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RED BLOOD CELL DIAMETERS

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RED BLOOD CELL DIAMETERS

BY

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PREFACE

A PREVAILING demand for reprints which I am unable to supply and an increasing desire on the part of clinicians and laboratory workers to be better acquainted with the uses of the red cell diameter distribution curves and with the methods employed in the measurement of red cell diameters, which has lately come to play a rather important part in the diagnosis and treatment of pernicious anaemia and other diseases, have induced me to collect and arrange in book form my published contributions to this subject, and in endeavouring to explain and illustrate the simple arithmetical and elementary statistical processes involved I hope I may encourage my fellow workers in a pursuit full of fascinating interest and usefulness.

I would like here to take the opportunity of saying how grateful I am for the hospitality of University College Hospital and Medical School which has made most of my work possible.

I wish also to express my thanks to the proprietors of the *Journal of Pathology and Bacteriology* and of the *Guy's Hospital Reports* for their kind permission to reproduce the papers and figures which I have published in their journals.

CECIL PRICE-JONES

April 1933
LONDON

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CHAPTER I

HISTORY

The history of red corpuscles starts at the beginning of the seventeenth century. About 1610 in the small Dutch town of Middlebourg an obscure optician named Zacharie Jans made spectacles and polished lenses. It is related that his children, while playing in his workshop, were directed by 'chance' to pile the lenses like bricks one on the top of the other, revealing unconsciously to the suddenly inspired father the inherent possibilities of the combination of lenses, and the birth of the microscope. The merit of the invention, at about the same time, is also given to Jacques Meticus of Alkmaar and to Cornelius Drebbel (Alkmaar, 1572–1634), the astronomer to James the First, who showed his instrument to the King in 1619; and there is support for this in a letter by Rubens to his friend Peiresc in 1629. Rubens was staying in London on a diplomatic mission from the Spanish Court to King Charles I; he says: 'I have only seen the famous philosopher Drebble in the street, and scarcely had time to exchange a few words with him. . . . I am assured that for many years he has produced only this optic instrument with a perpendicular tube, and which enlarges enormously the objects placed beneath it . . .' (*Rubens, Painter, Diplomat*, by Emile Cammaerts, 1932, Faber & Faber, Ltd.). The discovery is also attributed to Galilei (Pisa, 1564–1642), who was certainly the first to apply the instrument to physiological investigations.

Some forty years after this (1658) Swammerdam (Amsterdam, 1637–80) described the oval red blood cells of the frog, and Malpighi (Bologna, 1628–94) in 1661 observed the circular corpuscles in the blood of the hedgehog, but he thought they were globules of fat and did not pursue the matter further. In 1673, with an improved form of microscope devised by himself, Leeuwenhoek (Delft, 1632–1723) discovered the red cells in human blood. He noted that the blood corpuscles of many kinds of mammals had circular contours and were spherical whilst those of birds and fish were oval, but he was unable, with the instruments he used, to see any appreciable differences of size; his scale of measurement also was very rough. He selected minute grains of sand 'as nearly as possible alike' and, arranging them in a line, he counted the number of grains occupying the length of an inch. This principle was used by later microscopists who substituted spores of the puff-ball (*Lycoperdon bovista*), said to have the size of 1/3,500 of an inch, or the sporules of *Lycopodium* with a mean

diameter of 1/940 of an inch. The micrometer devised by Dr. James Jurin (1718) was scarcely more reliable. This observer twisted a hair, or fibre of silk or fine wire, round a pin or cylinder and, ensuring that the successive twists were closely in contact, he exposed the coil under the microscope, measured the interval between the first and last twist and divided this by the number of twists, and so obtained the diameter in inches of the hair. Blood was then spread thinly over the microscope field and he counted the number of red cells corresponding to a segment of hair. But hairs vary in thickness from root to tip, and he obtained most variable values, e.g. 7, 8, 12, and 13 red cells to the transverse section of the hair. After frequently repeated experiments he estimated the mean diameter of the hair as 1/324 of an inch and red cells as 1/10 part of the diameter of a hair or 1/3,240 of an inch, or about 7·75μ; from another set of measurements he noted the diameter of the red cell as 1/1,940 inch or 12·5μ.

de Senac (1693–1770) in 1749 found that the red cells were not spherical but lens-like or disks; he thought at first that the cells were equal in size and estimated the maximum measurement at 1/300 of a line; assuming a line=2·116628 mm., this corresponds to about 7·22μ; later he found that they varied from 1/250 to 1/300, or on average measured 1/275 of a line or about 7·69μ.

Little more was heard about the blood corpuscles until later in the eighteenth century William Hewson (1739–74), the celebrated partner of William Hunter, published in 1770 his remarkable work on the structure, form and dimensions of the blood corpuscles, an annotated edition of which was made by Dr. George Gulliver and published by the Sydenham Society of London in 1846. Hewson gives, in a plate, representations of red cells from the blood of different animals including man. They are represented 'of the size they appeared to my eye when viewed through a lens of 1/23 of an inch focus; which, allowing 8 inches to be the focal distance of the naked eye, magnifies the diameter 184 times.' He describes the cells as flat disks, 'as flat as a guinea'. Although he gives no values of size, he finds that not only are they of different sizes in different animals, they are not all of the same size in the same animal, and in the same species they differ in size at different periods of life; those of the chick on the sixth day of incubation were found to be larger than those in the blood of a full-grown hen; he could find no difference in size between the blood-cells of a child and those of an adult man. Hewson's work was soon discredited, and the corpuscles he figured were thought to be 'probably air bubbles'. The subject was again brought into notice in 1821 by MM. Prévost and Dumas. These observers published in the *Biblio-*

thèque universelle des sciences de Genève, 1821, t. xvii, p. 215, a paper on 'Examen du sang et de son action dans les divers phénomènes de la vie' which I have not been able to obtain in London, but which has been abstracted with abbreviations in the *Annales de chimie*, 1821, t. xviii, p. 280. From this it seems that these authors regarded the red cells as spherical with a central luminous point. For measuring the cells they spread very small drops of blood on glass slides so that desiccation was very rapid, and adopted the method devised by Capt. Kator, whereby the object under the microscope seen by the right eye is made to coincide with a scale divided into millimetres and half-millimetres placed laterally and seen with the left eye. Having established the magnification of the microscope—preferably 300 diameters—they deduced the real values and obtained a mean of ten observations equal to 1/150 millimetre or about 6·6μ. Capt. Kator's account of this method in the *Phil. Trans.*, 1818, says: 'a ruler divided into inches and tenths of an inch was placed on the box which supports the microscope; a mother-of-pearl micrometer scale, each division of which was equal to 1/200 of an inch, was placed under the microscope'; viewing this with both eyes open 'its image appeared to be projected on the ruler, and one division appeared to subtend the space of one inch. The micrometer scale being removed, blood sufficiently dilute was placed under the microscope [probably a dried film, but this is not stated] and, being viewed with both eyes open, a globule of blood (red cell) appeared to occupy in the first experiment 1/2 of 1/10 of an inch, and in the second experiment 1/3 of 1/10 of an inch upon the ruler; hence the size of the globule by the first experiment will be 1/2 of 1/10 of 1/200 of an inch or 1/4,000 of an inch, and by the second experiment 1/3 of 1/10 of 1/200 or 1/6,000 of an inch, the mean of which or 1/5,000 of an inch may be considered as about the mean diameter of a globule of the blood', or about 5μ. This is a similar value to that obtained by Wollaston, using the microscope scale he devised for Dr. Young (Milverton, Somerset, 1774–1829).

In *An Introduction to Medical Literature* (2nd edition, 1823) Dr. Thomas Young discusses the observations of Hewson, and, while accepting the existence of the red corpuscles, he does not regard them as 'flat as a guinea', and with respect to the central particle 'detached within a vesicle' described by Hewson as like a 'pea in a bladder', Dr. Young cannot doubt that 'Mr. Hewson was completely mistaken'. He then describes his 'eriometer'* (ἔριον=cotton, μέτρον=measure)

* This apparatus and method has in recent years been rediscovered by Pijper (1924), and explored by Millar (1926), Emmons (1927), Eve (1928), and others.

devised by him to measure the sizes of wool fibres. This instrument is based on the observation that when a luminous object is viewed through a fluid containing particles 'it is surrounded by rings of colours, somewhat resembling those of the rainbow'. These rings of colours may also be employed for measuring the comparative and the real dimensions of these particles. 'Immediately about the luminous object we see a light area terminating in a reddish dark margin, then a ring of bluish green, and without it a ring of red; and the alternations of green and red are often repeated several times when the particles or fibres are sufficiently uniform. I observed some years ago that the rings were the larger as the particles or fibres affording them were smaller, but that they were always of the same magnitude for the same particles. It is, therefore, only necessary to measure the angular magnitude of these rings, or of any one of them, in order to identify the size of the particles which afford them; and having once established a scale, from an examination of a sufficient number of substances of known dimensions we may thus determine the actual magnitude of any other substances which exhibit the colours.' Using the micrometer scale invented by Dr. Hyde Wollaston (*Phil. Trans.*, 1813, p. 119), the measurement he obtained for the diameter of the human red corpuscle was 5μ to 6μ.

About this period (1826–9) the celebrated optician Amici of Modena improved his microscope objectives by the addition of several achromatic systems one above the other by which chromatic and spherical aberrations of higher order were overcome; he also described a prism eyepiece attachment invented by Dr. Wollaston known as the camera lucida; to Amici also is attributed the first good immersion system. In the spring of 1827 Amici came to this country and showed his instruments to Mr. J. Jackson Lister, the father of Lord Lister. Using one of these new compound achromatic microscopes, Mr. Lister, in association with Dr. Hodgkin, made a number of observations and measurements of red blood corpuscles. The examinations were made on wet and dry preparations, as opaque and as transparent objects, under every variety of power and light and confirmed by frequent repetition. Adapting a camera lucida to the eyepiece, a stage micrometer placed in the focus of the object glass was drawn on a piece of paper at a known distance away;

The limitation of all these instruments, in common with the colour-index and volume-index methods, is that they measure the size of the corpuscles in mass and give average figures only; the details of the distribution are not known, and the variability cannot be accurately determined. But the diffraction method is obviously very convenient for quickly finding the mean diameter, especially in comparative rather than in absolute terms.

replacing the micrometer by a blood film the images of the red cells were drawn to scale on the same piece of paper. The diameter values obtained in this manner were 'pretty correctly' stated to be 1/3,000 of an inch or about 8μ; the number of cells measured to obtain this average is not stated. They found the ratio of the thickness of the disk to the diameter 1/45.

To George Gulliver, surgeon to the Horse Guards, we are indebted for the interesting *Life and Works of William Hewson* referred to above. In this book (1846) Gulliver gives a list of a number of measurements of blood corpuscles in man and animals; similar records are to be found in the *Proceedings of the Zoological Society* extending over a period of years from 1837 to 1846; they are also quoted by Milne-Edwards (p. 84). Gulliver measured the red cells of 485 species of vertebrates; his observations were made on perfectly fresh blood, and the corpuscles were measured either as 'they floated in the serum', or the cells thinly spread on glass and quickly dried. He used a compound microscope with achromatic object glass of 1/8 of an inch focal length made by Ross, and furnished with a micrometer eyepiece divided into spaces corresponding to 1/4,000 of an inch. The magnifying power was exactly 800 diameters with a clear definition. 'If one space and a quarter of this micrometer were occupied by a single globule, this would, of course, measure 1/3,200 of an inch; if three equally sized particles lying in a line and touching at their edges covered three spaces and a half the diameter of each of them would be 1/3,429 of an inch, if four spaces 1/3,000 of an inch. These measurements are mentioned because they are frequently obtained from the average-sized human blood disks, . . .'. I can find no mention in any of his papers of the number of cells he measured for an average estimation. In his list he gives only average diameters; he says, 'in any one of these there are red corpuscles about 1/3 smaller and 1/3 larger than the mean size.' Gulliver found that when the red cells of mammals are rapidly spread very thin and instantly dried on glass they are commonly slightly larger than when they are slowly dried, or than when they have been swimming for a few minutes either in liquor sanguinis, in the serum, or in dilute watery solutions of neutral salts. On the contrary, the red cells of birds and reptiles are usually rather smaller in the dried state however quickly it may have been done. Gulliver gives the diameter of human red cells as 1/3,200 of an inch (7·61μ); those of *Elephas indicus* 1/2,745 inch (9·1μ); whale (*Balaena boops*) 1/3,099 (8·03μ); musk deer (*Moschus javaicus*) 1/12,325 inch (2·02μ); cat (*Felis domesticus*) 1/4,404 inch (5·6μ); dog (*Canis familiaris*) 1/3,542 inch (7·05μ).

The earliest mention I can find of red cell diameter 'curves' is in a paper by L. Malassez in *Soc. de Biologie*,* series 9, January 5, 1889, entitled 'Sur la mésuration des globules sanguins, règle globulimetrique.' By means of a camera lucida he drew on a piece of paper the contours of 100 red cells at a magnification of 1,000 diameters; the diameters (two diameters if the cell is elliptic) of each cell are measured by a transparent scale of glass (gelatine, celluloid, or horn) on which are engraved circles progressing in size from 5 mm. to 15 mm. by intervals of 0·25 mm.; superposing this scale on the drawings the cell diameters are rapidly estimated, and by dividing by the magnification (i.e. 1,000) the mean diameter of the red cells is obtained. He apparently measured free cells in fresh preparations, and also cells dried on slides. He gives in the accompanying table (Table 1) distributions of red cell diameters of a healthy adult man; in a case of 'chlorosis'; and in a $4\frac{1}{2}$ months' foetus. Malassez says these diameters may also be expressed in the form of curves.

TABLE 1

Red cell diameter distribution (Malassez)

Diameter μ	*Numbers per cent.*		
	Adult man	*Chlorosis*	$4\frac{1}{2}$ *months' foetus*
6·50	1	0	0
6·75	2	1	1
7·00	7	0	1
7·25	13	0	1
7·50	18	2	1
7·75	24	8	1
8·00	18	10	5
8·25	9	14	3
8·50	5	25	8
8·75	2	19	11
9·00	1	10	11
9·25	..	9	15
9·50	..	0	12
9·75	..	2	11
10·00	..	..	10
10·25	..	..	4
10·50	..	..	3
10·75	..	..	2
11·00	..	..	1
Mean diameter 7·7μ		8·5μ	9·2μ

He does not estimate the degree of variability of the cells. I find, calculating from his figures, that the distribution of his adult man has $\sigma = 0{\cdot}46$, and $v = 6$ per cent.; for his case of 'chlorosis' $\sigma = 0{\cdot}49$

* *C. R. Soc. Biol.*

and $v = 5{\cdot}8$; and for the $4\frac{1}{2}$ months' foetus $\sigma = 0{\cdot}76$ and $v = 8{\cdot}3$ per cent.

In the preceding historical sketch I feel I have shortly but sufficiently indicated the line of advance along which our knowledge of the sizes of red blood cells has progressed from the primitive times of Leeuwenhoek to the more recent days of Malassez. He it was who first really established the art of haemocytometry on a scientific basis which has been adopted with additions and personal modifications by most modern workers in the subject.

CHAPTER II

THE MEASUREMENT OF RED BLOOD CELLS

I

Variations in the diameters of red blood cells are conveniently observed and measured in dried films. These are spread in the usual way—the thinner the better—dried in air without heat, fixed and stained with Jenner stain for two minutes, and, after washing with distilled water and drying, are superstained with weak aqueous solution of eosin for two minutes. These details should always be adhered to since it is found that alterations in the fixing and staining reagents can produce changes in the mean diameters of the cells.

Some simple form of projection apparatus adjusted for a magnification of 1,000 diameters is then arranged to project the microscope field on to a sheet of paper lying on the table. Having chosen a thin portion of the film, the red cells being well separated are outlined in pencil; I usually draw 500 cells. Two diameters, maximum and minimum, of each of these cells are then measured to 0·5 mm. with a glass millimetre scale and can be expressed directly in terms of μ; the mean of these two measurements is accepted as the diameter value of the cell. The diameter values are then arranged in groups progressing in order of magnitude by intervals of $0{\cdot}25\mu$ from the smallest to the largest cells. The mean diameter of 500 cells is taken to represent the mean diameter of the red cells for any sample of blood.

It will be useful to introduce here the 'artifice' which is commonly adopted for calculating the arithmetic mean (M), the standard deviation (σ), and the coefficient of variation (v) of any sample of variables. This is taken from Yule's *Introduction to the Theory of Statistics*, pp. 108–12, but I have substituted the example and figure given in that book by an actual blood-cell diameter distribution of a healthy man (Table 2), since the red cells of the blood are presumably no exception to the fact that most measurable properties and products of living things are arranged according to the normal curve of variation.

The *arithmetic mean*. Column 1 in this table shows the mid-points of class intervals ranging from 6μ to $8{\cdot}75\mu$ progressing by $0{\cdot}25\mu$ intervals (or $\frac{1}{4}\mu$ units); for practical purposes all the values in each class are regarded as if they were identical with the mid-value of the class interval. Column 2 gives the frequency (f) or the number of diameters corresponding in magnitude to the various class intervals.

TABLE 2

1	2	3	4	5
Mid-points of Class intervals $\frac{1}{4}\mu$	*Frequency* f	*Deviation from arbitrary mean* ξ	*Product* $f\xi$	$f\xi^2$
6·00	1	−5	5	25
6·25	6	−4	24	96
6·50	8	−3	24	72
6·75	42	−2	84	168
7·00	83	−1	83	83
7·25	123	0 (arbitrary mean)	−220	..
7·50	113	+1	113	113
7·75	68	+2	136	272
8·00	32	+3	96	288
8·25	17	+4	68	272
8·50	4	+5	20	100
8·75	3	+6	18	108
	$N=500$		+451	1597
			−220	÷ 500 = 3·194
			$\Sigma(f\xi)+231$	−0·213
			$\Sigma(f\xi)/_N = +0{\cdot}462\ (\frac{1}{4}\mu)$	$\sigma^2 = 2{\cdot}981$
				$\sigma = 1{\cdot}726$
				$\frac{1}{4} = 0{\cdot}4315\mu$

$$M = 7{\cdot}25+\frac{0{\cdot}462}{4}\mu$$
$$= 7{\cdot}25+0{\cdot}1155\mu$$
$$= 7{\cdot}3655\mu$$

$$v = \frac{0{\cdot}4315\times 100}{7{\cdot}3655}$$
$$= 5{\cdot}858 \text{ per cent.}$$

Column 3 shows the deviation (ξ) or distance of each group in class intervals from an arbitrarily chosen mean, in this example $7{\cdot}25\mu$, separating 5 groups of smaller cells from 6 groups of bigger cells. Column 4 gives the product ($f\xi$), each frequency being multiplied by its corresponding ξ value. Addition of these products gives -220 and $+451 = +231 = (f\xi)$; dividing this result by 500 or the total number of cells measured, $(f\xi)/N = +\ 0{\cdot}462\ (\frac{1}{4}\mu)$ or $0{\cdot}1155\mu$; add this to the arbitrary mean, $7{\cdot}25\mu+0{\cdot}1155\mu = 7{\cdot}3655\mu$ = the mean diameter of this sample of 500 red cells.

The *standard deviation* (σ) is the measure in μ of the dispersion of the diameters, their range in size, and the way in which the numerical frequencies of the different diameters are arranged. In order to obtain some quantity that shall vary with the dispersion it is necessary to average the deviations by a process that treats them as if they were all of the same sign, and squaring is the simplest process

for this purpose. Column 5 gives values of $f\xi^2$ (i.e. col. 3 multiplied by col. 4); addition of these results gives 1,597; divided by 500 = 3·194; the difference of the mean (M) from the arbitrary mean is shown in column 4 to be 0·462 ($\frac{1}{4}\mu$); this difference squared is 0·213 ($\frac{1}{4}\mu$) and must be subtracted from 3·194, giving 2·981 = σ^2, or σ (standard deviation) = 1·7262 ($\frac{1}{4}\mu$) or 0·4315μ.

The *coefficient of variation* (v) is the standard deviation expressed as a percentage of the mean, and forms a measure of the variability which is independent of the unit in which the measurements have been made. In the example given $v = 0{\cdot}4315 \times 100/7{\cdot}3655 = 5{\cdot}8$ per cent.

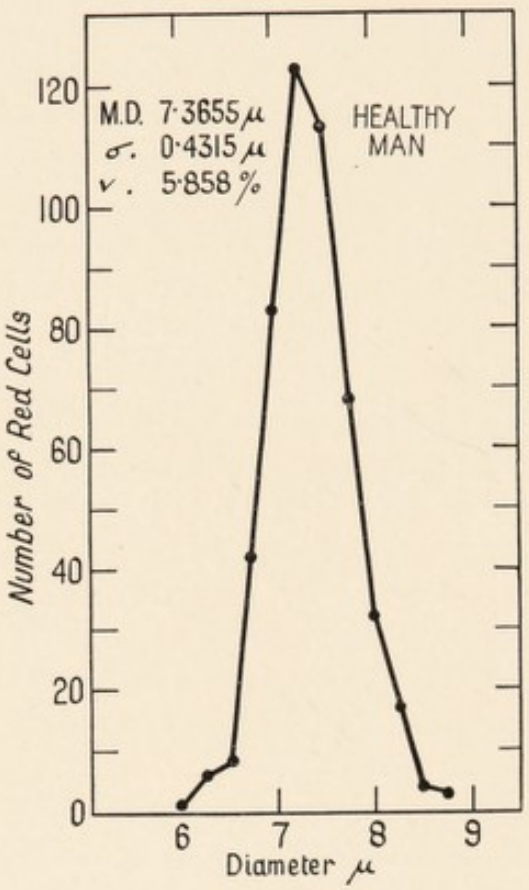

Fig. 1.

The *standard error of the mean** is the standard deviation (σ) divided by the square root of the number of cells measured $\left(\frac{\sigma}{\sqrt{N}}\right)$.

From columns 1 and 2 represented respectively by abscissae and ordinates a distribution curve can be drawn (Fig. 1).

II

There are in the circulating blood about 17×10^{12} red corpuscles, a number greater than the human population of the earth. These red cells vary in diameter from about 5μ to 9·5μ. To obtain the actual mean diameter measurement of all the cells is obviously impossible; it is necessary to take a sample of the population, and 500 cells is a convenient number.

If two samples of 500 cells each are taken simultaneously, fixed and stained and measured, and the frequency with which each diameter occurs ascertained and the mean diameter calculated, it is plain that the two means will only rarely be identical; one will be larger than the other owing to the error inherent in sampling a variable population. To such a difference no real meaning is attached

* In my published papers I have used the 'standard' error and have mistakenly referred to it as 'probable error' (v. Yule, p. 311). Probable error = standard error $\times$ 0·6745.

and it is regarded as non-significant. If, on the other hand, the two samples are not taken simultaneously from the same population a similar difference will probably be found. This may be due either to the error of sampling or to the diameter of the cells composing the population having changed in the interval between taking the two samples.

In Table 3 are given the data of seven samples of blood taken at intervals over a period of twenty-four hours from the same person.

The difference between samples C and E is $0{\cdot}614\mu$, between F and G is $0{\cdot}007\mu$. The natural presumption is that the former may mean much and the latter mean nothing. The meaning of the differences of intermediate value cannot, however, be determined by such subjective tests, and for their evaluation it is necessary to proceed by statistical rules.

To test the difference between two means add together the squares of their standard errors, take the square root of this sum and divide it into the difference between the two means; if the figure thus obtained is 3 or more the difference is regarded as real and significant, if less than 3 it is non-significant.

For example, taking the samples D and E (Table 3):

Standard error squared $\left(\frac{\sigma}{\sqrt{N}}\right)^2$ $D = 0{\cdot}000605$

" " " $E = 0{\cdot}000548$

add $= 0{\cdot}001153$

$\sqrt{\text{of sum}}$ $= 0{\cdot}033955$, say $0{\cdot}034$

Mean of $D = 7{\cdot}633$

" " $E = 7{\cdot}048$, $D-E$ $= 0{\cdot}585$

$0{\cdot}585/0{\cdot}034 = 17{\cdot}2$, therefore significant.

Taking samples F and G:

Standard error squared $\left(\frac{\sigma}{\sqrt{N}}\right)^2$ $F = 0{\cdot}000449$

" " " " $G = 0{\cdot}000428$

add $= 0{\cdot}000877$

$\sqrt{\text{of sum}}$ $= 0{\cdot}0029614$, say $0{\cdot}003$

Mean of $F = 7{\cdot}326$

" " $G = 7{\cdot}333$, $F-G$ $= 0{\cdot}007$

$0{\cdot}007/0{\cdot}003 = 2{\cdot}3$, which is not significant.

In like manner $A/B = 2{\cdot}6$, not significant.

$A/C = 6{\cdot}3$, significant.

$B/C = 3{\cdot}4$,,

$E/F = 8{\cdot}8$,,

$C/D = 0{\cdot}8$, not significant.

TABLE 3

Samples of blood taken at intervals from same person

μ	A	B	C	D	E	F	G
5·50	..	..	..	..	2	..	..
5·75	..	..	..	..	5	..	..
6·00	1	2	5	1	13	3	3
6·25	6	7	2	5	25	8	6
6·50	7	11	5	13	60	23	20
6·75	32	38	14	27	82	52	53
7·00	83	53	48	36	90	76	90
7·25	92	79	69	72	85	108	84
7·50	98	92	97	94	78	104	109
7·75	80	77	82	85	32	64	76
8·00	65	68	84	83	18	40	40
8·25	21	43	50	38	7	15	15
8·50	9	15	32	29	3	6	4
8·75	5	11	9	11	..	1	..
9·00	1	3	2	4	..	..	..
9·25	..	1	1	2	..	..	..
Totals	500	500	500	500	500	500	500
Mean	7·461	7·546	7·662	7·633	7·048	7·326	7·333
σ	0·477	0·552	0·526	0·551	0·523	0·474	0·462
v	6·4	7·3	6·9	7·2	7·4	6·5	6·3
			Standard error of mean				
$\left(\frac{\sigma}{\sqrt{N}}\right)$	0·0214	0·0248	0·0236	0·0246	0·0234	0·0212	0·0207
$\left(\frac{\sigma}{\sqrt{N}}\right)^2$	0·000458	0·000615	0·000557	0·000605	0·000548	0·000499	0·000428

This 'three times' rule is based on a great mass of varied experience as well as on theoretical considerations; it is, however, only valid if sources of error other than random sampling can be reasonably excluded.

To test this point, two series of measurements were made each of a number of films made as nearly as possible simultaneously from the same individual; in each film 500 cells were measured (Tables 4 and 5). In the first trial 8 films were made (Table 4) yielding 28 possible pairs for comparison; 17 of the differences are less than 3 times the standard error, 7 are between 3 and 4 times, 2, A/G and

D/E, between 4 and 5 times, and A/D and D/H 5·3 and 5·4 times respectively.

In the second series 10 films were made (Table 5) giving 45 differences for comparison. Of these 28 were less than 3 times, 5 between 3 and 4 times, 11 between 4 and 5 times, and 1, A/B, 5·1 times.

TABLE 4

Eight films—or 28 pairs for comparison

	Mean	*Standard deviation*		*Mean*	*Standard deviation*
A	7·401	0·483	*E*	7·376	0·470
B	7·304	0·476	*F*	7·294	0·474
C	7·339	0·470	*G*	7·269	0·574
D	7·242	0·458	*H*	7·400	0·470

TABLE 5

	Mean	*Standard deviation*		*Mean*	*Standard deviation*
A	7·521	0·492	*F*	7·389	0·474
B	7·364	0·488	*G*	7·458	0·499
C	7·493	0·469	*H*	7·375	0·473
D	7·513	0·511	*I*	7·468	0·460
E	7·382	0·462	*J*	7·444	0·467

It is evident, therefore, that the whole process involves errors other than those inherent in the sampling of a variable population; these are presumably due to differences in the making and rate of drying of the films, errors in measurement, and so forth. It would be possible to dissect some of them out for further analysis, but for my present purpose it is sufficient to know that an allowance of 5 times covers the whole of them in 95 per cent. of the differences tested. In other words, a difference between two means which is more than 5 times its standard error may, therefore, be regarded as a real and significant difference. For practical purposes, when, as in the present series of measurements, the means and standard deviations are all fairly close together, it is convenient to have a standard of difference which, based on the method of testing outlined above, is expressed in absolute values and the calculations omitted. With means of about 7·4μ and standard deviations of about 0·48μ and 500 cells measured, the standard error of the difference between any two means is about 0·03μ; 5 times this or 0·15μ is, therefore, probably a significant difference. To be on the safe side I have allowed more than this, and the smallest difference on which any stress is laid is

0·2μ; differences between 0·15 and 0·2 are taken as probably but not conclusively real; differences below 0·15 are disregarded.

