THE CLINICAL EXAMINATION OF THE HÆMIC LEUCOCYTES

BY

ARTHUR G. PHEAR, M.D.

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I. INTRODUCTORY.

THE object of this communication is to draw attention to the advantages of fluid preparations over the cover-slip method more commonly in vogue in the study of the white corpuscles of blood. In the cover-slip method a thin film of blood is spread on the cover-slip, fixed, and subsequently stained. An initial difficulty is the preparation of a film in which the corpuscles are evenly arranged, so that the cells may be individually observed; the relatively scanty leucocytes are apt to be obscured by the overwhelming numbers of the red corpuscles.

Then, however carefully the film is prepared, there is inevitably some flattening of the cells with distortion of outline, and the preparation is thereby rendered useless for the purpose of accurate drawing or measurement. Further, in making the differential count, there is the uncertainty as to whether the leucocytes are fixed on the cover-slip in the proportion in which they exist in the blood; some may be destroyed in the process of making the film; some may be washed away; others are obscured by the red corpuscles; the outcome in practice is that counts of different films taken from the same blood often give materially different results.

These difficulties are, for the most part, met by examining the blood as a fluid preparation, having previously diluted it with a suitable fluid medium. A fluid should be used of a kind to preserve the white cells as far as possible without distortion, and to this a stain should be added of a strength to bring out clearly the structural detail of the cell without too far modifying its translucency. The thickness of the unflattened cell treated in this manner is an advantage, since, by gradually focussing from above downwards through the depth of the cell, views are obtained at different focal planes which are equivalent to a series of horizontal sections, and a good idea is gained of the true shape and disposition of the nucleus and other structures within the cell.

In one respect the cover-slip film is preferable to the fluid preparation. The fixed film of blood can be mounted and preserved as a permanent object, whereas the fluid preparation is in good condition only for a limited time, beginning to deteriorate in the course of a few days. In clinical work, however, all that is usually desired is to ascertain and record the numerical proportions of the several kinds of leucocyte; no permanent preparation is needed. It is only when unusual forms of leucocytes are present that permanent specimens are required, and in this event they can readily be made, though in every

case it is desirable to supplement the permanent film by *camerâ lucidâ* drawings of the undistorted corpuscles, as they appear in a fresh fluid preparation.

Of the innumerable stains and solvents that are available, I find that the best for general use is a .2 per cent. solution of methylene blue (Grübler) in alcohol 40 per cent.¹ In this solution the distinctive features of the leucocytes are preserved, while the red corpuscles lose their colour and become relatively inconspicuous.

II. METHOD.

(a) A small quantity of the methylene blue solution is drawn up into a pipette. The pipette for the enumeration of the white blood corpuscles supplied with the Thoma-Zeiss apparatus is convenient for this purpose. The fluid should be drawn up to the mark 0.5 on the pipette, since this quantity forms a film of convenient depth when the operation is completed.

(b) The finger, having been carefully cleaned, is pricked with a sharp needle, and a fairly large drop of blood is allowed to exude.

(c) A glass slide is brought into contact with the drop of blood, and as much blood removed as adheres to the slide. Care should be taken to keep the glass slide from touching the finger.

(d) The methylene blue solution is now slowly blown out from the pipette on to the drop of blood, and the two are allowed to mingle. When the fluid has all been expelled, the end of the pipette is wiped, and a current of air is directed through the pipette *on to the surface* of the drop which lies on the slide. By this means eddies are set up, and in a few seconds the blood and methylene blue solution are thoroughly and uniformly mixed; as

¹ A dilute solution of methylene blue in 40 per cent. alcohol, to which a trace of caustic potash and of osmic acid is added, is recommended by Kanthack and Hardy, who found it especially valuable for the study of the finely granular basophile cell. See 'Journal of Physiology,' vol. xvii, p. 88 ("Wandering Cells of Mammalia").

they mingle the colour of the fluid changes from blue to a deep green.

(e) A cover-glass, seven eighths of an inch square, is placed over the fluid, and the latter spreads out as a thin film, none of which exudes beyond the margin of the cover-slip if the above directions have been closely followed. The edges are then sealed with vaseline, and a preparation is obtained which may be examined at leisure with an immersion lens. The whole process takes about three minutes.

