The blood, how to examine and diagnose its diseases.

THE BLOOD:

HOW TO EXAMINE AND DIAGNOSE ITS DISEASES.

THE BLOOD:

HOW TO EXAMINE AND DIAGNOSE ITS DISEASES.

BY

ALFRED C. COLES, M.D.

B.Sc. of Public Health, Edinburgh;
Fellow of the Obstetrical Society, London;
Formerly Senior House-Surgeon at the County Hospital, York.

WITH SIX COLOURED PLATES.

LONDON:
J. & A. CHURCHILL,
7 GREAT MARLBOROUGH STREET.
1898.

PREFACE.

DURING a somewhat prolonged investigation into the microscopic appearance of the blood in certain pathological conditions, I was at an early period struck with the difficulty of finding any exhaustive account of the methods employed in, and the help to be derived from, the examination of the blood.

Various papers written by Drs. Gulland, Kanthack, and Muir have appeared in different journals, but these only deal with one department; and a full and critical account of the methods, especially those dealing with the fixing and staining of films, was incomplete or absent.

Most of the literature relating to the microscopic examination of the blood was in German, some in French, but very little in English, excepting on Malaria.

Hayem's *Du Sang*, a stupendous work, treats more particularly of the red corpuscles, but the account of the occurrence of nucleated red blood corpuscles and the development of the cellular elements, is either out of date or not generally accepted as correct, whilst very little mention is made of the variety of leucocytes, classified according to Ehrlich's investigation on the staining reaction of the granules contained in their protoplasm.

Von Limbeck's *Grundriss einer Klinischen Pathologie des Blutes* gives a much fuller account of the latter, but the language in which it is written—German—prevents some taking advantage of the information it contains.

Having regard to the importance and value of a careful examination of stained preparations of the blood, I have collected what information I could from the German, French, and scattered articles in the English medical journals.

When near the conclusion of my work, an excellent book was brought out in English by Dr. Cabot, of America, on *The Clinical Examination of the Blood.* To my mind this work is deficient in the matter of technique, particularly in the staining of dry films, and no mention is made of a subject which has quite recently attracted some attention in England, viz., Splenic anæmia.

This small work has no claim to originality, being more or less a compilation from other literature. I have followed most of the observations mentioned, and after repeated failures, wish to draw attention to an efficient and reliable method of blood examination which answers all the requirements of the physician.

Certain parasites of the blood—*e.g.*, filaria sanguinis hominis, the spirochaetae of relapsing fever—as well as the symptoms of the diseases described, except splenic anæmia, have been omitted, as these are dealt with at length in all text-books on medicine.

No mention is made of the question of the chemical examination of the blood, partly because it is work that

can only be done in a laboratory, and partly that up to the present the results so obtained have not proved to be of that clinical value which they may ultimately reach.

All the illustrations, except Plate VI., are taken from films which I have made during the last five years.

I take this opportunity of expressing my thanks to Dr. Gulland and Dr. Kanthack for kind suggestions as to methods, especially in the earlier days.

I have given a list of books and articles from which I have extracted freely, and would particularly mention my indebtedness to the following: Hayem, von Limbeck, and an article by Stengel in the *Twentieth Century of Medicine.*

BOURNEMOUTH.

COLOURED PLATES

CONTENTS.

Pathology of the Blood—

THE BLOOD:

HOW TO EXAMINE AND DIAGNOSE ITS DISEASES.

METHODS OF EXAMINATION OF THE BLOOD.

A COMPLETE and systematic examination of the blood would include the enumeration of the corpuscular elements, the estimation of the amount of haemoglobin, and the examination of films microscopically.

The first two procedures have been generally recognized for a considerable period, but the last, especially the use of differentially-stained preparations, has received an attention disproportionate to its value.

THE ENUMERATION OF THE CELLULAR ELEMENTS OF THE BLOOD.

Two instruments are used in England for counting the corpuscles, viz., the Thoma-Zeiss and Gower's Haemocytometer. The latter is recommended in almost all the English textbooks, but it is not as convenient or accurate as the Thoma-Zeiss apparatus.

The Thoma-Zeiss Haemocytometer.

This consists of a small pipette for counting the red corpuscles, the stem of which is divided into tenths, and

marked 0·5 and 1·0. Just above the mark 1· the tube expands into a bulb containing a small glass bead, at the end of which the number 101 is marked. The second pipette (when supplied with the instrument) for counting the white corpuscles, is very similar, but slightly larger.

The lobe of the ear or tip of the finger is cleaned, dried, and then pricked with a needle.

When a drop of blood appears, the point of the pipette is placed into it, and gentle suction made till the blood reaches the point marked 1·0 at the commencement of the bulb. It is well not to commence to draw up the blood till the drop is sufficiently large, or otherwise the mark may not be reached, and this would render a fresh attempt necessary. The point of the pipette should not be pressed on the surface of the skin, or the flow may in that way be stopped; and lastly, the point of the pipette should never be allowed to escape from the drop of blood till the desired mark is reached, or an air bubble will be drawn in and the result vitiated.

Some writers advise that the blood be only drawn up to the point 0·5, so that the dilution being 1 in 200, instead of 1 in 100, shall facilitate the counting of the corpuscles. According to these observers, if the mark be overstepped, it is possible to lower the column of blood to the point 0·5 by gently tapping the pipette on a towel.

I invariably draw the blood up to the mark 1·, as the leucocytes can then be counted on the same film as the red corpuscles; and further, that although with a dilution of 1 in 100 about $12\frac{1}{2}$ corpuscles in health are seen in each square of the counting chamber, yet in

most anæmias, and it is with these that we are chiefly dealing, half, frequently less than half, that number are present. Another point in favour of the latter method of procedure is, that the less the multiplier the less the error in the calculation.

As soon as the blood has reached the mark the outside of the point of the pipette is quickly wiped, to remove any blood adhering to it, and immediately immersed in the diluting fluid, and this is drawn up to the mark 101. Any of the diluting solutions hereafter given may be used.

In doing this it is advisable to roll or shake the pipette whilst suction is being applied, in order to prevent the blood floating on the top of the diluting fluid. Care should also be taken that the point of the pipette is kept under the fluid, or a bubble of air may be drawn in.

It is extremely easy to overstep the mark 101, as the bulb seems to fill suddenly.

So far the whole process should be performed as quickly as the precautions necessary to be observed will allow. As everything depends on the accuracy of the preceding technique, I will briefly mention the possible or probable errors that may at first occur.

1. The blood column should not be allowed to coagulate before the diluting fluid is drawn up.

2. An air bubble or break in the column of the blood, usually due to starting before the drop is large enough or allowing the point of the pipette to slip out of the drop of blood or neutral solution whilst suction is being made, may occur, which would render the preparation useless. If the puncture be deep enough a sufficient quantity of blood will quickly appear.

3. The blood column may suddenly stop, probably either because the top of the pipette is touching the skin, or because the capillary bore of the pipette is not dry, and coagulation has taken place.

The pipette now contains one part blood and 100 parts of the neutral solution, and may safely be left for some time before counting. There is no need to carry the microscope to the bedside. All that is necessary is the pipette, which can be carried in a small case a little larger than that containing a clinical thermometer, and a small bottle holding about thirty drops of the diluting fluid.

In order to carry about the filled pipette Cabot suggests that a rubber band be placed longitudinally round it, in order to close the ends. At first I tried to obviate any danger of escape of the contents by placing india-rubber caps on both ends, but found that this had the disadvantage of driving the fluid in the capillary bore into the bulb, making the dilution one in 101, instead of one in 100. I find now that the best method is to carefully detach the india-rubber tubing, after the pipette is filled, without compressing it—any pressure at this end forces a small drop of the liquid out at the point end—and then the tube is placed in its case, and kept horizontally till used.

The Counting.

The slide containing the counting chamber consists of a disc of glass, on which parallel lines are engraved, which by their intersection form 400 squares, the length of a side of each of which is $\frac{1}{20}$ of a millimetre. The squares are further divided by double lines into groups of 16. When the coverglass is applied each cell has a depth of $\frac{1}{10}$ of a millimetre.

The charged pipette is thoroughly shaken, previously closing both ends with the thumb and ring-finger—the india-rubber tubing having been removed—and then a drop is blown out, which consists only of the diluting fluid. It is well to blow out three or four large drops before charging the counting cell. After this a small drop is placed on the centre of the ruled disc and covered with the coverglass. The drop should only cover the ruled area, and should not run into the little channel around it. Care should be taken to avoid air bubbles appearing in the film, and lastly, the coverglass should fit so closely to the outer table of glass that Newton's rings can be seen. The slide may then be placed under the microscope and kept in a horizontal position. In a few minutes the corpuscles have sunk to the bottom and may be counted. A magnification of about 200 (*i.e.*, $\frac{1}{4}$ or $\frac{1}{6}$ inch objective) is very suitable. The light should be so arranged that the lines as well as the corpuscles are seen. It sometimes happens—I have, however, only noticed it in the Gower's ruled chamber—that the parallel lines are invisible; and Hawkesley, in a private communication, stated that this is easily remedied by rubbing a lead pencil over the ruled area, and then wiping gently with a soft handkerchief before filling the cell.

The number of red corpuscles found in several groups of 16 squares should be counted and then the slide washed, cleaned, and thoroughly dried, and another drop counted. The greater the number of corpuscles counted the less the error.

Some corpuscles will be seen on the lines and not in the squares, and in such cases it is usual to count in all corpuscles touching the top and right lines, and to

leave out of the count those touching the lower and left lines.

Calculation.—Each square has a cubic capacity of

$$\frac{1}{20} \times \frac{1}{20} \times \frac{1}{10} = \frac{1}{4000} \text{ cubic millimetre,}$$

therefore the formula for calculation will be :—

$$\frac{\text{No. of corpuscles counted} \times \text{dilution } (100) \times 4000}{\text{No. of squares counted.}} = \text{No. of corpuscles per cubic mm. of blood.}$$

Example.—Supposing 2000 corpuscles were counted in 10 groups of 16 squares, and the dilution was 1 in 100, then,

$$\frac{2000 \times 100 \times 4000}{10 \times 16} = 5{,}000{,}000 \text{ red cells in 1 cubic millimetre of blood.}$$

Counting the White Corpuscles.

There are two methods of counting the leucocytes : 1st, with the special pipette ; and 2nd, with the ordinary pipette and at the same time as the red blood corpuscles.

With the Special Pipette.—Here the process is similar to that just described for counting the red corpuscles. The diluting solution used is generally a $\frac{1}{3}\%$ solution of glacial acetic acid in water, which renders the white cells evident, but the red cells somewhat transparent. It requires a much larger quantity of blood than the ordinary pipette.

With the Ordinary Pipette.—I find the red corpuscle pipette sufficiently accurate for clinical purposes, and it further necessitates much less discomfort to the patient, as the puncture required for filling the ordinary small pipette, the capillary tubes of Fleischl's haemometer, and the preparation of half a dozen films on the glass slides, need not be very deep.

Ewing, who has made numerous observations on the occurrence of leucocytosis, especially in pneumonia, commends the use of the ordinary pipette for counting the white as well as the red corpuscles. He states that the employment of the large pipette or leucocytometer has several disadvantages. "The amount of blood required is inconveniently large. Acetic acid obscures the outline of the leucocytes, and it is often difficult to distinguish them from *débris* left by the red blood cells. The leucocytes become very cohesive, and appear in the counting-chamber in considerable masses, in spite of persistent efforts to secure the even distribution necessary for an accurate count." (*New York Med. Journal*, Dec. 16, 1893.)

It is very advantageous to use a tinted diluting solution, as the white corpuscles are then sharply defined. I find Toisson's solution answers excellently, provided it is always filtered before use.

With a dilution of 1 in 100 only about $7\frac{1}{2}$ leucocytes will be found in normal blood in the whole of the 400 squares, and it is obviously necessary to somewhat modify the process.

Under the microscope it will be seen that the area of ruled squares only occupies a small part of the whole of the cell, and it is advisable to include the whole area under observation.

The method which I use is the following, suggested by Stengel. The area of the field of vision of the microscope is first determined. The ruled area on the slide is focussed with, say, $\frac{1}{4}$ inch objective, and the tube of the microscope drawn out till "one of the parallel lines of the ruled slide accurately coincides with either side of the field of vision." In my own microscope with

$\frac{1}{4}$ inch objective, eyepiece 2, and the tube drawn out to 162 mm., the diameter of the field of vision corresponds to 10 squares, each of which is $\frac{1}{20}$ mm., so that the diameter $= \frac{1}{2}$ mm. and the radius $\frac{1}{4}$ mm.

From the formula $R^2\pi$, where $\pi = 3{\cdot}1416$ and R = radius, in this case $\frac{1}{4}$ mm., then

$$\tfrac{1}{4} \times \tfrac{1}{4} \times 3{\cdot}1416 = 0{\cdot}19635 \text{ square mm.}$$

But the depth of the cell is $\frac{1}{10}$ mm., therefore the cubic capacity of a field of vision will be :—

$$\tfrac{1}{4} \times \tfrac{1}{4} \times \tfrac{1}{10} \times 3{\cdot}1416 = 0{\cdot}019635 \text{ cubic mm.}$$

Then the formula for calculation will be the following :—

$$\frac{\text{No. of leucocytes counted in field of vision} \times \text{dilution (100)}}{\text{area (0·019635)} \times \text{No. of fields counted.}}$$

As two of these factors are constant—viz., the dilution and the cubic area of the field of vision—we can simplify it thus :—

$$\frac{100}{\cdot 019635} = 5092{\cdot}946$$

Then the simplified formula will in this instance be the following :—

Working with $\frac{1}{4}$ inch objective, eyepiece 2, tube length 162 mm., and the dilution of 1 in 100 :—

$$\frac{\text{No. of leucocytes counted} \times 5092{\cdot}946}{\text{No. of fields in which they were counted}} = \text{No. of leucocytes per 1 cmm. of blood.}$$

Example.—Suppose that 40 microscopic fields were examined and 64 leucocytes seen :—

$$\frac{64 \times 5092.946}{40} = 8148 \text{ leucocytes per cubic millimetre of blood.}$$

In the same way I find that my $\frac{1}{4}$ inch objective when used with eyepiece No. 4, and tube length of 173 mm., includes 8 cells of $\frac{1}{20}$ mm.

Therefore the diameter $= \frac{8}{20}$ mm. and the radius $= \frac{8}{40}$ or $\frac{1}{5}$ mm.

$$\therefore \frac{1}{10} \times \frac{1}{5} \times \frac{1}{5} \times 3{\cdot}1416 = {\cdot}0125664,$$

the dilution being 1 in 100:—

$$\frac{100}{{\cdot}0125664} = 7957{\cdot}728.$$

The formula in this case would therefore be:—

$$\frac{\text{No. of leucocytes counted} \times 7957{\cdot}728}{\text{No. of fields counted}} = \text{No. of leucocytes in cubic millimetre of blood.}$$

When counting the leucocytes in the field of vision, instead of in the ruled areas, care must be taken to go regularly from one circular area to another, and to include an entirely fresh field at each count. The areas counted must not be selected, but should be taken just as they come. When the leucocytes are very numerous, as in leucocythemia, in which 45 to 50 white cells may be seen in one field with the $\frac{1}{4}$ inch objective and No. 2 eyepiece, it is much easier to estimate their number over some part of the ruled area, not necessarily over the squares.

Cleaning the Blood Pipette.

It is most important that the capillary pipette should be cleaned immediately after it has been used. The directions generally given are that, first some of the diluting fluid, then water, then alcohol, and lastly, ether should be sucked into the pipette, and after shaking, blown out. On account of the fineness of the capillary bore this is a very tedious proceeding, and involves considerable labour.

I have found the following method of cleaning invaluable, and it involves no apparatus other than that which every physician possesses, viz., an aspirator. I attach

the blood pipette by a small piece of india-rubber tubing to the inlet tube of a Potain's aspirator, and then, after exhausting the air in the bottle, allow a large quantity of water (or at first, normal saline solution), then alcohol, and finally ether, to be drawn through it. If the instrument is wanted at once, warm air from a spirit lamp may be drawn through, so as to rapidly dry every part of the tube. Before use the pipette must be absolutely clean and dry.

Gower's Haemocytometer.

This instrument is in more general use in England than the above mentioned, but after considerable experience with it, I am convinced that Gower's instrument cannot be compared with the one just described. It has no advantages over the Thoma-Zeiss apparatus, and is much more complicated and costly.

In using this form of haemocytometer 995 cubic millimetres of the diluting solution, measured in the larger pipette, are placed in the small mixing jar supplied with the instrument. Into the capillary pipette 5 cubic millimetres of blood are drawn, and then blown into the diluting fluid. The two liquids are well mixed with the glass rod stirrer, and then a drop of the diluted blood is placed in the counting-cell and covered with a coverglass, which is held in position by two clips. The corpuscles are allowed to settle and then counted.

Each square measures $\frac{1}{500}$ cmm., and the dilution is 1 in 200; therefore the formula for the calculation would be—

$$\frac{\text{No. of corpuscles counted} \times \text{dilution (200)} \times 500}{\text{No. of squares counted}} = \text{No. of corpuscles in 1 cmm. of blood.}$$

Neutral Diluting Fluids.

For diluting the blood for the purpose of enumeration various preserving or neutral solutions may be used, the simplest being a 2 or 3 % solution of salt in water. Amongst others the following may be mentioned :—

Gower's Solution consists of a solution of sodium sulphate in distilled water, having a specific gravity of 1025, or any of the following formulæ may be used :—

Sodium Sulphate	grs. 104.
Acetic Acid	℥j.
Distilled water	℥iv.

Hayem's Solution consists of—

Hydrarg. Perchlor.	0·5 grms.
Sodium Sulphate	5·0 „
Sodium Chloride	1·0 „
Distilled water	200·0 „

Pacini's Solution—

Hydrarg. Perchlor.	1·0 grms.
Sodium Chloride	2·0 „
Glycerine	13·0 „
Distilled water	113·0 „

The last mentioned is allowed to stand for a considerable time (two months), and when about to be used is diluted with three times its volume of water, and filtered.

The indifferent solutions may also be tinted with some of the aniline dyes, in order to render the white blood corpuscles more apparent.

The 2 or 3 % aqueous salt solution may be coloured with gentian violet, about 1 % of the saturated alcoholic solution being added.

Toisson's Diluting Solution is probably one of the most convenient for clinical purposes. It consists of the following :—

Aqua. distill.	160 cm.
Glycerine (neutral)	30 cm.
Sodium Sulphate	8 grm.
Sodium Chloride	1 grm.
Methyl. Violet	0·025 grm.

All these indifferent solutions require very thorough filtration before being used. Toisson's fluid is particularly adapted for diluting, as it stains the leucocytes very clearly, and thus facilitates their enumeration.

A Differential Count of the Leucocytes can only be satisfactorily made by means of stained films. In such specimens 500 to 1000 leucocytes are counted and classified according to their character into small uninucleated, large uninucleated, multinucleated, eosinophile leucocytes and in pathological conditions, marrow cells or myelocytes.

ESTIMATION OF THE AMOUNT OF HAEMOGLOBIN.

Various instruments have been devised for estimating the amount of haemoglobin, but probably the most satisfactory for clinical purposes are Von Fleischl's haemometer and Gower's haemoglobinometer. They are both colorimetric, the blood being in each case diluted, and the resulting colour compared in the one case with a tinted-glass wedge, in the other with a standard solution of some pigment.

Von Fleischl's Method.

The apparatus consists of the following :—

1. A metal cylinder, closed at one end by glass and divided into two equal compartments by a vertical septum.

2. A stage somewhat resembling that of a microscope, which carries a wedge of red glass. The latter is graduated and can be moved horizontally along beneath the stage.

3. Small capillary tubes, which fill automatically and hold a definite quantity of blood.

4. A small glass pipette for filling the compartments with water.

Full instructions of the method of using are supplied with the instrument, and I shall only briefly describe the most important points. The cylinder is partially (about one-fourth) filled with water by means of the glass pipette, and as soon as a fairly large drop of blood has appeared at the point punctured, the capillary tube, held by the stem in which it is fixed, is brought in contact with it, and the blood immediately fills it by capillarity, provided that it is held in a position other than the vertical. The tube containing the exactly-measured quantity of blood is quickly cleared of any blood which may be adhering to its outer surface and immediately placed in the water in one compartment of the cylinder. It is then moved about in order that the water may dissolve out the colouring matter of the corpuscles, and then, whilst it is held vertically, drops of water from the pipette are allowed to fall on and run through it until the capillary tube is quite clean. With the handle end of the capillary

tube the blood and water are thoroughly mixed, and then water added till the compartment is quite full. The other side is filled with water only, so that on one side we have a mixture of water and blood and on the other water alone. The cylinder is then placed on the circular opening of the stage, so that the coloured part is directed away from the finger screw, whilst the side containing the water is over the wedge of coloured glass.

Artificial light, such as that afforded by a lamp or gas (daylight, electric, or incandescent light cannot be used), is reflected upwards from the plaster of Paris reflector, and it is generally better to use as little light as possible.

The coloured glass is then moved along, preferably in jerks, and not too gradually, till the red tint in both compartments appears equal and the percentage noted. Daylight must be excluded as much as possible, and it is easier to estimate the intensity of the two colours when the axis of the eyes is at right angles to the septum, which divides the cylinder into two equal compartments, the lateral portions of the retina being more sensitive to colour impressions. One eye only should be used, and that at the ordinary reading distance from the stage. Sometimes a piece of paper rolled into a tube may be looked through, as this, by cutting off any light in the room, renders a sharper definition possible.

The whole process only occupies one or two minutes, and the results are very fairly accurate. It renders the greatest assistance in watching the progress of cases, especially chlorosis, and sometimes obviates the necessity of a long count of the red corpuscles.

The instrument should be used soon after the blood is measured and diluted, but it is possible when the cylinder is carefully covered with the small circular glass disc supplied with the instrument, to delay a short time. I generally use an elastic ring to keep on the glass disc whilst I am carrying it.

Certain fallacies occur in using Von Fleischl's haemometer. Firstly, the readings are rather low, as many healthy individuals only show 85 to 90 % of haemoglobin with this instrument. Secondly, when the haemoglobin is very low it is extremely difficult to determine exactly at what point the two colours are quite equal. I have found this particularly noticeable in pernicious anæmia. Under such conditions I use two capillary tubes of blood instead of one, and therefore take one-half the percentage as the approximate measure of the haemoglobin. I find that Stengel also advises this procedure. Thirdly, in cases of leucocythemia the enormous increase in the white cells renders the mixture of blood and water turbid. Leichtenstern recommends the addition of a very small quantity of alkali to the water in these cases.

Gower's Haemoglobinometer.

This instrument is much used in England, and gives approximately good results. It is, however, not as convenient as Von Fleischl's apparatus, but is somewhat less bulky, and costs only about one-third the price of the latter.

It consists of two glass cylinders of equal size placed vertically side by side on a small stand. One contains a tinted solution equal to a 1 % watery solution

of blood. The other, open at the top, is graduated in tenths up to 120. In addition there is a capillary pipette, measuring 20 cmm., and a small drop-bottle for diluting the blood.

A few drops of water are first placed in the graduated cylinder and 20 cmm. of blood are drawn up into the capillary pipette and quickly discharged into the cylinder. The pipette is then filled with water in order to dissolve any blood which may be adhering to the surface of the tube, and this is added to the mixture of blood and water. The cylinder is immediately shaken to mix the blood and water before the former coagulates, and water is added drop by drop with the dropper, till the tint in both tubes is equal. This is easily ascertained by placing a piece of white paper behind the tubes, or by holding them up to the light from a window. The percentage of haemoglobin is then seen by the scale on the graduated cylinder.

The amount of haemoglobin as determined by either of these methods is usually expressed as so much per cent., 100 being taken as the normal, and it is advisable to add the name Von Fleischl or Gower after it, in order to show which apparatus was used.

Hayem, however, expresses the amount of haemoglobin in terms of normal red corpuscles.

For example, in describing the results of an examination he states that the number of corpuscles was three million per cubic millimetre, and the amount of haemoglobin was equal to that of two million normal corpuscles, *i.e.*, 40 %.

The amount of haemoglobin that each corpuscle contains—the so-called colour index or corpuscular

richness in Hb—Hayem indicates this by the letter G —may be estimated thus :—

The number of healthy corpuscles to which the haemoglobin was equivalent divided by the number of corpuscles counted.

Supposing the number of red corpuscles = three million, and the Hb = two million of normal corpuscles, *i.e.* 40 %, then the amount of Hb each corpuscle contains is equal to $\frac{2}{3}$ or 0·6—the normal being 1. Or the percentage of Hb divided by the percentage of corpuscles present will give the same result. In this case the number of red corpuscles = 60 % of the normal and the Hb = 40 %, therefore the colour index per corpuscle = $\frac{40}{60} = \frac{2}{3}$ or 0.6.

The *Examination* of the *Corpuscular Elements* of Blood may be made in three ways :—

1. The examination of fresh undiluted blood.
2. The examination by means of diluting or indifferent solutions.
3. The examination by means of dry films prepared and stained according to Ehrlich's method.

All these can be used, but for clinical purposes the examination of the blood in the fresh and dry films is especially advised.

The Examination of Fresh Blood.

This is the ordinary procedure adopted by almost all physicians, and if certain precautions are taken is very reliable as far as it goes.

The tip of the finger or lobe of the ear, thoroughly cleansed and as thoroughly dried, is pricked with a needle, preferably triangularly pointed. Puncture of the lobe of the ear is less painful, but the manipulation

of the blood is somewhat more difficult in this position than when the finger is pricked. Slight hyperaemia may be produced before puncturing by gentle friction over the part, but the blood must not be squeezed out.

The drop thus obtained is allowed at once to fall on the slide and the coverglass put over it, avoiding undue pressure; or we may touch the exuding drop with the coverglass and then immediately place it upon the slide.

The blood should be in as thin and uniform a layer as possible, the elements protected against any moisture or foreign matter in the form of dirt, and the process carried out as quickly as possible. If the specimen be rung round with oil or vaseline it will be protected from evaporation, and therefore remains longer unaltered.

When the blood is to be examined for micro-organisms the place of puncture must be well washed with soap, then with 1 in 1000 Hg. perchloride, and, lastly, with alcohol and ether. The needle should be sterilized by heat, and the slides and coverglasses scrupulously clean.

Some observers maintain that the pressure of the coverglass is injurious, as it tends to unduly spread out the corpuscles, and adopt means by the intervention of a layer of paper, or drop of wax at the corners of the coverglass, to prevent this.

This is, however, seldom necessary if ordinary care be taken. The thinnest films are essential to the study of the nuclear structure of the leucocytes.

Hayem uses a very thin ringed cell in order to follow any modifications of coagulation in inflammatory conditions.

Specimens obtained in this simple manner show the size, shape, and colour of the red blood corpuscles,

the blood plates of Hayem, and the approximate number of leucocytes present, with a slight insight into the condition of their nuclei and granules. The parasites of the blood, *e.g.*, those of malaria and recurrent fever, are also well seen in these preparations, in fact many writers prefer fresh unstained blood for the examination of the haematozoa.

The measurement of the size of the corpuscular elements is only reliable when made in this fresh condition.

The Examination with Preserving Fluids.

These neutral or indifferent solutions — stained or unstained—are used more particularly in pure histological work. Amongst them the following may be mentioned :—

Hayem's and Pacini's solutions, formulæ already given above.

Bizzozero's Fluid—physiological salt solution ($\frac{3}{4}$ %) —stained with methyl. violet (1:5000) or with gentian violet (1:3000).

Arnold uses salt solution 0·6 %, stained with methyl. green, whilst Mosso uses a 1 % methyl. green in physiological salt solution.

A solution of osmic acid, 1 % or 2 %, tinted with methyl. green is very suitable for the examination of the blood plates.

These solutions may be used in any of the following ways. A drop of the diluting fluid is laid on the cleansed finger and a prick made through it, so that the blood at once mixes with the neutral solution; or some of the blood may be allowed to flow into the preserving fluid in the proportion of about 1 to 100,

and then, after letting the preparation stand, the supernatant fluid is poured off and the elements are then mounted. They may be washed after being exposed to the preserving liquid, stained, and then mounted permanently.

The Examination of Stained Films.

In this method, introduced by Ehrlich, the blood is spread out in a very thin layer on the coverglass or slide, the film is then fixed and subsequently stained, so that permanent preparations are thus obtained. The success of the preparation depends here, as in other histological work, very largely on the quality of the film.

Making Films.

The usual procedure is to prepare such films on *coverglasses*. The drop of blood is allowed to fall on a perfectly clean and dry coverglass, and this is covered by another. These are then separated by gently sliding one over the other, avoiding pressure.

In order to obviate the presence of any moisture from the fingers, Erhlich uses a pair of forceps to hold the coverglasses whilst spreading.

Other writers use a thin glass rod or platinum needle to spread the blood, or with a rapid shaking movement attain similar results.

I have always used *slides* in preference to coverglasses for the purpose of preparing films of sputum, etc., and have found this method particularly adapted to blood films. The slides used should be the best, as the better the surfaces and edges the more uniform the film will be. These should be thoroughly cleaned by

means of strong sulphuric acid, then with water, and lastly with ether. The slides should be perfectly dry, but not warmed before using. Immediately the drop of blood appears at the spot punctured, it is allowed to touch one end of the surface of a slide held between the finger and thumb of the left hand. Another slide, held in the right hand, is used to spread this drop over the surface of the glass by passing it along the slide—holding the glass at an angle of about 45°—in a quick

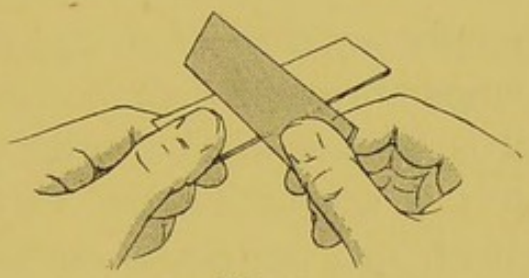


FIG. 1.
Author's Method of Spreading Blood Films on Slides.

but steady movement from left to right. (See Fig. 1.) If the drop be large enough and the glass slide perfectly true a thin uniform layer of blood is thus spread over the lower slide. The slide used as the spreader should only pass over the surface of the blood once.

The following points should be attended to :—

1. The films should be as thin and uniform as possible. Very thin layers of blood dry in one or two seconds, before any alteration in the blood takes place.

2. The time that elapses from the moment that the blood issues from the puncture, to that of a completely spread film, should be as short as possible, otherwise the corpuscles tend to undergo alterations, particularly crenation. If several films are required the drop from the puncture should be wiped away and a fresh drop obtained.

3. Undue pressure should be avoided, as this tends to alter the shape and size of the elements.

4. One should also very carefully prevent the access of any moisture to the films.

The method of spreading films on slides meets these requirements much better than the use of coverglasses. The film so obtained is about $1\frac{1}{2}$ inches long and generally of the breadth of the slide, and thus presents a larger area from which a workable field can be selected.

One can generally say that a film that does not dry at the ordinary temperature of the room in two or three seconds after it is spread, will be rather too thick. In cases in which the corpuscles are very considerably reduced in number, *e.g.*, pernicious anæmia, it is sometimes advisable to spread films somewhat thicker than those ordinarily required, and these can be quickly dried by swaying them in the air.

If the blood films be examined without a coverglass under the microscope, the red corpuscles should appear uniformly separated one from another, otherwise if in contact, no true idea of their form is obtained. The leucocytes also suffer by thickness of the films, as they seem reduced in size, and when stained have a shrunken appearance. In manipulating the slide with its unfixed film on the stage of the microscope, it is important that the fingers should not approach the film part, or otherwise the heat and moisture from the hand or even the breath, tend to disintegrate it. When the film is successfully made, the red corpuscles are fixed in their normal shape, but the white corpuscles are flattened, lamelliform, and are generally larger than in the moist condition. (Hayem.)

The slide method commends itself particularly on account of its convenience for clinical purposes, the only requisites being a box containing half a dozen clean slides and a needle. A number of such films can be prepared and will keep indefinitely—provided that moisture and dust are excluded—without any further fixing. I have stained such films three years after they were made, and have found that their reaction to stains, and their appearance under the microscope, were all that could be desired. Films prepared on slides, as opposed to coverglass preparations, have a further advantage in the fact that they are easily transmitted by post.

Method of Fixing Blood Films.

In order to study the effects of various stains on the elements of the blood, these air-dried films must be fixed.

If any aqueous or glycerine solution of a stain be applied before they are fixed, the whole film either washes off or is converted into a granular *débris*. On the other hand, air-dried preparations unfixed can be stained at once, provided that the stain used be in alcoholic solution.

Solution of iodine with potassium iodide does not dissolve out the haemoglobin, and is, according to Hayem, one of the most useful methods of examining for nucleated red corpuscles in blood. If films be exposed to the vapour of a 1 % solution of osmic acid the haemoglobin is rendered insoluble; but this somewhat impairs their reaction to certain of the aniline stains.

According to Hayem, when films have been prepared for a month or more the haemoglobin adheres more firmly to the corpuscles than at the time of preparation.

For practical purposes it is necessary to use one of the following methods in order to fix the haemoglobin of the red cells of the film.

By means of Heat.—Ehrlich, to whom we are indebted for the film method of examination of blood, subjected the air-dried preparation, placed on a copper plate, to a temperature of 110°–120° C. for two hours or more. Löwit placed the films—which had first been allowed to dry very thoroughly in the air—in an oven at a temperature of 110°–115° C. for one to two hours, allowing a gradual increase and decrease in the heat. He pointed out that too great heat, or heat extending over too long a time, destroys the structure of the nuclei.

This method of treatment takes too much time for clinical purposes, and many of the recent observers agree that much of the finer detail of structure is altered by it.

No doubt passing the coverglass film two or three times through the flame of a spirit lamp or bunsen burner, as commonly advised, answers the purpose fairly well. After trying the latter method of heating for some years I found that for certain stains, particularly Ehrlich's acid haematoxylin solution, very thorough fixation was necessary; and moderate application of heat was usually followed by disintegration or total disappearance of the film when treated with such stains as the above, whilst too great heat spoils some of the nuclear structure.

Muir's Method of Fixing is probably the best for all fine histological preparations, but is somewhat tedious for clinical work.

"Films are placed at once, before any drying can occur, in a saturated solution of corrosive sublimate with $\frac{3}{4}$ % of sodium chloride added, preferably heated to a temperature of 50° C., though this is not essential, and are allowed to remain for about half an hour. They are then thoroughly washed in $\frac{3}{4}$ % common salt solution, taken through successive strengths of alcohol, and then stained in the same way as sections. I also add salt in the same proportion to the weaker strengths of alcohol." (*Journal of Anat. and Phys.*, vol. xxvi., 1892, p. 393.)

Gulland has recently suggested a modification of this process, which I shall describe later.

Fixing by Alcohol and Ether.—Nikiforoff's Method gives the same results as the application of heat, and for clinical use is much more convenient.

Films dried in the air are placed for a short time in a mixture of equal parts of absolute alcohol and ether. Von Limbeck and others advise an immersion of fifteen to thirty minutes, but I find three to five minutes quite sufficient for the staining with eosin and haematoxylin. If preparations are slightly heated after the film has dried in air, and when cold placed in the above solution, a few minutes' immersion seems sufficient, but this is not usually necessary.

It is convenient for clinical work and gives sufficiently accurate results, but for detailed structure it is not as good as that recommended by Muir or Gulland. Absolute alcohol alone may be used instead.

Formol promises to be very useful, and as its action is rapid, most convenient for clinical examinations. [Formol is a 40 % solution of formic aldehyde in water.] Benario, for fixing blood preparations, uses a 10 % watery solution of formol, and of this stock solution he dilutes one part with ten of alcohol when it is required for use. The preparations remain in it for one minute, and can then be stained without drying. The mixture—formol one part, water nine, alcohol ninety parts—could be used if well stoppered to prevent evaporation.

For detailed study of nuclear structure, and for the demonstration of karyokinesis, fixation by Flemming's solution or by Muir's method may be used. I find the following very satisfactory :—

Muller's Method.—The films are heated, and when cold placed in a saturated aqueous solution of picric acid for twenty-four hours. They are then washed for several (twelve to twenty-four) hours, to remove as much as possible of the acid, and subsequently stained for several hours in very diluted Bohmer's haematoxylin solution. They are finally washed, and any overstaining with haematoxylin removed by weak hydrochloric acid alcohol, under frequent examination with the microscope. They should be cleared with xylol or cedar oil, and mounted in Canada balsam.

I have obtained very fine specimens by this means. They may, however, be stained for a short time in undiluted borax carmine, instead of haematoxylin.

Method of Staining Films.

Ehrlich has divided aniline stains into three classes, viz., acid, basic, and neutral dyes.

The acid stains are, as Kanthack has pointed out, "chemically neutral salts, yet tinctorially they react like free acids." They include eosine, aurantia, indulin, picrate of ammonium, orange G, etc.

Among the basic stains are found fuchsin and its derivatives, saffranin, methyl-green, methylene-blue, etc. Neutral stains are formed by the junction of an acid with a basic stain, *e.g.*, a mixture of acid fuchsin with methylene-blue, or methylene-green, etc.

The same observer has classified leucocytes, according to the manner in which the granules in their protoplasm react to aniline dyes, into the following :—

i. Eosinophile—leucocytes containing α granules, which stain deeply with eosine or other acid aniline stains.

ii. Mastzellen—cells which contain large coarse granules basophile in nature = γ granules.

iii. Basophile—cells with fine δ granules, which stain with basic aniline dyes, *e.g.*, methylene-blue.

iv. Neutrophile, or ϵ granules, those which stain with a mixture of a basic and acid stain.

The *Eosinophile* leucocytes may be easily seen by staining fixed films in a concentrated solution of eosine in glycerine, or better in 5 % carbolic-glycerine. The preparation is exposed to the stain for several hours, washed, dried, and mounted in Canada balsam. Glycerine solutions of eosine have a selective, and carbolic acid increases this eosinophilous action. The eosinophile granules are stained a deep red colour.

Glycerine solutions of eosine give beautiful results, but they are not as generally useful or as easily manipulated as the aqueous or alcoholic solutions. Aqueous or weak alcoholic solutions of eosine, or aqueous solutions of orange G may also be used.

The *Mastzellen*, or leucocytes containing γ granules are stained by Ehrlich's acid dahlia solution, which consists of :—

Absolute Alcohol	50 cc.
Distilled water	100 „
Glacial Acetic Acid	12·5 „

Dahlia is added to saturation.

Müller recommends that preparations before staining should be heated for several hours, and then further fixed in absolute alcohol and ether; but this is not essential. Stain films for several hours, wash in water, remove the excess of stain by acid alcohol, and finally wash, dry, and mount in balsam. The γ granules appear stained of a reddish violet.

They may be much more conveniently demonstrated by staining for a minute or so in an aqueous solution of methylene-blue, when they have a purplish tint, whilst all the other elements are stained blue.

The *Basophile* cells, or leucocytes with δ granules, are best stained by saturated aqueous solutions of methylene-blue. Löffler's methylene-blue solution, consisting of concentrated alcoholic solution of methylene-blue 30 cc., distilled water 30 cc. and 100 cc. of $\frac{1}{10000}$ caustic potash solution, answers very well. Preparations are stained for about one minute. The so-called *Neutrophile*, or ϵ granules, may be stained by Aronson and Phillips' mixture, Ehrlich's triple stain (formula given below), or Ehrlich's neutral mixture. The last is made by adding one volume of strong watery solution of methylene-blue gradually with constant stirring, to five volumes of a saturated watery solution of acid fuchsin, and then five volumes of dis-

tilled water are added. After allowing the mixture to stand for a few days it is filtered.

Preparations are stained for two to five minutes, rapidly rinsed with water, and quickly dried and mounted. The red corpuscles are stained red, the ϵ granules violet, whilst the α granules appear of a purple colour.

For clinical purposes it is desirable to use two or more stains, either separately or combined, which will show all these elements on one and the same film.

Kanthack advises the following procedure:—"Films allowed to dry in the air are passed three times through the flame, and stained in a solution of eosine (0·5 gram of eosine, alcohol 70 cc., water 30 cc.) for half a minute or less. They are then thoroughly washed in distilled water, dried between blotting paper, and then passed again 3–5 times through the flame, and then counter-stained in Löffler's methylene-blue solution for a few seconds, again washed, dried, and mounted in Canada balsam." (*Medical Chronicle.*)

Such films should show the eosinophile granules stained deep red, the basophile granules blue, the ϵ, or so-called neutrophile granules—which Kanthack and Hardy have shown are really fine eosinophile or oxyphile granules—light red, the nuclei of the leucocytes a brilliant blue colour, and the red corpuscles a pale red colour.

Notwithstanding many very kind and suggestive hints from Dr. Kanthack, I have rarely succeeded in obtaining, or rather constantly obtaining, what I should consider satisfactory results.

I prefer to use Kuhne's carbolic methylene-blue in preference to Löffler's solution.

After trying all the usually-described methods of double staining with eosine and methylene-blue, either separately or combined in mixture, *e.g.*, Plehn's* or Canon's,† I have never succeeded sufficiently to recommend these stains for clinical use. No doubt the results depend very largely on the time the film is subjected to the action of the methylene-blue solution, and particularly on the rapidity with which the films are dried after being stained.

Ehrlich's new *Triple Stain* seems to be very generally used. It consists of the following formula. Saturated watery solutions of orange G, acid fuchsin, and methyl-green are first prepared.

Saturated watery solution of	Orange G . .	24–27 cc.
	Acid fuchsin . .	16–33 „
	Methyl-green . .	25 „
Then add—	Water . . .	60 „
	Absolute alcohol .	40 „
	Glycerine . .	20 „

The mixture should stand for one or two weeks before being used.

Films fixed by heating or prolonged (twelve hours) action of the alcohol and ether mixture are said to give the best results. Stengel found that as a rule a rather larger quantity of methyl-green was required.

* Plehn's, or Czenzinsky's Mixture, has the following formula:—

Methylene-blue concentrated aqueous solution .	40 parts
Eosine, ½% solution in 70% alcohol . . .	20 „
Distilled water	40 „

† Canon's solution, very closely resembling this, is composed of:—

Methylene-blue concentrated aqueous solution .	40 parts
Eosine, ¼% solution in 70% alcohol . . .	20 „
Distilled water	40 „

Film preparations fixed by alcohol are stained in either of these mixtures for several hours (3–6 according to Müller) rinsed in water, dried, and mounted in Canada balsam.

After staining for from two to eight minutes the preparation is rapidly rinsed in water, dried, and mounted. The nuclei of the leucocytes are stained a greenish blue, the eosinophile granules red or brownish red, and the neutrophile granules violet or lilac colour. The red blood corpuscles are coloured yellow, and their nucleus when present green.

Gulland speaks very highly of the *Ehrlich Biondi* mixture, and prefers it to other triple stains. It is best to obtain Grubler's solution ready-made, or the powder for making it from one of the London agents (*e.g.*, Baker or Kanthack).

Preparations are stained in a few minutes (from ten to thirty) in the concentrated solution.

Cabot recommends the following modification of the triple stain :—

* Ehrlich Biondi (or Grubler's Biondi-Heidenhain)	
Powder	grs. xv.
Absolute alcohol	1 cc.
Distilled water	6 cc.

Preparations fixed by heat are stained for about one to five minutes. He points out that the time required for staining depends on the length of time which the specimens have been heated. If the preparations be over-heated the red blood corpuscles have a faint colour, if under-heated they are of a brownish grey tint, instead of a brilliant yellow.

Personally I cannot recommend the use of any of the triple stains for clinical use. The colours are more or less blurred, and as the green colour of the nuclei is

* This formula is taken from Cabot's book, but I doubt whether gr. xv. of Grubler's powder, which contains little or no fuchsin, is soluble in 7 cc. of dilute alcohol.

very indistinct, there is considerable difficulty in obtaining a good insight into the nuclear structure.*

Dr. Gulland, in a private communication on the subject, advised staining preparations for thirty minutes in the undiluted stain, washing with distilled water very rapidly, and dehydrating with absolute alcohol as quickly as possible. The preparations are then cleared with xylol and mounted in balsam.

To overcome the faint colouring of the nucleus, he suggests staining very lightly with haematoxylin first, then with the Ehrlich Biondi solution.

Dr. Gulland, in *British Medical Journal*, March 13th, 1897, has suggested a method of fixing, and, if necessary, partially staining, blood films at the same time. Fresh films before becoming dry in the air are placed in the following solution :—

Absolute alcohol, saturated with eosine	25 ccm.
Pure ether	25 ccm.
Sublimate, in absolute alcohol (2 grms. to 10 ccm.)	5 drops (more or less).

Films are fixed in three or four minutes. They are then thoroughly washed and stained for one minute in a saturated watery solution of methylene-blue, washed in water, quickly dehydrated in absolute alcohol, cleared in xylol, and mounted in balsam.

* Even such an authority as Bolles Lee makes the following statement in regard to the Ehrlich Biondi mixture as a stain for general work :—

"After a very careful study of this admired stain, I find I cannot commend it for any but the most special objects. It must in the first place, I think, be acknowledged that a reagent that can neither be prepared nor preserved without the minute precautions detailed above, is something much too great and good for human nature's daily food. The stain is a very fine one when successful, but the most minute precautions will not ensure its being successful. It is very capricious, it seldom gives the same result twice running." (*Microtomist's Vade-mecum*, 4th edition, 1896.)

He suggests that any acid stain which is soluble in alcohol, and not precipitated by sublimate, may be used instead of eosine, or the stain may be omitted altogether from the fixing mixture, and films subsequently stained in any of the aniline dyes.

I have not generally used this method, chiefly because the absolute alcohol and ether mixture answers very well for practical purposes. To my mind this method has one disadvantage if haematoxylin is to be the subsequent stain, that is, all the sublimate must be very thoroughly washed out from the preparation, otherwise a precipitate will be formed when brought into contact with haematoxylin. It fixes the elements much better than the alcohol and ether solution, and for detailed study of blood and film preparations generally, it answers excellently.

Müller, in a very extensive paper (in *Centralblatt fur Allgemeine Pathol. u. Pathol. Anatomie*, 1892) discussing methods of blood examination, repeatedly draws attention to *haematoxylin* as being undoubtedly the best nuclear stain, and particularly recommends eosin-haematoxylin. This can be used either as Ehrlich's eosin-haematoxylin solution, or by first staining in eosine and then in haematoxylin. [As haematoxylin solutions require to be kept some time to mature, it is better to obtain them ready prepared from Grubler's agents.] Preparations require to remain in the solution some hours.

Formerly I stained fixed films for several hours in Ehrlich's acid haematoxylin solution, then washed, and, if necessary, decolourised in weak acid alcohol, and then stained in glycerine or aqueous solution of eosine. The great disadvantage of this method, independently of the time required for the stains to act, was that the

eosinophile granules never would stain as well after immersion in the acid haematoxylin as before, and hence it is advisable to stain first with eosine and then with haematoxylin.

I have modified this process, and now always use the following method.

Eosine and Haematoxylin.

Films of blood on slides dried in the air are fixed in the alcohol and ether mixture for a minute or so. They are then stained in a dilute alcoholic solution of eosine, viz., eosine one part, water one hundred, alcohol one hundred [the eosine soluble in water is used in preference to that soluble in alcohol], for about half a minute or longer. They are then washed in water and stained with Bohmer's or Delafield's haematoxylin solution (which should always be filtered before use), for about ten to fifteen seconds, washed in water, and examined whilst wet to see if the blue of the haematoxylin in the nuclei of the leucocytes is sufficiently deep; if not, they are again exposed to haematoxylin. The films are then washed—tap water being sufficiently alkaline to develop the blue colour of the haematoxylin—dried, cleared with xylol, or cedar oil, and mounted in Canada balsam.

The staining with eosine requires no comment. The exact time matters little, the longer films are stained the deeper red will the corpuscles appear. As to the haematoxylin solution, the old formula of Bohmer, or Mayer's Haemalum, answers perfectly well. It is advisable to procure it ready-made. The time it requires to stain depends very largely on its maturation.

Until one knows the staining power of the haematoxylin it is better to be on the safe side, and under rather than over-stain. We aim at having the nuclei of the leucocytes stained clearly and distinctly without displacing the eosine in the protoplasm of the multi-nucleated leucocytes.

The time required for staining with haematoxylin may be anything from a few seconds to ten minutes. I find a half-minute's immersion gives perhaps the best results.

By this means films can be made, fixed, stained, and mounted in about ten to fifteen minutes. Of all methods it is, as far as my experience goes, the most constant and reliable.

The red blood corpuscles appear of a yellow-pink, or red colour, according to the time that the film is exposed to the eosine. The nuclei of the multi-nucleated leucocytes stain distinctly and sharply of a dark blue colour, the threads of chromatin connecting the nuclei being clearly seen. The nuclei of the small uninucleated leucocytes are of a somewhat deeper blue. The protoplasm of the leucocytes is of a pink colour, whilst the granules of the eosinophile cells are stained deep red.

The eosin-haematoxylin method of staining is particularly adapted for the examination of the nucleated red blood corpuscles. The nuclei and, when present, the mitotic figures are very conspicuous, as they are stained of a dark blue, almost black colour. Degenerative changes in the red blood corpuscles, "polychromatophilia," are well seen.

This stain is also particularly suited for the demonstration of the malaria organism, as I have pointed out when dealing with this subject.

Coverglass films are usually stained by placing them film downwards in a watch glass containing the stain, or, in cases in which the stain acts very quickly, by simply pouring on the solution of the dye for a few minutes and then washing.

In order to fix and stain slide films I use wide-mouthed, glass-stoppered bottles, about $1\frac{1}{2}$ inches in diameter, $3\frac{1}{2}$ inches in height, holding two ounces. (See Fig. 2. These have been specially made for me by the York Glass Co.) They are large enough to hold two slides (or even four) back to back. This is a very convenient, economical, and clean method of using fixing and staining solutions. Such bottles contain alcohol and ether solutions, eosine, triple stain, etc. They are not particularly adapted for haematoxylin solutions, as these always need filtering before using.

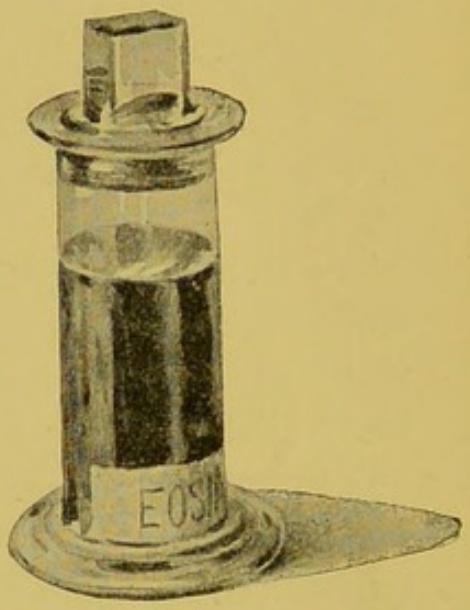


FIG. 2.

Bottle in which Blood Films made on Slides can be conveniently fixed and stained.

All the aniline stains used should be Grubler's, and these, as well as the instruments for counting the corpuscles and estimating the haemoglobin, can be obtained of Baker, 244, High Holborn, W.C.

With regard to the microscopic magnification necessary for clinical work, practically everything can be seen with a $\frac{1}{6}$ or $\frac{1}{8}$ inch objective; but a $\frac{1}{12}$ inch oil immersion lens is almost essential for the study of the malarial organism, and is in all cases of the greatest value.

General Morphology of the Blood.

Blood consists of a fluid, the plasma, or liquor sanguinis, containing cellular bodies, the corpuscles. Three forms of elements may be recognized—the red blood corpuscles, by far the most numerous, giving to blood its characteristic colour; the white blood corpuscles, or leucocytes; and the blood plaques, or blood plates.

THE RED BLOOD CORPUSCLES, OR ERYTHROCYTES.

In man these are biconcave, non-nucleated, flattened discs, having a circular outline. (See Plate I., Fig. 1.) Their surface is smooth, permitting them to glide easily past each other, and although very extensible they are equally elastic. When seen in a thin layer they are of a yellow rather than red colour, and in consequence of their shape appear darker in the centre than at the margins when seen at a distant focus, but lighter in the middle and dark at the periphery when viewed with a near focus. The colour of the corpuscle is due to the presence of haemoglobin, and in health practically all the red cells contain exactly the same amount of colouring matter.

As regards their *size* it is possible, according to Hayem, to recognize, firstly, large elements measuring 8 to 9 μ, constituting about $12\frac{1}{2}\%$; secondly, medium-sized corpuscles, having an average diameter of 7·5 μ, constituting about 75 %; and thirdly, small corpuscles measuring 6 to 6·6 μ, which constitute about $12\frac{1}{2}\%$ of

all the erythrocytes. It is thus seen that by far the greater number of red blood corpuscles have a mean diameter of 7·5 μ. (The Greek letter μ is equal to $\frac{1}{1000}$ part of a millimetre, and is usually known as a micro-millimetre.)

In addition to these, the same observer speaks of dwarf corpuscles, "globules nains," having a diameter between 3·5 and 6 μ, and giant corpuscles, "globules géants," above 9·5 μ. These are both occasionally found in health, but the latter only in the newly-born. Alterations in size are, however, very conspicuous in several pathological conditions, which will be noted later.

The erythrocytes are of an extremely delicate structure, and undergo considerable alterations when exposed to moisture, foreign material, or injury. Alterations in their form, due to artificial causes, must not be confounded with changes resulting from pathological causes.

One of the earliest and most marked *artificial* deformation is that in the presence of moisture the disc-like corpuscle assumes a spherical form, or under similar conditions loses part of its colouring matter, becoming much paler, constituting the chlorocytes of Hayem; whilst others, losing all their haemoglobin, become completely decolourised, and are termed achromacytes by the same author; and it is probable that the so-called invisible corpuscles, which Norris considered as the early form of the red corpuscles, are none other than these altered by external agencies.

Further, the corpuscles may become spinous, undergo fragmentation, or become crenated or vacuolated, and hence the desirability of paying considerable attention to the method of examination before regarding these changes as of pathological importance.

Hayem considers that the coloured elements consist of a part insoluble in water, constituting the stroma of Rollett, having no apparent structure, the surface of which becoming somewhat hardened constitutes a sort of membranous envelope; and a part soluble in water, an albuminoid material containing haemoglobin.

