TABLE V.—*Comparative Results with Various Antimalarials in Therapeutic Doses (Most et al., 1946)*

Drug	Latent Period in Days and Percentage of Relapses			
	30	40	50	120
Chloroquine ..	Nil	Less than 1%	11%	70%
Mepacrine ..	9%	28%	40%	80%
Quinine ..	54%	67%	72%	85%
Quinine and pamaquin ..	—	—	—	11%

and pamaquin has been found by Fairley (1946) in the same theatre almost to double the latent period obtained by a combination of quinine and pamaquin, although the percentage of relapses remained the same in both series. Also in the same theatre Pullman *et al.* (1948) found the relapse rate in severe B.T. infections to be four and a half times that in moderately severe infections with a combination of quinine and pentaquine, the superiority of which, therefore, still has to be proved.

Toxicity

Blood and urine examinations which were carried out showed no changes, and camoquin was well tolerated by all patients without giving rise to any complaint. Toxic symptoms with mepacrine and proguanil were also very rare. A few patients complained of abdominal pain after proguanil, and some vomited after mepacrine.

Summary

Camoquin has been found definitely superior to proguanil and mepacrine in the treatment of malaria. In M.T. malaria it seems to be superior to chloroquine in controlling fever and asexual parasitaemia. In B.T. malaria, fever is controlled earlier with camoquin, but in a few cases there is a slight difference in the rate of clearance of parasitaemia in favour of chloroquine. Evidence suggests that with larger doses of camoquin relapses are few, and are delayed. Although further experience is required to establish a world-wide superiority, especially over chloroquine, the use of a single dose and the smaller cost are distinct advantages.

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OBSERVATIONS ON METHODS OF MEASURING THE ERYTHROCYTE SEDIMENTATION RATE

BY

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The most popular methods of measuring the erythrocyte sedimentation rate (E.S.R.) are those described by Westergren (1921) and Wintrobe and Landsberg (1935); the latter is commonly known as the Wintrobe method. Technically, these procedures differ mainly in the nature of the anticoagulant and the length of the sedimentation tube; the bore of the tube is the same in both. Many investigators have compared the methods to assess their reliability, and Davis (1946), in a critical survey, concluded that the one of choice for general purposes is that of Westergren, because it is less sensitive to minor influences and more responsive when sedimentation is rapid. Recently, Gilmour and Sykes (1951) found, in a large series of cases, that a raised Wintrobe result was always accompanied by a raised Westergren; but a raised Westergren was not accompanied by a raised Wintrobe in 27% of female and 28% of male cases. They considered that the Westergren method is the more efficient as a pointer to organic disease.

In the course of routine haematological investigations we observed, within a short space of time, two patients in whom the E.S.R. by the Wintrobe method was normal whereas very high readings (over 100 mm. in one hour) were obtained by the Westergren. In both instances an abnormal protein was subsequently discovered in the blood, and if only the former method had been used an important diagnostic clue would have been lost. Because of these paradoxical results we decided to compare the two methods in a small series of cases. This investigation confirmed the confidence of Davis (1946) in the superiority of the Westergren method. It is, however, convenient to be able to perform a full haematological examination, including the E.S.R., on 5 ml. of oxalated blood as recommended by Wintrobe (1933). A series of experiments was therefore designed to explore the possibility of adapting the Westergren method, in which sodium citrate is the anticoagulant, to the use of oxalated blood.

Technical Methods

All needles and syringes were dry-heat sterilized and the sedimentation tubes were scrupulously cleaned and dried before use. Care was taken to ensure that the tubes were set in a strictly vertical position with air-bubbles excluded.