This arbitrary standard would not be applicable if the means differed widely from about 7·5μ (e.g. goat's corpuscles 4·5μ), or if the red cells were much more variable and had a standard deviation higher than about 0·6, or if the number of cells measured were not 500. The precision of the answer varies with the square root of the number of cells measured; if for 500 the necessary difference is taken as 0·2, for 200 it would be 0·32, for 100 cells 0·45, and for 1,000 0·15. A number between 200 and 500 gives reasonable accuracy, and does not involve too disproportionate labour.

CHAPTER III

THE RED CELL DIAMETERS OF HEALTHY PERSONS

I

In my first communication (1922) I reported the measurements of 500 red cells from each of 20 healthy persons (10,000 cells). The mean diameter ranged from 6·96μ to 7·49μ with a mean of 7·210μ. Considering that this sample was probably too small to express adequately the distribution of mean diameters in a population of healthy persons I extended the series by 80, and so obtained measurements of 500 red cells from each of 100 persons (50,000 cells). From these measurements I was able to estimate the mean of the 100 means, their standard deviation and coefficient of variation, and was then in a much better position to know the range of normality and to judge the probability of any figure being abnormal.

These 100 persons were in no way selected, they were just haphazard, presumably healthy adults from 19 to 92 years of age, and, with the exception of 4 who were old and leading sedentary lives, they were all in ordinary occupation at the hospital, which in no case involved heavy muscular work. There were 92 males and 8 females; there is no evidence to suggest that either age or sex has any influence on the size of the cells.

The dry film method described above was followed in all its details. The specimens were all obtained as nearly as possible at the same time in the forenoon. Red cell counts and haemoglobin estimations were made in most instances and corresponded to accepted normal standard limits. The complete record of the 50,000 measurements is set out in Table 6. The cell diameters ranged from 4·75μ to 9·50μ, the mean diameter was 7·202μ, the standard deviation 0·487μ, and the coefficient of variation 6·325 per cent. The 100 mean diameters of 100 persons are summarized in Table 7. They give a mean of 7·202μ, standard deviation (σ) = 0·172μ, and coefficient of variation (v) = 2·3 per cent.*

* There is presumably a personal factor in these measurements, and the differences between the healthy means found by various observers no doubt depend to some extent on individualities of technique, the calibration of micrometers, and other details.

Using dry films, Grosh and Stifel (1925) got 7·4μ, Bell Thomas and Means (1926) 7·7μ, Medearis and Minot (1927) 7·55μ, Ohno and Gisevius (1925) 7·9μ, Pohle (1927) 7·3μ, Wischnewsky (1928) 7·2μ, Silvette (1927) infants 7·4μ; using wet methods Thomas Young (1823) found 5 to 6μ, McCormick (1927) 7·3μ, Holler and Kudelke (1928) 7·6μ, Jorgensen and Warburg (1927) 7·6μ, Ponder and Millar (1924) 8·8μ.

TABLE 6

Distribution of Diameters

Mid-points of class intervals μ	*Case* 1 *Sex M* *Age* 56	2 *M* 45	3 *M* 30	4 *M* 19	5 *M* 39	6 *F* 79	7 *M* 79
4·75	..	..	..	..	..	..	..
5·00	..	..	..	..	..	..	..
5·25	..	..	..	..	..	..	..
5·50	..	..	..	..	..	..	..
5·75	..	2	..	1	..	..	2
6·00	2	..	7	3	5	3	14
6·25	10	3	10	14	24	15	14
6·50	19	12	25	31	50	28	35
6·75	54	39	56	61	81	70	68
7·00	64	63	88	77	97	96	91
7·25	119	100	106	93	82	119	94
7·50	100	112	84	96	84	76	95
7·75	68	79	50	66	47	52	43
8·00	39	57	45	37	21	31	25
8·25	20	23	16	16	7	6	12
8·50	5	6	11	3	2	4	4
8·75	..	3	..	2	..	..	3
9·00	..	1	1	..	..	..	..
9·25	..	..	..	..	..	..	..
9·50	..	..	1	..	..	..	..
Total	500	500	500	500	500	500	500
Mean diameter	7·339	7·443	7·302	7·280	7·124	7·211	7·186
Standard deviation	0·470	0·470	0·524	0·503	0·484	0·460	0·522
Variability per cent.	6·4	6·3	7·2	6·9	6·8	6·4	7·3

Mid-points of class intervals μ	*Case* 18 *Sex M* *Age* 35	19 *M* 50	20 *M* 55	21 *M* 25	22 *M* 25	23 *M* 24	24 *F* 29
4·75	..	..	2	..	..	..	..
5·00	..	..	..	..	..	..	..
5·25	..	..	..	..	3	..	..
5·50	..	1	..	2	..	..	..
5·75	..	3	2	..	..	1	1
6·00	2	9	4	9	5	5	7
6·25	9	14	4	31	18	16	8
6·50	16	43	31	55	43	53	23
6·75	64	63	59	113	87	72	50
7·00	99	94	96	103	112	109	93
7·25	107	105	114	104	112	95	121
7·50	84	87	86	55	77	85	99
7·75	79	48	68	24	25	37	61
8·00	27	23	22	2	15	22	24
8·25	7	7	11	2	3	4	10
8·50	5	2	1	..	..	1	2
8·75	1	1	..	..	..	..	1
9·00	..	..	..	..	..	..	..
9·25	..	..	..	..	..	..	..
9·50	..	..	..	..	..	..	..
Total	500	500	500	500	500	500	500
Mean diameter	7·280	7·160	7·231	6·942	7·074	7·119	7·257
Standard deviation	0·447	0·494	0·474	0·413	0·442	0·457	0·453
Variability per cent.	6·1	6·9	6·6	5·9	6·3	6·4	6·2

in 100 *healthy persons*

8	9	10	11	12	13	14	15	16	17
M	*M*	*M*	*M*	*M*	*M*	*M*	*M*	*M*	*M*
90	77	44	32	38	42	44	39	36	38
..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..
1	..	..	..	1	..	..	..	..	..
3	3	1	3	5	..	..	..	..	..
5	6	15	1	4	..	1	3	2	6
22	25	41	11	12	2	5	3	6	15
47	36	61	34	29	9	20	18	28	41
72	72	91	74	57	25	55	53	51	77
121	106	101	101	76	70	96	98	92	106
93	114	96	103	102	102	110	103	106	92
73	79	57	86	89	112	118	105	88	73
40	36	23	41	68	78	59	73	66	51
19	13	11	36	34	60	24	32	42	22
3	6	3	8	15	31	10	8	12	15
1	4	..	2	6	8	2	3	3	2
..	..	..	..	1	3	..	1	3	..
..	..	..	..	1	..	..	..	1	..
..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..
500	500	500	500	500	500	500	500	500	500
7·091	7·114	6·968	7·200	7·274	7·487	7·283	7·307	7·311	7·169
0·468	0·470	0·460	0·456	0·526	0·446	0·410	0·431	0·476	0·485
6·6	6·6	6·6	6·3	7·2	6·0	5·6	5·9	6·5	6·8

25	26	27	28	29	30	31	32	33	34
M	*F*	*M*	*F*	*M*	*F*	*F*	*F*	*M*	*M*
25	22	23	31	22	26	(?) 27	22	21	23
..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	1	..
..	..	1	..	..	..	..	..	2	..
..	..	1	1	..	..	2	..	5	1
4	1	2	..	..	1	8	1	15	..
14	5	22	5	3	2	25	3	56	11
40	18	41	15	8	13	50	17	73	32
73	42	72	48	25	41	86	37	115	53
89	68	112	57	66	62	114	70	100	88
111	127	111	117	100	109	111	133	70	124
92	118	80	99	121	115	69	106	48	88
36	63	40	75	87	77	19	71	10	68
25	34	13	57	50	50	11	39	5	26
11	16	4	17	27	26	3	18	..	8
4	6	1	9	7	4	2	3	..	1
1	2	..	..	4	..	..	2	..	..
..	..	..	..	2	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..
500	500	500	500	500	500	500	500	500	500
7·239	7·360	7·119	7·404	7·487	7·423	7·034	7·376	6·841	7·252
0·479	0·441	0·425	0·463	0·448	0·440	0·441	0·432	0·452	0·435
6·6	6·0	6·0	6·2	6·0	5·9	6·3	5·9	6·6	6·0

TABLE 6

Distribution of Diameters

Mid-points of class intervals μ	*Case* 35 *Sex M* *Age* 22	36 *M* 22	37 *M* 28	38 *M* 30	39 *M* 29	40 *M* 31	41 *M* 28
4·75	..	..	..	..	..	..	..
5·00	..	..	..	..	..	..	..
5·25	1	..	..	..	..	..	..
5·50	..	..	..	..	1	..	..
5·75	2	1	1	1	5	..	1
6·00	8	5	1	..	10	4	5
6·25	13	13	5	6	18	26	15
6·50	45	28	16	12	61	47	48
6·75	66	62	48	30	103	85	88
7·00	95	94	52	69	96	95	118
7·25	114	126	150	91	96	115	120
7·50	78	91	83	121	63	69	62
7·75	42	56	66	70	26	35	33
8·00	23	18	43	62	16	15	6
8·25	5	4	22	28	3	5	4
8·50	6	2	9	8	1	3	..
8·75	1	..	2	2	1	1	..
9·00	1	..	2	..	..	..	..
9·25	..	..	..	..	..	..	..
9·50	..	..	..	..	..	..	..
Total	500	500	500	500	500	500	500
Mean diameter	7·160	7·199	7·388	7·458	7·021	7·097	7·066
Standard deviation	0·501	0·438	0·482	0·469	0·477	0·470	0·410
Variability per cent.	7·0	6·1	6·5	6·3	6·8	6·6	5·8

Mid-points of class intervals μ	*Case* 52 *Sex M* *Age* 20	53 *M* 36	54 *M* 27	55 *M* 34	56 *M* 20	57 *M* 20	58 *M* 21
4·75	..	..	..	..	..	..	..
5·00	..	..	..	..	..	..	..
5·25	..	..	..	..	..	..	..
5·50	..	..	..	2	..	1	..
5·75	..	1	..	3	..	2	1
6·00	..	1	..	3	1	20	3
6·25	9	3	2	17	5	53	5
6·50	17	10	10	29	14	78	21
6·75	50	42	28	70	37	99	46
7·00	82	65	61	101	68	111	80
7·25	145	105	120	119	123	84	142
7·50	82	100	127	85	93	36	97
7·75	60	93	77	42	85	11	52
8·00	33	43	40	19	47	4	36
8·25	17	26	23	8	22	1	13
8·50	3	8	11	2	4	..	4
8·75	2	2	1	..	..	..	..
9·00	..	1	..	..	1	..	..
9·25	..	..	..	..	..	..	..
9·50	..	..	..	..	..	..	..
Total	500	500	500	500	500	500	500
Mean diameter	7·313	7·438	7·449	7·162	7·405	6·850	7·302
Standard deviation	0·495	0·467	0·426	0·466	0·448	0·437	0·441
Variability per cent.	6·8	6·3	5·7	6·5	6·0	6·4	6·0

—continued

in 100 *healthy persons—continued*

42	43	44	45	46	47	48	49	50	51
M	*M*	*M*	*M*	*M*	*M*	*M*	*M*	*M*	*M*
39	28	38	25	35	22	27	20	23	?
..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..
1	..	..	..	..	..	1	..	..	..
3	..	1	..	..	2	..	1	2	..
6	7	2	1	1	8	..	2	10	..
13	9	7	3	6	17	9	9	24	9
34	26	35	21	19	44	14	22	50	11
77	55	48	49	54	76	50	61	86	33
92	82	90	76	76	97	58	93	97	59
120	151	133	129	144	135	119	126	121	111
78	85	92	108	107	68	102	102	69	111
43	51	58	66	46	38	76	63	33	81
26	26	27	34	31	10	45	14	6	50
7	6	4	11	13	4	19	5	2	27
..	2	2	1	2	..	5	2	..	6
..	..	1	1	1	1	2	..	..	2
..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..
500	500	500	500	500	500	500	500	500	500
7·157	7·229	7·243	7·321	7·290	7·097	7·379	7·233	7·042	7·426
0·467	0·436	0·434	0·419	0·417	0·442	0·467	0·414	0·438	0·446
6·5	6·0	6·0	5·7	5·7	6·2	6·3	5·7	6·2	6·0

59	60	61	62	63	64	65	66	67	68
M	*M*	*M*	*M*	*M*	*M*	*M*	*M*	*M*	*M*
24	21	55	(?) 60	36	32	39	47	(?) 28	?
..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..
..	..	1	..	..	..	..	..	..	..
..	..	3	..	..	..	..	..	..	..
..	3	17	..	..	..	..	..	..	..
6	7	35	9	1	1	2	..	3	1
11	31	76	17	13	4	7	3	4	6
23	60	96	45	21	7	21	9	16	13
73	116	100	76	64	42	59	27	52	39
90	116	105	111	89	65	97	71	45	59
123	101	46	103	133	95	117	111	108	135
102	40	18	74	80	95	106	103	92	88
44	20	2	45	57	77	63	76	94	81
19	4	1	16	31	56	20	55	52	44
4	2	..	3	9	38	7	29	23	26
3	..	..	1	2	13	1	15	8	7
1	..	..	..	..	7	..	1	2	1
1	..	..	..	..	..	..	..	1	..
..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..
500	500	500	500	500	500	500	500	500	500
7·206	6·947	6·661	7·109	7·248	7·492	7·254	7·476	7·379	7·411
0·443	0·411	0·439	0·451	0·439	0·501	0·411	0·457	0·503	0·458
6·1	5·9	6·6	6·3	6·1	6·7	5·7	6·1	6·8	6·2

TABLE 6

Distribution of Diameters

Mid-points of class intervals μ	*Case* 69 *Sex M* *Age* 34	70 *M* (?) 25	71 *M* 26	72 *M* 22	73 *M* 24	74 *M* 37	75 *M* 45
4·75	..	..	..	..	..	..	..
5·00	..	..	..	..	..	..	..
5·25	..	..	..	..	..	..	..
5·50	..	..	..	..	..	1	..
5·75	..	..	2	..	1	4	1
6·00	6	4	2	1	5	5	..
6·25	15	8	16	6	15	37	7
6·50	36	19	26	8	36	60	22
6·75	54	58	74	42	64	98	67
7·00	91	77	85	83	104	111	82
7·25	121	137	124	123	111	107	125
7·50	100	92	85	113	78	50	106
7·75	48	64	54	68	61	20	53
8·00	21	26	21	32	17	5	26
8·25	6	11	9	17	5	2	8
8·50	2	3	2	4	2	..	2
8·75	..	1	..	3	1	..	1
9·00	..	..	..	..	..	..	..
9·25	..	..	..	..	..	..	..
9·50	..	..	..	..	..	..	..
Total	500	500	500	500	500	500	500
Mean diameter	7·198	7·280	7·202	7·365	7·178	6·961	7·261
Standard deviation	0·451	0·443	0·455	0·431	0·458	0·431	0·426
Variability per cent.	6·3	6·1	6·3	5·9	6·4	6·2	5·9

Mid-points of class intervals μ	*Case* 86 *Sex M* *Age* (?) 27	87 *M* 22	88 *M* (?)	89 *M* (?)	90 *M* 23	91 *M* 24	92 *M* 26
4·75	..	..	..	..	..	..	..
5·00	..	..	..	..	..	..	..
5·25	..	..	..	..	..	..	..
5·50	2	2	..	..	..	..	1
5·75	2	1	..	3	3	..	5
6·00	6	5	2	10	10	6	8
6·25	26	10	8	17	27	25	39
6·50	50	40	18	49	60	55	78
6·75	93	63	35	107	79	85	92
7·00	98	112	61	87	127	108	115
7·25	104	115	98	110	94	117	103
7·50	69	76	99	60	67	66	39
7·75	35	50	83	34	24	29	18
8·00	11	18	55	13	7	6	2
8·25	2	6	30	9	2	3	..
8·50	2	1	6	1	..	..	..
8·75	..	1	4	..	..	..	..
9·00	..	..	1	..	..	..	..
9·25	..	..	..	..	..	..	..
9·50	..	..	..	..	..	..	..
Total	500	500	500	500	500	500	500
Mean diameter	7·048	7·161	7·442	7·062	7·001	7·025	6·907
Standard deviation	0·463	0·456	0·498	0·472	0·438	0·436	0·422
Variability per cent.	6·6	6·4	6·7	6·7	6·3	6·2	6·1

—continued

in 100 *healthy persons—continued*

76 *M* 55	77 *M* 24	78 *M* 42	79 *M* 22	80 *M* 42	81 *M* 31	82 *M* 35	83 *M* 58	84 *M* 20	85 *M* 36
..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..
1	..	..	..	..	..	..	..	1	..
4	9	3	..	5	1	..	..	5	..
12	20	3	2	15	2	3	3	8	3
20	39	7	1	39	11	14	5	29	6
56	65	23	19	73	36	39	19	46	30
81	68	49	41	122	79	69	46	86	59
84	105	85	43	102	86	104	77	126	80
116	103	131	107	85	131	127	112	116	133
69	62	88	94	37	77	73	88	58	92
35	18	69	99	19	33	48	82	19	63
16	8	26	40	3	29	18	38	3	21
4	3	12	34	..	11	5	22	3	11
2	..	4	13	..	3	..	7	..	1
..	..	..	4	..	1	..	1	..	1
..	..	..	3	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	..	..
500	500	500	500	500	500	500	500	500	500
7·064	6·944	7·279	7·481	6·889	7·191	7·159	7·367	6·994	7·254
0·489	0·489	0·456	0·503	0·433	0·463	0·428	0·473	0·426	0·437
6·9	7·0	6·3	6·7	6·3	6·4	6·0	6·4	6·1	6·0

93 *M* 27	94 *M* 48	95 *M* 42	96 *M* 25	97 *M* 26	98 *M* 23	99 *M* 26	100 *F* 25	*Total*
..	..	..	..	..	..	..	..	2
..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	6
..	..	1	..	..	1	..	2	29
1	1	3	1	1	5	1	3	149
4	8	10	2	4	7	9	19	517
11	15	30	12	8	22	11	48	1,530
30	37	61	28	19	45	37	92	3,429
76	63	97	63	58	75	72	106	6,568
84	109	94	83	77	113	117	116	8,956
127	100	113	118	120	97	102	71	11,214
83	83	52	98	104	75	79	33	8,388
52	53	24	53	59	42	46	9	5,154
21	18	8	30	35	10	20	1	2,570
8	9	5	9	11	4	4	..	1,070
3	4	2	2	3	4	1	..	319
..	..	..	1	1	..	1	..	81
..	..	..	..	..	..	..	..	17
..	..	..	..	..	..	..	..	..
..	..	..	..	..	..	..	..	1
500	500	500	500	500	500	500	500	50,000
7·200	7·173	6·994	7·244	7·297	7·083	7·145	6·815	7·202μ
0·451	0·477	0·466	0·457	0·453	0·480	0·455	0·415	0·487μ
6·3	6·6	6·7	6·3	6·2	6·8	6·4	6·1	6·325 per cent.

TABLE 7

Mean diameters of 100 *normal persons*

Mean diameter	*Number of persons*	
6·650–6·699	1	
6·700–6·749	0	
6·750–6·799	0	
6·800–6·849	2	
6·850–6·899	2	
6·900–6·949	4	
6·950–6·999	4	Minimum 6·661μ
7·000–7·049	6	Maximum 7·492μ
7·050–7·099	8	
7·100–7·149	6	
7·150–7·199	13	
7·200–7·249	12	
7·250–7·299	13	
7·300–7·349	7	
7·350–7·399	7	
7·400–7·449	9	
7·450–7·499	6	
	100	

Mean μ 7·202
Standard deviation (σ) 0·172
Coefficient of variation (v) 2·3 per cent.

The 80 fresh cases have extended my previously observed normal range from 6·968–7·487μ to 6·661–7·492μ, but only 10 of the diameters fall outside the range of the series of 20 persons, and the mean is emended only from 7·210μ to 7·202μ.

The distribution of the means is irregular; the curve (Fig. 2) ends abruptly on the right. This may represent the fact. If the distribution is compared by the χ^2 method with a 'normal' curve (calculated from the observed mean and standard deviation), P works out at 0·52,* so that the observed distribution may quite well represent the normal distribution which we should expect.

II

It is necessary here to consider the nature of the 'normal' curve and 'goodness of fit'.

As stated above, most measurable properties and products of living things are arranged according to the normal curve of variation, and it may be presumed that the red cells of the blood are no exception. In other words the majority of the cells will have a diameter

* This value of P means that in 52 trials in 100 we should get by random sampling a correspondence between observation and theory as bad or worse; other groupings of the figures gave $P = 0{\cdot}43$ and 0·78.

near to the mean diameter, and at increasing distances on either side of the mean the number of cells will be progressively fewer. Thus in a case where the mean diameter is 7·28μ, two-thirds of the cells are found to lie between 6·50μ and 8·0μ, and only about six per thousand are smaller than 6·0μ or greater than 8·50μ. More precisely the 'normal' or Gaussian curve is a symmetrical binomial curve, the nature and properties of which are discussed in books on statistics (Yule, 1919, chap. xv; Bowley, 1920, Bk. II, chaps. ii–iii).

An ideal symmetrical curve with its base-line bounds an area or polygon which is divided into two equal parts by the mean ordinate,

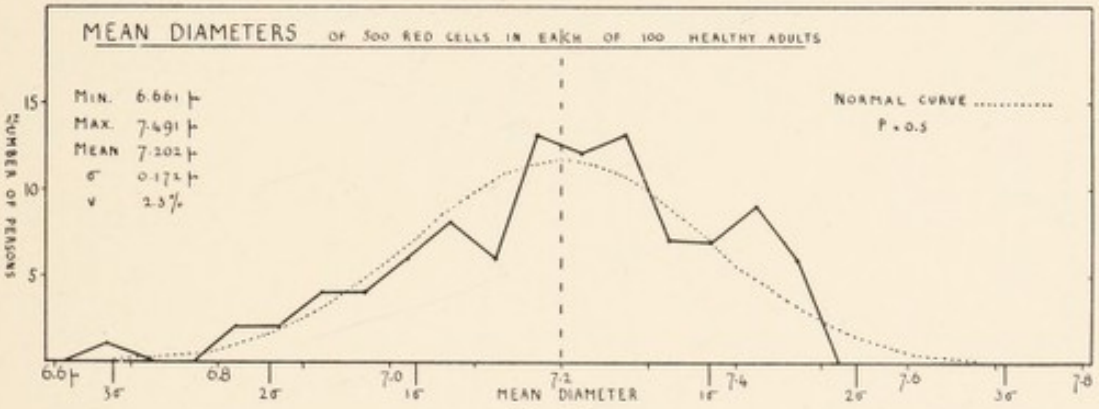

FIG. 2.

the mode, the median, and the mean being identical. Given the number of observations (frequencies), the mean value, and the standard deviation, it is possible to construct a corresponding normal curve for any sample of a population or collection of variables, from a calculation of the values of the ordinates at different distances from the mean ordinate.

Since the mean ordinate divides the polygon into two equal parts, each portion is 50 per cent. or 0·50. With an ordinate at a distance of 0·1 times the standard deviation from the mean, there will be 0·53983 of the whole area on one side and 0·46017 on the other. With an ordinate at a distance from the mean equal to the standard deviation, about 16 per cent. of the area will be cut off and the greater portion will be 0·84134. With an ordinate at a distance from the mean equal to twice the standard deviation, only 2·3 per cent. will be cut off and, therefore, the greater fraction will be 0·97725 and the rest of the area will be 1—0·9772 = 0·0228 = $2\frac{1}{4}$ per cent., or about 23 per 1,000, i.e. 43·4 to 1 against. Again, with an ordinate at a distance equal to 3 times the standard deviation the greater fraction will be 0·9987 and the rest of the area will be 1—0·9987 = 0·0013 = 13 per 10,000 or 770/1, which means that the chances of

any frequency occurring in that small area are 770 to 1 against.* Tables have been constructed giving the calculated values for the areas at any distance from the mean (Yule, loc. cit., p. 310); the symmetrical curve formed by these ordinates is the normal curve.

The theoretical curve which is calculated in this way could be expected to correspond exactly with the curve of the observed data

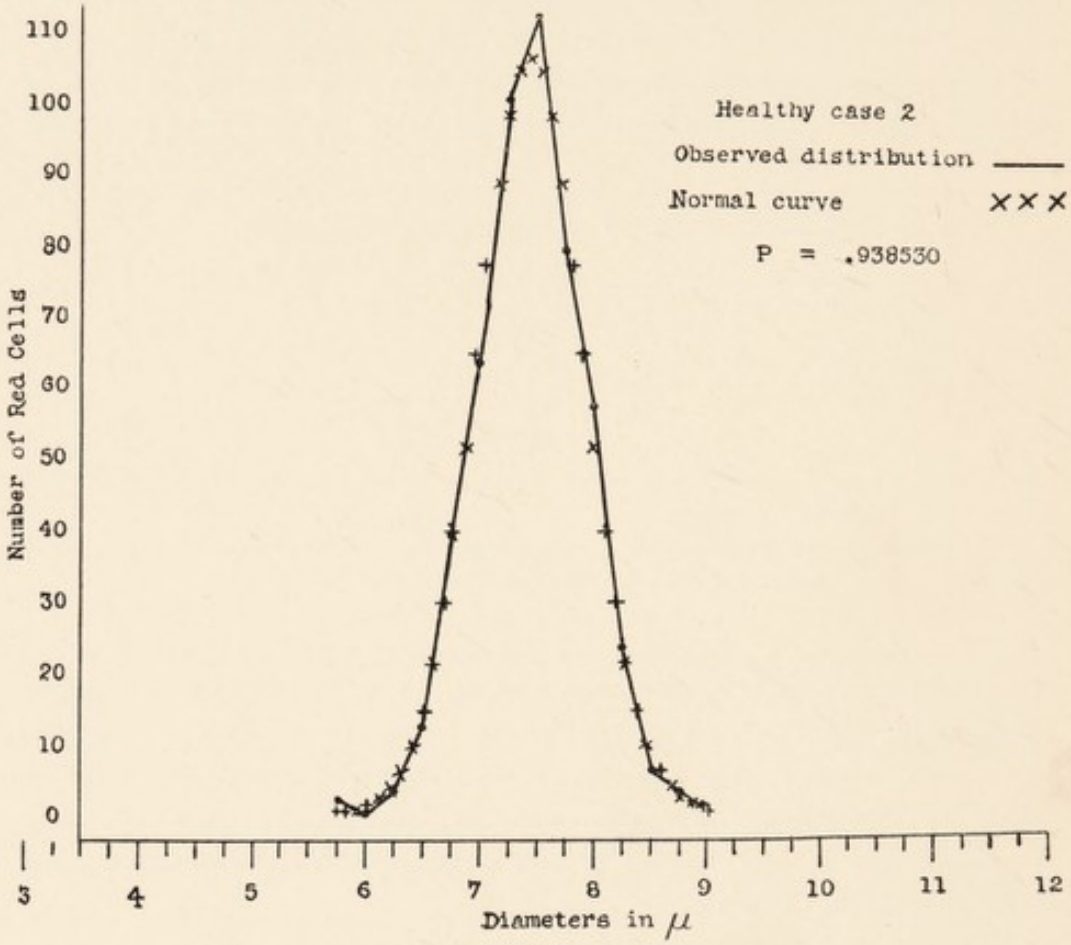

FIG. 3.

only if the whole of a large population (in this case all the red cells in the whole blood) had been measured. In practice it is possible to examine only a sample, and 500 cells is a convenient number, bearing in mind, as stated above, that the precision of the result varies as the square root of the number measured.

