THE HAEMOGLOBIN CONCENTRATION OF NORMAL ENGLISH MALES AND FEMALES[1].

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The determination of the mean normal haemoglobin concentration has received more attention in America than in this country. Recently Price-Jones (1931) has compared the values in London with those found in America. He used the Haldane haemoglobinometer and his results are as accurate as is possible having regard to the method employed. In America his results were 3 per cent. above those of the Haldane gas analysis apparatus (1901) by which the clinical instrument is supposed to be calibrated. Twenty comparisons were made in America and showed that the Haldane gas apparatus gave results averaging 2·6 per cent. below those of the Van Slyke apparatus. Price-Jones considers this to have no statistical significance. From the standpoint of experimental accuracy the difference is appreciable, as all methods for the measurement of oxygen combined with Hb are essentially pragmatic.

Price-Jones finds that the male mean in London is 105 per cent. in Haldane's scale. The American value, determined by numerous observers using gas analysis and other methods, is about 114 per cent. He suggests that the difference is due to the universal use of motor cars in America, particularly closed cars, causing chronic carbon monoxide poisoning, and this in turn causing a compensatory output of Hb to balance that portion liable to be put out of action by the intake of the gas.

In a preliminary report it is stated that Sokhey (1929), using the Van Slyke method, found that the mean of 121 Indian males in Bombay was 111·3 per cent.

One of us (C. E. J., 1932) has already investigated this theory and finds it inadequate to explain the fact that the mean Hb concentration of males in Salford is the same as that in America. In the Salford investigation 53 males, evenly distributed throughout the age period 20 to 50, were examined by the flicker haemoglobinometer. The mean was found to be 115·2 per cent. in Haldane's scale with a standard determined by a number of Van Slyke estimations. In this paper it was shown that besides not meeting the experimental observations, the CO theory was opposed to historical fact. The position then was that America, Salford and Bombay had a comparatively high concen-

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tration, whereas that of London was considerably lower, a matter sufficiently peculiar to warrant further investigation. It was decided to examine the English mean over a wide area including London. We have shown (1931) that the flicker Hb-meter is, where comparable, slightly superior in accuracy even to the Van Slyke apparatus, but in the present work we have thought it proper to use the primary method of gas analysis, so all our results are Van Slyke determinations. Blood was obtained from London, Cambridge, Liverpool, Sheffield, Salford and Altrincham, and we wish to record our deep obligation to the numerous volunteers and to Dr Carmichael of St Bartholomew's Hospital, London, to Professor Dean of Cambridge, to Professor Dible of Liverpool, and to Drs Platt and Brodie of Sheffield for their kindness in collecting the specimens.

The Van Slyke apparatus used was the original model without mechanical shaker or water jacket and the standard technique of Van Slyke and Stadie (1921) was followed except for two minor variations. We found that two minutes' shaking did not evolve quite the whole of the gas, so after the ferricyanide had been added and the mercury evacuated, the blood was allowed to stand for three minutes as the formation of methaemoglobin was not instantaneous. The apparatus was then taken out of its stand and shaken by hand, vigorously and unceasingly, for three minutes. This procedure always results in constant volume, but further shaking after a reading is not omitted as it is also a test for leakage. The second point concerns the temperature: it was found that the temperature of the mercury and of the reduced blood was always about two degrees higher than that of the surrounding atmosphere. The reading was therefore always taken from the mercury reservoir. The effect was due to absorption of heat from the hands during the various manipulations required.

Oxalated blood was used and the pipette was calibrated for delivery of oxalated blood.

METHOD OF CALIBRATION.

A piece of pressure glass tubing of suitable length is drawn at one end to a short fine point. From any pipette 2·4 c.c. of water is placed in an evaporating dish and the water drawn up into the pipette to be calibrated. The meniscus is marked with a file and the essential procedure is to ascertain the exact value of the mark for capacity, and deliveries of distilled water, and of oxalated blood. The pipette is filled to the mark with mercury, which is then weighed and the volume calculated, taking the density of mercury at room temperature to be 13·590. The pipette is filled with distilled water and the weight of water delivered calculated, assuming the density of water to be 0·998 at room temperature.

The pipette is then dried successively in spirit and ether. A small bottle containing about 5 c.c. of oxalated blood and a piece of folded filter-paper are placed in one pan of a balance and weighed. Blood is drawn into the

pipette to the mark and any blood adhering to the outside of the pipette is wiped off with the paper which is returned to the pan. The blood is next delivered from the pipette into a watch-glass of known weight. It is done by holding the pipette upright and allowing it to empty by gravity. The point will hold a small amount of blood and this is blown out whilst the pipette touches the watch-glass. In this way a drop that may have formed on the outside is removed as well as the short column. In actual use the pipette is filled, then wiped, and the point allowed to touch the inside of the cup of the Van Slyke apparatus at a point a little distance from the actual drain hole. The mercury is made to fall, so the air is being drawn into the apparatus but no actual suction is used on the pipette, the latter being emptied by gravity alone. The last drop that clings to the point is removed by placing the pipette for a moment over the drain hole and this corresponds to the act of blowing in the calibration. In this way the method of emptying the pipette is reproduced as faithfully as possible. If suction is used to empty the pipette throughout, a larger amount of blood will cling to the sides and a smaller volume will be delivered.

