Two of the three Group A strains isolated from ward flies proved to be of Type 4. Since a close check was being kept on all wound and throat infections in these wards, all Group A streptococci being typed by the kind co-operation of Dr. S. D. Elliott, it is possible to trace a hypothetical relationship between a carrier condition in these flies and infections in the human population of the wards. Type 4 had been identified only once previously—in a sore throat in a nurse ten days before the infected flies were caught; it is possible that this nurse conveyed the infection to a wound in which it escaped recognition. During the following two months Type 4 was recovered from eight further persons, six having wound and two throat infections. All these cases occurred in one of the two wards studied, and it was in this ward that both of the Type-4-carrying flies had been caught. After this stage two patients—one admitted to each ward—were found to have a Type 4 wound infection on admission, and 15 further cases in the two wards during an ensuing period of three months from that time are therefore not necessarily traceable to the earlier cases.

These observations do not prove the transmission of haemolytic streptococcal infection by flies, but they strongly suggest its possibility. When infection spreads otherwise inexplicably from one part of a hospital to another this agency should perhaps be considered.

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THE HALDANE HAEMOGLOBINOMETER

I. IRON, OXYGEN, AND THE BRITISH STANDARDS INSTITUTION COLOUR STANDARD

BY

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For more than 40 years the Haldane-Gowers haemoglobinometer has been in general use in this country, and for most clinical purposes it has been a satisfactory instrument. When, however, its results, changed by means of the traditional conversion factors into units of oxygen capacity and thence into grammes of haemoglobin, are compared with those of other methods in other countries, a curious discrepancy is apparent which has led to much confusion. Added to this, there is now a demand for a high degree of accuracy, resulting from the widespread adoption of the haemoglobin level as an index of nutrition and of haemoconcentration in shock. Judged by this more exacting requirement, it has become only too clear that the instrument as generally used may give dangerously inaccurate and variable results, and that a complete investigation of its efficiency is urgently needed.

The establishment by Donaldson, Harding, and Wright (*J. Path. Bact.*, 1943, **55**, 205) of a permanent B.S.I. Haldane colour standard, together with a means for accurate comparison at the National Physical Laboratory, has, for the first time, provided the essential fixed point on which such an investigation must be based. We have taken advantage of this standard in certain observations, which, though they have no claim to the desired completeness, may serve to guide further work. The problem of haemoglobinometry has been solved to their own satisfaction by a succession of workers during the past 60 years, but it still exists in all its complexity. We have at least avoided this one pitfall, of supposing our results to be final.

The first step in the present investigation has been an attempt to determine the mean oxygen capacity and iron content of blood of a colour equal to 100% of the B.S.I. colour standard. This attempt, which has met with unexpected difficulties, is here briefly described. The variability of the results of haemoglobin estimation by the Haldane-Gowers method under different conditions and in the hands of a large number of trained observers will be dealt with in a second communication. Finally, the puzzling systematic discrepancy between the results of this method and those of others, bound up as it is with the history of the Haldane standard, will later be discussed in the form of a review of the literature.

Method of Investigation

Material.—Blood was taken by venepuncture, a tourniquet being applied for the minimum time necessary to produce adequate venous distension and being released at the instant of puncture. The blood was heparinized, and within a few minutes of collection was divided into the required number of sub-samples, the vessel being shaken continuously to ensure even sampling.

The subjects were healthy adults (members of the hospital staff and medical students), 29 being males and 20 females. Unfortunately these were not evenly distributed in chronological order, most of the females being towards the end of the series—a fact that has complicated analysis of the results. The figures obtained in one other case, including an obviously erroneous iron estimation, have been omitted.

The following determinations were made on each blood sample and (except for iron determination) on the day of collection.

1. *Oxygen Capacity* (H. M. Jope).—This was estimated by the constant-volume van Slyke method, all measurements being made with carefully calibrated apparatus. Duplicate or triplicate estimations were made and the mean figure taken. In 20 blood specimens the CO capacity was also determined as a check on oxygen measurements. In addition 11 estimations of oxygen capacity have been made by Prof. C. G. Douglas, using the Haldane blood-gas apparatus, in parallel with the van Slyke estimations. The results of the two methods are in good proportional agreement, though the Haldane method gives results averaging 0.35 ml. % lower.