The corpuscles have no cell membrane, no contractile protoplasm, and do not, therefore, undergo amoeboid movement, such as is seen in some of the leucocytes.

They are by far the most numerous of the elements found in the blood, and the general consensus of opinion is, that there are five million red corpuscles per cubic millimetre in the blood of a healthy man, and $4\frac{1}{2}$ million in that of a woman.

Physiological Alterations of the Red Blood Corpuscles.

The Effects of Age.—The number of red corpuscles shortly after the birth of a child is relatively high, being greater than that of the mother. Hayem found a maximum of 6,262,000 and a minimum number of 4,340,000. In a child 10 hours old Otto found 6,912,000, and in another 25 hours old 6,496,000.

After the first day there is a gradual decrease in their number, whilst the weight of the body undergoes an increase. It appears very doubtful whether the time at which the umbilical cord is tied, influences the number of corpuscles. Hayem, however, found in six children in whom the cord was tied immediately, an average of 5,087,000, whilst in eight children in whom the cord was not tied until the umbilical artery had ceased, an average of 5,576,000 per cmm., and this difference persisted for forty-eight hours.

The colour of the capillary blood for the first few days after birth is darker than that of the adult condition, and more closely resembles the colour of venous blood.

The size of the corpuscles appears much more unequal in infancy, the maximum being 9–10 μ, the smaller measuring only 5·5 μ, and there is a marked alteration in their diameter from day to day. They more readily undergo changes in form under the influence of moisture, the small corpuscles readily becoming spherical. The amount of haemoglobin contained in the individual corpuscles at this early age is greater than in the adult—the colour index on an average being 1·1. These alterations are probably due to developmental conditions.

"In spite of the presence of large corpuscular elements in the blood, nucleated red blood corpuscles are never found in the newly-born." (Hayem.) They disappear at about the 6th or 6½ month of intra-uterine life.

Influence of Sex.—There are fewer coloured corpuscles in the adult female than in the male. This may possibly be due to menstruation, as Sterlin found that in children from 9 months to 15 years the red corpuscles were slightly more numerous in the female.

Constitution.—The robust have, as would be expected, a larger number of coloured elements than the feeble.

Effects of Food.—The taking of food causes a slight decrease in the number of red corpuscles, as opposed to an increase in the number of leucocytes—probably

due to the dilution of the blood during digestion. A mixed diet would seem to be the most suited for the renovation of the blood. A fast of 24 hours' duration produces a very distinct increase in the number of the corpuscles, and a prolonged fast has much the same effect. Hayem quotes a case in which a dog was totally deprived of food until its death, which took place after 25 days, the red corpuscles steadily increasing till the end. This apparent increase is evidently the result of thickening of the blood when the corpuscles become concentrated. Exactly the same thing occurs in diarrhœa.

Prolonged fatigue causes a diminution in their number. Menstruation produces a slight anæmic condition, which quickly returns to the normal. Pregnancy is followed by some degree of diminution of the red corpuscles and haemoglobin, but the alteration varies very much in different individuals. Young women and primipara show the greatest diminution in the corpuscular elements, especially in the latter two months. Lactation does not cause any appreciable anæmia. The effects of parturition depend very largely on the quantity of blood lost, and on the resistance and vigour of the individual. Generally there is some diminution in the red cells, but reparative process slowly sets in.

Hayem states that the number of red corpuscles and the quantity of haemoglobin are the same in all parts of the vascular system.

Pathological Alterations in the Red Blood Corpuscles.

Alterations in Number.—Polycythemia is relatively infrequent, and practically only occurs—in addition to fasting and want of nourishment—in conditions in which

concentration of blood occurs as the result of considerable loss of liquids, particularly from the intestinal tract, *e.g.*, cholera and diarrhœa. Hayem points out that 6·2 to 6·5 millions of red corpuscles may be counted during the algide stage of cholera. He does not believe that the plethoric condition could be attributed to an actual increase in the number of red corpuscles in the blood.

Diminution in the number of the erythrocytes, oligocythemia, is of very frequent occurrence. When produced rapidly, as by repeated haemorrhages, anæmia cannot pass certain limits without proving fatal, and patients are in a very serious condition when the number of red cells approaches one million. (Hayem.) "When the anæmia is progressive the body seems to adapt itself to the impoverished condition of the blood, and not infrequently patients are met with, active and working for their living, in whom the blood may only show two million corpuscles per cmm., or even less. This fact is still more remarkable when one realizes that often the haemoglobin in these corpuscles is at the same time much less than in normal elements." (Hayem.)

Hayem, as a result of his extended experience, says that an anæmia may be curable when the number of erythrocytes is not lower than half a million, but does not think that life could continue when they fall lower than 3 to 400,000.

In fatal cases of anæmia some authors have found less than 300,000 corpuscles per cmm.

Alterations in Diameter.—Not only may the red cells undergo alterations in number, but alterations in size are often seen in pathological conditions. There may be

Plate I.

Red Blood Corpuscles and their Alterations.

Film preparations stained with eosine and haematoxylin. Examined with eyepiece 2. Objective $\frac{1}{12}$" oil immersion and tube length of 160 mm.

Fig. 1.—Normal red blood corpuscles showing the slight variation in size found in health.

Fig. 2.—Blood plates, some isolated, some in clusters. They are usually coloured with a mixture of eosine and haematoxylin.

Fig. 3.—Small red corpuscles or Microcytes, of which two are deeply stained—the so-called Eichhorst's corpuscles.

Fig. 4.—Large red corpuscles or Megalocytes, two of which show some degree of vacuolation. (*a*) Normal corpuscles.

Fig. 5.—Poikilocytes of varying size, shape, and colour.

Fig. 6.—Other degenerative changes in the corpuscles. (*a*) Cracked or vacuolated elements. (*b*) Pale or shadow corpuscles. (*c*) Polychromatophile corpuscles.

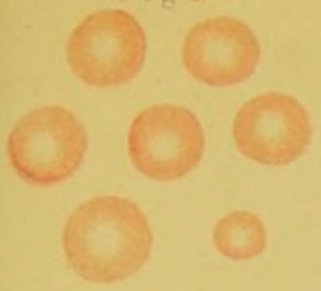
Fig. 7.—Nucleated red corpuscles of about the size of the normal elements—Normoblasts—from blood. In some the nucleus is single, in others double, and in a few treble. In some the nucleus is partially extruded. (*a*) Normoblast dividing. (*b*) Nucleus partially free, with only a slight amount of protoplasm surrounding it. (*c*) Nucleus of normoblast free. (*d*) Microblast.

Fig. 8.—Large nucleated red corpuscles or Megaloblasts, generally showing a somewhat indistinct large nucleus. The central figure also illustrates polychromatophile degeneration.

Fig. 9.—Nucleated red corpuscles from the marrow of a kitten two days old. Two karyokinetic figures (*a*) are seen.

PLATE I.

Fig 1.



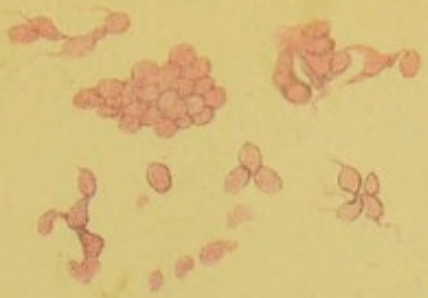


Fig. 2.

Fig 3.

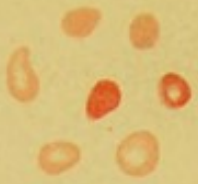


Fig 4.

Fig 5.

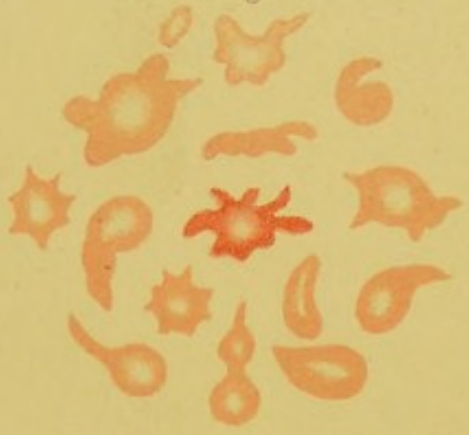


Fig 6.

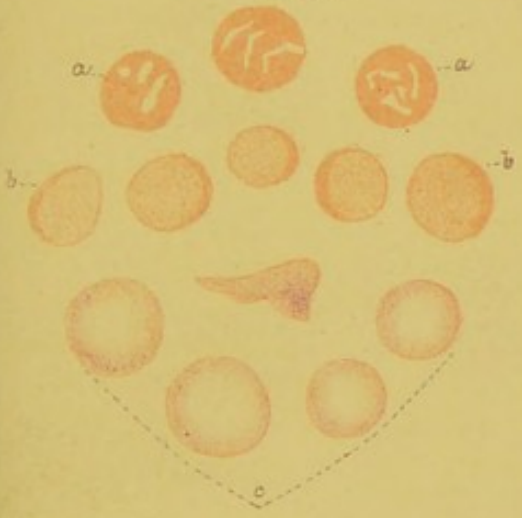


Fig 7.

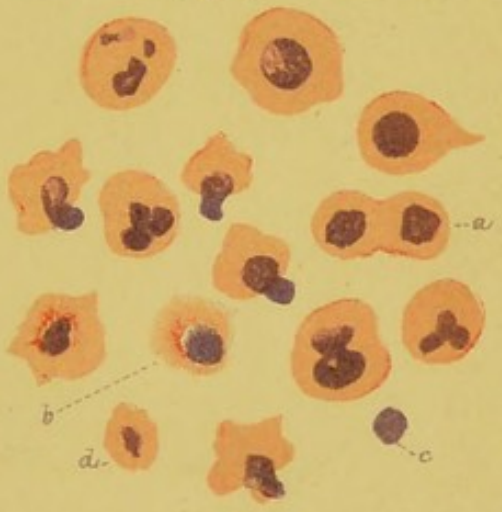


Fig 8.

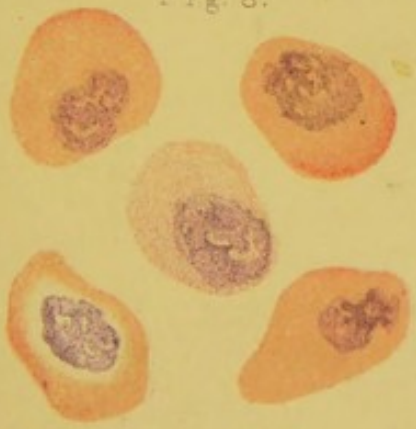
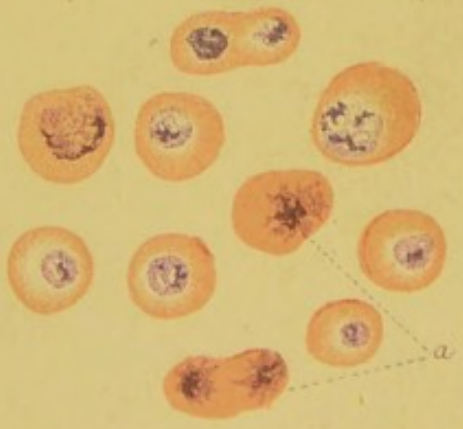


Fig 9.



F.L. Coles del

West, Newman. Chr.

a diminution in the diameter, *e.g.*, the dwarf corpuscles or microcytes (see Plate I., Fig. 3), measuring 3·5 to 6 μ, or an increase in size when numerous giant cells or megalocytes, generally 9·5 to 12 μ, or even 14 μ in diameter, may be found. (See Plate I., Fig. 4.)

Eichhorst's corpuscles are small deeply-stained bodies, about 3 μ in diameter, generally spherical in form. They were considered by their discoverer to be characteristic of pernicious anæmia; but this is generally regarded as incorrect, and the opposite, viz., the occurrence of megalocytes in large numbers, is more important, though not characteristic of pernicious anæmia.

The term macrocythemia has been applied to the condition in which the large elements prevail, microcythemia when the small corpuscles are in excess. Hayem states that megalocytès rather than microcytes point to a severe anæmia.

Alteration in size is usually associated with *changes* in the *form* of the corpuscles. The elements losing their disc-like form may show irregular thickenings or projections from their border, giving rise to oval, pear-shaped or peculiarly elongated bodies, which have been termed poikilocytes by Quincke, or schistocytes by Ehrlich. (See Plate I., Fig. 5.)

These are met with in nearly all forms of anæmia, and are by no means characteristic of pernicious anæmia, as Quincke originally supposed.

Fragments of corpuscles are certainly seen in some severe conditions, although, as Hayem very strongly points out, these may occur in normal blood if due care has not been taken in the avoidance of moisture, pressure, etc.

The same observer has noticed the occurrence in

some cases of anæmia of very small rod-shaped, red corpuscles, to which he has given the name pseudo-bacilli. They have been mistaken for bacteria on account of their small size, their shape, and the very active movements which they undergo, probably partly molecular and partly amoeboid in nature. Whether these latter changes are due to artificial conditions, or whether the corpuscular elements in disease are more susceptible to injury, seems a little doubtful.

Normally the amount of haemoglobin contained in a corpuscle is in direct proportion to the size of the element; but when pathological alterations in size and shape occur, there is frequently no relation between these two factors.

Diminution in the amount of haemoglobin is usually more pronounced in the small than in the large or giant corpuscles, and not infrequently the amount of colouring matter in the latter is increased, and this accounts for the high colour-index frequently found in pernicious anæmia.

Sometimes, in spite of all care used in the method of examination, partially decolourised chlorocytes, or totally colourless corpuscles, achromacytes or "shadow corpuscles," may be seen, showing a diminished resisting power of the coloured elements. These are said to occur particularly in infectious diseases, adynamic typhoid, haemorrhagic small pox, haemoglobin-anæmia, etc.

Gabritschewski found that the red blood corpuscles have an elective staining reaction with eosine, rather than with other aniline dyes. In the circulating blood the living red corpuscles do not stain, *achromatophile;* but as they die—*e.g.*, when fixed—they stain only with

one of the dyes—the acid stain—of a mixture of aniline colours—*e.g.*, eosine and haematoxylin; this is termed *monochromatophile.* In pathological conditions, however, especially in severe anæmia, measles, scarlet fever, small pox, typhus, and purpura, a few red blood corpuscles lose their elective staining property, and from a mixture they stain with two or three colours, either diffusely or in streaks or spots, the last especially in their central part, *polychromatophile.* (See Plate I., Fig. 8.) Thus when eosine and haematoxylin are used the diseased erythrocytes appear of a violet or purple, instead of the normal pink or red colour. These alterations Ehrlich considered as signs of the death of the corpuscle.

Maragliano and Castellino divide these changes, showing the death of a corpuscle—necrobiotic alterations—into endoglobular and total transformations.

The endoglobular changes commence by diminution of the haemoglobin in the centre, with an increase in that at the periphery, causing an apparent enlargement of the central depression of the corpuscle, so that eventually only the margin of the red corpuscles is coloured. This gives the cell an appearance of a figure of 8 when seen on its side.

Sometimes the decolourisation does not proceed so uniformly, and the blood corpuscle may show variously-shaped white patches, with fairly definite margins. This condition corresponds to vacuolation of a corpuscle, as described by Hayem and others. (Plate I., Figs. 4 and 6.) The decolourised areas not infrequently show amoeboid changes, and have been mistaken for unpigmented malaria parasites.

As the process advances, movement of the particles

within the decolourised zones becomes apparent, and increases in rapidity, and finally the whole of the corpuscle degenerates into a granular mass.

The changes in the corpuscle as a whole—total necrosis—appear later than the endoglobular alterations. The corpuscles show little projections as points or round knobs, which, increasing in size, constitute the condition described as poikilocytosis. Some of these projections become detached and float in the plasma. At the same time decolourisation occurs, and though at first starting from the centre, ultimately affects the whole corpuscle.

Limbeck, in confirming this account, remarks that reagents of various kinds will produce similar results, especially the endoglobular changes, yet they may be seen in the blood drawn freshly under certain pathological conditions, proving that these diseases may be associated with necrosis of the red corpuscles.

In addition to pathological alterations in the blood plasma—*e.g.*, that resulting from cancer, severe tuberculosis of the lung, parenchymatous nephritis, etc.—various poisons, chloroform, antifebrin, dilute acids and alkalies, and organic acids may produce similar results.

Ehrlich drew attention to the fact that in anæmic conditions, the appearances of degeneration were very closely connected with those of regeneration of the blood corpuscles. He considers poikilocytosis as essentially a sign of degeneration in anæmias. Quincke, who first introduced the term, regarded it as characteristic of pernicious anæmia, but as it occurs in the blood in many other conditions much of its significance is lost.

Hayem mentions the occurrence of increased viscosity and diminished elasticity of the red corpuscles in hyper-

trophic cirrhosis of the liver with jaundice, and in certain cachectic conditions. Under such circumstances the corpuscles run together, fuse into a mass, and lose their individual outline.

Other important alterations of the corpuscles, which occur in malarial fevers, will be fully described when we speak of this disease.

Nucleated Red Blood Corpuscles.

The occurrence of nucleated red blood corpuscles in the blood is the most interesting and important of the numerous alterations which the red cells may undergo in disease. Although present during intra-uterine life, it has been generally accepted that they are never seen in the normal blood of man at birth. Associated with signs of degeneration of the blood in certain pathological conditions, are the signs of regeneration, and amongst these the appearance of nucleated red cells.

These elements cannot be detected in fresh, unstained blood, but are best seen in dry films stained with a solution of methylene-blue, or better, with eosin-haematoxylin, and even then careful examinations of many films may be necessary before they are met with.

They have been termed, according to their size, normoblasts, microblasts, and megaloblasts.

The *Normoblasts* (Plate I., Fig. 7) are nucleated red cells of about the same size as the ordinary red corpuscle, varying from 7·5 to 8 μ in diameter.

The nucleus is usually single, round or oval, and shows an intra-nuclear network. It stains very deeply with most nuclear stains, so that in films coloured with eosin-haematoxylin, even under a low magnification,

they are seen to be the darkest objects in the preparation. It is smaller than the nucleus of a lymphocyte, which, although it stains deeply, does not stain as intensely as the nucleus of these elements. (Plate III., Fig. 1.)

Its size bears no constant proportion to that of the whole corpuscle, but it seldom exceeds one-half the diameter of the red cell. The nucleus is usually eccentrically situated, but is nearly always completely surrounded by protoplasm. Sometimes, however, appearances are met with, which suggest the extrusion of the nucleus.

Occasionally, instead of a single nucleus, two or three apparently separate nuclei may be seen lying very near each other, or connected by delicate strands of nuclear structure, so that a figure of a dumb-bell or trefoil results.

Mitotic figures are sometimes, though very rarely, seen in the nuclei of normoblasts. The *protoplasm* surrounding the nucleus is homogeneous, and contains haemoglobin ; it stains uniformly with the acid aniline dyes, but is somewhat paler in colour than the ordinary red corpuscle. Sometimes it shows polychromatophilia, and appears of a purplish tint when stained with eosin-haematoxylin.

The normoblasts are not infrequently crenated, and in my experience in most cases of secondary anæmia they seldom show a perfectly regular outline.

Microblasts (Plate I., Fig. 7, d) are nucleated red corpuscles, smaller than the ordinary coloured corpuscle. They are less frequently met with than either of the other varieties.

The *Megaloblasts* (Plate I., Fig. 8) are considerably

larger than the normoblasts, being nearly always over 10 μ, not infrequently 14 to 16 μ in diameter.

The nucleus is often extremely large, occupying $\frac{2}{3}$ of the cell. It stains much less deeply than that of the normoblast, presenting at all times a very indistinct appearance. The protoplasm contains more haemoglobin than the small variety and stains deeply with eosine, and the whole cell has a swollen or distended appearance.

It sometimes shows signs of degeneration, and polychromatophilia, vacuolation, etc., may occasionally be seen.

Megaloblasts and normoblasts may undergo poikilocytosis, constituting poikiloblasts.

I can confirm the statement that the normoblasts are less resistant to, or more easily deformed by, pressure, and have noticed that in some films three or four small free nuclei, with little or no surrounding protoplasm, may be met with (Plate I., Fig. 7, b and c); but whether they existed as such in the blood, or whether this was the result of the slight pressure they were subjected to in spreading the film, remains uncertain.

Limbeck and Ehrlich state that the normoblasts differ from the megaloblasts in that the nucleus of the latter is not extruded, but undergoes degeneration and disintegrates by degrees in the cell. Normoblasts are found in the bone marrow of a healthy adult (Plate I., Fig 9), whilst megaloblasts never occur in the adult, only in the marrow and blood-forming organs of the embryo. Ehrlich, therefore, regards the appearance of the latter in pathological conditions in an adult as a return to the embryonic type.

The conditions under which nucleated red cells may

be met with, and still more the importance from a clinical aspect of their occurrence, still seem somewhat uncertain.

Hayem (*du Sang*, 1889) expresses the following opinions regarding them :—

"This interesting lesion appears to me rare, notwithstanding the assertion of certain authors. I have only met with it in extreme anæmias and in leucocythemia." (p. 386.) "Nucleated red cells are constantly found in leucocythemia at some time in its course" (p. 857), "when the haemopoietic organs, especially the spleen and marrow, are full of embryonic elements, proving that in those cases in which numerous nucleated red corpuscles are formed in the haemopoietic organs, these elements can enter into the blood without losing their nuclei, and that they persist in the general circulation with their own characteristics, just as in the embryo." (p. 609.) "Excluding leucocythemia, the appearance of nucleated red corpuscles is a rare condition in human blood. I have only seen it in patients suffering from extreme anæmia, almost at the point of death, and their number has never been conspicuous. These elements have always been infrequent, and their appearance has coincided with an aggravation in the condition of the patient; they have never acquired the significance of a reparative process on the part of the blood. When nucleated red corpuscles, however scanty, are found in a patient suffering from spontaneous or symptomatic anæmia the most serious prognosis ought to be given, and a near fatal termination expected. Would this be so if these elements were destined to regenerate the blood?" (p. 609.)

"They only appear in the blood of intense and

extreme anæmia, and seem to take only a very limited part in its regeneration. It is a final and insufficient attempt at the restoration of the blood." (p. 610.)

Speaking of chlorosis Hayem says, "Whatever be the intensity of the anæmia in this disease, I have never been able to find a single nucleated red corpuscle. For some years past a certain number of foreign pathologists have asserted that nucleated red cells are met with frequently enough in chlorosis. I have re-examined with reference to this statement my collection of preparations, taking care to stain the elements, and the result of this inquiry was absolutely negative." (p. 623.)

"Towards the end of pernicious anæmia nucleated red corpuscles may be seen, but they are not found in all cases." (p. 794.)

As to their occurrence in post-haemorrhagic anæmia, he states "that exceptionally nucleated corpuscles may be seen in anæmia, the result of haemorrhage; firstly, in cases in which repeated haemorrhages have reduced the patient to a condition threatening existence, but they are extremely scanty and require very careful examination before they are found; secondly, when repeated haemorrhages, or even a single large loss of blood, occur in a patient already suffering from an organic disease, and their presence under these conditions is of very grave import." (p. 826.)

Muir, in the *Journal of Anat. and Physiology*, 1891–92, states that up to that time he had found these elements in twelve cases, viz., four cases of pernicious anæmia, six of leucocythemia, one of traumatic anæmia, and in one case of secondary anæmia complicated with haemorrhages.

In half of these they were numerous, whilst in the

remainder they were scanty, and required careful search. In traumatic anæmia they were generally normoblasts; in pernicious anæmia, megaloblasts or poikiloblasts.

As to their importance he mentions that in one case of post-haemorrhagic anæmia, and two of pernicious anæmia in which they were most numerous, the patients ultimately recovered, whilst in cases of most extreme anæmia they were absent immediately before death. He states that Ehrlich records their absence before death in a case in which the red blood corpuscles fell to 213,360 per cmm., and "there is therefore reason in Ehrlich's supposition that their presence indicates that the bone marrow is still producing red blood corpuscles."

Von Limbeck considers the occurrence of normoblasts as a sign of the existence of new formation of the blood. They are usually only found for any length of time in variable numbers in severe anæmias, and decrease in number with continued improvement in the blood. Very rarely they occur in groups in the blood, a condition Von Norden has termed "blood crisis." The megaloblasts are, according to Ehrlich, a sign that the formation of blood is not following a normal manner. Megaloblasts and megalocytes are, according to this observer, the criteria of genuine pernicious anæmia, whilst he regards poikiloblasts as decided signs of severe alterations in the blood.

Some recent writers, contrary to Hayem, state that nucleated red corpuscles, especially and probably only normoblasts, may occur in severe chlorosis, and many record their presence in severe anæmias.

Stengel expresses the opinion that "the smaller forms usually present themselves first, and in the more

moderate grades of anæmia," whilst megaloblasts may be conspicuous in the severe cases.

"Nucleated corpuscles are undoubtedly the expression of active haemogenesis, the smaller forms being particularly significant. Occasionally they appear in separate crops (blood crisis, Von Norden), followed by increase in the number of red corpuscles in the blood. The larger forms, according to Ehrlich, are expressions of imperfectly conducted haemogenesis; but this view, it seems to me, cannot be well maintained. On the contrary, they would appear to be the expression of unusual demands upon the haemogenetic function, in which the large as well as the small forms escape from the bone marrow before their development has been completed."

I have quoted the opinions of Hayem, Muir, Von Limbeck, and Stengel as to the importance and frequency of nucleated red corpuscles, in order to emphasize the importance of these elements in the blood.

My experience is, however, quite at variance with Hayem's as regards these two points, and I consider that the two forms—the normoblasts and megaloblasts—differ entirely in their importance. I have frequently found the former in the most varied forms of primary and secondary anæmias when the oligocythemia is pronounced, and should expect to find them, or rather should make prolonged search for them, in cases in which the red corpuscles were less than two and a half million per cmm.

I quite agree with Hayem that they are constantly found, usually in large numbers, in spleno-medullary leukaemia, but are frequently absent from the lymphatic form of this disease.

With regard to their importance I am of the same opinion as many other observers, that normoblasts originate from the bone marrow, and their presence in the blood indicates a severe anæmia, but one in which an effort is being made by the marrow to improve the blood.

Megaloblasts are entirely absent from healthy adult marrow, although found in foetal red medulla, and their presence in the blood must point to a profound alteration, possibly of a degenerative nature, in the bone marrow.

It would seem that normoblasts either represent a call on the blood-forming organs — the marrow — in which premature nucleated corpuscles are precipitated into the circulation, and which, in readily losing their nucleus, increase the number of corpuscles, or that their presence indicates the degree of the increased activity on the part of the blood-forming function of the marrow.

On the other hand, megaloblasts are signs of perversion of the haemogenetic action of the marrow, and in that they apparently do not readily lose their nucleus, and do not form mature corpuscles, are indicative of retrogression rather than progression.

When megaloblasts and normoblasts are present in the blood the anæmia is always severe, and when the former are more numerous than the latter, and continue to remain so, the condition is probably one of pernicious anæmia.

The diagnostic importance of the presence of nucleated red corpuscles, especially megaloblasts, in the blood, necessitates a very careful examination of several films before their absence is determined. Sometimes

they are very numerous and may be easily found, whilst at other times their discovery occasions much laborious searching.

The Development of the Blood.

1. *In the Embryo.*—In the vascular area of the embryo a mass of cells, probably formed from the mesoblast, are found, to which the term "blood islands" has been given. From these cells, which contain nuclei, the earliest blood vessels and blood are formed. They multiply by indirect division and assume a red colour.

White blood corpuscles appear later, being formed in the venous capillaries of the liver, and, as in the adult, in the marrow of the bones, the spleen, and lymphatic glands.

The red bone marrow consists of the following elements:—Marrow cells, nucleated red blood corpuscles containing haemoglobin, eosinophile cells, giant cells, and mastzellen, or cells containing γ granules.

In the adult the bone marrow is the most important seat of blood formation.

The spleen is not generally regarded as an important factor in the formation, but rather as the seat of destruction of the red blood corpuscles.

The Malpighian corpuscles are, however, said to be one of the chief centres of the formation of leucocytes, which pass into the splenic pulp, and from thence into the general circulation.

The lymphatic glands are probably concerned in the formation of lymphocytes, as small uninucleated leucocytes often showing division by mitosis are seen there.

As the age of the foetus increases the number of

nucleated red corpuscles in the blood decreases, whilst the non-nucleated increase, probably due to the transformation of the former into the latter. This change may, according to some observers, take place in the liver, according to others in the circulating blood or spleen, and writers differ in their view of the method by which this is brought about.

Some assume that the nucleus simply disappears (Bizzozero and Neumann); others, that there is an actual separation of the nucleus from the cell. (Rindfleich and Howell.) Hayem states that the nucleated and non-nucleated red blood corpuscles have a distinctly separate origin.

Ehrlich believes that some of the nucleated red corpuscles, those known as normoblasts, lose their nuclei by a process of extrusion, whilst others, the megaloblasts, lose them by a method of endocellular disintegration.

2. *Formation of the Red Corpuscles in the Adult.*—The nucleated red corpuscles of the bone marrow are generally considered as the precursors of the ordinary non-nucleated erythrocytes of the blood.

In support of this view Neumann and Bizzozero adduce the following facts:—That a great number of mitotic forms of these cells are seen under normal conditions in the marrow, that they disappear with rapidity, and that in all periods of life division by mitosis or karyokinesis is seen.

On the other hand, to what extent the spleen and lymphatic glands take part in the formation of red blood corpuscles opinions differ.

After loss of blood in a previously healthy individual,

signs of rapid regeneration appear, and when the haemorrhage has been excessive, nucleated red cells are seen in the circulating blood. The time necessary to replace the corpuscles is naturally in proportion to the amount of the haemorrhage. Löwit believes that the spleen, lymphatic glands, and marrow take an equal share in the formation of both the red and white corpuscles, and states that in these organs two different forms of cells, the erythroblasts and leucoblasts, both characterized by an absence of haemoglobin, arise.

These differ in that the leucoblasts possess amoeboid movement, and multiply by direct or amitotic division, whilst the erythroblasts possess no amoeboid movement, and increase by indirect or mitotic division.

Latterly, however, he thinks the leucoblasts divide by indirect division by granules. The nucleated red corpuscles arise from the erythroblasts containing no haemoglobin, by endocellular means in the blood. Müller considers that the red and white corpuscles have a common origin, and a common mode of division. Denys assumes, as Löwit does, two separate sources of development of white and red corpuscles, which multiply by mitosis.

Hayem thinks that the red corpuscles arise directly from the blood plates, or as he terms them the haematoblasts, which increase in size and acquire haemoglobin. In favour of his theory he adduces the facts that normally a number of erythrocytes are found in the blood, smaller than the ordinary corpuscles, and these are increased in number when there has been a loss of blood, and assumes that they are the early stages of new corpuscles. He has noticed all transitional forms between these haematoblasts and mature red corpuscles.

He points out that there are two varieties of erythrocytes in the embryo and adult. The ordinary non-nucleated which occur in normal blood, and which increase in cases of renovation of the blood of haematoblastic origin; and secondly, the nucleated red corpuscles, which occur in the embryo and in many of the lower animals, but are not normally present in man after the sixth month of intra-uterine life. They are never present in the healthy newly-born child, but appear in extreme anæmias, and seem to take only a limited part in the regeneration of the blood. These he considers are formed by the haemopoietic organs.

Hayem's theory of the haematoblastic origin of the red blood corpuscles is not generally received as correct.

Engel considers nucleated red cells have originated from embryonic cells containing haemoglobin :—metrocytes. These divide by mitotic division into two, each containing a nucleus. Each of these again divides into a nucleated and non-nucleated cell, forming nucleated and non-nucleated red blood corpuscles. The non-nucleated corpuscles occur in mammals, whilst the nucleated forms decrease in number till the end of intra-uterine life, when they are entirely absent. They lose their nuclei with a little surrounding protoplasm containing haemoglobin, or the latter may be dissolved by the serum of the blood. The cell, having lost part of its contents, by pressure in the vascular system becomes concave.

THE BONE MARROW.

Having regard to the importance which nucleated red corpuscles and marrow cells play in pathological

conditions of the blood, it may not be out of place to give a short description of the red bone marrow.

I have examined the bone marrow of the mouse and newly-born kitten. Films were made on the slides by gently spreading out the fresh marrow in as thin layers as possible. They were then fixed in the usual way, and stained with eosine (preferably glycerine solutions) and haematoxylin. Films heated and then fixed in picric acid solution, as described under "methods," gave beautiful results as regards the karyokinetic figures, but the eosinophile cells stained imperfectly.

The best description in English of the marrow is that given by Muir and Drummond in the *Journal of Physiology*, 1892, and I have somewhat followed their description.

The Marrow Cells (Plate V., Fig. 2).—Large uninucleated cells, measuring 12–14 μ in diameter, are the most conspicuous elements present. Their protoplasm contains very fine granules, neutrophile, or finely oxyphile in nature, which are well seen in preparations stained with the triple stain. (Plate III., Fig. 5.) When films are stained with eosine and haematoxylin the protoplasm of the marrow cells, as in the blood of leucocythemia, colours a bluish colour, and not pink, as is the case with the multinucleated leucocytes, and little or no sign of granules can be seen. Their nucleus is of large size, generally round or oval in shape, and frequently has an indented or lobed appearance. It is poor in chromatin, stains somewhat faintly, and sometimes shows nucleoli in its interior. All stages in their division by the formation of karyokinetic, or mitotic figures, are beautifully seen, especially in the marrow of

young animals. They are stained somewhat deeper than the nuclei of other leucocytes. (Plate III., Fig. 9.)

The *eosinophile*, or coarsely granular marrow cells, possess a single or lobed nucleus, and sometimes show mitotic figures. Their protoplasm contains coarse eosinophile granules. They differ from those of normal blood, not only by their size, but also by the fact that they are mononuclear, not polynuclear. (Plate II., Fig. 7; Plate III., Fig. 6.)

Nucleated red corpuscles, termed haematoblasts by Neumann, nucleated haematoblasts by Hayem, and erythroblasts by other writers, are seen in the red marrow of all mammals. (Plate I., Fig. 9.) They vary considerably in size, some being about the same as that of the ordinary red corpuscle, whilst many are nearly twice as large. Between these extremes all intermediate sizes are seen. Their protoplasm is coloured with haemoglobin, but less deeply than the non-nucleated variety. It is homogeneous, stains with eosine and other acid aniline dyes, and is usually of spherical form, but tends to undergo alterations in shape more readily than the ordinary erythrocyte. The larger nucleated cells, the youngest elements, are only faintly coloured with haemoglobin. Their nucleus is large, and often shows a well-marked intra-nuclear network, or a somewhat dotted appearance. The smaller forms are older, *i.e.*, nearer the non-nucleated condition, and contain a larger amount of colouring matter. Their nucleus is smaller, and stains very deeply with haematoxylin, so that little beyond a granular appearance can be made out.

In both forms the nucleus is usually situated in the centre or towards the periphery, but in some of the smaller varieties the nucleus may appear almost entirely

pushed out of the cell, and occasionally small free nuclei may be seen without, or with extremely little, protoplasm around them. In the larger elements karyokinetic figures are often apparent, whilst in the smaller forms two nuclei, or a budding nucleus, may be frequently met with.

In addition to these red nucleated corpuscles there are colourless nucleated corpuscles, containing no haemoglobin.

Although all intermediate forms between the larger erythroblasts and the marrow cells proper are seen, Muir states that they are probably two distinct elements, and that the marrow cells belong to the leucocyte class.

Giant cells are very large, conspicuous elements, many measuring over 60 μ in diameter. (Plate V., Fig. 2.) According to Muir they belong properly to the haemopoietic tissue, and have no relation to the osteoblasts. They are generally of somewhat irregular, or lobed appearance, containing one or many nuclei. The nucleus, which is very lobulated, is arranged somewhat in the form of a circle, having one or more apertures in the centre. The nuclear portion of the cell stains a pale blue with haematoxylin, and not infrequently red blood corpuscles are seen in its interior.

According to Hayem these cells are concerned with the destruction rather than the formation of the red blood corpuscles.

In addition to the marrow cells proper and the giant cells, multinucleated and other leucocytes may be seen in red marrow, and are probably the ordinary elements of the blood, which have become mixed with the marrow in preparing films.

There is still another class of cell which I have

frequently seen, both in marrow and in the blood of leucocythemia. These are larger than the ordinary marrow, but do not reach the size of the giant cells. They are stained very faintly with haematoxylin, and seem to consist of a nucleus without any surrounding protoplasm. They appear to be made up of a very loose, open network, and contain several vacuoles or unstained parts, which appear quite homogeneous.

In the marrow they are very plentiful, but are not nearly as numerous in leucocythemic blood.

THE WHITE BLOOD CORPUSCLES, OR LEUCOCYTES.

When blood is examined in the fresh state, in addition to the red corpuscles, pale, white, or greyish bodies will be seen—the leucocytes, or white blood cells.

These are small spherical masses of protoplasm, containing no haemoglobin, and although larger in size, are much less numerous than the coloured elements.

Some appear almost homogeneous, others slightly granular, whilst a few contain large, highly refractile granules. If the slide be maintained at the temperature of the body it will be noticed that the smaller elements remain at rest, retaining their spherical shape, whilst the larger undergo various alterations in their form and outline, due to amoeboid movement, which they, unlike the erythrocytes, possess.

The white blood corpuscles have been variously classified. Wharton Jones, in 1846, recognized three varieties, viz., leucocytes containing fine granules, leucocytes with coarse granules, and nucleated leucocytes without granules. Max Schultze later, 1865, pointed out the occurrence of four forms:—

1st. Small round cells with nucleus surrounded by little, clear protoplasm, non-amoeboid;

2nd. Larger cells with nucleus and clear protoplasm, amoeboid;

3rd. Cells containing many nuclei with finely granular protoplasm, amoeboid; and

4th. Leucocytes containing coarse granules in their protoplasm, also amoeboid.

Hayem describes the leucocytes under the following heads:—

(*a*) Those of the 1st variety—the smallest cells generally spherical, finely granular, possessing a very large nucleus, which occupies nearly the whole of the cell. He includes in this class the larger or intermediate forms with an indented nucleus.

(*b*) Those of the 2nd variety—larger spherical cells with finely granulated protoplasm, and lobed or divided nucleus. These are the most numerous in normal blood.

(*c*) Those of the 3rd variety—including cells conspicuous by their very granular appearance; the granules replacing two-thirds or three-fourths of the element.

Ehrlich further elaborated this structural classification, by pointing out that the white cells react peculiarly to various aniline dyes according to the nature of the granules which they contain; and according to their staining reaction, he divides the leucocytes into:—

1. Cells with α or eosinophile granules, which stain deeply with acid aniline dyes, *e.g.*, eosine. (Plate II., Fig. 5.)

2. Cells with β or amphophile fine granules, staining both with acid or basic dyes. We are not concerned with these in blood.

3. Cells with γ or coarse basophile granules, the

PLATE II.

The Leucocytes.

Blood films stained with eosine and haematoxylin. Examined with eyepiece 2. Objective $\frac{1}{12}$" oil immersion and tube length of 160 mm.

Fig. 1.—Lymphocytes or small uninucleated elements. The nucleus is deeply stained—the protoplasm small in amount, or apparently absent. (*a*) Two large lymphocytes approaching in size the large uninucleated elements.

Fig. 2.—Large uninucleated Leucocytes or Hyaline cells. The nucleus is somewhat indistinct, the protoplasm slightly coloured with haematoxylin.

Fig. 3.—Transitional forms intermediate between the large uninucleated and multinucleated elements.

Fig. 4.—Multinucleated or Neutrophile leucocytes. The nuclei numerous, showing distinct intranuclear network. The protoplasm with this stain (eosine and haematoxylin) shows no granules.

Fig. 5.—Normal Eosinophile cells having usually two or more nuclei. The protoplasm contains large granules deeply stained with eosine.

Fig. 6.—Marrow cells or Myelocytes from leukaemic blood. Large uninucleated elements.

Fig. 7.—Eosinophile Myelocytes or Marrow cells containing eosinophile granules, showing single large nucleus surrounded by and imbedded in coarse granules. One is partially ruptured.

Fig. 8.—Marrow cells from leukaemic blood containing fine oxyphile granules, and therefore intermediate between the ordinary myelocytes and the eosinophile myelocytes.

Fig. 9.—Marrow cells from leukaemic blood, showing karyokinetic figures.

PLATE II.

Fig. 1

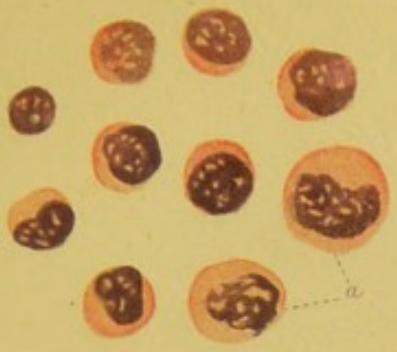


Fig. 3.

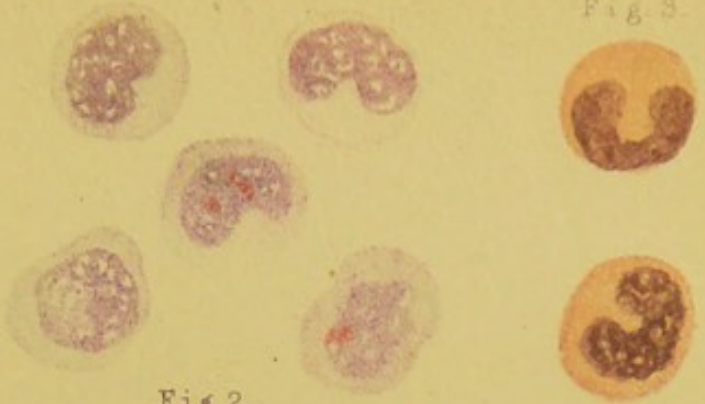


Fig. 2.

Fig. 4.

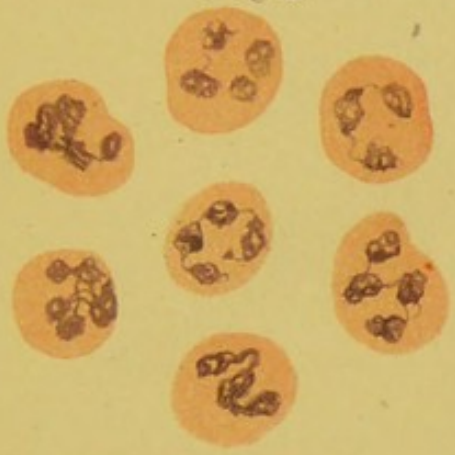


Fig. 5.

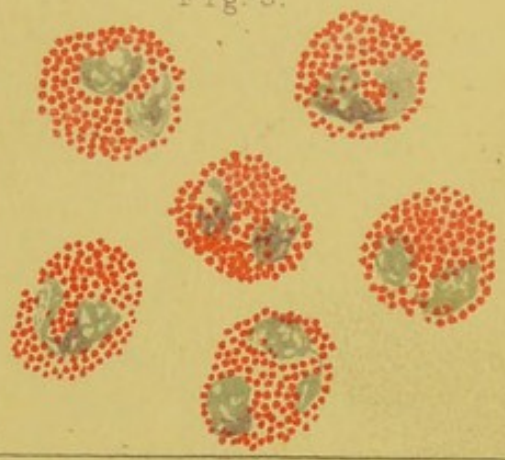


Fig 6.

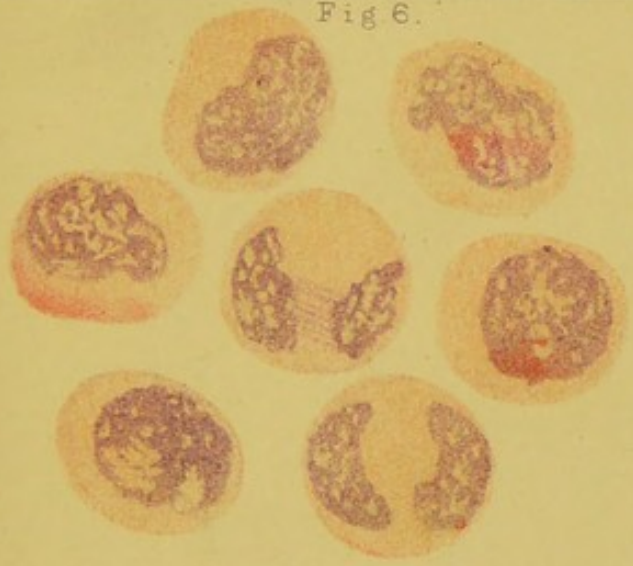


Fig. 7.

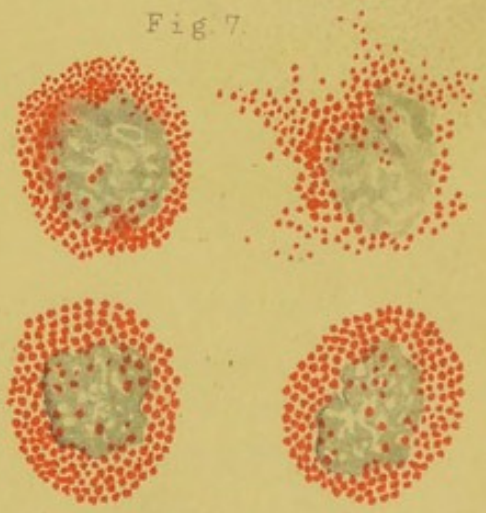


Fig. 8.

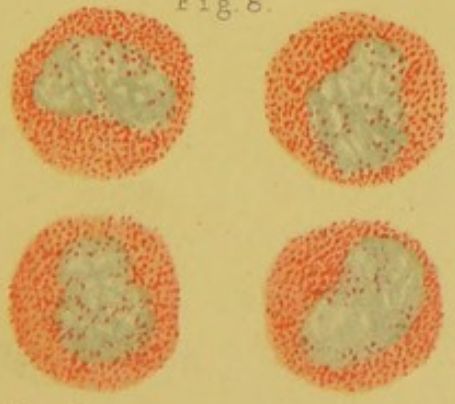
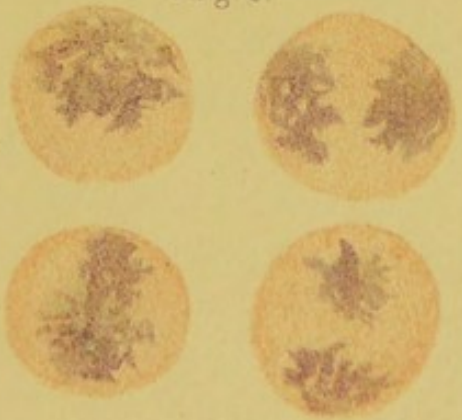


Fig. 9.



F.L. Coles del.

West, Newman Chr

nuclei of the lymphocytes are coloured before those of any other leucocytes, and this staining characteristic, together with their size and shape, render these cells easily recognizable.

The protoplasm of the smaller lymphocytes is insignificant, the nucleus frequently appearing bare and utterly destitute of a surrounding body. When visible the protoplasm rarely exceeds the appearance of a slender ring, one point of which may be thickened.

Occasionally the cell body of the larger forms of this variety contains very fine granules, basophile in nature, which stain with methyl-blue. (Plate III., Fig. 7.)

These leucocytes appear to be more resistant, *i.e.*, undergo less deformation in shape than the other varieties, and unlike these they are neither amoeboid nor phagocytic.

Their number is very variable, being increased after food, and diminished during starvation. They generally constitute about 15 to 20% of the leucocytes present in the blood. They are considerably increased in lymphatic leucocythemia, a disease affecting the lymphatic glands, and are then associated with an increase in the number of the large uninucleated elements. They are usually diminished in diseases in which the activity of the lymphatic glands is impaired.

The lymphocytes, as their name implies, originate from the lymphatic tissue, and are probably immature forms of the large uninucleated leucocytes, as all intermediate forms between the small and large cell are seen. Gulland considers them to be the early form of all the other leucocytes. They are sometimes spoken of as the young white corpuscles.

Lymphocytes are met with considerably larger than

the typical form just described. These are often indistinguishable from, and are probably intermediate forms of, the hyaline cell.

Large Uninucleated Leucocytes.

Synonyms.—Hyaline cell, Kanthack; large hyaline cell, Sherrington; large lymphocytes; larger types of the first variety, Hayem; macrophagocytes, according to Metschnikoff. These are considerably larger than the small variety or lymphocytes, being sometimes the largest of all forms of leucocytes met with in normal blood. They measure generally about $8\frac{1}{2}$ to 12 μ in diameter. (Plate II., Fig. 2; Plate III., Fig. 2.)

The cell is usually oval or spherical in outline, but this is by no means constantly the case in dry preparations, and frequently they have the most irregular form, possibly due to their arrested amoeboid movement, or possibly from the fact of their being less resistant to pressure than the other leucocytes.

Their protoplasm, which is usually abundant, contains no granules, although it has a somewhat ground-glass appearance. It stains slightly with the basic aniline dyes, so that in films stained with eosine and haematoxylin, or methyl-blue, the protoplasm surrounding the nucleus has a faint bluish tinge instead of pink, as is the case with most of the other leucocytes.

The nucleus, which is relatively large, has a round or oval shape, and not infrequently shows a deep notch, giving it a kidney-shaped appearance. The intranuclear network is very diffuse and the nucleus as a whole stains faintly with the usual dyes, and I have frequently noticed that haematoxylin colours it a peculiar greyish-blue tint.

These leucocytes undergo active amoeboid movement, and are phagocytic.

The large size of these cells and their often indented nucleus, their somewhat irregular outline, as well as the faint blue colour which nucleus and protoplasm alike stain with eosine and haematoxylin, make the typical forms easily recognizable; but I quite agree with Sherrington's statement that it is often extremely difficult to decide whether some leucocytes should be classified as small or large uninucleated forms. (Plate II., Fig. 1, a.)

Large uninucleated leucocytes, in which the nucleus is indented or distinctly horse-shoe shaped, and which show a well-marked intranuclear network, approach in appearance the multinucleated elements, and are often classified separately under the term *transitional forms.* (Plate II., Fig. 3.)

The number of the large uninucleated elements is estimated at about 6–8 % of all the leucocytes.

Sherrington has noticed that in the blood of dogs and cats an increase in the number of both the large and small forms is associated with a diminution in the haemoglobin and red corpuscles.

Hayem estimates the number of the leucocytes of "the 1st variety," which includes the large and small uninucleated forms, as 23 %, and remarks that in chronic cases, in which the lesion is due to an advanced cachectic state, there is an increase in these forms.

Multinucleated Leucocytes.

Synonyms.—Neutrophile cells or leucocytes with ϵ granules, Ehrlich; finely granular oxyphile or acido-

phile cells, Kanthack; the oxyphile cells, Gulland; microphagocytes, Metschnikoff; cells with finely granular protoplasm, Max Schultze; leucocytes of the 2nd variety, Hayem.

These are by far the most numerous of all leucocytes present in normal blood.

They are considerably larger than the red corpuscles and lymphocytes, but not quite as large as the large uninucleated and eosinophile cells. They vary in size. Hayem gives their measurement as from 7·5 to 9·5 or 10, and other writers as from 8 to 10 μ in diameter. (Plates II. and III., Fig. 3.)

The protoplasm contains numerous very fine granules, which Ehrlich found to be ϵ or neutrophile in man, but amphophile (β granules) in the rabbit. Kanthack and Hardy consider them to be faintly oxyphile or acidophile, *i.e.*, staining with the acid aniline dyes, and this view is generally accepted at the present time.

The neutral mixture of Ehrlich, as well as the triple stains, give these granules a lilac or violet colour. According to Kanthack, when film preparations are stained with eosine and methyl-blue, these granules take on the eosine, and are therefore eosinophilous in nature.

In the method of staining films previously recommended, viz., with eosine first and then with haematoxylin, the protoplasm of these cells appears uniformly stained pink, and no evidence of granules can be made out with $\frac{1}{12}$ inch oil immersion objective.

If, however, films be exposed to the fumes of osmic acid before they dry, as Kanthack has suggested, their granular appearance may sometimes, though according to my experience rarely, be seen. Their nuclei, which are not very easily seen in the spherical condition of the

living unstained cell, become very evident when the leucocyte is flattened in the film and stained.

They usually consist of several round or oval nuclei, connected by thin threads of chromatin. They vary in number, but usually three, sometimes five or six, may be seen in a single leucocyte. In some the nuclei are so closely in contact that they appear almost fused into one irregular nucleus, and are therefore spoken of as multipartite nuclei, or as leucocytes with polymorphous nuclei. These transitional forms often show a deeply indented nucleus.

The nuclei show a very marked intranuclear network, the chromatin threads and nodes of which appear clearly defined with haematoxylin, methyl-blue, etc. Together they form variously-shaped figures, such as 3 or 5, or resembling the letters s, z, u, etc.

The irregularity of the nucleus, considered by Löwit as evidence of degeneration, and by other early writers as a sign of multiplication, was shown by Arnold to be due to amoeboid movement of the cell.

According to Sherrington, "if the cell is allowed to quiet down before it is killed, the nucleus often returns to the spherical form."

Many writers have objected to the term "multinucleated" applied to these cells, and Gulland in particular has raised strong arguments against the classification of leucocytes according to the nature of their nuclei; in fact, he states that "no one shape of nucleus is associated invariably with any one type of cell body, or with granules of a special kind." As this term, however, represents a condition of things usually seen in dry films, and as these leucocytes are so very generally spoken of by it, we prefer for purposes

of clinical enumeration to retain it, qualifying its use by the names "neutrophile or finely oxyphile."

These leucocytes are actively amoeboid, and possess the power of ingesting particles, whether these be of the nature of bacteria—"phagocytosis"—or products of the degeneration of the red blood corpuscles, *e.g.*, the malarial granules in cases of malaria, or the black pigment granules of melanotic tumours.

Pus consists for the most part of neutrophile cells, but in order to study their characteristics it is essential that the pus should be as fresh as possible, otherwise they appear as degenerated cells, and their granules may show Brownian movement. This latter condition, according to Sherrington, is a sign that the cell is nearly dead, and although present in some of the pus corpuscles, is always absent from the leucocytes of healthy blood.

The *number* of multinucleated leucocytes in the blood is generally about 70–75 % of all the leucocytes. Hayem gives the proportion as 70 %, Von Limbeck 70–80 %. They are, however, subject to considerable variation in number. Generally speaking when leucocytosis, *i.e.*, an increase in leucocytes, is present the multinucleated elements are those which are chiefly concerned.

