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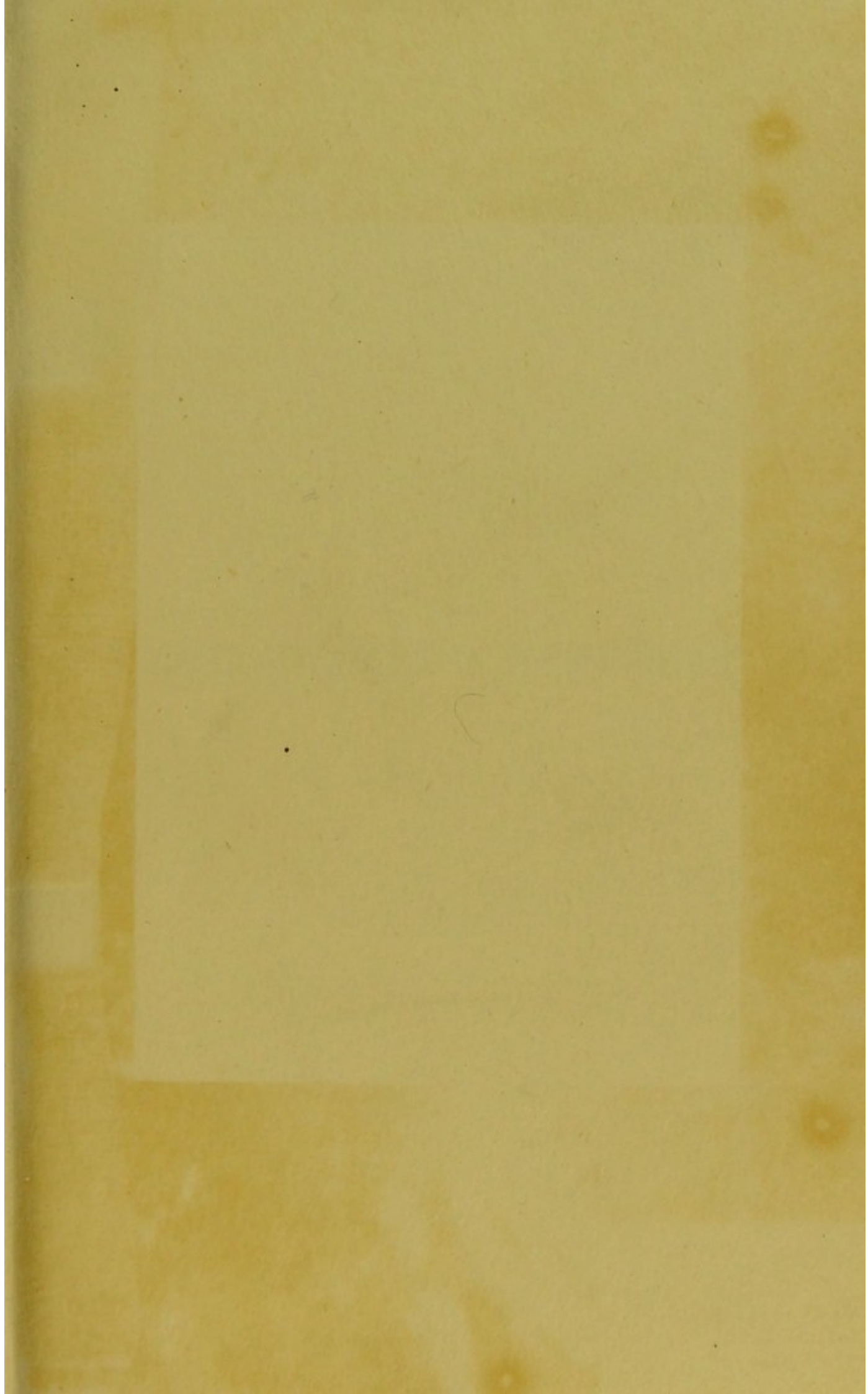
RECENT ADVANCES IN HÆMATOLOGY.

DESCRIPTION OF PLATE.

MAGNIFICATION ABOUT 700 DIAMETERS.

(For fuller description, see text.)

Figs. 1-9. *Red corpuscles* (Jenner's stain).
>   Fig. 1. Erythrocyte, rather smaller than normal.
>   ,,   2. Megalocyte, unusually large.
>   ,,   3. Erythrocyte showing change in shape, and polychromatic staining.
>   ,,   4. Erythrocyte, with granular basophilia.
>   ,,   5. Normoblast.
>   ,,   6. Normoblast, with granular basophilia.
>   ,,   7. Larger normoblast.
>   ,,   8. Megaloblast.
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Figs. 27-29. *Eosinophile cells.*
>   Fig. 27, triacid stain; Fig. 28, Jenner's stain; Fig. 29, Leishman's stain.)

Figs. 30-33. *Neutrophile myelocytes.*
>   (Fig. 30, Leishman's stain; Figs. 31, 32, triacid stain; Fig. 33, Giemsa's stain.)

Figs. 34-38. *Myeloblasts.*
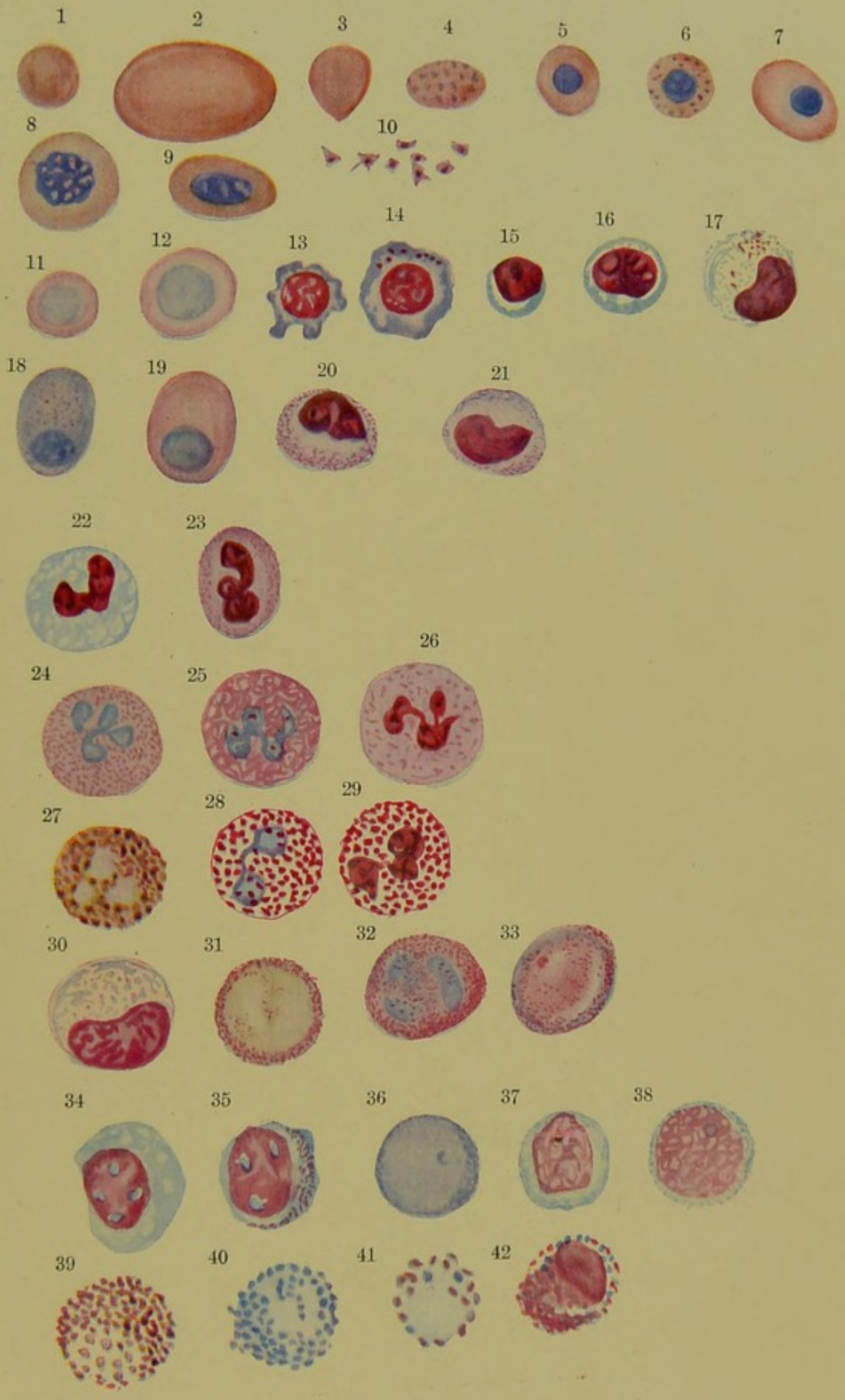>   (Figs. 34, 35, Giemsa's stain; Fig. 36, Jenner's stain; Figs. 37, 38, Leishman's stain.)

Fig. 39. *Eosinophile myelocyte* (triacid stain).

Figs. 40-42. *Mast cells.*
>   (Figs. 40, 41, eosin and methylene blue; Fig. 42, Giemsa's stain.)
>   Fig. 42 shows mature (purple) and immature (blue) granules.

Note.—Figs. 11-14, 18, 19, 22, 25-30, and 37-39 are after those figured by R. M. J. Buchanan (*The Blood in Health and Disease*); those stained with Giemsa's stain are after Ehrlich and Lazarus (*Anæmia*, 1910).

1 2 3 4 5 6 7
8 9 10
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30 31 32 33
34 35 36 37 38
39 40 41 42

RECENT ADVANCES

IN

HÆMATOLOGY

BEING

THE DR. JAMES WATSON LECTURES FOR 1910.

BY

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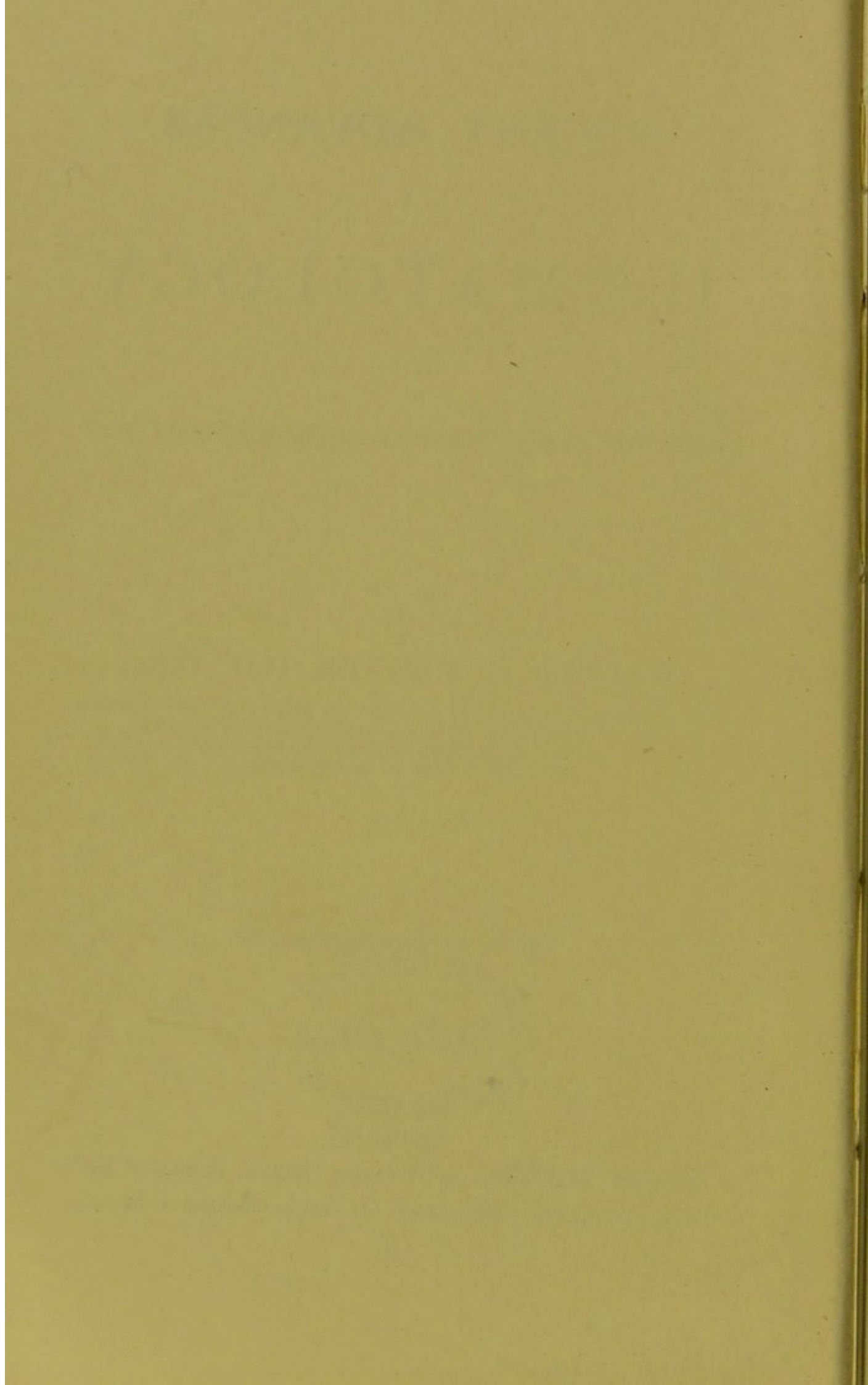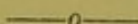IN PRACTICE OF MEDICINE, GLASGOW UNIVERSITY.

WITH A COLOURED PLATE.

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CONTENTS.

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PREFACE.

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THESE lectures were delivered before the Royal Faculty of Physicians and Surgeons of Glasgow on the eleventh, fifteenth, and eighteenth of November, nineteen hundred and ten, and they were originally published in the *Glasgow Medical Journal*. They are now re-issued in book form, so that they may have the continuity and convenience of access which they are apt to lose when scattered through several numbers of a monthly journal.

In preparing the lectures, I have consulted most of the standard works on hæmatology—Ehrlich and Lazarus, Cabot, Da Costa, Ewing, Buchanan—as well as the articles on diseases of the blood in the systems of medicine edited by Nothnagel, by Allbutt and Rolleston, and by Osler and M'Crae. Many papers in current periodical literature have also been laid under contribution. To all of these I have to express my indebtedness. I am specially indebted to Dr. Florence Sexton, who has drawn for me the figures in the coloured plate: any fault that may be found with these last must be ascribed to the process of reproduction, and not to the artist.

WALTER K. HUNTER.

GLASGOW, *April, 1911.*
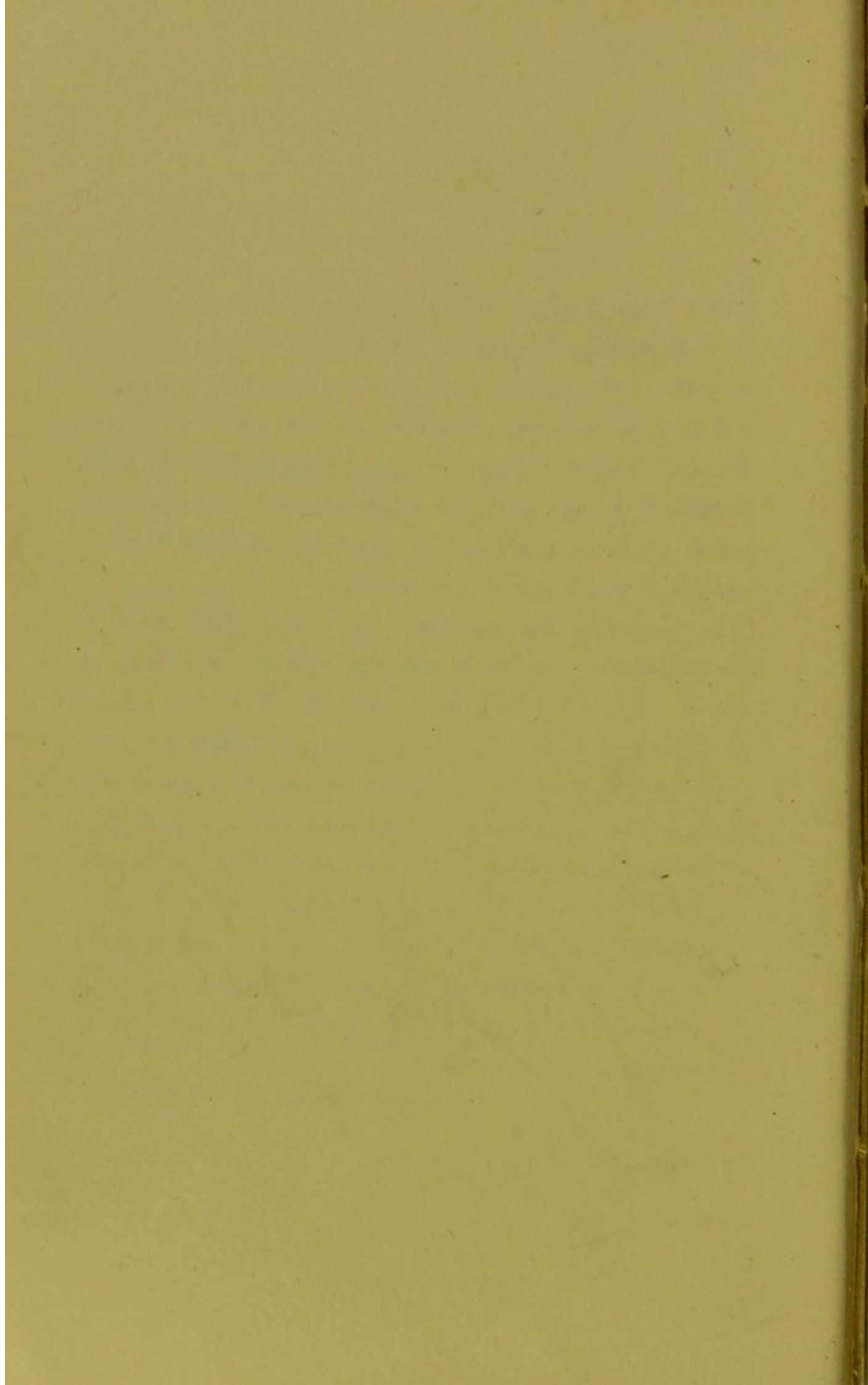
RECENT ADVANCES IN HÆMATOLOGY.

LECTURE I.

MR. PRESIDENT AND GENTLEMEN,—My first duty is to thank the Watson Trust for the honour they have done me in inviting me to deliver the Dr. James Watson Lectures for 1910. I have also to thank them for the very congenial theme which they have given me for this prelection.

Hæmatology, as the term is now understood, is a comparatively recent study; but the amount of work that has been done in the past ten years in regard to the blood, both in health and in disease, is very great, and the subject has now a not inconsiderable literature. The task, therefore, of dealing with this, of compressing it into the space of three lectures, has been one of some difficulty, and I would crave your indulgence if the result should prove not altogether satisfactory. I have made no attempt to trace the progress of hæmatology during these ten years; my aim has been to give you some account of the present position of our knowledge in regard to the various problems of this branch of medicine, rather than a survey of the steps by which the knowledge has been reached.

A

The blood, as you know, is composed of a fluid plasma in which are suspended its more consistent elements, namely, the red corpuscles, the white corpuscles, and the blood platelets. The clinical examination of the blood, as at present in use, confines itself mainly to an examination of these elements, or at least of the red and white cells and the hæmoglobin; and it is the diseases associated with disturbances of the corpuscles that we have chiefly to do with in hæmatology. But before passing on to that part of the subject, I wish to consider very briefly some of the points in regard to the clinical examination of the blood as a whole or of the blood plasma, such, for example, as the specific gravity of the blood, its reaction, coagulability, viscosity, &c. This might also include a study of such substances as toxins and antitoxins, agglutinins, hæmolysins, bacteriolysins, opsonins, and other antibacterial bodies, for they are all present in the blood plasma; but a consideration of these, it seems to me, falls within the domain of bacteriology rather than that of hæmatology, and so I propose to leave them to be dealt with at some future time by some other Watson lecturer.

Specific Gravity of the Blood.—The blood has a specific gravity which averages about 1060 in the male and 1056 in the female, and variations from the normal are dependent to a large extent on variations in the number of the red corpuscles. This will be readily appreciated if one considers the chemical composition of the blood. For example, the blood plasma contains only 10 per cent of solid substances, consisting chiefly of the proteids fibrinogen, serum albumen, and serum globulin, whereas the red corpuscles have 37 per cent of solids; and so the specific gravity of the blood plasma is 1026 to 1030, as compared with 1056 to 1060,

the specific gravity of the whole blood. Further, of the 37 per cent of solids in the red corpuscles, only 8 per cent is represented by proteins, and 90 per cent by hæmoglobin. It will be clear, therefore, that the amount of hæmoglobin in the cell is the chief factor in determining the specific gravity of the blood, and the red cell by itself counts for comparatively little, it being represented by the 8 per cent of protein, which is the chief constituent of the cell stroma.

The composition of the plasma changes but little in the various primary blood conditions, and so the specific gravity of the plasma varies little in such diseases. The specific gravity can, therefore, be taken as a rough index of the amount of hæmoglobin in the blood, it being estimated that every 4·46 per 1000 of specific gravity is equal to 10 per cent of hæmoglobin. Any considerable excess of white corpuscles in the blood, such as is met with in leukæmia, would determine an increase in the specific gravity, and in such conditions the specific gravity could not be taken as an index of the hæmoglobin. But white blood corpuscles contain only about 10 per cent of solid matter, and hence slight variations in their numbers disturb but little the specific gravity of the blood as a whole.

It will thus be seen that the estimation of the specific gravity of the blood is, speaking generally, an estimation of its hæmoglobin, and as this can be measured more accurately by the hæmoglobinometer, a knowledge of the specific gravity is of little clinical importance.

The various contrivances for taking the specific gravity are comparatively simple, and need not be here described.

Alkalinity of the Blood.—The reaction of the blood as it flows from the puncture wound is faintly alkaline, due to the presence in the blood of sodium bicarbonate and sodium

phosphate. A proportion of these substances is combined
with the proteids of the plasma, and the remainder is
present, chiefly in the red corpuscles, in the form of
diffusible salts. The alkalinity of the whole blood is higher
than that of the serum only, and the alkalinity is increased
with an increase in the number of the red corpuscles,
and *vice versa*.

The alkalinity is said to be slightly higher in men than
in women, to vary somewhat with the time of day, being
lowest in the early morning, rising in the afternoon, to
fall again in the evening. It is said to be lessened in the
fevers and other acute infections; also in general debility,
in the severer anæmias, diabetes, uræmia, and scurvy.
Increased alkalinity is said to increase the immunity of
the tissues against bacterial invasion.

