CHAPTER XXIII.

THE BLOOD.

Examination of the Blood.

The essentials of blood examination as a part of physical diagnosis are as follows:

I. *Hæmoglobin test* (Tallqvist in all cases).
II. Study of a *stained blood film* in most cases.
III. *Total leucocyte count* (Thoma-Zeiss) in many cases.
IV. Count of *red corpuscles and Widal reaction* in a few cases.
V. Coagulation time, rarely.

I will now give a brief account of each of these methods and of the interpretation of the data obtained by them.

I. Hæmoglobin.

(*a*) The Tallqvist scale consists of ten strips of red-tinted paper corresponding to the tint of a filter paper of standard quality when saturated with blood containing ten per cent, twenty per cent, thirty per cent, etc., hæmoglobin up to one hundred per cent. To perform the test we puncture the lobe of the ear with a glover's needle (*not* with sewing needle), saturate a strip of the filter paper which is bound up with the scale, in the blood of the patient to be examined, and compare the tint of this strip with the different standard tints in the scale. Always saturate at least half a square inch of filter paper with blood and allow it to dry until the gloss has disappeared. *Do not blot it*, and do not delay in making the comparison after the humid gloss has disappeared. Stand with the light behind you or at one side of you; use daylight always.

The test is not accurate within ten degrees, but a degree of accuracy greater than this is very rarely required for any purpose of diagnosis, prognosis, or treatment. In rare cases, when a more accurate reading is needed, we may use the instrument of Gowers as modified by Sahli.

(*b*) Sahli's instrument see (Fig. 209) must be obtained from one of the firms recommended by him,[1] else the standard solution is likely to be inaccurate in color. To use the instrument we first put a few drops of water[2] into the empty tube (Fig. 209, *B*), then suck up blood with the pipette (Fig. 209, *C*), until the mark 1 is reached. Wipe the point of the pipette and immediately blow out the blood into the water at the bottom of the tube (*B*). Suck this mixture of blood and water back into the pipette and blow it out again twice to cleanse the pipette. Next add water from the dropper (*D*), a few drops at a time, until the tint of the mixture of the blood and water is the same as that of the standard solution, when both are looked at with transmitted light. After each addition of water close the end of the tube with the thumb and invert it twice, then scrape the thumb on the edge of the tube so as to rub off any moisture deposited there during the process of inversion. As the tint of the mixture of blood and water approaches that of the standard solution, add the water two drops at a time, and close the eyes for a few seconds between each two attempts at reading. When the colors in the two tubes seem to be

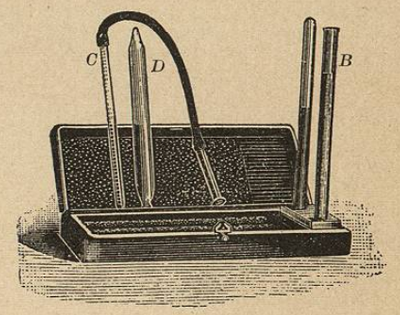


FIG. 209.—Gowers' Hæmoglobinometer. *B*, Diluting tube; *C*, pipette; *D*, dropper.

[1] Holtz or Büchi of Berne.

[2] The description here given follows Gowers.

Sahli—"*Fill concavity* of *empty tube* with decinormal HCl solution," and blow the blood into this—then dilute with water as above.

identical, read off the figure corresponding with the meniscus of the column of fluid in the tube. The resulting figure represents the percentage of hæmoglobin.