As to the origin of these leucocytes authorities differ. Some, including Ehrlich, maintain that they are developed from the lymphocytes, which are formed in lymphatic tissue, and the large uninucleated cells.

Gulland has shown that in the early life of the embryo there are no leucocytes present in the blood, that the lymphocytes are the earliest stage of all the varieties of leucocytes, and that multiplication and

division of the white cells by mitosis takes place in adenoid tissue.

Kanthack maintains that karyokinesis or mitosis occurs in the neutrophile cells, and adduces this as an argument against their being merely transitional stages of the uninucleated cells. He considers that they appear in the blood as distinct elements, and have an individual existence.

These leucocytes are considered by many to be the most mature form of the colourless elements, and are therefore referred to as the "adult" cells.

Eosinophile Cells.

Synonyms.—Coarsely granular oxyphile or acidophile cells, Kanthack; leucocytes with α granules, Ehrlich; coarsely granular cells, Max Schultze and Wharton Jones; leucocytes of the 3rd variety, Hayem.

When normal blood is examined in the fresh condition, or by means of dry films, a few leucocytes may be seen conspicuous by their very granular appearance. They are seen as a mass of highly refractile granules, which occupy nearly the whole of the cell, and under a high magnification the granules appear to be spherical, brighter in their centre than at the periphery.

When stained with one of the acid aniline dyes, particularly eosine, they are very prominent features in the film, and on this account received from Ehrlich the name of eosinophile cells. (Plate II., Fig. 5; Plate III., Fig. 4.) They are generally spherical in shape, but on account of the granules contained within their protoplasm, their margin is frequently irregular. In a badly-prepared film, in which much pressure has been used, occasionally the cells are seen to have

ruptured and the granules extruded. I do not consider that they resist external pressure so well as the lymphocytes and multinucleated, but probably better than the hyaline cells.

Their *size* varies, usually they are larger than the lymphocytes and multinucleated, but in dry films at least are not as large as the large uninucleated leucocytes.

Von Limbeck states that they are intermediate in size between a large and small uninucleated cell. Hayem gives their average diameter as 8–9·5 μ, Kanthack as 10–11 μ.

The *nucleus* may be single, double, or deeply indented. It is generally somewhat horse-shoe shaped, the transverse part being thinner than the extremities, and is usually directed towards the periphery of the cell. At other times it is irregularly lobed, consisting of two egg-shaped nuclei, connected by a thin bridge of nuclear structure, which may be almost invisible.

The nucleus appears to have been pushed to one side of the cell, as it is scarcely ever central in position. It does not stain as deeply as that of the multinucleated leucocyte, and the intranuclear network is not as evident.

The protoplasm containing the granules occupies about three-fourths of the whole of the cell. The number and size of the granules vary in different leucocytes. For the most part they are relatively large spherical bodies, highly refractile, often showing in fresh preparations a greenish-yellow tint.

In some the granules are very large, and the clear and frequently unstained matrix in which they are embedded allows of their being clearly defined. In

Plate III.

The Leucocytes—*continued.*

Figs. 1 to 6.—Blood films stained with Ehrlich-Biondi's stain.

Fig. 1.—Four Lymphocytes contrasted with (*b*) nucleated red and (*a*) normal corpuscle. Protoplasm contains no granules.

Fig. 2.—Large uninucleated or Hyaline cell. Protoplasm contains no granules.

Fig. 3.—Multinucleated or Neutrophile Leucocytes showing the indistinct outline of several nuclei. The protoplasm contains fine neutrophile granules stained a violet colour.

Fig. 4.—Normal eosinophile cells containing two or more nuclei surrounded by large eosinophile granules.

Fig. 5.—Marrow cells or Myelocytes from leukaemic blood containing a single large nucleus surrounded by fine neutrophile granules stained a violet colour. The granules in Figs. 3 and 5 are the same; the elements differ, however, in their size, and the shape of their nucleus.

Fig. 6.—Marrow cells containing eosinophile granules, or Eosinophile Myelocytes, from leukaemic blood. Figs. 4 and 5 contain the same kind of granules, but differ in their size and the shape of their nucleus.

Fig. 7.—Marrow cells and two small uninucleated leucocytes from leukaemic blood, showing fine basophile or δ granules stained with methylene blue.

Fig. 8.—Mastzellen or cells containing coarse basophile or γ granules. Film preparation from the peritoneum of a mouse stained with methylene blue.

Fig. 9.—Marrow cells or Myelocytes, from the marrow of a kitten two days old, showing all stages of karyokinetic division. Film preparation fixed in picric, stained with dilute haematoxylin and then with eosine according to Muller's method.

Plate. III.

Fig. 1.

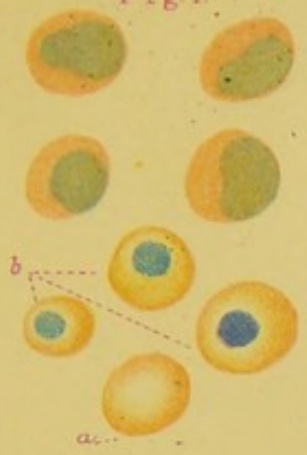


Fig. 2.

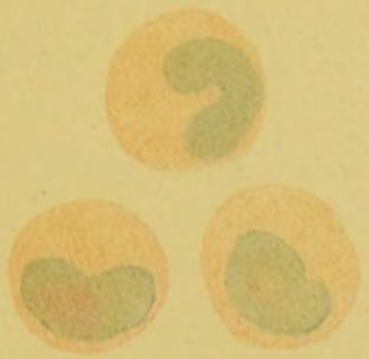


Fig. 3.

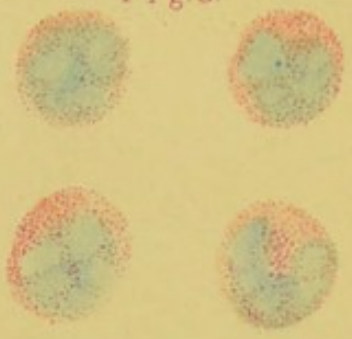


Fig. 4.

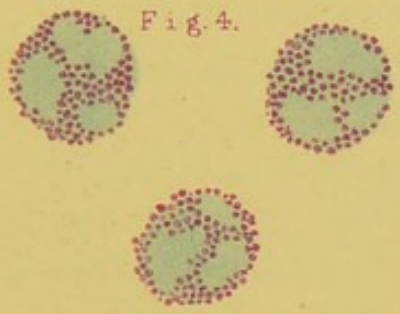


Fig. 5.

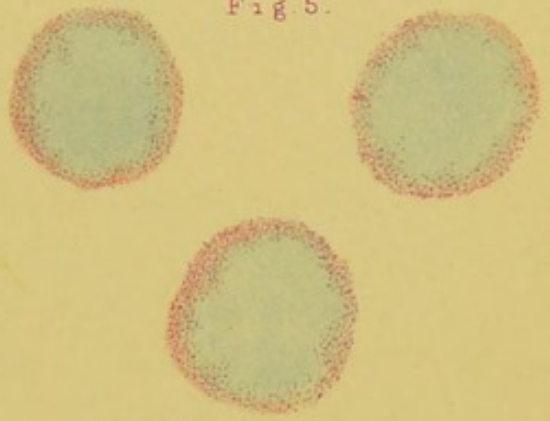


Fig. 6.

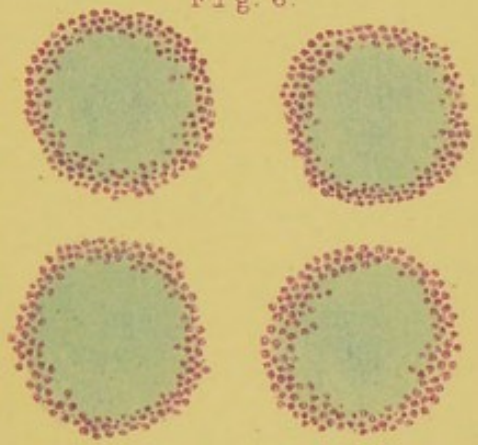


Fig. 7.

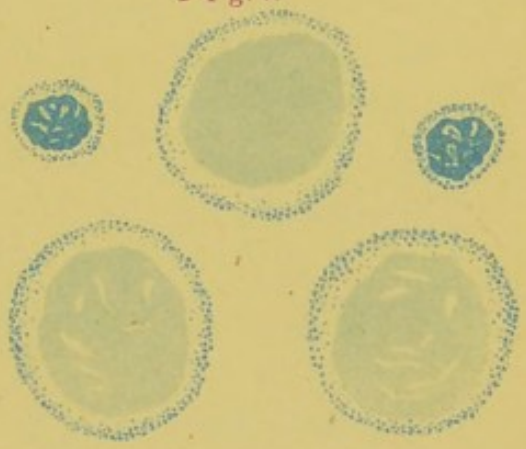


Fig. 8.

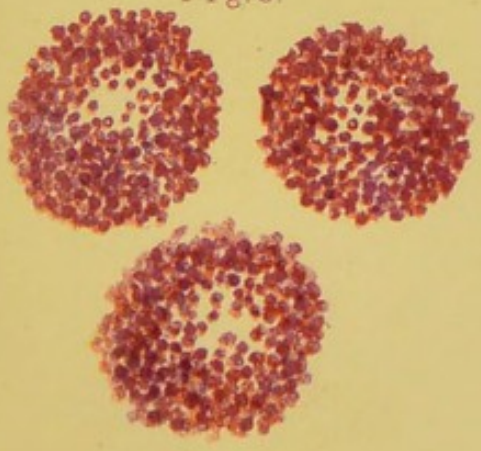
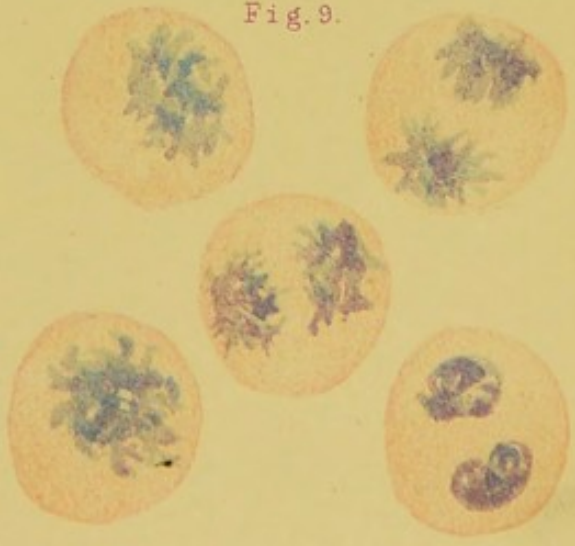


Fig. 9.



F.L. Colen del.

West, Newman Chr.

others they are small and more numerous, so that when stained with eosine the protoplasm takes on a very deep red colour, which renders the individual granules indistinct.

Their oxyphilous reaction is much greater than that of the finely granular cells, and, according to Kanthack, this is increased by corrosive sublimate or the application of heat.

Sherrington has shown that the granules, which he considers are situated in a cortical layer of the cell, never exhibit Brownian movement in the normal leucocyte, this only occurring in abnormal conditions.

The nature of these granules is still undetermined. Hayem states that although they have a particular affinity for eosine they are not haemoglobin, for they are more highly refractile than that substance, and are not dissolved by liquids in which haemoglobin is soluble; but he thinks that the black granules, which he states are often associated with them, are possibly altered haemoglobin or its derivatives.

They are not of the nature of fat, as osmic acid does not blacken them.

Ehrlich considers them to be an albuminoid substance.

It is stated that Barker has recently found that they contained iron.

Sherrington mentions that they give the reaction for phosphorus faintly, and suggests that they are of the nature of nucleo-albumin.

The eosinophile cells are amoeboid, but not phagocytic. Ehrlich considers that they are derived from the bone marrow; Kanthack and Hardy that they originate in the connective tissue and serous spaces. An argument against their origin from the bone marrow is that one

observer, according to Adami, has found numerous eosinophile cells in the ray, which possesses no bone marrow. As Kanthack points out, marrow after all consists only of specially developed connective tissue cells.

Some writers assume that they are the mature forms of leucocytes, and consider the comparatively small size of the nucleus, and the small amount of chromatin that it contains, evidence in favour of this view.

The eosinophile cells are comparatively rare in adult human blood, although abundant in areolar tissues and bone marrow.

Hayem states that they constitute 7 % of all the leucocytes, Stengel 2–4 %, and Von Limbeck, quoting Zappert, gives their number in healthy adult man as varying from 0·67 to 11 %.

Their absolute number, according to Zappert, varies from 55 to 784 per cmm. They are relatively numerous in healthy children up to 13 or 14 years of age, whilst in old age as well as middle life they are subject to great variations.

Von Limbeck remarks that the eosinophile cells have taken an important place in recent literature relating to the blood. Many writers have attached diagnostic and prognostic value to the increase or decrease of their number. Present experience, however, hardly justifies the unqualified acceptance of this view.

Amongst conditions attended with numerical alterations Von Limbeck mentions the following :—

Pregnancy and menstruation have no effect on their number. In leukaemia they vary greatly, but usually constitute about 2–6 % of all the leucocytes present. Their absolute number is generally increased, but

relatively they may be normal or even less than normal.

In chlorosis and severe anæmia they are frequently increased, but sometimes during the later stages are diminished, but do not afford any diagnostic or prognostic value.

Cardiac failure is usually attended with a normal number.

During the course of non-febrile tuberculosis of the lung a diminution may sometimes be seen.

Bronchial asthma and emphysema of the lungs are often associated with an increase in their number, as is frequently the case in affections of the liver and inflammation of the kidney.

Functional neuroses, as well as skin diseases, may also show an increase, whilst malignant tumours, excepting sarcomata, frequently present a diminution. Ehrlich considered that an increase in the number of these cells indicated a chronic disturbance of haemogenesis, and particularly an implication of the bone marrow, as in spleno-medullary leukaemia. This view is no longer entertained, for in leukaemia there may be a relative diminution in the number of eosinophile cells.

Many infectious diseases attended with a high temperature also show a diminution. Croupous pneumonia is especially noticeable by the great diminution, or even entire disappearance, of these cells from the blood.

Injection of tuberculin was followed by a diminution of the eosinophile cells, which again increased on the disappearance of the leucocytosis.

Von Limbeck, in commenting on the immense amount of literature on the subject, states that from his experi-

ence, only two facts are constant, viz., an increase of the eosinophile cells in asthma, and a decrease, or even entire disappearance of them, in croupous pneumonia; and adds that the reappearance of them in the blood affords a means of foreseeing the approach of the crisis with great probability.

Eosinophile myelocytes, or marrow cells containing eosinophile granules, which are almost pathognomonic of spleno-medullary leukaemia, will be fully described under this heading. (Plate II., Fig. 7; Plate III., Fig. 6.)

Marrow Cells, or Myelocytes.

Synonyms.—Markzellen, of German writers; globules blancs hypertrophiés, Hayem; large uninucleated cells with neutrophile or ϵ granules.

These cells are never present in normal blood, although they occur in large numbers in the red marrow of bones. They are very characteristic of spleno-medullary and mixed leukaemia, sometimes constituting the greater number of leucocytes found in that disease.

They are very large cells, attaining a diameter, according to Hayem, of even 20 μ. (Plate II., Fig. 6; Plate III., Fig. 5.)

Their protoplasm contains fine granules, which Ehrlich considered were neutrophile, Kanthack finely oxyphile. In preparations stained with eosine and haematoxylin the protoplasm almost invariably stains a faint blue colour with the haematoxylin, rather than pink with the eosine, but does not show any granules.

Stengel makes the following allusion to them: "According to Ehrlich the myelocytes are indistinguishable from the large uninucleated or transitional form of

leucocytes, except for the presence of neutrophile granules. In some instances, undoubtedly, Ehrlich's description applies, but the typical myelocyte differs from the ordinary large uninucleated leucocyte in the much greater size of the corpuscle, and in the unusual pallor of the nucleus. These leucocytes may be distinguished by their morphology alone in many instances, but the small forms approach so nearly the ordinary uninucleated elements, that differential stains are necessary to distinguish the nature of the individual corpuscles, and even after this test, doubt may exist as to the proper classification."

The nucleus is usually single, often indented or somewhat lobed. It is very poor in chromatin, stains only faintly with nuclear stains, and shows very little intranuclear structure.

They are said to be identical with Cornil's "cellules medullaires."

Marrow cells are the chief diagnostic feature of spleno-medullary leucocythemia, where they are almost as numerous as the multinucleated leucocytes.

Till recently it was thought that they were only to be found in this disease, but it is now known that they occur also in severe anæmias, particularly pernicious, myxœdema, syphilis, and other chronic cachectic conditions; but their percentage in all diseases except leukaemia is extremely small.

We shall speak more fully of their characters and relationship to marrow cells proper, when we describe the condition of the blood in leucocythemia.

Mastzellen, or *cells containing γ granules*, or coarsely granular basophile cells. (Kanthack.)

Sherrington states that he has never met with them

in normal mammalian blood, but has seen a few in the reaction stage of Asiatic cholera. According to Limbeck they sometimes occur in leukaemia; according to Müller they are very numerous in this disease.

They occur in health, however, in the coelomic fluid and connective tissue of the body, and in the marrow of bones. They can readily be seen in preparations made from the peritoneal fluid of the mouse, and stained with Ehrlich's acid dahlia solution, or with methylene-blue. (Plate III., Fig. 8.)

The nucleus stains very faintly, and with either of these stains appears rather as a cavity, which is situated somewhat out of the centre of the cell.

It is poor in chromatin, no intranuclear structure being visible by the ordinary methods of procedure.

The cell protoplasm is filled with very numerous dense granules, which obscure the nucleus, and at the periphery give the outline of the cell an irregular margin. They stain very deeply with dahlia and methylene-blue, and appear of a purplish-blue colour, contrasting with the surrounding blue cells.

They are not very refractile, and in unstained preparations are indistinguishable from other cells except by their size.

They are very large cells, often exceeding 20 μ in diameter.

According to Kanthack they are non-amoeboid, but Gulland states that they possess this power.

They are not phagocytic.

These cells originate in the connective tissue, but "owing to their large size cannot get further in the blood stream than the first capillaries." (Gulland.)

Some writers speak of the finely and coarsely granular

basophile leucocytes, *i.e.*, cells with δ and γ granules respectively, under the general term mastzellen, and state that they may be found in small numbers in normal blood.

Von Limbeck expresses doubt whether even the small finely granular basophile cells occur in the blood of a healthy adult. Canon points out that they are of constant occurrence, especially in healthy and sick children.

I have frequently found the δ, or finely granular basophile cells, which are especially numerous in leucocythemia, but in my whole experience have never met with true mastzellen cells in the blood of this or any other condition.

The Number of Leucocytes.

The number of all forms of white corpuscles found in normal blood, depends on various conditions, and writers differ as to what should be considered the average. Hayem gives their number as 6000 per cmm. Limbeck as 8–9000, allowing an increase or decrease of a thousand, and the proportion of white to red corpuscles as about 1 to 5 or 600. Afanassieu gives the proportion as 1 to 600, and Muir states that the proportion of 1 to 500 is rarely exceeded without some pathological condition being present.

Under many physiological as well as pathological conditions they may undergo an increase in number, constituting leucocytosis or hyperleucocytosis, or a diminution in number, known as hypoleucocytosis, leucopenia, or leucolysis. The last expression is used by Löwit to indicate a destruction of the leucocytes.

When only the lymphocytes are increased it is sometimes called lymphocytosis. Leucocytosis is usually, however, attended with an increase of the multinucleated or neutrophile cells.

The proportion of the various forms of leucocytes present in normal adult blood is as follows :—

	According to Rotch.	According to Stengel.
Multinucleated or neutrophile cells	60–75%	65–70%
Small uninucleated cells or lymphocytes	24–30%	25%
Large uninucleated or hyaline cells	3– 6%	3– 6%
Eosinophile cells	1– 2%	not above 3%

Other writers give very similar figures.

The Influence of Physiological Conditions on the Leucocytes.

Digestive Leucocytosis.—After a meal the number of leucocytes increases, reaching a maximum at the time that intestinal digestion is taking place. An increase of 18 to 20 % may be found about this time. This increase depends largely on the nature of the diet; thus proteid food causes a much greater increase than a mixed diet. The lymphocytes and multinucleated leucocytes are chiefly concerned in this form of leucocytosis, the eosinophile and large uninucleated forms either remain unaffected or tend to diminish. During fasting there may be a slight diminution in the number of white cells, but this is seldom pronounced. Von Limbeck records the result of Luciani's examination of the blood of Succi, the fasting-man, in whom the leucocytes sank from 14,530 to 861 per cmm. in seven days, but on the eighth day increased

to 1530, and remained at about that figure till the end of the fast.

There is said to be a relative increase in the eosinophile, but a diminution in the lymphocytes and multinucleated elements, during starvation.

During pregnancy there is usually, though not invariably, an increase in the leucocytes; at delivery there is very little alteration; whilst during lactation there may also be a slight rise in their number. In the *early days of childhood* there is a condition of leucocytosis. During the first two or three days of life, the white cells are more numerous than in the adult, but when the weight of the body is at its minimum, about the third day, the leucocytes decrease considerably, whilst the red corpuscles increase. The leucocytosis observed in the first few days of life, is occasioned by an increase in the number of lymphocytes, and not the multinucleated elements. The eosinophile cells are also more numerous at this period.

The condition of the blood during infancy and early childhood, will be more fully dealt with when the diseases of children are described.

Other physiological conditions, such as fatigue, differences in sex, etc., apparently have very little influence on the number of leucocytes. Hayem noticed an increase in their number in the Esquimaux, and attributes it to their fatty diet, milk producing similar results.

Pathological Alterations in the Leucocytes.

Increase in the *number* of the leucocytes is of very frequent occurrence in pathological conditions. It is found in acute inflammatory diseases, in which the

leucocytes may attain the number of 36,000, but more usually 15 to 20,000 per cmm., as well as in chronic diseases. Of the latter the most striking illustration is leukaemia. Usually the multinucleated leucocytes are the form that are increased; but sometimes, especially in advanced cachectic states, *e.g.*, rickets, syphilis, etc., the lymphocytes are chiefly involved. Occasionally in cases of lymphosarcoma, malignant disease, and in terminal leucocytosis, as well as in the post haemorrhagic forms, the uninucleated leucocytes are increased, but this is not generally the case.

It is rather difficult to say what number of leucocytes should be considered pathological. Hayem and Stengel state that when the leucocytes exceed 10,000 per cmm., *i.e.*, a proportion of 1 to 500, the condition is one of leucocytosis, and the latter very rightly points out that the proportion of white to red corpuscles is not nearly as reliable a test of the existence of this condition, as the actual number of leucocytes.

Decrease in the *number* of leucocytes, a much rarer condition, is found in many long-continued fevers, not complicated by inflammatory conditions, such as typhoid fever. It is also seen in extreme anæmias. In fact, those diseases in which the lymphatic organs are involved, are usually attended with a decrease in the number of the white corpuscles.

Alterations of the leucocytes other than numerical may occur in various pathological processes.

In extreme anæmia some of the multinucleated elements are found to contain haemoglobin in their protoplasm, which gives them a yellow tint, especially at their margins. Leucocytes containing haemoglobin, are said to have been mistaken for giant nucleated

red corpuscles; but the granular condition of their protoplasm, and the multipartite character of their nuclei, should in stained preparations prevent any mistake.

Under certain conditions, particularly leucocythemia, some of the leucocytes may lose their amoeboid power of movement.

In cases in which marrow cells appear in large numbers in the blood, *e.g.*, splenic and mixed leukaemia, remarkably pale and indistinct leucocytes may be found, containing vacuoles of very varying size. Mention of these has already been made in connection with the bone marrow.

In most forms of malarial fevers the white blood corpuscles often contain pigment granules—the so-called "melaniferous leucocytes"—and a similar condition has been noticed in general melanosis. These are generally regarded as the product of the destruction of the red blood corpuscles.

Inflammatory Leucocytosis and Phagocytosis.

Excellent accounts have been given by Kanthack and Hardy, Sherrington and Adami, on the part the leucocytes take in inflammation and against bacteria. All that is stated here is largely culled from the writings of these authors, as well as those of Metschnikoff.

"The process of inflammation is essentially the endeavour on the part of the organism to promote the migration of leucocytes, and to aid in the inclusion and destruction of the irritant."

When inflammation attacks, or poisons of the nature of bacteria are inoculated into the body, two conditions result—a temporary diminution, quickly followed by

an increase in the number of the white cells of the blood.

The initial decrease of the leucocytes, termed "leucolysis" or "leucopenia" by Löwit, or "hypoleucocytosis," sets in immediately after the irritant has been applied. The variety of leucocytes which are chiefly diminished is, according to Sherrington and others, the multinucleated or neutrophile cells.

Although this stage is of brief duration, the diminution is sometimes very marked.

The cause of this phenomenon has received many explanations. Löwit considers that it is owing to a destruction of these cells. Some assume that the leucocytes are drawn away to other parts of the circulation, whilst others think that the colourless elements accumulate in the vessels of the liver, spleen, and marrow.

Sherrington has not found that the leucocytopenia bears any constant relation to the extent of the fever.

This primary stage is quickly followed by leucocytosis, and Kanthack considers it a rule "that previous to recovery from bacterial products, an increase in the number of leucocytes takes place." Löwit considers this is due to an increase of the lymphocytes; but many other observers, including Kanthack and Sherrington, attribute it to the multinucleated leucocytes, the granules of which, according to the former, become more eosinophilous, but not larger. The eosinophile cells are said to be greatly diminished, or to actually disappear from the blood in this as in the former stage. It has been suggested that they may be drawn to some particular part of the circulation, or that they pass to the seat of injury.

The increase in the multinucleated cells is, according to Romer, due to an actual reproduction of these cells in the blood.

Very similar results are to be found in many infectious diseases. In pneumonia all writers recognize the occurrence of leucocytosis in favourable cases, and in pleurisy, erysipelas, scarlet fever, measles, etc., the same condition is observed. In pneumonia, and most acute inflammatory affections, leucocytosis is regarded as a favourable, and the absence of it an unfavourable sign. Kanthack states "that in very acute diseases where the process runs a rapid course, where we have resolution by crisis—in fact, processes which are comparable with artificial infections or intoxications—a well-marked leucocytosis always ushers in the crisis or recovery, or at any rate is a most favourable sign of great prognostic value." (*Medical Chronicle*, 1894.)

Phagocytosis.—For a considerable time it has been known that the leucocytes can take up foreign matter into their interior, and more recently it was found that they could attack and incorporate bacteria. All the leucocytes do not, however, possess this phagocytic power, the multinucleated or neutrophile, and the large uninucleated or hyaline cells are the most important elements concerned in this process. Although a leucocyte may take up bacteria, it does not necessarily follow that it digests all. Thus in subacute and chronic diseases, such as gonorrhœa, tubercle, etc., organisms are found in their protoplasm in a living condition. In many cases the microbes are not only seized and incorporated in the substance of the leucocyte, but they are subjected to a process analogous to digestion. If the

organisms are very virulent, exceedingly few are taken up by the leucocytes, and it is a long time before phagocytosis comes into action.

Chemiotaxis, a term used to denote the attraction by which the leucocytes are drawn to the point of injury, explains why the wandering cells of the body accumulate at the part which is attacked by the micro-organisms. When the latter are not very virulent, the leucocytes are attracted in large numbers, "positive chemiotaxis," but when very virulent, the leucocytes are not drawn to the point of infection, "negative chemiotaxis," but a negative may eventually become a positive chemiotaxis. Positive chemiotaxis is very variable in amount, and many writers doubt the occurrence of negative chemiotaxis, assuming the absence of the leucocytes to be due to their destruction by the microbes.

"The cure of zymotic or mycotic disease, whether localised or general, and the immunity also, are mainly brought about by the activity of special cells—the phagocytes—and are primarily dependent on the attraction existing between these cells and the products of bacterial metabolism." (*Allbutt's Medicine*, vol. i. p. 87, 1896.)

Kanthack and Hardy have found an increased number of eosinophile cells at the seat of injury, as, for instance, in blisters, etc., and as these cells are very numerous in fresh pus from acute gonorrhœa, purulent sputum of tubercular disease, and possibly in pneumonic sputum, they consider that these cells migrate whenever noxious influences—inflammatory in nature—are to be counteracted. They have suggested the theory that although the eosinophile cells are not phagocytic, yet they assist in the process by possibly preparing the

particles for digestion, and recognize a stage of "eosinophile attack and that of phagocytic ingestion."

The Leucocytosis of Malignant Disease.

Many malignant growths, particularly sarcomata—osteo- and lympho-sarcoma—as well as carcinomata—scirrhus and encephaloid—are attended with an increase in the number of leucocytes. It is usually more marked in the sarcomata than the carcinomata, varying in number from normal to 50,000 or more.

In cases of sarcoma, involving the lymphatic structure, the appearance of the blood may closely resemble that of lymphatic leukaemia, in that the same variety of white cells, viz., the small uninucleated leucocytes, may be increased in the former as well as the latter.

Hayem states that epitheliomata are the form of malignant neoplasms, which may show no increase in the number of the leucocytes, and notes that cancers of the stomach may be divided into those attended with and those without leucocytosis, and suggests that the latter are probably epitheliomatous in nature.

Hayem lays down the following rule, "that all tumours —independent of inflammatory or suppurative complications—accompanied with an increase of leucocytes are of a malignant nature."

Cachectic leucocytosis may occur in a variety of chronic affections, particularly in syphilis and rickets. As the spleen and glands may be enlarged in these diseases, and as the increase in leucocytes may affect chiefly the uninucleated forms, the differential diagnosis between it and lymphatic leukaemia, may present considerable difficulty.

Agonal, or *terminal leucocytosis*, noticed towards the end of life in many chronic cachectic diseases, may sometimes be very pronounced. It has been noted in pernicious anæmia, and usually involves the small uninucleated leucocytes. It is said to be due to a reduction in the blood pressure, and the discharge of leucocytes from the lymphatic organs into the circulation. (Stengel.)

Post-haemorrhagic leucocytosis occurs soon after loss of blood, and persists some few days according to the quantity of blood lost. Sometimes the uninucleated, but more usually the multinucleated, elements are increased.

THE BLOOD PLATES.

Synonyms. — Blood plaques, or haematoblasts of Hayem.

The third corpuscular elements of the normal blood were first discovered by Hayem, and termed by him "the haematoblasts, or the germs of the red blood corpuscles," and were later described by Bizzozero as the blood plates.

In fresh blood preparations made and examined as quickly as possible, very small round or oval bodies, measuring about 2 to $3\frac{1}{2}$ μ in diameter are seen.

These are of a faint yellow colour, and are slightly granular in appearance. In a short time—a few seconds —they run together into grape-like clusters, and, losing their rounded form, become spinous. At the same time they appear more granular and highly refractile, and ultimately very thin filaments of fibrin are seen starting from their angular projections.

They are remarkable for their adhesiveness, in virtue

of which they adhere to red corpuscles or adjoining blood plates.

In order to examine them in detail it is necessary to use Hayem's solution, or the 1 % osmic acid solution mentioned before. They may also be well seen in dry film preparations, provided that the film has been quickly made and dried. (Plate I., Fig. 2.) On account of their adhesiveness, the blood plates will be most numerous in that part of the film where the blood first came in contact with the slide. An exact enumeration of them is not easy, as they tend to run together into little masses.

They stain faintly with aniline dyes, having an amphophile reaction, so that in dry preparations stained with eosine and methyl-blue, or eosine and haematoxylin, they generally appear pale violet colour. This colour is, however, variable.

Löwit considered them at first to be artificial products of the blood plasma, or of the degeneration of the white blood corpuscles ; but Bizzozero proved that they could be seen in the circulating blood of the bat. The majority of writers now consider that they exist as such in the blood. In the circulating blood in the web of the frog, blood plates, which are here very large and easily mistaken for leucocytes, can be readily seen.

Their high specific gravity, together with their great adhesiveness, explain their most important function—the formation of the white blood clot. Whether they have any other use is still undetermined.

Hayem's theory that they are the early forms of red corpuscles is not generally accepted. According to Hayem, in man the blood plates number 250,000 per cmm. Afanassieu gives their number as 200,000 to 300,000, and Fusari from 180,000 to 250,000.

The first-named observer considers that a *diminution* in their number is a serious sign. Among the causes which may lead to this, he mentions prolonged fast, long-continued typhoid fever, progressive anæmia, and advanced cachexia, especially cancerous cachexia. Hayem's statement that "there is only one condition in which the decrease in the number of blood plates can be considered favourable, and that is where it is temporary and associated with an increase in the number of the red corpuscles," is evidently based on his peculiar theory of blood formation.

Diminution in their number may also occur after certain poisons—pyrogallic acid, glycerine, etc., in high fevers, *e.g.*, typhus and erysipelas, but not in tuberculosis nor in pneumonia.

Pizzini points out that in all infectious fevers there is a diminution in the number of the blood plates, which is inversely proportional to the height of the temperature —the higher the fever the more marked is the diminution of these elements. After the cessation of fever they often increase, reaching a maximum six to seven hours after the crisis. They are sometimes said to disappear entirely from the blood in malarial fever.

Increase in the number of haematoblasts is of frequent occurrence. It may be noted in all anæmias unattended with fever, especially at the time that regeneration of the blood occurs, and is said to be a favourable sign. It also occurs after loss of blood, and in leucocythemia.

According to Hayem a temporary increase in their number is concerned in a renovation of the blood, and augmentation of their transverse diameter is said by Fusari to occur in anæmia as soon as improvement takes place.

In inflammatory conditions in which the film is increased very viscid masses occur, having a granular appearance and containing many blood plates, as well as red and white corpuscles. These have been termed "plaques phlegmasique" by Hayem. In certain cachectic conditions, somewhat similar granular masses are found in the blood, differing from the former in the following particulars :—Their outline is more defined, they contain few but large plates, and include a very limited number of coloured and colourless corpuscles. These he speaks of as "plaques cachectique."

The same observer also mentions the formation of crystals in very severe anæmias, due to modifications, probably of a chemical nature, of the haematoblasts.

Pathology of the Blood.

Anæmia.

The term anæmia is used to imply a diminution in the quantity of blood—"oligaemia," in its corpuscles—"oligocythaemia," or in its haemoglobin or colouring matter—"oligochromaemia," or a combination of these conditions.

The pallor noticeable in anæmic conditions is due to a decrease in the amount of haemoglobin, whether that be from a diminution of the actual amount of blood, of the corpuscles, or of the colouring matter of the red cells.

It must be noted that the degree of pallor is not necessarily a sign of the extent of the anæmia, and that in some individuals pallor, due to alterations of the capillary supply to the skin, or to deficient pigment, may exist without any departure of the blood from the normal. This has been termed pseudo-anæmia.

From this it is apparent that the red blood corpuscles and their contained haemoglobin are only concerned in this condition, and that, although alterations in the number of leucocytes may and do occur, yet they cannot be regarded as factors in the process.

Absolute diminution in the quantity of blood, "oligaemia," occurs after haemorrhage, but except in those cases in which the loss has been so great as to cause an immediately fatal result, the mass of blood

is maintained at nearly the same bulk, owing to the relationship existing between the liquid of the various tissues and the vascular system.

Decrease in the number of red corpuscles—oligocythaemia—is an exceedingly common condition, but diminution of the haemoglobin—oligochromaemia—may occur without any numerical alteration of the coloured elements, as is seen in some of the slighter forms of anæmia, and particularly in chlorosis.

Otto found that after haemorrhage the number of corpuscles increased before the haemoglobin, and some authors consider mere diminution in the haemoglobin a sign of regeneration of the blood.

Diminution of the red corpuscles may be due either to lessened blood formation, "haemogenesis," as in conditions of starvation, unfavourable hygienic surroundings, new growths, etc., or to excessive destruction of the red cells, "haemolysis," as in fevers, etc., or to a combination of these factors.

Admitting that in health the red corpuscles number about five million per cmm., and the amount of haemoglobin present is represented by five million normal corpuscles, or 100 %, and taking into consideration the individual variations of the blood in health, what condition constitutes anæmia? Hayem considers patients to be suffering from anæmia when they complain of functional troubles evidently connected with the state of the blood, and when the amount of haemoglobin is only equivalent to four instead of five million normal corpuscles, *i.e.*, 80 %.

Stephen Mackenzie states that anæmia commences when the corpuscles constitute 80 %, or four million per cmm.

Hayem classifies anæmias generally according to their severity into four groups :—

1. *Anæmia of the 1st degree, or slight anæmia.*—The haemoglobin of the blood is equal to three or four million red corpuscles, *i.e.*, 60–80 %. The red corpuscles are in some cases normal or slightly altered, and their number is greater than is expressed by the value of the haemoglobin. In other cases their number is proportional to the amount of haemoglobin, and both show slight changes in the form of the red cells. If the amount of haemoglobin each corpuscle contains—*i.e.*, the colour index—be represented in health as 1, then in this degree of anæmia it will most frequently be represented as from 0·65 to 0·9. This, according to Hayem, is not the form which is most improved by iron.

2. *Anæmias of the 2nd degree, or of average intensity.*—This is the most common type. The haemoglobin is equal to 2–3 million corpuscles, *i.e.*, 40–60 %. The number of red corpuscles varies from 3–5 million, and the small corpuscles, "globules nains," are frequent. Therefore, although their number is not reduced, their average size is diminished, and the individual value in haemoglobin or their colour index, is notably lower than the normal, and varies from 0·3 to 0·8, generally 0·5.

Large corpuscles may be found, but they are not numerous enough to raise the average diameter of the red cells to the normal. It is very rarely that giant cells are seen. Chlorosis belongs to this type of anæmia, which is essentially characterized by the disproportion between the amount of haemoglobin and the number of corpuscles.

3. *Anæmias of the 3rd degree, or intense anæmia.*—This includes cases in which the anæmia, although severe, does not *per se* threaten life.

The haemoglobin is equivalent to from 2 million to 800,000 red cells, *i.e.*, 40 to 16 %. The red corpuscles number between 800,000 and 4 million, and the amount of haemoglobin which each corpuscle contains varies considerably, sometimes being as low as 0·4, but often equals 1·0 or even above.

Whilst small corpuscles are especially characteristic of anæmia of the 2nd degree, large and giant corpuscles (megalocytes) are essentially features of the more intense anæmic conditions. In some cases the red cells are small, but relatively numerous, whilst in other and severer types they are, on an average, larger than in normal blood, but their number is considerably reduced, whilst in both, large and even giant cells are frequently seen.

4. *Anæmias of the 4th degree, or extreme anæmia.*—This includes cases in which the anæmia may *per se* cause the death of a patient. In it the amount of haemoglobin is equal to about 800,000 normal corpuscles, *i.e.*, 16 %, and the red corpuscles fall to 800,000 and below. Although death is not inevitable when the number of red cells is only equal to 800,000, yet Hayem from his experience expresses the opinion that anything below this, places the life of the patient in extreme danger, on account of the condition of the blood alone.

As the average diameter of the corpuscles is greater than, and their colour almost as great as the normal, the amount of haemoglobin that each contains is often above

1, the figure expressing the amount in a healthy corpuscle, and frequently varies from 0·88 to 1·7.

Large corpuscles are the prevailing feature of this form of anæmia, and giant cells—megalocytes—reaching even 16 μ in diameter, are frequent, constituting 3 to 12 % of the red cells present. Sometimes dwarf corpuscles, "globules nains, or petits"—microcytes—are present, and the irregularity in the size, form, and colour of the elements is a striking feature.

According to Hayem, nucleated red corpuscles *only* occur in some cases of extreme anæmia, but as we have previously mentioned, our experience regarding the occurrence of these elements is totally opposed to this view.

It is in this type of anæmia especially that vacuoles in the corpuscles, as well as crystals, are seen.

The *leucocytes* play a very secondary part in anæmia, and undergo very little alteration in the first two degrees, but are not infrequently modified in the severer forms.

Their actual number is usually diminished, and some may be found to contain haemoglobin. Sometimes the small uninucleated leucocytes are increased, at other times—these are less numerous—the other forms of white corpuscles are increased in number, and occasionally marrow cells may occur in severe anæmia. The blood plates usually remain as numerous as in the normal condition, and are only diminished in extreme anæmia. (Hayem).

Some observers, *e.g.*, Muir and Eichhorst, do not agree with Hayem's statement, that the presence of large corpuscles indicates a severe, and small forms a mild, anæmia. It was the last-named writer who assumed

that very small deeply-coloured corpuscles were characteristic of pernicious anæmia. Hayem expresses the opinion that contrary to the statements of Eichhorst, Quincke, and others, anæmia associated with small red corpuscles, instead of being characteristic of the most serious of all anæmias—progressive pernicious anæmia—indicates rather the most benign of the severer anæmia—and from my experience I regard this as the correct view.

Stephen Mackenzie, in the Lettsomian Lectures (*B. M. Journal*, January 24th, 1891), makes the following scale of anæmia :—

Anæmia commences at 80 %, *i.e.*, 4 million red corpuscles ; it is decided between 65 and 50 %, *i.e.*, $3\frac{1}{4}$ to $2\frac{1}{2}$ million ; grave at 50 %, *i.e.*, $2\frac{1}{2}$ million ; very grave at 35 %, or $1\frac{3}{4}$ million ; and fatal at 7·5 %, *i.e.*, when the red corpuscles number only 375,000 per cmm.

It is usual for clinical purposes to divide anæmia into two classes—the primary or idiopathic, and the secondary or symptomatic ; but it must be borne in mind that many conditions which cause symptomatic anæmia may affect the blood-forming organs, and so give rise to a condition of the blood closely resembling that of primary anæmia.

This classification is convenient, but not necessarily scientific, and some cases of anæmias, which are at present termed primary, may eventually be proved to be really secondary to some organic condition, which so far remains undetermined.

Primary, idiopathic or *essential anæmias* are those in which the condition of the blood or blood-forming organs is the main characteristic of the disease, and include chlorosis, simple primary anæmia, pernicious anæmia, leucocythemia, Hodgkin's disease, and splenic anæmia.

Secondary or *symptomatic anæmias* include those conditions in which the state of the blood is not the essential characteristic of the disease, but is due to other causes outside the haemopoietic system, such as those resulting from haemorrhage, malaria, malignant growths, and organic diseases generally.

Primary Anæmia.

Chlorosis.

Synonyms.—Chloraemia, or chloranaemia; greensickness; cachexia virginum; morbus virgineus; bleichsucht of German writers.

Chlorosis is a form of primary anæmia affecting chiefly, though not exclusively, the female sex during the time of puberty or early womanhood. It is usually characterized by a peculiar pale, sallow complexion, having a somewhat greenish tint, and by a diminution in the amount of haemoglobin greater than that of the number of the red corpuscles.

This definition requires to be somewhat modified, as this form of anæmia occurs also in boys in a somewhat modified form, and the so-called "chlorotic condition of the blood"—the greater reduction of haemoglobin over that of the corpuscles—is by no means confined to chlorosis proper, but occurs in many other forms of anæmia.

Character of the Blood.

The blood as it proceeds from a puncture is usually pale in colour and remarkably fluid. It coagulates normally, and there is no evidence that the amount of fibrin is other than normal.

The total amount of blood is probably not reduced; its specific gravity, however, is somewhat less than that of healthy blood.

According to Duncan, who was the first to investigate both the number of the corpuscles and the amount of haemoglobin, chlorosis consists in a diminution in the amount of the latter—oligochromaemia—whether the number of corpuscles are normal or less than normal; and Graeber even went further, and stated that diminution in the number of red cells was a complication of chlorosis with anæmia. In favour of this view he mentions that during treatment, the number of corpuscles increases before any increase in the amount of haemoglobin takes place.

Laache divides chlorosis into two classes, one in which there is no diminution in the number of the erythrocytes —"pseudo chlorosis"—and the other in which the corpuscles as well as the haemoglobin are deficient, which he terms "true chlorosis."

Most writers consider that there is a diminution in the amount of haemoglobin usually associated with a decrease in the number of the red corpuscles, but that the former is more conspicuous and characteristic than the latter.

Thus, out of 247 cases of chlorosis examined by various writers, in 40 % the corpuscles numbered four million and upwards, whilst in the remaining 60 % they were less than that number.

Hayem found that in 60 cases of chlorosis

20 cases showed anæmia of 2nd degree, red b. corp. 4 mill., Hb = 2·7 mill. or 54 %, G = 0·65.

39 cases showed anæmia of 3rd degree, red b. corp. 2·9 mill., Hb = 1·5 mill. or 30 %, G = 0·52.

1 case showed anæmia of 4th degree, red b. corp. 937,360, Hb = 796,756 or 16 %, G = 0·85.

G = individual corpuscular value in haemoglobin or the so-called colour index.

In all these cases the amount of haemoglobin was always less than the number of corpuscles, and the individual corpuscular value in haemoglobin was reduced as a rule to 0·5 or 0·3, the normal being = 1.

The so-called chlorotic condition of the blood occurs in many chronic secondary anæmias, in cases recovering from haemorrhage, and in the most varied pathological conditions; but it is seldom as constant or as pronounced as in chlorosis.

Regeneration of the blood, which occurs in chlorosis during treatment with iron and arsenic, as in anæmias the result of haemorrhage, does so, as Otto and Graeber pointed out, by a primary increase in the number of red corpuscles, and then by an increase in the amount of haemoglobin.

Alterations in the size, form, and colour of the corpuscles, as well as mere numerical change, are seen in the blood of chlorotics, and for clinical examinations Hayem recommends the use of dry films, which can be compared with those of normal blood. (See Plate IV., Fig. 1.)

In shape the corpuscles may be more or less altered, according to the degree of oligocythemia. Poikilocytes are frequently present in all forms of chlorosis, and are generally more pronounced in cases in which the corpuscles are considerably reduced in number.

Muir, however, does not consider that the poikilocytosis always varies proportionately to the diminution of the number of the red corpuscles, and states that it may be very well marked when the number is very little reduced. Hayem points out that alterations in

Plate IV.

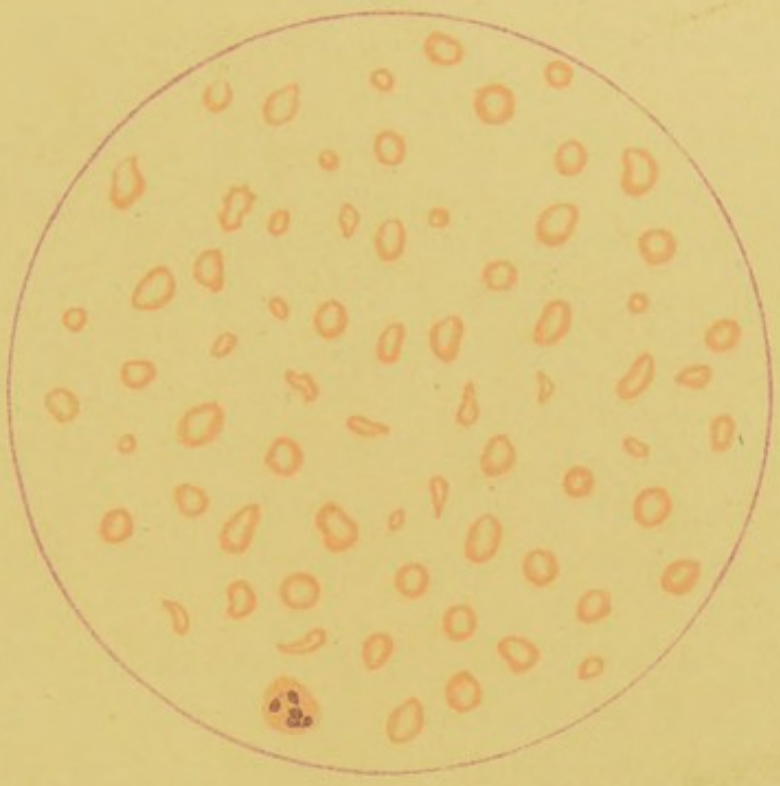
Film preparations stained with eosine and haematoxylin. Both examined with eyepiece 2. Objective $\frac{1}{8}''$.

Fig. 1.—**Chlorosis.** The red blood corpuscles show moderate poikilocytosis. They are, on the average, somewhat below the size of normal corpuscles. Microcytes are numerous. The cells are stained faintly and the central depression very marked. At the lower part of the film a multinucleated leucocyte is seen.

Fig. 2.—**Pernicious Anæmia.** The red blood corpuscles have an average diameter considerably above the normal, and Megalocytes are numerous. Poikilocytosis is pronounced. The corpuscles are deeply stained, and the central depression almost, if not completely, absent. Two large nucleated red blood corpuscles, Megaloblasts, are seen.

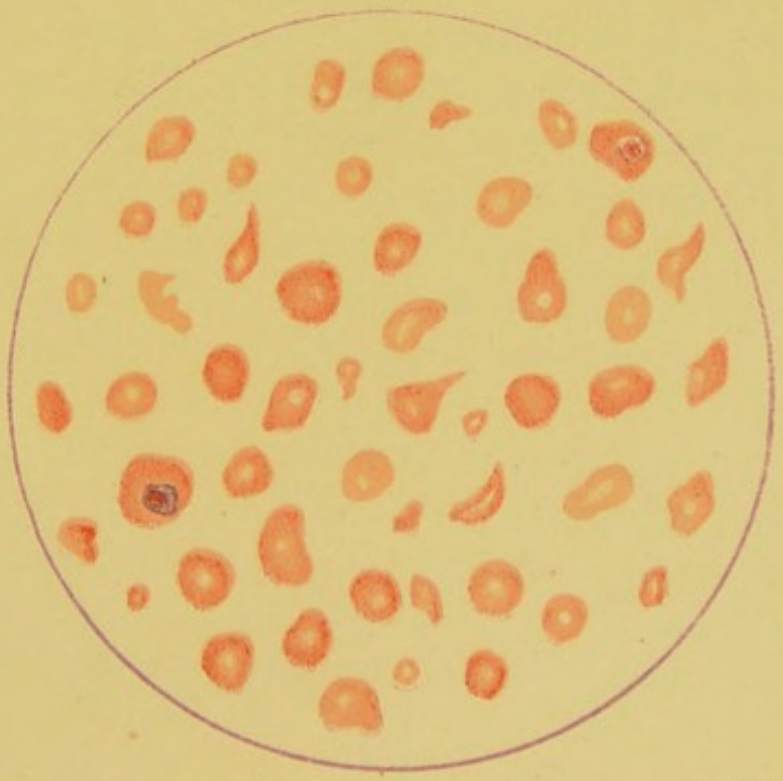
PLATE IV.

Fig. 1.



Chlorosis.

Fig. 2.



F.L.Coles del.

Pernicious Anæmia.

West, Newman Chr.

form occur most frequently in the smaller corpuscles present.

Alterations in the size of the corpuscles are seen in many forms of chlorosis, and generally speaking the smaller corpuscles—microcytes—are more conspicuous than the larger elements, so that the average diameter of the red cells may fall from the normal 7·5 μ to 7 or 6·5 μ. (Hayem.)

Some writers consider this very characteristic of chlorosis. When the chlorotic condition is very severe large corpuscles—megalocytes—may be comparatively numerous.

The colour of many individual corpuscles is perceptibly lessened, and the pallor of the red corpuscles, seen either in fresh preparations or in dry films, justifies Hayem's description of the disease as a chlorosis of the individual corpuscles. In stained films some corpuscles are much less deeply stained than others, whilst in some, signs of degeneration described by Maragliano and Castillino are also apparent. Normal blood corpuscles when fixed, stain pink with the eosine out of a mixture of eosine and methyl-blue, or eosine and haematoxylin —"monochromatophile"—whilst those corpuscles which are undergoing necrobiotic changes are coloured with both the eosine and haematoxylin, or methyl-blue, a purple colour, "polychromatophile." Examples of this may occasionally be seen in chlorosis. (Plate I., Fig. 6.)

As to the occurrence of *nucleated red blood corpuscles* in cases of chlorosis, opinions differ considerably. Hayem, notwithstanding the opinion of other writers, has carefully looked for, but never discovered them, even in the most severe types of chlorosis, although

he mentions a case in which the corpuscles numbered only 937,360 per cmm.

Muir, in his earlier writings, states that he met with no nucleated red corpuscles in a long series of cases of chlorosis, in which all the elements were carefully enumerated.

Kanthack observes that nucleated erythrocytes are rare in chlorosis, and are a sign of severe anæmia.

According to Von Limbeck nucleated corpuscles occur in severe chlorosis, as they do in any other form of severe anæmia, occasionally in large masses, constituting, according to Neudorfer, a blood crisis, and their presence is often followed by an increase in the red cells. These are for the most part normoblasts, *i.e.*, nucleated red cells of the same size as the healthy corpuscle. (Plate I., Fig. 7.)

Stengel mentions the occurrence of normoblasts, which are said by some to indicate a favourable prognosis. He, however, admits that they are rarely conspicuous in cases of moderate severity, and are never marked except in the severest forms of the disease.

Large nucleated red cells—megaloblasts or gigantoblasts—have also been met with in this disease, but are never as numerous as normoblasts.

In one case of chlorosis in a girl of 16, in whom all the typical symptoms of this disease were present, I found numerous nucleated red corpuscles, although the red cells present numbered 3·5 million per cmm. These were apparently absent from time to time, but when present were fairly numerous, so that in a thin film with $\frac{5}{8}$ inch coverglass as many as 8 to 11 would be found. They were mostly normoblasts, and nearly all showed extensive crenation and appeared less resistant to external

injury, as some free nuclei were also seen. In many the nucleus was pushed to one side, and in some almost extruded; the protoplasm in a few stained purple with eosin and haematoxylin—polychromatophile. It is only right to mention that the patient was subject to slight attacks of bronchial asthma.

We must conclude from the later writers, as well as from our own experience, that nucleated red blood corpuscles do occur in chlorosis, but probably only in the severer forms of that disease, and when present are usually of the normoblast variety. Their presence may indicate a severe type, but also one in which a reparative effort on the part of the haemopoietic organs, probably the bone marrow, is being made. I cannot but regard the megaloblasts, should they occur, as a sign of much more serious disturbance of the blood and blood-forming organs.

The *blood plates* are generally somewhat increased in number, although in the very severe forms they may, according to Hayem, be diminished.

The *leucocytes* are very little affected in uncomplicated cases of this disease, their number and variety being usually normal. In severe cases Hammerschlag has, however, found a few marrow cells.