It is difficult by the administration of drugs to produce
more than a temporary change in the reaction of the blood,
for an excess of acid or alkali is very quickly eliminated
from the circulation by the kidneys, skin, and other channels
of excretion.

In testing the reaction of the blood, litmoid (resorcin blue),
and not ordinary litmus, paper should be the indicator
employed. The degree of alkalinity is expressed in terms
of the number of milligrammes of sodium hydrate to be
neutralised in 100 c.c. of blood, and, in estimating the
alkalinity, various acids are used to neutralise the alkali.
For example, Engel employs $\frac{1}{75}$ normal solution of tartaric
acid, and 1,000 c.c. of this solution neutralises 530 mgr.
of sodium hydrate. Small quantities of the acid are added
to a known amount of blood till the blood is no longer
alkaline. The amount of acid added will be the measure
of the alkalinity of the specimen of blood, and from the
above formula the number of grains of sodium hydrate in

100 c.c. of the blood can readily be calculated. Sir Almroth Wright uses dilute sulphuric acid as the neutralising fluid. He takes equal quantities of blood and different dilutions of the acid, and the dilution that exactly neutralises the alkali of the blood is the measure of the alkalinity of the blood. He expresses the alkalinity in terms of the dilution of the acid. Thus, normal blood = $\frac{N}{35}$, *i.e.*, a quantity of blood is neutralised by an equal quantity of acid diluted thirty-five times. With increased alkalinity the dilution is less; with lessened alkalinity the dilution is greater.

With Dare's method laked blood is titrated with $\frac{1}{2000}$ normal solution of tartaric acid till the absorption bands in the spectroscope characteristic of oxyhæmoglobin disappear to be replaced by the bands of methæmoglobin in neutral or acid solutions. The amount of tartaric acid added is then noted, and by a table of equivalents the alkalinity of the blood is read off in terms of milligrammes of sodium hydrate per 100 c.c. of blood.

The estimation of the reaction of the blood has not so far proved of any real value in clinical medicine, and it is commonly omitted in a systematic examination of the blood. This is in part to be accounted for by the fact that the different methods of estimating the alkalinity seem to give very different results. It is possible that some of these methods liberate certain bases not normally free in the blood, and so increase the alkalinity; but whatever the explanation be, it is the case that in the various text-books on hæmatology you will find widely different figures regarding the normal alkalinity, and equally conflicting statements as to its variation in disease.

Coagulation of the Blood.—When blood is withdrawn from the vessels it coagulates—that is, it resolves itself into

(*a*) a coagulum or clot, consisting of fibrin and red blood corpuscles; and (*b*) a straw-coloured fluid, the blood serum. This serum (specific gravity, 1026) has much the same composition as blood plasma, but in place of the fibrinogen of the latter, which is now absent, there is added a new substance called serum globulin. The formation of fibrin depends on the splitting up of the fibrinogen of the blood plasma by the action of a coagulin called thrombin. Thrombin, in turn, is supposed to be produced by the interaction of a pro-thrombin (present in the blood), thrombokinase (present in the tissues and blood corpuscles), and the calcium salts of the blood. Thrombin is not present in normal blood, and only appears when the blood is shed, or when there has been some injury to the vessel wall, both of which conditions will determine the disintegration of white corpuscles and the liberation of their contents. Calcium, it is to be noted, is present in the blood in two forms—(*a*) combined with the protein molecules and (*b*) in a dissociable form; it is in the latter form only that it has to do with the production of thrombin.

Now, up till quite recently we have been taught that the coagulability of the blood is greatly influenced by the amount of calcium present in the circulation, and so, when it seemed advisable to increase this coagulability one would prescribe considerable doses of calcium chloride or calcium lactate. A lessened coagulability could, it was said, be produced by similar doses of citric acid, and so this drug would be substituted when a diminution in the coagulability was indicated. It is still recognised that these two drugs have a definite control over the coagulability of the blood; but Addis[1] has shown that, while the amount of ionizable calcium in the blood may be increased by dosing

[1] *Quarterly Journal of Medicine*, January, 1909.

with calcium salts and lessened by giving citric acid, yet the increase or decrease so induced, even when large doses are given, is considerably less than that required to alter the blood's coagulability. The erroneous statements formerly published, he says, are due to faults in technique. There are two errors in, for instance, Wright's coagulometer. In the first place, the arrangements for maintaining a constant temperature are not satisfactory, and slight variations in temperature cause considerable variations in the rate of coagulation. For example, at 10·25° C. the coagulation time is twenty-one and a half minutes, whilst at 20·5° C. it is five minutes twenty-two seconds. The other error is in regard to taking the first appearance of fibrin, on blowing the blood from Wright's capillary tube, as an indication of coagulation. The appearance of this fibrin, Addis says, "is a phenomenon which is due to the chance concomitance of external physical conditions, and one which bears no relation to the coagulation of the blood as a whole."

Addis [1] has devised an apparatus which is a modification of Brodie and Russell's coagulometer, and he maintains that in his instrument the process of coagulation "is fairly comparable to that which occurs within a blood-vessel on account of the presence of a foreign body." There is an arrangement for keeping the blood at an even temperature in successive observations, and its only contact with foreign matter is where the drop of blood under observation comes in contact with the glass cone from which it is suspended. The moment at which there is stoppage in the main flow of blood and there appears a definitely laminated clot— that moment is taken as being the most accurate indication of coagulation. By this method the average coagulation time is found to be about seven and a half minutes in the

[1] *Quarterly Journal of Exper. Physiology*, November, 1908.

healthy individual, and there are practically none of the
daily variations described by some writers.

As to the coagulability of the blood in disease, this has
now to be re-investigated, for in view of Addis's criticisms,
one cannot accept the results obtained in working with the
older coagulometers. I might add, however, that Addis's
coagulometer is not applicable for testing the coagulation
time in pathological conditions in which the corpuscles
agglutinate by virtue of their own serum, this hinders the
flow, and it is almost impossible to estimate when coagu-
lation has actually taken place.

We have just seen that the administration of calcium
salts by the mouth increases the amount of ionizable calcium
in the blood, and whilst the increase is not sufficient to
affect the coagulability of the blood, there seems evidence
that it may lessen considerably the excitability of the
nervous system. MacCallum and Voegtlin,[1] in their work
on experimental tetany, have shown that the calcium in the
blood and tissues of the animals affected was reduced to
one-half of the normal, and that administration of calcium
had a marked effect in controlling the tetany spasm; and
Kinnicutt[2] has recorded a case of gastric tetany in the
human subject where the spasms were apparently com-
pletely controlled by doses of 30 to 60 grains of calcium
lactate.

Excision of the parathyroids in dogs has been shown to
lessen the calcium in the tissues whilst at the same time
it produces tetany, and there is some reason, therefore, for
the view that the parathyroids have a controlling influence
on calcium metabolism, though in what way this is brought
about is not yet understood. It all points, however, to the

[1] *Journal of Experimental Medicine*, 1909, vol. xi, p. 118.
[2] *Trans. Assoc. American Physicians*, vol. xxiv, p. 475.

advisability of studying the calcium content of the blood, particularly in conditions which show undue excitability of the nervous system. Blair Bell,[1] of Liverpool, has devised a means for doing this which is applicable for clinical work. By this method the ionizable calcium in a specimen of blood is precipitated in the form of minute crystals of calcium oxalate. The red corpuscles are then destroyed by the addition of acetic acid, and the calcium crystals counted on a Thoma-Zeiss hæmocytometer. An average of one crystal to each square is regarded as a calcium index of 1, and this is equal to 1 part of calcium oxide in 6,000 parts of distilled water.

Viscosity of the Blood.—This is a condition to which some attention has recently been paid, and it is quite apparent that the degree of viscosity must have an effect on the circulation as a whole. The viscosity depends mainly on the number of corpuscles present in the blood. Hence in polycythæmia the viscosity is greatly increased, whereas in the severer anæmias it is greatly lessened. The failure of the corpuscles to form rouleaux in these anæmias is an illustration of lessened viscosity.

The viscosity of the blood in health is estimated as being about five times that of distilled water at body temperature, and the viscosity can be measured by the instrument used by Dunning and Watson, and described in *The Lancet* of 14th July, 1906. The estimation of the viscosity of the blood has not, so far, come into general use in clinical medicine.

The Volume of the Blood.—There are several methods for estimating the total volume of the blood. The method

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[1] *British Medical Journal*, 20th April, 1907.

of Haldane and Lorrain Smith[1] is perhaps the most convenient, and it seems to be reasonably accurate. The patient inhales a known volume of carbon monoxide gas, and the percentage of saturation of blood with the gas is estimated by comparing its colour with that of ox blood of a known saturation. From this the amount of carbon monoxide which will be necessary to saturate the whole blood is calculated; and as the percentage capacity of the whole blood to absorb carbon monoxide is known to be about 18·5 per cent, the total volume of the blood is readily estimated.

The relative volume of corpuscles and plasma may be determined by centrifuging a specimen of blood in a graduated capillary tube which has previously contained cedarwood oil. As the corpuscles separate from the plasma, the percentage volume of each may be read off. By using this hæmatocrit, as it is called, in conjunction with the hæmocytometer, a measure of the average size of the individual red corpuscles may be arrived at. For instance, if the hæmocytometer gives the percentage of the red corpuscles as 50, and the centrifuge the volume of corpuscles as 80 per cent, then the average "volume index" of the red cells will be $\frac{80}{50}$, i.e., 1·6. In pernicious anæmia the volume index tends to be over normal on account of the large proportion of megalocytes, but in the secondary anæmias, where a large proportion of the red cells are smaller than normal, the index is usually below normal.

Cryoscopy.—By cryoscopy is meant the determining of the freezing point of a fluid as compared with that of distilled water. And as the freezing point of any fluid

[1] *Journal of Physiology*, vols. xxii and xxv.

is in proportion to the number of its contained molecules, the greater the concentration of the blood the lower will be its freezing point. The freezing point of blood is very constant in health, ranging from $-0.55°$ C. to $-0.57°$ C., but when there is much renal insufficiency, the freezing point may be as low as $-0.65°$ C. to $-0.70°$ C. It is chiefly as a test of the functional capacity of the kidney that cryoscopy is used; but in other diseases there may be variations from the normal freezing point, *e.g.*, in diabetes the freezing point is low, whilst in pernicious anæmia it is high.

There are a number of different makes of cryoscope, but they are all modelled on that of Beckmann; and their manipulation requires some care and skill if one is to obtain accurate results. Cryoscopy has not come into general use in hæmatology, and, indeed, it does not seem to have fulfilled the usefulness anticipated for it when first it was introduced.

Fat is present in the blood in small proportions (1.6 per 1,000 of blood) in the form of a very fine emulsion. But the amount may be greatly increased in physiological as well as pathological conditions, so that excess of fat, or lipæmia, as it is called, has no great clinical significance.

The amount of fat may be estimated by straining blood films with Sudan III, when the fat globules show as brick-red dots scattered throughout the film.

Spectroscopic Examination of the Blood.—The spectroscope is used in medico-legal work as a means of detecting the presence or absence of blood; but it is also of considerable service in clinical medicine in determining the presence or absence of carbon monoxide in the blood, as well as the conditions called methæmoglobinæmia and

sulph-hæmoglobinæmia. In carbonic oxide poisoning, carbonic oxide hæmoglobin takes the place of the normal oxyhæmoglobin. The spectroscopic appearances of these two forms of hæmoglobin are somewhat similar, but they differ in that in the former the two absorption bands of oxyhæmoglobin are displaced slightly towards the violet end of the spectrum, and the addition of ammonium sulphide fails to produce the broad band of reduced hæmoglobin as it does when oxyhæmoglobin has been present.

In certain toxic conditions, for instance, poisoning with potassium chlorate or potassium permanganate, the oxyhæmoglobin of the blood is replaced by methæmoglobin, and the spectroscope gives the characteristic bands of the latter. The addition of ammonium sulphide changes methæmoglobin into oxyhæmoglobin, and later to reduced hæmoglobin, and in this way distinguishes methæmoglobin from acid solutions of hæmatin.

The spectroscopic appearances of sulph-hæmoglobin are very similar to those of methæmoglobin, but the addition of a small quantity of ammonium sulphide does not discharge the absorption bands from the red as it does with methæmoglobin. On the other hand, carbon monoxide added to solutions of sulph-hæmoglobin displaces the bands as it does not when passed through solutions of methæmoglobin.

Red Blood Corpuscles.—We must now pass on to consider the various corpuscular elements in the blood, and first of all the red corpuscles. In normal blood this corpuscle is by far the most numerous cell, there being in the male, 5,000,000; and in the female, 4,500,000 per c.mm.

The red corpuscle is a thin, flattened biconcave disc, with regular outline and smooth surface. It is made of a highly elastic material, which allows of the cell undergoing

changes in shape as it passes along in the circulation. Its diameter ranges from 6·5 to 9 μ; but in normal blood the size is very uniform, 75 per cent of the corpuscles measuring 7·5 to 8·5 μ. The cell seems homogeneous throughout, and has no nucleus. It is probably made up of a hyaline network, in the meshes of which is contained the hæmoglobin, a finely granular substance, the exact composition of which is not known. Hæmoglobin is the important part of the cell, for it is by means of hæmoglobin that oxygen is carried to all the tissues of the body, and without hæmoglobin the red corpuscle to a large extent becomes useless. If the cell contains the normal amount of hæmoglobin, we say its colour index is 1, and variations in the amount of hæmoglobin are represented in fractions of this. The colour index is roughly estimated by dividing the percentage of hæmoglobin by the percentage of red corpuscles in a given specimen of blood. But this only gives the average colour index of any single cell. In staining the red corpuscle, however, it is not the cell stroma but the hæmoglobin that reacts to the oxyphilic stain, and so the depth of the staining is some indication of the proportion of hæmoglobin in each cell. For the same reason the periphery of the cell being thicker, and containing therefore more hæmoglobin, stains deeper than the central parts. It is to be noted that in staining a blood film with a double stain—that is, a stain which contains both an acid and a basic dye — the red corpuscle stains with the acid element only, and if there are two such acid dyes present, it reacts to only one of them. Further, the intensity of the staining is influenced to a considerable extent by the method of fixing that may have been employed.

The red corpuscle has an existence, it is said, of from three to four weeks, at the end of which time it gets

broken up in the liver, spleen, or some other gland. In the same drop of blood, therefore, there must be corpuscles of different ages, and we are told that the older the corpuscle the greater is the amount of hæmoglobin it contains. This may be so, but experience shows that the nearer the blood is to the normal, the more uniform is the staining of its individual corpuscles, and that it is in the severer anæmias that the variation in the intensity of staining is most in evidence.

In disease, then, the individual red corpuscle may have its hæmoglobin content lessened or increased, the cell staining with lesser or greater intensity as the case may be.

But in addition to this we sometimes find that in certain of the anæmias the red corpuscles most deficient in hæmoglobin have a tendency to *polychromatic staining, i.e.,* they react to basic as well as acid stains, and so, instead of appearing pink when stained with eosin and methylene blue, they take on a greyish-purple tint, staining apparently with both these dyes. One meets with all shades of polychromatic staining, from the cell almost normal to the cell so blue as to have only the slightest recognisable tinge of pink.

Different interpretations have been given to this polychromatic staining. Some hold that it is an indication of the youth of the cell, for it is recognised that in early embryonic life the analogue of the red blood corpuscle is a nucleated cell without hæmoglobin, and whose protoplasm stains with basic dyes. Others maintain that the polychromatic staining is a sign of degeneration of the cell, and that as it degenerates it loses the capacity for retaining hæmoglobin, and at the same time acquires an affinity for basic stains. It is true that polychromatic staining is mostly met with in normoblasts, megaloblasts, and other

immature cells, but at the same time it must be recognised that such cells, being immature, will the more readily undergo degeneration when they enter the blood-stream. It is probable that both the explanations contain a measure of truth, and that polychromatic staining is met with in immature cells, and also in cells in process of degeneration.

Polychromatic staining may affect the whole of the cell protoplasm, or it may be patchy in distribution. Sometimes it gives the cell a stippled appearance, there being a number of basophile dots scattered throughout the cytoplasm. This condition has been called *granular basophilia*; it is probably a form of degeneration, and is chiefly found in lead-poisoning, pernicious anæmia, and other toxic states.

But in addition to these variations in staining, the size and shape of the red cell may be markedly altered, and the more extreme the anæmia the more marked are such changes. The size only may be affected, or both the size and shape. When the red cell is larger than the normal limits, it is called a *megalocyte*, and measures from 9 μ to 12 μ, though sometimes it is as large as 20 μ in diameter. When smaller than normal the cell is called a *microcyte*, and measures 3 to 5 μ in diameter. *Poikilocyte* is the name given to the cell when its shape is altered. This change may take a variety of forms. For example, it may be pear-shaped, spindle-shaped, dagger-shaped, horseshoe in shape, or even spherical. It is chiefly the cells smaller than round that show poikilocytosis, but a megalocyte may also be a poikilocyte.

The significance of the poikilocyte is not certain. Possibly it leaves the marrow as such, being a hastily-formed, and therefore an ill-formed, corpuscle. It has also been suggested that it is derived from the splitting up of normal erythrocytes, an endeavour on the part of the organism to increase

the number of corpuscles in the circulation. The majority
of the megalocytes leave the marrow as such, being derived
from a megaloblastic marrow; but a proportion of the
megalocytes may be ordinary red corpuscles which have
become swollen or dropsical — possibly a compensatory
increase in size of the individual cell to make up for a
deficiency in the number of cells. Like the megalocyte,
the microcyte may leave the marrow as such, being formed
from a microblast or small normoblast. Some microcytes,
however, are probably derived from ordinary erythrocytes by
a process of gemmation.

In most of the severer anæmias a proportion of nucleated
red corpuscles are to be met with in the peripheral circulation.
These are not normally present in blood, but they are found
in abundance in red bone-marrow: it is from the nucleated
corpuscles of the marrow that the non-nucleated cell of the
blood is derived. In anæmia there may be such an urgent
call for red corpuscles that the store of non-nucleated cells is
insufficient to supply the demand, and so nucleated cells are
hurried into the circulation before they have had time to lose
their nucleus and become non-nucleated cells.

Just as there are three sizes of non-nucleated red
corpuscles, so we speak of three sizes of the nucleated
corpuscles, namely, the normoblast, the megaloblast, and
the microblast.

The *normoblast* has the size and shape of the ordinary red
corpuscle, but it has, in addition, a round or oval nucleus with
a diameter rather more than half that of the whole cell. The
nucleus has a perinuclear membrane, and a reticular network
to which the chromatin seems to be attached. It stains very
intensely, more so than the nucleus of any of the white
corpuscles. There is no nucleolus visible. Variations in the
form of the nucleus are not uncommon, and so it may be

lobulated or split up into two, three, or more portions, often of unequal size. The protoplasm of the cell stains like that of the ordinary erythrocyte, possibly rather deeper, and not unfrequently it shows signs of polychromatophilia.

The *megaloblast* has a diameter ranging from 10 to 20 μ, but it usually measures about 12 μ. It may be described briefly as a nucleated megalocyte. The nucleus is larger than that of the normoblast, and does not stain so intensely or uniformly. It also shows more structure, and its margin is not so well defined. There is no nucleolus. With certain methods of staining a colourless ring may be seen separating the nucleus from the surrounding cytoplasm. This cytoplasm frequently shows polychromatic staining, and sometimes granular basophilia.

Megaloblasts are present normally in the blood and marrow of the fœtus, and if present in the marrow of the adult it must be in very small numbers. When, therefore, megaloblasts appear in the circulation of the adult they are taken as an indication that the marrow has reverted, in part at least, to the megaloblastic type of growth. In the same way the presence of the normoblasts in the blood is a sign that the marrow is normoblastic in its type of growth, and, further, they are an indication of the functional activity of the red bone-marrow. Both normoblasts and megaloblasts are frequently found in the blood at the same time. The dividing line, however, between these two cells is not always very well defined, and it is sometimes exceedingly difficult to say of an individual cell whether it should be classed as normoblast or megaloblast. The size of the cell is not the sole distinction, and the characters of the nucleus must also be considered. Sometimes a very large cell has the nucleus of the normoblast, and, again, a much smaller cell may have the nucleus of the megaloblast. Indeed, there would seem

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to be intermediate forms connecting these two types of cell, and such forms are typical neither of the normoblast nor yet of the megaloblast.

The *microblast* is but rarely met with in blood films. It has a diameter of 5 to 6 μ, or less. Its nucleus is like that of the normoblast, and there is only a very small amount of cytoplasm surrounding it. The microblast is probably a normoblast which, for some reason or other, is deficient in its cytoplasm, but the significance of this cell is not quite certain.

White Blood Corpuscles.—We now pass to consider the white corpuscles. In normal blood these cells range in number from 5,000 to 10,000, with an average of 7,500 per c.mm.; and, unlike the red cells, the white cells consist of several varieties.

In considering the minute structure of the various white corpuscles we find that each has a reticular nucleus, inside of which one or more nucleoli may be found. The nucleus stains with varying degrees of intensity in the different types of cell, the intensity of the staining depending largely on the closeness of the nuclear network and on the amount of its chromatin, irregular masses of which seem to be adherent to the strands of the network. The nucleus of the less mature leucocytes is usually comparatively large, and poor in chromatin. As the cell grows older, the nucleus as a rule grows smaller, and becomes richer in chromatin. The nucleus of the older cells therefore stains deeper than that of the younger ones.

The cell protoplasm has also a meshwork of fine fila-ments continuous with that of the nucleus, from which it is separated by the perinuclear membrane. With appropriate staining it may be shown that, at a point close to the

nucleus, this reticulum appears denser, forming a structure called the archoplasm. In the centre of the archoplasm lies the centrosome.

Certain of the white corpuscles contain granules in their protoplasm, which, according to their staining reaction, are spoken of as being basophile, oxyphile, or neutrophile.[1] These granules were thought by Gulland[2] to be the knots of the cell network, the coarsely granular cells having a coarse reticulum with large knots, whilst in the finely granular cells the reticulum was thought to be fine and the knots small. According to this view, the "granules" of the cell would not really be granules lying more or less free in the cell protoplasm, but they would belong to the stroma of the cell, and would form as essential a part of the cell as the nucleus itself.

Ehrlich, however, does not take this view. He regards the granules as products of cellular metabolic activity deposited in a solid form, and constituting a reserve material to be given off from, or used up by, the cell as occasion might demand. It has been pointed out that the granular cells are the most actively amoeboid of all the leucocytes, and that the lymphocyte, which is non-granular, is probably quite passive in its activities. Kollmann, too, has shown that on starving certain animals the granules disappear

[1] In a double stain, made up of a basic (methylene blue) and an acid (eosin) dye, the oxyphile granules stain intensely with the acid dye, and the basophile granules with the basic dye. The neutrophile granules were originally thought to stain with a neutral stain, i.e., a mixture of the acid and basic dyes. But Kanthack and Hardy (1894) have shown that no such neutral dye can be formed by the mixture of these two stains, and that the neutrophile granules are really faintly oxyphile. The term neutrophile, however, is still retained to designate this type of granule.

