CLINICAL PATHOLOGY IN GENERAL PRACTICE

THE NORMAL BLOOD COUNT

BY

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A blood count offers figures from which the clinician hopes to confirm his diagnosis. Unfortunately, the errors in even the best-conducted blood count are enormous, and in an expert's hands a determination of haemoglobin and an examination of a stained film are usually more informative.

Haemoglobin Level

Methods.—The estimation of haemoglobin is done by one or other colorimetric method, nowadays usually comparison of dilute (1 in 200) oxyhaemoglobin with a grey screen, viewing both through a green filter (M.R.C. photometer) or photoelectric determination of the absorption of light by a dilution of blood in water (oxyhaemoglobin) or in N/10 NaOH (alkaline haematin). The classical Haldane method is dying out because of its inherent inaccuracy, although it and the M.R.C. photometer are the only methods which can be calibrated by the National Physical Laboratory.

Reproducibility.—The modern methods are about as accurate as are the graduated pipettes used for them. In time past, the errors of calibration of haemoglobin pipettes were great: nowadays they are usually bought to British Standard Specification No. 797 and tolerance is $\pm 3\%$, so that a series of replicates taken from a bottle of blood will vary by no more than this. Unfortunately, replicates taken from a patient at different times vary much more than this, for any individual may show fluctuations of 7% in his haemoglobin level. A convenient estimate is that two estimations of haemoglobin made at different times on the same patient differ significantly if the difference exceeds a tenth of the average of the two determinations. The old Haldane method is much less accurate than are the modern methods mentioned above, and with this method the difference should exceed 15% for it to be significant.

Standards.—It was only during the war that E. J. King in London and R. G. Macfarlane in Oxford discovered that Haldane's original calibration of his "100% = 13.8 g." standard had been inaccurate, and that the 100% Haldane standard actually corresponded to 14.8 g. haemoglobin per 100 ml. This explained a series of observations which had puzzled haematologists—for example, why Americans appeared to average about 1 g. haemoglobin per 100 ml. more than Englishmen. The new Haldane calibration is that 100% = 14.8 g. per 100 ml. The Medical Research Council has recently organized a scheme whereby samples of blood of accurately known haemoglobin content are supplied at regular intervals to large laboratories so that they can standardize their instruments. With this and a secondary standard such as a grey glass or wire mesh screen, a haemoglobino-meter can be maintained in accurate calibration for years, even if a photoelectric cell has to be changed at short notice.

Normal Values.—For men these can be taken as 14.8 ± 1.5 g. per 100 ml. (13.3–16.3 g.) or 90–110%, which will include more than 19 out of 20 normal subjects. Occasionally normal subjects have lower or higher figures than this, but such subjects are rare: only about 1 in 300 have values below 12.6 g. or over 17 g. (85%–115%). For women, the level chosen depends on the definition of normal. If "normal" is equated to "optimal," then the level for women is the same as that for men; but many women can maintain this only when their average iron intake is much greater than normal—some 30–60 mg. in place of the usual 10–15 mg. If "normal" is equated to "usual,"

however, the range extends much lower: it is quite common to find apparently healthy women with haemoglobin levels of 85% or even 80% (12.6 or 11.8 g. per 100 ml.). Such women nearly all show a rise to levels within the male range if they receive a supplement of 65 mg. ferrous iron daily for 30–60 days (one ferrous sulphate compound tablet, N.F.), though they often fall again as soon as the iron supplement is withdrawn. In such women the menstrual loss is usually excessive or the diet is poor.