Owing to the error of random sampling the correspondence between the actual and calculated curves of distribution will in the great majority of these samples be more or less imperfect. When the correspondence is so slight that it would seem unlikely or impossible that the measured sample could belong to a population of cells distributed according to size exactly on the normal curve, there is

* See Pearl, *Medical Biometry*, 1923, pp. 244 and 365.

strong presumption that the population is not homogeneous but is composed of a mixture of two or more groups of individuals, each group varying according to its normal curve. If, on the other hand, the correspondence between the observed and the calculated curves is good there is no prima facie reason to suspect heterogeneity.

Examples of normal curves calculated by the method given by

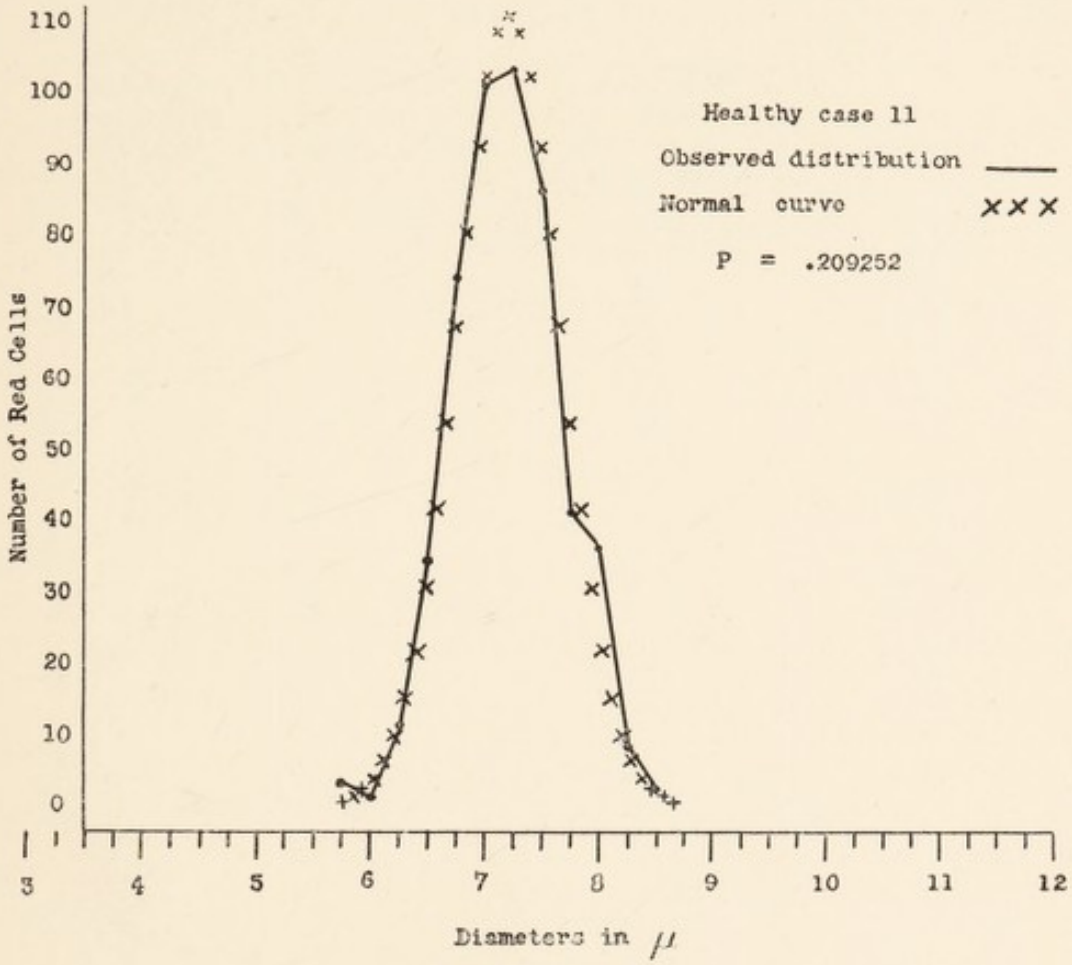

FIG. 4.

Yule (p. 307) are shown in Figs. 3 and 4; the observed curves of red cell diameters from healthy persons are superposed on their respective normal curves. It is well to emphasize the fact that the comparison of curves by the mere ocular inspection of the graphs is apt to be extremely misleading, and excepting in cases of wide and obvious divergence such comparisons would be of small value.

The quantitative measure of the goodness or badness of correspondence or 'fit' was carried out by Karl Pearson's 'chi square' (χ^2) method (*Biometrica*, 1914, part 1, p. 85). There finally emerges from this procedure a figure 'P' which is a measure of the goodness of fit. When $P = 0{\cdot}50$ it means that 50 out of 100 samples from a

population varying according to the normal curve would diverge more from the normal distribution than the sample under consideration, in other words, the fit is fairly good. When $P = 0{\cdot}999999$ it means that with 999,999 out of 1,000,000 samples there would be a worse fit, or the sample under consideration is practically a perfect fit. When $P = 0{\cdot}001$ it means that only once in 1,000 samples would

TABLE 8

Units Diameters μ	*Frequencies* -3σ $M = 6{\cdot}686$ $= 7{\cdot}202 - 3 \times 0{\cdot}172$	*Frequencies* $+3\sigma$ $M = 7{\cdot}718$ $= 7{\cdot}202 + 3 \times 0{\cdot}172$	*Frequencies Healthy mean* $M = 7{\cdot}202$ $\sigma = 0{\cdot}487$
5·25	1	..	..
5·50	5	..	..
5·75	16	..	2
6·00	39	..	5
6·25	69	1	15
6·50	95	5	34
6·75	102	14	66
7·00	84	34	90
7·25	52	65	112
7·50	25	93	84
7·75	9	103	51
8·00	2	87	26
8·25	1	56	11
8·50	..	28	3
8·75	..	10	1
9·00	..	3	..
9·25	..	1	..
9·50	..	..	..
	500	500	500
M	6·686	7·718	7·202
σ	0·480	0·484	0·487
v	7·1	6·2	6·3

a worse fit be met with, and the divergence is so great that it is impossible to imagine that the sample belongs to a normal population. The value of P depends to some extent upon the judgement used in forming the groups of figures for the calculation. Inasmuch as 'good' and 'bad' are relative terms, the interpretation of P is dependent on the stringency of the criteria which are chosen for dealing with the question of chances. It seems reasonable to regard $P + 0{\cdot}5$ and over as a good fit, and when P is less than 0·1 it is a bad fit. On the whole the goodness of fit of the observed curves of the red cell diameters of healthy persons with their respective normal curves is satisfactory.

We are now in better state to discuss the distribution curves of 100 healthy persons as expressed by Fig. 2. The highest observed

mean diameter is 7·492μ, but it would be rash to assume that this is the highest limit of normality. By the ordinary + or — 3σ rule for normal or near normal distributions this should be 7·202+ (3×0·172) = 7·718μ, and it would be unwise to be quite sure that any mean diameter less than 7·8μ was abnormally large. Similarly 7·202—(3×0·172) = 6·686μ will represent the smallest (—3σ) mean diameter within normal limits. We noted above that with 3 times σ,

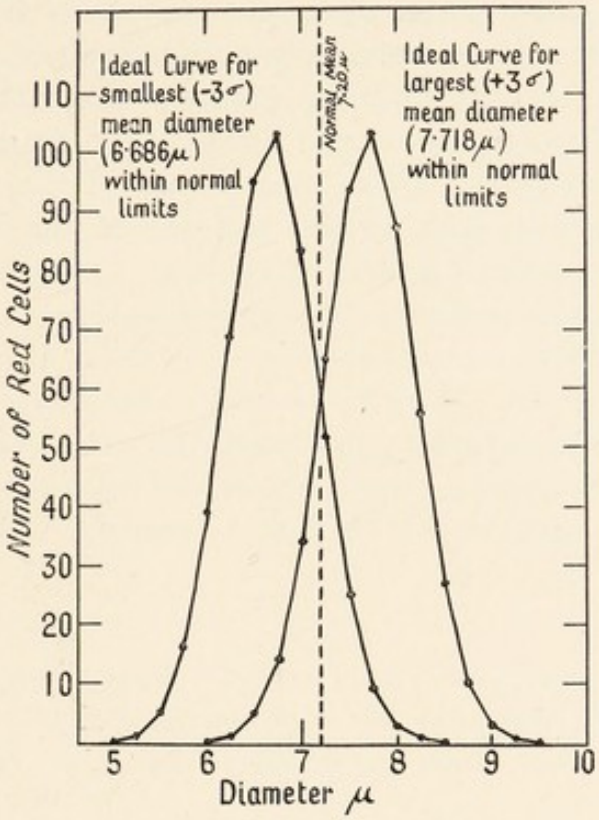

FIG. 5.

the smallest area of the polygon would be 1—0·9987 = 0·0013, so that with 1,000 healthy persons we should expect to get about 1 with a mean diameter greater than 7·718μ or less than 6·686μ. In other words, the chances against 7·718μ and 6·686μ being normal diameters are 13 per 10,000, or over 770 to 1. With 2·5σ we should expect 6 diameters greater than 7·632μ or less than 6·772μ, the chances against normality being 157 to 1; and about 23 per thousand with + or —2σ, the chances of 7·546μ and 6·858μ being normal are about 43 to 1.

There is no arbitrary limit to normality. Which limit anybody chooses for working purposes depends on their psychological attitude towards questions of chance. I believe, however, that all experience goes to show that it is not safe to trust to a less stringent criterion

than a deviation from the mean of 3 times the standard deviation, though this will sometimes involve the classification of really abnormal bloods as normal. I conclude therefore that *the normal range of mean diameters determined by my method is from* 6·686μ *to* 7·718μ; a mean diameter outside these limits is almost certainly abnormal and one which is larger than 7·546μ or smaller than 6·854μ is open to suspicion.

In Table 8 I have set out the calculated frequencies for normal curves ranged about mean diameters of -3σ (6·686μ) and $+3\sigma$ (7·718μ) ideal curves, and contrast them with the observed frequencies of diameters in the healthy mean (7·202μ). From these frequency distributions three curves can be drawn; they are represented in Fig. 5. To simplify the chart I have omitted the mean healthy curve, indicating it only by the ordinate of the healthy mean (7·202μ). To the right is drawn the ideal normal curve for the largest $+3\sigma$ mean diameter (7·718μ) within normal limits, to the left the ideal curve for the smallest -3σ mean diameter (6·686μ) within normal limits. These curves define the limits of normality, and cells occurring outside the boundaries on either side are regarded as abnormal: by counting those outside the bounds it is possible to estimate the degree of microcytosis or megalocytosis as the case may be.

III

The coefficient of variation of these 100 mean diameters is 2·3 per cent. The figure seems low, but I do not know of any measurements of linear dimension which are exactly comparable, except those of Wischnewsky.*

The variation from one person to another is perhaps in part due to factors to be described later. The red cells are not always the same size in the same person. By my method they are shown to be smallest when the blood is most alkaline at the end of a deep sleep and largest after violent exercise; the extreme mean diameters so expressed

* Wischnewsky (1928; kindly translated for me by Prof. V. Korenschewsky) has measured the red cell diameters by the dry film method in 179 persons of various races—Mongols, Persians, Turks, Finns, and Caucasians—who were collected in Moscow and examined after several months' stay, by which they were presumably acclimatized to their new surroundings. He concludes that racial and climatic differences have no appreciable influence. He measured varying numbers of cells, seldom less than 200. Allowing for some personal factor, from which the method can scarcely be wholly free, his results are in good agreement with mine. His average mean diameter is 7·248μ, or 0·046μ greater than the mean of my 100 cases, which is not significant; only two cases—one at each end—fall outside my calculated normal range. The variability of his series is 3·0 per cent., of the same low order as that of my series. Some of his results are set out in my 1929 paper, *Journ. Path. Bact.* xxxii, p. 482, and are here repeated in Table 9, p. 29.

in one individual may differ by as much (1·0μ) as the extremes found in the series of 100 healthy people. In making the present observations these exaggerated conditions have been carefully avoided. From errors of sampling and method, films made from the same person at the same time may give means differing by as much as 0·158μ, and repeated observations on the same person show that in the ordinary course of the working day differences of 0·2μ–0·3μ may occur. Variations due to these causes presumably on the whole cancel one another and in any case they can account only for a small part of the normal range which has been found.

It seems, therefore, that red cells are inherently larger in some healthy persons than in others, and to further this view I have selected two persons whose mean red cell diameters on the first examination were 6·815μ and 7·492μ respectively, and I have examined a series of films taken from them under approximately similar conditions extending over periods of time. The results are

TABLE 9 (see footnote, p. 28)

Mean diameters of 170 *healthy persons in Moscow* (*Wischnewsky*)

Mean diameter	*Number of persons*	
6·600–6·649	1	
6·650–6·699	0	
6·700–6·749	1	
6·750–6·799	0	
6·800–6·849	2	
6·850–6·899	3	
6·900–6·949	8	
6·950–6·999	10	
7·000–7·049	13	
7·050–7·099	13	
7·100–7·149	8	
7·150–7·199	12	Minimum 6·64μ
7·200–7·249	13	Maximum 7·83μ
7·250–7·299	15	
7·300–7·349	17	
7·350–7·399	17	
7·400–7·449	12	
7·450–7·499	10	
7·500–7·549	8	
7·550–7·599	5	
7·600–7·649	8	
7·650–7·699	1	
7·700–7·749	1	
7·750–7·799	0	
7·800–7·849	1	
	Total 179	

Mean	7·248μ
Standard deviation (σ) . .	0·218μ
Coefficient of variation (v) .	3·0 per cent.

set out in Table 10. In the case of *N* the mean diameters taken at intervals are not significantly different over a twelve months' period; with *V* a similar fairly constant value is maintained for three months. Two other cases, *T* and *B*, show similar constancy after ten years' interval.

TABLE 10

Red cell mean diameters

N

Date	*Mean diameter of 500 red cells*
xii.28	6·815μ
ii.29	6·798μ
12.iv.29	6·885μ
24.x.29	6·955μ
30.x.29	6·859μ
6.xi.29	6·950μ
15.xi.29	6·869μ
15.xi.29 (after exercise)	7·094μ
15.xi.29 (after forced respiration)	6·804μ
27.xi.29	6·749μ
3.xii.29	6·950μ
Average	6·884μ

V

Date	*Mean diameter of 500 red cells*
xii.28	7·492μ
6.iii.29	7·403μ
9.iii.29	7·390μ
11.iii.29	7·230μ
13.iii.29	7·678μ
13.iii.29 (2nd film)	7·586μ
15.iii.29	7·260μ
16.iii.29	7·533μ
18.iii.29	7·233μ
20.iii.29	7·346μ
23.iii.29	7·436μ
27.iii.29	7·482μ
Average	7·422μ

T

Date	*Mean diameter of red cells*
29.iii.20	6·968μ
12.xi.29	6·974μ

B

Date	*Mean diameter of red cells*
11.vi.19	7·443μ
12.xii.29	7·476μ

CHAPTER IV

THE COEFFICIENT OF VARIATION

THE VARIABILITY OF RED CELL DIAMETERS IN ONE POPULATION

The coefficients of variation of the populations, each of 500 cells, from 100 healthy persons are summarized in Table 11, and expressed in Fig. 6. The mean is 6·326 per cent. and by the $\pm 3\sigma$ rule the normal range should be 5·333 to 7·319 per cent., which corresponds fairly well with the observed range, 5·64 per cent. to 7·26 per cent. The distribution compared with its normal curve gives $P = 0{\cdot}40$.

The coefficient of variation may be greater than the extreme limit of the healthy range under three conditions: (1) the range of red cell diameter may be increased, the distribution remaining normal, as in some examples of anaemia following haemorrhage (Fig. 12); (2) the range of size may be normal but the distribution within it too irregular; (3) the range of sizes may be too great and their distribution irregular, as happens in most cases of active pernicious anaemia. A population of red cells which shows an excessive variability may be homogeneous or heterogeneous.

Table 11

Coefficients of variation of 100 *healthy persons*

Coefficient	*Number of persons*	
5·60–5·69	2	
5·70–5·79	4	
5·80–5·89	5	
5·90–5·99	9	
6·00–6·09	10	
6·10–6·19	7	
6·20–6·29	15	Minimum 5·64
6·30–6·39	11	Maximum 7·26
6·40–6·49	5	
6·50–6·59	9	
6·60–6·69	7	
6·70–6·79	7	
6·80–6·89	2	
6·90–6·99	2	
7·00–7·09	2	
7·10–7·19	1	
7·20–7·29	2	
	Total 100	

Mean	6·326
Standard deviation . .	0·331
Coefficient of variation .	5·2 per cent.

The coefficient of variation is important because it is probably the most delicate test for a departure from the normal in red cell diameters, and the great majority of active cases of pernicious anaemia show an abnormally high variability as well as an excessive mean diameter. The two do not necessarily go together. As will be

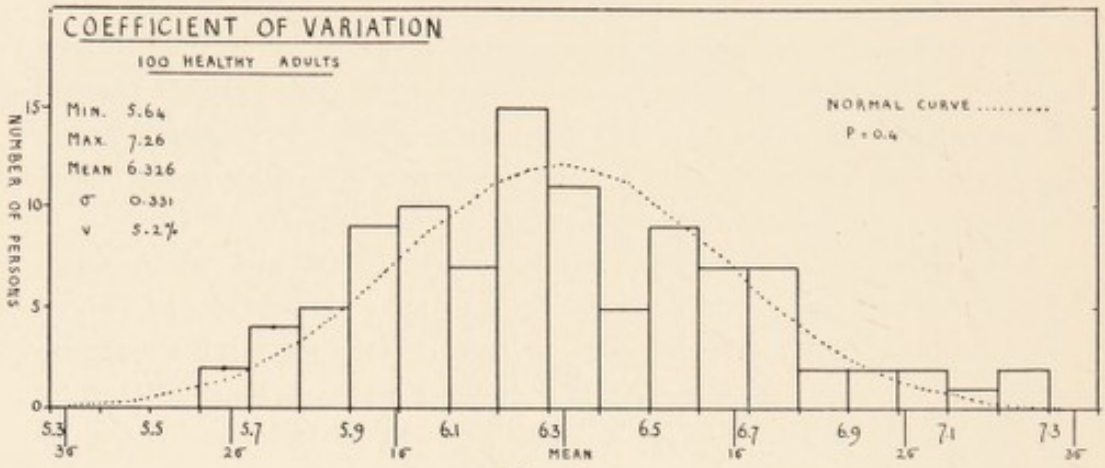

FIG. 6.

shown later (p. 41) in emphysema the mean diameter may be considerably greater than normal with a variability within the healthy range, and (p. 39) if the blood-cells are swollen by violent exercise their variability remains unchanged. Contrariwise, as in anaemia from haemorrhage (p. 43), the red cells may on the whole be smaller than usual and the variability very high. In pernicious anaemia a high variability is almost more constant (though less characteristic) than a high mean diameter.

CHAPTER V

DIURNAL VARIATION

That there is a diurnal variation in the diameters of red cells, namely, a gradual increase during the day and a diminution during sleep, appears from Fig. 7 and Table 12, which show three sets of observations taken during ordinary working days, the first film in the morning being taken immediately on waking.

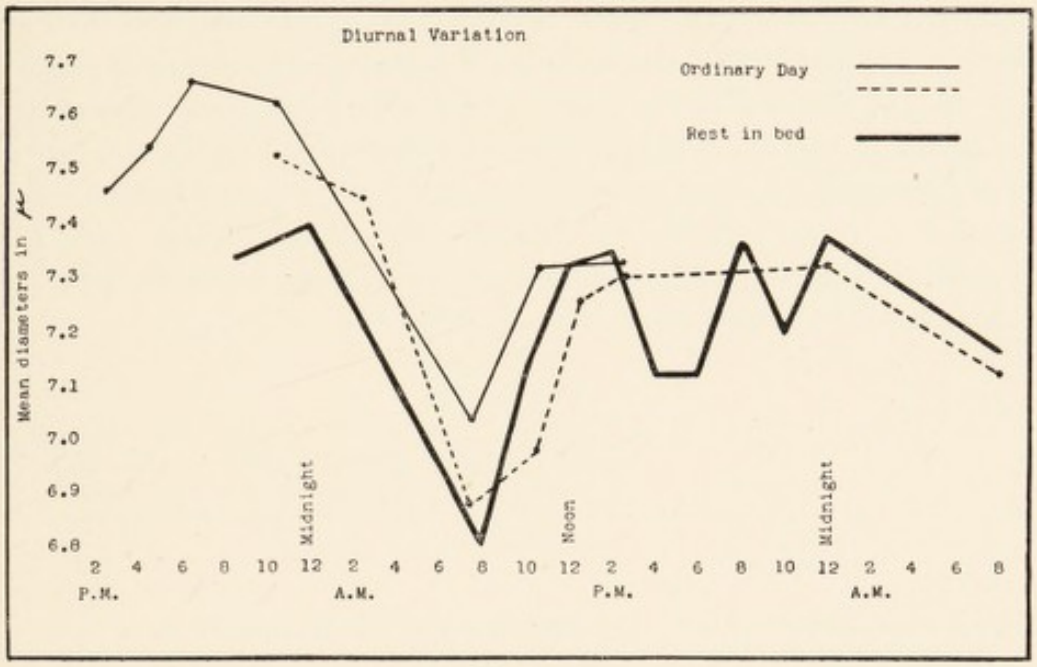

Fig. 7.

Table 12

1		2		3	
Time	*Diameter*	*Time*	*Diameter*	*Time*	*Diameter*
2.30 p.m.	7·461	10.30 p.m.	7·526	11 p.m.	7·267
4.30 ,,	7·546	2.30 a.m.	7·452	6.45 a.m.	7·019
6.30 ,,	7·662	7.30 ,,	6·904	8.20 ,,	7·040
10.30 ,,	7·633	10.30 ,,	6·983	9.20 ,,	7·508
7.30 a.m.	7·048	12.30 p.m.	7·265	12.30 p.m.	7·422
10.30 ,,	7·326	2.30 ,,	7·315	3.30 ,,	7·194
2.30 p.m.	7·333	Midnight	7·332	Midnight	7·349
		8 a.m.	7·135	6.30 a.m.	6·925
				1 p.m.	7·281

These particularly striking variations, amounting in some cases to as much as 0·6μ, obviously suggest that the red cells swell and shrink in association with bodily activity. For example, in series 3 of the preceding table, the quick rise of 0·46μ between 8.20

and 9.20 a.m. was recorded after a short push-bicycle ride with a trailer.

By experiment it has been found that (*a*) violent exercise increases these changes, (*b*) gentle exercise has no apparent influence, and (*c*) rest in bed does not abolish these diurnal variations.

(A) *Violent Exercise increases Diameters.* In these experiments 'violent' exercise meant running as quickly as I could up and down six flights of steps three times both ways, until I felt unable to run any more and was distressed in breathing. Films were made immediately before and after the exercise. From the observations recorded in Table 13 it appears that it is possible by means of violent exercise to produce in the course of a few minutes quite remarkable increases in the diameters of the red cells; in one case the increase was followed by a much greater diminution, the increase indeed being only of doubtful significance, but the time of day (3 p.m.) and the possibility that the diameters were relatively large before the exercise may account for this small increase (Fig. 8).

TABLE 13

No.	*Time*	*Diameter before Exercise*	*Diameter after Exercise*	*Change in Diameter*
1	11.0 a.m.	6·868	..	..
	11.10 „	..	7·330	0·46
2	11.10 „	6·884	..	..
	11.30 „	..	7·197	0·313
	12.10 p.m.	..	6·736	0·46
3	3.0 „	7·173	..	..
	3.20 „	..	7·330	0·16
	4.0 „	..	6·786	0·6

The violent exercise in this experiment (3) was with two 12 lb. dumb-bells. The results of these three experiments are derived from the measurement of 200 cells.

(B) *Gentle exercise has no special influence.* I walked from Gower Street to Jack Straw's Castle, Hampstead, about 4 miles, and back, in $2\frac{1}{2}$ hours; it was a hot day. Films were made immediately before and after the walk, and the mean diameters were 7·22μ and 7·27μ respectively. On another occasion I walked along the Portsmouth Road at Hindhead, a measured 8 miles, in about 2 hours. It was a fresh morning and I did not get hot. The diameters before and after were respectively 6·93μ and 7·26μ, an increase of 0·28μ. This

rise, however, is no greater than that recorded in series 1 of Table 13 between 7.30 and 10.30 a.m. on an ordinary working day, or so great as the rise of 0·32μ on July 10, 1919, between 8 and 10 a.m., while I was resting in bed (see below). Other observations on another subject with moderate exercise that caused no sense of fatigue gave similar results.

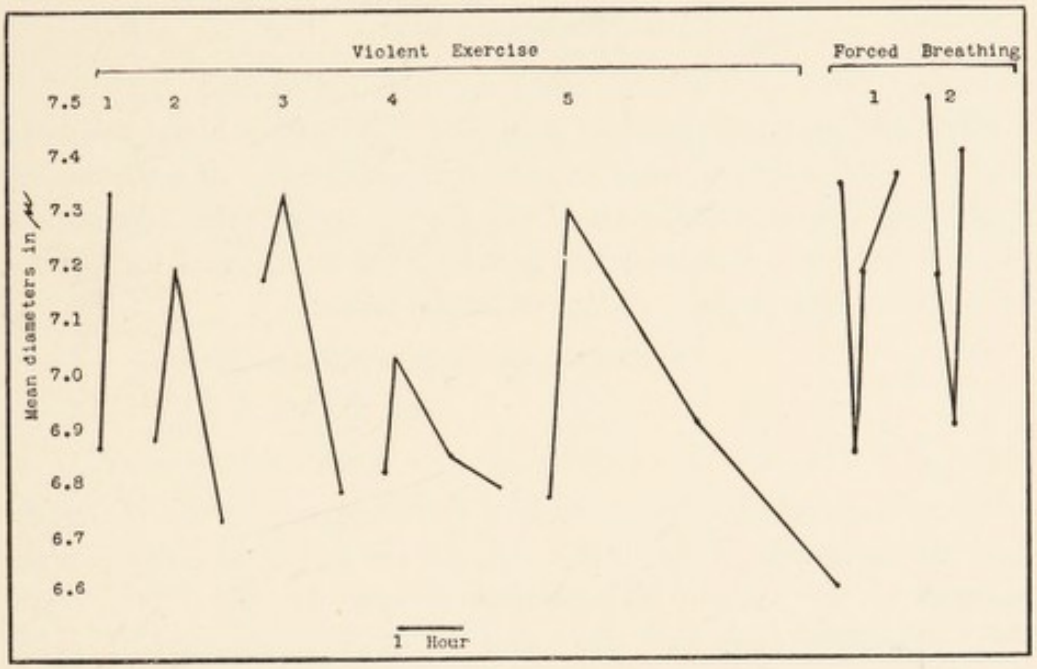

Fig. 8.

In one experiment to demonstrate the effect of violent exercise on the diameters, both dry and moist specimens were measured; the diameters in both showed an increase of over 0·55μ, followed by a diminution of about the same amount.

Table 14

Dry	*Moist*	
6·81 (100)	7·744 (100)	Before exercise
7·40 (108)	8·300 (107)	Immediately after
6·86 (100)	7·731 (100)	Two hours later

(C) *Rest in bed does not abolish the diurnal variations.* On two separate occasions I remained lying down in bed for twenty-four hours; I took my usual meals, and employed my time in reading. The results which are recorded in Table 15 show, especially on one occasion, remarkable variations which are in similar direction and in no less degree than the variations recorded from an ordinary working day.