[2] *Journal of Physiology*, vol. xix.

from the granular leucocytes. It looks, therefore, as if some nutritive function might be attributed to these granular elements, and that they may, in a measure, represent the potential energy of the cell. It is also to be kept in mind that the individual leucocyte has only a short life, not more than a few days at the longest. It may be that in dying the leucocyte gives up its granules to the circulation, and this may be of material value to the organism; indeed, it has been shown that increased bactericidal power of the blood is associated with the discharge of both eosinophile and neutrophile granules.

The exact chemical composition of the cell granules is not yet fully determined, but Ehrlich holds that they form a specific secretion for the type of cell to which they belong. He also states that the granules in any one leucocyte are uniformly basophile, neutrophile, or oxyphile, and that any apparent exceptions are to be attributed to the unequal staining of granules which are not equally ripe.

If these granules, then, are a secretion of the cell pro-toplasm they cannot be regarded as forming part of the cell reticulum, though it is probable that they are in some way attached to it.

It must here be noted, however, that in certain "non-granular" cells, *e.g.*, in certain lymphocytes, the cytoplasm has a granular appearance which *is* due to the reticular network and not to the presence of a specific granulation. According to Ehrlich these cells, and only such cells, would answer to Gulland's views as to the nature of cell granula-tion, namely, that the "granules" are the knots of the cellular reticulum.

Now, just as red corpuscles may show pathological changes, so may the white corpuscles. Indeed, in the majority of toxic conditions a proportion of the white cells appear to

be undergoing some retrograde process. Such changes seem to be most common in the polynuclear leucocytes and in the neutrophile marrow cells, and to be comparatively rare in the lymphocytes and eosinophile cells.[1] They seem to be more frequently met with, too, in the cells of the marrow than in the similar cells of the blood-stream. The changes mentioned may either affect the nucleus or the protoplasm of the cell, or both, at about the same time. In the polynuclear cell the nucleus shows what might be called an increased polymorphism—that is, the nucleus becomes divided up into a larger number of lobes (12 to 20 [2]) which tend to arrange themselves at the periphery of the cell, giving it a somewhat rosette-like appearance. Such a nucleus stains rather faintly. In the myelocyte the nucleus may have its chromatin condensed into little masses, usually arranged just inside the perinuclear membrane; or else the nucleus may become swollen and vacuolated, and possibly have the chromation diffused through the nucleus, staining in an ill-defined manner. In both cases the nuclear membrane ultimately ruptures, and its contents become lost in the cell protoplasm. When the cytoplasm is affected it has a swollen appearance, and its reticulum stains more intensely, giving a definitely granular appearance to the cell. Vacuoles appear, and increase in number and in size, till ultimately only a skeleton of a cell remains. This framework then breaks up, and so the cell becomes dissipated into endless fragments.

It is not certain that all the above changes should be regarded as evidence of degeneration in the cell; possibly some are due to stimulation. Buchanan [3] has noticed the

[1] Carnegie Dickson, *The Bone Marrow* (Longmans, Green & Co.), 1908, p. 56.

[2] Buchanan, *The Blood in Health and Disease*, p. 135.

[3] *Ibid.*, p. 134.

increased "polymorphism" in cells with which he has been taking the opsonic index, and he suggests that the increased lobulation of the nucleus has some association with phago-cytosis. It is to be noted that the eosinophile cells are very liable to rupture as a result of traumatism, so that the presence of eosinophile granules free in a blood film may be simply an artefact, the result of spreading the film, and in no way due to degeneration of the cell.

In chronic suppuration the leucocytes of the blood are sometimes found to contain fat granules (visible on staining with Sudan III). These may be the result of cellular degeneration, or else due to absorption of fatty matter from the blood plasma by the healthy phagocyte cells.

In similar conditions an iodine reaction may be obtained in dried films, due to the presence of glycogen in the cell. The glycogen may show as little masses of varying size and shape deposited in the cell protoplasm, or the whole cytoplasm may seem to give a diffuse iodine reaction. It is chiefly in the polynuclear cells that the glycogen can be found, and much less often in the lymphocytes and marrow cells.

In addition to such suppurative conditions as pneumonia and pyæmia, this glycogen reaction may be met with in various toxæmias, such as diabetes, pernicious anæmia, leukæmia, &c. It may also be obtained at times from small particles lying free in the blood plasma, but these are probably glycogen-containing fragments of disintegrated leucocytes, for they are never met with when the reaction is absent in the leucocytes.

Up to about 90 per cent of the polynuclear cells in a film may show the presence of glycogen, and it is said that a patient with a well-marked glycogen reaction is always

seriously ill, the amount of glycogen being some indication of the severity of the illness.

There is a difference of opinion as to whether the glycogen reaction is to be regarded as a degenerative change, or as evidence of an increased functional activity in the leucocyte. Dr. W. H. Brown,[1] who has made a careful study of this reaction in a variety of diseases, is strongly of opinion that the glycogen in the cell is an expression of increased phagocytic activity, and is not of a degenerative nature.

Ehrlich divides the white corpuscles normally present in the blood into six different groups, and this classification is now very generally followed. Ehrlich's grouping is as follows :—

1. Lymphocytes.
2. Large mononuclear (hyaline) cells.
3. Transitional cells.
4. Polymorphonuclear (neutrophile) cells.
5. Eosinophile cells.
6. Mast cells.

The cells in the first three groups are sometimes spoken of as the non-granular, and those in the last three as the granular leucocytes.

1. *The lymphocyte* measures on an average about 7·5 μ in diameter, but the size may range from 5 to 10 μ, or even more than that. It has a single round nucleus which fills

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[1] *The Practitioner*, January, 1910.

N.B.—This iodine reaction is obtained in moist films, but not in dry films, of normal blood. In disease, however, the reaction is present in dry films. The substance which gives the reaction is said not to be glycogen, but to be related to the amyloids (see *Anæmia*, Ehrlich and Lazarus, English translation, 1910, p. 96).

the greater part of the cell. This nucleus stains deeply with basic dyes, and may show a reticular formation. Within the nucleus one or two nucleoli, with thick limiting membrane, are to be found.

The cell protoplasm with the Romanowsky stain takes up the basic dye, and it has a somewhat granular appearance, due, certain writers believe, to the presence of fine basophile granules. Ehrlich, however, maintains that the lymphocyte has no such granulation and that this appearance is to be ascribed to a condensing of the reticular structure of the cytoplasm, which is basic in its staining reaction. Immediately surrounding the nucleus there is a clear area in which the cell reticulum is very slightly represented. In this zone lie the fuchsinophile granules or rods, which are visible (coloured a yellowish-crimson red) on staining by Schridde's method. The cell protoplasm usually stains less intensely with basic dyes than the nucleus, though this varies in different cells as well as with the stain employed. With the triacid stain no granules of any sort are to be seen, and the cytoplasm either remains colourless or else takes on a faint pink tinge. With Giemsa's stain the cytoplasm stains a pure blue colour. With certain stains (Leishman, Giemsa) some ruby-red (azurophile) granules are to be found in the larger lymphocytes scattered throughout the cytoplasm. Sometimes these granules are very few in number and large in size, or they may be smaller and more abundant.

The margin of the lymphocyte has often a frayed-out appearance, and may show at times little bud-like excrescences. These, however, are artefacts produced by the pressure in spreading the films.

It is to be noted that the larger the lymphocyte the paler is its nucleus, and the cell protoplasm is proportionately

more abundant. In the larger cell, too, the nucleus may be oval in shape and eccentric in position. It is sometimes also seen to be indented, or even divided up into two or more fragments. The largest lymphocytes are only met with in pathological conditions, such, for example, as in lymphatic leukæmia.

The lymphocyte is generally believed to be non-phago-cytic, and it is a matter of dispute whether or not it possesses active amœboid movement.

It represents from 20 to 25 per cent (in infancy about 70 per cent) of all the white corpuscles in normal blood.

2. *The large mononuclear (hyaline) leucocyte* is two or more times the size of the lymphocyte and has a diameter of 11 to 15 μ. There is a large oval or kidney-shaped nucleus which occupies about half the volume of the cell. The nucleus shows a reticular formation and it stains rather feebly with basic dyes. It is often placed towards the margin of the cell, and there is no nucleolus to be seen. The cytoplasm, which has also a basophile reticulum, stains even more faintly than the nucleus, and with Giemsa's stain has a slate-grey colour. With the triacid or Giemsa stain a number of very fine neutrophile granules are to be seen in the meshes of the reticulum. They are not uniformly present throughout the whole of the cell, but sometimes they are so closely packed together as to give the cytoplasm a diffuse red colour.

The staining reactions of the large mononuclear cell may, with certain stains, be similar to those of the lymphocyte. But Ehrlich insists that the two constitute quite different types of cell, and that transitional forms from one to the other are not observed. He says that azurophile granules are not present in the large mononuclear cell. This cell

is phagocytic and actively amœboid. It represents 1 to 2 per cent of all the white corpuscles in normal blood.

3. *The transitional cell* in size and general appearance is somewhat similar to the large mononuclear cell. The nucleus of the former, however, is deeply notched, and may be quite horseshoe in shape. It stains, too, more intensely than the nucleus of the latter. The cytoplasm of the transitional cell with Giemsa's stain shows the same staining reaction as the mononuclear, only the neutrophile granulation of the former is more abundant.

The exact relationships of this transitional cell are not determined. It has been claimed as an intermediate stage between the large mononuclear and the polynuclear leucocyte, and, again, as an intermediate stage between the neutrophile marrow cell and the polynuclear leucocyte. In normal blood the transitionals are not numerous, and probably do not represent more than 1 per cent of all the white cells present.

4. *The polymorphonuclear cells* measure about 10 to 12 μ in diameter, but it is not uncommon to find them smaller or larger than this. The cell has a convoluted or lobed nucleus, which is rich in chromatin, and which stains deeply with basic dyes. There is no nucleolus visible. The protoplasm of the cell stains faintly with acid stains, and it contains fine granules, of somewhat irregular shape, which are neutrophile in their staining reaction. Their colour varies in tint, ranging through different shades of violet and red, depending on the method of fixation and the pigments that have been employed. The granules are most abundant at the periphery of the cell, and scanty in the perinuclear area. Individual cells, however, may vary a good deal in the amount of granules they contain.

The polymorphonuclear cell is actively phagocytic and amœboid. It contains oxydising and proteolytic ferments. It represents about 70 per cent of all the white corpuscles in normal blood.

5. *The eosinophile cell* is about the size of the poly-nuclear leucocyte, though not infrequently rather larger. It has a similar polymorphous nucleus, but usually not so much broken up. The staining, too, is less intense than that of the nucleus of the polynuclear cell. The cell proto-plasm seems to remain unstained, but it contains large spherical granules which are strongly oxyphile, and which colour different shades of brown, red, or yellow, according to the special methods of staining.

The eosinophile cells are easily ruptured, hence in a blood-film eosinophile granules are not unfrequently met with free in the blood plasma.

This cell is phagocytic, and actively amœboid; it represents from 2 to 4 per cent of the white corpuscles in normal blood.

6. *The mast cell* varies greatly in size, its diameter averaging from 9 to 12 μ, though it may range from 7 to 22 μ. There is a relatively large, round, ovoid, or slightly lobulated nucleus which is situated rather towards one side of the cell. It stains faintly with basic dyes, and is often some-what ill-differentiated from the surrounding cytoplasm, which is also basophile. The striking feature of the cell, however, is its granulation. These granules, as a rule, are coarse, but they vary a good deal in size and shape. Some-times they are smaller than the eosinophile granules, but frequently they are considerably larger. They may have a spherical, oval, or cubical form. The granulation is usually

not so abundant as with the eosinophile cell, and its distribution may be limited to one part of the cytoplasm. Generally the granules are most in evidence at the periphery of the cell, but the variations in this respect are very great. The granules show an intense affinity for basic dyes. They stain, however, metachromatically; for example, with methylene blue they stain a violet tint instead of the blue colour of the stain. But they are soluble in water and hence may disappear, or remain only as fragments, on staining with watery solutions of any of the dyes. They are not visible on staining with the triacid mixture.

The mast cells have some amœboid movement. They are rare in normal blood—not more than $\frac{1}{2}$ per cent of all the white corpuscles ; but in a disease like myelogenous leukæmia they may be present in considerable numbers. In leukæmia the mast cell, or mast myelocyte as it is then called, has much the same characters as the mast cell of the normal blood. In the mast myelocyte, however, the cell granules do not always stain metachromatically, and if they are sufficiently immature they are but little soluble in water.

Some hæmatologists[1] distinguish two types of basophile granular cells, and the term "mast cell" has been used to include both of these. In the first form the nucleus is definitely polymorphous and stains with moderate intensity. The cell granules are small (δ granules of Ehrlich), and they are not so definitely metachromatic as in the other form. In the second type the nucleus is oval, or at most trilobed, and it stains faintly. The granules, as a rule, are large (γ granules) and metachromatic in their staining. But it is not easy to draw a line between these two types, for in an individual cell there may be a considerable variation

[1] *E.g.*, Da Costa.

in the size of the granules, some being very large and others small; also, as we have just seen, large granules in the mast myelocyte do not always stain metachromatically.

In addition to the above six forms of leucocyte which are present in normal blood, there are several others found in the circulation only in disease. The most important of these is the *neutrophile myelocyte*. This cell has a diameter of from 10 to 20 μ, but it is occasionally smaller or larger than that. There is a single large nucleus which is round, oval, or slightly indented, and which stains rather feebly. There is no nucleolus to be seen in the mature myelocyte. The cell protoplasm shows a fine reticulum, faintly basophile, which contains ripe neutrophile granules similar in size and staining reaction to those of the poly-nuclear leucocyte. These granules are most abundant at the periphery of the cell.

The neutrophile myelocyte is very similar in appearance to the large mononuclear cell of normal blood, and the larger size of the granules in the former seems to constitute the main point of difference between the two. Both are probably derived from the myeloblast.

The myelocyte is not generally regarded as being actively amœboid, but it has been described as taking part in phagocytosis.[1] It is present in the blood in large numbers in myelogenous leukæmia. It is also met with in certain of the anæmias of childhood, in the infectious fevers, especially in children, and in diseases of the bone-marrow.

There is another cell similar to the neutrophile myelocyte in size and in the appearance of its nucleus, but differing in having in its cytoplasm the eosinophile granules of the eosinophile leucocyte in place of the neutrophile granules

[1] Rowley, *Journal of Experimental Medicine*, January, 1908.

of the polynuclear leucocyte. It is called the *eosinophile myelocyte*, and it is found in the blood in myelogenous leukæmia, in certain of the anæmias of infancy, and in any marked eosinophilia.

Both the neutrophile and the eosinophile myelocyte are, according to Ehrlich, originally derived from a cell to which the name *myeloblast* has been given. This cell is very abundant in the marrow of the embryo, but it is only found in small numbers in that of the healthy adult. In certain of the cases of acute myelogenous leukæmia, however, the marrow may revert to the embryonic type, and the myeloblast may then be the prevailing marrow cell, not only in the marrow, but also in the blood. There are larger and smaller forms of the myeloblast, and the former in particular are very similar in appearance to the myelocyte, differing chiefly in there being an absence of granules from their cytoplasm.

Some hæmatologists do not seem to recognise the existence of the myeloblast as such. Some call it a leucoblast, and some confuse it with the large (pathological) lymphocyte. But Ehrlich insists on the distinction between this last cell and the myeloblast, and he says that transitional forms connecting the two types of cell are never found. The two, he says, have a quite separate origin, the myeloblast arising from bone-marrow, and the pathological lymphocyte from lymphatic gland tissue.

The following features, according to Naegeli,[1] serve to distinguish the myeloblast from the large lymphocyte:—The nucleus of the myeloblast stains with moderate intensity, staining much deeper with the triacid stain than does the nucleus of the pathological lymphocyte. With Giemsa's stain

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[1] *Anæmia*, by Ehrlich and Lazarus, translated by Armit, second edition (Rebman, Limited), 1910, p. 102.

three to four nucleoli are to be seen as blue rings inside the myeloblast nucleus. The cell protoplasm in the myeloblast has a basophile reticulum which comes close up to the nuclear membrane, and leaves no clear perinuclear zone as in the lymphocyte. Azurophile and fuchsinophile granules are absent from the myeloblast. Certain of the myeloblasts, too, show a commencing neutrophile granulation, and all gradations between such forms and the mature myelocyte are to be met with. The nearer to the myelocyte the less basophile is the cell reticulum, and as the myelocyte stage is reached the nucleoli of the myeloblast disappear.

But, whilst these distinguishing features may be useful in the hands of the skilled histologist, they have not proved of the same service to the clinician. And so one turns with a measure of relief to another possible means of distinguishing the myeloblast from the large lymphocyte. We have just seen that the polymorphonuclear leucocyte contains oxidising and proteolytic ferments, and, as Dr. Shaw Dunn[1] demonstrated to the Medico-Chirurgical Society last winter, these ferments, whilst absent from the lymphocyte, are present in both myelocyte and myeloblast. Tests have therefore been devised whereby this ferment action of the myeloblast can be readily demonstrated, and it is claimed that a positive result with these tests serves to distinguish the myeloblast from the lymphocyte in which the reaction is negative.

The myeloblast may show some resemblance to the large mononuclear leucocyte, but in the latter the nucleus is most often kidney-shaped, and it contains no nucleoli. Its cell protoplasm, too, has a grey-blue colour when stained with Giemsa's stain, in place of the bluer blue of the myeloblast.

[1] *Glasgow Medical Journal*, October, 1910; see also *Journal of Pathology and Bacteriology*, July, 1910.

There are still two other forms of leucocyte frequently described, but they are rarely met with, and only in disease. They are not really additional varieties of the white corpuscle, for the first seems to be nothing more or less than a portion of a fragmented polynuclear leucocyte, and the other a myeloblast undergoing degeneration. The first has been called the *small neutrophile pseudolymphocyte*. It is the size of the small lymphocyte, and it has a large round deeply-stained nucleus with neutrophile granules in the narrow zone of surrounding protoplasm. The cell is found in the blood in small-pox, and in pleural exudations. It is supposed to be, as I have just said, a portion of a broken-up polynuclear leucocyte—that is, there is a part of the nucleus with some of the granular protoplasm adherent to it.

The other type of cell is the "*irritation form*" (Reizungs-formen of Türk), which is chiefly met with in leukæmia, and which Ehrlich says is a pathological myeloblast; at one time it was regarded as an abnormal nucleated red corpuscle. The cell measures some 8.5μ in diameter, but is often considerably larger. There is a central round or oval nucleus which is homogeneous throughout, and stains with moderate intensity. There are no nucleoli. The cell protoplasm is strongly basic, and is often seen to be vacuolated. It stains deep blue with Giemsa's stain and deep brown with the triacid stain.

LECTURE II.

In the first lecture I described to you the various types of corpuscles met with in the blood in disease as well as under normal conditions; but in order to fully understand the significance of the abnormalities, it is essential that we should have some sort of conception of the origin of all these corpuscles as well as of their mode of production.

The two tissues specially concerned with the formation of blood corpuscles are (1) the bone-marrow, and (2) the lymphatic glands, with which we include the lymphatic tissue throughout the body. Ehrlich's view, which is now very generally accepted, is to the effect that the lymphocytes are produced almost exclusively in this lymphatic tissue, whilst all the other white corpuscles, as well as the red corpuscles, have their origin in the bone-marrow.

In the healthy adult only a part of the **bone-marrow** is functionally active, but in the fœtus, and during the first years of life, the whole of the marrow is highly vascular (red marrow) and takes part in the formation of blood corpuscles. As age advances, however, the red marrow lessens in amount, being replaced by fat; and in the adult the red marrow is limited chiefly to the ends of the long bones, to the ribs, and bones of the skull. But the amount of active marrow varies not only with the age of the individual, but also in different individuals of the same age. It varies, too,

in different bones and in different parts of the same bone. Its amount varies most of all in health and disease, for when there is a great demand for fresh red corpuscles, as in anæmia, or for white corpuscles, as with a leucocytosis, then the amount of the active marrow increases so that it may supply the increased demand. And not only does white marrow in this way become transformed into red marrow, but the red marrow itself may become much more active than under ordinary circumstances. The conversion of white to red marrow may affect larger or smaller areas of marrow simultaneously, but usually it is a matter of local spread from the margin of the active to the adjacent latent tissue. When all the fat cells of the white marrow have been replaced and still more blood-forming tissue is required, an absorption of bone begins to take place so as make room for the increasing number of marrow cells.

On microscopic examination normal marrow is seen to be made up of a congeries of large fat cells held together by a fine framework of retiform tissue. In this reticulum lie the blood-forming and formed cells of the marrow. White marrow being latent contains but few of these blood cells. Red marrow, on the other hand, is exceedingly cellular, and, if specially active, may be so overrun with cells as to have the fat spaces entirely obliterated.

Certain of the narrow cells, it is found, are concerned with the production of red blood corpuscles, and constitute what Professor Muir [1] has called the *erythroblastic* part of the marrow. Other cells have to do with the formation of white corpuscles, and so they are called the *leucoblastic* marrow cells. In normal red marrow the erythroblastic and leucoblastic cells are usually found more or less mixed up with each other. In new-formed red marrow, however,

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[1] *Journal of Pathology and Bacteriology*, 1901, p. 161.

an arrangement of cells into erythroblastic and leucoblastic areas can often be distinctly made out. When this is so the erythroblastic tissue is seen to consist of wide capillaries lined [with nucleated red blood corpuscles, whilst towards the centre of the capillary are the ordinary non-nucleated red cells. Amongst the nucleated corpuscles are seen micro-blasts, normoblasts, and cells similar in appearance to the normoblast but rather larger (8 to 10 μ). In the marrow of the fœtus, and in early infancy, megaloblasts are also present, and certain hæmatologists maintain that a small proportion of megaloblasts are likewise to be seen in normal adult marrow. Ehrlich, however, does not take this view, for he insists that the normoblast and megaloblast are quite different types of cell, and that the former is never derived from the latter.

The non-nucleated red corpuscle of the blood is formed from the nucleated red cell by loss of its nucleus. According to some authorities the nucleus disappears by a process of extrusion, and according to others by fragmentation and solution in the cell protoplasm. Ehrlich believes that the nucleus of the normoblast is lost by extrusion whilst the nucleus of the megaloblast disappears by fragmentation and solution.