In working with a high power, it is important to regulate the light, as many details of the cell structure are seen to best advantage with a comparatively dim light. The diaphragm should be adjusted until the most favourable illumination is obtained.

On examination of the fluid film, it is found that the red corpuscles have lost their colour, and are visible in outline only as transparent discs, which do not interfere with the study of the white corpuscles. The white corpuscles are therefore the only conspicuous objects in the field, and of these the several varieties can readily be distinguished, notwithstanding the absence of an acid stain. That the cells are free from the distorting influence of pressure may be shown by measuring the distance from the upper surface to the lower surface of the cell by means of the fine adjustment. This can readily be done in the case of the coarsely granular cell, of which both the upper and the lower limits can be easily and clearly defined. The diameter from above downwards, in every case that I have measured, corresponds almost exactly with the diameter from side to side; the cell is therefore approximately spherical.

The rapidity with which the fluid film can be prepared and the uniformity of the result render the method well suited for clinical work. The aspect of the leucocytes is, however, in certain respects, peculiar and different from their appearance in a dried film made in the more ordinary way, and I therefore ask leave to add a short

description of the leucocytes as they appear in a fluid film made from normal blood, as a preliminary to the employment of the method in the study of forms that may deviate from the normal.

III. THE NORMAL LEUCOCYTES.

Five kinds can be distinguished in such a preparation. In order of frequency, these are :—

(1) Rounded cells, with polymorphous nuclei and finely granular protoplasm (“*finely granular oxyphile cells*” or “*polymorphonuclear cells*”).

(2) Rounded cells, with single rounded nuclei and clear protoplasm which stains feebly or not at all with methylene blue. The group includes the *lymphocyte*, the *large hyaline cell*, and intermediate grades.

(3) Large cells, usually oval, with single nuclei of irregular shape, and protoplasm which stains diffusely with methylene blue.

(4) Rounded cells, with protoplasm containing large refractile granules (“*coarsely granular oxyphile cells*,” or “*eosinophile cells*”).

(5) Small cells, usually rounded, with lobed nuclei, and protoplasm which stains a diffuse mauve or purple tint with methylene blue, and contains granules of medium size, and deep blue or violet colour. The granules are often aggregated at one or more points peripherally (“*finely granular basophile cells*”).

The Polymorphonuclear Cell.

Contour, rounded.

Diameter,¹ 9 to 10 μ .

Numerical proportion, 45 to 60 per cent.

The *protoplasm* of this cell does not stain with methylene blue, and shows no structure beyond a fine granula-

¹ Measurements were taken of 100 cells. The diameter was 8.5 μ in 2, 9 μ in 16, 9.5 μ in 41, 10 μ in 38, 10.5 μ in 2 cells, and 11 μ in 1 cell.

tion. The granules are minute, numerous, and evenly diffused throughout the protoplasm. When in focus they appear as dark dots. They have no affinity for methylene blue, being no more conspicuous in an alcoholic solution of methylene blue than in an alcoholic solution of similar strength in which no methylene blue is dissolved.

The *nucleus* is a complex body consisting of several rounded lobes, disposed irregularly in the central part of the cell. At first sight the lobes may seem to be separate from one another. If, however, the focus be slowly changed, it can, in almost every instance, be made out that connecting strands, of varying thickness, pass from one lobe to another. There is, in fact, no break in the continuity of the parts of the nucleus, which exists as an elongated body, much contorted, in places deeply constricted, elsewhere bulged into rounded swellings, of which there are three, four, or five, rarely a greater number. As the focus is altered, one part after another of the nucleus comes into view, and it is appreciated as a structure of substantial thickness, the limits of which can be defined in the vertical as well as in the horizontal plane (see Fig. 1).

FIG. 1.

Drawings of a polymorphonuclear cell at different focal planes, to show the continuity of the parts of the nucleus. The letters *a* to *d* indicate the focal planes from above downwards. The loose coil of the elongated nucleus can be traced by following the numbers 1 to 8.

The cells represented in this and subsequent figures were all drawn in outline with camera lucida.

