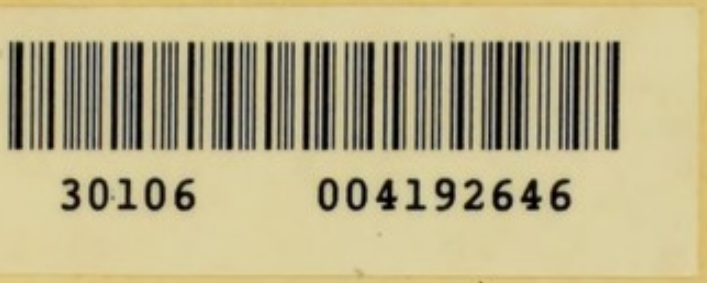
The morphology of normal and pathological blood / by George A. Buckmaster.



THE MORPHOLOGY OF NORMAL AND PATHOLOGICAL BLOOD

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PREFACE

THIS volume contains the substance of a course of lectures recently delivered in the Physiological Institute of the University of London.

I am indebted to Dr C. Slater and Mr C. T. Dent for permission to make use of the material collected during our work on polycythaemia at high levels.

All the illustrations and charts are original, with the exception of Figures 6 and 9*a*. The coloured plates were drawn and painted by my wife, and each represents a single selected field of the specimen viewed in daylight. My thanks are due to Mr Murray for these plates, and for the excellent way in which they are produced. The photographs of the blood-changes in leukaemia are reproduced from negatives taken by Mr Gordon Webb.

During the past fifteen years I have had abundant opportunities of investigating, not only the normal, but also those abnormal features of human blood that are met with in disease, and this work, together with other considerations, has led me to clearly recognise that both the closely related sciences of physiology and pathology may be studied either as subjects which have a purely academic interest, or what appears to be of far greater importance—as subjects which have a direct bearing on the advancement of medicine. In these lectures, where it was desired to give only an outline of the subject, this view as to the close relationship of physiology and pathology to medicine has been kept in mind.

A complete knowledge of the literature on the blood is practically beyond the reach of anyone, but all the references which are given have been actually consulted. The various excellent volumes on the blood which exist in English have not been used for reference, since they are easily accessible. Possibly these lectures may be of service as an introduction to larger works on haematology.

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THE MORPHOLOGY OF NORMAL AND PATHOLOGICAL BLOOD

LECTURE I

INTRODUCTORY—SPECIFIC OXYGEN CAPACITY—QUANTITY OF BLOOD IN THE BODY IN HEALTH AND DISEASE—STAINING BEHAVIOUR OF THE RED CORPUSCLES—POLYCHROMASIA—BASOPHILIA

Of the fluids which directly or indirectly bathe the fixed cells of the Metazoa, the caelomic is both phylogenetically and ontogenetically the first to appear. This, of which a part in higher animals is termed lymph, occurs as an almost cell-free fluid in the lowest phyla and into it wandering cells may enter, and thus a primitive sporadic mesoblast or hypoblast (Beard) may be formed, the complexity of which stands in direct relation to the degree of differentiation of the cells of the organism. The less differentiated are the cells of any organism, the simpler is found to be the composition both of this fluid and the blood. The amaeboid caelomic cells are leucocytes, and these, though varying somewhat in different phyla, are met with in all animals from Echinodermata and Vermes to man.

A completely closed blood-vascular system canalised in the mesoblast is found in both oligochaeta and polychaeta; in some of these worms haemoglobin is dissolved in the plasma, while the closed haemal system of another family, the Nemertines, contains a blood that is devoid of leucocytes but possesses coloured corpuscles floating in colourless plasma. The colour

is due to haemoglobin; in Amphiporus the elliptical discs are red or yellow, and in Euborlasia they are yellow, spotted with red. The fluid in the blood-system of insects appears to possess some physiological functions of the lymph and to be concerned with the distribution of food material alone, oxygen being introduced directly to the cells of the organism by chitinous tracheae. Coloured blood in which colourless corpuscles float, is found in many arthropoda and mollusca, its red or bluish tint is due to haemoglobin or haemocyanin, and the metal in the latter pigment is known to be copper and not iron.

The blood system of vertebrates is an open one, a free communication exists between the lymph and blood, either by the main trunks of one system opening into the other, or by an admixture of the two fluids in such regions as the bone-marrow, haemolymph organs, and possibly the spleen, though this has been regarded as a haemal gland (T. Lewis).[1] It is in vertebrates alone that the blood acquires those morphological characters, which permit of a sharp distinction into chromocytes and leucocytes. The non-nucleated red disc of man and mammals is to be looked upon as the most highly differentiated unit of the body, since it apparently possesses but a single function, that of transporting gas molecules from one region of the body to another. This function is secured not only by the presence of haemoglobin and by the rapidity with which blood moves in the body, but especially by the enormous corpuscular surface of the blood, which, on the assumption that 3·5 litres is the normal volume and 5,000,000 the number of corpuscles per c.mm., will be about 2500 square metres, or over 1000 times that of the body surface. The specific oxygen capacity (Bohr) is the ratio between the number of grammes of iron and the number

[1] The researches of T. Lewis, Warthin, Weidenreich, Dayton and others have added greatly to our knowledge of these bodies which Leydig discovered in 1851. Human haemolymph glands are freely distributed in the body; some 40 or 50 may be found in the retro-peritoneal region at the sides of and behind the great vessels. The glands may possess blood-sinuses which communicate directly with lymph-sinuses, or short capillary connections may exist between the blood-vessels and lymph sinus. Their function is considered to be haemolytic. Large phagocytic mononuclears, mast cells and eosinophil leucocytes are present. Warthin ascribes an excessive haemolytic activity to haemolymph glands in some cases of pernicious anaemia.

of c.c. of oxygen in a given volume of blood, blood corpuscles, or a solution of haemoglobin saturated with air at a given temperature and pressure. Blood corpuscles outside the body certainly differ in their oxygen capacity, samples of the same blood may differ by more than 20 per cent., and Bohr considers that blood corpuscles alter in their specific oxygen capacity during their transit through the systemic and pulmonary capillaries. If this is so, the blood must contain various haemoglobins of different specific oxygen capacities.

The red corpuscle may be regarded as possessing no single feature that can with certainty be relied upon as evidence of its life, for the adult red disc has apparently lost its power of reproduction and active growth, shows no signs of metabolic activity, and exhibits a function which is purely chemical and physical in character, that of an oxygen carrier, which it is conceivable could be quite well performed, as is the case with certain other animals, by a solution of haemoglobin in the blood, if that pigment could only remain in solution in the plasma and not be destroyed by the liver or treated as an abnormal constituent by the kidneys.

Except the undoubted fact that the haemoglobin-content of the disc may vary, and that after a severe haemorrhage, as v. Lesser, Hühnerfauth, and Otto have shown, a restoration of the normal number of corpuscles precedes that of the haemoglobin, as the accompanying chart (p. 4) proves, little or nothing is known of the behaviour of the normal undegenerated red corpuscles within the body, apart from their function as carriers of oxygen and possibly also of carbon-dioxide (Bohr).

The red corpuscles as seen in a drop of blood are of various ages, some are quite young, some are old; they vary also in size and in haemoglobin-content; further, some succumb earlier to laking agents than others, and if blood is examined at high levels, this fact is particularly obvious, for a specimen of Sherrington's fluid which slowly but completely laked human blood in 24 to 36 hours at the sea-level at a temperature of 16° C., was found at the same temperature not to affect about $\frac{1}{20}$ of the total number after a few weeks' residence at an altitude of 7000 feet.

Between the red corpuscles and the hypertonic plasma which bathes them a many-sided exchange exists, electrolytes

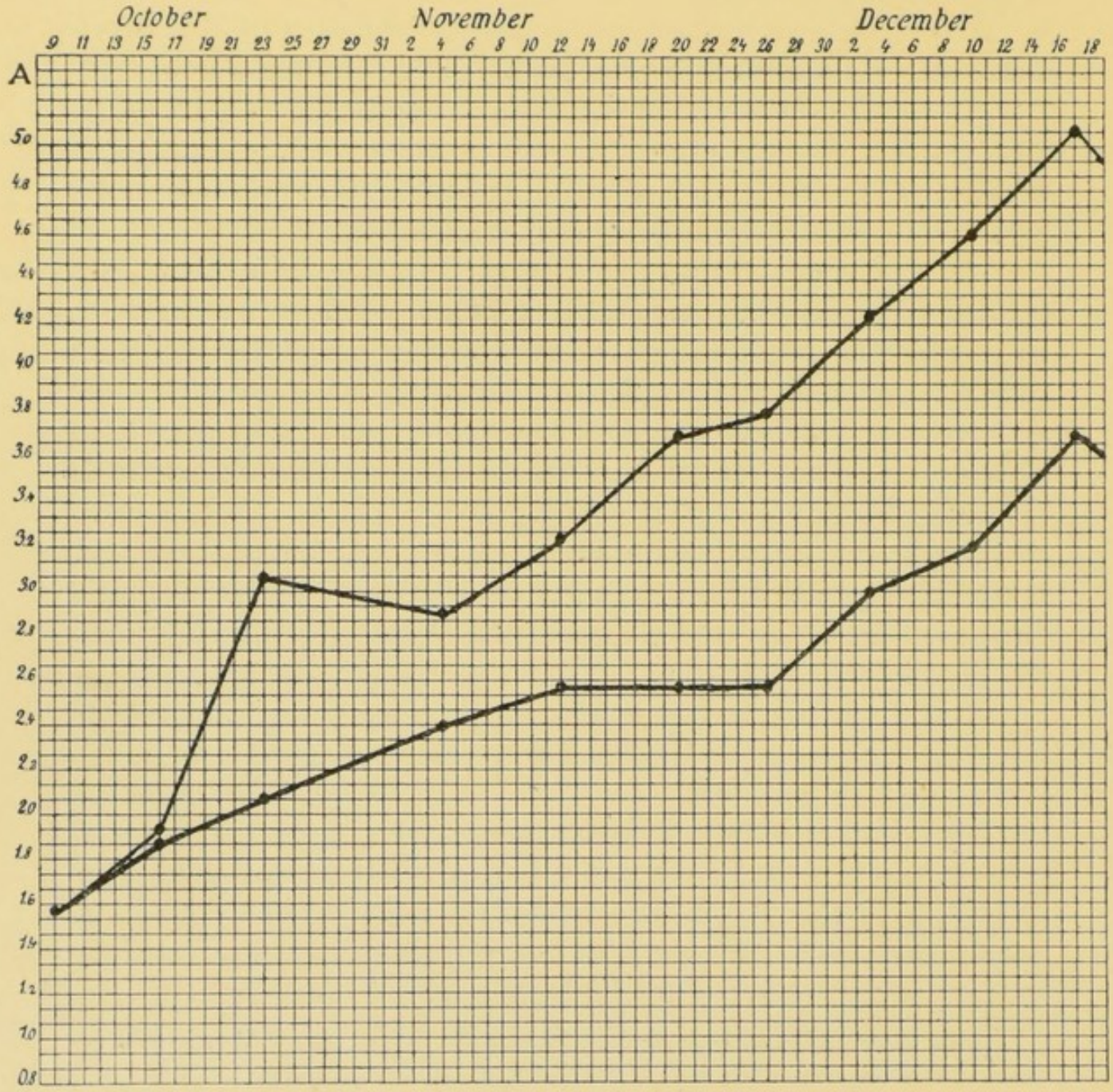
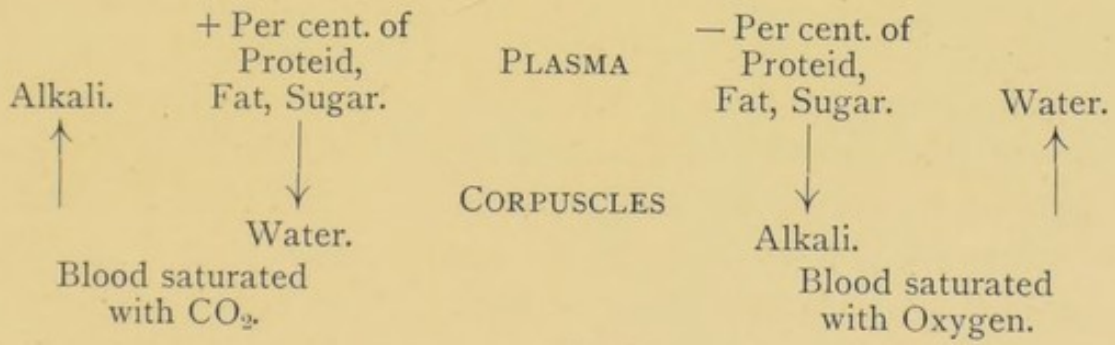


FIG. 1.—Chart (from Laache) showing the gradual rise in corpuscular-content of the blood in a girl 16 years of age after a severe haemorrhage. During the course of this post-haemorrhagic anaemia the number of corpuscles rose from 1·4 to 5·2 millions per c.mm. in about 2 months. The lower line shows the value of the corpuscles as far as their haemoglobin-content is concerned, expressed in numbers of normal corpuscles. The colour-index of the corpuscles is reduced, so that the blood in this respect somewhat resembles that of many cases of chlorosis.

may pass with ease either out of or into the corpuscles, and this may be accompanied by an alteration in their volume, for when de-

fibrinated blood is saturated with carbon-dioxide the potassium ions of electrolytes pass from the corpuscles into the serum, so that the molecular concentration of this is increased (Hamburger, G. N. Stewart), but at the same time water apparently enters the corpuscles, for these become obviously swollen and may burst. The serum therefore increases in basicity, and owing to its loss of water the percentage of proteid and sugar and fat augments. Exactly the converse is stated to occur when the blood is saturated with oxygen. In another experiment blood corpuscles were suspended in normal saline and the fluid saturated with CO_2. The corpuscles are scarcely permeable to bivalent CO_2 anions, but the chlorine ions easily enter the disc, so that the formation of Na_2CO_3 causes the fluid to become distinctly alkaline. Doubtless during life a continual movement of electrolytes occurs between the red disc and the plasma, which varies as blood is in the systemic or pulmonary circulation, or in the arteries, veins, or capillaries. The following diagram, constructed from the results of Hamburger and G. N. Stewart, shows the exchange which may take place :—



A physical explanation has even been suggested by W. Myers in order to account for the peculiar biconcave shape of the red corpuscles. The surface is greater for a biconcave than for a flat disc; this is probably an advantage, and hypothetically the condition might be due to the existence of a membrane which was capable of extension but not of contraction, and since the osmotic pressure within the corpuscles is less than that of the plasma, a movement of water from the corpuscles is followed by an amount of shrinking which produces biconcave faces to the red corpuscles.

Although the histology of normal and abnormal human blood

has received the almost exclusive attention of many observers during the last twenty years, it must be allowed that our knowledge of the origin of the red disc, of its duration of life, of its actual structure, or of its chemical nature, is still limited and uncertain, and the clearest proof of this is the quantity of contentious literature which exists, for in proportion as certainty obtains with reference to any question so does the range of discussion become narrowed. It is on account of the complexity of such a fluid as the blood, which may be studied from many various aspects, and to the undeniable fact that the blood of any given organism possesses characters which belong to that organism alone, that an explanation is partly to be found for the many conflicting views which are encountered in connection with almost every inquiry in the physiology or morphology of the blood.

It is only possible to approximately estimate the volume of blood circulating in the body by any of the ingenious methods suggested by Vierordt, Buntzen, or Thibaut. These as well as the so-called clinical methods proposed by Quincke or by Tarchanoff are of theoretical rather than practical value, and for any given case, a guess as to the quantity of blood in the body has to be made by taking into account such symptoms as the aspect of the patient or fulness of the pulse; the first of which is of little or no value, and the second only when well marked. In disease the absolute volume of the blood may be permanently increased or diminished, but in health, there is abundant experimental evidence to show that the maintenance of a constant volume of blood in circulation is of the greatest importance, even though this takes place at the expense of such tissues as muscle or blood plasma itself. An additional quantity of blood thrown into the circulation is, after a short preliminary fall, soon followed by a rise in the specific gravity of the blood plasma with a fall in that of the muscles, and conversely a diminution in the volume of blood, is succeeded by a rise in the specific gravity of the muscles and a fall in the plasma (Lazarus-Barlow, Sherrington and Copeman).

The total quantity of blood in the body can, however, be estimated by a method devised by Haldane and Lorrain Smith,

but I believe only the latter and Houston have published papers in which this has been employed in clinical investigations. Since the capacity of haemoglobin for oxygen and carbon-monoxide is identical, though its affinity for the latter gas is 140 times greater than for oxygen, their method ascertains to what extent the blood completely absorbs a known volume of carbon-monoxide, and, on the assumption that none of this gas is oxidised in the body, and no other substance in the blood except haemoglobin unites with it, the experiment enables the carbon-monoxide or oxygen-capacity of blood to be measured. If the percentage oxygen-capacity of the individual's blood is estimated by comparing its colour with that of a blood of which the percentage oxygen-capacity has been determined by the blood-pump or the potassium ferricyanide method or by means of Haldane's modification of the Gowers' haemoglobinometer, then it is easy to connect the measure of the total oxygen-capacity with the volume of the blood, or with the mass of this fluid which is the product of the volume and specific gravity.

Saturation of about one-fifth of the haemoglobin of the blood with carbon-monoxide has no harmful effects, the time required for an observation is about 10 to 15 minutes, and information as to the mass of blood is obtained by the carbonic oxide method which certainly ranks in accuracy with that yielded by the haemocytometer where the error may be anything between 5 to 20 per cent. For actual details as to the method, the original papers must be consulted, but it has yielded the following interesting results. Instead of the mass of the blood in man being 7·7 per cent. of the body-weight, or about $\frac{1}{13}$ (the figures obtained by Bischoff in 1855 and 1857), it is only 4·9 per cent., or $\frac{1}{20{\cdot}5}$ of the body-weight. A ratio as high as this is not given by individuals who possess a large amount of fat, but is then $\frac{1}{30}$, for the gross weight in this case does not fairly represent the actual body-weight; to obtain this the estimated amount of fat must be deducted. If we assume that these results are, as I believe, correct, it is evident that not a physiological theory, which notoriously has often only a short existence, but an inaccurate physiological fact has held its ground for more than fifty years. An estimate of $\frac{1}{13}$ of the body-weight is much too

high, and must therefore have impaired the conclusions of all work in which it has been employed as the figure for the total quantity of blood in the body.

Information as to how much blood a man may lose in a single haemorrhage without a fatal result is uncertain. Experiments on animals only allow a partial conclusion to be formed, for not only do different species show marked differences in respect to the amount that can be abstracted, but it appears as if a man will stand a larger percentage loss of the total quantity than most animals (Lazarus). Children can only withstand a moderate loss from which an adult would easily recover (Laache), but in any haemorrhage not only the total amount lost, but the rapidity with which blood escapes from the body is of cardinal importance.

Tolmatscheff while studying metabolism, noticed a fact well known to breeders of cattle, that small withdrawals of blood cause a deposition of fat, a condition which also obtains in some forms of anaemia, in which an increased discharge of nitrogen is observed. He found that within 60 days an amount of blood almost equal to the total mass could be abstracted from the dog without harm. For an adult man 30 per cent. of the haemoglobin can be combined with carbon-monoxide before any symptoms due to want of oxygen occur (Haldane). The loss of half the mass of blood in one haemorrhage is probably fatal (Panum), but if calculations are made from a remarkable case of haemorrhage from the aorta recorded by Sydney Phillips, it will be found that a man may in a period of 15 months lose almost eleven times the whole blood-content of his body. If this averaged 3·5 litres, an amount equal to this would repeatedly leave the body every 50 days.

By an extended use of the carbonic oxide method, Lorrain Smith has succeeded in proving what other observers have only been able to infer as to the quantity of blood in the body in various diseases. His conclusions are of exceptional importance, and show how fallacious it is to build up theories as to the nature of a disease by determining the percentage corpuscular-content and haemoglobin-value in drops of blood.

In that form of anaemia known as chlorosis, the volume of

the blood is always increased in proportion to the severity of the disease, while the total haemoglobin-content remains normal. Therefore the volume-increase must depend upon an increase of the plasma, and since each individual red disc is deficient in haemoglobin, the number of red corpuscles must be absolutely augmented. The same is true for the leucocytes, these are absolutely increased, though their relative numbers alter since the lymphocytes are augmented.

In pernicious anaemia the total haemoglobin-content is diminished in proportion to the severity of the disease, while the volume of blood may be either increased or diminished.

As might have been anticipated the volume of blood in post-haemorrhagic anaemia is nearly normal.

An extended investigation by the carbon-monoxide method into the changes which the blood exhibited in a case of heart lesion similar to those which have been described in valvular disease associated with cyanosis and plethora, showed that not only was the volume of the blood enormously increased, but that both the absolute number of corpuscles and amount of haemoglobin augmented; as far as the latter was concerned this was actually doubled.

I have briefly referred to this work, not only because I have myself employed the carbon-monoxide method, but for the reason that it appears to be the only recent mode of observation which has really advanced the clinical pathology of the blood; to the counting of red corpuscles I have always attached comparatively little importance, and with the introduction of this method I am more than ever confirmed in this opinion.

The table on p. 10 is slightly modified from the papers by Haldane and Lorrain Smith.

The figures given in the various columns are obtained as follows:—

1. By direct weighing.
2. By the haemocytometer (Thoma-Zeiss).
3. By administration of a known volume of CO gas.
4. Determined by Haldane's carmine method.
5. Calculated = volume of CO absorbed $\times \frac{100}{\%\text{ saturation.}}$

1	2	3	4	5	6	7	8	9	10	11
Body-weight in kilogrammes.	Red Corpuscles in millions per c.mm.	Volume of Dry CO absorbed in c.c. at 0° and 760 mm.	Percentage saturation of Haemoglobin with CO.	Total Oxygen capacity of Blood in c.c.	Oxygen capacity per 100 c.c. of Blood in c.c.	Percentage of Haemoglobin. Normal = 100.	Total Mass of Blood in grammes.	Grammes of Blood per 100 grammes of Body-weight.	c.c. of Oxygen per 100 grammes of Body-weight.	Observers.
Normal Individuals.										
72·9	...	116	18·9	614	18·7	...	3455	4·74	·84	Haldane and Lorrain Smith.
89	...	116	22·7	511	18·2	...	2970	3·34	·57	
Moderate Chlorosis.										
49	...	51·9	13·4	387	...	82	2528	5·1	·79	Lorrain Smith.
50	...	55·2	13·3	415	...	65	3401	6·8	·83	
Severe Chlorosis.										
41	2.040	77·5	28	277	...	29·1	5129	12·4	·67	
49·5	4·287	73·4	19·3	380	...	40	3653	7·3	·76	
Pernicious Anaemia.										
62·7	1·616	42	16·8	250	...	43	3125	4·9	·45	

6. Determined by comparison of the colour of human blood with that of ox-blood the oxygen capacity of which had been ascertained by the blood-pump or the ferricyanide method.

7. Determined with the haemoglobinometer (Haldane-Gowers').

8. Calculated = the total oxygen capacity $\times \frac{100}{\%\text{ oxygen capacity.}}$

9. Calculated = $\frac{\text{mass of blood}}{\text{body-weight.}}$

10. Calculated = $\frac{\text{total oxygen capacity}}{\text{body-weight.}}$

Human red corpuscles are grouped into erythrocytes (chromocytes, xanthocytes) and normoblasts or erythroblasts[1] while in pathological blood, larger, smaller, or distorted forms of the corpuscle known as megalocytes, microcytes, poikilocytes or schistocytes, a term indicative of their origin (Ehrlich), are found, together with abnormal nucleated cells, the megaloblasts and metrocytes (Engel). Mitotic figures are not infrequent in nucleated red corpuscles; I am not aware that the chromosomes in any cell of human blood have been actually counted, but their number is probably 32 like other somatic cells of the body. The forms in pathological blood will be considered in a subsequent lecture. At present it is sufficient to point out that though very little is learned about the essential structure of the red corpuscles by histological methods of fixing and staining, much interest centres around their behaviour towards various stains.

The unfixed red disc acquires a deep orange colour in iodine vapour, becomes tinted with such a dye as neutral red, but does not stain at all with acid or basic dyes; it is achromatophil. According to the nature of the fixing agent the haemoglobin of the disc varies in its affinity for such dyes as eosin, aurantia, or orange G, but stains orthochromatically, and may therefore be spoken of as acidophil or oxyphil. The disc, if it stains diffusely with a basic dye, such as logwood or methylene-azur, is said to show polychromatophilia or polychromasia, a condition which, according to Ehrlich, is due to a coagulation-

[1] This term is generally used as a synonym for normoblasts, but is employed by Löwit as a term for those haemoglobin-free mother cells of normoblasts, which he has described in the bone-marrow.

necrosis of the discoplasm of the corpuscle, which consequently loses its specific property of retaining haemoglobin. This state, which is also known as the anæmic degeneration of the disc, is regarded as a process that is dependent upon a vitiated nutrition produced by the altered condition of the plasma (Cohn), it may be a sign of senescence, but the studies of Pappenheim and others on the transformation of the normoblast to the non-nucleated corpuscle have shown that the disintegration of the nucleus within the disc may, in consequence of the distribution of chromatin particles, cause polychromatophilia, and also show instead of a diffuse staining a number of discrete points, blue-black in colour, which cause the disc to present a granular appearance, like that known as the basophil granulation of the red corpuscles.

These peculiar staining features of the disc have received various interpretations. They may be signs of age, they may be signs of youth. The leucocytes are believed to have only a short duration of life, existing for a few days in the blood, and then by their death furnishing the alexines, cystases, or complements of that fluid, in virtue of which this becomes possessed of bactericidal and haemolytic properties (Metchnikoff); but the red corpuscles, on the other hand, have probably a longer existence, and are very likely destroyed in the liver and in haemolymph organs (Warthin). As to the duration of their life no certain knowledge exists, it has been surmised to be from about three weeks to a month (Quincke). In every specimen of blood that is examined, there must exist side by side young, adult, and aged corpuscles. Are there any signs by which the corpuscles can be ranked in order of their age? Could this question be answered, and if in any specimen, fresh or stained, it were possible to state, even roughly, the percentage of, say, the young forms, a genuine advance in knowledge would certainly ensue. Fresh blood, as is known, lakes in hypotonic solutions of electrolytes, and those corpuscles are to be considered the more resistant the weaker the percentage of salt which leaves them uninjured. But even this behaviour is not a feature that can be used as a criterion of age. Although those discs which succumb earliest to weak laking reagents

may be less resistant than others, a variable number of them remaining unlaked 24 to 36 hours after the rest have disappeared, it is not known whether it is an old or young corpuscle that has laked more readily. Madsen in his researches on tetanolysin has shown by very exact experiments, that *in vitro*, blood corpuscles behave to varying amounts of this haemolysin, in such a way that for a given strength and amount of poison, the percentage of laked discs is so constant that it is possible to demonstrate the existence of a definite scale of resistance to toxins from various sources. However, it is known that such a scale is specific and not applicable to other laking agents, so that corpuscles may resist one poison but be destroyed by another, but whether mature or immature corpuscles have been laked is unknown. I hold the view that no opinion as to the age of corpuscles can be formed by any laking method, chiefly because fresh blood corpuscles behave towards hypotonic solutions of salt and other laking agents in a manner precisely similar to what is seen with blood corpuscles which have been killed by formaldehyde, and the disc so altered that its content is no longer haemoglobin, but methaemoglobin. The different sizes of the discs is also useless for the determination of age. But with staining methods a certain amount of evidence with reference to the degeneration of corpuscles can be obtained, and, moreover, according to some observers an estimation of the amount of haemoglobin in the disc will give a clue to its age.

The normoblast, which in embryonic blood, can be seen to change into a non-nucleated corpuscle by extrusion of the nucleus (this can easily be induced by irrigation with 3 per cent. salt solution) or by karyorrhexis, fragmentation of the nucleus (this can be studied by the addition of a minute amount of neutral red to living blood), is admitted to be the youngest of the red corpuscles. In early embryonic life the nucleated reds are of large size, metrocytes, and a normoblast in transformation to an erythrocyte becomes smaller (Pappenheim). The amount of haemoglobin in the larger discs is frequently less than in the smaller ones, but this is not constant, and the same is true for the nucleated cells. Although the evidence on this point

is insufficient, it is possible, if not probable, that there is some relation between age and haemoglobin-content, such as M. B. Schmidt and others have attempted to show is true for nucleated corpuscles in the embryo. His observations lend support to the view that the haemoglobin-content has no relation to the size of the cell, but a higher content is found in those with degenerating nuclei, and, within limits, the older the corpuscles become the richer do they grow in colouring matter.

Evidences of the degeneration of the disc, as seen in stained preparations, are to be found in certain necrotic features of the corpuscles met with in disease, when the disc appears eroded at the edge, or clefts occur on its surface. Schüffner's dots, which appear as fine red granules by Romanowsky staining in the discoplasm of swollen red cells infected by benign tertian parasites of malaria, also probably indicate degeneration, but the significance of Maurer's dots or clefts in the red corpuscles, which are infected by malignant tertian parasites, is doubtful. As a diagnostic feature, both Schüffner's and Maurer's dots are of importance, though the latter only occur in cells infected by young segmenting forms, and are only to be found in the peripheral blood at certain stages of the fever.

Since many observers, such as Ehrlich, E. Grawitz, and Pappenheim, regard polychromatophilia, basophil granulations, and the presence of a discrete accumulation of haemoglobin in the disc, the haemoglobinaemic inner-body, as undoubted signs of degeneration of the red corpuscle, the features of these changes, and the conditions under which they occur, is of some considerable interest.

In my own preparations of normal blood, though particularly searched for this special purpose, I have never seen basophil granulations in a red disc, but polychromatophilia is not uncommon, and is peculiar to certain corpuscles which are somewhat larger than the average. Discs which stain in this way are so rare as to be of no use as a means for distinguishing old from young chromocytes. The polychromatophilic condition of the red disc seen occasionally in normal blood is more frequent in that of anaemia. In chlorosis I have not seen it, but in pernicious anaemia it often reaches a

high grade of intensity, which may entirely disappear under treatment. Regarded by many observers as a degeneration of the disc, it is also known to occur as a physiological process in the development of blood in the embryo. Even though this is the case, the aspect of the disc, the size, and the ragged contour of polychromatophil discs give the impression that this feature is a sign of the degeneration and not regeneration of the corpuscles. But it is impossible to dispute the fact, that in the blood of newly born animals, such as the guinea pig, rat or mouse, some of the discs are polychromatophil, but if, as some observers believe, these are transformed normoblasts, this view is not easy to reconcile with the statements of Gabritschewsky and Dominici, that the youngest normoblasts contain no haemoglobin and are basophil, the older ones polychromatophil, and those which are just about to lose their nuclei, oxyphil. Again, if Ehrlich's contention that the normoblasts lose their nuclei by extrusion is correct, it is also quite impossible that an admixture of nuclear material can be the cause of the polychromatic staining of the disc. P. Schmidt's figures also show that in the blood of normal animals the discs show both polychromasia and haemoglobin-inclusions; but, as the latter are uniformly accepted as a degenerative feature of the blood corpuscles, the associated polychromatic discs are conceivably of the same nature. Those who regard polychromatophilia as a sign of degeneration point out:—

1. That the discs often show other signs of degeneration, such as the necrobiotic changes described by Maragliano.

2. The condition is seen in inanition, when the bone-marrow shows no sign of activity.

3. Discs with this feature appear in the blood after a severe haemorrhage, and earlier than the appearance of normoblasts.

4. Megaloblasts, but not normoblasts, may show this change; in other words, young cells which are normal constituents of blood or bone-marrow are free from polychromasia (E. Grawitz).

With these views I am in agreement, for from a study of anaemic conditions of the blood, it appears that the graver is the type, the more marked is the polychromatophilia. The following observation also supports the view that polychromatic

staining is a sign of degeneration. A few drops of human blood are received into a sterile tube and defibrinated by shaking with a globule of mercury. It is then allowed to sediment. After 4 to 5 days many of the red corpuscles will be found to show a polychromatophilia which was not present when the blood was shed. I think it is a fair inference that similar appearances in stained blood-films are due to comparable necrotic changes.

I would, however, hazard the suggestion that possibly both those observers who have regarded polychromatophilia as a degenerative, and those who are convinced it is a regenerative change, may be correct. I believe I am right in saying that we are entirely ignorant either as to the place or manner of formation of haemoglobin, and it is possible that an immature disc may stain polychromatically until it possesses some amount of haemoglobin. A development of this pigment may occur in the blood stream (Löwit, Giglio-Tos, Stassano). The experimental evidence in support of this view has not commanded much attention, but since we can indicate no definite region of the body where a corpuscle poor in haemoglobin can acquire a greater amount, it may be that this increase occurs within the blood stream and at the expense of the plasma, which is the natural habitat of the corpuscle. Again, towards the end of its existence the disc might lose haemoglobin or a part of this proteid, and with the loss of or change in this colouring matter, the corpuscle would show polychromatophilia.

Considerable clinical importance is also assigned to the basophil granulations of the red corpuscle, and as to the occurrence of this condition numerous observers have confirmed the observations of Askanazy who, in 1893, first demonstrated these intra-corpuscular granules in cases of severe anaemia. In chlorosis, in certain cases of lead poisoning, in coprostasis, in leukaemia, and especially in pernicious anaemia, where basophil granules may be the most striking feature of a blood film, this abnormal appearance of the red disc may be seen. It is most marked in cases of pernicious anaemia where, at times, I have seen more than 50 per cent. of the corpuscles so affected, although not a single punctated disc will be seen in other cases. The blood in post-haemorrhage anaemia 24 hours after the

haemorrhage, and also in typhoid fever, may show basophil granules. In the red marrow, an identical condition is stated to occur (Naegeli). This I have never seen, although human marrow, absolutely fresh and normal, has been repeatedly investigated for the purpose of determining this particular fact. On this question as to the existence of healthy red cells which possess basophil granules, I hold the same opinion as Grawitz, who has never seen these forms in normal marrow. Experimentally it can be shown that normoblasts, polychromatic discs, and red corpuscles with basophil granules, occur in the blood of mammals which have been treated with toxic doses of phenyl-hydrazine, nitro-benzol, or lead, all of which induce a severe and progressive type of anaemia. The effect of lead upon the blood is placed beyond doubt by an observation of W. Pepper, who found basophil granules in the red corpuscles of his own blood twenty-five hours after taking seven and a half grains of lead acetate. It is difficult to come to any other conclusion than that this feature of the corpuscles is a degenerative change. The majority of recent researches on this question, those of Naegeli, P. Schmidt, Sabrazès, and Lutoslawski, appear to support the view that all the three conditions of the red corpuscles mentioned above stand in genetic relationship, and either separately or together are evidence of blood regeneration in blood-forming areas in response to a blood-destruction. The difficulties which are always encountered in trying to interpret the information yielded by stained films, for unstained blood is practically useless, are particularly apparent in attempting to settle this question. Even if the contention of these observers is accepted that nuclear material disintegrated in varying amounts is the cause of basophilia, the undoubted fact that many nuclear stains, such as methyl-green, do not demonstrate the granules must be borne in mind.

In malaria (Plehn), in paroxysmal haemoglobinuria (Guyot), in black-water fever (P. Schmidt), when invariably profound blood destruction is occurring, basophil granulations are always to be found, though normoblasts are unknown in the blood of such patients. Moreover, in chlorosis, punctated discs are rare, normoblasts by no means uncommon, and the regeneration

of blood, or, at any rate, its improvement under treatment in grave forms of anaemia, is marked by the disappearance of basophil granules, although nucleated discs rarely entirely disappear.

The most conclusive evidence that basophil granules are not necessarily nuclear fragments is that they occur in nucleated megaloblasts. As to the diagnostic and prognostic value of these punctated discs in blood there is not a consensus of opinion. The impression obtained by observation of a disc that is both polychromatophil and granular is that it is degenerated. Contrasted with other discs it is often swollen and bloated ; such a corpuscle has the appearance of being functionally an entirely useless constituent of the blood. A chronic anaemia which shows only moderate poverty in haemoglobin may show marked polychromasia, and *vice versâ*.

The haemoglobinaemic inner-body described by Ehrlich appears as a small rounded particle of haemoglobin which stains intensely with eosin or Israel's fluid, while the rest of the corpuscle possesses a lighter tint than those discs which do not show an inner-body. This can be extruded, and then does not disintegrate but persists for some time as an abnormal constituent of the plasma (Heinz). In phenyl-hydrazine or nitrobenzol poisoning inner-bodies can be seen both inside and outside the discs. It seems that a profound injury to the living blood produces a condition similar to what is to be seen when Wlassow's fluid is added to blood *in vitro*. The extrusion of the inner-body is by some observers regarded as giving rise to blood-platelets (J. Arnold, Heim).

LECTURE II

STRUCTURE OF THE RED CORPUSCLES—POLYCYTHAEMIA OF HIGH ALTITUDES

If the view is correct, that by micro-chemical study only comparatively few facts as to the structure of the red corpuscle have been obtained, we are compelled to use other methods of investigation. Those of physiological chemistry are also of little use for the purpose, since the chemist can only indicate what substances are present, and in what proportion, and moreover can only isolate and describe the constituents of killed cells or the metabolites of living ones. Physical methods, therefore, alone remain available, and owing to the work of G. N. Stewart, Hedin, Overton, Koeppe, Hamburger, Oker-Blom, and Gryns among others on purely physical lines, our conceptions as to the structure and permeability of the red corpuscles, even if limited, rest upon a solid basis.

The chief constituent of the disc, haemoglobin, forms about 84 to 96 per cent. of the total organic matter of human corpuscles. The study of this colouring matter has been the subject of much recent research; here I can only mention that, unlike most proteids, haemoglobin is dextro-rotatory, and this is due to the nuclein moiety of the histone in haemoglobin.[1]

[1] Both nucleo-proteids and haemoglobin are dextro-rotatory, for :—

Oxyhaemoglobin . . .	$a_{(D)} = +10{\cdot}2°$.
CO-haemoglobin . . .	$a_{(D)} = +10{\cdot}8°$.
Nucleic acid	$a_{(D)} = +67°$ to $73{\cdot}5°$ (Th. Osborne).
CO-haemoglobin . . .	$a_{(D)} = +10{\cdot}4°$ (Gamgee and Croft Hill).
Haemoglobin	
Oxyhaemoglobin . . .	
Nucleo-histone from Thymus .	$a_{(D)} = +37{\cdot}5°$ (Gamgee and Jones).

Laidlaw has also shown the ease with which the iron can be split off from haemochromogen, or auto-reduced haemoglobin, and the ease with which this metal can be reunited to pure haematoporphyrin prepared from haemin by Nencki and Fr. Sieber's method. The average composition of the red corpuscles (pig) according to Bunge, is as follows:—

Haemoglobin	32·05
Water	63·21
Other substances: salts, cholesterin, of which only 1 per cent. are electrolytes	4·74

From the results of this table we can affirm that haemoglobin as it is known when separated from the corpuscles, cannot be in solution in the disc, for 32 parts of haemoglobin cannot be held in solution by 63 parts of water, since only 18 per cent. of haemoglobin can be held by 100 parts of water at 37° C. Neither is the pigment in a crystalline form, for the corpuscles are isotropous. Part of the haemoglobin may be in solution, the rest can only be in some amorphous condition. Whether, as Bohr affirms, several kinds of haemoglobin form the substance as we know it, it is certain that haemoglobin from different animals varies in solubility, and under certain artificial conditions the pigment can be caused to crystallise inside the corpuscles. Hamburger has shown that this occurs when the red corpuscles of the carp are treated with 8 per cent. cane sugar. This observer, as the result of elaborate researches into the permeability of the corpuscles, considers the entire framework of the disc is permeable, and that haemoglobin is retained by this in a state similar to but not identical with that of a true solution. Almost the last paper published in 1900 by Rollett, who had made the blood a subject of especial study, is devoted to a consideration of the structure of the red corpuscle. In his view, which does not appear to greatly differ from that advanced by Brücke fifty years earlier, the red corpuscle possesses a hyaline porous stroma, in the spaces of which an endosoma exists. The haemoglobin is held in an amorphous condition by this endosoma, while the electrolytes are an integral part of the stroma. According to Rollett the laking action of a hypo-isotonic fluid is

due to a swelling of the stroma consequent upon the passage of water towards the electrolytes. The endosoma and its contents are therefore pressed out by this turgidity of the stroma.

The red corpuscles have a low electrical conductivity compared with that of the serum or plasma, and defibrinated blood has a greater electrical resistance than that of serum. This I can show you with human blood, by measuring the resistance of equal columns of the following fluids placed in one of the arms of a Wheatstone's bridge. Roth and G. N. Stewart were the first to make accurate measurements of the electrical resistance of blood by Kohlrausch's method, in which a telephone replaces the galvanometer, and electrodes coated with platinum black, dip into the fluids, the resistance of which is to be measured. The conductivity for a given temperature is the reciprocal of the resistance at that temperature, expressed in mhos or gemmhos.

I.—HUMAN BLOOD FROM A CASE OF CYANOSIS TAKEN ON OCTOBER 27TH. DEFIBRINATED AND KEPT AT 0° C. EXAMINED ON OCTOBER 28TH.

Temperature, 18·3° C. Tube, 57 mm. long, 1·6 mm. diameter.

	Resistance in Ohms.
1. Defibrinated Blood	109,000
2. Serum (slightly red)	103,000
3. Blood laked with a trace of Saponin . .	53,000
Resistance of Electrodes before experiment	4,330
,, ,, after experiment .	3,900

II.—CAT'S BLOOD AT 19° C.

Tube, 50 mm. long, 1·6 mm. diameter.

	Resistance in Ohms.
1. Serum	28,330
2. Deposit of Corpuscles	117,300
3. Defibrinated Blood	39,820
4. Defibrinated Blood laked with Saponin .	26,850
5. Serum (Frog)	35,800
6. Defibrinated Blood (Frog) . . .	51,800
Resistance of Electrodes before experiment	12,040
,, ,, after experiment.	13,620

From the figures placed on the board as the result of each of these experiments, it is obvious that (1) the conductivity of the blood is raised by laking with saponin; (2) the conductivity of the corpuscles is low; (3) the presence of corpuscles in blood serum diminishes the conductivity of this fluid, just as is seen on the addition of non-electrolytes like haemoglobin and other proteids.

Since conductivity in the above instances depends entirely on the grade of dissociation of the electrolytes both of the serum and corpuscles, the resistance which is exerted by the corpuscles might be due to the fact that they are surrounded by an envelope that is either quite impermeable to the electrolytes of the serum, or to whatever ions are in the fluid of the corpuscles. It is a fair assumption that the surface of every cell of the body possesses the characters of an envelope which is permeable to certain extra- and intra-cellular fluids that are in contact with it, and on the properties of this the chemical nature of the contents of the cell and the osmotic pressure within it must depend.

From the behaviour of such laking agents as saponin, water, or foreign serum towards the red corpuscles, which do not necessarily induce disintegration of the discs, since it is possible to stain the ghosts or shadows, we learn that certain laking agents, such as eel or frog serum, act in such a way that only haemoglobin passes out of the corpuscle. The ghosts or shadows of the disc remain visible, and the electrolytes of the disc can subsequently be made to pass out by saponin. Further light is thrown on the degree of permeability by the use of sugar, which produces an exactly opposite effect; it is then found that the electrolytes pass out, while the haemoglobin remains within the corpuscles. By the subsequent addition of saponin the haemoglobin can be caused to escape.

The experiments just shown point to the conclusion that the relations of the haemoglobin and the electrolytes to the other constituents of the corpuscle and to the envelope are such that under certain conditions the colouring matter may be liberated and the electrolytes retained, or, conversely, under other conditions the pigment may be retained and electrolytes pass out, but in general it is easier for haemoglobin, in spite of its large

molecular weight, which lies between 13,000 and 16,000, to escape than it is for the electrolytes (G. N. Stewart).

The most probable view, which must at present necessarily be only an hypothesis as to the structure of the red disc, would seem to be, that within the envelope none of the haemoglobin is in solution as such, but that this proteid and most of the electrolytes are in combination with the stroma or endosoma, which forms not more than 3·5 per cent. of the disc. Incidentally it may be mentioned that haemoglobin in the serum never passes into the corpuscles, though in the case of the electrolytes this is possible. As to the conditions that underlie the peculiarities in the behaviour of the red corpuscles to certain substances such as NaCl and NH_4Cl, a research by G. N. Stewart, in which the electrical conductivity of blood was measured after the addition of one or the other of these salts, shows that the relative permeability of the envelope or whole stroma of the corpuscles is not dependent on their life but on their structure. Thus laking agents such as saponin or water have the same effect on the conductivity of blood, whether these are added to fresh blood, stale unlaked blood, or blood fixed by formaldehyde. The corpuscles remain permeable to NaH_4Cl, when they are undoubtedly killed by formaldehyde, and the haemoglobin of the disc converted into methaemoglobin. No change of any importance is noticed in the conductivity of blood dependent upon the so-called "vital" properties of the corpuscles, and these under a variety of conditions incompatible with life, continue to preserve their relative impermeability to the electrolytes of the serum. The preservation of the shape and aspect of the corpuscles in plasma may therefore be dependent, not upon the vital, but the physico-chemical features of the envelope and stroma.

The views of Overton may also be briefly mentioned. He considers that the surface layer of the protoplasm of all animal, and also vegetable cells, is impregnated with a layer of a compound of cholesterin and lecithin, which permits of a slow or rapid exchange of substances between the cell and its medium. What he terms the static osmotic features of a cell, apart from its inherent protoplasmic activity, depends upon the solubility

of substances in cholesterin-lecithin, or on the coefficient of distribution between this and water; in other words, depends upon an elective solution-affinity of the protoplasmic surface. As an example he instances the entrance into cells of the sulpho-basic dyes, toluidine blue, neutral red, or chrysoidin, while sulpho-acid dyes like eosin will not enter, and therefore do not stain the living disc. The envelope of cells is absolutely different to such colloid membranes as are found around muscle tubes; these are found to be permeable to nearly all inorganic salts, which is certainly not true for the envelope of the red corpuscles. Applying these views to the nutrition of cells, he points out that the envelope of cells is permeable to most organic poisons, to alkaloids, and also to such bodies as phenol or antipyrin, while the essentially nutritive substances such as proteids or carbohydrates cannot conceivably enter the cell in a similar manner.

These conceptions of the structure of the red corpuscle are in accord with those advocated by Professor Schäfer, who, quite recently, has recapitulated his reasons for regarding the corpuscles as vesicles bounded by a membrane enclosing fluid contents. The behaviour of the red corpuscles towards laking agents such as saponin, solanine, and tetanolysin is conditioned by the nature of their envelope. The ingenious experiments of O. Pascucci might have been predicted. He has shown that artificial corpuscles can be made by closing the ends of small glass tubes containing solutions of haemoglobin or cochineal with silk impregnated with a mixture of lecithin and cholesterin. The tubes are immersed in various liquids, and it is found that their contents do not escape unless the cholesterin is chemically attacked by saponin, solanine, or cobra-poison. According to Pascucci the stromata of red corpuscles are composed of about one-third lecithin-cholesterin and two-thirds cell proteids.

In 1897 a most elaborate and excellent paper on the permeability of the red corpuscles was published by Hedin. His method depends upon the well-known fact that a lowering of the freezing point of a solution depends upon its molecular concentration in electrolytes, though it is an established physical fact that the depressions of the freezing points of solutions of

salts, strong acids, and bases are greater than the values calculated from their molecular concentration. For non-electrolytes such as alcohols, glycerine, acetone, formaldehyde, or any non-dissociable substances, the lowering of the freezing point Δ is proportional to the molecular concentration, thus ·1 gram-molecule solution in 1000 c.c. water $-\cdot 187°$ C. Where dissociation occurs, Δ depends not only on the molecular concentration but on the grade of dissociation, since a dissociated ion behaves like a molecule, and for any dissociable substance will be $\cdot 187 \times 1 + (k-1)a$, where a = the grade of dissociation and k = number of ions.

When a substance is dissolved in serum, the freezing point is lowered by an amount generally equal to that produced in an equal volume of water by the addition of the same amount of substance. Hedin therefore used ·85 per cent. of salt solution instead of blood plasma, and for blood, that of the ox after defibrination. When plasma was employed, this was obtained from 1 per cent. oxalated blood.

To equal volumes of blood a and plasma b, the same amount of a substance, such as urea, ammonium chloride, or sugar, is added and mixed. The blood is centrifugalised, and Δ estimated for the supernatant liquid with Beckmann's apparatus. For example, suppose the blood corpuscles equal 50 per cent. of the blood, and for a, $\Delta = -\cdot 306°$, and for plasma b, $\Delta = -\cdot 287$, it is obvious that $a = b$, or,

Case 1, $$\frac{a}{b} = 1, \text{ since } \frac{306}{287} = 1.07$$

in other words, the dissolved substance is distributed equally in the blood and plasma, or the corpuscles are permeable to the dissolved substance, of which about half has disappeared into their structure. To this group belong NH_4Cl, urea, antipyrin, and urethane.

Case 2, $a > b$, or,

$$\frac{a}{b} > 1, \text{ since } \frac{515}{358} = 1.44$$

The dissolved substance has practically not entered the corpuscles at all, such as NaCl or sugar.

Case 3, $a < b$, or,

$$\frac{a}{b} < 1, \text{ since } \frac{479}{564} = .8$$

The dissolved substance has been taken up in larger quantity by the corpuscles than by the plasma. The corpuscles are therefore very permeable to such substances which include aldehydes, ketones, and alcohols.

From these results we may infer that the corpuscles not only possess a permeable envelope; and whatever its actual nature may be, it is certainly not a semi-permeable membrane nor a colloid membrane. The surfaces of other cells doubtless also have selective properties, and that such substances as the alcohols, aldehydes, or a narcotic like urethane can and actually do enter the cells of the body, may be regarded as established.

POLYCYTHAEMIA.—The number of red corpuscles as determined in a drop of the peripheral blood may vary greatly, both in health and disease. In the former case an individual with only 3,900,000 per mm. may enjoy perfect health and show no anaemic symptoms (Manuel), and on the other hand a persistent polycythaemia may exist of 6 to 7,000,000 corpuscles without any symptoms from which such an increase might be inferred. Except the polycythaemia of newly-born children, and that noticed at high levels, most of the examples of both a physiological and pathological increase of corpuscles are due to an inspissation of the blood, as may be proved by taking the specific gravity of both the blood and the serum. Even that attributed to phosphorus poisoning is probably due to the loss of water by vomiting, for the condition does not occur until this symptom appears. Elsewhere I have given reasons why I do not attach much importance to a simple count of red corpuscles, and in examining any case clinically I consider that the haemoglobin value, alkalinity, and coagulability, together with an examination of fresh and stained films, should precede the counting of red corpuscles.

As remarkable figures, the following table gives a few instances of a quite extraordinary polycythaemia. In some of these cases it is hardly conceivable that the peripheral blood

really indicates the true value. Koeppe's results with the haematocrit give the average total volume of corpuscles in human blood as 52·6 per cent. My own average on some forty or fifty cases works out at 48·2 per cent.; oil or liquid paraffin being used as the diluting fluid. If this volume corresponds to 5,000,000 corpuscles, a polycythaemia of 10,000,000, unless the excess of corpuscles over the normal are all peculiarly minute discs, is an impossibility, for the blood would be an almost solid mass of corpuscles.

Observer.	Case.	Red Corpuscles.	Leucocytes.	Haemoglobin (Sahli's Haemoglobinometer).
Reinhold .	Grave anaemia, with carbon-monoxide poisoning .	11,200,000	14,000	91
Fromherz .	Congenital heart disease .	9,800,000	...	....
Rosengart .	Splenic tumour. . .	10,000,000	...	...
Parkes Weber	Erythromelalgia (Weir Mitchell) . . .	9,000,000	...	...

The pallor of Europeans in the tropics does not appear to be connected with oligocythaemia, though Grawitz, as the result of experiments on animals exposed to high temperatures, considers that this produces degeneration of the red corpuscles and oligocythaemia. His experiments, however, admit of other interpretations, as both Eijkman in Batavia and Marestang in New Caledonia have shown, by most careful work, that a slight polycythaemia is generally found in Europeans within the tropics. From Nansen's expedition it has also been learnt that the long polar night of many months duration does not perceptibly affect the blood so long as the hygienic conditions as to food and ventilation remain good.

The blood at high altitudes shows polycythaemia. Viault's original discovery in 1890 that the blood, both of man and animals, at an elevation of 14,000 feet in the Andes of Peru showed a persistent increase of corpuscles per mm., has been confirmed by many observers. This increase disappears within 24-36 hours on a return to the sea-level, and the normal

corpuscular-content begin to augment again on a reascent to 1000 feet or more. This polycythaemia is an entirely unique phenomenon which occurs under physiological conditions, and as a permanent state cannot be induced in any other way, although a temporary but intense polycythaemia, it is true, may be caused by profuse sweating (Durig), intense light (Kronecker, Meyer), or conditions which establish an apoplasmia of the blood. If there is no dispute as to the fact that high altitudes induce a state of polycythaemia, this is by no means true of its cause, which has been, indeed continues to be, a subject of much contention, and one which can hardly be considered to have been finally solved by the various scientific expeditions made by physiologists of Switzerland, Germany, Italy, and France, for the particular purpose of arriving at some sufficient explanation of the increase of haemoglobin and corpuscles in a unit volume of blood. In particular, a great impulse was given to this inquiry by Miescher of Basle, not only by improving the methods of research, but also by specially training those who actually made the observations.

There is a general agreement that both the number of corpuscles and the percentage of haemoglobin augment during residence at high levels. According to Abderhalden's recent work, not on man but animals, both increase *pari passu* and an increase of haemoglobin does not precede that of the corpuscles, a fact which appeared to have been established by the observations of Egger, Koeppe, and others. Descent from a height to the sea-level is also followed by a return to the normal corpuscular-content and haemoglobin-value in two or three days. Miescher and his school regard the polycythaemia as an adaptation of the organism to the diminished oxygen tension of high altitudes: it is a reaction on the part of the organism, by which an increased flow of red corpuscles into the blood-stream is brought about by an increased activity of the bone-marrow or other regions where blood-formation is possible. The polycythaemia is, therefore, real and not apparent. E. Grawitz declares the increase is apparent, and the recent work of Abderhalden supports this view. The polycythaemia is only an expression of the fact that the blood has become more

which may actually leave the body. A state exactly the converse of hydraemic plethora is produced, and this is the cause of the increase of corpuscles per c.mm. In my own opinion the question is not yet decided, but it is improbable that any necessity for an increase of corpuscles really exists, since we are more that sufficiently supplied with oxygen at the sea-level, and even at the height of Mont Blanc, where the percentage of oxygen would equal an atmosphere at the sea-level of 11 to 12 per cent., there is abundance of oxygen for the needs of the organism.

During two successive years, Mr Dent, Dr Slater, and myself, studied this question of polycythaemia in the Alps, and I will draw your attention both to our methods and also to some of the results of our unpublished work. We are of opinion that the counting of blood corpuscles at high levels requires an amount of attention which no one can give when at a level of anything over 10,000 feet. Sustained mental work is out of the question at this level, and is performed with difficulty in Europe at a level of even 6000 feet. This statement, we are certain, would be confirmed by those who have experienced the cold and discomfort, together with the nausea, and other slight symptoms of *mal du montagne* which affects most people in some degree. Our observations were taken at the following places :—

		Height (Feet).
1st year	London	56
	Grimsel	6,148
	Sierre	1,765
	St Beatenberg	3,767
2nd year	London	56
	Montanvert	6,303
	Chamounix	3,455
	Mont Blanc (Vallot Hut)	14,600

It appears to us that a single count upon one counter is useless in any haemacytometric method. Scarcely any observer gives details as to his procedure in this respect, and the same is true for most clinical observations. Photography as an aid in blood counting is obvious. The records are permanent, and can be verified by any number of observers. All our photo-

inspissated; large amounts of water leave the vessels, much of graphs taken by Mr Dent were generally glass negatives. Films were not so satisfactory. Alferow[1] was the first to use photography as an aid to counting corpuscles. Danilewsky,[2] and more recently MacMunn,[3] have drawn attention to this as a method, but no observer has employed it in a systematic research.

We do not believe that our food in any way modified our results, for it is a well-known fact that in Switzerland the food is of the same kind everywhere. Mr Dent, Dr Slater, and myself each furnished a set of observations every day, taken at the same time. These included:

1. Weight taken with a spring balance.

2. Temperature in the mouth, 7 A.M.; pulse; respiration; temperature in the mouth, 10 P.M.; pulse; respiration.

3. Hours and amount of exercise.

4. Amount of sweating (none, slight, free or profuse).

5. Urine excreted (total amount).

   Total urea, by Dupré's apparatus. Corrections of gas made for 0° and 760 mm.
   Specific gravity.
   Reaction to litmus paper.

6. Blood corpuscles were counted in a pair of selected Thoma-Zeiss chambers which were practically similar. Dilutions, 1 : 100 or 1 : 200 with isotonic Hayem's or Sherrington's fluids. All observations were made in duplicate and the slides photographed. A single field contained 64 squares, and all the corpuscles in these were counted at leisure in London, and all duplicates rejected which did not agree within 10 per cent.

7. The number and kinds of leucocytes were also counted in some cases, but it was soon found that these did not show any variation from the normal.

8. The volume of corpuscles and plasma was estimated with

[1] Alferow, *Arch. de Phys.*, 3rd ser., t. iii., p. 269, 1884.

[2] Danilewsky, *Pflüger's Archiv f. Phys.*, Bd. 61, p. 264, 1895.

[3] MacMunn, *Proc. Phys. Society*, 1901. Leonard has photographed the diapedesis of leucocytes, *Amer. Journ. of Med. Sciences*, cix. 1895.

the haematocrit (Gärtner's). The diluting fluid was Sherrington's, isotonic with human plasma.

9. Specific gravity of the blood.

10. Specific gravity of the serum. This and that of the blood were taken by Hammerschlag's method, using a special tested set of specific gravity beads ranging from 1018 to 1880, made by Casella; with these, determinations to the fourth decimal place could be made.

11. Haemoglobin determined by Oliver's haemoglobinometer, with artificial light (small candle).

12. Films of blood fixed over osmic acid were taken each day. Subsequently these were stained in the same way by Israel's stain for haemoglobin, and logwood (Ehrlich's) for nuclei. In cases of doubtful nucleated red corpuscles, the companion film was specially stained with a modification of Ehrlich-Biondi-Heidenhain's fluid.

I cannot here enter at all fully into the work of other observers—time does not allow of this—but I will throw on the screen some of the results given in Viault's original paper, which contained fifteen separate observations on men, women, and animals. He used the Malassez counter.

VIAULT.

Lima (430-650 feet).		Morococha, Andes (14,000 feet).	
Viault	5,000,000	After 14 days on the Andes	7,100,000
		8 days later	8,000,000
		Companion	7,300,000
		A German	7,960,000
Paris.		His Wife	7,081,000
Dog[1]	6,850,000	Dog	9,000,000
Llama[1]	13,186,000	Llama	16,000,000

[1] Counted by Hayem.

The next table shows that each of the four cases in which we examined the number of corpuscles, the specific gravity of the blood, and the haemoglobin-content of individuals who had resided 4 to 6 weeks at a level of 6148 feet, gave figures

much above the normal. In three observations the number of corpuscles slightly exceeded 8,000,000 per c.mm. and the haemoglobin-content in seven varied between 120 and 125.

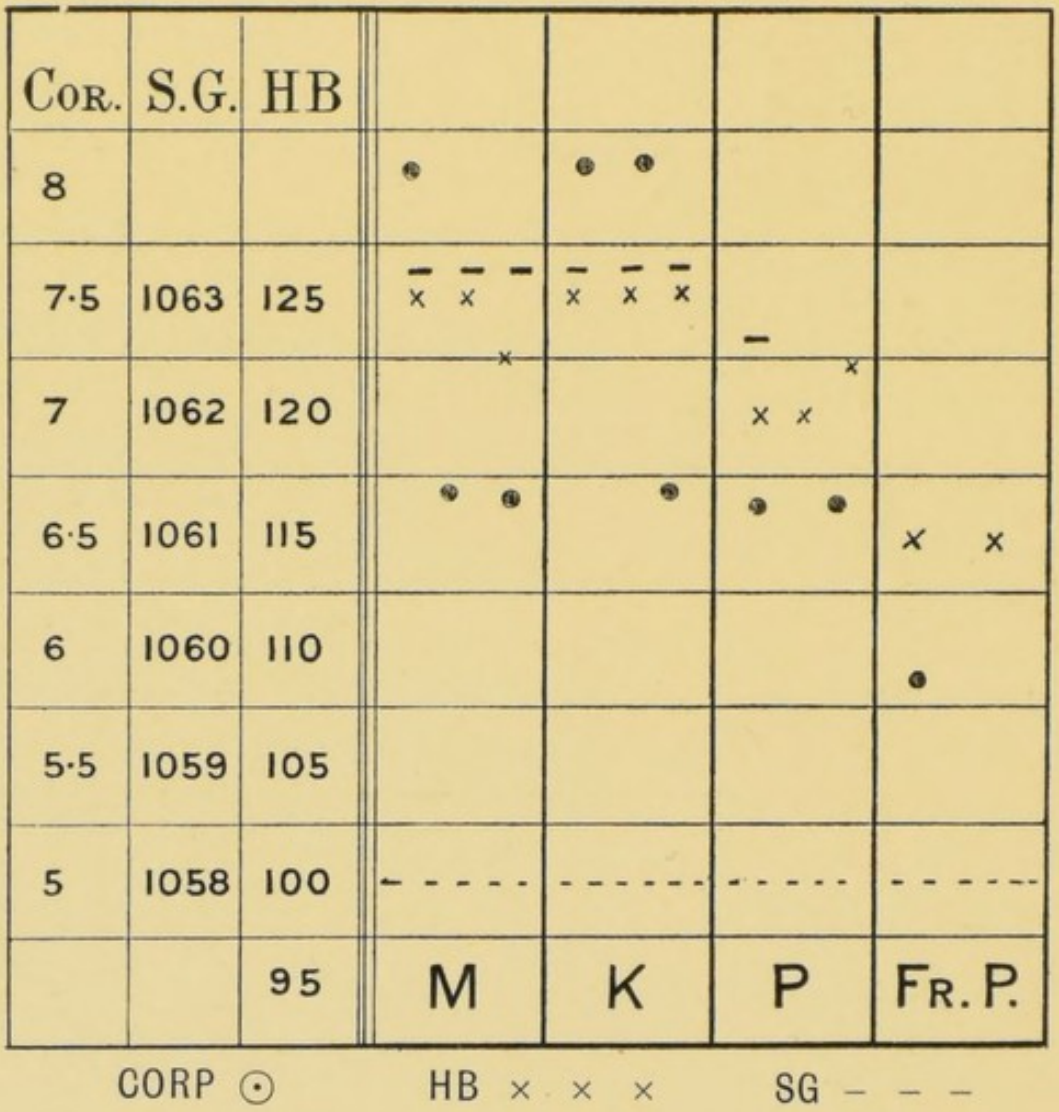


Fig. 2.—Chart showing polycythaemia, high haemoglobin readings, and specific gravity of the blood in twenty-five observations on three men (M, K, and P) and one woman (Fr.P.), all of whom had resided at a level of 6145 feet for 4 to 6 weeks, and one of them (K) for 10 months.