True mitosis is not commonly seen in the nucleated red corpuscles, but, according to Dickson,[1] it is much more frequent in the larger normoblast than in the normoblast of normal size; and so in normal adult marrow this larger normoblast is probably the cell from which the non-nucleated red corpuscle is derived, the normoblast of normal size being an intermediate stage in the process of development.

The leucoblastic areas of the marrow are made up of white corpuscles, and they are said to be always extra-vascular.

[1] *Ibid.*, p. 64.

The prevalent leucocyte in this leucoblastic tissue is the neutrophile myelocyte. It represents from 50 to 60 per cent of all the cells and shows the most active mitosis. Then there is a smaller and somewhat variable proportion of polynuclear neutrophiles; these last are found lying nearer to the blood-stream than the myelocytes from which they are derived. A small proportion, too, of eosinophile cells, both mononuclear and polynuclear, are present, scattered amongst the other cells. About 1 per cent of mature and immature mast cells and a very few myeloblasts are also met with. In addition to these a larger proportion (15 to 20 per cent) of cells which have the appearance of small lymphocytes[1] are to be seen; but it is rare in normal adult marrow to find them arranged so as to form definite lymph follicles.

Besides the above white corpuscles, any of which may be present in the blood-stream, at least two other types of cell are met with in bone-marrow. The first of these is the giant cell. It is large (20 to 50 μ in diameter), varies greatly in size, and is very irregular in shape. The nucleus is complex in structure, sometimes described as being basket-shaped, but sometimes it seems to be polynuclear; it stains rather deeply. The cell protoplasm is non-granular and in the mature cell stains with acid dyes.

The giant cells are increased in number in pneumonia, septicæmia, and other such diseases. They are phagocytic, and are often seen to contain leucocytes in various stages of disintegration.

This giant cell is probably derived from the cells of the

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[1] Naegeli says these cells do not contain fuchsinophile granules, and are, therefore, not lymphocytes ; he regards them as myeloblasts. He says the bone-marrow does not normally produce lymphocytes (*Anæmia*, Ehrlich and Lazarus, 1910, p. 117).

marrow reticulum, but possibly also from certain of the marrow cells themselves.

In addition to the giant cell there are other phagocytic cells, which have been called pigment cells. They vary greatly in size, and contain a pale nucleus, somewhat oval in shape, and usually two nucleoli. The cytoplasm is non-granular, and may contain numbers of red blood corpuscles, as well as other cells, and also pigment granules. These cells are not only phagocytic but also amœbic, and are found in the marrow in considerable numbers in pernicious anæmia and other diseases where there has been much destruction of blood corpuscles. They are probably derived from the endothelial cells of the capillaries as well as from the branching cells of the marrow reticulum.

Now we have just seen that when there is an increased demand for white or red blood corpuscles that the bone-marrow assumes an unwonted activity. This, to begin with, seems to affect simultaneously both the erythroblastic and leucoblastic cells of the marrow, so that there is an initial increase of red and white corpuscles. Soon, however, the activity limits itself to one special type of cell. For example, in a secondary anæmia where there is need of an increased production of red corpuscles there is now a special activity of the erythroblastic cells, and the erythroblastic areas are proportionately increased. If, on the other hand, there is a demand for white cells, as in abscess formation, it is the leucoblastic elements that show the increase. In this leuco-blastic tissue, too, one type of leucocyte may show much greater productive activity than the others. In a poly-nuclear leucocytosis it is the neutrophile myelocytes[1] that

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[1] *Note.*—From the neutrophile myelocytes are formed the polynuclear cells. But there seems seldom to be a great reserve of these latter cells in the marrow; hence with a polynuclear leucocytosis if the myelocytes

are increased in number, whilst in enteric fever and small-pox the chief increase is in the cells resembling lymphocytes. Then, again, there may be an eosinophile leucocytosis met with in such diseases as helminthiasis, asthma, urticaria, and pemphigus. This must mean an increased production of eosinophile cells in the blood-forming tissues, but, as far as I know, it has not been conclusively determined that there is always an increase of eosinophile cells in the marrow corresponding to the increase of eosinophiles in the blood, although one would expect that it would be so.[1]

It seems clear, therefore, that in certain morbid conditions one type of marrow cell may be stimulated to greater pro-ductive activity than the others, and that the stimulus must be a specific one, for individual diseases have their specific forms of leucocytosis. The various marrow cells, then, apparently react differently to different stimuli, and it is found that the same stimulus may produce an increase of one type of leucocyte and at the same time an absolute diminution of another. By way of illustration one might quote the observation that eosinophile cells may disappear from the blood-stream during a polynuclear leucocytosis to return again when the leucocytosis is ended.

The stimuli which are responsible for these reactions in bone-marrow are probably generated at the seat of the infective process. It is well known that bacteria or their products may, on entering a tissue, have an attracting or repelling (positive or negative chemiotactic) action on the

cannot produce the polynuclears sufficiently quickly to meet the demand, they (the myelocytes) may themselves appear in the circulation. In long-standing leucocytosis there may not only be a great increase of myelocytes in the marrow, but also an increase of myeloblasts.

[1] Dickson suggests that the marrow is only one of the several sites where eosinophile cells proliferate, and that these cells may be formed in certain of the other hæmopoietic tissues (*Ibid.*).

leucocytes of the blood; and certain of these chemiotactic substances doubtless enter the circulation and produce corresponding effects in the analogous marrow cells. We must note, however, that it is not always only one type of marrow cell that is stimulated in this way, for in myelogenous leukæmia we may have several forms of leucocyte—polynuclears, neutrophile and eosinophile myelocytes, and mast cells, all greatly increased in number, both in the marrow and in the bloodstream. In the more acute cases of leukæmia, again, these cells may to a large extent disappear from the blood and marrow, to be replaced by the very active increase of a more primitive marrow cell, the non-granular myeloblast. A similar sort of reaction occurs in pernicious anæmia where the erythroblastic areas not only become greatly extended, but take on the megaloblastic or embryonic type of growth. In a condition to which the name leukanæmia has been given we seem to have an increased activity both in the primitive erythroblastic and leucoblastic areas, for megaloblasts as well as myelocytes are found present in the blood.

In contrast, however, to the hyperplasias of bone-marrow, certain degenerative changes fall to be recorded, and these have almost always associated with them a lessened production of blood corpuscles. They may therefore determine an anæmia, or a leukopenia, or a faulty leucocytosis, or all of these.

In the first place, the extent of the red marrow of the adult may be considerably restricted, the conversion of the red marrow of the child to the white marrow of the adult becoming more extensive than normal. As a result, areas in the adult, normally red, are replaced by fat, and so are useless as blood-forming tissue.

The marrow may also undergo a gelatinous degeneration,

the red or yellow marrow being replaced by a gelatinous-looking substance allied to mucin. This condition is met with after undue activity of the marrow, as with a prolonged suppuration, also in starvation, and in general debility. Drs. Stockman and Charteris[1] have produced similar changes in animals after a lengthy administration of such drugs as arsenic, mercury, or lead.

Then there may be an overgrowth of the connective tissue elements of the marrow, producing a sclerosis similar to what may be met with in the liver or kidneys. Such a change has been seen in syphilis, old age, and long-standing debility.

In addition to these changes extensive hæmorrhages have been found in the bone-marrow, destroying the function of considerable areas of marrow tissue. There may likewise be tumour growths infiltrating the bone-marrow and displacing the erythroblastic and leucoblastic cells. Carcinoma is perhaps the most common of these neoplasms. A tumour growth of this sort, in the process of replacing the marrow, seems to disturb its cells in some unusual way, for it is not uncommon in such cases to find not only myelocytes, but also megaloblasts appearing in the circulation. In lymphatic leukæmia, too, the marrow gets so completely replaced by the proliferating lymphocytes that in many cases not more than 3 to 4 per cent of granular leucocytes are to be met with in the blood.

We have seen, then, that the bone-marrow is the tissue chiefly concerned in the formation of blood corpuscles. But the lymphatic glands also play a part, and it is they that are responsible for the production of most of the lymphocytes that are present in the blood.

[1] *Journal of Pathology and Bacteriology*, 1903.

In its general structure the lymph follicle of the **lymphatic gland** resembles somewhat the bone-marrow. As in the marrow there is a very fine reticular tissue in the meshes of which lie numerous cells, the cells in the case of the lymph follicle being entirely lymphocytes. These cells present something of a concentric arrangement, the ones at the centre (germ centre) being larger and showing active mitosis, whilst those at the periphery are the smaller, mature lymphocytes. From the periphery of the follicle the lymphocytes pass into the lymph sinuses, thence into the lymphatic vessels, and so to the blood-stream. It has been demonstrated that there are more lymphocytes in the efferent than in the afferent vessels of the lymphatic gland; hence more lymphocytes leave the glands than enter them. It seems quite clear, therefore, that the lymphatic glands supply lymphocytes to the blood of the general circulation.

Under ordinary circumstances blood-vessels do not open into the lymph channels of the lymphatic glands; but certain of the prevertebral glands form an exception to this statement, for their sinuses may be seen to contain red blood corpuscles. Such glands are called hæmolymph glands, and seem to be concerned in the destruction of red corpuscles; in this respect they have a resemblance to the spleen.

In lymphoid tissue, as in bone-marrow, the cells of the reticulum and the endothelial cells lining the sinuses are actively phagocytic. Indeed, one of the chief functions of the lymph glands is to act as a filter to the lymph stream, and to deal with such foreign substances as dust, disintegrating blood corpuscles, tumour cells, micro-organisms, &c., such as may be present in the lymph channels. In any infection of the lymphatic glands there is nearly always a proliferation of the endothelial cells; and in enteric fever the proliferation may be largely confined to these cells, and

may be so considerable as to produce a distinct glandular enlargement.[1]

The most common enlargement of the glands, however, is due to a hyperplasia of the lymphocytes themselves; and so one sees enlargement of the germ centres, more active mitosis, increase in the size of the follicles and in the number of lymphocytes in the sinuses. The entrance into the gland of foreign material seems at times to act as a stimulus to this hyperplasia. If the stimulus reaches the gland by the lymphatic vessels not more than a group of glands will be enlarged. If, on the other hand, it comes by the blood-stream there may be a general glandular enlargement, such as one sees in glandular fever and in other blood infections.

But the hyperplasia of lymphoid tissue, even when wide-spread, does not always determine an increase of lymphocytes in the blood. Ehrlich has always taught that lymphocytes have no amœboid movement, and that they do not respond to chemiotactic influences. When a lymphocytosis does take place there is, he says, increased functional activity of the gland, but the movements of the lymphocytes are entirely passive, for they are washed out of the glands into the lymph stream, an increased flow of lymph carrying out an increased number of lymphocytes. This view, how-ever, is not universally accepted, some hæmatologists holding that the lymphocyte has amœboid movement and that it does respond to chemiotactic influences.

A lymphocytosis is, on the whole, rather a rare occurrence, at least when compared with the polynuclear leucocytosis. Perhaps the best example is the lymphocytosis of whooping-cough, which may reach from 20,000 to 40,000 per c.mm. If such a lymphocytosis is passive it would be explained

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[1] Muir, *Trans. Path. Soc. of Lond.*, vol. liii, part 3, 1902.

as due to swelling and irritation of the peribronchial glands during the paroxysms of coughing. It is to be noted that in infancy, where there is greater activity of the lymphoid tissues, the proportion of lymphocytes in the blood is always considerably larger than in the adult. In the illnesses of infancy the blood readily shows both a relative and absolute lymphocytosis.

But in contrast to the lymphocytosis there may be an absolute as well as a relative diminution in the number of lymphocytes in the blood. Such a condition may be produced by a widespread disease of the lymphoid tissues. There may be simple atrophy, as met with in old age, in anæmia, and cachexia. There may be a fatty or hyaline degeneration, or a fibrosis or calcification of the glands. Any of these, if widespread, might determine a diminution in the production of lymphocytes. Tumour growths may act in the same way by displacing the lymphoid tissues of the glands: secondary sarcomatous, carcinomatous or endo-theliomatous growths are not uncommon in lymphatic glands. The primary neoplasms met with are more often associated with hyperplasia rather than with destruction of the lymphoid elements. With these primary growths I include lympho-sarcoma, lymphatic leukæmia, and pseudo-leukæmia. There seems to be all degrees of malignancy in such hyperplasias, ranging from the simple hyperplasia to what might be called the hyperplasia of lymphosarcoma which infiltrates the gland capsule and extends to the neighbouring tissues. In lymphatic leukæmia there is usually a very great increase of the lymphocytes in the circulation. The same may apply to lymphosarcoma, but in the other growths the numbers of white corpuscles remain more or less normal. Finally, in myelogenous leukæmia the lymphoid tissue may in part be transformed into a tissue resembling red

bone-marrow, and so different varieties of granular leucocytes may thus be produced in the lymphatic glands.

I have thus described to you the two tissues chiefly concerned in the production of white and red blood corpuscles, as well as some of the changes that may take place in these tissues as a result of disease. Incidentally, too, we have seen that when one type of cell has been proliferating to an excessive degree, it may reach what seems to be the limits of its reproductive power. The proliferation is then taken up by a more primitive cell, which now becomes the prevailing cell in the marrow or lymphatic gland and probably also in the blood. In pernicious anæmia, for example, where there is a great increase in the activity of the erythroblastic part of the marrow, the normoblasts become to a large extent replaced by megaloblasts. In the same way in myelogenous leukæmia the proliferating neutrophile myelocyte may give place to the non-granular myeloblast. The more acute the process the more primitive seems to be the proliferating cell. This is well illustrated not only in myelogenous but also in lymphatic leukæmia, in the most acute forms of which the prevailing cell is the large immature lymphocyte rather than the normal lymphocyte of the blood. In the anæmias of infancy, too, there seems to be a special tendency for these embryonic cells to proliferate, and the younger the subject the more readily do the marrow cells assume their embryonic activity. This leads us, then, to enquire as to the nature and origin of these primitive blood corpuscles, and as to their relationships to each other and to the more mature blood cells.

Now we know that the bone-marrow and lymphatic glands are not functionally active till the fourth or fifth month

of fœtal life, and, as blood corpuscles appear in the fœtal circulation considerably before this, it is quite apparent that they must be originally produced in some tissues other than those just mentioned.

The earliest appearance of at least the red corpuscles seems to be associated with the formation of the blood-vessels. To begin with, red corpuscles are probably formed in several different organs, but at a slightly later period the liver is their chief seat of origin. At this period of embryonic life it is exceedingly difficult to differentiate between the primitive red and the primitive white corpuscles; and indeed certain hæmatologists (Pappenheim and others) hold that originally there is only one type of blood corpuscle, resembling in appearance the large lymphocyte, and that this cell is the common ancestor of both the red and white corpuscles. Further, it is also said that before the third or fourth month of embryonic life there is only one type of white corpuscle, from which all others are ultimately derived, and that it has the general character of this large lymphocyte. Ehrlich, however, disputes this view, and he maintains that the lymphocytes and marrow cells have no common ancestor, and that they are genetically distinct. Naegeli[1] supports Ehrlich's conclusions, and he claims to have proof that the myeloid tissue develops first, and that at a considerably later period lymphoid tissue appears as a quite separate phenomenon. He says that cells of the myeloid series are in the blood before any lymphocytes can be found.

We may say, then, that it is a matter of dispute whether the red corpuscle has a common ancestor with the white corpuscle or is genetically distinct. Whichever it be, the earliest recognisable form of red corpuscle is a nucleated

[1] *Anæmia*, Ehrlich and Lazarus, 1910, pp. 119 and 148.

cell without hæmoglobin and in general appearance not unlike a large lymphocyte. This red corpuscle next acquires hæmoglobin and then has the characters of the megaloblast. About the fourth or fifth month the megaloblast seems to settle down in the bone-marrow, and there it proliferates, forming the erythroblastic (megaloblastic) part of the marrow. At birth the megaloblasts have to a large extent disappeared from the marrow and been replaced by normoblasts. There is a difference of opinion as to whether or not the normoblasts are derived directly from the megaloblasts. The normoblast then loses its nucleus, and so the mature red corpuscle is evolved.

It is likewise in dispute as to whether the various white cells are derived from one common ancestor, the primitive lymphocyte; or from two separate cells, one of myeloid origin and the other of lymphoid origin. Whichever it be, some of these primitive cells settle down in the lymph glands and these produce lymphocytes. Others proliferate and produce myeloid tissue, first in liver, spleen, and other tissues, and later in the bone-marrow. The original marrow cell is the myeloblast. From this are derived the neutrophile and the eosinophile myelocytes, the mast myelocyte, and the large hyaline cell. From the neutrophile and eosinophile marrow cells the polynuclears and eosinophiles of the blood are respectively derived. A small proportion of polynuclears probably also take origin from the hyaline (large mononuclear) cells, the transitional cell being the intermediate stage between the two. It is in dispute as to whether or not the marrow normally sends any number of lymphocytes into the blood-stream; and it is very doubtful if the mature lymphocyte, or even a primitive lymphocyte, ever becomes transformed into one of the granular cells or into the hyaline cell. Neither is there any

good reason for thinking that the neutrophile cells ever become eosinophile cells, or mast cells, or *vice versa*.

It is a matter of some difficulty to determine what exactly takes place when lymphoid tissue gets replaced by myeloid tissue, as it does in certain cases of myelogenous leukæmia; or when myeloid cells of the bone-marrow are replaced by lymphoid cells, as in lymphatic leukæmia. Ehrlich would deny the possibility of the transformation of one type of cell into the other by, for example, the germinal centres of lymph follicles producing myeloid cells. And there are reasons in favour of the view that there is not a transformation of one tissue into the other but a replacement of one by the other.[1]

There seems reason, too, for believing that this replacement does not altogether depend on a transplantation of the myeloid or lymphoid cell by, for instance, the blood-stream, but that the growth takes place from a pre-existing cell. The pre-existing cell, Naegeli[2] seems to think, is an undifferentiated cell lying in the tunica adventitia of the vessel wall. Whether there is only one type of cell, which may differentiate into a myeloid or a lymphoid cell according to the stimulus, or two types of indifferent cell, one promyeloid and one prolymphoid, must at present remain in doubt.

Another suggestion by Naegeli[3] is to the effect that such undifferentiated cells may previously have been differentiated and have returned to their embryonic or indifferent state, to again, under suitable stimulus, assume their former function. We know that in early embryonic life the liver, spleen, and lymphatic glands show myeloid growth which

[1] *Anæmia*, Ehrlich and Lazarus, 1910, p. 148.

[2] *Ibid.*, p. 150.

[3] *Ibid.*, p. 151.

disappears on the maturing of the bone-marrow. The myeloid metaplasia in these organs, met with in myelogenous leukæmia, certainly looks like a return of these tissues to their former type of activity.

Blood Platelet.—In addition to the red and white corpuscles in the blood there is a third element that has to be considered, namely, the blood platelet or blood plate. This body is round or oval in shape, and measures about 3μ, with a range from 1 to 5μ. With the Romanowsky stain it takes a bluish tint at the periphery, whilst at the centre it has a granular appearance and stains a reddish-purple colour. The blood plate, however, has no true nucleus, and indeed no definite structure whatever. Its staining reaction is alkaline, and it contains glycogen.

The blood plates in normal blood are said to range in number from 200,000 to 700,000 per c.mm., but the methods of estimation are not satisfactory, and these figures may not be accurate. The platelets are increased in pneumonia, secondary anæmia, chlorosis, and in most wasting diseases, whilst they have been said to be lessened in purpura hæmorrhagica and hæmophilia. The idea that they are reduced in the last two diseases has suggested the view that the blood plates have to do with the coagulability of the blood, and it has been said that blood will not coagulate in the absence of blood plates. They seem also to have adhesive properties, and they tend to form clusters whenever the blood is shed.

Ever since the discovery of the blood plates there has been much difference of opinion as to their origin and significance. Hayem, who was the first to describe them, regarded them as the ancestors of the red corpuscles. Others maintain that they are artefacts produced after the blood

has been drawn, and that they have no existence in the circulating blood. Others, again, regard them as little buds nipped off from the red corpuscle, or as extrusions from this corpuscle, remnants possibly of its lost nucleus. Some authors have thought the plates to be extrusions from the white corpuscle; and, lastly, J. H. Wright has brought forward evidence in favour of their being derived from the processes of the giant cell of the marrow. It is difficult to be certain what is the true origin of the blood plates, but the consensus of opinion seems to take the view that they are derived from one or other of the cells of the blood or of the marrow; but whether they have any definite function, and, if so, what exactly it may be, remains in doubt.

Nearly allied to the blood plates is the **blood dust** or **hæmoconia**. This consists of minute, colourless, refractile bodies which show active Brownian movement. They resemble in appearance micrococci in the blood, and they have been thought to be granules escaped from some of the granular leucocytes. They have no relationship to the coagulability of the blood, and in this respect they possibly differ from the blood plates.

I propose now in the time that remains at our disposal to pass in review, very briefly, some of the recognised diseases and disorders of the blood, and to try to indicate to you the present position of our knowledge in regard to their etiology and pathogenesis.

Secondary Anæmia.—Secondary anæmia is not in itself a disease, but rather a symptom occurring in the course of many different diseases. It may, therefore, be due to a variety of morbid conditions. There may be a diminished

production of blood corpuscles due to some fault of the bone-marrow. There may be loss of blood due to one or more hæmorrhages, or increased destruction of red corpuscles as in the septic infections, rheumatism, malaria, and, indeed, with almost any form of intoxication. There may be both increased destruction and defective formation of blood acting at the same time.

The condition of the blood in a typical case of secondary anæmia is fairly characteristic. There is a moderate diminution in the number of red cells, and a greater diminution in the hæmoglobin. The individual red cells tend to be smaller than normal, and the number of poikilocytes and normoblasts is in proportion to the degree of the anæmia. There is usually a slight leucocytosis.

Some of the severer secondary anæmias may closely resemble pernicious anæmia, and hæmatologists are not agreed as to the distinction that should be drawn between the two. Certain writers hold that all anæmias of known cause should be regarded as secondary anæmias, whether or not there are megalocytes and megaloblasts present in the blood. Others take the view that the presence of these cells indicates a specific change in the bone-marrow, and that all cases with megalocytes and megaloblasts in the blood should be classed as pernicious anæmia. I shall discuss this point more fully later on.

Chlorosis.—Chlorosis still remains a disease of unknown etiology, and whilst it has many of the characters of secondary anæmia it is convenient to keep the two conditions separate. A notable feature which might serve to distinguish chlorosis from the other anæmias is that in the former there is a marked increase in the total amount of blood plasma, so that the blood-vessels are fuller than

normal. So far, there is no easy method of estimating the total volume of the blood, at least such as would be suitable for use in clinical medicine, although the method of Haldane and Lorrain Smith has been used for this purpose in some of the London hospitals.