Mean Corpuscular Haemoglobin Concentration

The packed cell volume (P.C.V.) indicates the percentage of the volume of the blood which is occupied by the red cells; it is determined with the haematocrit. The standard practice is to collect venous blood into Wintrobe's anti-coagulant (potassium and ammonium oxalate) and centrifuge a sample in Wintrobe's haematocrit tube for 30 minutes at 3,000 r.p.m. The normal range for men is 42–48% (mean 45%) and reproducibility is about the same as for the estimation of haemoglobin. Estimation of the P.C.V. gives the same sort of information as does that of the haemoglobin—in fact, one is often substituted for the other, but usually the haemoglobin is slightly more informative. The two estimations can, however, be combined to yield an index of great value—the mean corpuscular haemoglobin concentration (M.C.H.C.), obtained by dividing haemoglobin concentration expressed in grammes per litre by the percentage of blood occupied by cells (P.C.V.). The M.C.H.C. is a direct indication of iron deficiency. Most normal subjects have M.C.H.C. values between 32% and 35%; patients with marked iron deficiency have values below 30%, while values between 30% and 32% may occur in normal subjects, but are suggestive of mild iron deficiency. Because haemoglobin and P.C.V. can both be determined with high and comparable accuracy, the M.C.H.C. is subject to quite a small experimental error, and is reproducible with a high degree of consistency.

Red-cell Count

The red-cell count is greatly overrated as a diagnostic aid, because the errors inherent in it are immense and the labour required by it is considerable. First, the tolerance on volume in the counting chamber is $\pm 5\%$, and that of dilution pipettes $\pm 3\%$, so that duplicate counts by different workers could be performed with instruments differing by as much as 16%. So extreme a variation would, of course, be rare, but in addition to this error of calibration is the uncertainty due to chance variations in the number of cells which settle on unit area; this random error was computed theoretically as long ago as 1879 and demonstrated practically in 1881: it is enormous.* Thus if a count of 4,000,000 per c.mm. is estimated by counting 400 cells, then there is 1 chance in 20 that the true count is less than 3,600,000 or more than 4,400,000. This range can be reduced only by counting more cells: it is halved whenever the number of cells counted is increased fourfold.

In my own laboratory I deprecate the performance of red-cell counts unless 1,000 cells are counted; my clinical colleagues rarely insist on counts when they appreciate how unreliable they are and how much labour they require.

*One count in every 20 may be expected to lie more than $(200/\sqrt{N})\%$ from the true value, where N is the number of cells counted: if $N=400$, then $200/\sqrt{N}=10\%$.

Colour Index

There are three indices which depend on the red-cell count and share with it its unreliability: (1) The colour index (C.I.) (Hb, expressed as percentage of normal ÷ red cells, expressed as percentage of normal; or, since normal red blood cells approximate 5,000,000 per c.mm., $\text{Hb}\% \div 20 \times \text{red-cell count in millions per c.mm.}$). (2) The mean corpuscular haemoglobin (M.C.H.), which is the average Hb content of a cell (Hb contained in 1 ml. blood ÷ 1,000 × red cells per c.mm.: mean 29×10^{-12} g. or 29 $\gamma\gamma$). (3) The mean corpuscular volume (M.C.V.), the average volume of a cell, computed from P.C.V. and red-cell count, as follows: $\text{P.C.V.} \div 10 \times \text{red cells in millions per c.mm.}$ The normal M.C.V. lies between 82 and 95 μ^3 . Of these three indices the M.C.V. is the most important.

The colour index and M.C.H. do not discriminate between the volume of a cell and the concentration of haemoglobin therein. For example, a patient's count might be $\text{Hb} = 42\%$ or 6.2 g., red cells = 2,000,000 per c.mm., $\text{P.C.V.} = 24\%$, when $\text{C.I.} = 1.05$ and $\text{M.C.H.} = 31.2 \gamma\gamma$, well within the normal range, leading to a diagnosis of simple or aplastic anaemia. When the M.C.V. is found to be 120 μ^3 , considerably increased, and the M.C.H.C. is 26%, a subnormal value, one is led to consider a combination of pernicious and iron-deficiency anaemias—a combination less rare than might be expected and quite clearly shown in the stained film. It is its inherent inaccuracy and inability to discriminate between changes in volume and changes in haemoglobin concentration which has made haematologists spurn the colour index and adopt instead Wintrobe's "absolute indices," which facilitate precise diagnosis by separating volume changes from changes in concentration of haemoglobin within the cell. Personally, I usually reduce labour by estimating Hb and P.C.V., computing the M.C.H.C. to assess the degree of iron deficiency, and examine a film to exclude gross changes of size and shape in the cells: I find that only about one erythrocyte count need be performed for 12 to 15 determinations of haemoglobin.