TABLE 15

1				2	
Time	*Diameter*	*Time*	*Diameter*	*Time*	*Diameter*
8.30 p.m.	7·348	4 p.m.	7·128	Midnight	7·349
Midnight	7·403	6 „	7·126	8 a.m.	7·061
8 a.m.	6·811	8 „	7·374	2 p.m.	7·429
10 „	7·136	10 „	7·208	11·30 p.m.	7·434
12 Noon	7·335	Midnight	7·384		
2 p.m.	7·350	8 a.m.	7·174		

(D) *Local cyanosis increases diameters.* In taking blood from the finger it is of practical value to note that application of a tourniquet producing venous congestion of the part increases the diameter of the red cells and should either not be used or allowed for. Three examples of this increase are given in the table:

	Without tourniquet	*With tourniquet*	*Increase*
1 . . .	6·749μ	7·157μ	0·408μ
2 . . .	7·476μ	7·680μ	0·204μ
3 . . .	7·168μ	7·359μ	0·191μ

All the facts obtained from the observations recorded above suggest that the variations in size of the red cells are due to differences in the reaction of the blood. Experiments *in vitro* in which CO_2, lactic acid, or sodium carbonate was added to defibrinated rabbits' blood showed that the red cells swell with an increase of acidity and shrink when the blood is made abnormally alkaline. The reactions of the blood were determined by the method described by Boycott and Chisolm (1910), in which whole blood is used and CO_2 counts as an acid; the results are given as c.cm. N/10 acid per 100 c.cm. blood required to produce flocculation of the nucleo-protein in the red cells. The results of these *in vitro* experiments are given in Table 16. The relations of the changes in the reaction of the blood and the corpuscular volume are well shown, but the changes produced in the diameters as measured from the dried films are not so marked, especially in those specimens to which saline, lactic acid, or sodium carbonate were added. The addition of these solutions appears to affect the preparation of the films, which showed many crenated and distorted cells, so that an error of selection was unavoidably added to the error of sampling. This notwithstanding, it is seen that the changes produced in the diameters moved in the same direction as the changes in volume. Similar experiments were made by Gürber (1895), von Limbeck (1895), and by Hamburger (1897) by which they showed that the addition of CO_2 to blood increased the volume of the red cells; Hamburger and also von Limbeck found that the

TABLE 16

No.	*Tube*	*Procedure*	*Reaction*	*Volume*	*Diameter*
10	1	Addition of 0·9 per cent. saline .	27	100	6·260
	2	,, lactic acid . .	13	112	6·445
	3	,, sodium carbonate .	39	93	6·314
14	1	Addition of 0·9 per cent. saline .	26	100	6·123
	2	,, lactic acid . .	12	111	6·325
	3	,, sodium carbonate .	42	92	6·077
15	1	Addition of 0·9 per cent. saline .	22	100	6·543
	2	,, lactic acid . .	6	112	6·657
	3	,, sodium carbonate .	48	87	6·364
11	1	Blood alone	38	100	6·169
	2	Addition of expired air . .	30	103	6·436
	3	,, CO_2 . . .	18	115	6·712
13	1	Blood alone	12	100	6·389
	2	Addition of expired air . .	7	102	6·535
	3	,, CO_2 . . .	2	114	6·796

red cells of venous blood are bigger than the red cells of arterial blood. Von Limbeck points out that the swollen blood-cells in the lung give up CO_2 and become smaller, and he regards this as a morphological expression of chemical and physiological processes analogous to the volume changes in gland-cells before and after secretion. The swelling of red cells in venous blood may perhaps be considered as an ingenious arrangement for slowing the passage of the blood through the lungs until the cells have got rid of CO_2.

Several simultaneous determinations of blood reaction and red-cell diameters have also been made on myself before and after short violent exercise. The results are given in Table 17.

TABLE 17

No.	*Time*	*Reaction of Blood*	*Diameter before Exercise*	*Diameter after Exercise*
4	2.10 p.m.	43	6·823	. .
	2.26 ,,	35	. .	7·038
	3.0 ,,	40	. .	6·830
	3.45 ,,	40	. .	6·791
5	12.30 ,,	46	6·770	. .
	12.50 ,,	37	. .	7·301
	2.45 ,,	40	. .	6·912
	5.5 ,,	42	. .	6·603

In each case an increase in diameter has been associated with a diminution in alkalinity. The observations of Ryffel (1910) have shown that there is a considerable increase in the lactic acid in the

blood at the end of short violent exercise (running to exhaustion in the laboratory) but not with moderate exercise of longer duration (walking in competition), which corresponds with my findings in respect to the variations in the red cell diameters. The increase of alveolar CO_2 which is caused by violent exercise suggests that under these conditions there is also an accumulation of CO_2 in the blood, which is got rid of during the hyperpnoea which lasts for a short time after the end of the exercise, and Campbell, Douglas, and Hobson (1920) have also concluded that during severe muscular work CO_2 is dammed back in the body. If this is the cause or a cause of the swelling of the red corpuscles, a diminution of the CO_2 in the blood by forced breathing should lead to a diminution in diameters. Such I have found to be the case in two experiments (Table 18) (Figs. 8 and 9).

TABLE 18

No.	*Time*	*Blood Reaction*	*Diameter*
1	1.47 p.m.	28	7·347
	1.51 to 1.57½ p.m. forced breathing		
	1.58 p.m.	34	6·852
	2.5 ,,	29	7·185
	2.37 ,,	26	7·365
2	1.40 ,,	23	7·508
	1.41½ to 1.47 p.m. forced breathing		
	1.47½ p.m.	30	7·175
	2.3 ,,	30	6·908
	2.10½ ,,	22	7·408

These results show that after about six minutes forced breathing the mean diameter of the red cells has diminished by about 0·5μ; subsequently, as the CO_2 reaccumulates and the alkalinity of the blood returns to normal, there is a rapid restoration of the normal diameter.

It will be gathered from all these experiments and observations that the diurnal variation in the diameter of red blood cells is presumably due to altered reaction of the blood, though of the details of this alteration there is little direct evidence. It is, however, known that at the dreary moment of first awakening the blood is a good deal more alkaline than later on during the day (Boycott and Chisolm, 1910, p. 29); and Leathes (1919) shows that while the kidney produces an acid urine during the night, in the morning the reaction swings strongly over to the alkaline side, the plan presumably being to have the blood more alkaline during the night and less alkaline during the day.

These diurnal variations of red cell diameter, and those observed

with violent exercise and after forced breathing, oscillated within the normal diameter limits. The distribution curve retained more or less its original form and was shifted *en masse* to the right or to the

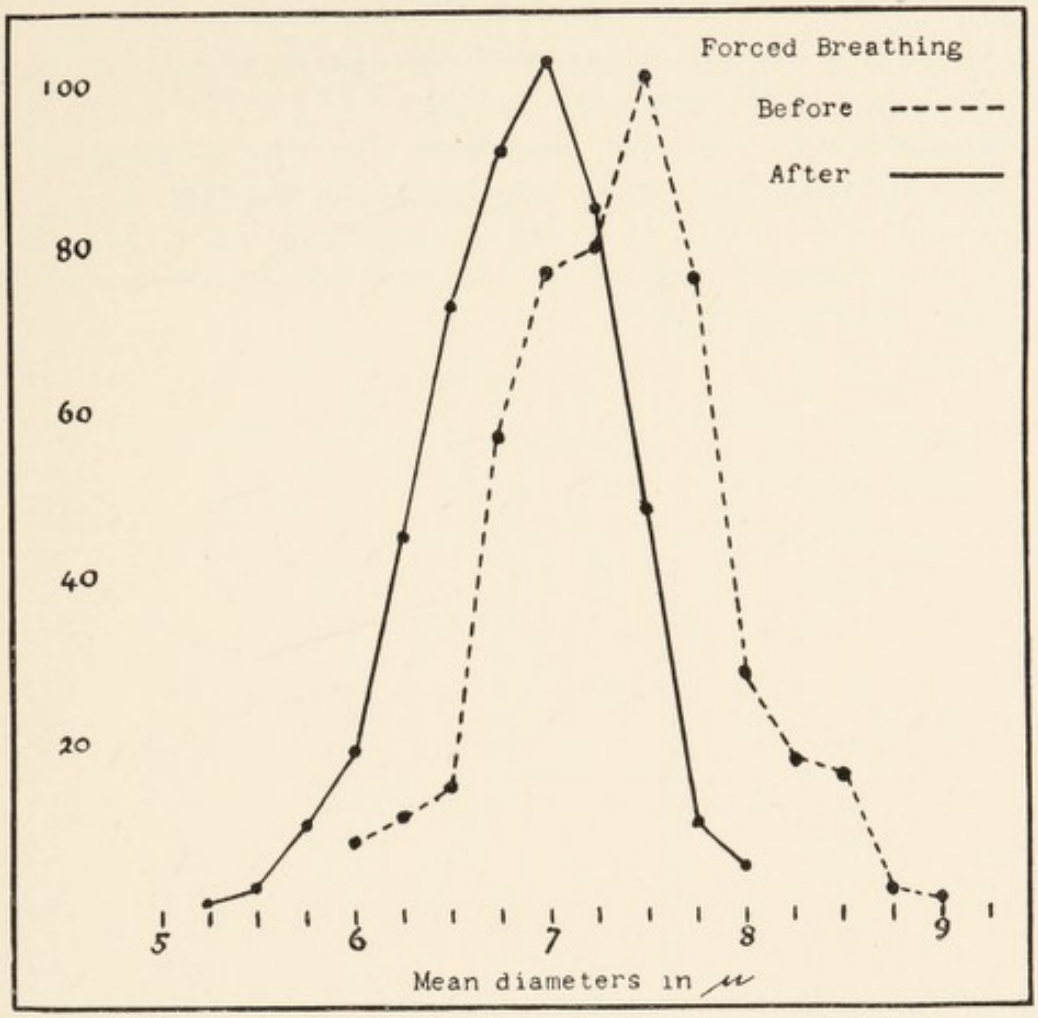

FIG. 9.

left as the case might be, supporting the notion that the effects were produced by some factor acting simultaneously on all the circulating cells, and probably not influencing the haemopoietic organs. The slight changes of the coefficient of variation which were not always parallel with those of the mean diameter may perhaps be explained by some difference in the degree or rate of response by different cells in the samples.

CHAPTER VI

EMPHYSEMA

Whilst collecting samples for the measurement of red cells from healthy persons my attention was directed to five men whose cells were significantly larger than the others. On further investigation it was found that these men were suffering from emphysema. I then examined the red cells of 22 cases of emphysema and found that the mean diameters ranged from 7·33μ to 8·17μ, the mean diameter of the series being 7·69μ.

Table 19

Emphysema				
No.	*Age*	*Mean Diameter*	σ	*v per cent.*
o. 1	35	8·17	0·49	5·9
b. 2	43	7·60	0·51	6·7
o. 3	48	7·59	0·51	6·7
o. 4	50	7·80	0·55	7·0
o. 5	53	7·23	0·61	8·4
o. 6	53	7·63	0·48	6·5
b. 7	54	7·41	0·47	6·3
o. 8	56	7·78	0·53	6·5
o. 9	60	8·05	0·61	7·7
o. 10	62	7·63	0·54	7·0
o. 11	63	7·70	0·56	7·5
o. 12	63	7·91	0·65	8·2
o. f. 13	64	7·87	0·56	7·1
b. 14	64	7·93	0·55	6·9
o. 15	66	7·90	0·57	7·8
b. f. 16	71	7·33	0·52	7·0
b. f. 17	73	7·80	0·55	7·4
b. 18	75	7·68	0·55	7·1
b. 19	76	7·61	0·51	6·7
b. f. 20	78	7·45	0·52	6·7
b. f. 21	80	7·36	0·52	7·0
b. f. 22	100	7·73	0·56	7·2
Means	..	7·69	0·54	7·06

f. = Female. *o.* = Up and about. *b.* = Confined to bed.

The results are set out in Table 19, and a curve of one case is given in Fig. 10, which shows the characteristic shift to the right of a healthy curve without any extension of the diameter range or increase of the coefficient of variation.

Diurnal variation in diameter also occurs in these patients; their large red cells are quite as mobile as healthy red cells. A man aged 35, suffering from severe emphysema, when first examined

had red cells with mean diameter 8·17μ. After admission the diameters varied from 8·53μ (p.m.) to 7·34μ (a.m.), a range of 1·19μ. On four successive days the measurements at

5 a.m. were	7·87	and at	9 p.m.	8·19
,,	7·87	,,	,,	8·53
,,	7·94	,,	,,	8·23
,,	7·92	,,	,,	8·40

The mean coefficient of variability of the red cell diameters of these

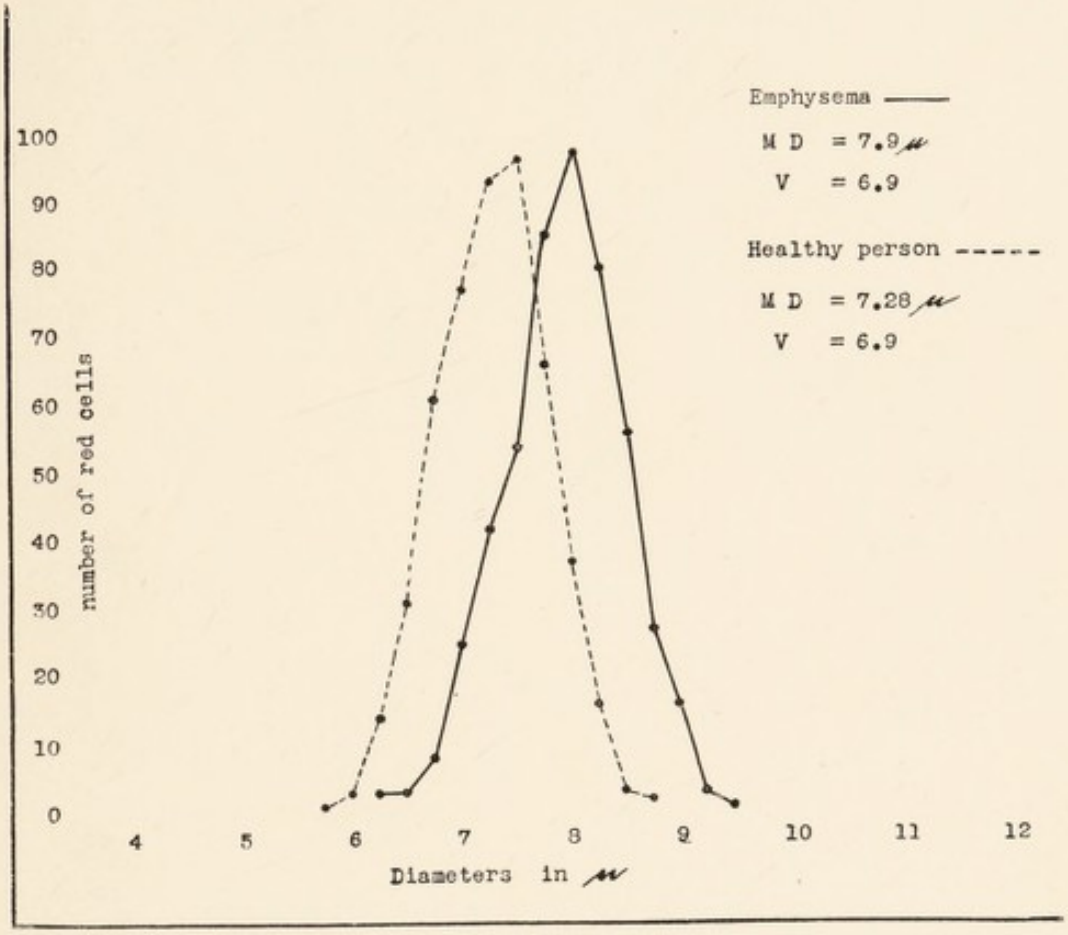

FIG. 10.

emphysema cases was 7·06 per cent., which is not significantly different from the healthy mean variability coefficient.

The distribution curve, like that of diurnal variation, has been moved *en masse*, retaining its original shape but shifted to the right of the mean healthy curve; all the circulating red cells have been acted on by some common agent; there is no evidence of any influence on the haemopoietic organs. The curves are symmetrical, and, like the healthy curves, show great similarity among themselves; they all fit fairly well with their respective 'normal curves'.

It seems probable that the increased size of the red cell diameters in emphysema is associated with an increased quantity of CO_2 in the blood, in spite of normal reaction (Scott, 1920). It follows that CO_2 must have a special swelling action on the corpuscles out of proportion to its acidity, analogous to its action on the respiratory centre, described by Scott (1918) as that of a 'specific respiratory hormone'.

CHAPTER VII

THE RED CELL DIAMETERS AFTER HAEMORRHAGE

I HAVE examined only 10 cases of haemorrhage. They were all suffering from varying degrees of anaemia, at various periods after the haemorrhage from different sources—gastric and uterine—and of presumably varying amounts. Only one examination was made in each case. This collection of people is too small and too varied to give very useful results from statistical treatment. I have set out the data of the examinations in Table 20.

TABLE 20

Anaemia after Haemorrhage

	Number										
	1	2	3	4	5	6	7	8	9	10	
Haemoglobin	20	20	26	30	38	40	40	42	44	60	..
Red cells, mill.	1·80	2·50	3·45	2·80	3·60	3·30	2·64	2·13	3·97	3·07	..
Colour index	0·55	0·40	0·37	0·53	0·53	0·60	0·75	0·98	0·56	0·99	..
Mean diameter	6·319	7·225	6·900	6·611	7·179	6·891	7·215	6·943	6·702	6·813	6·850
Standard deviation	0·79	0·69	0·70	0·57	0·92	0·76	0·61	0·51	0·66	0·65	0·75
Variability	12·5	9·5	10·1	8·6	12·8	11·0	8·4	7·4	9·8	9·5	10·9
Microcytosis per cent.	27·8	0·6	2·2	8·6	0·6	13·0	0·2	1·0	6·6	2·4	..
											Total
3·50μ	2	..	..	..	..	..	..	..	..	..	2
3·75	1	..	..	..	..	1	..	..	..	..	2
4·00	..	..	..	1	..	1	..	..	..	1	3
4·25	4	.	..	..	..	2	..	..	1	2	9
4·50	2	..	..	..	..	2	..	3	..	..	7
4·75	9	1	1	..	..	6	1	1	2	3	24
5·00	13	1	..	1	1	2	..	1	2	2	23
5·25	20	1	3	3	3	9	1	..	5	4	49
5·50	33	1	10	12	5	12	2	1	15	6	97
5·75	49	7	19	29	5	38	6	1	21	14	189
6·00	68	19	29	58	25	53	9	15	48	26	350
6·25	61	25	47	61	44	51	17	30	58	41	435
6·50	71	40	65	97	54	78	32	58	83	83	661
6·75	58	52	78	78	61	77	60	102	79	86	731
7·00	37	64	71	69	77	72	85	104	55	87	721
7·25	24	75	58	45	61	42	92	90	50	64	601
7·50	23	85	47	26	47	29	82	54	35	34	462
7·75	10	46	32	13	33	8	50	26	26	25	269
8·00	12	40	21	5	22	7	36	10	13	11	177
8·25	1	26	14	1	16	3	18	3	3	3	88
8·50	1	6	1	1	4	3	5	..	2	5	28
8·75	..	5	..	..	12	2	3	1	2	3	28
9·00	1	3	..	..	7	1	..	..	..	..	12
9·25	..	2	..	..	8	..	1	..	..	..	11
9·50	..	1	2	..	3	1	..	..	..	..	7
9·75	..	..	1	..	4	..	..	..	..	..	5
10·00	..	..	1	..	3	..	..	..	..	..	4
10·25	..	..	..	..	1	..	..	..	..	..	1
10·50	..	..	..	..	..	..	..	..	..	..	0
10·75	..	..	..	..	2	..	..	..	..	..	2
11·00	..	..	..	..	2	..	..	..	..	..	2
Total . .	500	500	500	500	500	500	500	500	500	500	5,000

Averages: diameter, 6·879; standard deviation, 0·686; coefficient of variation, 9·98.

From this it appears that the mean diameter of the red cells after haemorrhage is lower than the mean of healthy persons, averaging 6·879μ and ranging from 6·3μ to 7·225μ. The distribution curve is shifted to the left of the healthy curve, e.g. case 1 (Fig. 11). The curves are usually of a less symmetrical type than the healthy curves, they show less similarity among themselves, and do not fit so well with their respective normal curves. Fig. 12 shows the

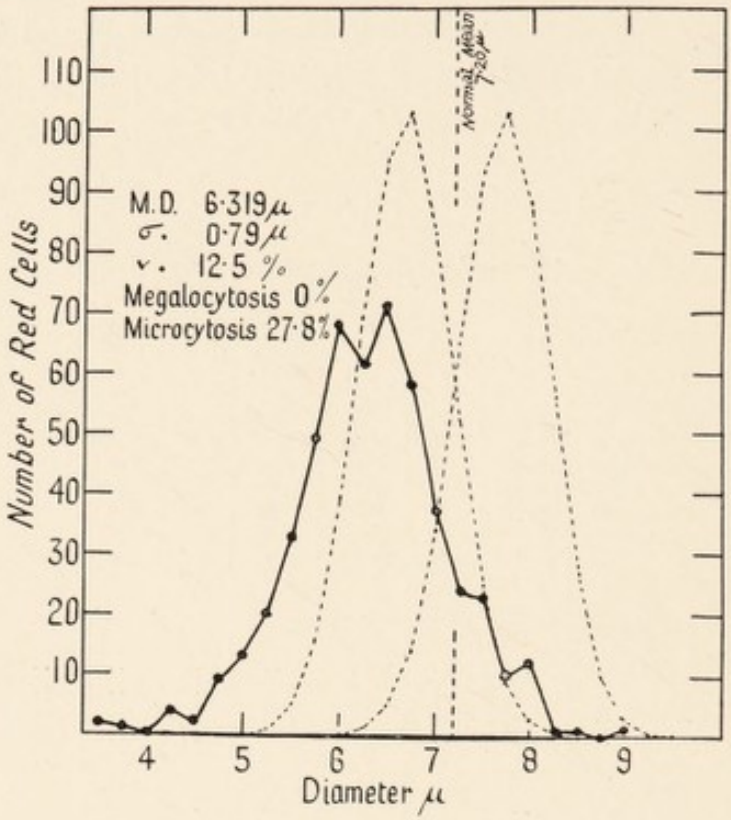

Fig. 11.

distribution of case 3 superposed on its normal curve and giving a very good fit; $P = 0{\cdot}95$, or in other words there would be a worse fit 95 times out of a 100. This contrasts with the distribution of case 6 (Fig. 13), where the fit is very bad, in fact there could not be a worse fit in 1,000,000 times. The coefficient of variation is raised in all these cases, averaging about 10 per cent., and ranging from 7·4 to 12·5 per cent. This increase in the variability might be expected if we assume, as seems legitimate, that there may be two classes of cells in the blood after haemorrhage, the sample being heterogeneous, each component being distributed according to its respective normal curve; (*a*) small cells newly formed by the sudden over-stimulation of the bone marrow following the anaemia caused by the loss of corpuscles, and (*b*) normal cells still remaining in the

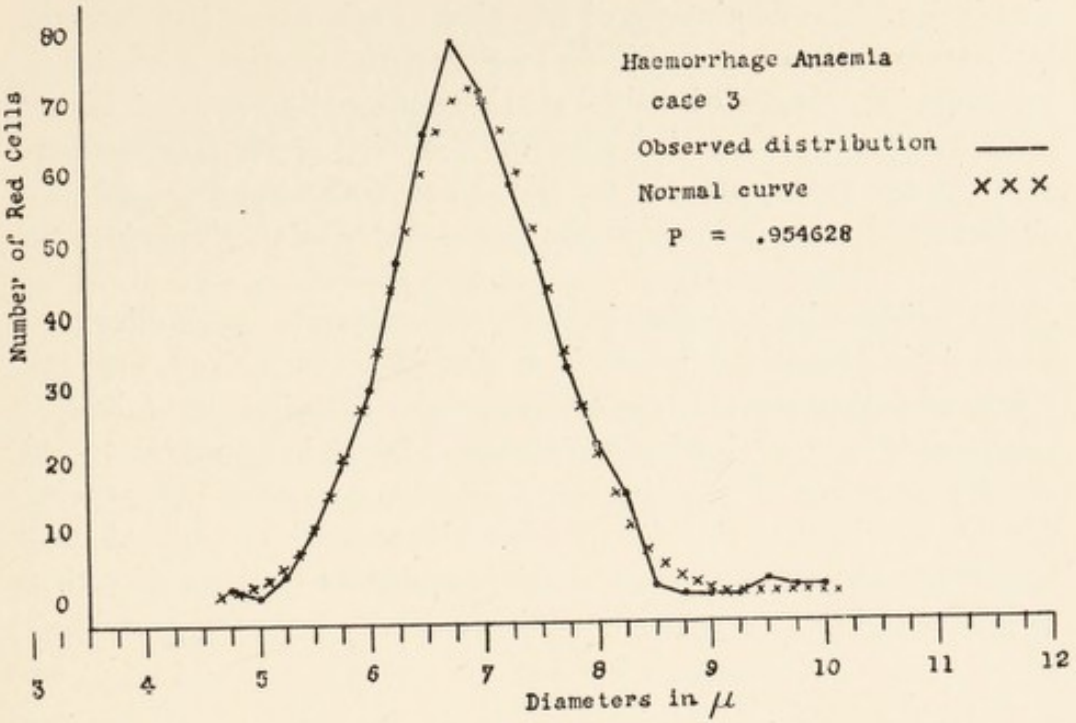
Haemorrhage Anaemia
case 3
Observed distribution
Normal curve
P = .954628
Number of Red Cells
Diameters in μ

FIG. 12.

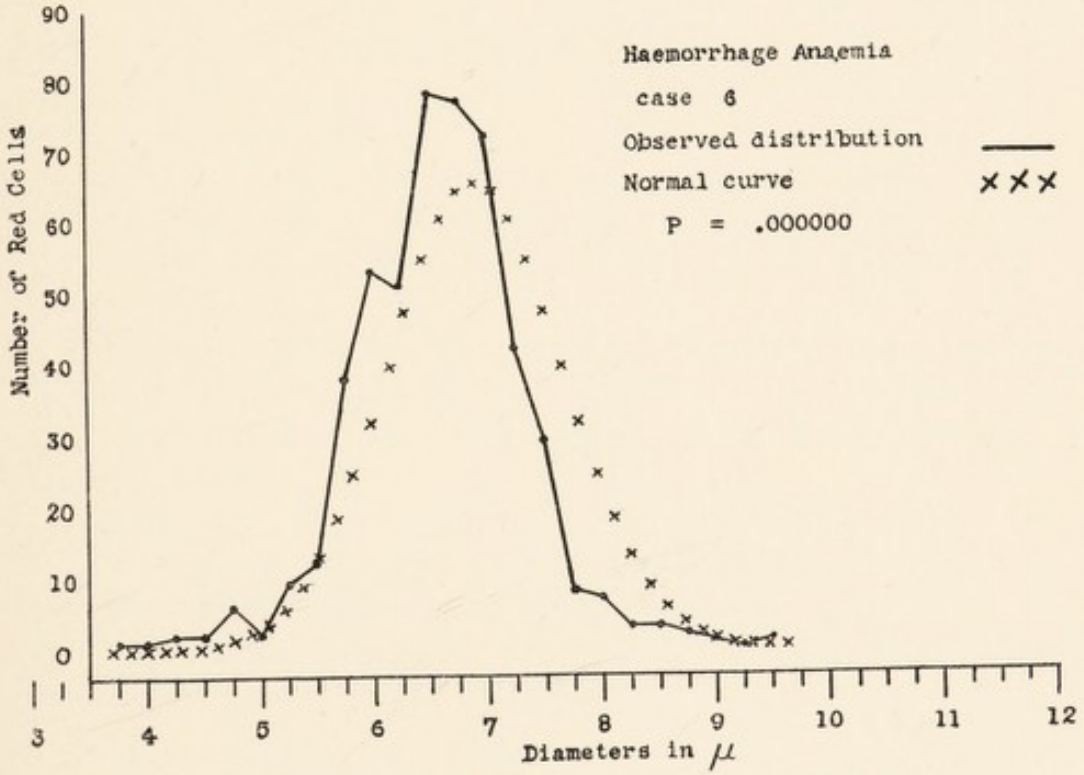
Haemorrhage Anaemia
case 6
Observed distribution
Normal curve
P = .000000
Number of Red Cells
Diameters in μ

FIG. 13.

circulation. The dominance of one kind of cell would be expressed by a more or less symmetrical curve, and the relative share taken by these two classes of cells would depend on the amount of the haemorrhage, on the period after the cessation of the haemorrhage at which the examination of the blood was made, and also probably on the specific rate at which the individual was able to return to health.

