early in embryonic life; and they were found to be very constantly present in the marrow of all the various animals referred to above.

(d) *The fine oxyphile or neutrophile granular cells* differ from the preceding by the much greater size of their nuclei, which are often notched or reniform or tending to divide, and by the much smaller size of their granules. These granules vary extremely in their number; occasionally no granules can be detected, the reticular protoplasm of the cell substance is stained faintly neutrophile, and the cell is only recognizable by its general similarity in size and nuclear characters to those cells that possess granules. This variety constitutes about 30 to 40 per cent. of all the white cells. It is the "myelocyte" of the clinicians. It is regarded as the prototype of the polymorphonuclear leucocyte of the blood. In man the granules take a neutrophile stain.

(e) *The amphiphile granular cells* contain two kinds of granules; some that stain oxyphile and others that stain basophile. These granules are coarser than those of the fine oxyphile cells, but finer than those of the coarse granular cells. This variety has not been met with in human marrow examined by this method.

(f) *The coarse basophile cells* resemble the coarse oxyphile cells, but the granules, which vary greatly in size in the same cell, take a basophile stain. This variety has not been met with in human marrow examined by this method.

(g) *The fine basophile granular cells* resemble the coarsely-granular oxyphile cells in the character of their nuclei, but the granules are of the fine variety, and stain deeply basophile. This variety constitutes about 1.5 per cent. of all the white cells.

(h) *The polymorphonuclear leucocytes* are mostly brought to the marrow by the blood, and should not be regarded as true myelocytes. They comprise the neutrophile and eosinophile leucocytes of the blood, and need no description here.

It is to be noted that the large mononuclear hyaline cells of the blood have never been observed in any specimen of marrow examined by this method. The same remark also applies to the mast cell.

Results of Differential Counts.

The subjoined table exhibits examples of differential percentage counts of the cells of red bone marrow, prepared and classified according to the method that has been detailed above.

Source.	Giant Cells.	Lymphoid Cells.	Coarse Oxyphile Cells.	Fine Oxyphile or Neutrophile Cells.	Amphiphile Cells.	Coarse Basophile Cells.	Fine Basophile Cells.	Polymorphonuclear Cells.	Nucleated Red Cells.
Man,* healthy ...	0.2	50.2	3.3	39.3	0	0	0.2	6.7	8.2
Newborn infant ..	1.5	70.7	6.7	10.2	0	0	0	10.7	57.25
Rabbit, healthy ...	0.3	55.6	0	32.8	0.9	0.6	2.1	7.2	10.0
Dog, healthy ...	0.16	45.8	2.0	22.6	0.16	0.16	0.66	29.9	22.0

* This man died from internal hæmorrhage, the result of a carriage accident.

NOTE.

A short description of the above method was given, and microscopical specimens were shown, at a laboratory meeting of the Pathological Society of London, held at Guy's Hospital, in May, 1904.

CLINICAL METHODS OF ENUMERATING LEUCOCYTES.

By EDWARD TURTON, M.D., B.Sc., M.R.C.P.,
Hull.

THE methods of estimating the number of leucocytes in the blood, which are described in nearly all the medical textbooks and even in the excellent treatises on the blood, are generally very lengthy and tedious, and in most cases involve the use of complicated calculations. Now that the custom of making careful blood counts is becoming more common I am convinced, after a lengthy study of the procedures usually described, that some of the simpler methods which are available for quickly and easily performing a leucocyte count are

deserving of more universal practice. Rapidity, simplicity, absence of involved calculation, combined with sufficient accuracy, are the essentials of good bedside clinical examinations.

The first practical point to consider is, whether it is advisable to count the red corpuscles and leucocytes in the same preparation, or to make two slides and count the two varieties of cells separately. Haematologists seem about equally divided on the subject. It is certainly a great convenience, as well as a great saving of time, to require only one drop of blood, and to have to clean only one mixing pipette and slide. But with blood in which the ratio of red cells to leucocytes approaches to 500 to 1 or so, it is almost impossible by using one slide to avoid overcrowding of red cells on the one hand or a big margin of error, due to too few leucocytes, on the other. Cabot¹ says there should be at least a hundred leucocytes actually counted, to use as a basis for calculating the number present in unit volume of blood. Employing the method I shall describe later, I prefer to count the two varieties of cells in different slides, using the "white" Thoma-Zeiss pipette for the leucocytes with a dilution of 1 in 20, and the other pipette for the red cells with a dilution of 1 in 200. In any case where the leucocytes are increased to 25,000 or more per cubic millimetre, and especially when the red cells are also reduced, it is easy to count both varieties of cell in one preparation, using a dilution of 1 in 100. Sherrington² in his investigations used a dilution of 1 in 50, and emphasizes the importance of counting the two kinds of cells together.