Blood samples for the Wintrobe and Westergren tests were taken by venepuncture. First, with the needle left *in situ*, 5 ml. of blood was delivered from a syringe into a glass bottle containing dry oxalate mixture (ammonium oxalate 6 mg., potassium oxalate 4 mg.). A second syringe containing 1 ml. of 3.8% sodium citrate was then attached to the needle and 4 ml. of blood withdrawn; this blood-citrate mixture was transferred to a clean dry test-tube. Both specimens were mixed by gentle shaking for approximately one minute. For straightforward testing the sedimentation tubes were charged immediately, the Wintrobe with the

TABLE I.—*Details of Cases in which the Westergren and Wintrobe Results were Dissimilar*

Case No.	Sex	Diagnosis	Initial E.S.R. (mm.)						Remarks
			Wintrobe			Westergren			
			$\frac{1}{2}$ hr.	1 hr.	2 hr.	$\frac{1}{2}$ hr.	1 hr.	2 hr.	
1	F	Myelomatosis	6	15	61	145	151	154	Reversal of A/G ratio. Rapid clumping of red cells in Westergren tube Abnormal protein of undetermined nature in peripheral blood Plasma g'obulin 11.6 g. per 100 ml. Plasma proteins normal " " " " " " " " "
2	F	Unknown	0	0-1	2	110	115	130	
3	F	Myelomatosis	1	5	—	—	145	149	
4	M	Tuberculous pericarditis	1	2	10	18	36	70	
5	M	Myocardial infarction	1	10	21	10	19	28	
6	M	" "	2	6	27	7	25	38	

blood-oxalate mixture and the Westergren with the blood-citrate. Readings were taken at half, one, and sometimes two hours.

Comparison of the Wintrobe and Westergren Methods

Tests by the Wintrobe and Westergren methods were carried out simultaneously on 52 patients suffering from a wide variety of medical complaints. Since the measured length of the Westergren tube is twice that of the Wintrobe, standards of comparison must inevitably be arbitrary. In most cases, however, the first-hour readings in both methods corresponded fairly closely, and they were classified into four groups—namely, normal, up to 10 mm. (Westergren), and 15 mm. (Wintrobe); slightly raised, normal to 30 mm.; moderately raised, 31 mm. to 50 mm.; and high, above 50 mm. The results by the two methods were thought to be either similar or dissimilar depending on whether they fell into the same or different groups. No correction was applied for low packed-cell-volume values; doubts about the wisdom of applying such a correction have recently been discussed by Terry (1950).

Similar Results

Forty-six cases gave similar first-hour results; these were normal in 12, slightly raised in 16, moderately raised in 9, and high in 9. In all 12 cases with normal results the Wintrobe and Westergren readings agreed closely. Of the 25 cases with slightly or moderately raised results, 22 gave Wintrobe readings higher than Westergren, whereas the opposite obtained in 7 of the 9 with high results.

Dissimilar Results

The results were dissimilar in six cases, normal Wintrobe being associated with raised Westergren readings. The discrepancy was gross in three cases, marked in one, and slight in two; brief details are shown in Table I. In all instances the Westergren result was more compatible with the clinical condition of the patient. The observations which originated the investigation were made in Cases 1 and 2.

Tests on the first four cases were repeated at intervals over a period of ten days, and the Westergren results never altered appreciably. The Wintrobe method, however, lacked this consistency, and in Cases 1, 2, and 4 the readings varied from test to test. In Case 2 there were actually gross fluctuations, for on some occasions, as in the initial test, sedimentation was negligible, whereas on others it was maximal in half an hour. The readings in Case 4 rose gradually until after nine days they were almost identical with the Westergren figures.

Influence of Anticoagulant and Tube.—To determine whether the anticoagulant or the sedimentation tube was primarily responsible for the dissimilarity in results, citrated and oxalated blood samples from Cases 1 and 4 were tested in both Wintrobe and Westergren tubes. The results, which are recorded in Table II, show that in both tubes the citrated blood sedimented more rapidly than the oxalated. In these cases, therefore, it was the anticoagulants and not the tubes that were decisive; the oxalate seemed to act as a retarding agent. In Case 1 the oxalated blood sample in the Wintrobe tube gave a first-hour reading, at the time of the experiment, of 50 mm., but sedimentation was still much delayed in the first half-hour compared with the citrate specimen.