Occasionally the nucleus is S-shaped, or arranged in the form of a horseshoe, and lies in one plane; it is then obviously an undivided body. Less commonly the parts of the nucleus are closely coiled and form a rounded mass resembling the simple rounded nucleus of the hyaline cell; but on close observation the intricacy of its structure has always been evident; I have never observed in my preparations, in which the death of the corpuscle is almost instantaneous, the rounded nucleus described by Sherrington¹ in cells which he allows to die slowly.

The nucleus is coloured deeply with methylene blue, though not so deeply as in the case of the hyaline leucocytes. One or more points, more strongly stained than the surrounding parts, indicate the position of nucleoli.

The group of polymorphonuclear cells is sharply defined; no cells are present in normal blood intermediate in structure between the cells of this and other groups of leucocytes.

Rounded Cells, with single rounded Nuclei and clear Protoplasm.

The group includes:—

(a) **Lymphocytes**, in which the nucleus, occupying nearly the whole cell, is surrounded by only a thin envelope of protoplasm.

(b) **Large hyaline cells**, in which the diameter of the cell is at least twice the diameter of the nucleus.

(c) **Intermediate forms.**

Diameter, varies from 6 to 10 μ , rarely more.

Numerical proportion, 35 to 45 per cent. of total number of leucocytes.

A prominent feature of this group is the great variability in the size of the cells which compose it. All gradations between the lymphocyte and the large hyaline cell are present and readily found in normal blood, and

¹ 'Proc. Internat. Congress of Physiologists,' Liège, 1892.

it is impossible to split the series into any but the most arbitrary divisions, since the members of the group resemble one another in every essential point except that of size. The cells of intermediate size are probably rightly regarded as transitional forms, or as stages in the growth of the lymphocyte into the larger hyaline cell. The size of the nucleus is remarkably constant throughout the series; its diameter is seldom less than $4.5\ \mu$ or more than $5.5\ \mu$. The growth of the cell is, therefore, chiefly an increase of the extra-nuclear protoplasm.

Cells showing gradations from the lymphocyte to the large hyaline cell.

The larger cells of the group correspond with the "myelocytes" of Sherrington.¹

The *protoplasm* of these cells is always clear, free from granules, and remains in the methylene blue solution for some time without taking the stain, or at most being faintly tinged with blue. Eventually an exceedingly fine

¹ Vide 'Proceedings of the Royal Society,' vol. lv, p. 193. The name "myelocyte" is more commonly given to certain cells that are not represented in normal blood.

granulation becomes visible, probably due to staining of the nodes of the protoplasmic network. There is never a strong diffused blue coloration of the protoplasm, as in certain cells to be described.

The *nucleus* is a simple, undivided structure, with a round outline, which in many cases is broken by an indentation, more or less deep. The notch is more often present than at first sight appears; for it is only conspicuous when in profile, *i.e.* when the position of the cell is such that the notch is situated laterally. If, from the position of the cell, the notch lies, not at one side, but above or below, the visible outline of the nucleus is not broken by the notch. Occasionally in the fluid preparation a lymphocyte, whose nucleus appears round, rolls over, and with the change of position an indentation comes into view. The nucleus stains with methylene blue more strongly in this than in any other kind of leucocyte. All parts of the nucleus do not, however, take the stain equally well; the colour is concentrated at certain points and areas within the nucleus, and the result is a patchy or mottled appearance.

Large Oval Cells, with irregular Nuclei, and Protoplasm which stains diffusely with Methylene Blue.

The cells about to be described are not usually recognised as forming a distinct group of the hæmic leucocytes. They are probably classed in most accounts with the large hyaline cells, though in several respects they differ remarkably from the large hyaline cell as it is commonly figured. In the plate accompanying the communication on "Wandering Cells of Mammalia," by Kanthack and Hardy, the large hyaline cell appears as a rounded body, with rounded nucleus, the latter being deeply stained with methylene blue, while the cell protoplasm is not stained. In the case of the cell to which attention is now drawn, the contour of neither cell nor

nucleus is round, and the protoplasm is readily stained in solutions of methylene blue.

The cells are remarkable both for their size and shape ; they are as a rule oval, and larger than the leucocytes of any other group ; in length they vary from 10 to 14 μ , and in breadth from 7 to 10 μ . They are always to be found in normal blood, and their proportion to the total number of leucocytes in circulation is fairly constant, ranging from 5 to 10 per cent.