The exact *nature* of *chlorosis* is still undetermined. It is almost universally admitted that it is a primary anæmia, depending upon some disturbance of the blood-forming organs, and not merely a symptom of some general disease. "The character of the blood, its poverty in haemoglobin, comparative richness in albuminates, the marked renovating influence of iron, all indicate a defective haemogenesis." (Stephen Mackenzie.) That it is not due to an increased blood

destruction—"haemolysis"—seems to be established by the absence of signs of blood destruction taking place in the body; and hydrobilirubin or urobilin pigments, (resulting from the disintegration of red corpuscles), in urine and faeces, and marked degeneration of the corpuscles, as well as the presence of jaundice, are all wanting in chlorosis, although present in pernicious anæmia.

What conditions give rise to the defective haemogenesis, whether menstrual, nervous, or vascular derangements, or constipation attended with absorption of toxic products from the intestines, are still unknown.

Von Noorden and others maintain that want of nutrition from abnormal gastric digestion or diminished amount of nourishment cannot—*per se*—account for the defective regeneration of the blood, since fasting causes neither oligocythemia nor oligochromaemia.

Diagnosis.—The diagnosis of chlorosis must not depend on the examination of the blood alone, as the so-called chlorotic condition of the blood may occur in other forms of anæmia, and may occasionally be absent from chlorosis.

Anæmia occurring in young females, attended with yellowish-green pallor, cardiac, digestive and menstrual disorders, which rapidly improves under suitable dietetic and ferruginous treatment, and in which there is marked reduction in the amount of haemoglobin, usually with, more rarely without, diminution in the number of red blood corpuscles, renders the diagnosis of chlorosis usually very easy.

Sometimes secondary anæmias, resulting from malig-

nant disease, syphilis, loss of blood, etc., very closely resemble chlorosis, and here we have to rely on a careful consideration of the history, symptoms, and physical signs. The chlorotic type of the blood is not usually as marked in these conditions as in chlorosis, but we must insist on the fact that the description of the blood given above is not absolutely characteristic of, or peculiar to chlorosis.

Leucocytosis generally occurs in secondary anæmia, but is absent from chlorosis, except when complications are present.

A very important point is the diagnosis of chlorosis from simple primary, and particularly pernicious anæmia; but this will be considered later, when these diseases have been described.

Simple Primary Anæmia.

Many cases of anæmia originating without any discoverable pathological cause, and therefore primary, are met with, in which the essential features of chlorosis are absent, and which, under suitable hygienic and medical treatment, rapidly improve, and therefore cannot be included under the definition of progressive pernicious anæmia.

Many writers refuse to consider this as a special type of anæmia, and we must admit the difficulty of distinguishing some cases, which are not typical, from the anæmia just described, and pernicious anæmia.

This form of anæmia includes many cases which are due to unfavourable conditions, but it is somewhat difficult to say exactly what element, whether want of fresh air or light, is most concerned in its production.

In the milder type of cases there is no evidence of blood destruction, and hydrobilirubin and jaundice are absent; but some of the most severe forms, which border very closely on pernicious, show indications of disintegration of the red blood corpuscles, jaundice, etc.

The number of red cells and the amount of haemoglobin usually diminish in the same proportion, but whilst this is true of the less severe forms, exceptions are found in the more aggravated types. Laache pointed out that sometimes the amount of haemoglobin is lower than the number of corpuscles, whilst in cases of severe anæmia it is higher, and the individual corpuscular value in haemoglobin, *i.e.*, the colour index, may approach or even exceed the normal.

Von Limbeck describes a case of severe simple primary anæmia occurring in a woman aged 40, who was living in the greatest misery, subject to hard work, and who had suffered for a long time from want of food. On admission the number of red corpuscles was 306,000 and the haemoglobin 18 %, whilst under treatment in hospital at the end of three months the corpuscles reached 2,690,000 and the haemoglobin 60 %, when she left the hospital. She was readmitted a month later, and during this short time the corpuscles had fallen to 700,000, haemoglobin 26 %; but at the end of six weeks' residence in hospital they numbered 4,280,000, and the haemoglobin 65 %. Jaundice and other signs of blood destruction were noticed during part of her illness. In this case the reduction in the number of the erythrocytes was not always proportionate to the amount of haemoglobin.

The number of red cells vary considerably from slight diminution to those cases, as the above, in which

there is an enormous reduction. The corpuscles may undergo alterations in size and shape, microcytes, megalocytes, and poikilocytes being often present.

Hayem considers, as we have previously mentioned, that the predominance of small corpuscles occurs in slighter, whilst megalocytes belong to the severer forms of this anæmia; and he divides cases into those in which the red corpuscles are small, but relatively numerous, (these are the least serious), and those in which they are on the average larger in size, but very reduced in number; in other words, the average size of the corpuscles is inversely proportional to their number.

The corpuscles and haemoglobin have probably a development independent of each other, and the increase in the number of corpuscles may be suspended whilst renovation of the haemoglobin is taking place, or *vice versa.* (Von Limbeck.)

As signs of degeneration are associated with those of regeneration, and as the degree of the anæmia fluctuates, so the microscopic condition of the blood varies from time to time. During regeneration nucleated red corpuscles of the normoblast type appear. Megaloblasts, except in severe cases, are not numerous in this form of anæmia. According to Von Limbeck all these conditions may appear in any severe anæmia, and are therefore not characteristic of any one particular form.

The *blood plates* are, especially in severe cases of primary anæmia, frequently increased in number. (Von Limbeck.)

The *leucocytes* usually show no important or characteristic alteration, either in number or in quality. Commonly, according to Hayem, their number is somewhat diminished, probably owing to the impaired

function of the haemopoietic organs. Sometimes the number of lymphocytes, at other times the multinucleated leucocytes, are relatively increased, and the latter may occasionally contain a small quantity of haemoglobin in their protoplasm.

Any conspicuous change in the number or nature of the leucocytes usually indicates the presence of some complication.

The *diagnosis* of this form of anæmia is in some cases a simple matter. The fact that simple primary anæmia may occur at almost any age in either sex, that in typical cases the chlorotic condition of the blood is absent, and the haemoglobin and corpuscles are usually diminished in the same proportion, is sufficiently characteristic to distinguish it from chlorosis.

The condition of the blood, and the improvement which takes place under efficient hygienic and medicinal treatment, generally aid in the diagnosis of simple primary from pernicious anæmia.

Cases, however, may occur, in which it is impossible at once to say, even after a careful examination of the blood, as well as a clinical history of the patient, which of these forms we have to deal with. Though evidence of blood destruction is always present in true cases of pernicious, it is absent from any but exceptionally severe forms of simple primary anæmia.

The prevalence of megalocytes, and particularly the presence of a large number of megaloblasts, are in favour of pernicious anæmia, especially when associated with increasing oligocythemia and a general downward tendency of the case.

Improvement under treatment, especially if permanent, indicates simple primary anæmia.

Progressive Pernicious Anæmia.

Synonyms.—Primary, essential or idiopathic anæmia; Addison's anæmia; Biermer's disease; anæmatosis. (Pepper.)

Although this disease had been previously recognized by Andral and others, it was not till 1843 that Addison accurately described cases of anæmia occurring without any discoverable cause, and resulting almost invariably in death, under the term idiopathic anæmia. Later, Biermer in 1868 drew attention to the existence of severe anæmia, and more fully, in 1872, to some of its pathological characters. He named it progressive pernicious anæmia, and included severe primary anæmia as well as those secondary to pregnancy, lactation, and haemorrhage.

Nearly all the earlier writers recognized its fatality, but differed considerably as to its being primary, *i.e.*, without a recognizable cause, or secondary to other minor pathological conditions. Thus Eichhorst, who collected all the literature relating to it up to 1877, divided cases into those which are essential, primary, or idiopathic, (of these he counted seventeen), and those which were secondary to other causes. In the latter group he included twenty-nine cases in which the anæmia followed pregnancy, ten after confinement, twenty-four from affections of the alimentary canal, seven from loss of blood, and seven were attributed to bad hygienic surroundings. He defined pernicious anæmia as a condition of severe anæmia, which defies all treatment, proceeds without being checked, and usually ends in death.

Hayem describes it as a morbid condition, characterised by extreme anæmia, which most frequently ends fatally.

"Thus the element of fatality seems to be regarded as the most important feature, and the absence of cause became of secondary importance. Now, however, there seems to be a tendency to attach more weight to the absence of apparent cause, and to consider those cases only to be pernicious anæmia in which no such cause can be recognized. But even in such cases of fatal anæmia as are preceded by some definite lesion which may rank as a cause, there may remain much to explain if the supposed cause, as sometimes happens, ceases to operate long before death, and only leaves the patient in a condition which never improves, but gradually—it may be in the course of months—deteriorates until death ensues. Thus, it seems to me, it will be too great restriction of the term to apply it only to apparently quite idiopathic cases, and it ought to include, or at any rate we must still recognize as very obscure, those cases in which a temporary cause is followed by a persistent anæmia with a fatal tendency." (Taylor, *Brit. Med. Journal*, 1896, Sept. 19, 719.)

Modern English writers are still at variance as to what constitutes the nature of pernicious anæmia.

Dr. Stockman maintains that pernicious anæmia is not a disease in itself, but a symptom of several exhausting conditions, which induce an initial anæmia followed by degenerative changes in the vessels, resulting in capillary haemorrhages, and these cause the excessive anæmia.

Dr. William Hunter holds that pernicious anæmia is a specific, definite disease, in which the blood is

destroyed in the portal circulation, and excess of iron is deposited in the liver as a result of this haemolysis.

From the condition of the blood and clinical aspects of the disease we shall consider it as a disease arising in most cases without any determinable cause, sometimes from insignificant causes, and characterised by extreme anæmia and marked changes in the blood, which almost invariably terminates in death.

Condition of the Blood.

The blood usually flows freely from a puncture, but sometimes, especially in emaciated subjects, in whom the tissues are dry and the peripheral circulation diminished, it is somewhat difficult to obtain. It appears remarkably thin and watery, and is generally very pale, sometimes yellowish in colour. It has been described as of a dirty pinkish-red colour, resembling the washings of a vessel which had contained blood, or the water used in washing meat, an appearance said to be very characteristic. Exceptionally it has been noted of a reddish-brown or coffee colour.

So extremely thin and fluid is the blood that I have frequently noticed a difficulty in obtaining a drop large enough to suck up into the Thoma-Zeiss pipette, or to fill the automatic capillary tube of Von Fleischl's haemometer, as the blood from the puncture tends to run or spread out, and seldom forms a large globule.

Films of blood from a case of pernicious anæmia are frequently very thin, as the cellular elements are so scanty, and it is well, particularly with a view to the differential count of the leucocytes and the examination for nucleated red cells, to spread the films rather

thicker than usual, and to hasten their drying by moving them rapidly about in the air, or as some suggest by slightly heating them.

On standing, the corpuscles in a drop of blood soon separate and fall to the bottom, leaving clear serum above. The blood has apparently lost its cohesiveness, rouleaux formation is absent, or when present defective, and coagulation is slow—a condition resembling, according to Hayem, a certain degree of haemophilia, and is found in all severe anæmias.

The specific gravity is usually lower than in the normal condition.

If a drop of blood be examined in the *fresh condition*, taking care that the film is not too thin, characteristic alterations are seen. The corpuscles group themselves into little masses composed of two to six elements, which, owing to their great dissimilarity in size, give the rouleaux a very irregular appearance, or they lie isolated in the field. The red blood corpuscles appear unequal in size and are deformed. Many giant cells—megalocytes—are conspicuous in each field, often associated with the presence of microcytes.

The *numerical decrease* of the *red cells* is extraordinary, in fact usually under no other conditions is this alteration known to be so excessive.

Even when the disease is first apparent the corpuscles number only $2\frac{1}{2}$ million, more frequently $1\frac{1}{2}$ million, and usually when the disease is pronounced they frequently fall considerably below one million, and towards the end they may sink below 500,000 per cmm. Lepine records a case in which the corpuscles fell to 378,000, Müller one in which they numbered 360,000, and Quincke mentions a case in which they were only

found to be 143,000 per cmm. This is certainly the lowest on record.

Temporary increase in the number of red cells may occur several times in the course of the disease, but relapses as frequently follow. During periods of temporary improvement, the corpuscles may even reach the normal number. Thus one case is mentioned by Dr. Gibson in which at the commencement, the corpuscles numbered 500,000 to 600,000; under treatment they rose to over five million, and the patient was discharged from the hospital in apparent health, only to return in a few weeks feeling unwell. He died within a week after readmission.

The *haemoglobin* is always greatly reduced in amount, though usually not to the same extent as the corpuscles, so that the individual corpuscular value in haemoglobin is usually greater than normal, varying, according to Hayem, from 0·88 to 1·7, the normal being 1·0. This is due to the fact that the corpuscles, though enormously reduced in number, are well coloured, and their average size considerably increased.

It is said that Von Fleischl's instrument is inaccurate for determining the small amount of haemoglobin present in this disease. In one case I found the haemoglobin = 15 %, and in order to satisfy myself, after cleaning the instrument, I filled two instead of one of the capillary tubes with blood, and found the reading for the two exactly equal to 30 %. This apparently proved that error, if present, is unimportant.

Laache and Hayem concluded that the characteristic feature of the disease, consisted in an increase in the size of, and the relative excess of haemoglobin in, the corpuscles. Nearly all writers agree that this condition is

very characteristic of pernicious anæmia, although von Limbeck remarks that the apparent richness of the red corpuscles in haemoglobin, advanced by Laache as pathognomonic of this disease—a statement which he says is unjustly found in text-books—is met with in nearly all cases of severe oligocythemia.

The *alterations in the size* of the blood corpuscles is a conspicuous feature in pernicious anæmia, the average diameter of the elements being considerably increased. (Plate IV., Fig. 2.)

It was previously mentioned that in health large corpuscles, measuring 8·5 μ in diameter, are found, constituting about 12½ % of all the red cells; but in this disease giant cells—megalocytes—measuring upwards of 8·5 μ, occur. These are never found in health, with the exception of the newly-born, and belong exclusively to pathological conditions. (Plate I., Fig. 4.)

Laache found the average measurement of the megalocytes about 11–13 μ, but has seen some as large as 15 μ. Hayem's results closely correspond with those of Laache. He states that the giant cells usually measure about 9·5 to 12 μ. The most numerous are about 10 μ; those of 12 μ are rarer, but in exceptional cases he found them as large as 14 μ and even 16 μ in diameter.

Megalocytes are not, however, numerous, seldom constituting more than 3 to 12 % of the red corpuscles; so that increase in the average diameter of the elements is due to the prevalence of large-sized cells, not exceeding 10 μ. They are frequently oval or pear-shaped.

Small or dwarf corpuscles—microcytes—occur in this disease, but they are by no means as conspicuous or as characteristic as the megalocytes. They vary in size from that of a blood plate to an erythrocyte, but are

usually about 3 to 4 μ in diameter, and generally have about the same colour as the ordinary red corpuscles.

Eichhorst thought that the occurrence of small round corpuscles, measuring about 3 or 4 μ, conspicuous on account of their deep colour and high refractive index, peculiar to idiopathic anæmia. "Eichhorst's corpuscles," as they are called, are, however, met with in other forms, and are frequently absent from pernicious anæmia. (Plate I., Fig. 3.) They are of no diagnostic value whatever. Muir noticed their absence from nine cases of pernicious anæmia which he examined. He mentions that in one case of this disease in which the corpuscles were much below one million, the red cells were practically of the same size, and considerably larger than the normal, having a diameter of 9 μ.

Changes in the form of the coloured elements are commonly seen in this form of anæmia. Poikilocytes are very frequent, and Quincke, who first drew attention to them, thought characteristic of pernicious anæmia. Many of these are oval or fusiform, as if one end had been drawn out into a point; others are lanceolate, with one end broad and round, the other tapering. (Plate I., Fig. 5.) In some the outline is irregularly indented or broken; others are stellar or fragmented; whilst many are so irregular as to defy description. They vary in size from a blood plate to a figure measuring 15 μ or more in its greatest diameter.

These changes in the outline of the erythrocytes are probably degenerative signs, constituting that variety described by Maragliano and Castellino as "total necrosis."

The *colour* of the individual corpuscles varies considerably. The megalocytes are often pale in colour,

with an absence of the central depression or concavity, although the central portion may appear somewhat faintly stained with eosine, owing to the diminution of the haemoglobin in this part of the cell. Frequently, however, the giant corpuscles are somewhat deeply coloured, with no diminution in the colour of their centre. (Plate IV., Fig, 2.)

The smaller cells often appear rich in colouring matter, and although the smallest poikilocytes are pale, yet the larger forms are frequently deeply coloured, except in their projections. Many writers point out that the cell depression or concavity is either entirely wanting, or only partially present, in many corpuscles.

Very faint, almost colourless red cells, termed "shadow corpuscles," are met with in pernicious anæmia, but are not usually abundant. (Plate I., Fig. 6a.) They are sometimes only recognized by a pale circle of colour.

According to Stengel, faint granular matter may occasionally be seen within them, or near their periphery.

In films stained with eosine and haematoxylin, most of the corpuscles, some with, many without a central concavity, are stained a pink colour of varying intensity according to the amount of haemoglobin contained; a few of them, however, especially the larger megalocytes, are coloured partly with the eosine, partly with the haematoxylin, a purple colour, *i.e.*, Polychromatophile—a sign, as was previously mentioned, of degenerative or necrotic alterations in the corpuscles. This is often seen in the protoplasm of the nucleated red corpuscles, especially in that of the megaloblasts. (Plate I., Figs. 6 and 8.)

Other signs of degeneration — the endoglobular, necrotic changes of Maragliano, may be met with, in

which decolorization occurs in the centre of, or in patches in the cell, forming vacuoles and finally general granular disintegration of the interior of the red cells.

Amoeboid movements of some of the poikilocytes, and other red corpuscles, have been observed by several authorities, and Hayem has drawn attention to the occurrence of rapid movements on the part of some of the very small microcytes, which have assumed the shape of little rods, closely resembling, and are said to have been mistaken for, bacteria.

Nucleated red blood corpuscles or erythroblasts are usually, though not constantly, present in the blood of cases of pernicious anæmia.

Stengel speaks of them as a constant feature in this disease, although varying considerably in number. Hayem mentions their frequent occurrence towards the end of the disease, but remarks that they are not present in every case, and notes their absence in one out of three fatal cases which he has fully described.

Muir found them in four out of eleven cases, and remarks that "the two patients in whom they were most numerous, ultimately recovered, whilst in cases of most extreme anæmia, they were absent before death." He mentions the fact that "Ehrlich found them absent before death, in a case in which the corpuscles had fallen to 213,360 per cmm.

Limbeck states that when, at least partial, regeneration occurs, nucleated red cells appear.

These may occur as normoblasts, *i.e.*, nucleated red cells about the size of an ordinary erythrocyte, or smaller as microblasts, or larger as megaloblasts. When a poikilocyte contains a nucleus, it is spoken of as a "poikiloblast." The normoblast usually measures 7·5 to 8 μ, but it never

exceeds 9·5 μ. The nucleus which is of small size is always stained deeply with haematoxylin. It is generally situated towards the periphery of the cell. It frequently shows division into two or three smaller parts, and occasionally, though extremely rarely, karyokinetic figures may be found. (Plate I., Fig. 7.)

I would add, a point not usually emphasized by most writers, that frequently free nuclei, easily distinguished from other elements by their small size and deep-staining reaction, are met with in blood films of cases of pernicious anæmia. These are no doubt derived from the normoblasts. Von Noorden describes the occurrence of blood crisis in this disease, *i.e.*, the occasional appearance of a large number of normoblasts, followed in a few days by a marked increase in the red blood corpuscles.

Microblasts, very small nucleated red cells, may be found in this form of anæmia, but they are very infrequent. (Plate I., Fig. 7d.)

Megaloblasts are considerably larger than an ordinary erythrocyte, being nearly always over 10 μ, and often 14–16 μ in diameter. The nucleus, which is usually more centrally placed, is larger, but not nearly so deeply stained as that of the normoblast, and when found in a cell, whose protoplasm shows polychromatophilic degeneration, is somewhat indistinct. (Plate I., Fig. 8 ; and Plate IV., Fig. 2.)

It is impracticable to estimate the number of nucleated red cells present in the blood. In some cases they appear extremely numerous, in others they require considerable looking for.

Approximately their number per cubic millimetre may be determined by, first, ascertaining the number of white

cells per cmm. by the Thoma-Zeiss pipette; and, second, by finding how many nucleated red cells are present in a large number of leucocytes counted on the dry-stained preparations. The formula for the calculation will be:—

$$\frac{\text{No. of leucocytes per cmm.} \times \text{number of nucleated red corpuscles counted in the dry film.}}{\text{No. of leucocytes counted in dry film.}}$$

For example, in one of my cases the leucocytes numbered 1944 per cmm. In films stained with eosine and haematoxylin I found 2 nucleated red cells whilst counting 1000 white cells.

Therefore $\frac{1944 \times 2}{1000} = 3{\cdot}8$ nucleated red cells per cmm.

I agree with many observers that the megaloblasts are more characteristic, and more important as a prognostic sign, than the normoblasts.

The Blood Plates are, according to Hayem, Muir, etc., usually diminished in number. Other writers, Limbeck, Stengel, etc., maintain that they are not diminished, but rather increased, and are found grouped together in thick masses. I can confirm the latter statement.

The Leucocytes, like the blood plates, undergo unimportant alterations in this disease.

Von Limbeck considers that the white corpuscles in this, as in the greater number of severe anæmias, are not increased in number.

Hayem indicates that they are usually diminished, that the uninucleated forms are most numerous, and that some of the leucocytes show vacuolation of their protoplasm, whilst others contain a small amount of haemoglobin.

Stengel remarks that in the earlier stages they may be diminished, although they are more frequently normal, or even increased; and points out that before death there may be a marked terminal leucocytosis.

Most observers are of the opinion that the number is somewhat diminished, and that the small uninucleated elements or lymphocytes are usually relatively greatly increased.

Many recent writers mention the presence of a small number of myelocytes or marrow cells, and I have found them occasionally in severe cases of pernicious anæmia.

Nature of the Disease.

The majority of observers have agreed that pernicious anæmia depends on excessive blood destruction, rather than defective blood formation; and that this haemolytic nature of the disease, so ably emphasized by Dr. Hunter, is supported by the occurrence of ferruginous blood pigments in various organs, the presence of jaundice, an excess of urobilin in the urine, and the occasional occurrence of haemoglobinaemia.

Where this destruction takes place, whether in the portal circulation, liver or spleen, or what exact condition gives rise to it, is not yet ascertained.

Many consider it to be due to intestinal auto-intoxication, and bring forward the fact that cases of severe anæmia have been benefited by intestinal antiseptics, and that indican has been found in excess in the urine, and occasionally peptonuria, cadaverin, and other products of intestinal putrefaction.

The changes which occur in the bone marrow in pernicious anæmia are essentially a return from the

adult condition to that of the embryo; the fat being displaced by red marrow, containing a large number of nucleated red cells, and also megalo- or giganto-blasts, which are not normally present. The marrow changes, probably secondary and compensatory in nature, help to somewhat explain the condition of the blood in this disease.*

The Diagnosis.—In some cases the diagnosis may be comparatively easy, frequently, however, it is extremely difficult, and often impossible without an examination of the blood by means of dry-stained films. The clinical history, and the results of a careful physical examination, must in all cases be taken into consideration.

The following clinical features are very suggestive of the presence of this disease:—The absence of any evidence of organic disease, the severe progressive anæmia, the inefficiency of ferruginous treatment, the peculiar, flabby condition of the subcutaneous tissue, and the absence, in most cases at least, of emaciation, especially if associated with extreme weakness, lemon-yellow complexion, and the occasional occurrence of slight pyrexia and haemorrhages, particularly retinal.

* In a *post-mortem* examination of a case of pernicious anæmia in a man aged sixty-three years, I found all the characteristic alterations in the bone marrow described by Muir.

One inch of the middle third of the shaft of the femur was removed, and it was seen that the yellow fat of normal marrow was entirely replaced by a deep blood-red marrow having the appearance of recently coagulated blood. The medullary canal of the bone was enlarged.

Microscopically—in films as well as sections—this red marrow consisted in a great increase in the number of nucleated red corpuscles, many of which were of enormous size.

The nuclei of these megaloblasts were single, double, or fragmented. The non-nucleated red corpuscles present showed all the alterations found in the blood of pernicious anæmia, viz., megalocytes, poikilocytes, and various forms of cells undergoing degeneration.

The blood should be carefully examined, and will in many cases clear up the diagnosis. There is, however, in the blood, no one absolutely pathognomonic sign, although the occurrence of several alterations just described are together very significant.

The enormous decrease in the number of red corpuscles, seldom occurs as constantly in any other condition as in progressive idiopathic anæmia.

After severe haemorrhage, and during the course of malignant diseases, the corpuscles may, however, sink below one million, but this reduction is exceptional. The relative increase in the amount of haemoglobin, combined with marked oligocythaemia, is often a striking feature, but it is not pathognomonic, as some undoubted cases of pernicious anæmia have been described, in which the haemoglobin had undergone a greater reduction than the number of corpuscles; whilst in other severe cases of anæmia—not pernicious in nature—the high individual corpuscular value in haemoglobin has been noticed.

Poikilocytosis is of very frequent occurrence in all varieties of anæmia, and I have noticed it, certainly in a slight form, in very moderate degrees of oligocythaemia. The very large irregular poikilocytes are of more significance, and the presence of these, with comparatively large numbers of megalocytes, megaloblasts, and particularly poikiloblasts, is very strong evidence of this disease.

Generally speaking, nucleated red corpuscles are more frequent in this than in any form of anæmia, with the exception only of leucocythaemia; and of the three varieties the megalo- or giganto-blasts are of the most diagnostic importance.

Megaloblasts, associated with the presence of normoblasts, do occur in other diseases, but in practically no other than pernicious anæmia are the former more numerous than the latter.

The peculiar staining reaction—polychromatophile—of some of the red blood corpuscles is of little diagnostic value.

Von Limbeck regards the occurrence of signs of rapid destruction of the blood cells, which is shown in the marked degeneration of the corpuscles, the excess of hydrobilin or urobilin in the urine, and the evidence of slight jaundice, as the only condition which distinguishes pernicious from any other severe anæmia.

From Chlorosis.—Pernicious anæmia can usually be easily distinguished from chlorosis, in that the former generally shows great diminution in the number of the corpuscles, and a relative increase in the amount of haemoglobin, *i.e.*, the individual corpuscular value is above the normal, whilst the latter is characterized by relatively moderate oligocythaemia, but great reduction in the haemoglobin, so that the corpuscular value is below the normal.

The corpuscles in pernicious anæmia are generally larger than in health, megalocytes and large poikilocytes being frequently seen, and nucleated red corpuscles are almost always present. The megaloblasts are more numerous than the normoblasts. (Compare Fig. 1 and Fig. 2, Plate IV.)

In chlorosis the corpuscles are, on the whole, somewhat smaller and paler than in the normal; they do not show such marked alteration in their form; poikilocytes, though commonly present, are not as irregular; and nucleated red blood corpuscles only

occur in severe forms, and then are usually of the normoblast type. Megaloblasts may occur in exceptionally severe cases of chlorosis, but are never more numerous than the normoblasts.

Marrow cells are more frequently met with in pernicious anæmia than in chlorosis, but in neither disease are they ever numerous.

From Secondary Anæmia, especially that resulting from malignant disease and haemorrhage.

Cases of severe anæmia, particularly those resulting from malignant disease, *e.g.*, carcinoma of the stomach, may frequently present clinical features closely resembling pernicious anæmia, but the examination of the blood will usually render their distinction possible.

In secondary anæmia the red blood corpuscles are seldom below one million, and oligochromaemia is generally more pronounced than oligocythaemia, so that a chlorotic condition of the blood is found. The erythrocytes are usually smaller in size and paler in colour. Nucleated red cells are not infrequently present, especially in the severer forms, but they are principally of the normoblastic type. In those exceptional cases in which the corpuscles are very considerably reduced, leucocytosis, usually involving an increase in the multinucleated cells, is almost invariably present.

In pernicious anæmia, on the other hand, the reduction of the red cells is always extreme, and oligocythemia is in excess of oligochromaemia. The corpuscles exceed the average in size, and are usually well coloured. Megaloblasts are present, and generally form the greater number of nucleated red cells. The leucocytes are diminished, and there is usually a relative increase of the lymphocytes.

The following case illustrates the improvement, too often only temporary, which may take place in the course of Pernicious Anæmia under suitable treatment:

Woman, aged 47, presented all the clinical features of the disease, viz.: lemon-tint complexion, slight jaundice, bile pigments in the urine, progressive weakness without emaciation, and irregular pyrexia. She complained of loss of strength, shortness of breath, and buzzing or swimming in the head. Physical examination revealed no cause for the condition.

The Blood appeared of a dirty colour. The red corpuscles numbered 914,583 per cmm.; their average size was increased, and megalocytes were very numerous.

Poikilocytosis and other degenerative alterations in the red cells, *e.g.*, vacuolation, crenation, and polychromatophilia were extremely frequent.

Nucleated red corpuscles were fairly numerous, and at first the megaloblasts were considerably in excess of the normoblasts. The haemoglobin was about 15 %.

The leucocytes were diminished, numbering only about 1914 per cmm. The lymphocytes were relatively greatly increased, whilst the multinucleated and eosinophile cells diminished.

A few myelocytes, or marrow cells, were seen.

The following table shows the progress which took place under treatment with bone marrow, etc.

The increase in the corpuscles was attended with a diminution and ultimate disappearance of the megaloblasts, and latterly all forms of nucleated corpuscles were absent.

At the last examination the red cells numbered $4\frac{1}{2}$ million, but their average diameter was greater than the normal.

CASE OF PERNICIOUS ANÆMIA DURING TREATMENT.

	April 24th.	May 1st.	May 8th.	May 17th.	May 22nd.	May 29th.	June 12th.	June 26th.	July 10th.
Red Blood Corpuscles	914,583	705,147	666,666	1,201,785	1,485,833	2,077,500	3,670,000	3,581,250	4,400,000
Leucocytes	1,944	2,919	2,801	3,961	3,060	4,752	7,300	5,873	9,166
Haemoglobin	15 %	15 %	14 %	24 %	30 %	37·5 %	53 %	55 %	50 %

DIFFERENTIAL COUNT OF THE LEUCOCYTES.

Multinucleated	51 %	46·5	51	46·4	48	34·5	57	60	50
Lymphocytes	46 %	51·3	44	51	49	56·5	31	35	40
Large Uninucleated	2 %	1·5	3	1·4	2·5	7	6·5	4	5
Eosinophile	1 %	0·7	1·5	1·1	2·5	2	5·5	1	5

NUCLEATED RED BLOOD CORPUSCLES.

Proportion to Leucocytes	29 in 1500	23 in 1500	15 in 1500	5 in 1500	52 in 1500	1 in 1500	0	0	0
Number per cmm.	38	45	28	12	104	3	0	0	0
Normoblasts	11	16	6	5	52	1	0	0	0
Megaloblasts	18	6	9	0	0	0	0	0	0
Microblasts	0	1	0	0	0	0	0	0	0

Leucocythaemia.

Synonyms.—Leukaemia, or leuchaemia; lymphadénie leucémique (Trousseau).

Leucocythaemia is a chronic disease, characterized by an immense increase in the number of the white, and usually slight diminution in the red blood corpuscles, associated with alterations in the bone marrow, spleen, or lymphatic glands, either singly or all of these structures together.

Hughes Bennett, of Edinburgh, was the first to draw attention to this disease. He recorded in the *Edinburgh Medical Journal* of 1845, a case of enlargement of the spleen, in which the blood was found to contain a large number of pus cells, a condition which he at first termed "suppuration of the blood."

In the same year, but a few weeks later, Virchow recorded a similar case in which he found that the pus cells were identical with the leucocytes, or white cells of the blood, and gave to the disease the name of "white blood," or "leukämie."

In 1846 Fuller and Walshe found the same condition of the blood in the living patient.

Although the discovery of this disease is almost universally attributed to Hughes Bennett, yet earlier writers—Bichat in 1801, Velpeau in 1827, Hodgkin in 1832, Donné in 1839, and others—had described cases which were very similar, if not identical with leuchaemia.

Virchow distinguished two forms of the disease—one in which large white corpuscles preponderated, which he termed "Splenaemia," lienal or splenic leukaemia, owing to the supposed origin of these cells from the spleen; and the other in which small leucocytes or lymphocytes

were most numerous, "Lymphaemia," or lymphatic leukaemia.

As probably the large leucocytes arise from the bone marrow and not from the spleen, modern writers consider that the so-called splenaemia of Virchow should be termed "myelaemia," or myelogenic leukaemia.

Later, three types of the disease were recognized—the splenic or lienal, the lymphatic, and the medullary or myelogenic, according to the tissue primarily affected. Usually, however, pure forms of leukaemia are very rarely met with, as the disease generally affects more than one of the blood-forming organs.

True cases of myelogenic leucocythaemia are extremely rare, and their existence doubted, till Beatty of Dublin, described what is considered to be a reliable form of this disease.

Recently, most observers recognize only two forms—the lymphatic, in which the lymphatics are generally enlarged; and the spleno-medullary, spleno-myelogenic, or lieno-medullary form, which is usually attended with enlargement of the spleen, and is of more frequent occurrence than the former.

Condition of the Blood.

The blood from a puncture is usually of normal colour, but occasionally it has a rather suggestive appearance. Sometimes it is merely very pale and more fluid than the normal, at other times it resembles a mixture of pus and blood, or may be so white that it looks like milk or pus, hence the name given to the condition.

Rarely, however, is the colour dark, although in certain cases it has been said to be of a chocolate

colour. It flows easily from a puncture, sometimes there being a distinct haemorrhagic tendency. The specific gravity is generally lower than normal. Its reaction was thought to have been acid, but it is now known that as in healthy blood it is alkaline, but may after standing undergo decomposition, and may then give an acid reaction. Notwithstanding the great increase of the leucocytes, coagulation is, according to Hayem, normal, and the amount of fibrin not increased unless inflammatory complications are present.

Most other observers, however, maintain that the blood does not coagulate as readily as in health, and some have attributed this to the presence of peptones in the blood. Fano asserted that blood containing peptone may be restored to the normal coagulability by passing carbonic acid gas through it, although similar treatment renders the coagulation of healthy blood slower.

Rywosch and Berggrün found this to be the case with leukaemic blood.

If a drop of blood be examined in *the fresh state*, amongst the rouleaux of red corpuscles the enormous number of white cells is a conspicuous and characteristic appearance. Even in such unstained preparations it may be possible to determine the existence of leukaemia, and the form of disease present, by the size of the leucocytes — as these are of the small uninucleated variety in lymphatic, whilst in spleno-medullary leukaemia they are mainly very large uninucleated elements associated with the presence of cells containing highly refractive granules.

It is, however, usually necessary to examine stained films before this distinction can be determined.

The Leucocytes.

The *number of white corpuscles* is in all cases very greatly increased, and the occurrence of 500,000 is of no special rarity. Even in cases of moderate severity they usually reach as high as 100,000 or 200,000 per cmm.

Earlier writers usually speak of the proportion of the white to red corpuscles. In health there are approximately 8000 to 9000 white, and five million red corpuscles in a cubic millimetre, or a proportion of about 1:600, and under normal conditions a proportion of 1:500 is rarely exceeded. In leucocythaemia the relation of white to red elements is frequently 1 to 8, but may be considerably higher, thus Virchow has found them to be as 2 to 3, Vogel as 1 to 2, and according to Robin the leucocytes may be twice as numerous as the coloured cells.

The proportion of the two forms is not as important as the actual number present in the blood, because, obviously, the ratio may be influenced, not only by an increase in the white, but also by a diminution in the red corpuscles.

It is extremely difficult and usually impossible to say what number of leucocytes or proportion of white to red, justifies the condition being called leucocythaemia. Magnus Huss and others considered that the proportion of 1 to 20 and above warranted a diagnosis of this disease. That this is unreliable is proved by the fact that Von Jaksch found in cases of anæmia in children that the proportion of leucocytes to red corpuscles was as 1 to 12, 1 to 17, and 1 to 20, and in adults he found the proportion of 1 to 8·1, and yet the conditions were not leucocythaemia.

There is no doubt that cases of leucocytosis do occur in which the white cells may be exceedingly numerous, as for instance, one case of cancer in which Hayem found 70,000 leucocytes, and the number of white, or their ratio to red cells, is by no means a characteristic sign of the disease. Of much greater importance is the quality rather than the quantity of cells present, and this can only be determined satisfactorily by means of stained films.

Not only does the number of colourless cells vary considerably in different cases, but it undergoes marked fluctuations in the same case from time to time. Hygienic surroundings, suitable diet, and medicinal treatment—arsenic tonics, etc.—are frequently attended with an improvement (too often temporary) in the general health, and a diminution in the number of the leucocytes.

A remarkable fact observed by several writers, is that the occurrence of an infectious disease in a patient is often attended with a marked diminution in the white cells. Eisenlohr records a case of mixed (splenomedullary-lymphatic) leukaemia, in which, during the febrile period of typhoid, the spleen, lymphatics, and the leucocytes of the blood, rapidly diminished, but that at the end of the fever they returned to their original condition.

Quincke found a similar diminution in a case of leucocythaemia, in which miliary tuberculosis supervened, and Kovacs, during an attack of febrile influenza. Müller found that in a case of leukaemia, the occurrence of chronic sepsis was attended with a fall in the number of the white corpuscles, from 246,900 to 57,300 per cmm., but when the sepsis was cured they returned to their former number.

In most of these cases, complicated with infectious diseases, the uninucleated elements were diminished, whilst the multinucleated and polymorphous forms were relatively increased in number.

So far little is known as to the significance of this remarkable condition, although Müller regards the process as one of "irritation," the result of the virus of infection, and considers it a sign of improvement. An analogous state of things occurs in other diseases, *e.g.*, the effect of erysipelas on malignant tumours, and this suggests, as von Limbeck remarks, the possibility of our regarding leucocythaemia in the light of a new growth.

It is to be noticed that the temporary beneficial action of infectious diseases does not always occur in leukaemia, and Müller describes a case of the lymphatic form of the disease, in which an attack of septicaemia caused no decrease, but an actual increase of the leucocytes from 180,000 to 400,000 per cmm.

The various Forms of Leucocytes found in Spleno-medullary Leucocythaemia.

The enormous numerical increase of the leucocytes in this disease is associated with alterations in individual forms and varieties of these elements, and we are indebted to Ehrlich for our knowledge of the nature and methods of observing these various cells. As the white cells differ in the two forms of the disease, we shall first describe the spleno-medullary type.

In the absence of a case of leukaemia, all the elements we are about to mention may be readily

Plate V.

Film preparations stained with eosine and haematoxylin. Examined with eyepiece 2. Objective $\frac{1}{8}''$.

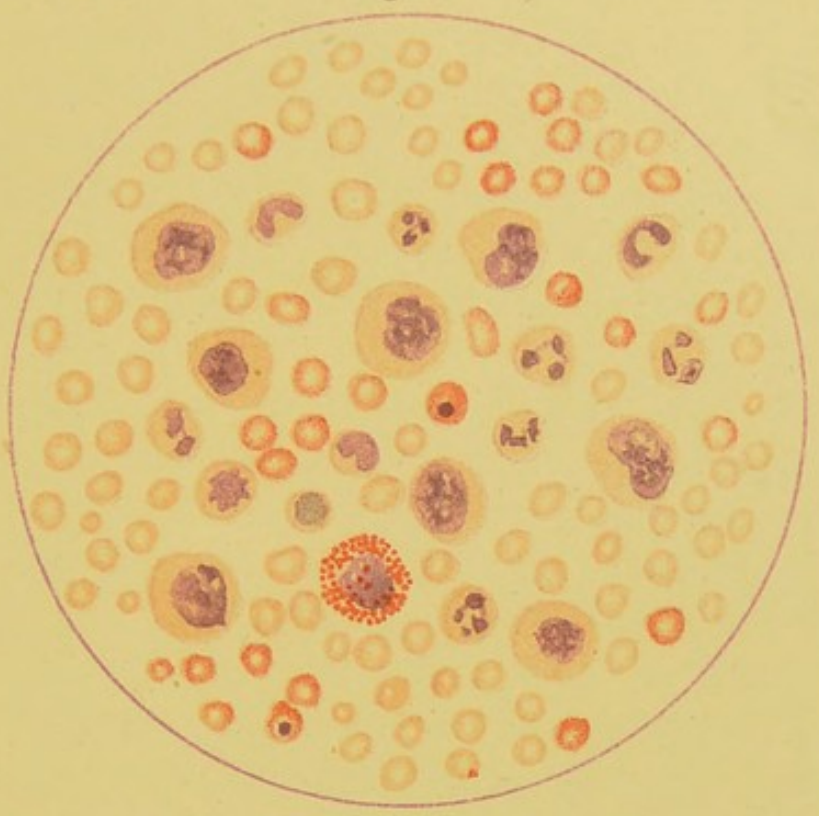
Fig. 1.—**Spleno-medullary Leucocythaemia.** The leucocytes are enormously increased—about 1 to 5. Marrow cells are the prevailing form of leucocytes present. One eosinophile myelocyte is seen. The red corpuscles show no very marked alteration, but two nucleated red corpuscles, or normoblasts, with deeply stained nuclei are present.

Fig. 2.—**Marrow** of the femur of a kitten two days old. Marrow cells are very numerous, and are practically identical with those seen in Fig. 1. Two marrow cells undergoing karyokinetic division are seen. Nucleated red corpuscles varying in size, but all of the normoblast type, are very numerous.

At the top of the field is a giant cell, and the peculiar arrangement of its nuclei is well seen. Three corpuscles are contained within its substance.

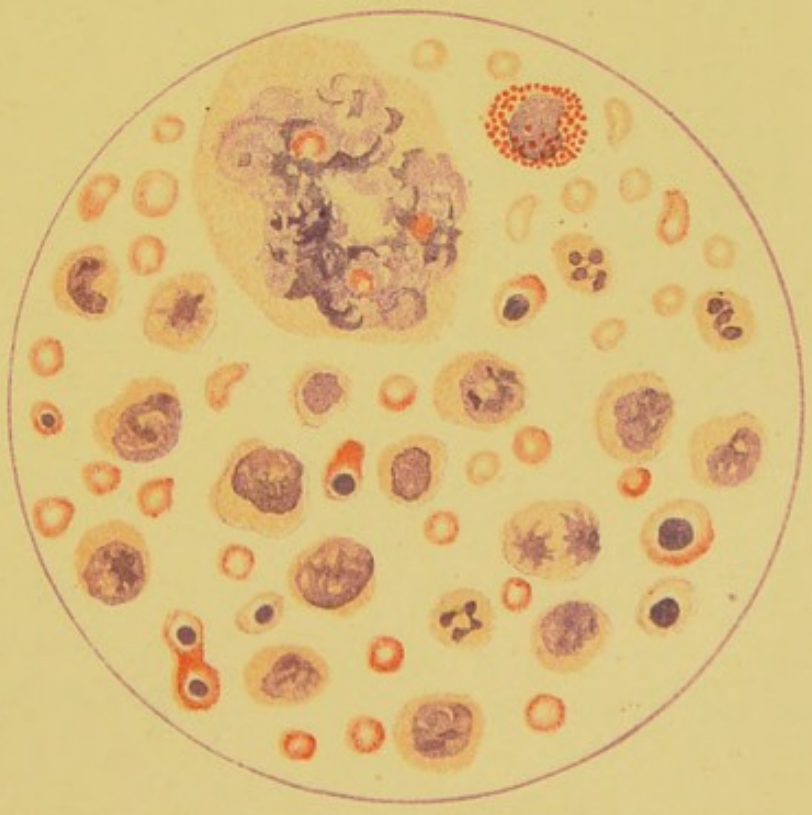
PLATE V.

Fig. 1.



Spleno-medullary Leucocythæmia.

Fig. 2.



F.L. Coles del.

Normal Marrow.

West, Newman Chr.

seen in the marrow of a kitten, prepared by the method previously mentioned; and if with this some of the blood of the animal be mixed, a condition resembling the spleno-medullary form of the disease may be seen. (Compare Figs. 1 and 2, Plate V.)

Myelocytes, or *Marrow Cells;* synonym: Leucocytes hypertrophies (Hayem).

Large uninucleated leucocytes, having distinctive staining reaction, are the most characteristic elements in this form of leukaemia. They are very large cells, measuring sometimes as much as 16 μ, or even 20 μ in diameter, and generally having a more or less circular or oval outline. (Plate II., Fig. 6.) Some of the marrow cells are very small, not much larger than a red corpuscle, and indistinguishable from the large or small lymphocytes, except that they are the only form of white cell present in the blood, which has a single nucleus, and whose protoplasm contains fine granules which stain with Ehrlich's neutral mixtures. (Plate III., Fig. 5.)

All forms of the myelocytes possess no power of amoeboid movement.

The nucleus is of large size, and although lobed, it is nearly always single, and does not show the deep indentations of the polymorphous leucocytes. It is usually round or oval, sometimes indented at one side, or horse-shoe or kidney shaped, and stains faintly with nuclear stains.

The nucleus is poor in chromatin, but a fine irregular network may in some cases be seen. It occupies a somewhat eccentric position, although it may sometimes be in the centre of the cell.

The surrounding protoplasm is abundant, and con-

tains very fine granules, which were formerly said to be only neutrophile, as they stain with Ehrlich's neutral stain, but are now generally considered to be also finely oxyphilous or eosinophilous. Myelocytes may in the same film contain neutrophile and fine oxyphile granules, and the latter may show all gradations, up to coarsely granular oxyphile or eosinophile cells. (Plate II., Figs. 6, 7, and 8.)

In specimens stained with eosine and haematoxylin, the protoplasm is almost invariably tinted blue with haematoxylin, and the granules are not discernible even with the high power of the microscope, but these are clearly demonstrated by using one of the triple stains of Ehrlich. (Plate III., Fig. 5.)

Marrow cells may be easily distinguished from lymphocytes and multinucleated leucocytes, by their large size, faintly stained large nucleus, and absence of amoeboid movement; and typical examples are easily separated from the large uninucleated or hyaline cells; but sometimes even when stained with the triple stain, it is extremely difficult and to me occasionally impossible to say whether the leucocyte is a large hyaline or a marrow cell.

Some of these elements may show evidence of karyokinetic division, especially when the blood film has been previously fixed with Flemming's solution. (Plate II., Fig. 9.)

Marrow cells were till recently thought to occur only in leucocythaemia, and von Limbeck states "that their presence in other diseases, though frequently asserted, is even now doubtful."

It is now known that they are occasionally present in very small numbers, as we have mentioned before, in

pernicious and some chronic anæmias and wasting diseases, and I have found elements closely resembling them in the blood of two cases of myxoedema in 1892. Zappert is said to have met with them in some cases of skin diseases, and Neusser after an attack of uraemia. These statements do not diminish their importance and diagnostic value in leukaemia, as in no other do they ever reach the relatively large number that they attain in this disease.

In spleno-medullary leucocythaemia they generally constitute about 30 to 50 % of all the leucocytes present, whilst in other diseases, in which they sometimes occur, they never, according to Cabot, exceed 9 % and usually not 2 %.

In three typical cases of this form of leukaemia, I found that the marrow cells constituted 37·2, 54·5, and 46 % respectively of all forms of leucocytes present.

Mosler, who first discovered this form of leucocyte, found them in the marrow obtained from a puncture of the sternum, and their origin from, and identity with, the marrow cells of the bone, have been universally confirmed. The cells described by Cornil and Ranvier as "cellules médullaires" and "medulocelles," were proved by Müller to be identical with these leucocytes.

Eosinophile or Coarsely Granular Oxyphile Cells.

Three forms of these leucocytes may be recognized in leucocythaemia. 1st. The ordinary eosinophile cells of normal blood (Plate II., Fig. 5, Plate III., Fig. 4), spherical cells with usually two nuclei connected by a band of chromatin, placed somewhat eccentrically in the protoplasm, and surrounded by, and embedded in a

mass of coarse, highly refractile granules, which stain deeply with the acid aniline dyes, *e.g.*, eosine aurantia, etc. Their nuclei stain fairly deeply with haematoxylin. These cells have an average diameter of about 10 to 11 μ. In three cases, I found that the ordinary form of eosinophile cells, constituted 43, 13, and 38 % respectively, of all the eosinophile cells present.

2nd. Small eosinophile cells deeply stained with eosine, which do not occur in the normal blood are extremely rare, if they ever occur, in any other disease. They are about the size of a red blood corpuscle or even smaller. In the same three cases I noticed that the relative number of small eosinophile cells varied considerably. In one they counted 2 %, in another 7 %, and in the third case 6 % of all forms of eosinophile cells.

3rd. Marrow cells containing eosinophile granules or eosinophile myelocytes. These never occur in health and probably in no other disease, although it is stated that they are occasionally seen in very small numbers in pernicious anæmia. (Plate II., Fig. 7, and Plate III., Fig. 6.)

They exactly resemble the ordinary marrow cell, but have coarse eosinophile instead of fine neutrophile (or oxyphile) granules. All stages from the finely granular oxyphile to the coarsely granular eosinophile myelocytes may be seen. (Plate II., Figs. 6, 7, and 8.)

Their large nucleus is always single, often indented at one side, and usually in contact with the wall of the cell at one part. It stains faintly with haematoxylin, and is poor in chromatin. Karyokinetic division of the nucleus can occasionally be seen. These elements are of very large size, measuring often 13 μ and

sometimes 16 or 18 μ in diameter. I have frequently noticed the presence of large vacuoles in the leucocytes, especially in the marrow cells or eosinophile myelocytes.

The eosinophile myelocytes, which are identical with the form found in normal marrow, are the most numerous of all the eosinophile leucocytes, and together with the ordinary myelocyte, are the most characteristic of all the elements present in the blood of leucocythaemia.

I found that in three cases of this form of the disease, they numbered respectively 55, 80, and 56 %, *i.e.* an average of $63\frac{2}{3}$ % of all the eosinophile cells.

The nature of the granules present in these three forms of eosinophile cells is still a matter of uncertainty. They were formerly regarded as fat granules, and Litten and Müller are stated to have stained them black with osmic acid, but it has not yet been proved that everything blackened with osmic is necessarily of the nature of fat. The majority of observers regard them as more of the nature of an albuminoid material.

The absolute number of eosinophile cells is always increased, but the relative number is sometimes above, sometimes below the normal of 2–4 %. Von Limbeck states that their relative number compared with the other forms of leucocytes is not raised, and mentions that Zappert found in ten cases of leukaemia not more than 8 % of eosinophile cells, a proportion which he says is often exceeded in health. [Von Limbeck and Zappert give a higher percentage for the eosinophile cells in health than other observers, their average being from 0·6 to 11 %.] I counted the relative number of all forms of eosinophile cells in three cases of this disease, and found that in one patient—a child of 11 (and it is well known that these cells are easily increased in child-

hood even in health) they were equal to 14½ %, but ultimately fell to 7 %. In another, that of an adult, the average was 5 %, whilst in a third they only reached 3 % of all the white cells present.

Many, particularly the earlier writers, considered that a relative increase in these cells was a characteristic sign of leukaemia, but this is certainly not the case, as frequently they are diminished, whilst in asthma, skin diseases, and a number of other pathological conditions they may be markedly increased.

Mere numerical increase in the proportion of eosinophile cells is of no assistance whatever in the diagnosis of the disease, but qualitative alterations — more especially the appearance of eosinophile myelocytes— are of the highest importance.

Multinucleated or Neutrophile Leucocytes.

The ordinary multinucleated cells are seen in leukaemia, but are not such conspicuous features on account of their size, as the marrow cells. (Plate II., Fig. 4, and Plate III., Fig. 3.)

They are increased in absolute number, but are relatively less numerous than in health, constituting about 50 % of all the white cells present. In some cases they only number about 20 or 10 %, sometimes even less. In appearance they differ from each other in size, staining characters and the shape of their nuclei, and are not so regular as in health, or even in leucocytosis. In most the protoplasm is faintly but uniformly stained with eosine, whilst in a few it is quite colourless, taking on neither the eosine nor haematoxylin, and the latter frequently show very irregular nuclei, suggestive in some

cases of mitotic division. Not infrequently very small multinucleated leucocytes are seen, not much larger than an ordinary red blood corpuscle, with the protoplasm darkly stained. In many of these cells the amount of chromatin in the nucleus is diminished and the intra-nuclear network is not nearly as well seen, as in the ordinary multinucleated elements, and in some, the distinction from marrow cells is almost impossible.

Sometimes the fine granules appear to be distinctly more like fine eosinophile than neutrophile, and usually they are less pronounced than in normal blood.

The multinucleated leucocytes in three of my cases constituted 43·3, 35·5 and 48·3 %, an average of about 42 % of the leucocytes present.

A marked increase in the relative number of multi-nucleated elements occurring in the course of the disease, would suggest the presence of some inflammatory complication.

Small Uninucleated Leucocytes or Lymphocytes.

These forms—small elements about the size or slightly larger than the red blood corpuscles, with very deeply stained nucleus, surrounded by a small border of non-granular protoplasm, are greatly reduced in their relative number. In three cases I found that the lymphocytes constituted only $4\frac{1}{2}$, 5 and $2\frac{2}{3}$ % respectively of all forms of white cells, instead of the normal 15 to 25 %. In appearance these elements have practically the same characters as those occurring in health, and also occasionally contain fine basophile granules. (Plate II., Fig. 1, Plate III., Fig. 1 and Fig. 7.)

Fraenkel drew attention to larger forms of lympho-

cytes, having a large, sometimes deeply indented, or even polymorphous nucleus, poor in chromatin, which he considered were of lymphatic origin. Mitosis is seldom seen in the small uninucleated elements.

Large Uninucleated Leucocytes—Hyaline Cells.

These are probably mature forms of the small lymphocytes, and in health contain no granule in their protoplasm. (Plates II. and III., Fig. 2.) The nucleus is large, single, or indented, poor in chromatin, and stains faintly with nuclear stains, and shows indistinct signs of an intranuclear network. The protoplasm stains very faintly with eosine, and usually takes on some of the stain of the nucleus, appearing of a very faint bluish pink with eosine and haematoxylin.

In the spleno-medullary form of the disease they are of very little importance, being usually relatively diminished.

Transitional Leucocytes.