The general features of a progressive increase in the haemoglobin of the blood, as shown in the case of Dent, Slater, and myself, can be seen in Fig. 3, together with the marked fall that occurs when we descended from 6000 to 1700 feet. A reascent of 2000 feet is followed by a distinct though small increase. It will be noticed that the first observations read respectively 100, 102·5, 90·5. This is accounted for by the fact that the two first readings were taken after four days'

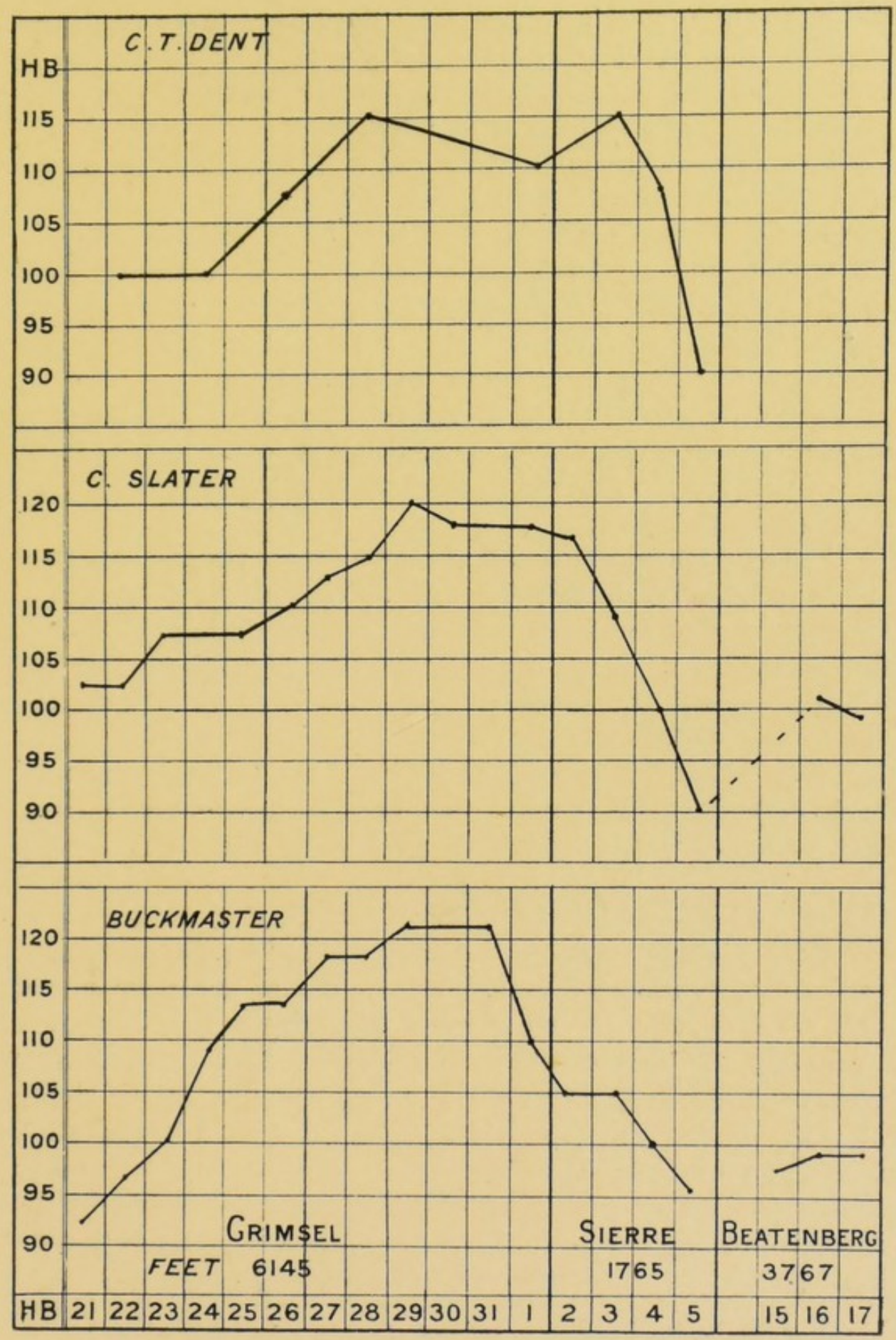


FIG. 3.—Chart showing curves of the haemoglobin-content of our blood at high levels, taken each day from August 21st to September 5th.

residence at 6000 feet, and the third the first day after reaching that level. With the haemoglobinometer used it is rare to find that the blood of any normal individual who lives in London gives a reading as high as 100.

The corpuscular-content per c.mm. and the volume, estimated with the haematocrit, are shown in Fig. 4. There is a progressive increase, marked, it is true, by some fluctuations in the number and volume of the corpuscles. The maxima of the curves very closely correspond with those of the haemoglobin-content (Fig. 3), and the fall after a descent of 4300 feet and slight rise on the reascent is well marked.

In the next chart (Fig. 5) the haemoglobin and number of corpuscles are given during a residence at 6300 feet, 3445, in the Vallot hut on Mont Blanc at 14,000, and again at 3445 feet (Fig. 5, p. 37).

The general results are identical with those already described by other observers, but the fluctuations in the corpuscular-content in each one of us are very evident during our stay at the Montanvert. I am inclined to attribute this to the fact that the amount of exercise taken this year was not kept so constant in amount as was the case during the time we were at the Grimsel. What is noticeable in the curve is, that the fluctuations in both the haemoglobin and number of corpuscles are uniform. The same fact is obvious in that part of the chart which shows the observations made on Mont Blanc. The following tables (pp. 36 and 38), give the variations in weight, specific gravity of the blood, specific gravity of the serum, and haemoglobin-content for Slater, Dent, and myself. The output of urine, amount of urea excreted, and amount of sweat lost, are also indicated.

Before stating the conclusions to our observations, it may be mentioned that a temporary polycythaemia can be easily induced during the few hours occupied by an ascent in a balloon (Gaule, Jolly, Bensaude, v. Schrötter and Zuntz). Some observers have also described histological appearances in the blood which might account for a polycythaemia, such as normoblasts, some of which showed mitosis (Gaule, Schauman and Rosenquist), or numbers of microcytes may enter the blood-stream (Mercier, Römisch, Köppe).

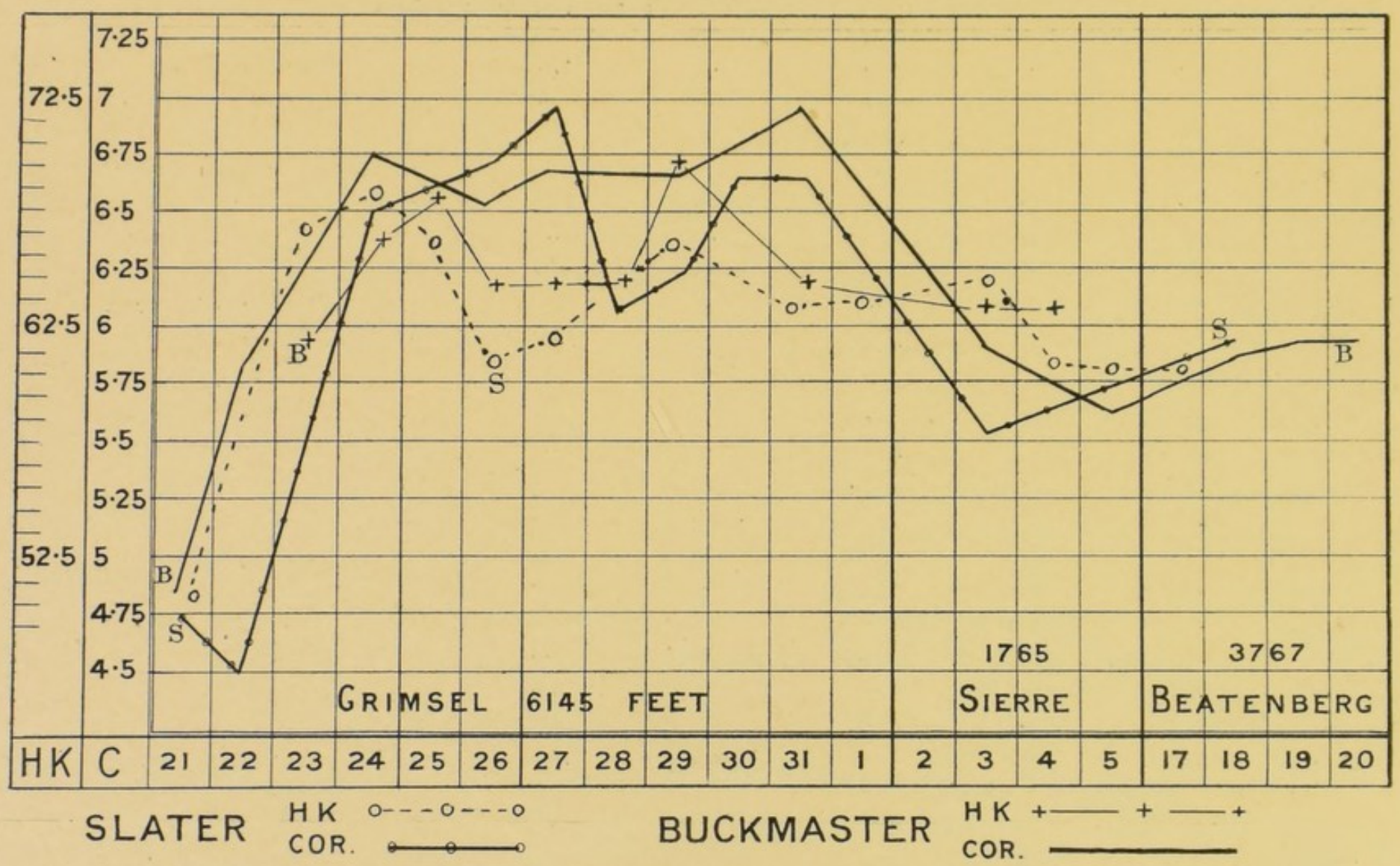


FIG 4.—Chart showing the fluctuations in the amount of haemoglobin and volume of the red corpuscles. The latter taken with Gärtner's haematocrit.

TABLE I.—(G. A. B.).

Date.	Place and Altitude.	Weight, in lbs.	Hb.	S.G. Blood.	S.G. Serum.	Urine, c.c.	Urea, grammes.	Sweat.
August								
21	Grimsel (6148 feet) .	163	92·5	1059	...	1020	25	Slight
22	,, ,, .	160	96	...	...	1205	29·1	0
23	,, ,, .	164	100	...	...	1460	31·1	Free
24[1]	,, ,, .	162	106	1062	1029	1505	28·1	0
25	,, ,, .	...	108	...	...	1295	31·2	0
26	,, ,, .	...	112	...	...	1300	31·2	0
27	,, ,, .	...	112	1064	...	1280	25	0
28	,, ,, .	162	118	1063·5	...	1500	31·2	0
29	,, ,, .	...	118	1063	1031	1370	32·1	0
30	,, ,, .	...	...	...	1028·5	1515	33·2	0
31	,, ,, .	161	120	1063	1029	1381	...	0
Sept.								
1	,, ,, .	...	...	...	1029·5	...	...	0
2	Sierre (1765 feet) .	...	...	...	...	1290	...	0
3	,, ,, .	160	105	1061	1029·5	1389	32·1	0
4	,, ,, .	...	...	...	...	1290	30·2	0
5	,, ,, .	...	100	1060	...	...	...	0
9	Beatenberg (6303 feet)	...	98	...	1028	...	...	0
12	,, ,,	160	...	...	1028·5	...	...	0
15	,, ,,	...	100	1061	1029	...	...	0
18	,, ,,	...	105	...	...	...	...	0

[1] From this date onwards no exercise was taken, owing to an injured ankle.

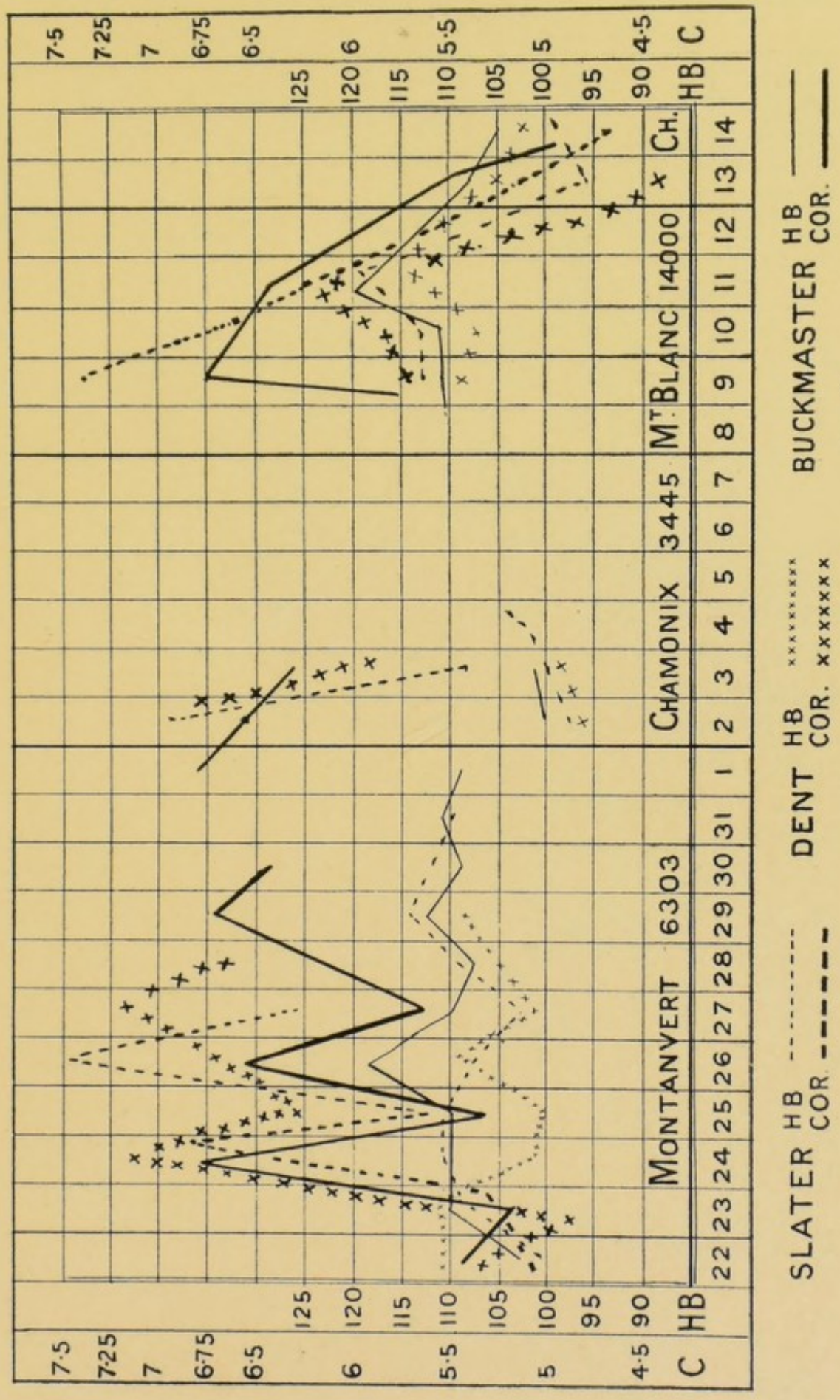


FIG. 5.—Chart showing fluctuations in amount of haemoglobin and number of corpuscles per c.mm. at Montanvert, Chamounix, and the Vallot hut on Mont Blanc.

TABLE II.

Place and Altitude.	C. T. D.				C. S.			
	Weight in lbs.	Hb.	S. G. Blood.	S. G. Serum.	Weight in lbs.	Hb.	S. G. Blood.	S. G. Serum.
Grimsel (6148 ft.)	169	100	...	...	166	102·5	1061	...
,, ,,	...	...	...	...	165	102·5	1061·5	1030
,, ,,	...	100	1060	1027	163	105	...	...
,, ,,	168	...	...	...	160	110	...	...
,, ,,	...	115	1063·5	...	...	112	1063	1028
,, ,,	...	...	...	...	...	115	1063·5	...
,, ,,	170	110	1063	1030	163	120	1064	1030
Sierre (1765 ft.) .	...	105	1061	...	...	108	1062	...
,, ,,	...	90	...	...	...	100	...	...
,, ,,	...	...	...	...	159	90	1059	1028

Certain observers (Gottstein, Schroeder, Meissen, and Starcke) have regarded the Thoma-Zeiss chamber as unreliable, since at high levels the included air, according to Gottstein, causes the apparatus to behave like an aneroid barometer, and produces a bulging of the cover-glass so that the volume-space above each square on the floor of the counter is increased. It is stated by Schroeder that a given definite dilution of blood corpuscles yeast-cells, or lycopodium spores gives much higher figures when prepared and counted in a chamber at a pressure of 624 mm., compared with a similar preparation at 745 mm. This difference in pressure would cause the depth of the cell to be ·1078 mm. instead of ·1 mm., and would increase a count of 5,000,000 to 5,440,000. The Schlitz-Kammer devised by Meissen is intended to avoid this source of error. But with a cover-glass ·41 mm. thick, it is physically inconceivable that this should be bulged out to any extent; and if the cell is filled at 624 mm. pressure, as in Schroeder's experiments, the apposition of the cover-glass cannot include air at a greater pressure. Turban, Sokolowski, and Gaule believe that no source of error can lie in this factor of altered barometric pressure, and it is immaterial whether the Schlitz-Kammer be used or not. Observations made with the ordinary Thoma-Zeiss are, as Abderhalden has shown, identical with those made with Meissen's modification. Although in a question of this kind our reason appears to me

to be quite as safe a guide as an experiment, the following figures show that when the Thoma-Zeiss cell is filled and counted first at 760 mm. and then again in an apparatus at a reduced pressure, there is no difference in the counts which could possibly explain the polycythaemia of high levels.

1. BLOOD DILUTION, 1 : 200, HAYEM'S FLUID. Thickness of Cover-glass, ·455 mm.

	Pressure.			No. of Corpuscles.
(*a*)	758 mm.		=	4,680 000
	260 mm.	= 27,000 feet	=	4,700,000
(*b*)	758 mm.		=	5,200,000
	260 mm.	= 27,000 feet	=	5,250,000

2. BLOOD DILUTION, 1 : 200, HAYEM'S FLUID. Thickness of Cover-glass, ·44 mm.

Pressure.			No. of Corpuscles.
760 mm.		=	5,310 000
330 mm.	= 18,000 feet	=	5,410,000

3. BLOOD DILUTION, 1 : 200, HAYEM'S FLUID. Thickness of Cover-glass, ·46 mm.

Pressure.			No. of Corpuscles.
760 mm.		=	5,100,000
340 mm.	= 16,000 feet	=	5,080,000

Our observations, therefore, show that :

1. The polycythaemia of high levels is an undoubted fact, independent of any error in the method. In men at a height of 6000 feet the number of corpuscles may reach 7 to 8,000,000 per c.mm.

2. It is established rapidly. Signs of an increase both of haemoglobin and corpuscles can be detected within 12 to 24 hours at a height of 6000 feet, and with slight fluctuations tends to augment for some weeks.

3. Both the haemoglobin-content and the corpuscles augment in the same degree. The latter does not, as Egger and others have believed, precede the former. The steady increase of the haemoglobin is easier to follow with accuracy than the corpuscular-content. The mean figure is about 125 to 128 (Oliver's

haemoglobinometer) for men, and 116 for women, who have resided some months at a level of 6000 feet.

4. Return to a lower level is followed within 12-24-36 hours by a distinct fall both in the haemoglobin and number of corpuscles per unit volume of peripheral blood. This, in turn, is set aside by a reascent.

5. The phenomena seen at 6000 feet are paralleled by those observed at 14,000; four days' residence at that level does not produce a more marked polycythaemia than at 6000 feet. We are disposed to believe that there is a certain level at which this condition is at its maximum, probably 6000 to 8000 feet in Europe. Double this height certainly does not produce a more evident polycythaemia or richness in haemoglobin.

6. The specific gravity of the serum does not show any marked differences at the sea-level or at the height of 6000 feet.

7. The specific gravity of the blood augments at high levels, and falls after an descent of 4000 or 11,000 feet.[1]

8. Several hundred microscopic specimens of blood made at high levels showed neither normoblasts, microcytes, nor any features which indicated that at high levels an excessive activity of the bone-marrow occurred. In this we are in agreement with Jolly, Loewy, and Abderhalden. Neither in preparations freshly made nor in stained films were any abnormal number of blood-platelets observed, though these have been stated to augment (Kemp).

Not only for man, but for mammals (Jaquet, Abderhalden) and birds (Giacosa), the existence of a polycythaemia of high altitudes is placed beyond dispute, but both the significance and explanation of this condition is difficult. There is no sufficient evidence either that this is due to an increased formation of corpuscles (Miescher and his pupils) or a diminished destruction of these (Fick). Observations on animals have shown that a polycythaemic condition is established in all parts of the vascular system, so that an altered distribution of the blood in the

[1] Our highest figures are well below those given by G. N. Stewart. Using Hammerschlag's method, the average of 165 male students was 1054·4, but 9 of these showed a specific gravity ranging from 1066-1070.

peripheral parts of the body can be excluded. The comparatively rapid appearance of the polycythaemia can therefore only be due to an exit of the plasma from the vessels comparable to what is seen under other conditions, such as abdominal shock (Roy and Cobbett) or certain acute inflammatory lesions. This exit of the plasma in our observations is certainly not secondary to an excessive loss of water from the body (Grawitz), for the complete set of observations made at 6000 feet were conducted in an atmosphere of cloud and mist, the air was saturated with moisture, and the amount of sweat lost was very small. The output of urine varied but little, and the body weight also remained practically constant. With this exit of the plasma the percentage of haemoglobin would also augment in the blood, but whether, as is probable, the total amount in the body remains constant, can only be ascertained for man by observations with Haldane's carbonic-oxide method. We may, however, infer that this is true for man, since it has been conclusively proved by Abderhalden to be the case in animals. This observer has also found that a small but definite increase in the total amount of the haemoglobin of the body is present in animals which are born at high levels, or in those which have remained for a long time at an altitude of 6000 feet.

LECTURE III

HAEMOLYSIS WITHIN AND OUTSIDE THE ORGANISM

IN the previous lecture I have shown that the red discs possess a surface layer or envelope of varying permeability, but we must realise that the introduction of such terms as stroma, endosoma, discoplasm, envelope, to indicate the various parts of which the corpuscle is conceivably composed, gives no precise information as to its structure. Still, upon the structure of the corpuscle its existence in the plasma must depend. Alterations in size and shape may, and actually do, occur without any obvious alteration in structure; thus the volume of the individual corpuscles is greater in venous than in arterial blood (Hamburger), and poikilocytes, which are regarded as fragmentation-products (schistocytes) of the red corpuscles, preserve the same structure, for a piece of the discoplasm "possesses an inherent tendency to assume the typical biconcave form in a state of equilibrium" (Ehrlich). Damage to the structure of the disc is followed either by separation of the haemoglobin from a colourless shadow—often spoken of as a ghost—a process which, within the vessels, produces haemoglobinaemia; or the red corpuscles extrude pieces of their substance and undergo fragmentation. Phenyl-hydrazine produces this latter condition, and causes an intense anaemia, from which a mammal can recover whether it possesses a spleen or whether this has been excised, and Heinz, the author of these experiments, therefore concludes that the regeneration both of corpuscles and haemoglobin can be perfectly brought about in the absence of this organ. Another drug, toxic for the red corpuscles, but one that does not

necessarily cause haemolysis, is toluylenediamine. An intense anaemia, which, according to Stadelmann, resembles megaloblastic pernicious anaemia as seen in man, is set up by this drug, both when given by the mouth or injected into the body. He also points out that recovery from the anaemia takes place in dogs when the spleen has been removed. It is the bone-marrow which shows the features of a blood-regenerating area.

The appearance of haemoglobin or its derivatives in the urine is inconceivable without a pre-existing haemoglobinaemia, but the exit of the colouring matter into the plasma is not necessarily followed by its appearance in the urine, for this depends first upon the number of corpuscles that are damaged, and secondly upon the part of the vascular system in which this takes place. According to Hayem, a physiological haemoglobinaemia exists, since, without exception, the serum which separates from blood taken from any vessel shows traces of haemoglobin. This, seen *in vitro*, is insufficient proof that it occurs in the body. Schäfer, in order to determine this point, examined the blood of the splenic vein, where, it has been stated, free haemoglobin is always present; but neither in this nor in arterial blood was he able to detect any haemoglobin spectroscopically in the serum of the rabbit, cat, dog, or monkey.[1] An occasional positive result was due probably to the chloroform or ether which had been used as an anaesthetic.

The destiny of dissolved haemoglobin in man is largely a matter of inference from experiments on animals,[2] but many of these, such as intraperitoneal or subcutaneous injections of dissolved haemoglobin, would appear to be of little use in

[1] Oxyhaemoglobin can be identified in a solution 1 cm. thick, when this contains ·01 per cent. (Hoppe-Seyler). By conversion into CO-haemoglobin, and examination of a long column of fluid, much smaller percentages can be detected (Haldane's method). I find when blood with a haemoglobin reading of 90 per cent. (Haldane-Gowers' haemoglobinometer) is diluted with distilled water, 1 : 1,000,000, that 10 to 20 c.c. of this will suffice to oxidise guaiaconic acid in the presence of H_2O_2. In my opinion, no reaction for haemoglobin exceeds this in delicacy.

[2] Minute traces of haematoporphyrin occur in most urines, but the existing evidence with reference to cases of marked haematoporphyrinuria in chronic poisoning by sulphonal, trional, tetronal, shows that this is due to a perverted metabolic change which haemoglobin undergoes, rather than to an haemoglobinaemia (A. E. Garrod). In a case of *acute* sulphonal poisoning, I could find no excess of haematoporphyrin.

helping towards a conclusion. Laked blood (Ponfick) or solutions of haemoglobin from the horse in isotonic saline (Stadelmann), when injected into the vessels of dogs, is followed by no excretion of pigment so long as the amount does not exceed $\frac{1}{60}$ or even $\frac{1}{28}$ of the total haemoglobin-content of the body. The recent figures of Camus show that $\frac{1}{37}$ of the total haemoglobin of the body, or, calculated for a man of 65 kilogrammes, a destruction of all the red corpuscles in 85 c.c. of blood, may pass into the plasma when such organs as the liver, spleen, and marrow will retain the pigment in the form of haemosiderin, but with a larger amount of haemolysis this function ceases, for the capacity of these organs to deal with the pigment is exhausted. Haemoglobin, then, appears first in the bile, and if still greater destruction occurs, not only does this appear in the urine, but the convoluted tubules of the kidney may become choked with a mass of fibrin and haemoglobin, the formation of which is attributed to the raised coagulability of the blood, consequent upon a leucolysis such as that which is described by Jacob, Krönig, and others, as the result of the presence of free haemoglobin in the plasma. After haemolysis, without the loss of haemoglobin from the body, a regeneration of the blood occurs rapidly, since the products of disintegration which are retained in the organism are directly available. If haemoglobin leaves the body, the regeneration then follows Otto's law, the corpuscular value reaching the normal before the haemoglobin-content (Tallqvist).

An experiment first demonstrated by Ehrlich in cases of paroxysmal haemoglobinuria, consists in plunging the finger of the patient in iced water; blood from this finger will then be found to yield after coagulation a serum strongly tinted with haemoglobin, while the serum from another finger of the same individual is normal. This observation has been frequently verified, but the phenomenon is not always seen even at the haemoglobinuric crisis. Similar observations, which are of interest, have been made by Murri and Justus in cases of syphilis. The blood of thirty syphilitic patients obtained by venesection was allowed to clot either at a temperature of 20° C. or at 2-3° C. From the former set a clear serum separated, but from the

latter twenty-eight out of the thirty showed a reddish serum. Murri further showed that by the application of this method 90 per cent of those whose blood gave a positive result had a syphilitic history, and clinically, haemoglobinaemic attacks are known to occur in such patients after chilling the body. Justus has recorded even still more interesting evidence that haemoglobinaemia can be established in syphilitic individuals by simply causing a stagnation of blood in the vessels. The blood removed from the median basilic vein in these patients yields a reddish serum after tightly constricting the arm for a few minutes. This observer has drawn particular attention to the destruction of corpuscles which probably occurs when 1-6 milligrammes of corrosive sublimate are introduced into the veins of patients with marked syphilis; for not only does the haemoglobin-percentage of the blood, as determined with v. Fleischl's haemometer, show a fall which he believes is a diagnostic sign of syphilis, but in several instances blood drawn from another vein shows, after coagulation, a characteristic red serum, while a similar experiment on normal individuals is accompanied by no haemolysis. The following curve shows the fall in the percentage of haemoglobin which follows each intravenous or intramuscular injection of mercury in cases of syphilitic cachexia; but it will be noticed that if the treatment is continued, each injection is followed after a time by general improvement, and a steady rise in the amount of haemoglobin.

This reaction of the organism, observed in 1897, is claimed by Justus to be of great diagnostic value in doubtful cases of syphilis. The test, however, has shared the fate of many others, being confirmed by some and contradicted by other observers. Fuerstein, in his criticism on the value of this test, agrees with Grawitz and Biernacki that it is of doubtful value, since out of forty-five cases only five gave a positive result. My own observations in two cases are not so unfavourable, neither are those of others (A. Whitfield), and I believe that a renewed study of the test is most desirable; the specific gravity of the blood, serum, and corpuscles should each be taken, so as to control the values obtained with the haemoglobinometer.

In such haemoglobinaemic conditions as have been referred

to, the simplest, as it is also the oldest conception, would be to attribute the escape of colouring matter into the plasma as due to an altered state of the corpuscles, to a diminished

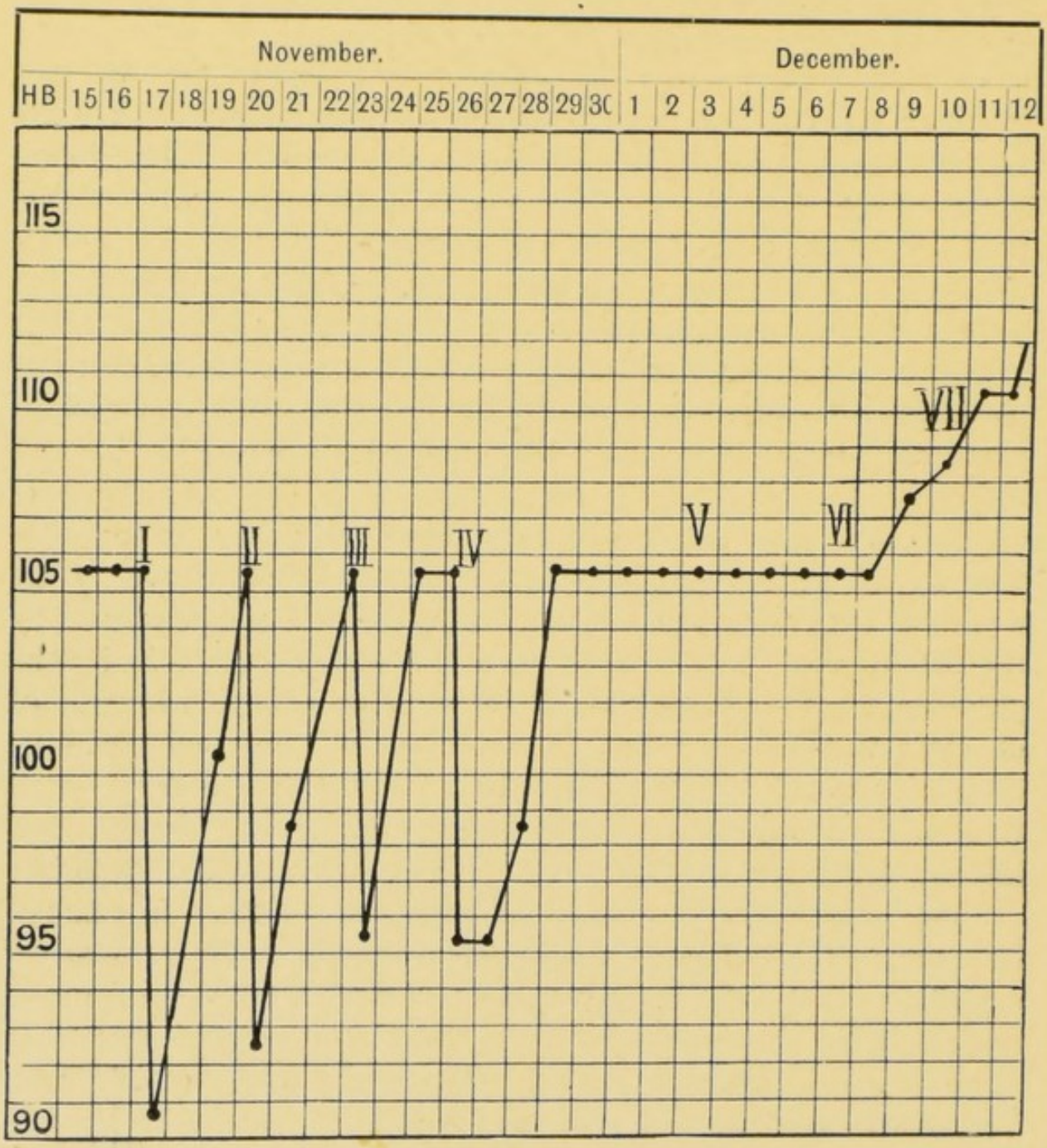


FIG. 6.—Chart of fluctuations in the percentage of haemoglobin as determined with v. Fleischl's haemometer in a case of syphilis treated with seven intravenous injections of 5 mgrms. of $HgCl_2$ (J. Justus).

resistance of these to laking influences, or to direct corpuscle-poisons. But with the resistance of the corpuscles this phenomenon of haemolysis may have no relation, since solanine,

digito-toxine, and other lytic agents cause laking in isotonic suspensions of blood corpuscles. The whole question of haemolysis is moreover a complicated one ; such simple explanations as those just offered will be found to be insufficient, and I propose to indicate the more important methods of effecting haemolysis outside the body, many of which I can demonstrate to you ; and next, to show how by the behaviour of the red corpuscles to laking agents and towards those substances to which the disc is permeable, views as to the nature of haemolysis have been evolved which have had a marked influence on the development of that theory of antitoxin immunity which we owe to Ehrlich.

1. The knowledge that distilled water causes haemolysis is familiar to everyone. Both haemoglobin and the electrolytes leave the corpuscles, and the phenomenon is seen equally well whether the experiment is made with a deposit of fresh corpuscles or a film of dried blood. The laking effect produced by alternately freezing and thawing blood is also doubtless due not to a destruction of the disc by the expansion of its water but to the action of distilled water, for on thawing a frozen saline solution it is pure water that at first separates out from the salts, which are entirely unaffected by freezing. The above laking agents are not toxic in any sense, since their action is dependent simply on physical causes.

2. Red corpuscles when broken to pieces by prolonged trituration with sand are found to lose their haemoglobin (Rywosch). Solutions of this detritus in ·9 per cent. NaCl, and in distilled water, are of the same depth of colour. From this experiment we may infer that the colouring matter is not in intimate union with the stroma, and that mechanical damage to the envelopes of the disc is followed by a loss of haemoglobin.

3. Certain substances penetrate into the corpuscles and conceivably disorganise them, as for example, urea, ammonium chloride, glycerine, alcohol, or ether. As you can see, this haemolysis by urea and also that by NH_4Cl does not occur if the salt is added to defibrinated blood in ·9 per cent. NaCl, and although I do not demonstrate this to you, I may point out that this haemolysis within the blood-vessels can be produced

by the injection of 10 per cent. solutions of urea, but not if the solution is made isotonic to that of blood plasma by sodium chloride.

4. The glucosides to which Kobert first drew attention, saponine, cyclamine, githagine, and also solanine, possess an intense haemolytic action. As you can see, the effect of saponine in minute amount haemolyses a large amount of blood with great rapidity. It is quite immaterial whether the corpuscles are or are not in isotonic saline, and a solution of saponine 1 : 125,000 will effect a rapid haemolysis; this is retarded at 0° C. The action of these toxic haemolytic glucosides is believed to be due to their union with the cholesterin of the corpuscle, for it has been shown that cholesterin has the power of protecting the corpuscles from saponine (Ransom) or agaricine. Corpuscles in isotonic salt solution can be protected against agaricine not only by cholesterin but by the serum of any animal, in consequence of the presence of this substance (Hideyo Noguchi). Whether the cholesterin or compound of cholesterin in the red disc forms the surface of the corpuscle or is distributed in the stroma, we do not know; but if the first is the case these glucosides could act without really entering the corpuscles.

The action of saponine involves three steps. At first the envelope of the corpuscles is affected, next the pigment escapes, and finally the electrolytes (G. N. Stewart). Solanine largely loses its haemolytic power if bile or cholesterin or frog's serum is added, so that tadpoles and fish that would be poisoned by solanine and cyclamine are protected by serum added to the water, or by bile, if the poisoning is by saponine.

5. Haemolysis can also be produced by substances of a much more complicated nature than any of those hitherto enumerated. They are the products of living cells, and are either toxic phyto-albumoses such as ricin, crotin, and abrin, or they are toxic bodies found in the filtrates of cultures of micro-organisms. To this class belong tetanolysin, megatheriolysin, pyocyaneus lysin, staphylococcus lysin, and others. With reference to the haemolysin in the filtrates of bouillon cultures of bacterium pyocyaneum, W. Bulloch and W. Hunter found this lytic substance in three out

of eight different cultures, and the lysin was toxic for the red corpuscles of the ox, sheep, rabbit, monkey, cat, dog, and rat. An antihaemolytic substance was found to exist in the serum of a goat immunised against this bacterium, and the chief interest of their work lies in the paradoxical results of neutralisation of the lysin with the antihaemolytic body. With small doses of the latter, haemolysis occurs; with medium doses none, since the lysin is neutralised, but with larger doses haemolysis again occurs. Haemolysins are also to be found, together with numerous other toxic bodies, in snake-venom,[1] and some of these (for example, cobra lysin: the lysin in the venom of the rattlesnake, water-moccasin, and the copper-head), have been extensively studied by W. Myers, Flexner and Hideyo Noguchi. By the kindness of Captain Rogers, I can show you on the screen the rapid haemolysis produced in human blood by a minute quantity of Daboia venom. You can also see that this contains a much more powerful haemolytic body than the poison of Enhydrina, a venomous salt-water snake found abundantly on the coast of Orissa.

6. Haemolytic sera. Of these there are possibly two distinct

[1] Snake-venoms, according to their source, possess great variations in the proportions in which the different groups of toxic substance occur. No venom possesses all the groups which have been found to exist in different venoms. Different venoms have been found to contain one or more of the following:—

1. A powerful fibrin ferment. (Lamb, Martin.)
2. A neurotoxin, with in many cases a special affinity for the cells of the respiratory centre.
3. A neurotoxin with an affinity for nerve-endings in muscle, and those in the diaphragm in particular.
4. Various cytolysins, for example, for red blood cells, endothelium of vessels, leucocytes, nerve cells, and the cells of many other tissues. (These cytolysins are distinct for each cell, and are of the nature of amboceptors.—Flexner and Noguchi.)
5. An antibactericidal body. (Welch and Ewing.) (These are of the nature of anticomplements.—Flexner and Noguchi.)
6. An antifibrin ferment. (Cunningham, Lamb.)
7. Agglutinin for red blood cells, etc. (Weir Mitchell and Reichert, Flexner and Noguchi.)
8. A proteolytic ferment. (Weir Mitchell, Flexner and Noguchi.)
9. A substance which causes systolic standstill of the isolated heart. (Elliot.)

This list is taken from a paper by C. J. Martin.

types which may act in an entirely different way as destroyers of the red corpuscles. The existence of sera which may contain a lysin for certain red corpuscles has been long recognised, for the transfusion of sheep's blood into the veins of human beings, as an attempted therapeutic measure, dates from 1667 (Jean Denis), though some years earlier the replacement of a part or the whole of the blood of one animal by that of the same or an allied species had been successful. Exact experiments by Landois showed that the fever and severe haemoglobinuria which follows the transfusion of sheep's blood into a man, or human blood into rabbits, is due to a specific globulicidal action. Simple experiments which are quite easy to make, can enable any one to observe for himself that the serum of most horses is haemolytic for the corpuscles of the guinea-pig, and that of the dog for the rabbit, though the converse does not occur.

The serum of certain cases of chlorosis and myelogenous leukaemia, I have noticed is haemolytic for normal human corpuscles, and this effect of chlorotic serum can be set aside by the addition of a definite amount of sodium chloride. Similar observations have been recorded by Maragliano, Ascoli, and more recently by Pagniez. Their observations for chlorosis and pernicious anaemia are conflicting, but they are mentioned here to show that if, as is possible, the haemolytic substances are the product of cell destruction within the blood-stream, that the serum of any abnormal individual may show at one time a positive and at another a negative action.

Apart from the haemolysins of normal sera, these bodies can be caused to appear in the serum by intraperitoneal, subcutaneous or intravascular injections of the corpuscles of one species into a suitable animal of another species such as the rabbit or guinea-pig. The original observation was made by Belfanti and Carbone in 1889, who noticed that on injection of the blood corpuscles of a rabbit into a horse, in the manner of an immunity experiment, the serum of the horse became toxic for the rabbit, and the corpuscles of this animal could be extensively haemolysed both in the body and *in vitro*.

Similar facts were rediscovered by Bordet in 1898, and others by Ehrlich and Morgenroth in 1899. All these observers demonstrated that various specific haemolytic sera could be obtained, such as one for the corpuscles of the ox by injections or defibrinated ox-blood into rabbits, or another for the corpuscles of fowl's blood by injecting these into a rabbit.

Not only do haemolysins appear in sera by the procedure just described, but agglutinins, which cause a clumping of corpuscles, may also be, and generally are, associated with them. What constituent or constituents of the corpuscles are concerned in causing the formation of haemolysins and agglutinins is uncertain. Nolf's view, that with water-laked discs the agglutinogenic property is a function of the stromata and the haemolysinogenic a function of the liquid into which haemoglobin and electrocytes have escaped, is not confirmed by Stewart, who finds that either the stromata or liquid can cause the formation both of agglutinins and haemolysins.

7. A number of drugs cause not only haemolysis but also a conversion of haemoglobin into methaemoglobin in man and animals. The blood-colouring matter then appears in this form in the urine.[1] Though I have looked through a good deal of literature, I have not yet met with any information as to the existence of methaemoglobin in human blood plasma or serum. The drugs that have been most studied in this connection are:—1. Chlorates of potassium and sodium. 2. Nitrites and nitrobenzol. 3. Phenylhydroxylamine, a poison which acts with exceeding rapidity in minute doses (Lewin). 4. Derivatives of anilin, such as phenacetin and antifebrin or acetanilide. 5. Quinine, according to Koch, Plehn, and most other observers, induces those attacks of blackwater fever which are seen in tropical countries where a pernicious type of malaria is endemic. A single five-grain dose of this drug will often suffice to cause the appearance of blood pigment in the urine of infected individuals (Stephens and Christophers).

In 1888 Mosso pointed out the intensely haemolytic power

[1] Variable amounts of methaemoglobin are nearly always present in haemoglobinuria.

of eel serum, and both *in vitro* and in the body this serum is powerfully haemolytic. I find, however, considerable differences in the various specimens of eel sera; some are much more haemolytic than others. In these normal sera which contain haemolysins, the most obvious feature in their chemistry is that the ratio of globulin to albumin varies; thus the ratio of serum-globulins (eu-globulin and pseudo-globulin) to serum-albumin yields the following quotients:—

$$\text{Man} \quad \frac{3{\cdot}10}{4{\cdot}52} = {\cdot}66$$

$$\text{Rabbit} \quad \frac{1{\cdot}79}{6{\cdot}22} = {\cdot}4$$

$$\text{Eel} \quad \frac{5{\cdot}28}{1{\cdot}45} = 3{\cdot}57$$

$$\text{Frog} \quad \frac{2{\cdot}18}{{\cdot}366} = 6{\cdot}33$$

A single paper—that by Krompecher—is the only one that I have seen which deals with the action of frog's serum, which he found on intravenous injection was toxic and haemolytic for rabbits; Landois had previously shown that the serum was haemolytic for rabbits' blood. What observations I have made were prompted by the belief that I should, from its high percentage of globulin, find that the serum exerted a powerful haemolytic action, and indeed frog's serum does possess an intense action on human blood. Experiments with this I will demonstrate, but need not say more about the method of procedure beyond calling to your notice that in the experiments care was taken that the observations were carried out with sterile fluids and tubes, and that washed human blood corpuscles suspended as a 5 per cent. solution in ·85 per cent. NaCl was the test substance, though the haemolysin lakes the blood of guinea-pigs, cats, rabbits and monkeys, being indeed one of the most powerful of haemolytic sera. The activity of the serum is destroyed by heating to 55° C. for half an hour; it is not impaired by drying, so that a small flake of dry serum acts like the fluid. What I may point to as further matters of interest are:—1. That the haemolytic action is set aside by a certain percentage of various salts. 2. The haemolysis occurs

at a temperature of 2° C., and I believe in several sets of experiments that it took place at 1° or even 0° C. Its occurrence at such an exceedingly low temperature is remarkable, but I am certain as to the facts, since the laked fluid was oxyhaemoglobin, and there was not any bacterial growth, nor any entrance of water into the tubes, which might have lowered the salt-content. This haemolysis at a very low temperature was unexpected, and is not in accord with the observations on other haemolytic sera where haemolysis is stated not to occur at 0° C. An estimation of the amount of haemolysis was made by comparison with a set of eight tubes with varying dilutions of CO-haemoglobin. To determine the haemolysis for any tube, this was shaken up, centrifugalised and a measured amount of the laked supernatant fluid removed, the haemoglobin converted into CO-haemoglobin and compared with the standards. I have also used the graphic method which Bashford has suggested and employed for estimating the extent of haemolysis by the stain the fluid gives to white filter paper. For future work, from the few experiments I have made I believe the extent of the haemolysis effected by any laking agent can be accurately determined by Kohlrausch's method for measuring the conductivity of solutions, since the escape of haemoglobin, which is not an electrolyte, from the discs must lower the conductivity of a solution in proportion to the amount which is present.[1]

On the much debated point, whether the haemolysin present in various normal sera or those in various snake-venoms as

[1] Proteids, like colloidal inorganic and organic substances, are apparently not in a state of true solution, since their effect is negligible in modifying the freezing point or vapour pressure of the "solvent." So-called solutions of proteids are fine suspensions, and possess no osmotic pressure (Waymouth Reid). The appearances of these suspensions in the ultra-microscope negatives the idea of a true solution (Raehlmann).

Haemoglobin differs from other proteids. It is a feeble electrolyte (Gamgee). Assuming the molecular weight of haemoglobin to be 16669 (Jaquet) and no dissociation to occur, 1 per cent. concentration of this substance in water at 15° C. would give an osmotic pressure of 10 to 11 mm. of mercury. With a gelatine membrane a fairly constant osmotic pressure in relation to concentration of the substance is obtained, and this, together with the appearances of the solutions in the ultra-microscope, leads to the conclusion that haemoglobin in water exists in a state of true solution (Waymouth Reid).

well as those which are developed in any serum, as the result of an injection of blood corpuscles, are or are not truly specific, there is a divergence of opinion, but a certain amount of evidence exists to show that the haemolysins are largely specific, and in this conform to the specific features of the various precipitins, bacteriolysins, and agglutinins.

The chief results of some experiments which I have carried out on the haemolytic action of frog's serum on human blood are shown in the following tables. In obtaining the serum it is of the utmost importance that this is not contaminated with the secretion from the skin of the animal, as this is actively haemolytic.

TABLE I.

Tube	C.mm. of Frog-Serum, 24 hours old.	Human Blood, 76 c.mm. in 424 c.mm. of ·85 per cent. NaCl.	Kept for 24 hours at Temperature C.	Tubes shaken, centrifugalised, and laked fluid estimated by scale, and tubes arranged in that order	Amount of Laking, the maximum reckoned as 100.
1	7·5	·5 c.c.	0° to 1°	Tube 10	112
2	8	·5 c.c.	0° ,, 1°	,, 11	110
3	7·5	·5 c.c.	0° ,, 1°	,, 9	110
4	16	·5 c.c.	0° ,, 1°	,, 4	95
5	0	·5 c.c.	0° ,, 1°	,, 8	72
6	8	·5 c.c.	0° ,, 1°	,, 6	70
7	0	·5 c.c.	16·8°	,, 3	50
8	5	·5 c.c.	16·8°	,, 2	40
9	10	·5 c.c.	16·8°	,, 1	40
10	15	·5 c.c.	16·8°	,, 5	0
11	20	·5 c.c.	16·8°	,, 7	0

From this table, which embodies the results of one of six similar experiments, it appears that:—

1. The serum of the frog is powerfully lytic for human blood corpuscles at temperatures 0° to 16·8° C.

2. Minute amounts of serum are efficient.

3. A large amount of serum lakes more corpuscles than a smaller quantity at a low temperature.

4. This difference is not so marked at 16·8° C.

TABLE II.

Tube	C.mm. Frog-Serum.	Human Blood, 70 c.mm. in 430 c.mm. of ·9 per cent. NaCl.	24 hours at Temperature C.	Result.
1	5	·5 c.c.	16°	Laked.
2	5	·5 c.c.	16°	Laked.
3	Heated to 55° C., ½-hour	·5 c.c.	16°	Unlaked.
4	Heated to 55° C., ½-hour	·5 c.c.	16°	Unlaked.
5	5	·5 c.c.	16°	Laked.
6	0	·5 c.c.	16°	Unlaked.
7	5	·5 c.c.	0°	Slight laking.
8	0	·5 c.c.	0°	Unlaked.

This table shows :—

1. That the haemolytic action is destroyed by heating to 55° C. for half an hour.

2. Slight laking occurs at very low temperatures.

TABLE III.

Tube	Frog's Serum in c.mm.	Frog's Plasma in c.mm.	5 per cent. of defibrinated Human Blood Corpuscles in ·9 per cent. NaCl.	Temperature for 20 hours.	Result.
1	5	...	·5 c.c.	21°	Good laking.
2	5	...	·5 c.c.	21°	Good laking.
3	...	5	·5 c.c.	21°	Good laking.
4	...	5	·5 c.c.	21°	Good laking.
5	0	0	·5 c.c.	21°	Unlaked.
6	0	0	·5 c.c.	21°	Suspicion of laking.
7	...	5	·5 c.c.	0° to 1°	Laked.
8	...	5	·5 c.c.	0° ,, 1°	Laked.
9	5	...	·5 c.c.	0° ,, 1°	Laked.
10	5	...	·5 c.c.	0° ,, 1°	Laked.
11	0	0	·5 c.c.	0° ,, 1°	Unlaked.

From this table it would appear :—

1. That both serum and plasma lake equally well, though naturally at a temperature of 21° the plasma soon becomes serum, but probably does not at 0° to 1° C.

2. Both serum and plasma lake human blood at 0° to 1° C.

The haemolytic action of plasma and serum in other instances has been shown to be similar (A. W. Hewlett, Bellei, Dömeny).

TABLE IV.

Tube	C.mm. Frog-Serum.	5 per cent. of defibrinated Human Blood in ·85 per cent. NaCl.	Temperature for 69 hours.	Amount of saturated $(NH_4)_2SO_4$.	Result.
1	0	·5 c.c.	26° to 30°	...	A suspicion of laking.
2	10	·5 c.c.	26° ,, 30°	...	Good laking.
3	5	·5 c.c.	26° ,, 30°	...	Marked laking.
4	15	·5 c.c.	26° ,, 30°	...	Less than 2.
5	20	·5 c.c.	26° ,, 30°	...	Less than 4, but quite obvious.
6	20	·5 c.c.	26° ,, 30°	10 c.mm.	Unlaked.
7	20	·5 c.c.	26° ,, 30°	5 c.mm.	Unlaked.
8	0	·5 c.c.	26° ,, 30°	...	Just like Tube 1.
9	20	·5 c.c.	26° ,, 30°	5 c.mm.	Unlaked.

From this table it would appear:—1. That laking by frog's serum is set aside by the presence of neutral salts. 2. That for some of the corpuscles ·85 per cent. NaCl was slightly hypotonic.

TABLE V.

Tube	50 c.mm. Human Blood in 1·5 c.c. of ·85 NaCl.	Material added.	Temperature for 69 hours.	Result.
1	·5 c.c.	5 c.mm. frog-serum . . .	0° to 3·5° C.	Laked.
2	·5 c.c.	Nothing	0° ,, 3·5° C.	Unlaked.
3	·5 c.c.	Flake of dried serum . . .	0° ,, 3·5° C.	Laked.
4	·5 c.c.	Flake of dried serum . . .	0° ,, 3·5° C.	Laked.
5	·5 c.c.	Frog's skin well washed with sterile ·6 per cent. NaCl	0° ,, 3·5° C.	Laked.
6	·5 c.c.	Do. do.	0° ,, 3·5° C.	Laked.
7	·5 c.c.	Boiled frog's skin . . .	0° ,, 3·5° C.	Unlaked.
8	·5 c.c.	Boiled frog's skin . . .	0° ,, 3·5° C.	Unlaked.
9	·5 c.c.	Nothing	0° ,, 3·5° C.	Unlaked.

From this table it is evident that:—

1. Dried serum preserves its haemolytic power at least for one week.

2. The skin of the frog possesses a haemolytic action on human blood corpuscles.

3. The laking occurs at temperatures 0° to 3·5°.

TABLE VI.

Tube	5 per cent. defibrinated Blood in ·85 per cent. Salt.	Frog's Serum.	C.mm. of saturated NaCl.	Temperature for 65 hours.	Result.
1	·5 c.c.	50 c.mm.	50	0° to 2°	Slight laking.
2	·5 c.c.	50 c.mm.	40	0° „ 2°	Slight „
3	·5 c.c.	50 c.mm.	30	0° „ 2°	More „
4	·5 c.c.	50 c.mm.	20	0° „ 2°	Laked.
5	·5 c.c.	50 c.mm.	100	0° „ 2°	Unlaked.
6	·5 c.c.	50 c.mm.	250	0° „ 2°	Unlaked.
7	·5 c.c.	50 c.mm.	0	0° „ 2°	Laked.
8	·5 c.c.	50 c.mm.	100	0° „ 2°	Broken tube.
9	·5 c.c.	50 c.mm.	250	0° „ 2°	Unlaked.
10	·5 c.c.	50 c.mm.	0	0° „ 2°	Laked.

That the presence of salt in certain proportions hinders or abolishes the haemolytic action of frog's serum on human blood, is shown by Table VI.

Investigations into the mode of action of those haemolysins which can be artificially produced in sera have engaged the attention of some of the most distinguished observers, and the study of their effect on red corpuscles has had a marked influence on that conception as to natural and acquired immunity against infection which was advanced by Ehrlich some six years ago; and whatever may be the ultimate fate of Ehrlich's theory of immunity, it has undoubtedly exerted a profound influence on many branches of research.

The only test for a haemolysin—for though probably a proteid, its real nature is a matter of conjecture—is that which is given by its behaviour towards certain red corpuscles; for while some haemolysins can only lake the corpuscles of a definite species of animal, so that the blood corpuscles of a guinea-pig mixed with these of another animal can be distinguished by treatment of the mixture with a haemolysin, others, such as those in the serum of eels, lampreys, or frogs, will dissolve the haemoglobin out of the discs of many species. A haemolysin is

therefore a definite toxic substance for a specific body, the red corpuscle. All haemolysins are destroyed by a temperature of 55° C. maintained for half an hour. Toxins which act upon the nervous system, such as tetanospasmin in the neurotoxin of cobra-venom or enhydrina-venom, are destroyed at higher temperatures (100° C.), while such ferments as pepsin, lactase, and others, the metabolites of cells, are rendered inactive at 65° C. A haemolysin retains its activity for a variable time when the serum is dried, and in this respect resembles snake-venoms and most enzymes.

Bordet's researches on haemolysins first showed the mechanism by which all sorts of foreign cells or proteids introduced into the living body are destroyed. The necessary agents are cytases[1] (complements or alexines), which are probably the products of leucocytes, and these can only act when a second body or amboceptor anchors them to the object of attack. Amboceptors are to be regarded as specific bodies which can attach themselves even at a temperature of 0° C. to the cell that is to be destroyed. The whole process can be studied *in vitro*, and a haemolytic or bacteriolytic serum loses its power by heating to 55° C. for half an hour, since this destroys the cytase; but since all sera contain cytases, the heated serum can be activated, and regain its haemolytic power by the addition of unheated serum from any normal animal of the same or an allied species. Haemolysis by cytolysins, produced by an injection of heterogeneous blood, therefore occurs only with the co-operation of three bodies, the corpuscles, amboceptor, and cytase (alexine).[2]

[1] The term alexine was introduced by Hans Buchner in 1891. An alexine or defensive proteid (Hankin) was subsequently shown to be a complex of two substances —true alexine, or complement, and amboceptor. Cytase is a term originally employed by Metchnikoff. He considers this cell-destroying ferment (?) as identical with Buchner's alexine. I have followed later French writers, who use cytase as a synonym for true alexine or complement.

[2] Haemolysis by venom also requires the co-operation of three bodies, and venom-haemolysis is of the nature of an amboceptor *plus* complement action. The venom only contains the former, the latter is supplied by the cells and fluids in which the cells are suspended.—(Flexner and Teruyo Noguchi.) Natural sera, *e.g.*, those of the dog, ox, goat, or rabbit, all of which haemolyse guinea pig's blood, probably act in the same way. Lecithin (an intracellular complement), according to Kyes, may act as complement for red corpuscles and cobra-venom.

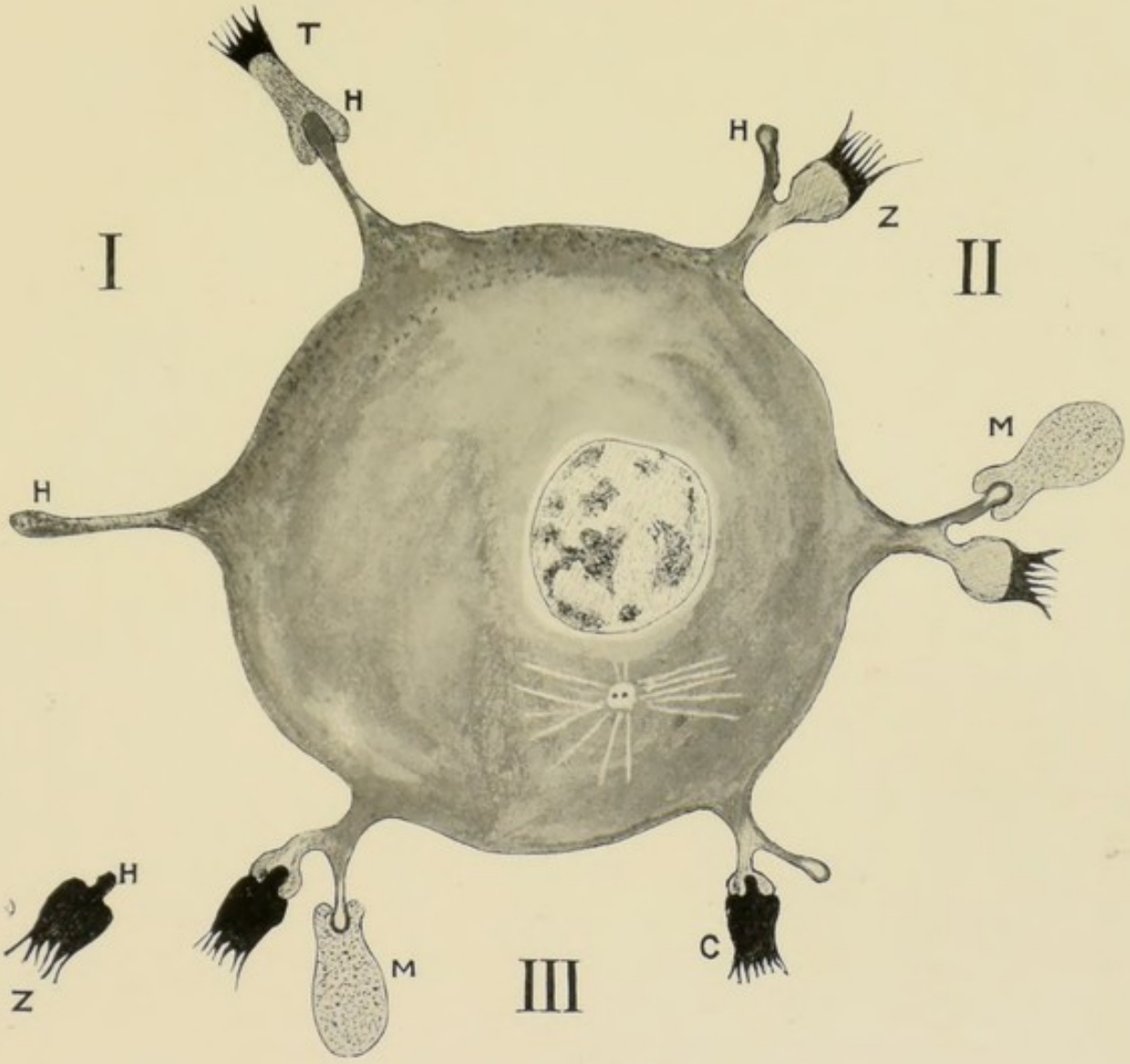


DIAGRAM OF A CELL, SHOWING THE PERIPHERY FURNISHED WITH THREE DIFFERENT ORDERS OF RECEPTORS (CONSTRUCTED FROM EHRLICH'S FIGURES).