2. *Iron Determinations* (J. R. P. O'Brien and P. Quelch).—Haemoglobin iron was estimated by the Klumpp titanous chloride method, the results being the average of duplicate determinations. The method was standardized against pure iron wire, and all apparatus was carefully calibrated.

3. *Ultra-violet and Visible Absorption Spectra* (E. M. Jope).—A Hilger E3 medium quartz spectrograph and a Spekker photometer with ribbon filament lamp were used. The plates were matched on a photo-electric microphotometer. The results in the visible range were checked by two estimations made in parallel with the National Physical Laboratory spectrophotometer. To save space only the ultra-violet readings are quoted, and these have been expressed as a percentage of the B.S.I. standard on the basis of six determinations in parallel with the N.P.L. comparator.

4. *Haemoglobin Estimations* (B. Amos and R. G. Macfarlane).—The Haldane-Gowers haemoglobinometer was used. B.S.I. standards, diluting tubes, and pipettes were employed, and the necessary correction factors were applied. Each estimation was the mean of independent determinations by both observers, except for a period when one was not available. About half-way through the series both observers made many observations in parallel with colorimetric determinations by the National Physical Laboratory to eliminate personal errors, and this procedure was followed by rather higher readings being obtained from this point onwards. At the time of each estimation the observers were in ignorance of any other estimation on that blood sample—a matter of importance in view of the great susceptibility of this method to bias. In addition to the estimation of colour by the Haldane-Gowers method, six samples of blood were sent to the National Physical Laboratory for direct comparison with the colour standard by means of the special comparator. The figures so obtained were then

correlated with the other estimations which had been simultaneously carried out in Oxford. This procedure was designed as a check on the visual estimations of colour, which were considered to be most liable to error.

5. *Red Cell Counts* (B. Amos and R. G. Macfarlane).—Red cells were counted, using N.P.L. certified pipettes and counting-chambers. The recorded figures were the means of duplicate independent determinations by both observers.

6. *Packed Cell Volume*.—Duplicate determinations were made in N.P.L. certified haematocrit tubes, spun for 30 minutes at 3,000 revolutions a minute at a radius of $5\frac{1}{2}$ in.

Results

(Analysed by R. G. Macfarlane and R. H. Mole)

Space does not allow more than the means and coefficients of variation to be given, and these have been arranged in tabular form. The complete figures have been deposited with the Medical Research Council, and can be seen if desired.

TABLE I.—*Determinations (V = Coefficient of Variation)*

Estimation	(A) Series of 49		(B) 29 Males	(C) 20 Females	(D) 6 N.P.L. Samples
	Mean	V (%)	Mean	Mean	Mean
Haemoglobinometer reading (% B.S.I. standard)	101.40	9.00	106.80	92.95	103.40 (By N.P.L. comparator)
Spectrophotometer reading (% B.S.I. standard)	102.50	9.38	109.30	93.20	103.40
Red cells (10^6 per c.mm.)	5.23	8.39	5.51	4.82	5.30
Packed cell volume (%)	44.80	7.61	47.03	41.55	44.60
Oxygen capacity (ml./100 ml.)	20.11	9.15	21.30	18.37	20.34
Iron content (mg./100 ml.)	50.40	9.98	53.96	45.24	49.83

The means and coefficients of variation are given for the 49 sets of six estimations in Column A. Then follow the means for males and females separately, and finally those of the six samples in which the haemoglobin was estimated by the N.P.L. comparator. From these six samples it was calculated that $\text{HbO}_2 E_1^{1.000 \text{ cm.}} (\lambda = 414 \text{ m}\mu) 1.18$ was equivalent to 100% of the B.S.I. standard, and the spectrophotometric readings are given as a percentage of this extinction coefficient.