It is noteworthy that even in those cases where the mean diameter was not significantly different from that of healthy blood-cells, the degree of anisocytosis (v) was always greater than in health. In this condition the influencing factor, the loss of blood, is acting not on the circulating cells and affecting them all in common, as occurs with the changes in diameter found after violent exercise, after forced breathing, and in emphysema, but operates as a direct disturber and exciter of the haemopoietic system.

CHAPTER VIII

PERNICIOUS ANAEMIA

The diameters of the red cells in this condition vary from 3·75μ to 13·0μ; taking samples of 500 cells the average mean diameter of 68 observations was 8·31μ* or over 1μ greater than the average mean diameter of healthy red cells, or about 0·6μ greater than the normal $+3\sigma$ limit (7·718μ).

The means of the 68 observations ranged from 7·487μ to 9·673μ; the mean diameter of 50 consecutive cases, which had the same range, was 8·263μ, and the coefficient of variation of these means was 4·9 per cent.

The curves of distribution of diameters in all these cases lie to the right of the healthy mean and usually to the right of the healthy curve, and, depending on the degree of megalocytosis, are more or less extended outside to the right of the $+3\sigma$ limit of normality.

In Fig. 15 is an example of a distribution curve from a case with mean diameter 8·060μ, and $v = 16{\cdot}3$ per cent., showing its relation to the area of normality. In this case the excess of big cells was 202 so that the degree of megalocytosis is 40·4 per cent. In this case also there was an extension of the curve to the left of the area of normality, the number of extra small cells was 24, i.e. a microcytosis of 4·8 per cent. Megalocytosis occurred in all the samples of pernicious anaemia blood, varying in degree from 5 per cent. to over 66 per cent.; microcytosis was also present in about 75 per cent. of the cases.

Fig. 15 also shows the relation of the distribution of diameters in this case to its calculated ideal curve to which it has no fit ($P = 0{\cdot}000000$), and suggests the heterogeneous nature of its population of red cells.

Similar curve relations are shown in Fig. 16, where there is a megalocytosis of 22·8 per cent. and a microcytosis of 1 per cent.; the correspondence with the ideal curve is not so bad as in Fig. 15, yet it is not at all good: $P = 0{\cdot}095947$ or a worse fit would be expected in 96 out of 1,000 samples.

* Jorgensen and Warburg (1928), in their interesting and valuable monograph, point out that S. T. Sorensen as long ago as 1876 maintained that megalocytosis was characteristic of pernicious anaemia. Direct micrometric measurements were used by him and others, but the method was soon neglected and superseded by the easier estimation of the colour index and less commonly the volume index (e.g. Capps, 1903; Campbell, 1922; Haden, 1924).

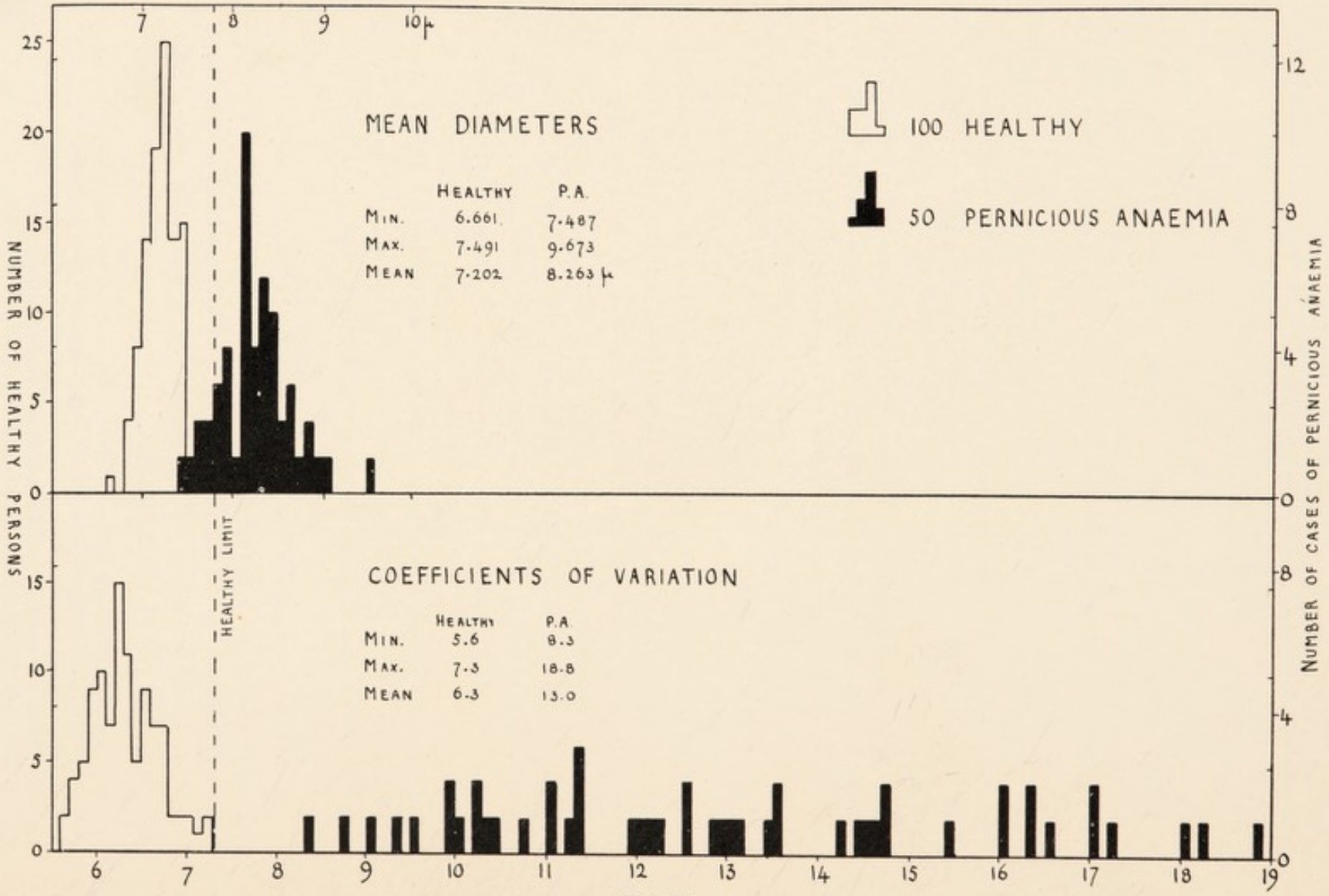
7
8
9
10μ
MEAN DIAMETERS
HEALTHY
P.A.
MIN. 6.661 7.487
MAX. 7.491 9.673
MEAN 7.202 8.263 μ
100 HEALTHY
50 PERNICIOUS ANAEMIA
NUMBER OF HEALTHY PERSONS
NUMBER OF CASES OF PERNICIOUS ANAEMIA
HEALTHY LIMIT
COEFFICIENTS OF VARIATION
HEALTHY
P.A.
MIN. 5.6 8.3
MAX. 7.3 18.8
MEAN 6.3 13.0

FIG. 14.

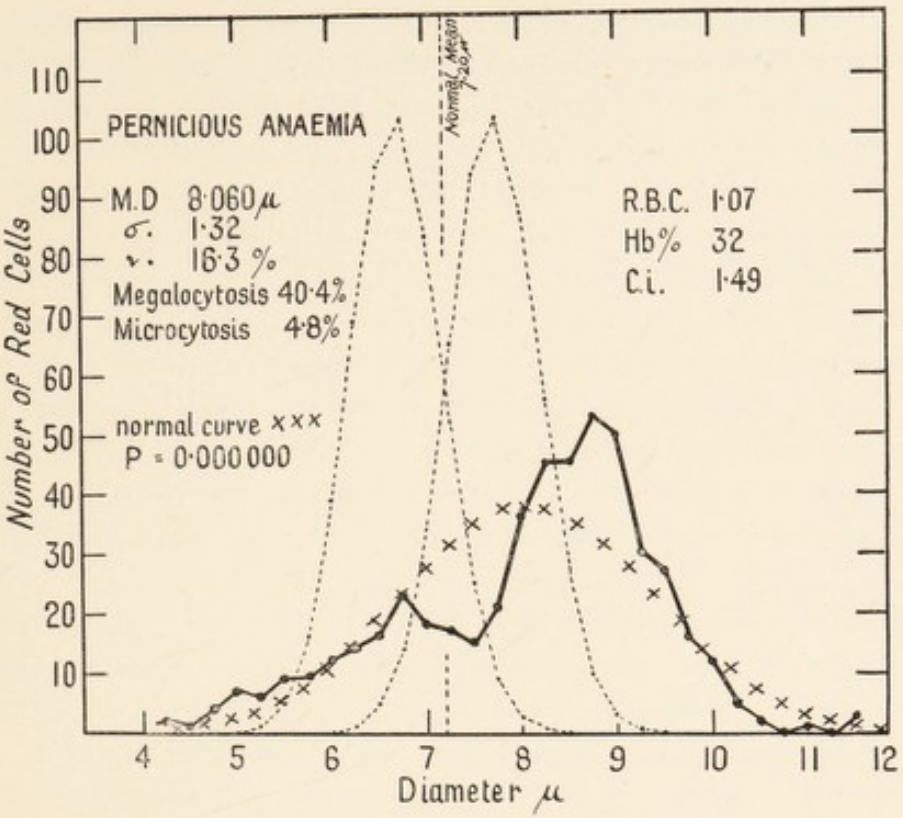
PERNICIOUS ANAEMIA
M.D 8·060μ
σ. 1·32
v. 16·3 %
Megalocytosis 40·4%
Microcytosis 4·8%
normal curve xxx
P = 0·000 000
R.B.C. 1·07
Hb% 32
C.i. 1·49
Normal Mean 7·20μ
Number of Red Cells
Diameter μ

FIG. 15.

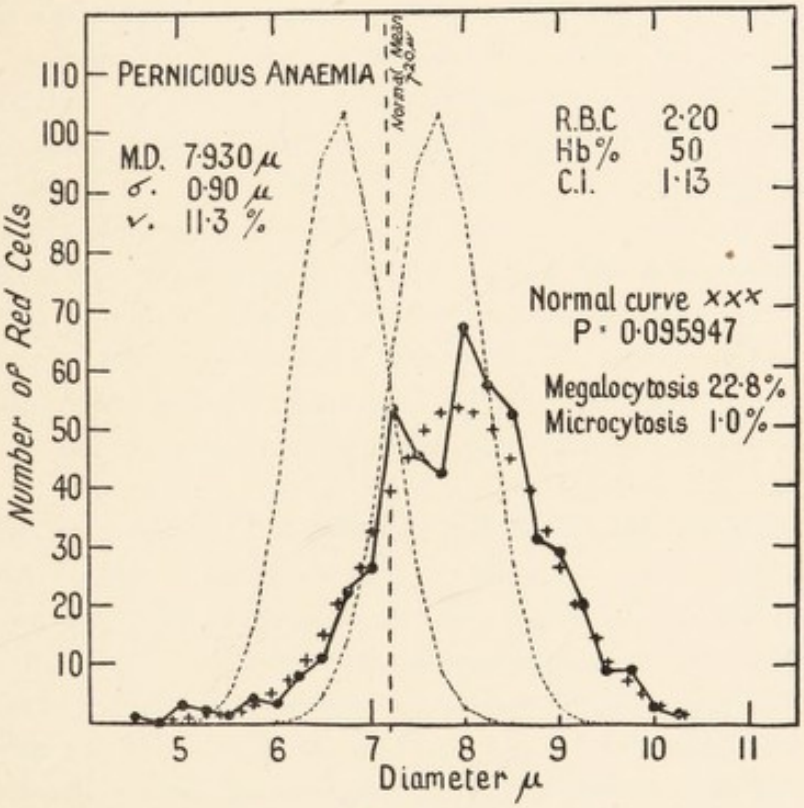
PERNICIOUS ANAEMIA
M.D. 7·930μ
σ. 0·90 μ
v. 11·3 %
R.B.C 2·20
Hb% 50
C.I. 1·13
Normal curve xxx
P · 0·095947
Megalocytosis 22·8%
Microcytosis 1·0%
Normal Mean 7·20μ
Number of Red Cells
Diameter μ

FIG. 16.

This want of correspondence of the observed curves with their respective ideal curves is characteristic of the majority of cases of pernicious anaemia. Among 20 cases that were examined by the 'χ^2' method only 2 gave $P = 0{\cdot}5$ or over; the best fit was $P = 0{\cdot}597$, and 9 cases gave $P = 0{\cdot}000000$, that is to say that generally the correspondence was very bad.

The symmetrical curves from healthy persons are generally in good agreement with their respective ideal curves; the curves of haemorrhage anaemia have only a fair though variable agreement; the curves of pernicious anaemia, which are mostly asymmetrical, often grotesque, in shape, agree very badly with their ideal curves and strongly suggest that they are composite curves such as might arise from two or more sets of cells in a heterogeneous population of red cells.

From this suggestion I have been led to regard the composite appearance of these curves as compatible with the assumption that the blood in these cases contains three classes of red cells; (1) abnormally large cells derived from megaloblasts which fail to mature owing to the absence of the 'intrinsic' factor; (2) normal sized cells derived from normally acting marrow; for I conceive it is probable, at least at the earlier stages of the disease, that the deficiency of the liver ('intrinsic') substance is not extremely developed and that there may yet be sufficient to activate a part of the marrow to function in a healthy way; (3) small cells resulting from the extra stimulation of the bone marrow to combat the anaemia caused by the abnormal destruction of red cells.

Most of my cases were examined only on one occasion; by repeated examinations it would be possible to observe the changes that take place in the distribution curves of the cell diameters during the course of the disease, and by the consideration of the component curves some conception might be formed of the relative activities of the three groups of cells at any particular period or clinical condition.

A prominent feature of the blood-cells in pernicious anaemia is the high degree of variability or anisocytosis; this is even more constant, though perhaps less characteristic, than the high mean diameter which is also almost always present. In Fig. 14 I have set out the values of 50 consecutive cases at their first examination (before treatment) and compared them with those of the 100 healthy people. It shows that the lowest variability among the pernicious anaemia cases is well to the right of the healthy range and differs from the healthy mean by 6σ (Table 11) ($6 \times 0{\cdot}331 = 1{\cdot}9$). But five

of the mean diameters are within the healthy range and might pass for normal were it not for their high variabilities and the presence of too many large cells (Table 21).

TABLE 21

Mean diameter μ	*Difference from healthy mean in terms of* σ	*Variability*	*Difference from healthy mean in terms of* σ
7·487	1·7	11·3 per cent.	15
7·577	2·2	12·5 ,,	18
7·650	2·6	18·2 ,,	36
7·670	2·7	9·9 ,,	11
7·717	3·0	14·2 ,,	24

If Fig. 14 was drawn on a scale of standard deviations, the spread of the variabilities would be reduced to about one-half (0·172/0·331), but on any method of comparison they show a larger proportionate variation than the mean diameters.

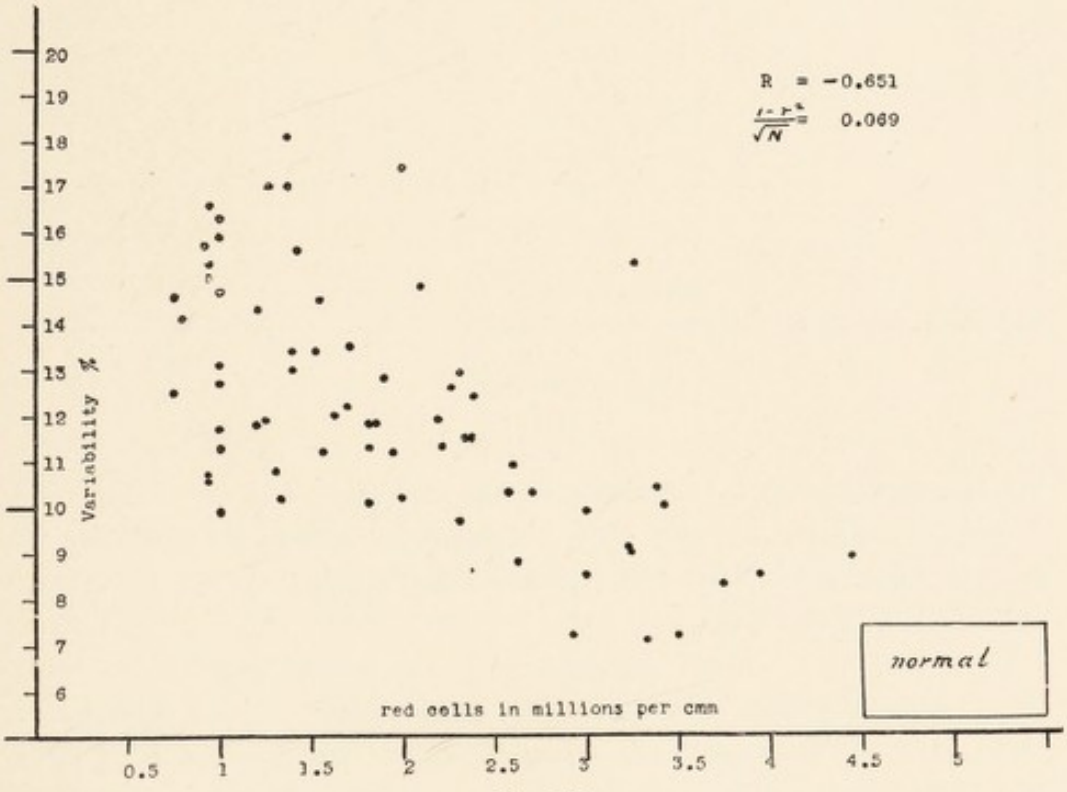

FIG. 17.

The coefficients of variation of these 50 samples varied from 8·3 to 18·8 per cent., giving a mean of 13 per cent., or just twice that of the healthy cells.

In Fig. 17 I have plotted out 68 red cell counts according to their respective coefficients of variation. In the right-hand corner of the chart are shown the corresponding relations and limits for healthy

red cells. It is at once seen that there is probably some correlation between the number of red cells per c.mm. and the variability of the red cell diameter. On calculating the coefficient of correlation (Yule, pp. 183–6) I found $R = -0{\cdot}651$, and the standard error (Karl Pearson) of this value is 0·069, so that R is over nine times

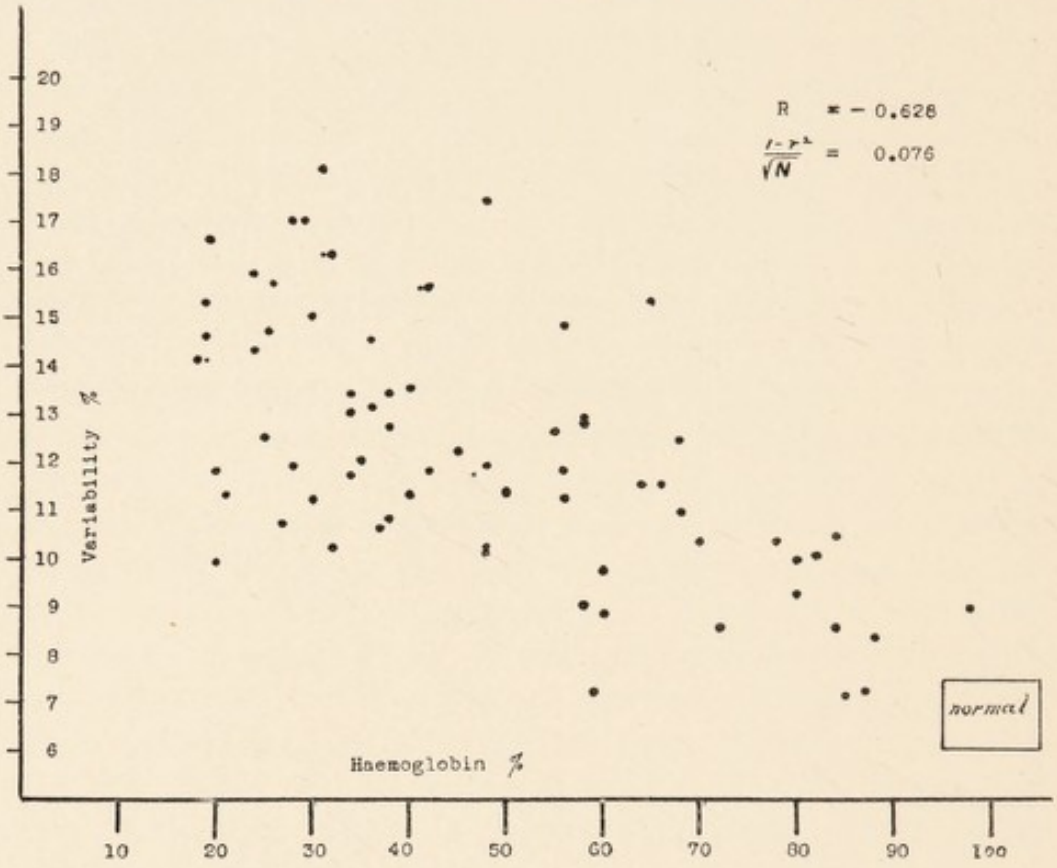

Fig. 18.

the standard error, or in other words the coefficient of correlation −0·651 is significant and may be regarded as a good correlation. It expresses the fact that the greater the variability the fewer are the number of red cells.

In Fig. 18 is a similar plotting of haemoglobin percentages and their respective red cell diameter variabilities. In the right-hand corner are the corresponding relations and limits for healthy blood. Here again a definite correlation is suggested. On calculation it is found that $R = -0{\cdot}628$, and the standard error is 0·076, so that R is over eight times the standard error, and may be regarded as a significant and good correlation, expressing the fact that the greater the variability the lower is the haemoglobin percentage.

Taken together these two charts seem to establish conclusively that the degree of anisocytosis varies directly with the degree of anaemia.

Fig. 19 shows a similar plotting of mean diameters and their respective variabilities. The presence of any correlation is only doubtfully suggested. On calculation $R = -0{\cdot}27$ with a standard error of 0·112, so that R is only just over twice the standard error, and therefore on the three times rule it cannot be regarded as significant.

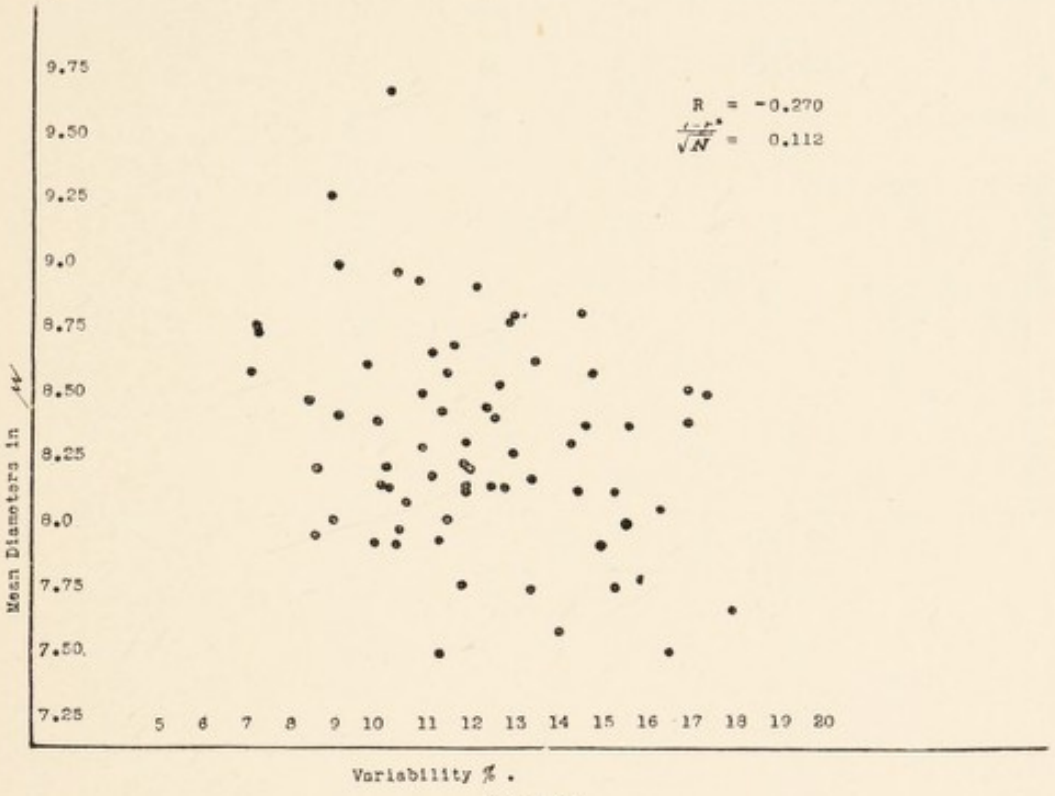

Fig. 19.

Fig. 20 is a plotting of mean diameters and their respective red cell counts. There is obviously no correlation, and on calculation $R = 0{\cdot}115$ with a standard error of 0·119. Similarly in Fig. 21, showing plottings of mean diameters and their respective haemoglobin percentages, there is obviously no correlation. $R = 0{\cdot}126$ and the standard error is 0·119.

The absence of correlation in these last two charts establishes a remarkable characteristic of the blood in this condition, viz. that the *red cell diameters are independent of the severity of the anaemia.* This is also exhibited in Table 22, in which it appears that the large mean diameter of the red cell persists throughout the illness, and even when the patient is apparently convalescent. In case 4, when first examined by me, the red cell count was 4,440,000 per c.mm., Hb per cent. 98, and colour index 1·1. From these values it would have been difficult to diagnose 'pernicious anaemia'. Measurement of the red cell diameters gave a mean of 8·02μ, with a variability of

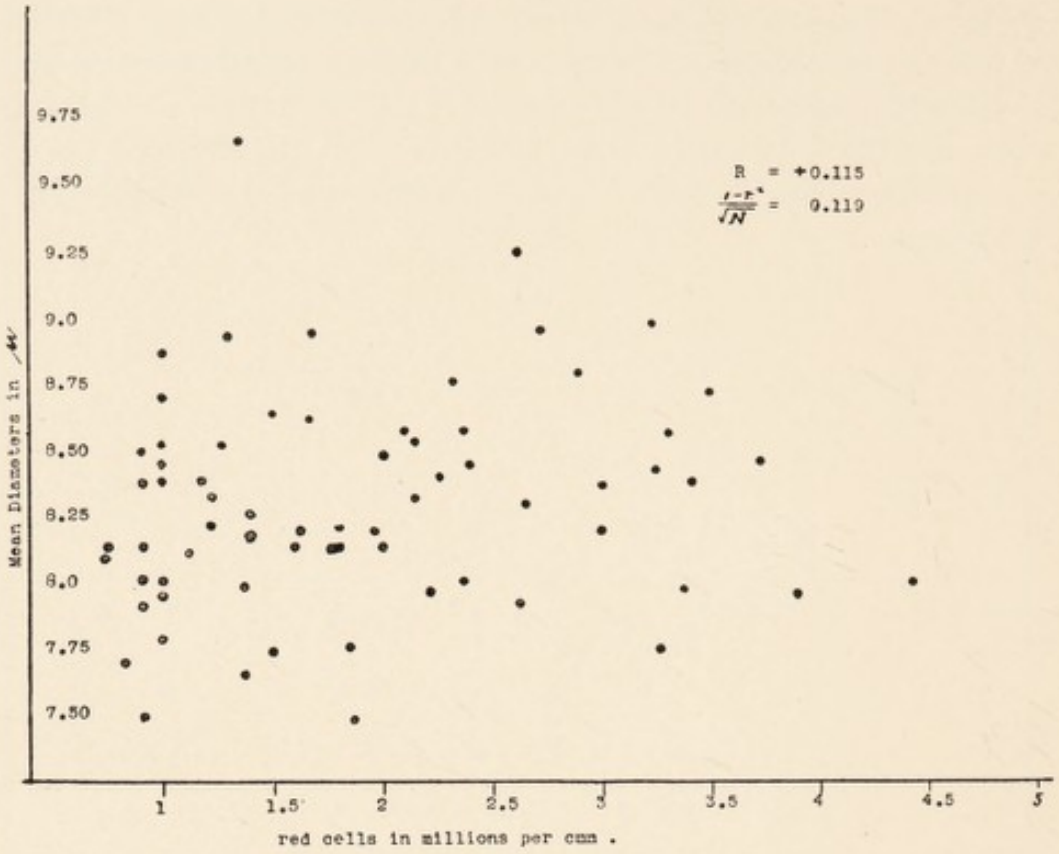
R = +0.115
$\frac{1-r^2}{\sqrt{N}}$ = 0.119
Mean Diameters in μ
9.75
9.50
9.25
9.0
8.75
8.50
8.25
8.0
7.75
7.50
1
1.5
2
2.5
3
3.5
4
4.5
5
red cells in millions per cmm .