Receptor I.—The haptophore complex (H) shows the fixation of a toxine molecule with its toxophore (T) to the receptor. A detached unsatisfied receptor may become an antitoxine molecule or an antiferment molecule.

Receptor II.—This shows the haptophore (H) and zymophore group (Z), also the fixation of a proteid by a receptor. Detached receptors yield agglutinins or precipitins.

Receptor III.—To this class belong the haemolysins; each consists of a cytase (C (alexine or complement), which has a haptophore (H) and zymotoxic group (Z). The receptor detached from the cell and without a cytase is an amboceptor.

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This theory of haemolytic action, which is a fragment of the much larger one of immunity, has been in part built up from the facts just detailed. Further, the nutrition of a cell, at any rate as far as its proteid is concerned, is a normal process of fixation which is paralleled by an abnormal, similar process which is seen on the introduction into the organism of foreign proteid, such as red corpuscles, bacterial toxins, or blood proteids. The response of the organism to an invasion by these substances is the development of specific anti-bodies or cytotoxins, antitoxines, or precipitins.

These are not cell-products or metabolites discharged by living cells, but are complicated particles which become detached from the surfaces of fixed somatic cells. Like metabolites, sooner or later they appear in the blood plasma, and confer on that fluid for a time specific characters, in virtue of which it possesses haemolysins, assuming that red corpuscles were the proteids introduced. If substances such as alkaloids or salts are introduced into the organism, these, unlike proteids, do not become fixed by the cell, since they are not of direct nutritional value.

The protoplasm of a living cell, according to Ehrlich's view, must be looked upon as a vital complex of molecules, the centre of which differs from the periphery in that the latter is furnished with side-chain molecules. The surface of the cell, which is the part by which the whole cell is related to its medium, is provided with a receptor apparatus, and any member of this apparatus may either have fixed some proteid, when that receptor is satisfied, or may be free, that is ready to fix, or may be detached from the cell and float freely in the surrounding medium. The diagram on Plate I. shows the three orders of receptors which on Ehrlich's theory of immunity may be conceived to exist in large numbers at the surface of the cells of an organism.

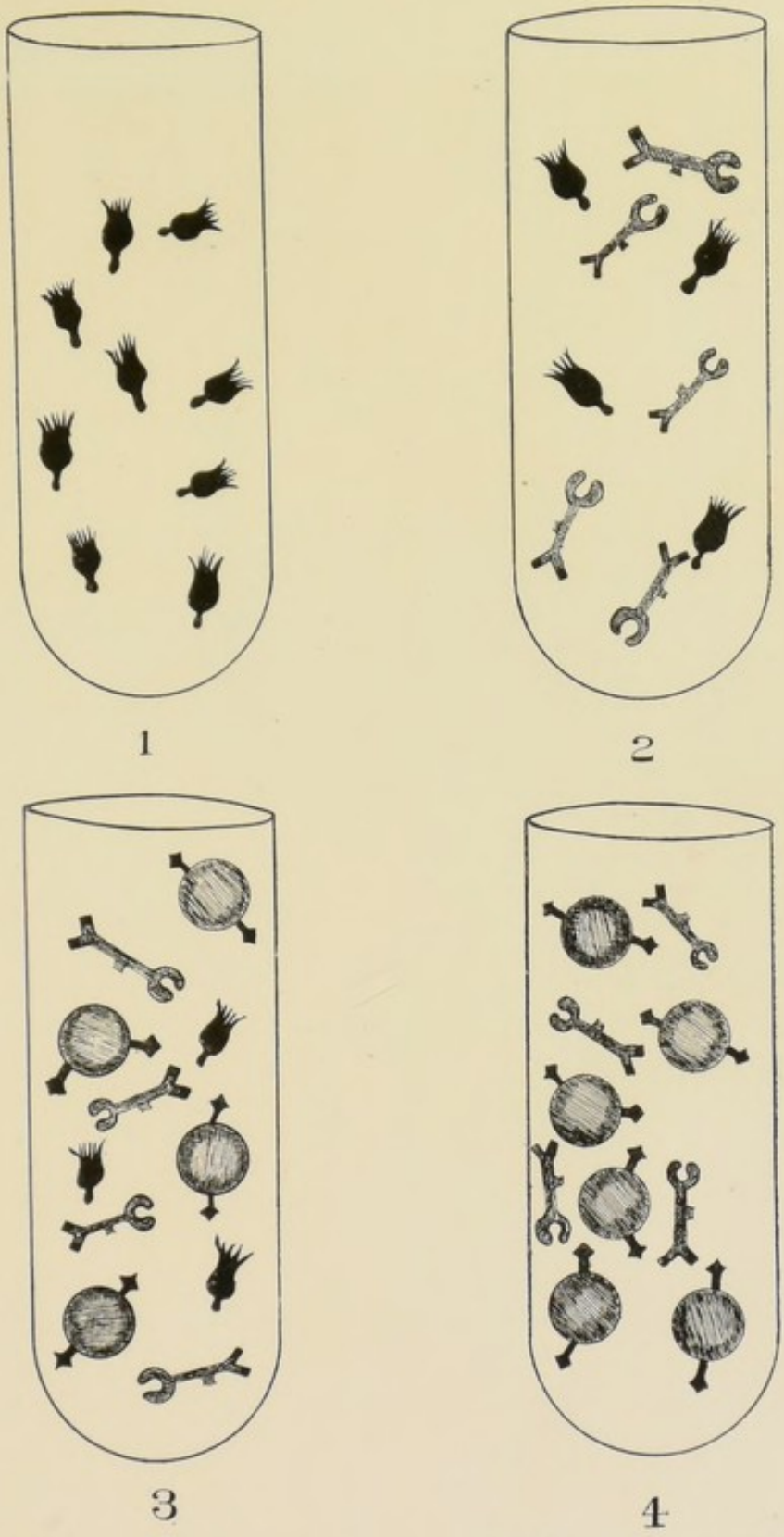
A serum originally non-haemolytic becomes so by the detachment of receptors of the third order. When present in sera their attack upon the red corpuscles is comparable to their fixation of these, when, as foreign bodies, they are introduced into the organism, and destroyed by the cytase after this has attached itself to the haptophore group of the receptor. The

corpuscles on their part also possess receptors, by means of which they can become fixed to the receptors of the cell.

The entire explanation of haemolysis on Ehrlich's theory can best be understood by describing the sequence of events in one of his most ingenious experiments, which is easy to follow if we remember that the cytase is the essential lytic agent, which is powerless to haemolyse unless joined to the red corpuscle by an amboceptor, while the latter alone is incapable of directly haemolysing the disc. The experiment is explained diagrammatically in the accompanying figures (Plates II., III.).

According to Ehrlich, the haemolytic action of such sera as those of the frog or eel are comparable in their action to artificial sera.

Fixed cells of the body, it would seem, may also either lose existing receptors or develop new ones; thus Kossel discovered that during the immunisation of rabbits with eel serum the corpuscles of this animal acquired a great resistance towards this toxic substance, a fact which may be explained by a loss of receptors by the corpuscles.



DIAGRAMMATIC REPRESENTATION OF THE FIRST STAGES OF AN EXPERIMENT BY EHRLICH ON THE HAEMOLYSIS OF SHEEP'S BLOOD BY GOAT'S SERUM.

1. Normal serum of a goat. Cytase (alexine) is a constant constituent.

2. Immune serum of a goat; that is, the serum of the animal after injections of sheep's red corpuscles. Amboceptors (immune bodies) are developed in this, being tne detached receptors of fixed body cells.

3. Mixture of sheep's red corpuscles with receptors or haptins and immune goat's serum. This mixture would become haemolysed if left for some hours; it is, however, heated for fifteen minutes to 55° C.

4. Condition after heating; the cytase has been destroyed. This mixture is kept at 37° C. for fifteen minutes.

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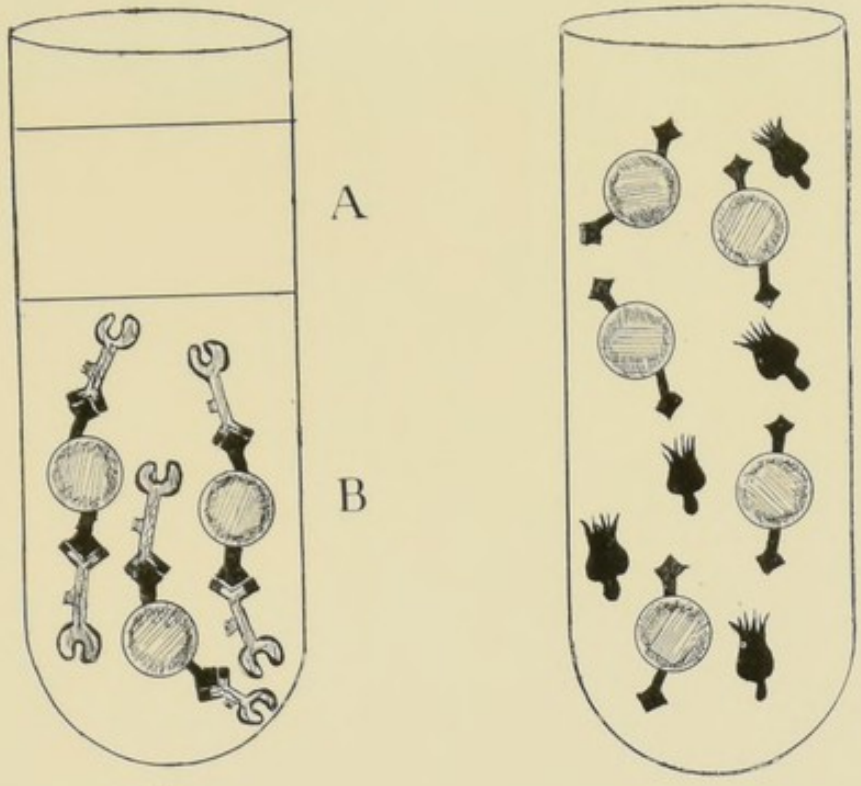

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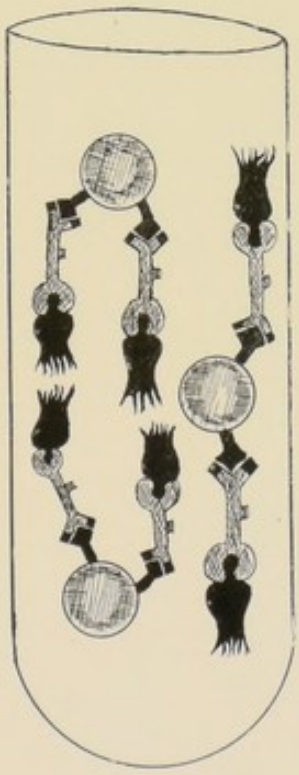

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SECOND PART OF THE SAME EXPERIMENT REPRESENTED DIAGRAMMATICALLY.

5. Tube 4 after this has been centrifugalised. A, cell-free liquid ; B, deposit.

6. Supernatant liquid A *plus* normal goat's serum *plus* sheep's red corpuscles. There is no haemolysis.

7. Deposit B *plus* normal goat's serum. Haemolysis occurs.

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LECTURE IV

THE WHITE CORPUSCLES OF THE BLOOD

IT will be obvious to those of you who recognise the extensive amount of work which has been published on the nature, relationships, origin, and functions of the leucocytes, that it will only be possible within the limits of a lecture to briefly indicate the present state of our knowledge. It is well known how great an impulse was given to haematology by Ehrlich's discovery of the methods of heat-fixation of blood-films and the differential staining of these with aniline dyes, a discovery which was made in the same year, 1878, that Weigert demonstrated his methods for staining bacteria in tissues by the use of such aniline dyes as methyl violet and fuchsin. The following table may serve as a short introduction to the subject of the present lecture :—

1773. HEWSON.—Discovery of the white corpuscles of human blood.[1]

1845. VIRCHOW.—Recognition post-mortem of "weisses Blut" (leukaemia).

1846. WHARTON JONES.—Recognition of granular leucocytes,

[1] "The secretion of lymphatic glands is a white mucus-like fluid . . . if we dilute it with a solution of Glauber's salts in water, or with serum . . . with a lens of $\frac{1}{25}$ of an inch focus, we observe numberless small white solid particles resembling those central particles found in the vesicles of the blood (pp. 66, 67) . . . we have proved that vast numbers of central particles made by the thymus are poured into the blood-vessels through the thoracic duct (p. 133), and if we examine the blood attentively we see them often floating in it." The exact date of this paper is uncertain. Hewson died in 1774, at the age of thirty-five, and the volume which contains the above quotation was edited by Magnus Falconer, and published by Longmans in 1777.

and description of the amoeboid movement of white corpuscles in the blood of the skate.

1850. DAVAINE.—Discovery of amoeboid movement in human leucocytes.

1843. W. ADDISON.
1846. A. WALLER.
1867. J. COHNHEIM. } Discovery of diapedesis.[1]

1863. v. RECKLINGHAUSEN showed that pus cells are amoeboid, and can ingest finely divided material.

1865. MAX SCHULTZE.—Classification of human leucocytes.

1878. EHRLICH.—Specific staining of granular and non-granular leucocytes.

1882-1883. METCHNIKOFF.—Phagocytic behaviour of embryonic mesoderm cells in the larvae of Echinoderms (Bipinnaria), demonstrated to Virchow at Messina. Discovery of phagocytic blood-cells in Daphniae infected with Monospora infestans.

The comparatively small number of white corpuscles in the blood is quite compensated for by the important part which they play in physiological and pathological processes. In the adult an average number of 7680 per c.mm. was obtained by Rieder from a review of the existing literature, 5000 to 6000 (E. Grawitz, Da Costa) or 6000 to 9000 (Türk) is generally considered as roughly indicating the physiological variation. The ratio of red to white is 1 : 600, and the ratio 1 : 300 given in many books is probably too high (Jolly, Malassez).

In newly born children there is a definite leucocytosis during the first few days, but apart from this, neither age nor sex modifies the absolute number of white corpuscles. Fehrsen confirms the work of previous observers, and shows in a series of forty cases of newly born (children, the counts being done up to the twentieth hour after birth) that the average number per c.mm. is 18,400 and the highest 32,500. Whether the weight of the child is going up or down, by the tenth day there is still, as a rule, a distinct increase above the normal number. Had Fehrsen's excellent paper also contained data as to the

[1] See notes in the References to literature of this lecture.

temperature of the children, it might have explained some of the high numbers which are occasionally seen a few days after birth. I find that a selected quarter of his cases gives an average of 18,270 per c.mm. on the tenth day. The cause or causes of this leucocytosis in the first weeks of life is unknown, but from a study of the papers by Carstangen, Fehrsen, and Jolly, it appears that during the first twenty-four hours the increase is due chiefly to polymorphonuclear leucocytes, but after this period to lymphocytes and large mononuclear cells.

A satisfactory classification of leucocytes, even assuming that such exists, is only possible by studies on stained films, in which particular attention is devoted to the granulations of the cytoplasm rather than to the structure of the nucleus. To the actual shape of the nucleus I attach less importance than to the amount and disposition of the chromatin, which varies considerably both in nucleated red and white corpuscles. A young nucleus in Pappenheim's view is round, pale, and poor in chromatin, or amblychromatic, and in all kinds of leucocytes the process of ageing takes place by the round nucleus first becoming lobed and then polymorphic, while its increased richness in chromatin is evidenced by more intense staining of the trachychromatic nucleus. For example, large lymphocytes may show polymorphic nuclei such as are figured in Rieder's Atlas and often observed in leukaemic blood (Rieder's cells). Our knowledge of nuclear chromatin in the leucocytes of normal and pathological blood is most imperfect, but that some nuclei such as those of Türk's cells are entirely free from chromatin is beyond dispute. Since the aspect of leucocytes, both those with and those without granules, varies considerably according to the methods employed for fixation and staining —for leucocytes only stain as they are dying, and take up no colouring matter in solution while alive (Rosin and Bibergeil)— the nomenclature of these cells and the systems of grouping them so as to exhibit their relationships is in some confusion, and not infrequently when some general idea of the varieties of white corpuscles has been obtained, this becomes obscured by discussions largely of theoretical interest concerning the physical or chemical action of simple or mixed dyes; such for

example as the still disputed point whether a mixture of the two rosaniline salts fuchsin S. and methyl-green with the acid azo-derivative orange G. does or does not form a neutral combination.

The table on page 65 may be of interest as indicating the growth of our knowledge of the morphology of the blood, especially that of the white corpuscles.

A classification of leucocytes which could be universally accepted would be one that was founded on their origin. Unfortunately, the sites and modes of origin vary in different classes of vertebrates, and it is obvious that the extirpation of such regions of blood-formation as the bone-marrow or lymphatic glands is beyond the range of experiment. We are therefore compelled to draw our inferences as to the origin of leucocytes from clinical and morbid anatomical researches, though it must be recognised that the purely morphological study of leucocytes is an insufficient method for determining their origin. It is, however, known that in the human embryo of two and a half months, excepting a few cells identified as lymphocytes, the blood is free from leucocytes ; only nucleated and non-nucleated spherical red corpuscles which vary in size and in the amount of nuclear chromatin are found in that fluid. In the marrow, spleen, and blood, non-granular leucocytes precede the granular forms. Neutrophil myelocytes and eosinophil myclocytes are found from the fourth month onwards. The leucocytes figured by Engel in the blood of the human embryo at four and a half months comprise neutrophil myelocytes, eosinophil myclocytes, polymorphonuclear cells, small lymphocytes, and non-granular cells with a tri-lobed nucleus. Kölliker, in 1879, expressed the opinion that leucocytes arose in the thymus from the original hypoblastic cells of this organ ; Gulland, in 1891, stated that the first leucocytes to be seen in the embryo appeared in the neighbourhood of the thymus ; and four years ago, Beard's researches on elasmobranch embryoes confirmed these views—that the original site of leucocyte formation was in the hypoblastic rudiments of the thymus, whence the cells wander all over the body and into the blood. This occurs at an early period when there are no leucocytes in

MORPHOLOGICAL ELEMENTS OF THE BLOOD.

J. MÜLLER . .	1839	red bodies	lymph and chyle corpuscles			...	...	...
WHARTON JONES.	1846	...	nucleated cells		finely granular cells	...	coarsely granular cells	...
MAX SCHULTZE .	1865	...	small hyaline	large hyaline	finely granular	...	coarsely granular	...
HAYEM . . .	1877	...	small round nucleated non-amoeboid cells	larger amoeboid cells	finely granular amoeboid	...	cells with highly refracting granules	Haematoblasts.
EHRLICH . .	1877	...	lymphocytes	...	neutrophil cell	basophil cell	eosinophil cell	...
METCHNIKOFF .	1892	...	lymphocyte	mononuclear leucocyte	polynuclear cell	...	eosinophil cell	...
SHERRINGTON (Cat and Dog) .	1893	chromocytes nucleated and non-nucleated.	small hyaline	large hyaline	finely granular	...	eosinophil	ppt. (?)
KANTHACK and HARDY	1894	...	lymphocyte	hyaline	finely granular oxyphil	finely granular basophil	eosinophil	...
E. GRAWITZ. .	1896	...	*Type I.* mononuclear cells		*Type II.* large cells with bowed or horse-shoe nucleus		*Type III.* eosinophil	Blood-platelets.
			small lymphocytes	large lymphocytes	young forms with faint granulations			...
					older forms with marked granulations or neutrophil polymorphonuclears.			

the blood or coelomic cavity, the spleen is undeveloped, and no lymphoid tissue exists in any part of the embryo. According to Beard, these emigrant cells from the thymus are the source of all the lymphoid structures in the body.

The relation of the human spleen to the formation of leucocytes has always been a subject of controversy. If a recent article by Litten, and one by Ehrlich and Lazarus, in Nothnagel's *Handbuch der specielle Pathologie und Therapie* is consulted, it will be noticed that the former regards the spleen as a region where an initial development of leucocytes takes place, while the latter authors regard that organ as entirely functionless in this respect. Four recorded cases exist of congenital absence of the spleen in man. Its non-development is probably due to an altered blood-supply during foetal life. In Sternberg's case, the blood had been examined during life and found to be normal. When death occurred at seventy-five years of age, the autopsy showed no other abnormality except congenital absence of this organ. There was no enlargement of lymphatic glands; the bone-marrow was normal, and from its aspect not exceptionally active. We may therefore infer that the spleen is not essential for the production of haemic leucocytes. Extirpation of this organ in animals and man is followed within four to eight weeks by a moderate lymphocytosis, and months later by some degree of eosinophilia. Most observers consider that a certain number of large mononuclear leucocytes originate in the spleen.

Most workers have, with slight modifications, accepted the classification proposed by Ehrlich, founded on an examination of permanent stained specimens. In normal blood six and possibly seven well-defined varieties of leucocytes can be distinguished, and the percentage of these forms is fairly constant.

Normal human blood of an adult contains the following varieties, and relative percentage of leucocytes:—

1. Cells with abundant neutrophil granulations or polymorphonuclears, 70 per cent.
2. With few neutrophil granules (transitionals), 1 per cent.
3. With eosinophil granulations, 3 per cent.
4. With basophil granulations, ·5 per cent.

5. Without granulations (lymphocytes), (*a*) small forms, (*b*) large forms, 23 per cent.

6. Without granules (large mononuclear cells), 2 per cent.

The number, 70 per cent. (Ehrlich), given above for the polymorphonuclear cells is probably higher than that which is found in many individuals. Boycott states that the average number is about 57 per cent., while Phear gives a still lower figure, 54 per cent. This observer, from a large series of observations, considers that the following table gives the approximate percentages of the varieties of leucocytes :—

Mononuclear leucocytes (include groups 2, 5, and 6 of the list given above), 43·4 per cent.
Polymorphonuclears, 54·0 per cent.
Eosinophils, 1·9 per cent.
Mast cells (group 4 of list given above), ·7 per cent.

Any statements as to the kinds of leucocytes are best expressed in absolute numbers per c.mm. ; in this volume of blood an average count of 7500 leucocytes will show :

5000 polymorphonuclears,
2000 lymphocytes,
350 large mononuclears,
150 eosinophils.

The ratio of polymorphonuclears to lymphocytes is therefore for the adult about 3 : 1 or 5 : 2. This ratio does not hold for children ; in the first year the ratio of P : L = 1 : 3 and subsequently 1 : 2, and of these lymphocytes 5 to 10 per cent. of the total are large non-granular cells with a round or lobed nucleus.

The excellent cytological work of Gulland led him, some ten years ago, to the idea regarded by Ehrlich and his school as a retrograde step, that the lymphocyte is the ancestral non-granular form of all varieties of leucocytes. The certain relationships of the polymorphonuclear and eosinophil to slightly granular or non-granular cells in the bone-marrow is, however, no longer a matter of dispute, and his recent views are that leucocytes may be grouped in four series. In each series the cells undergo a cycle of changes, in the sense that any cell may

grow in size, its nucleus alter in consequence of amoeboid movement, and the cytoplasm of the cell body become more and more granular. He considers—and I am of the same opinion—that the granules are not reserve materials nor metabolic products for secretion, neither is the granular leucocyte in any sense comparable to a unicellular gland (Ehrlich). The granules are plasmatic not paraplastic, if we use v. Kupffer's nomenclature, and belong indeed to the spongioplasm (Leydig), not to the hyaloplasm. J. Arnold and Hesse hold somewhat similar views. A framework or system of plasmosomes forms the granular structure of the cytoplasm, and from the highly variable microchemical reactions of the granules within the same leucocyte Arnold considers that some granules carry iron and others fat. A neutrophil granule may become eosinophil by the taking up of haemoglobin (St. Klein). That any granular cell always contains the same kind of granules, is not in accord with facts. A cell from the human marrow may, and often does, contain granules which vary in their affinities for dyes, and neutrophil granules appear oxyphil by certain methods, or even take up methylene blue (Hirschfeld, Schur).

The leucocytes found in normal human blood may be arranged in four groups or series:—

1. LYMPHOCYTE SERIES.
   - Small lymphocytes.
   - Large lymphocytes.
   - Large mononuclears (Ehrlich).
   - Transitional forms with a variable degree of granulation.
2. NEUTROPHIL SERIES.
   - Non-granular and granular cells.
3. EOSINOPHIL SERIES.
   - Non-granular and granular cells.
4. BASOPHIL SERIES.
   - Non-granular and granular cells.

All the forms of the first group may occur in normal blood, but only the terminal stages of the others; the initial stage of all

is a non-granular cell. Lymphoid tissue, at any rate that in the lymphatic glands as well as the lymph, shows every variety of the lymphocyte series. The macrophages found on the walls of the lymph sinuses are probably swollen and bloated mononuclear cells.

For any given stained leucocyte of normal blood it is easy to assign differential features, but with reference to the origin, relationship, duration of life, and destiny of any cell, we often find a statement of opinions rather than a statements of facts; and even when the latter are beyond question, the conclusions drawn from them are generally unconvincing. It has not been possible to study the life-history of any leucocyte, and therefore any conceptions as to the relations of one form to another, or the phases through which any cell may pass, must be received with a critical mind, and until some method is devised by means of which the life-histories of the various cells of the blood can be studied with the same facility as those of bacteria or protozoa, much of what is taught and stated in books as to the relations of one form of leucocyte to another, must be regarded as the expression of views rather than the statement of an unquestionable truth.

The Lymphocyte Series

1. *The Lymphocytes.*—A cell resembling the human lymphocyte is found in nearly all animals (Meinertz). In the foetus non-granular cells can be recognised earlier than the eosinophils, and these again earlier than the neutrophil leucocytes. The smallest lymphocytes are about the diameter of a red corpuscle; the largest are double this size. In a thickly spread film of normal blood, the smaller forms exceed the larger, and I think that many large lymphocytes are crushed smaller ones. It is generally held that the small lymphocytes are young forms, but unfortunately this is only one view directly opposed by another which maintains that the older lymphocyte is the smaller of the two. There is really no convincing evidence in support of either opinion. I have never seen any definite amoeboid movements of a lymphocyte, but a limited amount of movement even for the smallest forms is described by Wlassow and Sepp.

Many observers do not regard the lymphocyte as capable of moving from place to place, and Hirschfeld, who has studied the behaviour of these cells on Deetjen's agar-medium, considers that they do change their shape but not their position.

The structure of the lymphocyte is best studied in the small uncrushed cell. The cytoplasm is not homogeneous, but composed of a densely reticulated non-granular basophil protoplasm disposed as a thin hull around a single nucleus, which generally stains less intensely than the protoplasm with a basic dye. Both pyronin (Pappenheim) and the Mylius' reaction display the protoplasm of lymphocytes in a manner that is almost specific. The nucleus often appears surrounded by a thin clear zone, and the protoplasmic edge is more distinct in the larger forms. These possess a protoplasm that at times is frayed out, and may show some degree of azurphil granulation with Romanowsky staining, so that recently the lymphocytes have been described as granular cells (Schridde). The nuclei of small cells stain more intensely than those of larger forms ; in the latter the shape of the nucleus may become irregular and even lobed, and one or more nucleoli are to be observed near its periphery. Even from these features it is not always easy to positively class a cell as a large lymphocyte. The shape and staining of the nucleus is an insufficient feature on which to decide. The large mononuclear cells which constitute the chief leucocytes in acute lymphatic leukaemia or chloroma, though they occur in variable numbers in other forms of leukaemia, do not appear to me to be the same nature as the larger lymphocytes. Indeed this is the opinion, as will be shown later, of other observers.

2. *Large mononuclear cells.* — These, though grouped here as members of the lymphocyte series, differ histologically from ordinary haemic lymphocytes. Mononuclear leucocytes (Ehrlich) are best recognised by the aspect of the nucleus. The cells are often twice or three times the size of a red corpuscle, and possess a large oval eccentric nucleus which stains comparatively feebly with basic dyes, while the relatively abundant protoplasm appears only faintly basophil. In size the nucleus equals about half the volume of the protoplasm, and is often found situated close against the edge of the cell,

and shows a vesicular appearance. Eosin gives the protoplasm a lilac tint, and the general aspect of the nucleus is best shown by methylene blue; the cell is amoeboid. The blood of children contains more of these cells than that of the adult. Apart from the fact that an excess of mononuclears is a constant blood change in malaria (L. Rogers), an increase is often met with in leukaemia and in acute septic conditions.

In Ehrlich's opinion these cells have no relationship whatever with lymphocytes, but though morphologically mononuclears markedly differ from lymphocytes, there are sufficient reasons, independent of their supposed places of origin, for considering them as members of the lymphocyte series.

3. *The transitional forms* are regarded as large mononuclear cells in the protoplasm of which a fine scanty neutrophil granulation has developed, the staining of the nucleus has improved, and the shape changed to that of a horse-shoe or hour-glass. This is a rare cell in normal blood, and difficult of recognition under any circumstances. The terminology of this cell is unfortunate. The "Uebergangsform" of German writers is the original and no better name, though it does indicate a view that in the blood and probably elsewhere the large mononuclear cells change by becoming granular into transitionals, and these, again, by a continuation of the change and alterations in shape and chromatin-content of the nucleus become fully developed polymorphonuclear neutrophil leucocytes (Ehrlich). This progressive development has never been witnessed, but is surmised to occur from a study of stained specimens; this may, indeed often does, give an impression that such a change might occur, but such an observation is not positive evidence. I have frequently seen both large mononuclears and cells indistinguishable from transitionals in lymphoid tissue, and the granulation of the cell is I believe a feature connected with its age rather than with its nutrition.

As to the origin of the cells of the lymphocyte series, everyone is agreed that lymphocytes are passed into the blood-stream by the lymphatics, or reach that fluid by an admixture of blood and lymph in haemolymph organs. Lymph-adenoid tissue (His) is widely distributed in the body, and the cells chiefly

present are identical with haemic lymphocytes. Active mitosis of somewhat larger mononuclear cells (lymphocytes) I have seen, after suitable fixation, particularly well in the villi, solitary follicles, and lymphatic glands. In the latter the germ-centres show fluctuating periods of activity (Flemming), the dividing cells are somewhat large lymphocytes ; each of these has a large clear nucleus in an abundant protoplasm, which stains strongly with basic dyes, a dense zone of small lymphocytes, the nuclei of which are in the resting state, surrounds each centre, a condition well seen in the cortex of the gland, in the tonsil, or solitary follicles. These latter structures, after mitotic activity, stand out as obvious, isolated swellings just beneath the endoderm of the gut. Human marrow contains lymphocytes—often a large number in the case of children—a fact of which I have satisfied myself by frequent observation.

The large mononuclear cells do not, according to Ehrlich and most German authorities, arise in the lymph glands, and their site of origin though doubtful is certainly not in these structures. The matter is one on which judgment may be suspended ; for my own part, I consider that cells similar to these do occur in lymphatic glands, and that macrophages are possibly the same kind of leucocyte. Both eosinophil and basophil cells have been described as existing also in these glands (Dominici), though the presence of such cells in these structures is, of course, no evidence at all as to their origin. The same objection applies, but with somewhat less force, to the mononuclear cells, and consequently the evidence which exists as to the place or places of origin of these is not absolutely conclusive. Possibly the bone-marrow (Ehrlich), lymphatic glands (Gulland), and the spleen (Türk), each contribute a small supply to the blood.

THE NEUTROPHIL SERIES

The polymorphonuclear leucocyte varies in size, the average diameter is 10 μ ; it is an amoeboid, actively phagocytic cell with fine granules, which are the nodal points or microsomes of a protoplasm which possesses a strong attraction for stains, as eosin aurantia or acid fuchsine. The granules take either a red

tint with eosin (oxyphil) or a violet red with tri-acid (neutrophil), but the varying colour of the granules is due in part to the solvent of these dyes, and even more to the method of fixation. With Jenner's stain the granules must be described as oxyphil.

Most observers regard the granules of the polymorphonuclears as neutrophil, but the granules may appear basophil, oxyphil, or colourless in the human leucocyte according to the choice of method. The granules do not stain at all if a trace of alkali is added to Jenner's stain, but those of the eosinophil cells still stain pink (Scott). Marino states that all the granules of man or monkey are oxyphil or basophil, and never neutrophil, but though the size of the granules is more distinctive than their behaviour to dyes, it is quite easy to pick out the granules of the eosinophil with eosin, while those of the polymorphonuclear remain unstained, and therefore, for this among other reasons we may consider that the granules of these two leucocytes are specifically different. The single nucleus is rich in chromatin and formed of several lobes, joined by fine chromatin strands. The shape may resemble a V, U, Z, or E. The wreathed appearance, figured by Sherrington, for the neutrophil of the dog in oxalated blood incubated for forty-eight hours is not common, though I have once noticed a similar appearance in almost every leucocyte in a case of septic leucocytosis. Nucleoli are absent in the nuclei of polymorphonuclear leucocytes (Rosin and Bibergeil).

The staining features of the cytoplasm are inferior to the shape of the nucleus as a means of identification. In a fresh preparation, the granules of a living cell do not show the Brownian movement so common in dead polymorphonuclears, and which is a feature of many pus cells. A basophil reaction of all or some granules is stated to occur in normal blood (Simon), or to be of pathognomonic importance (Kolisch), but most observers consider this is due to faulty methods. The so-called glycogen or iodophil granules in the cell-protoplasm are not infrequent in degenerated cells.

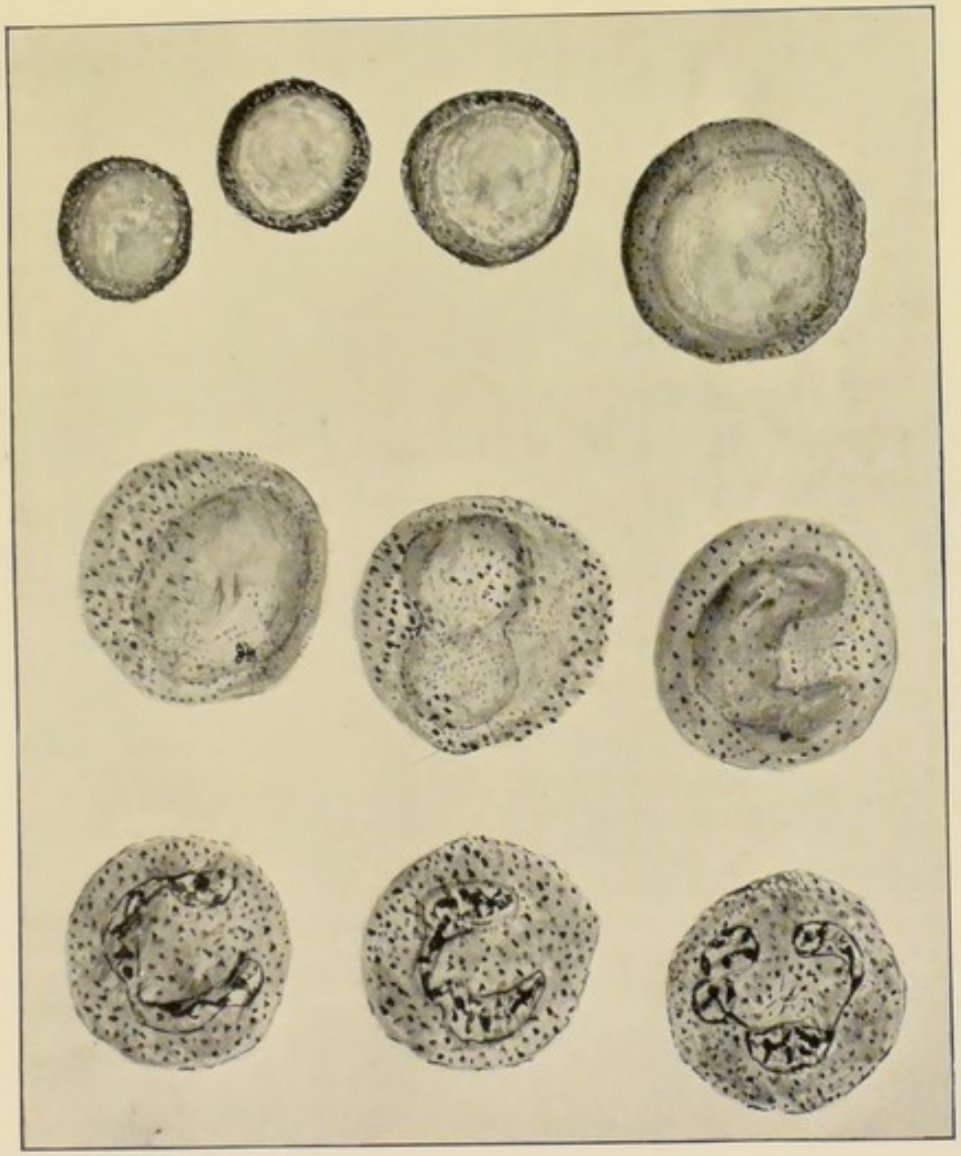
The origin of this cell is, beyond doubt, the bone-marrow. The leucoblastic mother-cells of the bone-marrow which become neutrophil myelocytes are non-granular cells with large ambly-

chromatic nuclei and a variable amount of faintly basophil cytoplasm. These mother-cells are considered by Pappenheim and Benda to be lymphocytes; Naegeli speaks of them as myeloblasts, Banti as hyaline marrow-cells, and M. Wolff as indifferent lymphoid cells, while Ehrlich regards them as mother-cells of the leucocytes which belong to the myeloid group; Grawitz speaks of them as "Stammzellen," and Türk as "lymphoid Stammzellen." These cells divide, and the daughter-cells, by division or transition, pass into mononuclear cells, which possess first a scanty and then a profuse neutrophil granular protoplasm (myelocytes of Ehrlich and Uthemann). The further transformation of this cell into the polymorphonuclear leucocyte also occurs normally in bone-marrow, and this terminal phase alone under normal conditions passes into the blood (Ehrlich, R. Muir, Dominici, Engel, and others). Plate IV. shows the generally accepted metamorphosis of an initial non-granular cell of the bone-marrow into a polymorphonuclear leucocyte. The cells of the early and middle stages may divide by karyokinesis.

The Eosinophil Series

The eosinophil leucocyte generally exceeds the polymorphonuclear in size; the granules are large, discrete, highly refracting, and give certain micro-chemical reactions which are distinctive, such as those for phosphorus, iron, and a colour change with vanillin and aldehyde (Weiss). The granules are oxyphil, since they stain deeply with eosin and other acid dyes. The cell is amoeboid, and to some extent phagocytic. The cell body is rarely vacuolated, a condition not uncommon in the neutrophil cell, and a feature possibly connected with its age, if we may rely on the behaviour of other cells as a guide for this opinion. The single nucleus contains less chromatin than that of a neutrophil, and its shape may be a crescent or trefoil. Human blood often appears to contain several varieties of these cells.

The origin of these cells, according to Ehrlich, is the bone-marrow. Pappenheim considers that an initial non-granular marrow cell with basophil protoplasm becomes transformed into



Transformation of a leucoblastic marrow-cell (stammzelle, lymphocyte, lymphoid cell) into a neutrophil myelocyte, which subsequently becomes a polymorphonuclear leucocyte.

[*To face page* 74.

an eosinophil myelocyte (H. F. Müller), and by a development of granules subsequently appears as the perfected eosinophil of the blood, though this cell is far more widely distributed in the coelomic fluids of the body. This relation of the marrow cell to the haemic cell would appear to have much probability, for the eosinophil granules in the leucocytes of the blood of the dog and rabbit are spheres, of the horse cuboids, and of the dog short rods, while the oxyphil myelocytes of each of these animals have granules which are of the same aspect and shape as those of the haemic cells (Sherrington).

The Basophil Series

The mast cell of normal human blood is always to be found in any film, provided that this is spread somewhat thickly and appropriately stained (Boycott). It is a rare cell in the blood, and I think most easily found two to three hours after the first meal of the day. This leucocyte varies in size; generally it is somewhat smaller than a neutrophil cell. The granules vary in number in different cells and in size in the same cell, but their average size equals that of the eosinophil granules. In fresh specimens the granules are practically invisible, and in this respect contrast with those of an eosinophil leucocyte. Mast-cell granules are soluble in water, watery stains, and solutions of acid dyes. In the opinion of some observers the granules are mucin (Williams). They do not stain at all with tri-acid, but do so metachromatically with basic dyes; that is, would appear violet with methylene blue. Polychrome methylene blue (Goldhorn), pyronin or dahlia (Westphal), display the granules admirably. They are well preserved, and appear a deep black with Türk's iodine and methylene blue stain. The lobed or tri-lobed nucleus stains much more feebly than the granules. This leucocyte is amoeboid (Gulland), an observation I have been unable to confirm for the mast cells of leukaemic blood. In connective tissue, mast cells are to be found, but they are rare in marrow except in conditions where they abound in the blood; but, as will be shown in a subsequent lecture, the mast cells of myelogenous leukaemia differ from those met with in normal

blood. As to their origin, it is surmised that basophil myelocytes may be their immediate antecedent cells. "We shall not err in deriving the haemic mast cell solely from bone-marrow, or in believing that their origin is not from the connective tissues, even when they are excessively increased there" (Ehrlich and Lazarus, *Die Anaemie*, 1901).

The following table, slightly modified from H. Hirschfeld, shows the varieties of leucocytes met with in some mammals. The granules are distinguished by tri-acid, or some modification of this stain, after heat-fixation at 120° C. Considered generally, the leucocytes of man exceed those of all other animals in size. It may be added that the reactions of the granules in many myelocytes resemble those of the blood of the animal: thus, neutrophil, eosinophil, basophil, for man; amphophil, eosinophil, basophil, for the rabbit; nigrosinophil for the guinea-pig.

TABLE SHOWING OCCURRENCE AND COLOUR-AFFINITIES OF THE GRANULES IN LEUCOCYTES OF DIFFERENT ANIMALS.

	Mononuclear Cells.			Polynuclear Cells.						
	Without Granules.	Basophile Cytoplasm.	Oxyphile Cytoplasm.	Without Granules.	Eosinophil.	Indulinophile.	Basophile.	Neutrophile.	Neutrophile.	Amphophile.
Man	+	+	...	...	+	...	+	+	...	...
Sheep	+	+	...	...	+	...	+	...	+	...
Goat	+	+	...	...	+	...	+	...	+	...
Ox	+	+	...	...	+	...	+	...	+	...
Pig	+	+	+	...	+	...	+	...	+	...
Horse	+	+	+	+?	...	...	+	+?	...	...
White Mouse	+	+	...	+	+	...	+	...	...	...
Rabbit	+	+	...	...	+	...	+	...	...	+
Guinea-pig	+	+	...	+	+	+	+	...	...	...
Dog	+	+	...	+	...	...	+	+?	...	...
Cat	+	+	...	+	...	...	+	...	...	+
Rat	+	+	+	...	+	...	+	...	+	...

A + mark indicates that the cell is present in blood.

It will be noticed that many animals possess non-granular polynuclear leucocytes. The neutrophil granules considered

by Ehrlich as distinctive for human-blood cells are stated by Hirschfeld to occur in human polymorphonuclears of a size, arrangement, and staining behaviour which is absolutely characteristic, and enables this cell to be easily differentiated from the homologous cells of other mammals. The basophil, lymphocyte, and coarsely granular oxyphil cell occur in all animals hitherto examined.

In pathological conditions, not only do the forms which have been described occur in altered relative and absolute numbers, so that a drop of blood may show a leucopenic or leucocytotic state, but the following leucocytes may make their appearance in circulating blood as well as other forms of cells, which it is often difficult to assign to any of the following groups:—

1. Myclocytes, in the sense that such cells normally occur in bone-marrow, and abnormally may appear in circulating blood.
   - (*a*) Free from granules (myeloblastic cell of Naegeli).
   - (*b*) With neutrophil granules. These are the "Markzellen" of Franz Mosler, or myclocytes of Ehrlich and Uthemann.
   - (*c*) With eosinophil granules. These are the eosinophil myclocytes of H. F. Müller.
   - (*d*) With basophil granules.
   - (*e*) With some basophil and some neutrophil granules in one cell.
2. Pseudo-leucocytes.
3. Mononuclear non-granular cells (Türk).
4. A cell described as a plasma cell (Weil).
5. Mononuclear non-granular cells, which are often identified as large lymphocytes. Many of these cells are non-granular myelocytes, and correspond closely in all their features to myeloblasts (Naegeli) or lymphoid marrow-cells (Türk).

Myclocytes are plump mononuclear cells which at an early stage of existence are probably non-granular, but which develop a variable quantity of fine or coarse granules, which collect in a small number at one pole of the cell, or the protoplasm may be so densely speckled that the existence of a nucleus can only be inferred. These cells are the distinctive feature of

mycloid as contrasted with lymphoid tissue, and whereas the latter is widely distributed in the body, the former is restricted to the interstices of bone. The leucoblastic tissue of bone-marrow may occur in distinct areas, and the evidence that is available, together with an extended examination of bone-marrow in health and disease, gives the idea that non-granular mononucleated cells are to be regarded as the brood-cells, "Stammzellen," or ancestral cells from which the neutrophil, basophil, eosinophil, and mixed granular forms are derived. This non-granular form therefore becomes developed into members of a neutrophil, eosinophil, and basophil series.

1. *Neutrophil myclocytes.*—These are occasional immigrants into normal blood, and may be seen in post-haemorrhagic anaemia (Lazarus, Engel). This cell is the one for which the term "myclocyte" is generally reserved; it is the myclocyte seen by H. F. Müller, Robin, and Cornil, and identified by Ehrlich and Uthemann as possessing the following characters, which are also well defined by Engel. The cells are generally larger than polymorphonuclear leucocytes: some may measure 26 μ in diameter (Sabrazès) or more than three times the size of the red disc; the nucleus of such a large cell is pale, and lies to one side of the very neutrophil granular cytoplasm. This large form is seen only in myelogenic leukaemia, and has been identified by some haematologists as Cornil's myelocyte. The large cells are always associated with smaller myelocytes, of the size or smaller than polynuclears. The single plump nucleus, which is poor in chromatin, almost fills the cell; it is as a rule spherical, but may be hour-glass shaped or indented. I consider the myelocyte is never motile, but Jolly describes both the neutrophil and eosinophil myclocytes of leukaemia as showing amoeboid movements. This question as to movement is of importance, especially as this property of the myclocyte is necessary if a myeloid leucocytosis is to be ranked with those other kinds, which are regarded as an active chemotactic emigration from bone-marrow. Though I have repeatedly observed leukaemic blood on the warm stage, I have never convinced myself that the myelocytes exhibited any movement at all. Even if they did, being quite familiar with the behaviour

of red corpuscles, which often show a spurious type of movement, I should hesitate to consider the change in shape as evidence that the cell was exhibiting amoeboid movements, or even alive. To come to a positive decision on this point is, however, difficult. Excellent observers have denied this property both to lymphocytes and myelocytes (Ehrlich, Grawitz, Rieder, Löwit, H. F. Müller, E. Neumann, Renaut). Even from Jolly's account, it is clear that the movements of myelocytes are minute, though in order to account for their emigration from the bone-marrow, in response to a positive chemotactic influence Ehrlich now considers that their capacity for movement is proved, and quite sufficient to explain the appearance of these cells in the blood. Even for lymphocytes, with the greatest care I cannot obtain any evidence of movement, and any irregularities in contour which the larger ones may show, are widely dissimilar from the behaviour of either the polymorphonuclear or eosinophil cells.

The neutrophil myelocytes appear in human embryonic marrow at the fourth to fifth month (Engel), and in blood during the seventh or eighth month. They occur in the blood during extra-embryonic life in the following pathological conditions :—

(*a*) Generally their large numbers characterise myelogenous leukaemia, but the percentage varies greatly from day to day, or even from hour to hour, and may entirely disappear from the blood, even though the disease progresses and terminates fatally.

(*b*) In septic and infectious diseases, particularly of children, as in diphtheria, where 3 to 16 per cent. have been recorded ; 4 per cent. of myelocytes augurs an unfavourable prognosis (Engel). Also in pneumonia, especially at the crisis (Türk), though as a general rule the leucocytosis diminishes at this period.

(*c*) In most forms of grave and persistent anaemia. In pseudo-leukaemica infantum (v. Jaksch). I have also seen myelocytes in cases of rickets, and once in Addison's disease.

(*d*) In confluent or haemorrhagic variola (Ferguson).

2. *Eosinophil myelocytes.* — These mononuclear cells, with large prominent oxyphil granules, approximate in size to the

neutrophil myelocytes, with which cells they are associated. Their granulation is generally denser and their size more constant. Occasionally mammoth examples are met with nearly four times the size of a red corpuscle. Eosinophil myclocytes form most conspicuous cells in myelogenous leukaemia, and may even form the largest percentage of leucocytes. In forms of leukaemia where few or no neutrophil myelocytes are found, the eosinophil myclocyte is very rare. This cell is a feature of myelaemia.

3. *Basophil myclocytes* are generally small mononuclear cells; the smallest may resemble a lymphocyte in size, and possess a single spherical nucleus with a zone of basophil granules. In larger cells, the amount and size of the granulations is variable; often this is collected at one pole of the cell. A satisfactory distinction between this myclocyte and a mast cell is often a matter of difficulty; no doubt it is at times described as a mast cell. Both this myclocyte and the mast cell may occur in myelogenic leukaemia; large numbers of the latter, 15 or even 40 per cent., often form a marked feature of this disease.

4. *Myclocytes with hetero-chromatic granules* form the last of the group of myclocytes. The granules are generally large ones, and the cell gives the impression that it is a transformed eosinophil myclocyte.

5. *Small neutrophil pseudo-leucocytes* (Ehrlich) or *pseudo-neutrophil lymphocytes* (Weil).—These are degenerated fragments of polymorphonuclear cells, a spherical fragment of a nucleus may have some half-dozen neutrophil granules adhering to it. These cells occur when rapid fragmentation of the neutrophiles occur in the blood-stream, as in confluent small-pox.

6. Türk's stimulation forms. Described as a mononuclear cell by many observers. Türk drew attention to them as "Reizungs Zellen," in 1898. They occur in pathological blood, generally associated with myelocytes: 4 per cent. may occur in a differential count. They are non-granular cells, generally about the size of a lymphocyte or neutrophil cell. The feature of the cell is a moderately large eccentric spherical structureless nucleus, destitute of chromatin, and an intense red-brown (with tri-acid) vacuolated protoplasm, which forms a zone around the

nucleus broader than what is seen in lymphocytes. With haematoxylin-eosin the protoplasm stains reddish violet. It is not beyond question whether the cell is a leucocyte or an abnormal nucleated chromocyte (Engel).

7. The cell described by Weil as a *plasma cell*, I have never seen in blood. He states that it is an oval mononuclear cell, the non-granular protoplasm of which stains deeply with thionine.

8. Large non-granular mononuclear cells, considered to be identical with large lymphocytes, often occur in large numbers in several types of leukaemic blood. Numerically, these characterise acute (lymphatic) leukaemia. In all cases when an enumeration of leucocytes is made, data as to the absolute numbers per cubic millimetre are more valuable than information as to the percentage number; for it is obvious that in leukaemia, where the ratio of white to red may be 1:4, a low percentage may be associated with a high absolute value.

The large lymphocytes (?) may reach nearly 327,000 per c.mm. (Phear.) Descriptions of these cells are conflicting. According to some observers they are colourless cells, variable in size, resembling large lymphocytes. The cell body forms a narrow zone around a large round nucleus, poor in chromatin, which shows no indication of mitosis. The protoplasm is free from granules, and shows no marked affinity for either neutral or acid dyes. It shows protuberances and frayed edges. The cells are easily disorganised, as may be seen by slightly raising the temperature. They are "unriefe Formen," the destiny of which is not to develop in the blood but to disintegrate (E. Grawitz). Another view of these cells is that they are large lymphocytes, of a size 8μ to 24μ, and exactly resemble the small lymphocytes in the staining of their nuclei and protoplasm. The edge of the cells may protrude or bud off and circulate in the plasma as free elements; a clear halo surrounds the nucleus, which may be spherical, lobed, or pear-shaped, often shows several clear spaces, and one or more nucleoli. This description of Ehrlich, Engel, and others is responsible for the identification of these cells as large lymphocytes.

The cells differ from ordinary lymphocytes in other respects

than size; with methylene blue the nucleus stains less intensely than that of the other lymphocytes, the cell protoplasm on the other hand stains less deeply. This possesses an exceedingly fine compact granulation, but with triacid no genuine neutrophil granules can be demonstrated. The relative amounts of nucleus and protoplasm vary considerably; most cells possess an eccentric nucleus. Ehrlich's description of this cell is identical with that given recently by Pinkus, who adds that the cytoplasm shows no structure in fresh or stained specimens, but is frequently more basophil than the nucleus, and sometimes slightly granular with a froth-like appearance. The nucleus of the smaller forms may show a structure consisting of two or three central and five to ten peripheral chromatin masses joined together by coarse chromatin threads; the network so formed is more or less dense according as the nucleus is small or large (Pinkus).

Many of the "Markzellen" of older authors (Fraenkel, Müller) are identical with the so-called large lymphocytes; but since the recognition of acute leukaemia by Ebstein and Fraenkel, the contention that the preponderating leucocytes in this disease are large lymphocytes, rests almost entirely on Ehrlich's authority, though other observers who have studied these large cells which Benda speaks of as myelogonien derived from the marrow, consider that his view is untenable. Benda's view that lymphogonien and myelogonien represent two distinct series of cells originating respectively in lymph-adenoid and myeloid tissue can be supported by weighty evidence.

I have myself frequently examined the blood in three, and the blood, marrow, and lymph glands in one case of undoubted acute leukaemia. One of these resembled a case described by Grawitz,[1] for it occurred in an adult, and presented certain chronic features. The large lymphocytes in the blood were exactly similar to those in his figures. I incline to the opinion that many of the cells identified as large lymphocytes in leukaemia are never seen in normal blood, and that no lymphocyte comparable to these exists. Lymphocytes as a group are resistant cells, stain typically, and the non-granular protoplasm gives a sharp Mylius' reaction.

[1] Grawitz, *Klinische Pathologie des Blutes*, Berlin, 1896, p. 123.

The large leukaemic lymphocyte certainly differs from lymphocytes in staining properties, it smears so easily that this is a feature of the cell, and the protoplasm, though often a very thin zone, gives a reaction for alkali, never intense but resembling that given by the polymorphonuclears. The protoplasm also often stains a faint pink colour with methylene blue and eosin, and a few very fine neutrophil granules may also be seen in the protoplasm. Apart from these features, the cell gives a general idea that it is of feeble consistence and stains badly. In fresh preparations the cell appears to belong to the largest of leucocytes (12 to 15 or 20 μ), and the red discs seem quite dwarfed. Most recorded cases of acute leukaemia are associated with a pathological change in the bone-marrow; this structure is often loaded with leukaemic lymphocytes. In many parts of the marrow these cells are more numerous than the chromocytes, and are genuine constituents of the marrow, not of the blood (Phear). According to Melland, the typical cells of acute lymphatic leukaemia are not identical with large lymphocytes. Post-mortem, the neutrophil and eosinophil myelocytes of the bone-marrow are found to be almost entirely replaced by cells resembling those seen in the blood-stream. Moreover, the protoplasm of these tends to stain with acid dyes and to exhibit the remains of a few neutrophil granules, and I believe that the cells are either degenerated or undeveloped neutrophil myclocytes, but most probably the latter.

As far as histological evidence is of value, this view that the leukaemic lymphocyte is not necessarily connected with purely lymphoid tissue has much probability. The lymph glands in many cases show but slight changes compared with the constant abnormal state of the marrow (Bradford and Shaw); Phear's plates also emphasise this fact. The leukaemic lymphocyte, may probably be regarded as a myelogenic cell of abnormal blood, the feature of the blood in acute, and a constituent of the blood in myelogenous leukaemia.

It will be noticed that of the abnormal cells which may be found in blood, the majority appear to have emigrated from the bone-marrow, and indeed a positive diagnosis of certain diseases in which this is a site of pathological change is only possible by

an examination of the blood. That in man bone-marrow is a site of blood-formation both for the red and some white corpuscles, is admitted by every observer. Into the contentious subject of blood-formation I do not propose to enter. It is sufficient to point out that a set of statements with reference to this question made for any animal is by no means necessarily correct for another. It is known that bone-marrow has no existence in many vertebrates, and that the angioblasts of the chick are of hypoblastic and not mesoblastic origin, as in all mammals hitherto investigated.

In the human foetus and during the first year of infancy the marrow is red and very vascular, filling the shafts of the long bones, where it is apparently in full activity. In the adult, a fatty non-myeloid tissue replaces the red marrow, which becomes restricted to the epiphyses, to the ribs, sternum, and bones of the skull. In the adult, the marrow may be regarded as a region where great fluctuations in activity occur, so that the encroachments of the red on the yellow marrow, or the conversion of one into the other, can be seen with the naked eye, a condition which is observed better in the frog than in man. The frog manufactures red corpuscles only after the breeding season (Carl Marquis, Neumann), and during the period of maximal nutritional activity, from April to August. The long bones of these animals show a lymphoid fatty marrow after the period of blood-formation from September to March, which changes to a striking red tissue, in which active mitosis is in progress during the period of blood-formation.

I may say that I have found it is not possible to bring about this phase of activity in winter frogs by subjecting them for long periods of time to diminished atmospheric pressure (fourteen or eighteen days at 420 mm., which equals 1600 feet altitude).

Accounts of the structure of marrow are conflicting, and moreover most of the published work deals with the structure of this in animals other than man. Marrow is a troublesome tissue for study. Quantities of large fat-cells appear to be held together in a fine framework of retiform tissue, that is so loaded with cells that it recalls the structure of the cortex of a

lymphatic gland, except for the fact that the cells are larger, more varied, and often contain large numbers of granules. Some areas appear to be chiefly erythroblastic, others to be chiefly leucoblastic. The relation of the cells to the capillaries is also difficult to ascertain, but I have at times seen the lumen of the larger vessels choked with giant cells. Injections are stated to fill the capillaries and not reach the extra vascular areas, but local inoculations of the marrow of the rabbit with small rods of Indian ink, thickened with gum, is followed by the appearance of quantities of cells loaded with pigment in the circulating blood (J. Arnold), and an accumulation of these in the spleen, liver, and lungs.

For examining the cell-contents of marrow, I find that the best results are obtained by using thin sections of elder-pith, as was independently suggested by Arnold. The split epiphyses of long bones removed in operations yield abundant material when squeezed in a vice, and on lightly touching a drop with a pith-section, which is at once dropped into saturated corrosive sublimate in .9 per cent. salt, the cells are perfectly fixed (Muir, Gulland). Occasionally absolute alcohol was used, and also formol-alcohol. Other fixing methods gave no results so good as sublimate. I soon found that the use of a great variety of staining methods did not materially help in grouping the cells, for many cells quite recognisable when stained in one way, become almost unrecognisable when stained in another. I have used Phear's 2 per cent. methylene blue in 40 per cent. alcohol for fresh films, and for fixed specimens the stains I habitually use for blood, those of Leishman, Ehrlich-Biondi-Heidenhain, Ehrlich's tri-acid, Goldhorn's polychrome methylene blue, and Jenner's stain. The Ehrlich-Biondi stain, used in full strength and carefully washed out with water, gave the most uniformly useful results.

I believe no method for the examination of fresh marrow is equal in ease to that with elder-pith. There is no distortion of the cell, and often a single cell will be found lying quite alone in one of the spaces of the pith parenchyma. In normal adult human marrow, with this method, the following cells can be identified.

I.—Erythroblastic Group

1. Chromocytes which exactly resemble those of the circulating blood. Polychromatophilia is, in my experience, very rarely seen.

2. Some smaller chromocytes, 6 μ in diameter, which are spheres and not discs.

3. Normoblasts with a nucleus which stains more intensely with basic dyes than the nucleus of any other cell in the bone-marrow. The contour of the nucleus is sharp, and it is difficult to recognise any differentiation into nucleo-plasm and chromatin.

4. Cells containing haemoglobin, which show mitosis.

5. Free nuclei (classed here, since some are probably the extrusions of normoblasts).

6. Small normoblasts (microblasts).

7. A few large cells with haemoglobin, and a large nucleus which stains deeply, like that of the normoblast. This cell is not a megaloblast, but apparently corresponds to the cell described by Malassez as "L'hematie à large noyau," or the metrocyte of Engel.

II.—Leucoblastic Group

1. Cells indistinguishable from small lymphocytes.

2. Cells indistinguishable from large lymphocytes.

3. Cells resembling large lymphocytes, with a few neutrophil granules in the protoplasm.

4. Cells generally larger than any lymphocyte, with a round or bean-shaped nucleus. The cell protoplasm is well developed and free from granules; it is a cell which may show mitotic phases of the nucleus. These are probably lymphoid marrow-cells (Türk) or "Stammzellen."