(*c*) *The Color Index.*—The data to be obtained by these instruments stand for the amount of the coloring matter in a given unit of blood when compared with the amount in a similar unit of normal blood. When the hæmoglobin percentage is low, anæmia is always present, and the degree of anæmia is measured by the amount of reduction in the hæmoglobin per cent. But the percentage of hæmoglobin is not a measure of the number of corpuscles present in a given unit of blood, for if the corpuscles are large and contain each of them a relatively large amount of hæmoglobin, they may be considerably diminished in number and yet furnish a normal bulk of hæmoglobin, as tested by either of the instruments described. Thus in pernicious anæmia the corpuscles are often so large that they contain nearly one-third as much again as a normal corpuscle, so that even though their number is considerably diminished they may carry a normal amount of hæmoglobin. This condition is known as a "*high color index.*" On the other hand, the number of red corpuscles may be normal, yet each corpuscle so deficient in hæmoglobin that the hæmoglobin in a given quantity of blood is as low as forty or fifty per cent. This state of things is often found in chlorosis or in any form of secondary anæmia (see below, page 475). When the diminution in the number of red corpuscles is greater than the diminution of hæmoglobin, we say that the color index is high, meaning that each corpuscle carries more hæmoglobin than normal. Thus if we have a red count of two millions and a half of red cells, and each cell contained the normal amount of hæmoglobin, the hæmoglobin percentage would be fifty, representing a reduction in hæmoglobin proportional to the reduction in the red cells; but if with the same count we had a hæmoglobin percentage of seventy-five, this would mean that each corpuscle contained half as much again as compared with the hæmoglobin in normal red cells. Here we should say that the color index is 1.5. Five million red cells and one hundred per cent of hæmoglobin give a color index of 1; so do four million red cells with eighty per cent of hæmoglobin, three million and sixty per cent, two million and forty per cent, and so on. An example of low color index would be four million red cells with forty per cent hæmoglobin, representing a color index of 0.5; or three million red cells with thirty per cent hæmoglobin, representing again a color index of 0.5.

The diagnostic significance of the color index is briefly this: Any diminution in hæmoglobin means anæmia, but a diminution in hæmoglobin with a high color index suggests, though it does not prove, pernicious anæmia, while a low color index points to chlorosis or secondary anæmia of any type. Normal color index, despite anæmia, is most often found immediately after hemorrhage.

II. Study of the Stained Blood Film.

To recognize the *presence* and the *degree* of anæmia one needs only the *hæmoglobin test*, but to determine the *kind* of anæmia, to study the leucocytes, or to search for parasites we need the stained blood film. Two processes are now to be described:

1. Preparing the film.
2. Staining.

1. *Blood films* may be spread on slides or on cover glasses. The first method is the easier; the second gives better preparations. To prepare blood films on slides, dip two slides in water and rub them clean with a towel or handkerchief; put a drop of blood near one end of one slide, put the other slide against the drop, and rest it evenly upon the first, as shown in Fig. 210. Next draw the upper slide along horizontally, so as to spread the drop over the whole surface of the lower slide. The process may then be repeated, reversing the slides and using as a "spreader" the one on which the

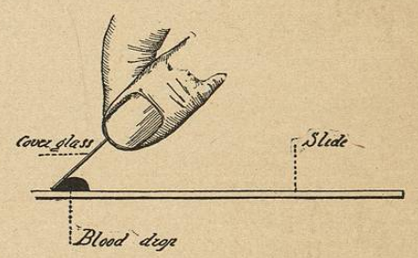


FIG. 210.—Method of Spreading Blood Films.

film has already been prepared. This method is so simple that one can usually succeed with it at the first attempt, but the corpuscles are not spread quite so evenly as in cover-glass preparations and it is somewhat more difficult to get a perfect stain.

The *cover-glass method* requires a much greater degree of cleanliness and manual dexterity than the slide method. Cover glasses must be washed in water and then *thoroughly* polished with a *silk* (not cotton or linen) handkerchief. The success of the whole process depends upon the thoroughness of the polishing. Every part of the glass must be thoroughly gone over, taking care not to omit the corners. This is rather tedious and often drives us to use slides, which can be much more quickly prepared. With cover glasses we must remove not only all dirt and grease, but also every speck of dust or lint which may settle upon them. The use of silk as a polisher reduces this difficulty to a minimum.

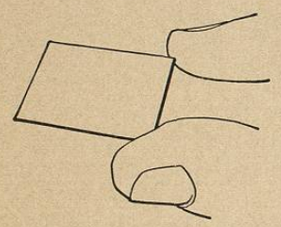


FIG. 211.—Proper Method of Holding a Cover Glass.

Having prepared the cover glasses in this way, the next point is to keep them both clean and dry during the process of spreading the blood. We must always hold them as in Fig. 211, and never touch any part of their surfaces with the fingers. Any one whose fingers tend to get moist must handle the cover glasses with forceps, but most of us will always use our fingers, despite the warnings of our Teutonic brethren. Holding a cover glass as in Fig. 211, touch the centre of it with the tip of a drop of blood as it issues from a puncture, taking care not to touch the skin of the ear itself; then drop this cover glass (blood side downward) upon a second cover glass in such a position that their corners do not match. If the covers are quite clean and free from dust, the blood drop will at once spread so as to cover the whole surface of the glasses. The instant it stops spreading, take hold of the upper cover glass by one corner and slide it rapidly off without lifting it or tilting it at all. This needs some practice, and some men never learn it; hence the use of slides.