Large oval cells, showing various forms of the irregular nuclei.
The faint shading indicates the staining of the protoplasm.

The *protoplasm* is peculiar in its affinity for methylene blue ; it takes the stain rapidly, becoming coloured a uniform and rather opaque blue, without at first a trace of granulation. The coloration is visible in the most recently prepared specimen, and is not subsequently deepened to any extent, though a fine granulation may become evident, perhaps due to the more intense staining of the nodes of the protoplasmic network. The colour reaction with methylene blue is characteristic ; in the polymorphonuclear cell the finely granular protoplasm remains unstained ; in the lymphocyte and larger hyaline cells the staining of the protoplasm is comparatively faint, and is not evenly diffused, but appears as a fine blue granulation ; in the coarsely granular leucocyte the staining of the large granules gives to the cell, as a whole,

a greenish tint, while the protoplasm of the "basophile" cell acquires a mauve or purple tint, not likely to be confounded with the opaque blue coloration of the large oval cell under notice.

The *nucleus*, always irregular, varies much in shape. It is large, occupying a considerable part of the cell, and is usually elongated in the direction of the long axis of the cell. The form commonly seen is that in which the outline on one side forms an unbroken curve, while on the other side it is interrupted by one, two, or three notches, giving to the whole structure a lobed or reniform shape. Sometimes there appear to be two separate oval nuclei, lying obliquely across the cell, one at either end; as the focus is lowered, a broad connection comes into view between the two lobes, which are, in fact, the bulged extremities of a single reniform nucleus, lying with the notch upwards (see Fig. 4). The lobes of the nucleus are occasionally arranged in an irregular cup-like manner around a central depression. Other forms are drawn in outline (Fig. 3), from which it is seen that the general

Large oval cell. The same cell is drawn at two focal planes. At the upper plane (a) there appear to be two nuclei. On lowering the focus these are seen to be the extremities of a large single nucleus.

characters of the nucleus are constant, though in detail of outline it exhibits much diversity.

The nucleus stains with methylene blue, but not so

strongly as in the preceding groups. Two or more nucleoli can usually be distinguished.

Transitional Forms between the Lymphocyte and the Cell just described.

Cells intermediate in structure between the lymphocyte and the large oval cell are found circulating in the bloodstream, and, indeed, are so far from uncommon as to give reason for supposing that a certain number of the lymphocytes develop while in circulation into the larger and more differentiated cells. The transitional forms include cells which resemble lymphocytes in size and every other respect except that the nucleus, which nearly fills the cell, is not rounded, but elongated, and sharply bent on itself in the form of a half spiral. One of these cells is figured (Fig. 5), the coiled arrangement of the nucleus being shown by camera lucida drawings taken at different

a, b, and c are drawings of one cell at three focal planes, showing the closely coiled nucleus. In *d* the parts of the nucleus are shown superimposed.

focal planes. In other cells, no larger than lymphocytes, the nucleus has a cup-like form, the nuclear indentation

(see p. 339) being deepened, with thickened and irregular margins. Other cells may be found, larger than the lymphocyte, in which there is a broader margin of protoplasm, and in which the protoplasm stains diffusely with methylene blue, and surrounds a nucleus of irregular form.

The lymphocyte is the only cell to which the large oval cell has obviously close relations; no transitional forms are found between the large oval cell and any other kind of leucocyte in normal blood.

It has already been pointed out that the lymphocyte is closely related to the large hyaline cell. From the study of the leucocytes in circulation, it would therefore appear that the lymphocyte is an immature form which may develop either into the large hyaline cell with rounded nucleus and clear protoplasm, or into the larger oval cell with irregular nucleus and protoplasm which stains opaquely and diffusely with methylene blue. Moreover, it is probable that the growth of the lymphocyte into either of these two forms occurs while it is actually in circulation, since the intermediate stages of its growth are commonly present and readily found in the bloodstream. It may be remarked here that, so far as the study of the hæmic leucocytes goes, there is no ground for supposing that the lymphocyte develops into any form of leucocyte other than the two mentioned. Intermediate forms are not present in normal blood between the lymphocyte on the one hand and the polymorphonuclear cell, the coarsely granular cell, or the "basophile" cell on the other.