TABLE II.—*The Mean Equivalent of 100% B.S.I. Standard, as determined by the Haldane-Gowers Haemoglobinometer (H), the Spectrophotometer (S), and, in Column 5, by the N.P.L. Comparator in Six Cases*

I	2	3			4			5
		Series of 49			Last 24			
		All 49	29 Males	20 Females	All 24	11 Males	13 Females	
Oxygen capacity (ml. per 100 ml.)	H	19.89	19.97	19.78	19.60	19.48	19.71	19.70
	S	19.55	19.46	19.70	19.70	19.51	19.88	
Iron content (mg./100 ml.)	H	49.81	50.59	48.68	48.66	49.16	48.24	48.26
	S	48.97	49.29	48.50	48.90	49.25	48.67	
Red cells (10^6 per c.mm.)	H	5.17	5.16	5.18	5.14	5.12	5.16	5.19
	S	5.09	5.03	5.18	5.17	5.11	5.22	
Packed cell volume (%)	H	44.37	44.11	44.75	43.60	42.80	44.30	43.72
	S	43.60	42.98	44.59	43.80	42.80	44.70	
Spectrophotometer reading	H	101.70	102.50	100.20	99.40	99.80	99.10	100.00

In this table the results have been expressed as the mean equivalents of 100% haemoglobin measured by the haemoglobinometer, the spectrophotometer (indicated by the letters H and S respectively in Column 2), and, in the last column, by the N.P.L. comparator on six samples. The values for the whole series and for males and females are given separately in Column 3. It will be seen that the haemoglobinometer ratios are consistently higher than those of the spectrophotometer, suggesting that the average readings of the haemoglobinometer were about 2% too low. Analysis of the series in chronological groups shows a reduction in this discrepancy

about half-way through the series when estimations in parallel with the N.P.L. were begun. The resulting change in the statistics affected the sexes unequally, since there were more females in the second half of the series than in the first. It is for this reason that the results for the last 24 samples have been given in Column 4, and here it will be seen that the two methods of estimation agree to about 1% or less.

Despite these variations, the following points are clear from Table II: (a) The B.S.I. standard is equivalent to about 19.7 ml. O_2 per 100 ml. and about 49 mg. of haemoglobin iron per 100 ml., instead of the expected 18.5 ml. O_2 per 100 ml. and 46.25 mg. Fe. (b) There seems to be a sex difference in the relations between iron, oxygen, and colour.

TABLE III.—*The Coefficients of Variation (V) of the Ratios of Haemoglobin as estimated by the Spectrophotometer (S) and Haemoglobinometer (H) to Oxygen, Iron, Red Cells, and Cell Volume Estimations, and of Iron to Oxygen*

Ratio:	$\frac{\text{O}_2}{\text{S}}$	$\frac{\text{C}_2}{\text{H}}$	$\frac{\text{Fe}}{\text{S}}$	$\frac{\text{Fe}}{\text{H}}$	$\frac{\text{O}_2}{\text{Fe}}$	$\frac{\text{S}}{\text{H}}$	R.B.C. S	R.B.C. H	P.C.V. S	P.C.V. H
Series of 49	3.12	4.27	2.42	4.04	3.46	3.48	4.66	4.00	3.46	3.68
Series of 24	1.81	3.68	2.47	3.92	2.85	2.95	5.31	4.28	3.58	3.36

Some idea of the correlation of two sets of variables is given by the coefficient of variation (V) of their ratios. Table III contains the values for V of the ratios given in Table II. A range of twice the coefficient of variation above and below the mean will probably be found in a series of 22 such ratios, and serves as a useful index of the "probable range" of variation. On the figures for the whole series, and taking $\pm 2V$ as the probable range, in 22 blood samples with the same spectrophotometric reading two estimates of the oxygen capacity may differ by as much as 12%, or 17% if the haemoglobinometer is used instead of the spectrophotometer. It will be seen in this series that the best agreement is between iron estimation and the spectrophotometer, with a "probable range" of less than 10%.

The figures for the last 24 subjects show a considerable reduction in the variability of the ratios in which oxygen estimation takes part, particularly in that of oxygen to spectrophotometer in which the "probable range" has been reduced to about 7%. This improvement is presumably due to technical advance in the oxygen measurements. The other ratios show variable changes.

