normally low; the same stimuli also produced a small increase when applied at night, when the number of microfilariae in the blood is normally high. The mechanism of production of these effects is unknown.

Increase in the concentration of carbon dioxide in the alveolar air had no significant effect on the microfilarial count during either day or night.

Vasodilatation produced by the intravenous injection of hexamethonium iodide had no significant effect on the microfilarial count, but severe exercise in one monkey produced an increase during the daytime.

Variation of temperature, pulse rate, and respiratory rate had no effect.

There is much resemblance between the cyclic changes in the concentration of phosphates in the blood and urine (as recorded in the literature) and those in the number of microfilariae in the blood; but no direct relationship between the two could be demonstrated experimentally.

ADDENDUM.—Since this paper was written Stanbury and Thomson (1951) have shown that in the diurnal cycle of excretion of sodium, potassium, chloride, and bicarbonate ions, a maximum was reached at midday and a minimum during sleep, and in some cases the fall began in the afternoon. There were, however, no parallel changes in the plasma levels of these substances during the 24 hours.

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The first meeting took place recently of a working party set up in Scotland to consider a series of reports issued by the Advisory Council on Education in Scotland on education for handicapped children. These reports cover the needs of children suffering from handicaps in sight, hearing, and speech, physical and mental impairment, and maladjustment, as well as those whose deprivation lies more in the social field. The working party, under the chairmanship of Dr. John Jardine, will study these reports and see how best to produce a co-ordinated plan on a national basis, and how education, training, and care can be brought to every handicapped child no matter where he is situated. The reports of the Advisory Council show that the provision to meet the needs of handicapped children is far from adequate, and a great deal remains to be done, unfortunately at a time when resources of all kinds are depleted. Priorities will therefore have to be determined. The working party is anxious to hear of the results of recent inquiries and investigations bearing upon any aspect of its work. All relevant information of this kind will be received most gratefully by Mr. James B. Frizell, secretary to the working party, at the City Education Department, 12, St. Giles Street, Edinburgh, 1.

EFFECT OF HYPOCHROMASIA ON THE SPECIFIC GRAVITY AND SEDIMENTATION OF RED CELLS*

BY

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As the red blood cells and haemoglobin content of the blood can be measured by relatively accurate methods, it would seem unquestionable that any variations of these should neither be measured by such a relatively inaccurate technique as the sedimentation rate nor be allowed to become a second active factor influencing the result that this test sets out to provide. The sedimentation rate is used normally as an indirect measure of change in the proportions or total quantity of the plasma proteins, especially of a rise in plasma fibrinogen or globulins; these plasma protein changes occur in pregnancy and in many diseases. The alteration in the plasma proteins is reflected by changes in the suspension stability of the red cells. Movement of the patient's red cells, suspended in his own plasma, is observed and recorded, and the red cells are the only indicators in the test. In no other laboratory experiment would the indicator be allowed to vary and provide a different result, unless the answer was corrected by the factor appropriate to the change in the indicator. There would appear to be no valid reason why an exception to this generalization on indicators should be made in the test known as the sedimentation rate.

Correction Charts

There are still conflicting opinions on the use of the correction charts employed in this test. One of the most recent criticisms is that of Terry (1950), who suggested that the small abnormal cells of hypochromic anaemia resist rouleaux formation, or that the plasma of anaemia is deficient in rouleaux-forming properties. The mean corpuscular haemoglobin concentration (M.C.H.C.) values of the bloods he examined were extremely low. His Tables I and III showed 38 bloods with M.C.H.C. values between 16 and 23%, and 10 between 24 and 29%.

Other workers have demonstrated that the blood from cases of "chronic anaemia" (hypochromic anaemia) sediments with abnormal slowness, and that the use of accepted correction charts provides abnormally low corrected rates.