The Coarsely Granular Cell.

Contour, rounded.

Diameter, 9.5 to 10.5 μ .¹

Numerical proportion, 1 to 2.5 per cent.

¹ Measurements were taken of 50 cells. The diameter was 9 μ in one cell, 9.5 μ in 8, 10 μ in 35, 10.5 μ in 5 cells, and 11 μ in one cell. The diameter usually given is too large, perhaps because the measurements are taken from dried films, in which the cells are necessarily flattened.

Drawings of coarsely granular cells (the lower ones in outline only), showing various forms of nuclei.

In fluid preparations made with methylene blue the attention is immediately arrested by the coarsely granular cells, not only on account of the large refractile granules which they contain, but by reason of their colour.

The *granules* have some affinity for methylene blue, and in the fluid preparations are stained, not blue, but a peculiar green or yellowish-green colour, which gives to the whole cell a striking appearance. The individual granule, when its upper surface is in focus, appears as a glistening object, rounded or slightly oval, with dark outline; as the focus is lowered the glistening appearance is lost, and at the focus of its equatorial plane the granule is seen as an opaque body of a uniformly dark green, almost black colour.

The *nucleus*, often placed excentrically, is not so complex as that of the polymorphonuclear cell; it is usually

bi-partite, each part being rounded, and containing a nucleolus. A connecting strand between the two parts can sometimes be distinguished; often it is obscured by the granules. Less commonly the nucleus is tri-partite, or assumes other forms, some of which are figured. The deep blue stain of the nucleus is in marked contrast to the greenish colour of the rest of the cell.

The "Basophile" Cell.

Contour, rounded, rarely oval.

Diameter, 8μ .¹

Numerical proportion, .3 to 1 per cent.

"Basophile" cells, showing lobulation of nucleus. In the detailed drawing the peripheral aggregation of the granules is indicated.

The basophile cell is among the smallest and is the rarest of the leucocytes present in normal blood. In

¹ Measurements were taken of 29 cells. Of these the diameter was 7μ in one cell, 7.5μ in 2, 8μ in 15, 8.5μ in 4, and 9μ in 3 cells. Four cells were oval; two of these measured $7\mu \times 8\mu$; the remaining two were $7\mu \times 9\mu$

outline it is usually round, exceptionally oval. The *protoplasm* stains diffusely with methylene blue, the resulting tint being not a pure blue, but a mauve or purple. This colour reaction is distinctive, though the cause of it is not clear; it is probable that the basophile cell has a special affinity for methylene red, which is often present in small quantity in specimens of methylene blue. The protoplasm contains granules, usually described as fine, but in human blood, at any rate, intermediate in size, being coarser than the fine granules of the polymorphonuclear cell, and finer than the large granules of the coarsely granular cells. The granules stain deeply with methylene blue, and are apt to be massed peripherally in one or more dark violet clumps, close to the surface of the cell.

The *nucleus* is a complex structure, consisting of rounded lobes, of which there are seldom less than four, often five, connected with one another, and usually aggregated in a compact mass near the centre of the cell. The parts of the nucleus are stained a dull slate or grey colour with methylene blue.

The above account of the leucocytes is based on preparations made with a .2 per cent. solution of methylene blue in alcohol 40 per cent. Other stains and other media may be used in the preparation of the fluid film without sacrificing the advantages of the fluid method, and although I believe an alcoholic solution of methylene blue to be the most useful medium for clinical work, yet for special purposes, such as the investigation of the staining affinities of certain granules, it is necessary to vary the nature of the stain, and sometimes of the fluid used for dilution.

In **alcohol 40 per cent.** (without stain) the red corpuscles are laked. The *polymorphonuclear leucocytes* show much detail of structure. The fine granules appear as small, distinct, dark points; they are as conspicuous as in a methylene blue preparation. The outline of the complex

nucleus is sharp, and the nuclear network is often beautifully clear, more so than in most stained specimens. The nuclei of the *lymphocytes* and *larger hyaline cells* are also remarkable for their sharp outline with distinct nuclear reticulum and nucleolus. The protoplasm of these cells is clear and free from granules. The protoplasm of the *large oval cells* displays an immense number of dark granular points, very fine, a few about the same size as, but the larger number finer than, the granules of the polymorphonuclear cell. The *coarsely granular corpuscles* are striking objects. The large granules, owing to their refractile nature, appear as glistening bodies when their upper surface is in focus, changing to rounded black dots as the focus is lowered to that of the equatorial plane.