Cabot has made the interesting observation that in America chlorosis is disappearing, and that there are now not nearly so many cases as there were five to ten years ago; he cannot offer any explanation for this decrease in number. I know of no figures which would show the same state of matters in this country, but the subject might be worthy of some inquiry.

Splenic Anæmia.—Splenic anæmia is another anæmia of which the etiology is unknown. The blood has the characters of a secondary anæmia with leucopenia; but the distinguishing feature of the disease is the very considerable enlargement of the spleen, which enlargement seems to precede the anæmia. There is also a tendency to hæmorrhage, especially from the stomach. Many of the cases terminate with cirrhosis of the liver, ascites, and sometimes jaundice, to which symptom-complex the term *Banti's disease* is given.

It is doubtful if splenic anæmia is a specific disease, and not just a grouping of symptoms which may be caused by several different morbid conditions. The enlargement of the spleen is the one constant change found *post-mortem*, but on microscopical examination the histological appearances of this organ may be somewhat different in the different cases. It is customary to classify these changes under one of two different types.

1. In the first and more common type there is a general hyperplasia and fibrosis of the whole organ, involving

capsule, reticulum of pulp, and Malpighian bodies. The blood sinuses usually show proliferation of their endothelial cells, and these cells probably go to form the new fibrous tissue of the reticulum. There are strikingly few blood corpuscles enclosed in the altered pulp. Associated with this change in the spleen there is frequently a moderate cirrhosis in the portal areas of the liver; also, the portal vein, or one of its branches, may be found occluded by an old thrombus. The hæmolymph glands, too, may be enlarged, and Warthin[1] has described a marked endothelioid hyperplasia in these glands in four cases of Banti's disease examined by him. There is no constant change in the marrow in this type of splenic enlargement other than such as may be met with in any other secondary anæmia.

2. In the second type, that described by Gaucher, the appearances are very distinctive, and cannot properly be regarded as an earlier stage of type 1. On microscopical examination of the spleen there are seen to be a congeries of little rounded spaces filled with large cells, of 20 to 40 μ diameter, each cell having one or more small, deeply stained nuclei, and an abundant homogeneous-looking cytoplasm. Surrounding these spaces there are bands of coarse connective tissue. Similar groups of cells have been found in this type of splenic enlargement in the liver, lymphatic glands, and in the bone-marrow. There has been considerable difference of opinion as to the nature and origin of these cells, but the most recent writers seem to regard them as derived from the reticular or endothelial cells of the tissue in which they are growing. It is uncertain if they ultimately form fibrous tissue.

It is said that this Gaucher type of splenic anæmia differs somewhat in its clinical features from the ordinary type

[1] *Trans. Assoc. Amer. Physicians*, vol. xxiv, p. 286.

of the disease. In the first place, it has a tendency to affect several members in a family. The anæmia, too, appears later, and it and the general symptoms of debility are less pronounced. The liver tends to be larger than in the ordinary type.

There is no satisfactory explanation of the splenic enlargement in splenic anæmia. The fibrosis of the spleen, the tendency to hæmorrhages, and the associated cirrhosis of the liver suggest a resemblance to a primary cirrhosis of the liver. From that point of view the splenic enlargement would be regarded as toxic in origin; and the anæmia would be due to the same toxin, for the fibrosed spleen cannot very well be associated with an increased hæmolysis. The fact that excision of the spleen seems to cure the anæmia gives some colour to Banti's suggestion that the enlarged spleen elaborates toxic substances, and that the anæmia is due to the action of these toxins. There is, however, no proof of the formation of such toxins in the spleen; and so the pathogenesis of splenic anæmia must, in the meantime, remain obscure.

It is interesting in this connection to note that H. B. Day and A. R. Ferguson have recorded "A form of Splenomegaly with Hepatic Cirrhosis endemic in Egypt,"[1] which seems to have the clinical features of Banti's disease. They have not determined the cause of the condition, but they associate it with a toxæmia of intestinal origin.

Kala-azar, or Tropical Splenomegaly.—Kala-azar, or tropical splenomegaly, is a form of anæmia with enlargement of the spleen met with in India. The symptoms are fever of an irregular type, great enlargement of the

[1] *Annals of Tropical Medicine and Parasitology*, November, 1909, vol. iii, No. 3.

spleen, enlargement of the liver, and secondary anæmia. There is a very marked leucopenia (about 1,000 white corpuscles per c.mm.), and the decrease is chiefly in the polynuclears, there being a relative increase of both lymphocytes and large mononuclear cells. As the disease advances emaciation becomes very marked, and sometimes there are symptoms of obstruction in the portal circulation. The duration of the illness is usually from six to nine months, although some patients live for two to three years. It is practically always fatal, the mortality being from 96 to 98 per cent. *Post-mortem* there is usually found some cirrhosis of the liver, both intralobular and multilobular. The spleen, whilst greatly enlarged, rarely shows much fibrosis.

It is now generally accepted that the disease is due to the presence of a protozoön, to which the name " Leishman-Donovan body " has been given, after its discoverers. This organism is found in great abundance in the spleen, liver, bone-marrow, and, indeed, in most of the other tissues as well. It has also been seen in the blood, inside the polynuclear cells. It is thought to be conveyed from one person to another by means of the bed bug.

The anæmia and splenic enlargement of kala-azar are thus due to a definite and a known cause, and they must be regarded as analogous to the anæmia and splenomegaly of, for instance, enteric fever rather than to that of ordinary splenic anæmia. Kala-azar is, therefore, more correctly classed with the specific fevers than with the diseases of the blood.

Infantile Splenic Anæmia.—Infantile splenic anæmia (von Jaksch's splenic anæmia or anæmia pseudo-leukæmica infantum) is a condition affecting children between the

ages of 9 months and 2 years, and it is characterised by anæmia of the secondary type, leucocytosis, and enlargement of the spleen. The blood usually shows a proportion of nucleated red cells, both normoblasts and megaloblasts. The increase in the white corpuscles may affect, more or less uniformly, all varieties of the white cells normally present in the blood, but there is nearly always in addition a proportion of neutrophile myelocytes. It must, however, be remembered that in infancy the number of white corpuscles in the blood, as well as the proportions of their several varieties, is not the same as that of the adult. In children under 2 years the white corpuscles number in health from 12,000 to 14,000 per c.mm., and of these, the lymphocytes represent from 50 to 70 per cent, and the polynuclears from 30 to 40 per cent.

Opinions are divided as to whether infantile splenic anæmia is a specific disease, or simply a secondary anæmia occurring during infancy, a period at which the spleen and lymphatic glands readily enlarge, and the blood-forming organs generally are easily disturbed. There is no proof of the disease being due to a specific cause; and the arguments in favour of this view are based on clinical grounds, that is, on the more or less characteristic clinical picture the cases present to us. Further, it has been pointed out that the same causes as produce secondary anæmia in the adult are found associated with the origin of infantile splenic anæmia, and that the *post-mortem* appearances in no way suggest a disease *sui generis*. Histological examination of the spleen in some of the cases shows thickening of its capsule and fibrous reticulum, but in a number of other cases there is no evidence of such a fibrosis. The pulp most often shows a simple hyperplasia,

and only in a proportion of the cases has there been noted any increase of endothelial cells. The liver presents no constant or characteristic change; and beyond some hyperplasia of the lymphoid elements there is nothing special to note on examination of the lymphatic glands. In the bone-marrow there is seen such hyperplasia as one meets with in any secondary anæmia or leucocytosis. In some cases there has been found a certain amount of myeloid transformation of the liver, spleen, and lymphatic glands, but it is doubtful if these cases are not, as suggested by Ledingham,[1] rather examples of leukæmia (leukanæmia) than of the typical infantile anæmia. In support of this view I would quote a case, reported by me two years ago,[2] of an infant, seven weeks old, who had most of the signs of an infantile splenic anæmia, and yet on *post-mortem* examination proved to be a leukæmia.

If suitably treated the ordinary case of infantile splenic anæmia is readily curable, although the cure may be very slow. Death when it occurs is due most often to bronchopneumonia or some other complication. The enlargement of the spleen, however, may persist for a long time even after the anæmia has disappeared; or, again, the anæmia may persist, in varying degrees of intensity, for many years—for twenty years in one of my cases[3]—and yet there may be ultimate recovery, although the spleen usually remains enlarged.

It is difficult to determine what relationship a case of this sort has to the cases of splenic anæmia appearing in childhood (from 2 to 15 years of age), such, for example, as the two cases recorded by Dr. Cowan[4] in 1907. Possibly

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[1] Allbutt and Rolleston, *System of Medicine*, 1909, vol. v, p. 783.

[2] *Lancet*, 23rd January, 1909; Case 10.

[3] *Ibid.*, Case 1.

[4] *Quarterly Journal of Medicine*, October, 1907.

some of these latter are cases of infantile splenic anæmia where the splenic enlargement and anæmia had not been previously noted, and others may belong to the Gaucher type of splenic anæmia already referred to. On all these points, however, fuller information is required.

Finally, it is to be noted that in five cases of infantile splenic anæmia occurring in Naples and three in Tunis,[1] an organism similar to the Leishman-Donovan body has been found, and this suggests that in these cases the anæmia and splenomegaly have a common origin with the kala-azar of the adult.

[1] Quoted by Ledingham, *ibid.*, p. 785.
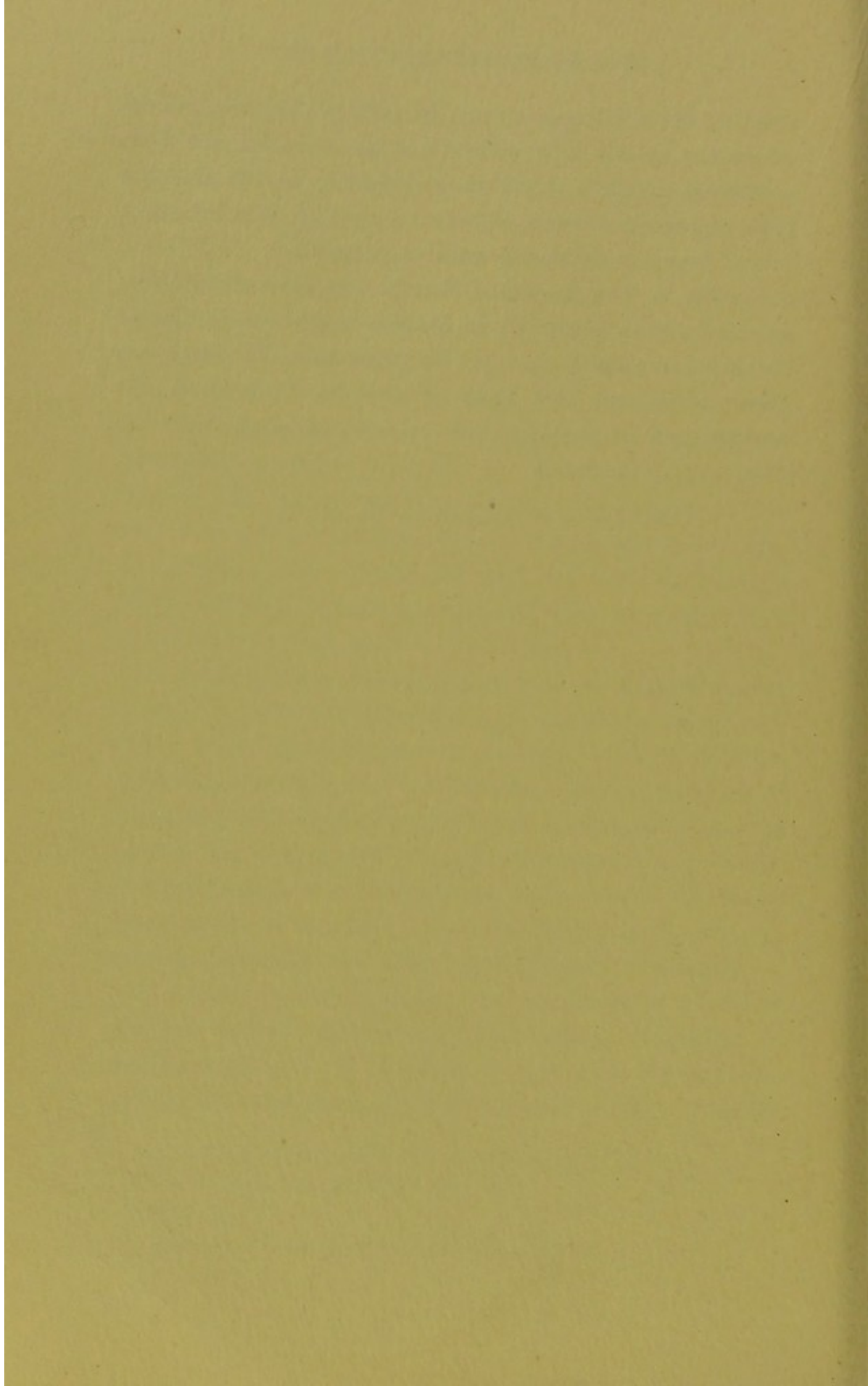
LECTURE III.

Pernicious Anæmia.—This anæmia we must consider with rather more detail, for there is some difference of opinion as to what constitutes pernicious anæmia, as well as regards its etiology and pathogenesis.

But neglecting for the moment such a consideration, and using the term in a general way, one might describe pernicious anæmia as a severe and progressive anæmia, insidious in onset, and showing usually one or more striking remissions, but ultimately going on to a fatal termination; as being more common in men than in women, and much more common after than before 35 years of age. Characteristic blood changes are also present, there being great diminution in the number of the red corpuscles (2,000,000, or less, per c.mm.), high hæmoglobin index, absence of leucocytosis, and the presence of a considerable proportion of megalocytes, and possibly some megaloblasts. On *post-mortem* examination the two outstanding changes met with are (1) the great excess of iron (hæmosiderin) in the liver, and, to a less extent, in the kidneys, spleen, and marrow, and (2) the hyperplasia of red bone-marrow, this growth being, in part at least, megaloblastic in type. A description such as this, I take it, will be accepted by most hæmatologists as a sufficiently accurate characterisation of at least the average case of pernicious anæmia.

Now, it is not disputed that the anæmia is due to an abnormal destruction of red corpuscles which has been going on in some part of the circulation, nor is there any doubt that the increase of iron in the liver is the result of this hæmolysis. It is also recognised that it is the large proportion of megalocytes that determines the high hæmoglobin index, and that the megalocytes, in turn, are derived from the megaloblastic marrow. So far, there is fairly general agreement; but when we come to inquire into the relationship of the hæmolysis to this megaloblastic marrow we find ourselves in the midst of an active controversy. Briefly, the main dispute is as to which of these two morbid processes is primary, as to whether the disease begins as a hæmolysis or as a megaloblastic degeneration of the bone-marrow.

The former view is the older one, and it was formulated by William Hunter more than twenty years ago. According to this theory a hæmolytic toxin is elaborated in some part of the alimentary tract, from whence it passes into the portal circulation, producing there a great destruction of red corpuscles. The iron pigment liberated from the corpuscles is then carried to the liver and stored up in the liver cells, giving the hæmosiderin reaction which Hunter regards as pathognomonic of Addisonian anæmia. The hæmolytic toxin he regards as a specific substance, and in its origin closely associated with an inflammation of the tongue and similar lesions which he finds *post-mortem* in the gastric and intestinal mucous membranes.

Hunter finds glossitis as an early symptom in all his cases. Sometimes it is acute, sometimes chronic and degenerative in character; but when once contracted it is exceedingly difficult to cure. He finds, too, that an exacerbation of the glossitis is frequently the forerunner

of a period of increased hæmolysis. Further, this hæmo-lytic toxin, he says, has a distinctly stimulating action on the bone-marrow, and the hyperplasia of the marrow he regards as compensatory and secondary to the destruction of red corpuscles.

Besides this, he draws a definite distinction between Addisonian anæmia and what he calls septic anæmia, the form of secondary anæmia which clinically resembles most closely Addisonian anæmia. In septic anæmia there is often an oral sepsis, but no hæmolysis confined to the portal area, and no excess of iron in the liver. In this anæmia, too, the septic toxin has an aplastic rather than a hyperplastic action on the bone-marrow. Glossitis is absent in septic anæmia. But these two forms of anæmia may run concurrently in the same patient, and the great improvement which sometimes takes place on curing the oral sepsis is due to the removal of the septic anæmia with its inhibitory action on the marrow. The removal of the oral sepsis does not cure the glossitis, and, therefore, does not cure the Addisonian anæmia; but, on the other hand, the oral sepsis is favourable to the development of the glossitis and the other lesions of the alimentary tract with which Hunter associates the production of the specific hæmolytic toxin.

The other theory[1] in regard to the production of pernicious anæmia is to the effect that a toxin is produced in some part of the body, not necessarily the digestive tract, and that it acts primarily on the marrow, producing a megalo-blastic rather than a normoblastic hyperplasia. The result of this megaloblastic marrow is that immature and ill-formed red cells are sent into the circulation, and these being more

[1] See, for example, paper by Gulland and Goodall, *Journal of Pathology and Bacteriology*, January, 1905.

vulnerable than normal corpuscles there is increased hæmolysis. The evidence of this hæmolysis is said to be widespread, and not limited to the portal area.

Both theories, therefore, recognise the action of a toxin, and both recognise a hæmolysis; but the first theory maintains that the toxin acts primarily as a hæmolytic agent, whilst the second takes the view that its initial action is to produce a megaloblastic marrow. The point at issue may not seem of very great consequence; but the view one takes as to the essential lesion in pernicious anæmia makes a considerable difference in the grouping of the various cases that may present themselves for consideration. Most hæmatologists who accept the first theory seem to regard pernicious anæmia as a specific disease, caused by a specific toxin, and they would include only the cryptogenic cases under the term, *i.e.*, the cases that Hunter would call Addisonian anæmia. Most of those who accept the second theory seem to regard pernicious anæmia as a group of symptoms resulting from various causes, any of which will produce a megaloblastic marrow. According to this view, "pernicious anæmia" is much more comprehensive, and would not be a disease *sui generis*, although some writers seem to think that the toxin which produces the marrow change is a specific poison.

But let us consider some of the arguments that have been advanced for and against these different views. We have seen that Hunter associates the origin of the toxin with certain lesions in the alimentary tract; his conclusions, however, are not generally accepted as proved. In not more than about 50 per cent of the cases collected by the various writers on the subject has there been evidence of sore tongue, or sickness, or vomiting. In 372 of Cabot's[1]

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[1] Osler and M'Crae's *System of Medicine*, vol. iv, p. 622.

collected cases careful enquiry was made regarding the state of the mouth and tongue, and in only 42 per cent was there any evidence of a lesion in the buccal mucous membrane. And it is always to be remembered that the alimentary disturbances may be the result of the treatment with arsenic, or may be secondary to the anæmic condition instead of the cause of it.

Hunter insists that the glossitis is an initial symptom; but this must be difficult to determine, for pernicious anæmia is so essentially insidious in its onset that one almost never sees a case in the initial stage of the disease. Indeed, in the majority of cases there is an anæmia of about 2,000,000 red corpuscles per c.mm. before the patient presents himself for diagnosis and treatment.

But apart from the attacks of vomiting and diarrhœa which a proportion of the cases complain of, and which are often paroxysmal in character, the digestive tract presents little evidence of disease. The appetite may be poor, but the digestion is usually good, and the absorption of proteins, carbohydrates, and fats shows little abnormality. There is seldom excess of ammonia or sulphates in the urine, such as would suggest undue intestinal putrefaction. *Post-mortem*, too, no constant or specific lesion is to be found in the mouth, stomach, or intestine—at least, judging from the majority of recorded cases; and any such lesion as is found might equally well be secondary to the toxæmia and general anæmia.

But the alimentary tract has been claimed to be the seat of origin of the toxin because hæmolysis is said to take place in the portal area, and the amount of iron pigment in the liver is reckoned the measure of the hæmolysis that has taken place. There is no doubt of the great increase of the iron in the liver in all the Addisonian cases of pernicious

anæmia. In the normal liver there is about 0·09 per cent of iron, whilst in pernicious anæmia it may be over 1 per cent. The iron in the liver, however, may be increased in any condition in which there is increased destruction of blood corpuscles, as, for example, in pyæmia; but the increase of iron is much greater and much more constant in pernicious anæmia than in any other disease.

There is also increase of iron in the urine, in the kidneys, and in the spleen in many of the Addisonian cases. In the urine the iron may be three to four times as much as in health. In the kidneys there is normally 0·01 per cent of iron, but in pernicious anæmia there may be as much as 0·09 per cent. The spleen in health contains about 0·18 per cent, but in pernicious anæmia it may be up to 0·3 per cent.[1]

But whilst increase of iron means increased hæmolysis, it does not necessarily mean that it is due to a toxin passing from the alimentary tract into the portal circulation. It is known that changes similar to those met with in pernicious anæmia, i.e., hæmosiderin in the liver and a megaloblastic marrow, can be produced by certain hæmolytic drugs (ricin, saponin) injected into the general circulation; so that there is no good reason for insisting that the excess of iron in the liver means a toxin more or less limited to the portal area. Besides this, we sometimes find cases of anæmia with what seems to be a toxic degeneration in the spinal cord, and which show symptoms of the spinal disease prior to the anæmia. Dr. Byrom

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[1] It is to be noted in passing that the intensity of the ferrocyanide test for iron pigment is not necessarily in proportion to the amount of iron present in these tissues. This reaction, if positive, indicates an excess of iron, but it specially indicates that the iron is present in such simple form as to respond to ordinary chemical tests.

Bramwell[1] has recently reported such a case where the spinal symptoms were present long before there was any appearance of the anæmia which ultimately developed, and which clinically and on *post-mortem* examination had all the features of a typical Addisonian anæmia. If this spinal lesion is produced by the same toxin as the anæmia, it is apparent that the toxin has been acting on the tissues of the general circulation before it has shown much effect on the blood in the portal area. Dr. Bramwell's case, too, may be quoted as an argument against the view that combined degeneration of the cord in pernicious anæmia is due to the anæmia. But, on the other hand, we cannot insist that the degeneration is produced by a specific toxin, for the degeneration is also met with in severe secondary anæmias as well as in leukæmia.

M'Neil[2] has shown that the blood serum from the nine cases of pernicious anæmia examined by him had no hæmolytic action on washed red corpuscles; and Cabot suggests that the toxin of pernicious anæmia does not act directly as a hæmolytic agent, but that it stimulates to an excessive activity the hæmolysis that normally goes on in the spleen, hæmolymph glands, and bone-marrow, and that the iron liberated is changed into hæmosiderin in the liver and kidneys. Iron in any case is normally stored by the liver as well as by the spleen and bone-marrow; and it is reasonable to think that with excessive hæmolysis there should be an excessive amount of iron in the liver and these other organs. Pathologists are not agreed as to whether or not hæmolysis actually takes place in the liver itself in pernicious anæmia.