With regard to the diluting fluid, when counting both red cells and leucocytes on one slide, a 3 per cent. solution of common salt deeply coloured with gentian violet is usually quite sufficient, though Toisson's or Sherrington's fluids may be used. The stained nuclei of the leucocytes render counting easy, and help is given by the suggestion of Gowers³ of raising the objective slightly out of focus when the white cells are distinguished by their greater refracting power.

When counting leucocytes alone, with the "white" pipette, I use a fluid containing 1 c.cm. of glacial acetic acid, or 3 c.cm. of the acidum acetum B.P. in 100 c.cm. of water, which gives a solution containing 0.99 per cent. of hydrogen acetate. This is stronger than Thoma's fluid, which is 0.3 to 0.5 per cent., but it serves to dissolve the red cells in half a minute, and leaves the leucocytes deeply stained by the gentian violet. Stronger solutions are liable to affect the white as well as the red cells. It is possible with this fluid to distinguish roughly the relative proportions of mononuclear and polynuclear cells, though for a proper differential count fixed and stained blood slides only are of any use. I have been unable to obtain properly stained specimens using Ehrlich's triacid or Leishman's Romanowski stain with fresh blood films, nor do I consider the enumeration of fresh eosinophile cells as done by Zappert⁴ or Elzholz⁵ a satisfactory clinical procedure.

The Thoma-Abbé Zeiss instrument, or one of the many modifications of it, as those of Zappert and Türck, is that which is now almost universally employed in this country as well as in America and on the Continent, and I shall in consequence confine my remarks to this apparatus. The procedures adopted in counting leucocytes may be divided into two classes—(1) the method of "squares," and (2) the method of "fields."

1. The Method of "Squares."

On the Thoma-Zeiss instrument, as is well known, an area of 1 sq. mm. is divided into 400 small squares, each with a side of $\frac{1}{20}$ mm. and an area of $\frac{1}{400}$ sq. mm. These small cells are arranged by additional lines into 16 groups each of 16 small cells. By means of a cover glass a chamber of $\frac{1}{10}$ mm. is formed. The cubic content of the space above each square is thus $\frac{1}{4000}$ of a c.mm. If, then, the leucocytes be counted in a number of cells and the average found, it is necessary to multiply the number thus obtained by 4,000 and by the dilution (whatever this may be); this gives the total number per c.mm. To facilitate matters it is better to count the cells in groups of sixteen squares, but even so this is a long and tedious method, and involves some eyestrain.

In the Prague clinic it is customary to count the leucocytes in the whole 400 squares; this, though taking a good deal of time, involves no calculation, as with a 1 in 10 dilution you multiply by 100 by simply adding two terminal cyphers to the number obtained.

Ewing⁶ counts both the red and white corpuscles in one specimen, using Toisson's fluid with a dilution of 1 in 100 and a modified Zappert chamber, which is ruled so that the cells in an area of 9 sq. mm. can be counted.

Preitetschensky⁷ describes a modified Zeiss slide, on which

there are ruled 100 large squares subdivided into 1,600 small ones. In counting leucocytes he multiplies the number found in the 100 large squares by 50 when the blood is diluted 1 in 20, and by 25 when it is diluted 1 in 10; this gives the cells in 1 c.mm. of blood.

Türk's⁸ modification has nine large squares ruled on the slide, each being 1 sq. mm. in area.

2. The Method of "Fields."

Here, instead of laboriously counting the leucocytes in the ruled squares on the floor of the counting chamber, the squares are ignored and the stained cells counted in a certain area, which may consist of the whole field of the microscope or some portion specially demarcated.