TABLE II.—*Influence of the Anticoagulant and the Tube on the E.S.R. in Two Cases in Which the Classical Methods gave Dissimilar Results*

Case No.	Time (Hours)	Tube	E.S.R. (mm.)	
			Citrated Blood	Oxalated Blood
1	$\frac{1}{2}$	West.	134	6
	$\frac{1}{2}$	Wint.	75	6
	1	West.	140	20
	1	Wint.	76	50
4	2	West.	142	60
	2	Wint.	77	65
	$\frac{1}{2}$	West.	18	1
	$\frac{1}{2}$	Wint.	16	1
	1	West.	36	2
	1	Wint.	26	2
	2	West.	70	13
	2	Wint.	40	10

Use of Oxalated Blood in the Westergren Test

Blood, oxalated as for the Wintrobe test, was diluted with 3.8% sodium citrate in the usual proportion of 4 to 1; the E.S.R. was then measured in a Westergren tube. This procedure is referred to as the modified Westergren method.

Comparison of the Classical and Modified Westergren Methods. (a) *Immediately after Withdrawal of Blood.*—Thirty patients were studied. Citrated and oxalated blood samples were prepared in the usual way, and for the purpose of the modified test the latter were immediately diluted with citrate. The tubes were set up as soon as possible and the first-hour readings noted, that by the classical method being accepted as the correct sedimentation rate for each case. The results, which were classified into the same four groups as previously, are shown in detail in Table III. In general there

TABLE III.—*Comparison of E.S.R. by the Classical and Modified Westergren Methods*

E.S.R. (mm. at 1 hr.)	E.S.R. Group							
	Normal		Slightly Raised		Moderately Raised		High	
	a	b	a	b	a	b	a	b
	2	2	20	15	38	34	104	117
4	4	4	20	17	41	43	75	72
8	10	25	25	37	37	62	65	65
2	2	15	12	38	30	85	77	77
2	2	28	30	40	15	80	65	65
7	7			35	28	80	80	80
						136	140	140
						78	90	90
						88	103	103
						90	75	75
						65	79	79
						145	150	150
						130	130	130
Total cases	6		5		6		13	

a = the classical Westergren method. b = the modified Westergren method.

was fairly close agreement; the only serious discrepancy was in one case in the moderately raised group, when the readings varied from 40 mm. (classical method) to 15 mm. (modified method). Only one of the cases (Case 3) which gave

dissimilar results by the classical Westergren and Wintrobe methods was included in this series. Here the readings by the three methods, all performed at the same time, were 145 mm. (Westergren), 5 mm. (Wintrobe), and 150 mm. (modified Westergren).

(b) *At Intervals after Withdrawal of Blood.*—Ten further cases were studied by withdrawing from each (a) 25 ml. of blood, which was divided equally among five bottles containing dry oxalate mixture, and (b) 8 ml. of blood, which was added to a tube containing 2 ml. of 3.8% sodium citrate. After the usual gentle mixing 4 ml. of oxalated blood was extracted from one of the bottles and diluted with 1 ml. of citrate solution. This specimen was used for the modified test, which was set up immediately, and at the same time the classical test was performed on some of the citrated blood. The remaining four bottles of oxalated blood and the rest of the citrated blood were then stored at room temperature and the tests repeated after 3, 6, 12, and 24 hours. First-hour readings were recorded, and for comparison the correct E.S.R. for each case was taken as that obtained by the classical method performed immediately after the withdrawal of blood. Results were accepted as accurate only if the difference between the actual and correct readings did not exceed the following:

2 mm. when the correct E.S.R. fell within the normal range (0–10 mm.).

5 mm. when the correct E.S.R. fell within the slightly raised range (11–30 mm.).

10 mm. when the correct E.S.R. fell within the moderately raised range (31–50 mm.).

15 mm. when the correct E.S.R. fell within the high range (above 50 mm.).