The bottle and paper are weighed again and it follows that the loss must be the weight of the blood inside the pipette. The watch-glass and blood is next weighed and gives the weight of blood delivered. The capacity volume of the pipette is the volume of the blood taken up, therefore a simple calculation will give the volume delivered. The pipette after use is always washed in water, spirit and ether in order that it may be not only dry but free from grease. The amount delivered in relation to the capacity volume depends upon the viscosity of the particular sample of blood and also upon the shape of the pipette and the relation of the area of its wall to its volume. For this reason it is not advisable to make an empirical allowance: each pipette should be calibrated separately.

We used the same pipette throughout our work and it had the following values:

		c.c.
Capacity volume		2·320
Delivery volumes:	Distilled water	2·265
	Four different samples of blood	2·170
		2·165
		2·163
		2·150
	Mean	2·162

The difference between the mean of 2·162 c.c. and the delivery of water is 4·8 per cent. We call attention to this because it is sometimes assumed that an empirical allowance of 2 per cent. represents the probable difference between the delivery of water and of blood. It may be true for large pipettes, but if used for small volumes such as are used here it will cause the percentage readings of Hb to be nearly 3 per cent. too low.

A normal individual we defined as one who appeared to be in normal

health, made no complaint, had no immediate history of illness, and carried out his usual work. We are unable to concur with the suggestion that the highest values obtained in America indicate a form of polycythaemia. It would be equally permissible to reject the lowest readings on the score of anaemia. The suggestion also begs the main question.

We have stated our results to 0·5 per cent. in Haldane's scale, as the variations in the delivery volumes of blood from the pipette demonstrate that statements of smaller differences have no experimental significance (see Table I).

Table I. *Determination of the normal haemoglobin concentration by the Van Slyke method and expressed in Haldane's scale.*

Males.

LONDON.

106·5	122·5	112·0	123·5	120·0	104·5	109·5	122·5	126·0	117·5
114·0	133·0								

CAMBRIDGE.

102·5	128·5	118·0	123·0	106·0	114·5	113·5	120·5	115·0	124·5
117·0	120·5	124·0	111·5	115·5					

LIVERPOOL.

113·5	128·5	108·0	112·5	116·5	123·5	118·0	103·0	119·5	108·5
104·0	112·0	110·0	137·5	101·0	108·0	125·5	109·0	122·0	119·5

SHEFFIELD.

123·0	117·5	104·0	116·0	111·0	119·0	118·5	114·0	109·5	122·5
119·0	118·0	113·0	116·0	113·5					

SALFORD AND ALTRINCHAM.

119·5	106·5	131·5	110·5	118·5	118·5	125·5	118·0	107·5	115·0
112·5	123·5	109·5	109·5	121·5	122·0	115·0	119·5	107·0	102·0
108·5	118·5	121·5	109·5	121·5	120·0	119·5	116·0	117·0	116·0
107·5	115·0	103·5	112·5	113·5	113·5	119·5	103·5	125·5	113·5
117·5	109·0	112·5	111·0	118·5	116·0	113·5	114·5	115·5	113·5
119·5	121·0	115·5	118·0	107·5	104·0				

Analysis of data.

Total number of individuals examined	118
Mean haemoglobin concentration	114·76 %
Haemoglobin in g. per 100 c.c.	15·85

Number of cases of	100·0 to 102·0 % inclusive	... ...	2
,,	102·5 ,, 104·0 ,,	... ...	7
,,	104·5 ,, 106·0 ,,	... ...	2
,,	106·5 ,, 108·0 ,,	... ...	8
,,	108·5 ,, 110·0 ,,	... ...	9
,,	110·5 ,, 112·0 ,,	... ...	7
,,	112·5 ,, 114·0 ,,	... ...	14
,,	114·5 ,, 116·0 ,,	... ...	14
,,	116·5 ,, 118·0 ,,	... ...	12
,,	118·5 ,, 120·0 ,,	... ...	16
,,	120·5 ,, 122·0 ,,	... ...	8
,,	122·5 ,, 124·0 ,,	... ...	9
,,	124·5 ,, 126·0 ,,	... ...	5
,,	126·5 ,, 128·0 ,,	... ...	0
,,	128·5 ,, 130·0 ,,	... ...	2
,,	130·5 ,, 132·0 ,,	... ...	1
,,	132·5 ,, 134·0 ,,	... ...	1
,,	134·5 ,, 136·0 ,,	... ...	0
,,	136·5 ,, 138·0 ,,	... ...	1

Females.