The simplest method is that ascribed by Cabot⁹ to Dr. Franklin White of Boston. A circular piece of paper or cardboard just fitting into the eyepiece of the microscope has a square hole cut out of the middle. This square aperture is of such a size as to admit a view of a portion of the field equal to 100 small squares, or $\frac{1}{4}$ sq. mm. Using a $\frac{1}{4}$ -in. objective, I have found it convenient to cut the square aperture equal to a group of sixteen small squares. The necessary calculation in these cases is very much simplified. Methods allowing the use of the whole circular field of the microscope are to be preferred to the above, as they allow a larger field for search with the same magnification. The area of the microscopic field, and hence the volume of blood in sight, can be determined by a simple measurement of the diameter of the field. By using the same combination of eyepiece and objective and the same length of tube this diameter remains a constant. Having obtained this, we can estimate the cubic contents of the cylindrical volume of blood from the formula:

Cubic content of cylinder = $r^2 \pi h$ or $\frac{d^2 \pi h}{4}$, where r = radius, d = diameter, and h = the height of the cylinder, which in the Thoma-Zeiss counting diameter is $\frac{1}{10}$ mm.

The diameter of the field may be conveniently measured by means of the ruled lines of the Thoma-Zeiss instrument, the lines being $\frac{1}{10}$ mm. apart. The draw-tube of the microscope can be moved up and down until the sides of the field exactly correspond to a certain number of squares. In the first method described by Stengel¹⁰ the sides of the field are made to correspond exactly to eight small squares, as in Fig. 1.

Fig. 1.

The diameter of the field is then $8 \times \frac{1}{10}$ mm. and the radius $\frac{4}{10}$ mm. The area of the circle then is $r^2 \pi = \pi \left(\frac{4}{10}\right)^2 \times 3.1416$ sq. mm., and the cubic contents of the space = $\frac{1}{10} \times \left(\frac{4}{10}\right)^2 \times 3.1416$ c.mm.

If, now, 25 of these fields be counted, the total cubic space will be $\frac{1}{10} \times 3.1416$ c.mm. = 0.31416 c.mm. With a dilution of 1 in 10 the volume of the blood containing the leucocytes will be $\frac{0.31416}{10}$ c.mm. = 0.031416 c.mm.

Therefore, to obtain the number of leucocytes present in 1 c.mm. of blood it will be necessary to divide the number in 25 fields by 0.031416, or multiply by 31.838.

Butler¹¹ applies this method by employing the following factors (Ostheimer), which may be used to multiply the total number of white cells in 25 fields according to the number of squares contained in the diameters of the field employed, the dilution in these cases being 1 in 20.

Diameter of field	5 squares	multiply by	162.96
"	6	"	113.16
"	7	"	83.12
"	8	"	63.54
"	9	"	50.28
"	10	"	40.74

Another method described by Stengel is carried out as follows:

The tube of the microscope is moved up and down, altering the magnification until the corners of a square group of the small squares just touch the circumference of the field of vision at four points. The area of the field of vision may be readily determined by its relation to the area of the enclosed square. Thus in Fig. 2, the radius of the circle $A B$ will be

Fig. 2.

equal $\frac{A C}{\sqrt{2}}$, this being so the area of the field will be = $\left(\frac{A C}{\sqrt{2}}\right)^2 \pi$.

This method, however, again involves the use of a long calculation.

Professor A. E. Wright¹² has described a method by which only a plain cell of known depth—as that provided with Gowers's or the Thoma-Zeiss haemocytometer—is required for the enumeration of the blood corpuscles. The diameter of the field is estimated by a micrometer scale. Professor Wright manufactures his own mixing pipette from drawn-out glass tubing, which he calibrates by a simple method, or uses an air bubble index as in diluting serum in Widal's reaction. The application of these methods might be useful with a class of students where economy is necessary, but the ordinary mixing pipettes, after the original of Potain, are more useful in clinical work.

The above methods involving the use of somewhat complicated calculations may well be replaced by one of the following, which involve no calculation, after the size of the field has once been determined and a mark made on the draw-tube of the microscope.

Professor O. Grünbaum¹³ finds the exact diameter of the field by basing his calculation on the length of the tube of the microscope.