FIG. 20.

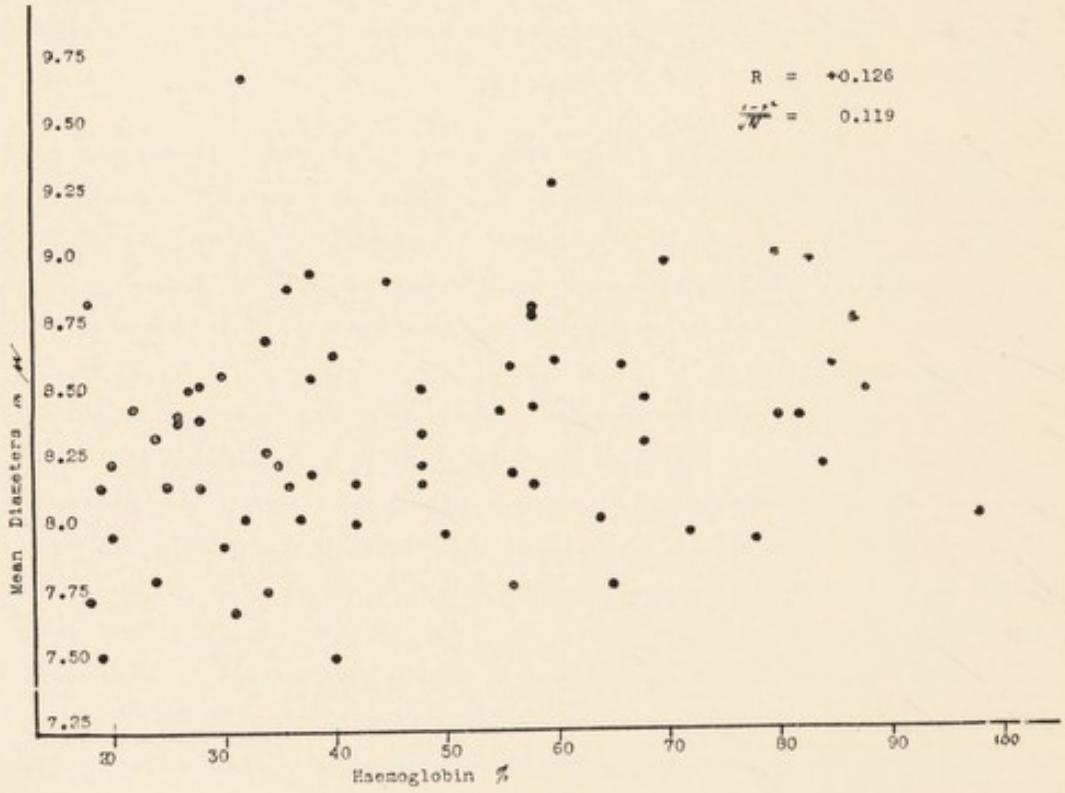
R = +0.126
$\frac{1-r^2}{\sqrt{N}}$ = 0.119
Mean Diameters in μ
9.75
9.50
9.25
9.0
8.75
8.50
8.25
8.0
7.75
7.50
7.25
20
30
40
50
60
70
80
90
100
Haemoglobin %

FIG. 21.

TABLE 22

Case	*Date*	*Red cells in millions per c.mm.*	*Hb per cent.*	*Mean red cell diameter*	*Variability per cent.*	
1	12.xi.21	1·37	28	8·369	17·08	
	3.v.22	2·29	60	8·585	9·7	
	13.xii.22	1·06	38	8·533	12·7	
	16.i.23	1·40	38	8·172	13·4	
	31.i.23	1·40	34	8·259	13·0	
	14.ii.23	1·26	28	8·118	11·9	
2	27.x.22	1·59	36	8·133	14·5	
	6.xii.22	2·00	48	8·478	17·4	
	22.xii.22	2·66	68	8·290	10·9	
	8.i.23	3·06	80	8·365	9·9	
	31.i.23	3·00	84	8·190	8·5	
	28.ii.23	3·90	72	7·940	8·5	
3	6.ii.22	2·15	48	8·314	11·9	
	13.ix.22	2·38	66	8·570	11·5	
	17.i.23	3·39	84	7·965	10·4	
4	6.x.21	4·44	98	8·022	8·9	
	28.iii.22	2·62	78	7·913	10·3	
	22.iv.22	2·02	48	8·131	10·2	
5	22.viii.22	0·75	25	8·137	12·5	
	31.viii.22	0·93	30	7·915	15·0	2 days after transfusion.
	16.ix.22	1·03	36	8·856	13·1	
	29.ix.22	1·31	38	8·919	10·8	
	12.x.22	1·04	34	8·685	11·7	
	1.xi.22	0·93	37	8·093	10·6	
	20.xi.22	0·93	27	8·487	10·7	2 days before death.
6	31.i.21	3·25	58	8·418	9·0	
	26.iv.22	1·21	24	8·310	14·3	
	23.v.22	0·90	26	8·363	15·7	
7	16.xi.22	2·28	55	8·408	12·6	
	27.xi.22	2·40	68	8·440	12·4	
	4.xii.22	2·36	64	8·036	11·5	
	13.xii.22	1·78	58	8·124	12·8	
	20.xii.22	1·94	56	8·176	11·2	
	27.xii.22	1·85	56	7·751	11·8	
8	20.ii.22	1·37	31	7·650	18·18	
	29.xi.22	2·10	56	8·577	14·8	
9	16.ix.22	1·82	48	8·198	10·1	
	11.x.22	1·82	42	8·133	11·8	
	27.xii.22	1·53	34	7·737	13·4	
10	23.x.22	3·27	65	7·748	15·3	
	30.x.22	1·04	24	7·787	15·9	
	9.xi.22	0·93	19	8·125	15·3	
11	11.x.23	1·34	32	9·673	10·2	
	24.x.23	2·63	60	9·250	8·8	
	21.xi.23	2·90	58	8·748	7·2	
12	19.vii.22	3·30	85	8·572	7·1	
	24.x.22	3·50	87	8·719	7·2	
	20.iii.22	3·74	88	8·456	8·3	
13	29.xi.22	2·30	58	8·764	12·9	
	31.i.23	3·41	82	8·374	10·0	
	14.iii.23	3·24	80	8·982	9·1	

8·9 per cent. Five months later the patient was readmitted to hospital, and a blood examination then gave red cells 2,620,000, Hb per cent. 78, colour index 1·5. The mean diameter of the red cells was 7·91, or practically unchanged. The variability had risen to 10·3 per cent. in association with the increased anaemia.

Table 22 comprises measurements from 13 cases which I have examined on several occasions during the course of the disease.* It also exhibits fairly constantly the correlation demonstrated above between the variability and the degree of anaemia. In case 1 a rise in the red cell count of 900,000 per c.mm. and Hb per cent. of 32 is accompanied by a fall in the variability of 7·3 per cent., and a subsequent fall in the red cell count of 1,230,000 and Hb per cent. of 22 was associated with a rise of 3 per cent. in variability. In case 2 a rise in red cell count of 1,900,000 per c.mm. and Hb per cent. of 24 is accompanied by a fall in variability of 6·9 per cent. In case 6 a fall in the red cell count from 3,250,000 to 906,000 per c.mm., and Hb per cent. from 58 to 26 is associated with a rise in variability from 9 per cent. to 15·9 per cent. The same principle holds in most of the other cases, with the marked exception of case 10, where the variability remains constant in spite of a very great alteration in the degree of anaemia.

In case 5 the examination made two days after transfusion showed, as might be expected, a lowered mean diameter of the red cells and an increased variability owing to the introduction of normal-sized cells.

* These cases were observed before the introduction of liver treatment.

CHAPTER IX

THE EFFECT OF LIVER TREATMENT ON PERNICIOUS ANAEMIA

The effect of liver treatment on the red cell diameters is illustrated by 12 cases (summarized in Table 23 and set out in detail in Table 24) treated at University College Hospital under the haematological care of Dr. Janet Vaughan (1928) at first as in-patients and afterwards for long periods as out-patients and partly with various liver extracts but mostly with whole liver. Two cases (3 and 9) were complicated with symptoms of subacute combined degeneration of the cord.

Anaemia. After treatment the anaemia was cured in all cases, in the sense that anaemic symptoms had disappeared. The haemoglobin percentage was above 80 in all and above 90 in 7.

Mean diameter. In 11 cases the mean diameter became smaller and in 9 it came back to within the normal range (less than 7·718μ); after treatment 3 mean diameters differ from the healthy mean (7·202μ) by less than the standard deviation (0·172μ), that is they are smaller than 7·374μ, and 3 mean diameters by less than twice the standard deviation that is smaller than 7·546μ. But, with one exception, and that only on one occasion, no mean diameter was brought to the left of the healthy mean. If the red cells were completely restored to the healthy condition and if pernicious anaemia occurs in persons whose mean diameters before the onset of the disease were distributed in a normal fashion, one would have expected some at least of these 9 cases to show mean diameters at and below the healthy mean (7·202μ). The failure to reach the healthy mean is perhaps most striking in those cases (8, 10, 11) which before treatment had mean diameters within the healthy range.

In 3 patients the mean diameter remains outside the healthy limit. In case 3 (Fig. 22) it has fallen 0·586μ but is still very high at 7·939μ; the megalocytosis in this case, which was as much as 55·4 per cent. (more than half the cells being too large and distributed outside to the right of the normal limit), though much reduced is still 18·4 per cent.; there is no anaemia and the variability is normal. The general condition of the patient is excellent; he is a Thames pilot and is able to carry on his work; his symptoms of cord degeneration have also improved; he says he takes liver regularly.

In case 9 (Fig. 23) which, like case 3, was complicated with subacute combined degeneration of the cord, after fifteen months' treatment,

TABLE 23

Cases of pernicious anaemia before and after treatment

	Case	1	2	3	4	5	6	7	8	9	10	11	12
	Sex	*M*	*F*	*M*	*M*	*F*	*F*	*F*	*F*	*F*	*M*	*M*	*F*
	Age	45	46	58	74	58	31	52	50	50	27	54	16
Before treatment	Red cells in millions	1·70	2·65	2·64	1·70	2·63	1·00	1·04	1·04	1·52	1·96	1·56	1·20
	Hb per cent.	41	68	78	44	62	22	24	22	42	48	34	29
	Colour index	1·20	1·28	1·50	1·30	1·19	1·10	1·20	1·10	1·40	1·23	1·09	1·20
	Mean diameter in μ	8·622	8·132	8·525	8·334	7·946	8·136	7·738	7·670	7·823	7·717	7·577	8·357
	v per cent.	10·4	11·0	8·7	11·0	10·2	12·1	16·0	9·9	14·7	14·2	12·5	13·4
	Megalocytosis	57·8	36·8	55·4	44·0	22·4	29·2	19·6	7·4	26·0	18·4	8·8	48·6
After treatment	Liver treatment in months	8	14	10	10	16	6	17	8	15	8	4	16
	Red cells in millions	5·2	5·0	5·0	4·3	4·7	4·7	4·05	4·34	5·30	4·93	4·24	5·20
	Hb per cent.	110	90	96	96	84	84	80	82	92	90	90	82
	Colour index	1·05	0·90	0·96	1·1	0·89	0·89	1·0	0·9	0·86	0·90	1·07	0·78
	Mean diameter in μ	7·557	7·453	7·939	7·732	7·590	7·372	7·922	7·552	7·265	7·407	7·392	7·276
	v per cent.	6·0	5·7	5·7	8·7	7·2	6·5	8·2	7·1	6·0	8·2	7·9	8·5
	Megalocytosis	0·2	0·0	18·4	9·6	1·8	0·0	16·6	0·6	0·0	1·8	0·2	0·0

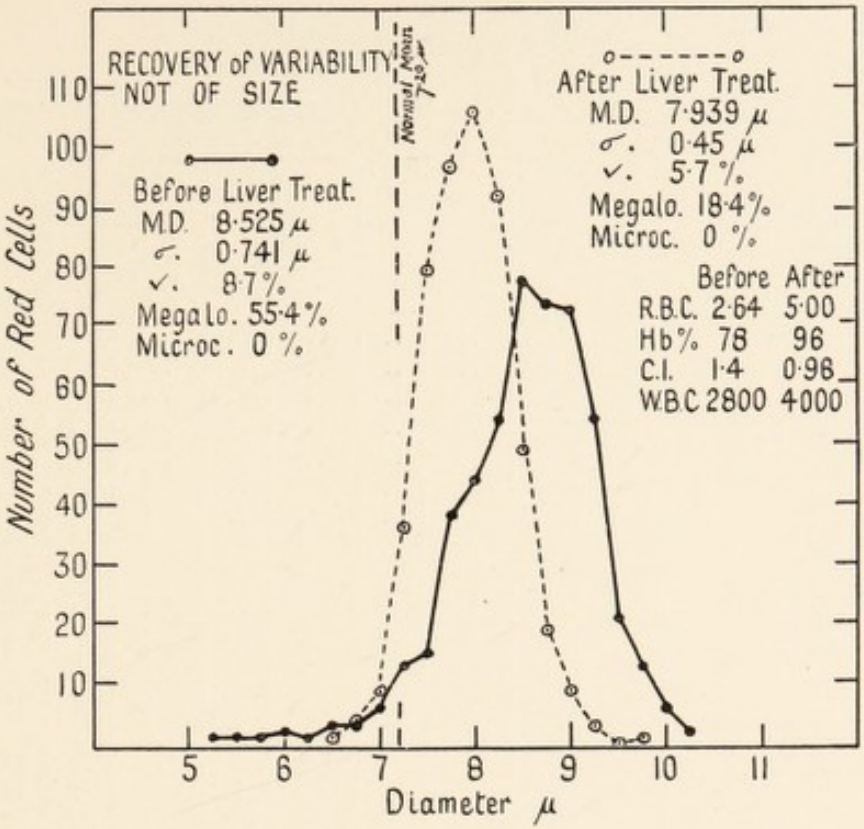

FIG. 22. Case 3.

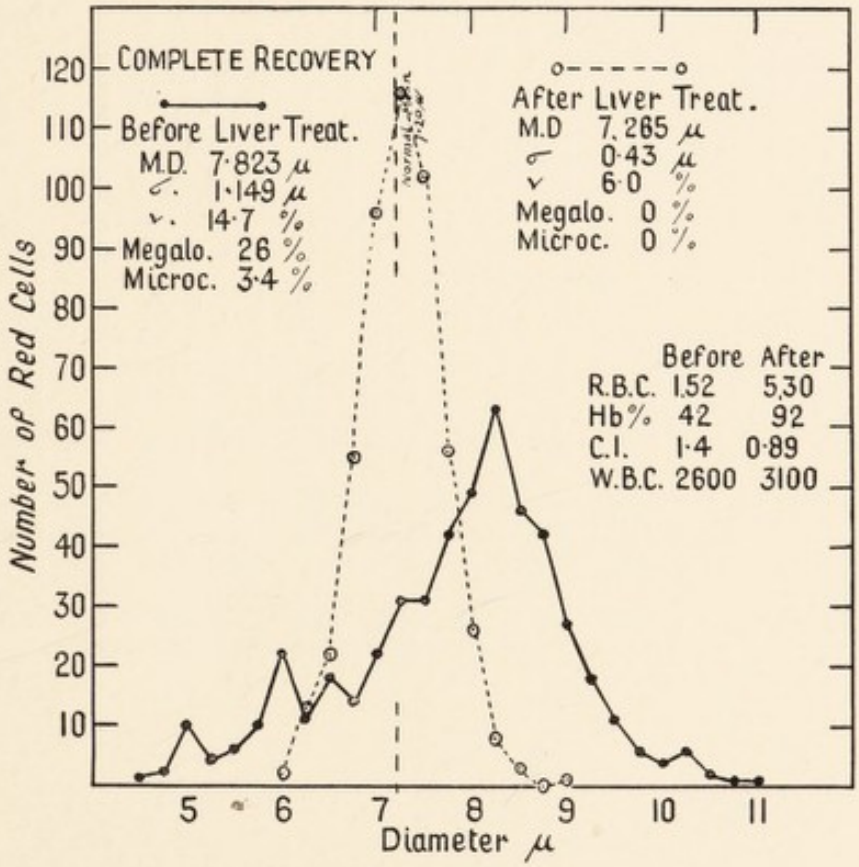

FIG. 23. Case 9.

TABLE 24

Cases of pernicious anaemia

Mid-point of class intervals μ	*Case* 1 *Age* 45 *Sex* *M*		2 46 *F*		3 58 *M*		4 74 *M*		5 58 *F*	
	Before	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*
4·00	..	..	..	..	..	..	..	..	1	..
4·25	..	..	..	..	..	..	..	..	0	..
4·50	..	..	..	..	..	..	..	..	1	..
4·75	..	..	..	..	..	..	..	..	1	..
5·00	..	..	1	..	..	..	..	..	1	..
5·25	1	..	0	..	1	..	1	..	2	..
5·50	2	..	7	..	1	..	5	..	1	..
5·75	1	**1**	4	..	1	..	2	..	3	..
6·00	5	**1**	6	..	2	..	6	..	3	..
6·25	4	**6**	9	**4**	1	..	6	**6**	6	**4**
6·50	2	**5**	13	**11**	3	**1**	6	**19**	6	**12**
6·75	4	**12**	8	**29**	3	**4**	7	**22**	10	**31**
7·00	8	**37**	13	**69**	6	**9**	15	**41**	22	**53**
7·25	18	**107**	26	**98**	13	**36**	18	**57**	35	**85**
7·50	18	**125**	23	**111**	15	**79**	31	**101**	50	**84**
7·75	18	**101**	66	**101**	38	**95**	42	**55**	66	**90**
8·00	40	**57**	50	**50**	44	**105**	52	**54**	83	**61**
8·25	49	**28**	49	**19**	54	**91**	48	**67**	57	**31**
8·50	57	**11**	67	**5**	77	**48**	63	**38**	51	**27**
8·75	68	**5**	63	**3**	73	**19**	64	**16**	43	**13**
9·00	74	**2**	43	..	72	**9**	48	**8**	33	**9**
9·25	52	**2**	23	..	54	**3**	27	**5**	14	..
9·50	29	..	14	..	21	**0**	24	**5**	6	..
9·75	16	..	10	..	13	**1**	13	**4**	4	..
10·00	13	..	5	..	6	..	10	**2**	0	..
10·25	9	..	..	..	2	..	8	..	0	..
10·50	6	..	..	..	..	..	2	..	0	..
10·75	3	..	..	..	..	..	1	..	0	..
11·00	2	..	..	..	..	..	0	..	0	..
11·25	0	..	..	..	..	..	0	..	1	..
11·50	1	..	..	..	..	..	1	..	..	..
11·75	..	..	..	..	..	..	..	..	..	..
12·00	..	..	..	..	..	..	..	..	..	..
Total	500	**500**	500	**500**	500	**500**	500	**500**	500	**500**
M.D.	8·622	**7·557**	8·132	**7·453**	8·525	**7·939**	8·334	**7·732**	7·946	**7·590**
σ	0·90	**0·46**	0·90	**0·43**	0·75	**0·46**	0·92	**0·68**	0·81	**0·55**
v	10·4	**6·0**	11·06	**5·7**	8·7	**5·7**	11·0	**8·7**	10·2	**7·2**
Megalocytosis per cent.	57·8	**0·2**	36·8	**0·0**	55·4	**18·4**	44·0	9·6	22·4	**1·8**

TABLE 24 (*contd.*)

before and after treatment

6 31 *F*		7 52 *F*		8 50 *F*		9 50 *F*		10 27 *M*		11 54 *M*		12 16 *F*	
Before	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*
..	..	..	..	..	..	..	..	1	..	..	..	..	..
..	..	..	..	..	..	..	..	1	..	..	..	..	..
..	..	1	..	..	..	1	..	1	..	2	..	..	..
..	..	5	..	1	..	2	..	2	..	2	**1**	..	..
1	..	6	..	0	..	10	..	1	..	1	**2**	3	**1**
1	..	5	..	1	..	4	..	2	..	6	**0**	3	**0**
2	..	10	..	4	..	6	..	8	..	6	**3**	6	**1**
2	..	9	**1**	4	..	10	..	14	..	14	**1**	5	**4**
8	**5**	15	**0**	5	**1**	22	**2**	16	**3**	10	**4**	4	**7**
6	**7**	17	**0**	9	**2**	11	**13**	16	**9**	14	**9**	7	**20**
9	**19**	26	**4**	16	**13**	18	**22**	16	**28**	18	**23**	17	**40**
13	**45**	18	**19**	28	**38**	14	**55**	27	**49**	23	**32**	14	**63**
15	**66**	32	**30**	38	**56**	22	**96**	29	**73**	39	**80**	16	**65**
32	**107**	43	**43**	55	**77**	31	**116**	33	**94**	47	**75**	20	**85**
43	**104**	40	**63**	77	**103**	31	**102**	47	**87**	57	**101**	21	**73**
59	**73**	46	**79**	57	**78**	42	**56**	55	**68**	75	**70**	22	**60**
68	**45**	42	**81**	73	**58**	49	**26**	53	**36**	51	**56**	28	**36**
54	**21**	46	**67**	54	**42**	63	**8**	45	**24**	50	**30**	50	**20**
59	**6**	30	**54**	32	**17**	46	**3**	47	**6**	34	**6**	58	**18**
41	**2**	18	**29**	20	**8**	42	**0**	26	**10**	14	**3**	51	**3**
32	..	27	**15**	11	**3**	27	**1**	21	**7**	13	**3**	50	**3**
21	..	12	**5**	9	**2**	18	..	11	**1**	11	**0**	47	**1**
4	..	21	**4**	3	**2**	11	..	7	**3**	5	**0**	30	..
4	..	8	**1**	2	..	6	..	8	**0**	5	**1**	23	..
3	..	7	**3**	1	..	4	..	5	**2**	1	..	9	..
5	..	4	**0**	..	..	6	..	0	..	1	..	7	..
5	..	5	**1**	..	..	2	..	3	..	1	..	4	..
5	..	3	**0**	..	..	1	..	4	..	..	..	2	..
3	..	2	**0**	..	..	1	..	1	..	..	..	1	..
2	..	0	**0**	..	..	..	..	..	..	..	..	2	..
2	..	2	**1**	..	..	..	..	..	..	..	..	..	..
0	..	..	..	..	..	..	..	..	..	..	..	..	..
1	..	..	..	..	..	..	..	..	..	..	..	..	..
500	**500**	500	**500**	500	**500**	500	**500**	500	**500**	500	**500**	500	**500**
8·136	**7·372**	7·738	**7·922**	7·670	**7·552**	7·823	**7·265**	7·717	**7·407**	7·577	**7·392**	8·357	**7·276**
0·99	**0·48**	1·24	**0·65**	0·76	**0·54**	1·15	**0·44**	1·1	**0·61**	0·95	**0·59**	1·12	**0·62**
12·1	**6·5**	16·0	**8·2**	9·9	**7·1**	14·7	**6·0**	14·2	**8·2**	12·5	**7·9**	13·4	**8·5**
29·2	**0·0**	19·6	**16·6**	7·4	**0·6**	26·0	**0·0**	18·4	**1·8**	8·8	**0·2**	48·6	**0·0**

the curve shows complete recovery, the mean diameter is 7·265, the variability 6·0 per cent., and there is absence of megalocytosis.

In case 7 (Fig. 30) the mean diameter has increased, though hardly significantly, to 7·922μ and the megalocytosis has been reduced only from 19·6 to 16·6 per cent.; this woman took liver badly. However, the haemoglobin has risen from 24 to 80 per cent., and the variability has fallen from 16 to 8 per cent. Case 4 (Fig. 24) has taken liver

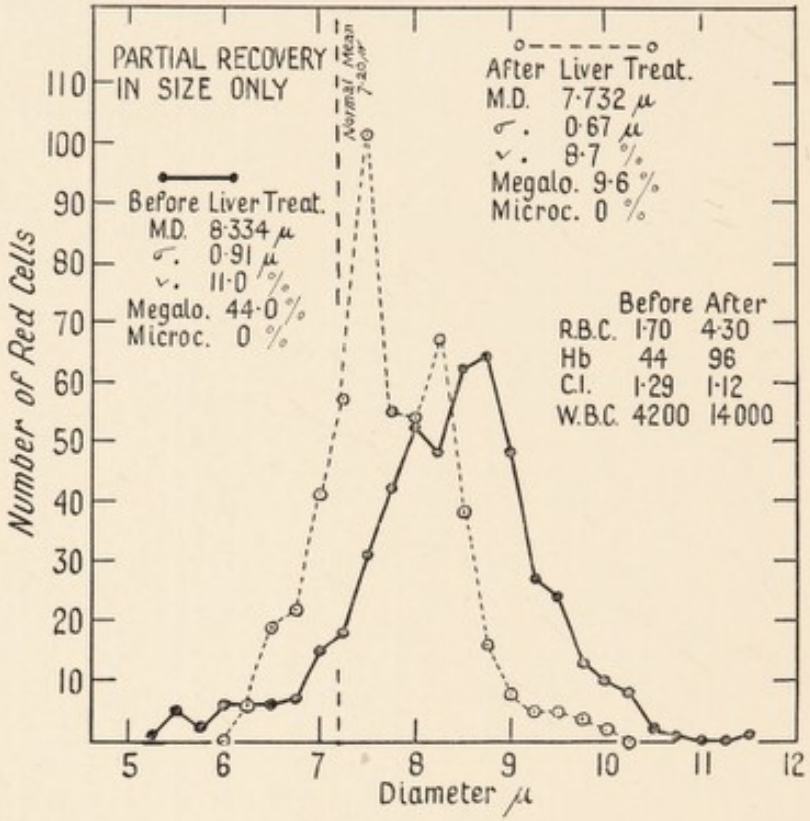

Fig. 24. Case 4.

conscientiously, is clinically well; his mean diameter has fallen by 0·602μ but is still too high (7·732μ); the megalocytosis has been reduced from 44 to 9·6 per cent. He is an elderly man, a retired chemist.

Coefficients of Variation. The variabilities before treatment were all well outside the healthy maximum (7·3 per cent.). After treatment 7 came within the healthy range, and 4 fell below the healthy mean (6·2 per cent.). Of the other 5, 2 have excessive mean diameters and 3 have diameters compatible with health. On the other hand, the 'cured' case (3), Fig. 22, with the largest mean diameter has a variability nearly down to the healthy minimum.

In considering these reciprocal relations it should be borne in mind that the variability is influenced as much by the presence of

cells which are too small (as in anaemia from haemorrhage) as by megalocytes, and in different specimens of pernicious anaemia bloods the relative share of the two groups varies a good deal.