Price-Jones Curve

An index of great value, which is unfortunately too expensive to perform as a routine, is the Price-Jones curve. This is performed by classifying cells according to their average diameter, and drawing the curve showing how frequent are the cells of different sizes. A normal blood yields a symmetrical narrow-based curve because the variation in size of cells (anisocytosis) is small. Severe iron-deficiency anaemia yields a curve in which the peak has moved to a smaller diameter and the base is wider because cells are more variable (anisocytosis increased); pernicious anaemia yields a relatively flat curve, with a peak shifted to a higher diameter and a very wide base (gross anisocytosis). Price-Jones curves are at present mainly of academic interest, though they may become a routine investigation when a new automatic particle counter and measurer comes into use, because this instrument should allow red-cell counts to be done automatically on several thousand cells, which could also be classified automatically into groups differing in diameter by about 1 μ .

The halometer, introduced by Pijper, of Pretoria, is a convenient instrument by which the modal cell diameter ("peak of the Price-Jones curve") can be measured accurately, and the degree of variation in size can be roughly assessed. There are many instruments available, but the simplest is that of the Association of Clinical Pathologists: it consists of a board on which are fixed two flash-lamp bulbs 28 in. (71 cm.) apart. If a thin blood film is held to the eye while looking at the bulbs in a darkened room, each light is seen to be surrounded by (from within outwards) a yellow halo, a dark zone, and a circular rainbow, the red being outside. The observer moves to and from the lights in a line perpendicular to that joining them until the outer edges of the two circular rainbows just touch. The distance from the

blood film to the midpoint between the lights, measured in feet, is equal to the modal cell diameter (peak of Price-Jones curve) in microns. Three unstained films should be measured. If anisocytosis (variation in size) is marked, the rainbows will be less distinct, and in severe pernicious anaemia anisocytosis may be so great that the rainbow cannot be clearly seen and the halometer cannot yield any measure of cell diameter, though even here it does inform one that anisocytosis is excessive.

The ingenious practitioner can devise his own halometer: in emergency I have made a white mark on brown cardboard, made a pencil hole 14 in. (35.5 cm.) from the mark, and hung the card in front of an electric light: I retire until the red of the rainbow touches the white line; then my eye is N feet from the hole, where N is the modal cell diameter of the specimen of blood.

Appearance of the Red Cell

The measures so far considered permit one to estimate the concentration of haemoglobin in the blood, and the size and haemoglobin saturation of the cell. The shape of the cell has been considered only in relation to the Price-Jones curve, and its functional activity not at all.

The shape of the cell is best assessed by examining a stained blood film which is so thin that the cells are separate. Variation in size is described as anisocytosis; it is slight in normal subjects, moderate in iron-deficiency anaemia, and extreme in pernicious anaemia. Misshapen cells are called poikilocytes, and if they are numerous poikilocytosis is said to be present and is always an indication of serious haematological disorder. An estimate of whether the cell tends to be larger than normal, about normal size, or smaller than normal can be made by eye, and these are described by the terms macrocytosis, normocytosis, and microcytosis respectively. If the film is well stained the peculiarities of staining can be seen: when the normal cell is a pinkish yellow, a few cells of slaty blue colour can sometimes be seen; if the colour is uniformly distributed, the cell is polychromatic (shows polychromasia) and polychromatic cells appear to be the same as reticulocytes. Sometimes the slaty blue colour is due to the presence of dots of blue pigment—that is, of methylene blue or other basic dye from the stain—and this is punctate basophilia, common in marked anaemias but rare in the absence of gross anaemia except in patients with lead-poisoning.