These are intermediate between the large lymphocytes and the multinucleated leucocytes, differing from the former by the irregularity of the nucleus, which tends to become multipartite, by the presence of a larger quantity of chromatin and a more distinct intranuclear network. (Plate II., Fig. 3.) As we have previously mentioned, it is difficult, frequently impossible, to distinguish between these different forms of cells, as all intermediate types are met with.

Basophile Cells.

In films stained with methylene blue alone, some of the small leucocytes, as in health, contain fine basophile

or δ granules, but in addition to these many large marrow cells have these granules.

Atypical basophile cells are very commonly met with in spleno-medullary leucocythaemia. (Plate III., Fig. 7.)

Buchanan has pointed out the occurrence of mixed granules—basophile and finely granular oxyphile—in the same cell, and this, he says, is particularly noticeable in the large uninucleated or marrow cells.

It seems that myelocytes may be met with containing neutrophile, basophile, fine oxyphile, and coarse oxyphile or eosinophile granules, and all of these forms I have frequently seen, but so far have been unable to confirm Buchanan's interesting statement of the occurrence of mixed granules.

"Mastzellen," or cells containing coarse basophile or γ granules which stain deeply with basic stains, are said to occur in leukaemia. (Plate III., Fig. 8.) They are, however, as von Limbeck states, seldom found in the spleno-medullary form of the disease, and their presence is by no means characteristic, as they have been seen in the blood of patients suffering with hereditary syphilis.

Mitotic or Karyokinetic Division of the Nuclei.

In films of blood quickly prepared as we have described, *i.e.*, by fixing for a short time in a mixture of ether and alcohol, and staining in eosine and haematoxylin, occasionally all stages in the division of a leucocyte may be seen. This is more clearly demonstrated in films specially prepared by Flemming's, Muir's, or Müller's method as previously described. Indirect division of the nucleus — mitosis — is by no means frequently met with, and Flemming, who first

drew attention to them, found but one among several thousands of white cells. They are said to be seen in any of the leucocytes, but are much more frequently met with in the marrow cells or myelocytes.

I have occasionally seen these karyokinetic figures, either in the stage of the stellar bodies with radiating, fairly deeply stained filaments, or in that of two rosette figures at either end of the cell, which in further stages show constriction of the protoplasm. (Plate II., Fig. 9.) Some of these are quite as distinct as the karyokinetic figures found in the bone marrow of young animals. (Plate III., Fig. 9.) In other cells, especially those previously mentioned, in which the protoplasm is almost colourless, the nucleus is irregularly arranged in the form, and very suggestive of, the earlier stages of division, but I am not convinced that they are actually mitotic figures.

Löwit, in accordance with his theory of blood formation, does not regard these as white cells, but as erythroblasts, *i.e.*, the first step in the formation of erythrocytes.

The majority of observers, however, consider them to be undoubtedly leucocytes, undergoing multiplication by indirect nuclear division.

The Red Blood Corpuscles.

The red corpuscles are usually decreased in number, and it is rarely that cases of leucocythaemia are met with, in which there is no oligocythaemia present, but it is in very few cases excessive.

It is stated (Quincke and Litten) that they may be normal, or even above the normal number, but this is

an unusual condition. Most frequently they constitute about 2 to 3 million per cmm., but may reach as high as $4\frac{1}{2}$ or even 5 million, and as low as, according to Eichhorst, 316,000.

The amount of haemoglobin is usually diminished in proportion to the reduction of the red elements, but not infrequently it is lower than the number of the latter.

It is not easy to determine exactly the percentage with von Fleischl's instrument, on account of the turbidity produced by the number of white cells present. This may be somewhat overcome by adding, as previously mentioned, a little caustic soda or potash to the water, as suggested by Leichtenstern.

It is a very remarkable fact that patients suffering with leucocythaemia frequently show little pallor; in fact, as Wilks pointed out, they have often a good colour on the lips and face, and when seen in bed do not appear to be very ill.

Alterations in the size, shape, and staining reaction of the red corpuscles occur in this as in other forms of anæmia, and are more marked in those cases in which the oligocythaemia is pronounced. In cases in which the anæmia is slight, according to Hayem, small corpuscles prevail, whilst in severer types of the disease large red cells, and even megalocytes may be often seen. In one of my cases, in which the corpuscles numbered $3\frac{1}{2}$ million, megalocytes were frequent, but in another, having about 3 million red cells per cmm., they were much less numerous. Often the cells show very little alteration in their shape, but when the anæmia is intense, extreme degrees of poikilocytosis may be observed.

I have occasionally met with corpuscles showing

Maragliano's degenerative signs, such as vacuoles, within the cells, and the peculiar staining reaction—Polychromatophile—due to necrobiotic changes.

Amoeboid movements on the part of the red cells have been observed by Friederich, similar to those sometimes found in pernicious anæmia.

The most conspicuous feature on the part of the red corpuscles is the presence of *nucleated red cells*, and although they may be seen in severe anæmic conditions, this is the only disease in which they are present where there is very slight oligocythaemia. (Plate V., Fig. 1.)

Hayem, who so emphatically denies the existence of nucleated red corpuscles in chlorosis, etc., admits that they are a constant feature in some stage or another of this form of leukaemia. In cases in which the red corpuscles number four million or more, they may be found in almost every field of the microscope. They are mostly normoblasts, about the same size as the ordinary erythrocytes, sometimes megaloblasts, and occasionally I have met with the most perfect examples of microblasts. Signs of direct—amitosis—and indirect—mitosis—division are frequently seen in the nuclei of the normoblasts. (Plate I., Figs. 7 and 8.)

The Blood Plates.

These elements are sometimes increased in number, and Pruss found that in four cases they reached two million, the normal being about 200,000 to 300,000 per cmm. In some cases they are however diminished, and Hayem gives their figures in one case as 128,650, and in another as 271,800 per cmm.

Charcot-Leyden Crystals.

Many observers, amongst others Neumann, Westphal, and Eberth, have frequently found these crystals in the blood of patients suffering from leukaemia. They are probably identical with the crystals found in the sputum during an attack of asthma. Charcot and Vulpian described them as octahedral, whilst Cohn thought them hexagonal pyramids.

They are obtained by allowing a small quantity of blood to stand for some time in order to partially dry.

Their origin seems as yet undetermined.

Westphal considers that they are formed in the spleen, and obtained them by puncture of that organ in leukaemic patients, whilst other writers think that these are a crystallized product from the blood plasma or leucocytes. According to Schreiner they are chemically a compound of phosphoric acid with an organic base. They are said to be found in the normal bone marrow.

They are by no means constant in the blood of leucocythaemia, and von Limbeck and von Jaksch have repeatedly failed to find them, and in dry films I have never met with any crystalline forms.

Lymphatic Leucocythaemia, or Lymphaemia.

This form of leukaemia is of much less frequent occurrence, runs a more acute course, and usually terminates fatally in shorter time than the previously described type of the disease. It is attended with enlargement of the lymphatic glands, and occasionally, though not generally, with some slight increase in the

size of the spleen, but the latter never reaches, in unmixed cases of the disease, the enormous size that is met with in the spleno-medullary forms. (Plate VI., Fig. 1.)

The Leucocytes.

The white cells are greatly increased in number, but not to the extent that they are in the spleno-myelogenous form. The average proportion of white to red corpuscles is usually about 1 to 15, or 1 to 45, instead of 1 to 2, or 1 to 10, as is frequently the case in the latter variety of leukaemia.

The actual number of leucocytes varies considerably, but is usually about 40,000 to 250,000 per cubic mm. Hayem records one case in which they numbered 476,000 per cmm., and in four cases described by Muir they averaged about 441,600.

The prevailing form of leucocyte is almost entirely that of the large and small uninucleated cells or lymphocytes, and they may constitute 95 % of all forms of white corpuscles present.

In some cases the elements are almost all of the small lymphocytic variety—cells about the size of the red blood corpuscles containing a relatively large nucleus, which stains deeply with nuclear stains, and shows signs of a dense intranuclear network. (Plates II. and III., Fig. 1.) The nucleus may in some cases be deeply indented. The surrounding protoplasm is very small in amount, and is usually devoid of any granules. In other cases the leucocytes are nearly exclusively, large uninucleated lymphocytes with large nuclei, which stain faintly, and their protoplasm, which is abundant, generally also contains no granules, but is frequently somewhat

Plate VI.

Film preparations stained with eosine and haematoxylin.

Fig. 1.—**Lymphatic Leucocythaemia.** The leucocytes are increased, but not to the same extent as in Fig. 1, Plate V. They are all—with the exception of one multinucleated leucocyte—of the lymphocyte variety. No eosinophile cells or nucleated red corpuscles are seen. Examined with eyepiece 2. Objective $\frac{1}{8}''$.

Fig. 2.—**Malarial Parasites.**

A. **Parasites** of **Quartan Fever,** showing stages in their development, from a small intra-corpuscular pigmented body to that of segmentation. The red blood corpuscles are of about the normal size. In the segmenting stage the pigment is situated in the centre, and around it are eight spores.

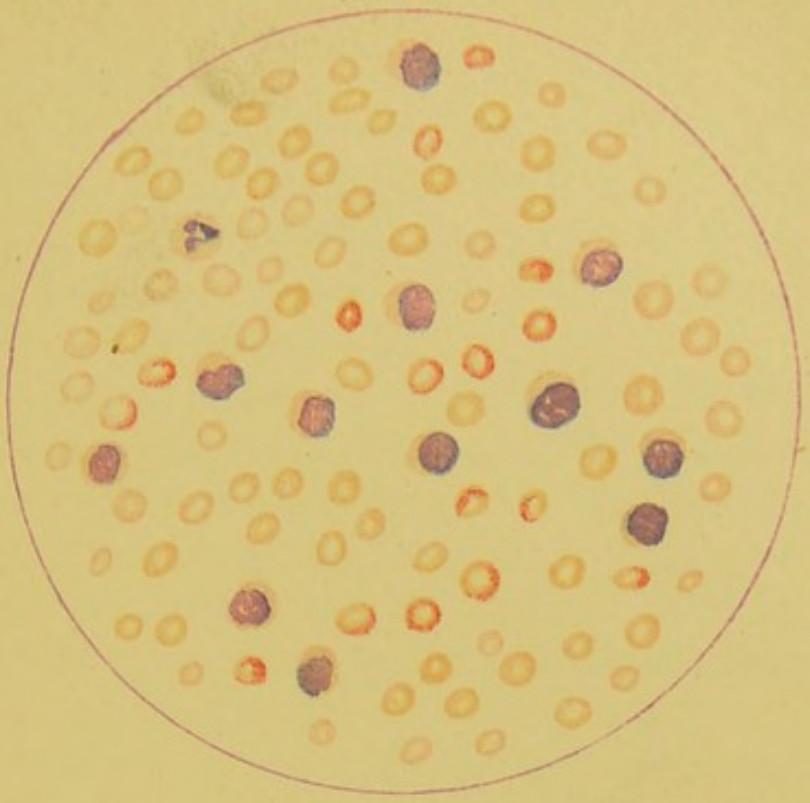
B. **Parasites** of **Tertian Fever,** showing stages in development. The red blood corpuscles are somewhat larger than the normal, and the number of spores found in the segmenting forms is more numerous than in that of quartan fever.

C. **Parasites** of **Irregular** or **Aestivo-Autumnal Fever.** The amoeba is seen to assume the form of a ring. The red corpuscles are smaller and more deeply stained. Two Laveran corpuscles or crescentic bodies are shown.

Examined under rather higher magnification than previous plates. Eyepiece 4. Objective $\frac{1}{12}''$ oil immersion. Tube length 160 mm.

Plate VI

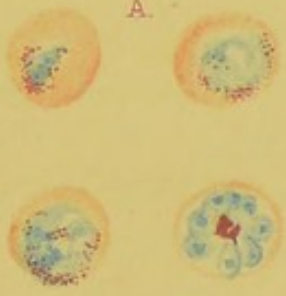
Fig. 1.



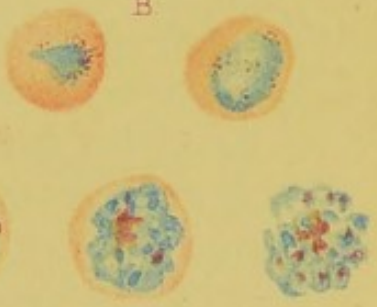
Lymphatic Leucocythæmia.

Fig. 2.

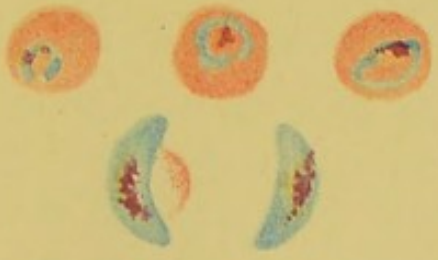
A.



B.



C.



Malarial Parasites.

F.L.Coles del.

West, Newman Chr

coloured with the nuclear stain. (Plates II. and III., Fig. 2.) Not infrequently cases are met with in which both the small and large hyaline cells or lymphocytes are seen, as well as intermediate sizes.

When stained with methyl-blue alone some of the small and large elements show fine basophile granules, arranged in a ring round the nucleus. (Plate III., Fig. 8.) Signs of mitotic division of the nuclei are very rarely seen in lymphatic leukaemia, and this is remarkable, as these uninucleated leucocytes multiply by indirect mitotic division in the lymphatic glands.

The multinucleated or neutrophile leucocytes are relatively and absolutely diminished in number, and the eosinophile cells are extremely infrequently seen.

Marrow cells or myelocytes may or may not be present in the lymphatic form of the disease. Muir saw them in one out of four cases, and von Limbeck found them wanting in his case of lymphaemia. Stengel remarks that large uninucleated cells, corresponding to marrow cells, are rarely seen, although he quotes Fraenkel's statement as to the occurrence of uninucleated hyaline cells larger than the ordinary large lymphocyte. Generally speaking, marrow cells when present are so in very small numbers.

The Red Blood Corpuscles.

The number of the erythrocytes is almost invariably diminished, and usually to a greater extent than in the spleno-medullary form of the disease. Hayem records a case, in which they numbered 3·72 million; Müller, 2·55 million; and von Limbeck two cases, in which they reached 3·8 and 3·4 million per cmm. In Muir's

four cases they did not average 1·5 million per cmm. Notwithstanding the fact that the oligocythaemia is generally more pronounced in lymphatic leukaemia, yet nucleated red corpuscles are extremely rare in this form, although abundant in the spleno-myelogenic type of the disease.

Müller, Limbeck, and Wertheim have met with a few isolated forms, and Muir found them in one case, in which the red corpuscles were below one million per cmm. The latter states that he has only found them when the anæmia has been severe. Most observers agree that nucleated red cells are frequently absent, and when present are never found in large numbers.

The Pathology of Leucocythaemia.

Virchow and many other observers regard leucocythaemia as a primary disease of the blood-forming organs, and consider that the blood is only affected secondarily, whilst Löwit and some others maintain that the condition of the blood is primary, and that the haemopoietic organs are secondarily affected. The former is most generally accepted as the correct view of the process, and is based upon the fact that organic changes of the nature of proliferation have been found in the blood-forming organs.

Those who maintain that the blood is the primary seat of the disease, consider that the proliferative changes found in the haemopoietic organs are due to elements in process of multiplication, being carried there by the general circulation, and not due to an actual proliferation taking place in the organs themselves.

In favour of the former and in opposition to the latter theory, is the fact that signs of multiplication of the cellular elements, *i.e.*, karyokinetic figures, are much more numerous in the organs concerned in blood formation than in the blood itself.

The earlier writers, especially Hughes Bennett, looked upon the condition as one of "suppuration of the blood"; whilst latterly some observers, Kottmann and Bard, consider the process as one of "new growth or cancer of the blood."

Löwit maintains that the excessive number of leucocytes found in both forms of the disease is not due to increased proliferation, but rather to diminished destruction of the leucocytes, or an arrest in the development of the uninucleated, into more mature white corpuscles. This view is not generally entertained, because although uninucleated elements may prevail in both types of the disease, yet there is no evidence that diminished destruction of the leucocytes actually occurs, some even maintaining that leucolysis is increased.

(*a*) The *spleno-medullary Leukaemia* is essentially characterized by the occurrence of certain elements which are not found in the normal blood, viz., myelocytes, eosinophile myelocytes, and nucleated red corpuscles, and these in health are only found in the bone marrow. (Compare Figs. 1 and 2, Plate V.)

In this disease the marrow of the spongy and long bones is found to have undergone considerable alteration, and the fat is largely replaced by purulent, or, as Neumann terms it, pyoid, material. This is of a yellowish colour, either distributed uniformly or in patches throughout the marrow, and which, though

at first firm, becomes softer and more liquid, and eventually closely resembles pus.

Microscopically it is seen to consist very largely of marrow cells of both varieties, and proliferation is evident from the large number of cells in process of division, and all stages of karyokinesis may be seen.

It seems very probable that all the elements found in the blood in this form of the disease may be explained by excessive proliferation of the marrow cells and their overflow into the blood. The nucleated red corpuscles which, as Muir points out, lie for the most part at the periphery of the marrow near the vessels, might easily escape into the blood stream.

Flemming and others maintain that the proliferation occurs in the marrow, and that the elements in process of division found in that situation are far too numerous to be accounted for by the assumption that they are carried there by the blood, and in the blood, mitotic figures, though occasionally found, are far too few to be regarded as the primary factors.

What is the primary cause of the proliferation of the cellular elements in the marrow seems still a matter of the greatest obscurity.

At present there is no evidence that bacteria are the exciting cause, although in acute cases it seems possible that infection may play an important part.

(β) *Lymphatic leukaemia.*—Partly owing to the greater rarity of this form of leukaemia, and partly to the fact that the elements present are not abnormal leucocytes, it is extremely difficult to determine the primary cause of the disease.

In this type of leukaemia the bone marrow proper is largely displaced by lymphoid tissue, consisting of small

uninucleated leucocytes, and a few nucleated red cells. The spleen is usually somewhat enlarged owing to the presence of an enormous number of lymphocytic elements. The lymphatic glands are frequently, but not always, enlarged in the lymphatic form of the disease. They are softer than the normal, owing to a proliferation of the cellular rather than the connective tissue elements.

Mitotic figures are frequently seen, pointing to rapid multiplication of the cells.

The lymphatic glands are occasionally not enlarged, and Leube, according to Muir, records a case in which neither the lymphatic glands nor the spleen were hypertrophied, but in which a lymphoid condition of the bone marrow was present. Muir further states that there may be enormous enlargement of the spleen, and yet the blood may contain an excessive proportion of small uninucleated cells. He considers that the term lymphatic, applied to this type of leucocythaemia, should refer to the variety of leucocytes present, rather than to their origin.

The condition seems to depend on proliferation of the lymphoid elements in the lymphatic tissue throughout the body.

Wertheim is of the opinion that the two forms of leukaemia are quite different, whilst Müller, on the other hand, considers the process in them analogous, but that in the lymphatic form the lymphatic system is chiefly involved.

Von Limbeck points out the close resemblance that exists between lymphatic leukaemia and lymphosarcomata, and considers the temporary improvement that occasionally takes place in a leukaemic patient attacked

with an infectious disease as analogous to the influence of erysipelas on some forms of malignant disease.

What cause gives rise to the cell proliferation in both forms of the disease is at present entirely unknown.

Diagnosis of Leucocythaemia.

The diagnosis of this disease is in some cases an extremely easy matter, but in others, especially in the lymphatic form, it may be exceedingly difficult to say whether we have to deal with leucocythaemia or lympho-sarcoma, and in either type of the disease stained film preparations are, in my opinion, of much greater value than mere enumeration and proportion of the red and white cells.

1. *Diagnosis of the Spleno-medullary Form.*

This, by far the commoner type of the disease, is characterized by a large increase in the number of leucocytes, a slight variable diminution of the red corpuscles, the constant appearance of nucleated red cells not accounted for by the degree of anæmia; and, above all, by the presence of abnormal elements, namely, the marrow cells and atypical eosinophile cells or eosinophile marrow cells.

All observers agree that the leucocytes may in certain cases be so enormously in excess of the normal as to equal, and occasionally to exceed, the number of red blood corpuscles present; but at times the number of white cells, though increased, are not sufficiently so to justify the diagnosis of leucocythaemia.

As previously mentioned, there is no absolute number of leucocytes, nor is there any figure representing the

ratio of white to red which entitles us to distinguish between leucocytosis, from whatever cause, and leucocythaemia.

Some recent writers — Gilbert and Hayem — have placed the limit of leucocytosis at 70,000 white cells per cmm., others have asserted that when the proportion of white to red corpuscles exceeds 1 to 20 the condition is one of leukaemia rather than leucocytosis. This is, however, by no means reliable.

Mere relative increase in the number of eosinophile cells, although mentioned as an early diagnostic sign by von Jaksch, is not characteristic, as frequently these elements are relatively diminished.

The only diagnostic features of this form of leucocythaemia, are the occurrence of marrow cells and eosinophile myelocytes, and although the former may be found occasionally in pernicious anæmia, and in a few other pathological conditions, yet they are present in extremely small numbers, whilst in leucocythaemia they constitute a very large proportion of all the elements present.

It must be mentioned that during the course of this disease the splenic enlargement may to a great extent subside, and the number of leucocytes undergo a marked diminution ; yet if such a case be seen for the first time in this stage, myelocytes are present in sufficient numbers to warrant a diagnosis of the condition.

Nucleated red blood corpuscles are present in large numbers, quite out of proportion to the degree of the oligocythaemia, and these, and the occasional presence of mitotic figures, help to clinch the diagnosis.

As an example of the importance of stained film preparations of blood, and the comparative insignificance of the enumeration of the cellular elements and the

examination of blood in the fresh unstained condition, I will describe one of the last cases of spleno-medullary leukaemia, which quite recently I saw in consultation.

A girl, aged 11. Spleen very much enlarged, occupying the greater part of the left side of the abdomen, extending anteriorly to the linea alba, and at the back as far as the posterior axillary line, and reaching within one and a half inches of the symphysis pubis.

The notch was felt just below the umbilicus. The organ measured about 10 inches in length, and $8\frac{1}{2}$ inches in its greatest breadth.

The lymphatic glands in the groin and axilla were just palpable. There was no tenderness on deep percussion of the bones.

The blood had been carefully examined in the fresh condition, and an enumeration of the corpuscles made by her medical attendant, but the comparatively small number of leucocytes apparently excluded the diagnosis of leukaemia, and the case appeared rather one of splenic anæmia.

I found on my first examination that the red corpuscles = 3,458,333; the haemoglobin 37 % (von Fleischl); the white blood corpuscles, 44,315 per cmm., or a proportion of white to red as 1 to 78.

From my enumeration I could make no diagnosis, nor did the examination of fresh blood films render this possible; but in dry films stained with eosine and haematoxylin I found that the leucocytes present very largely consisted of typical marrow cells, and large eosinophile myelocytes were frequent. Nucleated red blood corpuscles, especially of the normoblastic type, were also present in large numbers, although the red cells were fairly numerous and well shaped.

A differential enumeration of the leucocytes showed that the myelocytes were 37·2 %, and a certain diagnosis of spleno-medullary leukaemia was given.

The proportions of the other elements will be seen in the table below.

An examination of the blood made a week later showed that the leucocytes had slightly increased, reaching 55,385 per cmm. After a lapse of five weeks the leucocytes reached 555,795, whilst the red numbered 2,143,333, or a proportion of white to red cells as 1 to 3·8, or practically 1 to 4.

The following table shows the remarkable increase in the number of white cells, and the proportion of the various forms of leucocytes found at intervals in the course of the disease :—

CASE OF SPLENO-MEDULLARY LEUCOCYTHAEMIA.

	April 18, '97.	April 25.	May 29.	June 5th.
Red Blood Corpuscles	3,458,333	3,576,562	2,143,333	3,029,166
White Blood Corpuscles	44,315	55,385	555,795	696,960
Haemoglobin (von Fleischl)	37 %	44 %	40 %	30 % ?
Proportion of White to Red Corpuscles	1:78	1:64½	1:3·8	1:4½

DIFFERENTIAL ENUMERATION OF THE LEUCOCYTES.

Multinucleated	43·3 %	36	49 %	52
Marrow Cells	37·2	33	43	35
Lymphocytes	4·7	11	1	2
Eosinophile	14·6	20	7	11

VARIETIES OF EOSINOPHILE CELLS.

Myelocyte Eosinophile	43 %	45	70 %	66
Ordinary Polynuclear Eosinophile	55	55	28	34
Small Eosinophile	2	0	2	0
Nucleated Red Corpuscles to Leucocytes	41 in 2000	50 in 2000	15 in 2000	14 in 2000

It is possible that at the time the first examination was made, and previous to it, some complication might have caused a temporary diminution in the number of the leucocytes, and the subsequent increase may have been merely a reversion to the former state of the blood. The large size of the spleen would fully permit this opinion.

Against it must be mentioned that no signs of recent inflammatory complications existed, and the temperature chart for some weeks previously only showed the usual irregular rise so frequently found in this disease.

On the other hand the condition first seen might have been merely an incipient form of the disease.

In this case the qualitative rather than the quantitative alteration in the leucocytes as seen in stained films, rendered the diagnosis of spleno-medullary leukaemia certain from the first examination.

(*a*) *Diagnosis from Leucocytosis.* As a general rule, the number of white corpuscles in leucocytosis does not exceed 60,000, but it may in exceptional cases reach over 100,000 per cmm., whilst commonly in leukaemia these cells are over 200,000 per cmm.

In leucocytosis usually the multinucleated leucocytes are the prevailing type, although in some cases the uninucleated lymphocytes may predominate, but these are chiefly of the small variety. In leukaemia the abnormal elements so frequently alluded to, are present in large proportions, whilst the multinucleated forms are relatively diminished in number.

(*β*) *From Hodgkin's Disease and Splenic Anæmia.*— These diseases are attended with, in the one case, enlargement of the lymphatic glands, and in the other, usually slight enlargement of the spleen, but the latter

may be greatly increased in size, and the condition may somewhat resemble this form of leukaemia.

The blood, however, in these two diseases is usually more or less normal, and the leucocytes, if increased, are not very numerous, and the multinucleated forms generally predominate, and very few, if any, of the abnormal leucocytes, so characteristic of the latter disease, are seen.

(γ) *From Malarial enlargement of the Spleen.*—The condition of the blood, the absence of marked leucocytosis, the presence of malarial organisms, or of pigment granules in the leucocytes, as well as the entire absence of marrow cells, render the distinction from splenomedullary leukaemia very easy.

(δ) *Tumours in the region of the Spleen or Hydronephrosis*, or malignant disease of the kidney, may clinically resemble leukaemic enlargement of the spleen, but the absence of the notch, and the condition of the blood, which may in malignant disease of the kidney show marked leucocytosis, involving however almost entirely the multinucleated leucocytes, render the diagnosis certain.

2. *Diagnosis of Lymphatic Leukaemia.*

The diagnosis of this, fortunately the less common form of leukaemia, presents greater difficulty than the former. In an article in the *British Medical Journal*, Dec. 5th and 12th, 1896, on the "Relation between Leukaemia and Pseudo-leukaemia," Martin and Mathewson take a very pessimistic view of the value of the examination of the blood as a means of distinguishing these two diseases. I must mention, however, that they

refer throughout their paper to the lymphatic form of leukaemia.

The essential diagnostic feature of this form of leucocythaemia is the occurrence of permanent leucocytosis, which varies considerably in degree, but usually there are about 130,000 white cells per cmm., involving almost exclusively the large and small uninucleated elements or lymphocytes, whilst marrow and eosinophile cells are extremely few. The red blood corpuscles are diminished usually to about half the normal number, but nucleated red cells are exceedingly rare.

(*a*) *Diagnosis of Lymphatic from Spleno-medullary Leukaemia.*—In the lymphatic type of the disease, the leucocytes are not increased to the same extent as in the spleno-medullary form. In the former 130,000 white cells, with a ratio of white to red corpuscles of 1 to 30, are usually seen, whilst in the latter the leucocytes frequently number 400,000 or 500,000 per cmm., with a ratio of 1 to 6. In lymphatic leukaemia the leucocytes are almost entirely normal elements—lymphocytes; and the other white cells—the multinucleated and eosinophile cells, are considerably reduced in number. Marrow cells may occasionally be present in some few cases, but even then constitute only a small percentage, not more than 0·5 %. (Compare Fig. 1, Plate V., with Fig. 1, Plate VI.)

In the spleno-medullary form, the leucocytes present are largely abnormal elements, large uninucleated neutrophile cells or myelocytes, constituting 30 to 50 % of all forms of white cells seen. The multinucleated elements are relatively reduced, but may constitute 50 %, whilst the eosinophile cells, though not usually relatively increased, are present in fair numbers, and many of them

are eosinophile myelocytes. The lymphocytes are considerably diminished in number.

As regards the red corpuscles, they are usually somewhat less numerous in the lymphatic than in the spleno-medullary form, but nucleated corpuscles though extremely numerous and constant in the latter, are absent or very infrequent in the former.

(β) *From Hodgkin's Disease, or Pseudo-leukaemia and Splenic Anæmia.*—In both Hodgkin's disease and lymphaemia, enlargement of the lymphatic glands is the prominent clinical feature, but the blood in the former, as well as in splenic anæmia, is usually normal; and although there may be an increase in the number of the leucocytes, yet this very rarely attains the high degree seen in typical lymphatic leukaemia. The leucocytosis in pseudo-leukaemia, when present, involves chiefly the multinucleated cells, but sometimes the lymphocytes are the elements concerned. Osler has stated that occasionally the leucocytes in Hodgkin's disease are greatly increased, and the character of the blood becomes that of lymphatic leukaemia.

(γ) *From Sarcomata and other Malignant Diseases.*—As a general rule leucocytosis, when it results from carcinomata, is usually attended with an increase in the number of multinucleated elements, but this is not always so in sarcomata, and cases have been recorded by Palma and others in which lymphocytosis occurred.

When multinucleated leucocytes are present in excess, lymphatic leukaemia is easily excluded; and in other cases, in which the lymphocytes are the prevailing type, the number of the white cells seldom reaches that found in lymphaemia.

Yet many authorities, particularly von Limbeck,

admit that some cases of lymphosarcomata may occur, in which the leucocytes are very considerably increased, and are mainly of the lymphocytic type, a condition which it is practically impossible to distinguish from true lymphatic leukaemia. We shall reserve the question of the diagnosis of both forms of leukaemia occurring in children, from what has been termed "anæmia infantum pseudo-leukaemica," till we have described this disease.

Acute Leukaemia.

A form of leucocythaemia, characterized by its rapid course, has been described under this name, and Ebstein collected an account in 1889 of seventeen such cases. Since then additional cases have been recorded, so that at the present time their number has reached to thirty-five or forty.

The duration of the disease varies from six days to nine weeks. Most observers, however, limit it to six weeks, whilst Fraenkel, in a recent account (1895) of ten cases, includes those which lasted four months.

In all cases the blood and blood-forming organs showed the characteristic appearances of leucocythaemia, but unfortunately many of the descriptions give merely the number and not the variety of leucocytes present.

According to Leyden, Greiwe, and Kossler, all forms —the splenic, spleno-medullary, and lymphatic—may occur in acute as in chronic leukaemia. Thus Eichhorst's case was pure splenic, Leyden and Kossler's spleno-medullary, whilst two other cases described by Kossler were the lymphatic variety; and the last-named author noticed that in one of his cases—a woman aged

twenty-seven years, who died within seven days—myelocytes were the prevailing elements, whilst the multinucleated leucocytes were very infrequent, and the lymphocytes and nucleated red corpuscles entirely absent. The proportion of white to red corpuscles was 1:60. In Greiwe's and in two other cases of Kossler, the increase in the white cells took place at the expense of the small lymphocytes.

According to Fraenkel, the characteristic condition of the blood is that of lymphaemia, *i.e.*, the small uninucleated are enormously increased, whilst the multinucleated leucocytes are considerably diminished in number. He pointed out that the nuclei of the lymphocytes were somewhat poorer in chromatin than the normal, and their protoplasm frequently contained basophile, never neutrophile, granules. Mitotic figures were occasionally seen, as well as a few nucleated red corpuscles.

The erythrocytes are usually diminished in number, and in some cases the anæmia is pronounced. Von Limbeck considers that the anæmia, which is sometimes at the commencement a prominent feature of the disease, and which later is followed by leucocytosis, may depend on the haemorrhagic tendency—a marked feature in acute leukaemia—and this might also account for a leucocytosis of 30,000 per cmm., but would not explain the occurrence of 300,000 or more leucocytes.

It seems extremely difficult to explain the nature of this form of the disease.

Possibly some of the cases described by the earlier writers, especially where no exact investigations of the blood were made, were of the nature of a high degree of leucocytosis, whilst in others it is probable that acute

symptoms might have supervened in a patient affected with chronic, latent, or unrecognized leukaemia.

There are many clinical features present, *e.g.*, sudden onset with rigors, irregular fever, enlargement of the lymphatic glands, and occasionally of the spleen, the frequent haemorrhagic tendency, and the acute course of the illness, which all point to this as a separate form of leucocythaemia, in which infection may play an important part.

Hodgkin's Disease.

Synonyms: Pseudo-leukaemia (Cohnheim), Lymphatic anæmia (Wilks), Adénie (Trousseau), Lymphosarcoma (Virchow), Malignes Lymphom (Billroth), Desmoid-carcinom (Schulz), Anæmia splenica (Greisinger).

Hodgkin's disease is defined by Osler as "an affection characterized by progressive hyperplasia of the lymphatic glands with anæmia, and occasionally the development of secondary lymphoid growths in the liver, spleen, and other organs."

The disease was first recognized by Hodgkin in 1832, and Wilks in 1865 described a number of cases under the name of "Hodgkin's disease." Cohnheim in 1857 described cases under the term Pseudo-leukaemia, which closely resembled leucocythaemia, only lacking the characteristic condition of the blood in that disease.

French writers included Hodgkin's disease and leukaemia under the general term "Adénie."

A splenic form of the disease was recognized by the earlier writers, and recently described by Banti and Bruhl under the names anæmia splenica, spleno-megalie primitive, and splenic anæmia. We shall describe this later.

There is no doubt that tubercular, syphilitic, inflammatory, and malignant enlargement of the lymphatic glands have been included under the term Hodgkin's disease.

Clinically, tubercular enlargement of the lymphatic glands may usually be distinguished from pseudo-leukaemia by the fact that the former is more common in the young, the disease is more or less localized, it involves the submaxillary rather than the glands in the anterior and posterior cervical triangles, and the glands often fuse together and tend to suppurate.

Syphilitic enlargement of the glands is usually distinguished by the history of the disease and the moderate size, though often universal distribution of the glandular hyperplasia.

Malignant diseases of the lymphatics when secondary, are easily distinguished if the primary growth is found, but when the glands are primarily affected, the distinction is often impossible, many considering Hodgkin's disease as a primary malignant lymphoma.

Anatomically and pathologically, pseudo-leukaemia closely resembles leucocythaemia, and some observers maintain that the former may develop into the latter. A few writers have recorded such cases, but while recognizing that transformations may undoubtedly occur, yet some of the cases published may probably be instances of terminal leucocytosis rather than true leukaemia.

The Condition of the Blood.

The examination of the blood is of the greatest importance, although characteristic alterations in the number and nature of the elements are conspicuously

absent. At the commencement of the disease the blood is practically normal, but when the condition is advanced, and the patient has become cachectic, then all the signs of the anæmia associated with cachexia are present.

Generally the number of red cells is somewhat below five million, but in severe cases they may be reduced to two million per cmm.

The haemoglobin usually undergoes a greater diminution, so that a chlorotic condition of the blood is frequently seen. In cases in which the anæmia is pronounced, all the alterations in size and shape of the red corpuscles may occur as in any other form of severe anæmia. Nucleated red corpuscles may occasionally be seen when the oligocythaemia is a marked feature of the disease, but these are very few in number and nearly always of the normoblast type.

The *Leucocytes* are present either in normal numbers or sometimes below, more usually above, the number found in health. Von Limbeck states that the condition of the blood depends upon the nature of the lymphoid growths; thus he found in two cases of splenic anæmia (it is evident that he includes splenic anæmia under the term Hodgkin's disease) that a chlorotic condition of the blood was associated with a normal number of leucocytes, whilst cases in which the lymphatic glands are enlarged are almost always attended with an increase, if only a moderate one, of he white cells of the blood.

The presence of inflammation in the lymphatic glands usually results in an increase of the leucocytes.

Most observers are agreed that, generally speaking, the variety of the white cells present does not materially

differ from that seen in health, and any marked leucocytosis is owing to an increase in the multinucleated or neutrophile elements; occasionally, however, it is stated that the small uninucleated lymphocytes may predominate. Von Limbeck considers that the nature of the leucocytosis is variable, and states that if the multinucleated cells are increased this would be in favour of the inflammatory character of the lymphatic tumours, whilst increase of the small lymphocytes gives the condition an appearance similar to that found in sarcoma of the lymphatic glands.

Occasionally, as in some other forms of chronic anæmia, a few myelocytes may be seen, but these are always infrequent, and in most cases absent.

Acute Pseudo-leukaemia, analogous to acute leukaemia, has been described, in which the disease takes a very acute course.

These cases are attended with all the usual characters of the chronic form, but are usually associated with a marked haemorrhagic tendency and moderate pyrexia.

Lannois and Courmont, in their cases, found, in addition to enlargement of the lymphatic glands, certain organisms, viz., staphylococcus pyogenes aureus, and streptococcus pyogenes, in the blood and lymphatics.

Von Limbeck states that it is extremely doubtful whether this is a separate disease, as all the symptoms —oligocythaemia, reduction in the amount of haemoglobin, slight leucocytosis, haemorrhagic tendency, enlargement of the spleen and lymphatic-glands, occasionally jaundice, and usually slight fever, may be occasioned by various pathological conditions, especially acute sarcomatosis (Fagge) as well as chronic pyaemia.

Diagnosis of Hodgkin's Disease.

The blood shows very little alteration, and is at first practically normal, although later all the signs of anæmia may appear. Leucocytosis, if present, is usually due to an increase in the multinucleated elements.

The negative character of the blood is of the greatest assistance in distinguishing this disease from both forms of leukaemia, as was previously mentioned.

Splenic Anæmia.

Synonyms: Spleno-megalie primitive; Anæmia splenica (Banti); Pseudo-leukaemia splenica. We shall speak of this as Splenic Anæmia of adults (or children), in contradistinction to that described later as "Anæmia pseudo-leucaemia infantum," or the splenic anæmia of infants.

Attention has recently been drawn to this disease in England by West, and more particularly by Frederick Taylor at the British Medical Association in London in 1896.

Banti, an Italian observer, in 1882, and Bruhl, of Paris, in 1891, have both written a short account of the disease, and it is more especially from the article by Bruhl in *Archives générales de Médecine*, 1891, that I have gathered the following information.

Splenic anæmia is characterized by a marked increase in the size of the spleen, progressive anæmia, an absence of leucocytosis, and a similar absence of enlargement of the lymphatic glands. Such a definition excludes leucocythaemia, and the ordinary forms of Hodgkin's disease or pseudo-leukaemia.

No separate account of this disease is given by Limbeck and Stengel, who regard it as merely a variety of Hodgkin's disease, whilst Hayem and Cabot make no mention at all of its occurrence.

Some writers have divided Pseudo-leukaemia into a splenic and a lymphatic form, according as the spleen or the lymphatic glands are involved, and consider this disease belongs to the former group.

The *Symptoms* and *Signs* of the disease vary. Sometimes anæmia is the first symptom which causes the patient to seek medical advice, whilst at other times the presence of a splenic tumour, partly on account of its size, partly also from the pain resulting from an attack of perisplenitis, calls attention to the disease.

The symptoms of anæmia present no peculiarity. Pallor, progressive feebleness without emaciation, and palpitation and breathlessness on exertion are present, as in all other forms of anæmia.

At other times attacks of colic-like pain in the left hypochondrium, nausea, vomiting, and diarrhœa occur. These occur in paroxysms, and are generally followed by an interval of varying duration, in which the patient feels comparatively well.

The spleen on examination is always found enlarged, sometimes reaching as far as the iliac crests. It is uniformly increased in size, and its shape is unaltered. The liver is either normal or slightly enlarged, and in the latter case often associated with the presence of jaundice.

Haemorrhages are not frequent, but occasionally epistaxis may be an early and prominent symptom. The entire absence of any enlargement of the lymphatic glands is the point of distinction between splenic anæmia and Hodgkin's disease.

The Condition of the Blood.

The examination of the blood alone will not distinguish the disease from pseudo-leukaemia, as there is nothing pathognomonic of the condition to be found. On the other hand, it is impossible to differentiate between leucocythaemia and splenic anæmia without a blood examination.

The red corpuscles are nearly always diminished in number, frequently falling to two and a half million, and occasionally to one million per cmm. or even less.

Generally speaking, the anæmia corresponds to the second or third degree of anæmia according to Hayem's classification, which has been previously mentioned. Bruhl states that the corpuscles undergo little or no alteration in shape, and poikilocytes are absent. Banti, however, describes the red cells as being deformed, fusiform or oval. They are paler than the normal corpuscles, owing to the marked diminution in the amount of haemoglobin.

The size of the erythrocytes is generally diminished, and microcytes measuring 3 to 4 μ are not infrequently seen. Bruhl states that the red corpuscles are never increased in size in this disease.

The amount of haemoglobin undergoes a much greater diminution than the number of corpuscles, giving the blood a very pronounced chlorotic character. It often falls to 50 %, and may even reach only 13 %. The individual corpuscular value in haemoglobin—the colour index—is considerably below the normal, in this way resembling chlorosis, but in marked contrast to that found in pernicious anæmia, and Bruhl regards this pronounced oligochromaemia as very suggestive of the disease.

The *leucocytes* in the majority of cases are normal, and any marked increase points to some complication. In none of the cases described by Banti and Bruhl is a differential enumeration of the variety of leucocytes given. The former, however, mentions that the multinucleated elements were, as in health, the most numerous, and in one case found the eosinophile were increased, and a few leucocytes were seen containing granules which stained deeply with methylene-blue, these probably being basophile in nature.

Speaking generally, no marked alteration in the number or quality of the leucocytes has been found, and they may be regarded as practically normal.

The disease, in the majority of cases, progresses till a fatal termination occurs.

The duration of the illness varies from six months to four years, but more usually from half to two years. Some cases have a very rapid course, analogous to that sometimes seen in acute leukaemia and pseudo-leukaemia.

The following table shows the number of the red and white corpuscles, and the amount of haemoglobin in a few cases that have been recorded of this disease.

CASES OF SPLENIC ANÆMIA.

Name of Observer	Bruhl.	Banti.	Banti.	Banti.	Potain.	Taylor.
Age and Sex	Man, 54	Man, 18	Girl, 16	Woman, 72	Man, 50	Girl, 13
Red Corpuscle	2,851,350,	3,999,000,	3,948,000	3,720,000,	2,000,000	2,700,000
White Corpuscle	10,757	4704	6876	19,805	no leucocytosis	no leucocytosis
Haemoglobin	1,246,500, or 43 %	2,400,000, or 48 %	68 %	2,594,000, or 52 %	33 %	chlorotic diminution
Colour Index	0·43	0·632	0·86	0·697	0·825	diminished

In the case recorded by Dr. West, *British Medical Journal*, June 13, 1896, the patient was a man aged 36; the red corpuscles and haemoglobin were diminished, and the leucocytes slightly increased, but not more than

the fever would account for. Dr. Taylor described another case in a boy, aged 19, in which red corpuscles and haemoglobin were 55 % of the normal, but no leucocytosis at first.

The Diagnosis.

A careful examination of the blood, not merely the enumeration of the elements, is of the greatest value in distinguishing splenic anæmia from other pathological conditions associated with an enlargement of the spleen.

A correct diagnosis is of the utmost importance, as there is some hope that the disease may be cured by splenectomy. Whilst this operation has been almost invariably attended with a fatal result in leucocythaemia, at least three cases of splenic anæmia have been recorded in which a cure followed removal of the spleen.

The essential characteristics of the disease are the marked enlargement of the spleen, the absence of enlargement of the lymphatic glands, and the condition of the blood, viz., a diminution in the red corpuscles, associated with a greater diminution in the amount of haemoglobin, the absence of leucocytosis and abnormal white blood corpuscles.

From Leucocythaemia.

In both forms of leukaemia the leucocytes are usually, though not invariably, enormously increased. In the spleno-medullary type, marrow cells and eosinophile myelocytes are numerous and characteristic, whilst in the lymphatic form the small and large uninucleated elements predominate. I must again draw attention to the fact that an absence of a great increase in the leucocytes does not exclude leucocythaemia. In a case

previously mentioned of a girl of 11 with very enlarged spleen, I found the leucocytes only numbered 44,313, the red cells about $3\frac{1}{2}$ million per cmm., and without stained films leucocythaemia would have been excluded, and a diagnosis of splenic anæmia given.

From Hodgkin's Disease, or Pseudo-leukaemia.

The condition of the blood will not distinguish these two diseases. The presence of enlarged lymphatic glands without, or with only slight enlargement of the spleen, points to Hodgkin's disease.

From other *conditions* in which *an enlarged spleen* is present, the fact that the spleen in splenic anæmia is always considerably enlarged uniformly, without losing its form and direction, and that its surface presents no irregularities, enables us to exclude *hydatid cysts* and *cancer* of the spleen.

Waxy disease of the spleen, which might somewhat resemble it, is distinguished by a history of suppuration or syphilis, and by similar disease in other organs, especially the intestine and kidney, giving rise in the one instance to diarrhœa, and in the other albuminuria.

Malarial enlargement of the spleen is recognized by the history of residence abroad, or of previous attacks of ague.

Before considering the various forms of secondary anæmia which occur in different pathological conditions, it will be well to glance at the following table which I have compiled. It will show the main features found in the blood of the most important forms of anæmia, and includes secondary anæmia, considered generally, in order to accentuate the differences which exist between it and all forms of primary or idiopathic anæmia.

DIFFERENTIAL CHART OF THE MOST

	1.—PRIMARY		
Red Blood Corpuscles—	**Chlorosis.**	**Simple Primary Anæmia.**	**Pernicious Anæmia.**
NUMBER . .	Usually diminished, rarely below 2 million.	Always diminished, but extent variable.	Generally enormously diminished.
SIZE and COLOUR	Diminished in size, microcytes frequent. Paler in colour.	Diminished in size in slight, but may be larger in severe cases. Colour variable.	Increased in size. Megalocytes frequent. Colour usually not diminished.
SHAPE . .	Poikilocytosis seldom excessive.	Poikilocytosis pronounced in severe cases.	Poikilocytosis always extremely pronounced.
Haemoglobin .	Relatively greater diminution than number of corpuscles.	Diminished proportionate to number of corpuscles.	Generally relatively high, in excess of the corpuscles.
Colour Index .	Always low.	Variable, generally about normal.	Frequently high.
Normoblasts .	Present in severe cases, generally in small numbers.	Present in severe cases.	Almost always present.
Megaloblasts .	Absent or extremely rare.	Only found in severe cases, and not as numerous as the Normoblasts.	Nearly always present and more numerous than the Normoblasts.
Leucocytes— Number . .	Generally normal.	Generally normal, sometimes diminished.	Usually diminished.
LYMPHOCYTES .	Sometimes increased relatively.	Sometimes increased relatively.	Usually relatively increased.
MULTINUCLEATED or Neutrophile .	Usually normal, sometimes diminished.	Sometimes increased, sometimes diminished.	Usually diminished.
MYELOCYTES or Marrow Cells .	Absent or extremely rare.	Absent or extremely rare.	A very small percentage frequently present.

IMPORTANT FORMS OF ANÆMIA.

ANÆMIA.			2.—SECONDARY ANÆMIA.
Spleno-medullary Leukæmia.	**Lymphatic Leukæmia.**	**Hodgkin's Disease and Splenic Anæmia.**	
Moderately diminished.	Diminished to about half the normal, lower than in spleno-med. leukaemia.	Slight diminution except in severe cases.	Diminution constant, but variable in amount.
Size variable, small in slight, large in severe cases, colour usually diminished.	Size and colour variable, generally both diminished.	Generally smaller and paler in colour.	Usually not increased in size. Colour generally diminished.
Poikilocytosis slight in mild, marked in severe cases.	Poikilocytosis present but variable.	Poikilocytosis seldom excessive.	Poikilocytosis variable, but pronounced in severe cases.
Diminished proportionately to, or greater than corpuscles.	Diminished proportionately to or greater than corpuscles.	Diminution is greater than number of corpuscles.	Diminished, usually greater than corpuscles.
Generally normal, sometimes lower.	Not usually above the normal.	Nearly always less.	Usually below the normal.
More numerous in all degrees than in any other disease.	Extremely rare, usually absent.	Absent, except when anæmia is very severe.	Commonly present when anæmia is pronounced.
May occasionally be found, but not numerous.	Absent.	Absent.	Rare except in severe cases, and then not as numerous as Normoblasts.
Enormously increased.	Greatly increased, but not to the extent found in sp.-med. leukaemia.	Normal or slightly increased.	Usually increased.
Relatively diminished.	Enormously increased, sometimes the small, sometimes the large forms.	Generally normal, but may be increased.	Usually diminished.
Relatively diminished.	Considerably diminished.	Generally normal, may be increased.	Usually increased.
Very numerous and characteristic, often 50%.	In most cases absent, if present extremely few.	Almost always absent.	Absent or extremely rare.

Secondary or Symptomatic Anæmia.

This includes all forms of anæmia, the result of some organic change, situated outside the haemopoietic system, in which the condition of the blood is merely symptomatic or secondary, and is not the essential characteristic feature of the disease.

It embraces anæmias resulting from organic disease, acute or chronic infectious diseases, and those due to parasites and haemorrhages.

Condition of the Blood in Secondary Anæmias Generally.

The blood usually shows a greater reduction in the amount of haemoglobin than in the number of the corpuscles, *i.e.*, oligochromaemia is in excess of oligocythaemia, or, in other words, a chlorotic type.

Many earlier writers maintain that the haemoglobin and red cells undergo an equal and proportionate decrease, but although this may be true in some cases, it is certainly the exception rather than the general rule.

In cases of moderate severity the number of corpuscles is frequently normal, while the haemoglobin, as estimated by von Fleischl's haemometer, varies from 60 to 75 %.

The corpuscles in the slight forms simply appear somewhat paler than in the normal condition, and in cases of moderate anæmia, although their number may not be diminished, yet alterations in their size and shape often occur.

In severe cases the red corpuscles undergo a diminution, which may in some forms, particularly those in which actual destruction of the coloured elements occurs, such as malaria, haemorrhages, etc., be considerable. The haemoglobin may be very greatly diminished, sometimes falling to 40 or even 20%.

The disproportion between the haemoglobin and corpuscles is in some cases not conspicuous, whilst in others, especially in cases of tuberculosis, syphilis, carcinoma, etc., it may be extremely well marked, and as such conditions resemble chlorosis, they have received the term of "chloro-anæmia."

Alterations in size and form of the red corpuscles in some of the moderate as well as in the severe degrees of anæmia may be very noticeable. The small red cells—microcytes—are frequently met with, as well as the large corpuscles, megalocytes, but the former are usually much more numerous than the latter. According to Hayem the severer the anæmia the more numerous are large corpuscles, whilst the prevalence of small elements usually indicates a milder condition.

Poikilocytosis and other evidences of the necrobiotic changes described by Maragliano, viz., crenation, vacuolation, and the peculiar staining reaction which we have so frequently alluded to under the name of "polychromatophilia," may be met with in this as in any other form of severe anæmia. (See Plate I.)

In cases of very severe symptomatic anæmia, in addition to the signs of degeneration just described, evidence of regeneration, the presence of nucleated red corpuscles, may be seen. These are usually nor-

moblasts, *i.e.*, nucleated cells of the size or slightly larger than the normal red corpuscle, but never exceeding 10 μ in diameter, with a small nucleus usually somewhat eccentrically placed, generally less than one half the size of the corpuscles, which always stains very deeply with haematoxylin and other nuclear stains. It sometimes shows signs of division, and not infrequently a two, three, or four lobed figure may be found. (Plate I., Fig. 7.)

The outline of these cells is often irregular or crenated. In cases which have developed rapidly, especially those resulting from haemorrhages, normoblasts may be moderately numerous. Stengel remarks, and other writers agree, that in ordinary degrees of anæmia occurring in young children under five years of age, they are rarely absent.

Large nucleated erythrocytes—megaloblasts or gigantoblasts (Plate I., Fig. 8)—with faintly stained nucleus are occasionally seen, but only in the severest forms, and even then in very small numbers, and are never as numerous as the normoblasts, except in the very severe anæmia which occasionally results from intestinal parasites. Askanazy even found them in larger numbers than the latter in the blood of a patient suffering from bothriocephalus latus.

It is stated by Stengel that Kraus and Herz have noticed the occurrence of swelling of the red corpuscles, probably a result of alterations in the density of the blood serum. "The former observer found acute swelling of the corpuscles in typhoid fever and peritonitis, and after haemoptysis; among the causes of chronic swelling of the corpuscles are various forms of cachectic anæmia."

The Leucocytes.

The number of white corpuscles found in secondary anæmias varies considerably, depending on the nature of the primary pathological cause. Usually they are somewhat increased, often normal, and very rarely below the number found in health. The leucocytosis usually consists of an increase in the neutrophile or multinucleated variety. In some chronic cases of long duration there may be a diminution in the white cells, and it is in these cases that the uninucleated elements may be relatively increased.

In inflammatory conditions, particularly those attended with the formation of pus or fibrino-purulent exudation, the leucocytosis may be very pronounced, whilst in non-inflammatory conditions it may be only moderate, but is sometimes considerable. According to Hayem, a moderate increase to from 10,000 to 20,000 per cmm. is somewhat suggestive of cancer.

The number of blood plates is also variable, but an increase rather than a decrease is more generally seen.

Diagnosis.

In order to distinguish secondary from primary forms of anæmia, especially chlorosis and pernicious anæmia, it is necessary to take into account the clinical history of the illness, as well as a thorough physical examination of the patient.

Typical cases of *chlorosis* will be recognized by the age, sex, and menstrual disorders, and the absence of any organic cause sufficient to produce the condition.