5. Ehrlich's myelocytes, varying in size, with scanty or abundant neutrophil granulations.

6. Eosinophil myelocytes.

7. Myelocytes with basophil granules.

8. Mast cells.

9. Polymorphonuclear leucocytes.

10. Eosinophil cells similar to those in the blood.

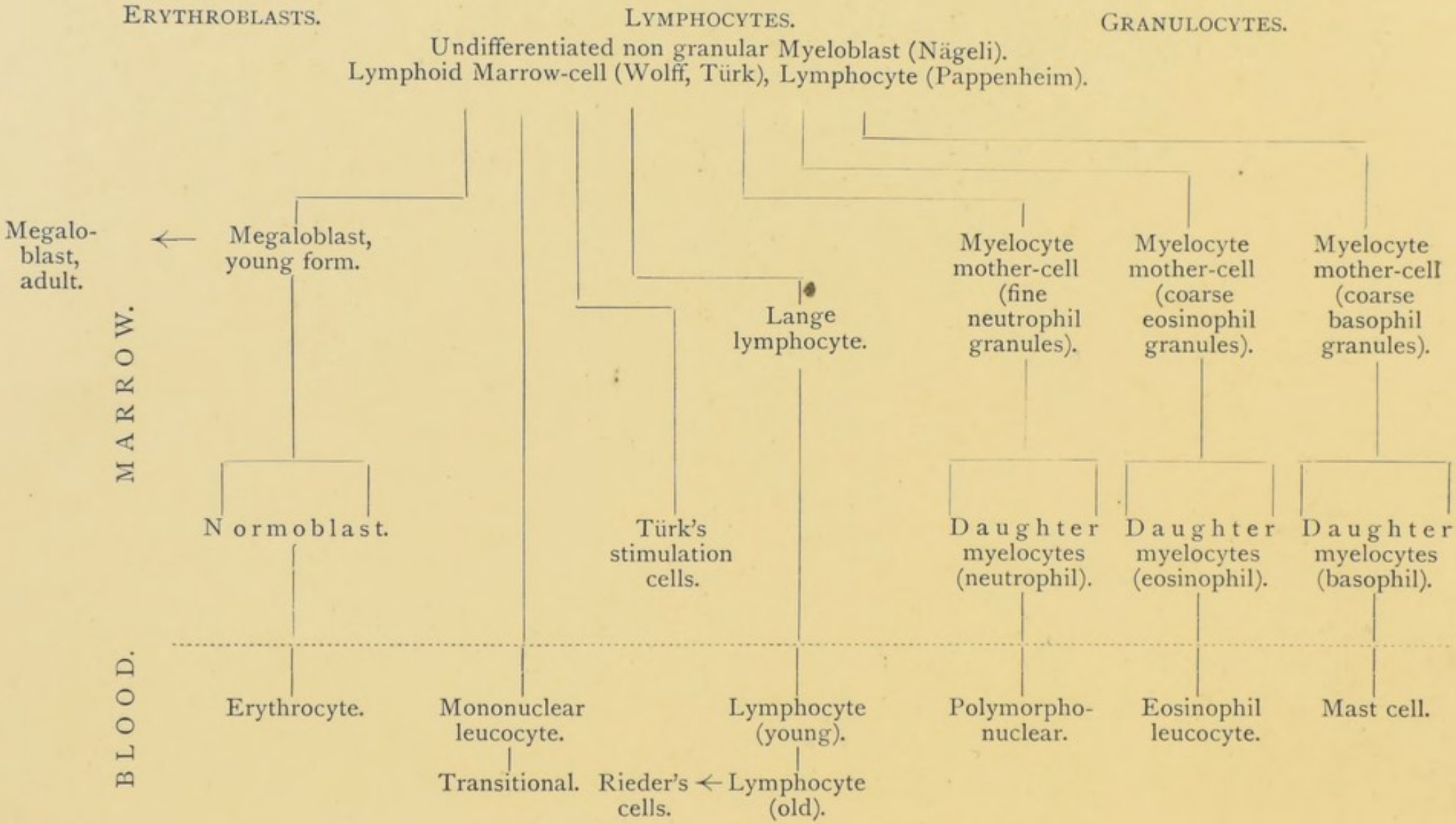
11. Giant cells (myeloplaxes, megacaryocytes). The cell-body is free from granules, and may measure 30 μ. The protoplasm is sometimes oxyphil, sometimes basophil. It is amoeboid and phagocytic. The nucleus is rosette-shaped, or more often several nuclei are seen separate or joined by fine threads of chromatin. The cells lie against or even inside the capillary walls. Several centrosomes may occur in these giant cells.

Of the relations of these several forms to each other and to those of normal blood, our knowledge is still uncertain. The granule-bearing cells far outnumber the non-granular; the latter are probably the ancestral forms or "Stammzellen" of the others.

The following table (p. 88) may provisionally serve as a partial guide to the views held by Pappenheim, Grawitz, Wolff, and Türk, as to the relationships of the cells of the bone-marrow. In the opinion of these observers there is not the slightest need for a separation of cells into the myeloid and lymphocyte groups suggested by Ehrlich. According to Pappenheim, all the members of the three groups of erythroblasts, lymphocytes, and granulocytes originate from an ancestral lymphocyte.

[TABLE.

SCHEMATIC TABLE OF THE BONE-MARROW CELLS, SIMPLIFIED FROM THE VIEWS OF PAPPENHEIM, WOLFF, AND TÜRK.



LECTURE V

LEUCOCYTOSIS, LEUCOPENIA, AND LEUCOLYSIS

QUITE apart from the structure of the leucocytes their general biological interest is very great, since of all the cells of the body they can be most easily studied alive for long periods after separation from the other units of the organism. During life these amoeboid cells transport material from place to place, and may exhibit phagocytic behaviour to such an extent, that fifty or more micro-organisms, *e.g.* bacillus typhosus, can be seen by Leishman's method to be included in a single polymorpho-nuclear leucocyte. This function has invested the leucocytes with a peculiar interest; for if Behring's statement is accurate, that tubercle bacilli are always present in the human body, we should succumb to tuberculosis or other diseases of bacterial origin but for the phagocytic activity of these cells. In other words, we remain in health not because of the absence of attack, for this is constant and persistent, but because an efficient means of defence normally exists.

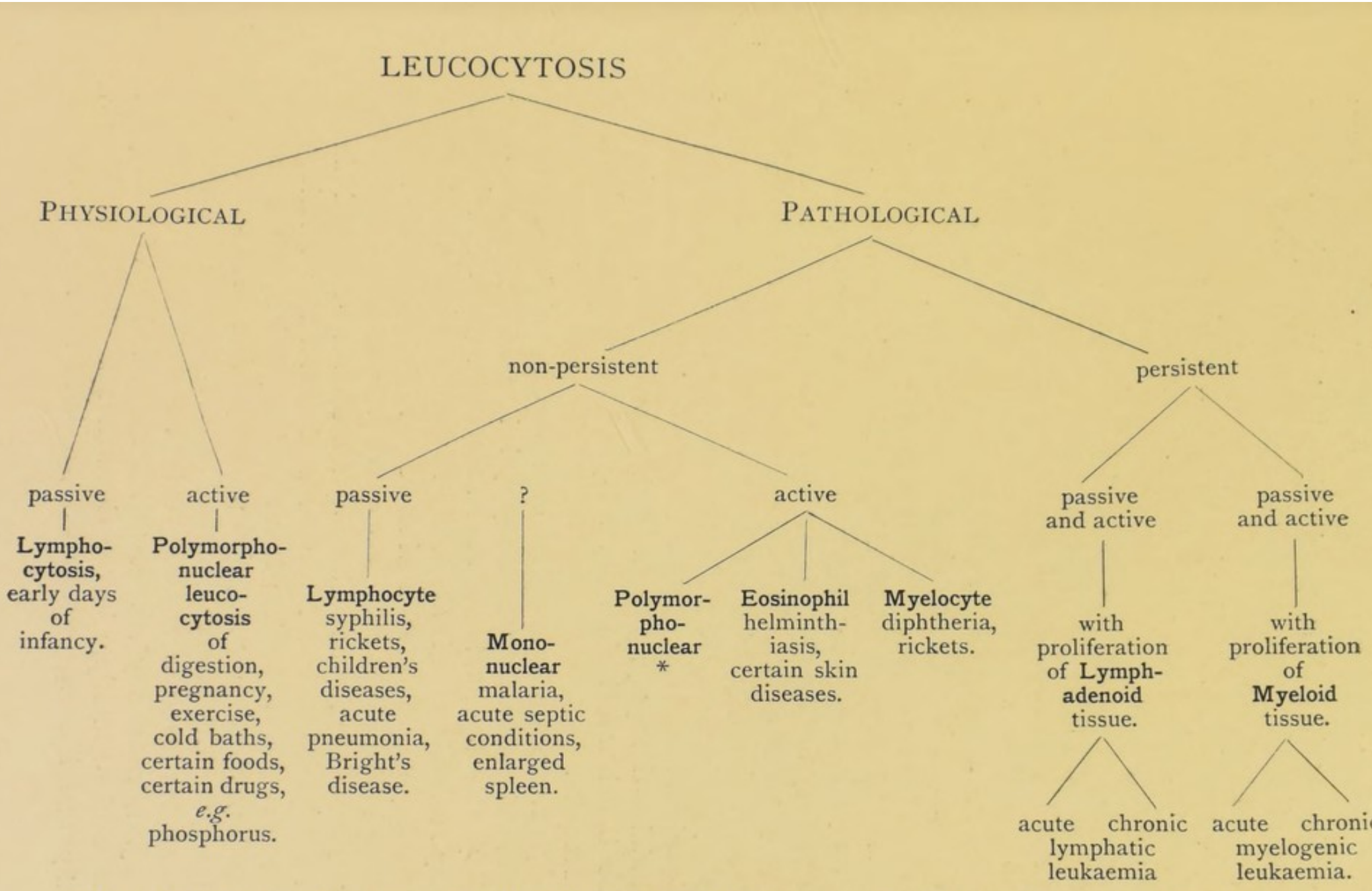
A transient or persistent variation in the number of leuco-cytes in the blood is generally of the nature of an increase—a leucocytosis; occasionally a decrease is seen—leucocytopenia, or leucopenia. In leucocytosis, from whatever cause, there is an absolute increase of the total leucocytes, and in this the normal ratio of the varieties of white cells to each other may be, but generally is not preserved. In the latter case, the type of leuco-cytosis will be indicated by that leucocyte which, owing to its relative increase, gives the distinctive feature to the blood change; and when the assured fact of an increase of cells has

been established by the use of paired counters, the relative numbers and characters of the cells in any leucocytosis can be definitely ascertained by the differential counting of stained films. If an obviously altered ratio only exists, this is expressed as either a percentage, or an absolute increase of any given cell, information which often has a greater diagnostic value than that of a leucocytosis. Some observers point out that in leucocytosis and leukaemia one is transient, the other persistent; and though this is generally true, especially when the physiological or infective leucocytoses are studied, a more or less persistent aleukaemic leucocytosis may exist for a long time; and conversely, I have seen an undoubted myelogenous leukaemia which showed for months a typical myelocyte leucocytosis disappear entirely, and be replaced by a perfectly normal number of normal leucocytes, a condition which existed for weeks until death.

It is generally laid down, somewhat arbitrarily, that a leucocytosis exists when the absolute number per c.mm. exceeds 10,000, but it is this very statement which is responsible for much confusion. A leucocytosis only exists when there is a definite excess of leucocytes in any given individual, compared with the average possessed by that individual in health. In some individuals the number may be about 10,000, in others 8000, or even 4000; my own number for the past two years averages 11-12,000 per c.mm. These constant variations are especially obvious where numbers of students are repeating Leishman's method for estimating phagocytosis, and actual enumerations prove that as much variation exists in the physiological leucocyte-count of different people as in their pulse-rate, weight, or frequency of respiration.

Just as attempts, more or less successful, have been made to form the leucocytes into groups, so the following table (p. 91) represents an attempted classification of the various leucocytoses which is founded on Ehrlich's theory of their origin, but which is one that has no relation to their causation.

If we consider the diagnostic value that is attached to leucocytosis as a symptom of disease, it may be of use to realise under what circumstances this might theoretically occur. It is



* Most of the pathological, and practically all of the experimental leucocytoses, show both an absolute and relative polymorphonuclear increase. Injections of pilocarpin are stated to produce a lymphocytosis (Waldstein)

conceivable that a leucocytosis might be induced by any one or more of the following conditions, and in disease several of these often exist together.

1. The circulating blood may become inspissated as a result of the loss of water, which, *plus* the dissolved salts and some proteids, passes to the extravascular districts, and by this means an apoplasmia of the blood is produced. The specific gravity of the blood rises, the absolute number of all the morphological elements of the blood goes up, the haemoglobin-value augments. The viscosity of the blood will increase, and hence the heart will eject a fluid with a high friction-coefficient. A leucocytosis thus established would be due to an apparent rather than a real change in the blood.

2. The evidence which exists seems to indicate that many leucocytes do not live in the blood-stream for more than three to five days. The average number therefore is maintained by a supply which equals the loss; an alteration of this balance in one direction would be indicated by a leucocytosis due to the supply being steady, while the destruction or emigration is diminished or does not occur, or there might be a normal or diminished supply, and no emigration or loss. In this case the blood would show little beyond an absolute increase of leucocytes.

3. The destruction or loss of white cells might be constant or even increased while the supply is in excess.

4. The distribution of the leucocytes in the vascular system may resemble what is frequently noticed in examining blood for bacteria, protozoa, or filariae. Some vascular districts may abound in white cells which are absent elsewhere. The admixture of red and white corpuscles must be more intimate in a large vessel with the blood moving at a high velocity than in the narrow capillaries; it is therefore possible that a drop of capillary blood may not, and probably often does not, always give accurate information as to the average number of leucocytes in other vascular regions.

5. Without any change in the inflow or loss of leucocytes, these might multiply in the blood-stream. To my knowledge, only one isolated observation has been recorded where the

actual mitotic division of a leucocyte has been witnessed in the blood. It is at any rate exceedingly rare. Mitotic figures occur at times sparingly in the blood of myelogenic leukaemia, but it is the myelocytes alone which show these. An observation of Spronck is often quoted. He stated that in the circulating blood of mammals (man and rabbit) 2 per 1,000,000 of the leucocytes exhibit mitotic figures; by calculation, it would seem that about one-twentieth of the total leucocytes could be accounted for on the assumption that this is a constant normal feature in blood.

6. Leucocytosis, in the sense that the total number of leucocytes in the blood, as in cases of chlorosis or Bright's disease may augment, will not be apparent in a drop of peripheral blood, owing to an increase in the volume of the plasma. The work of Lorrain Smith has shown that this actually often is the case. We should therefore bear in mind that although a drop of peripheral blood may, it also may not, yield any evidence of a leucocytosis that really does exist.

Under physiological conditions, either a slight or well-marked leucocytosis may occur. In newly-born children it is distinct—22,000 per c.mm. twenty-four hours after birth, or 18,000, which is the average of forty counts up to the twenty-fourth hour (Fehrsen). The feature of the leucocytosis is an increase of polymorphonuclears, which subsequently decrease, so that by the tenth day there is an absolute and relative increase of lymphocytes. Digestive leucocytosis is usually inconsiderable in man, the number of cells rarely exceeding 15,000. Some individuals show practically no post-prandial leucocytosis, but when seen, it reaches its maximum about four hours after a meal. The increase appears to me to be sometimes due to an increase in the numbers of lymphocytes, sometimes of polymorphonuclear cells. That of children is generally well marked, and is indeed a lymphocytosis. The phenomenon, whatever view may be taken as to its origin, is considered by Rieder, Poehl, and others to chiefly follow the ingestion of proteids rather than other foodstuffs. Recent work on animals shows that a constant and pronounced lymphocytosis reaching its maximum four hours after food, succeeds a transient leucopenia

of the first hour. The inflow of leucocytes into the blood is probably from the bone-marrow, since the leucocytosis is not modified by splenectomy, and is not due to transport from the wall of the gut, since the number and varieties of leucocytes are identical in the mesenteric artery and vein, and no special activity of the germ-centres of the lymphoid tissue of the intestinal wall can be noticed (Goodall, Gulland, and Noel Paton). In order to determine the existence of a pathological leucocytosis, blood should not be examined a few hours after food. It is also established that blistering, massage, and perfectly aseptic operations, especially on the abdomen, are followed by a leucocytosis (20,000 per c.mm., according to Wassermann); this soon disappears, but persists and augments should septic infection occur. Both the temperature-curve and the leucocyte-content of the blood must be considered together as evidence of post-operative complications.

It is chiefly in connection with pathological and experimental leucocytoses that theories as to their causation have been evolved, for no entirely satisfactory explanation has been given for the physiological form, and the views which have been advanced have been made to fit in with those which have been obtained from a study of those experimental leucocytoses which may be induced by a vast variety of procedures, among which the intravenous injections of albumoses, bacterial filtrates, or bacterial proteids may be mentioned. In 1888 Landerer showed that a marked leucocytosis was produced in many animals by injections of cinnamate of soda. Since such injections have been recommended and employed as a therapeutic measure in phthisis, the action of this drug has been the subject of many investigations. A perusal of these is chiefly of value in showing the caution which must be exercised before any set of experimental facts can be accepted as permanent additions to knowledge. Batty Shaw has injected cats with this drug, and while confirming the work of previous observers (Richter and Spiro) as to the marked leucocytosis which occurs, regards his experiments as affording support to the view that lymphocytes are stimulated to become transformed into transitional and polymorphonuclear leucocytes. He noticed that the percentage of eosinophils were diminished,

and no cells showed any mitotic figures. It is quite certain that these are exceedingly rare in all haemic leucocytes, though Roemer, some years ago, asserted that the increase of leucocytes was due to a rapid division of these cells in the blood, especially in that of the veins. F Charteris and E. P. Cathcart find that the drug produces in rabbits only a slight but permanent leucocytosis; the polymorphonuclear cells are relatively diminished, while the lymphocytes, and especially the large mononuclear cells, are both relatively and absolutely increased. For the purpose of discovering some drug that would produce leucocytosis without causing much constitutional disturbance, Herbert French has made experiments upon himself, and recorded that neither the ingestion nor subcutaneous injection of nuclein raised the number of his leucocytes per c.mm., and when cinnamate of soda was administered hypodermically this never produced any leucocytosis.

In pathological conditions leucocytosis is so exceedingly common that the converse state of leucopenia, which is rare, is a symptom of some diagnostic value in uncomplicated typhoid or measles, when contrasted with the almost constant leucocytoses of other infective diseases. The following types of leucocytosis have been recognised.

Polymorphonuclear Leucocytosis

This form, which is more frequent than any other, is commonly met with in post-haemorrhagic anaemia, and in acute rather than chronic infectious diseases, especially those accompanied with septic conditions, which are almost uniformly accompanied by a polymorphonuclear leucocytosis.[1] In acute suppurations this is the rule, but the extent of the increase of leucocytes bears no relation to the intensity of the process. The total leucocytes per c.mm. may reach 70,000, but I am certain that the existence of pus may remain undetected or even unsuspected, owing to the absence of any leucocytosis.

[1] Virchow is regarded as the discoverer of leucocytosis. The earliest observation which I can find is one by W. Addison, who pointed out in 1843 that the white cells were very abundant in blood taken from an inflamed surface in the neighbourhood of a boil.

The condition of the blood in appendicitis has been the subject of much research. In the papers of Loeper, Da Costa, Wassermann, and Cazin there is sufficient evidence to show that appendicitis with and without suppuration, gangrene, or peritonitis differs in the leucocyte count, which rarely exceeds 15,000 per c.mm. in uncomplicated cases, but may reach 20,000 or even 45,000 (Cazin) where the toxines of an abscess are being absorbed or a septic peritonitis is developing. Cases of appendicitis certainly cannot be differentiated from other abdominal inflammations, such as pyosalpinx, by the recognition of a leucocytosis.

In erysipelas, diphtheria, acute rheumatism, and especially pneumonia, where it is an early symptom, the absolute number of leucocytes augments. The increase is well marked in pneumonia, indeed it is a grave sign, if there is a leucopenia (Hayem and Gilbert). The leucocytosis suddenly disappears at the crisis. In severe forms of septic diphtheria in children 3 to 4 per cent. of neutrophil myelocytes may be found; but the appearance of these, especially in children, is not restricted to diphtheria, for myelocytes are not uncommon in cases of rickets. A leucocytosis with myelocytes necessitates a bad prognosis (C. S. Engel). The occurrence of a leucocytosis in influenza is disputed; it has never been very marked in my own observations.

In the protoplasm of polymorphonuclear leucocytes, particularly in those of a leucocytosis associated with suppuration or pneumonia, iodophil granulations or spherules may be seen, if dried films of blood or pus are exposed to iodine vapour for fifteen to thirty minutes, and then mounted in cedar oil (Salmon) or saturated laevulose solution (Ehrlich). The nature of this iodophil substance, which may also exist as irregularly-shaped masses in the plasma, is a matter of dispute. It is generally regarded as glycogen, but micro-chemical reactions are insufficient to absolutely determine this point. In an intense leucocytosis the so-called glycogen reaction, which some observers speak of as the glycogenic degeneration of leucocytes, may be present not only in polymorphonuclears but in large mononuclears, and Loeper has described iodophil granules in

the cells of pus and marrow. In experimental aseptic suppurations established in animals identical granules are seen in the leucocytes (Sabrazès and Muratet). In diabetes the iodophil reaction is frequent, but not constant; its appearance is possibly due to some associated suppurative process. Some leucocytes of normal blood may show iodophil granules (Zollikofer, M. Wolff). According to American observers marked iodophilia is diagnostic of inflammatory suppurations (Dunham, Locke). Blood-platelets show a mahogany tint with iodine, and if on account of this reaction leucocytes are considered to possess particles of glycogen, we cannot deny the existence of this in platelets.

Eosinophil Leucocytosis

Eosinophilia

The relative increase of coarsely granular eosinophil cells, known as eosinophilia, may reach 30 per cent. or more, and should this occur at the expense of other leucocytes, no actual leucocytosis will exist. On the other hand, by the addition of an excess of eosinophils to the blood, a condition of eosinophil leucocytosis would be established.

An excess of these leucocytes in the blood is not infrequent, especially in children. The blood of persons suffering from bronchial asthma, certain skin diseases such as pemphigus, prurigo, and psoriasis, may also show an excess of eosinophils, but this is specially noticeable when either the blood itself or the gut is the habitat of nematoid worms. That the conditions within the gut profoundly modify the blood, is no longer a matter of doubt, and this fact is particularly obvious in cases where intestinal worms die or multiply in the alimentary canal. In ankylostomiasis and in trichinosis eosinophilia is a frequent if not a universal symptom, and in bothriocephalus-anaemia the blood gives a picture which closely resembles that of pernicious anaemia. In filariasis, one species of parasite, Filaria nocturna (T. Lewis), occupies the systemic vessels during the night, and can therefore be found in the circulating blood, while during the day the parasites remain lodged in the pulmonary vessels. Gulland has shown that this corresponds with the

fluctuation in the eosinophil cell-content of the blood. The percentage number rises *pari passu* with the gradual invasion of the system by the embryo parasites.

TIME.	TOTAL EOSINOPHILS PER C.MM.
10-11 A.M.	276
4 P.M.	476
10-20 P.M.	508
11 P.M.	1500
12 midnight	1200

Though eosinophilia occurs in helminthiasis it is not pathognomonic of this condition, indeed, in my own opinion it is pathognomonic of no single disease. In ankylostomiasis the blood shows a marked eosinophilia similar to that described by Brown, Kerr, Blumer, and Newman in trichinosis. Miner's anaemia, as investigated particularly in the Dolcoath mine by Haldane and Boycott, is a symptom of ankylostomiasis, and the blood apart from other features of anaemia shows a marked eosinophilia. In 94 per cent. of the cases examined a percentage of eosinophils above 8 per cent. was found, and the average for infected individuals was 18 per cent. The eosinophilia is pronounced and persistent for a long time, even after treatment and removal from insanitary surroundings.

It is generally taught that an excess of eosinophils is common in the blood of patients with various skin diseases. I cannot say that any marked eosinophilia has been noticed in the majority of those cases which I have seen, and the recent observations of H. S. French compel an entire change of opinion on this question. Certainly the alleged increase of these cells in most cutaneous diseases is not in accord with facts. On the assumption that any percentage less than five is within normal limits, then a higher figure of 10 per cent. will constitute a slight but marked eosinophilia. French has reviewed the work of previous observers who have described changes in the blood associated with certain skin diseases. In sixty-nine cases which are recorded he finds that "only twenty-one, or less than one-third, showed eosinophilia; three out of eight with eczema; three out of ten with lupus vulgaris; one out of five with

measles ; four out of six with pemphigus ; three out of four with psoriasis ; two out of nine with scarlet fever ; two out of three with sclerodermia ; two out of thirteen with syphilis ; and none at all with acne vulgaris, cutaneous burns, erythema from salol, erythema multiforme, herpes zoster, lichen ruber planus, or chronic urticaria. And the only cases in which it was marked, six in all, were one case out of eight with eczema ; one out of ten with lupus vulgaris ; three out of six with pemphigus, and one out four with psoriasis." At the commencement of his work French appears to have held the generally accepted view that eosinophilia is a frequent blood change, and one of diagnostic value. His results, however, led to quite a different conclusion, for out of ninety patients in only four was a marked eosinophilia present, a few cases showed some, while the great majority showed none at all. In many patients a lymphocytosis existed, but was not a constant feature except in cases of congenital syphilis and urticaria. In pemphigus and dermatitis herpetiformis a certain amount of eosinophilia was noticed.

LYMPHOCYTOSIS

In children during the first few years of life the number of lymphocytes in blood is high, at six months the maximum exists (8000 to 9000), which is four to five times the number found in the adult. This is the normal lymphocytosis of infancy, which after the first week is associated with about 5000 polymorphonuclear—the number found per c.mm. in adult life. Should a septic leucocytosis occur in children, this as in the adult is indicated by an increase not of lymphocytes but of polymorphonuclears. Comparatively few diseases are accompanied by a definite lymphaemia. In the convulsive stage of whooping-cough, the number is said to be four times the normal. The leucocytosis of post-haemorrhagic anaemia in a few cases has been chiefly a lymphocytosis (Lazarus). Pneumonia in the adult may show a blood change with 96,000 leucocytes, of which 82,000 were lymphocytes (Houston).

In Hodgkin's disease I have noticed both an increase of leucocytes and also the opposite. Drysdale's recent work on

fourteen cases show that the changes in the blood are moderate in extent. The average leucocyte count was 10,340 per c.mm. In seven cases the polymorphonuclears were above 5000 and seven were below. The same variations held for the lymphocytes. Seven cases gave numbers above and seven below 2000 per c.mm. It would appear that scarcely any diagnostic information can be obtained from a blood examination in cases of lympho-sarcoma, tubercular adenitis, or Hodgkin's disease. A distinctive feature of the blood in cases of variola is a progressive increase of lymphocytes, and a corresponding diminution of the polymorphonuclears (Ferguson). A leucocyte count of 20,000 showed 45 per cent. of polymorphonuclears and 51 per cent. of lymphocytes.

General information as to an increase of large mononuclear cells has been given by Houston, who points out that they may be increased in splenic enlargement, malaria (L. Rogers), certain acute septic conditions, some cases of chlorosis, and in the anaemia of typhoid fever. A marked lymphocytosis is frequent in Bright's disease.

The immediate cause or causes which are concerned in the production of a leucocytosis in man has been the subject of much dispute. Theoretical ideas as to the origin of this, as of other subjects, are of little value, unless they are the direct outcome of experimental work. When the conditions of an acute suppurative process are realised — where ounces of pus form and ooze from the body in a few days, each c.mm. of pus containing about 1,000,000 polymorphonuclear cells, so that the leucocytes of about $3\frac{1}{2}$ litres of blood would yield only an ounce of pus (R. Muir), and that, further, there is also maintained a steady leucocytosis—it is evident that an enormous supply of these cells must originate from somewhere. The formation of pus is from the blood alone, which fluid is enriched with an influx of leucocytes from other organs. We are aware of no places whence this supply could come except the bone-marrow and lymph-adenoid tissue, which is distributed widely in the body, and also forms the bulk of such organs as lymphatic glands, haemolymph organs, the spleen, and thymus. Of these, the spleen and thymus alone can be completely excised, though

the results of such experiments is conflicting. The experimental study of leucocytosis therefore resolves itself into an inquiry as to what agents may produce leucocytosis, supplemented with researches which are chiefly clinical and anatomical, for with the exception of experimental aseptic leucocytoses, it may be considered that all these are directly due to the absorption of bacteria or their products, or of certain chemical materials from the surfaces of the body, especially that of the gut-surface. Unlike the essential blood-poisons, phenyl-hydrazine, salts of lead, or chlorates, which produce a genuine toxic leucocytosis, these substances may conceivably excite the manufacture of leucocytes in the blood-forming organs, and also in virtue of the amoeboid behaviour of these cells, attract them first into the blood and subsequently to the foci where an emigration of leucocytes is in progress. This attraction, known as positive chemotaxis, is the basis of the chemotactic theory of leucocytosis which Erhlich and his school have grafted on to the phenomenon of phagocytosis.

Our knowledge of chemotaxis dates from the studies of Pfeffer and Stahl in plant physiology. Among other experiments, they showed that the antherozoids of mosses and some vascular cryptogams reach the oosphere in virtue of an attractive stuff, such as cane-sugar, malic acid, or its salts, which arises from the disintegration of the canal-cells of the archegonia. The chemotactic agents which attracted or repelled bacteria were subsequently studied, and Leber was the first to show that a suppuration without micro-organisms, such as that produced by turpentine, silver nitrate, or tannic acid is a chemotactic phenomenon. If sealed capillary tubes containing these substances are introduced under the skin or into the anterior chamber of the eyeball and then broken, a plug of leucocytes collects in the open end of the tubes which is greater than that found in control tubes containing normal saline. The metabolic products of pyogenic bacteria, and still more the substances obtained from their disintegration, are for the most part chemotactic substances, and it is these bodies which bring about an active leucocytosis of polymorphonuclear cells, of eosinophils, or of myelocytes, while lymphocytosis is a pheno-

menon unconnected with chemotaxis, and is a simple passive increased flow of non-motile lymphocytes into the blood. In the first type the bone-marrow is the place of exit, in the other the lymph-adenoid tissue of the body. In certain experimental leucocytoses of septic origin the course of events, according to the latest views, is that the following stages may occur. An initial transient leucocytopenia, lasting only a few hours, is succeeded by a polymorphonuclear phase, which in turn is followed by an increase in lymphocytes, and lastly an eosinophil leucocytosis of short duration is seen. The last stage is evident, since when the polymorphynuclears are at their maximum the eosinophils are absent or diminished, but subsequently reappear. A substance which at a given time is positively chemotactic for the former is negatively chemotactic for the latter (Ehrlich).

The changes in the bone-marrow which precede an active leucocytosis have been fully described by R. Muir and Roger. There is in fact a reaction of the bone-marrow, especially well marked when a suppurative process is in progress. In this tissue an excessive activity of the parent cells of the bone-marrow can be demonstrated while the germinative encroaches on the yellow marrow to such an extent that in a few days the shaft of the femur contains the latter in diminished amount. The mitotic centres in lymphatic tissue also, according to Muir, may so react in certain infections that lymphocytes and large mononuclear cells may multiply and enter the blood-stream. These reactions, in the light of present knowledge, are due to a stimulating influence exerted in these regions by the circulation of substances that have entered or formed in the blood. The passage of the motile leucocytes into the blood-stream is therefore, according to Ehrlich, a positively chemotactic phenomenon. His views are easily accessible in the translation of the first volume of *Die Anaemie*, 1900, but like other theories of leucocytosis and other theories generally, this chemotactic view has been much criticised. Ehrlich has recently reaffirmed his opinion that both an active and passive leucocytosis occurs, and that in eosinophil leucocytosis, specific substances which arise from the disintegration of haemoglobin or epithelial cells

attract these amoeboid cells from the bone-marrow (*Versammlung Deutscher Naturforscher und Aerzte*, Breslau, 18—24th September 1904).

Both in physiological and pathological conditions phagocytosis occurs; all forms of the blood cells, the lymphocytes proper excepted, possess in a variable degree the power of ingesting other cells, finely divided carbon, certain chemical substances, and bacteria. That red blood corpuscles may be devoured by leucocytes has been known for a long time; mononuclear cells of the spleen may even show a haematoidin crystal as the residue of an ingested red corpuscle, and cells in other haemolymph glands may possess particles of bilirubin. The ingestion of granular leucocytes by non-granular may also confer upon the latter a spurious granulation. With reference to the intake of pigment which has originated from haemoglobin by injury to the red corpuscle by malaria parasites, in the vast majority of cases this is found not in the polymorphonuclear but in the large mononuclear cells, which are distinctly increased in malarial disease.[1]

That the leucocytes of the blood are actual or potential phagocytes is the fundamental fact in Metchnikoff's theory of immunity, and by Leishman's method a quantitative estimation of this phagocytic power may be obtained. In phagocytosis it would appear that for certain micro-organisms to be devoured by cells, substances in the serum (opsonines) are of cardinal importance; these acting upon the bacteria or other bodies render them a more easy prey for phagocytes, which are found to be actually unable to ingest certain micro-organisms which were the subject of experiment, if the opsonines of the serum are absent or have been destroyed by heat.

The papers of A. E. Wright, in which he has detailed his methods and propounded his views, are in the strictest sense of the word original, a term which I may point out is often misapplied. Confirmation of his work is not a matter of difficulty, and the observations of Bulloch are in absolute accord

[1] A percentage increase of fifteen large mononuclear cells is proof of an actual or recent malarial infection. If 20 per cent. are present, the actual parasites or pigmented leucocytes are always to be found in the blood (Stephens and Christophers).

with those of Wright, whose method I will give in almost his own words. It has been found by experiments made by Wright and Stewart Douglas, that there exists in normal serum, but in much larger quantity in the serum of those who have been inoculated against the attacks of such bacteria as staphylococci, tubercle-bacilli, or other micro-organisms, a protective substance or opsonine (Lat. *opsōno*, I prepare food for table), which either enters into combination with, or so affects the bacteria as to render them easy of destruction by leucocytes.

Phagocytosis cannot take place if the specific opsonine is absent, or if this has been destroyed by a temperature of 60° C. "Accurate measurements of the opsonic power of any serum is possible by Leishman's method, or a modification of this where (1) one volume of washed leucocytes obtained from the citrated blood[1] of a normal man, (2) one volume of a suspension of some definite micro-organism, and (3) one volume of the serum of a patient whose opsonic index for the same micro-organism requires to be determined, is mixed in capillary tubes and kept at 37° C. for fifteen to twenty minutes. Films are now prepared and treated with Leishman's stain. The first thirty or forty white corpuscles which come into view are examined, and the number of ingested bacilli noted for each cell. The total number of intracellular bacilli divided by the number of leucocytes yields the phagocytic count." This compared with that obtained by exactly the same precedure, using the serum of a normal man, gives the "opsonic index," the latter count being taken as unity (Wright). The above outline of the method shows that in studying the behaviour of the blood not only must its agglutinating, bacteriolytic, and phagocytic power be measured, but that a determination must be made of its opsonic power. The opsonic power of the blood can be artificially raised so that the resistance of the individual can be augmented with respect to specific pathogenic organisms; this as a therapeutic measure far exceeds the repeated failures which have marked the efforts

[1] Phagocytosis is stopped in a medium with 3 per cent. citrate of soda, but goes on actively where the percentage does not exceed 1·5 per cent.

of those who have attempted the destruction of micro-organisms by bactericidal drugs. An opsonic index as high as 1·3 is frequent in cases of chronic lupus which are improving under treatment with the Finsen light; the index may even reach 2 (Bulloch). To the question whether the opsonines, which Wright has discovered are substances hitherto unknown or unsuspected in sera, or whether they are identical with the whole or a part of the bacteriolytic material which is known to exist in the liquids of the organism, it is premature to give a definite answer. In a recent paper G. Dean considers that the substance which prepares bacteria for phagocytosis is thermo-stabile, and identical with an opsonine or fixateur of French writers.

Recent work has considerably augmented our knowledge of the absorption of chemical substances which was originally studied by Kobert and his pupils of the Dorpat school. He demonstrated that soluble salts of iron are picked up by leucocytes and retained in the liver, spleen, and red marrow, whether the substances are introduced into the blood, peritoneum, or subcutaneous tissue. Subsequently leuco-cytes laden with iron wander to the wall of the gut and escape into the cavity of the bowel, from the surface of which iron, like calcium, is in part excreted. A similar ingestion and transport by leucocytes has been shown for a variety of other drugs, such as salts of mercury (Stassano), silver (Samoiloff), salicylate of soda (Arnozan and Montel), and iodine (Labbé and Lortet), but the arguments which are advanced to prove that in this transport material is carried to specific places are to my mind unconvincing.

Since the ferment actions ascribed to leucocytes can only be demonstrated *in vitro*, it is difficult to be certain whether the properties ascribed to them are really displayed within the body. It may be accepted that leucolysis occurs within the blood-stream and in glands, and this has for many years been regarded as the source of fibrin-ferment or plasmase (Duclaux). It is believed that the anti-body to this, thrombase, possibly also of a ferment nature, effects a neutralisation of the plasmase, which by leucolysis is always being formed, and consequently prevents

intravascular clotting. The complicated question of the formation of alexines, cytases, or destroyers of cells (bactericidal ferments), can only be mentioned here. Portier, Brandenberg, and others have also demonstrated *in vitro* the presence of oxydases in leucocytes by the oxidation of guaiaconic acid and salicyl-aldehyde. It is probable that the ferment properties of the blood may be either intracellular or become extracellular, and a feature of the serum, owing to leucolysis. S. G. Hedin considers that since the proteases of the spleen are probably contained in the leucocytes, it is probable that the tryptic and antitryptic enzymes of ox serum—the former of which is attached to the eu-globulin fraction, and the latter to the albumin fraction (E. P. Cathcart)—of the precipitated serum-proteids are derived from leucocytes. An intracellular protease which requires a slightly acid medium as one condition of its activity, could do its work within a leucocyte, for not only is the reaction of the nucleus considered to be acid, but the fluid in the vacuoles of the cell is also acid (Metchnikoff).

Though Löwit, who was the first to draw attention to the condition of leucopenia, referred this to a leucolysis within the blood-stream, a view not generally entertained at the present time to be a sufficient explanation, there is no doubt that decay and death of leucocytes does actually occur within the blood, and this is augmented by a high body-temperature. Outside the body, a temperature of 40° C. causes a rapid disintegration of the polymorphonuclear cells, but on the other hand the leucocytes are uninjured by a temperature of 0° C. (E. Botkin). In certain diseases, especially in haemorrhagic variola, degeneration of the leucocytes, as Ferguson has shown, may reach a high grade of intensity. As signs of this degeneration, the protoplasm becomes vacuolated, the nuclei stain feebly, the granules of polymorphonuclear cells diminish or may disappear, while the nuclei may fragment and pass into the plasma and circulate as free bodies. The polymorphonuclear cells seem to be more liable to degenerate than other leucocytes. Probably the above description is a rapid pathological picture of what occurs more slowly under normal conditions in the blood-stream.

The following results obtained by K. Boden are of interest

as showing the varying resistances of the blood-cells, and the features of their degeneration outside the body; it is not improbable that his observations give a fairly accurate picture of what actually occurs during life in the blood or spleen. Boden examined blood which had been allowed to sediment in sealed sterile tubes for varying periods of time, from one and a half hours to one hundred days. Red corpuscles become smaller, subsequently show polychromatophilia, next become eroded, and finally refuse to stain at all (achromasia). After one hundred days, undoubted red corpuscles can still be recognised: they are the most resistant to destruction of all the elements of the blood.

White corpuscles disintegrate much more rapidly. Plasmolysis can be recognised in the polymorphonuclear cells within one and a half hours. These become irregular in shape, the granules stain metachromatically, and the nuclei become pyknotic—that is, stain deeply—owing to a formation of nucleic acid which raises their affinity for basic dyes. By subsequent disintegration, free granules, but never any which give the reactions of glycogen or fat, are found for some weeks in the sediment. The lymphocytes outlast all the other varieties of white cells, and may be recognised after six or seven weeks.

LECTURE VI

THE BLOOD-PLATELETS

If the amount of controversial literature about a subject is any measure of its importance, then the bodies which were originally discovered by F. Arnold in 1845, described at length by Max Schultze in 1867, by Hayem in 1877 under the name of haematoblasts, and known since Bizzozero's work in 1882 as blood-platelets, must be regarded with considerable interest.

The actual existence of these in normal blood has, however, been denied. To my knowledge, only mammalian blood possesses platelets; the "Spindeln" of amphibian blood are certainly not their homologues, but are antecedent stages in the development of the red corpuscles (Neumann, C. Marquis). Their absence in frog's blood is beyond question, as Löwit and Druebin point out; neither of these observers, nor Mosen, could demonstrate them in other coagulable fluids, such as lymph. I cannot find any platelets in frog's blood, the blood of fish or pigeons, nor in the serum of man nor any mammal. Serous exudations, such as hydrocele, pericardial or ascitic fluids, possess none of these bodies, nor can they be demonstrated by any of the methods which have been suggested for the purpose of preserving them. In a recent paper Eisen states that the blood of amphibia possesses both "Spindeln" and platelets, but to my knowledge he is the only observer who has described the latter bodies in amphibian blood.

Now, in the history of science, and of physiology in particular, it may be noticed that the more controversial the literature which deals with any particular subject becomes, the more un-

certain for the time is our knowledge; for in proportion as the subject in dispute tends to settle down into accepted truths, so do the number of papers written to support any particular view steadily diminish. Both physiology and pathology are simply overburdened with a rapidly growing literature, part of which is produced by those who do work for the subject's sake and part by those who do work in order to publish; further, there is a tendency among some of us to only pay attention to the latest investigations, and to forget that earlier observers, though they may not have employed the most recent methods, were as careful and accurate as those of to-day. Without neglecting the literature, it is of the first importance to fix our thoughts not on what is written about a subject, but on the subject itself. These remarks came into my mind in thinking over the history of the blood-platelets. Stated to have no pre-existence in normal circulating blood by Löwit, Ranvier, Weigert, Wlassow, and others, Sherrington expresses the opinion that the platelets are not living elements of the blood, but rather of the nature of precipitates containing proteids, which are shed either from the corpuscles or from the plasma, views which are regarded by Ehrlich and Lazarus as "erroneous, on the grounds of their own extensive observations."

In human blood mixed with Hayem's fluid or ·6 per cent. peptone in ·65 per cent. NaCl (Affanassiew's fluid), large numbers of platelets may be seen; on an average, 635,300 per c.mm., or at a lower estimate 180,000. In number they therefore rank second among the morphological constituents of the blood. Kemp and Calhoun, who used a mixing fluid containing 2·5 per cent. of formic aldehyde, state that the average number for the blood of men is 862,000, and for that of women 833,000. A year later, Kemp's number for his own blood in Paris is stated to be 457,000, which he found rose to 1,206,900 per c.mm. when he examined his blood seventy-two hours later on the Görner Grat, at a height of 10,000 feet. These high values I am inclined to attribute to the amount of formic aldehyde in the diluting fluid; for though I have examined the blood of ten individuals and made over two hundred observations at heights varying from 6000 to 14,000 feet, I observed no increase of plate-

lets. In anaemic conditions of children complicated with splenic enlargement, the number may rise to 829,000 (van Emden). In chlorosis and in post-haemorrhagic anaemia their number is stated to increase, but even this is not constant, and no relation exists between the degree of anaemia and the absolute number of platelets. The figures by R. Muir for chlorosis are often quoted, but they are well below the average number for healthy men. Van Emden's number for pernicious anaemia is less than a twentieth of the average number, while in myelaemia he finds they may be present in normal amount, or in half or double the normal. In this disease they are figured by Engel as arising from the rupture of erythrocytes, and described by Hayem as swollen and hypertrophied in appearance. The disintegration of red discs that may occur in toxic haemoglobinaemia leads to an increase in the number of platelets, but, as Lazarus points out, the blood in such cases may be entirely destitute of them.

The statement that an increase in number is related to a diminished coagulation-time, cannot be uniformly true. Even if the first condition obtains in chlorosis, the second certainly is not constant. I have often seen an increased time (twelve to fourteen minutes) in chlorosis, and A. E. Wright has shown that the coagulability of the blood may be considerably reduced in this disease; von Noorden is also of this opinion, so that the comparatively rare thromboses in chlorosis are probably due to causes other than a conglutination of blood-platelets, although an aggregation of these seems to occur when the endothelial lining of an artery is injured (Wlassow). From this plugging of a vessel by masses of platelets they have been regarded as specific agents for inducing coagulation of the blood; and, according to Dekhuyzen, there is found in worms, echinoderms, molluscs, crustacea, and all vertebrates, with the exception of mammals, a cell or thrombocyte which possesses the following features. It is an amoeboid, finely granular, spindle-shaped cell with an oval nucleus, the protoplasm of which is abundantly furnished with fine radiating processes which, running into others from neighbouring thrombocytes, form natural thrombi, plug injured vessels, and arrest the loss of blood. The

morphology of mammalian thrombocytes (blood-platelets) is peculiar, but their function is similar: they stick together and close vessels. On these views, organisms are furnished with special cells for a specific purpose, and the closure of vessels is due to a phenomenon which is entirely different to a coagulation of blood. The introduction of the term appears to me of doubtful value, for in the first place it is certain that the thrombocytes of amphibian blood are not in any way homologous with human haematoblasts (Schwalbe, Arnold, Eisen, Löwit, Neumann, Marquis, Engel, Maximow, Pappenheim, and others); and secondly, one may ask why it is that the shed blood of some animals, which, if Dekhuyzen's statement is correct, must contain thrombocytes, shows no clumping together of any of its cells and remains uncoagulated for several days (Delezenne, A. E. Wright). In the opinion of Morawitz, platelets pre-exist in blood, and he regards them as the sole source of thrombogen, which is a material indispensable for the formation of fibrin, and one that cannot be obtained from the red or white corpuscles. Apart from the often-quoted work of Schimmelbusch and Eberth, and the less known papers of Wlassow, who found that experimental clots were masses of platelets, the histology of thrombi has been but little investigated, although it offers a fine field for research. There is probably a causal relationship between bacteria and thrombosis. Proteus vulgaris is certainly found as a pure culture in venous clots, and this organism may be responsible for clotting in cases of cystitis or puerperal sapraemia (Pakes).

The following observations by Bizzozero are of interest in the history of the blood-platelets. During the defibrination of blood by whipping, he states that two periods can be distinguished; in the first, a thick layer of platelets collects on the bunch of wires, while in the second these coalesce into a granular mass on which layers of fibrin collect. The preliminary whipping of the blood therefore only removes the platelets. He also noticed that if half the total volume of blood in a dog is withdrawn from the carotid artery, kept warm, defibrinated, and then returned to the animal by the jugular vein, that the number of platelets in a sample from the vein of the ear is

much diminished. On repeating this experiment nine times in succession, the platelets entirely disappear, and the blood no longer coagulates. Blood without platelets therefore suffices for existence, and the regeneration of these bodies appears to be accomplished in about five days. The sedimentation of morphological elements from cold peptone-plasma,[1] which thereafter becomes incapable of coagulating, was discovered by Wooldridge in 1885, and this observation together with the one just described, shows, in my opinion, not that the platelets exist in living blood, but that mechanical and chemical injuries to protoplasma or living plasma may render it incoagulable; for at any rate part of the peptone in living plasma is treated as a foreign substance, just as is the case with dissolved haemoglobin, being excreted by the kidneys with great rapidity.

Enough has been said to show that the platelets have played a considerable part in the physiology and pathology of blood. Puzzling as is their origin, the descriptions of them are fairly uniform, if we except the presence or absence of haemoglobin. In human blood they appear as granular bodies, about 3 to 3·5 μ in their longest diameter. Their shape varies considerably; some are biconvex structures, others are flatter, of a faint greenish tint, and possess numerous processes. They may be isolated, or hang together in groups of two or three. Some agglutinate into large clumps. The tendency to adhere to the surface of the glass varies; a large number move freely in a slow current; shape, size, aspect, all vary when blood is examined after admixture with different proportions of fixing fluids; thus in Hayem's fluid they nearly all appear as biconvex bodies.

I find that they persist after the red discs are carefully haemolysed by hypotonic solutions of various salts. Weak acetic acid causes the platelets to appear homogeneous and to strongly refract light. They give an intense Mylius' reaction with iodine-eosin, contain a nuclein centre, and stain to some extent with eosin, but better with basic dyes, iodine green, pyronin, dahlia, and Spiller's purple; indeed, they react in a

[1] The substance actually used to render blood non-coagulable is chiefly deutero-albumose.

manner somewhat similar to that of the basic granules of mast cells.

The earliest figure known to me which accurately shows the aspects of the platelets of shed human blood without any mixing fluids, is given by Schiefferdecker; both the elliptical, granular bodies, and those which show fine processes extending from a clear contour around a granular centre, are well shown; the former tend to collect into heaps, the latter often hang together loosely, in sets of three or more. The elliptical bodies may or may not throw out processes, and simulate the second type. Osler in 1874 figured platelets within an excised venule. His drawings of their changes in shed blood resembles what I have seen in oxalated blood.

In 1897 H. Deetjen described a new method for the study of platelets. When human blood is streaked upon agar with ·7 per cent. NaCl, and examined on a warm stage, not only do the leucocytes exhibit particularly lively movements, but innumerable amoeboid platelets can be seen, separate, or in heaps of from ten to twenty. In 1901 he employed another medium, and since the introduction of this was really a new method in blood investigation, the composition may be given here, for it is essentially by the introduction of fresh methods that advances in knowledge may be expected.

Agar	2 grammes.
Distilled water . . .	100 c.c.
NaCl	·6 grammes.

When this is dissolved and filtered, 7 c.c. of a 10 per cent. solution of metaphosphate of soda and 5 c.c. of a 10 per cent. solution of bipotassium phosphate are added. The medium must not be boiled after the addition of these salts.

Blood is allowed to run on to a thin sheet of solid agar, and the preparation covered. An enormous number of platelets, each provided with numerous processes, are then seen; many of them stick to the cover-glass, and in Deetjen's words appear like a miniature starry heaven. He describes them as nucleated, amoeboid bodies; at any rate amoeboid in the sense

that they extrude processes, though from their stickiness they may, but do not easily shift their position.

Around these observations a renewed interest in the platelets has arisen, and figures of these bodies are to be found in recent text-books, where they appear as isolated nucleated cells, furnished with seven or eight well-defined processes. Dekhuyzen, Kopsch, and Argutinsky have verified Deetjen's

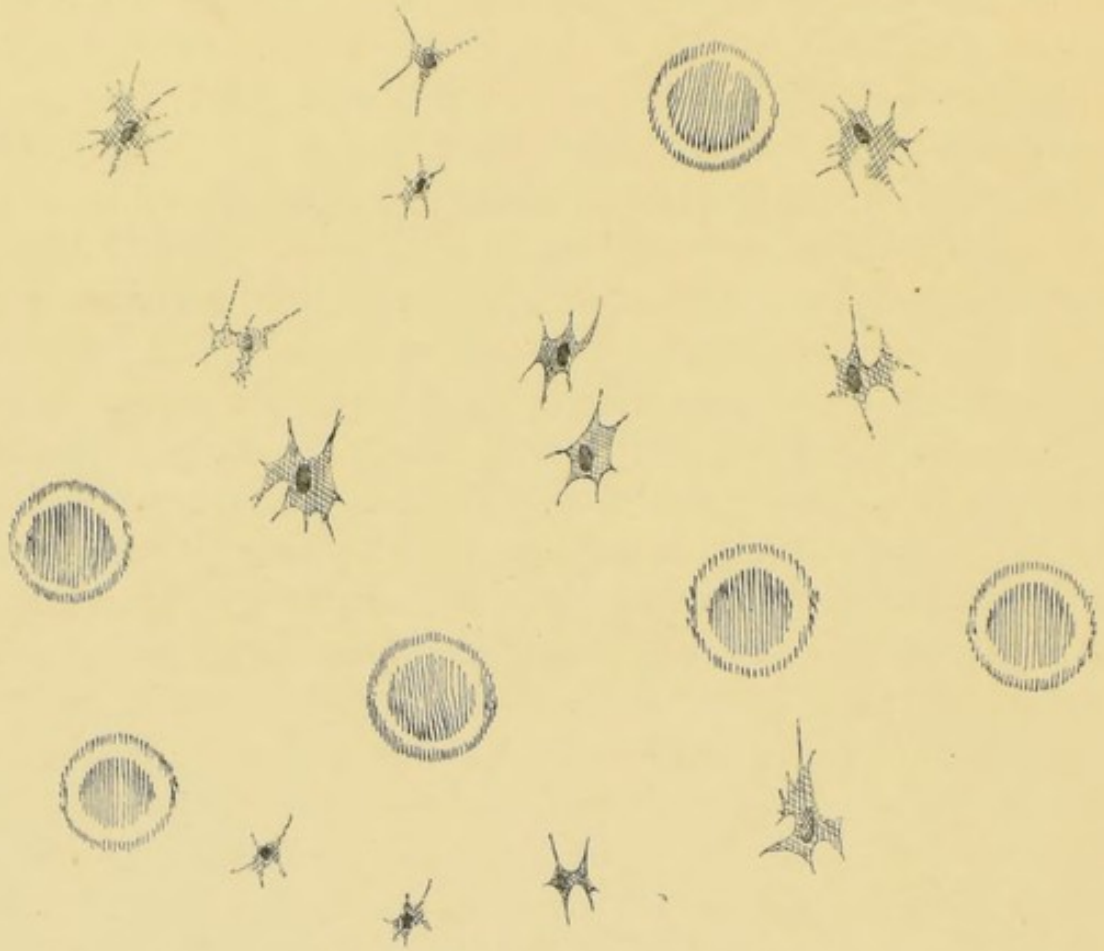


FIG. 7.—Aspect of blood-platelets when blood is examined by Deetjen's metaphosphate-agar method.

results. By simply receiving blood into a saline solution absolutely isotonic with that of the serum, and carrying out the whole observation in an absolutely sterile manner, Dekhuyzen has also obtained evidence of the amoeboid characters of the thrombocytes. After fixation with osmic acid, the peripheral hyaline part with the pseudopodia stains faintly with eosin; the spherical, strongly refractile centre stains with basic dyes, and therefore is regarded as chromatin. The platelets show no sign of haemoglobin (Kopsch). Puchberger allows weak solu-

tions of brilliant kresyl-blue to dry on a slide, and examines fresh blood on this surface. He finds that Deetjen's bodies stain particularly well, and his drawings show that probably some of these arise from the red discs. He states that the blood in pernicious anaemia, purpura haemorrhagica, and certain other cases of purpura is almost destitute of platelets, while in myelogenous leukaemia they may be hypertrophied to nearly the size of a chromocyte.

Most observers are agreed that in the histology of the coagulation process threads of fibrin spread out from points where clusters of blood-platelets may be observed, but, apart from their apparent behaviour in the coagulation of some fluids, it is only possible to come to a conclusion as to their real nature by renewed experimental work. Unfortunately the results of recent work are not concordant, and three different views, each of which has received strong support, are current as to the nature and origin of the platelets.

First view. The platelets or thrombocytes are independent bodies or cells existing in normal human blood, of equal morphological value to the leucocytes and chromocytes. The nucleated spindle-shaped cells of frog's blood are the homologues of mammalian platelets.

Second view. They do not exist in normal blood but are artefacts, that is, they are precipitates from the plasma, either of nucleo-proteid, globulin, or fibrinogen.

Third view. They exist to a variable amount in normal blood, being fragmented off from the red or white corpuscles. They therefore may or may not contain haemoglobin, and the platelets may or may not contain a central inner-body which stains with basic dyes. If separated from the white cells they might be amoeboid, for Klemensiewicz has observed that non-nucleated pieces of leucocytes exhibit movements.

A partial compromise of these views is that blood-platelets may be true or false, terms which are equivalent to true platelets and Arnold's bodies, or platelets and microcytes (Pappenheim). The homogeneous bodies, which are distinctly granular with a nuclein centre, are genuine constituents of the blood, whether these rank as cells or as cell-fragments, while the

homogeneous non-granular bodies seen in blood-preparations are false platelets, or separations from the plasma.

It would be easy to give the names of those who advocate any one of the above views, but this would be of little help in reaching a decision as to the nature and origin of the platelets.

While repeating Deetjen's work soon after it was published, I, for the first time, saw the platelets in enormous numbers exactly as he had described them. Though quite familiar with the general appearance and staining properties of these structures as they are seen in fresh films or blood obtained by puncture through fluids such as osmic acid, Hermann's fluid, Hayem's fluid, saturated potash, or Affanassiew's fluid, the ease and certainty with which they were displayed by Deetjen's method was most striking. I next noticed that the solution of salts without the agar acts equally well; but should the agar or the fluid become contaminated with bacteria the salt-content is so rapidly altered that the platelets, though present in small amount, are shown better by other common methods. After fixation of the platelets, which adhere to the cover-glass, with osmic acid, the general methods of blood-staining give the impression that the platelets are genuine constituents of the blood, and Leishman's stain, allowed to act for half an hour, gives beyond question the best permanent specimens (rapid Romanowsky method).

I have worked almost exclusively on my own blood, considered as normal, and with that of patients with chlorosis, leukaemia, and pernicious anaemia. I can add but a single remark about the amoeboid behaviour of the platelets: I have never seen a pseudopodium retracted, but have often seen one extended to a length of 16-20 μ. I consider their locomotive power so slight that I cannot be positive that this exists. In a recent paper Schneider also denies that the platelets are amoeboid.

Here, it must be mentioned, that in any blood investigation it is most difficult to repeat exactly the conditions of any observation, but though with practice this error diminishes, I have never rested content with less than twenty observations for determining any given point. With the view of attempting

to come to some definite idea about the nature and origin of the blood-platelets in man, it soon was evident that a study of the existing literature would prove almost useless, since a good deal of this describes the behaviour of these bodies in large amounts of mammalian, but not in drops of human blood, and the controversial character of some of the papers referred to often impugned even the accuracy of other observations; moreover, for the solution of scientific questions we require evidence and not arguments. Even if it is admitted as beyond question that in the circulating blood of the bat, mouse, or guinea-pig, platelets exist, these facts do not prove that they exist in human blood. This statement is one that is frequently quoted, and rests chiefly on the authority of Bizzozero and Laker. Those who have repeated their experiments, and among others J. Arnold, have added an important piece of information; the more carefully the observations on the living mesentery are carried out, the fewer are the number of platelets. With reference to the existence of platelets in the capillaries of the patagium of the bat, one must not overlook the fact that this structure is examined with difficulty, since light has to pass through two layers of skin loaded with pigments and a lens of $\frac{1}{4}$ inch is the highest objective which can be used. A. F. Alcock has spent much time in attempting to verify Bizzozero's original observation, but in several bats, with every advantage of illumination, and using the highest apochromatic objectives which were possible, was unable to observe any platelets in the living blood. I am indebted to Dr Alcock for his kindness in giving me this information, which entirely accords with my own view, that the wing of the bat is a most unsuitable object for a study of the circulation in capillary districts; and the current idea that the platelets are extraordinarily difficult to see in any amount in human blood unless certain precautions are taken, that they are something like the explosive blood cells described by Hardy in the crayfish,[1] and, like these, rapidly disintegrate unless the drop of blood as it issues mixes with an indifferent or fixing

[1] Metchnikoff and Hardy noticed these explosive cells, which disintegrate within a few seconds owing to contact with glass. The products of the explosion appear to cause the discharge of other cells in their vicinity.

fluid, is the statement of an opinion rather than of a fact. It is not only the case that a drop of fresh blood may not show a single platelet, but it is capable of proof that the very methods which are supposed to demonstrate them, do actually produce as artefacts enormous numbers of bodies, which are certainly the bodies enumerated in blood-counts and described as morphological constituents of that fluid. Thus, speaking of the action of 33 per cent. potash, some observers have stated that in this fluid "the platelets are better preserved . . . but of no use, because of its destructive effect upon the red cell."

I hold the view that platelets do not exist in normal human blood, and the evidence which I will now give is in my opinion absolutely conclusive on this point. I may say this view is the exactly opposite one to that which I held for a long time, both before and after I began to work at the subject. Indeed, blood inside the body, and blood injured by contact with any surface which is wetted by it, are two absolutely different things. Blood alters with great slowness if received under oil into a vessel coated with vaseline, and the plasma which collects above the sediment of corpuscles may be stirred freely without injury, with an oiled glass rod. This experiment made by Freund in 1886 was subsequently confirmed by Haycraft, and this, together with many of the devices contrived by Löwit for his study on the origin of platelets, so as to avoid contact of the blood with glass, I have repeated.

But as neither oils nor odourless paraffin are indifferent fluids for blood, I use a method suggested by Neumann, in which the blood lies between two cover-glasses, one of which is half the diameter of the other. The lower disc hangs towards a moist hollow cell in the slide, and by vaseline the larger cover is fixed as a roof to the cell. Another and much better method of my own, so as to avoid any contact of blood and glass, is to examine blood-films on loops of thin platinum wire. It is not easy, but quite possible to obtain perfectly good films of blood which can be examined in a moist chamber, and in such films I find on examining with $\frac{1}{6}$-inch objective of long working distance, that as a rule not a single platelet is to be seen. In making films which shall show this result, not

only should the utmost cleanliness be observed, but the sterilised loop should not touch the surface of the skin, nor be swept violently through the drop of blood. Neither must the film be shaken. The advantages of the film method are obvious. Though exposed to moist air, the blood is touched to only a slight extent, and with a perfectly horizontal position the corpuscles are in a state of absolute rest. The film, in fact, is in a state of tension. If the film is vertical, the corpuscles subside within half a minute, so that a clear sheet of plasma can be observed. Attempts which were made to artificially produce platelets in these thin films were too difficult to repeat sufficiently often to afford assured results, but these film experiments do not support the view that platelets exist in normal human blood.

The statement that platelets are not seen is of course opposed by the equally unproven assertion that they disappear during the few seconds which elapse between pricking the finger and examination. But Bizzozero's own work, where he states that they can be collected by whipping blood with a bunch of wires, shows that this theory of their rapid disappearance is not in accord with the facts. Hayem also finds the platelets preserved for a long time, when blood is received into a capillary space between two cover-glasses, and kept at a temperature of 1° to 1·5° C. But any one who repeats Bürker's observations can satisfy himself that the current statement as to the rapid disappearance of the platelets in shed blood is incorrect. This observer allows a drop of blood to fall on to the surface of a perfectly clean paraffin block kept in a moist chamber. The drop of blood at a temperature of 18° C. does not coagulate for thirty to forty-five minutes, during which time the corpuscles settle, and plasma loaded with platelets forms a cap on the surface. This plasma layer can be removed, examined, and stained. Bürker regards these bodies as genuine morphological constituents of blood, and considers they are at first distributed in that fluid, and being lighter, subsequently rise to the surface.[1] The plasma therefore continues to show more and more plate-

[1] Blood-platelets are stated to have a high specific gravity (v. Limbeck, P. Schiefferdecker). This is not the case.

lets as time goes on. If a drop of blood prepared in this way is touched after thirty minutes with a cover-glass, the preparation shows myriads of platelets, leucocytes, and red discs in rouleaux. I find that a drop of oxalated blood shows a similar appearance, though no rouleaux are formed. Any stratum of the drop will also show platelets; and it is not from Bürker's facts but from his inferences that I dissent, for reasons which I shall attempt to make clear later on. In any specimens of blood which contain platelets, I am satisfied that they are bodies even more resistant than many red corpuscles to the action of haemolysing agents, and that it is not in accord with the facts to consider them as bodies whose rapid disintegration is a special feature.