If the diameter of the field be 8 divisions of the Thoma counting chamber the radius will be 0.2 mm., and by using the formula $r^2 \pi h$ the volume of the mixture seen at one time is $(0.2)^2 \times \pi \times \frac{1}{10} = \frac{1}{10} \pi$. Therefore, with a dilution of 1 in 100 the average number per field must be multiplied by 7951 in order to ascertain how many leucocytes there are in a cubic millimetre of blood. As the number 7951 is obviously inconvenient, it is arranged that the size of the field be diminished until this is increased to 10,000. The diameter of the field must be such that $r^2 \pi h = \frac{1}{100}$, that is, $r = 0.178$ mm., or the diameter = 0.357 mm. This may be contrived in the following way: Suppose, with a $\frac{1}{4}$ in. objective, No. 4 compensating ocular, and 175 mm. tube, the diameter of the field is 8 squares, or 0.4 mm., on extending the tube to 200 mm. the diameter of the field becomes 7 squares, or 0.35 mm. Since the diameter of the field varies inversely as the length of the tube, and an increase of 25 mm. in the latter causes a diminution of 0.05 mm. in the former, it follows that an increase of 21.5 mm. will reduce the diameter of the field from 0.4 mm. to 0.357 mm. Therefore, with a tube length of 196.5 mm. the average number of white cells per field must be diluted by 10,000, with a dilution of 1 in 100, or by 1,000 with a dilution of 1 in 10.

The method I have used for some time is similar to that recently described by Dr. A. E. Jones.¹⁴ I endeavour to find a field of such diameter that the cubic contents shall be equal to $\frac{1}{100}$ th of a c.mm. With a field of such dimensions it will be readily seen that, using a 1 in 20 dilution of blood and counting 20 of these fields, it will be necessary only to multiply the number of leucocytes in these 20 fields by 100, or simply add 2 cyphers to the end. The method is one of great simplicity, and the 20 fields are counted in a minute or two.

The cubic capacity of the cylinder with the required diameter is $\frac{1}{100}$ c.mm., but this = $\frac{d^2}{4} \times \pi \times h$ c.mm. = $\frac{d^2}{4} \times 3.1416 \times \frac{1}{10}$ c.mm.

$\therefore \frac{1}{100} = \frac{d^2}{4} \times 3.1416 \times \frac{1}{10}$, from which it will be found that $d = 7.136$ squares on the Thoma-Zeiss scale; this is equal, roughly, to $7\frac{1}{2}$ squares. As every fifth square on the slide is divided in two by another ruled line, we have thus to take only a quarter of a half-square thus marked off, which with

seven whole squares will give the diameter of the field required (see Fig. 3). By a suitable eyepiece and a $\frac{1}{2}$ in. objective, it is quite easy, by moving

Fig. 3.

the draw-tube up and down, to arrange a field with a diameter of just $7\frac{1}{2}$ squares; this having been found, it is only necessary to make a mark on the draw-tube, and the arrangement can be used without any further calculation beyond counting twenty fields, with a dilution of 1 in 20, and adding two cyphers to the number so obtained.

Dr. A. E. Jones uses a field with a diameter of $6\frac{1}{2}$ squares, the capacity of which is $1\frac{1}{8}$ c.mm. Twenty-five fields are counted, and with a dilution of 1 in 20 it is only necessary to multiply the number obtained by 100.

Arrangements suitable for any number of fields or any dilution of blood can be contrived on the above plan, or that described by Professor Grünbaum; these methods are much more quickly performed than those involving the use of squares, and require no complicated calculations. As it is not necessary to restrict the count to the ruled spaces, a much larger area can be searched than by any other method.

In cases of marked leucocytosis, when the red cells and leucocytes are counted in the same slide, the red cells are counted by using the ruled squares, and the leucocytes by the "field" method.

Now that easier and more rapid methods of enumerating leucocytes are available, it is to be hoped that in all diseases of the blood leucocyte counts will be more frequently repeated than has previously been the case, for the results of isolated examination are most misleading.

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MEMORANDA:

MEDICAL, SURGICAL, OBSTETRICAL, THERAPEUTICAL, PATHOLOGICAL, ETC.

OVAROTOMY IN A CHILD.

THE unusually early age of the patient—she was only 11½ years old—impels me to report this case, to which I was called in consultation by Dr. McCandless, of Rathfriland. She had a temperature of 101° F., and was suffering from colic in the lower part of the abdomen with gastro-intestinal disturbance. These symptoms she had complained of more or less for the previous five months, but they had become much aggravated during the last few days with the elevation of the temperature. There was an obvious fullness of the hypogastrium and a tumour was plainly felt extending above the umbilicus, dull, fluctuating, very tense, and with clearly defined outlines. There was no dullness in the flanks, and the tumour was unaltered on evacuating the bladder. There was a tuberculous family history, and it was only this fact, coupled with the youth of the patient, who had shown no signs of early puberty, that cast any doubt upon the diagnosis of a frank ovarian cyst. I urged an immediate operation, as the patient was in a serious condition, but it was impossible to secure an experienced surgeon, as her people were of very humble means and lived far away from the hospital, so that I had to undertake it myself. I did so with some diffidence, as neither the surroundings nor our equipment were ideal; but I had the

advantage of valuable assistance from my friend, Dr. Radcliffe; and Dr. McCandless gave chloroform.