TABLE IV.—*Other Ratios: Colour Index; Mean Corpuscular Volume; Mean Corpuscular Oxygen and Iron; Oxygen and Iron Concentrations; Oxygen per gramme of Iron; Mean Corpuscular Haemoglobin; and Haemoglobin Concentrations*

Ratio	Series of 49		29 Males	20 Females	N.P.L. Samples
	Mean	V (%)	Mean	Mean	Mean
Hb/R.B.C. (C.I.)	0.967	4.00	0.969	0.965	0.97
P.C.V./R.B.C. ($\text{M.C.V.} \times \text{c.}\mu$)	85.8	4.03	85.33	86.46	84.52
O_2 /R.B.C. ($\text{M.C.O}_2 \times \text{c.}\mu$)	38.48	4.08	38.69	38.17	37.98
Fe /R.B.C. ($\text{M.C.Fe} \times \gamma\gamma$)	0.0964	4.87	0.098	0.0939	0.093
O_2 /P.C.V. (O_2 : ml./100 ml.)	44.85	3.76	45.29	44.22	44.96
Fe /P.C.V. (Fe : mg./100 ml.)	112.35	3.82	114.76	108.84	110.02
O_2 /Fe (O_2 : ml./g. Fe)	399.5	3.46	394.8	406.3	408.1
Hb/R.B.C. ($\text{M.C.Hb} \times \gamma\gamma$)	28.7	4.00	28.76	28.64	28.67
Hb P.C.V. (Hb: g/100 ml.)	33.5	3.68	33.6	33.2	33.95

In this table the means of other ratios are given, usually in a form that is already familiar. The ratio of colour estimation to red cell count, for instance, is given as the ordinary "colour index"; packed cell volume to red cell count as the "mean corpuscular volume." The figures for mean corpuscular haemoglobin and haemoglobin concentration have been calculated from the haemoglobinometer readings by the use of Hufner's factor, and on the assumption that 100% colour is equivalent to our observed figure of 19.7 ml. oxygen per 100 ml. Such a conversion, included here for comparison with the previous findings in the literature, is arbitrary and gives a false impression of accuracy. The oxygen capacity per gramme of corpuscular iron is very near the theoretical figure, but again it is of interest to observe the sex difference.

Discussion

A difficulty in attempting to define the British Standards Institution colour standard in any terms except those of colour is the fact that other measurable attributes of haemoglobin, such as iron content and oxygen capacity, are not, as determined by us, strictly proportional to the readings obtained with the Haldane haemoglobinometer and the spectrophotometer. Part of this lack of proportionality is, of course, due to instrumental and technical defects, and it is evident that as our series progressed some of these difficulties were overcome to an extent that materially lessened the variability. Nevertheless, even in the latter part of our series blood samples of the same extinction coefficient as measured by the spectrophotometer might show estimated oxygen capacities differing by 7% and iron contents differing by nearly 10%, while if the haemoglobinometer is used as a measure of haemoglobin these differences are increased to nearly 15% and 16% respectively.

A further part of this variability may be due to the presence of inactive haemoglobin and/or non-haemoglobin iron, the presence of which in larger amounts in men than in women might explain our observed sex difference. It ought to be pointed out, however, that this series was not planned to determine the extent of such sex differences, which were, in fact, not expected. In consequence our efforts to arrive at a satisfactory estimate of their statistical significance have proved fruitless, and the usual criteria, being in this series misleading, have been omitted. It is therefore difficult, if not impossible, to say how much of the observed variation between colour, oxygen capacity, and iron content was due to technical error, and how much to a genuine difference from one sample to another. Thus it is desirable to repeat the observations in a further series that has been carefully planned beforehand to make such an analysis possible.

It is quite clear that, in our hands, there is a large variability in haemoglobin estimation by the Haldane-Gowers haemoglobinometer. If a further series is attempted some other and more precise method for estimating colour must be employed—preferably the efficient comparator as used by the National Physical Laboratory.

Despite these variations there seems little doubt that the B.S.I. colour standard is equivalent to a greater mean haemoglobin, oxygen capacity, and iron content than was expected, though it is still conveniently close to the haemoglobin level associated with 5 million normal red cells per c.mm. This finding has an interesting bearing upon the supposed difference between English and American normal haemoglobin values. It also raises the questions why and when the standard changed from Haldane's original value, or whether in fact the Haldane standard has always been underestimated. These matters will be fully discussed in the third paper in this series.