Fresh blood cannot be examined without damage, even if oil is not an indifferent fluid, still less so are any of the suggested indifferent media. In my own blood I can succeed in making specimens which are entirely free from platelets, and then subsequently in the same specimen produce such a quantity that they equal or exceed the number given by those observers who have concerned themselves with an estimation of the absolute amount in a c.mm. of normal blood. Platelets produced in this way are undoubtedly the bodies described and figured by Deetjen, Kopsch, Puchberger, and others. That it is difficult to prove a negative, is an accepted truth; but after repeated failures by other methods, I have succeeded in satisfying myself and others to whom I have demonstrated the experiment, that whereas a wet specimen of blood generally does show a few platelets, a specimen made in the following manner does not contain a single one of these bodies. Though the methods suggested by Löwit and Wlassow have been unfavourably criticised, it is quite easy to make preparations free from platelets by carefully following their directions. In my own method both the slide and cover-glass are wetted with a drop of sterile human serum, and the finger is pricked through a large drop of serum placed on the thoroughly cleansed skin. The wet cover-glass touches the drop which is mounted; pressure of the cover-glass upon corpuscles is prevented by pieces of glass or mica. The specimen is rimmed with vaseline.

As a rule, not a single platelet is to be seen if this manœuvre is properly carried out and attention paid to the question of temperature, for both the slides and cover-glasses should be kept warm at a temperature of 40° C., or slightly above that of the body. Even after some five minutes only one or two suspicious objects, which might possibly be platelets, can be seen. If a control experiment is made exactly in the same way, except that the finger is pricked through a drop of meta-phosphate solution, an almost countless number of platelets is obtained. Either human blood-serum causes an entire disintegration of existing platelets in human blood, or Deetjen's fluid preserves them better than does serum, but the same line of reasoning applies to the use of 1 per cent. osmic acid, or 33 per cent. caustic potash, and it is difficult to believe that these fluids are capable of fixing in a c.mm. of blood half a million bodies which suddenly disintegrate in serum.

The real point at issue is this. Either certain fluids fix and preserve something pre-existent in living blood, or they cause the appearance of certain morphological bodies which are only visible after the fluid is added. The bodies are, in my opinion, artefacts, and have no existence in normal blood; should any film show a large number of platelets, this is not physiological but pathological. I do not deny that some specimens of blood may show a certain number, but only a few, which may be due to disintegration of the red corpuscles *in vitro*. Just as Burdon Sanderson, more than twenty years ago, indicated that damage to cells is the chief efficient cause in establishing that sequence of events which comprises inflammation, so I hold that platelets are produced both inside and outside the body by damage to the blood-plasma. To the actual chemical nature of these bodies I have paid no attention, but on the hypothesis that they are separations of proteids, their staining features may be contrasted with the staining behaviour of proteid precipitates, which is fully described by G. Mann in his *Theory and Methods of Physiological Histology*.

Doubts have been expressed whether the bodies described

as platelets by different observers are all of the same nature. For example, are the bodies figured by Osler and G. Eisen or Bizzozero, demonstrated by Deetjen, photographed by Kopsch, observed by Wooldridge, counted in normal and pathological conditions by Hayem and his school, and more recently by van Emden and Kemp, who finds that a c.mm. of blood at high levels contains an excess of platelets, identical morphological structures? Among the half-million platelets in a unit volume of blood, are some of these cells and others mechanical separations from plasma or conglutinations of proteid molecules? Though the platelets may, and actually do, vary in shape and aspect, I have come to the conclusion that while the vast majority are produced from the plasma (Deetjen's platelets), some, but a comparatively small number, arise from the red corpuscles, under conditions which I will presently describe.

It is easy to see platelets in enormous numbers. T. G. Brodie and A. F. Russel tried a large number of solutions with a view to the enumeration of these bodies; about thirty different solutions are enumerated in their paper, all, or most of which, on admixture with blood, show varying amounts of blood-platelets. Two views only are possible to explain the appearance of platelets, when blood and other miscible fluids come in contact: either pre-existent bodies are preserved, or bodies previously non-existent are manufactured. It is this question which requires an answer. Such fluids as solutions of peptone, 14 per cent. solution of magnesium sulphate (Bizzozero), Hayem's fluid, Determann's fluid, 1 per cent. osmic acid, 33 per cent. solution of caustic potash, solutions of formaldehyde or those which contain glycerine, can in no sense be regarded as either indifferent or normal; they are actually damaging to such a fluid as blood, and even the admixture of a drop of blood with one of isotonic salt solution probably at once alters the blood, so that a few platelets are to be seen. The fact that red corpuscles do not apparently alter their aspect, and that leucocytes may still show movements, is not a fair criterion that the blood-plasma is not, as I believe it is, profoundly altered. The fluids mentioned above, as well as those specially

devised by Hayem[1] and Marcano[2] and also by Acquisto[3], for demonstrating haematoblasts (platelets), when added to a film of blood that shows no platelets, can be seen to produce a crowd of these bodies.

It is admitted by everyone that the platelets are admirably shown by Deetjen's metaphosphate-agar medium, and for the sake of argument we may regard this as the accepted method for their demonstration. A drop of blood about 2 c.mm. contains about a million of these bodies, which, though liable to rapid disintegration, at any rate require more than one or two seconds for this to occur. If the Deetjen's medium is not used, an almost total destruction of these occurs in this space of time. What causes this? Not changes of temperature, since no precautions are necessary. The contact of the blood with air is the same in all experiments. The contact of glass with the blood might cause this, but it is seen that the platelets adhere to glass and are not destroyed. Now, since in my opinion the bodies which appear when the edge of a drop of drawn blood and Deetjen's fluid metaphosphate-medium, or Affanassiew's fluid, or 1 per cent. oxalate of potassium, are identical in all respects with Deetjen's bodies on agar, I consider that they arise from an admixture of blood with the medium. Many observers do not deny that these precipitates

[1] Hayem's fluid :—

Distilled water	200 c.c.
NaCl	1 gramme
Na_2SO_4	5 grammes
Iodine in iodide of potassium	3·5 c.c.

The last is made by an excess of iodine in 5 per cent. solution of potassium iodide.

[2] Marcano's fluid :—

Na_2SO_4 solution in water, specific gravity 1020	100 c.c.
Formaldehyde, 40 per cent. in water	1 c.c.

[3] Acquisto has employed a fluid with the following composition : Eight parts of water are added to one part of a mixture of equal volumes (1 c.c.) of ·5 per cent. chromic and picro-sulphuric acid, 1 per cent. mercuric chloride, and 33 per cent. glacial acetic acid in 67 parts of alcohol. In this fluid it is stated that the platelets are well preserved. Those in the blood of the triton and salamander may divide by karyokinesis. Human platelets are, however, in no sense homologous with those of amphibia.

have the greatest resemblance to platelets; the difficulty in distinguishing one from the other may be due to the fact that they are the same bodies.

Here it may be mentioned that the change of temperature in a drop of blood is probably responsible for the clumped bodies which may be seen in blood films. These are groups of platelets which I believe clump together simply in consequence of a fall in temperature. Observations on this point are not yet completed; but in conformity with other work, cooled human blood-plasma alone shows typical clumps of platelets. If blood films be prepared on slides and cover-glasses warmed to the temperature of the body, and the entire preparation made and observed at a temperature of 37° C., then no platelets of any kind are seen at first, though some may appear as the blood dies and coagulates; but even this is not constant.

I soon noticed that the number of platelets produced by different fluids varied considerably with the amount of the diluting fluid; as a general procedure, about twice the volume of fluid was added to a drop of my own blood. The diluting fluids most frequently used were Deetjen's fluid without agar, 1 per cent. ammonium chloride, 1 per cent. potassium oxalate, 1 per cent. sodium chloride, 2 per cent. citrate of soda, 1 per cent. calcium chloride, 10 per cent. potassium iodide. The question of the relative permeability of the red discs to these fluids has been discussed in a former lecture, and, moreover, only partial information can be obtained by microscopic work. If a drop of human blood be placed on a slide and allowed to come in contact by its edge with a drop of 1 per cent. potassium oxalate twice the size, a hair free from fat being placed in the drop so as to avoid pressure of the cover-glass, and then examined immediately, a steadily increasing number of platelets like those described by Deetjen, and figured by Kopsch, Puchberger, and others can be seen to spread out from the edge of the blood; and as the fluids mix and the corpuscles settle, it is easy to satisfy oneself that these bodies do not at first adhere in any quantity to the cover-glass, but are forming and streaming about in a stratum above the red discs.

Parts of the specimen where the blood alone exists show no platelets until the diluting fluid has diffused so far. Often the platelets remain quite free from each other, or only two or three hang together. Large clumps are often entirely absent; when they do occur I can offer no explanation, but it may be due to an imperfectly cleaned glass.

Identical appearances occur with Deetjen's fluid, or 1 per cent. ammonium chloride, but the platelets in the latter show fewer amoeboid bodies. The most certain fact in these observations is that it takes time for the platelets to appear; at first a few, subsequently more, finally large numbers are seen when the fluid slowly mixes with the blood, the last stage however is rapidly reached—in about a minute, if the blood is placed on a thin sheet of metaphosphate agar.

Apart from this phenomenon, which is one connected with the blood-plasma, oxalate of potassium possesses the power of inducing a change in the red discs, which resembles but is not quite similar to that described so fully by J. Arnold. This observer used 10 per cent. potassium iodide; the blood was diluted ten times, and kept in sealed tubes for varying lengths of time. Such preparations show numerous platelets, and the extrusion of processes from the red discs can also be observed. Many of these are granular bodies provided with fine filaments, by one of which they may remain connected with the disc for a long time. After fixing drops of the mixture in osmic acid, Arnold then embedded in celloidin, cut sections, and stained them in a variety of ways by triacid, methylene-blue, eosin, and Weigert's fibrin stain. A large number of these separated bodies stain with eosin and show a bluish central part, which stains with basic dyes. Max Schultze, Hünefeld, Hayem, and Stricker, to mention only a few observers, have also pointed out that the red corpuscles can be induced to alter their shape and extrude processes, and any one who has performed Leishman's method of estimating the phagocytic power of leucocytes has probably, as I have myself, often seen the phenomenon described by Arnold.

The influence of poisons within the circulation also produces changes in the shape and aspect of the red disc, parts of which

fragment off, and Schwalbe has figured those which are produced by injecting toluylendiamine into the vessels of dogs. Besides an intense anaemia, icterus, and blood destruction, the extrusion of bodies which may or may not be cut off from the disc occurs in the circulating blood. There is little doubt that these are comparable to Arnold's bodies, some of which have been termed a basic nucleoid or inner-body, that has been described as occurring in the red disc at a certain stage of its existence. Schwalbe considers these extruded bodies are identical with Deetjen's blood-platelets, and believes that all varieties of platelets can arise from the red discs. He has also seen them arise from leucocytes, when blood is examined in thin sections of pith, a method which was devised by Arnold, and independently by myself, so as to avoid any pressure on the blood.

The views of Wlassow, Schneider, Preisich, and Heim are to some extent in accord, since these observers regard the platelets as arising from the red discs. Wlassow denies their existence in normal blood, and especially emphasises their absence in specimens, where precautions are taken to examine films of blood between glass surfaces smeared with a viscid layer of paraffin and vaseline, so as to prevent any destruction of the red corpuscles. On the other hand, damage to the blood by mechanical injury to the wall of a vein gives rise to the formation of a white thrombus, which is formed by an adhesion of red corpuscles to the wall of the vessel; these next extrude granular bodies (platelets) which are nucleo-proteid in nature, and by collecting together form thrombi. If fresh blood is treated with a solution of saturated mercury perchloride diluted five times with water, the fragmentation of the red discs and extrusion of platelets rich in haemoglobin can be observed (Wlassow-Sacerdotti phenomenon). This in my opinion is exactly comparable with what is seen in blood after poisoning with nitro-benzol; many of the red corpuscles then resemble those in the figures of Preisich and Heim, which show one to three stained bodies within a red corpuscle, from which they may subsequently be liberated as platelets; these they consider to be nuclear material, but in specimens stained to demonstrate

the haemoglobin-content of the corpuscles they appear to me to be condensed masses of haemoglobin which correspond to a nucleoid (Pappenheim). Apart from the nucleoid, a red disc may show other inner-bodies which may also be extruded.

In the opinion of Preisich and Heim the extruded platelets stain like the granules of polymorphonuclear cells, and the

FIG. 8.—Aspect of human blood corpuscles treated with Wlassow's fluid. The disrupted and partially extruded pieces of the red corpuscles stain intensely with stains that are specific for haemoglobin, such as that used by Israel and Pappenheim.

largest may equal the diameter of a red disc, or even exceed this in the blood of pernicious anaemia.

As far as an opinion may be formed on the work just described, it seems that we are simply presented with facts relative to pathological rather than physiological processes, and that even if platelets do arise from the red corpuscles it is an abnormal and not a normal phenomenon. Differential staining is an insecure basis for drawing deductions as to the origin of these doubtful bodies, even if the structures described by various observers are of uniform nature, which is by no means certain.

My own observations on blood *plus* oxalate of potassium have brought out distinctly the difference between living plasma and dead serum. It is a pure assumption, but one that is often made, that plasma and serum are two nearly similar fluids. Our knowledge of the former is almost as meagre as is our knowledge of protoplasm, and the reasons why this is the case are sufficiently obvious.

If a drop of oxalate of potassium, or Deetjen's fluid, be placed on a slide between isolated drops of blood and serum, and covered so as to avoid pressure, then the three fluids give two contact edges, and a convincing proof is obtained that platelets only appear where the blood and oxalate touch. Their comparatively slow formation can also be observed.

About half an hour later the platelets will be found to have assumed a shape which for a long time led me to think that some of them were extruded from the red corpuscles. Tailed bodies are seen in large numbers, generally separated from each other. Some possess two to five long processes, four or five times the diameter of a red corpuscle; sometimes a platelet possesses a set of them, so that in miniature it resembles the spermatozoon of a crayfish. The following figure, from a stained preparation, gives a picture of the aspect of these bodies, which are certainly transformations of the platelets, for two reasons:—

(1) The change can be followed if the specimen is watched for about twenty or thirty minutes.

(2) If the platelets are collected alone free from corpuscles these bodies are easily seen while the deposit of corpuscles never shows any forms like these. In making permanent preparations I never press or smear the blood, but simply allow the fluid to dry in the air, otherwise distortion and damage to the disc frequently occurs.

Leishman's stain demonstrates these bodies perfectly. They are for the most part pin-shaped; the head stains a deep purple, the stem is much fainter. Any stain which demonstrates the mast-cell granules picks out these bodies with great distinctness. The tailed platelets may lie together in a small group, but generally they show no tendency to stick together or adhere to the glass. The filament or filaments cannot possibly be

fibrin, unless we allow that a formation of this occurs in oxalate plasma. (*Cf.* Osler's plate, *Proc. Roy. Soc.*, 1874.)

Earlier workers at oxalate plasma have shown that cell-free oxalated frog's plasma remains non-coagulable even if calcium be added. From mammals Druebin could not obtain a cell-free oxalate plasma, but notes that the more platelets

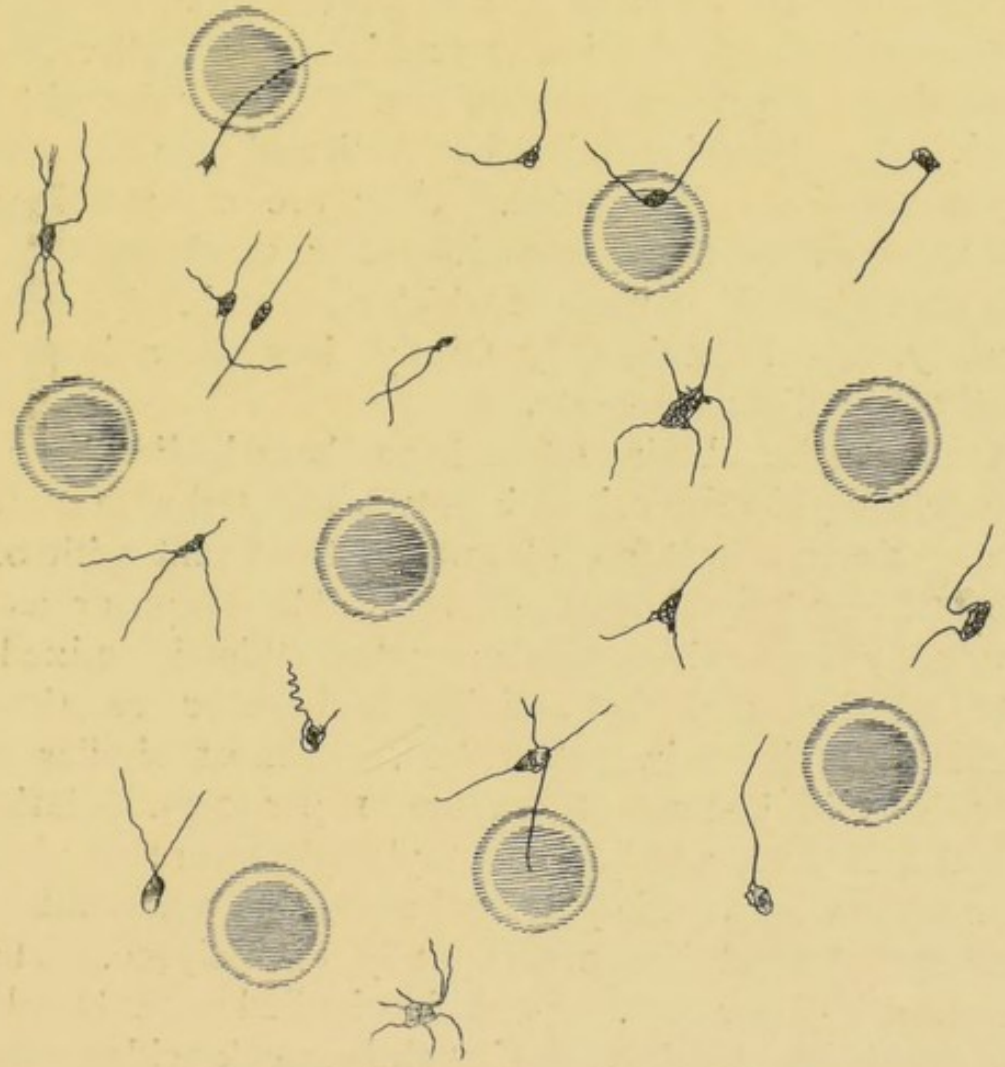


FIG. 9.—Appearances in blood half an hour after mixture with 1 per cent. potassium oxalate. These tailed structures were originally platelets or Deetjen's bodies.

there are in the fluid the better it coagulates with calcium salts. Serum yielded no platelets of any kind whatever when mixed with varying preparations of oxalate.

There are indeed, I believe, two kinds of platelets, both of which are artefacts, in that they do not exist normally in plasma (never in serum), and the statement as to their absence in lymph is often quoted. Oxalated blood shows (1) bodies which

resemble Deetjen's platelets; (2) bodies which can be shown to be extruded from the red discs.

The first may be spoken of as separations from plasma, the second as extrusions from the red corpuscles.

The first type of platelets may occur in vast numbers, and are of lighter specific gravity than the red or white corpuscles collecting above the layer of leucocytes when blood and 1 per cent. oxalate in the proportion of 1 : 4 is centrifugalised. Some platelets clump together, some do not. They stain with basic dyes, give the Mylius' reaction for alkali, and often the so-called glycogen reaction with iodine. They are clearly shown by Rabl's method, by methylene-blue after osmic acid, and by Unna's methylene-blue, none of which dyes stain the red discs. Conversely, Israel's stain only faintly tints these bodies, but stains the red discs intensely.

Plasma can be obtained free from these bodies by rapidly centrifugalising blood received into tubes wetted with castor oil. Such a specimen of cell-free plasma on treatment with oxalate shows the irregularly shaped platelets. These appear as usual in gradually increasing numbers when this is mixed with oxalate solution, and the staining and other reactions are identical with those just described. Almost similar results are obtained if, instead of oxalate, 1 per cent. solutions of KCl, NaCl, NH_4Cl, $CaCl_2$, or metaphosphate are employed in suitable proportions. They are to be seen in great numbers, and persist when the red discs are carefully haemolysed. This can be observed if ·6 per cent. peptone in distilled water be added to blood. Weak alkalies do not affect them, weak acids cause them to alter somewhat in aspect and partly to disappear. Time does not allow me to enter into the probable chemical nature of these plasma separations.

Among the deposit of red corpuscles in a centrifugalised mixture of blood and oxalate, quite different bodies can be seen. They are extruded from fresh red corpuscles, but are not to be seen in corpuscles obtained from clotted blood. The process of formation of platelets can easily be followed. In about half an hour after blood and oxalate is mixed, even at ordinary temperatures, but still better at 38° C., the extrusion of these bodies from

the discs can be seen in progress. Only a certain number of corpuscles exhibit this phenomenon. I think this is possibly due to the different ages of the corpuscles, for in any specimen of blood some cells must be young and some senile.

As far as staining may be relied upon, some of these extruded bodies, but not all, appear to stain better with logwood than eosin. Some also appear to possess a central particle, the so-called inner-body (Immermann) or nucleus of some observers. However, into this disputed point I will not enter; what is quite certain is that these bodies contain haemoglobin. Apart from their origin, these platelets always are developed later than those separated from plasma, but I am not certain whether a few red discs may not disrupt directly blood is shed, though I have never happened to see this in normal blood. In pernicious anaemia fresh blood may give this impression, schistocytes conceivably originate in this way (Ehrlich).

I have never seen, nor am I acquainted with any entirely satisfactory evidence that any one else has seen, the leucocytes give rise to platelets, though no doubt the fact is possible, and Hirschfeld considers this does take place in myelogenous leukaemia. Most observers, though they recognise the differences in the aspect of the platelets, have not distinctly indicated that these differences are related to their mode of origin.

It is certain that platelets may pre-exist in pathological blood; experimental evidence is conclusive on this point; but either profound destruction of the blood corpuscles must occur, *e.g.*, poisoning with pyrogallol, phenyl-hydrazine, salts of lead (P. Schmidt), or injuries to the vessel walls or to the plasma, as by transfixion of a vessel with a needle (Zahn), may cause the pathological appearance of platelets.

The results of my own observations satisfy me that the platelets are artefacts or pathological bodies, which, according to their origin, fall into four groups:

1. Platelets containing haemoglobin.
2. Platelets destitute of haemoglobin.
3. Platelets with an inner-body.
4. Platelets without an inner-body.

According to its origin from the living plasma, or living

red disc, so does each individual platelet possess its own features, which it shares with others of similar nature. In conclusion, the following statements embody the views I hold as to the nature of the platelets :—

1. Normal living blood contains either none at all or extremely few platelets, the few bodies which may be seen are generally clumped. They separate from plasma, partly by contact with foreign bodies, partly on account of a lowering of the temperature from 37° C. to one of 18° to 20° C. Blood commences to change or die immediately it leaves the body.

2. The addition of so-called fixing and indifferent fluids does not preserve but produces enormous quantities of platelets, or the structures which are described and enumerated as platelets.

3. Under certain conditions platelets continue to make their appearance for some length of time after blood is shed. The shape, aspect, and numbers of such bodies vary somewhat with the nature and amount of the diluting fluids.

4. With Deetjen's metaphosphate, the platelets appear very suddenly and are nearly all of the same aspect. Many of them conform to an amoeboid type.

5. With oxalate of potassium one type of platelets which resemble Deetjen's bodies form from the plasma. About half an hour later they have assumed the aspect of pin-like and tailed bodies. Subsequently extruded bodies may arise from the red discs. These fragments stain differently, and resemble the bodies found in coagulating blood after the administration of certain blood poisons. It is just conceivable that a few of these latter bodies may exist in normal blood. In abnormal blood they certainly exist. There is no evidence given by my experiments that the leucocytes fragment.

6. No convincing proof has yet been given that normal human blood contains a third morphological element.

LECTURE VII

THE GUAIACUM, HAEMIN, AND BIOLOGICAL TESTS FOR BLOOD

IT must be admitted that, until a few years ago, none of the proposed methods by which attempts were made to give a positive opinion as to the precise nature of any blood could be regarded with much favour. The differentiation of human from mammalian blood is difficult, if not impossible, from a study of the average size of the chromocytes, which differ in various mammals, though it is easy to recognise the biconvex corpuscles of the Camelidae and the minute discs of the musk-deer. The idea of M. Bethe, that preparations of the blood of man might be distinguished from those of other mammals, depends upon the well-known fact that in any specimen of blood the red corpuscles vary in size. If the corpuscles for any animal are classified according to their size, and the percentage determined for each class, a nearly constant proportional graphic curve will be obtained. These curves were found to vary for different animals, though the curve for man closely resembled that for the guinea-pig, and by this means it was claimed that preparations of human blood and those of other animals might be distinguished from each other. I have mentioned this attempt on the part of an excellent histologist to solve this question, but it is rather of the nature of a histological feat than a valuable and practicable method, and it is certainly much easier to distinguish the blood of a cat or guinea-pig from that of a man, by attention to the features of stained leucocytes, than to the size of red

corpuscles (Hirschfeld). Moser has also suggested the possibility of forming a judgment on this point, from a study of the shape and size of haemoglobin crystals in different animals, a subject which has also been investigated by Henocque. To my knowledge, however, but little use has been made of this method, which is one that evidently can have but a limited application, when it is realised that the crystallisation of haemoglobin and its derivatives often depends upon a variety of favourable conditions; and in the case of some animals this proteid often will not crystallise at all, or does so with difficulty.

In a mixture of the red corpuscles of two animals of different species, the corpuscles of one can be laked by a given haemolytic serum, and Deutsch has even proposed this as a method for identifying a specimen of blood, but this method is clearly inapplicable except in those few instances where the corpuscles have undergone no change.

Among the various tests for blood, that known as the guaiacum reaction is of considerable interest, and as I shall hope to show, is entitled to a much higher rank as a positive test than is generally allowed. Previously to 1842, when the absorption-spectrum of haemoglobin was discovered, the haemin test was chiefly used for the detection of blood-pigment, and with the introduction of the guaiacum test the general consensus of opinion inclined to the view that its real value as a test for blood depended rather upon a negative than a positive result. It was, and is still, taught that the production of a blue colour was not a certain proof of the presence of blood, but, if a suspected fluid did not yield the colour, probably blood or haemoglobin was absent.

Tincture of guaiacum used for this test should be made from the unoxidised central portions of the resin. When made, the solution should be kept in the dark. Among other substances, three acids, guaiacetic, guaiacic, and guaiaconic, have been isolated by Oscar Doebner and Lücker. The blueing of guaiacum tincture is due to the oxidation of guaiaconic acid, $C_{20}H_{24}O_5$, to guaiacum-blue. To this substance, prepared in quantity by the action of Fe_2Cl_6 on guaiaconic acid, he gives

the formula $C_{20}H_{22}O_6$. The oxygen in guaiacum-blue is easily lost at 100° C., and also by the action of a number of oxidising agents. Doebner regards guaiaconic acid as a condensation product of tiglic aldehyde and phenols. That solutions of guaiacum became a sapphire-blue colour on oxidation, was originally discovered by Schönbein in 1847. Guaiaconic acid is a yellow powder soluble in chloroform, ether, alcohol, or 36 per cent. of spirit. The solution in chloroform alone easily becomes blue by shaking with air. An alcoholic solution of guaiaconic acid is oxidised to guaiacum-blue by—

1. Various inorganic substances. With iodine, chlorine, bromine, nitric or chromic acids, ferric chloride, salts of copper, and many peroxides, but not persulphates, guaiaconic acid yields a blue colour, which may be seen to develop at once, or after a few minutes.

2. By living cells, or by their intracellular enzymes. By extracellular enzymes, which act best, not in a neutral or slightly alkaline medium, but in an acid one.

3. By the action of certain substances which decompose H_2O_2. Some of these are enzymes (catalase, peroxydase), the whole group of which possesses this property, others are metals in a finely divided state or in colloidal solution.

With reference to the last group, according to O. Loew, catalases are enzymes in vegetable and animal cells (especially those of the liver, while the liquids of the organism possess but little—F. Batelli and Mdlle. L. Stern), which decompose H_2O_2 with evolution of molecular oxygen. He considers that H_2O_2 is a metabolite produced by oxidation, and subsequently decomposed by catalase. Among the enzymes we may distinguish (1) direct oxidases, which are specific; (2) peroxidases, or indirect oxidases, which decompose H_2O_2 and turn guaiaconic acid blue; and (3) catalases, which act in a similar way, but do not blue guaiaconic acid. Pieces of potato which give no blue reaction with guaiaconic acid do so at once on the addition of a drop of H_2O_2, and the following experiment, which is uniformly successful, is of interest. A piece of potato that by itself does not oxidise guaiaconic acid is wetted with this, five or ten minutes later it is dropped into a test-tube, covered with H_2O_2

and alcoholic guaiaconic acid floated on to this liquid. The potato turns blue (peroxydase action), the liberated oxygen (catalase action) ascends through the liquids, but the supernatant acid is unaffected and does not turn blue.

Not always, but occasionally, it is possible to repeat the following experiment by A. Schmidt, which, twenty years ago, was considered to support his view that the oxygen linked to haemoglobin possessed the properties of ozone. When a piece of filter paper was moistened with tincture of guaiacum, and a drop of blood diluted 1 : 20 was added, a blue stain appeared on drying in the air, which did not occur if haemoglobin had lost its respiratory oxygen, or if this had been replaced by carbonic oxide. It is said that this reaction does not succeed with some specimens of guaiacum, but I have satisfied myself that the test succeeds or fails, not because of differences in the guaiacum, but on account of the richness or poverty of the blood in leucocytes; and I believe it is due to the presence of guaiac-oxydase in the polymorphonuclear cells; remove these, and the blood will no longer give a blue colour.

The guaiacum test where the tincture, together with either ozonic ether, hydrogen peroxide, or oil of turpentine which has been exposed to the air, is used, is an exceedingly delicate reaction for blood. In Germany the test is known as van Deen's reaction, and, as I have seen, is generally performed with the use of turpentine-oil. In carrying out the test, an alcoholic solution of Merck's guaiaconic acid has many advantages over an alcoholic solution of the resin. To the suspected fluid add about one-eighth its volume of hydrogen peroxide (20 vols. per cent.),[1] mix, and at once float on to the surface a solution of guaiaconic acid, when a blue colour will commence to develop just above the resinous ring and slowly diffuse into the clear zone above this. I find that with a dilution of blood 1 : 10,000, an amount of this dilution containing 200 red corpuscles will suffice to give the distinctive blue colour. The test succeeds with old blood-stains, putrefied blood, CO-haemoglobin, and

[1] Though not necessary for the ordinary test, in my own work I have used chemically pure hydrogen peroxide. That generally employed is strongly acid to litmus, and contains hydrochloric acid.

methaemoglobin. If a weak solution of blood is boiled, the brown filtrate of impure haematin gives a positive result. Haemin crystals suspended in water, ethereal solutions of acid haematin, and solutions of haematoporphyrin, give the guaiacum test. Solutions of chlorophyll, from which substance a crystalline phylloporphyrin almost identical with haematoporphyrin can be obtained, or of bilirubin or haematoidin, do not give the guaiacum reaction. I find also that absolutely pure haemin crystals prepared by Schalfijew's method and recrystallised from solution in chloroform and pyridin, give the guaiacum reaction. Under the microscope a few hundred dried or fresh blood-corpuscles or crystals of pure haemin can be seen to cause the liberation of oxygen—the gas is not ozone—from hydrogen peroxide.

A current explanation of this test is, that some constituent of the red disc—in my opinion haemoglobin rather than any cell proteid—a nucleo-proteid, but certainly not an enzyme, liberates oxygen ions, which then oxidise guaiaconic acid to guaiacum-blue. It is evident that in order to understand the guaiacum test as applied to blood, the behaviour of this fluid or its pigment to H_2O_2 must be considered. It is known that solutions of hydrogen peroxide slowly change into water and oxygen, but a more rapid and visible decomposition is produced by such inorganic bodies as colloidal platinum, peroxide of lead, platinum black, and manganese dioxide. Living cells, and most, if not the whole group of enzymes, effect the decomposition of hydrogen peroxide, though no evidence at present exists that the specific actions of any of the latter are in any way related to their catalytic effect on H_2O_2, which is ascribed by O. Loew to their contamination with another ferment, known as catalase. Molecular oxygen is evolved from peroxide of hydrogen with varying degrees of intensity by a large number of complex substances, such as the blood of all vertebrates,[1]

[1] Some observations by Cotton are of interest. He states that when 1 c.c. of the blood of different animals, as man, horse, pig, or guinea-pig, are treated with 12 c.c. of hydrogen peroxide (18 vols. per cent.) and the oxygen evolved from this mixture is collected, great and constant differences are found in the quantity of gas which is liberated. His figures in c.c. are, for the horse 320 to 350, guinea-pig 115, pig 300 to 320, and for human blood 580 to 610. However, it is certain that the relations of blood to peroxide are exceedingly complicated, and until considerably more is known

milk from any animal, fibrin, some kinds of pus, watery infusions of malt or potatoes, and, as I have found, by pure haemin crystals. Senter considers that haemoglobin itself does not appreciably affect H_2O_2, but that blood decomposes this at 0° C., since it possesses a catalytic enzyme or haemase which is easily destroyed by hydrocyanic acid, an action which certainly is not noticed in the case of all other ferments. On a superficial examination the change undergone by peroxide is identical, no matter which of these substances is employed; but this is not so, for, with the exception of blood or haemin, the action of all the bodies just enumerated is permanently destroyed by boiling or by heating to 65° C. for one hour. A current hypothesis offered as an explanation is, that in the case of milk, pus, or potato, a ferment (peroxydase or catalase—Loew) action takes place which is modified by temperature, the presence of electrolytes or alcohol, while in the case of blood, some constituent of the red corpuscle, such as nuclein or histone (Spitzer), effects the decomposition of H_2O_2. I think that a slight evolution of oxygen may be more advantageously observed under the microscope than in a test-tube; but apart from the fact that free molecular oxygen is disengaged by fresh milk, it is easy to prove in both boiled and unboiled milk that there is, apart from any ferment action, a slow disappearance of peroxide, due to the oxidation of some other constituent or constituents. Just as the enzymes and anti-enzymes of blood serum appear to be linked respectively to euglobulin and albumin (Hedin, Landsteiner, Cathcart), so the peroxydases of milk are attached to caseinogen (Raudnitz), or at any rate are carried down with this proteid when it is precipitated by acids or salted out.

The oxygen liberated from peroxide, if molecular oxygen, does not oxidise guaiaconic acid. This I can show by decom-

about the nature of the reactions which take place, the above observations, some of which I have repeated, are only of interest in showing that probably the evolution of oxygen from peroxide by blood is due to some chemical change rather than to a catalytic action by the blood. In some respects mixtures of blood and peroxide behave like mixtures of blood and milk; the peroxide is in part decomposed with liberation of oxygen, while some of the oxygen of the peroxide is employed in the oxidation of substances in these fluids (Raudnitz).

posing H_2O_2 with potato or blood and allowing the disengaged oxygen to pass through a porous disc of plaster of Paris, which blocks one end of a test-tube containing guaiaconic acid, which under those conditions never becomes oxidised to guaiacum-blue. This experiment is of interest in another way, for if the mixture of blood and peroxide be now mixed with guaiaconic acid by pushing out the plaster disc, no blueing occurs, though it does so immediately if a minute amount of diluted blood is added. I find that a mixture of blood and H_2O_2 in contact for a few minutes absolutely loses the power of blueing guaiaconic acid, even though quantities of oxygen are being evolved. The test, therefore, depends not on a catalytic action exerted by some constituent of blood, but an undoubted chemical change is produced on the blood by peroxide, and *during* this change the oxidation of guaiaconic acid occurs. What the change actually is, I cannot at present definitely state. Peroxide of hydrogen always contains a trace of hydrochloric acid, but it is immaterial for the above experiments whether commercial or chemically pure peroxide, prepared by distillation in a vacuum, be used for the experiments just described.

Schönbein, the discoverer of ozone, showed that by the action of blood corpuscles hydrogen peroxide breaks up into water and oxygen, but that this does not occur in the presence of small amounts of hydrocyanic acid. As a deduction from this experiment, it was believed that hydrocyanic acid destroyed the function of the red corpuscles, so that these could no longer fix oxygen, a conception which subsequently appeared to be established by the researches of Geppert, who showed that when animals were slowly poisoned by HCN, all the cells of the organism lost the power of fixing and of utilising oxygen, even in the presence of an excess of this gas. Assuming that this view is probably correct for living cells, it is, I believe, no explanation of the effect of hydrocyanic acid on the red corpuscles. Since hydrocyanic acid is one of the most remarkable poisons with which we are acquainted, for a single drop of the pure acid may kill with almost the rapidity of a lightning stroke, I thought that a re-examination of its effect on the red corpuscles would be of interest. The addition of small

or large amounts of 2 per cent. solution of hydrocyanic acid that was freshly prepared, caused no obvious change in the appearance or spectrum of solutions of defibrinated blood that were made by diluting blood with ·9 per cent. sodium chloride. Moreover, the reduction and re-formation of oxyhaemoglobin is apparently in no way impaired by even a large amount of hydrocyanic acid, and the amounts of oxygen obtained from defibrinated blood by the pump are the same as that from blood treated with hydrocyanic acid. We must therefore believe that if the exchange of oxygen between the blood- and tissue-cells is destroyed by hydrocyanic acid, this does not at the same time impair either the association or liberation of oxygen by the corpuscles. When blood treated with HCN is tested by guaiaconic acid and peroxide of hydrogen, the reaction may succeed or fail. According to Kobert, the following reaction occurs—

$$2HCN + H_2O_2 = C_2H_4N_2O_2$$

oxamide is produced. It would seem that the guaiaconic acid is protected from oxidation unless an excess of H_2O_2 is present.[1]

Without denying the existence of oxydases in blood that may *in vitro* turn guaiacum blue, the work of Brandenburg, most of which I have repeated, may require us to modify our views as to the distribution of a specific oxydase ferment which can convert guaiaconic acid into guaiacum-blue, and Rosell in Hofmeister's laboratory was unable to find guaiacoxydase in the blood, pancreas, or other organs, though peroxydases which decomposed hydrogen peroxide, or oxydases that acted upon iodo-phenol, were present.

Outside the body certain cells can undoubtedly oxidise guaiaconic acid, guaiacol, or salicyl-aldehyde, and this action is probably due to a ferment. In other cases substances possess the power of decomposing H_2O_2, and such a property may or may not be destroyed by boiling; but if destroyed, though a

[1] The few observations which exist show that hydrogen cyanide has practically no effect on the hydrolytic action of enzymes; in the case of emulsin the hydrolysis is slightly accelerated (Aders Plimmer). However, a retarding influence of the poison on catalases and inorganic ferments (Bredig) is stated to occur. The fact that hydrocyanic acid does not inhibit the reaction between blood, peroxide, and guaiaconic acid, is an additional piece of evidence which shows that this is not a catalytic action.

ferment action may be inferred, it is not necessarily proved, as the following considerations will show.

The direct blueing of guaiaconic acid, not only by blood (A. Schmidt, Klein, Brandenburg, E. Meyer), but also by pus, saliva,[1] milk, semen, by extracts of the spleen, brain, muscle, and ovary, has been regarded as a function of an enzyme (guaiacoxydase) which is destroyed by heat, and belongs to that group of oxidases which includes tyrosinase (Bertrand, Gonnermann), which was obtained from dried mushrooms macerated in chloroform-water, or from the seedlings of Lupinus albus, and shown by Gessard to exist in quantity in the glandular tissue which produces the ink of Sepia officinalis; tyrosinase converts tyrosin into homogentisic acid, and this ferment is probably concerned in the pigmentation of the skin (Gessard, Durham). Another oxidase, known as laccase (Bertrand), changes hydroquinone to quinone. This is probably a catalytic action, since laccase effects the oxidation of hydroquinone to an extent that is dependent upon the amount of oxygen supplied.

I have never succeeded in obtaining the test with saliva, even though human saliva almost constantly contains KCNS, and sulphocyanates directly change guaiaconic acid to guaiacum-blue. With pus, the test may or may not succeed, and a specimen of pus that does not give it, may do so twenty-four hours later, and again in twenty-four hours the test may fail. The presence of reducing enzymes (reductases), such as those described by J. de Rey-Pailhade of Toulouse, in 1881-88, Olivier, and Pozzi-Escot, may possibly explain the failure of the test with pus. Mr J. A. Gardner and myself have found that perfectly fresh milk will give a blue colour if violently shaken for some minutes with guaiaconic acid, but the test only succeeded with milk despatched from the country in sterile bottles. No sample of London milk that we have examined ever gave this test.

Since I have made some observations on the guaiacoxydase of potato with the idea that they might throw some light on the behaviour of the oxydases of blood and pus, I will briefly

[1] In examining urine for blood pigment, no saliva must be present, nor must the patient be taking any salt of iodine (Mahomed).

mention a few of them. The guaiacoxydase of potato is distributed chiefly in the cells just beneath the corky layer of the tuber, and the amount or activity of this varies with the different races of potato-plants. From my own observations, I may mention that if we assume, as is probably the case, that living cells are destroyed by chloroform, arsenious acid (saturated), half-saturated perchloride of mercury, saturated ammonium fluoride, and a temperature of 65° C., then after treating the cortical layer of cells just below the corky layer of potatoes by any of the above agents, a guaiacoxydase can be subsequently extracted with water. This is also the case when the experiments are so conducted that the cells are *immediately* killed by shredding the cortex of the potato *under* the fluids. Unfortunately this neither proves nor disproves that the ferment exists as such in the cells. The action of this ferment in the acid liquid disappears when the reaction becomes markedly alkaline to litmus. The activity of the oxydase in the extract is not impaired by the addition of chloroform or perchloride of mercury, but is checked at 0° C., and destroyed at temperatures above 70°. Living potato does not blue guaiaconic acid if the cells are anaesthetised with chloroform vapour, or if they are deprived of oxygen (10 mm. barom. press.). The ferment extracted from potato is of course not inhibited.

In 1887 Vitali showed that pus turned guaiacum resin blue, but red corpuscles did not do this unless turpentine oil, ozonic ether, or peroxide of hydrogen was also added. It is easy to see that the reaction with pus is destroyed by previously heating to 65° C.; that with the red corpuscles is not, even by prolonged boiling. Various vegetable extracts, such as malt or potato-rind, behave like pus, and the action is destroyed at 65° C. Brandenburg considers that the effect of pus upon guaiacum-tincture is due to the nucleo-proteid of polymorphonuclear pus cells, but this proteid when separated from other organs such as the spleen, thymus, or liver, is found to be incapable of alone oxidising guaiacum, though the various nucleo-proteids, since they decompose H_2O_2, yield a blue colour with guaiacum and ozonic ether. In the case of organs rich in lymphocytes, like thymus or lymphatic glands, the guaiacum test, as Brandenburg

was the first to show, is negative, but organs rich in myelocytes or polymorphonuclear cells give an intense blue coloration. The marrow, in cases of pernicious anaemia, myelogenic leukaemia, sections of chloroma neoplasms, or edges of rapidly growing tumours, yield the guaiacum test. Even with ·04 c.c. of the blood of myelogenic leukaemia the test will succeed, if the leucocytes are collected on a filter paper and then touched with tincture of guaiacum. The nucleo-proteid which can often be separated from the urine in cases of leukaemia, icterus, or in febrile conditions, is probably derived from the kidney rather than the leucocytes, if the guaiacum test is any criterion, since the nucleo-proteid separated from urine does not turn guaiacum-tincture blue, while pus in urine or the nucleo-proteid prepared from it, will do so, provided care be taken to free it from the reducing substances which are constantly present in urine.

Brandenburg's work has received its fair share of hostile criticism (Klein and others). Any one who has worked at the blueing of guaiacum by living cells or their enzymes, is familiar enough with the difficulty of obtaining constant results. Some but not all of Brandenburg's results I can confirm, and therefore incline to the view that a ferment-like action is concerned. Recently Erich Meyer has demonstrated that a drop or two of diluted blood from a patient with myelogenous leukaemia can oxidise guaiaconic acid, a reaction which is a function not of lymphocytes but granular leucocytes.

In my opinion, the guaiacum test for blood when carried out with guaiaconic acid and H_2O_2, as a test for haemoglobin or any of its derivatives, exceeds in delicacy any single test with which I am acquainted, and until some other substance than haemoglobin can be shown to give this reaction when present in minute quantities, I believe, contrary to those who have been content to repeat the current statements of text-books, that, properly carried out, the test is absolutely distinctive for blood-pigments.

The following table proves that the reaction is exceedingly sensitive. A small amount of blood, laked with distilled water, is diluted in a haematinometer until the left absorption band just disappears. The solution is halved, and one portion con-

verted to CO-haemoglobin. The well-known pink tint, when viewed along the length of a 6-inch test-tube, is sufficiently intense to justify still further dilution, and water is now added equally to both solutions until the pink colour can just be recognised. Such a solution is practically colourless in a layer 1 cm. thick. 5 c.c. of each of these solutions is diluted with fresh normal urine, which was slightly acid but gave a slight precipitate of phosphates in boiling, a matter of no importance, since neither blood nor bile is entangled in a precipitate of phosphates.

The tests were all performed with quantities of ·5 c.c. in tubes 1 c.c. capacity.

No.	Amount used for Test, in c.c.	Dilution of Solutions in Urine.	Blue Colour.
1	·5	1 : 1 { Hb+O Hb+CO	Good. Good.
2	·5	1 : 2 { Hb+O Hb+CO	Good. Good.
3	·5	1 : 3 { Hb+O Hb+CO	Good. Good.
4	·5	1 : 4 { Hb+O Hb+CO	Quite evident. Quite evident.
5	·3	1 : 9 { Hb+CO in Test-tube	Faint, but evident.

As a reaction for blood or haemoglobin in urine, if the fluid is boiled, cooled, and tested with guaiaconic acid and peroxide of hydrogen, I believe the guaiacum test is a far more trustworthy reaction than is currently believed. In delicacy it exceeds the colour test for platinum with potassium iodide, and ranks with that of Nessler for ammonia. I have frequently demonstrated that if the test is performed in Nessler's glasses, that 1 part in 5,000,000 of a blood that contains 5,000,000 corpuscles per c.mm. and a haemoglobin value of 97 per cent. (Haldane-Gowers) can be detected with this test. The above figure is for laked blood; if unlaked, the test easily succeeds with 1 part in 1,000,000.

The following additional facts which I have observed are of some interest in connection with this test :—

1. Boiling the blood or any fluid suspected to contain this, in no way interferes with the reaction. It is advisable to always do this, since milk, pus, or fibrin after boiling do not yield the test. With this precaution the test is distinctive.

2. The reaction is accelerated by slightly warming the liquid.

3. If the fluid is markedly alkaline to litmus, the test fails ; if just alkaline, the test succeeds.

4. An acidity of ·3 to ·4 per cent. hydrochloric acid does not interfere with the test.

5. Ozonic ether is a slightly better reagent to use than 20 vols. per cent. solution of H_2O_2.

6. Fluids containing haemoglobin or most of its derivatives give the test when it is impossible to detect pigment by any other method.

7. Exceedingly weak solutions of oxyhaemoglobin, reduced haemoglobin, CO-haemoglobin, methaemoglobin, haemochromogen, ethereal solution of acid haematin, haemin crystals in solid form, haemin dissolved in alcoholic potassium carbonate, dried blood ten years old, all give the test.

8. Very dilute solutions of haematoporphyrin prepared as a pure product give a blue colour with guaiaconic acid and H_2O_2. I anticipated that the removal of the iron from haemoglobin might prevent the test. This is not so, for iron-free pigment gives a distinct blue colour. This reaction succeeds with solutions of haematoporphyrin which have been boiled.

9. Solutions of haematoidin in chloroform or in alcoholic potassium carbonate, and solutions of bilirubin, do not give the guaiacum test.

The haemin test is of such wide application, and so easy to perform, that it is worth briefly considering under what conditions this test is possible or impossible, for undoubted blood stains may occasionally give no positive haemin test, a fact which appears to be insufficiently appreciated. Though Funke was the first observer to show that blood can yield crystals of a brown tint, Teichmann, in 1853, quite accidentally realised the essential fact, that the demonstration of the crystals, which are

to-day named after him, is possible with minute traces of blood. The practical value of this discovery at once became apparent, but the expectation that one kind of blood might be distinguished from another by some variation in the crystals, has not been realised.

Teichmann's original method was the one described at the present time in most text-books, with the exception that no sodium chloride was used. The addition of this is unnecessary, except in the case of old blood-stains. An addition of salt was originally suggested by Virchow, but more than a trace of this interferes with, and may even prevent, the success of the test. A minute amount of potassium chloride appears to me to be preferable to salt, certainly the crystals are larger. The brown or black colour and the rhombic shape is distinctive for crystals of haemin, which can be easily prepared in quantity by Schalfijew's method. To four volumes of glacial acetic acid heated to 80° C. add one volume of strained defibrinated blood. This lowers the temperature to 50°-60°. Heat at once to 80° and set aside to cool. I find that 1000 c.c. of sheep's blood yields 3-5 grammes of haemin. The crystals are subsequently purified by thorough washing with water, absolute alcohol, and ether. Chemically pure haemin crystals can now be obtained by recrystallisation from their solution in chloroform and pyridin (Küster).

This substance is the hydrochloric-acid ester of haematin, and other acids than acetic, such as lactic, tartaric, and oxalic, in alcoholic solution, together with such salts as bromides, and iodides of sodium potassium, calcium, or barium, may be used in the preparation of various haemins (Bifalvi). That chlorine is built up into the molecule of haematin is probable, since haemin crystals yield HCl on treatment with concentrated sulphuric acid, and the chlorine of the compound unites with potassium when the crystals are dissolved in caustic potash. Although haemins prepared by different methods have been regarded as differing in structure, Küster states that all haemins examined by him have the following empirical formula—

$$C_{34}H_{33}O_4N_4FeCl.$$

The following are the general conclusions reached by Lewin

and Rosenstein as to the conditions under which this haemin test may or may not succeed. Dried blood is intimately mixed with a minute crystal of salt, covered and irrigated with glacial acetic acid, and heated to boiling point. With CO-haemoglobin and methaemoglobin prepared by any method the test succeeds; it uniformly fails if the blood is in the state of haemochromogen; this is the condition of most of the colouring matter in Valentine's meat juice, from which, as well as from haematoporphyrin, which is free from iron, crystals of haemin cannot be obtained. Rose was the first to show that when blood is mixed with rust the haemin test is negative. Blood stains on rusty metal may therefore be difficult to recognise; the explanation of which is, that in the presence of oxide of iron no hydrochloric acid is formed from sodium chloride by the action of acetic acid. If any thick layer of blood has collected on iron, only the superficial layer free from rust may give the haemin test. An explanation of the failure of the test for the deeper layers of blood, is probably due to the following chemical reactions—

(1) $Fe(OH)_3 + 3C_2H_4O_2 = Fe(C_2H_3O_2)_3 + 3H_2O.$
(2) $Fe(C_2H_3O_2)_3 + 3NaCl = FeCl_3 + 3NaC_2H_3O_2.$

It is not infrequently stated that the haemin test is negative for blood stains which have been treated with soap and water or fatty acids. This is of some medico-legal interest, but appears incorrect, since the treatment of blood stains with boiling potash for twenty to twenty-five minutes, or washing the stains with soap and water, in no way impairs the formation of typical crystals.

In 1898 Bordet, while engaged in studying the phenomena of haemolysis, showed that a haemolytic serum for rabbit's blood, which was obtained by repeated intraperitoneal injections of rabbit's blood into guinea-pigs, also contained agglutinins, which effected the clumping of red corpuscles in a manner similar to the agglutinins discovered in 1896 by Gruber in the serum of animals treated with cultures of bacillus typhosus, and which he found caused a specific clumping of typhoid bacilli. A year later, Bordet noticed that when rabbits were treated with intra-

peritoneal injections of fowl's blood, that not only was a serum obtained which was specifically haemolytic for the fowl, but that a fine coagulum was formed, and that this precipitate (precipitum) was produced by a specific substance (precipitin).

Previous to this, the observations of Kraus had shown that the sera of rabbits immunised against typhoid, plague, and cholera contained a substance which not only clumped the corresponding bacilli, but also produced a cloudiness, which was attributed to another agglutinin, when this serum was added to the filtrate of a corresponding bacterial culture. In other words, the sera contained specific agglutinins, or phyto-precipitins, and these substances may appear in sera as a result of the injection of plant organisms, and they are capable of inducing an aggregation of proteid molecules. The discovery of precipitins in 1899 is generally attributed to Tchistovitch. He worked with eel's serum—a powerfully toxic substance—and showed that animals can be immunised against this, and that the serum of these animals contained a zoo-precipitin which induced a cloudiness on admixture with the serum of the eel. This type of experiment is capable of great variation, and the formation of precipitins or coagulins, either by the injection of the blood of one animal into another species,[1] or by the introduction of various albumins, globulins, or peptones (W. Myers), into the peritoneum, is often spoken of as the Bordet-Tchistovitch phenomenon, which is the basis of the biological test for blood (Uhlenhuth, Ziemke), or of the method for recognising the specific proteids of blood sera and allied fluids (Wassermann and Schütze), or for determining the relationships which exist between different animals (Nuttall).

Blood serum must necessarily be looked upon as a product of the death of blood; it has the same relation to plasma that killed has to living protoplasm, but beyond that, it undoubtedly possesses in addition, or at any rate in excess, the products of leucolysis; so that the opinion has been held that serum is a genuine physiological solution of leucocytes. Normal and

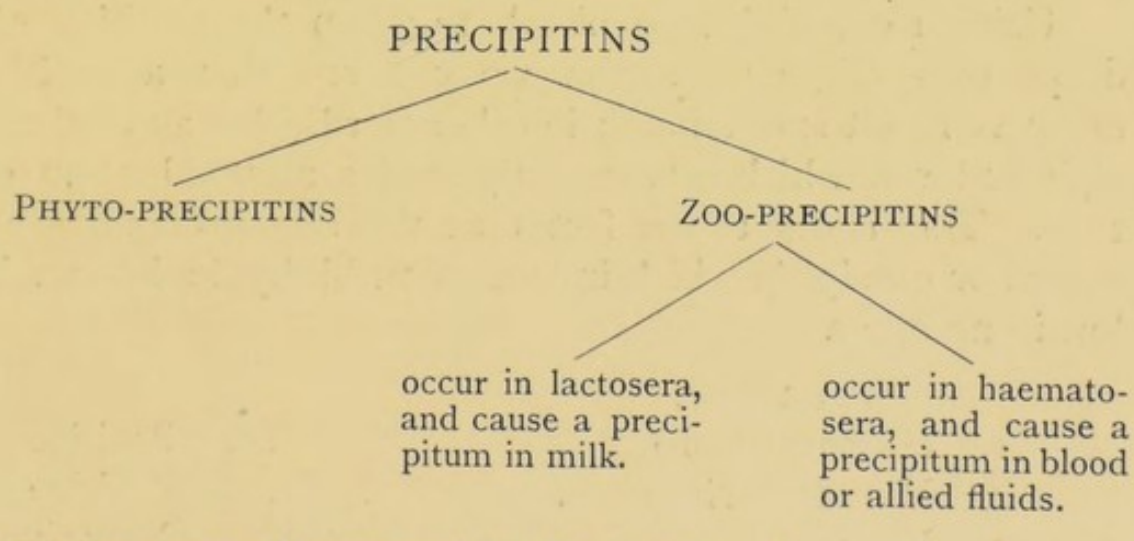
[1] In the case of closely allied animals, namely, rabbit and guinea-pig, or pigeon and fowl, practically no precipitins can be developed by injecting the blood of one into the other (Nuttall).

immune sera may both contain identical substances, the chemical and physical characters of which are only imperfectly known, and for the detection of these there is no test beyond that which is yielded by their specific actions.

In normal sera there may be found :—

Antitoxins.
Agglutinins.
Precipitins.
Ferments.
Anti-ferments.
Cytotoxins,[1] which include haemolysins and bacteriolysins.
Opsonins.[2]

Any of these may be developed in the serum of an animal which may be otherwise free from them, by treatment similar to that which induces active immunity. By the introduction of most, and probably all of the above substances, into animals, a development of anti-bodies in serum, for example, anti-ferments or antiprecipitins, can also be shown to take place. According to our present knowledge, the precipitins can be grouped with reference to their effects, or, as follows, with reference to their origin.



Lactosera were discovered by Bordet in 1899, and the precipitin which was developed in rabbit's serum after treatment by

[1] Cytotoxin, a term we owe to Metchnikoff, may be a substance in serum, venom, or cultures of animal or plant organisms which destroy any living cell. Lysins kill, but do not necessarily dissolve and destroy protoplasm.

[2] Opsonins are substances which prepare bacteria or other cells, *e.g.*, blood corpuscles for phagocytosis (A. E. Wright).

intraperitoneal injections of milk was found to precipitate caseinogen. Such a lactoserum possesses other bodies than milk precipitins, such as haemolysins, a precipitin for ox serum, and a lysin which immobilises the spermatozoa of the ox (Meyer and Aschoff).

Haematosera resemble in many respects the lactosera, and such sera may be looked upon as far as their precipitin effect is concerned, as partially or wholly specific. The rabbit is the animal that is generally used to supply any given haematoserum, and by injecting the blood of other animals, a variety of antisera may be obtained, which, following the terminology of Nuttall, may be spoken of as anti-human, anti-pig, or anti-avian.[1]

Through the kindness of Dr d'Este Emery, who has given me some anti-human serum obtained by the intraperitoneal injection of human ascitic fluid into a rabbit, I am enabled to show you that this anti-human serum when added to the following dilutions of blood, rapidly induces a cloudiness in human blood alone, a discovery made independently by Uhlenhuth, and by Wassermann and Schütze. One paper was published within a few hours of the other.

Using 1 c.c. of diluted blood, 1 : 40, on the addition of two drops of anti-human serum we can see that a positive or negative result is obtained ; in other words, that an obvious precipitate forms which subsequently settles at the bottom of the tube. This is due to the fact that the serum contains a substance or precipitin which induces a turbidity in human blood, but in no other.

	Diluted Blood.	
1.	Human (fresh)	Positive
2.	Human (dried)	Positive
3.	Frog	Negative
4.	Fowl	Negative
5.	Guinea-pig	Negative

[1] A few months after this lecture was given, Nuttall's admirable work on *Blood Immunity and Relationship* was published. The subject is so excellently dealt with in this book, and the literature so thoroughly tabulated, that I feel inclined to regret the time which I gave to the literary study of the biological test for blood.

Diluted Blood.

6.	Cat	Negative
7.	Original ascitic fluid that had been used for injection + antiserum .	Positive
8.	Original ascitic fluid alone . .	Negative
9.	Human serum + antiserum . .	Positive
10.	Human serum alone . . .	Negative

In Uhlenhuth's original list nineteen different bloods were examined with anti-human serum. A precipitate occurred with human blood, but not with that of the dog, cat, pig, or sheep, deer, fallow-deer, horse, donkey, hare, rabbit, guinea-pig, rat, mouse, and four different birds.

In the original list of Wassermann and Schütze, details of observations on twenty-three different bloods are given; only that of man and the baboon gave a precipitate. Subsequent lists of twelve different bloods (Ziemke) and nine (Schirokich), confirmed these results.

Time does not permit me to enter at length into the purely theoretical views which have been advanced as to the production of precipitins within the body, or as to the mode of formation of the characteristic precipitate; but, although it is probable that the production of precipitins depends upon a process that is no doubt similar to the production of antitoxin, in response to the injection of toxin, why a precipitin in relation with the proteid, which on injection gave rise to it, should form an insoluble substance, is not so clear.

Toxin and antitoxin, ferment and antiferment, give no precipitate; their resulting molecules are probably small, and it is improbable that even the union of large molecules, such as those of a globulin, would produce a precipitate. The phenomenon is possibly a complicated one, in which proteid molecules are clumped, just as bacilli or blood-corpuscles may be by the action of agglutinins. Indeed several observers consider that although both agglutinins and precipitins may co-exist in the same serum, they are fundamentally similar bodies. The action of both is diminished at 60° C. and destroyed by a temperature of 68° C.; moreover, the loss of their distinctive actions cannot be restored by the addition of any other serum,

unless this normally contains agglutinins or precipitins. Such features distinguish these substances from the cytases (alexines) that conceivably play a part in such phenomena as haemolysis or bacteriolysis, where a temperature of 55° C. abolishes the lytic action, which can be restored by fresh serum.

How precipitins originate is unknown; whether their genesis is from leucocytes or other cells is largely a matter of conjecture. Our knowledge of their action rests almost entirely on experiments *in vitro;* a precipitum obtained by treating a solution of

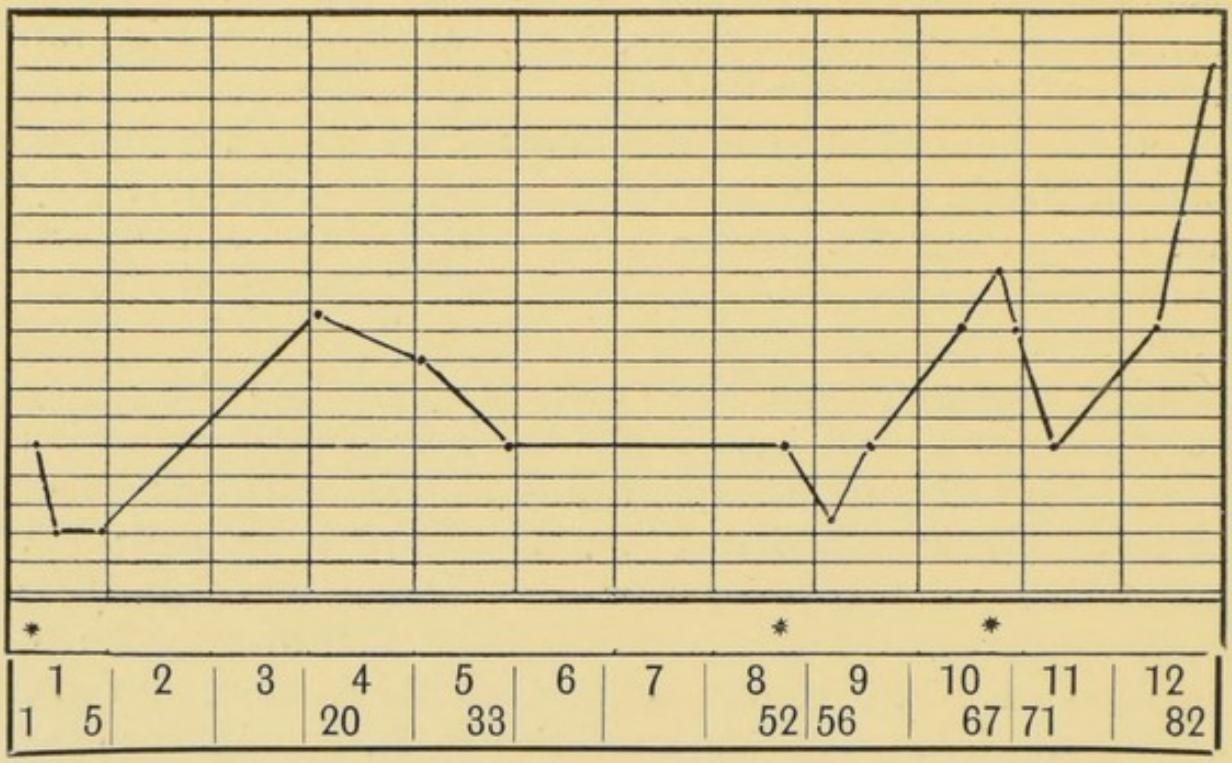


FIG. 9*a*.—Curve of immunisation-reaction, *i.e.*, of the antitoxin content of milk after three successive injections of tetanus toxin (Brieger and Ehrlich). An immediate fall and subsequent rise follows each injection.*

peptone with an antipeptone precipitin when injected into the rabbit causes embolism, which does not occur if peptone is injected into a rabbit with antipeptone precipitin in its blood (Bashford).