Schuster (1938) remarked that certain cases of hypochromic anaemia had a slow sedimentation rate and the diminution of cells had but little accelerating effect. Gregg (1937) produced artificial anaemia in rabbits and observed that repeated bleedings, resulting in a "chronic anaemia," gave this slowing of the sedimentation rate as distinct from acute anaemias, which caused an acceleration proportional to the degree of anaemia. Smith (1938) noted that the blood in nutritional anaemia sedimented very slowly and did not "correct" either by Rourke and Ernstene's (1930) or by Wintrobe's (1946) chart. MacFarlane and O'Brien (1946) stated that in certain cases of chronic anaemia the sedimentation rate was comparatively slow despite the low packed cell volume, and that there appeared to be a natural compensation for the tendency of anaemic bloods to sediment quickly. Wintrobe and Landsberg (1935) stated that the specific gravity of the red cells had no effect on the sedimentation rate. Ham and Curtis (1938) quoted Neuberg and Snapper as having found a correlation between the colour index and the sedimentation rate, but in their own experi-

*This work formed part of the thesis approved by the University of London for the M.D. degree.

ments produced a correlation between the sedimentation rate and the mean corpuscular volume (M.C.V.), and did not find any correlation between it and the M.C.H.C.

Theoretically, because of the high molecular weight of the haemoglobin molecule, if the amount of haemoglobin is reduced proportionally more than is the amount of cell stroma—that is, there is a low M.C.H.C.—then the specific gravity of the red cells should fall and tend to approach that of the plasma in which they are suspended.

Stokes's law shows that this should produce great slowing in the rate of fall, as a numerator is markedly reduced. According to Stokes's law, the velocity of fall of a small sphere in a viscous fluid (V) can be calculated by the formula:

$$V = \frac{2r^2 (d_1 - d_2) g}{9 \eta}$$

V = velocity
g = acceleration due to gravity
d₁ = density of settling particles
d₂ = density of fluid medium
η = viscosity of fluid medium
r = radius of settling spheres

If d₁ approaches d₂, V will be markedly reduced.

Technique

Five ml. of blood was oxalated with the correct amount of Heller and Paul's (1934) mixture. Blood counts were performed and a haematocrit tube was filled and centrifuged at the same time as one of the 3-mm. tubes described below. A 12-cm. length of 3-mm. glass tubing had one end drawn out to give a narrowed thin glass neck, and this end was sealed with a large rounded mass of glass which would not injure the rubber pad of the centrifuge cup. When the haematocrit tube showed a constant corpuscular volume the 3-mm. tube was divided 1-2 mm. below the level of buffy white-cell layer, leaving the packed red cells in the lower portion of the tube. The thinned neck was now snapped off.

Pasteur pipettes were bent to give a 1-cm. length at right angles to the rest of the capillary, and one of these prepared pipettes was used to suck up a suitable quantity of the packed red cells from the 3-mm. tube. The bent end of the filled pipette was taken below the surface of a bottle of copper sulphate solution, where the red cells were expelled as a narrow ribbon which was broken off against a glass rod, placed in the bottle for this purpose. The red-cell ribbon was observed to see if it rose or fell in that particular bottle of copper sulphate. Care had to be taken to avoid a false reading due to sudden expulsion of the red cells, stirring of the bottle's contents, or inclusion of an air bubble in the red-cell column. A fresh pipette was used for each examination.

Readings from 37 different bloods

The copper sulphate solutions were made up as described by Phillips *et al.* (1943) to include a series providing specific gravities from 1075 to 1099. It was found that the packed red cells would not sink uniformly if dropped into the bottle from above the level of the solution.

The results are shown on the accompanying Chart. The points plotted consist of readings from 37 different bloods, in which the M.C.H.C. varied from 23 to 37% and the specific gravities measured from 1073 to 1096.