Films so prepared will keep for a long time without deteriorating, especially if the air is excluded.

2. *Staining.*—The introduction of the Romanowsky method of staining (Nocht's, Ziemann's, Jenner's, Leishman's, Wright's) enables us to dispense with all other blood stains and greatly shortens the time of the process. Wright's stain is identical with Leishman's except in the method of preparation, which Wright has considerably simplified, and as either of these mixtures can be obtained ready made of any of the larger dealers in physicians' supplies, I shall not describe the method of making it. Reliable stains can always be obtained from the Massachusetts General Hospital in Boston. An ounce bottle will stain hundreds of specimens.

To stain a cover-glass film, grasp it with Cornet's forceps, rest the forceps on the sink so that the film side is upward and is approximately horizontal. Draw a little of Wright's or Leishman's stain into a clean medicine-dropper and squeeze out upon the film enough to flood its surface.

(*a*) Allow the stain to *act for one minute;* during this time the methylic alcohol contained in it fixes the film upon the cover glass.

(*b*) Next add distilled water from a clean medicine-dropper until a greenish metallic lustre appears like a scum upon the surface of the stain. Usually about six or eight drops of water are needed if we are using a seven-eighths-inch cover glass. The stain, so diluted with water, should remain upon the cover glass about *two minutes*. The exact time does not matter.

(*c*) Next wash off the stain with water cautiously and let the film remain in clean water for about a minute more or until it takes on a light pink color. Dry gently with blotting paper and mount in Canada balsam.

This whole process can be completed inside of five minutes, and I know of no other staining method at once so rapid, so reliable, and so widely applicable. It brings out all the minutiæ of the red corpuscles, leucocytes, and blood parasites, and for clinical work no other stain is needed.

APPEARANCE OF FILMS SO STAINED.—1. The *normal red corpuscles* appear as round discs with pale centres. Their color depends

upon the length of time that we continue the washing with clear water after the staining mixture has been poured off, and varies from brown through pink to golden yellow.

(*a*) *Poikilocytosis* means the appearance in the blood of red cells variously deformed, sausage shaped, battledore shaped, oblong, pear shaped, etc. It is always associated with *abnormalities in the size* of the corpuscles, so that dwarf forms and giant forms appear.

(*b*) *Polychromasia* (or *polychromatophilia*) refers to abnormal staining reactions in the red corpuscles, whereby isolated individuals take on a brownish or purplish tint, sharply contrasted with the pink or yellow of the corpuscles around. If this brownish or purplish tint occurs in all the corpuscles, it has no pathological significance, but merely means that the staining has been incorrectly performed.

(*c*) "*Stippling*" refers to fine, dark-blue dots scattered over the pink surface of a red corpuscle, as if a charge of fine shot had been fired into it.

All the abnormalities just described are to be found in any of the types of severe anæmia, whether primary or secondary, but stippling may also be found *without anæmia* in some cases of *lead poisoning*, and is therefore useful as a confirmatory sign in cases of this disease.

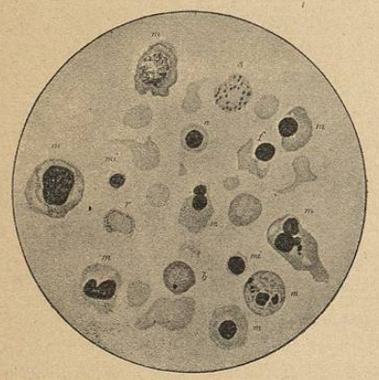
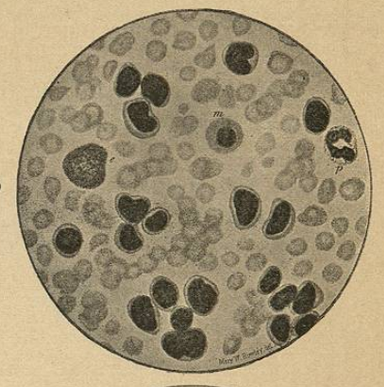
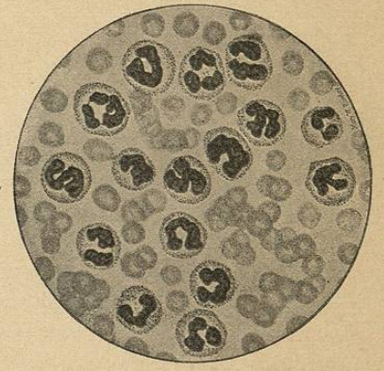


FIG. 212.—Nucleated Red Cells. *m*, *m*, Megaloblasts; *n*, normoblast; *s*, stippled cell.