The formation of precipitins in the organism doubtless follows the law that was first determined for the formation of antitoxin by Ehrlich and Brieger, a law the truth of which has been abundantly proved by the researches of A. E. Wright and others. Stated briefly, their experiments show that with

successive introductions of substances such as toxins, vaccines, or proteids into the organism, the blood and other fluids become, up to a certain point, progressively richer in anti-bodies, but that this takes place in such a manner, that for any given injection there is at first a negative and subsequently a positive phase.

The chart (Fig. 9*a*), which is taken from a paper by Brieger and Ehrlich (1893), shows the fluctuation in the amount of tetanus antitoxin present in the milk of an immunised milch goat who was treated with three doses of tetanus toxin administered in the first, eighth, and tenth week of observation.

An interesting preliminary note by A. Hunter shows that occasionally precipitins may be found in the serum of rabbits after a single injection of 5 c.c. of a mixture of the separated proteids of ox-serum, more is found after the third, and a considerable amount after the fifth injection. The relation of the polymorphonuclear leucocytosis to the precipitin-content, I have attempted to show from the data he has given (Fig. 10, p. 154).

The features of interest in the curve are that the acme of leucocytosis occurs as the precipitin-content of the blood diminishes, and with its disappearance the serum becomes progressively richer in precipitin. Whether one phenomenon is associated with the other, or whether the relationship is that of cause and effect, must at present remain a matter of uncertainty.

The Tchistovitch-Bordet phenomenon was first employed by Uhlenhuth of Griefswald as a medico-legal test for the detection of human blood, since, from what has been said, if human blood be introduced into rabbits, the rabbit serum ought to precipitate human serum, and human serum alone.

His actual original experiment was to obtain human blood, defibrinate this, and inject 10 c.c. into the peritoneal cavity of rabbits every six to eight days. After five injections, the rabbit's blood contained the specific precipitin. To carry out the test, he diluted human blood with an equal amount of 1·6 per cent NaCl, and added six to eight drops of the rabbit's serum : this rabbit's serum may be called, using Nuttall's nomenclature, anti-human serum.

The following method was employed by Uhlenhuth in the examination of old dried stains of human blood. The piece of rag or cloth is soaked in 6 c.c. of ·75 per cent. NaCl, ·5 c.c. of anti-

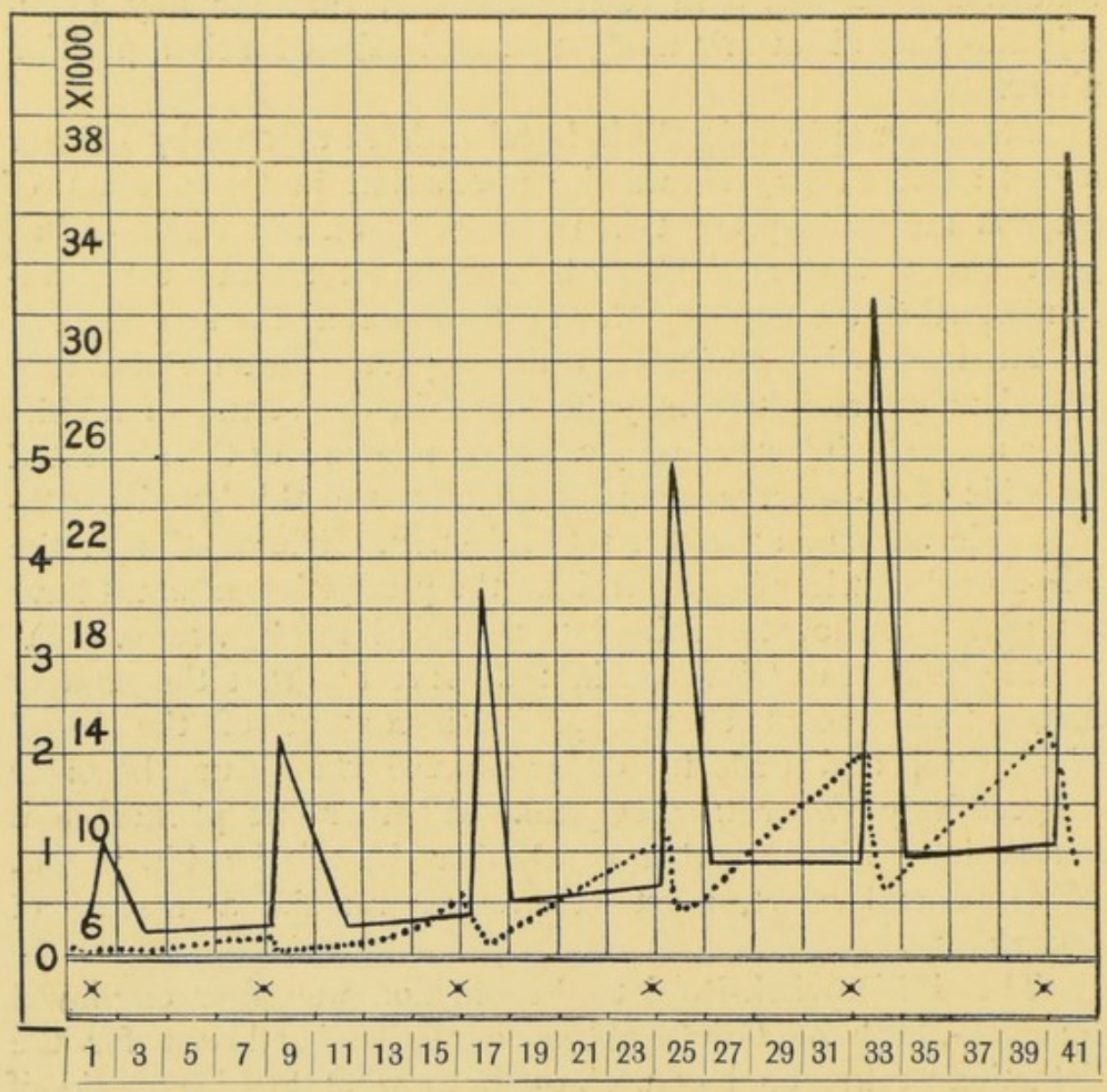


FIG. 10.—Chart contributed from data given by A. Hunter, of the leucocyte-count and formation of precipitin in the rabbit: marked leucocytosis (polymorphonuclear), and fall in precipitin-content succeeds each injection of a mixture of the separated proteids of ox-serum. Injections indicated by ×.

serum is added to the liquid, and the mixture kept at 37° C. for one hour.

In a number of papers Uhlenhuth has developed this method for the detection of the blood of man and other animals. The guaiacum and haemin tests are first applied, and subsequently by using various antisera which contain specific precipitins for

the blood of different animals, the kind of blood is identified. Any precipitate is compared as far as possible with control precipitates, made by using dried bloods, the age and origin of which is known.

In Germany the test has been widely employed in forensic practice, and has been recognised officially by the Justizministerium of Germany and Austria, and also by the Egyptian and Roumanian Governments. It may be of interest to quote a few cases. The following dozen specimens were transmitted to Uhlenhuth from the medico-legal institute of Bucharest :—

		Diagnosis.
1.	Articles A and B (blood on cloth) .	A Human B Pig
2.	Dried blood (shirt) . . .	Fowl
3.	„ (2 shirts) . .	Human
4.	„ (shirt) . . .	Human
5.	„	Human
6.	„	Human
7.	„ (stockings) . .	Human
8.	„ (piece of wood) . .	Fowl
9.	„ (cloth) . . .	Human
10.	„ (2 shirts, 2 stockings) .	Human
11.	„ (shirt) . . .	Human
12.	„ (maize-leaf) . .	Human

Subsequently the diagnosis made in Griefswald of these bloods was completely confirmed by intelligence from Bucharest. Examples of other cases are :—

No. 21.—Decision in a murder case at Strassburg.

Question.—Human blood, or that of a cow?

Answer.—The former;—which, from the evidence, was confirmed.

No. 22.—Blood stains on clothes were found to be due to human and sheep's blood. It was proved that the accused had killed a man, and had also been engaged in slaughtering sheep two weeks before the murder.

In this country Nuttall has independently used the precipitin test, for the purpose of determining doubtful relationships

between animals, and such investigations on purely physiological lines, may supplement, or even decide, specific or generic affinities. It is obvious that the rabbit can, on injection of the blood of another species, react and furnish a specific precipitin. The antisera obtained are negative except for that individual species of animal whose blood was used for injection.

In Nuttall's method dilutions of human blood 1 : 100 with normal saline, and equal volumes of 1 : 100 diluted anti-human serum are used. The reaction occurs not only with fresh blood, but also with—

1. Old blood (two months).
2. Putrefied blood.
3. Mixtures of human, and five or six other bloods.

With dilutions of blood 1 : 500 or even 1 : 16,000, the reaction succeeds, so that the test is a delicate and reliable one. However, the reaction appears not to be absolutely specific for human blood, since anti-human serum does contain a precipitin with which the following reactions are noticed. These are :—

1. Maximal for human blood.
2. Nearly maximal for the anthropoid apes (Simiadae).
3. Submaximal for the old-world monkeys (Catarrhini).
4. Minimal for the new-world monkeys (Platyrrhini).
5. Negative for Lemuridae, and also all other mammals, fish, and birds.

In connection with this part of the matter, Nuttall has shown that one or two antisera, those of the pig and sheep, may possess two features, since they will slightly precipitate many mammalian bloods, and therefore give a so-called *mammalian reaction* and also give a maximum specific precipitum with the blood of the same species.

Thus it was found that with the following antisera :—

1. Anti-horse serum, positive only for the horse and donkey.
2. Anti-dog serum, positive only for eight species of dogs.
3. Anti-pig serum, besides its specific action for the pig, may give a general *mammalian* but not an avian reaction.

The precipitin test for blood promises to be of great service. Like many other methods, except in the hands of those who

have specially studied the conditions under which the reaction may occur, be modified or absent, an erroneous judgment may be formed. Opalescent antisera are obviously useless. The cause of this opalescence is unknown, but such sera cannot be cleared by filtration through porcelain. A typical qualitative test may be made by using 5 c.c. of blood or serum diluted 1 : 20 with ·6 per cent. salt solution. To this add one to two drops of anti-serum. This falls to the bottom of the tube, and in a few minutes a white cloud appears, which subsequently forms a dense precipitate that finally settles after a few hours. The whole reaction is accelerated by a temperature of 37° C., but apparently not in any other way (Graham-Smith and Sanger).

Blood stains many years old can be shown to respond to the precipitin test, and not only blood but also saline extracts of organs, milk, pus, sputum (Wassermann), since these contain proteids related to those of the blood. The reaction is stated by Meyer to be obtained with the extract of pieces of an Egyptian mummy, guaranteed to be at least 5000 years old. Lastly, Merkel has observed that precipitins developed in the blood of the mother may pass to the foetus.

It has been shown that the precipitin test for blood has been gradually evolved from a study of the anti-bodies which cause a precipitum in various proteid solutions, such as the globulins, and albumins of eggs, meat, milk, or blood, and that by means of a precipitin, bodies which to the physiological chemist are so similar as the proteids of the blood plasm of man, ox, or horse, are in reality of different constitution. An extension of the test as applied to blood is found in papers by Uhlenhuth and Ascoli, the former of whom has shown that in a mixture of uncooked minced meat or sausage, the flesh of horse, cat, or dogs, if present, can be detected by precipitins, while the latter has made use of the reaction to recognise the presence of milk proteids or egg proteids in urine where in pathological cases these ingested proteids have passed into the circulation and been eliminated.

LECTURE VIII

THE MORPHOLOGY OF PATHOLOGICAL BLOOD

IF we consider the extent to which specialisation occurs, both in medicine and those sciences which are directly related to it, and, moreover, realise the danger which exists that those who are pursuing any particular study may over-estimate its importance, we shall avoid making the mistake of claiming more for any method of investigation or diagnosis than it really merits. It is true that investigations of the blood in disease have acquired considerable clinical value during the last ten or fifteen years, but examinations of the blood from the purely morphological point of view are, apart from the detection of bacteria or protozoa, unable, with a single exception, to do more than add additional support to a diagnosis. Except in leukaemia, where a diagnosis is made, indeed is only possible, by histological methods, the instances where a blood examination enables a diagnosis to be confidently given are exceedingly rare. Even for pernicious anaemia this statement I believe holds good, although the aspect of the blood in this disease is fairly constant and well known. The real value of clinical investigations on the blood depends therefore partly upon its aid to a diagnosis, but much more upon the slow accumulation of knowledge as to those conditions of the blood which are associated with, but do not necessarily characterise pathological processes; thus in diphtheria or rickets the blood may possess some myelocytes, in malaria an excess of large mononuclear leucocytes is frequent, and in advanced syphilis a few normoblasts may be found, but none of these diseases are identified

by the appearance of those cells in the blood. On the other hand, as a means by which a prognosis is obtained, investigations on the morphology of the blood possess a high value.

Though the organism strives by every means to maintain the blood in a normal state, both as regards its mass, its density, and its morphology, throwing out foreign material such as bacteria (Wissokowitsch and Werigo), glycogen, dissolved haemoglobin, or abnormal quantities of normal constituents such as dextrose or sodium chloride almost as fast as they are introduced, practically no disease exists which leaves the blood unaffected. It is in this way that most of the features of pathological blood arise; for it is still unproved that this fluid is ever the site of a primary pathological process, although the terminology of certain diseases indicates that this has been the view of some observers.

The introduction of the term anaemia into nosology we owe to Andral, who defined it as a special morbid state, in which there is a relative diminution in the mass of blood which at the present time is known as oligaemia (Gendrin). Early clinical observers regarded the anaemic condition as the converse of plethora, and only through the growth of our knowledge of the physiology rather than the pathology of the blood, was it possible to recognise that the clinical conceptions of anaemia were insufficient to appreciate the changes which the blood might undergo in volume, or in number of cells, or in amount of haemoglobin. It is in anaemic conditions particularly that blood examinations are required, but such work yields only an insufficient basis for any classification of the forms of anaemia, since our knowledge as to the causation of any type has, in the majority of cases, no pretensions to scientific accuracy. We shall not be in error in considering that disease may and does react upon the blood, sometimes in an obvious manner, at other times is not so obviously, though from recent observations on the serum, especially those of A. E. Wright, Stewart Douglas, and W. Bulloch, we obtain glimpses of profound changes in that fluid, which can only be discovered by methods which until quite recently have played no part in clinical investigations of the blood.

No satisfactory classification of anaemias, not even that recently suggested by Pappenheim, appears to me to be possible until our knowledge as to the origin of the red and white corpuscles and the region where and manner in which haemoglobin is built up is more definite and certain. However, pathological blood, when examined histologically, can be referred with tolerable accuracy to one or other of the following groups (p. 161). This scheme does not pretend to be a classification in any sense of the word, and no more than any other method of grouping does it permit of any inference being drawn as to the cause or causes of the morphological aspect of the blood.

In certain diseases, and also in some conditions which are physiological rather than pathological, histological preparations of human blood show definite distinctive features. Some of these I will demonstrate by means of photographs and stained films projected on the screen. The methods of preparing the latter are briefly described in the Appendix.

Acute Post-Haemorrhagic Anaemia

In this condition an examination of the blood possesses no diagnostic value, since the anaemia is caused by a considerable loss of blood within a few minutes or a few days. The specimen I show was taken ten hours after a great loss of blood (haematemesis). Morphologically, the blood has a normal aspect, both as regards the red corpuscles and the leucocytes; of the latter there is a slight increase in number. No normoblasts are to be seen. These cells from the bone-marrow indicate regeneration of blood corpuscles, but can only be seen for the first time twenty-four to forty-eight hours after the haemorrhage. Their appearance is transient, and their numbers variable. The coagulability of the blood of this patient was raised, the haemoglobin-percentage and number of corpuscles were diminished, but both these values would probably be even still lower a few days later, owing to the entrance of extra-vascular liquid into the blood. An acute swelling of the blood discs so that their volume markedly augments, polychromatophilia, and the appearance of poikilocytes one or two days after the loss of blood, have

1.
LEUCOCYTOSIS.

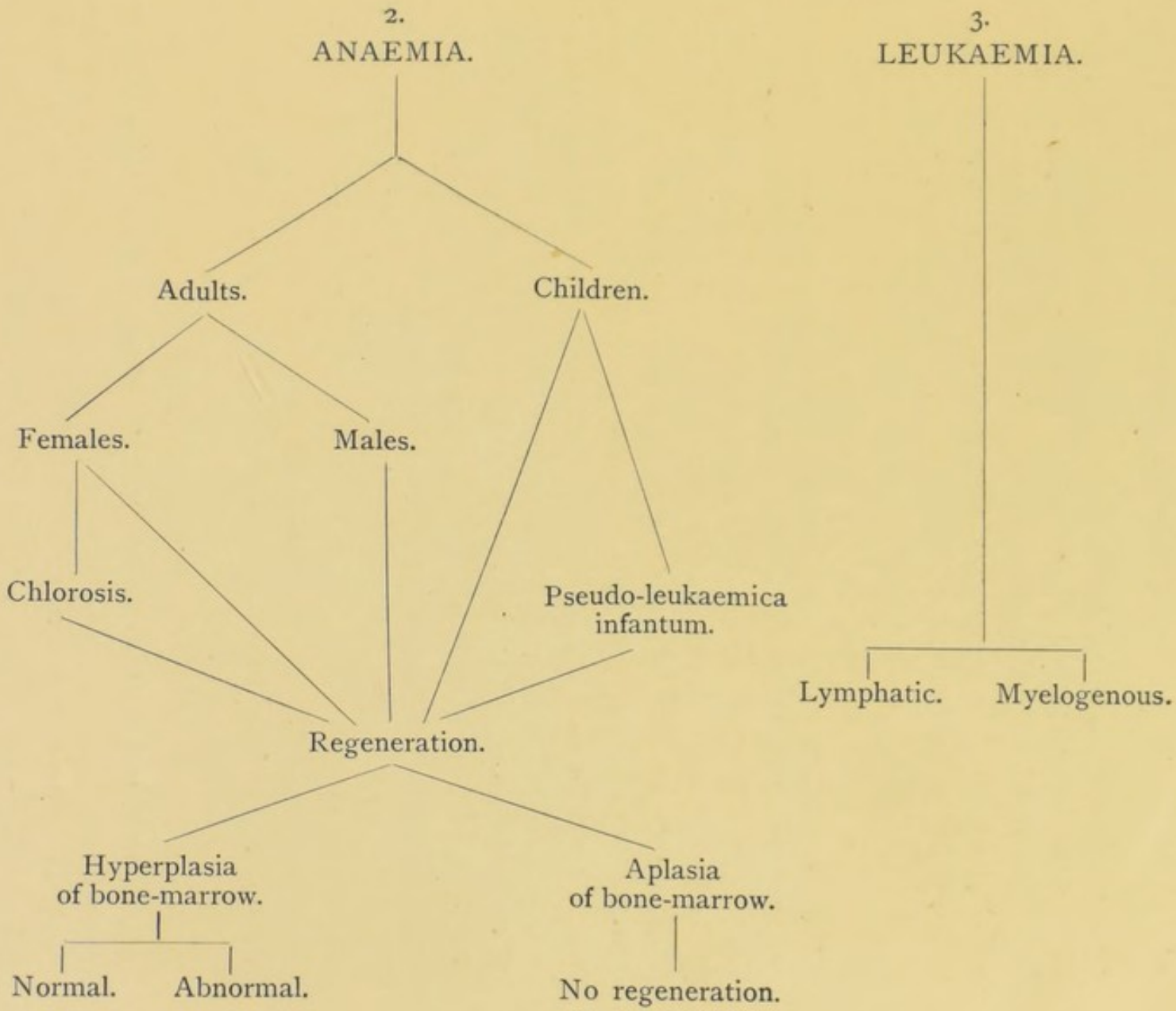
1. Polymorphonuclear.

2. Eosinophil
eosinophilia.

3. Lymphocytosis
lymphaemia.

4. Mononuclear.

5. Myelocyte
myelaemia



been recorded (Herz, Laache). In the leucocytosis of post-haemorrhagic anaemia an increased number of lymphocytes is not infrequent; myelocytes have been observed by Lazarus, while Ehrlich has recorded the appearance of non-granular polymorphonuclear leucocytes.

Secondary Anaemia

In a large number of those simple chronic forms of anaemia, which are generally referred to as secondary, any given case may possess its own peculiar features, which vary from a simple diminution in the number of red corpuscles generally accompanied by a corresponding poverty in haemoglobin, to cases where the morphology of the blood presents a picture resembling in many respects that of a pernicious type. The vast majority of cases of anaemia which are examined are those in which the changes in the blood are but a symptom of such diseases as fever, syphilis, malignant disease, malaria, or the result of abnormal hygienic conditions and bad nutrition. Most forms of secondary anaemia, as far as the morphological aspect of the blood is concerned, may I think be referred to one of the four following types. Under the microscope the blood may show:—

1. An almost normal appearance.
2. Features which recall those of chlorosis.
3. Certain features resembling those met with in pernicious anaemia.
4. A large number of red corpuscles, which are undoubtedly spheres and not discs. This is a rare condition.

In most forms of secondary anaemia frequent enumerations of the corpuscles, an estimation of the haemoglobin, and calculation of the haemoglobin-content of the individual red corpuscles are of more importance than an examination of stained blood films. By these methods also the progress of the anaemia is best followed. Of this extensive group, a secondary anaemia with many features of the blood of chlorosis is exceedingly common; on the other hand, another type may show a picture not unlike that of pernicious anaemia. Any individual case must be studied by itself. Blood examination is an aid to

diagnosis, indeed a valuable one; but in my opinion, as an auxiliary to clinical work, does not, except in a few cases, rank in importance with an examination of the urine, and certainly not with a bacteriological investigation.

General statements which shall cover the entire group of those forms of anaemia which are ranked as secondary are obviously liable to be wrong for any given case, and I feel satisfied that it is often most desirable to examine the blood of a patient without any reference either to his history or clinical symptoms, and for this purpose a classification of various forms of anaemia, where attention is particularly directed to the morphology of the red corpuscles, their number and haemoglobin-content, such as that proposed by Hayem, which does not pretend to be founded on etiological conditions, appears to me to be useful, especially so from the point of view of prognosis.

In cases of secondary anaemia the haemoglobin in a drop of peripheral blood is diminished; this may be slight or considerable (14 per cent. of the normal). The corpuscular content is diminished, but to a less degree. The density of the blood is diminished, as might have been inferred from the fall in haemoglobin. In many forms of anaemia, especially those seen in acute sepsis, the specific gravity of the blood rapidly falls. If the total solids diminish to 15 per cent. (normal = 21 to 22 per cent.), and the proteids of the serum fall to 6·5 per cent. (normal = 10·5 per cent.), recovery never takes place (Roscher with E. Grawitz). All cases of anaemia show an excess of water in the serum (Askanazy), and it is not improbable that the volume of the blood is actually increased, though estimations of the total solids of the sera in chronic anaemias are not concordant. The same is true for the degree of alkalinity, and the coagulation-time of the blood. Morphologically, the red discs when stained with Israel-Pappenheim's fluid show variable poverty in haemoglobin, so that the disc often appears as a ring. The shape is preserved, and the variations in size are almost always of the nature of a diminution (3 to 5 μ). Alterations of contour are common. Together with this, poikilocytosis and polychromatophilia are not infrequent even in mild forms of anaemia.

The blood of a secondary anaemia with the morphological

characters just enumerated may slowly or rapidly change, and in cases of syphilis, malaria, or malignant or chronic disease of the stomach, present an appearance like the photographs thrown on the screen, apart from the fact that the blood becomes impoverished in corpuscles and haemoglobin (1,100,000 and 16 per cent. Hb). The first photograph shows an extreme degree of poikilocytosis resembling that of pernicious anaemia; the poverty of the cells in haemoglobin is obvious, and there is some degree of leucocytosis, a condition which is often noticed at an earlier stage, when the blood change is less abnormal.

The next photograph (Plate II., Fig. 1) shows that in late stages of advanced syphilis the morphology of the blood may still more closely approximate to that of pernicious anaemia; besides marked poikilocytosis, a normoblast, some megalocytes rich in haemoglobin are to be seen, also two large mononuclear leucocytes. Without any history, on such a blood film it would be difficult to give an opinion. The presence or absence of megaloblasts is the chief distinguishing feature between the two diseases. To come to a decision, several specimens would need a thorough systematic examination. A remark of v. Limbeck, "Ich glaube dass es kaum ein besseres Beispiel als die Syphilis zur Erlauterung des Satzes gibt, dass kein anaemischer Blutbefund für irgend eine klinische Form der Anaemie characteristisch sein kann," expresses, I believe, the views of those who have attempted to gain their knowledge by laboratory work in preference to studying the literature of the subject.

In miner's anaemia (Haldane, Boycott), which is a secondary anaemia induced by ankylostomiasis, the most prominent clinical symptoms of the condition are pallor, cardiac distress, and shortness of breath. The cases in the Dolcoath mine were also accompanied by irritable, inflamed swellings of the skin, locally known as "bunches"; such lumps possibly indicated the position of larval forms of Ankylostoma duodenale. A feature of the blood in this anaemia, like other forms dependent on helminthiasis, is a pronounced and persistent eosinophilia. As to the causation of the anaemia, apart from the loss of blood at the surface of the gut, which in acute cases is considerable, there is some experimental evidence to show that it is

due to an absorption of toxic material from the intestinal tract.

A severe secondary anaemia in children, described by v. Jaksch in 1889, under the name of anaemia infantum pseudo-leukaemica, and by Ehrlich as pseudo-pernicious anaemia, is due to a variety of causes, among which syphilis, tubercle, and rickets may be mentioned. The blood in this type of anaemia shows:—

1. Variations in the size and intensity of staining of the red corpuscles; some of these are of a chlorotic type.

2. Nucleated red corpuscles, which are generally normoblasts.

3. Leucocytosis, which is chiefly due to an increase of lymphocytes and large mononuclear cells; one or two myelocytes may be seen if a stained film is thoroughly examined.

These features, however, characterise in a less degree many other forms of secondary anaemia in children.

CHLOROSIS

An examination of the morphology of chlorotic blood appears to me to have but slight interest; the disease is generally easily diagnosed without any blood examination, and I feel inclined to group this disease not among the anaemias, but in that class of metabolic diseases which includes such conditions as diabetes and gout.

The anaemia of chlorosis is generally rapidly established, varies from a comparatively simple to a grave type, yields more readily to treatment than other forms of anaemia, and tends to become re-established when treatment is suspended. The blood in chlorosis may occasionally show the number of corpuscles in a c.mm. and the percentage of haemoglobin diminished to an equal extent; but in the majority of cases this is not so, the individual corpuscles are poor in colouring matter, and hence the number per c.mm. falls but slightly, while the haemoglobin is considerably reduced (4,500,000 corpuscles, Hb = 52 per cent.).

The cardinal fact in chlorosis, and one which cannot be ascertained by the ordinary routine methods, is that the volume of the blood is augmented in proportion to the gravity of the

disease (Lorrain Smith). The total amount of haemoglobin in the blood is unaltered, and therefore, since the individual corpuscles are poor in haemoglobin, their absolute number in the blood must be increased; and as $\frac{R}{W}$ is constant, the total number of leucocytes are also augmented, even though this may

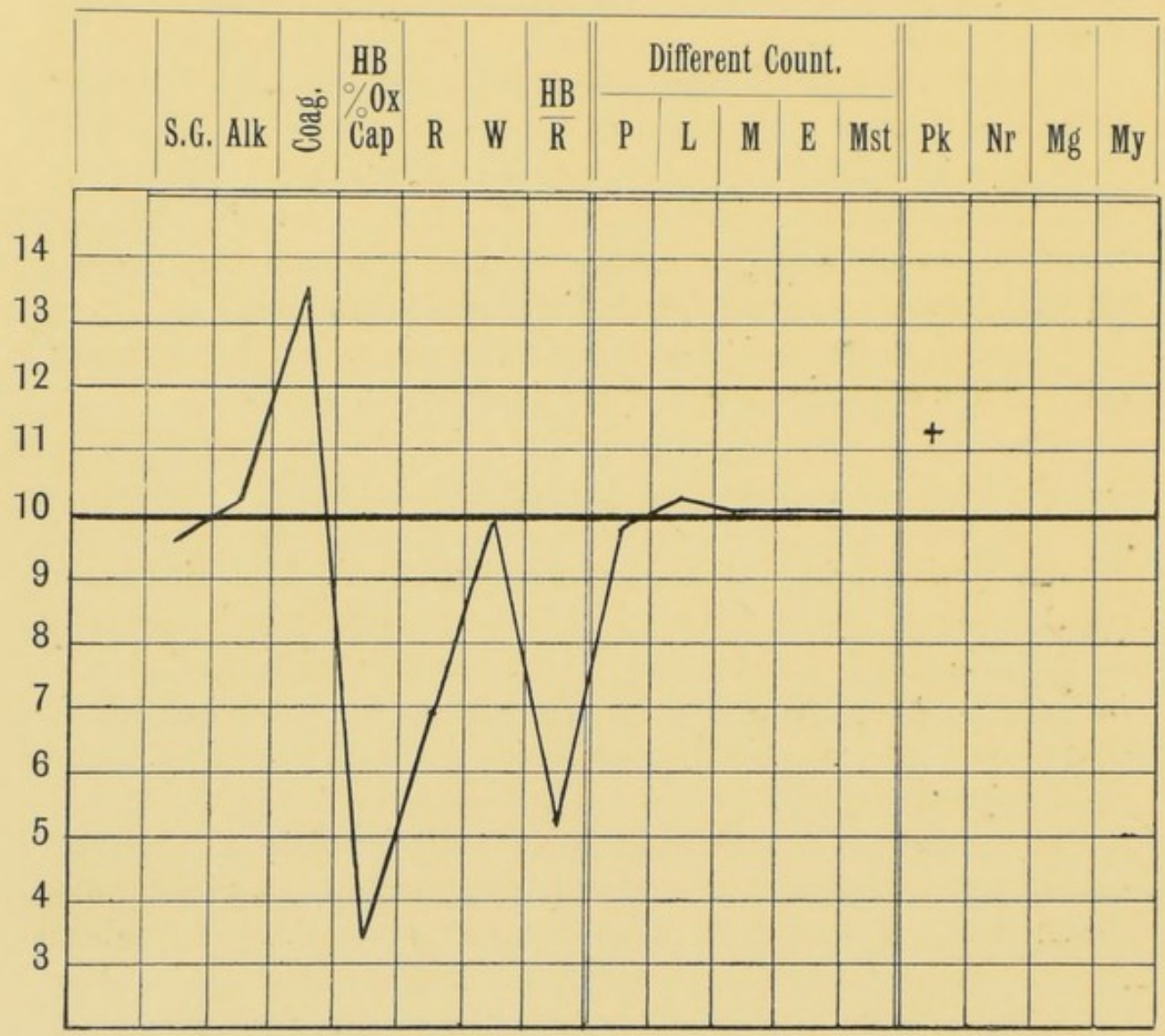


FIG. 11.—Curve showing the deviations from the normal in a typical case of chlorosis. The normal values lie along line 10.

not be evident in a drop of peripheral blood. In cases successfully treated with preparations of iron, the total haemoglobin of such patients is found to be the same as that of untreated or uncured cases. Salts of iron, therefore, apparently do not act by directly inducing a formation of haemoglobin (Lorrain Smith). Cases of chlorosis recognised clinically, appear to me

to show the following features in stained specimens (Plate I., Fig. 1):—

1. Ringing of the red corpuscles.
2. Unequal sizes of discs.
3. Variable degree of poikilocytosis.
4. Necrotic changes in the red discs.
5. Polychromatophilia.
6. Normoblasts, in very severe cases.
7. Leucocytes, normal both in appearance and relative numbers, but in a differential count the latter may be changed; on a single occasion I have seen an undoubted myelocyte.
8. Blood-platelets are increased.

On the preceding chart (Fig. 11) is shown the curve which was constructed from an examination of the blood in a case of chlorosis of moderate severity, compared with that of a healthy woman twenty years of age.

Progressive Pernicious Anaemia

The series of fifteen cases published by Biermer of Zurich in 1872 established the clinical features of pernicious anaemia, and though attempts were made by Eichhorst, Hayem, Laache, and H. F. Müller to complete his work by haematological researches these were only partially successful, until Ehrlich (1885-92) recognised megaloblasts and megalocytes in the blood, showed also that in this disease blood-regeneration in the bone-marrow proceeded on pathological and not physiological lines, and finally referred this specific megaloblastic anaemia to a perverted type of haematopoiesis which was comparable to that of embryonic life. Around the pathology and etiology of this form of anaemia much controversy has arisen, the view of W. Hunter that it is a haemolytic anaemia dependent upon a chronic infective process appears to have much probability. Contrary to the opinion of most other observers, he regards the changes in the bone-marrow as of secondary importance.

In any severe anaemia the regeneration of the blood may proceed normally on physiological lines, or abnormally on pathological lines, or be entirely absent. Typical cases of per-

nicious anaemia are by no means uncommon. The specimens I can show you are all cases of undoubted examples of this disease, the morbid anatomy of which conformed closely to the classical description given by Cohnheim and Geelmuyden for the bone-marrow, while such organs as the liver, spleen, kidneys, and pancreas showed a generalised siderosis (Quincke).

The features of the following specimens are :—

No. 1. Marked inequality in the size of the red corpuscles. Some are not discs but prolate spheres, the largest diameter of one of which is about 14 μ. These are megalocytes, and are intensely stained owing to their abundant haemoglobin. Two polychromatophil megalocytes are seen, also a similar condition in one or two red discs ; other discs show a chlorotic aspect ; several minute spheres or microcytes are present, these do not stain as intensely as the megalocytes.

No. 2. The following slide, from a specimen photographed for me by Mr Gordon Webb, shows that almost every red corpuscle is characterised by basophil granulations ; these are the "punktierte Erythrocyten" of German writers. In some cases of pernicious anaemia they are entirely absent ; even when present in the blood, I have not found this appearance of the corpuscles post-mortem in the bone-marrow (Plate III., Fig. 2A).

No. 3. Specimen showing a highly developed state of poikilocytosis ; some of the corpuscles are chlorotic in character, others are loaded with haemoglobin. In fresh blood some of these distorted cells showed spurious movements. An absence of the rouleaux formation is the rule, but not a universal one.

No. 4. A single normoblast is seen in the next specimen ; the red corpuscles are for the most part spheres and not discs.

No. 5. A megaloblast somewhat pentagonal in shape, with highly coloured protoplasm, and an irregular, rosette-shaped, faintly tinted nucleus, is seen among several poikilocytes and microcytes (Plate II., Fig. 2). On looking through this specimen the leucocytes are somewhat scanty in numbers ; two neutrophil myelocytes are also to be seen.

It is particularly noticeable in pernicious anaemia, that

Fig. 1.

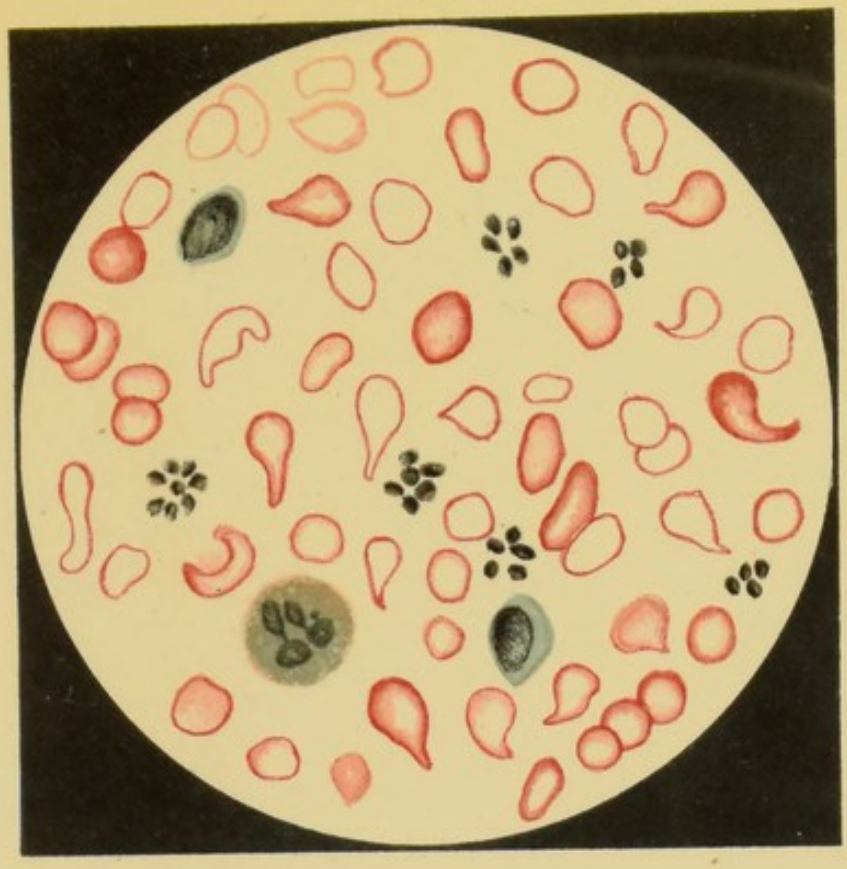
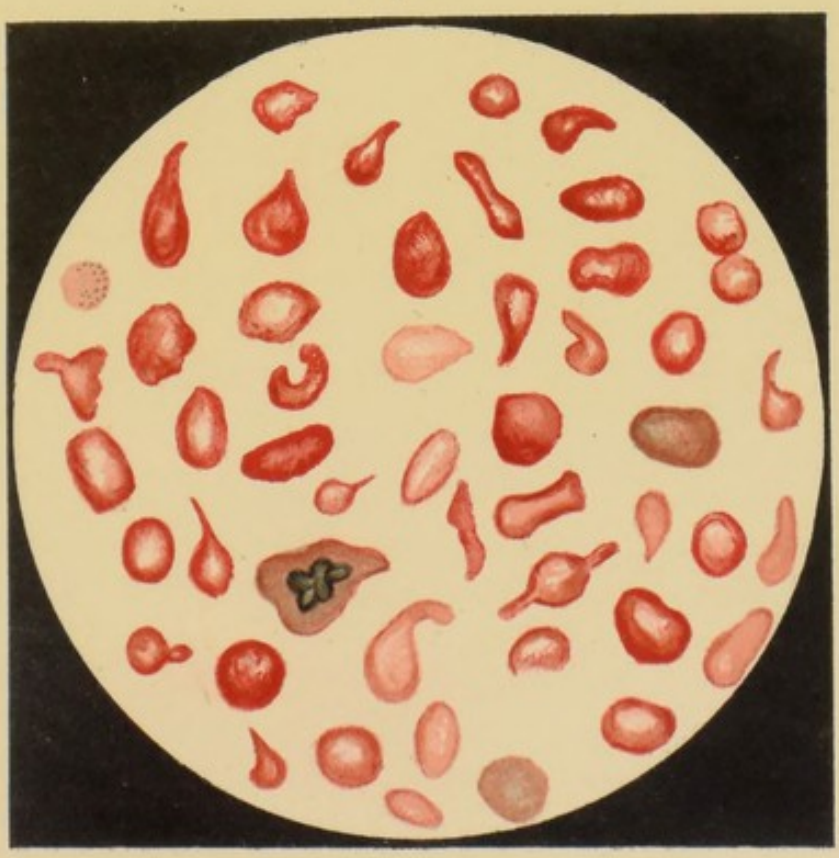


Fig. 2.



PL. I.

Fig. 1. Chlorosis. Fixation in absolute alcohol, stained with Israel-Pappenheim's fluid and Löffler's methylene blue. X 1000.

Fig. 2. Pernicious Anæmia. Fixation in absolute alcohol, stained with Israel-Pappenheim's fluid and Löffler's methylene blue. X 1000.

among a number of different cases each seems to have its own peculiar features. In some of the specimens just shown, variability in size, shape, and haemoglobin-content of the red corpuscles is at once obvious, in others excessive poikilocytosis; some show normoblasts, others megaloblasts. Basophil granulations are a feature of some cases. This protean character in the morphology of the blood is a feature of pernicious anaemia. A cell constantly found is the polychromatophilic megalocyte; compared with these, megaloblasts from which they arise or normoblasts are rare; when the former are seen normoblasts are frequently absent, and when these occur megaloblasts are uncommon. A megaloblastic anaemia without any definite clinical history that would throw light on its causation, such as carcinoma of the rectum, granular kidney, syphilis, or typhoid fever, in all of which diseases I have seen blood with some megaloblasts, is probably to be ranked as progressive pernicious anaemia. The persistent presence of megalocytes alone, especially if these are in sufficient numbers to explain the high colour index of the blood, which may reach 1·7 (Hayem), is in my opinion a feature as distinct as the presence of megaloblasts. My own observations lead me to believe that the latter not infrequently appear for the first time and persist during the last few days of life. Periods of temporary improvement not infrequently alternate with relapses.

In pernicious anaemia the number of corpuscles is considerably reduced. Hayem's original observation of a colour-index >1 has been abundantly confirmed (Laache and others). This may range from 1·2-1·7 (Hayem). In fresh blood the rouleaux formation, as in other severe forms of anaemia, is generally absent. According to Lorrain Smith the total haemoglobin of the body, as estimated by the carbonic oxide method, is diminished in proportion to the gravity of disease; the average is 48 per cent. of the normal. This contrasts markedly with another anaemia often classed as primary, chlorosis, where no absolute diminution of the colouring matter occurs (total average amount of Hb=95 per cent.). The volume of the blood may show either an increase or a diminution; the former is noticeable in severe cases.

The following chart shows the curve obtained by examining the blood in an undoubted case of pernicious anaemia.

If the blood is examined in cases of anaemia which are secondary to helminthiasis, then in cases where Bothriocephalus latus occurs in the gut, this parasite may or may not cause an

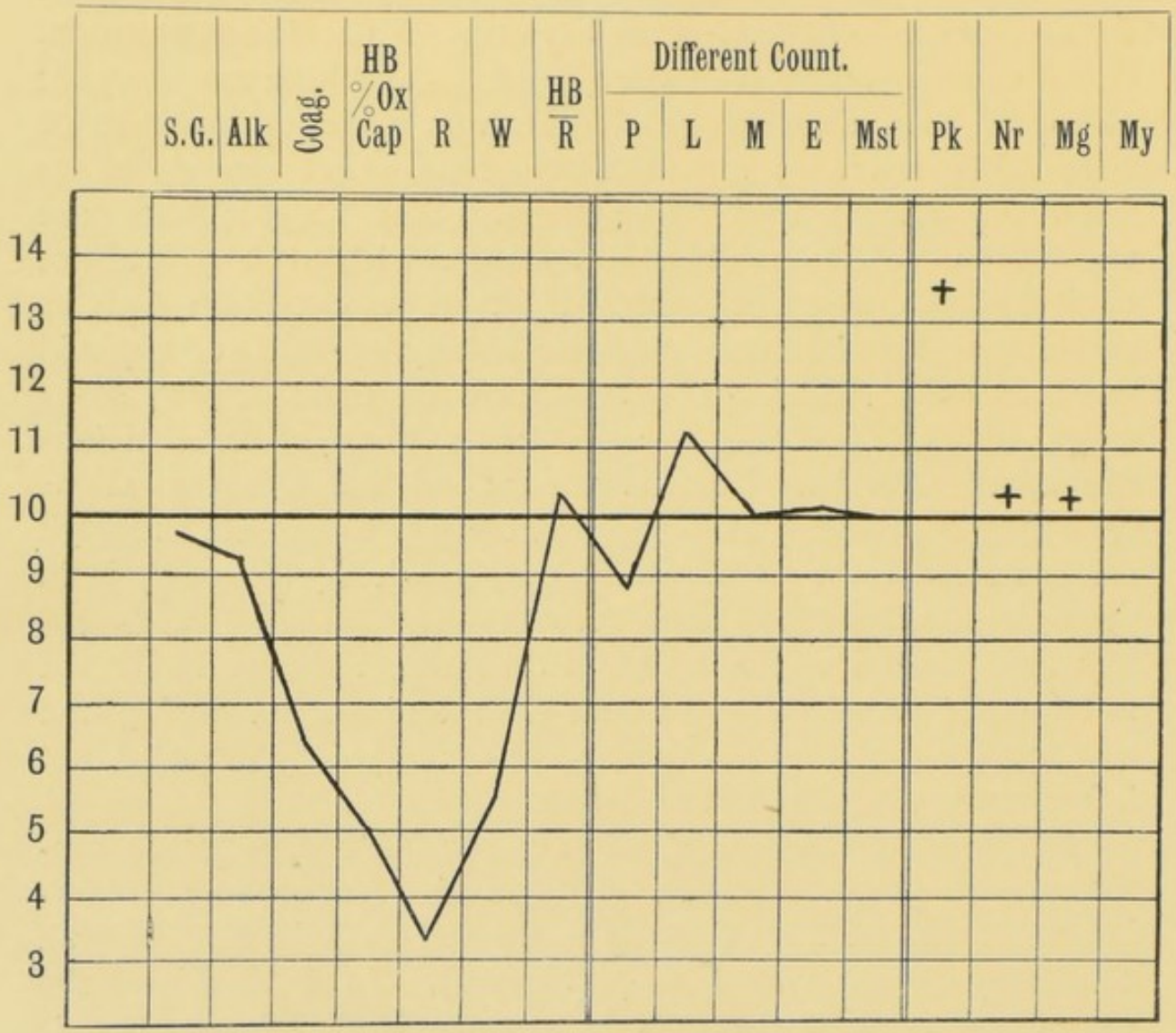


FIG. 12.—Curve showing deviations from the normal in a typical case of progressive pernicious anaemia. The normal values lie on line 10.

anaemia of a megaloblastic type. If this is observed, it is probable that the anaemia follows upon the absorption from the intestine of toxic material derived from dying or dead putrescent parasites (Schapiro, Wiltschur, Babes, Schauman, and Tallqvist). This anaemia may therefore be regarded as a progressive pernicious anaemia the etiology of which is known.

LEUKAEMIA

The growth of our knowledge concerning the group of diseases known as leukaemia possesses an interest both for the physiologist and pathologist. The disease is not peculiar to man, for it has been described by Wolff and Jöhne in calves a few weeks old. Probably Rokitansky, certainly J. Reid and Hughes Bennett, recognised a condition post-mortem which according to the last observer was fatal, "from suppuration of the blood." Virchow observed the same morbid appearance of the blood, in November 1845, but not only did he find no evidence of any inflammation of the veins, but the idea that pus spontaneously formed in the veins was directly contrary to the views which he held as to the nature and origin of inflammation. The disease, now for the first time recognised as "Weisses Blut," was held to be due to an excess of leucocytes secondary to a primary disease of the spleen. One month after Virchow's publication in *Froriep's Journal*, Dr Fuller of St George's Hospital, for the first time diagnosed during life the disease which is known to-day by the term suggested by Hughes Bennett—leucocythaemia, or, following the German synonym, leukaemia. In addition to a splenic form of leukaemia, Virchow described in 1847 a form associated with enlargement of lymphatic glands, and though differences in the character of the cell-content of the blood were recognised, the distinction of the two diseases was founded chiefly on morbid anatomy.

The observations of Neumann that in man the bone-marrow is the chief, if not the exclusive, site of blood-formation, both for the red and white corpuscles, together with the almost constant pathological changes of this structure which are associated with all types of leukaemia, were responsible for the introduction of a third form of leukaemia, the medullary or myelogenous type.

A histogenetic conception of the leukaemic process, together with that differentiation of the leucocytes which Ehrlich's methods have rendered possible, enables the view to be taken that in leukaemia the blood shows an absolute persistent leucocytosis; not only is the number of leucocytes absolutely

increased, but there is a quantitative deviation from the normal percentage which, together with an invasion of the blood by abnormal forms of white cells, constitutes the special pathognomonic sign of the disease. The changed morphology of the blood is therefore the cardinal symptom of a disease which, as a matter of fact, is often discovered quite accidentally. Additional clinical facts and post-mortem observations can only indicate the possible regions which are implicated in producing the specific blood change. My own view is that in both lymphaemia and myelaemia, the bone-marrow in the majority of cases of the former, and universally in the latter, is the primary site of those pathological processes which induce the changes in the blood. In the adult, myeloid tissue is restricted to the bone-marrow, while lymph-adenoid tissue is universally distributed (Arnold, Ribbert). It is therefore difficult to indicate any particular region which is primarily involved and produces a lymphaemia. There are undoubted cases of lymphatic leukaemia without any marked swelling of the lymphatic glands or tonsils, but in every case which I have seen, the bone-marrow showed a marked lymph-adenoid hyperplasia, loaded with cells of the size, shape, and staining properties of those which characterised the blood leucocytes.

Acute Lymphatic Leukaemia

This is a comparatively rare disease which rapidly runs its course accompanied with fever, so that to many observers its course resembles that of an acute infective process. An oligocythaemia, low haemoglobin-content, and an absolute increase of leucocytes is constant. $\frac{W}{R}$ varies, but averages 1 : 40. The feature of the blood is the appearance, in variable numbers, of cells which are regarded as large lymphocytes by most observers. The typical lymphocytes occur, but are less frequent. Polymorphonuclear cells are always diminished in number, 2-20 per cent., or entirely absent.

The nature of the large cells (lymphocytes?)[1] which

[1] In acute lymphatic leukaemia of children, the small lymphocytes may be numerically the chief cells (Theodor, *Archiv. f. Kinderheilk.*, xxii., 1897).

Fig. 1.

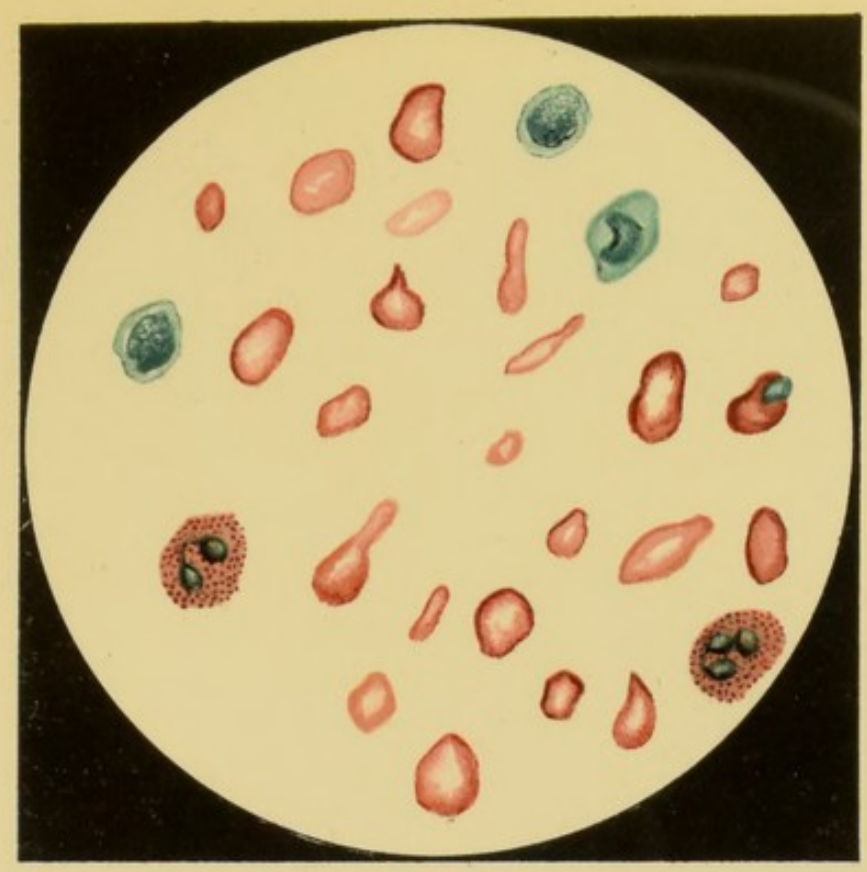
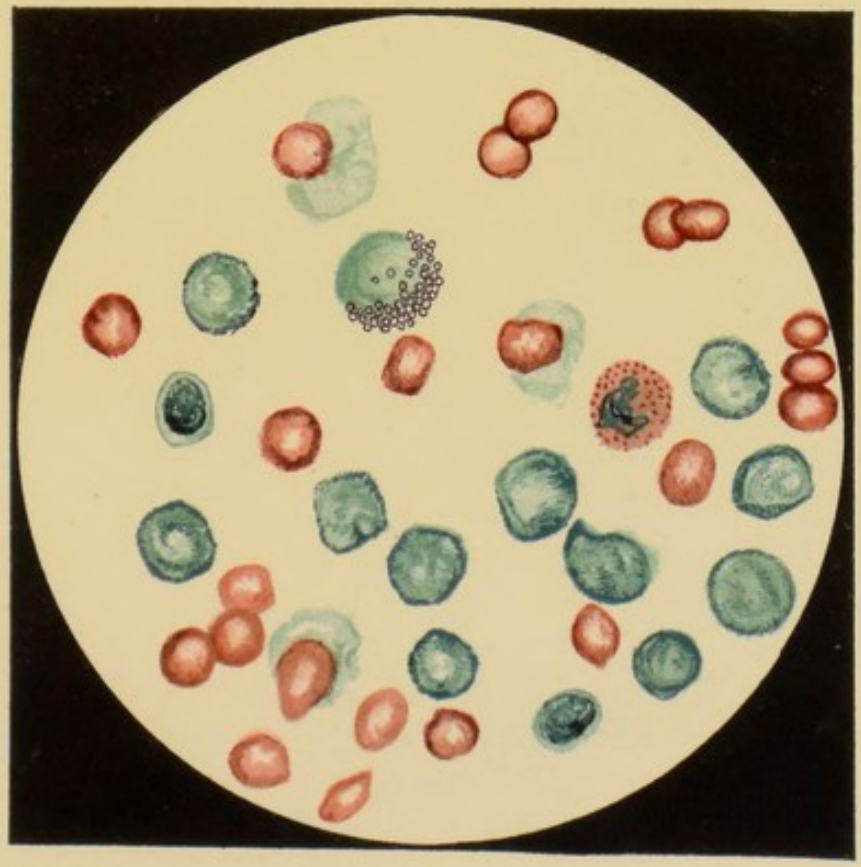


Fig. 2.



PL. II.

Fig. 1. Chronic Anæmia (syphilitic). Jenner's stain. × 1000.

Fig. 2. Acute Lymphatic Leukæmia. Jenner's stain. × 1000.

characterise acute lymphatic leukaemia appears to me by no means free from doubt; not only is the outline of these cells remarkable, but the transformation of the nucleus is unlike that of any lymphocyte. The size of the cell and the few granules the protoplasm may show, are peculiar to the disease. They also tend to smear and become froth-like to an extent never seen with the large lymphocytes of normal blood. Further, these cells appear quite different to those of similar size, which occur in comparatively few numbers in chronic lymphaemia. The term bone-marrow cell, or "unripe" form of myelocyte, indicates, I believe, the region whence these cells come.

The accompanying slide shows the features presented by a typical case of acute lymphatic leukaemia (Plate II., Fig. 2).

1. Predominance of large lymphocytes (?), a few as large as 18 μ, most of them from 10-12 μ, some smeared, some foam-like, flattened ghosts 20 μ or more in diameter. I feel convinced that cells similar to these are never seen in normal blood. Even though the term "large lymphocyte" is retained, it is well to remember that this leucocyte only occurs in pathological conditions, and probably arises from the bone-marrow. Some cells are spheres and show a faint granulation, others have flattened sides and an angular outline; an aspect certainly produced by pressure in making the preparation, for in fresh specimens all the cells are spheres. Some of these possess two nuclei. These leukaemic leucocytes very frequently show several protuberances, which I am inclined to think are induced by a changed condition of the blood-plasma. The nucleus of the cell is often bean-shaped, stains badly in fixed but well in fresh films, and occupies four-fifths of the cell.

2. Comparatively few, apparently normal polymorphonuclear leucocytes.

3. A few eosinophil leucocytes, one twice the normal size.

4. Very few small lymphocytes.

5. One or two neutrophil myelocytes. An eosinophil myelocyte (rare).

6. Red cells vary in size and in staining, a few are chlorotic; there is no poikilocytosis.

7. One normoblast the size of a red disc, and another twice the size, occur in this film. (These are of rare occurrence.)

Of this case the following figures may be given :—

Chromocytes	2,800,000
White corpuscles . . .	60,000
$\frac{W}{R}$ 1 : 45.	
Haemoglobin (Haldane-Gowers), 49 per cent.	
Differential count of leucocytes (percentage).	
Lymphocytes (?) . . .	90
Polymorphonuclears . . .	6
Eosinophil	2
Myelocytes	2

CHLOROMA

In this rare disease, which is chiefly one of early life in males, a multiple lymphoma, the nodules of which are of a bright pea-green colour, is found post-mortem infiltrating the ribs, the bones of the face (orbital and temporal), and also as an extensive green growth along the length of the vertebral column. This neoplasm has been described as orginating in the bone-marrow, and subsequently spreading under the periosteum. The blood shows the features of a marked anaemia, which subsequently changes to an appearance indistinguishable from that of acute lymphatic leukaemia (Dock, Gümbel). The ratio $\frac{W}{R}$ may be 1.

Two kinds of large lymphocytes are found—(*a*) non-granular cells 2-3 times the size of a red disc. The whole cell stains with methylene blue, but the nuclear membrane and the scanty chromatin of the large, swollen, oval, or bowed nucleus stains better than the scanty rind of cytoplasm. This is a cell apparently indistinguishable from the large lymphocyte (?) of acute or of myelogenous leukaemia. (*b*) A smaller cell, less frequent but common, is, as far as an opinion is possible from an examination of plates, a genuine large lymphocyte. The nucleus is large and somewhat indistinctly outlined in a protoplasm which stains less intensely than the nucleus with basic dyes.

In one case of chloroma which I have seen I examined the lymphatic glands, marrow, spleen, and blood (post-mortem), and found about 200,000 leucocytes per c.mm., of which only 2 per cent. were polymorphonuclears. The rest of the cells might have been grouped as large lymphocytes, but by a variety of methods of staining it was possible to identify nearly all of these as myelocytes. Every degree of fine neutrophil granulation could be distinguished. Those which were destitute of granules I consider were myeloblasts (Nägeli), or lymphoid marrow cells (Türk). Some of these cells contained two nuclei.

Chronic Lymphatic Leukaemia

The diagnosis of this disease is only positive when certain clinical symptoms, such as enlargement of the lymphatic glands, variable enlargement of the spleen, a tendency to haemorrhages, and not infrequently chronic skin diseases, are associated with obvious changes in the blood. To establish that these exist, the advice given by Mosler more than forty years ago, to examine the blood repeatedly in all doubtful cases of disease at short intervals of time, still holds as a sound rule of practice. A specimen of such a case shows a large relative increase of undoubted lymphocytes, which may reach 90-92 per cent. of the white cells. These are chiefly, but not entirely of the small variety, and the larger lymphocytes appear to me entirely different from the forms classed under this name in acute leukaemia. A certain number of the lymphocytes are swollen, stain badly, and are probably degenerating. There are no myelocytes or eosinophil cells in the specimen I show, and the ratio of white to red corpuscles, 1 : 45, is very high for this disease. The specimen shows the condition shortly before death, when the increase of lymphocytes is greater than during the progress of the disease ; the red corpuscles of this case were at one time 4,000,000, and the haemoglobin 77 per cent. (Haldane-Gowers), but later the number fell to 2,800,000 per c.mm., with 52 per cent. of haemoglobin.

Acute Myelogenic or Myeloid Leukaemia

The chronic form of this disease is more frequent than any other form of leukaemia, while an acute type is so exceedingly rare that its occurrence has been denied (Walz); but there are several recorded cases besides the one recently described by Billings and Capps in 1903. The disease can only be recognised by an examination of the blood, since the clinical symptoms are those of a severe progressive anaemia. The blood shows only an occasional normoblast; the leucocytes vary from 16,000-540,000, of which 25-96 per cent. are myelocytes. In the diagnosis of this rare disease, it must be remembered that in progressive pernicious anaemia an acute phase of this disease is often accompanied by an invasion of myelocytes into the blood-stream.

Chronic Myelogenous Leukaemia

Although various cases of myelogenous leukaemia possess different features, so that the blood of one patient by no means resembles that of another, and further, the blood pictures for any given case may vary from day to day, or even within a few hours, a general idea of the morphology of the blood can be obtained from the specimens I show you, which present the following features:—

1. The leucocytes are enormously increased— $\frac{W}{R} = \frac{1}{5}$ or $\frac{1}{20}$.

2. Neutrophil myelocytes of various sizes, and with a variable amount of neutrophil granulations, are constant; they may amount to more than 80 per cent. of the total leucocytes, or not exceed 10 per cent. These are the distinctive cells of this leukaemia, and the diagnosis is more positive the larger the percentage of them and the greater the ratio $\frac{W}{R}$.

3. Eosinophil myelocytes are constant in all the cases I have seen; often they form 6-10 per cent. of the total leucocytes.

4. An absolute and percentage increase of mast cells. These may exceed the number of myelocytes. Their presence is a

Fig. 1.