Application to the Sedimentation Rate

Case 1

Blood from a case showing a marked hypochromic anaemia and a very low corrected sedimentation rate (using the Wintrobe tube and a Hynes and Whitby (1938) correction chart) was carefully cross-grouped with a suitable normochromic blood showing a moderate increase in the corrected sedimentation rate. Forty ml. of blood was drawn from the chosen normochromic and the hypochromic case, and 20 ml. from each placed into the correct amount of Heller and Paul's mixture (Wintrobe blood), and 20 ml. added to 5 ml. of 3.8% sodium citrate solution (Westergren blood). After mixing, samples from the four blood specimens were placed aside; the remainder of each of the four bloods was centrifuged and the corpuscles were separated from the plasma. The cells of each blood were washed in three changes of the plasma, in which they were finally suspended; as described by Ham and Curtis (1938). The whole of these manipulations took about two hours.

The final plasma-red-cell mixtures were thoroughly mixed at the same time as the undisturbed samples which had been put aside before the bloods were centrifuged. Sedimentation tubes were filled and suspended in water at 20° C. (Rogers, 1946a, 1946b) and at the same time haematocrit tubes were filled with samples of the Westergren bloods.

TABLE I.—Effect of Hypochromasia on the Sedimentation Rate

Examination	Normochromic Blood. Case of Pulmonary Tuberculosis	Hypochromic Blood. Case of Duodenal Ulcer
R.B.C. (mil. per c.mm.) ..	6.4	4.2
Hb (g.%) ..	17.0	6.5
C.V. (c.cm.) ..	49.0	27.0
M.C.V. (μ ³) ..	77.0	64.0
M.C.H.C. (%) ..	34.7	24.0
Specific gravity of red cells blood " " whole	1093	1074
Specific gravity of plasma..	1058	1037
Total protein (g.%) ..	1027	1026
Albumin (g.%) ..	7.0	6.0
Globulin (g.%) ..	5.6	4.8
A/G ratio ..	1.4	1.2
A/G ratio ..	4:1	4:1

TABLE II.—Effect of Hypochromasia on the Sedimentation Rate

Corpuscles	Plasma	Fall in 1 hr. (mm.)	C.V. c.cm.	Corrected Rate (Hynes and Whitby)
Wintrobe Sedimentation Rate				
Normochromic	Normochromic	35	37	18
Hypochromic ..	"	5	40	1
Normochromic	Hypochromic	6	39	2
Hypochromic ..	"	2	38	½
Blood as taken:				
Same day ..	Normochromic	11	49	20
" " " ..	Hypochromic	9	28	½
3 days before	Normochromic	15½	46	18
" " " ..	Hypochromic	8	27	½
Westergren Sedimentation Rate				
Normochromic	Normochromic	24		31
Hypochromic ..	"	3		37
Normochromic	Hypochromic	7		31½
Hypochromic ..	"	1		35
Blood as taken:				
Same day ..	Normochromic	5		41
" " " ..	Hypochromic	5		24
3 days before	Normochromic	8		
" " " ..	Hypochromic	6		

At the end of one hour, after each tube was filled, readings were taken and the haematocrit tubes centrifuged, together with some of the unmanipulated "Wintrobe" blood samples which were used to fill the 3-mm. tubes previously described. The results are given in Tables I and II.

Case 2

It was not possible, in this case, to make such a full investigation to show the practical effect of marked reduction in the M.C.H.C. on the sedimentation rate.

A man of 79, with a carcinoma of the colon, was found to have a very low corrected Wintrobe sedimentation rate, but the blood count showed a marked hypochromic anaemia. Suitable normochromic red cells were washed once in the patient's plasma and then used to perform a Wintrobe sedimentation test when suspended in the patient's plasma. The results are given in Table III.

TABLE III.—Results in Man of 79 With Carcinoma of Colon

R.B.C.	2.8 mil. per c.mm.	Sp. G. of R.B.C.	1075
Hb	4.6 g. %	Plasma proteins	7.2 g. %
C.V.	20 c.cm.	Albumin	4.8 "
M.C.V.	71 μ^3	Globulin	2.4 "
M.C.H.C.	23%	A/G ratio	2:1

Patient's plasma was cross-grouped with K.B.R.'s red cells in tube at 37° C. for one hour and was compatible.