Still another view regarding the excess of iron in the

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[1] *British Medical Journal*, 11th June, 1910.

[2] *Journal of Pathology and Bacteriology*, July, 1910, p. 64.

liver is that of Professor Stockman. He holds that pernicious anæmia is due to multiple small hæmorrhages, and that the excess of iron pigment is derived from the extravasated blood. Pernicious anæmia would thus be a severer form of secondary anæmia in which hæmorrhages had occurred.

Most of these theories, then, presuppose a toxin in some part of the circulation, but whether or not the toxin comes from the alimentary tract is not certainly determined. And the excessive amount of iron in the liver indicates an excessive liberation of hæmoglobin. This may result from extravasation of blood; from increased hæmolysis going on in the general circulation, or in the portal circulation, or as an increased activity of the ordinary hæmolytic apparatus—all the result of some toxic agent; or, finally, the hæmolysis may depend on the marrow sending into the circulation immature red cells, which are possibly of little use to the organism, and are, therefore, quickly removed and broken up.

As to the bone-marrow in pernicious anæmia, there is little doubt that in the vast majority of cases it shows an active hyperplasia. The type of hyperplasia, however, varies considerably in the different cases. In some, the marrow is largely erythroblastic, much of which may be megaloblastic in its type of growth. In a larger number of other cases there is a great increase of the leucoblastic elements, and in many of the cases the white cells in the marrow are not only absolutely but also relatively increased. The increase in some cases seems to affect chiefly lymphocyte-like cells; in others, the myelocytes; and yet in others, again, both these types of cell about equally. But in spite of this leucoblastic overgrowth in the marrow, there is a lessened number of white cells in the circulation.

The number of the lymphocytes in the blood is little altered, although they are relatively increased, but the polynuclear cells are both absolutely and relatively diminished.

Now, we have seen that different interpretations are given to these changes in the marrow. According to one view (Ehrlich, Gulland, Ewing), the megaloblastic marrow is the primary and one constant lesion in pernicious anæmia. Ehrlich holds that megaloblasts constitute a separate type of cell from the normoblast, and that megaloblasts are not present in normal marrow. The appearance of megaloblasts in the marrow is therefore an expression of a preversion of the function of the bone-marrow. According to the other view (Hunter, Cabot, Bunten), the changes in the marrow are the result rather than the cause of the anæmia, and they indicate an over-activity of the bone-marrow in attempting to repair the anæmia, such an over-activity resulting in the marrow reverting to its embryonic (megaloblastic) type of growth. In this way the megaloblast would appear to be simply a less mature form of normoblast, with which it is said to be connected by several intermediate forms.

The chief argument advanced in favour of the second theory is the observation of Bunten that small doses of the hæmolytic drug, ricin, given over a considerable period, produces a megaloblastic marrow identical with that found in pernicious anæmia, whilst larger doses, given for a shorter time, produce a normoblastic marrow—that is to say, the marrow is megaloblastic or normoblastic according to the duration of the hæmolysis. If the megaloblastic marrow were a response to a specific toxin, the type of marrow should not have varied with the size of the dose.

The fact that megaloblasts very readily appear in the blood in the anæmias of infancy, and the more readily the profounder the anæmia, suggests that it is the excessive drain on the marrow that causes the reversion to this type of marrow growth. Also, the reaction in the marrow in pernicious anæmia cannot strictly be regarded as specific, for, as we have seen, in many of the cases the hyperplasia of the leucoblastic elements is greater than that of the erythroblastic.

Warthin[1] has reported an interesting case of fatal hæmorrhagic anæmia in the adult, the patient bleeding from a nasal "angiectatic polyp" almost continuously for three weeks. There were large numbers of megaloblasts in the blood, and *post-mortem* examination showed a megaloblastic marrow. If in this case all primary blood diseases can be certainly excluded, and this seems to be so, it is strong evidence in favour of the view that a megaloblastic marrow can be produced without the intervention of a specific toxin, and simply as the result of an excessive demand for red blood corpuscles.

Besides this, there seems to be no conclusive evidence of increased fragility of the red corpuscles in pernicious anæmia. The resistance of these cells to various strengths of saline solutions seems to be little different from normal, and M'Neil[2] has recently shown that their resistance to saponin hæmolysis, at least in the chronic cases, is very little diminished—indeed, it is much greater than the resistance of the red cells in certain cases of jaundice in which there was no anæmia.

Fuller information, however, is required regarding the relationship of megaloblastic and normoblastic marrow, and

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[1] *Trans. Assoc. Amer. Physicians*, vol. xxiv, p. 227.

[2] *Journal of Pathology and Bacteriology*, July, 1910.

regarding the relationship of both to the various types of anæmia.

It is thus apparent that in studying the pathological anatomy of pernicious anæmia it is exceedingly difficult to find any uniformity of opinion as to what constitutes the essential lesion in the disease. And, on the clinical side, it is equally difficult to get agreement as to what are the symptoms and signs on which one may found a diagnosis. If we define pernicious anæmia as a profound anæmia with high colour index and megalocytes and megaloblasts in the blood, then we include anæmias produced by apparently many quite different causes. If to the above definition we add that the anæmia must be cryptogenic in origin, the disease becomes much more restricted, and embraces only such anæmias as have no recognisable etiology. So that, till agreement as to what shall constitute the boundaries of "pernicious anæmia" is arrived at, it will be convenient to group the cases of profound anæmia according to their apparent, or their possible, etiology. This will give us the following groups:—

Group I.—*Addisonian anæmia*, and, according to many writers, it is the only true pernicious anæmia. Here there is no obvious explanation for the anæmia. Such cases almost invariably have great increase of iron in the liver as well as a megaloblastic marrow.

Group II.—Cases in which the anæmia seemed to begin during pregnancy or soon after childbirth. Cabot suggests that the anæmia is due to an auto-intoxication, evidence of which is not infrequently met with in pregnancy in the form of nephritis, eclampsia, or obstinate vomiting. The

pregnancy cases differ from the Addisonian cases in that whilst the latter are met with more often in men, and more often after 35 years of age, the former are all in women, and usually in those under 35 years. The pregnancy anæmias are more definitely progressive, and seldom show the characteristic remissions of the Addisonian cases; but the possibility of cure in this group of cases seems less remote than in those of Group I.

Group III.—Cases in which the anæmia seems to date from one or several hæmorrhages, and yet in which the anæmia is quite out of proportion to the amount of blood lost. Many of these cases may be Addisonian cases with insidious onset, in which the hæmorrhage is a result of the anæmia and not its cause. But, on the other hand, it is to be remembered that Professor Stockman regards all cases of pernicious anæmia as due to repeated hæmorrhages, and it is these hæmorrhages, he says, that convert a simple anæmia into a pernicious one.

Group IV.—Cases in which the anæmia is associated with cancer of the stomach. A secondary anæmia is an almost constant accompaniment of any cancer; but in cases of cancer of the stomach the anæmia may be unusually profound, and in some rare instances it has the characters of a pernicious rather than of a secondary anæmia. There is some doubt, however, if the tumour growth in these cases has any causal relationship to the anæmia.

In some of the cancer cases secondary growths have been found in the bone-marrow, inducing a certain amount of megaloblastic change in the marrow, and the appearance of megalocytes, megaloblasts, and myelocytes in the blood. Drs. Harrington and Teacher reported a

case of this sort at a meeting of the Medico-Chirurgical Society last winter, and they refer in their paper [1] to several other published cases. The anæmia in these cases may be, in part at least, aplastic, that is, due to displacement of marrow by the tumour growth. And the megaloblastic change of the remaining marrow may either be secondary to the anæmia, or, as Ehrlich suggests, due to toxins derived from the tumour acting on the marrow cells and inducing a megaloblastic degeneration. The hæmosiderin reaction in the liver in most of these cases seems to be very slight, if present at all.

Group V.—Cases in which an intestinal parasite is the apparent cause of the anæmia. The two intestinal parasites that produce the most profound anæmia are (1) bothrio-cephalus latus and (2) ankylostoma duodenale. From each of these worms a toxin has been recovered which has a definitely hæmolytic action on red corpuscles, and the anæmia they produce is almost certainly, in great part at least, toxic in origin.

With both these worms the anæmia may be secondary in type, but with bothriocephalus latus especially it is not uncommon to find the blood with a high colour index, and with megalocytes and megaloblasts also present. The course of the anæmia, too, is progressive and fatal unless the worm be removed.

In cases dying with bothriocephalus anæmia the marrow may be found to be megaloblastic in type, and in some cases at least the iron pigment in the liver has been increased in amount.

The vast majority of cases of ankylostomiasis have a low hæmoglobin index. But certain cases, on the other hand,

are reported as having a high index, and otherwise have a blood picture very like that of an Addisonian anæmia. In some of the fatal cases excess of iron has been found in the liver; in others the iron does not exceed the normal.

In bothriocephalus anæmia, but more especially in anky-lostomiasis, an excess of eosinophile cells is met with in the blood, and this helps to differentiate an anæmia due to intestinal worms.

Group VI.—Aplastic anæmia. This is a profound anæmia in which, instead of there being a hyperplasia of the bone-marrow, there is atrophy of the normally active marrow. The cases are somewhat rare, and differ clinically in certain points from Addisonian anæmia. Aplastic anæmia is more common in women than in men—two-thirds of Cabot's cases being women, and three-fourths of the cases were under 34 years of age. The course of the illness, too, is more acute, and it is progressive without remissions. Subcutaneous hæmorrhage and hæmorrhages from the mucous membranes are much more common than in the typical pernicious anæmia. Also, the blood is different. The hæmoglobin index varies, but it may be low; usually there is little poikilocytosis, anisocytosis, or polychromato-philia, and usually no nucleated red corpuscles. There is a leucopenia with a high percentage of lymphocytes (80 to 90 per cent). On *post-mortem* examination the red marrow is found to be less extensive than normal, and to be under-going a fatty degeneration.

It is difficult to be certain whether this is a pernicious anæmia of toxic origin in which the marrow has failed to react, or an anæmia secondary to the inactivity and atrophy of the blood-forming tissue. We have already seen that a drug like arsenic in small doses stimulates the

bone-marrow to greater activity, whereas in large doses it has an inhibitory and degenerative action. It may be that there is a toxin in this disease with a similar action to the large doses of arsenic. In 1900 Professor Muir[1] described a case with the characters of aplastic anæmia, and he then expressed the opinion that the marrow change was a primary one. In this case there was an increase of iron pigment both in the liver and kidneys, but this he regards, not as evidence of hæmolysis, but as resulting from the somewhat extensive hæmorrhages that were present in the tissues. In a somewhat similar case, recorded by Drs. James Carslaw and Shaw Dunn,[2] there was only a very slight deposit of hæmosiderin in the liver, but here there was but little extravasation of blood into the tissues. The marrow in this case was aplastic, except for a very few nodules which showed a megaloblastic activity.

Group VII.—Acquired hæmolytic ictero-anæmia: Widal's syndrome.[3] As the blood picture in Widal's cases of ictero-anæmia has a considerable resemblance to that in pernicious anæmia, it is convenient to group them along with the foregoing cases. The general course of this illness, however, is much less severe than in pernicious anæmia, and, indeed, ictero-anæmia seems to be never fatal. Nine cases have been recorded—six in females and three in males, with ages ranging from 16 to 67 years. The disease begins insidiously without any very apparent cause. There is

[1] *British Medical Journal*, 29th September, 1900.

[2] *Glasgow Medical Journal*, May, 1910. A somewhat similar case to this is recorded by Professor M'Weeney in the *Journal of Pathology and Bacteriology*, July, 1909.

[3] See paper by Barton, *Amer. Journ. of Med. Sciences*, August, 1910.

first general weakness, and then the pallor and yellow discolouration of conjunctivæ, skin, and mucous membrane appear. But there is no itching of the skin and no brady-cardia. The motions are normal in colour. Although the urine contains excess of urobilin, there is no appearance of bile pigments. The blood count during an exacerbation of the disease shows the red corpuscles ranging from 1,000,000 to 3,000,000 per c.mm., and with high colour index. There is poikilocytosis, anisocytosis, and poly-chromatophilia; nucleated red cells may form 5 per cent of all the red corpuscles, and both megaloblasts and normoblasts are represented. There seems to be usually a leucocytosis, but the counts have ranged from 4,500 to 40,000 white cells per c.mm. Neutrophile myelocytes are occasionally present. The coagulation time of the blood is normal, and the blood plasma is not unduly hæmolytic. The red corpuscles, however, show lessened resistance, as tested by varying strengths of saline solutions.

There is in every case some enlargement of the spleen, and its size seems to vary with the exacerbations and improvements which are characteristic of the course of the disease. The liver is sometimes enlarged. The illness tends to become chronic, and has a very variable duration, ranging from a week to several years. There has been one death in the nine cases recorded, and this patient died of an intercurrent pneumonia. On *post-mortem* examination there was found excess of pigment in the liver, and a megaloblastic hyperplasia of the bone-marrow.

The primary lesion in the disease is supposed to be the undue fragility of the red corpuscles, leading to increased destruction of these cells, and associated with this is the enlargement of the spleen and pigmentation of the tissues. Barton believes the pigment to be formed in the blood and

not in the liver, and he says that the condition differs from pernicious anæmia in that in the latter disease, as well as in jaundice, there is no increased fragility of the red corpuscles.

A congenital form of ictero-anæmia has been described under the titles *Congenital family cholæmia* [1] and *Congenital anæmia, with jaundice and enlargement of the spleen*,[2] but the symptoms in the acquired and congenital forms are practically the same. In the congenital form, however, the disease tends to affect several members in a family, and often several members in successive generations. The jaundice and anæmia date from before or soon after birth. The jaundice is essentially chronic, and may persist till middle life. The anæmia is usually less marked than in the acquired variety, and the hæmoglobin index in the majority of cases is lower than normal. There are almost always some normoblasts, megaloblasts, and myelocytes found in the films, but in the most of the cases there is a leucopenia—Buchan and Comrie's cases being an exception. There is the same enlargement of the spleen and absence of bile from the urine (except in Buchan and Comrie's cases), although bile has been found in the blood plasma in the majority of cases. The motions remain normal.

The general health, as a rule, seems to be little affected, but Buchan and Comrie record the death of three cases which lived two, three, and seven days respectively. *Post-mortem* they found signs of biliary obstruction in the liver, but no hæmosiderin reaction in this organ, although

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[1] Hawkins and Dudgeon, *Quarterly Journ. of Medicine*, January, 1909; and Hutchison and Panton, *ibid.*, July, 1909.

[2] Buchan and Comrie, *Journal of Pathology and Bacteriology*, vol. xxii, 1909.

it was present in the spleen. In both liver and bone-marrow there was evidence of increased megaloblastic activity.

As in the acquired, so in the congenital, form of acholuric jaundice, the increased fragility of the red corpuscles seems to be the most apparent cause of the anæmia. This fragility leads to increased destruction of these cells in the spleen, and gives rise to the hæmosiderin reaction in that organ. The increased fragility may depend on defective formation of red cells, and it may be that the primitive type of marrow persisting too long in part accounts for this by producing cells less mature, and, therefore, with less resistance. The jaundice is doubtless hæmolytic in origin, the products of hæmolysis possibly producing some catarrhal obstruction in the biliary ducts.

Paroxysmal Hæmoglobinuria.—Paroxysmal hæmo-globinuria is usually grouped with diseases of the urinary organs, but as the hæmoglobin in the urine is essentially dependent on there being free hæmoglobin in the blood, it seems better to speak of the condition as a hæmoglobinæmia, and to classify it along with the diseases of the blood.

We have already seen that even under normal conditions a destruction of red corpuscles is continually going on in the spleen, or some other part of the vascular system, and as a result a certain amount of hæmoglobin is constantly being liberated. The blood plasma, however, has not been shown to contain free hæmoglobin, and it would seem, therefore, as if the liberated hæmoglobin were quickly broken up into its component parts, globulin and hæmatin. From the hæmatin the bilirubin of the bile is doubtless derived; and the amount of bilirubin formed would seem to be some measure of the amount of free hæmoglobin—

that is, a measure of the destruction of red corpuscles that has been going on. When, however, the hæmolysis has been excessive—when, for example, one-sixtieth[1] of the total hæmoglobin of the blood has been liberated, then the free hæmoglobin apparently cannot be dealt with in this way, and it is therefore eliminated by the kidneys as hæmoglobin, giving rise to the condition called hæmoglobinuria. So that hæmoglobinuria not only means that there is a hæmoglobinæmia, but also that there has been a very large quantity of hæmoglobin liberated.

Any agent, then, that can produce an excessive hæmolysis will determine a hæmoglobinuria. Such drugs, for instance, as carbolic acid, chlorate of potassium, pyrogallic acid, and naphthol produce a hæmoglobinuria. Saponin, ricin, and certain snake venoms act in the same way. Hæmoglobinuria, too, may occur in the course of some of the acute infective fevers, such as scarlet fever or small-pox. It is also met with as a complication of malaria, constituting the "blackwater fever" of that disease; and sometimes it is one of the phenomena of Raynaud's disease. In paroxysmal hæmoglobinuria the hæmoglobin in the urine is the essential feature of the malady.

In all these conditions the hæmolysis would seem to take place in the general circulation; and it has been shown that the blood serum has a pink colouration (due to the presence of free hæmoglobin) before there is any appearance of hæmoglobin in the urine, and that the blood serum regains its normal colour prior to the disappearance of the hæmoglobinuria.

As the result of a paroxysm of hæmoglobinæmia the blood count may show loss of a million or more red cells, but this deficiency is usually very quickly made up again,

[1] M'Alister, *Quarterly Journal of Medicine*, July, 1909, p. 368.

the corpuscles being more quickly renewed than the hæmoglobin. The polynuclear leucocytes, too, are more actively phagocytic, as well as slightly increased in number, during a paroxysm, and there seems reason for believing that they take an active part in the destruction of the red cells. The red cells themselves, however, do not seem unduly vulnerable in paroxysmal hæmoglobinæmia.

The nature of the hæmolysis in paroxysmal hæmoglobinuria has been carefully studied by Eason[1] and others, and its pathogenesis is now much better understood than formerly. It has been shown, for example, that the blood serum of a patient subject to paroxysmal hæmoglobinuria is hæmolytic *in vitro* for the red corpuscles of a healthy individual, and that the hæmolysis only takes place when the serum and corpuscles are subjected first to a low temperature, and then raised to body temperature. The hæmolysis, it is said, depends on the presence in the serum of amboceptor and complement, the former becoming attached to the corpuscles at the low temperature, and the latter linking itself on to the amboceptor when the temperature is raised. The attack of hæmoglobinuria is most often precipitated or determined by the patient going out into the cold air, or putting his hands or feet into cold water. The cold reduces presumably the temperature of the surface blood, and permits of the union of amboceptor and corpuscle. When the surface blood reaches the internal organs the higher temperature there will determine the action of the complement, and so the hæmolysis is produced. It is possible that in certain cases of Raynaud's disease there is a similar hæmolytic toxin in the blood, and that during the attack of local syncope or congestion it becomes activated, much in the same way as does the toxin of

[1] *Journal of Pathology and Bacteriology*, 1906.

paroxysmal hæmoglobinuria when cold is applied.   The nature of the hæmolytic process in blackwater fever has not, so far as I know, been worked out.   Quinine is thought to sometimes determine the paroxysm of hæmoglobinuria, but in what way this drug produces or precipitates the hæmolysis has not yet been established.

In all these diseases, then, there is probably some toxic agent present in the blood serum which under the action of certain stimuli becomes active, and so the hæmolysis results. What the nature of the toxin may be, and from whence it is derived, is a matter for speculation.   In blackwater fever one naturally associates it with the presence of the plasmodium malariæ (malignant tertian parasite).   Further, it has been pointed out that a considerable proportion of the patients with paroxysmal hæmoglobinuria have suffered at one time or other from syphilis, and that the hæmolytic toxin is possibly para-syphilitic in nature.   The activating agent may vary in the different diseases, being, for instance, cold in paroxysmal hæmoglobinuria, some vasomotor disturbance in Raynaud's disease, and possibly quinine in blackwater fever.

Finally, we must note that by immunising guinea-pigs with the serum of a case of paroxysmal hæmoglobinuria Eason obtained an anti-hæmolytic serum which neutralised *in vitro* the hæmolytic action of the hæmolytic serum.   This would seem to suggest for the future a possible line of treatment for cases of paroxysmal hæmoglobinuria.

Splenomegalic Polycythæmia.—Polycythæmia means an increase in the number of the red corpuscles in the blood, and there is almost always an increase in the total volume of the blood as well.   In the majority of the cases there is enlargement of the spleen, and cyanosis is also a prominent

feature. The condition is apparently dependent on an undue activity of the erythroblastic elements of the bone-marrow.

Parkes Weber[1] divides the cases of polycythæmia into the two following classes:—

1. Where the polycythæmia is due to a recognised cause, such as imperfect oxygenation of the blood and tissues, as in cardiac and pulmonary disease.

2. Where no such cause is to be discovered.

The polycythæmia of Group I he calls an erythrocytosis, that of Group II an erythræmia.

It is mainly to the cases of the second group that the term splenomegalic polycythæmia is applied. In these the red corpuscles may number from 7,000,000 to 12,000,000 per c.mm., and the hæmoglobin register from 170 to 180 per cent. There is nearly always an associated leucocytosis, sometimes as many as 20,000 white corpuscles per c.mm.; in any case, there is always a relative increase of the poly-nuclear cells (75 to 90 per cent). A proportion of myelocytes, as well as normoblasts and megaloblasts, are frequently found in the blood films. The viscosity of the blood is always increased, and the blood pressure in some cases is very high. The resistance of the red cells seldom varies much from normal.

The liver as well as the spleen may be considerably enlarged. The enlargement of the spleen seems to be due in part to vascular engorgement and in part to hyperplasia of the splenic pulp. The hypertrophy of the spleen doubtless means increased hæmolysis, but on microscopic examination there is little evidence of this in any individual portion of the spleen. In some cases there has been found obstruction in the splenic vein, and in other cases the spleen is not enlarged at all.

[1] *Quarterly Journal of Medicine*, October, 1908.

The liver is engorged with blood, but otherwise shows little change; all the abdominal vessels are likewise engorged. In the bone-marrow there is great increase of the leucoblastic as well as the erythroblastic elements, and the fat in the shafts of the bones is invaded and replaced by red marrow.

The symptoms associated with polycythæmia can be quite satisfactorily explained by the increased amount of blood in the circulation, and this in turn is doubtless due to the hyperactivity of the bone-marrow. But what determines this increased hæmopoiesis still remains in obscurity.