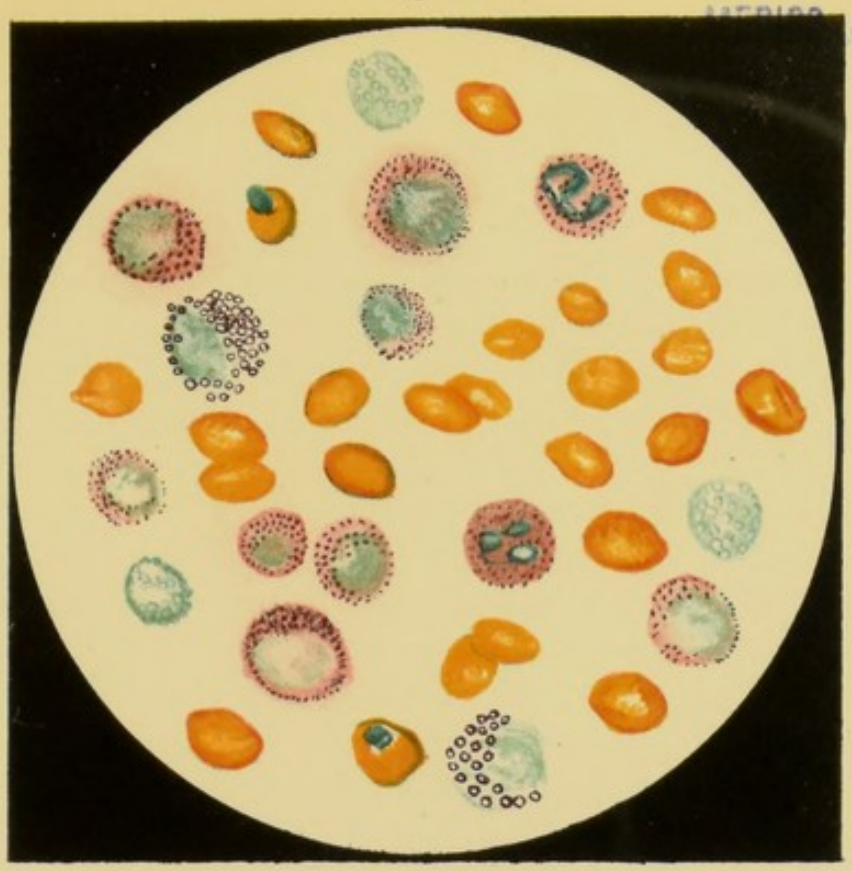
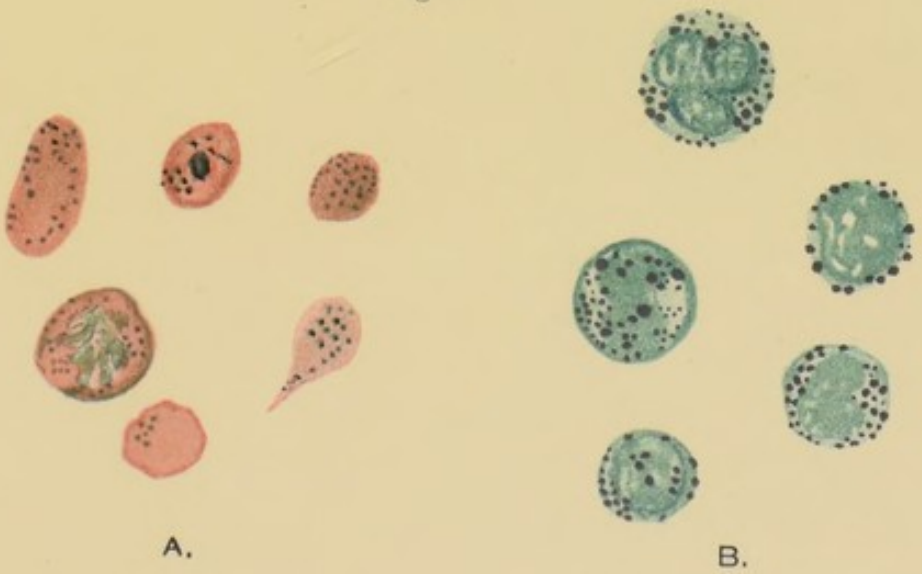


Fig. 2.



PL. III.

Fig. 1. Myelogenous Leukæmia. Heat-fixation at 130°C. Stain, Biondi-Ehrlich-Heidenhain (Israel's modification). X 1000.

Fig. 2. A. Basophil granules in a megaloblast and chromocytes of pernicious anæmia. Stain, eosin-logwood.

B. Varieties of mast-cells in chronic forms of myelogenous leukæmia. Stain, polychrome methylene blue.

prominent feature, often as marked as the presence of myelocytes.

5. Normoblasts in varying numbers.

6. No apparent increase of polymorphonuclear cells, though there is an absolute excess of these.

7. Eosinophil cells appear to be normal in amount, but variable in size.

8. Cells like those described in acute lymphatic leukaemia as large lymphocytes, are almost constantly seen.

9. The red corpuscles in shape are generally quite normal, and form rouleaux in fresh specimens. Stained films at times show some polychromatophil discs.

The pathological mast cells which often form such conspicuous objects in some types of myelogenic leukaemia vary considerably from those of normal blood. Some mast cells are large mononuclear structures, and probably are basophil myelocytes, but others show with triacid or methylene-blue eosin, neither of which stains display the granules of normal mast cells, stained granules some of which are red and some blue, in the same cell. Therefore of the granules in pathological mast cells, some are soluble in water and do not stain, while others do so with both acid and basic dyes. Mast cells of this type, though peculiar to the blood of myelogenic leukaemia, are not found in all cases of this disease (Plate III., Fig. 2B).

The Charcot-Leyden crystals, which have been erroneously regarded as a distinctive feature of myelogenic leukaemia, never occur in fresh blood, though they may appear as the fluid dries under the cover-glass. Post-mortem, the blood or spleen-juice often shows these crystals. Blood removed by puncture from the spleen during life is stated to show these crystals immediately (Westphal).

The following photographs (Plates IV., V., Figs. 1, 2, 3, and 4) taken of the blood in myelogenic leukaemia show how the blood may alter from week to week, but what is still more remarkable, as this case developed the blood became normal during the fortnight before death. The myelocytes and mast cells entirely disappeared. A very large number of specimens conclusively established this fact. Indeed, from the blood

examination at this stage of the disease it was impossible to state any pathological change.

PSEUDO-LEUKAEMIA

A variety of cases, chiefly on clinical and pathological grounds, have at some time or another been grouped under this name, which we owe to Cohnheim. Some forms, such as Hodgkin's disease, have been regarded as an aleukaemic phase of lymphatic leukaemia, since by means of blood examinations the transition of a pseudo- into an acute lymphatic condition has been observed in a few cases, others as malignant lymphoma, while the nature of the cell-infiltration of the lymphatic glands in other cases is often unable to be recognised clinically, but may be found post-mortem to be due to tubercular or malignant disease.

Although I have frequently examined the blood in Hodgkin's disease, malignant lymphatic lymphoma, and cases diagnosed a few years ago as splenic anaemia, it is impossible to say more than that the blood often appears normal, or may show the signs of a secondary anaemia of varying intensity. Similarly, there may be a moderate excess or diminution in the absolute number of leucocytes the relative proportions of which may be preserved, though at times the percentage of lymphocytes may be nearly twice the normal. In the present state of our knowledge it may be certainly affirmed that in pseudo-leukaemia it is quite impossible to predict what the blood examination of any case will show. It is, however, in these doubtful cases of enlargement of the lymphatic glands that much importance is attached to blood examination. The grouping suggested by Pinkus is that in true pseudo-leukaemia a generalised lymphoma exists, associated with a constant relative lymphocytosis, $\frac{W}{R} = 1 : 200$ or $1 : 100$. Such forms should be regarded as stages of lymphatic leukaemia. In a second class are those cases of malignant lymphoma which, apart from changes in the red corpuscles, show no leucocytosis but may show a characteristic diminution of the lymphocytes. Whether these changes

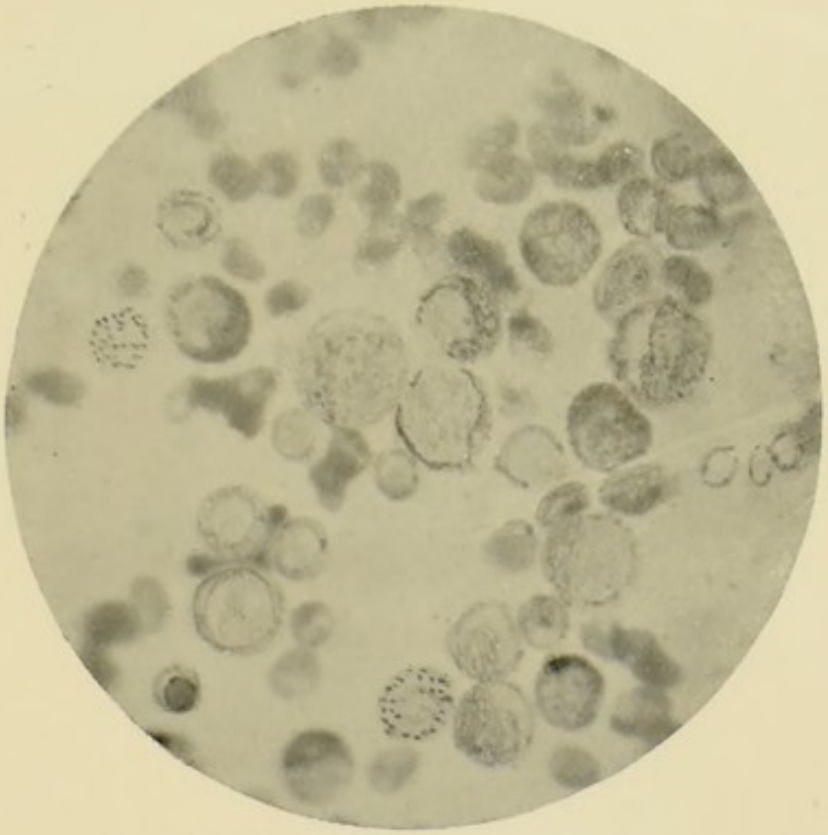


FIG. 1.—Myelogenous leukaemia. Condition of the blood on February 10, 1904. Jenner's stain. The blood contained many myelocytes, mast cells, and normoblasts.

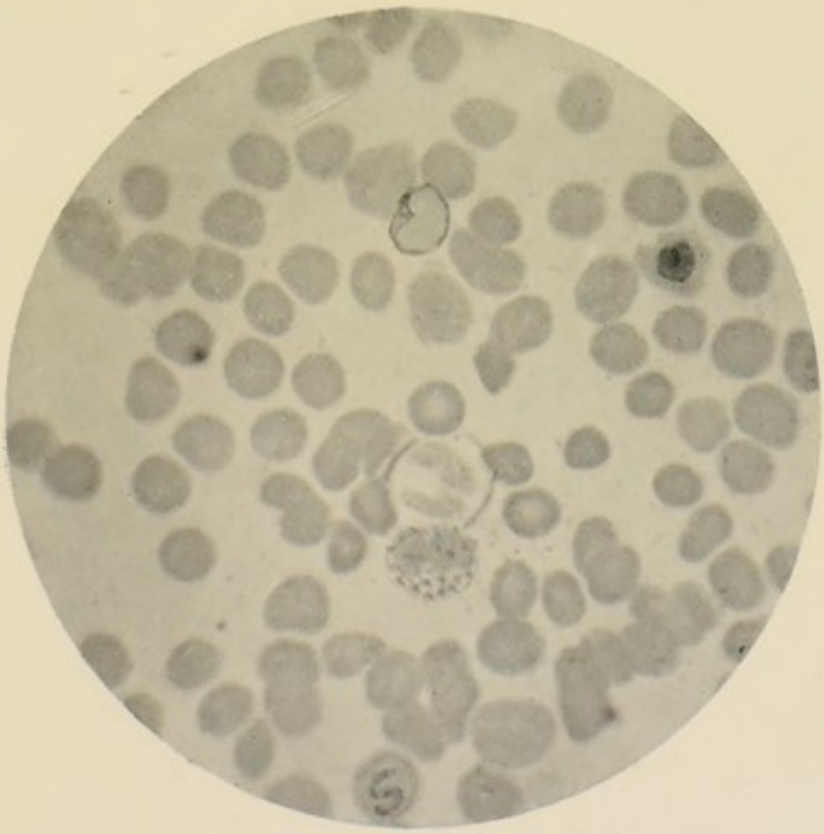


FIG. 2.—Myelogenous leukaemia. Condition of the patient's blood on May 5th. Leishman's stain. The blood contained few myelocytes, frequent normoblasts, and lymphocytes.

[*To face page* 178.

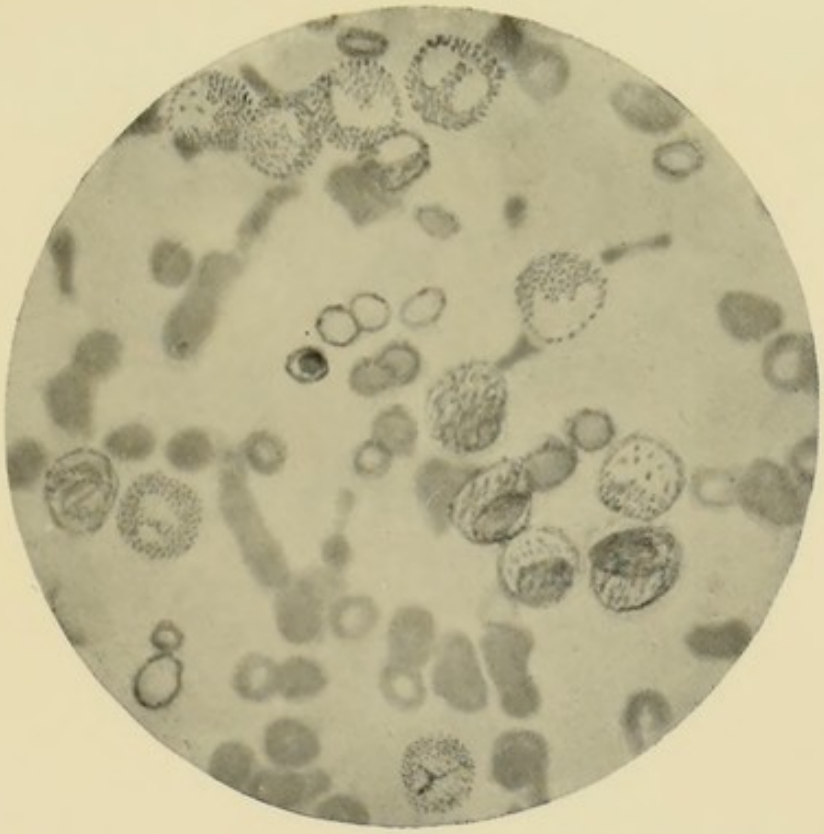


FIG. 3.—Myelogenous leukaemia. Condition of the blood of same patient on June 13th. Leishman's stain. The leucocytes were chiefly neutrophil and eosinophil myelocytes.

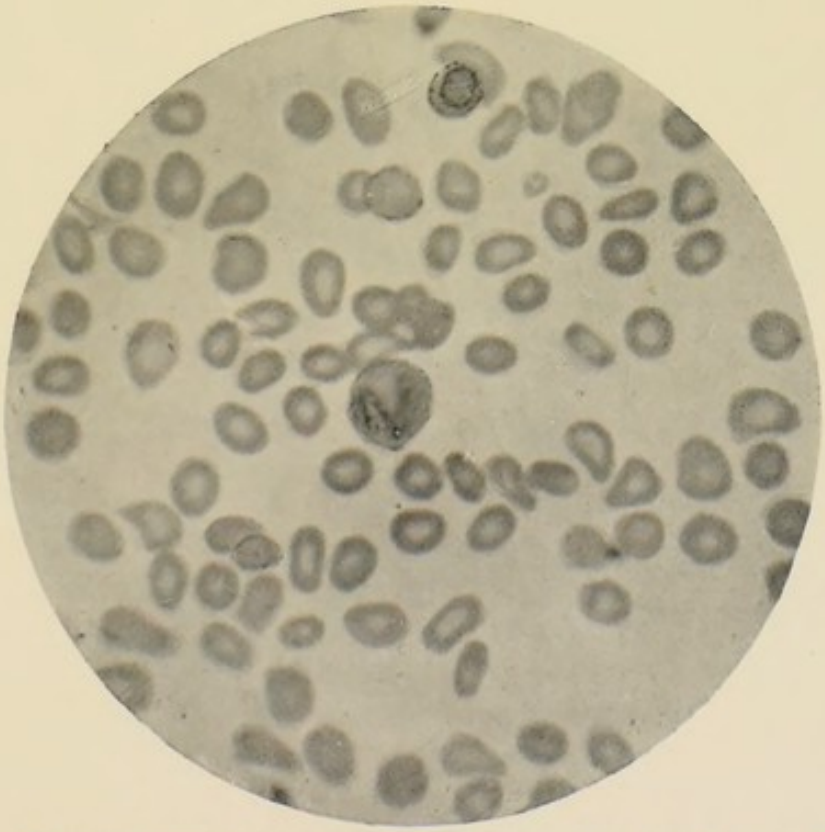


FIG. 4.—Myelogenous leukaemia. Condition of the blood on July 27th, during the last week of life. Leishman's stain. The blood contained no myelocytes nor mast cells.

[*To face page* 178.

in the blood are really symptomatic is one to which it would appear no positive answer can at present be given.

PROTOZOA IN BLOOD

Apart from the parasites of malaria puncture of the peripheral vessels, the spleen or liver may show the presence of Trypanosoma gambiense, a protozoon discovered in man by Dutton and Forde in 1902. From the same sources the parasites discovered by Leishman in 1900—Leishman's bodies (Leishmania donovani, *Ross*)—which have been regarded as an early developmental stage of trypanosoma (L. Rogers), may be obtained. Trypanosomiasis affects many vertebrates, and in man cases of this are generally fatal, and may show the symptoms which form the clinical features of sleeping sickness, a disease which has been studied in the Congo Free State and Uganda. The peripheral blood may or may not show active motile parasites, and febrile attacks are often but not always associated with their increase in the blood. Shortly before death large numbers may be present.

The photograph from one of my own specimens shows the relative size and shape of this parasite in human blood. It is an elongated, motile protozoon. Its granular, protoplasmic body contains a macro-nucleus in the middle, and a blepharoplast at the posterior extremity. An undulating membrane attached along the body is prolonged anteriorly into a flagellum. Excellent figures of this parasite are given by Stephens and Christophers (*The Study of Malaria*, 1904).

The Leishman-Donovan parasites were first described and figured by D. D. Cunningham in 1884, in the granulation tissue of Delhi sore. Subsequent research has shown that in Assam, India, Egypt, China, and the Barbary States there exists a non-malarial remittent fever associated with anaemia and marked fluctuations in the volume of the spleen which is wholly distinct from malaria. In this disease, known as kala-azar[1] (endemic in Assam), cachexial fever (India), the parasites are almost constantly found in the spleen, kidney, bone-marrow,

[1] Almost any epidemic disease in Assam is termed kala-azar.

intestinal ulcers, or in the peripheral blood when the temperature reaches 103° F. (Donovan, Laveran, and Mesnil). The Leishman-Donovan bodies are intracellular parasites occurring within large mononuclear cells of lymphoid tissue, of vascular endothelium or leucocytes (Castellani). A single parasite is elliptical in shape and 1·5 to 4 μ long. In the protoplasm an oval nucleus and a rod of chromatin is embedded. The large nucleus is often seen dividing or divided. Groups of parasites in a common capsule or cyst may be seen in macrophages, and by the disintegration of numbers of these cells the bodies form a zoogloea. The red corpuscle is not invaded by the parasite. The blood examination may show 2-300,000 red discs and a normal number of leucocytes (Donovan), while Leonard Rogers gives 4,000,000 red and 2000 white cells for the average count. Opinion is unanimous that there is a relative increase of large mononuclear cells, averaging 22 per cent. of the total leucocytes.

APPENDIX

SELECTED CLINICAL METHODS FOR THE INVESTIGATION OF THE BLOOD AND FLUIDS FROM SEROUS CAVITIES

1. Fixing and Staining Blood-films.
2. Blood-counting—Differential Count.
3. Alkalinity or Basicity of Blood (A. E. Wright).
4. Estimation of Haemoglobin and the Percentage Oxygen-capacity of Blood. Comparison of the Methods of Oliver, Haldane-Gowers and Fleischl-Miescher.
5. Specific Gravity.
6. Cytology of Serous Effusions.
7. Coagulation-time.

CLINICAL METHODS OF INVESTIGATION

In a study of the morphology of the blood, three different methods are employed which historically fall into as many periods. In the first of these, by the examination of fresh specimens, a certain amount of knowledge was definitely gained with reference to the forms of anaemia, and the existence of leukaemia was recognised as a distinct disease during life, after Virchow in 1845 had first discovered post-mortem the existence of "Weisses Blut." The investigations of Wharton Jones, Max Schultze, Rindfleisch, Hughes Bennett, and Neumann fall in this first period. In 1872 a method for the enumeration of corpuscles was introduced by Malassez, and

in 1877 by Sir William Gowers. During this second period, very little advance was made or indeed was possible until the year 1878. Advances in scientific knowledge are largely dependent on the discovery of new methods, and the publications of Ehrlich in 1878, 1879, and also in 1891 stand in the same relation to haematology as the introduction of solid culture-media by Koch stand to bacteriology. These branches of science were practically created by new methods.

This introduction of a new reliable method was immediately followed by the acquisition of a large amount of positive knowledge of the morphology of normal and pathological blood. The third period is therefore distinguished by the introduction of definite fixing and staining methods, and by the simultaneous development of theories as to the manner in which staining is effected, either with a combination of acid dyes or of basic dyes, or by neutral mixtures, in all of which purely chemical processes are concerned. In staining with colour-salts which are insoluble in water, there is a union between colour-acids and colour-bases, and solubility of a stain is obtained either by excess of one or other dye, or by the use of such a solvent as methyl alcohol.

A great variety of clinical methods have been devised in this country and abroad, which require amounts of blood varying from a good-sized drop taken from the lobe of the ear, or root of the nail, to 5 or 10 c.c. taken in a sterilised syringe from a vein. The most elementary study of any specimen of blood for clinical purposes should take note of the place of puncture, since the blood from capillary districts of the skin has a higher concentration than that of veins. The time of the observation is also of importance, since a physiological digestive leucocytosis is known to commence about two hours after a meal; the haemoglobin-content of the blood also shows a diurnal fluctuation, being higher in the morning than at night time. Even the character of the blood may alter slightly; for instance, the rare mast cell of blood is in my experience most easily found early in the day, three or four hours after a substantial breakfast. Lastly, conditions of sweating, diarrhoea, dropsical conditions, and the administration of drugs, have all a marked influence, which should be taken into account, and where daily blood examinations are made these should be carried out at the same times each day. The following methods are sufficient for the majority of cases:—

1. Microscopic examination of fresh films.

2. Examination of fixed stained preparations.

3. Determination of the haemoglobin in terms of its oxygen-capacity.

4. Estimation of the relations between the number of the red and white corpuscles, and also of the percentage of the different kinds of the latter. This is the differential count of an evenly spread stained film. If time and opportunity allow, determinations of the coagulation-time, specific gravity, alkalinity, and an enumeration of the white and red corpuscles may be made. To actual countings of erythrocytes I attach but little value, since even in the most expert hands considerable errors may occur. I am aware that this is a heterodox view, but absolutely reliable results can only be obtained by using a selected pair of counters which are exact, counting the diluted blood on both of these and rejecting any two determinations which do not agree within 10 per cent. As a matter of fact this procedure is rarely adopted, and consequently the possibility of error is considerable; again, the actual number of corpuscles in a sample of blood is of secondary importance to a haemoglobin determination. If the corpuscles are normal in character, it is a matter of indifference whether the number per c.mm. be six or four millions, the essential point to be determined is the capacity of the blood to associate oxygen, and this depends entirely on the haemoglobin, 70-75 per cent. of haemoglobin is amply sufficient for man. It may be called to mind that even with a haemoglobin-content of 100 or 120, the whole of this does not take up the maximum amount of oxygen; in other words, the oxygen capacity as determined by agitating blood with oxygen is in excess of that which obtains in arterial blood. In fact, inside the body arterial blood is not saturated with oxygen. The explanation of this is that probably the apical regions of the lungs are imperfectly ventilated. Venous blood contains only one-half the amount of carbon dioxide which can be taken up.

Examination of Fresh Specimens

Absolutely clean cover-glasses,[1] not thicker than ·08 to ·1 mm. in thickness, should be used. The glass is held by forceps, and

[1] Cover-glasses, especially in hot climates, tend to become coated with a layer of alkaline silicates. To remove this and to clean, they should be placed in warm water containing 1 per cent. chromic acid and 1 per cent. Nordhausen sulphuric acid for ten to twenty minutes; not longer, as this treatment makes them brittle. Rinse in distilled water, and dry with silk or tissue-paper. Or the glasses may be placed for ten minutes in glacial acetic acid, thoroughly washed in water, and dried.

the surface of the second drop of blood that exudes—the first being wiped off or blown away—from a puncture made by a Hagedorn needle or sharp steel nib in the skin, which has been previously cleaned, is lightly touched with the cover-glass. This is at once inverted on a cleaned slide which has been slightly warmed, and the blood will be found to spread out in an even film. The edges of the cover-glasses are fixed to the slide with a little vaseline or putty, and examined with a magnification of about 400 diameters. A small diaphragm should be used. This method, which appears to have fallen somewhat into disuse, is capable of yielding much information which can be obtained in no other way. The absence of rouleaux formation, a marked feature of many diseases, particularly of pernicious anaemia, definite information as to poikilocytosis, the spherical or discoid shape of the chromocytes, the determination of the living or dead condition of the granular leucocytes, and a certain amount of knowledge as to the time of onset of coagulation, can be obtained by this method. Though it is impossible to demonstrate some features of interest with a fresh instead of a stained film, the former should never be discarded where there is a suspicion of pernicious anaemia, chlorosis, or blood-parasites. The method is an ideal one for malarial blood. For the typing of leucocytes or nucleated chromocytes, estimation of necrotic changes in the red discs, or for the determination of the varying haemoglobin-content of the corpuscles, fresh specimens give information far inferior to that yielded by stained films. Of the suggestions made for staining fresh blood, though I have tried most of them, 2 per cent. solution of methylene blue in 40 per cent. of alcohol (Phear), and the addition of a fragment of solid neutral red to the blood (Pappenheim), seem to be the best.

Examination of Dried Stained Preparations

The second drop of blood as it escapes without pressure from a puncture is spread over a clean slide or cover-glass by one of the following procedures. Holding one cover-glass with forceps in the left hand, pick up another with forceps, lightly touch the drop of blood, and let this glass fall upon the other, so that the drop spreads out evenly in a regular capillary layer; now with two fingers slide, not lift, the upper glass from the under, and wave the glasses in the air so as to rapidly dry them.

Another method is to touch the blood-drop with cigarette-paper, let this momentarily adhere to the glass, and then pull the

paper so as to make a continuous smear. The corpuscles, however, occasionally tend to become somewhat distorted, and leucocytes, especially in leukaemic blood, smear and stain badly. This method is convenient, but does not in my hands yield uniformly good results. By touching a drop of blood with the surface of a clean slide, then placing the bevelled edge of another slide on the drop, waiting a moment to allow the blood to spread along the edge, and then drawing one slide along at an acute angle with the other, films can be made which are as perfect as those obtained by the first method, which is the one originally devised by Ehrlich. A. E. Wright[1] suggests that blood-films should be made on glass roughened with fine emery paper, and this is a good method to prevent distortion of leucocytes. However made, the blood-films should be dried rapidly in the air by waving them about; this also prevents the coagulation of proteids, which only occurs in the presence of moisture. The more quickly the film dries the more serviceable is the preparation, and the layer of blood should be so thin and spread so evenly, that when observed at a sharp angle a spectrum such as that obtained with a diffraction grating should be seen. Films properly prepared can be kept in the dark for months. To prevent mechanical injury to the films, they may be coated with a layer of melted paraffin which is removed subsequently with xylol. Films which have been kept for some weeks will be found to have become fixed, and react to stains as if they had been subjected to heat-fixation.

Fixation of the Dried Film

Having tried almost every published method for the fixation of films, I incline to the view that Ehrlich's original method of fixation by dry heat yields, on subsequent treatment by special stains, the best and most permanent results. The effects of heat as a method of fixation were first brought into prominence by Koch's method for staining bacteria. In the case of blood the procedure checks the absorption of water by proteids, and thereby prevents their decomposition; it prevents loss of haemoglobin and causes adhesion to the slide. Thin, wet films can be fixed by immersion in steam for two or three seconds, and dried films by immersion for half an hour. The ordinary process for fixing bacteria on a cover-glass by passing this through a flame is rarely successful for blood, which requires a

[1] A. E. Wright, *Brit. Med. Journ.*, July 9, 1902.

temperature above 110° C. and below 140° C. A copper plate about 30 cm. long, 10 cm. wide, and 3-4 cm. thick is heated at one end, and in a short time the flow of heat along this is constant, and three points can be fixed upon it by water, xylol, and oil of turpentine, which correspond to the boiling points of these liquids—100°, 139°, 150° C. Cover-glasses or slides, film-face on the copper, are placed in series along the plate from 100°-139° for half to two hours, the shorter time for the higher temperatures. It is safer to heat for at least half an hour, though at times good results may be obtained with five minutes' fixation at 120° C. In no case must a temperature of 140° C. be exceeded. The optimum temperature which gives the best results varies for the blood of different animals and also for different diseases.

Another method for the fixation of wet films is that advocated by R. Muir and Gulland. It is excellent for a study of the granules of leucocytes, but less satisfactory for red corpuscles. Films while still wet are floated on saturated mercuric chloride in ·75 per cent. NaCl for one hour, and then thoroughly washed in ·75 per cent. saline before staining.

Films may also be easily fixed in absolute alcohol for one hour, in osmic acid vapour for one minute, or 1 per cent. formaldehyde in alcohol for 1 minute, but all these methods yield preparations inferior in details to those obtained by dry heat. The best of our chemical methods of fixation is that with pure methyl-alcohol. The fixation is complete in three minutes, though one hour's treatment in no way interferes with subsequent staining. In my opinion, cell-granules are best displayed after heat-fixation or sublimate, the nuclei after alcohol. More complicated methods, such as allowing blood to fall into Hermann's or Flemming's fluid, and embedding the drop in paraffin and cutting sections, have no advantages for ordinary work over the preparation of films.

A method which I have used for many years without being aware that it had been suggested by J. Arnold, I have found of considerable use: it is reliable and satisfactory. For the study of both blood and marrow, especially the latter, it is unequalled. The difficulty of making really good preparations of bone-marrow is very great: the cells smear, are easily distorted, and will not form a thin layer except at the edges. I have used thin slices of elder-pith, lightly soaked in marrow or blood, and at once, while wet, floated on saturated mercuric chloride, in ·75 per cent. NaCl for half an hour, washed out thoroughly in

page 85 says "lightly touching" a drop with a pith-section.

·75 per cent. NaCl, and subsequently stained the slices with the Ehrlich-Biondi mixture. The most perfect preparations can be obtained in this way, and for a study of marrow or blood it is an excellent method, and one in which the cells are neither distorted nor subjected to pressure.

Staining of Fixed Films

Except for the determination of special points of interest, it is advisable to adhere to a single method of staining, since the appearances of blood are extremely variable according to the methods of fixation or staining. Although I have worked with almost every staining method which has been suggested, I prefer one method for normal blood, another for chlorosis, and others for leukaemic blood, since particular methods display different features. In my opinion, no method of staining equals the brilliancy of pictures obtained by the Ehrlich-Biondi or triacid mixtures, no method is so generally useful as that of Jenner or Leishman, and no method for intensely staining haemoglobin is comparable to that of Israel-Pappenheim. Again, the pyronin-methyl-green stain enables the structure of lymphocytes to be studied better than by any other method. Mast cells give a negative picture of their granules with many stains; especially is this the case with triacid, and they therefore require to be stained either with Jenner's fluid, or by some special method such as that of Westphal or Türk.

In the staining of a blood-film a large amount of positive knowledge is quickly obtained, and one is tempted to vary the common routine methods. All the existing staining processes are the result of much study, and have been possible by an exact knowledge of the chemical nature of the dyes which are employed. Though staining appears an entirely empirical process, this is obviously not the case; but at the same time, when it is remembered that to make a really serviceable preparation is never an easy matter, the best results are obtained by a rigid adherence to the several steps of the whole process. With methods of successive staining, especially where aniline dyes are used, it is difficult to get constant results, however carefully the preparation is treated. To demonstrate some particular feature in any given cell of the blood, *e.g.*, the structure of the nucleus, mitosis, or to study the relations of the mitoma of a cell, it is often of use to employ a single stain with subsequent differentiation. Simultaneous staining is generally

employed in methods of panoptic staining, when at least two and often three different dyes are employed to bring into prominence the greatest number of features presented by any one film. The full beauty of such staining is only to be seen with an oil-immersion or an apochromatic objective, used with most careful illumination. All stained films, when washed free of stain, are dried between folds of filter paper, taking care to use the smooth side of this. If examined with a dry lens, they are then mounted in neutral xylol-Canada-balsam, or the preparation can be examined when dry directly with an oil-immersion.

1. Jenner's method. The bottles to hold the stock solutions must be perfectly clean and dry, and are washed out with methyl-alcohol (Merck's methyl-alcohol, *puriss f. analyse*, free from acetone). 1 gramme eosin (water-soluble, Grübler) in 100 c.c. methyl-alcohol. 1 gramme methylene blue (Ehrlich's medicinal, free from zinc) in 100 c.c. methylic alcohol. The stain is made in small quantities of 22 c.c. as required, by mixing 10 c.c. of the first with 12·5 c.c. of the second.

Air-dried films are covered with, or floated on the stain for five to seven minutes; throw off excess of stain, wash rapidly in distilled water until a pink colour appears (about fifteen seconds), dry rapidly with filter paper, and mount in neutral balsam. By this method all the essential microscopical features of the blood are shown, also filaria, bacteria, and malarial parasites if present. The granules of every kind of leucocyte, including the mast cells, are well displayed. Films are best examined with daylight.[1]

In many respects the stain is similar to the eosinate of methylene blue, introduced in 1891 by Romanowsky, many modifications of which have been devised by Nocht, Maurer, Laurent, Rosin, Giemsa, and more recently by Leishman, whose method of rapid Romanowsky staining I believe to be the best for routine work. Several distinct colours are formed by the action of eosin and methylene blue, *e.g.*, methylene violet, methylene-azur; and the principle of the staining is that this takes place during the precipitation of the stain with water.

2. Leishman's method of rapid Romanowsky staining.[2] Use eosinate of methylene blue (Grübler), and dissolve ·15 grammes of this in 100 c.c. of methyl-alcohol (Merck's). It dissolves with

[1] Jenner's plates, showing the results of his method, are given in Allchin's *System of Medicine*, vol. ii., p. 297, 1900.

[2] *Brit. Med. Journ.*, Sept. 21, 1902.

difficulty, so that the solution is made by rubbing the sticky dye in a mortar with about 20 c.c. of liquid at a time until the whole 100 c.c. is used. An almost saturated solution is obtained. It is difficult to keep the stain in good condition.

Blood-films are covered with three drops of stain for half a minute to fix, then add six drops of distilled water, and allow the mixed diluted stain to act for five minutes. Quickly wash off with distilled water, and leave the specimen to soak in this for half a minute, dry and examine directly in cedar-wood oil, or mount in balsam. The dense nuclei of the polymorphonuclears or lymphocytes should appear as a deep purplish red, or ruby colour, and this forms the best index of the depth of staining. Should the red corpuscles appear bluish, the pink colour may be restored by washing in diluted acetic acid, 1 : 1500. The results are excellent as a nuclear and haemoglobin stain; mast-cell granules and malarial parasites are well shown, but the method is not a perfect one for differentiating granules. As far as my own judgment of colour is concerned, it will be found when the specimen is viewed by natural white light that the red discs are pink with a shade of green, polychromatophilic corpuscles a violet, while basophil granulations in the red discs appear as dark dots. The platelets are red, with spiny outgrowths, and a pale blue zone lies around the red centre. Chromatin stains a ruby red or purple colour; thus the nuclei of normoblasts are dark violet, those of lymphocytes an intense purple in a deep blue protoplasm. Large mononuclear cells have a faint blue protoplasm, with occasional red granules; the nucleus is poor in chromatin, and of a pale diffuse purple tint. Mast-cell granules are often brownish purple; that is, they stain metachromatically, and encrust a cell containing an obscure faint purple nucleus. The granules of myelocytes may stain in a variety of tints, red, blue, and purple, even in the same cell. Their nuclei stain a faint violet.

If Leishman's stain is used for the detection of haemamoeba or trypanosoma suspected in blood, the time of staining is ten instead of five minutes. In order to show details of structure the chromatin requires deep staining, and the film should subsequently soak in water three to five minutes. In trypanosoma the macro-nucleus appears red, the blepharoplast black, the flagellum red, basophil granules black, and the protoplasm of the parasite blue. In the case of the malaria parasite, the protoplasm stains blue, the chromatin a light ruby-red colour, the melanin granules retain their natural appearance. To

demonstrate Schüffner's dots in the red cells infected by benign tertian parasites, the stain should act for fifteen minutes. Maurer's dots in the red cells infected by malignant tertian parasites are as distinctive as the crescents, and occur only in cells infected by young segmenting forms when these are mature. The peripheral blood does not constantly show them. The stain requires to act for an hour in order to demonstrate these dots. For the Leishman-Donovan bodies, stain as for malaria parasites, ten minutes; both the large and small chromatin masses stain a ruby-red in a faint blue protoplasm.[1]

Should blood-films be old, it is possible to get excellent results if the following procedure is adopted. After the first staining, if the contrast colours are not good, treat the preparation with absolute alcohol for half an hour, and restain with dilution 1 : 2 of water, repeating this process until the result is satisfactory.

The method of Tulloch,[2] according to Leishman, gives results only slightly inferior to his own. When Merck's methyl-alcohol cannot be procured, to 25 c.c. of methylated spirit two drops of a 10 per cent. solution of potassium bicarbonate are added, and in this mixture a saturated solution of the dye is made in a mortar. After twenty-four hours it is ready for use. Films are air-dried, fixed in methylated spirit for fifteen minutes, and treated for five minutes with the staining fluid diluted with two volumes of distilled water. Wash in distilled water for half a minute, and then for a few seconds until pink in 1 : 1500 acetic acid, again wash in water, dry, and mount.

3. Israel-Pappenheim's[3] method. This is the most perfect stain for determining the haemoglobin-content of the corpuscles. It is especially useful for blood examinations in chlorosis or pernicious anaemia, and it also shows the structure of normoblasts and megaloblasts with great distinctness. It was originally introduced as a method for studying the fate of nucleated red corpuscle in foetal mice. It is the easiest of methods, and gives results of great excellence; the intensity of the stain stands in relation to the amount of haemoglobin in the discs, so that many grades of colour can be seen in these. I consider it the best stain we possess for a study of the films in cases of pernicious anaemia, and both the stages of extrusion and dissolution of the nuclei can be followed in nucleated red corpuscles.

[1] Leishman, *Journal of Royal Army Medical Corps*, June 1904.
[2] *Journal of Royal Army Medical Corps*, August 1904.
[3] *Virch. Archiv*, Bd. 143, 1896.

As a nuclear stain haematein is used, and for the haemoglobin in the cytoplasm a mixture of three acid aniline dyes.

Rose bengale	6 grammes
Orange G	2 grammes
Aurantia	1 gramme

The dry stains are mixed together and dissolved by warming and shaking until the fluid is just transparent, in the following liquid :—

Distilled water	10 volumes
Glycerine	1 volume
Absolute alcohol	1 „

The films are fixed in absolute alcohol for ten minutes to an hour. Heat-fixation is useless. Osmic acid vapour-fixation gives inferior results. Stain with haemalum (Meyer) for five minutes, thoroughly wash in tap-water until no more stain comes away, then stain in the Israel-Pappenheim fluid for five minutes, thoroughly wash again, dry, and examine in balsam. This method is useless for the granules of leucocytes; the cytoplasm of these is tinted a faint rose-colour, and the nuclei are violet or blue-black. If laked blood be examined by this method the plasma stains, and the ghosts of the red discs stand out as circular white bodies. These ghosts are therefore displayed as negative pictures.

I use Ehrlich's haematoxylin in preference to haemalum, since it is a more effective nuclear stain. Many authors lay stress on the aspect and character of the nucleus as a guide to the differentiation of leucocytes, and though I consider this feature is of small importance, it is undoubtedly advantageous to use an intense nuclear stain. Erhlich's haematoxylin is best made according to Mann's directions.[1]

Haematein	2-5 grammes
Absolute alcohol	100 c.c.
Glycerine	100 „
Water	100 „
Potash alum	10 grammes
Glacial acetic acid	10 c.c.

Mix the haematein with acetic acid and 25 c.c. alcohol; after complete solution add glycerine, and 75 c.c. alcohol. Shake thoroughly, and while hot add the solution of alum in water.

[1] G. Mann, *Methods and Theory of Physiological Histology*, Oxford, 1902.

4. Dried films fixed in absolute alcohol stain badly or not at all with basic aniline dyes; but with the "wet method," where the film directly it is made is dropped into absolute alcohol, methyl-alcohol, saturated mercury perchloride in ·6 per cent. NaCl, or formalin-alcohol 1 : 10, excellent results are obtained with Erhlich-Biondi, triacid, or Jenner's stain. The following are the details of a good "wet method," during which the film must never dry.[1] Hold the film of marrow, blood, peritoneal fluid, etc., with wet side towards the vapour of formalin or 2 per cent. osmic acid for five seconds; this coagulates the plasma, the corpuscles retain their natural shape and do not clump together; then place in absolute alcohol for fifteen minutes. Remove excess of alcohol with filter paper, since this checks the action of the methylene blue. Stain with Jenner's fluid not longer than two minutes, as a longer time results in too feeble staining with methylene blue and over-staining with eosin. Throw off excess of stain and rinse quickly in two dishes of distilled water. Drop the film in and out of absolute alcohol, then treat with three washings of xylol, and mount in Canada balsam.

Smears of organs or marrow require a wet method; dried films of these are often unsatisfactory. The Ehrlich-Biondi or triacid diluted 1 : 4 with water gives most reliable results. The latter stain should act for five minutes. Wash quickly through water, methylated spirit, absolute, and xylol (R. Muir).

5. Ehrlich's triacid and Ehrlich-Biondi's method. Fixation with heat, or the wet film is fixed with sublimate saturated in ·75 per cent. NaCl. Heat-fixed films—and for most specimens of human blood a temperature of 110°-120° C. for about one hour appears to be best—are, when cool, stained for five minutes in triacid which should not be filtered, then rapidly washed in distilled water and this is removed as quickly as possible by filter paper, since the acid-fuchsin of the stain is rapidly removed by water. Examine the specimen in cedar-oil or balsam. With the exception of the basophil granules of mast cells, every detail of any importance is better displayed by this method than by any other. The haemoglobin stains orange-red, nuclei green-blue to green-black, granules of the polymorphonuclear cells and most myelocytes violet, eosinophil granules chiefly red, and the lymphocyte protoplasm a rose colour. The nuclei of normoblasts stands out with especial sharpness in these preparations. The granules of the mast cells of normal

[1] "Formalin or other fixing vapour followed by absolute alcohol as a Wet Method for Blood-films," by the Hon. G. Scott, *Journ. of Path. and Bact.*, 1901.

blood (but not of abnormal) are soluble in water, consequently they appear as clear spaces in these cells by this method. It is not a very satisfactory stain for lymphocytes. Coloured plates which show the effects of triacid frequently give no idea of the behaviour of this stain, as may be seen by comparing plates 1, 8, and 10 in Ewing's book[1] with plates 2, 4, and 5 given by Da Costa,[2] which show the absolutely different results that can be produced. The figures of Cabot,[3] Engel,[4] and Simon[5] are much more accurate.

The stain prepared in the following way gives the best results. Triacid (Ehrlich) consists of two acid and one basic dye. The latter is generally methyl green, but methylene blue may be substituted with advantage. Use absolutely clean vessels, and prepare saturated watery solutions of Grübler's stains, orange G, acid fuchsin, and methyl green. Allow these solutions to stand for twenty-four hours, and then add in order, continuously shaking—

Orange G	12·5 c.c.
Acid fuchsin	6·5 „
Distilled water	15 „
Absolute alcohol	15 „
Methyl green	12·5 „
Absolute alcohol	10 „
Glycerine	10 „

Ehrlich - Biondi - Heidenhain stain. Use the 3-Farben-gemisch supplied by Grübler, and to 100 c.c. of a ·4 per cent. solution of this in water add 7 c.c. of a half-saturated aqueous solution of acid fuchsin. Shake well, and do not pour the stain out, but pipette off from the top the quantity required. Heat-fixed or sublimate-fixed films are stained one to twenty-four hours. The subsequent treatment is the same as for the triacid stain; the staining effects are almost identical, but in certain types of leukaemia the results with the Ehrlich-Biondi stain are better than with triacid.

An excellent rapid method is that of Michaelis. Dried films are immersed in Zenker's fluid and nitric acid for five seconds, thoroughly washed, then stained with either triacid stain in the

[1] Ewing, *Pathology of the Blood*, 1905.
[2] Da Costa, *Clinical Haematology*, 1904.
[3] Cabot, *Clinical Examination of the Blood*, 1900.
[4] Engel, *Leitfaden zur klinischen Untersuchung des Blutes*, 1898.
[5] Simon, *Clinical Diagnosis*, 1902.

ordinary way. I find the method most reliable, and regard it as a most expeditious one when we desire to use a triple stain.

For Zenker's fluid the following is the formula :—

Müller's fluid, or 2·5 per cent. pot. bichromate	100 c.c.
Sublimate	5 grams.

To 100 c.c. add 5 c.c. glacial acetic acid and 5 c.c. nitric acid, just before using.

6. Pappenheim's stain for lymphocytes.[1] Two basic dyes, methyl green and pyronin, are used, saturated in water. The dyes should be weighed out and mixed in the proportions of 1 part of pyronin to 3·5 of methyl green. The solution should have a distinct bluish tint.

Preparations can be fixed with heat or alcohol. Since triacid is not a serviceable stain for mast cells or lymphocytes, the method of Pappenheim is exceedingly useful. The nuclei of lymphocytes stain violet or lilac in colour, the mast-cell granules metachromatically with pyronin, and appear scarlet red ; neither neutrophil nor eosinophil granules stain, but the nuclei of these cells appear bright green. The cytoplasm of the marrow giant cells stains an intense carmine colour, like the plasm of lymphocytes, which shows clearly the frayed-out fringe which is distinctive of large forms of these cells.

7. Mast cells are particularly well shown by Jenner's stain. The irregular size and variable distribution of the intensely basophil granulations, which are soluble in water, are also well brought out by simultaneous fixing and staining with 10 per cent. polychrome methylene blue in methyl-alcohol. With many basic dyes the granules stain metachromatically and the nuclei stain faintly. It is difficult to improve upon Ehrlich's original method. The films are fixed with alcohol, and stained for one to two hours in a saturated solution of dahlia in

Glacial acetic acid . . .	12·5 c.c.
Absolute alcohol . . .	50 „
Distilled water . . .	100 „

Wash off in water, dry, and mount in balsam.

Türk's method.[2] In this the mast-cell granules are sharply defined and intensely black, with the nuclei pale blue. Basophil granules in the erythrocytes are well displayed, and polychromatophil corpuscles appear dark green. The film is fixed by

[1] *Virch. Arch.*, clvii., 1899. [2] *Weiner klin. Wochenschrift*, No. 18, 1901.

heat at 120° C. for one hour, or by methyl-alcohol, then stained in 1 per cent. alcoholic (60 per cent.) methylene blue (*medic. puriss* Grübler's), which is warmed to 60° C. and allowed to cool. The film is rapidly rinsed in distilled water, placed for half a minute in iodine solution (1 : 300), again rapidly washed in water, and mounted in the following fluid :—

Iodine	1 grammes
Potassium iodide . . .	3 gramme
Distilled water . . .	100 c.c.
Gum arabic, *q.s.* for a thick syrup. - - - - - -	50 grammes

8. Methods of simultaneous or successive staining with solutions of eosin and methylene blue have been extensively employed, but they are unreliable, for, though it is by no means difficult to obtain good preparations, success depends almost entirely on individual skill, and uniformly successful preparations are only possible after much practice. Observers working with these dyes in the form suggested by Plehn, Chenzinsky, or A. Klein, where a single solution of methylene blue and eosin is used, or by the use of successive solutions, have pointed out not only the uncertainty of the method,[1] but also the variations in staining of the granules; thus the granules may at one time appear to be neutrophil, at another oxyphil, and this will depend entirely on the mode of procedure in the method. Again, in many cases the granules which are stained and visible in water become invisible in balsam. The following is a reliable method of successive staining.

Gulland's method of successive staining with eosin and methylene blue.[2] The dyes employed are alcoholic eosin and Ehrlich's purified methylene blue, supplied by Grübler. I do not find water-soluble eosins of much value. Films while still wet are at once fixed by a modification of Muir's method, by dropping them on to the following fixing fluid :—

Absolute alcohol saturated with eosin .	25 c.c.
Ether	25 „
Sublimate in abs. alcohol (20 gr. per cent.)	25 „

Fixation is instantaneous, and after four minutes the adhesion of the blood to the glass is complete. Wash thoroughly and

[1] This is evident, since not only does the staining power of eosin vary greatly with the percentage of alcohol, but what is a more serious objection, the staining effect of methylene blue varies with the age of the solution. Freshly made solutions stain rapidly compared with old ones. I am also satisfied that the temperature of a room affects the results.

[2] Gulland, *Brit. Med. Journ.*, p. 652, 1897.

rapidly in distilled water, and stain for one minute in saturated acqueous methylene blue ; wash rapidly in water, then in absolute alcohol, when clouds of stain come away ; next in xylol, and mount in xylol-balsam. The whole process takes about seven minutes, but any one of the steps, except that with methylene blue, can be lengthened even to the extent of twenty-four hours. The red corpuscles stain pink, the nuclei a deep blue, the cytoplasm of the leucocytes in shades of pink, both the eosinophil and basophil granules are well seen ; platelets are a paler blue than the nuclei.

If pus or sputum is stained by this method, it is advisable to allow the fixing fluid to act for about fifteen minutes.

9. The glycogen reaction of the blood. Whether the substance which responds by a mahogany stain to iodine is really glycogen similar to that found in the liver, is not certain. It is, however, known that both in normal leucocytes (Zollikofer), and especially after the introduction of glycogen into the blood-stream (Gabritschewsky), that the polymorphonuclear leucocytes, the mononuclear cells, and blood-platelets may all respond to the test. Some observers describe this glycogen reaction as a degeneration of the leucocyte, since it is seen in diabetes and in the leucocytoses that are of suppurative origin. Minute brown masses, apparently detached from leucocytes, may also be observed. The cells of pus, or the actively growing cells of the embryo, or a malignant tumour, will often show the glycogen reaction.

The film is dried in the air. It is then placed in a bottle containing iodine crystals for fifteen minutes, removed, and mounted in saturated laevulose solution, or cedar oil. A less satisfactory result is obtained by mounting the film in iodine solution thickened with mucilage.

10. The reaction of Mylius for the determination of the distribution of free alkali in glass, has been employed by Ehrlich as a test for the amount of alkali in blood, which stains an intense red by the alkali forming a coloured salt with an ethereal or chloroform solution of iodine-eosin. This is prepared from erythrosin, the sodium salt of iodine-eosin, by acidulating a 1 per cent. solution with acetic acid. The precipitate is filtered off, thoroughly washed in water, and dissolved in ether. Dry films spread on perfectly clean glasses, are immersed in the solution. The specimen is well washed in ether, and mounted in neutral Canada balsam. The plasma is tinted red, the cytoplasm of the leucocytes stand out pink, the nuclei are unaffected. The

red discs do not stain. The blood-platelets give the most intense red colour. It is considered that this reaction, which I find is not altogether an easy one to do, indicates the relative amounts of free alkali in the blood.

11. Polychromatophil and basophil reaction of the red corpuscles is of considerable clinical interest. Apart from actual necrotic signs such as may be seen in the red disc by the use of such a stain as that of Israel-Pappenheim, the basophil reaction, if it is a sign of degeneration, is the best indication of decayed red corpuscles that at present exists; but "polychromatophilia is no sign either of youth or degeneration, though it can accompany both (Pappenheim)." The basophil reaction is only to be recognised in stained preparations. Many staining methods are useless or unreliable to determine either polychromasia or the punctated basophil features of the red corpuscle. Successive staining methods are of no use, but Leishman's stain, or any other of the same nature as that of Laurent or Nocht, all of which are modifications of the Romanowsky method, yield good results. Triacid stain or successive staining with eosin and haematoxylin, is useless. Ehrlich recommends haematoxylin-eosin, and Türk the method he has introduced, for mast-cell granules. By this the polychromatophil corpuscles appear dark green. I have frequently obtained good results with Chenzinsky's eosin-methylene-blue, which solution is best used at a temperature of 60° C.

Haemocytometry

Red corpuscles. To an actual enumeration of the red corpuscles, except in comparatively few cases, I attach but slight importance.[1] The error may be anything between 5 and 20 per cent. This varies with the observer and with the number of squares counted. Unless the same blood dilution is counted on two counters and all results rejected that do not agree within 10 per cent., the results are uncertain. Personally I find no diluting fluid better than Hayem's,[2] though unfortunately no stain can be added since all organic colouring matters are precipitated by sublimate. I find Toisson's fluid

[1] For recent methods which are considered to diminish the time and labour of counting blood corpuscles, see Strong and Seligmann, *Brit. Med. Journ.*, Nov. 21, 1904; also Bürker, *Pflüger's Archiv*, cvii., 1905. This observer has devised a special counting chamber, which is filled with fluid by capillarity. Only 80 instead of 200 squares are counted. The mean error equals ± ·6 per cent.

[2] HgCl ·5 gram., Na_2SO_4 5 grams., Sod. chloride 1 gram., H_2O 200 c.c. (Hayem).

nearly useless, and it is certainly hyperisotonic for some specimens of blood.

Use the Thoma-Zeiss pipette for diluting the blood for the red discs, and the counter with $\frac{1}{4000}$ factor. Suck up blood to mark 5 or more, wipe end of pipette clean with filter paper, accurately note length of the blood column, and then, with pipette immersed in the diluting fluid, suck this up slowly while the pipette is continuously rolled between the thumb and finger so as to well mix the fluids. Stop exactly at the 101 mark. Without removing the rubber tube, close both ends of the glass tube and shake vigorously for about a minute. Eject three or four drops, wipe the end of the tube, and now blow out a minute drop on to the centre of the ruled disc of the counter. Cover carefully. The fluid should not have escaped into the circular well around the centre disc, and Newton's rings should be seen as an interference phenomenon due to the accurate apposition of the cover-glass to the cell. In counting the corpuscles in any square, count not only those which are included but those which lie on the base and left side of each square. Cells which lie on the top and right-hand side do not belong to the square. Count five squares left to right, next lower five right to left, then five left to right, and so on.

As you proceed, note on paper the totals of each 5 or 10 squares. The number per cub. mm. =

$$\frac{4000 \times \text{degree of dilution} \times \text{total corpuscles counted}}{\text{number of squares counted.}}$$

In diluting, it is unnecessary that the blood should exactly reach 05 or 1, for it is easy to calculate the degree of dilution. Thus, at 03, 05·4, or 06 this is

$$\frac{3}{1000}, \frac{5 \cdot 4}{1000} \text{ or } \frac{6}{1000},$$

and instead of multiplying the number of cells counted by 200 or 100, they must be multiplied by the reciprocals,

$$\frac{1000}{3}, \frac{1000}{5 \cdot 4} \text{ or } \frac{1000}{6}.$$

White corpuscles. An actual enumeration of these ranks among the most important of all clinical methods, but to be

of the slightest value either for purposes of diagnosis, prognosis, or treatment, this investigation should essentially be the work of a trained observer. For the enumeration of leucocytes a pipette which gives a dilution[1] of 1 : 10 is used (in cases of leukaemia I often use the pipette for red corpuscles), with the counter of Zappert, Elzholz, or Turk,[2] which has a ruled floor of 9 sq. mm., 5 of these are each subdivided into 16 smaller fields. All the leucocytes in 3, 6, or 9 sq. mm. are counted. The number of white corpuscles per c.mm. will be

$$\frac{100 \times \text{degree of dilution}}{\text{No. of sq. mm. counted}}$$

Differential counting of fixed and stained films. In this I follow the directions given by Ehrlich,[3] which are as follows :— "Faultless specimens, evenly spread, fixed and stained, are indispensable. Quadratic ocular diaphragms (Ehrlich-Zeiss) are used and dropped into the eyepiece. These stops[4] have sides 1 : 2 : 3 : 4 : 6 : 8 : 10 mm.; the fields, therefore, are 1 : 4 : 9 . . . . 100 sq. mm. The leucocytes are counted in a field of 100 sq. mm. Without changing the field, replace diaphragm 10 by 1. Count the red discs in this area exactly as they are counted in a ruled square of the haemocytometer. That is, include the corpuscles which overlap the left and lower margin and omit those which overlap the right and upper margin. By shifting the specimen about, more fields are counted, and for accuracy 100 fields are counted. The average of the red × 100 : sum of the leucocytes. If the white corpuscles are excessive in number, so that counting with a field of 100 sq. mm. is difficult, smaller fields can be used, when the average of the red × 64 or 36 : sum of the leucocytes."

The percentage relation of the various leucocytes is obtained by a further determination on the same film. Several hundred leucocytes are counted in any convenient field by moving the specimen about. A field is chosen of such size that it is easy to class the 15-20 leucocytes which may lie in this. About 10 counts will enable a fair percentage estimation to be made,

[1] Diluting fluid : 3 per cent. glacial acetic acid in distilled water with 1 per cent. of a 1 per cent. aqueous solution of gentian-violet.

[2] *Wiener klin. Wochenschrift*, Nos. 28 and 29, 1902.

[3] *Histology of the Blood*, Ehrlich and Lazarus, translation by W. Myers, 1900, p. 31.

[4] An eyepiece (Leitz) made so as to give varying square fractions of the field can be employed.

and this is far easier to do than a count of leucocytes in the haemocytometer; further, in the latter case normoblasts are easily confused with small lymphocytes, and the separation of myelocytes into groups is exceedingly difficult. The estimation of the numerical relation of the nucleated reds with normoblasts and megaloblasts can also be determined. Three exceedingly valuable sets of data can therefore be obtained by the above method. The observer is not hurried, the counts can be made at any time, the determinations can be made in any order, and it is moreover the only really certain way of accurately classifying the leucocytes. Still more important is the fact that the results can be subsequently confirmed and objections refuted. To obtain accurate information of the percentage of different leucocytes is not easy in the haemocytometer, for apart from the tendency which leucocytes have to clump together in many diluting fluids, I have often seen the cells of pathological blood so distorted that identification is hopeless. In myelogenous leukaemia the blood may vary greatly from day to day, or even within eight hours, and the method just described enables these changes to be detected with the minimal amount of time and trouble.

Example.

W. D., clerk, age 36, King's Ward, Bed 18 (Dr Cyril Ogle's case of myelogenous leukaemia), March 28, 1904.

i. Total leucocytes in field (diaphragm 6 ∴ area 36) = 14.
Total reds in 90 fields (diaphragm 1 ∴ are 1 sq. mm.) = 360

$$\therefore \quad \text{average} = \frac{360}{90} = 4 \text{ per sq. mm.}$$

Total reds in field, 36 sq. m. = 4 × 36 = 144
∴ leucocytes : chromocytes = 14 : 144 = 1 : 10.

ii. Using a field of 36 sq. mm., 280 white corpuscles were counted and classed, which, expressed in percentages, gave:—

Polymorph.	Eosinophil.	Mononuclears.	Lymph.	Myelocyte.	Mast Cells.
42	4	1	8	37	8

Determination of the Alkalinity of Blood by Wright's Method

In the light of present knowledge as to the reaction of solutions, it appears that the fluids of the body are both alkaline and acid. This depends upon the presence of free $\overline{HO}$ ions and $\overset{+}{H}$ ions, and the grade of alkalinity or acidity depends entirely on the concentration of $\overset{+}{H}$ or $\overline{HO}$ ions in solution. Consequently the reaction varies according to the indicator employed.

INDICATOR.

	Litmus.	Methyl orange.	Phenol-phthalein.	Dimethyl-amido-azo-benzol.
Blood Plasma .	Alkaline	Alkaline	Acid	Alkaline
Urine . . .	Acid	Alkaline	Acid	Alkaline

Blood is both acid and alkaline, and the information as to its reaction obtained by any given indicator depends upon how this is affected by the ions at any given concentration (B. Moore).[1] The reason why titration, as in the methods of Engel, Löwy-Zuntz, Wright, and others, does not give information as to the real alkalinity of the blood is because when an acid such as tartaric or sulphuric is added the $\overset{+}{H}$ ions join to the $\overline{HO}$ ions, and thereupon more alkaline molecules (carbonates and phosphates) undergo dissociation with a fresh production of $\overline{HO}$ ions.

Though I generally use Engel's modification of the method of Löwy-Zuntz, the method of Wright[2] is so simple and can be carried out so quickly, that it is preferable. Since the basicity of blood has been studied in this country chiefly by Wright and his pupils, it is desirable that his method should be employed so as to obtain comparable results. He estimates the alkalinity of serum separated from blood which has clotted in a blood capsule or ordinary capillary pipette. The serum is mixed in a capillary tube with an equal volume of diluted normal H_2SO_4.

[1] *Proc. Roy. Soc.*, May 1905. [2] *Lancet*, Sept. 18, 1897.

Solutions are made of 20-, 30-, 40-, 60-fold dilutions of the normal acid, and equal volumes of serum and any dilution of acid are mixed by drawing into a fine capillary, first serum and then acid, so that the same length of capillary is successively filled. The mixture is then ejected, mixed on a glass slide, and tested with carefully-sensitised faintly-red litmus paper. Several trials are made with different strengths of acid until the mixture is just neutral. Normal blood is $\frac{N}{35}$, *i.e.*, the alkalinity of serum is such that it is just neutralised by its own bulk of normal acid diluted thirty-five times. Using the above dilutions, a mixture of equal parts of 30- and 40-fold dilutions would give a 35-fold dilution. $\frac{N}{35}$ equals 530 mgr. NaHO.