Nucleated red corpuscles are divided into two main varieties: (1) *normoblasts*, which are of the size of normal corpuscles; and (2) *megaloblasts*, which are larger than normal corpuscles (see Fig. 212). The nucleus of the normoblast is generally small and deeply stained, navy blue. In the megaloblast the nucleus may have the same characteristics or may be much larger and paler, with a distinct intranuclear network. The protoplasm of both varieties is often discolored, murky, gray, or even blue, and sometimes stippled, so that by beginners the cell may be mistaken for a leucocyte. The mistake may be avoided, however, after some experience. In the protoplasm of nucleated cells there are often concentric rings like the layers in an oyster shell, and their outline is usually more irregular than that of any leucoctye. Further points of differentiation must be learned by practice.



b



a

FIG. 213.—*a*, Leucocytosis (40,000); sixteen polynuclears in a field. *b*, Lymphatic leukæmia. *p*, Polynuclear; *m*, megaloblast; *e*, eosinophile. Twenty-one lymphocytes in this field.

2. *Leucocytes.*—In normal blood four main varieties may be distinguished:

(*a*) Polynuclears or polymorphonuclear neutrophiles.

(*b*) Lymphocytes (large and small).

(*c*) Eosinophiles.

(*d*) Mast cells.

(*a*) *Polynuclears.* — The deeply stained, markedly contorted nucleus assumes a great variety of shapes in different cells, and is surrounded by a pinkish protoplasm studded with spots or granules just large enough to be distinguished under the oil immersion and slightly deeper in tint than the protoplasm around them. These cells make up about two-thirds (*sixty to seventy per cent*) of all the leucocytes present in the blood (see Fig. 213, *a*).

(*b*) *Lymphocytes.*—The smallest variety is about the size of a red cell, and consists of a round nucleus stained deep blue and surrounded by a very narrow rim of pale, bluish-green protoplasm. In the larger forms the nucleus occupies much less space relatively, is often less deeply stained, and may be indented. The latter variety is sometimes burdened with the useless name of "transitional cell," a term which in my opinion should be given up, since all lymphocytes are transitional. In the protoplasm of the larger varieties of lymphocyte one often sees a sprinkling of fine pink granules. From *twenty-five to thirty-five per cent* (or about one-third) of all leucocytes belong to the lymphocyte group—classing all sizes together (see Fig. 213, *b*).

(*c*) *Eosinophiles.*—The nucleus is irregularly contorted and attracts very little notice, owing to the very brilliant pink color and relatively large size of the granules in which it is immersed. The outline of the cell is more irregular than that of any other leucocyte, and its granules often become broken away and scattered in the technique of spreading the blood. The eosinophiles make up approximately *one per cent* of the leucocytes of normal blood.

(*d*) *Mast Cells.*—The shape of the nucleus can rarely be made out, and the main characteristic of the cell is the presence of large dark granules, stained blue or plum color, sometimes almost black, and arranged most thickly about the margin of the cell. Mast cells are very scanty in normal blood and make up not more than *one-half of one per cent* of the leucocytes.

Other varieties of leucocytes which appear in the blood only in disease will be mentioned later.

3. *Blood Plates.*—In the normal blood film, stained as directed above, one finds, beside the red corpuscles and the different varieties of leucocytes, a varying number of bodies, usually about one-third the diameter of a red corpuscle, irregularly oval in shape, staining dark red or blue and tending to cohere in bunches. Occasionally larger forms occur, and in these a vague network and some hints of a nucleus may be traced.

The significance of these bodies is unknown and they have at present no importance in medicine, although they not infrequently

lead to mistakes, because, when lying on top of a red corpuscle, they bear a slight resemblance to a malarial parasite.