In a solution of **osmic acid 1 per cent.** (without stain) the red corpuscles are not laked. The fine granules of the *polymorphonuclear cells* are visible as dark dots, and are more sharply defined than in the alcohol 40 per cent. preparation. The position of the nucleus is indicated by a clear space, free from granules; there is no distinct nuclear outline nor reticulum. In many of the *lymphocytes* a few dark granules of large size are visible in the protoplasmic rim. I have not seen these in any but osmic acid preparations. The granules of the *coarsely granular cells* are conspicuous objects, but appear no darker in osmic acid than in alcohol preparations.

Osmic acid (1 per cent.) fixes the *red corpuscles* without distortion, and it is a convenient solution with which to prepare fluid films when red corpuscles of abnormal shape are present. Only a minute drop of blood should be taken, and this should be thoroughly mixed with the diluting solution, to which a small quantity (.2 per cent.) of methyl green may with advantage be added.

Acetic acid (.3 per cent., as recommended by Thoma) causes the red corpuscles to disappear, but is not a good medium in which to examine leucocytes, since it rapidly produces changes in their structure. In a solution of methylene blue (.2 per cent.) in acetic acid (.3 per cent.)

the structure of the *polymorphonuclear cells* is for a few minutes beautifully distinct; the cell then becomes, as it were, ballooned on one side, a crescentic space appearing between the main mass of the cell and an external envelope, which seems to have separated from the granular protoplasm. In many cells the change goes no further for a considerable time; in a few the cell-protoplasm quickly becomes swollen and ill-defined, the nucleus only retaining its form and stain; ultimately the nucleus swells, becomes misshapen, and its colour fades. Similar changes take place in some of the *lymphocytes*, a thin outer layer separating and standing away from the body of the cell. Lymphocytes that are swollen in this way acquire a likeness to the large hyaline cells, and are liable to be estimated as such in the course of a differential count. Of *coarsely granular cells* only a small number is found, and these are materially altered. The cell, as a whole, is swollen; the granules are not visible as discrete bodies, but are broken down into a homogeneous transparent substance of a yellowish-green colour; the nucleus is ill-defined and feebly stained. In differential counts made with acetic acid preparations, the percentage of coarsely granular cells is considerably below the average, and probably many of them are actually destroyed.

Eosin in alcohol (40 per cent.).—The appearance of the *coarsely granular cell* in fluid preparations made with a dilute solution of eosin is very remarkable. A solution of convenient strength may be obtained by drawing a saturated solution of eosin in 40 per cent. alcohol up to the mark 1 on the white corpuscle pipette (Thoma-Zeiss); the bulb is then filled to the mark 11 with alcohol 40 per cent. A dilution of 1 in 11 is thus obtained, and with this fluid the preparation is made in the ordinary way. The result is striking in normal blood, and still more so when there is an excess of coarsely granular cells. The large granules stand out as conspicuous objects, highly refractile and coloured a deep crimson, while the other leucocytes are none of them more than feebly stained.

It is noteworthy that the so-called "fine oxyphile granules" of the *polymorphonuclear cells* are not stained at all deeply in this solution. The red corpuscles are laked.

Methyl green (4 per cent.) in alcohol (40 per cent.).¹—The red corpuscles are laked. The *nuclei* of all the leucocytes take the stain brilliantly; the protoplasm in all cases remains unstained. Methyl green is one of the most satisfactory stains for the nuclei of the lymphocytes and the larger hyaline cells; they stain sharply and clearly with but little loss of translucency. It is remarkable that the *protoplasm* of the large oval cell and of the "basophile cell," both of which stain characteristically with methylene blue, is unstained in solutions of methyl green.

The small *granules* of the polymorphonuclear cell have no affinity for methyl green. The granules of the basophile cell are not stained, notwithstanding the fact that methyl green is a double salt in which the colouring principle plays the part of a base. The large granules of the coarsely granular cell are tinged with green.