The examination of the blood,—the almost constant occurrence of oligochromaemia in excess of oligocythaemia, which is more marked and more constant in this than in secondary anæmia, the occasional presence of nucleated red corpuscles in chlorosis, as compared with their more common appearance in symptomatic anæmia, and the absence of leucocytosis,—generally renders the diagnosis certain.

Pernicious anæmia may occasionally present some clinical features resembling secondary anæmia, but the condition of the blood usually enables us to differentiate between them. Rarely does the number of corpuscles suffer so great a reduction in the latter as in the former, and the amount of haemoglobin in pernicious anæmia, though variable, seldom shows a relative reduction, more frequently a proportionate increase.

Nucleated red cells may be present in both forms of anæmia, but in pernicious anæmia the megaloblasts are more numerous than the normoblasts, the reverse of this being the case in symptomatic anæmia.

The average size of the red corpuscles in pernicious anæmia is greater than the normal, whilst in secondary anæmia it is more often smaller than larger.

In all cases of severe secondary anæmia, in which the red blood corpuscles are less than 2 million per cmm., leucocytosis occurs (Cabot), and the multinucleated forms are most numerous.

In pernicious anæmia the leucocytes are usually diminished, and the relative number of the multinucleated elements decreased, whereas the lymphocytes are proportionately increased.

Secondary Anæmia due to Malignant Disease.

The condition of the blood in anæmia resulting from, and symptomatic of, malignant new growths, depends largely on the nature and position of the neoplasm, as well as the occurrence or absence of haemorrhage.

The Blood in Carcinoma.

The red corpuscles in the earlier stages usually undergo no diminution, and may even be somewhat increased in number, due probably to concentration of the blood. In the later stages they may fall very considerably, and von Limbeck records a case of a man with carcinoma of the pylorus, in which the red cells numbered only $1\frac{1}{2}$ million at the first examination, but fell in four and a half months to 950,000 per cmm. Cancer of the stomach is usually attended with the highest degree of anæmia.

Even in the earlier stages, before any noticeable oligocythaemia is present, the corpuscles may show alterations in size and shape, whilst in extreme cancerous cachexia they undergo, according to Hayem, more marked alterations in shape than in any other form of severe anæmia, and this is particularly true of carcinoma of the stomach, in which the nutrition of the body is so much impaired.

The haemoglobin is usually somewhat lower than the number of corpuscles would lead us to suppose, and the individual corpuscular value (colour index), is very rarely over 1. Bierfreund has found that after operation it naturally falls from the loss of blood, but never regains the percentage that it was before the operation.

The corpuscles are usually somewhat smaller than the normal, and although megalocytes are present as well as

microcytes, yet the former are seldom as large as in pernicious anæmia.

Poikilocytosis may be extremely well marked, and nucleated red corpuscles, particularly the normoblasts, common in advanced cases. Megaloblasts may be present, but, according to Cabot, are always fewer in number than the normoblasts, and this fact is important as distinguishing cancerous from pernicious anæmia.

The Leucocytes.

The number of white corpuscles found in cases of carcinoma varies considerably. In a few instances, as for example cancer of the œsophagus, causing stricture, and therefore starvation, they may be reduced in number, whilst much more frequently carcinoma, especially when large and of rapid growth, or when attended with repeated haemorrhages, *e.g.*, carcinoma of the uterus and stomach, gives rise to leucocytosis.

The following examples given by Hayem will illustrate this :—

i. Scirrhus of the Breast.—14 cases showed an average of 11,400 leucocytes—the highest being 21,700, the lowest 2360 per cmm. In ten of these the leucocytes were above 10,000.

ii. Encephaloid of Breast.—3 cases ; average leucocytosis, 11,300.

After operation the number of leucocytes decreased—

SCIRRHUS OF BREAST.

BEFORE OPERATION.	AFTER OPERATION.
Case 1. Leucocytes 21,700	6200 rose to 8990 (recurrence)
„ 2. „ 11,500—11,550	8500 to 6200
„ 3. „ 11,000—12,400	8400

ENCEPHALOID CANCER OF BREAST.

Leucocytes . 10,000	9000

Hayem thinks it is possible to diagnose recurrence by an increase in the number of leucocytes.

iii. In cancer of the stomach Hayem found in seven cases no leucocytosis, there was an average number of 7,600; in five other cases, leucocytosis was present, the average of the white cells being 17,600 per cmm.

Other observers have found somewhat similar results, and increase of leucocytes probably only occurs in some, not all, cancers of this organ.

The leucocytosis which occurs three or four hours after a meal in the normal condition, may be absent in cases of gastric cancer, and this has been urged as an aid to the diagnosis of cancer, as distinct from ulcer of the stomach, which is usually attended with "digestive" leucocytosis.

Cancer of the kidney is said to be frequently associated with a considerable leucocytosis, whilst generally speaking in carcinoma of other organs it may be either present or absent, but is seldom very pronounced.

The increase of leucocytes is almost entirely on the part of the multinucleated or neutrophile cells, whilst the eosinophile cells are sometimes diminished, seldom increased, in number.

Marrow cells, or myelocytes, may occasionally be seen in very small numbers.

The Blood in Sarcoma.

Sarcomata are usually attended with greater anæmia than that found in carcinomata, and the leucocytes are generally rather more numerous.

Von Limbeck found that in eighteen cases of osteo-sarcoma recorded by numerous observers, the average

number was 16,554, the highest being 52,700, the lowest 6000.

In nine cases of lympho-sarcoma, the average number of leucocytes was 20,894, the highest being 55,100, the lowest 10,540 per cmm.

In three cases of melanotic sarcoma quoted by von Limbeck, the leucocytes, in all cases being over 22,000, averaged 30,800. The prevailing type of leucocyte present is usually the multinucleated, but occasionally the uninucleated elements are relatively numerous.

Rieder maintains that in the leucocytosis of sarcoma the normal proportion of the different forms of white cells is maintained.

The eosinophile cells may be increased in number, particularly if the medulla of the bones becomes affected. Myelocytes may be present in small numbers.

The Diagnosis.

The examination of the blood may, in both forms of malignant disease, give some help in diagnosis, and Hayem makes the following suggestive remark: "Every tumour which, independent of inflammatory or suppurative complication, is associated with an increase in the number of the white cells is a cancerous growth. Yet the number of leucocytes having been found normal in a case of (non-ulcerating) tumour of the breast, the absence of leucocytosis does not entitle us to exclude the diagnosis of cancer of the breast."

The secondary anæmia resulting from malignant disease may in some cases be so extreme as to resemble pernicious anæmia, but the examination of the blood, especially the occurrence of leucocytosis involving the multinucleated cells, the relative diminution in

the haemoglobin, and the small number of megaloblasts as compared with normoblasts, will generally enable a diagnosis to be made even when the neoplasm is not otherwise recognizable.

Anæmia Resulting from Haemorrhage, or Post-Haemorrhagic Anæmia.

Haemorrhage may cause very slight or extreme anæmia according to the amount of blood lost. The haemorrhage which occurs from large wounds, or that arising from the lungs, uterus, or cancer, may produce intense anæmia or actual death. Slight but long-continued haemorrhage, such as that from haemorrhoids, etc., may also occasion an anæmia which, though slow in developing, may reach an extreme limit.

The Blood.

The condition of the blood depends largely on the nature of the haemorrhage. The first effect will be oligaemia, *i.e.*, a loss in the total amount of the blood, and in such cases an examination may show no signs of oligocythaemia, as the number of corpuscles found will only suffer the same diminution as the amount of the serum, but when recovery takes place the blood serum will increase by absorption of fluids from the tissues, and the corpuscular elements will then appear much reduced, so that oligocythaemia is not pronounced until a few hours after haemorrhage.

The red blood corpuscles may fall after severe haemorrhage to 3 or even 2 million per cmm., and according to Osler, even after very severe loss of blood, their number is not reduced as greatly as in forms of

idiopathic anæmia. Hayem, however, describes a case of a woman, 21 years of age, in which haemorrhage occurred after confinement on August 17th, and recurred from time to time till August 25th, when a piece of placenta was expelled. On August 26th, fifteen hours after the last haemorrhage, the corpuscles numbered only 550,000 per cmm., and yet the woman recovered. He considers that the red corpuscles may fall to half a million without a fatal termination, but regards repeated haemorrhages which keep the corpuscles at about one million for some days as dangerous.

The haemoglobin may show a reduction corresponding to that of the corpuscles, but usually, as in other forms of secondary anæmia, the oligochromaemia is somewhat more pronounced than the oligocythaemia.

The corpuscles are usually slightly less than the normal size, whilst in severe cases a few megalocytes, measuring up to $10\frac{1}{2}$ μ in diameter, may be seen, as well as small red cells as small as 3 μ.

Poikilocytes may be numerous, and in severe cases show extreme variation in size and shape. According to Muir, the number of poikilocytes depends on the duration and intensity, and is not proportional to the intensity of the anæmia alone.

Various signs of degeneration of the cells may be seen in this as in any other symptomatic anæmia.

Nucleated red corpuscles appear more or less frequently in severe post-haemorrhagic anæmia. They are in the majority of cases of the normoblast type.

Occasionally free nuclei, distinguishable from lymphocytes by their much smaller size, and by the absence or merely irregular shreds of surrounding protoplasm, are

found. (Plate I., Fig. 7.) It is rather interesting to contrast this, the generally accepted fact regarding the occurrence of nucleated red cells, with Hayem's statement, that "in the majority of cases the loss of blood is repaired by haematoblastic formation (*i.e.*, blood plates), without it being possible to see a single nucleated red corpuscle in the blood." His theory, that the red corpuscles are formed from the blood plates, is not entertained by the greater number of observers, who maintain, as we have previously mentioned, that the non-nucleated normal corpuscles are formed from the nucleated red cells of the bone marrow, and the latter may in pathological conditions like this, get into the general circulation without losing their nucleus.

The *Leucocytes* are usually increased in number—post-haemorrhagic leucocytosis. A severe haemorrhage occurring in a healthy individual, is followed usually in a short time (ten to fifteen minutes according to von Limbeck), by a moderate increase in the number of white cells. The multinucleated are usually increased, whilst the uninucleated elements diminished in number. The blood plates are frequently very numerous, especially in cases due to repeated haemorrhages.

The Blood in Haemorrhagic Diseases.

Purpura.

The condition of the blood in all varieties of purpura —P. simplex, P. haemorrhagica, and P. rheumatica—shows little alteration, except that of anæmia, the result of haemorrhage.

In some cases, especially in purpura simplex, the ex-

amination reveals a practically normal condition of the cellular elements of the blood, whilst in others, particularly after large haemorrhages, the red corpuscles may undergo considerable diminution. In ordinary cases their number varies from $2\frac{1}{2}$ to 4 million, but may fall after extensive haemorrhage to 1 million per cmm.

The haemoglobin undergoes a reduction proportionate to, but more usually in excess of, the red cells.

The corpuscles may show the signs of degeneration, which Maragliano has pointed out, and polychromatophilia as well as those of regeneration, viz., the appearance of nucleated red cells—normoblasts.

The leucocytes are generally slightly increased in number, as in all other forms of post-haemorrhagic anæmia.

This increase usually involves the multinucleated elements, but occasionally lymphocytes may prevail.

Hayem noticed the occurrence of "leucocytes hypertrophiés," a term with which he usually describes the marrow cells, but it is somewhat doubtful from his description whether these are simply unusually large leucocytes or myelocytes proper.

The blood plates are sometimes considerably reduced in number, and Hayem also mentions the presence of "plaques phlegmasiques," *i.e.*, masses of granular material, apparently formed around, possibly from the blood plates.

From this short description it is apparent that there are no characteristic alterations in the blood, which in some cases appears normal, and in others merely shows such alterations as occur in any post-haemorrhagic anæmia.

Scorbutus, or Scurvy.

In this, as in the above disease, the blood presents no characteristic features.

The red corpuscles are usually only slightly diminished in number, varying from 3 to $4\frac{1}{2}$ million.

Hayem describes a case in which they averaged about $4\frac{3}{4}$ million, but in severe cases they may fall to 2 or even 1 million, and Bouchut has recorded a case in which, after severe haemorrhage, they were less than 600,000 per cmm.

Stephen Mackenzie, in the "Lettsomian Lectures" (*Brit. Med. Journal*, 1891), mentions an acute case of "scorbutic anæmia" ending fatally after an illness of two months, in which the corpuscles sank to 13%, or 650,000 per cmm. He remarks that the erythrocytes varied much in size, and in some, cracks and fissures were seen.

Usually, when the anæmia is pronounced, poikilocytes and polychromatophilia are seen, and in cases in which extensive haemorrhage has occurred, alterations in size and shape, as well as the presence of numerous nucleated red cells, may be very conspicuous.

The haemoglobin undergoes a somewhat greater diminution than the red cells, giving a chlorotic type to the anæmia.

The leucocytes may be normal, but are usually somewhat increased in number, especially when considerable haemorrhage has taken place.

The blood plates are said to be frequently more numerous than in the normal condition.

In Hayem's case they varied from 252,600 to 716,800 per cmm.

Haemophilia.

The examination of the blood reveals nothing characteristic, its general appearance closely resembling that of purpura or scorbutus.

The corpuscles and haemoglobin are reduced, the leucocytes increased as a result, and vary in degree according to the amount of blood lost.

Stengel states that loss of blood is more readily borne by haemophilics than by persons in health.

Hayem considers that the alteration in the blood consists in a remarkable delay in coagulation, and this is independent of any anatomical alterations in the elements. Other observers, Grandidier, Schmidt, etc., confirm this statement regarding the decreased coagulability.

Haemoglobinaemia, Haemocytolysis, or Blood Destruction.

Under certain conditions the haemoglobin may separate from the red corpuscles, leaving these pale, ("shadow corpuscles") or in irregular fragments.

The liberation of a large quantity of haemoglobin, besides colouring the serum, causes an increase in the quantity of the bile, and so gives rise to haematogenous jaundice. Part of the haemoglobin is carried to the liver, spleen, and other organs, whilst a large quantity is excreted with the urine, constituting what is known as haemoglobinuria, or methaemoglobinuria.

Disintegration of the leucocytes is frequently associated with the destruction of the red cells, and excess of xanthin bases, or of uric acid in the urine, is, according to Stengel, a reliable sign of leucolysis.

The blood in haemoglobinaemia may show a marked diminution in the number of erythrocytes, and many of

these are pale, constituting what we have previously described as "shadow corpuscles." (Plate I., Fig. 6.) Fragments of corpuscles are frequently seen, and Ehrlich states that in some cells the haemoglobin has separated from the general stroma, and appears as rounded granules within the corpuscles.

The haemoglobin rapidly decreases, especially in the severer cases.

The leucocytes may be decreased; sometimes, however, slight leucocytosis is present, and not infrequently they are normal.

The conditions which cause haemoglobinaemia are somewhat varied, and three forms may be clinically recognized, viz., toxic, symptomatic, and paroxysmal haemoglobinaemia.

The red blood corpuscles may be destroyed, and the haemoglobin liberated, and amongst such causes may be mentioned poison of snakes, mushroom poison, arseniuretted hydrogen, burns, malaria, and the haemoglobinuria of the new-born.

Other conditions, especially certain poisons, *e.g.*, chlorate of potash, pyrogallic acid, anilin, antipyrin, antifebrin, etc., act chemically, transforming the haemoglobin into methaemoglobin, which is usually eliminated in the urine.

Carbonic oxide and nitrous oxide gases form definite compounds with the haemoglobin, as well as causing the destruction of the corpuscles.

Most of these conditions may be recognized by the occurrence of dyspnœa, cyanosis, and the presence of haemoglobin or methaemoglobin in the urine, which can be most easily determined by spectroscopic examination.

The presence of free haemoglobin in the blood may be detected, according to von Jaksch, by allowing a little of the blood to remain for twenty-four hours in a refrigerator, when if the blood be normal the serum which separates will be of a clear yellow colour; if haemoglobinaemia be present the serum is a ruby red colour.

Another method recommended by the same observer is as follows: Blood serum is coagulated by heating to 70–80 °C., and if haemoglobin be present it is of a more or less brown colour, normal serum being of a yellow colour and of milky turbidity.

Paroxysmal Haemoglobinuria.

In this form of disease haemoglobin or methaemoglobin is discharged paroxysmally in the urine.

The blood, according to Hayem, coagulates rapidly, but the coagulum dissolves again in about three hours. If examined in the fresh condition the blood at first appears normal, but, according to Hayem, in a few minutes the corpuscles are seen to gradually lose their colouring matter, becoming chlorocytes, *i.e.*, pale corpuscles, and finally achromacytes, *i.e.*, colourless or shadow corpuscles. This is only seen in a few of the erythrocytes, even after the preparation has been made some hours.

The blood plasma is of a reddish colour, due to the contained haemoglobin. Very pale or shadow corpuscles are frequently seen, whilst fragmented forms are much less common.

The haemoglobin, especially during the paroxysm, is considerably reduced. The leucocytes are apparently normal.

The condition is distinguished from haematuria by the absence of blood corpuscles in the urine, and from malaria by the examination of the blood.

The Malarial Parasites.

The importance of the examination of the blood, in cases presenting symptoms resembling malarial fever, cannot be over-estimated.

The presence of the organism bears to malaria the same position that the tubercle bacillus does to tuberculosis, *i.e.*, it is absolutely characteristic.

Much has been written concerning the best method suited to their recognition, and notwithstanding the full account of the technique of the blood examination previously described, I shall, even at the expense of repetition, describe what seems to me to meet the requirements of physicians, viz., a process ensuring rapidity, certainty, and the one which introduces the fewest possible errors.

Method of Examining the Blood.

The blood may either be examined in the fresh condition, in dry unstained films, or in stained films.

(*a*) *In the Fresh State.*—The lobe of the ear or tip of the finger should be washed with soap and water, then with alcohol, and thoroughly dried. The part is then pricked with a needle, and the first droplet of blood wiped away, and the subsequent drop received on a perfectly clean coverglass or slide. This is immediately covered and the blood allowed to spread itself out between the two glass surfaces.

The size of the blood-drop should be small and pressure avoided.

Such preparations may be rung round with a layer of vaseline or wax, to retard evaporation, and they will keep fresh in this condition for some hours. The layer of blood must be thin, so that the corpuscles are seen on the flat and not on their edge, as is the case when rouleaux have formed.

Many observers state that this is the easiest and best method of examination, as the amoeboid movement of the parasite may be watched. It however presents some disadvantages, viz., that such preparations necessitate almost immediate examination—or at least within three or four hours—and are neither portable nor permanent.

(β) *In Dried Unstained Films.*—Films of blood are spread on cover-glasses, or much better on slides as I have previously described, and fixed by heat, and examined dry, *i.e.*, with a layer of Canada balsam round the margins of the cover-glass so as to make the preparation permanent.

Mounting in Canada balsam renders the film too transparent, and the structure of the corpuscles is not easily discernible.

(γ) *Examination of Dried Stained Films.*—The dry films may be first fixed by heat, or more easily by immersion in a mixture of equal parts of alcohol and ether for a short time—five minutes is usually sufficient—and then stained.

Methylene blue alone is probably the easiest of all the single stains. Aqueous or Loeffler's solution of the dye may be used.

Manson and Malachowski recommend solutions of borax—methylene blue, the former gives the following formula :—

Borax . . .	5
Methylene blue .	0·5
Water to . .	100

Stain for half a minute.

Rotch mentions that the variety of methylene blue is of some importance, as some do not stain satisfactorily. He recommends Grubler's methylene blue, soluble in alcohol, and uses a few drops of a saturated alcoholic solution added to one ounce of water. After staining, the films are thoroughly washed in water, dried in the air, not with heat, as this tends to decolourize them, cleared with xylol, and mounted in Canada balsam.

Methylene blue alone is a rapid—20 to 40 seconds are usually sufficient time—and easily managed stain. It colours the red corpuscles greenish blue, the nuclei of the leucocytes a deep blue, and the parasites a pale blue.

By its means the organism is, to my mind, not sufficiently differentiated from the general body of the corpuscles in which it is situated.*

Double staining with *methylene blue* and *eosine* is very universally recommended, either separately with the eosine first, and then with the methylene blue, or with a mixture of these dyes.

Of the latter, Plehn's formula given previously is frequently used, but I must admit that I can seldom manage to get a good result with any of the mixtures of these two colours. Films may be stained separately, first in 0·5 % of eosine in 50 % alcohol for about half a minute, washed and then stained in methylene blue

* Thionin-blue—1 % solution in 1 in 40 carbolic acid solution—is an excellent stain for malarial films.

(aqueous or Loeffler's solution) for 15 seconds, washed quickly—prolonged washing dissolves the blue—dried, and mounted in Canada balsam.

As previously mentioned under the method of blood examination, I cannot recommend eosine and methylene blue for general use. Sometimes I can obtain results which are quite satisfactory, but only after repeated examination under the microscope whilst wet. To me the disadvantages are threefold: the preparations invariably under or over-stain; the great tendency on the part of the stain to form granules, notwithstanding careful filtration; and the inequality of the intensity of the stain in different parts of the film.

Double staining with *eosine* and *haematoxylin* has given me excellent, reliable, and clean preparations for general blood examination, and it answers admirably for malarial films.

The process which I always adopt for the examination of the blood in any pathological condition has been previously described, and I will only briefly mention the stages of the process:—

i. Films of blood are spread on slides and allowed to dry in the air.

ii. When dry, are fixed by immersion in a mixture of equal parts of alcohol and ether for three to five minutes.

iii. They are then stained in eosine (0·5 % solution in 50 % alcohol) for half a minute—time not at all important.

iv. Washed in water and whilst wet,

v. Stained in haematoxylin for half a minute, exact time not essential; it is however generally advisable to stain malarial films rather longer than ordinary blood preparations, say for about 10–15 minutes. Bohmer's or Delafield's matured solution should be used after careful filtration.

vi. Washed thoroughly with *filtered* water (not necessarily distilled), dried in the air or over a spirit lamp, cleared with xylol or cedar oil, mounted in Canada balsam.

Advantages of this method :—

1. The absolute reliability and equality of the intensity of the stain over all parts of the film.

2. The red blood corpuscles are clearly and brightly stained with the eosine, and never appear bluish-red, as is frequently the case with methylene blue and eosine.

3. The films withstand prolonged washing without losing their colour.

4. The entire absence of any granules of stain or dirt, except such as may result from the filtered water used in washing.

5. The time required for staining need not be very exactly determined.

6. Lastly, and to my mind the greatest advantage lies in the fact that this method is equally excellent for all pathological conditions, and by its means malaria may be discovered when it has not been suspected ; for example, in cases in which an enlarged spleen suggests the presence of leucocythaemia, other methods than a general one might prevent the diagnosis.

Time at which the Examination of the Blood should be made.

Malarial organisms are present in the blood in one stage or other of the disease during the attacks of fever, as well as during the apyrexial period, but they vary in number and size, as can be readily understood from the description given below. They are most numerous during pyrexia, but are then for the most part small and not easily recognized by one unaccustomed to this par-

ticular form of microscopic research, whilst at the end of apyrexia they are fewer in number, but are larger in size and pigmented.

Laveran advises that the blood should be examined before or at the beginning of an attack, and Mannaberg states that just before the paroxysm is a particularly favourable time.

An interesting discussion in reference to this question occurs in the *British Medical Journal*, October 19, 1895, where it is pointed out that the best time for the beginner to find the parasite is about the end of the apyrexia and the beginning of the fever paroxysm, when the parasites, though fewer in number, are pigmented and much larger in size.

The Parasites of Malarial Fever.

In the following description of the malarial parasite, I have largely followed the account given by Mannaberg, and to a less extent that by Marchiafava and Bignami (*New Sydenham Society*, 1894), and by Laveran (*New Sydenham Society*, 1893).

Malarial fevers may be conveniently classified in the following manner :—

A.—Simple Intermittent Fevers.

Malarial Parasites with spore formation without "crescents."	i. Tertian Parasite. ii. Quartan Parasite.

B.—Irregular Malarial Fevers.

Aestivo-autumnal. Malarial Parasites with spore formations, with "crescents." Includes Irregular, Intermittent, Continuous, Remittent, and Pernicious or Malignant Fevers.	1. Pigmented Quotidian Parasite. 2. Unpigmented Quotidian Parasite. 3. Malignant Tertian Parasite.

A.—The Parasites of Simple Intermittent Fever.

i. *The Tertian Parasite.* (Plate VI., Fig. 2, B.)—The duration of the development of this parasite covers 48 hours.

(*α*) If the blood of a patient be examined within one to twelve hours after the attack, small clear specks, varying in size, are seen in the red blood corpuscles.

At this, the earliest stage in the life-history of the organism, the pigment is almost invariably absent, though occasionally very fine granules may be seen, and it is therefore known as the "*Hyaline form.*"

These increase in size, and undergo active amoeboid movements, which can be followed at the temperature of the room for about an hour after the preparation of the blood has been made.

In dry stained films these bodies are coloured pale blue with methylene blue and haematoxylin.

(*β*) In twelve to twenty-four hours after the chill, the hyaline bodies have increased in size, filling about $\frac{1}{3}$ of the corpuscle. They still possess amoeboid movement, and at this stage fine pigment granules are seen, and the red blood corpuscles, in which they are present, have become paler and larger.

(*γ*) In twenty-four to thirty-six hours after the attack it is noticed that the parasites have become larger, occupying $\frac{2}{3}$ to $\frac{4}{5}$ of the corpuscle, their amoeboid movements have diminished, but active movements on the part of the granules are conspicuous. In stained preparations the irregular shape of the organism, the presence of pigment granules, and the pale swollen corpuscles in which they are contained, will be seen in this stage.

(δ) In thirty-six to forty-eight hours after the last attack, and within twelve hours of the next, the parasite occupies the whole of the corpuscle, which remains merely as a shell of almost colourless protoplasm, and the pigment has ceased its movements in parts, and gradually merges towards the centre of the body, where it finally collects in a compact mass.

At this stage segmentation commences, the organism dividing into a number of small bodies, or in other words into 15–20 round spores, resembling a rosette or mulberry. At first the segmenting figure is surrounded by a capsule, the remains of the corpuscle. Preparations stained with eosine and haematoxylin show this spore formation very clearly. (Plate VI., Fig. 2, B.) The shell surrounding the spheres bursts, and the liberated spores separate, some to impregnate other corpuscles, where they begin a new life-history, and pass through the stages which we have described to complete segmentation, whilst the pigment granules, which previously occupied the centre of the segmenting body, are taken up by the leucocytes.

Cabot states that the young plasmodia are rarely, if ever, seen in the peripheral circulation free in the plasma or in the act of entering fresh corpuscles, and mentions that Thayer, who is one of the most recent authorities on the subject of the malarial parasites, has never seen them in this stage.

It must be remembered that the stage of segmentation, or spore formation, corresponds to the febrile paroxysm, so that when these are present in prevailing numbers we can predict the occurrence of another attack.

Every hyaline body does not go on to segmentation;

only a fractional number of parasites, according to Mannaberg, reach this stage, and quinine retards or prevents this, giving rise to imperfect or "still-born" forms.

Double tertian fever is really a quotidian fever, which completes its cycle of development in twenty-four hours.

ii. *The Quartan Parasite.* (Plate VI., Fig. 2, A.)—These closely resemble the tertian, but in addition to the time required for their development (seventy-two hours) possess characteristics which render them distinguishable from the former.

(*α*) At first, twelve hours after an attack, they consist of unpigmented hyaline bodies, situated in the red corpuscles, with slight, frequently without any, amoeboid movement.

(*β*) About twenty-four hours after the attack they are about $\frac{1}{6}$ to $\frac{1}{5}$ the size of a red blood corpuscle, some with a few peripherally placed granules, others without any pigment.

(*γ*) At forty-eight hours they are deeply pigmented, and occupy about $\frac{1}{2}$ to $\frac{2}{3}$ of the red cell. The amoeba is usually round, and it, like the pigment, shows no sign of movement.

(*δ*) At the stage of sixty hours the parasite fills the whole corpuscle, which, unlike the tertian, is neither enlarged nor pale, but usually presents in unstained preparations a brassy colour.

(*ε*) At sixty-six hours—six hours before the next attack—only a shell of the corpuscle remains visible, and the pigment granules are either arranged radially or partly collected in the centre. Indications of segmentation are seen.

(*ζ*) Three hours before the next, or sixty-nine hours

after the last attack, fully developed segmented bodies, consisting of from six to ten oval or spherical spores, or parasites commencing spore formation, with centrally placed pigment, may be seen.

Flagellated forms of malarial parasites are seldom seen in tertian and quartan fever.

B.—The Parasites of Irregular, Malignant, or Aestivo-autumnal Malarial Fever.

Most observers recognize two forms, the quotidian and the malignant tertian. Mannaberg and others divide the quotidian into the pigmented and the unpigmented varieties.

1. *The Pigmented Quotidian* parasite completes its life-history in twenty-four hours. At first small unpigmented bodies are seen in the red corpuscles endowed with active amoeboid movement. Unlike the hyaline form of the tertian and quartan fever, these bodies are ring-like, with a darker centre. (Plate VI., Fig. 2, C.) Soon fine pigment granules collect, first at the periphery, later when the amoeboid movement ceases in the centre of the organism.

The parasite now breaks up into segments.

The Italian observers have, however, pointed out that spore formation rarely occurs in the peripheral circulation, usually taking place in the spleen, bone marrow, etc.

In the blood, organisms with centrally placed pigment are frequently seen. The parasite is smaller than that of simple intermittent fever, seldom occupying $\frac{1}{3}$ of the corpuscle, which often shrinks and becomes brassy or copper coloured.

When the disease has existed for several days, cres-

centic bodies—Laveran's corpuscles, and oval and spherical bodies may be seen. (Plate VI., Fig. 2, C.)

2. *The Unpigmented Quotidian* parasite closely resembles the pigmented variety, but possesses no pigment. Spore formation, as in the previous form, takes place almost entirely in the internal organs.

The crescents found in the unpigmented quotidian are almost always pigmented except, as Mannaberg observes, in cases in which "the patient catches the infection before the crescents have developed, one has to deal with a completely unpigmented malaria."

3. *The Malignant Tertian* parasite is almost identical with the pigmented quotidian amoeba, but differs from it, as Marchiafava and Bignani have pointed out, in the following particulars: the time of development is longer (forty-eight hours), the pigment often shows movement, the parasite is larger, and in the advanced pigmented stages the organism possesses active amoeboid movement.

Crescents, Crescentic bodies or Laveran's corpuscles, only occur in the irregular, never in the simple malarial fevers. They are generally found after the fever has existed for some time. (Plate VI., Fig. 2, C.)

As their name implies, they are crescentic or new-moon shaped bodies, somewhat longer than the diameter of an ordinary red corpuscle, being usually 8–10 μ, with a breadth in the middle of 2–3 μ.

Pigment is always present, sometimes scattered, more frequently limited to the centre. In the former condition it may show slight movement, in the latter position it is however always motionless.

These bodies, although possessing no amoeboid movement, may become oval or round, *i.e., spherical bodies,* but according to Mannaberg this change takes place in

the blood when out of the vessel. From the round body so formed, as well as from free amoebae, *flagellated forms* may develop.

Corpuscles containing an amoeba may rupture, setting free organisms which constitute the extra-corpuscular bodies, and these may, under certain conditions—Ross and others state that exposure to the air is necessary—become flagellated.

The crescent body is developed within a corpuscle, and under good illumination, a fine line, the remains of the corpuscles, is seen, extending from near the ends of the Laveran's corpuscles.

The Diagnosis or Recognition of the Malarial Parasite.

Having briefly described the various appearances which the haematozoa present in the blood, the question naturally occurs, are there any objects present in the blood which the uninitiated might mistake for malarial organisms?

In answer to this I would emphatically state, that anyone who is familiar with the ordinary appearances of the blood corpuscles, blood plates, and leucocytes in fresh and stained films, will experience very little difficulty in distinguishing any stage in the life-history of the amoeba. Although most forms of the parasite may be *seen* with the $\frac{1}{4}$ inch, yet it is necessary to use a higher objective, preferably a $\frac{1}{12}$ inch oil immersion to *find* them.

The early form of the parasite, particularly the non-pigmented hyaline body, may alone present some difficulty, but all confusion may be obviated by contrasting this stage of the haematozoa with the elements with which it is most likely to be confounded.

Vacuoles in the Red Corpuscles, resulting either from defective technique, or from pathological conditions which we have previously described, are the elements most likely to be mistaken for the hyaline forms, but these may be differentiated by the following particulars.

Vacuoles have a well-defined outline, which may show alterations in shape; they are remarkably brilliant, having a white colour; they are frequently too numerous in some parts of the field, whilst they are totally absent in other parts, and lastly, that which is of the greatest importance, they possess no structure.

In contradistinction to the above, the haematozoa have an ill-defined outline, which gradually merges into the surrounding substance of the corpuscle; they possess no great brilliancy, having a faint yellow colour in fresh preparations; they are never present in every corpuscle in the field of vision, and consist of a definite structure, which in dry films takes on the colour of the nuclear stain used.

Blood Plates may, when lying isolated, be mistaken for the free malaria spores, and when in little heaps, may suggest the spore-formation stage of the amoeba.

Speaking generally, one should always look for the malarial organism inside a corpuscle, and not free in the serum, and as Mannaberg states, "it should be a rule never to diagnose a free spore in an unstained preparation."

Blood plates lie outside the red cells, either free, or more frequently in little clusters, in which they are seldom round. They stain diffusely with the aniline stains, and with eosine and haematoxylin are usually coloured reddish-purple, and show no definite structure. (See Plate I., Fig. 2.)

Haematozoa are usually situated in a corpuscle, although free spores are also seen. They stain pale blue with eosine-haematoxylin, and show distinct structure. The spore-forming stage of the organism almost always contains some pigment in the centre of the body, whilst this is absent from a group of blood plates.

Leucocytes containing pigment, and in the fresh condition endowed with amoeboid movement, cannot be confounded with haematozoa, as the former show the presence of nuclei, with their intra-nuclear network, which is deeply stained with nuclear stains; whilst the latter are more faintly stained, showing no chromatin network, and in the stage of their development, which is most likely to suggest a resemblance to a leucocyte, *i.e.* the large intra-corpuscular body, they possess no amoeboid movement.

Melaniferous leucocytes remain in the blood for a short time after the fever has ceased, but usually in two or three days after the last paroxysm of tertian or quartan fever they disappear entirely, whilst in malignant forms of malaria they are met with for some time, in fact as long as crescents themselves. (Mannaberg.)

Particles of dirt, dust, etc. may simulate the malarial pigment, and Mannaberg states that although pigment found in the leucocytes in a fresh condition is of great service in the diagnosis of malaria, too much importance should not be laid on free pigment. He also adds that the existence of malarial infection should not be assumed from its presence in the free condition alone.

Absolute cleanliness is most important in the examination of the blood of cases which are thought

to be malarial in nature, and all the stains, as well as the water used for washing the films, must be carefully filtered. Care in this direction is essential to the tyro, and of the greatest assistance to the skilled examiner, as particles of dirt or stain when situated on a corpuscle, especially if the preparation is being examined under a low power, are very confusing.

If a single malarial parasite be found, the diagnosis of malaria is established, and it is possible from careful microscopic examination of the blood to determine, not only the existence, but also the species — simple or malignant, and the variety—tertian, quartan, quotidian, or malignant tertian, as well as the severity of malarial fever.

The prevalence of large pigmented intra-corpuscular bodies indicates simple, whilst numerous small pigmented or non-pigmented haematozoa suggest, and crescents or spherical bodies resulting from crescents are characteristic of, the malignant form of malarial fever.

I cannot do better than to place here the excellent differential chart of Mannaberg [*New Sydenham Society*, 1894, page 372], which very clearly shows the points of resemblance and difference of the various classes and sub-classes of the parasite.

The probable time at which the next paroxysm will occur may be ascertained in the simple intermittent fevers, by determining which form of organism prevails, and then consulting the table previously given, remembering that the stage of segmentation corresponds to that of another attack.

TABULAR CHART OF THE CHARACTERISTIC

		Duration of Disease.	Movement.	Pigmentation.
Simple Intermittent Fever.	Quartan Parasite.	72 hours.	Small movements in the immature form.	Coarse granules, little or no movement.
	Ordinary Tertian Parasite.	48 hours or less in anticipating types.	Active amoeboid movement in the immature and also in the middle-aged forms.	Fine granules in immature forms, often in the larger actively swarming.
Malignant or Aestivo-Autumnal Fever.	Pigmented Quotidian Parasite.	24 hours.	The unpigmented immature forms very actively amoeboid, less active when pigment accumulates.	Very fine, later coalesce in one or two lumps, does not swarm.
	Unpigmented Quotidian Parasite.	24 hours or less.	Very active amoeboid movement.	None.
	Malignant Tertian Parasite.	48 hours.	Active, the movement remains present in the pigmented bodies.	Moderately fine, often shows oscillatory movements.

SIGNS OF THE VARIOUS PARASITES.

Maximum Size.	Form of Spore Formation.	Number of Spores.	Crescent Bodies.	Alterations in the Infested Blood Corpuscles.
The size of the red blood corpuscle.	Daisy form, the single spores round, with distinct nucleolus.	6–12.	None.	Red blood corpuscles are little discoloured and do not alter their size.
Size of the red blood corpuscle, sometimes even larger.	Sunflower or grape-like, single spores small, round, nucleolus rarely seen.*	15–20, often less.	None.	Red blood corpuscles are often hypertrophied and lose their colour quickly and completely.
$\frac{1}{4}$–$\frac{1}{3}$ the size of a red blood corpuscle.	Irregularly formed heaps.	6–8, even more.	Present.	Red blood corpuscles shrink often, and are then either darker stained, copper coloured, or may be completely decolorised (Schleier).
$\frac{1}{3}$–$\frac{1}{4}$ size of a red blood corpuscle.	Star-shaped, or in irregular heaps.	6–8.	Present.	Red blood corpuscles shrink frequently, and are darkly stained.
$\frac{1}{3}$–$\frac{2}{3}$ the size of a red blood corpuscle.	Irregular heaps.	10–12, rarely 15–16.	Present.	Red blood corpuscles shrink frequently, they are darkly stained or may be perfectly colourless.

* N.B.—In a fresh condition; it is always seen in a stained preparation.

The same remarks apply to the malignant fevers, but here greater difficulty will be experienced.

The severity of the paroxysm is generally, according to Golgi—and this is more or less confirmed by Mannaberg—proportionate to the number of organisms present.

It must be mentioned that occasionally no parasites can be found, although malaria is present. This occurred three times in 130 cases which Mannaberg examined, and he says it is most likely to occur during the first days of the illness, and advises waiting "until one or more paroxysms have occurred, and thereafter to repeat the examination of the blood at a specially favourable time (before the paroxysm)."

Secondary Anæmia resulting from Malaria or Alterations in the Blood other than the presence of Haematozoa.

Anæmia, due to the destruction of the red blood corpuscles by the malarial parasite, and also possibly to the parasitic poison in the blood serum, is present in varying degrees, and is generally proportionate to the duration and intensity of the disease.

Kelsch, in one case, found the oligocythaemia so pronounced that the red corpuscles numbered only half a million, whilst in another patient he determined that two million corpuscles were destroyed in the course of four days.

Hayem and Halla saw the red cells reduced to 1,182,760 and 2,800,000 per cmm. These figures represent severe cases.

There is apparently a greater destruction of coloured elements after the earlier paroxysms, and Thayer states that when they have been reduced to two or even one

million, they often remain stationary. The haemoglobin is usually reduced proportionately to the red corpuscles. Haemoglobinuria sometimes occurs in severe cases of malaria, and also in post-malarial anæmia.

Kelsch affirms that during convalescence the corpuscles increase more rapidly than the haemoglobin, and Mannaberg found that sometimes the haemoglobin continued to decrease for several days after the cessation of the fever, whilst the corpuscles began to increase.

Dionsi has stated that the anæmia following malignant fever is more persistent, and resists treatment longer, than that following the simple forms of malaria.

The leucocytes are usually diminished in number, and this is most marked just after a paroxysm. In the apyrexial period they may increase in number, seldom however reaching the normal, till the next paroxysm. The decrease involves chiefly the multinucleated elements; the large uninucleated cells as well as the transitional forms are relatively increased. This is said to be particularly noticeable in advanced and severe cases.

The smaller lymphocytes are usually also increased, whilst the eosinophile cells are somewhat diminished, but have occasionally been found more numerous in post-malarial anæmia.

The large uninucleated, and to a less extent the multinucleated elements, according to Marchiafava and Bignami, are the leucocytes which assume a phagocytic action in malaria, and these, especially the former, are often seen containing pigment in little masses, or diffusely in the form of fine granules. These observers state that the multinucleated are not so often pigmented as the large uninucleated elements, while the lymphocytes and eosinophile cells never contain pigment.

Melaniferous leucocytes are found in the blood during the attack and for twelve to twenty-four hours after, and when the organisms cannot be discovered are very strongly suggestive, though not absolutely diagnostic of malarial fever.

Diagnosis.

The presence of the amoeba in any of its various phases, and to some extent leucocytes containing granules, are the characteristic features which distinguish malaria from many pathological conditions clinically resembling it, particularly forms of pyaemia, septicaemia, and miliary tuberculosis, in which rigors, profuse perspiration, etc., occur in paroxysms, with a temperature chart not unlike that of ague.

Chronic malarial cachexia, with enlarged spleen, may be differentiated from diseases presenting somewhat similar signs, such as leukaemia, Hodgkin's disease, and the so-called splenic anæmia of adults and infants, by the examination of the blood, and particularly the absence of leucocytosis.

The Blood in Acute Diseases.

Nearly all the infectious fevers are associated with a more or less marked increase in the number of the leucocytes.

The following are, however, the most notable exceptions to this general statement :—

Typhoid fever, malaria, influenza, tuberculosis, leprosy, and sometimes measles.

Fever alone may cause some alterations in the blood. According to Maragliano it may first produce contraction of the peripheral vessels, hence concentration of

the blood, aided also by the increased loss of water during febrile conditions by perspiration, etc., so that the corpuscles may be actually more numerous per cubic millimetre.

After the fever has disappeared, dilatation of the vessels occurs, and partly from this cause, but also from the destruction of the cellular elements due to the increased metabolism, the number of the red cells shows marked diminution.

The Blood in Pneumonia.

The red blood corpuscles suffer very little numerical alteration during the febrile period, and even after the crisis, and whilst convalescence is being established, the anæmia is usually not pronounced. Hayem considers that an attack of ordinary pneumonia is responsible for the destruction of about one million red cells.

The blood when examined, particularly in the fresh condition, shows an extremely well-marked increase in the amount of fibrin, which is probably only equalled by that seen in acute rheumatism. Hayem divides pneumonias according to the presence or absence of this condition of the blood, into those attended with a pronounced increase in the fibrin, which usually have a favourable prognosis, and those serious typhoid types, in which the "phlegmasique" (*i.e.*, inflammatory) characters are completely absent, the latter almost invariably ending fatally.

The Leucocytes.

Numerous observations have been made regarding the number of leucocytes in this disease. Most writers agree that, at least in favourable cases, there is a

marked leucocytosis, which is sometimes proportionate to the temperature—more usually, however, the constant parallelism between the degree of leucocytosis and the temperature is not seen.

When the crisis occurs, the leucocytes generally diminish in number; but when the temperature falls, but again rises—false crisis—they are somewhat diminished, but are still above the normal, according to von Limbeck.

Some cases show no leucocytosis, and these are generally regarded as unfavourable.

Ewing has given an excellent account of the subject in the *New York Medical Journal* (December 16, 1893). He examined the blood in one hundred cases of lobar pneumonia, and as a result he expresses the opinion that (*a*) leucocytosis may be absent in very mild cases, or in very severe cases in which the reaction of the system is absent; (*b*) that it is to some extent proportional to the extent of the lung involved, but usually depends on the degree of reaction of the system against the poison; and (*c*) that a "well-marked leucocytosis in lobar pneumonia, while in itself a favourable sign, does not assure that the disease will pursue a favourable course, but indicates usually a severe infection. A moderately low degree of leucocytosis in severe cases is an extremely unfavourable sign, whilst in some cases its absence indicates, with rare exceptions, that the disease will prove fatal."

It has been suggested by von Jaksch that leucocytosis should be artificially induced by the injection of irritants, *e.g.*, pilocarpin, turpentine, etc., but this has not been found to be of any real service. The following statement by Cabot seems to me well worth noting, viz.,

that "leucocytosis is checked by anti-pyretics (Hare), but not by cold bathing, which speaks in favour of the latter method of reducing temperature."

During the febrile leucocytosis the multinucleated neutrophile elements are increased, whilst the lymphocytes, and particularly the eosinophile cells—the latter may even be absent—are decreased, but after the crisis the multinucleated cells are relatively diminished, whilst the eosinophile forms are increased.

The Diagnosis.

The presence of leucocytosis will distinguish pneumonia from typhoid, malaria, and sometimes influenza. Ewing has also pointed out that in acute tubercular inflammations of the lung, and in acute apical lesions, the presence of leucocytosis is in favour of simple pneumonia, its absence pointing to the tubercular form of the disease.

As regards the prognosis, the absence of leucocytosis, except in the very mild cases, is admitted by all to be a bad omen.

Typhoid Fever.

Quite recently the diagnosis of enterica has been rendered comparatively certain by Widal's method of examining the blood serum. Various modifications of the process are constantly being introduced, but the following by Sheridan Delépine (in Allbutt's *Medicine*, vol. ii., p. 1145, and *British Medical Journal*, April 17, 1897) seems to be very suitable.

The Quick, or Microscopic Method.

1. The blood is obtained in a small pipette of such diameter that a platinum loop, having a diameter of

about one millimetre, may be introduced into it when it is broken transversely.

2. One drop of the blood, or serum, is diluted with nine parts of neutral bouillon, and they are thoroughly mixed.

3. One drop of this $\frac{1}{10}$ dilution of blood and bouillon is mixed on a glass slide, with one or more drops of a young (twenty-four hours old) culture of the typhoid bacillus. The latter may either be a growth on bouillon, or an emulsion of the bacillus on agar with a small quantity of bouillon or normal saline solution.

4. Cover with a cover-glass, and examine under a magnification of 200 to 300 diameter.

Delépine does not consider it necessary or advisable to separate the serum entirely from the corpuscles, and regards the presence of a few of the latter as advantageous, as they support the cover-glass, and cause the films to be of uniform thickness.

If the blood be from a patient suffering from typhoid, particularly after the first week, the bacilli rapidly, or slowly, according to the potency of the serum, become sluggish, and then frequently motionless. They are soon drawn together in little masses of varying sizes, the so-called "agglomeration" phenomenon. In most cases this occurs within five to thirty minutes, but in some rare cases it may take from one to two hours.

If the blood be from a non-typhoid patient the bacilli remain diffused throughout the film, moving freely, and showing no tendency to the formation of clumps or clusters.

Grünbaum (*British Medical Journal*, May 1, 1897) recommends a greater dilution, viz., 1 to 32. He first dilutes the blood serum 1 to 16, and then mixes one drop of this with one drop of the typhoid culture.

This writer further considers that a time limit is important. He prefers an emulsion of an attenuated culture of the typhoid bacillus from agar rather than a bouillon culture.

For collecting the blood he thinks capillary tubes, of not too fine a bore, are better than capillary pipettes.

Johnston and McTaggart (*British Medical Journal*, Dec. 5, 1896), instead of fresh blood or serum, use dried blood. A large drop of blood is caught on a piece of sterilized paper, and this is afterwards moistened with sterilized water. Then the solution of blood so obtained is mixed with a drop of broth culture of the typhoid bacillus. By this means the blood may be transmitted a distance for examination.

Wright and Semple (*British Medical Journal*, May 15, 1897), on the principle that blood serum of a typhoid patient produces agglomeration, even when brought into contact with a culture of the bacillus which has been killed by heat, have advised the use of dead bacilli instead of a fresh culture. They use an emulsion of fresh agar culture, in which the bacilli have been killed by exposure to a temperature of 60° C. for five to ten minutes. They consider that this method will obviate all necessity for culture apparatus, and the physician's "equipment in connection with the serum diagnosis may in fact be narrowed down to a supply of these capsules of dead bacteria and a small supply of glass tubing for pipettes."

The value of the serum reaction in typhoid fever cannot be over-estimated, but as to which method is the most convenient and reliable I am not in a position to state, as typhoid fever is very infrequent in and about Bournemouth.

The Blood in Typhoid Fever.

The red blood corpuscles show very little alteration in the earlier stages of enterica, in fact, owing to the fever as well as the diarrhœa and perspiration, they may be apparently increased, but generally vary from $4\frac{1}{2}$ to 5 million per cmm.

During, or towards the end of the third week a marked diminution may take place, and not infrequently they fall to $3\frac{1}{2}$ million, and towards the end of defervescence may number only $2\frac{1}{2}$ million.

During the course of this disease there is, according to Hayem, a loss of $2\frac{1}{2}$ million of corpuscles even without the occurrence of haemorrhage. Intestinal haemorrhage may occasion very considerable diminution in the cellular elements, so that anæmia may be a conspicuous and dangerous feature.

The duration of the disease is not the only cause of the oligocythaemia, the increased temperature playing an important part, and Hayem has mentioned that in cases in which the temperature has been kept low by antipyretics, cold baths, etc., the loss of corpuscular elements has been less than in those in which less active treatment was adopted.

The haemoglobin is reduced in amount corresponding to, or greater than, that of the corpuscles.

The leucocytes, which are never increased in number in uncomplicated cases, usually undergo a marked diminution. Hayem states that they frequently fall below 2000, sometimes numbering only 1000 per cmm.

The diminution is said to be most noticeable towards the end—Thayer states about the fifth week—of the fever.

According to most observers, if leucocytosis occur during typhoid, it is due to some complication, such as perforation, suppuration or abscess formation, pneumonia, haemorrhage, etc. Von Limbeck confirms this statement, but notices the fact that sometimes pneumonia or inflammatory complications may occur during or towards the end of typhoid fever without producing any leucocytosis. He records such a case in which the formation of abscesses did not raise the number of leucocytes above 5000 per cmm.

The multinucleated elements diminish, whilst the uninucleated leucocytes, particularly the larger forms, are relatively increased, especially towards the end of the illness. After the fever has disappeared the former begin gradually to increase, but do not become normal for some time. The eosinophile cells are present, but not numerous.

The blood plates are usually reduced in number, and according to Hayem they reach their minimum about the time when the high temperature begins to decline.

The fibrin is apparently normal, and never shows in uncomplicated cases the marked increase which is found in many inflammatory conditions. The coagulation of the blood is unaltered.

Diagnosis.

The entire absence of leucocytosis, and more usually the diminution in the number of white corpuscles, enable us to distinguish uncomplicated cases of typhoid fever from many inflammatory conditions, which may at one or other time closely resemble it clinically.

Perityphlitis, inflammatory and suppurative conditions, and most of the acute infectious diseases, are at once

eliminated, as leucocytosis is as constantly present in these as it is absent in enterica.

Malaria and tuberculosis are associated with a diminution in the leucocytes, and therefore indistinguishable by a mere count from typhoid fever.

The presence of the malarial parasite, or pigment bearing leucocytes in the blood, points conclusively to the presence of ague.

Scarlet Fever.

There is usually a slight diminution in the number of the red corpuscles, which according to Hayem is most marked in mild cases on the first day of defervescence, in severe cases—in which the fall in temperature is delayed—about twenty-four hours after cessation of the fever. The same observer considers that, as in pneumonia, about one million of red corpuscles are lost during scarlatina.

The haemoglobin decreases as the disease proceeds.

The Leucocytes are usually increased, and von Limbeck and others notice that the leucocytosis may persist for some days after the temperature has fallen to and remained at the normal. He records a case in which the white cells numbered 14,000 thirteen days after the thermometer showed a normal temperature.

The multinucleated are the form of leucocytes which are increased, and Kanthack and others state that the eosinophile cells are also more numerous, and may reach 8--15 % during the second and third weeks of the disease. Some writers—Kotschetkoff, Kanthack, and others—consider the eosinophile cells, except at the earliest stage of the fever, to be an unfavourable

sign. According to Neusser an increase in the eosinophile cells in scarlatinal nephritis is a favourable, and their absence an unfavourable sign.

The lymphocytes, Cabot states, are decreased proportionately to the severity of the case.

The presence of leucocytosis distinguishes scarlet fever from measles in some, but not in all cases.

Measles.

Mild cases scarcely show any alteration in the blood, and seldom more than half a million red corpuscles are destroyed during its course. (Hayem.)

Most observers—von Limbeck, Rieder, Pée, etc.—agree that there is no leucocytosis in uncomplicated cases, but when there is marked catarrhal affection of the mucous membranes, or bronchitis, the leucocytes as well as the fibrin are increased.

The multinucleated elements, Felsenthal states, are relatively increased, the eosinophile cells, unlike those in scarlatina, diminished in number.

The absence of leucocytosis helps to distinguish measles from scarlet fever, and from the roseola of syphilis.

Small-Pox or Variola.

Hayem considers that of all the eruptive fevers small-pox is attended with the greatest destruction of the red corpuscles, causing a loss of about two million during its entire course.

The corpuscles are normal during the eruptive stage, increased during suppuration, possibly owing to thickening of the blood, and rapidly decrease in number—from dilution of the serum, as well as actual destruction of the elements—after the temperature has fallen. Hayem

further remarks that in the haemorrhagic forms of small-pox the diminution of the corpuscles occurs immediately after, and is proportional to the amount of the haemorrhage.

During the occurrence of suppuration the inflammatory character of the blood—increase in fibrin and white cells—becomes most pronounced.

The *leucocytes* in slight cases occurring in vaccinated individuals may, according to von Limbeck, show no increase, but in ordinary or severe cases, in the discrete forms during the third and fourth day, and in confluent small-pox towards the end of suppuration, the leucocytes are increased, and may reach 28,000 or 30,000 per cmm. in the latter form of the disease. In haemorrhagic variola the leucocytes may show a great increase.

Hayem considers that the increase in leucocytes, when not due to suppuration, indicates a severe form of infection.

Diphtheria.

The red blood corpuscles are in the earlier stages usually normal, or even above the normal; later there may be slight anæmia. It is stated that antitoxin injections tend to diminish the amount of oligocythaemia, and to somewhat increase the rapidity of blood regeneration.

The haemoglobin, according to Billings, suffers a slight reduction—about 10%—which is, however, less pronounced when antitoxin has been used.