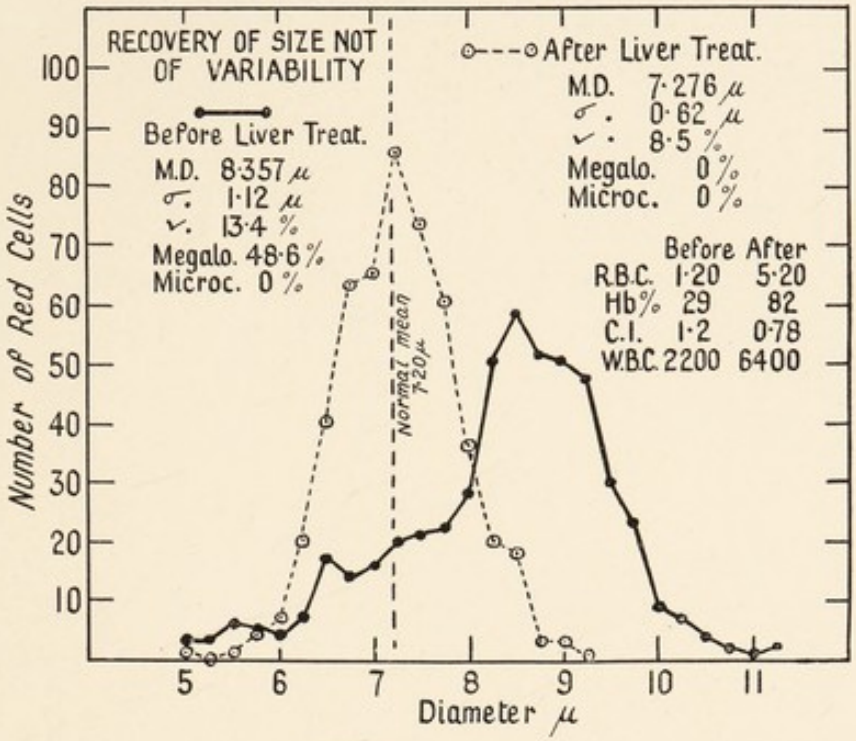

Fig. 25. Case 12.

Megalocytosis before treatment varied from 7·4 to 57·8 per cent. (average 31·5 per cent.). It disappeared after treatment except in the three cases 3, 4, and 7, which still had abnormally large mean diameters.

Taking one criterion with another, therefore, the red cells became normal in 6 of the 12 cases; the others are still abnormal in one or more particulars. Considering that liver provides something which the body lacks and does not remove the cause of the disease, this is perhaps what we should expect.

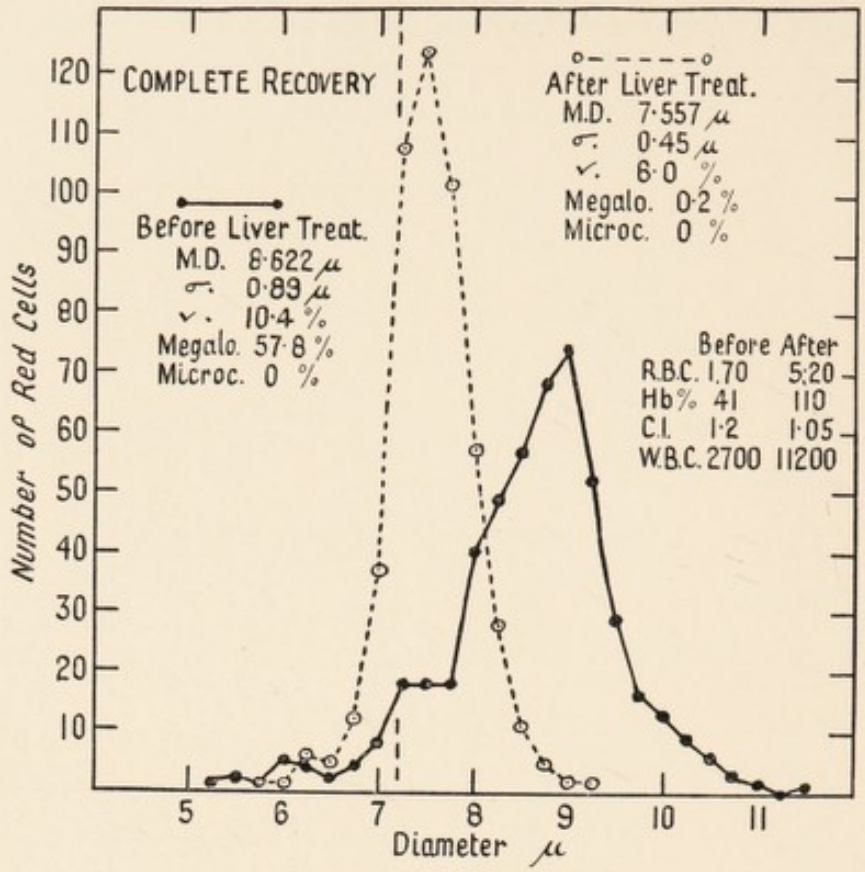

Fig. 26. Case 1.

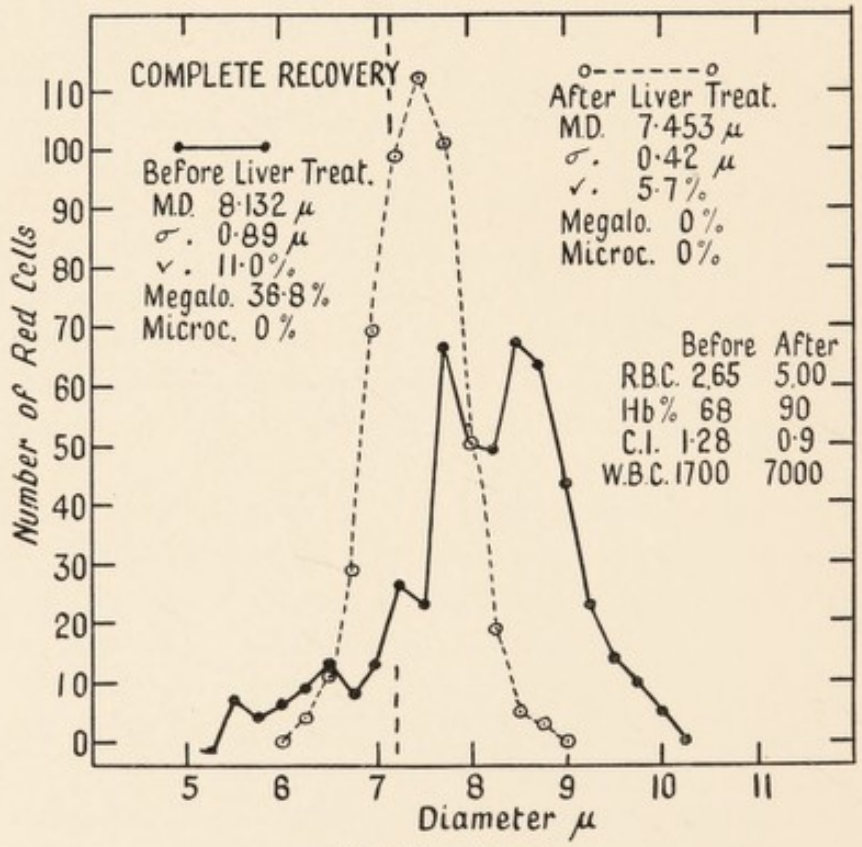

Fig. 27. Case 2.

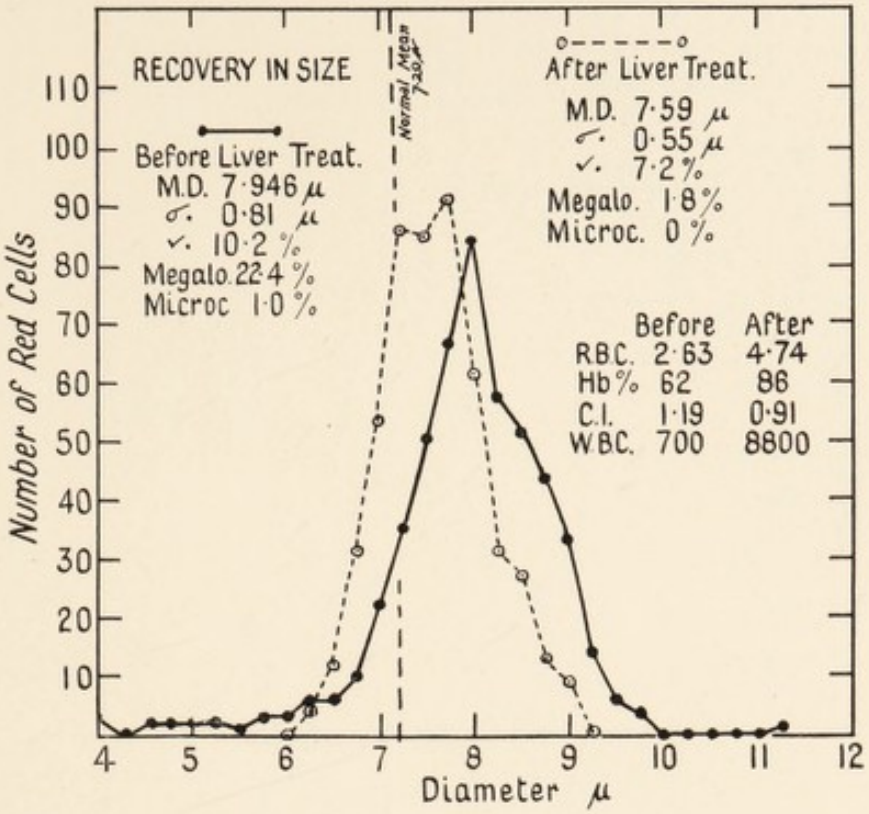

Fig. 28. Case 5.

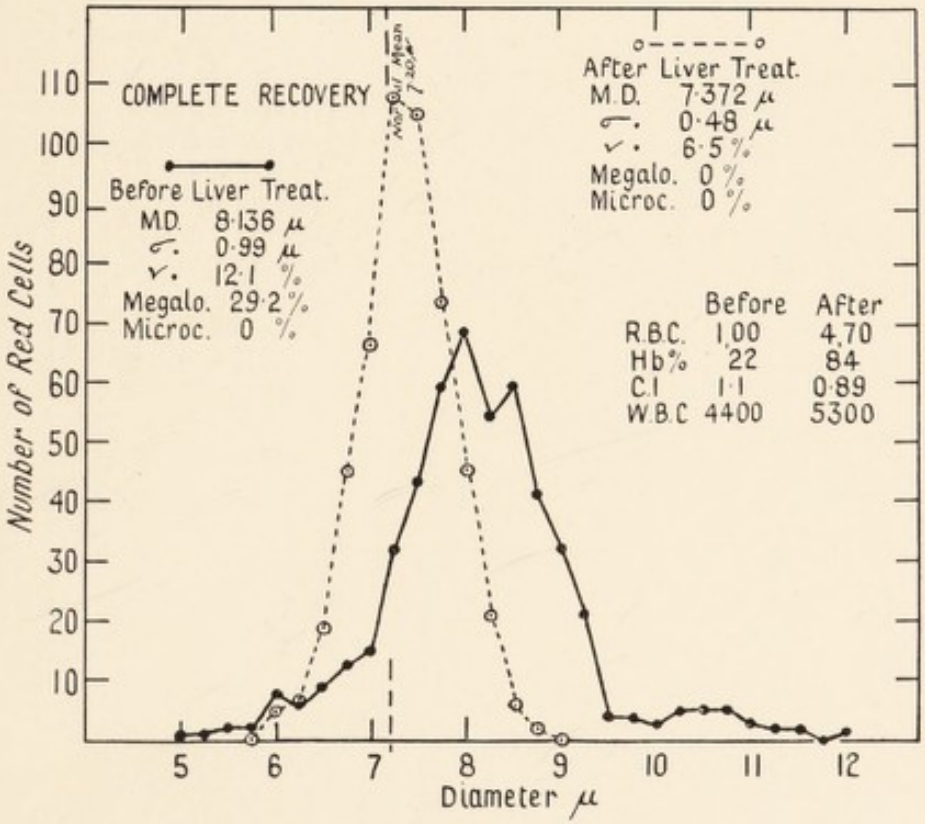

Fig. 29. Case 6.

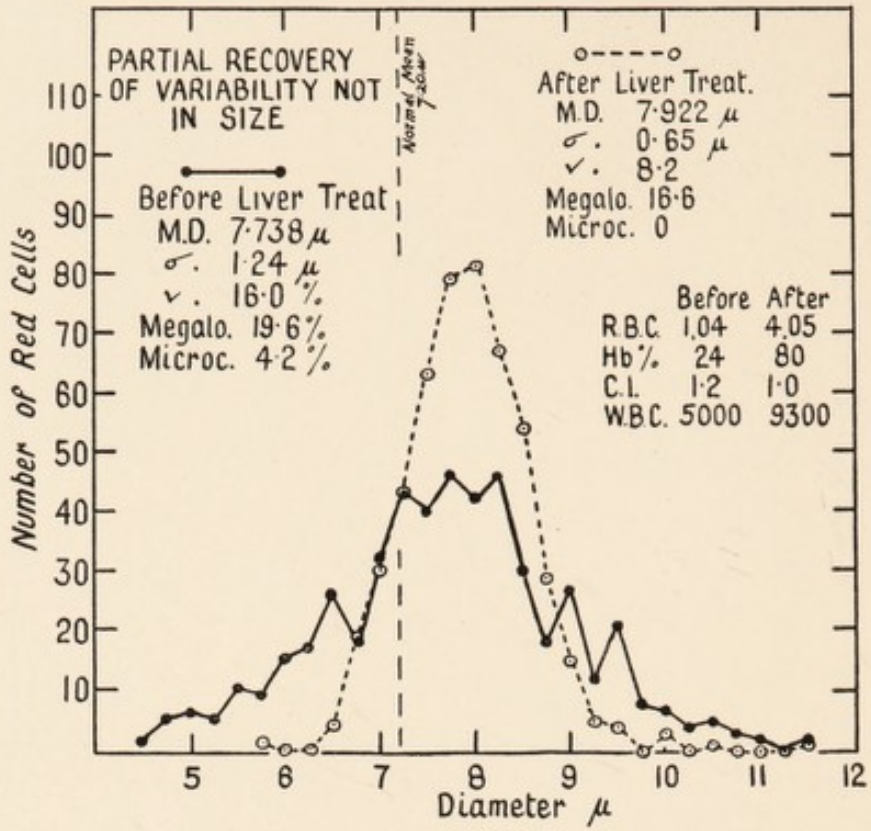

FIG. 30. Case 7.

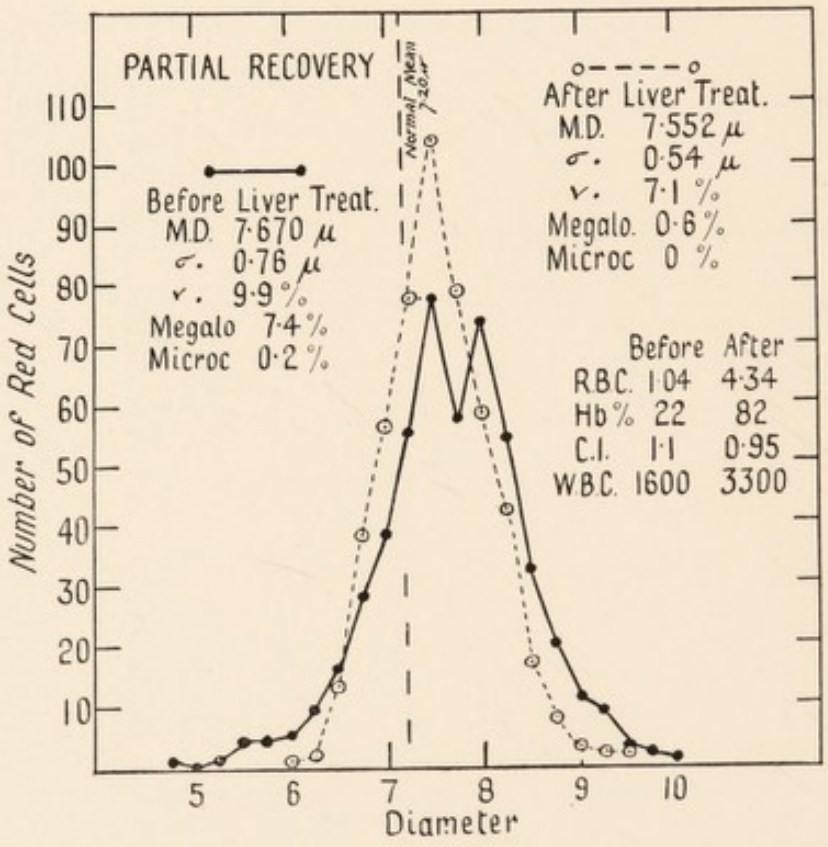

FIG. 31. Case 8.

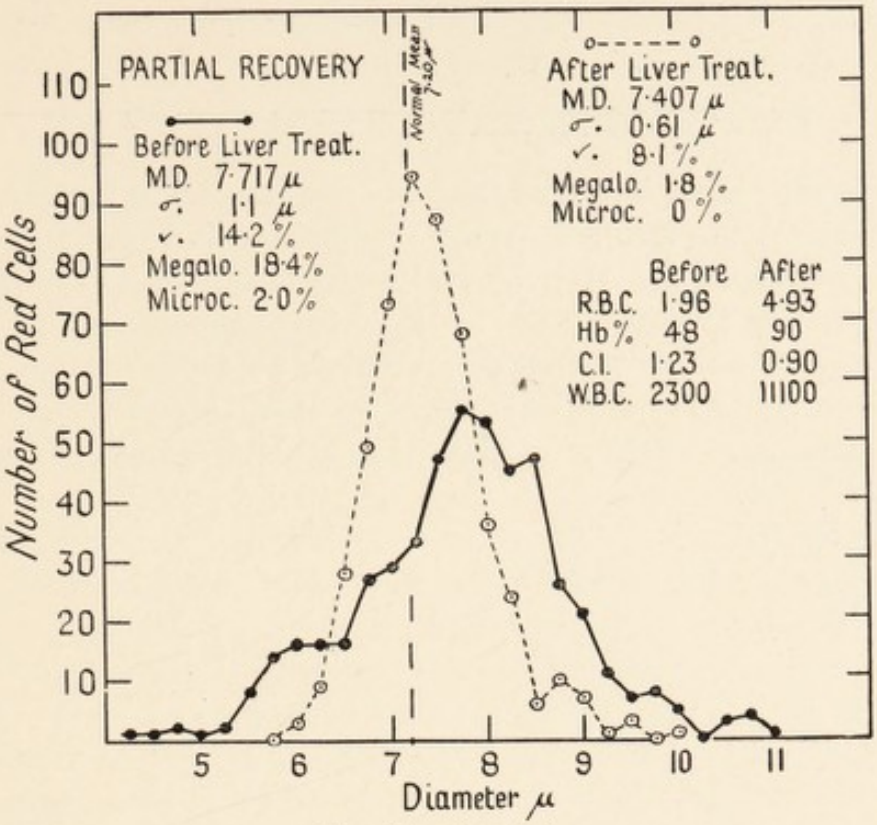

Fig. 32. Case 10.

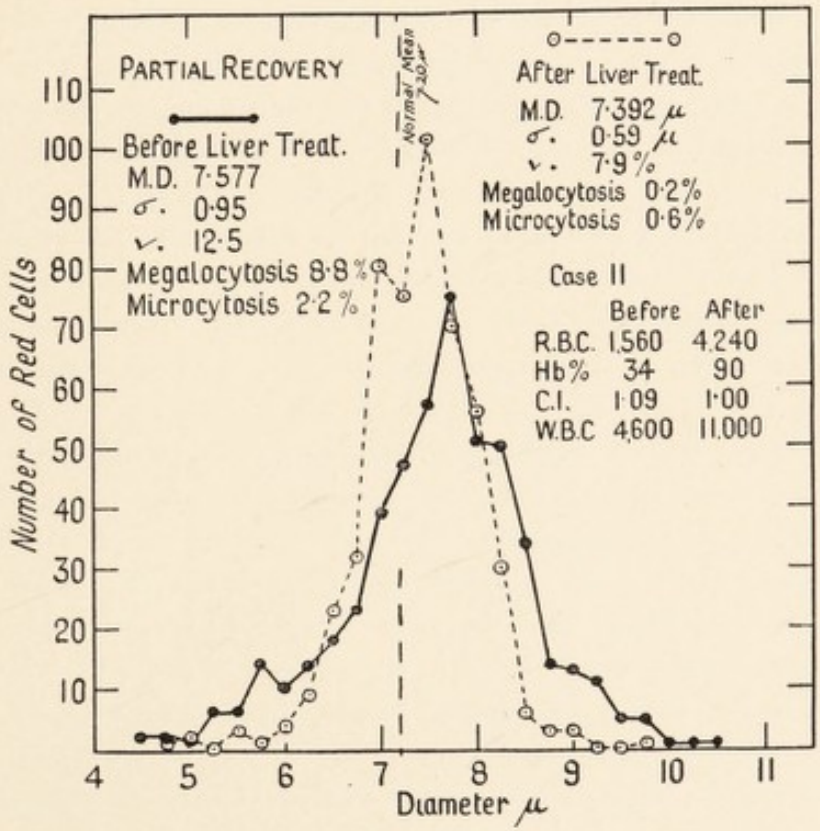

Fig. 33. Case 11.

CHAPTER X

THE RED CELLS IN MICROCYTIC ANAEMIA (WITTS)

This form of secondary anaemia has been brought to notice in this country by Witts (1930), who names it 'simple achlorhydric anaemia'; it has also been described by Kaznelson (1929) as 'achylic chloranaemia'; by Schulten (1930) as 'hypochromatic anaemia', and by Waugh (1931) as 'hypochromic anaemia with achlorhydria'. None of these names is quite satisfactory or specific, and their variety illustrates the difficulty of definitive as opposed to eponymous nomenclature.

The condition is a 'secondary' or 'chlorotic' anaemia resulting from a deficient production of haemoglobin. Whereas the red cell count is usually only moderately reduced, the Hb percentage is remarkably lower, so that the colour index is lowered to about 50 per cent. of the normal value. This form of anaemia occurs in women;* it is confined to the reproductive epoch and persists in varying intensity throughout the child-bearing period; it is much aggravated by pregnancy and tends to spontaneous cure after the menopause. It is frequently associated with hypochlorhydria or achlorhydria; according to Witts in 80 per cent. of cases. In the 8 cases under consideration there was complete achlorhydria in 5, a trace after histamine injection in 2, and later *hyper*chlorhydria in 1 case; these results do not suggest a causative association and do not bear out the attractive notion derived from the work of Mettier and Minot (1929) that, iron being more potent for blood formation when absorbed from an acid medium, there would with achlorhydria tend to be an iron deficiency and consequently an anaemia of this type.

The haematological features of this disease are anaemia, microcytosis, anisocytosis, and changes in the staining reaction of the red cells.

Anaemia. In all cases, examination before treatment shows a lowered red cell count (Table 26) usually only to a slight degree. In the eight cases I have studied the lowest count was 2·8 millions per c.mm., the highest 4·7 millions, and the average for the series was 4·03 (see Table 26). The Hb percentage was much more reduced: the lowest value was 24 per cent., the highest 56 per cent., the average 47 per cent. The colour index ranged from 0·42 to 0·72 with an average of 0·54.

Microcytosis. The sizes of the red cells are obviously smaller than

* Cases are reported of its occurrence in men.

in health. The mean diameters ranged from 6·2μ to 6·7μ with an average of 6·48μ. In all these cases there was microcytosis, 6 per cent. to as much as 37 per cent. of the cells lying outside the left-hand boundary of the -3σ curve.

Morphology. Blood films, stained and fixed with Jenner, and *superstained with aqueous eosin* (10 per cent.) for 5 minutes, show on microscopic examination that most of the red cells are badly stained, giving the appearance of unstained disks enclosed by pink rings of varying width, often barely distinguishable; many of these pale cells are oval in shape, some flattened to rods (biscuit shaped) with the stain limited to the ends of the rod; poikilocytes and fragments only occasionally occur.

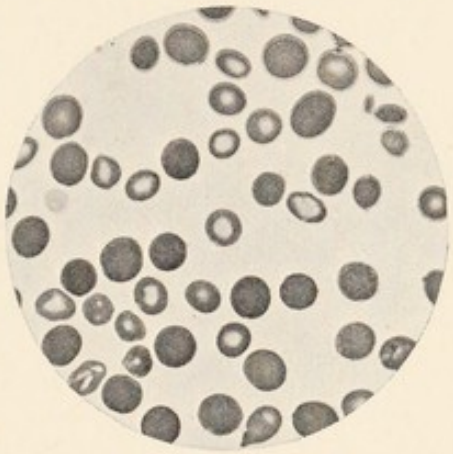

Fig. 34.

In striking contrast to these pale cells there are a few deeply stained cells; these are scattered irregularly through the film, but though on first examination before treatment they may be very few, yet I have observed them in all the cases, and have estimated their number, varying from 5 to 26 per cent. in counts of 500 red cells. They vary in depth of stain and size, and give the impression of being larger than the pale cells and hyperchromic. In films made from a mixture of my own blood and that of the patient, I was not able to distinguish these deep-stained cells from the healthy cells of my own blood, and I believed that these 'orthochromic' cells were healthy cells. If, however, I measured 500 of these cells and compared the distribution curve with that of 500 pale cells, it was at once clear (Fig. 35) that neither the pale cells nor the deep-stained cells are healthy; both are microcytic; the mean diameters are too small and the variability of their distribution or degree of anisocytosis is too high. Measurement of pale cells and dark cells for each case before or at commencement of treatment are given in Table 25.

The presence of 'well-stained' cells among faintly stained cells in the blood of secondary anaemia and chlorosis was recognized by Ewing (1901) and also by Cabot (1901); Waugh (1931) says there is in this hypochromic anaemia marked anisochromia of the red cells, which are poorly and irregularly stained. Watkins (1929) distinguishes 'hypochromasia' in which the red cells are uniformly pale, and 'anachromasia' in which there is a piling up of haemoglobin in

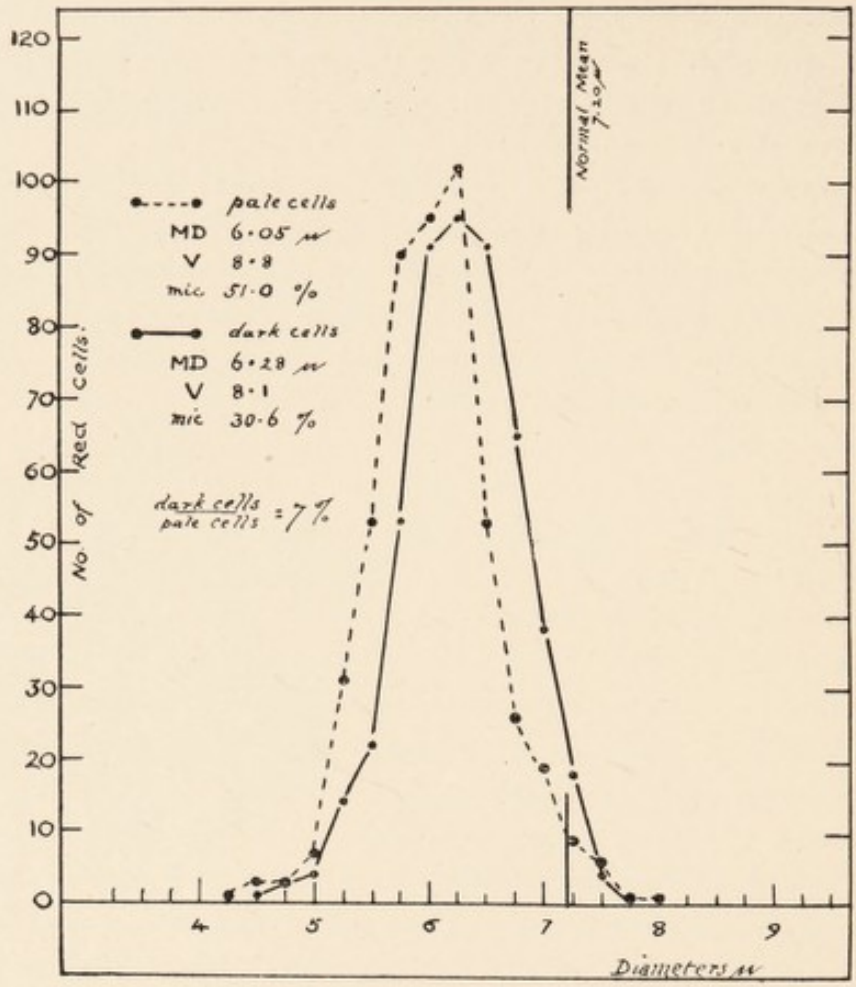

Fig. 35.