The well-stained normal red cell shows a slight central pallor, where the biconcavity is evident. Sometimes this is absent, when it is usually due to bad staining, though occasionally the cell may have a smaller diameter than usual and show no central pallor—this is described as microspherocytosis and is constantly found in congenital haemolytic anaemia (acholuric jaundice) and during haemolytic crises in haemolytic haemolytic anaemias. The central pallor may increase (hypochromia) until a narrow pink rim surrounds an unstained centre—the pessary cell of severe iron deficiency. Sometimes the periphery and the centre of the cell stain densely, but there is a zone of pallor between the two; this is the target cell, or Mexican hat cell, found in obstructive jaundice, in the severest iron-deficiency anaemia, in Mediterranean anaemia, and in sickle-cell anaemia; it is an abnormally thin cell, a leptocyte, the opposite of a spherocyte.

Typical mild or moderate iron deficiency shows no such extreme change. Some cells show increase in the central pale area, and such cells are usually rather small, but there are always normally staining cells of normal size present; if the size of the central pale area varies greatly from cell to cell, one may speak of anisochromia; this is a very sensitive indication of iron deficiency even if anaemia is only slight.

Some blood samples show other peculiarities of importance. Reticulocytes may be constantly increased in conditions in which blood cells are being produced more rapidly than normal, particularly in haemolytic anaemia and in

leuco-erythroblastic anaemia. Heinz bodies are refractile bodies detected within the erythrocyte after supravital staining: they appear after administration of haemolytic poisons such as phenylhydrazine, but can be produced *in vitro* by the action of ascorbic acid. Pappenheimer bodies are basophilic bodies seen in the red cells in rare forms of haemolytic anaemia. Siderocytes contain granules in which iron can be demonstrated. Howell-Jolly bodies are basophilic bodies 0.2–0.5 μ in diameter derived from chromosomes which have failed to fuse with either nucleus because they became separated from the mitotic spindle. They are particularly common after splenectomy, and are not rare in pernicious anaemia.

There are a few congenital diseases which must be mentioned. In some patients the erythrocytes are elliptical (elliptocytosis), in others crenated: both conditions may be associated with an increased rate of red-cell destruction. They are of considerable genetic interest and should always be referred to a specialist in genetics. In patients with thalassaemia (major or minor) many cells are target cells, and foetal haemoglobin persists in adult life. In sickle-cell trait or anaemia cells appear in films as target cells, but when reduced *in vitro* or *in vivo* the cell elongates and may throw out long terminal filaments; the change occurs because an abnormal haemoglobin is present and its reduced form is almost insoluble and crystallizes out. Since the Mediterranean trait (thalassaemia minor) may have a frequency of 20% in some populations (Cyprus) and sickle-cell trait 50% in others, such as the East African pigmoids, in certain areas they may come to be regarded as "normal"—that is, usual. There are other congenital anomalies involving leucocytes—hypersegmentation, or Alder's anomaly, and failure of segmentation, the Pelger-Huët anomaly—but these are extremely rare in all populations.

The Normal Blood Count

Haemoglobin		12–17 g. per 100 ml.
Erythrocytes		4.3–6.4 mill./c.mm.
Haematocrit		42–48%
M.C.V.		82–95 μ^*
M.C.H.C.		30–35%
Reticulocytes		0–2%
Total leucocytes		4,000–9,000 per c.mm.
Neutrophil	{ Juvenile	0–100 per c.mm.
	{ Staff	0–500 per c.mm.
	{ Segmented	2,500–6,000 per c.mm.
Eosinophils		0–400 per c.mm.
Basophils		0–100 per c.mm.
Lymphocytes		1,000–3,000 per c.mm.
Monocytes		0–800 per c.mm.
Platelets		250,000–500,000 per c.mm.