The *leucocytes* are, according to the majority of observers, usually increased, and generally the greater the severity of the disease, the greater the leucocytosis. Its absence may indicate a mild attack or a very severe condition. Antitoxin injections only cause a temporary fall

in the number of white cells shortly after they have been administered. Usually the multinucleated leucocytes, though sometimes the lymphocytes, are increased.

Ewing states that the staining reaction of the white cells is a measure of the degree of infection.

Myelocytes are said by Engel to be present in very severe cases, and he considers that when they occur in large percentages they constitute a bad sign.

Erysipelas.

Erysipelas produces much the same changes in the blood that pneumonia does, and the red corpuscles decrease, till they reach their minimum a few hours before the defervescence, and usually suffer a loss of from $\frac{1}{2}$ to 1 million or more in severe cases (Hayem). The haemoglobin is reduced about 10–20%.

Leucocytosis is usually present, and, according to von Limbeck, is proportionate to the temperature and the degree of severity of the case.

"The inflammatory character of the blood is a measure of the extent and intensity of the erysipelatous inflammation." (Hayem.)

In some cases occurring in scrofulous individuals this author found the leucocytes numbered only 7 to 8 thousand, whilst in ordinary severe cases they varied from 12 to 20 thousand per cmm.

Influenza.

Canon, who examined the blood in twenty cases of influenza, found a number of short bacilli about the thickness, but half the length, of the bacillus of mouse septicaemia. These bacilli are not easily found in the blood, but they are usually numerous in the

yellow masses seen in the sputum. He made cultivations from the blood on glycerine, or sugar agar, at 30° C. For staining the films he uses a mixture of eosine and methyl blue.

The majority of writers consider that the leucocytes are usually normal, although Hayem states that "la grippe" is attended with an increase in the fibrin and leucocytes.

Typhus Fever.

Leucocytosis is constantly absent, according to von Limbeck, whilst it is stated that Everard and Demoor have found it present.

Follicular Tonsillitis.

This generally shows slight leucocytosis, and Halla has noticed the same in the febrile period of angina tonsillaris.

Acute Rheumatism.

In all forms of acute rheumatism, whether the synovial membranes are involved or not, the blood shows a remarkable increase in the amount of fibrin, and this disease, and lobar pneumonia are attended with the most marked inflammatory condition. This "phlegmasique" character of the blood, as Hayem terms it, is best seen in blood examined in the fresh condition and in not too thin films. It is recognized by the delay in the coagulation, the enormous increase in the fibrin, which forms a dense fibrillar network, and by an increase in the leucocytes. When these conditions are very pronounced Hayem states that in all probability either pneumonia or acute rheumatism is present, and considers the examination of the blood in the fresh state of the greatest assistance in diagnosis.

The red blood corpuscles are diminished, and Hayem considers this disease one of those which cause the most marked destruction of the corpuscles, and notices that the sooner the attack is stopped by salicylates, the less the anæmia. Frequently one or two million corpuscles are lost during the fever.

The haemoglobin undergoes a diminution somewhat greater than that of the coloured elements, and not infrequently Hayem has noticed that when the corpuscles increase in number during convalescence, the haemoglobin may for a time diminish. The leucocytes are almost invariably increased, and generally proportionally to the severity of the attack.

In cases of moderate intensity they number 17 thousand to 18 thousand, in some cases as much as 25 thousand, whilst in subacute attacks they are just above the normal.

There is apparently no leucocytosis in chronic and muscular rheumatism.

Inflammation of Serous Membranes.

Non-tubercular inflammations of the serous membranes are usually associated with leucocytosis, and von Limbeck states that there is a relation between the cellular richness of the exudation and the number of the leucocytes in the blood.

Leucocytosis is always present in inflammations of these structures when they are due to streptococci, staphylococci, or pneumococci, but is usually absent in tubercular cases. He says that Rieder's observations are in agreement with his, in regard to the question of the differential diagnosis of tubercular from non-tubercular cases.

Pleurisy.

In simple acute pleurisy the fibrin and leucocytes are generally increased, but not to the same extent as in pneumonia. Hayem found that they varied from 7500 to 12,000, whilst von Limbeck records a non-tubercular case in which at first they numbered 13 to 15 thousand, but reached 22,000 per cmm. The leucocytosis is generally more pronounced during the fever, and may disappear when the temperature falls.

According to Hayem the inflammatory character of the blood, particularly the increase in the amount of fibrin, is less marked in tubercular and cancerous pleurisy than in simple or rheumatic forms, and most observers consider that leucocytosis is absent in tubercular cases.

When there is a large and continued increase in the number of the leucocytes, it suggests that a serous pleurisy has become purulent.

The red corpuscles and haemoglobin are but little altered, except in severe cases.

Peritonitis.

Serous or purulent, non-tubercular, and particularly septic forms of peritonitis, generally show a well-marked leucocytosis associated with a great increase in the amount of fibrin; but occasionally in very severe cases, as we saw was the case in grave forms of pneumonia, there may be no increase in the white cells.

According to Cabot, intestinal obstruction or malignant disease may cause an increase in the leucocytes, but rarely the fibrin; whilst tubercular peritonitis generally shows an entire absence of leucocytosis.

Pericarditis.

This follows the general rule, and shows leucocytosis in simple and septic cases, but it is frequently absent in tubercular forms of the disease.

Meningitis.

Hayem states that one always finds an increase in the leucocytes if not in the fibrin, and von Limbeck that leucocytosis is present in non-tubercular but absent in tubercular cases.

Osler, Zappert, Cabot, etc., found that leucocytosis occurs in some cases of tubercular meningitis, and the last-named observer considers that purulent meningitis shows a marked increase in the white cells, whilst the absence of leucocytosis would point to a tubercular form.

Rotch, in one case, an infant suffering from tubercular meningitis, found the leucocytes numbered 38,000, and considers that this increase pointed to some complication. In another case, also an infant, in which the white cells reached 37,500 per cmm., at the post-mortem examination, in addition to tubercular meningitis, an appendicitis was discovered, which accounted for the large number of leucocytes.

Hayem considers that the presence of leucocytosis would help to distinguish simple meningitis from hysteria, but lead "encephalopathy" and uraemic coma may be indistinguishable, as interstitial nephritis can cause similar alterations in the blood.

Cabot is of the opinion that cerebral tumours, hysteria, diabetic coma, sunstroke, etc., do not cause leucocytosis, whilst cerebral abscesses and apoplexy may.

Septicaemia and Pyaemia.

Numerous observers have found streptococci and staphylococci in cultures of blood taken, with the greatest antiseptic precautions, from patients suffering from many forms of septic infection. In some obscure cases, as for example septic endocarditis, very valuable assistance may be obtained by this method of research, but as the whole subject is somewhat outside the range of the blood proper, I must refer readers to those books dealing with bacteriology.

The red blood corpuscles are usually diminished, and in severe cases the oligocythaemia may be very pronounced.

The haemoglobin generally shows a diminution proportionate to that of the red cells. In some cases haemoglobinaemia, recognized by the reddish colouration of the blood serum, is present.

The Leucocytes.

Apparently contradictory accounts are given as to the number of white cells in all forms of septicaemia. Thus von Limbeck states that leucocytosis may be entirely absent in some cases, whilst in others it may reach a very high degree. As illustrating this he quotes a case of septic endometritis, ending fatally, in which the leucocytes were never higher than 5000, and on the other hand, a case of septic endocarditis, in which they reached 21,000. Rieder always found leucocytosis, and doubts the correctness of von Limbeck's observations. The latter, however, explains this difference on the ground that the whole question of leucocytosis, depends on the intensity of the infection and the power of

resistance of the body. Thus in dogs, in which inoculation of very virulent cultures of organisms caused no leucocytosis, death always resulted. He thinks that leucocytosis is particularly apt to occur in those cases in which exudation takes place, that high degree of leucocytosis is associated with exudation rich in cellular elements—or pus, and only slight in serous exudations, and that an absence of leucocytosis renders the presence of exudation formation improbable.

The occurrence and degree of leucocytosis in septicaemia is probably analogous to that seen in pneumonia, etc., that is, it is marked in most cases, but absent in very mild or in very severe cases which end fatally.

Its presence would in surgical cases, in which the temperature has gone up, point to sepsis, and would exclude fever resulting from hysteria or mental worry.

Suppuration or Abscess Formation.

The leucocytes generally increase proportionately to the intensity of the inflammatory condition, and reach their maximum when suppuration takes place. They suddenly fall when the pus escapes, but increase again when a new focus of suppuration occurs. As illustrating this point, Hayem mentions a case of suppurative pelvic peritonitis, in which the white cells numbered 21,000, and twenty-four hours after the evacuation of the abscess they fell to 9300. Similarly in an abscess of the forearm, in which they numbered 25,000, the next day, after incision and drainage, they were only 5000 per cmm., but subsequently became somewhat more numerous.

When the inflammation is disappearing, the leucocytes gradually diminish, and at the beginning of convalescence they may sink below the normal before definitely attaining the number found in health.

The increase of the white cells is almost invariably on the part of the multinucleated cells.

Associated with this leucocytosis there is usually an increase in the amount of the fibrin.

Hayem considers that the former is more essential, and is never completely absent from inflammatory conditions.

The examination of the blood in cases of obscure suppuration may be of the greatest diagnostic value, particularly in abdominal diseases.

Leucocytosis in such conditions would point to some inflammatory condition, *e.g.*, typhlitis, perityphlitis, peritonitis, pelvic suppuration, etc.

Malignant diseases usually show leucocytosis, but seldom an increase in the amount of fibrin. Speaking generally, absence of leucocytosis in uncomplicated cases would suggest typhoid, tubercular peritonitis, simple biliary or renal colic, obstruction from non-malignant disease, or fœcal accumulation, ovarian and pelvic neuralgias.

A perinephritic abscess would show an increase, whilst a cyst of the kidney usually presents no increase in the white cells.

Cabot, who has made extensive observations in cases of appendicitis, has stated that mild and very severe cases may show no leucocytosis, that an increasing leucocytosis may be the only evidence of extension of the process, whilst a persistently high increase of the white cells indicates a large abscess.

Secondary Anæmia in Chronic Infectious Diseases.

Syphilis.

Some cases of syphilis, especially in the earlier stages, show little or no alteration in the blood, but generally during the secondary period, more or less anæmia is present.

The red blood corpuscles vary considerably, being frequently somewhat diminished, but rarely in simple cases do they fall according to Hayem below $3\frac{1}{2}$ million. Usually the diminution in the haemoglobin is much more pronounced than that of the coloured elements, and, according to most writers, a chlorotic type of anæmia is frequently present.

Sometimes a very severe form of anæmia, termed by the early writers the "pernicious anæmia of syphilis," occurs. Müller describes a case of this kind, in which the haemoglobin was greater than the number of corpuscles, a condition frequently occurring in, and formerly considered characteristic of, pernicious anæmia. In this case the erythrocytes fell to 720,000, the haemoglobin being 18%. On the other hand, Lezius found that in twenty cases the red corpuscles were normal or nearly so, but the haemoglobin diminished.

Most observers state that syphilis produces much greater anæmia in women than in men.

Microscopically the corpuscles show little alteration except in the rare and exceptionally severe cases of this disease, in which all the signs of pronounced anæmia may be present. Thus Müller found in the severest form of syphilis various alterations in the elements, *e.g.*, megalocytes, microcytes, poikilocytes,

and nucleated red cells, usually normoblasts, but sometimes megaloblasts.

As von Limbeck states, the condition of the blood is most variable, and no one type characteristic of, or peculiar to the disease. The corpuscles may be normal, or may show a chlorotic condition, particularly in the earlier stages, whilst exceptionally in the severest forms of the disease, especially towards the end, the blood may resemble that seen in pernicious anæmia.

The leucocytes, according to von Limbeck, are generally normal in number in slight as well as severe cases, and Lezius found the uninucleated and multinucleated forms were present in the proportion seen in health.

Rille noticed leucocytosis, in which the lymphocytes and large uninucleated cells were increased, as well as the eosinophile leucocytes, especially when the specific roseola was present. He also found a few marrow cells. Bieganski observed an increase in the leucocytes, and whilst the multinucleated were diminished the uninucleated forms were increased. The administration of mercury, however, caused an increase of the former and a diminution of the latter.

It seems to me that there is no doubt that when the roseola appears, leucocytosis is generally present, and the uninucleated and eosinophile cells increased; whilst in the tertiary stage severe anæmia with pronounced lymphocytosis may occur.

According to Cabot the existence in adults of leucocytosis, with an increase in the proportion of the uninucleated and eosinophile cells, is in favour of syphilis, and opposed to the presence of tuberculosis, typhoid, or malignant disease.

Tuberculosis.

The blood shows exceedingly varied alterations in the different forms and stages of tubercle.

In the commencement, there may be no appreciable anæmia, notwithstanding the pallor, and Hayem remarks, that in cases in which the patients appear very anæmic, absence of any notable alteration of the red corpuscles is presumptive evidence of the presence of tubercle.

In phthisis it is not uncommon to find a normal number of red corpuscles associated with a diminution in the amount of haemoglobin; and these cases of tubercular pseudo-chlorosis are often, according to von Limbeck, indistinguishable from pure chlorosis.

In other cases oligocythaemia as well as oligochromaemia are present, whilst in exceptionally severe cases an extreme anæmia may accompany the disease. Thus von Limbeck records a fatal case of acute miliary tuberculosis, closely resembling pernicious anæmia, in which the red corpuscles numbered 730,000, the white 4300, and the haemoglobin 25%.

The red cells frequently show some degree of pallor, and poikilocytes may be present. Cabot states that nucleated red blood corpuscles are very rarely seen, except after haemorrhage, whilst in malignant disease they are sometimes present in large numbers.

The leucocytes are as a general rule not increased in pure uncomplicated cases of tuberculosis, and, according to Limbeck, tubercular meningitis and miliary peritonitis always show, even during the febrile period, a normal number of white cells.

Most observers agree as to the absence of leucocytosis

in pure tubercular diseases dependent on the presence of tubercle bacilli only, but when other organisms are present and secondary complications occur, then the blood presents all the signs of septicaemia, and leucocytosis may even be pronounced.

We shall briefly consider the effects of special forms of tubercular disease on the blood.

Phthisis.

In the early stages of apical catarrh, when fever is absent, the red corpuscles are normal, or somewhat below the normal, and the haemoglobin diminished. The leucocytes, when no complications attend the disease, show no increase.

When cavities are present, but fever absent, or present in only a slight degree, the red blood corpuscles are normal, or nearly so, and the white cells increased, often showing a leucocytosis of from 12 to 15 thousand.

When the fever is of the hectic type, the red corpuscles and haemoglobin are almost always diminished, the leucocytes variable. In cases of mixed infection, with high temperature, and of a generally septic character, leucocytosis is almost always found, whilst according to von Limbeck, in acute miliary or subacute miliary forms of the disease, it is usually absent.

Von Limbeck expresses the opinion that acute miliary or subacute granular forms can be distinguished from septic, and infiltrating or caseous pneumonia. According to this author, in cases of chronic septic phthisis, leucocytosis is usually moderate; in the infiltrating forms (tubercular pneumonias) it is often very pronounced, occasionally reaching that found in croupous pneumonia,

whilst in miliary tuberculosis of the lung leucocytosis is absent.

When leucocytosis is present, the multinucleated forms are increased, whilst the eosinophile cells are diminished. When it is absent the various forms of white cells may be present in their normal proportion, or there may be a relative increase in the small and large uninucleated elements. Marrow cells have been found in very small numbers in advanced cases.

Neusser notes, that in certain cases of phthisis, some of the large uninucleated, and multinucleated leucocytes contain a number of basophile granules, which he considers as a sign of the presence of a uric acid diathesis, and maintains that they justify a good prognosis. These cells, and their staining reaction, will be described under gout.

Acute miliary tuberculosis, tubercular forms of peritonitis, meningitis, pericarditis, and pleurisy, when uncomplicated, generally follow the rule of tubercular diseases, and show no increase—sometimes a diminution in the number of the leucocytes.

The Condition of the Blood in various General Diseases.

Constitutional Diseases.

Diabetes.

This disease is not attended with any constant or characteristic changes in the cellular elements of the blood.

The red corpuscles are sometimes normal, sometimes diminished, but may show an increase towards the end. The white corpuscles, though generally normal, may occasionally show a very pronounced digestive leucocytosis, even when there has been no prolonged fast previously. (von Limbeck.)

Recently two methods have been recommended for enabling a distinction to be made between normal and diabetic cases, by an examination of the blood alone.

1st. *Bremer's Film Method.* (*New York Medical Journal*, March 7th, 1896.)

(*a*) Film preparations of the blood are made and fixed by immersion for four minutes in a mixture of equal parts of alcohol and ether—which should be boiling.

(*b*) They are then stained for four minutes in the following solution :—

Saturated watery solutions of eosine and methyl-blue are mixed in equal proportions. The precipitate which forms is collected on a filter paper, washed and dried. When reduced to a powder, $\frac{1}{24}$ part of eosine and $\frac{1}{6}$ of methyl-blue are added.

Of this dry powder ·025 to ·05 grms. are dissolved in 10 grms. of a 33 % solution of alcohol.

This staining fluid does not keep well, and must be freshly prepared before use.

(*c*) After staining the films are washed in water and mounted.

The red blood corpuscles of diabetic blood are stained a green or greenish-blue; those of normal blood reddish violet.

Bremer has quite recently (*British Medical Journal*, July 3, 1897, Epitome) suggested the following modifications in the process, by which the alteration in the staining reaction can be seen with the naked eye.

Films of blood taken from a diabetic patient are made on the slide, and should not be too thin.

They are fixed by exposure to a temperature of 135° C. for six to ten minutes in a hot-air chamber.

The temperature and time mentioned must not be exceeded.

They can then be stained in a 1 % watery solution of methyl-blue for one to two minutes, after which they are washed and dried.

Diabetic blood is not stained, or only imperfectly, whilst non-diabetic blood stains of a bluish-green colour.

If the preparation be stained in Ehrlich-Biondi's solution for two to three minutes, the diabetic blood is stained orange, whilst normal blood is coloured violet.

If films are first stained in 1 % watery solution of methyl-green for one and a half to two minutes, then washed and stained in $\frac{1}{8}$ % of watery solution of eosine for ten seconds, according to this authority, the diabetic blood film remains green, whilst the non-diabetic blood takes on the eosine.

Methyl-blue and eosine give similar results.

2nd. *Williamson's Method* (*British Medical Journal*, Sept. 19, 1896) :—

i. Into a test tube 40 cmm. of water are placed.

ii. After puncturing, 20 cmm. of blood are added.

iii. Then 1 cmm. of a 1 in 6000 aqueous solution of methylene-blue are added.

iv. To this mixture 40 cmm. of liquor potassae are added, and the tube is thoroughly shaken in order to mix these solutions.

v. It is then allowed to boil for about four minutes.

This writer uses the capillary tube of Gower's haemoglobinometer, which holds 20 cmm., and the 1 cmm. pipette which accompanies Southall's ureometer, to measure these quantities.

The tube containing diabetic blood changes from a blue to dirty yellow or straw-colour, whilst that containing normal blood remains of a blue or bluish-green, but never decolorizes.

In both of these methods control experiments with normal blood should be made at the same time.

Gout.

The red corpuscles may show no alteration, whilst the leucocytes are usually normal according to Limbeck, although Neusser states that they are increased.

Uric acid crystals may be obtained by acidulating serum, obtained from a blister, with acetic acid (about six drops of 25 % acetic acid are added to one drachm of the serum). Crystals will form on a fibre of linen placed in the watch-glass in about twenty-four to forty-eight hours.

Neusser has drawn attention to the occurrence of

basophile granules in the large uninucleated and multinucleated leucocytes in cases of gout and uric acid diathesis.

Film preparations of blood fixed in the usual way are stained in the following solution, which is a slight modification of Ehrlich's triple stain :—

Saturated Watery Solution of	Methyl-green . .	80 cmm.
„ „ „	Acid Fuchsin . .	50 „
„ „ „	Orange-green . .	70 „
Distilled Water .	. . . .	150 „
Absolute Alcohol .	. . . .	80 „
Glycerine . .	. . . .	20 „

Mix.

These leucocytes are found in tubercular disease, gout, uric acid condition, and occasionally in diabetes and leucocythaemia.

Von Limbeck does not consider that they represent the existence of a uratic diathesis.

Janowski has found similar granules in pus corpuscles, and regards them as the result of degeneration of the leucocytes.

Myxoedema.

In a child suffering from myxoedema, von Lebreton found that before treatment the red corpuscles numbered $1\frac{3}{4}$ million, the haemoglobin 65 %, and the leucocytes 4500; after treatment with thyroid gland for forty days, the red cells increased to 2,450,000, the haemoglobin 68 %, and the white cells 9600 per cmm.

He found nucleated red cells before treatment, but these disappeared as the anæmic condition improved.

In a somewhat extensive examination of the blood of two cases of this disease, I found the red cells somewhat diminished, and noticed the prevalence of large uni-

nucleated leucocytes, a few of which were probably marrow cells. Since that time Thomas has also found the latter in myxoedema, but only in very small numbers.

Addison's Disease.

The anæmia seldom reaches a high degree, and dry preparations of the blood show little or no alteration.

Diseases of the Alimentary System.

In *Ulcer of the Stomach* the anæmia may, in some instances, be extremely pronounced, whilst in other cases only moderate oligocythaemia is present.

The haemoglobin is generally diminished to a greater extent than the number of corpuscles would suggest, but this is not always the case. Osterspey found that whilst in one case the red cells numbered 2,640,000, and the haemoglobin 30%, in another they had fallen to 1,900,000, with 31 % of haemoglobin.

The leucocytes are not usually increased except after haemorrhage, or a meal of albuminous food.

In *Cancer of the Stomach* severe anæmia, associated with the presence of extreme poikilocytosis and numerous microcytes, may be found, whilst nucleated red cells, especially normoblasts, are not infrequently seen.

The leucocytes are generally increased in number—the leucocytosis of malignant disease—particularly the multinucleated variety, and the number of the latter, according to Hartung, is directly proportional to the cachexia. The eosinophile cells are usually diminished.

The occurrence of digestive leucocytosis shortly after food is usually found in gastric ulcer, whilst its absence is, according to Limbeck, Hartung, and others, a strong indication of cancer of the stomach.

Intestinal parasites, particularly the bothriocephalus latus, may produce very severe anæmia, in some cases closely resembling that found in pernicious anæmia. Not only normoblasts, but even megaloblasts, have been seen in comparatively large numbers in a few cases.

Acute gastritis and gastroenteritis are usually associated with leucocytosis, but this is absent in typhoid fever.

In chronic gastritis there is usually no increase in the white cells, and, according to Cabot, digestive leucocytosis is sometimes absent.

Diseases of the Liver.

The red corpuscles vary in number considerably in different forms of hepatic diseases. In some cases, particularly towards the end of atrophic cirrhosis of the liver, very marked oligocythaemia may be found. Hayem has noticed that in many forms of cirrhosis, even when no jaundice is present, the red cells are more adhesive than in the normal condition, and vacuoles may be seen in their interior.

In catarrhal jaundice von Limbeck found that the red corpuscles were larger, and the haemoglobin more firmly attached to them than in health.

The leucocytes are also extremely variable in number. According to von Limbeck, in acute hepatitis and abscess of the liver they are always increased, in uncomplicated catarrhal jaundice they are never increased, whilst in cancer of the liver moderate leucocytosis is nearly always present.

The leucocytes, in different forms of cirrhosis of the liver, vary considerably according to the nature and progress of the case.

Diseases of the Respiratory System.

We have previously described the occurrence of well-marked leucocytosis in pneumonia, and its absence in the early stages of phthisis, miliary tuberculosis, and tubercular pleurisy.

Acute bronchitis is, at least during the febrile period, usually associated with an increase in the white cells, which generally reaches, according to Limbeck, 15,000 per cmm.

Pneumonia, empyema, and cancer of the lung are often attended with a greater increase in the number of white cells, than is generally found in serous pleurisy, whilst in tubercular pleurisy leucocytosis is absent in uncomplicated cases.

Malignant disease of the lung, although causing leucocytosis, is not usually associated with the marked increase in the amount of fibrin, which is found in inflammatory diseases of these organs.

In pulmonary emphysema and asthma the red corpuscles may show an apparent increase, due to cyanosis. The eosinophile cells are, according to many observers, more numerous in these two conditions, particularly just before, during, and immediately after a paroxysm of asthma. Increase of these cells is peculiar to pure bronchial asthma, and is absent from those cases of dyspnoea, or so-called asthma of renal or cardiac origin. I can confirm the statement, that an increase of eosinophile cells in the blood is associated with a similar increase of these cells in the sputum, during an attack of this disease.

Diseases of the Circulatory System.

Pericarditis and endocarditis, especially the septic forms, are attended with leucocytosis.

According to Hayem, endo- and pericarditis, when of rheumatic origin, are the forms which are associated with the most pronounced inflammatory character of the blood, *i.e.*, an increase in the leucocytes and fibrin, and he is of the opinion that, even in cases in which articular manifestations of the disease are absent, the occurrence of a dense network of fibrin points to rheumatism as the primary cause.

In infectious and septic forms of endo-pericarditis, although the leucocytes are frequently markedly increased, Hayem states it is rare to find an excessive proportion of fibrin.

In obscure cases of septic endocarditis, bacteriological cultures of the blood may give invaluable help in the diagnosis.

Valvular diseases of the heart, when compensation is present, usually show no alteration in the blood, but when this is imperfect or absent, and the effects of backward pressure occur, a diminution, or more frequently an increase of the red corpuscles, is found, owing to concentration of the blood serum, which results from œdema, etc. In the cyanosis of congenital heart disease, polycythaemia may be very pronounced.

The leucocytes are normal in organic valvular disease, except when complications are present.

Diseases of the Kidney.

In most forms of nephritis the red corpuscles are usually found to be normal, or somewhat diminished, but in some cases, according to von Limbeck, particularly those which are of a haemorrhagic type, very pronounced anæmia, in which the red corpuscles may fall to $1\frac{1}{2}$ or even 1 million per cmm., is sometimes found.

The haemoglobin undergoes a diminution proportional to, or somewhat greater than, that of the red cells.

The majority of observers state that the leucocytes are moderately increased in the greater number of cases of acute or chronic nephritis. According to Hayem the interstitial form is attended with the greatest increase in the amount of fibrin.

Von Limbeck states that there is frequently an increase in the eosinophile cells in nephritis.

In uraemic conditions the blood shows practically no alteration other than existed before in the number of the red or white blood corpuscles.

Malignant disease of the kidney is often attended with very pronounced leucocytosis.

In floating kidney the blood is normal, whilst in renal calculus there may be some degree of leucocytosis, especially when ulceration is present.

Nervous System.

We have previously mentioned the occurrence of leucocytosis in simple meningitis, and its frequent, although not constant absence, in the tubercular form of the disease.

In many functional diseases of the nervous system—hysteria and neurasthenia—a slight reduction in the amount of the haemoglobin is not infrequently seen, whilst in chronic organic nervous diseases one often finds a slight anæmic condition.

The eosinophile cells are occasionally increased in number in chorea, hysteria, and neurasthenia generally.

The Anæmias occurring in Infants.

The blood of infants, and to a less extent young children, presents certain differences from that of the adult.

The red corpuscles are more numerous at birth, constituting, according to Hayem 5,350,000, according to Schiff 5·8 million per cmm., and the former observer has noted greater variations in their size at this period of life.

The haemoglobin, though somewhat increased in amount, is less firmly attached to the corpuscles than in the adult.

Nucleated red cells, notwithstanding their presence in the earlier periods of intra-uterine life, are absent during the later months, and are rarely found in the blood of a healthy mature child at birth. Normoblasts, and even megaloblasts, may occasionally be seen in the blood of premature infants for a few days after their birth.

The leucocytes are also more numerous during the first week of life, constituting "the leucocytosis of the newly-born." Hayem places their number at birth at 21,000 per cmm. The uninucleated forms, as well as the eosinophile cells, are relatively numerous, whilst

the multinucleated, or neutrophile leucocytes, are diminished.

The number and proportion of the corpuscular elements during the early periods of life are shown in the following tables, taken from an excellent article on the blood of infants and children in *Rotch's Pediatric.*

At birth	red blood corpuscles			5,900,000,	leucocytes	21,000.
Seventh day	,,	,,	,,	5,000,000	,,	15,000.
First year	,,	,,	,,	,,	,,	10,000.
Sixth year and upwards	,,	,,	,,	,,	,,	7,500.

The Proportion of Leucocytes in Adults and Infants.

	Adults.	Infants.
Small uninucleated lymphocytes . .	24–30% ...	50–75%
Large uninucleated leucocytes . .	3–6 % ...	6–14%
Multinucleated, or neutrophile . .	60–75% ...	28–40%
Eosinophile cells	1–2 % ...	½–10%

Grundobin found that at the third year the multinucleated were equal in number to the uninucleated leucocytes, and after the eighth to tenth year there was little difference between the white cells of children and adults.

Under pathological conditions the blood of infants may show changes which do not ordinarily occur in the adult. In young children severe anæmia, associated with pronounced alteration in the size and shape of the red corpuscles, may result from very trifling causes.

Nucleated red corpuscles are not infrequently found in cases of slight oligocythaemia, and are generally more numerous in all forms of anæmia at this age.

The leucocytes are much more readily increased in number, and leucocytosis is often associated with many forms of anæmia. Whilst in adults leucocytosis is mainly

due to an increase in the multinucleated elements, in infants it involves an increase in the large or small uninucleated leucocytes, being in fact a lymphocytosis.

Marrow cells are much more frequently found in the severe anæmias of infants than in adults.

Anæmias, during the early periods of childhood, are often associated with an enlargement of the spleen.

Rickets, hereditary syphilis, and to a certain extent tuberculosis, are responsible for some of the severest forms of anæmia in infancy.

Anæmia Infantum Pseudo-leukaemica, or the Splenic Anæmia of Infants.

Synonym: "Anæmia splenica infettiva dei bambini," according to Italian writers.

In 1889 von Jaksch described under this term a form of anæmia occurring in children, which was characterized by marked enlargement of the spleen and at times slight enlargements of the lymphatic glands and liver. The blood showed considerable oligocythaemia, associated with pronounced and persistent leucocytosis. He found that some of these cases recovered.

Hayem and Luzet described cases resembling those of von Jaksch.

Luzet was, however, of the opinion that the disease usually ended fatally.

I will give a very brief outline of the clinical features of the disease, as it is not even mentioned in most of the text-books on medicine.

This form of splenic anæmia occurs most frequently in children under four years of age, but particularly between the ages of seven and twelve months.

It is not infrequently associated with the presence of rickets, and to a less extent inherited syphilis.

The disease begins insidiously, the anæmia gradually increasing till the waxy pallor of the skin is conspicuous. Sometimes the infants are emaciated, whilst at other times they are well nourished, but in all there is progressive weakness.

The spleen may be enlarged to such an extent as to occupy nearly the entire left side of the abdomen. More frequently, however, it is only moderately increased in size. The organ is firm and resistant to the touch. Post-mortem examination shows hyperplasia of the splenic elements.

The liver may be slightly enlarged, but frequently is of the normal size.

The lymphatic glands are in some cases enlarged.

The Condition of the Blood.

The red corpuscles are always considerably reduced in number, and are usually below three million. In one case von Jaksch found that the red corpuscles fell to 820,000, whilst the white cells numbered 54,666 per cmm.

The coloured cells frequently show signs of Maragliano's degeneration and polychromatophilia. The corpuscles are also of unequal size and shape, and all degrees of poikilocytosis may be seen.

Nucleated red cells are very numerous, and according to many observers characteristic.

They are for the most part normoblasts, and many show karyokinetic nuclear division, which Luzet considers very significant of the disease. Large nucleated

red corpuscles—megaloblasts—are sometimes found. The haemoglobin undergoes a marked diminution.

The leucocytes are always increased, generally ranging from 40,000 to 114,150 per cmm. Von Jaksch found the proportion of white to red corpuscles in three cases was as 1:12, 1:17, and 1:20. Usually the multinucleated elements predominate, but sometimes the uninucleated are in excess.

The eosinophile cells are, according to von Jaksch, diminished, but Luzet, Zappert, and others found them increased. Marrow cells have been found in small numbers in some cases.

Von Jaksch considered this a special disease of infants, from the occurrence of marked enlargement of the spleen, the absence of a leukaemic character of the blood, as well as the usually favourable prognosis.

Von Limbeck and many other observers regard it as not a special form of anæmia, much less a special disease of early life.

Stengel considers that it is not an independent disease, but probably a form of secondary anæmia, which owes its characteristics—enlargement of the spleen, leucocytosis, and the numerous nucleated red cells—chiefly to the age of the patient.

In favour of its being of secondary nature is the fact that Fischl found that in 18 cases of this disease 16 were affected with rickets, whilst two presented all the signs of inherited syphilis. In the latter two cases oligocythaemia and leucocytosis were very pronounced.

Diagnosis.

It would appear that sometimes so-called anæmia infantum pseudo-leukaemica shows all the signs of a primary anæmia—either pernicious anæmia, leukaemia, or Hodgkin's disease; and at other times it occupies a position intermediate between primary and secondary anæmia.

The presence of enlargement of the spleen does not exclude it from being pernicious anæmia, as although this disease in adults is usually associated with no enlargement of that organ, and no leucocytosis, yet any severe anæmia in infancy may be attended with both these conditions.

The increase in the number of leucocytes may not be considered sufficiently pronounced to suggest leukaemia, but a case of the spleno-medullary form of this disease—which we have previously described—an enlarged spleen, numerous nucleated red corpuscles, and only 44,000 leucocytes, would apparently be considered by many as one of splenic anæmia of infants. Here, however, the prevalence of marrow cells, to my mind, absolutely settled the diagnosis of leukaemia. In this particular case, within five or six weeks, the leucocytes increased from 44,000, or a proportion of white to red of 1 : 78, to 555,795, and a week later to 696,960, or a proportion of 1 : 4, and justified the diagnosis.

Given a case of anæmia in a young child, in which the spleen is considerably enlarged, the following diseases in addition to anæmia infantum pseudo-leukaemica suggest themselves: leucocythaemia,—spleno-medullary or lymphatic variety,—pernicious anæmia, or severe secondary anæmia associated with rickets and syphilis.

If blood films show that the marrow cells are numerous, constituting 30 to 50 % of all the leucocytes found, I should consider the case one of spleno-medullary leukaemia. As we have previously pointed out, qualitative alterations of the leucocytes are more important and characteristic of leukaemia, than mere numerical increase of the white cells.

If, however, the leucocytosis were very pronounced, but consisted in an almost exclusive increase in the lymphocytes, I should regard it as probably one of lymphatic leucocythaemia, although the diagnosis might be somewhat difficult.

Prevalence of large nucleated red corpuscles—megaloblasts—as well as megalocytes associated with very severe and progressive oligocythaemia, and to a less extent oligochromaemia, would suggest the presence of pernicious anæmia.

From Hodgkin's disease, especially when this is associated with leucocytosis, the diagnosis would present the greatest difficulty. In both the spleen and lymphatic glands may be enlarged, and in either, especially in the splenic anæmia of adults, the former may alone show enlargement.

I cannot help regarding some forms of anæmia infantum pseudo-leukaemica as similar to the so-called splenic anæmia of adults, and am of the opinion that both may be mere varieties of Hodgkin's disease, or pseudo-leukaemia.

Inherited Syphilis in Children.

The red corpuscles may be considerably reduced in number. The haemoglobin is sometimes diminished proportionately, sometimes less than, whilst not infrequently the reduction is greater than that of the red cells, constituting in the last case the so-called chlorotic condition of the blood. Loos and Fischl have noticed that microcytes, megalocytes, poikilocytes, and polychromatophilia are sometimes found, whilst in the severe forms of anæmia normoblasts and even megaloblasts may be seen.

The leucocytes vary in number, but are usually somewhat increased, ranging from 12,000 to 58,000.

Loos found the following percentage of the different forms of white cells: lymphocytes, 15·9 to 33·7; multinucleated elements, 26·4 to 58·7; and a marked increase in the transitional forms, which numbered 22 to 52·9 %.

Fischl noticed that the eosinophile cells were not increased, and Loos stated that they varied considerably, but were usually increased when the specific exanthemata appeared, and this is confirmed by Neusser and Zappert.

Marrow cells and leucocytes containing basophile granules were found by Loos.

Rotch points out that there is nothing characteristic of congenital syphilis in infants, the condition being that of severe secondary anæmia, frequently associated with the presence of an enlarged spleen. In an infant of three months he found the red corpuscles numbered 3,387,000, the leucocytes 20,000, and the haemoglobin 47 %.

The Anæmia of Rickets in Children.

Severe forms of rickets are nearly always associated with a marked diminution in the red corpuscles. Von Jaksch records a case in which they fell from 1,600,000 to 750,000 in three months. The corpuscles present all the signs of anæmia, and nucleated red cells, especially normoblasts, are frequently seen.

Slight forms of rickets may, however, present no signs of anæmia.

The haemoglobin generally suffers a reduction proportional to that of the red cells.

The leucocytes are almost invariably increased, and may reach as many as 45,000 per cmm.

According to von Limbeck the multinucleated forms predominate, whilst Rotch states that the lymphocytes were greatly increased. These apparently conflicting statements can be explained by the age of the child. The younger the child, the more numerous are the small uninucleated leucocytes, whilst in older children the leucocytosis consists chiefly of the ordinary multinucleated cells.

Marrow cells and karyokinetic signs of division of the nucleus of the leucocytes are sometimes seen.

Rotch mentions that in some cases the spleen is enlarged.

Inflammatory conditions of the alimentary tract, and tuberculosis, are usually attended with considerable anæmia in young children. Leucocytosis is almost invariably present, even if no oligocythaemia is found, and the lymphocytes are usually increased, whilst the multinucleated forms diminished.

In the various forms of tuberculosis affecting children the leucocytes, contrary to that found in the adult, are usually more numerous.

Monti and Berggrun found that, in a child of nearly seven suffering from tubercular disease of the lymphatic glands, the red corpuscles were 3·64 million, and the white 14,000; and in another child of about the same age, with chronic tuberculosis of the lung and peritoneum, the red numbered 3·23 million, and the white corpuscles 17,200 per cmm.

LITERATURE

TO WHICH REFERENCE HAS BEEN MADE.

Hayem, "Du Sang," Paris, 1889.

Von Limbeck, "Grundriss einer Klinischen Pathologie des Blutes," Jena, 1896.

Muir, "Contribution to the Physiology and Pathology of the Blood," *Jour. of Anat. and Phys.*, vols. xxv., xxvi., and xxvii., 1891.

Muir, "Method of Examining Blood, Bone Marrow," etc., *Journal of Anatomy and Physiology*, 1892, page 394.

Muir, "Observations on Leucocythaemia," *Journal of Pathology and Bacteriology*, vol. i. No. 2, Oct., 1892.

Muir and Drummond, "On the Structure of the Bone Marrow in Relation to Blood Formation," *Jour. of Anat. and Phys.*, Oct., 1893.

Muir, "On Changes in the Bone Marrow in Pernicious Anæmia," *Jour. of Path. and Bacter.*, vol. ii. No. 3, Feb., 1894.

Kanthack, "The Histological Changes of Blood in Diseased Conditions," *Medical Chronicle*, July, Aug., and Oct., 1894.

Kanthack and Hardy, "The Morphology and Distribution of the Wandering Cells of Mammalia," *Jour. of Phys.*, vol. xvii., 1894.

Kanthack and Hardy, "On the Characters and Behaviour of the Wandering Cells of the Frog, especially in relation to Micro-organisms," *Proceedings of Royal Society*, vol. lii., Jan., 1893.

Sherrington, "Note on some Changes in the Blood of the General Circulation consequent upon certain Inflammations of an Acute Local Character," *Proc. of the Royal Society*, vol. lv. No. 332, 1894.

Gulland, "Nature and Variety of Leucocytes," *The Report of the Laboratory of Royal College Physicians, Edinburgh*, iii., 1891.

Gulland, "On the Granular Leucocytes," *Jour. of Phys.*, vol. xix., 1896.

Gulland, "A Rapid Method of Fixing and Staining Blood Films," *British Medical Journal*, March 13, 1897.

Müller, H. F., "Die Methoden der Blutuntersuchung," *Centralblatt fur Allgemeine Pathologie u. Pathologische Anatomie*, No. 19 and 20, 1892.

Rieder, "Atlas der Klinischen Microscopie des Blutes," Leipzig, 1893.

Mackenzie, "The Lettsomian Lectures on Anæmia," *The Lancet*, Jan. 10th, 24th, Feb. 7th, 1891.

Ewing, "A Study of the Leucocytosis of Lobar Pneumonia," *New York Med. Journal*, Dec. 16th, 1893.

Bruhl, "De la Splenomégalie Primitive," *Archives Generales de Médecine*, 1891.

Laveran, "Paludism," *New Sydenham Society*, 1893.

Marchiafava and Bignami; Mannaberg, "Malaria and the Parasite of Malaria Fevers," *New Sydenham Society*, 1894.

Stengel, article on "The Diseases of the Blood" in the *Twentieth Century of the Practice of Medicine*, 1896.

Rotch, article on "The Blood in Infancy and Childhood" in *Rotch's Pediatrics*, 1896.

Buchanan, "Leukaemia," *Journal of Pathology and Bacteriology*, vol. iv. No. 2, 1896.

Williamson, "Method of Distinguishing Diabetic from Non-Diabetic Blood," *British Medical Journal*, Sept. 19, 1896.

Stockman, "The Nature and Treatment of Pernicious Anæmia," *British Medical Journal*, May 4, 11, 18, 1895.

Hunter, "The Pathology of Pernicious Anæmia," *British Medical Journal*, Feb. 8, 1896.

West, "Splenic Anæmia," *British Medical Journal*, June 13, 1896.

Oliver, "The Croonian Lectures: A contribution to the Study of the Blood and Circulation," June, 1896, *British Medical Journal.*

Manson, various contributions on "Malaria" in the *Brit. Med. Jour.*

Johnston and McTaggart, "Observations on the Serum Reaction in Typhoid," *British Medical Journal*, Dec. 5, 1896.

Taylor, "Discussion on Anæmia at the British Medical Conference," *British Medical Journal*, Sept. 19, 1896.

Martin and Mathewson, "Observations upon the Relation between Leukaemia and Pseudo-leukaemia," *Brit. Med. Jour.*, Dec. 5 and 12, 1896.

Von Jaksch, *Clinical Diagnosis*, 1893.

Delepine, "Technique of Serum Diagnosis, with special reference to Typhoid Fever," *British Medical Journal*, April 17, 1897.

Bremer, "The Examination of the Blood in Diabetes," *British Medical Journal*: Epitome, 1896, July 3, 1897, and Aug. 28, 1897.

Wright and Semple, "The Serum Diagnosis of Typhoid by means of Dead Typhoid Bacilli," *British Medical Journal*, May 15, 1897.

Cabot, "The Clinical Examination of the Blood." Longmans, Green, and Co., 1897.

Allbutt's *System of Medicine*, vols. i. and ii., 1896 and 1897.

Osler's, Fagge's and Reynolds' *Medicine.*

INDEX.

No. 3. *London, 7, Great Marlborough Street, November, 1898.*

A SELECTION

FROM

J. & A. CHURCHILL'S CATALOGUE,

COMPRISING

MOST OF THE RECENT WORKS PUBLISHED BY THEM.

N.B.—J. & A. Churchill's larger Catalogue, which contains over 600 works, with a Complete Index to their Subjects, will be sent on application.

Human Anatomy:
A Treatise by various Authors. Edited by HENRY MORRIS, M.A., M.B. Lond., F.R.C.S., Surgeon to, and Lecturer on Surgery at, the Middlesex Hospital. Second Edition. Roy. 8vo, with 800 Illustrations, nearly all original, and many of them in several colours, 36s.

Heath's Practical Anatomy:
A Manual of Dissections. Eighth Edition. Edited by WILLIAM ANDERSON, F.R.C.S., Surgeon and Lecturer on Anatomy at St. Thomas's Hospital, Examiner in Anatomy for R.C.P. and S. Crown 8vo, with 329 Engravings, 15s.

Wilson's Anatomist's Vade-Mecum. Eleventh Edition. By HENRY E. CLARK, M.R.C.S. Eng., F.F.P.S. Glasg., Examiner in Anatomy, F.P.S., and Professor of Surgery in St. Mungo's College, Glasgow. Crown 8vo, with 492 Engravings and 26 Coloured Plates, 18s.

An Atlas of Human Anatomy. By RICKMAN J. GODLEE, M.S., F.R.C.S., Surgeon and late Demonstrator of Anatomy, University College Hospital. With 48 Imp. 4to Plates (112 figures), and a volume of Explanatory Text. 8vo, £4 14s. 6d.

Human Osteology.
By LUTHER HOLDEN, Consulting Surgeon to St. Bartholomew's Hospital. Seventh Edition, edited by CHARLES STEWART, F.R.S., Conservator of the Museum R.C.S., and ROBERT W. REID, M.D., F.R.C.S., Regius Professor of Anatomy in the University of Aberdeen. 8vo, with 59 Lithographic Plates and 75 Engravings. 16s.

Also.

Landmarks, Medical and Surgical. Fourth Edition. 8vo, 3s. 6d.

The Student's Guide to Surgical Anatomy. By EDWARD BELLAMY, F.R.C.S. and Member of the Board of Examiners. Third Edition. Fcap. 8vo, with 81 Engravings. 7s. 6d.

Diagrams of the Nerves of the Human Body, exhibiting their Origin, Divisions, and Connections, with their Distribution to the Various Regions of the Cutaneous Surface, and to all the Muscles. By Sir W. H. FLOWER, K.C.B., F.R.S., F.R.C.S. Third Edition, with 6 Plates. Royal 4to, 12s.

A Manual of General Pathology, for Students and Practitioners. By W. S. LAZARUS-BARLOW, B.A., M.D., late Demonstrator of Pathology in the University of Cambridge. 8vo, 21s.

Pathological Anatomy of Diseases. Arranged according to the nomenclature of the R.C.P. Lond. (Student's Guide Series). By NORMAN MOORE, M.D., F.R.C.P., Assistant Physician and Lecturer on Pathological Anatomy to St. Bartholomew's Hospital. Fcap. 8vo, with 111 Engravings, 8s. 6d.

A Manual of Clinical and Practical Pathology. By W. E. WYNTER, M.D., M.R.C.P., Assistant Physician to the Middlesex Hospital, and F. J. WETHERED, M.D., F.R.C.P., Assistant Physician to the Consumption Hospital, Brompton. With 4 Coloured Plates and 67 Engravings. 8vo, 12s. 6d.

General Pathology:
An Introduction to. By JOHN BLAND SUTTON, F.R.C.S., Sir E. Wilson Lecturer on Pathology, R.C.S.; Assistant Surgeon to, and Lecturer on Anatomy at, Middlesex Hospital. 8vo, with 149 Engravings, 14s.

Atlas of the Central Nervous System. From the larger work of Hirschfeld and Léveillé. Edited by HOWARD H. TOOTH, M.D. F.R.C.P. With 37 Plates carefully coloured by Hand. Large Imp. 8vo, 40s.

The Human Brain:
Histological and Coarse Methods of Research for Students and Asylum Medical Officers. By W. BEVAN LEWIS, Medical Superintendent, West Riding Asylum. 8vo, with Engravings and Photographs, 8s.

A Contribution to the History of the Respiration of Man; being the Croonian Lectures delivered before the Royal College of Physicians in 1895, with supplementary considerations of the methods of inquiry and analytical results. By WILLIAM MARCET, M.D., F.R.C.P., F.R.S. With Diagrams, imp. 8vo, 12s. 6d.

The Physiology and the Pathology of the Cerebral Circulation: an Experimental Research. By LEONARD HILL, M.B., Hunterian Professor, R.C.S. With 41 Illustrations, Royal 8vo, 12s.

Elements of Human Physiology. By ERNEST H. STARLING, M.D., F.R.C.P., Joint Lecturer on Physiology at Guy's Hospital. Third Edition. Crown 8vo, with 140 Engravings, 7s. 6d.

Manual of Physiology:
For the use of Junior Students of Medicine. By GERALD F. YEO, M.D., F.R.S. Third Edition. Crown 8vo, with 254 Engravings (many figures), and Plate of Spectra, 14s.

Principles of Human Physiology. By W. B. CARPENTER, C.B., M.D., F.R.S. Ninth Edition. By HENRY POWER, M.B., F.R.C.S. 8vo, with 3 Steel Plates and 377 Wood Engravings, 31s. 6d.

Practical Lessons in Elementary Biology, for Junior Students. By PEYTON T. B. BEALE, F.R.C.S., Lecturer on Elementary Biology and Demonstrator in Physiology in King's College, London. Crown 8vo, 3s. 6d.

Medical Jurisprudence:
Its Principles and Practice. By ALFRED S. TAYLOR, M.D., F.R.C.P., F.R.S. Fourth Edition, by THOMAS STEVENSON, M.D., F.R.C.P., Lecturer on Medical Jurisprudence at Guy's Hospital. 2 vols. 8vo, with 189 Engravings, 31s. 6d.

By the same Authors.

A Manual of Medical Jurisprudence. Twelfth Edition. Crown 8vo, with 55 Engravings, 14s.

Hygiene and Public Health.
A Treatise by various Authors. Edited by THOMAS STEVENSON, M.D., F.R.C.P., Lecturer on Chemistry and Medical Jurisprudence at Guy's Hospital; Official Analyst to the Home Office; and SHIRLEY F. MURPHY, Medical Officer of Health of the County of London. In 3 vols., royal 8vo, fully Illustrated. Vol. I., 28s.; Vol. II., 32s.; Vol. III., 20s.

The Theory and Practice of Hygiene. By J. LANE NOTTER, M.D., Examiner in Hygiene and Public Health in the University of Cambridge and in the Victoria University, Professor of Hygiene in the Army Medical School; and R. H. FIRTH, F.R.C.S., Assistant Professor of Hygiene in the Army Medical School. With numerous Illustrations, Royal 8vo, 24s.

A Manual of Practical Hygiene. By the late E. A. PARKES, M.D., F.R.S. Eighth Edition, by J. LANE NOTTER, A.M., M.D. 8vo, with 10 Plates and 103 Engravings, 18s.

A Handbook of Hygiene and Sanitary Science. By GEO. WILSON, M.A., M.D., LL.D., F.R.S.E., D.P.H Camb., Medical Officer of Health for Mid-Warwickshire. Eighth Edition. Crown 8vo, with Engravings, 12s. 6d.

A Simple Method of Water Analysis, especially designed for the use of Medical Officers of Health. By JOHN C. THRESH, M.D.Vic., D.Sc. Lond., D.P.H.Camb., Medical Officer of Health for the County of Essex. Second Edition. Fcap. 8vo, 2s. 6d.

Elements of Health: an Introduction to the Study of Hygiene. By LOUIS C. PARKES, M.D., D.P.H. Lond., Lecturer on Public Health at St. George's Hospital. Post 8vo, with 27 Engravings, 3s. 6d.

Diet and Food considered in relation to Strength and Power of Endurance, Training and Athletics. By ALEXANDER HAIG, M.D., F.R.C.P. Crown 8vo, 2s.

The Prevention of Epidemics and the Construction and Management of Isolation Hospitals. By ROGER MCNEILL, M.D. Edin., D.P.H. Camb., Medical Officer of Health for the County of Argyll. 8vo. With several Hospital Plans, 10s. 6d.

Hospitals and Asylums of the World; their Origin, History, Construction, Administration, Management, and Legislation. By Sir HENRY BURDETT, K.C.B. In 4 vols., with Portfolio. Complete, 168s. Vols. I. and II.—Asylums: 90s. Vols. III. and IV.—Hospitals, &c., with Portfolio of Plans, 120s.

A Manual of Bacteriology, Clinical and Applied. With an Appendix on Bacterial Remedies, &c. By RICHARD T. HEWLETT, M.D., M.R.C.P., D.P.H. Lond., Assistant in the Bacteriological Department, British Institute of Preventive Medicine. With 75 Illustrations, post 8vo, 10s. 6d.

Mental Diseases:
Clinical Lectures. By T. S. CLOUSTON, M.D., F.R.C.P. Edin., Lecturer on Mental Diseases in the University of Edinburgh. Fifth Edition. Crown 8vo, with 19 Plates, 14s.

A Text-Book on Mental Diseases, for Students and Practitioners of Medicine. By THEODORE H. KELLOGG, M.D., late Medical Superintendent of Willard State Hospital, U.S.A. With Illustrations, 8vo., 25s.

Mental Physiology, especially in its Relation to Mental Disorders. By THEO. B. HYSLOP, M.D., Resident Physician and Medical Superintendent, Bethlem Royal Hospital; Lecturer on Mental Diseases, St. Mary's Hospital Medical School. 8vo, 18s.

The Insane and the Law: a Plain Guide for Medical Men, Solicitors, and Others as to the Detention and Treatment, Maintenance, Responsibility, and Capacity either to give evidence or make a will of Persons Mentally Afflicted. With Hints to Medical Witnesses and to Cross-Examining Counsel. By G. PITT-LEWIS, Q.C., R. PERCY SMITH, M.D., F.R.C.P., Resident Physician, Bethlem Hospital, and J. A. HAWKE, B.A., Barrister-at-Law. 8vo, 14s.

A Dictionary of Psychological Medicine, giving the Definition, Etymology, and Synonyms of the Terms used in Medical Psychology; with the Symptoms, Treatment, and Pathology of Insanity; and THE LAW OF LUNACY IN GREAT BRITAIN AND IRELAND. Edited by D. HACK TUKE, M.D., LL.D., assisted by nearly 130 Contributors. 2 vols., 1,500 pages, royal 8vo, 42s.

The Mental Affections of Children, Idiocy, Imbecility, and Insanity. By WM. W. IRELAND, M.D. Edin., formerly Medical Superintendent of the Scottish Institution for the Education of Imbecile Children. With 20 Plates, 8vo, 14s.

Mental Affections of Childhood and Youth (Lettsomian Lectures for 1887, &c.). By J. LANGDON-DOWN, M.D., F.R.C.P., Consulting Physician to the London Hospital. 8vo, 6s.

The Journal of Mental Science.
Published Quarterly, by Authority of the Medico-Psychological Association. 8vo, 5s.

Manual of Midwifery:
Including all that is likely to be required by Students and Practitioners. By A. L. GALABIN, M.D., F.R.C.P., Obstetric Physician to Guy's Hospital. Fourth Edition. Crown 8vo, with 271 Engravings, 15s.