Table 25

Before treatment

Number of case	*Dark cells per cent.*	*500 pale cells*		*500 dark cells*	
		M.D.	*v.*	*M.D.*	*v.*
1	8·0	6·66	8·4	7·03	8·6
2	17·0	6·67	9·2	7·02	8·5
3	26·6	6·44	8·8	7·11	12·8
4	19·0	6·51	8·4	6·49	7·5
5	10·8	6·65	6·9	6·51	9·4
6	5·4	6·27	9·0	6·38	10·2
7	7·0	6·05	8·8	6·28	8·1
8	7·6	6·45	10·3	6·45	10·4
Average . .		6·46	8·7	6·66	9·4

a narrow ring around the peripheral portion of the cell, leaving a wide colourless central zone. It is possible that his hypochromasic cells correspond to the orthochromic cells I have described above which had not been superstained with eosin, which I had found

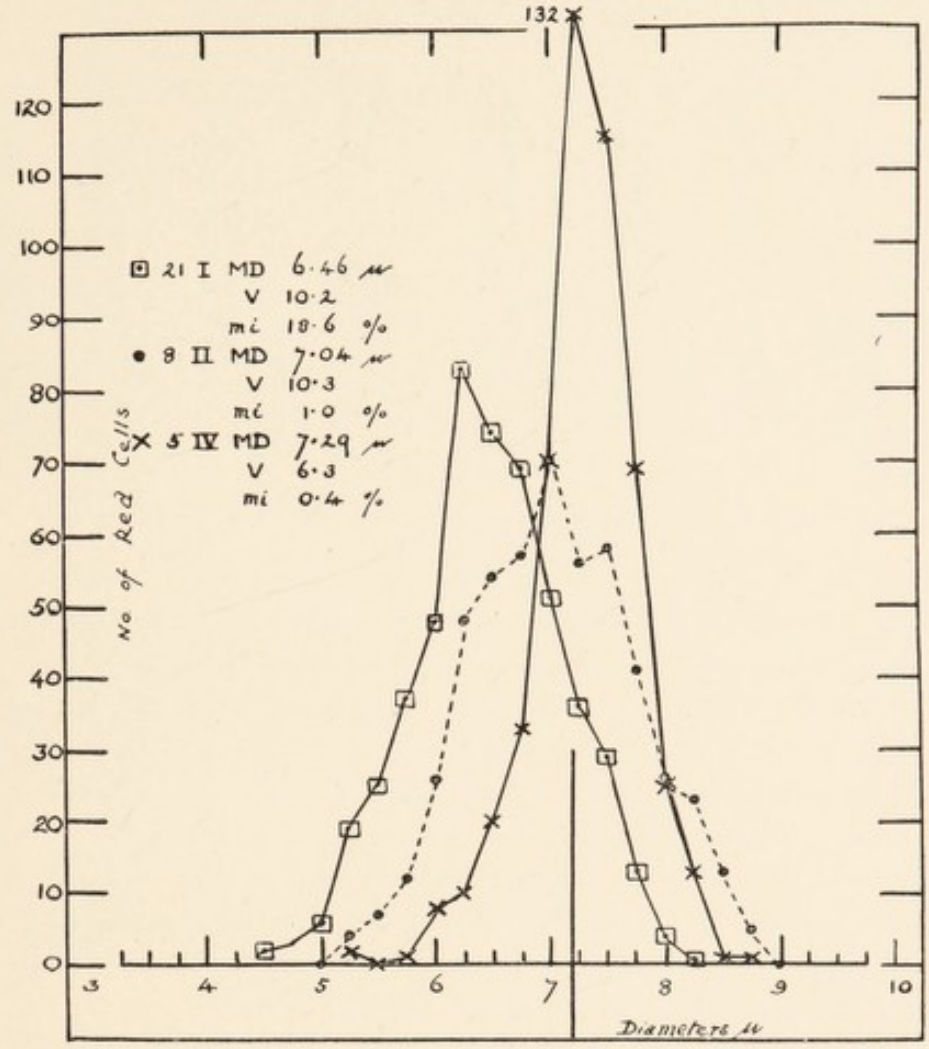

FIG. 36.

necessary to bring out the distinction clearly. I have not found any reference to these differently stained cells in the work of other observers.

Perhaps the most remarkable feature of this anaemia is the rapidity and degree with which it reacts to large doses of iron. The index of the reaction is the increase in number and size of the dark-staining (orthochromic) cells. Fig. 36 shows the progress of case 8 from January 21, when treatment (three 5-gr. Blaud's pills t.d.s.) was started, to April 5. At the first examination the deep-stained cells were only 7·6 per cent. in a count of 500 red cells, the mean diameter of the pale and dark cells together was 6·46μ, with a

variability of 10·2 and a microcytosis of 18·6 per cent. After 15 weeks' treatment the deep-stained cells had increased to 87 per cent.; the microcytosis had disappeared; the mean diameter had risen to 7·29μ with a variability of only 6·3, the estimated normal value for v, and the character of the distribution is that of an ideal normal curve.

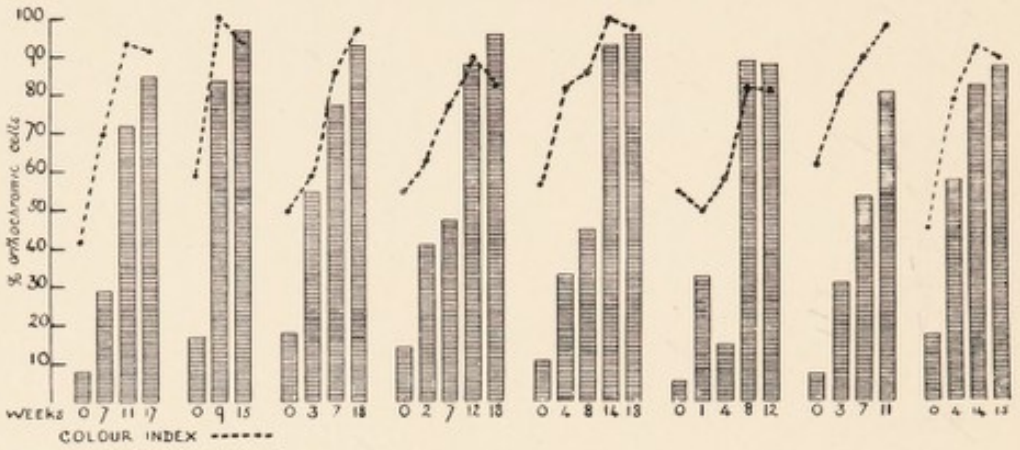

FIG. 37. Increase in percentage of orthochromic cells and colour index during treatment.

All the 8 cases show similar progresses. In Fig. 37 it is seen that corresponding with the increase of orthochromic cells there is a rise in the colour index. The effect of the treatment on the 8 cases is collected in Table 26. The mean diameter of the red cells is significantly moved to the right in every case and increased on average from 6·48μ to 6·99μ; the coefficient of variation decreased on average from 9·25 to 6·62 per cent. The number of orthochromic cells increased on average from 12 per cent. to 90 per cent. In all but one case the microcytosis had disappeared, but this case was only 11 weeks under treatment.

TABLE 26

Showing effect of iron treatment

Case	1		2		3		4		5		6		7		8	
	Before	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*	*Before*	*After*
Red cells in millions per c.mm.	2·8	5·8	4·7	5·27	5·28	5·05	4·12	4·53	4·6	4·95	3·49	4·64	4·03	5·3	5·1	5·49
Hb per cent.	24	102	56	98	55	98	45	75	53	94	38	75	49	98	46	96
Colour index	0·43	0·92	0·59	0·92	0·52	0·97	0·55	0·83	0·57	0·98	0·55	0·81	0·61	0·92	0·45	0·88
Mean diameter in μ	6·6	6·8	6·7	7·3	6·6	6·86	6·3	7·38	6·59	6·9	6·3	6·86	6·2	6·6	6·6	7·29
v (anisocytosis)	8·6	6·2	9·3	6·3	11·9	7·0	9·7	6·3	7·9	6·6	9·0	6·8	8·3	7·5	9·5	6·3
Microcytosis per cent.	3·8	0	5·8	0·4	12·6	0	27·2	0	7·8	0	22·8	0	37·2	5·8	9·2	0·4
Orthochromic red cells per cent.	8·0	85·8	17·0	97·0	18·2	93·0	14·4	96·4	10·8	95·8	5·4	88·2	7·0	80·8	17·0	87·2
Iron treatment in months	4		4		4		4		4+		3		2+		3	

APPENDIX

In the preceding pages I have stated the principles and methods involved in measuring the sizes of red blood cell diameters, and have given in some detail examples of the application of these measurements to diagnosis and prognosis. These examples, of course, could be extended to several other diseases, but as it is not my present purpose to write a textbook on haematology it may suffice if I include here a few curves from miscellaneous cases.

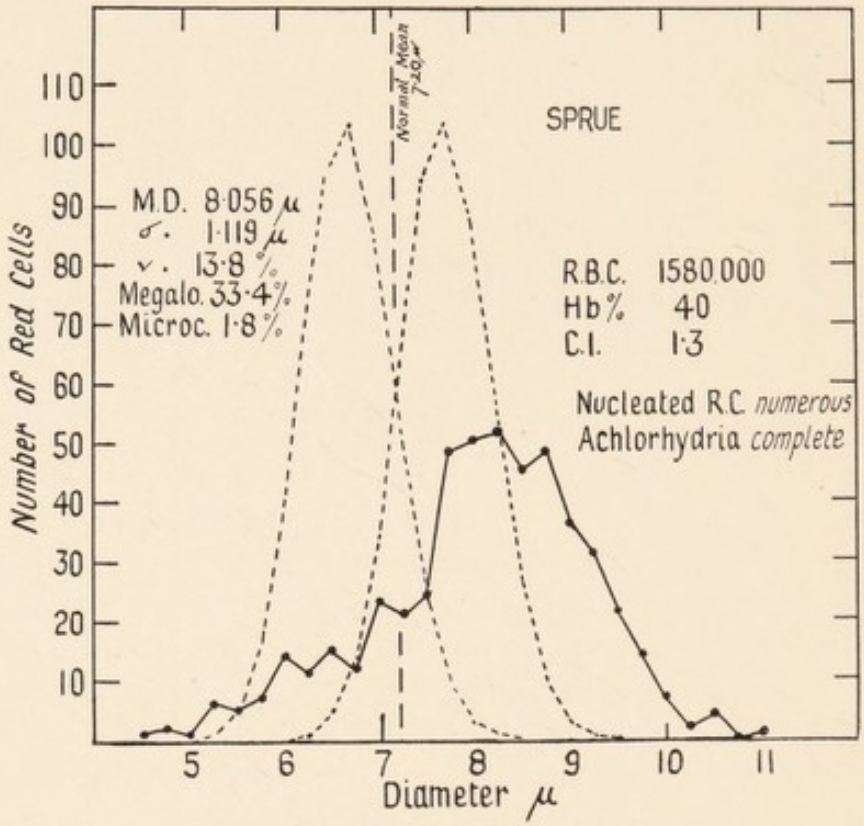

Fig. 38.

Figs. 38 and 39 are from cases of sprue, and Fig. 40 from a case of anaemia associated with steatorrhea. All these three curves show a high degree of megalocytosis and variability and a type of distribution indistinguishable from that of pernicious anaemia. Figs. 41 and 42 are from cases of aplastic anaemia both showing megalocytosis and high mean diameter.

Figs. 43 and 44 are from cases of Vaquez polycythaemia. Both curves show small mean diameter and raised variability, but the distribution is not characteristic or different from that of secondary anaemia; the degree of microcytosis in these cases is never very high. In spleno-megalic polycythaemia the bone marrow is intensely producing red cells. To enable the heart to overcome the increased work due to the greater viscosity of the blood the blood volume is increased. It was estimated in one of these cases that if the red cells had been of normal size the volume of his red cells would have been 93 per cent., in which case the blood would have been almost too thick to circulate; but the mean diameter was reduced by 0·6 μ. Throughout a long series of examinations the blood picture of these cases remained remarkably constant.

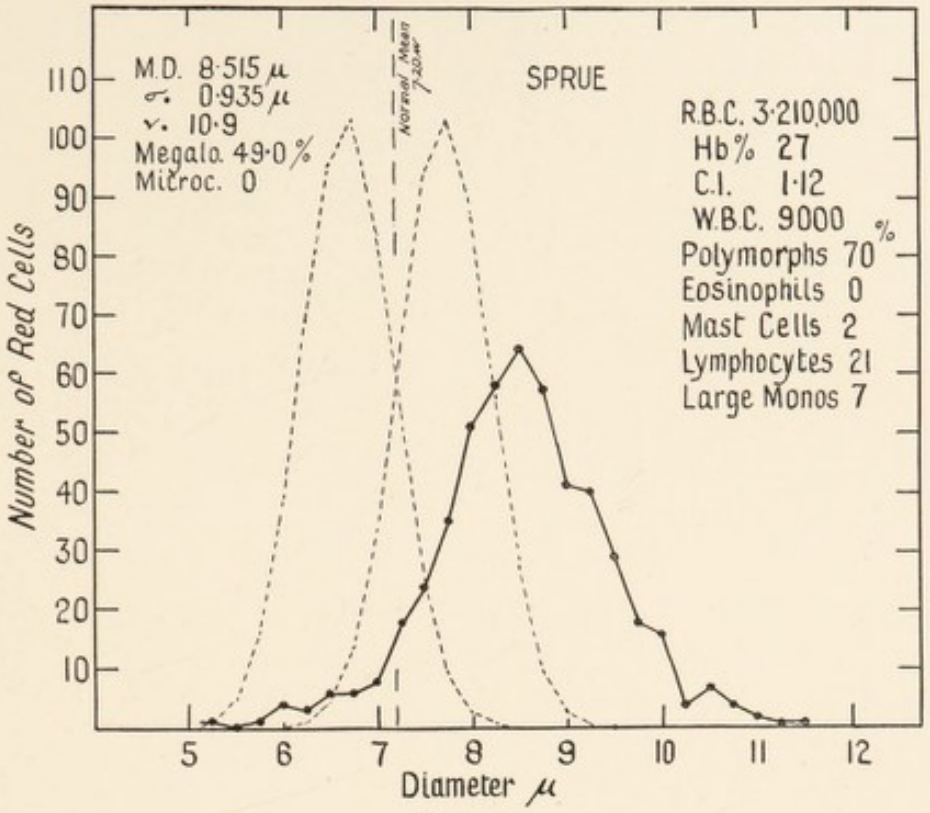
M.D. 8·515 μ
σ. 0·935 μ
v. 10·9
Megalo. 49·0 %
Microc. 0
Normal Mean 7·20 μ
SPRUE
R.B.C. 3·210,000
Hb% 27
C.I. 1·12
W.B.C. 9000
Polymorphs 70 %
Eosinophils 0
Mast Cells 2
Lymphocytes 21
Large Monos 7
Number of Red Cells
Diameter μ

Fig. 39.

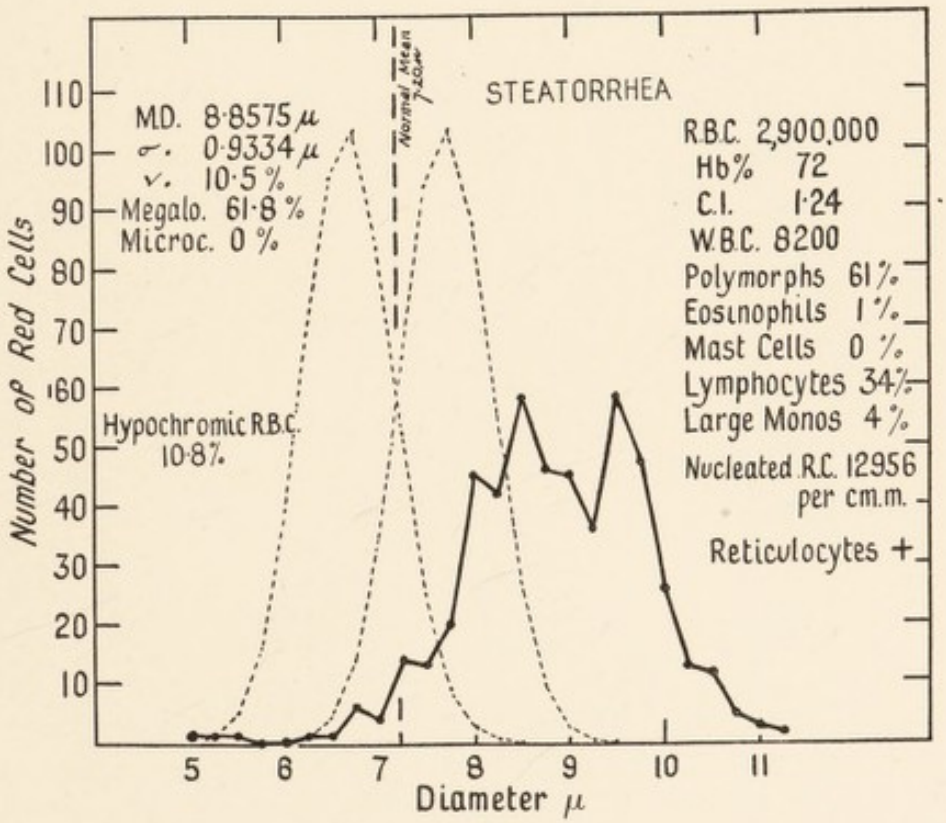
M.D. 8·8575 μ
σ. 0·9334 μ
v. 10·5 %
Megalo. 61·8 %
Microc. 0 %
Hypochromic R.B.C. 10·8%
Normal Mean 7·20 μ
STEATORRHEA
R.B.C. 2,900,000
Hb% 72
C.I. 1·24
W.B.C. 8200
Polymorphs 61 %
Eosinophils 1 %
Mast Cells 0 %
Lymphocytes 34 %
Large Monos 4 %
Nucleated R.C. 12956 per cm.m.
Reticulocytes +
Number of Red Cells
Diameter μ

Fig. 40.

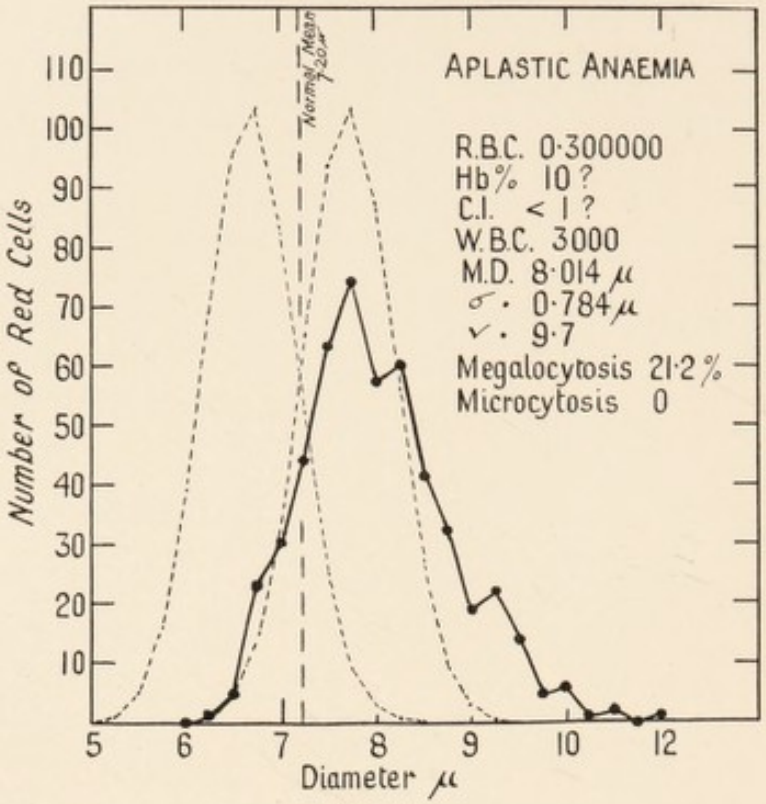
APLASTIC ANAEMIA
R.B.C. 0·300000
Hb% 10?
C.I. < 1?
W.B.C. 3000
M.D. 8·014 μ
σ· 0·784 μ
v· 9·7
Megalocytosis 21·2%
Microcytosis 0
Normal Mean 7·20 μ
Number of Red Cells
Diameter μ

Fig. 41.

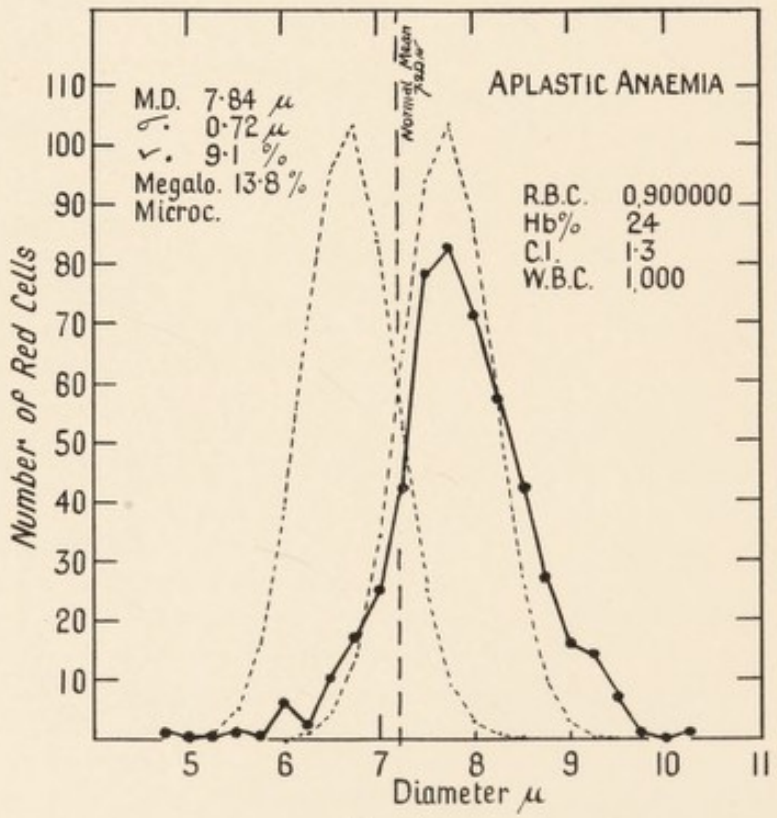
APLASTIC ANAEMIA
M.D. 7·84 μ
σ· 0·72 μ
v· 9·1 %
Megalo. 13·8%
Microc.
R.B.C. 0,900000
Hb% 24
C.I. 1·3
W.B.C. 1,000
Normal Mean 7·20 μ
Number of Red Cells
Diameter μ

Fig. 42.

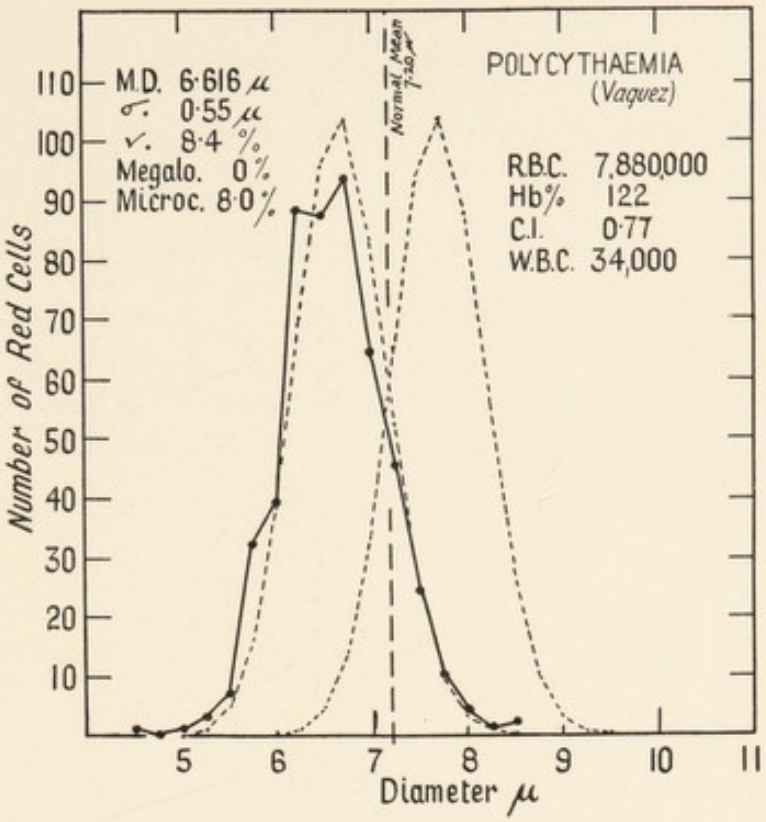
POLYCYTHAEMIA
(Vaquez)
M.D. 6·616 μ
σ. 0·55 μ
v. 8·4 %
Megalo. 0 %
Microc. 8·0 %
R.B.C. 7,880,000
Hb% 122
C.I. 0·77
W.B.C. 34,000
Normal Mean 7·20 μ
Number of Red Cells
Diameter μ

FIG. 43.

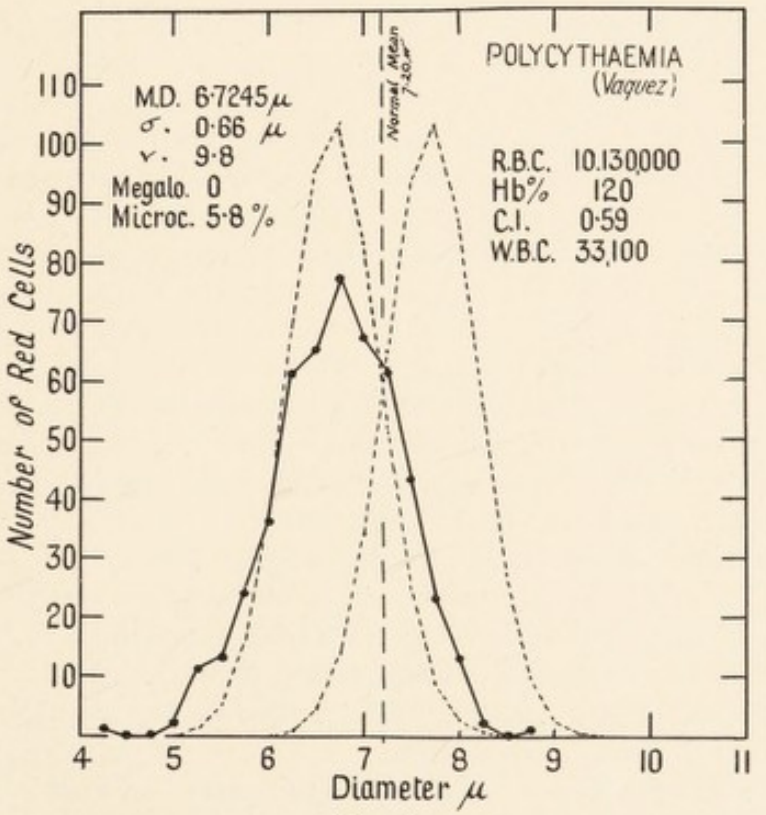
POLYCYTHAEMIA
(Vaquez)
M.D. 6·7245 μ
σ. 0·66 μ
v. 9·8
Megalo. 0
Microc. 5·8 %
R.B.C. 10,130,000
Hb% 120
C.I. 0·59
W.B.C. 33,100
Normal Mean 7·20 μ
Number of Red Cells
Diameter μ

FIG. 44.

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