The results (Table IV) show that the modified test maintained a higher degree of accuracy throughout the 24 hours than a classical one. After three hours' storage the former gave accurate readings in all ten cases, and even after 12

TABLE IV.—*Comparison of Classical and Modified Westergren Methods Immediately and at Intervals after Withdrawal of Blood*

	Time	Classical Westergren Method	Modified Westergren Method
No. of cases with accurate results	At 0 hours		10
	" 3 "	9	10
	" 6 "	5	8
	" 12 "	1	7
	" 24 "	0	2
Total cases		10	

The correct result for each case was the classical Westergren reading at 0 hours.

hours the results were still satisfactory in seven. As a final experiment the reliability of the modified test, after three hours' storage of oxalated blood, was further examined in ten additional cases. The previous findings were confirmed, for although the classical Westergren method was grossly inaccurate after three hours in five cases, the modified test gave accurate results both immediately and at three hours in all ten.

Discussion

The comparison of the Westergren and Wintrobe methods showed that the former was more reliable as a guide to the clinical condition of the patient; this agreed with the findings of Gilmour and Sykes (1951).

In three cases (Cases 1, 2, and 3) of the six cases in which the Wintrobe method proved misleading because of false low results, the Westergren figures were very high (over 100 mm. in one hour). These gross discrepancies were constantly associated with an abnormality of the plasma proteins, and in Case 1, which alone of the three was investigated further, the retardation of sedimentation in the Wintrobe test appeared to be a function of the oxalate anticoagulant. A feature common to all three cases was rapid clumping of erythrocytes in the Westergren tubes; this was immediately

followed by speedy descent. Citrate solution has an inhibitory influence on rouleaux formation (Hesser, 1924), but when, as in the cases under discussion, the auto-agglutinating properties of the plasma are very strong this effect must be largely neutralized. Probably, however, it is not negligible, for the absence of any inhibition with the solid oxalate anticoagulant could explain the low Wintrobe readings on the basis of rouleaux formation being so rapid and massive as to interfere with the swift and orderly descent of the red cells. The development of small areas of clear plasma at intervals along the length of the Wintrobe tubes was a striking feature. An important point which is unexplained is the alteration in behaviour, from time to time, of the Wintrobe tests; this was best exemplified by Case 2, in which on some occasions the reading was extremely low, whereas on others it was very high.

Although the cases which showed gross differences in results by the two methods all had abnormalities in plasma proteins, the converse did not hold, for others in the comparison series had a reversed albumin-globulin ratio and gave the expected high Wintrobe readings.

The modified test described in the second part of the investigation is both convenient and reliable, and therefore combines the main advantages of the classical Wintrobe and Westergren methods. It excels the former in accuracy, and is more convenient than the latter, for it can be performed, at the same time as other haematological examinations, on a single specimen of oxalated blood, which can be stored for a period of up to three hours before use. The test may therefore be useful in domiciliary practice when there must of necessity be a considerable time-lag before observations can be made on blood samples.

Just before this paper was submitted for publication, Goldberg, Glynn, and Bywaters (1952) reported preliminary observations on the simultaneous determination of the sedimentation rate by the Wintrobe and Westergren methods in patients with rheumatic disease. They found that in 10 out of 86 cases high Westergren were associated with normal or low Wintrobe readings.

Summary

The Westergren and Wintrobe methods of measuring the E.S.R. were compared in 52 patients suffering from a wide variety of complaints. In six cases raised Westergren readings, compatible with the clinical condition of the patients, were associated with normal Wintrobe readings.

This discrepancy between the two results was very gross in three patients, all of whom had an abnormality in plasma proteins.

A modified Westergren test which can be performed on oxalated blood after the addition of citrate is described.

When compared with the classical Westergren test, the modified test proved accurate in 49 out of 50 patients.

The modified test is reliable up to three hours after withdrawal of blood; because of this and the convenience of performing all haematological examinations on a single specimen of oxalated blood it may be suitable for use in domiciliary practice.

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