Summary and Conclusions

We have observed a lack of proportionality in the results of haemoglobin estimations by the Haldane-Gowers haemoglobinometer, the spectrophotometer, oxygen capacity, and iron determination on 49 blood samples.

Part of this variation is obviously due to technical error, and a further part seems to depend on a sex difference in the relative proportions of colour, iron content, and oxygen capacity of haemoglobin.

The British Standards Institution Haldane colour standard is apparently equivalent to 19.7 ± 0.2 ml. oxygen capacity per 100 ml. of blood, and 49 ± 0.8 mg. of iron per 100 ml. Because of the variation already mentioned, more exact figures cannot be given at present.

The Haldane-Gowers method for estimating haemoglobin has shown itself to be insufficiently exact in our hands for purposes of standardization, and, in any future attempt to define the B.S.I. standard, should be replaced by the N.P.L. comparator.

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THE HAEMOGLOBIN EQUIVALENT OF THE B.S.I. HALDANE STANDARD

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The classical procedure of Haldane (1900) for estimating haemoglobin in the blood has been widely used in this country for nearly half a century, and most of the records in the medical literature of haemoglobin concentrations are in terms of the Haldane standard. By estimating the oxygen capacity of the blood of 12 healthy male persons Haldane arrived at an average figure of 18.5 ml. of O_2 per 100 ml. of blood. This figure he took to be the oxygen equivalent of "normal" adult blood, and he defined his 100% haemoglobin standard in these terms.

The Haldane method consists in saturating a measured quantity of blood in water with carbon monoxide, and diluting the cherry-red colour so obtained until it matches a standard 1 in 100 dilution of 18.5 ml. O_2 blood which has been similarly gassed with CO. For convenience the standard CO-Hb solution has usually been sealed up in small test-tubes of standard dimensions, in which it has appeared to remain unaltered in colour for surprisingly long periods of time, in some cases for many years. The quality of the glass undoubtedly has an effect on the keeping quality of the CO-Hb solution, and where inferior glass has been used Haldane standards have been known to become unusable because of an obvious change in colour.

This and other shortcomings in the routine Haldane procedure led Clegg and King (1942) to adopt an alkaline haematin method of estimating haemoglobin, which, they showed, was readily adaptable to precise colorimetric measurement in either Duboscq or photo-electric instruments. For this method a haemoglobin concentration of 15.6 g. Hb, equivalent to 20.9 ml. O_2 per 100 ml. of blood (Haden, 1922), was used as the standard 100% of normal, and this figure appeared from modern work (e.g., Peters and van Slyke, 1932; Wardlaw, 1941) to be a more probable average of normal adults than the earlier, and lower, Haldane figure.

Previously, all haemoglobin estimations in the Hammersmith Hospital had been made by the Haldane method, and it was anticipated that the determined percentage values would show a sudden drop on introducing the new method and the higher standard. The change encountered was much smaller than was expected. This led us to suspect that our Haldane standards represented a higher haemoglobin concentration than the 13.8 g. per 100 ml. which is the equivalent of 18.5 ml. of O_2 . We have therefore calibrated our tubes of Haldane standard by reference to a number of bloods whose haemoglobin content has been assessed by accurate iron analyses (titanous chloride method of Delory, 1943) and comparisons with photometric haematin results. Our Haldane tubes have been compared by the National Physical Laboratory with the Haldane standard prepared by Donaldson, Harding, and Wright (1943). These authors have recently attempted to standardize the Haldane method and to define accurately the colour of the tubes of CO-Hb which are used as standards for the routine comparisons. As a result of our measurements we have determined that the colour of the British Standards Institution Haldane standard is in fact equal to that given by blood of 19.8 ml. O_2 capacity (14.8 g. Hb) per 100 ml., and not of its stated strength of 18.5 ml. O_2 or 13.8 g. haemoglobin.

Experimental Methods

Taking of Blood.—(1) Blood was drawn by venepuncture without stasis and delivered into bottles containing potassium oxalate. It was mixed by gentle inversion before measurement. (2) Free-flowing blood from the lobe of the ear was collected