The Practice of Midwifery: A Guide for Practitioners and Students. By D. LLOYD ROBERTS, M.D., F.R.C.P., Consulting Obstetric Physician to the Manchester Royal Infirmary, Physician to St. Mary's Hospital. Fourth Edition. Crown 8vo, with 2 Coloured Plates and 226 Woodcuts, 10s. 6d.

Manual of the Diseases peculiar to Women. By JAMES OLIVER, M.D., F.R.S. Edin., M.R.C.P. Lond., Physician to the Hospital for Diseases of Women, London. Fcap. 8vo, 3s. 6d.

By the same Author.

Abdominal Tumours and Abdominal Dropsy in Women. Crown 8vo, 7s. 6d.

A Short Practice of Midwifery, embodying the Treatment adopted in the Rotunda Hospital, Dublin. By HENRY JELLETT, M.D., Assistant Master, Rotunda Hospital. With 45 Illustrations, crown 8vo, 6s.

Obstetric Aphorisms:
For the Use of Students commencing Midwifery Practice. By JOSEPH G. SWAYNE, M.D. Tenth Edition. Fcap. 8vo, with 20 Engravings, 3s. 6d.

Economics, Anæsthetics, and Antiseptics in the Practice of Midwifery. By HAYDN BROWN L.R.C.P., L.R.C.S. Fcap. 8vo, 2s. 6d.

Lectures on Obstetric Operations: A Guide to the Management of Difficult Labour. By ROBERT BARNES, M.D., F.R.C.P. Fourth Edition. 8vo, with 121 Engravings, 12s. 6d.

By the same Author.

A Clinical History of Medical and Surgical Diseases of Women. Second Edition. 8vo, with 181 Engravings, 28s.

Gynæcological Operations:
(Handbook of). By ALBAN H. G. DORAN, F.R.C.S., Surgeon to the Samaritan Hospital. 8vo, with 167 Engravings, 15s.

The Student's Guide to the Diseases of Women. By ALFRED L. GALABIN, M.A., M.D., F.R.C.P., Obstetric Physician to Guy's Hospital. Fifth Edition. Fcap. 8vo, with 142 Engravings, 8s. 6d.

A Practical Treatise on the Diseases of Women. By T. GAILLARD THOMAS, M.D. Sixth Edition, by PAUL F. MUNDÉ, M.D. Roy. 8vo, with 347 Engravings, 25s.

A First Series of Fifty-four Consecutive Ovariotomies, with Fifty-three Recoveries. By A. C. BUTLER-SMYTHE, F.R.C.P. Edin., Surgeon to the Samaritan Free Hospital, Senior Surgeon to the Grosvenor Hospital for Women and Children. 8vo, 6s. 6d.

Sterility.
By ROBERT BELL, M.D., F.F.P. & S., Senior Physician to the Glasgow Hospital for Diseases peculiar to Women. 8vo, 5s.

Notes on Gynæcological Nursing. By JOHN BENJAMIN HELLIER, M.D., M.R.C.S., Lecturer on the Diseases of Women and Children in the Yorkshire College, and Surgeon to the Hospital for Women, &c., Leeds. Cr. 8vo, 1s. 6d.

A Manual for Hospital Nurses and others engaged in Attending on the Sick, with a Glossary. By EDWARD J. DOMVILLE, Surgeon to the Devon and Exeter Hospital. Eighth Edition. Crown 8vo, 2s. 6d.

A Manual of Nursing, Medical and Surgical. By CHARLES J. CULLINGWORTH, M.D., F.R.C.P., Obstetric Physician to St. Thomas's Hospital. Third Edition. Fcap. 8vo, with Engravings, 2s. 6d.

By the same Author.

A Short Manual for Monthly Nurses. Revised by M. A. ATKINSON. Fourth Edition. Fcap. 8vo, 1s. 6d.

Lectures on Medicine to Nurses.
By HERBERT E. CUFF, M.D., F.R.C.S., Superintendent, North Eastern Fever Hospital, London. Second Edition. With 29 Illustrations, 3s. 6d.

Antiseptic Principles for Nurses.
By C. E. RICHMOND, F.R.C.S. 8vo, 1s.

Diseases of Children.
For Practitioners and Students. By W. H. DAY, M.D., Physician to the Samaritan Hospital. Second Edition Crown 8vo, 12s. 6d.

The Diseases of Children.
By JAS. F. GOODHART, M.D., F.R.C.P., Physician to Guy's Hospital. Fifth Edition. Fcap. 8vo, 10s. 6d.

A Practical Treatise on Disease in Children. By EUSTACE SMITH, M.D., F.R.C.P., Physician to the King of the Belgians, and to the East London Hospital for Children, &c. Second Edition. 8vo, 22s.

By the same Author.

Clinical Studies of Disease in Children. Second Edition. Post 8vo, 7s. 6d.

Also.

The Wasting Diseases of Infants and Children. Fifth Edition. Post 8vo, 8s. 6d.

On the Natural and Artificial Methods of Feeding Infants and Young Children. By EDMUND CAUTLEY, M.D., Physician to the Belgrave Hospital for Children. Crown 8vo, 7s. 6d.

Materia Medica,
Pharmacy, Pharmacology, and Therapeutics. By W. HALE WHITE, M.D., F.R.C.P., Physician to, and Lecturer on Pharmacology and Therapeutics at, Guy's Hospital. Third Edition, based upon the B.P. of 1898. Fcap. 8vo, 7s. 6d.

Materia Medica
And Therapeutics. By CHARLES D. F. PHILLIPS, M.D., F.R.S. Edin.
Vegetable Kingdom — Organic Compounds — Animal Kingdom. 8vo, 25s.
Inorganic Substances. Second Edition. 8vo, 21s.

Recent Materia Medica, and Drugs Occasionally Prescribed; Notes on their Origin and Therapeutics. By F. HARWOOD LESCHER, F.C.S., Pereira Medallist. Fifth Edition. 8vo, 4s.

Practical Pharmacy:
An Account of the Methods of Manufacturing and Dispensing Pharmaceutical Preparations; with a chapter on the Analysis of Urine. By E. W. LUCAS, F.C.S., Examiner at the Pharmaceutical Society. With 283 Illustrations. Royal 8vo, 12s. 6d.

Galenic Pharmacy:
A Practical Handbook to the Processes of the British Pharmacopœia. By R. A. CRIPPS, M.P.S. 8vo, with 76 Engravings, 8s. 6d.

The Galenical Preparations of the British Pharmacopœia: A Handbook for Medical and Pharmaceutical Students. By CHARLES O. HAWTHORNE, M.B., C.M., Lecturer on Materia Medica and Therapeutics, Queen Margaret College, University of Glasgow. 8vo, 4s. 6d.

Practical Pharmacy.
By BARNARD S. PROCTOR, formerly Lecturer on Pharmacy at the College of Medicine, Newcastle-on-Tyne. Third Edition. 8vo, with 44 Wood Engravings and 32 Lithograph Fac-Simile Prescriptions, 14s.

A Companion to the British Pharmacopœia. By PETER SQUIRE, Revised by his Sons, P. W. and A. H. SQUIRE. Sixteenth Edition. 8vo, 12s. 6d.

By the same Authors.

The Pharmacopœias of the London Hospitals, arranged in Groups for Easy Reference and Comparison. Sixth Edition. 18mo. 6s.

Pocket Formulary
And Synopsis of the British and Foreign Pharmacopœias. By HENRY BEASLEY. Eleventh Edition. 18mo, 6s. 6d.

By the same Author.

Druggist's General Receipt-Book. Tenth Edition. 18mo, 6s. 6d.

Also.

Book of Prescriptions:
Containing upwards of 3,000 Prescriptions from the Practice of the most eminent Physicians and Surgeons, English and Foreign. Seventh Edition. 18mo, 6s. 6d.

Selecta è Prescriptis:
Containing Terms, Phrases, Contractions and Abbreviations used in Prescriptions, with Explanatory Notes, &c. Also, a Series of Abbreviated Prescriptions with Translations and Key. By J. PEREIRA, M.D., F.R.S. Eighteenth Edition, by JOSEPH INCE, F.C.S., F.L.S. 24mo, 5s.

Year-Book of Pharmacy:
Containing the Transactions of the British Pharmaceutical Conference. Annually. 8vo, 10s.

Royle's Manual of Materia Medica and Therapeutics. Sixth Edition, including additions and alterations in the B.P. 1885. By JOHN HARLEY, M.D., Physician to St. Thomas's Hospital. Crown 8vo, with 139 Engravings, 15s.

Southall's Organic Materia Medica: a Handbook treating of some of the more important of the Animal and Vegetable Drugs made use of in Medicine, including the whole of those contained in the B.P. Fifth and Enlarged Edition. By JOHN BARCLAY, B.Sc.Lond., some time Lecturer on Materia Medica and Pharmacy in Mason College, Birmingham. 8vo, 6s.

Manual of Botany.
By J. REYNOLDS GREEN, Sc.D., M.A., F.R.S., Professor of Botany to the Pharmaceutical Society. Two Vols. Cr. 8vo.
Vol. I.—Morphology and Anatomy. Second Edition. With 778 Engravings, 7s. 6d.
,, II.—Classification and Physiology. With 415 Engravings, 10s.

The Student's Guide to Systematic Botany, including the Classification of Plants and Descriptive Botany. By ROBERT BENTLEY. Fcap. 8vo, with 350 Engravings, 3s. 6d.

Medicinal Plants:
Being descriptions, with original figures, of the Principal Plants employed in Medicine, and an account of their Properties and Uses. By Prof. BENTLEY and Dr. H. TRIMEN, F.R.S. In 4 vols., large 8vo, with 306 Coloured Plates, bound in Half Morocco, Gilt Edges, £11 11s.

Climate and Fevers of India, with a series of Cases (Croonian Lectures, 1882). By Sir JOSEPH FAYRER, K.C.S.I., M.D. 8vo, with 17 Temperature Charts, 12s.

By the same Author.

The Natural History and Epidemiology of Cholera: Being the Annual Oration of the Medical Society of London, 1888. 8vo, 3s. 6d.

Psilosis or "Sprue," its Nature and Treatment; with Observations on various Forms of Diarrhœa acquired in the Tropics. By GEORGE THIN, M.D. Second and enlarged Edition, with Illustrations, 8vo, 10s.

A Manual of Family Medicine and Hygiene for India. Published under the Authority of the Government of India. By Sir WILLIAM J. MOORE, K.C.I.E., M.D., late Surgeon-General with the Government of Bombay. Sixth Edition. Post 8vo, with 71 Engravings, 12s.

By the same Author.

A Manual of the Diseases of India: With a Compendium of Diseases generally. Second Edition. Post 8vo, 10s.

The Prevention of Disease in Tropical and Sub-Tropical Campaigns. (Parkes Memorial Prize for 1886.) By Lieut.-Col. ANDREW DUNCAN, M.D., B.S. Lond., F.R.C.S., Indian Medical Service. 8vo, 12s. 6d.

Practical Therapeutics:
A Manual. By EDWARD J. WARING, C.I.E., M.D., F.R.C.P., and DUDLEY W. BUXTON, M.D., B.S. Lond. Fourth Edition. Crown 8vo, 14s.

By the same Author.

Bazaar Medicines of India,
And Common Medical Plants: With Full Index of Diseases, indicating their Treatment by these and other Agents procurable throughout India, &c. Fifth Edition. Fcap. 8vo, 5s.

A Commentary on the Diseases of India. By NORMAN CHEVERS, C.I.E., M.D., F.R.C.S., Deputy Surgeon-General H.M. Indian Army. 8vo, 24s.

Hooper's Physicians' Vade-Mecum. A Manual of the Principles and Practice of Physic. Tenth Edition. By W. A. GUY, F.R.C.P., F.R.S., and J. HARLEY, M.D., F.R.C.P. With 118 Engravings. Fcap. 8vo, 12s. 6d.

The Principles and Practice of Medicine. (Text-book.) By the late C. HILTON FAGGE, M.D., and P. H. PYE-SMITH, M.D., F.R.S., F.R.C.P., Physician to, and Lecturer on Medicine in, Guy's Hospital. Third Edition. 2 vols. 8vo, cloth, 40s.; Half Leather, 46s.

Manual of the Practice of Medicine. By FREDERICK TAYLOR, M.D., F.R.C.P., Physician to, and Lecturer on Medicine at, Guy's Hospital. Fifth Edition. 8vo, with Engravings, 16s.

The Practice of Medicine (Student's Guide Series). By M. CHARTERIS, M.D., Professor of Therapeutics and Materia Medica in the University of Glasgow. Seventh Edition. Fcap. 8vo, with Engravings on Copper and Wood, 10s.

A Dictionary of Practical Medicine. By various writers. Edited by JAS. KINGSTON FOWLER, M.A., M.D., F.R.C.P., Physician to Middlesex Hospital and the Hospital for Consumption. 8vo, cloth, 21s.; half calf, 25s.

A Text-Book of Bacteriology, for Medical Students and Practitioners. By G. M. STERNBERG, M.D., Surgeon-General, U.S. Army. With 9 Plates and 200 Figures in the Text. 8vo, 24s.

How to Examine the Chest: A Practical Guide for the use of Students. By SAMUEL WEST, M.D., F.R.C.P., Assistant Physician to St. Bartholomew's Hospital. Second Edition. With Engravings. Fcap. 8vo, 5s.

An Atlas of the Pathological Anatomy of the Lungs. By the late WILSON FOX, M.D., F.R.S., F.R.C.P., Physician to H.M. the Queen. With 45 Plates (mostly Coloured) and Engravings. 4to, half-bound in Calf, 70s.

By the same Author.

A Treatise on Diseases of the Lungs and Pleura. Edited by SIDNEY COUPLAND, M.D., F.R.C.P., Physician to Middlesex Hospital. Roy. 8vo, with Engravings; also Portrait and Memoir of the Author, 36s.

The Student's Guide to Diseases of the Chest. By VINCENT D. HARRIS, M.D. Lond., F.R.C.P., Physician to the City of London Hospital for Diseases of the Chest, Victoria Park. Fcap. 8vo, with 55 Illustrations (some Coloured), 7s. 6d.

Uric Acid as a Factor in the Causation of Disease. By ALEXANDER HAIG, M.D., F.R.C.P., Physician to the Metropolitan Hospital and the Royal Hospital for Children and Women. Fourth Edition. With 65 Illustrations, 8vo, 12s. 6d.

Medical Diagnosis (Student's Guide Series). By SAMUEL FENWICK, M.D., F.R.C.P., and W. SOLTAU FENWICK, M.D., B.S. Eighth Edition. Crown 8vo, with 135 Engravings, 9s.

By the same Author.

Outlines of Medical Treatment. Fourth Edition. Crown 8vo, with 35 Engravings, 10s.

Also.

Clinical Lectures on Some Obscure Diseases of the Abdomen. Delivered at the London Hospital. 8vo, with Engravings, 7s. 6d.

Also.

The Saliva as a Test for Functional Diseases of the Liver. Crown 8vo, 2s.

The Microscope in Medicine. By LIONEL S. BEALE, M.B., F.R.S., Consulting Physician to King's College Hospital. Fourth Edition. 8vo, with 86 Plates, 21s.

By the same Author.

The Liver. With 24 Plates (85 Figures). 8vo, 5s.

Also.

On Slight Ailments: And on Treating Disease. Fourth Edition. 8vo, 5s.

Myxœdema and the Thyroid Gland. By JOHN D. GIMLETTE, M.R.C.S., L.R.C.P. Crown 8vo, 5s.

The Blood: How to Examine and Diagnose its Diseases. By ALFRED C. COLES, M.D., B.Sc. With 6 Coloured Plates. 8vo, 10s. 6d.

The Physiology of the Carbohydrates; their Application as Food and Relation to Diabetes. By F. W. PAVY, M.D., LL.D., F.R.S., F.R.C.P., Consulting Physician to Guy's Hospital. Royal 8vo, with Plates and Engravings, 10s. 6d.

Medical Lectures and Essays. By Sir G. JOHNSON, M.D., F.R.C.P., F.R.S. 8vo, with 46 Engravings, 25s.

By the same Author.

An Essay on Asphyxia (Apnœa). 8vo, 3s.

Also.

History of the Cholera Controversy, with Directions for the Treatment of the Disease. 8vo, 3s.

Bronchial Asthma: Its Pathology and Treatment. By J. B. BERKART, M.D., late Physician to the City of London Hospital for Diseases of the Chest. Second Edition, with 7 Plates (35 Figures). 8vo, 10s. 6d.

Treatment of Some of the Forms of Valvular Disease of the Heart. By A. E. SANSOM, M.D., F.R.C.P., Physician to the London Hospital. Second Edition. Fcap. 8vo, with 26 Engravings, 4s. 6d.

The Schott Methods of the Treatment of Chronic Diseases of the Heart, with an account of the Nauheim Baths and of the Therapeutic Exercises. By W. BEZLY THORNE, M.D., M.R.C.P. Second Edition. 8vo, with Illustrations, 5s.

Guy's Hospital Reports. By the Medical and Surgical Staff. Vol. XXXVII. Third Series. 8vo, 10s. 6d.

St. Thomas's Hospital Reports. By the Medical and Surgical Staff. Vol. XXIV. New Series. 8vo, 8s. 6d.

Westminster Hospital Reports. By the Medical and Surgical Staff. Vol. X. 8vo, 8s.

Medical Ophthalmoscopy: A Manual and Atlas. By SIR WILLIAM R. GOWERS, M.D., F.R.C.P., F.R.S. Third Edition. Edited with the assistance of MARCUS GUNN, M.B., F.R.C.S., Surgeon to the Royal London Ophthalmic Hospital. With Coloured Plates and Woodcuts. 8vo, 16s.

By the same Author.

A Manual of Diseases of the Nervous System. Roy. 8vo.

Vol. I. Spinal Cord and Nerves. Second Edition. With 179 Engravings, 15s.

Vol. II. Brain and Cranial Nerves: General and Functional Diseases. Second Edition. With 182 Engravings, 20s.

Also.

Clinical Lectures on Diseases of the Nervous System. 8vo, 7s. 6d.

Also.

Diagnosis of Diseases of the Brain. Second Edition. 8vo, with Engravings, 7s. 6d.

Also.

Syphilis and the Nervous System. Lettsomian Lectures for 1890. Delivered before the Medical Society of London. 8vo, 4s.

The Nervous System, Diseases of. By J. A. ORMEROD, M.D., F.R.C.P., Physician to the National Hospital for the Paralysed and Epileptic. With 66 Illustrations. Fcap. 8vo, 8s. 6d.

Text-Book of Nervous Diseases, for Students and Practitioners of Medicine. By CHARLES L. DANA, M.D., Professor of Nervous and Mental Diseases in Bellevue Hospital Medical College, New York. Fourth Edition, with 246 Illustrations, 8vo, 20s.

Handbook of the Diseases of the Nervous System. By JAMES ROSS, M.D., F.R.C.P., Professor of Medicine in the Victoria University, and Physician to the Royal Infirmary, Manchester. Roy. 8vo, with 184 Engravings, 18s.

Also.

Aphasia: Being a Contribution to the Subject of the Dissolution of Speech from Cerebral Disease. 8vo, with Engravings. 4s. 6d.

Diseases of the Nervous System. Lectures delivered at Guy's Hospital. By SIR SAMUEL WILKS, BART., M.D., F.R.S. Second Edition. 8vo, 18s.

Stammering: Its Causes, Treatment, and Cure. By A. G. BERNARD, M.R.C.S., L.R.C.P. Crown 8vo, 2s.

Secondary Degenerations of the Spinal Cord (Gulstonian Lectures, 1889). By HOWARD H. TOOTH, M.D., F.R.C.P., Assistant Physician to the National Hospital for the Paralysed and Epileptic. With Plates and Engravings. 8vo, 3s. 6d.

Diseases of the Nervous System. Clinical Lectures. By THOMAS BUZZARD, M.D., F.R.C.P., Physician to the National Hospital for the Paralysed and Epileptic. With Engravings, 8vo. 15s.

By the same Author.

Some Forms of Paralysis from Peripheral Neuritis: of Gouty, Alcoholic, Diphtheritic, and other origin. Crown 8vo, 5s.

Also.

On the Simulation of Hysteria by Organic Disease of the Nervous System. Crown 8vo, 4s. 6d.

Gout in its Clinical Aspects. By J. MORTIMER GRANVILLE, M.D. Crown 8vo, 6s.

Diseases of the Liver: With and without Jaundice By GEORGE HARLEY, M.D., F.R.C.P., F.R.S. 8vo, with 2 Plates and 36 Engravings, 21s.

Rheumatic Diseases, (Differentiation in). By HUGH LANE, Surgeon to the Royal Mineral Water Hospital, Bath, and Hon. Medical Officer to the Royal United Hospital, Bath. Second Edition, much Enlarged, with 8 Plates. Crown 8vo, 3s. 6d.

Diseases of the Abdomen, Comprising those of the Stomach and other parts of the Alimentary Canal, Œsophagus, Cæcum, Intestines, and Peritoneum. By S. O. HABERSHON, M.D., F.R.C.P. Fourth Edition. 8vo, with 5 Plates, 21s.

On Gallstones, or Cholelithiasis. By E. M. BROCKBANK, M.D. Vict., M.R.C.P. Lond., late Resident Medical Officer at the Manchester Royal Infirmary and the Birmingham General Hospital. Crown 8vo, 7s.

On the Relief of Excessive and Dangerous Tympanites by Puncturing the Abdomen. By JOHN W. OGLE, M.D., Consulting Physician to St. George's Hospital. 8vo, 5s. 6d.

Headaches: Their Nature, Causes, and Treatment. By W. H. DAY, M.D., Physician to the Samaritan Hospital. Fourth Edition. Crown 8vo, with Engravings, 7s. 6d.

A Handbook of Medical Climatology; embodying its Principles and Therapeutic Application, with Scientific Data of the Chief Health Resorts of the World. By S. EDWIN SOLLY, M.D., M.R.C.S., late President of the American Climatological Association. With Engravings and Coloured Plates, 8vo., 16s.

The Mineral Waters of France And its Wintering Stations (Medical Guide to). With a Special Map. By A. VINTRAS, M.D., Physician to the French Embassy, and to the French Hospital, London. Second Edition. Crown 8vo, 8s.

Canary Islands Health Resorts, in their Climatological and Medical Aspects. By J. CLEASBY TAYLOR, M.D., M.R.C.S., Las Palmas. 8vo, with Maps, 3s. 6d.

Illustrated Ambulance Lectures: To which is added a NURSING LECTURE. By JOHN M. H. MARTIN, M.D., F.R.C.S., Honorary Surgeon to the Blackburn Infirmary. Fourth Edition. Crown 8vo, with 60 Engravings, 2s.

Surgery: its Theory and Practice. By WILLIAM J. WALSHAM, F.R.C.S., Senior Assistant Surgeon to, and Lecturer on Anatomy at, St. Bartholomew's Hospital. Sixth Edition. Crown 8vo, with 410 Engravings, 12s. 6d.

The Surgeon's Vade-Mecum: A Manual of Modern Surgery. By R. DRUITT, F.R.C.S. Twelfth Edition. By STANLEY BOYD, M.B., F.R.C.S. Crown 8vo, with 373 Engravings, 16s.

Operations on the Brain (A Guide to). By ALEC FRASER, Professor of Anatomy, Royal College of Surgeons in Ireland. Illustrated by 42 life-size Plates in Autotype, and 2 Woodcuts in the text. Folio, 63s.

The Operations of Surgery: Intended for Use on the Dead and Living Subject alike. By W. H. A. JACOBSON, M.A., M.B., M.Ch. Oxon., F.R.C.S., Assistant Surgeon to, and Lecturer on Anatomy at, Guy's Hospital. Third Edition. 8vo, with 401 Illustrations, 34s.

A Course of Operative Surgery. By CHRISTOPHER HEATH, Surgeon to University College Hospital. Second Edition. With 20 coloured Plates (180 figures) from Nature, by M. LÉVEILLÉ, and several Woodcuts. Large 8vo, 30s.

By the same Author.

The Student's Guide to Surgical Diagnosis. Second Edition. Fcap. 8vo, 6s. 6d.

Also.

Manual of Minor Surgery and Bandaging. For the use of House-Surgeons, Dressers, and Junior Practitioners. Eleventh Edition. Fcap. 8vo, with 176 Engravings, 6s.

Also.

Injuries and Diseases of the Jaws. Fourth Edition. By HENRY PERCY DEAN, M.S., F.R.C.S., Assistant Surgeon to the London Hospital. 8vo, with 187 Wood Engravings, 14s.

Also.

Lectures on Certain Diseases of the Jaws. Delivered at the R.C.S., Eng., 1887. 8vo, with 64 Engravings, 2s. 6d.

Also.

Clinical Lectures on Surgical Subjects. Delivered in University College Hospital. Second Edition, Enlarged. Fcap. 8vo, with 27 Engravings, 6s.

Surgery. By C. W. MANSELL MOULLIN, M.A., M.D., Oxon., F.R.C.S., Surgeon and Lecturer on Physiology to the London Hospital. Large 8vo, with 497 Engravings, 34s.

The Practice of Surgery: A Manual. By THOMAS BRYANT, Consulting Surgeon to Guy's Hospital. Fourth Edition. 2 vols. crown 8vo, with 750 Engravings (many being coloured), and including 6 chromo plates, 32s.

Surgical Emergencies: Together with the Emergencies attendant on Parturition and the Treatment of Poisoning. By PAUL SWAIN, F.R.C.S., Surgeon to the South Devon and East Cornwall Hospital. Fifth Edition. Crown 8vo, with 149 Engravings, 6s.

Diseases of Bones and Joints. By CHARLES MACNAMARA, F.R.C.S., Surgeon to, and Lecturer on Surgery at, the Westminster Hospital. 8vo, with Plates and Engravings, 12s.

Abdominal Surgery. By J. GREIG SMITH, M.A., F.R.S.E. Sixth Edition. Edited by JAMES SWAIN, M.S., M.D. Lond., F.R.C.S. Eng., Assistant Surgeon to the Bristol Royal Infirmary, Professor of Surgery, University College, Bristol. Two Vols., 8vo, with 224 Engravings, 36s.

The Surgery of the Alimentary Canal. By ALFRED ERNEST MAYLARD, M.B. Lond. and B.S., Surgeon to the Victoria Infirmary, Glasgow. With 27 Swantype Plates and 89 Figures in the text, 8vo, 25s.

Ovariotomy and Abdominal Surgery. By HARRISON CRIPPS, F.R.C.S., Surgical Staff, St. Bartholomew's Hospital. With numerous Plates, 8vo, 25s.

The Physiology of Death from Traumatic Fever; A Study in Abdominal Surgery. By JOHN D. MALCOLM, M.B., C.M., F.R.C.S.E., Surgeon to the Samaritan Free Hospital. 8vo, 3s. 6d.

On Anchylosis.
By BERNARD E. BRODHURST, F.R.C.S., Surgeon to the Royal Orthopædic Hospital. Fourth Edition. 8vo, with Engravings, 5s.

By the same Author.

Curvatures and Disease of the Spine. Fourth Edition. 8vo, with Engravings, 7s. 6d

Also.

Talipes Equino-Varus, or Club-foot. 8vo, with Engravings, 3s. 6d.

Also.

Observations on Congenital Dislocation of the Hip. Third Edition. 8vo, 2s. 6d.

Surgical Pathology and Morbid Anatomy. By ANTHONY A. BOWLBY, F.R.C.S., Assistant Surgeon to St. Bartholomew's Hospital. Third Edition. Crown 8vo, with 183 Engravings, 10s. 6d.

By the same Author.

Injuries and Diseases of Nerves and their Surgical Treatment. 8vo, with 20 Plates, 14s.

The Deformities of the Fingers and Toes. By WILLIAM ANDERSON, F.R.C.S., Surgeon to St. Thomas's Hospital. 8vo, with 18 Engravings, 6s.

Short Manual of Orthopædy.
By HEATHER BIGG, F.R.C.S. Ed. Part I. Deformities and Deficiencies of the Head and Neck. 8vo, 2s. 6d.

The Human Foot:
Its Form and Structure, Functions and Clothing. By THOMAS S. ELLIS, Consulting Surgeon to the Gloucester Infirmary. With 7 Plates and Engravings (50 Figures). 8vo, 7s. 6d.

Face and Foot Deformities.
By FREDERICK CHURCHILL, C.M. 8vo, with Plates and Illustrations, 10s. 6d.

Royal London Ophthalmic Hospital Reports. By the Medical and Surgical Staff. Vol. XIV., Part 2. 8vo, 5s.

Ophthalmological Society
of the United Kingdom. Transactions, Vol. XVII. 8vo, 12s. 6d.

Manual of Ophthalmic Surgery and Medicine. By W. H. H. JESSOP, M.A., F.R.C.S., Ophthalmic Surgeon to St. Bartholomew's Hospital. With 5 Coloured Plates and 110 Woodcuts, crown 8vo, 9s. 6d.

Nettleship's Diseases of the Eye: A Manual for Students. Sixth Edition, revised and edited by W. T. HOLMES SPICER, M.B., F.R.C.S., Ophthalmic Surgeon to the Metropolitan Hospital and the Victoria Hospital for Children. With 161 Engravings and a Coloured Plate illustrating Colour-Blindness, crown 8vo, 8s. 6d.

Diseases and Refraction of the Eye. By N. C. MACNAMARA, F.R.C.S., Surgeon to Westminster Hospital, and GUSTAVUS HARTRIDGE, F.R.C.S., Surgeon to the Royal Westminster Ophthalmic Hospital. Fifth Edition. Crown 8vo, with Plate, 156 Engravings, also Test-types, 10s. 6d.

Diseases of the Eye: a Practical Handbook for General Practitioners and Students. By CECIL EDWARD SHAW, M.D., M.Ch., Ophthalmic Surgeon to the Ulster Hospital for Children and Women, Belfast. With a Test-Card for Colour-Blindness. Crown 8vo, 3s. 6d.

On Diseases and Injuries of the Eye: A Course of Systematic and Clinical Lectures to Students and Medical Practitioners. By J. R. WOLFE, M.D., F.R.C.S.E., Lecturer on Ophthalmic Medicine and Surgery in Anderson's College, Glasgow. With 10 Coloured Plates and 157 Wood Engravings. 8vo, £1 1s.

Normal and Pathological Histology of the Human Eye and Eyelids. By C. FRED. POLLOCK, M.D., F.R.C.S. and F.R.S.E., Surgeon for Diseases of the Eye to Anderson's College Dispensary, Glasgow. Crown 8vo, with 100 Plates (230 drawings), 15s.

Convergent Strabismus, and its Treatment; an Essay. By EDWIN HOLTHOUSE, M.A., F.R.C.S., Surgeon to the Western Ophthalmic Hospital. 8vo, 6s.

Refraction of the Eye:
A Manual for Students. By GUSTAVUS HARTRIDGE, F.R.C.S., Surgeon to the Royal Westminster Ophthalmic Hospital. Ninth Edition. Crown 8vo, with 104 Illustrations, also Test-types, &c., 6s.

By the same Author.

The Ophthalmoscope: A Manual for Students. Third Edition. Crown 8vo, with 68 Illustrations and 4 Plates. 4s. 6d.

Methods of Operating for Cataract and Secondary Impairments of Vision, with the results of 500 cases. By G. H. FINK, H.M. Indian Medical Service. Crown 8vo, with 15 Engravings, 5s.

Atlas of Ophthalmoscopy.
Composed of 12 Chromo-lithographic Plates (59 Figures drawn from nature) and Explanatory Text. By RICHARD LIEBREICH, M.R.C.S. Translated by H. ROSBOROUGH SWANZY, M.B. Third Edition, 4to, 40s.

Glaucoma:
Its Pathology and Treatment. By PRIESTLEY SMITH, Ophthalmic Surgeon to, and Clinical Lecturer on Ophthalmology at, the Queen's Hospital, Birmingham. 8vo, with 64 Engravings and 12 Zinco-photographs, 7s. 6d.

Eyestrain
(commonly called Asthenopia). By ERNEST CLARKE, M.D., B.S. Lond., Surgeon to the Central London Ophthalmic Hospital, Surgeon and Ophthalmic Surgeon to the Miller Hospital. Second Edition. 8vo, with 22 Illustrations, 5s.

Hints on Ophthalmic Out-Patient Practice. By CHARLES HIGGENS, Ophthalmic Surgeon to Guy's Hospital. Third Edition. Fcap. 8vo, 3s.

Diseases and Injuries of the Ear. By Sir WILLIAM B. DALBY, F.R.C.S., M.B., Consulting Aural Surgeon to St. George's Hospital. Fourth Edition. Crown 8vo, with 8 Coloured Plates and 38 Wood Engravings. 10s. 6d.

By the same Author.

Short Contributions to Aural Surgery, between 1875 and 1896. Third Edition. 8vo, with Engravings, 5s.

Diseases of the Ear,
Including the Anatomy and Physiology of the Organ, together with the Treatment of the Affections of the Nose and Pharynx, which conduce to Aural Disease. By T. MARK HOVELL, F.R.C.S.E., Aural Surgeon to the London Hospital, and Lecturer on Diseases of the Throat in the College, &c. 8vo, with 122 Engravings, 18s.

A System of Dental Surgery.
By Sir JOHN TOMES, F.R S., and C. S. TOMES, M.A., F.R.S. Fourth Edition. 8vo, with 289 Engravings, 16s.

Dental Anatomy, Human and Comparative: A Manual. By CHARLES S. TOMES, M.A., F.R.S. Fifth Edition. Crown 8vo, with 263 Engravings, 14s.

Dental Materia Medica, Pharmacology and Therapeutics. By CHARLES W. GLASSINGTON, M.R.C.S., L.D.S. Edin.; Senior Dental Surgeon, Westminster Hospital; Dental Surgeon, National Dental Hospital; and Lecturer on Dental Materia Medica and Therapeutics to the College. Crown 8vo, 6s.

A Manual of Dental Metallurgy.
By ERNEST A. SMITH, F.I.C., Assistant Instructor in Metallurgy, Royal College of Science, London. With 37 Illustrations. Crown 8vo, 6s. 6d.

A Practical Treatise on Mechanical Dentistry. By JOSEPH RICHARDSON, M.D., D.D.S. Seventh Edition revised and Edited by GEORGE W. WARREN, D.D.S. Roy. 8vo, with 690 Engravings, 22s.

A Manual of Nitrous Oxide Anæsthesia, for the use of Students and General Practitioners. By J. FREDERICK W. SILK, M.D. Lond., M.R.C.S., Anæsthetist to the Royal Free Hospital, Dental School of Guy's Hospital, and National Epileptic Hospital. 8vo, with 26 Engravings, 5s.

Diseases of the Skin:
A Practical Treatise for the Use of Students and Practitioners. By J. N. HYDE, A.M., M.D., Professor of Skin and Venereal Diseases, Rush Medical College, Chicago. Second Edition. 8vo, with 2 Coloured Plates and 96 Engravings, 20s.

Skin Diseases of Children. By GEO. H. FOX, M.D., Clinical Professor of Diseases of the Skin, College of Physicians and Surgeons, New York. With 12 Photogravure and Chromographic Plates, and 60 Illustrations in the Text, Roy. 8vo, 12s. 6d.

A Handbook on Leprosy.
By S. P. IMPEY, M.D., M.C., late Chief and Medical Superintendent, Robben Island Leper and Lunatic Asylums, Cape Colony. With 38 Plates and Map, 8vo, 12s.

Leprosy in British Guiana.
By JOHN D. HILLIS, F.R.C.S., M.R.I.A., late Medical Superintendent of the Leper Asylum, British Guiana. Imp. 8vo, with 22 Lithographic Coloured Plates and Wood Engravings, £1 11s. 6d.

Diseases of the Skin (Introduction to the Study of). By P. H. PYE-SMITH, M. D., F. R. S., F.R.C.P., Physician to, and Lecturer on Medicine in, Guy's Hospital. Crown 8vo, with 26 Engravings. 7s. 6d.

Sarcoma and Carcinoma: Their Pathology, Diagnosis, and Treatment. By HENRY T. BUTLIN, F.R.C.S., Assistant Surgeon to St. Bartholomew's Hospital. 8vo, with 4 Plates, 8s.

By the same Author.

Malignant Disease of the Larynx (Sarcoma and Carcinoma). 8vo, with 5 Engravings, 5s.

Also.

Operative Surgery of Malignant Disease. 8vo, 14s.

On Cancer: Its Allies, and other Tumours; their Medical and Surgical Treatment. By F. A. PURCELL, M.D., M.C., Surgeon to the Cancer Hospital, Brompton. 8vo, with 21 Engravings, 10s. 6d.

Cancers and the Cancer Process: a Treatise, Practical and Theoretic. By HERBERT L. SNOW, M.D., Surgeon to the Cancer Hospital, Brompton. 8vo, with 15 Lithographic Plates. 15s.

By the same Author.

The Re-appearance (Recurrence) of Cancer after apparent Extirpation. 8vo, 5s. 6d.

Also,

The Palliative Treatment of Incurable Cancer. Crown 8vo, 2s. 6d.

Cancerous Affections of the Skin. (Epithelioma and Rodent Ulcer.) By GEORGE THIN, M.D. Post 8vo, with 8 Engravings, 5s.

By the same Author.

Pathology and Treatment of Ringworm. 8vo, with 21 Engravings, 5s.

Diagnosis and Treatment of Syphilis. By TOM ROBINSON, M.D., Physician to the Western Skin Hospital. Crown 8vo, 3s. 6d.

By the same Author.

Eczema: its Etiology, Pathology, and Treatment. Crown 8vo, 3s. 6d.

Also.

Illustrations of Diseases of the Skin and Syphilis, with Remarks. Fasc. I. with 3 Plates. Imp. 4to, 5s.

BY SIR HENRY THOMPSON, F.R.C.S.

Diseases of the Urinary Organs. Clinical Lectures. Eighth Edition. 8vo, with 121 Engravings, 10s. 6d.

Surgery of the Urinary Organs. Some Important Points connected therewith. Lectures delivered in the R.C.S. 8vo, with 44 Engravings. Student's Edition, 2s. 6d.

Practical Lithotomy and Lithotrity; or, An Inquiry into the Best Modes of Removing Stone from the Bladder. Third Edition. 8vo, with 87 Engravings, 10s.

The Preventive Treatment of Calculous Disease, and the Use of Solvent Remedies. Third Edition. Crown 8vo, 2s. 6d.

Tumours of the Bladder: Their Nature, Symptoms, and Surgical Treatment. 8vo, with numerous Illustrations, 5s.

Stricture of the Urethra, and Urinary Fistulæ: their Pathology and Treatment. Fourth Edition. 8vo, with 74 Engravings, 6s.

The Suprapubic Operation of Opening the Bladder for the Stone and for Tumours. 8vo, with 14 Engravings, 3s. 6d.

Electric Illumination of the Bladder and Urethra, as a Means of Diagnosis of Obscure Vesico-Urethral Diseases. By E. HURRY FENWICK, F.R.C.S., Surgeon to London Hospital and St. Peter's Hospital for Stone. Second Edition. 8vo, with 54 Engravings, 6s. 6d.

By the same Author.

Tumours of the Urinary Bladder. The Jacksonian Prize Essay of 1887, rewritten with 200 additional cases, in four Fasciculi. Fas. I. Royal 8vo., 5s.

Also.

The Cardinal Symptoms of Urinary Diseases: their Diagnostic Significance and Treatment. 8vo, with 36 Illustrations. 8s. 6d.

Atlas of Electric Cystoscopy. By Dr. EMIL BURCKHARDT, late of the Surgical Clinique of the University of Bâle, and E. HURRY FENWICK, F.R.C.S., Surgeon to the London Hospital and St. Peter's Hospital for Stone. Royal 8vo, with 34 Coloured Plates, embracing 83 Figures. 21s.

Lectures on the Surgical Disorders of the Urinary Organs. By REGINALD HARRISON, F.R.C.S., Surgeon to St. Peter's Hospital. Fourth Edition. 8vo, with 156 Engravings, 16s.

Chemistry of Urine;
A Practical Guide to the Analytical Examination of Diabetic, Albuminous, and Gouty Urine. By ALFRED H. ALLEN, F.I.C., F.C.S. With Engravings, 8vo, 7s. 6d.

Clinical Chemistry of Urine (Outlines of the). By C. A. MAC MUNN, M.A., M.D. 8vo, with 64 Engravings and Plate of Spectra, 9s.

Urinary and Renal Derangements and Calculous Disorders. By LIONEL S. BEALE, F.R.C.P., F.R.S., Consulting Physician to King's College Hospital. 8vo, 5s.

Male Organs of Generation (Diseases of). By W. H. A. JACOBSON, M.Ch. Oxon., F.R.C.S., Assistant Surgeon to Guy's Hospital. 8vo, with 88 Engravings. 22s.

The Surgical Diseases of the Genito-Urinary Organs, including Syphilis. By E. L. KEYES, M.D., Professor in Bellevue Hospital Medical College, New York (a revision of VAN BUREN and KEYES' Text-book). Roy. 8vo, with 114 Engravings, 21s.

Diseases of the Rectum and Anus. By ALFRED COOPER, F.R.C.S., Senior Surgeon to the St. Mark's Hospital for Fistula; and F. SWINFORD EDWARDS, F.R.C.S., Senior Assistant Surgeon to St. Mark's Hospital. Second Edition, with Illustrations. 8vo, 12s.

Diseases of the Rectum and Anus. By HARRISON CRIPPS, F.R.C.S., Assistant Surgeon to St. Bartholomew's Hospital, &c. Second Edition. 8vo, with 13 Lithographic Plates and numerous Wood Engravings, 12s. 6d.

By the same Author.

Cancer of the Rectum.
Especially considered with regard to its Surgical Treatment. Jacksonian Prize Essay. 8vo, with 13 Plates and several Wood Engravings, 6s.

Also.

The Passage of Air and Fæces from the Urethra. 8vo, 3s. 6d.

Syphilis.
By ALFRED COOPER, F.R.C.S., Senior Surgeon to St. Mark's Hospital for Fistula. Second Edition. Edited by EDWARD COTTERELL, F.R.C.S., Surgeon (out-patients) to the London Lock Hospital. 8vo, with 24 Full-page Plates (12 coloured), 18s.

On Maternal Syphilis, including the presence and recognition of Syphilitic Pelvic Disease in Women. By JOHN A. SHAW-MACKENZIE, M.D. 8vo, with Coloured Plates, 10s. 6d.

A Medical Vocabulary:
An Explanation of all Terms and Phrases used in the various Departments of Medical Science and Practice, their Derivation, Meaning, Application, and Pronunciation. By R. G. MAYNE, M.D., LL.D. Sixth Edition by W. W. WAGSTAFFE, B.A., F.R.C.S. Crown 8vo, 10s. 6d.

A Short Dictionary of Medical Terms. Being an Abridgment of Mayne's Vocabulary. 64mo, 2s. 6d.

Dunglison's Dictionary of Medical Science: Containing a full Explanation of its various Subjects and Terms, with their Pronunciation, Accentuation, and Derivation. Twenty-first Edition. By RICHARD J. DUNGLISON, A.M., M.D. Royal 8vo, 30s.

Terminologia Medica Polyglotta: a Concise International Dictionary of Medical Terms (French, Latin, English, German, Italian, Spanish, and Russian). By THEODORE MAXWELL, M.D., B.Sc., F.R.C.S. Edin. Royal 8vo, 16s.

A German-English Dictionary of Medical Terms. By FREDERICK TREVES, F.R.C.S., Surgeon to the London Hospital; and HUGO LANG, B.A. Crown 8vo, half-Persian calf, 12s.

A Manual of Chemistry, Theoretical and Practical. By WILLIAM A. TILDEN, D.Sc., F.R.S., Professor of Chemistry in the Royal College of Science, London; Examiner in Chemistry to the Department of Science and Art. With 2 Plates and 143 Woodcuts, crown 8vo, 10s.

Chemistry,
Inorganic and Organic. With Experiments. By CHARLES L. BLOXAM. Eighth Edition, by JOHN MILLAR THOMSON, F.R.S., Professor of Chemistry in King's College, London, and ARTHUR G. BLOXAM, Head of the Chemistry Department, The Goldsmiths' Institute, New Cross. 8vo, with nearly 300 Illustrations, 18s. 6d.

By the same Author.

Laboratory Teaching;
Or, Progressive Exercises in Practical Chemistry. Sixth Edition. By ARTHUR G. BLOXAM. Crown 8vo, with 80 Engravings, 6s. 6d.

Watts' Organic Chemistry.
Edited by WILLIAM A. TILDEN, D.Sc., F.R.S., Professor of Chemistry, Royal College of Science, London. Second Edition. Crown 8vo, with Engravings, 10s.

Practical Chemistry
And Qualitative Analysis. By FRANK CLOWES, D.Sc. Lond., late Professor of Chemistry in the University College, Nottingham. Sixth Edition. Post 8vo, with 84 Engravings and Frontispiece, 8s. 6d.

Quantitative Analysis.
By FRANK CLOWES, D.Sc. Lond., late Professor of Chemistry in the University College, Nottingham, and J. BERNARD COLEMAN, Assoc. R. C. Sci. Dublin; Professor of Chemistry, South-West London Polytechnic. Fourth Edition. Post 8vo, with 117 Engravings, 10s.

By the same Authors.

Elementary Quantitative Analysis. Post 8vo, with 62 Engravings, 4s. 6d.

Also.

Elementary Practical Chemistry and Qualitative Analysis. Post 8vo, with 54 Engravings, 3s. 6d.

Qualitative Analysis.
By R. FRESENIUS. Translated by CHARLES E. GROVES, F.R.S. Tenth Edition. 8vo, with Coloured Plate of Spectra and 46 Engravings, 15s.

By the same Author.

Quantitative Analysis.
Seventh Edition.
Vol. I., Translated by A. VACHER. 8vo, with 106 Engravings, 15s.
Vol. II., Parts 1 to 5, Translated by C. E. GROVES, F.R.S. 8vo, with Engravings, 2s. 6d. each.

Inorganic Chemistry.
By SIR EDWARD FRANKLAND, K.C.B., D.C.L., LL.D., F.R.S., and FRANCIS R. JAPP, M.A., Ph.D., F.I.C., F.R.S., Professor of Chemistry in the University of Aberdeen. 8vo, with numerous Illustrations on Stone and Wood, 24s.

Inorganic Chemistry
(A System of). By WILLIAM RAMSAY, Ph.D., F.R.S., Professor of Chemistry in University College, London. 8vo, with Engravings, 15s.

By the same Author.

Elementary Systematic Chemistry for the Use of Schools and Colleges. With Engravings. Crown 8vo, 4s. 6d.; Interleaved, 5s. 6d.

Valentin's Practical Chemistry and Qualitative and Quantitative Analysis. Edited by W. R. HODGKINSON, Ph.D., F.R.S.E., Professor of Chemistry and Physics in the Royal Military Academy, and Artillery College, Woolwich. Ninth Edition. 8vo, with Engravings and Map of Spectra, 9s. [The Tables separately, 2s. 6d.]

Practical Chemistry, Part I.
Qualitative Exercises and Analytical Tables for Students. By J. CAMPBELL BROWN, Professor of Chemistry in Victoria University and University College, Liverpool. Fourth Edition. 8vo, 2s. 6d.

Analytical Chemistry,
Notes for Students in Medicine. By ALBERT J. BERNAYS, Ph.D., F.C.S., F.I.C. Third Edition. Cr. 8vo, 4s. 6d.

The Analyst's Laboratory Companion: a Collection of Tables and Data for Chemists and Students. By ALFRED E. JOHNSON, A.R.C.S.I., F.I.C. Second Edition, enlarged, crown 8vo., cloth, 5s., leather, 6s. 6d.

Volumetric Analysis:
Or the Quantitative Estimation of Chemical Substances by Measure, applied to Liquids, Solids, and Gases. By FRANCIS SUTTON, F.C.S., F.I.C. Seventh Edition. 8vo, with 112 Engravings, 18s. 6d.

Commercial Organic Analysis:
A Treatise on the Properties, Modes of Assaying, Proximate Analytical Examination, &c., of the various Organic Chemicals and Products employed in the Arts, Manufactures, Medicine, &c. By ALFRED H. ALLEN, F.I.C., F.C.S. 8vo.
Vol. I.—Alcohols, Neutral Alcoholic Derivatives, Sugars, Starch and its Isomers, Vegetable Acids, &c. Third Edition. 14s.
Vol. II.—Fixed Oils and Fats, Hydrocarbons, Phenols, &c. With Illustrations. Third Edition in the press.
Vol. III.—Part I. Aromatic Acids, Tannins, Dyes, and Colouring Matters. Third Edition in preparation.
Vol. III.—Part II. Amines and Ammonium Bases, Hydrazines, Bases from Tar, Vegetable Alkaloids. Second Edition. 18s.
Vol. III.—Part III. Vegetable Alkaloids (concluded), Non-Basic Vegetable Bitter Principles, Animal Bases, Animal Acids, Cyanogen and its Derivatives. Second Edition. 16s.
Vol. IV.—Proteïds and Albuminous Principles, Proteoïds or Albuminoïds. Second Edition. 18s.

Cooley's Cyclopædia
of Practical Receipts, and Collateral Information in the Arts, Manufactures, Professions, and Trades: Including Medicine, Pharmacy, Hygiene and Domestic Economy. Seventh Edition, by W. NORTH, M.A. Camb., F.C.S. 2 Vols., Roy. 8vo with 371 Engravings, 42s.

Chemical Technology:
A Manual. By RUDOLF VON WAGNER. Translated and Edited by SIR WILLIAM CROOKES, F.R.S., from the Thirteenth Enlarged German Edition as remodelled by Dr. FERDINAND FISCHER. 8vo, with 596 Engravings, 32s.

Chemical Technology;
Or, Chemistry in its Applications to Arts and Manufactures. Edited by CHARLES E. GROVES, F.R.S., and WILLIAM THORP, B.Sc.

Vol. I.—FUEL AND ITS APPLICATIONS. By E. J. MILLS, D.Sc., F.R.S., and F. J. ROWAN, C.E. Royal 8vo, with 606 Engravings, 30s.

Vol. II.—LIGHTING BY CANDLES AND OIL. By W. Y. DENT, J. MCARTHUR, L. FIELD and F. A. FIELD, BOVERTON REDWOOD, and D. A. LOUIS. Royal 8vo, with 358 Engravings and Map, 20s.

Vol. III.—GAS AND ELECTRICITY. [*In the press.*

Technological Handbooks.
EDITED BY JOHN GARDNER, F.I.C., F.C.S., and JAMES CAMERON, F.I.C.

BREWING, DISTILLING, AND WINE MANUFACTURE. Crown 8vo, with Engravings, 6s. 6d.

BLEACHING, DYEING, AND CALICO PRINTING. With Formulæ. Crown 8vo, with Engravings, 5s.

OILS, RESINS, AND VARNISHES. Crown 8vo, with Engravings. 7s. 6d.

SOAPS AND CANDLES. Crown 8vo, with 54 Engravings, 7s.

The Microscope and its Revelations. By the late WILLIAM B. CARPENTER, C.B., M.D., LL.D., F.R.S. Seventh Edition, by the Rev. W. H. DALLINGER, LL.D., F.R.S. With 21 Plates and 800 Wood Engravings. 8vo, 26s. Half Calf, 30s.

The Quarterly Journal of Microscopical Science. Edited by E. RAY LANKESTER, M.A., LL.D., F.R.S.; with the co-operation of ADAM SEDGWICK, M.A., F.R.S., and W. F. R. WELDON, M.A., F.R.S. Each Number, 10s.

Methods and Formulæ
Used in the Preparation of Animal and Vegetable Tissues for Microscopical Examination, including the Staining of Bacteria. By PETER WYATT SQUIRE, F.L.S. Crown 8vo, 3s. 6d.

The Microtomist's Vade-Mecum:
A Handbook of the Methods of Microscopic Anatomy. By ARTHUR BOLLES LEE, Assistant in the Russian Laboratory of Zoology at Villefranche-sur-mer (Nice). Fourth Edition. 8vo, 15s.

Photo-Micrography
(Guide to the Science of). By EDWARD C. BOUSFIELD, L.R.C.P. Lond. 8vo, with 34 Engravings and Frontispiece, 6s.

An Introduction to Physical Measurements, with Appendices on Absolute Electrical Measurements, &c. By Dr. F. KOHLRAUSCH, Professor at the University of Strassburg. Third Edition, translated from the Seventh German Edition, by THOMAS HUTCHINSON WALLER, B.A., B.Sc., and HENRY RICHARDSON PROCTER, F.I.C., F.C.S. 8vo, with 91 Illustrations, 12s. 6d.

Tuson's Veterinary Pharmacopœia, including the Outlines of Materia Medica and Therapeutics. Fifth Edition. Edited by JAMES BAYNE, F.C.S., Professor of Chemistry and Toxicology in the Royal Veterinary College. Crown 8vo, 7s. 6d.

The Veterinarian's Pocket Remembrancer: being Concise Directions for the Treatment of Urgent or Rare Cases, embracing Semeiology, Diagnosis, Prognosis, Surgery, Therapeutics, Toxicology, Detection of Poisons by their Appropriate Tests, Hygiene, &c. By GEORGE ARMATAGE, M.R.C.V.S. Second Edition. Post 8vo, 3s.

Chauveau's Comparative Anatomy of the Domesticated Animals. Revised and Enlarged, with the Co-operation of S. ARLOING, Director of the Lyons Veterinary School, and Edited by GEORGE FLEMING, C.B., LL.D., F.R.C.V.S., late Principal Veterinary Surgeon of the British Army. Second English Edition. 8vo, with 585 Engravings, 31s. 6d.

Human Nature, its Principles and the Principles of Physiognomy. By PHYSICIST. Part I., Imp. 16mo, 2s.

INDEX TO J. & A. CHURCHILL'S LIST.

(Continued on the next page.

N.B.—J. & A. Churchill's larger Catalogue of about 600 *works on Anatomy, Physiology, Hygiene, Midwifery, Materia Medica, Medicine, Surgery, Chemistry, Botany, &c. &c., with a complete Index to their Subjects, for easy reference, will be forwarded post free on application.*

AMERICA.—*J. & A. Churchill being in constant communication with various publishing houses in America are able to conduct negotiations favourable to English Authors.*