SALFORD, ALTRINCHAM AND ECCLES.

98·5	96·5	103·0	86·0	105·0	108·5	116·0	105·5	100·5	100·0
99·5	103·0	100·5	116·5	103·5	93·5	112·0	102·0	100·5	98·5
99·5	107·0	97·0	106·0	109·0	107·0	101·0	119·0	102·0	92·0
112·5	99·5	102·0	105·5	92·5	93·0	96·0	111·0	87·0	102·5
87·5	113·0	94·0	92·0	99·5	108·5	102·0	83·0	102·0	96·5
87·5	100·5	100·5	99·5	96·5	104·5	105·0	92·5	99·0	92·5
99·0	98·0	96·5	87·5	86·0	86·5	83·0	108·5	109·5	104·5
100·5	112·0	110·5	100·5	99·0	110·0	105·5	100·0	92·0	99·0
77·0	97·0	99·5	98·0	98·5	82·0	104·0	109·5	90·0	110·5
99·0	98·0	105·5	123·0	90·5	93·5	100·5	101·5	100·0	90·0
104·0	110·0	96·0	109·5	106·0	93·5	100·0	94·0	121·0	110·5
104·5	99·5	92·0	112·0	106·5	101·5				

Analysis of data.

Total number of individuals examined	...	...	116
Mean haemoglobin concentration	...	...	99·66 %
Haemoglobin in g. per 100 c.c.	...	...	13·80

Number of cases of	72·5 to	75·0 % inclusive	...	...	0
,,	75·5 ,,	77·0 ,,	...	...	1
,,	77·5 ,,	79·0 ,,	...	...	0
,,	79·5 ,,	81·0 ,,	...	...	0
,,	81·5 ,,	83·0 ,,	...	...	3
,,	83·5 ,,	85·0 ,,	...	...	0
,,	85·5 ,,	87·0 ,,	...	...	4
,,	87·5 ,,	89·0 ,,	...	...	3
,,	89·5 ,,	91·0 ,,	...	...	3
,,	91·5 ,,	93·0 ,,	...	...	8
,,	93·5 ,,	95·0 ,,	...	...	5
,,	95·5 ,,	97·0 ,,	...	...	8
,,	97·5 ,,	99·0 ,,	...	...	11
,,	99·5 ,,	101·0 ,,	...	...	20
,,	101·5 ,,	103·0 ,,	...	...	10
,,	103·5 ,,	105·0 ,,	...	...	8
,,	105·5 ,,	107·0 ,,	...	...	9
,,	107·5 ,,	109·0 ,,	...	...	4
,,	109·5 ,,	111·0 ,,	...	...	9
,,	111·5 ,,	113·0 ,,	...	...	5
,,	113·5 ,,	115·0 ,,	...	...	0
,,	115·5 ,,	117·0 ,,	...	...	2
,,	117·5 ,,	119·0 ,,	...	...	1
,,	119·5 ,,	121·0 ,,	...	...	1
,,	121·5 ,,	123·0 ,,	...	...	1

DISCUSSION.

The ultimate utility of an ascertained mean is as a basis for comparison in order to form an opinion as to whether a given sample differs so widely from it as to be considered abnormal. The analysis of the results in both sexes shows that the mean value is not of as much practical importance as might be supposed. When allowance is made for the error of sampling it will be seen that two-thirds of the male results lie between the limits of 108 and 122 per cent. and that a given sample may fall indifferently at any value between these limits. Similarly, two-thirds of the female results lie between 93 and 107 per cent., although in this case there is a small tendency for the incidence of values to concentrate near the arithmetical mean.

We therefore suggest that a truer picture of the normal Hb concentration

in adult males is presented by the statement that whilst the mean is about 115 per cent., the limits of the normal are 100 and 138 per cent. and that the concentrations of two-thirds of all individuals lie indifferently between 108 and 122 per cent., influenced to a small extent by age.

The results obtained by us are in excellent agreement with the American determinations based upon gas absorption and colorimetric methods. Williamson's spectrophotometric work (1916) gave a mean of 122 per cent. Haldane. In this work dried haemoglobin was used as the standard and Price-Jones suggests that the high results may be due to the deterioration of the haemoglobin with age. There is no evidence available that this actually happened and it is equally possible that the factor 1·34, used for converting weight of Hb into volume of oxygen, may be too high. If this should be so, then Williamson's results would agree very well with those obtained by the other methods.

CONCLUSIONS.

1. The mean Hb concentration of 118 normal English males has been examined by two methods of precision and has been found in each case to be about 115 per cent. in Haldane's scale when the standard is determined by the Van Slyke gas apparatus.

2. In 116 women the mean determined by gas analysis has been found to be 100 per cent. in the same scale.

3. There is no foundation for the belief that the English mean differs materially from the American.

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