On opening the abdomen the tumour was found beneath the omentum; it had a smooth surface, was very tense, and filled the lower half of the abdominal cavity. On puncturing with a trocar about 1½ pints of slightly blood-stained fluid escaped. The cyst was then found to spring from the left ovary; a few recent adhesions to the intestines were easily separated by the fingers. The appendages on the left side were removed along with the tumour. The right ovary appeared healthy and was left. On opening the tumour a mass of hair presented, revealing a dermoid cyst, this, I suppose, accounting for its development at so early an age.

The patient stood the operation well, although it was rather prolonged (one hour and a half), and made a good recovery. I removed the stitches ten days later, and the wound was quite healed. The little girl is now running about and seems perfectly well.

Banbridge, co. Down.

J. HOPE REFORM, M.D.

SUCCESSFUL HERNIOTOMY IN A WOMAN AGED 86.

I HAVE read in the *BRITISH MEDICAL JOURNAL* of January 28th, p. 183, the case related by Mr. James A. Watts. Perhaps the following would interest your readers.

When I was *interne* at St. Antoine Hospital, Paris, on July 7th, 1899, I performed a radical cure of strangulated inguinal hernia in a woman aged more than 86. The hernia had been irreducible for two days, and the patient was in very low condition. Cocaine was injected locally and the operation performed rapidly; all was done in thirteen minutes, stricture divided, sac opened, ligatured, and cut off, and the inguinal ring and skin sutured. The cicatrization was perfect eight days after without complication.

I insist upon the necessity to operate rapidly, and to use cocaine in such cases when patients are in a precarious condition.

Nice.

JACQUES VAILLANT, M.D. Paris.

INCUBATION PERIOD OF MUMPS.

I AM induced to publish the following note, because, in that valuable publication *Code of Rules for Prevention of Infectious and Contagious Diseases in Schools*, the quarantine period of mumps is given as twenty-four days. No doubt there are many practitioners, like myself, who have regarded the periods of isolation and incubation of infectious diseases therein contained as authoritative and reliable:

On December 9th Miss E. L. was exposed to the infection of mumps by a child. On December 29th her brother was at breakfast with her. He left the house, and did not return till December 31st, and saw her no more, for on the former date she went to bed with an attack of mumps, for which she was isolated. On January 23rd, in the evening, at a theatre, the brother felt slightly ill with a little headache. He was expecting the disease, but as he had no pain at the side of the jaw he concluded he had a "chill," which, in the form of influenza, was prevalent at the time. On January 24th he had slight pain and tenderness on the right side, which developed into a typical attack of mumps. This history shows that at the extreme end of twenty-five days the attack commenced, though it could not be recognized as mumps till the twenty-sixth day. I have looked up the length of the incubation period in such books as I have at hand and find that in Bain's *Textbook* and Holt's *Diseases of Children* it is given as twenty-five days; while Donkin's, Money's, Jacobi's, and Thomson's treatises give three weeks as the limit.

Sevenoaks.

JAMES E. BLOMFIELD, M.A., M.B. Oxon.

ILLNESS FOLLOWING A TICK BITE.

SHORTLY after my arrival in East London in 1903 I noticed a very painful mark on my upper right arm. It resembled a flea bite, but was bigger, and was excessively tender. A friend told me it was a tick bite. A day or so later the glands in my right axilla became very painful—excruciatingly so. Examination showed no redness of the skin, but enlargement and excessive tenderness of the glands. Two days later I felt very ill. My temperature was 102°. For about a week I was indisposed. My illness was somewhat peculiar. Every morning on waking, and up till about 2 p.m., I felt splendid. Then my temperature went up to 101° or 102°, and I felt wretched. By evening, in addition to temperature, I had an excessively stiff, sore neck. The pain in the neck radiated from enlarged painful post-cervical glands. I made an excellent recovery, and have not since suffered in a similar fashion. Occasionally