III. Counting the White Corpuscles.

The instrument used all over the world at the present day is the pipette of Thoma-Zeiss, in which the blood is diluted either ten or twenty times. The diluting solution is one-half of one per cent glacial acetic acid in water. This diluting solution often accumulates spores and becomes cloudy. As soon as this happens a fresh bottle should be prepared. After a rather deep puncture blood is sucked up to the mark point .5 on the pipette, which is then immersed in the diluting solution and suction exerted until the mixture is drawn up to the point marked 11. This gives a dilution of one to twenty. By drawing blood up to the point marked 1, instead of to the point marked .5, we obtain a dilution of one to ten. After this the ends of the pipette can be closed with a rubber band, and the blood, so shut in, can be kept or transported without loss or change.

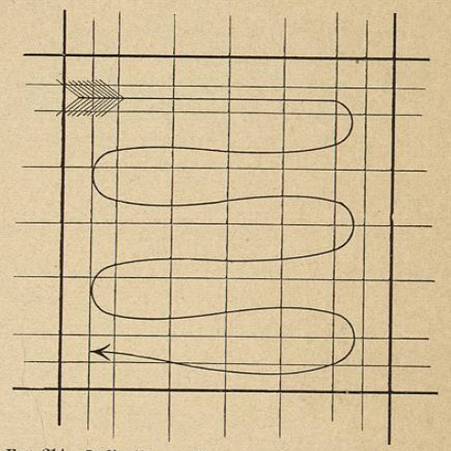


FIG. 214.—Indicating an Order in which the Squares may be Counted.

When we are ready to make the count, the rubber band is removed and the pipette rolled in the fingers rapidly back and forth for about one minute, to mix up the contents of the bulb thoroughly and evenly. Next blow out three drops, in order to get rid of the pure diluting solution which is in the shank of the pipette. Then put upon the circular disc of the counting chamber a drop of the

mixture from the bulb of the pipette. This drop must be of such a size that when the cover glass (see Fig. 215 *B*) is let down upon it[1] the drop will cover at least nine-tenths of the circular disc and not spill into the moat around it. The size of this drop can only be learned by practice. After about five minutes the leucocytes will have settled upon the ruled space which occupies the centre of the floor of the counting chamber, and the count can then be begun, using preferably a No. 5 objective of Leitz or a DD of Zeiss. The whole ruled space should be counted, and after a little practice this takes not more than five minutes. I usually begin my count in the left upper corner of the ruled space and proceed in the direction indicated by the serpentine arrow in Fig. 214. In normal blood one finds from thirty to fifty leucocytes in the whole ruled space. The number of leucocytes per cubic millimetre is obtained by multiplying this figure by 200. Thus if the number of leucocytes counted is 35, the number in a cubic millimetre of blood is $35 \times 200 = 7{,}000$. If great accuracy is needed, a second count with a fresh drop should be made and the average of the two taken; but in ordinary clinical work this does not seem to me necessary, for the amount of error, although considerable, is not such as to affect our diagnostic inferences.

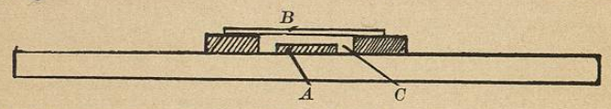


FIG. 215.—Thoma-Zeiss Counting Slide. *A*, Ruled disc: *B*, cover-glass; *C*, moat.

IV. Counting the Red Corpuscles.

Perhaps once in every twenty-five or fifty cases that one sees it is well to know the number of red corpuscles. They can then be counted with the Thoma-Zeiss pipette which is made for the purpose, and so arranged that the blood may be diluted one to two hundred. The technique is exactly that described in the last section, except that we need less blood and use a different diluting solution. I am accustomed to use a mixture suggested by Gowers, made up as follows:

Sodium sulphate	gr. cxii.
Dilute acetic acid	ʒ i.
Water	ʒ iv.

Blood is sucked up to the mark 0.5 and then Gowers' solution to the mark 101. After the drop has been adjusted in the counting chamber and the corpuscles have settled upon the ruled space, we usually count a field of twenty-five small squares at each of the four corners of the whole ruled space. The figure so obtained is multiplied by 8,000. The result is the number of corpuscles per cubic millimetre.

[1] To avoid air bubbles lower the cover glass with aid of a needle as in mounting microscopic specimens. This must be done as quickly as possible after the drop has been adjusted on the counting disc.

INTERPRETATION OF THE RESULTS SO OBTAINED.