The White Blood Cells

There are five or six different types of cell included under the generic term leucocyte, and each appears to have a special function. It is the absolute rise or fall of each type which is important, not the proportion which that type bears to the total. This makes it essential to express the differential count as N cells per c.mm.—the absolute count—and not as a percentage as was once the practice. In this way the analysis of a count is actually simplified. If one blood sample showed a leucocyte count of 20,000 and a second a count of 2,000 per c.mm., and both had differential counts of 20% neutrophils and 70% lymphocytes, one would have to think carefully to realize that in the first blood the neutrophils are normal and the lymphocytes greatly increased (suggesting pertussis) and that in the second the neutrophils are greatly reduced. If one sees the counts expressed as: (1) neutrophils 4,000, lymphocytes 14,000 per c.mm.; (2) neutrophils 400, lymphocytes 1,400 per c.mm.; the difference at once leaps to the eye.

The neutrophil polymorphonuclear cells in resting normal adults range from 2,500 to 6,500 per c.mm. The ordinary neutrophil has a segmented nucleus. Some 10% of neutrophils have, however a nucleus with the form of a bent rod without constrictions: these are the "staff forms" or "stab-

kernige" cells, a slightly less mature form; and about 1% of neutrophils have a kidney-shaped nucleus—the "juvenile" or metamyelocyte stage.

The neutrophils increase (1) on exercise—walking about may cause a rise of 500–1,000 and violent exercise a rise of 20,000–30,000; (2) irregularly during pregnancy, though rarely to above 10,000 per c.mm.; (3) when tissue is breaking down, as a result either of trauma or of necrosis due to infarction or to infection.

The eosinophil polymorphonuclear cells are usually said to range from 0 to 200 in normal subjects. Recently techniques for direct counting in a haemocytometer have been developed, and these are far more precise than the differential count; the new techniques reveal a few counts in normal subjects up to 800 per c.mm., and analysis suggests that there is no definable upper limit of normal,* although counts over 600 per c.mm. are more likely to be abnormal than normal. Eosinophils are increased in a variety of conditions in which foreign proteins enter the body—the allergic diseases (asthma, hay-fever, and some eczemas), infections with trichinellae and ascarides while the larvae are passing through the body, some abnormal antigen-antibody reactions such as exfoliative dermatitis, and collagen diseases in some patients.

The basophil polymorphonuclears number 0–100 per c.mm. and are of uncertain function.

The lymphocytes appear to be involved in the production of antibodies. Normally they number 1,500–3,000 per c.mm. and are increased in infancy to 4,000–6,000 per c.mm.; they are also increased in whooping-cough, acute infectious lymphocytosis, and lymphatic leukaemia. Abnormal lymphocytes characterized by foamy cytoplasm and an immature nucleus are numerous (500–10,000 per c.mm.) in glandular fever, and occur in smaller numbers in a variety of virus infections, notably in infective hepatitis, as well as in normal infants during the first 14 days of extrauterine life.

The monocytes are normally 0–800 per c.mm.; they are increased in the recovery phase of acute infections and in many chronic infections.

The sum total of the leucocytes is very variable, but in young men at rest it varies between 3,600 and 8,000 per c.mm., with a very few as low as 3,100 or as high as 9,300. Diurnal variation averages 1,600, but may reach 2,500. There is a slight rise between noon and 3 p.m., but no true digestive leucocytosis. The neutrophilia of exercise and of other conditions is reflected in the total leucocyte count. In infants of over 3 months and children under 4 years, lymphocytes exceed the neutrophils and may reach 6,000 per c.mm.; these two cell types are equally numerous by the age of 4 years, and thereafter slowly progress towards adult levels.

Platelets range from 200,000 to 500,000, the range varying somewhat according to technique. Increases are common in polycythaemia rubra vera and chronic myeloid leukaemia, decreases in thrombocytopenic purpura, acute leukaemia, and, more frequently each year, as a result of the administration of drugs.

Next article on Clinical Pathology.—"Laboratory Investigations in the Diagnosis of Iron-deficiency and Simple Chronic Anaemia," by Dr. George Discombe.

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*The logarithm of the eosinophil count is normally distributed about a mean.