Enterogenous Cyanosis.—A cyanosis (enterogenous cyanosis) may also result from the partial conversion of the oxyhæmoglobin of the blood into methæmoglobin or sulph-hæmoglobin. These hæmoglobin compounds in the blood give the skin and mucous membrane the purple-blue appearance of cyanosis.

In this form of cyanosis there need be no change in the number of red or white corpuscles in the circulation; but in some of the cases the spleen has been found enlarged and clubbing of the fingers and toes noted. The cyanosis is usually chronic and may last for many years, although in varying degrees of intensity. In most of the cases it has been associated with some intestinal disturbance; in some there has been constipation and in some diarrhœa.

The methæmoglobinæmia seems to be due to the presence of nitrites in the blood acting on the oxyhæmoglobin and converting it into methæmoglobin, and the sulph-hæmo-globinæmia to the presence of hydrogen sulphide producing in a similar manner sulph-hæmoglobin. It is not certainly determined whether the nitrites and the hydrogen sulphide

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are derived from the alimentary tract or originate in the blood. Gibson and Carstairs Douglas [1] cultivated an organism like the bacillus coli from the blood in their case, and they associate this bacterium with the production of the nitrites.

Leukæmia.—The essential feature of leukæmia is the malignant hyperplasia that affects the leucocyte-forming tissue and which results in an enormous increase in the production of white blood corpuscles. The hyperplasia may affect only one type of cell, and when that is so there may be an absolute diminution of the other leucocytes, or the increase may affect several varieties, although one variety may be considerably more increased than the others. In lymphatic leukæmia it is the lymphocytes only that show this great increase, and it is dependent on a hyperplasia of the lymphoid tissue throughout the body, but especially in the lymphatic glands and bone-marrow. In myeloid leukæmia there is an absolute increase of several varieties of white cells, but chiefly the granular cells; there is, however, nearly always an absolute increase of the lympho-cytes as well. In certain of the more acute myeloid leukæmias the granular cells may, to a large extent, be replaced by a more primitive cell, the non-granular myelo-cyte (pre-myelocyte or myeloblast). This cell closely resembles, and according to certain writers is indistinguish-able from, the large lymphocyte; and it is probable that a number of cases that have been grouped as lymphatic leukæmias, owing to the predominance of this non-granular cell, are really cases of acute myelogenous leukæmia. The difference in the staining reactions, however, between the

[1] *Lancet,* 14th July, 1906 ; see also *Quarterly Journal of Medicine,* October, 1907.

large lymphocyte and the myeloblast have already been referred to.

The bone-marrow in myeloid leukæmia shows great hyperplasia of the myeloid elements; and just as in lymphatic leukæmia, where the normal marrow is replaced by lymphocytes, so in myeloid leukæmia the spleen and lymphatic system generally may be converted into a myeloid tissue with precisely the same microscopic appearances as the myeloid tissue of the bone-marrow.

In some cases of lymphatic leukæmia there is no enlargement of lymphatic glands, and the lymphatic hyperplasia seems to begin in the bone-marrow, in other cases there is little or no involvement of the marrow, the hyperplasia being confined chiefly to the lymphatic glands. In the same way in myelogenous leukæmia there need be but little change in the spleen or lymphatic glands, the myeloid overgrowth being entirely in the marrow.

It would seem, therefore, as if this hyperplasia might begin in any part of the blood-forming tissue, whether it be spleen, lymphatic gland, or bone-marrow, and it may possibly begin simultaneously in several parts of this tissue. Ultimately the whole blood-forming system tends to become involved, although the hyperplasia may predominate in one part more than another. It is to be noted, however, that certain writers maintain that the primary lesion in leukæmia, whether myelogenous or lymphatic, is always in the bone-marrow.

The method of spreading of the morbid process throughout the tissues cannot be certainly determined. It may be by metastasis as in tumour growth, or it may be that various parts of the blood-forming organs respond simultaneously, or in turn, to the stimulus which produces the primary hyperplasia. And it is to be remembered that the liver, as

well as certain other organs, is the seat of active blood formation in the embryo, so that the myeloid transformation that is found in these organs in leukæmia might be regarded as a return of a latent blood-forming tissue to its embryonic activity. In support of this suggestion is the observation that in certain cases of lymphatic leukæmia a myeloid transformation has been found in lymphatic glands, this doubtless being compensatory for the loss of myeloid tissue in the bone-marrow. The aggregations of leucocytes in the liver, kidneys, lungs, and other organs in leukæmia are probably due in part to infiltration, in part to local proliferation of the infiltrating cells, and in part to the proliferation of pre-existing cells.

Ehrlich draws a definite distinction between the form of hyperplasia in the two types of leukæmia. He regards lymphatic leukæmia as of the nature of a tumour growth, or perhaps it is more accurate to say as a functional over-activity of the lymphoid tissues. Myelogenous leukæmia, on the other hand, he holds is a form of leucocytosis where several varieties of the granular leucocytes respond to some chemiotactic or stimulating influence. The tendency of the present day, however, seems to be to give to the two forms of leukæmia a common pathogenesis rather than to regard them as separate morbid entities.

But this opens up the whole question of the essential nature of leukæmia, and one which at present cannot be determined. The etiology of the disease is unknown. No specific micro-organism has yet been isolated, and there is no satisfactory proof that the disease is infective in origin. Is it then a sarcoma, or sarcomatosis, of the blood-forming tissues, as Banti has suggested? Leukæmia is a malignant, and apparently purposeless, hyperplasia of certain tissue cells, and in this respect it resembles sarcoma. But, then,

we do not know the cause of the overgrowth in sarcoma. It is doubtless due to some stimulus, but where this stimulus comes from, and whether or not it is of a similar nature to the stimulus which is present in leukæmia, we have at present no means of determining. We speak of the overgrowth in sarcoma as being neoplastic, and of that in leukæmia as hyperplastic, but it is difficult to formulate exactly the essential difference between the two.

From the histological point of view the relationship between lymphatic leukæmia and lymphosarcoma seems fairly close. Indeed, a series of cases[1] might be collected which would show all gradations from benign lymphoma, or pseudoleukæmia, to lymphatic leukæmia and lymphosarcoma. Such cases are all grouped under the term lymphocytoma, and they have as a common feature enlargement of lymphatic glands, the enlargement being due to overgrowth of cells of the lymphocyte type. In some of the cases the glands involved are limited in number, whilst in other the glandular enlargement is widespread. In some cases (leukæmia and pseudoleukæmia) there is infiltration of the liver and other organs with lymphocytes, and in other cases no such infiltration; in others, again (lymphosarcoma), the lymphocytes infiltrate the capsule of the gland and invade the surrounding tissues. In all the cases the disease tends to be progressive, although in some the growth is so slow as to make such cases comparatively benign. All degrees of malignancy, however, are met with, and cases that have seemed benign for years may suddenly become malignant.

Warthin[2] regards these different types of lymphocytoma as genetically related, as being, indeed, the same disease

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[1] Warthin; Osler and M'Crae's *System of Medicine*, vol. iv, p. 827.

[2] *Ibid.*

with different degrees of severity. Some of the lympho-cytomata, he would say, are leukæmic, that is, they have an excess of lymphocytes in the circulation, and some, on the other hand, are aleukæmic; but the tissues in the aleukæmic cases may show precisely the same infiltrations with leucocytes as the leukæmic cases, and cases originally aleukæmic may ultimately become leukæmic.

Regarding these cases of glandular enlargement (due to proliferation of lymphocytes) from the clinical side, we may have the following types:—

1. Aleukæmic cases, with no increase of white corpuscles, and no relative increase of lymphocytes, in the blood.

2. Cases where the number of white corpuscles is within normal limits, but where there is an absolute and relative increase of lymphocytes. I reported a case of this sort at a meeting of the Medico-Chirurgical Society[1] a few weeks ago. The patient had a widespread glandular enlargement, but only 7,916 white corpuscles per c.mm.; 94 per cent of these, however, were lymphocytes.

3. Cases of typical lymphatic leukæmia with great increase of white corpuscles in the blood, 90 per cent or more of which are lymphocytes.

4. Cases of lymphosarcoma with increase in the number of white corpuscles in the blood, a large proportion of which are lymphocytes. Dr. Mackenzie Anderson permits me to quote a case of this sort that was in his wards in 1908. The patient had enlargement of the glands in the neck, axillæ, and groins, as well as a tumour occupying practically the whole of the anterior mediastinum. The white corpuscles numbered 28,000 per c.mm., 80 per cent of which were lymphocytes. The tumour, on microscopical examination, proved to be a lymphosarcoma.

[1] *Glasgow Medical Journal*, February, 1911.

Cases of chloroma (lymphatic type) may be included here, as they have many of the features of a lymphosarcoma. Chloroma, I might add, is a form of leukæmia (or sarcoma) in which there is hyperplasia of one or other of the marrow cells. The tumours which result have a green colour, and they penetrate the bone containing the marrow from which they grow. Metastases are common in liver, kidney, spleen, lymphatic glands, and the marrow of the shafts of the long bones. The blood is similar to that met with in leukæmia.

Somewhat analagous conditions to those of lymphatic glands may be met with in the hyperplasias of bone-marrow. There may be (1) cases with extensive marrow hyperplasia of the type seen in myelogenous leukæmia, and yet little increase in the number of leucocytes in the blood; (2) aleukæmic cases with tumour growth from bone (myeloma), the tumour consisting of myeloid cells; (3) cases of myeloid chloroma (tumour growth of bones of the head) where the blood and marrow have the characters met with in myelogenous leukæmia.

It is apparent, therefore, that whilst in a typical case of leukæmia the increase of white corpuscles in the blood-forming tissues determines a corresponding increase in the blood, there are certain atypical cases where there is no such increase. In some of the latter the proportions of the various white corpuscles remain normal, in others, one type of cell may be absolutely and relatively increased, the remaining cells being correspondingly diminished. Further, in lymphosarcoma and in lymphatic chloroma the blood may have the characters of lymphatic leukæmia, and in myeloid chloroma the characters of myelogenous leukæmia.

Errors of diagnosis, however, may occur. For example, in whooping-cough there may be a considerable lympho-cytosis, and this may occur with the complications as well

as during the paroxysmal stage of the disease. Cabot [1] records the case of a boy, aged 12, who during an attack of broncho-pneumonia, complicating whooping-cough, had a leucocytosis of 94,000 per c.mm., 75 per cent of which were small lymphocytes. The blood count became normal on recovery from the illness.

A large percentage of myelocytes, even with a low total leucocyte count, would, in most cases, be quite correctly regarded as indicative of myelogenous leukæmia. But Simon [2] records a case which forms an exception. The case was one of fracture of the leg in which the blood presented the picture of myelogenous leukæmia (50,000 white corpuscles with 15·2 per cent myelocytes), and yet on the mending of the leg the blood returned to normal.

The term "mixed-cell leukæmia" has been applied to cases in which the two forms of leukæmia were thought to co-exist at the same time. Probably many of the cases described as such are cases of myelogenous leukæmia in which the non-granular myelocytes (myeloblasts) have been counted as large lymphocytes; and the cases of myelogenous leukæmia which have been said to have become transformed into lymphatic leukæmia are possibly to be explained in the same way—that is, that the proportion of non-granular myelocytes has greatly increased at the expense of the granular cells.

In almost all the cases of myelogenous leukæmia a proportion of lymphocytes (usually an absolute increase) is found in the blood films, so that in this sense all myelogenous leukæmias are mixed-cell leukæmias. Professor Muir [3] sums up the matter in the following words:—"So far as we

[1] Osler and M'Crae's *System of Medicine*, vol. iv, p. 670.

[2] *American Journal of Medical Sciences*, 1906, p. 444.

[3] Clifford Allbutt and Rolleston's *System of Medicine*, vol. v, p. 796.

know, there is no instance in which the essential changes of myeloid and lymphoid leukæmia were present concomitantly both in the blood and in the tissues."

It is customary to classify cases of leukæmia as acute or chronic according to the rate with which the disease runs its course : the distinction, however, is more or less arbitrary. Cases which die within two or three months from the first appearance of symptoms are regarded as acute, and the others as chronic. In acute cases the symptoms are more severe, there is fever, and frequently hæmorrhages from the mucous membranes and into the skin. Hæmorrhages are said to be more common in the lymphatic forms, but the most profuse bleeding that I have seen was in a case of the myelogenous variety, a case which I brought before the notice of the Medico-Chirurgical Society last winter.[1] Speaking generally, the more acute the lymphatic leukæmia the larger is the proportion of large lymphocytes, and the more acute the myelogenous leukæmia the larger the proportion of myeloblasts.

In all leukæmias there is, sooner or later, a diminution in the number of red corpuscles, but the colour index is usually low, and the general characters of the blood are those met with in any secondary anæmia. Nucleated red cells are present in both forms of leukæmia, but they are far more abundant in the myelogenous. The nucleated red cells include both normoblasts and megaloblasts, the proportion of the former, however, is usually considerably greater than that of the latter.

Occasionally in acute leukæmia, and towards the termination of a chronic leukæmia, the anæmia becomes extreme, with high hæmoglobin index, and megaloblasts more numerous than normoblasts. The white corpuscles may number not

[1] *Glasgow Medical Journal*, January, 1910.

more than 10,000 per c.mm., with only 10 to 15 per cent of myelocytes. Such cases have, therefore, some of the features both of leukæmia and of pernicious anæmia, and it is difficult to know whether they are to be classed with the former or with the latter disease, or in a group by themselves to which the name *leukanœmia* has been given. In the majority of cases the *post-mortem* appearances are those of myelogenous leukæmia rather than of pernicious anæmia, and in particular the hæmosiderin reaction in the liver is not obtained. The most of the cases, therefore, grouped as leukanæmia are probably cases of leukæmia with a profound anæmia, although a smaller proportion of cases may be cases of pernicious anæmias with some myelocytes (up to 10 per cent) present in the blood. It is to be remembered that the number and proportions of the various white corpuscles in the blood may be greatly affected by some intercurrent infection, and we frequently see such a terminal feature in cases of leukæmia.

Little real advance has been made in the *treatment* of leukæmia. Arsenic is still the drug that seems to do most good, but any benefit is most often only temporary. Roentgen ray treatment in a certain number of cases produces a symptomatic cure, but it seems never to be permanent, and it rarely lasts more than a few months to two or three years at the longest. With this treatment there is great diminution in the size of the spleen and lymphatic glands as well as in the number of white corpuscles in the blood. Indeed, the white cells may ultimately come to be normal in number. The patient's general health improves, and he feels fit for greater exertion. It is thought that the *x*-rays have a destructive action (due to a leucolytic toxin) on white corpuscles, especially the less mature cells. As a rule, more success is gained with the myelogenous than

with the lymphatic leukæmias; in acute cases *x*-ray treatment seems at times to do more harm than good.

Purpura.—This is a term which is given to the spontaneous hæmorrhages of small extent which are sometimes met with in the skin or mucous membranes. It is a symptom occurring in the course of many different diseases, such, for example, as scarlet fever, small-pox, cerebro-spinal fever, Bright's disease, heart disease, leukæmia, the severe anæmias, and jaundice; it may also attend the administration of such drugs as iodine and quinine. Sometimes, however, the purpuric eruption is the essential feature of the disease, and then the term is applied to the morbid condition that produces the symptom as well as to the symptom itself. It is in the latter sense, that is, as the disease purpura, that this disorder takes its place amongst the diseases of the blood.

The etiology and pathogenesis of purpura still remain obscure, and hence the grouping of the various clinical types is not altogether satisfactory. We speak, for example, of *purpura simplex* when the eruption is limited to the skin; of *purpura hæmorrhagica* when there are, in addition, hæmorrhages from the mucous membranes; of *purpura rheumatica* when an arthritis accompanies the purpuric eruption; and of *Henoch's purpura* when certain abdominal symptoms are superadded, particularly vomiting and colic. But all these so-called varieties probably only represent degrees of severity, or variations in the symptomatology, of the same disease. Even the mechanism by which the hæmorrhages are produced is unknown. An explanation for this has been sought for in the vessel wall, and, again, in the blood itself. No constant change, however, is to be found in the vessels, and in a number of cases careful

microscopic examination at the seat of hæmorrhage has failed to show any abnormality; in such cases the escape of blood is probably by diapedesis. In other cases, again, localised degenerations have been found in the vessels, and these are thought to give rise to thrombosis with subsequent rupture. Purpuric spots may be produced experimentally by the injection of certain toxic substances, such, for example, as some of the snake venoms, and it has been suggested that these toxins have a destructive action on the endothelial cells forming the capillary wall, and in this way give rise to the minute hæmorrhages.

Examination of the blood of patients suffering with purpura usually shows a diminution in the number of red corpuscles, but the anæmia is apparently secondary to the hæmorrhages and in proportion to the amount of blood lost. There may also be a moderate leucocytosis. But the most notable abnormality seems to be the diminution in the number of blood platelets. This may be very considerable, as low as 10 per cent of the normal, and it seems to be a fairly constant change in purpura. It is said that there is a relationship between the diminution of platelets and the hæmorrhagic tendency.[1] There is some uncertainty whether this diminution of platelets depends on deficiency of production or on their increased destruction.

In the majority of cases of purpura the coagulation time of the blood is about normal. It is doubtful if there is any very definite relationship between the number of blood plates and the coagulation time of the blood. It has been said that blood will not coagulate in the absence of the platelets and that the addition of platelets hastens coagulation. At one time it was thought that the prothrombin necessary for the coagulation of the blood was contained

[1] Coe, *Journal of American Medical Association*, 1906, p. 1090.

in the platelets, but now the view seems to be that pro-thrombin is present in the blood plasma; and in purpura, as we have just seen, there is a considerable diminution in the number of platelets, and yet the coagulation time is about normal. Hayem,[1] however, has pointed out that the blood-clot formed from the blood of a patient with purpura does not contract so as to extrude its serum as does the clot of normal blood, and he seems to think that this loss of contractility has some relationship to the deficiency of blood plates.

But the pathogenesis of purpura may be viewed from another standpoint. Professor Osler has drawn attention to the close association of purpura with erythema, urticaria, and angio-neurotic œdema. Sometimes a patient may suffer from both urticaria and purpura at the same time, or the one condition may follow the other. The colic in Henoch's purpura is regarded by many as an angio-neurotic œdema of a part of the intestine, and according to these writers Henoch's purpura would form an example of purpura and angio-neurotic œdema occurring in the same patient at the same time. From this point of view purpura would be regarded as vasomotor in origin, due to some toxin producing vaso-dilatation of the smallest vessels, with diapedesis of red corpuscles, instead of the serous exudation of urticaria or angio-neurotic œdema.

Hæmophilia.—Hæmophilia is a hereditary disorder, the chief feature of which is the tendency to severe hæmorrhages into the tissues and cavities of the body, as well as from the skin and mucous surfaces. Certain cases of hæmophilia may show purpuric spots, and may thus closely resemble the more chronic purpuras. In hæmophilia, however, there is

[1] Pratt; Osler and M'Crae's *System of Medicine*, 1908, vol. iv, p. 690.

usually a history of there being "bleeders" in the family; and the recurrence and persistency of the hæmorrhages is a much more definite factor in hæmophilia than in purpura.

As in purpura, so in hæmophilia, the cause of the hæmorrhages has been referred to undue fragility in the blood-vessels, and, again, to an abnormality in the blood itself. But in the vast majority of cases histological examination has failed to show any structural change in the vessels, and dry cupping does not seem to produce any greater transudation through the capillaries of the hæmophilics than of healthy individuals. Proof, therefore, of any structural alteration in the vessel walls is so far wanting, and so the explanation of the hæmorrhagic tendency must be sought for in the blood itself.

Examination of the blood shows a secondary anæmia proportionate to the amount of bleeding that has taken place. The leucocytes are usually somewhat lessened in number, being sometimes as low as 3,000 to 4,000 per c.mm. This diminution affects chiefly the polynuclear cells, which are not only absolutely, but also relatively, lessened; they are frequently not more than 50 per cent of all the white cells present. The blood platelets, according to Hayem, are not diminished in number, and the retraction of the blood-clot takes place as in normal blood. Indeed, the clot of hæmophilic blood when once formed is as firm as that of the healthy individual, and it is difficult to distinguish the one from the other. The diminution in the number of platelets, and the special characters of the clot, may serve, therefore, to distinguish the blood of purpura from that of hæmophilia.

But the most important change, and perhaps the only constant one, to be found in the blood in hæmophilia is its lessened coagulability. In the majority of cases the

coagulation time is much greater than normal, being frequently as much as thirty to sixty minutes, and sometimes longer than this. Addis[1] has shown that there is also some correspondence between the length of time the the blood takes to coagulate and the severity of the clinical symptoms. The present tendency, therefore, is to explain hæmophilia as due to this deficiency in the coagulability of the blood. Addis[2] says that the prolongation of the time required for coagulation "is the sole proximate cause of hæmophilia, sufficient in itself to explain all the symptoms," and he holds that "hæmorrhage is no more easily induced in a hæmophilic than in a normal person," the distinction, he says, "is not in the occurrence but in the amount of the bleeding." He points out that thrombokinase is the one substance necessary for coagulation of the blood not contained in the blood plasma itself. Thrombokinase is present in the tissues generally, and as blood escapes from the ruptured vessels it comes in contact with this thrombokinase. Thrombin is thus formed, and subsequently a blood-clot. The amount of thrombin formed depends on the amount of thrombokinase, and the greater the amount of thrombokinase the more rapid is coagulation. Addis found that by adding thrombokinase to hæmophilic blood the coagulability was greatly increased, but even with considerable quantities of thrombokinase the thrombin seemed to take an unusally long time to form. Large quantities of thrombokinase were therefore necessary to produce in hæmophilic blood the rapid clotting of normal blood.

In hæmophilia the extravasated blood no doubt does form a clot, but for the arrest of hæmorrhage it is necessary that the clot should occupy the orifice in the wounded

[1] *Quarterly Journal of Medicine*, October, 1910.
[2] *Ibid.*

blood-vessel. If the blood clots only after it has left the vessel the wound in the vessel will still remain open and the bleeding still go on. The clotting in hæmophilia is apparently not sufficiently rapid to seal up the injured vessel, and so the flow of blood washes away from the vessel the products of this slower coagulation before it has had time to form a clot.

Thrombokinase, as we have seen, is contained in the white blood corpuscles, and Wright[1] suggests that the diminution in the number of polynuclear cells in the blood of hæmo-philics has possibly some relationship to the lessened coagulability.

Sahli, several years ago, recognised the deficiency of thrombokinase, but he thought it was due to a functional defect in the vessel wall, and that it was the ruptured vessels that normally secreted the thrombokinase. There is, however, as far as I know, no definite proof of this functional defect in the vessels in hæmophilia.