1. *Secondary Anæmia.*

The hæmoglobin is usually reduced more than the count of red corpuscles, giving a *low color index*. In mild cases the hæmoglobin may fall as low as forty per cent before the red corpuscles show any considerable diminution. In severe cases the red cells fall to 3,000,000, 2,000,000, and occasionally even to 1,000,000 or below it; but the hæmoglobin usually suffers even more severely.

The leucocytes may be normal, increased, or diminished, depending on the cause of the anæmia. Thus in anæmia due to chronic suppurative hip-disease the leucocytes are often increased to 20,000 or 30,000, while in malarial anæmia the leucocytes are often subnormal. There are no characteristic changes in the differential count, which varies with the underlying disease.

The changes seen in the stained blood film are briefly: Poikilocytosis, abnormal staining of the red corpuscles, and the presence of nuclei either in normal-sized corpuscles (normoblasts) or in giant corpuscles (megaloblasts). The degree of poikilocytosis and abnormal staining reaction is proportional to the severity of the anæmia. In mild cases we find only normoblasts, and those only after a long

search; in severe cases we may find megaloblasts as well, but almost invariably these cells are fewer than the normoblasts.

The commonest causes for secondary or symptomatic anæmia are as follows:

(*a*) Hemorrhage—gastric, hemorrhoidal, traumatic, puerperal, etc.

(*b*) Malaria, more rarely sepsis or other infections.

(*c*) Malignant disease.

(*d*) Chronic suppurations.

(*e*) Chronic glomerulo-nephritis.

(*f*) Cirrhosis of the liver.

(*g*) Poisons, especially lead.

(*h*) Chronic dysentery.

(*i*) Intestinal parasites.

It is important to remember that insufficient food or even starvation does not produce anæmia, and so far as we know no form of bad hygiene has any notable effect upon the blood. Persons may grow very pale under bad hygienic conditions, but their blood is usually not affected unless one of the diseased conditions mentioned above is present.

2. *Chlorosis.*

The blood is practically identical with that just described, though the color index is sometimes lower, poikilocytosis less marked, and nucleated red cells fewer. The pallor of the centres of the cells ("achromia") is often very marked. The leucocytes are generally normal and the differential count practically so, although the percentage of polynuclear cells is often low with a corresponding relative increase of lymphocytes.

3. *Pernicious Anæmia.*

The number of red cells is usually below 2,000,000 when the case is first seen. The color index is high and the leucocyte count subnormal. The stained specimen shows very marked deformities

and abnormal staining reactions in the red cells, with a tendency to the predominance of large forms. Many of the latter contain nuclei ("megaloblasts"), and a smaller number of normal-sized cells also contain nuclei ("normoblasts").

The polynuclears are relatively diminished, with a corresponding relative increase in the lymphocytes.

In the remissions which form so important a feature of the course of pernicious anæmia, the blood is generally transformed until it is almost or quite normal. In the subsequent fall it may take on all the features of secondary anæmia or chlorosis, and lead to unavoidable errors in diagnosis and prognosis. Fortunately cases are rarely seen for the first time at this (non-characteristic) stage.

Interpretation of the Results of the Leucocyte Count and Differential Count.

By combining the facts obtained by the total white count and the differential count, we can estimate the number of each variety of leucocyte contained in a cubic millimetre of blood. Thus with 10,000 white corpuscles, 70 per cent of which are polynuclear (as seen in the stained film), we have 7,000 polynuclear cells per cubic millimetre, which may be considered the upper normal limit. Any number greater than this should be considered as a *leucocytosis*. In a similar way we can say that any number greater than 3,500 is above the normal limit for lymphocytes and constitutes a *lymphocytosis*, while *eosinophilia* is present whenever the number of eosinophiles is more than 400 per cubic millimetre. It is much better to use these absolute numbers than to rely upon percentages. If we say, for example, that 3 per cent of eosinophiles is within normal limits, we shall make an error now and then in cases of myelogenous leukæmia, in which, with a total count of 500,000 leucocytes, 3 per cent of eosinophiles would amount to a total of 15,000 per cubic millimetre, or nearly thirty times the normal number. Errors are also common in the opposite direction. For example, in typhoid, with a total leucocyte count of 3,000, the lymphocytes may reach 60 per cent and yet be well within the normal limits, for 60 per cent of 3,000 is