Wintrobe Sedimentation Rate—Both Cells Suspended in Patient's Serum

	Patient's Cells	K.B.R.'s Cells
Fall in 1 hour	47 mm.	52 mm.
C.V.	20 c.cm.	34 c.cm.
Corrected rate (by Hynes and Whitby's graph)	5 mm.	28 mm.

In this experiment it was felt that a misleading "normal" corrected sedimentation rate was due to the low M.C.H.C. of the patient's red cells.

Discussion

A hypothesis that red cells with a reduced M.C.H.C. would have a lowered specific gravity has been found to be correct. Experiments showed that hypochromic red cells sedimented much more slowly than normochromic cells, suspended in the same plasma. In these experiments there was a correlation between both M.C.V. and M.C.H.C. and the sedimentation rate, which agrees with the findings of Ham and Curtis on the correlation between the sedimentation rate and the M.C.V., and with Neuberg and Snapper's correlation between it and the colour index. It does not agree with Ham and Curtis regarding the lack of correlation between sedimentation rate and M.C.H.C., or with Wintrobe's observations on the specific gravity of red cells and the sedimentation rate.

This work has provided an explanation of Terry's results, as all his cases had marked hypochromic anaemia, many with M.C.H.C. values far below those encountered in the present investigation. It does not substantiate Terry's suggestion that the plasma of such patients is deficient in rouleaux-forming powers.

Terry argued that his slowly sedimenting hypochromic anaemic bloods proved that the sedimentation rate was not increased by anaemia and therefore the observed rate had the same significance in anaemia as in normal blood states. It might be more correct to state that he showed that two factors—the slowing due to the red cells having a low specific gravity, and the accelerating effect of the lowered packed cell volume—may nearly cancel the influence each will have on this test. Terry's work does not prove that correction charts are fallacious in any other type of anaemia; but the fact that correction charts may be misleading in such hypochromic anaemias does not mean that the uncorrected rate is any more informative. Many workers would hesitate to state that the results Terry showed in his Table III (the Westergren sedimentation rates of cases with a slightly raised E.S.R.) were indications of any condition other than that of anaemia.

It is obvious that in cases with a very marked hypochromic anaemia the sedimentation rate may provide an inadequate indication of active disease.

Summary

Evidence has been given that the specific gravity of red blood cells falls very appreciably when the M.C.H.C. becomes low. This might explain the slow sedimentation of blood from some cases of "chronic anaemia," as it was shown that markedly hypochromic cells sediment more slowly than do normochromic cells, and these red cells with a very low M.C.H.C. and low specific gravity may not be good "indicators" in sedimentation rate estimations.

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TATTOOING AS POSSIBLE MEANS OF TRANSMITTING VIRAL HEPATITIS

BY

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The object of this paper is to provide statistical evidence to show that tattooing should be regarded as an important means of dissemination of viral hepatitis among Service personnel in Hong Kong. This investigation was initiated after observing that the incidence of tattooed patients in the hepatitis wards appeared to be higher than in other wards.

The term "viral hepatitis" was proposed by Neefe *et al.* (1946) to describe those forms of hepatitis caused by filterable infectious agents which have not yet been routinely isolated and identified. Evidence arising from extensive experiments, using human volunteers, has shown that there are at least two virus-like agents concerned in the aetiology of viral hepatitis.

Transmission of Virus

These two agents have been called (a) virus IH and (b) virus SH (Neefe, 1949). Virus IH is present in the faeces and blood, and is concerned with infectious hepatitis (incubation period of 15–40 days), while virus SH is found only in the blood and is concerned with homologous serum hepatitis (incubation period of 50–150 days). It will be noticed that both viruses appear in the blood, and, according to Neefe, parenteral transmission of such blood may cause a hepatitis following an incubation period typical of virus IH—that is, 15–40 days—or of virus SH—that is, 50–150 days, according to the infecting virus.