IV. THE DIFFERENTIAL COUNT.

For making a differential count of the leucocytes the fluid film prepared with an alcoholic solution of methylene blue is convenient, since in this the red discs are inconspicuous, and the white corpuscles, which it is desired to enumerate, alone attract the attention. The method also appears to fulfil the conditions necessary to an accurate enumeration. It is essential to accuracy that the blood should be uniformly mixed with the diluting fluid. That this is so in the fluid preparation, I have satisfied myself by making separate counts over different areas of the same preparation; the results have always been close, the variation in the case of the commoner forms amounting to no more than 2 or 3 per cent. I find also that different preparations made from the same individual, at

¹ The solution rapidly undergoes changes and therefore must be freshly made.

the same time, tally closely in the relative proportion of the several leucocytes; from this uniformity it may be inferred that the percentage of the leucocytes is substantially the same in the fluid film as in the blood, and that the preparation is a reliable index to the state of the blood.

The greater the number of leucocytes brought under notice, the more accurate are the results arrived at. No fewer than 500 should be counted. A mechanical stage is almost a necessity; it not only ensures that all the leucocytes within a given area are included in the count, but it further prevents the risk of going over the same ground twice. It has been my practice to count the cells with an immersion lens; the extra time involved is well spent, since it is seldom that any uncertainty is felt as to which group a given leucocyte should be placed in when a one-twelfth immersion lens is used; whereas, in working with a lower power, this difficulty not infrequently arises.

With a view of testing the somewhat varying statements on the relative proportions of the several kinds of leucocytes in healthy human blood, I have made a series of forty-nine observations, in the course of which a total of over 26,600 leucocytes were counted. Dealing with these figures as a whole, the percentages were as follows:

Polymorphonuclear cells, 54.

Rounded hyaline cells, 36 (of these 18 per cent. were lymphocytes).

Large oval cells, 8·4.

Coarsely granular cells, 1·9.

Cells with basophile granules, ·7.

For each kind of leucocyte a further calculation was made, the mean being taken, in the first place, of the percentages which were in excess of the average for that leucocyte, and, in the second place, of the percentages which were below the average. In this way what may be termed a mean maximum and a mean minimum percentage has been estimated for each leucocyte.

TABLE I.

Total number of leucocytes counted, 26,644.

Number of observations, 49.

			Mean minimum percentage.		Mean maximum percentage.
Polymorphonuclear cells	.	.	48	.	59
Rounded hyaline cells	.	.	30	.	40
Large oval cells	.	.	6·8	.	11
Coarsely granular cells	.	.	1·2	.	2·5
"Basophile" cells	.	.	·4	.	1·1

In estimating the percentage of the various leucocytes, some authorities take no account of the lymphocytes on the ground that they are immature forms. There is, however, great difficulty in deciding which corpuscles are immature and which have reached their full development. It is likely that, of the group of cells with hyaline protoplasm, all but the largest hyaline cells are as properly termed immature as the lymphocytes, and we have no certain knowledge, even with regard to the largest hyaline cells, as to whether they have attained the final stage of their growth. I have, therefore, preferred not to discriminate between adult and immature forms, but to include in the count all the leucocytes that were seen.

The observations from which the figures in Table I are computed were made on more than one individual, and not all at the same hour of the day. Among them is included a series of observations, made on one individual and at the same hour of the day, the time chosen being that at which the influence of meals is least likely to be felt, viz. immediately before breakfast. In this series 6000 leucocytes were counted in the course of ten observations, and the results are summarised in Table II.

TABLE II.

Total number of leucocytes counted, 6000.

Number of observations, 10; made on one individual, on each occasion before the first meal of the day.

	Smallest percentage observed.	Mean minimum percentage.	Average percentage.	Mean maximum percentage.	Largest percentage observed.
Polymorphonuclear cells . . .	47	49	50	53	54
Rounded hyaline cells . . .	37	38	40	42	45
Large oval cells . . .	4.5	5.7	6.9	8	10
Coarsely granular cells . . .	.8	1.5	2	2.6	3.4
"Basophile" cells . . .	.2	.3	.45	.6	.8

It is interesting to note the small range within which the percentages of the two common forms of leucocyte varied—in the case of the polymorphonuclear cell between a mean minimum of 49 per cent. and a mean maximum of 53 per cent., and in that of the rounded hyaline cells between 38 and 42 per cent. The range of variation is naturally much greater proportionately for the leucocytes of rare occurrence than for the commoner forms. In the case of the coarsely granular corpuscle and the basophile corpuscle, the numbers that actually passed under observation in the course of the second series of ten counts were so small as to afford no secure basis for the calculation of averages. I prefer, therefore, to rely on the larger numbers dealt with in Table I, as indicating more accurately the average occurrence of these rarer leucocytes.

