ESR, I prefer not to correct the ESR as it is a measurement of the blood and it will return to normal when the red cell mass returns to normal (*see* figures 12 and 13).

The ESR in the past has been most useful in assessing the activity of chronic disease such as pulmonary tuberculosis (Westergren 1921b), rheumatic fever (Payne and Schlesinger 1935), rheumatoid arthritis (Gibson 1946) etc. It is an indication of modern medical progress that so few chronic cases have been included in my series (*see* table I). I have found the ESR most useful in diagnosing and following such diseases as pneumonia, iron-deficiency anaemia and toxic hepatitis.

Summary

A new micromethod for estimating the ESR is described and evaluated in 100 patients seen in general practice and compared with a standard technique.

The normal values for the new method are found to be 0–15 mm./1 hr. in women and 0–9 mm./1 hr. in men.

As a screening test for organic disease and for following the pro-

gress of an illness, the new technique is found to be adequate.

Acknowledgements

I should like to express my gratitude to all the patient patients who took part in this trial. To my partners, L. F. and G. P. T. I acknowledge my thanks for allowing me to attend their patients. To the following I express my appreciation for help in planning the trial: W. J. D. F. of the Royal Free Hospital and G. W. C. of the Hospital for Sick Children, Great Ormond Street. My wife, as in previous studies, has shown considerable understanding when the midnight oil has been burned.

REFERENCES

Babington, B. G. (1830). *Trans. med.-chir. Soc. Edinb.*, **16**, 293.
Bach, F., and Gray Hill, N. (1932). *Lancet*, **1**, 75.
Balachowsky, S. (1925). *Ann. méd.*, **18**, 201.
Bannick, E. G., Gregg, R. O., and Guernsey, C. M. (1937). *J. Amer. med. Ass.*, **109**, 1257.
Beaumont, G. E., and Maycock, J. W. (1935). *Lancet*, **2**, 19.
Biernacki, E. (1894). *Zbl. inn. Med.*, **15**, 713.
Brinckman, R., and Wastl, F. H. (1921). *Biochem. Z.*, **124**, 25.
Cecil, G. W. (1964). Personal communication.
Chandler, A. B. (1960). *Nature (Lond.)*, **185**, 697.
Collins, D. H., Gibson, H. J., Race, J., and Salt, H. B. (1939). *Ann. rheum. Dis.*, **1**, 333.
Craddock, D. (1958). *An Introduction to General Practice*, 2nd ed., p. 37, London: Lewis.
Cutler, J. (1926). *Amer. J. med. Sci.*, **171**, 882.
Cutler, J. (1927). *ibid*, **173**, 687.
Cutler, J. (1940). *J. Lab. clin. Med.*, **26**, 542.
Dawson, J. B. (1960). *Brit. med. J.*, **1**, 1697.
Della Vida, B. L. (1942). *Brit. med. J.*, **2**, 278.
Fahraeus, R. (1918a). *Hygiea (Stockh.)*, **80**, 369.
Fahraeus, R. (1918b). *Biochem. Z.*, **89**, 355.
Fahraeus, R. (1921). *Acta. med. scand.*, **55**, 1.
Fahraeus, R. (1929). *Physiol. Rev.*, **9**, 241.
Gaskell, H. S. (1945). *Brit. med. J.*, **2**, 940.
Gibson, H. J. (1946). *Practitioner*, **157**, 28.
Goldberg, A., and Conway, H. (1952). *Brit. med. J.*, **2**, 315.
Haskins, H. D., Trotman, F. E., Osgood, E. E., and Mathieu, A. (1930). *J. Lab. clin. Med.*, **16**, 487.
Hunter, J. (1794). *A Treatise on the Blood, Inflammation and Gun-shot Wounds*, p. 50, London: Nicol.
Kato, K. (1938). *J. Lab. clin. Med.*, **23**, 980.
Landau, A. (1933). *Amer. J. Dis. Child.*, **45**, 692.
Langer, H., and Schmidt, W. (1926). *Z. Kinderheilk.*, **41**, 72.
von Limbeck, R. R. (1901). *The Clinical Pathology of the Blood*, p. 6, London: The New Sydenham Society.
Linzenmeier, G. (1923). *Munch. med. Wschr.*, **70**, 1243.
Linzenmeier, G. (1925). *ibid.*, **72**, 5.
McSweeney, C. J. (1934). *Lancet*, **2**, 756.
Nichols, R. E. (1942). *J. Lab. clin. Med.*, **27**, 1317.
Obermer, E. (1943). *Practitioner*, **151**, 43.
Payne, W. W. (1932). *Lancet*, **1**, 74.
Payne, W. W., and Schlesinger, B. (1935). *Arch. Dis. Childh.*, **10**, 403.
Peters, J. T. (1945). *Arch. intern. Med.*, **75**, 105.
Rappaport, F. (1943). *J. Lab. clin. Med.*, **28**, 746.

Shannon, F. T., and Bywaters, E. G. L. (1957). *Brit. med. J.*, **2**, 1405.
Smith, C. H. (1936). *Amer. J. med. Sci.*, **192**, 73.
Terry, R. (1950). *Brit. med. J.*, **2**, 1296.
Walton, A. C. R. (1933). *Quart. J. Med.*, **2**, 79.
Westergren, A. (1921a). *Acta med. scand.*, **54**, 247.
Westergren, A. (1921b). *Brit. J. Tuberc.*, **15**, 72.
Westergren, A. (1926). *Amer. Rev. Tuberc.*, **14**, 94.
Whitby, L. E. H., and Britton, C. J. C. (1963). *Disorders of the Blood*, 9th Ed. p. 269, London: Churchill.
Wintrobe, M. M., and Lansberg, J. W. (1935). *Amer. J. med. Sci.*, **189**, 102.
Zeckwer, I. T., and Goodell, H. (1925). *Amer. J. med. Sci.*, **169**, 209.

WHAT MANNER OF WOMEN MIDWIVES OUGHT TO BE

" As concerning their persons, they must be neither too young nor too old, but of an indifferent age, between both; well composed, not being subject to diseases, nor deformed in any part of their body; comely and neat in their apparell; their hands small and fingers long, not thick, but clean, their nails pared very close; they ought to be very chearfull, pleasant, and of a good discourse; strong, not idle, but accustomed to exercise, that they may be the more able (if need require) to watch, etc. Touching their deportment, they must be mild, gentle, courteous, sober chast, and patient; nor quarrelsome nor chollerick neither must they be covetous, nor report anything whatsover they hear or see in secret, in the person or house of whom they deliver; for, as one saith, it is not fit to commit her into the hands of rash and drunken women, that is in travel of her first child. As concerning their minds, they must be wise and discreet; able to flatter and speak many fair words, to no other end but only to deceive the apprehensive women, which is a commendable deceipte, and allowed, when it is done, for the good of the person in distress."

William Sermon, *The Ladies Companion or the English Midwife* quoted from *English Midwives their History and Prospects.* J. H. AVELING, M.D. London. J. & A. Churchill. 1872. Pp. 42.