In most hæmophilics there are from time to time exacer-bations of the disease, and Wright says that such exacerba-tions correspond to periods during which the coagulability of the blood is still further lessened. Wright also makes certain suggestions for the treatment of hæmophilia. He advises giving thymus gland tabloids, or some other form of nucleo-albumin, to increase the number of white corpuscles and at the same time to increase the coagulability of the blood. He advises the giving of the salts of calcium and magnesium, as he says they likewise increase coagulability. Carbonic acid gas, too, may be given to reinforce the action of the above drugs. As a local styptic he uses a watery extract of the fresh thymus gland, and he finds it very efficacious.

[1] Allbutt and Rolleston's *System of Medicine*, 1909, vol. v.

Scurvy.—The etiology and pathogenesis of scurvy still remain but imperfectly understood. The older theory, that it was due to an acid intoxication, has in recent years raised many objections, the chief being that in a considerable number of what seem to be cases of genuine scurvy there is no reduction in the alkalinity of the blood, and little abnormality as regards its coagulability. At present there is a tendency to regard the disease as infective in origin, and Babes has isolated a bacillus which he appears to claim as the specific micro-organism of scurvy. Hutchison [1] seems to favour the view that scurvy is an infective disorder, and he suggests that unsuitable food, exposure to cold and damp, fatigue, insanitary surroundings, and other conditions which formerly were thought to produce the disease, do not act as exciting causes, but rather by lessening resistance predispose to the growth of some specific virus.

The blood in scurvy has the characters of a secondary anæmia, and in the majority of cases there seems to be little if any leucocytosis. The coagulability of the blood, we have seen, is not materially altered, so that the hæmorrhages in scurvy cannot have the same explanation as in hæmophilia. Microscopic examination, too, has failed to show any definite lesion in the blood-vessels.

From this point of view, then, it would seem as if scurvy should be classed with diseases of infective origin rather than with diseases of the blood.

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[1] Osler and M'Crae's *System of Medicine*, vol. i.

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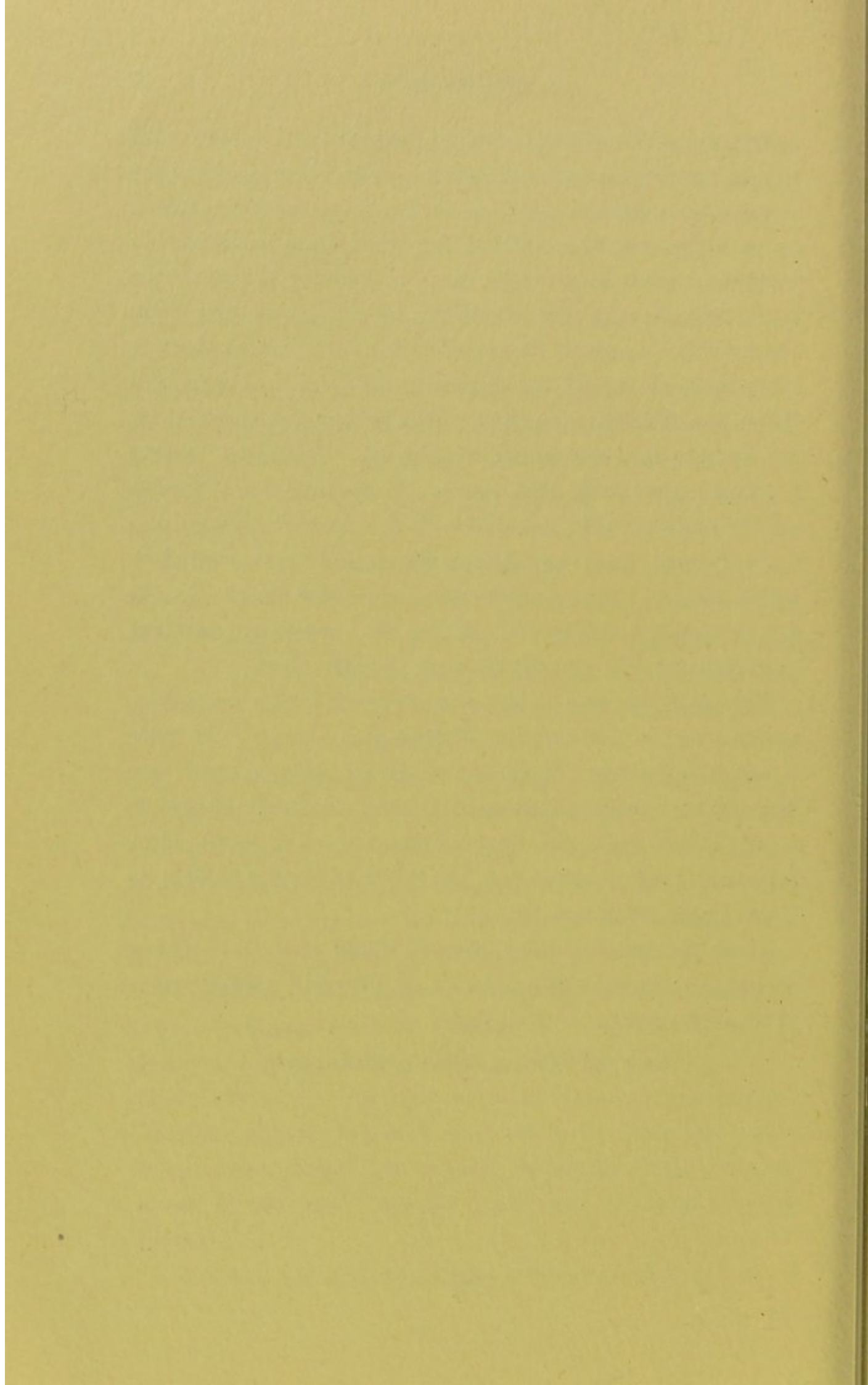
APPENDIX.

THE FIXING AND STAINING OF BLOOD FILMS.

In making blood films it is essential that the glass slides or cover-slips that are used should be absolutely clean, and in particular they must be free from grease of any sort; the films spread more evenly, and adhere much more intimately, when the surface of the glass is perfectly clean.

Cover-slips and slides may be conveniently cleansed by immersing them for a short time in a 25 per cent solution of nitric acid. They are then washed in water and rubbed dry with a soft cloth. They can now be stored in a vessel containing methylic alcohol, but they must again be carefully dried before being used.

The films may be spread on cover-slips or on slides. The former method probably gives the more perfect film, but one can usually obtain sufficiently satisfactory smears on the slides, and slides are more convenient to work with, for they break less easily and provide a more extensive film-surface for examination.

When cover-slips are employed they should not be thicker than 0·10 mm., and they must also be of the best quality obtainable, for if there is any unevenness of either cover the film will not spread uniformly, and the one cover will not slide from off the other without undue force, such as might

spoil the film. When making a film, then, two cover-slips are used, and each should be held by means of a pair of forceps rather than in the fingers. One cover is brought in contact with the drop of blood and is at once applied to the other cover, so that the blood spreads itself uniformly between the two surfaces. The upper cover is then slid off from the lower without raising or putting pressure on either cover. The films are allowed to dry in the air and are then ready for fixing and staining. It is to be noted that the drop of blood taken up by the cover-slip should be small, so that in spreading it does not reach quite to the margin of the cover; too large a drop makes the film unduly thick. Of the two films obtained by the above method the lower is usually the better one, though both may be sufficiently satisfactory.

When slides are used, the drop of blood is placed towards one end of a slide and the smear made by means of another slide. The end of this second slide is brought in contact with the drop of blood and the blood allowed to flow across the breadth of the slide. The second slide is then slowly drawn over the whole length of the first slide, and in so doing the blood film spreads itself over the surface of this slide. The second slide should be slightly concave, the concavity being downwards; and in making the smear the blood should follow this slide rather than that the slide be drawn over the blood, for with the latter method various artefacts may be produced.

Films should be thin, so that they quickly dry and prevent the shrinking that takes place before the cells become adherent to the slide. The perfect film should have all the cells in one plane, separate from each other, and without overlapping. Blood films if kept free from moisture, dust, and light seem to remain unaltered for quite a long

time, and weeks later they fix and stain almost as well as films freshly prepared.

The finger from which the specimen of blood is drawn must also be carefully washed with spirit or ether, so as to remove grease from the skin before the puncture is made. The first drop of blood that appears should be discarded as possibly containing some epithelial *débris*, and in taking up a drop of blood care must be taken not to touch the skin of the finger with the slide or cover-glass. The less pressure the finger is subjected to in obtaining blood the better, for such pressure is apt to express certain elements of the blood more readily than others, and in this way may possibly alter the composition of the film.

The Fixing of Blood Films.—Fixing of the various elements of the corpuscle is essential if the films are to be satisfactorily stained. The fixative seems to coagulate the albuminous structures in the cell and so renders them insoluble in the staining solution. It also makes them firm, giving them sufficient consistence to take up and retain the stain. There are many different fixing agents that might be employed, but as the staining may vary somewhat with the method of fixation it is better to work with but few, and the same, fixatives; in this way one's standard of comparison is less liable to be at fault.

The fixing agents used most in clinical work are (1) dry heat and (2) methylic alcohol. These two have this special merit that they preserve the cell in a more or less normal state and they do not affect its staining reactions.

1. Fixation by *dry heat* may be carried out on a metal plate or in a metal oven kept at a temperature of from 110° C. to 140° C. for ten minutes to half an hour. For several of the watery stains a temperature of 110° C. for

ten minutes fixes the films quite sufficiently well, but for more complex staining the temperature should be higher and the time longer. Fixation at 120° C. for half an hour is the method most often recommended, but some authors prefer simply to raise the temperature to 150° C., then extinguish the flame, removing the films from the oven when it has become cool again.

Fixation by heat is the method employed when staining with Ehrlich's triacid stain.

2. *Absolute methylic alcohol* (*pure*) fixes films in from two to five minutes; but absolute alcohol or equal parts of absolute alcohol and ether are also good fixatives. Several stains, such, for example, as those of Leishman and Jenner, are made up with methylic alcohol and so they serve to fix and stain the films at the same time.

Staining of Blood Films.—The different stains used in hæmatology depend for their action almost entirely on a chemical combination which takes place between the stain and the tissue stained. And so certain stains combine with one element in the cell and another group of stains with another element. Further, this specificity in staining has served as a means of differentiating and of designating the various parts of which the cell is composed. Thus we speak of the granules in the cell as being "oxyphile," "basophile," or "neutrophile," according as they react to acid, basic, or neutral dyes; and most of the staining reactions are comparable to what takes place when a salt is formed —that is to say, a chemical union is formed between the basic element in the cell and the acid element in the dye, and between the oxyphile cell element and the colour base. Sometimes, however, the staining is affected by the reaction of the fluid in which the stain may be dissolved.

Most of these dyes are true salts containing salt-forming groups, and the acid dyes are those salts in which it is the acid element that stains, whilst the basic dyes are those in which it is the base that is the staining agent. Eosin, for example, is the sodium salt of the coloured acid tetrabrom-fluorescein, whilst methylene blue is the chloride of the coloured base tetramethyl-diphenthiazin.

The mixture of an acid dye with a basic dye may form a neutral dye, *i.e.*, a colour acid is joined to a colour base, and such a dye will stain neutrophile elements which are not supposed to react to acid or basic stains by themselves. Eosin and methylene blue have been so combined as to form an "eosinate" of methylene blue which is neutral in its staining reaction, and which, therefore, stains the neutrophile elements of the cell; this means apparently that the neutral dye is taken up by the tissue as a salt without being broken up into the ions of which it is composed.

Certain mixtures of acid and basic dyes will thus stain at one time the oxyphile, basophile, and neutrophile parts of the cell; Jenner's stain (which is a compound of eosin and methylene blue) is a good example of such a mixture. It is to be noted, however, that the combination of eosin and methylene blue may also form a chemical substance (methylene azure)[1] which stains nuclear structures, but not with the tint of either of the original colours; the special colouration of the nucleus in cells stained by, for example, Giemsa's method depends on the presence of this methylene azure.

[1] Rosin states that five distinct colours may be formed by the mixing of eosin and methylene blue, viz., eosinate of methylene blue, methylene violet, methylene azure, methylene orange, and black dye. Quoted by G. Mann, *Physiological Histology*, 1902, p. 442.

There are a large number of acid and basic dyes that have been employed at one time or another in hæmatological work, but of the former eosin, acid fuchsin, and orange G., and of the latter methylene blue, methylene green, and gentian violet, are those most in use.

Formerly when a film was double-stained the two stains were used in succession, first the one, and after the film had been washed and dried then the other as a counter-stain. With this method, however, it was difficult to obtain constant or satisfactory results, and it has now been almost entirely discarded for a simultaneous staining with two or more dyes combined together in one solution. There are now many such stains in use, so many, indeed, that it is difficult for one individual person to be familiar with any considerable proportion of them. But in any case it seems better for the clinician to employ only a few well selected stains with the technique and staining reaction of which he has had experience, for in this way he will more readily recognise any abnormality that may appear. I propose, therefore, to describe only four methods of staining blood films, as these, taken in conjunction with each other, would seem adequate for demonstrating any of the morbid changes that may be met with in ordinary hæmatological work.

Jenner's stain.—A 1 per cent watery solution of methylene blue is added to an equal amount of a 1·25 per cent watery solution of eosin. The precipitate which forms is dried, washed, and dried again. This precipitate is the "eosinate" of methylene blue, and is now dissolved in pure methylic alcohol, when the stain is ready for use. It is more satis-factory, however, to buy the precipitate in the form of a powder, as supplied by Grübler, or as a "soloid" (tabloid), as made by Burroughs Wellcome & Co. The powder is

dissolved in the proportion of 0·5 grm. to 100 c.c. methylic alcohol.

The solution is dropped on to the dried film, and allowed to stain for from a half to five minutes, evaporation being prevented by covering the film with a watch-glass. The film is then well washed in distilled water, dried, and mounted in Canada balsam.

With this stain the red corpuscles appear bright red in colour. The nuclei of the various cells are blue. The eosinophile granules are bright red, and the neutrophile granules a paler red. The mast cell granules are a deep violet blue. Parasites and micro-organisms stain blue. The longer the stain acts the better do the oxyphile elements show; with a shorter time the parts stained blue are more in evidence.

Jenner's stain is one of the most convenient for use in general hæmatological work. It does not, however, certainly differentiate the neutrophile granules, and the nuclei of some of the leucocytes are not so well defined as with, for example, Leishman's stain.

Leishman's stain.—This is a modification of the Romanowsky stain, and the special mixture of eosin and methylene blue that is used is to be obtained in the form of a powder, prepared by Grübler; it may also be had as a "soloid" from Burroughs Wellcome & Co. Of the powder 0·15 grm. is dissolved in 100 c.c. methylic alcohol (pure). There is, however, some difficulty in obtaining complete solution of the dye, and so it is well to use a mortar and pestle in making up the stain. The powder is placed in the mortar and 20 c.c. of the methylic alcohol added. These are worked up by the pestle, and then the rest of the alcohol is gradually added, stirring with the pestle all the while.

In staining a film 7 to 8 drops of the stain are allowed to flow over the surface of the smear; this fixes the corpuscles in from a half to one minute. From 14 to 16 drops of distilled water are then added, and the water and stain mixed together by gently moving the smear from side to side. The water seems to precipitate the dye in some way, and the now diluted stain is allowed to act for five to ten minutes. The film is then washed in water, very briefly in dilute acetic acid (1 to 1,500), again in water, and then dried with blotting paper. It is mounted in the same way as in Jenner's staining method.

With Leishman's stain the red corpuscles take a rich red colour, the eosinophile granules are pink, the neutrophile granules varying tints of purple, and the basophile granules a bluish-brown colour. The protozoal parasites stain a pale blue, with their nuclei maroon. Micro-organisms are blue, and the platelets blue with a pink centre.

Leishman's stain is excellent for demonstrating the structure of the various protozoa met with in the blood; it also stains well the nuclei of the different white corpuscles as well as the azurophile granules of the lymphocytes. It does not, however, always differentiate the neutrophile granules in, for instance, the neutrophile marrow cells, and this is perhaps its chief defect as a general stain for hæmatological work.

Giemsa's stain.—The preparation of this stain is rather complicated, and it is therefore better to buy it in the form of a solution as supplied by Grübler.[1]

The films are first fixed for two to five minutes in methylic

[1] Burroughs Wellcome & Co. make "soloids" of "eosin azure;" each "soloid" is dissolved in 5 c.c. of equal parts of glycerine and pure methylic alcohol.

alcohol. One to 2 drops of the stain are then added to 1 c.c. of distilled water. This mixture is now used for staining the films, and it is allowed to act for five to ten minutes. The films are washed in distilled water, dried, and mounted.

The red corpuscles stain a pale red colour. The nuclei of the polynuclear cells are a violet red, and the nuclei of the mononuclear cells bright red. The protoplasm of the lymphocytes is blue, and of the large mononuclears a slate blue. The eosinophile granules are brownish red, the neutrophile granules violet red, and the mast cell granules mauve. Parasites stain blue.

This is a useful stain for general hæmatological work, for it differentiates the neutrophile granules of the polynuclear and marrow cells, and may serve to distinguish the large mononuclear cells from the large lymphocytes. It also stains azurophile granules.

Ehrlich's triacid stain.—This stain is a mixture of two acids (orange G. and acid fuchsin) and one base (methylgreen). Saturated solutions of these three dyes are made, and may be used after standing for twenty-four hours. They are combined in the following order and in the following proportions [1]:—

Saturated watery solution of orange G.,	13-14 c.c.
Saturated watery solution of acid fuchsin,	6-7 ,,
Distilled water,	15 ,,
Absolute alcohol,	15 ,,
Saturated watery solution of methyl-green,	12·5 ,,
Absolute alcohol,	10 ,,
Glycerine,	10 ,,

[1] This triacid stain can also be bought made up ready for use, and it is much more convenient to get it in this form, as there is a certain amount of trouble involved in compounding the stain.

The ingredients are measured in the same measure, and as each is added the mixture should be shaken, especially after the addition of the methyl-green. The stain must not be filtered, and it is ready for use as soon as it is made up.

The films are fixed by heat and stained in the triacid mixture for five to ten minutes. They are then washed in running water, dried, and mounted. The red corpuscles take a copper colour, the nuclei of the white cells a bluish-green, the neutrophile granules a violet brown, and the eosinophile granules violet red. These tints, however, differ slightly with different specimens of the mixture. The protoplasm of the lymphocytes stains a pale pink, and parasites, micro-organisms, and basophile (mast) granules do not stain at all.

The triacid stain is rather poor as a nuclear stain, but it is specially useful for demonstrating the neutrophile granules of the polynuclear cells and neutrophile marrow cells.

The four staining methods described above are, under most circumstances, quite adequate for the ordinary requirements of clinical medicine. There are, however, four supplementary methods which I wish to refer to, for they might possibly prove of use in certain special cases.

Altmann-Schridde's method [1] for demonstrating the fuchsinophile granules in the lymphocytes. The smears, which must be thin, are fixed for one to two hours in formol-Müller (1 in 9). They are then washed for some minutes in tapwater, and then in distilled water. They

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[1] Described in *Anæmia*, by Ehrlich and Lazarus, English translation, 1910, p. 91.

are transferred to a 1 per cent solution of osmic acid, and left there for half an hour in the dark. Next wash in water, and then pour on the film some drops of Altmann's anilin acid fuchsin solution (20 grms. acid fuchin added to 100 c.c. of a saturated solution of anilin in distilled water, the anilin solution being filtered after it is cold). The film with the stain on it is warmed over the flame till steam rises. It is then allowed to cool. Excess of stain is removed with blotting paper, and the film differentiated with a solution of picric acid (1 part saturated alcoholic solution of picric acid in 7 parts of 20 per cent alcohol); the film should take on a pale yellow colouration. It is then rapidly washed in absolute alcohol, passed through xylol, and mounted in Canada balsam.

The fuchsinophile granules or rods appear a yellowish-crimson-red colour, the eosinophile granules are dark red, and the neutrophile granules a brownish-red; the mast cell granules do not stain.

"*Indophenol-synthesis*" *test for oxydase.*[1]—The films are fixed in alcohol for five minutes. They are then floated face downwards for two to five minutes on a freshly prepared mixture of equal parts 1 per cent watery solutions of alpha-naphthol and of dimethyl-para-phenylendiamin. The films are next washed in running water, and examined mounted with water or glycerine. The granular leucocytes stain a dark opaque blue, owing to precipitation of indo-phenol by the oxygen in the cell protoplasm; the nuclei remain unstained. The eosinophile cells show the most marked reaction, but the neutrophile leucocytes react almost as well. The reaction in the large mononuclear

[1] See paper by Shaw Dunn, *Journal of Pathology and Bacteriology*, 1910, vol. xv, p. 20.

(hyaline) cells is less marked, and in the mast cells still less so. A considerable proportion of the non-granular myeloblasts have been found to give the reaction, but the lymphocytes, red corpuscles, and blood platelets show no blue deposit whatever. When examining cells in which the reaction is slight, it is well to prolong the action of the stain for fifteen to twenty minutes, as the blue colour in such cells takes longer to appear than in cells in which the reaction is more marked.

Specimens mounted in water or glycerine fade in a short time, and the films cannot be mounted in Canada balsam, as the indophenol is soluble in alcohol and in xylol. Mounted in undiluted water-glass the films retain their appearances for many weeks.[1]

This method should be of considerable value in clinical work as a means of distinguishing the non-granular myeloblasts from the large lymphocytes, which is sometimes a matter of no small difficulty, as, for instance, in some of the more acute leukæmias.

Iodine reaction for glycogen in the blood.—Dried films are placed for a few minutes—that is, till they take on a deep brown colour—in a stoppered bottle containing iodine crystals. The film is then mounted in a saturated solution of lævulose; any elements containing glycogen are recognised by their deep mahogany-brown colour.

Mylius test for alkali in blood.—Freshly prepared dry films are placed in a solution of free acid iodide of eosin in chloroform. The film becomes dark red in colour, and is then washed in pure chloroform. It is mounted while still wet in Canada balsam. The slides, cover-slips, and

[1] Shaw Dunn, *ibid.*, p. 22.

vessels used should be first washed in a weak acid to remove all trace of alkali.

The iodide of eosin, when brought into contact with free alkali, gives a bright red colouration, and so the cellular elements in the film which show this red colour are said to be alkaline in reaction. By means of this stain the protoplasm of the white corpuscles are shown to be alkaline, the lymphocytes containing a greater amount of alkali than the other white cells. The blood platelets are also definitely alkaline. The red corpuscles and the nuclei of the white cells show no reaction, and must, therefore, be either acid or neutral in reaction.

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