Further observations that I have made all tend to show that for one individual the daily variation in the relative proportions of the leucocytes is not great, provided the disturbance occasioned by meals be avoided. Thus in one case the percentage of the polymorphonuclear

cells on four consecutive days was 47, 48, 46, and 44; the percentage of the hyaline cells was 42, 40, 42, and 43, including lymphocytes, the numbers of which were 14, 10, 12, and 12 per cent.; large oval cells were present in the proportion of 9, 11, 9, and 9 per cent.

I believe it to be an essential condition that a long interval be allowed to elapse between the last meal and the occasion of making the count. The most convenient time is doubtless before breakfast; at no other hour of the day can the disturbing influence of meals be eliminated, and for no other time is a normal standard available for comparison.

For a healthy individual the following figures may be taken as representing the limits within which the percentage of the several cells usually lies at the time stated; it is not to be inferred that transgression of these limits is necessarily of pathological import, only that under circumstances of health it is exceptional for the limits to be exceeded in either direction.

Polymorphonuclear cells 45 to 60 per cent.

Rounded hyaline cells, including lymphocytes, large hyaline cells, and intermediate forms, 35 to 45 per cent.

Lymphocytes, in which nearly the whole cell is occupied by the nucleus, constitute from 10 to 20 per cent. of the total number of leucocytes.

Large hyaline cells, arbitrarily defined as those in which the diameter of the whole cell is at least twice the diameter of the nucleus, are not present in greater numbers than 2 per cent.

Large oval cells 5 to 10 per cent.

Coarsely granular cells 1 to 2·5 per cent.

Cells with basophile granules ·3 to 1 per cent.

The percentage for the polymorphonuclear cells (45 to 60 per cent.) is considerably below that stated by most authorities. Copeman¹ gives 75, Schäfer² 70 to 90, and

¹ 'System of Medicine,' Allbutt (1898), vol. v, p. 416.

² 'Text-book of Physiology' (1898), p. 152.

Kanthack and Hardy¹ 75 to 90, as the normal percentage in human blood. The latter authors excluded "immature" cells from their count; this partly explains the discrepancy between their results and mine. Also the figures given by me are based on observations made at the hour when the proportion of the polymorphonuclear cells is at its lowest. There is a fairly constant decrease of about 10 per cent. between 10 o'clock at night (*i. e.* about two hours after dinner) and 8.30 the next morning, and at no other time of day is the percentage of these cells so low as in the early morning before breakfast. I do not, however, propose to deal with the diurnal variation of the hæmic leucocytes, either as regards their total number or their relative proportions. The problem relating to the part played by meals and other factors in influencing their variation is an intricate one, and I have already occupied too much time in the discussion of matters that may seem to be more of physiological than of pathological interest; yet I must claim that from the clinical standpoint they are of importance, and on this ground I ask your indulgence.

¹ Loc. cit., p. 92.

(For report of the discussion on this paper, see 'Proceedings of the Royal Medical and Chirurgical Society,' Third Series, vol. xi, p. 109.)

DESCRIPTION OF PLATE VIII

Illustrating the Clinical Examination of the Hæmic Leucocytes
(ARTHUR G. PHEAR, M.D.).

The leucocytes are shown as they appear in fluid films prepared with the methylene blue solution described in the text.

The reaction of the several cells with methylene blue is indicated. The protoplasm of the polymorphonuclear cell (*a*) and of the lymphocyte (*b*) is unstained; that of the large hyaline cell (*c*) shows a faint blue granulation; the protoplasm of the large oval cell (*d*) is stained a diffuse blue colour; the granules of the coarsely granular cell (*e*) have a greenish tint; the protoplasm of the cell containing basophile granules (*f*) is mauve.