That the alkalinity of serum is a real measure of that of the blood is probable, since Wright points out that the serum, citrated plasma and blister-lymph of the same individual yield identical values. The maximal alkalinity may be $\frac{N}{25}$ the minimal $\frac{N}{200}$, which is met with in scurvy, and can be corrected by the administration of such drugs as lactate of soda and bicarbonates.[1]

Estimation of Haemoglobin

In clinical work the quantity of haemoglobin is not expressed in the same way by all observers; thus the amount is indicated by Henocque and Malassez in weights per cent., 13·8 grammes being the normal, while Hayem reckons the haemoglobin-content of the blood, which he terms "la richesse globulaire," by the number of the corpuscles, each of which should possess a normal load of haemoglobin that is necessary to confer a tint equal to the specimen examined. Gowers, v. Fleischl, Dare, Oliver, Haldane, express the amount for any given case on a centesimal scale above or below a standard which reads 100 for the normal.

Of all methods, it is probable that the spectro-photometer in the hands of a trained observer gives the most accurate information as to the amount of haemoglobin in the blood. But for clinical methods the Haldane-Gowers' instrument is the best of the numerous haemoglobinometers which have been devised. I

[1] *Lancet*, Aug. 25, 1900.

think it even exceeds in accuracy Dare's haemoglobinometer,[1] which is largely used in America and has the advantage that no dilution of the blood is required, while it is easy to use and gives quick results. The Haldane-Gowers' instrument is in every way preferable to that of Oliver, or the haemometer of Fleischl-Miescher. Under my direction, Mr Gordon Webb has carefully compared the readings obtained by using these three instruments. The results are shown in Fig. 13, p. 204. Considerable differences clearly exist, and it is most desirable either that all clinical determinations should be made with one standard type of haemoglobinometer, or if other forms are used, the readings should if necessary be raised or lowered.

By means of the Haldane-Gowers' haemoglobinometer the amount of haemoglobin in terms of its oxygen-capacity can be easily determined colorimetrically with the help of a standard solution of CO-haemoglobin. The average oxygen-capacity of normal human blood is 18·5 per cent. for men, 16·5 per cent. for women, and 16·1 per cent. for children. The standard consists of a 1 per cent. solution of ox or sheep's blood of 18·5 per cent. oxygen-capacity in a tube sealed up in coal gas. The exact percentage of haemoglobin which corresponds to 18·5 per cent. oxygen-capacity is still uncertain, but according to Hüfner it would be 13·8 per cent. The standard tube is marked at levels which correspond to 0·40 c.c. and 2 c.c. of 1 per cent. ox-blood solution, and these marks correspond to the 20 per cent. and 100 per cent. marks on the graduated tube; and in any pair of tubes the distances between the marks on the standard and between the 20 per cent. and 100 per cent. on the other tube must be the same, if the instrument is to give very accurate results. If in any pair of tubes the distances are unequal, then a correction must be made. This is about half the percentage difference. Therefore if the distance between the marks on the graduated tube is 4 per cent. less than that of the standard, the instrument will read 2 per cent. too high.

In using the instrument, partly fill the tube with water, add 20 c.mm. of blood from a pipette, and convert the mixture into CO-haemoglobin by saturation with a stream of coal gas. In about a minute the liquid appears pink, and is now diluted with successive drops of water until the tint equals that of the

[1] *Philaa. Med. Journ.*, Sept. 1900. The error does not exceed 2 per cent. (H. Dare). The haematograph of Gärtner (*Münch. med. Wochenschrift*, 1904) depends upon a fact noticed by Finsen, that solutions of haemoglobin wholly or partially cut off the actinic rays of the spectrum.

standard. A minute later the percentage is read off on the tube, another drop is added, and if necessary another, until the

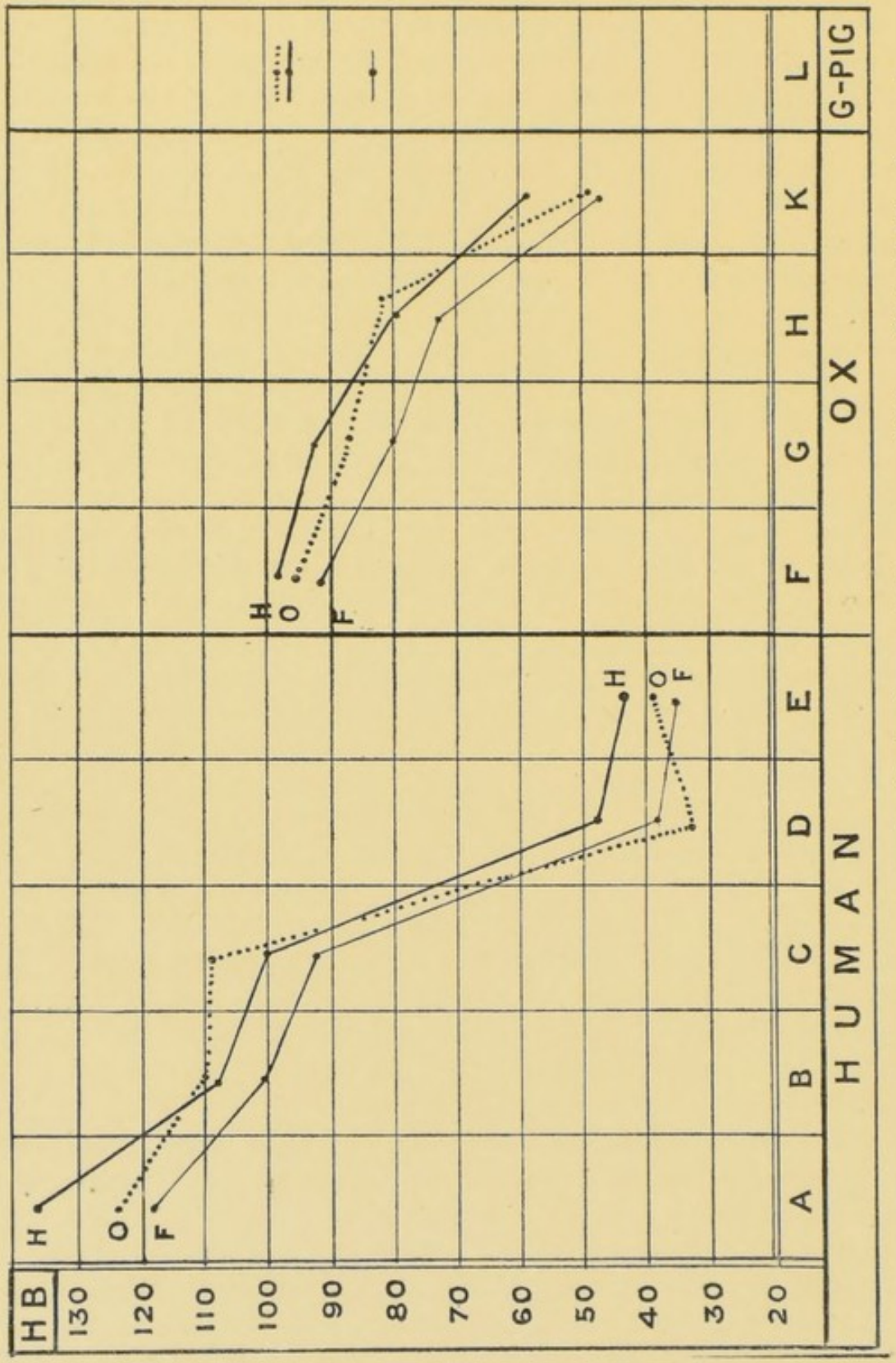


FIG. 13.—The different values obtained with the haemoglobinometers of Oliver, Fleischl-Miescher, and Haldane-Gowers, when various dilutions of human, ox, and guinea-pig blood were used.

tints appear just unequal. The mean of the readings, which give equality, is taken as the correct result. In comparing the

tints of the two tubes, which may be done with natural or artificial light, it is best to hold them up against the light and repeatedly transpose the tubes from side to side during the observations. Should the blood fail to give an exact match when sufficiently diluted and saturated with coal gas, some abnormal bililary pigment or methaemoglobin is certainly present in the plasma, and Haldane has pointed out that saturation of blood with carbonic oxide affords a delicate method of detecting abnormal pigments in the blood.

In estimating the proportion of haemoglobin in the blood of women as percentages of the average normal blood, it is necessary to add $\frac{1}{8}$ to the percentage which is actually read, and for the blood of children $\frac{1}{7}$ should be added. These corrections are necessary, since the average percentage of haemoglobin in the blood of women is about 10 per cent. and of children 13 per cent. below that of men.

The *colour-index* is a factor which indicates the average amount of haemoglobin which exists in a single red corpuscle for any given case compared with the normal, which is unity. The index may $= 1$, or be > 1, or < 1. The colour-index is therefore the ratio of the actual percentage of haemoglobin found in any specimen to the amount that would be present if all the corpuscles in a c.mm. contained their normal haemoglobin-content. That is, the

$$\text{Colour-index} = \frac{\text{Hb}}{x}$$

where Hb is the actual percentage of haemoglobin found by the haemoglobinometer and x is calculated from an enumeration of the corpuscles. It is clear that x always equals the number of corpuscles in a c.mm. $\times \frac{2}{100{,}000}$.

Specific Gravity of the Blood and Serum

For taking this by Hammerschlag's method with a mixture of benzol and chloroform (Inchley uses chloroform and Pratt's petrol A), I have for years used a standard set of 100 specific gravity beads made by Casella; with these I can read to the fourth place of decimals. A drop of blood ejected from a capillary tube under the surface of the liquid becomes a solid sphere, and alterations in the density of the mixture are made by adding benzol or chloroform until the drop and a single bead are suspended in the liquid at the same level. The fluid is

exceedingly sensitive to differences of temperature. At the following temperatures the specific gravity of the same mixture varied from 1057-1064. Therefore handling the vessel should be avoided, and the whole observation carried out as rapidly as possible.

Cent.	Sp. Gr.
17·8	1057
17·25	1058·5
16·85	1059
15	1060
14	1062
13·8	1063
13·2	1064

I consider the use of specific gravity beads gives a more accurate result than that yielded by the hydrometer. O. Inchley[1] has recently suggested a method similar to the one just described.

At birth the specific gravity (Hammerschlag's method) is 1066, and falls to 1050 in the third year of infancy. In 165 male students the average is 1054·4. In nine of these the figures are 1066-1070. 57 per cent. of the total gave 1054-1060 (G. N. Stewart).

Ziegelroth[2] has pointed out that the specific gravity of the body may vary considerably in different individuals while the specific gravity of the blood varies but little. The mean value of the latter in twenty-two adult men was 1057 (the range being 1061-1054). The mean specific gravity of the body was 1055. The highest value was 1069, while in fat individuals and in those with severe forms of anaemia the figures were as low as 1050 or 1046. The same observer finds that whereas the removal of 200 c.c. of blood from patients with chlorosis or secondary anaemia due to syphilis was followed by an immediate fall of about eight degrees in the specific gravity of the blood, which is succeeded in six to eight hours by a rise above the normal, that the withdrawal of 600 c.c. of water in the form of sweat in half an hour had practically no effect on the specific gravity of the blood.

Cytology of Serous Effusions

The recent work of French observers,[3] among whom F. Widal and Ravaut, Marcel Labbé, Sabrazès and Muratet may

[1] *Proc. of Physiol. Soc.*, Aug. 1904. [2] *Virch. Archiv*, cxlvi., 1896.
[3] Labbé, *Le Cytodiagnostic*, Paris, 1903.

be mentioned, on the nature and condition of the cells found in pleural effusions, has led them to assign considerable importance to this line of research in questions of diagnosis and prognosis; but though their results are of interest, these do not I think possess quite so high a diagnostic value as is claimed for them, and certainly do not rank in accuracy with the information which is more rapidly obtained by clinical examination.

The endothelium which lines the pleural cavities is composed of a sheet of cells each of which has a cuticle or face, the edges of which abut one on the other, while the deeper portions of the cells form a reticulated, confluent, protoplasmic plasmodium, in which the nucleus belonging to each cell is embedded (Kolossow, Ranvier). This lining may be inflamed, thickened, covered with a false membrane, or be the site of septic or other bacterial infection, which may originate new growths. Effusions in these cases differ greatly in their cytology from those met with in the course of cardiac and renal disease.

The fluid of the normal pleura, pericardium, or peritoneum always contains both red and white corpuscles; the ratio of these is higher than in the blood, and, with the exception of the mast cell, which is absent, the varieties of leucocytes are identical with those of the blood, but the eosinophil cells are twice as numerous. Synovial fluid is almost cell free. When the fluid of blisters is examined about twelve hours after their formation, this may contain 19 to 25 per cent. of eosinophil cells, almost the same percentage of polymorphonuclears as the blood (65-75 per cent.), together with a few lymphocytes and some large bloated mononuclear cells. Normal cerebro-spinal fluid may be considered as a secretion from the epithelial cells which cover the choroid plexuses. When obtained by lumbar puncture, this liquid is practically destitute of cells; occasionally I have found a few polymorphonuclears.

A cytological examination of any effusion is best carried out with 15 c.c. of fluid, or even with 2 c.c. from an exploratory puncture. This is defibrinated by shaking for half an hour in a sterilised flask containing glass beads. The turbid fluid is then decanted from the fibrin, centrifugalised, and the deposit examined, either unstained, or films may be made; the fixation and staining for these is the same as for blood. Defibrination somewhat modifies the cell-contents of the effusion; shed endothelial cells are not entangled, the lymphocytes only slightly, while many polymorphonuclear leucocytes are retained by the coagulum.

Since my own observations are insufficient in number to afford reliable data, the chief conclusions of French observers will be briefly stated.

In aseptic pleurisies of cardiac or renal origin the fluid of a recent hydrothorax is characterised by an abundance of desquamated patches of endothelial cells; at this stage little else is to be seen in the field of the microscope. In a later stage these cells still persist, but are swollen, die, and slowly disintegrate. Associated with these are many lymphocytes, a few red discs, and a few polymorphonuclear leucocytes. If the lungs become congested, an increase of the cells last mentioned occurs, and the numbers of these stand in direct relation to the intensity of the inflammation. Should the pleurisy be secondary to pulmonary embolism, the percentage of polymorphonuclears may rapidly reach 95.

Early tubercular infection of the pleura is characterised by a definite lymphocytosis, together with a few red corpuscles. The number of polymorphonuclear cells does not exceed 15 per cent. Endothelial cells are rarely found, and then only isolated one from another, never in patches.

The fluid drawn off in cases of pleurisy and hydro-pneumothorax occurring in the late stages of phthisis shows dead and deformed polymorphonuclear cells, together with free nuclei, many of which stain intensely. This pyknotic condition of the nucleus is a sign of degeneration. Endothelial cells are absent.

Septic effusions and those which are associated with streptococci or Fraenkel's pneumococcus present the general characters which are found in polymorphonuclear leucocytoses; endothelial cells and red discs are also uniformly found in pleurisies of this type.

The records of the cytology of effusions in cases of rheumatism, malignant diseases, and leukaemia have not, up to the present, yielded information of much clinical value. In leukaemia, an effusion which is aseptic shows a leucocyte-content which closely resembles that of the blood, and therefore contrasts in a striking manner with any pus which may be formed in this disease. As Fraenkel first pointed out, suppuration in a leukaemic individual is an accumulation of polymorphonuclear cells. On several occasions I have satisfied myself of the accuracy of this statement; the contents of an abscess examined during life is devoid of myelocytes, mast cells, or lymphocytes.

Cerebro-spinal fluid to the amount of 30 cub. cent. may be obtained by lumbar puncture in the interspace between the

4th and 5th lumbar vertebrae, half an inch to the left of the middle line, the spinal column being flexed as much as possible. If removed without admixture of blood, it is normally a perfectly clear liquid, free from any morphological elements. It differs from lymph or other exudations from the blood, contains only a trace of serum-globulin, while albumin, fibrinogen, and unorganised ferments are absent. A reducing substance is present, and this has recently been shown to be dextrose, not pyrocatechin. The average amount of cerebro-spinal fluid is 100 to 150 c.c., and it is a secretion from the single layer of cubical cells which cover the choroid plexuses.[1] Post-mortem, the fluid is invariably clouded by excess of leucocytes. Pathologically, the cell-contents found in the sediment after centrifugalisation of 3 c.c. of liquid varies with the condition of the meninges of the brain and cord. In tubercular meningitis many lymphocytes are found, a condition which contrasts with other acute inflammations, where the leucocyte-count shows a large percentage of polymorphonuclear cells. It is stated by Monod and Nageotte[2] that in general paralysis and tabes, diseases which the researches of Mott have shown to be essentially neuron-degenerations caused by syphilis, the presence of lymphocytes in cerebro-spinal fluid is a constant and early pathognomonic sign. Alcohol and the virus of syphilis both induce slow changes in the pia-arachnoid with a high percentage of lymphocytes in the serous fluid, a feature which is absent in peripheral neuritis, deeply seated tumours of the brain, and various functional neuroses and psychoses.

In trypanosomiasis the examination of centrifugalised cerebro-spinal fluid has acquired importance since the symptoms of "sleeping sickness" have been shown to be accompanied by the presence of Trypanosoma gambiense (Dutton) in the blood and serous fluids. In the opinion of D. Bruce[3] this uniformly fatal disease is directly caused by this parasite, a view which, however, has not met with universal acceptation. After death from Congo sleeping sickness, actually motile trypanosomes are invariably found, not only in the blood but in the fluid of the large serous cavities. During life the parasites may be freely distributed in the blood, and they have been seen in hydrocele or cerebro-spinal fluid when an examination of the peripheral

[1] F. W. Mott, "The Cerebro-spinal Fluid in relation to Disease of the Nervous System," *Brit. Med. Journ.*, Dec. 10, 1904. In this paper full references to the literature will be found.

[2] *Münch. med. Woch.*, p. 321, 1901. [3] *Brit. Med. Journ.*, Aug. 20, 1904.

blood was negative (Christy).[1] Together with the existence of the parasites the fluid becomes richer in leucocytes, and towards the termination of the disease the fluid frequently abounds with pyogenic bacteria and pus.

Coagulation-rate of the Blood

The oldest method applicable to small quantities of blood is that devised by Vierordt.[2] Blood is drawn into a capillary tube containing a white horse-hair which is withdrawn at intervals. The coagulation-time is considered to be the time which elapses between drawing the blood and its adherence to the hair. Some observers, for instance Hayem and his school, content themselves with noticing the average time which elapses between the shedding of a series of drops of blood on to a cleaned slide, or into a very small test-tube, and the moment the drop ceases to move on altering the position of the slide or tube. The observation is made in a moist chamber at the temperature of the room, and the average time is found to be about ten minutes.[3]

In this country most of the recent data for the coagulation-time have been obtained by A. E. Wright's method.[4] The pricked ear or finger is caused to bleed freely, and some eight or ten capillary tubes, each of which is provided with a rubber cap, are filled one after the other up to the 5 c.mm. mark; the end of each is wiped clean, and the blood aspirated a little farther up the tube. The tubes are kept at a constant temperature of 67° F. The exact time of filling each tube is noted, and the observation is continued by blowing out the content of the first tube on to a piece of paper after three minutes; by trials with the other tubes at varying intervals, it will be found that from one the blood cannot be expelled; the time which elapsed between the filling and blowing into of this particular tube gives the coagulation-time. The personal equation is an important factor in the use of this instrument, which requires considerable technical skill, and the results must be received with caution, unless the observer has had much practice.

In the method of Brodie and Russell[5] the periphery of a drop

[1] *Brit. Med. Journ.*, Aug. 20, 1904.
[2] Vierordt, *Archiv f. Heilkunde*, xix., 1878.
[3] Bezançon et Labbé, *Traité d'Hématologie*, p. 33, Paris, 1904.
[4] *Brit. Med. Journ.*, July 20, 1893, and Feb. 3, 1894 (figure of apparatus). *Lancet*, Aug. 1, 1898.
[5] *Journ. of Phys.*, 1897, p. 403.

of blood is caused to rotate by a jet of air, which plays gently and for short intervals of time on the edge of a film of blood held on the inverted summit of a glass cone. The observation is carried out at a constant temperature in a moist chamber, and the rotation of the blood is viewed with a low-power microscope. The coagulation-time is taken to be that between dipping the glass cone into the blood and the moment the rim of blood at the periphery becomes solid, and the rim is indented but not rotated.

It is difficult if not impossible to state the exact coagulation-time, the results of different observers are most conflicting; as far as I can gather, Wright's figures for normal blood at a temperature of 20° C. is 4 min. 50 sec. to 5 min.

The tables given by Carstairs Douglas,[1] who used Wright's method, bring out quite clearly the differences which may exist even when the greatest care is taken. In a series of 300-400 observations made at a temperature of 19·4° C., it will be seen that even in healthy women the time may vary from 5 minutes to twice that length of time.

	Average Coagulation-time in Minutes.	Shortest.	Longest.
I. Albuminurics—			
(*a*) Pregnant . . .	5.60	4.70	7·5
(*b*) Puerperal . . .	7.00	5.60	10.0
II. Eclampsias—			
(*a*) Pregnant . . .	7·40	4·50	9.0
(*b*) Puerperal . . .	7.00	4·50	9·5
III. Normal puerperal . . .	7·30	4·75	9·5
IV. Healthy pregnant women .	7·40	5.00	9.0
V. Healthy non-pregnant women	7·75	5.00	10.0

Brodie and Russell's figures for normal men at 20° C. vary between 8·40 and 7·42 minutes, but the average of their

[1] *Brit. Med. Journ.*, March 26, 1904.

measurements at this temperature is 7·8 min., which is practically identical with the figures just quoted for healthy women. Bürker, who has devised a new method for measuring the rate of coagulation, finds that the average time for man is 6 to 7·5 min. at 20°-21° C. He also states that the rate of coagulation varies during the day, being 12 min. at 6 A.M. (maximum), and 5-6·5 min. at 2 P.M. (minimum).[1]

Since in the method of Wright a small volume of blood is exposed to a relatively large surface of glass, which is difficult to clean properly, and in Brodie's method considerable movement of the blood must occur, I have for some time past used the following simple method, which gives results that appear to be as reliable as any others.

While studying the formation of blood-platelets I frequently examined thin films of blood stretched on a wire ring in a moist chamber. In such a transparent film of stretched blood-plasma rouleaux seldom form, every cell remains absolutely at rest and is not in contact with glass, while the preparation can be treated with minute traces of reagents, which remain localised on the film for some time. It is not easy to tell when the plasma clots; incidentally I may mention, that there is no change recognisable between coagulated and fresh films when these are examined with polarised light and quartz or selenite plates. I tried this, since it was possible that threads of fibrin might yield the violet-blue tint on a purple field which is given by connective tissue when this is examined with crossed Nicols and an interposed quartz plate (Ambronn and Held).

On placing the film in a vertical position, it is, however, easy to follow the process of clotting, and it appeared worth trying if vertical films could be used for measuring the rate of coagulation.

An elliptical ring about 6 or 6-7 mm. in the long diameter, and 4 in the short, is made of fine platinum, silver, or iron wire. The kind of metal does not affect the result. The loop, sterilised in a flame, and free from grease or dirt, picks up approximately the same mass of blood, and forms a film if the loop is swept through a drop of blood shed from the finger, the skin of which has been carefully cleaned. Milian[2] has demonstrated the existence in human skin of substances which accelerate coagulation, and in making the film the skin

[1] *Pflüger's Arch.*, cii., 1904.
[2] Milian, *Comptes rend. Soc. de Biologie*, t. 53, 1901.

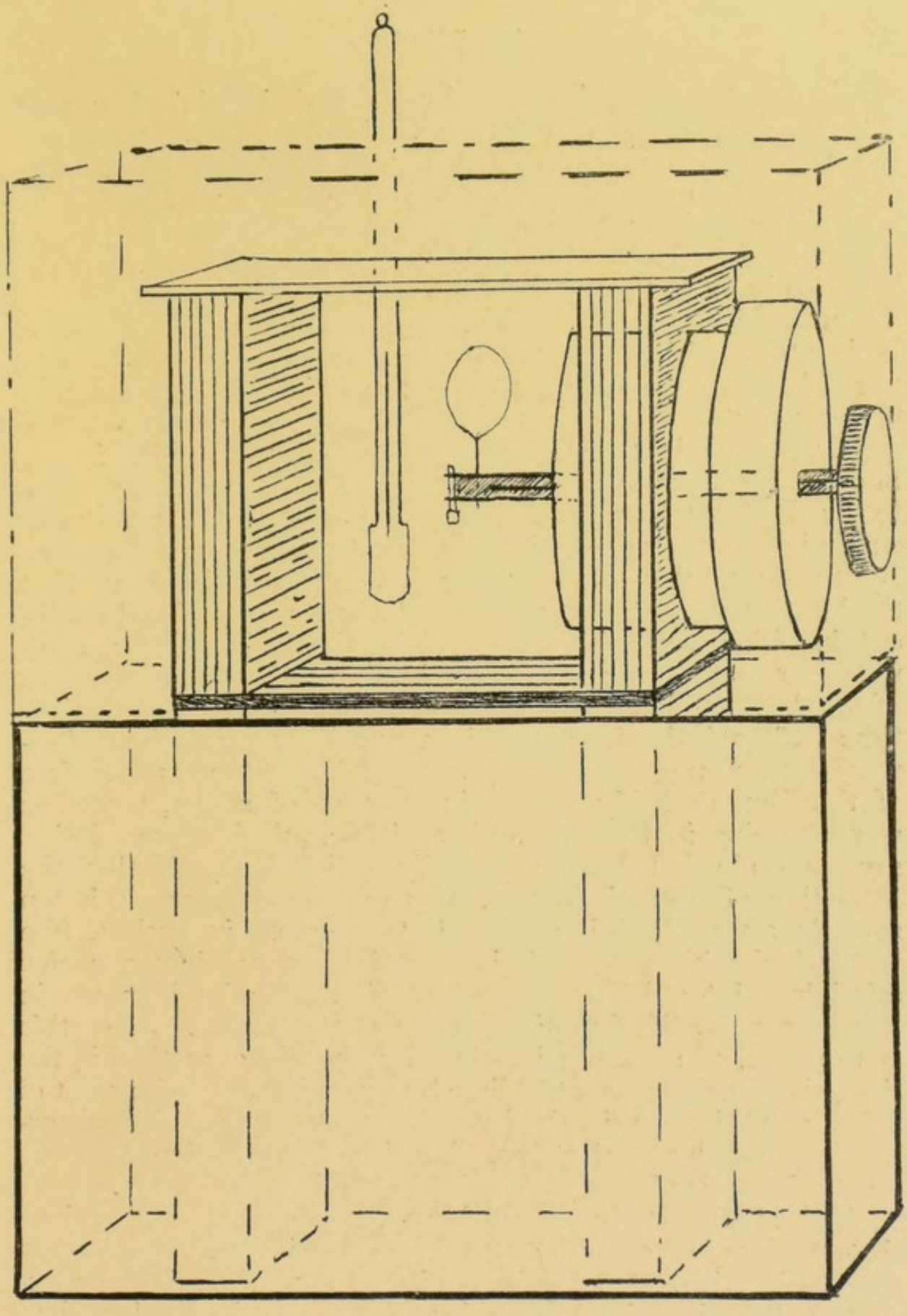


FIG. 14.—A rough sketch of the actual size of the apparatus. The outer metal case is made of similar parts, one of which fits on to the other. The lower half holds water, the upper half is covered with thick felt, and has two small windows cut in opposite sides. Inside this box is the moist chamber. This has a floor and two sides made of brass; the latter are grooved so as to take two plates of glass, which with another for the roof completes the chamber. The floor is lined with wetted filter paper, and the sides of the box are prolonged into four thick feet. The wire loop, the arrangement for rotating it, and the large plug, which can easily be removed and replaced, is shown. The thermometer is arranged as in the figure. The apparatus stands on a tripod, and can be heated to any given temperature. Flaps of felt in the outer case can be lifted up when observations are made.

should not be touched by the loop. A shake of the loop now detaches some blood, and leaves a thin film of even thickness, except at the contact edge, where it is slightly thicker. The shank of the loop is clamped in a holder so that it can be kept either horizontal or rotated upwards or downwards through a right angle, so as to be vertical. The observations are made in a small moist chamber (Fig. 14, p. 213).

In making an observation, commence with the film in a horizontal position, and at the end of two minutes rotate upwards into a vertical position, and examine with a magnification of about 10 diameters. The corpuscles will be seen to fall slowly, and a minute clear space will be noticed forming in the film a little distance from the upper edge of the loop; rotate to the horizontal at once, and in a few seconds rotate downwards into a vertical position, when a clear space again appears. By repeating this series of manœuvres, it will be found that the blood quite suddenly remains of the same opacity throughout in either vertical position. The time taken with a stop-watch or water-clock between this phase and the appearance of the drop of blood on the finger is taken as the coagulation-time. Should the loop remain vertical for some time afterwards, the corpuscles still very slowly fall about over the surface of the film, probably in a thin stratum of serum, but there is no spacing in the film such as is described above. Using my own blood I have repeatedly satisfied myself that the formation of fibrin in a film, may often commence within ten seconds after blood is shed. If the surface in such a film is touched with a fine platinum point, or still better a fine celloidin point, a delicate thread of film, several millimetres in length, can be lifted vertically up from the surface. Since this is the case, the coagulation-time by the above method is the time elapsing between shedding blood and the development of a film of fibrin sufficiently dense to check a *sudden* fall of corpuscles through a vertical film. A series of observations made on human blood is given in the following table, pp. 215, 216.

The results of this method may, I think, be held to show that, under the conditions of the experiment, the average coagulation-time at 31° C. is 5 min. 45 sec. As might be expected, the time is shortened at higher and lengthened at lower temperatures. Most of the observations were made on my own blood, but I should not be surprised to find marked differences of as much as two to three minutes in other normal individuals.

TABLE OF ALL THE OBSERVATIONS MADE IN MARCH AND APRIL 1904. Sets of successive observation are bracketed together. Diameter of the loop, 6·75 millimetres before the loop is made elliptical. Some loops made of iron, some platinum wire.

Observed Blood.	Temperature.	Coagulation-time.		Average.	
	Degrees Cent.	Min.	Sec.	Min.	Sec.
B.	31	5	5	5	5
B.	31	5	2		
B.	31	5	7		
B.	31	5	42	5	38
B.	31	5	46		
B.	31	5	25		
B.	31	5	25	5	20
B.	31	5	6		
B.	31	5	4		
B.	31	5	45		
W.	31	5	30	5	32
W.	30	4	40		
W.	30	6	30		
W.	30	5	30		
B.	33	4	20	4	56
B.	33	3	50		
B.	33	3	25		
B.	33	4	0		
B.	33	4	10		
B.	37·5	3	50	3	56
B.	37·5	3	48		
B.	37	4	10		
B.	39·2	2	50	2	56
B.	39·0	2	38		
B.	38·5	3	20		
B.	35	4	20	4	20
C.O.	34	5	30	5	17
C.O.	35	5	5		
C.O.	37·5	5	30		
C.O.	39	5	2		

TABLE OF OBSERVATIONS—*Continued.*

Observed Blood.	Temperature.	Coagulation-time.		Average.	
	Degrees Cent.	Min.	Sec.	Min.	Sec.
B.	36	5	20		
B.	36	5	20		
B.	35	5	30	5	23
B.	34	5	35		
B.	37	5	10		
B.	17·5	11	10	11	10
B.	19·5	10	20		
B.	21	10	30	10	33
B.	21	10	50		
B.	20	8	45	8	45
B.	20	8	45		
B.	44	3	0		
B.	42	3	10	3	30
B.	40	3	50		
B.	37·2	5	5		
B.	37	5	2	5	4
B.	35·8	5	7		
B.	36	5	25		
B.	36	5	30		
B.	5·35	5	40	5	35
B.	34·5	5	45		
B.	32·5-30	6	5	6	5
B.	31	5	45	5	45
B.	31	5	40	5	40
B.	35·32	5	20	5	20
B.	40	3	30	3	30
B.	39·38	3	5	3	5
B.	40	3	2	3	2
B.	42	4	10	4	10
B.	22·5	10	50	10	50
B.	21	11	1	11	1
B.	20	12	25	12	25
B.	20	12	15	12	15

Apart from salts of lime, which both *in vitro* and on internal administration increase coagulability, Dastre and Floresco[1] first

[1] *Arch. de Physiologie*, 1896.

showed that a similar effect was caused by 5 per cent. of gelatine in artificial serum. The action has been attributed to the lime salts present, or to the intense leucocytosis it causes, which induces an abnormal development of fibrin ferment, or plasmase of French authors. Since fluoride plasma possesses no thrombin, Arthus and Dastre regard the ferment not as a product of disintegrated cells, but as a true secretion of these, which is expelled as a consequence of osmotic-pressure variations in the plasma. This view may be considered to accord with the theory that non-coagulation of blood in the body is due to the neutralisation of a constantly present plasmase, produced by leucocyte-disintegration by an anti-body or thrombase.[1] The substance secreted from the leucocytes, according to Morawitz[2] and Fuld, is thrombokinase, which, in the presence of lime salts, acts upon thrombogen derived from blood-platelets in the plasma.

More than fifteen years ago, Lord Lister[3] drew attention to the fact that blood may clot, and not shrink, in a vessel rendered sterile by heat, or by washing it out with weak perchloride of mercury. This condition is frequently seen in the collection of antitoxic sera. In 1896 Hayem[4] drew attention to the absence of serum from the clotted blood of certain anaemias. He ascribed this phenomenon to the richness of the blood in haematoblasts. In pernicious anaemia, and many of the varieties of purpura haemorrhagica and Werlhof's disease, this non-retraction of the clot has been described. The French observers lay special stress on this condition for the positive diagnosis of pernicious anaemia; they even regard it as of greater value than a histological examination.[5]

It is known that coagulation is influenced by a variety of conditions, many of which are but imperfectly understood, thus Delezenne[6] has shown that bird's blood from a wound clots with great rapidity in half to ten minutes, but remains unclotted two to eight days if removed from the vessel with every precaution against admixture with lymph. A. Wright[7] has also investigated this point in the blood of man, dogs, and rabbits, and finds that the admixture of fresh lymph, muscle juice, or blister fluid markedly accelerates the coagulation-time.

[1] *Comptes rend. Soc. de Biologie*, t. 55, 1903.
[2] Article, "Blutgerinnung," in *Ergeb. der Physiol.*, 1, 1905. (Full literature.)
[3] *Lancet*, p. 1082, 1891. [4] *La Semaine médicale*, Nov. 1896.
[5] Hayem et Bensaude, *Comptes rend. Soc. de Biologie*, Jan. 1901.
[6] *Archives de Physiologie normal et Pathol.*, 1897.
[7] *Journal of Physiol.*, vol. xxviii., pt 4, 1902.

Arthus[1] has also shown the influence which is exerted by a wound on the rate of coagulation, and the short coagulation-time of a mixture of blood and lymph from a squeezed superficial puncture, contrasts with the much longer time when the blood freely issues from a deeper one. In the case of successive bleedings the blood has been noticed to clot more rapidly.

The viscosity of blood as determined by Hürthle[2] for animals, and Hirsch and Beck[3] for man, is found to be inversely related to the coagulation-time, and the less the viscosity the greater the time.

Hayem has drawn attention to the fact that in the same animal on different days, or even at different times in the same day, the coagulation-time may vary greatly. Dog's blood taken simultaneously from a vein in the ear and from one in the leg, may clot in nine and two minutes respectively.

In the same man I am satisfied that the coagulation-time on different days may, at the same temperature, vary by at least five to six minutes. Wright's extended researches have shown that an increased percentage of carbonic acid in the blood, or the administration of sufficient calcium chloride by the mouth, accelerates the coagulation of blood, and that the opposite occurs with the ingestion of alcohol, large quantities of liquid, or diminished amount of solid food.

[1] *Comptes rend. Soc. de Biologie*, Jan. 1902.
[2] *Pflüger's Archiv f. Phys.*, 1900.
[3] *Deutsches Archiv f. klin. Med.*, lxxx., p. 308.

REFERENCES

LECTURE I

BEARD.—"The Source of the Leucocytes and the true Function of the Thymus," *Anat. Anzeiger*, p. 550, 1900.

BOHR.—*Skandinav. Archiv f. Physiologie*, iii., p. 101, 1892.

COHN, M.—*Münchener med. Wochenschrift*, No. 6, 1900.

DAYTON.—*Amer. Journ. of Med. Sciences*, p. 448, 1904.

DOMINICI.—*Comptes rendus Soc. de Biologie*, 1899.

DRUMMOND.—"Haemolymph Glands," *Journ. of Anat. and Phys.*, 1900.

EHRLICH, P.—"Degeneration und Regeneration rother Blutscheiben," *Verhandl. d. Gesellschaft d. Charite-Aerzte*, June 10 and Dec. 9, 1880; and *Die Anaemie*, Ehrlich-Lazarus, 1900.

ENGEL.—"Die Blutkörperchen d. Schweins in d. erste Hälfte d. Embryonal. Lebens," *Archiv f. mikros. Anat.*, xvi., 1899.

GABRITSCHEWSKY.—*Archiv f. exp. Path. u. Pharm.*, xxviii., 1891.

GIGLIO-TOS.—*Arch. ital. de Biologie*, xxvi., 1896, and xxvii., 1897.

GRAWITZ, E.—*Berliner klin. Wochenschrift*, No. 9, 1900, and No. 46, 1901.

HALDANE, J. S.—"Carbonic Oxide and Oxygen-tension," *Journ. of Physiology*, xviii., p. 201, 1895; and "Action of Carbonic Oxide," p. 430, 1895.

HALDANE, J. S., and LORRAIN SMITH.—"Oxygen-tension of Arterial Blood," *Journ. of Physiology*, xx., p. 497, 1896; and "Mass and Oxygen Capacity of the Blood in Man," *Journ. of Physiology*, xxv., p. 333, 1900.

HAMBURGER.—*Osmotischer Druck und Ionenlehre*, Wiesbaden, 1902.

HAMEL.—*Deutsches Archiv f. klin., Med.*, lxvii., 1900.

HEINZ.—*Virch. Arch.*, cxxii., 1900; and *Handbuch der exp. Pathologie u. Pharmakologie*, 1904.

HÜHNERFAUTH.—*Virch. Archiv*, lxxvi., 1879.

ISRAEL, O., and PAPPENHEIM.—*Virch. Archiv*, cxliii., 1896.

LAACHE.—*Die Anämie*, Christiana, 1883.

LAZARUS.—"Die Anaemie," Bd. viii., *Nothnagel's Specielle Path. u. Therapie*, 1900.

LAZARUS-BARLOW.—*Proc. Physiol. Soc.*, May 1894.

V. LESSER—*Archiv f. Anat. v. Phys. (Phys. Abth.)*, 1878.

LEWIS, T.—*Journ. of Anat. and Phys.*, xxxviii., 1904 ; *Internat. Monatssh. f. Anat. u. Phys.*, xx., 1901.

LORRAIN SMITH.—*Trans. of Path. Society, Lond.*, p. 311, 1900, and p. 136, 1902.

LÖWIT, M.—*Sitzungsberich. d. k. Akad. der Wissenschaft.*, xcv., 1887.

LUTOSLAWSKI.—Inaugural Dissertation, Zurich, 1904. (Full literature on basophil granulations of the red corpuscles.)

MADSEN.—"Uber Tetanolysin," *Zeits. f. Hygiene*, xxxii., 1899.

MYERS, W.—*Journ. of Anat. and Phys.*, April 1900.

NAEGELI.—*Münch. med. Wochenschrift*, p. 195, 1904.

OTTO.—*Pflüger's Archiv*, xxxvi., 1885.

PANUM.—*Virch. Archiv*, xxix., 1864 (contains all earlier literature).

PAPPENHEIM, A.—"Bildung rother Blutscheiben," Inaugural Dissertation, Berlin, 1895.

PHILLIPS, S.—*Medico-Chirurg. Trans.*, lxxxi., 1898.

PLEHN.—*Deutsche med. Wochenschrift*, Nos. 28, 29, 1898.

QUINCKE.—(Duration of Life of Erythrocytes), *Deuts. Arch. f. klin. Med.*, p. 215, lxxv., 1880.

SABRAZÈS, BONNET, et LÉGER.—*Journ. de Phys. et de Path. générale*, Nov. 1900.

SCHMIDT, M. B.—*Ziegler's Beiträge*, ix., 1893.

SCHMIDT, P.—*Experimentelle Beiträge zur Pathologie des Blutes*, Jena, 1902.

SHERRINGTON and COPEMAN.—*Proc. Royal Society*, 1890.

STASSANO.—*Compt. rend. Soc. de Biologie*, cxxxi., 1900.

STENGEL, WHITE, and PEPPER.—*American Journ. of the Medical Sciences*, 1902.

STEWART, G. N.—*Journ. of Phys.*, xxiv., 1899.

TOLMATSCHEFF.—"Notiz über der Einfluss wiederholter Aderlasse auf die Ernahrung," *Medicinisch-chemische Untersuchungen*, heraus. Hoppe-Seyler, p. 396, Heft iii., 1868.

WARTHIN.—*American Journ. of Med. Sciences*, Oct. 1902, and p. 211, 1904.

WEIDENREICH.—*Anat. Anzeiger*, xx., p. 188, 1902.

WHITE and PEPPER.—*American Journ. of Med. Sciences*, 1901 ; and *Journ. of the Assoc. of American Physicians*, p. 410, 1901.

WOLFF.—*Berl. klin. Wochenschr.*, Sept. 8, 1902.

LECTURE II

BUNGE.—*Lehrb. d. Phys. v. Path. Chemie*, Leipzig, 1900 ; *Zeits. f. Biologie*, xv., 1876.

EIJKMAN.—"Blutuntersuchungen in den Tropen," *Virch. Arch.*, cxliii., 1896 ; and *Pflüger's Arch. f. Phys.*, 1897.

GAMGEE and CROFT HILL.—*Proc. Royal Soc.*, lxxi., 1903.
GAMGEE and JONES.—*Proc. Royal Soc.*, lxxi., 1903.
GRYNS.—*Pflüger's Arch. f. Phys.*, lxiii., 1896.
HAMBURGER.—*Osmotischer Druck und Ionenlehre*, Wiesbaden, 1902.
HEDIN.—*Pflüger's Archiv f. Phys.*, 1897.
KÖPPE.—*Pflüger's Archiv f. Phys.*, 1895.
LAIDLAW.—*Journ. of Phys.*, xxxii., 1905.
MARESTANG.—*Rev. de Médecine*, x., p. 468, 1900.
NOGUCHI.—"Antihaemolytic action of Blood Serum, Milk, and Cholesterin against Agaricin, Saponin, and Tetanolysin," *Med. Bulletin*, 1902.
OKER-BLOM.—*Pflüger's Archiv*, lxxix., lxxxi., 1900.
OSBORNE, TH.—*Amer. Journ. of Phys.*, 1903.
OVERTON.—*Pflüger's Arch. f. Phys.*, 1901.
PASCUCCI.—*Hofmeister's Beiträge*, vii., 1905.
RANSOM.—*Deutsche med. Wochenschr.*, No. 3, 1903.
ROLLETT.—*Pflüger's Arch. f. Phys.*, lxxxii., 1900.
ROTH.—*Centralblatt f. Physiol.*, July 1897.
SCHÄFER, E. A.—*Anat. Anzeiger*, xxvi., s. 587, 1905.
STEWART, G. N.—*Journal of Phys.*, xxiv., 1899, and xxvi., 1901.

POLYCYTHAEMIA

ABDERHALDEN, E.—"Über den Einfluss des Höhenklimas auf die Zusammensetzung des Blutes," *Zeits. f. Biologie*, xliii., 1902; *Pflüger's Archiv*, xcii., 1902.
BENSAUDE.—*Sem. Med.*, No. 53, 1901.
COHNHEIM, O.—"Die Alpinismus," reprint from *Die Ergebnisse der Physiologie*, 1902 (full literature).
DURIG.—*Pflüger's Archiv*, lxxxv., 1901.
EGGER.—*Centralbl. f. klin. Med.*, xiv., 1893; and *Archiv f. exp. Path. u. Pharmak.*, xxxix., 1896.
FICK, A.—*Pflüger's Arch.*, lx., 1895.
FROMHERZ.—*Münch. med. Wochenschr.*, p. 1718, 1903.
GAULE.—"Die Blutbildung im Luftballon," *Pflüger's Archiv*, lxxxix., 1902.
GIACOSA.—*Zeits. f. phys. Chem.*, xxiii., 1897.
GOTTSTEIN.—*Berl. klin. Wochenschr.*, Nos. 20, 21, 1898; *Münch. med. Wochenschr.*, 1898.
GRAWITZ, E.—*Berl. klin. Wochenschr.*, pp. 715 and 740, 1895.
JAQUET.—*Korrespondenzblatt f. Schweitz. Aerzte*, No. 15, 1900.
JOLLY.—"Examens histol. der Sang au Cours d'une ascension en ballon," *Compt. rend. Soc. de Biolog.*, liii., 1901.
KOEPPE.—*Münch. med. Wochenschr.*, No. 39, 1895.
KRONECKER, H., and A. MARTI.—"Comment agissent des irritations de la peau sur la formation des globules rouges du sang," *Arch. ital. de Biologie*, xxvii., 1897.

MEISSIN and SCHRÖDER.—"Zur Frage der Blutveranderungen im Gebirge," *Münch. med. Wochenschr.*, p. 610, 1897.

MERCIER.—*Archives de physiol.*, ser. 5, t. vi., 1894.

MIESCHER.—*Korrespondenzblatt f. Schweizer Aerzte*, p. 809, 1893.

MEYER, C. F.—"Einfluss des Lichtes im Höhenklima," Inaug. Dissert., Basel, 1900.

MOSSO, A.—"Laboratoire scientifique international du Monte Rosa," *Travaux de l'année* 1903, Turin, 1904.

REGNARD, P.—*La Cure d'altitude*, Paris, 1897.

REINHOLD.—*Münch. med. Wochenscrift*, No. 17, 1904.

RÖMISCH.—*Festschr. z. Feier d.* 50 *Jahr. Best. d. Stadt-Krankenhauses zu Dresden-Friederichstadt*, p. 245, 1900.

ROSENGART.—Quoted by Reinhold.

SCHAUMANN and ROSENQUIST.—*Pflüger's Archiv*, lxviii., 1897.

SCHRÖTTER and N. ZUNTZ.—"Ergebinesse zweier Ballonfahrten zu physiologischen Zwecken," *Pflüger's Archiv*, xcii., 1902.

WEBER, PARKES.—*Brit. Med. Journ.*, April 1904.

WEINZIEL.—*Amer. Journ. of Med. Sciences*, p. 120, 1903. This paper refers polycythaemia of high levels to cold.

WEISS, J.—"Über den angeblichen Einfluss des Höhenklimas auf die Hb-Bildung," *Zeitschr. f. physiolog. Chemie*, xxii., 1896.

VAN VOORNVELD.—"Das Blut im Hochgebirge," *Pflüger's Arch.*, xcii., 1902, and xciii., 1902 (complete literature).

VIAULT.—*Compt. rendus*, cxi., 1890, and cxiii., 1892.

ZUNTZ, N. A. LOEWY, FR. MÜLLER, and W. CASPARI.—*Höhenklima und Bergwanderungen in ihren Wirkung auf den Menschen*, Berlin, 1905.

LECTURE III

BASHFORD.—*Journ. of Path. and Bact.*, iv., 1904.

BELLEI.—"Hämolyse durch Blutplasma und Blutserum," *Münch. med. Wochenschr.*, p. 55, 1904.

BELFANTI and CARBONE.—Quoted from Metchnikoff, *Ann. de l'Inst. Pasteur*, xiv., 1900.

BIERNACKI.—*Sammlung klin. Vorträge*, No. 306, Leipzig, 1901.

BORDET.—*Ann. de l'Inst. Pasteur*, xii., 1898, xiii., 1899, and xiv., 1900.

BULLOCH, W., and W. HUNTER.—*Trans. Path. Soc., London*, 1900.

CAMUS.—"Les hémoglobinuries," Thèse, Paris, 1903.

DENIS, J.—*Journ. des Savants*, pp. 69 and 134, 1667.

DÖMENY.—*Annal de l'Inst. Past.*, 1901.

EHRLICH.—"Über paroxysmale Haemoglobinurie," *Verein f. innere Med.*, März, 1881; Croonian lecture, *Proc. Roy. Soc.*, 1900; "Schlussbetrachtungen," Bd. viii., *Nothnagel's Specielle Path. u. Therapie*, 1901.

EHRLICH and MORGENROTH.—*Berl. klin. Wochenschr.*, pp. 6 and 481, 1899; pp. 450, 681, 1900; p. 251, 1901.

FEUERSTEIN, L.—*Archiv f. Dermat. u. Syph.*, Dec. 1, 1903 (full literature). Critical research on value of this test: he agrees with Grawitz and Biernacki that the test is of doubtful value—out of 45 cases only 5 positive.

FLEXNER and NOGUCHI.—*Trans. of the College of Physicians, Philadelphia*, p. 25, 1902.

GAMGEE, A.—*Proc. Royal Society*, lxx., 1902.

GARROD and HOPKINS.—"On Haemato-porphyrinuria," *Journ. of Path. and Bac.*, iii., p. 434, 1896.

GRAWITZ.—*Allgem. med. Zentralzeitung*, 1895.

HEWLETT, A. W.—*Archiv f. exp. Path. u. Pharmak.*, p. 303, xlix., 1902-3.

JACOB.—*Zeits. f. klin. Med.*, xxx., 1896.

JUSTUS, J.—"Ueber die durch Syphilis bedingten Blutveranderungen in Hinsicht ihrer diagnostichen und therapeutischen Bedeutung," *Virch. Archiv*, cxl., 1895, and cxlviii., 1897.

KROMPECHER.—*Centralbl. f. Bakt. u. Parasit*, p. 588, 1900.

KRÖNIG.—*Berl. klin. Wochenschrift*, No. 46, 1895.

KYES.—*Berl. klin. Wochenschr.*, 1904; and *Zeits. f. physiol. Chemie*, lxi., 1904,

LANDOIS.—*Centralbl. f. d. med. Wissenschaft*, 1873 and 1874; *Die Transf. des Blutes*, Leipzig, 1875.

LEWIN.—*Archiv f. exp. Path. u. Pharmak.*, xxxv., 1895.

MARAGLIANO.—*Berl. klin. Wochenschrift*, No. 31, 1892.

MARTIN, C. J.—*Brit. Med. Journ.*, p. 514, Sept. 1904.

MOSSO, A.—*Archiv f. exp. Path. u. Pharmak.*, xxv., 1888

MURRI.—Quoted by Justus.

MYERS, W.—*Trans. Pathol. Soc., London*, 1900.

NOGUCHI.—*Med. Bulletin*, 1902.

NOLF.—*Annales de l'Inst. Pasteur*, xxii., 1902.

PAGNIEZ.—"Action exercée sur les globules rouges par quelques liquids normaux et pathologiques," Thèse de Paris, 1902.

PONFICK.—*Verhandl. d. Kongress f. inn. Medicin*, 1883; and *Virch. Arch.*, lxxxviii., 1882.

RANSOM.—*Deutsche med. Wochenschr.*, No. 3, 1901.

REID, WAYMOUTH.—*Journ. of Physiol.*, xxxiii., 1905.

ROGERS, L.—*Proc. Roy. Soc.*, May 1903.

RYWOSCH.—*Zentralbl. f. Physiol.*, No. 12, 1905.

SCHÄFER, E. A.—*Proc. Physiol. Soc.*, xi., 1890.

STADELMANN.—*Der Ikterus*, Stuttgard, 1891.

STEPHENS and CHRISTOPHERS.—*The Study of Malaria*, 1905.

STEWART, G. N.—*Amer. Journ. of Physiol.*, p. 103, 1902; p. 72, 1903; and No. 4, 1904.

TALLQVIST.—*Über experimentelle Blutgiftanaemieen*, Berlin, 1890.

WHITFIELD, A.—*Trans. Path. Soc., London*, p. 338, 1900.

LECTURE IV

ADDISON, W.—(1) "Experimental and practical researches on the Structure and Function of Blood-corpuscles; on Inflammation; and on the origin and nature of Tubercles in the Lungs," *Trans. of the Prov. Med. Assoc.*, vol. xi., p. 233, 1843. (2) "The Actual Process of Nutrition in the Living Structure, demonstrated by the Microscope. and the Renewal of the Tissue and the Secretions from the Blood thereby illustrated," *Trans. of the Prov. Med. Assoc.*, vol. xii., p. 235, 1844. (3) *Consumption and Scrophula*, p. 82, London, 1849.

ARNOLD, J.—*Virch. Archiv*, cxl., 1895, and clvii., 1897.

BANTI.—*Archivio di Fisiologia*, 1904.

BEARD.—*Anat. Anzeiger*, 1900.

BENDA.—*Arch f. Anat. and Phys.*, 1896.

BOYCOTT and HALDANE.—"Ankylostomiasis," *Journ. of Hygiene*, iii., p. 111, 1903; and iv., p. 437, 1904.

BRADFORD, J. R., and SHAW.—*Medico-Chirurg. Transactions*, London, 1898.

CARSTANJEN, M.—*Jahrbuch f. Kinderheilkunde*, lii., 1900.

COHNHEIM, J.—"Ueber Ertzündung und Eiterung," *Virch. Archiv*, xxxix., 1867. It is clear from a perusal of Cohnheim's paper that he was unaware of Waller's observations. His attention was directed to Addison's volume on *Consumption and Scrophula*, from which he quotes, by Virchow, who also had at that time apparently no knowledge of Waller's work.

DOMINICI.—*Comptes rend. Soc. de Biologie*, lii., 1900.

DAVAINE.—*Comptes rend. Soc. Biologie*, ii., p. 103, 1850. Davaine accurately observed and faithfully described what any student can at the present time easily verify. He noticed the rush in eddying currents of the red corpuscles past and around the stationary leucocytes, which latter cells during an observation of about twenty minutes changed both their shape and position. Since these movements outside the body were identical to those witnessed within the capillaries of the frog, Davaine correctly inferred that these were a vital and not a mechanical phenomenon.

DA COSTA.—*Clinical Haematology*, 1905.

EBSTEIN.—*Deutsches Archiv f. klin. Med.*, xliv., 1889.

ENGEL, C.—*Leitfaden zur klinischen Untersuchungen des Blutes*, Berlin, 1898. (Figs. 2, 3, 4, Tafel iv.).

EHRLICH.—*Verhandlungen d. Physiol. Gesellschaft zu Berlin*, No. 8, 1878-1879.

EHRLICH and LAZARUS.—*Die Anämie*, 1900.

FEHRSEN, A.—*Journ. of Physiol.*, xxx., 1903.

FERGUSON.—*Journ. of Path. and Bact.*, viii., 1903.
FRAENKEL.—*Deutsche med. Wochenschr.*, 1895.
FLEMMING, W.—*Archiv f. mikros. Anat.*, xxiv.
GRAWITZ, E.—*Klinische Pathologie des Blutes*, p. 28, 1896.
GULLAND.—*Journ. of Physiol.*, xix., 1896.
HAYEM.—*Du Sang et de ses alterations anatomiques*, Paris, 1889.
HEWSON, W.—*Experimental Inquiries*, Part the Third, being the remaining part of the observations and experiments of the late Mr William Hewson, F.R.S., by Magnus Falconer, 1777.
HESSE.—*Virch. Archiv*, clxvii., 1902 (literature); *Anat. Anz.*, 1902.
HIRSCHFELD.—*Virch. Archiv*, cxlix., 1897; *Berl. klin. Wochenschrift*, 1901.
JOLLY, A.—*Comptes rend. de la Soc. de Biologie*, v., 1898.
JONES, WHARTON.—*Phil. Trans.*, 1846.
KANTHACK and HARDY.—*Journ. of Physiology*, xvii., p. 81, 1894.
KLEIN, ST.—*Centralblatt f. innere Medizin*, Heft 4 and 5, 1899.
LAZARUS.—"Post-haemorrhagic Anaemia," *Nothnagel's Spec. Path. u. Therapie*, 1900.
LITTEN.—*Nothnagel's Spec. Path. u. Therapie*, Bd. viii.
MARINO.—*Annal. de l'Inst. Pasteur*, 1903.
MARQUIS, CARL.—"Das Knockenmack der Amphibien," Inaug. Dissert., Dorpat, 1892.
MEINERTZ.—*Arch. f. Path. Anat.*, clxviii.
MELLAND.—*Brit. Med. Journ.*, Sept. 1904.
METCHNIKOFF, E.—"Ueber die Immunitäl bei Infections krankheiten, mit besonderer Berüsitigung der Cellular-Theorie," *Ergebnisse der Allg. Ätiologie der Menschen u. Thierkrankheiten*, Lubarsch-Ostertag, 1896, p. 315; and *Virch. Archiv*, Bd. xcvi., p. 177; *L'Inflammation*, Paris, 1892.
MOSLER, FR.—*Die Path. u. Therapie der Leukaemia*, Berlin, 1872.
MUIR, R.—*Journ. of Path. and Bact.*, vii., 1901.
MÜLLER, H. F.—*Deutsches Archiv f. klin. Med.*, xlviii., 1891.
MÜLLER, JOHANNES.—*Elements of Physiology*, trans. by W. Baly, London, 1839.
NAEGELI.—*Deutsche med. Wochenscrift*, No. 18, 1900.
NEUMANN.—*Virch. Archiv*, cxliii., 1896.
PAPPENHEIM, A.—*Virch. Archiv*, clvii., 1898; and clix., clxiv., 1899.
PHEAR.—*Med.-Chir. Trans.*, lxxxii., p. 381, 1899.
PINKUS.—"Die lymphatische Leukaemie," *Nothnagel's Specielle Path. u. Therapie*, viii.
v. RECKLINGHAUSEN.—"Ueber Eiter-und Bindegewebe Korperchen," *Virch. Archiv*, xxviii., 1863.
RIEDER.—*Atlas der klin. Mikrosopie des Blutes*, Leipzig, 1893; *Beitrage zur Kentniss der Leucocytose*, Leipzig, 1893.
ROGERS, L.—*Brit. Med. Journ.*, Sept. 1904.
ROSIN and BIBERGEIL.—*Virch. Archiv*, 1905 (literature).

SCHULTZE, MAX.—*Archiv f. mikros. Anat.*, i., 1865.
SCHRIDDE.—*Münch. med. Wochenschrift*, No. 96, 1905.
SCOTT, Hon. A.—*Journ. of Path. and Bact.*, 1901.
SHERRINGTON.—*Proc. Roy. Soc.*, vol. lv., 1893.
SIMON.—*Amer. Journ. of Med. Sciences*, Feb. 1899.
STERNBERG.—*Virch. Arch.*, clxxiii., 1903.
TÜRK, W.—*Wiener klin. Wochenschr.*, No. 18, 1901 ; *Vorlesungen über klin. Hämatologie*, Bd. i., p. 401, 1904 ; and *Klin. Untersuchungen über das Verhalten des Blutes bei acute Infections krankheiten*, Wien and Leipzig, 1898.
WALLER, AUGUSTUS.—"Microscopic Observations on the Perforation of the Capillaries by the Corpuscles of the Blood, and on the Origin of Mucus and Pus-globules," *Phil. Mag.*, p. 1, Nov. 1843.
WEIGERT, K.—*Bericht über die Sitzungen der Schlesischen Gesellschaft f. Vartterb. Cultur*, Dec. 10, 1878.
WEIL.—*Comptes rend. Soc. de Biologie*, June 23, 1901.
WEISS.—*Centralbl. f. d. med. Wissensch.*, xxix., 1891.
WILLIAMS.—*Münch. med. Wochenschrift*, Feb. 1902.
WLASSOW and SEPP.—*Virch. Archiv*, clxxvi.
WOLFF.—*Deutsche Zeitsch. f. klin. Medizin*, xlv., 1902.
VIRCHOW, R.—"Weisses Blut," *Neue Notizen aus dem Gebiete der Natur und Heilkunde*, von Froriep (Weimar) u. Froriep (Berlin), No. 780, Nov. 1845.

LECTURE V

ARNOZAN and MONTEL.—*Congrès internat. de méd.*, Paris, 1900.
BARRATT.—*Proc. Royal Soc.*, October 1905.
BLUMER and NEWMAN.— *Amer. Journ. of Med. Sciences*, 1900.
BODEN, K.—*Virch. Archiv*, clxxiii., 1903.
BOTKIN, F.—*Virch. Archiv*, cxli., 1895.
BOYCOTT.—*Journ. of Hygiene*, iii., p. 111, 1903 ; and iv., p. 437, 1904.
BRANDENBURG.—*Münch. med. Wochensch.*, No. 6, 1900.
BROWN.—*Johns Hopkins Hosp. Reports*, April 1897.
CATHCART, E. P.—*Journ. of Physiol.*, xxxi., 1904.
CAZIN.—*XV^e Congrès français de chirurgie*, October 1902.
CHARTERIS and CATHCART.—*Journ. of Path. and Bact.*, x., 1905.
DA COSTA.—"The clinical value of blood examinations in Appendicitis," *Amer. Journ. of Med. Sciences*, November 1901.
DEAN, G.—*Proc. Royal Soc.*, 1905.
DRYSDALE.—*Brit. Med. Journ.*, September 1904.
DUNHAM.—*Boston Med. Journ.*, June 1901.
EHRLICH.—*Die Anämie*, 1900.

ENGEL, C. S.—*Verhandl. d. Vereins f. klin. Med.*, Berlin, 1896, 1897; and *Kong. f. innere Med.*, 1897.
FEHRSEN, A.—*Journ. of Physiol.*, xxx., 1903.
FERGUSON.—*Journ. of Path. and Bact.*, 1904.
FRENCH, H. S.—*Brit. Med. Journ.*, April 30, 1902; *Guy's Hospital Reports*, 1903.
GOODALL, GULLAND, and NOEL PATON.—*Journ. of Physiol.*, xxx., 1903; and Goodall and Paton, *ibid.*, xxxiii., 1905 (full references).
GULLAND, A.—*Brit. Med. Journ.*, 831, 1902.
HAYEM and GILBERT.—*Arch. génér. de Med.*, p. 257, 1884.
HEDIN, S. G.—*Journ. of Physiol.*, xxx., 1903.
HOUSTON.—*Brit. Med. Journ.*, September 10, 1904.
LANDERER.—*Münch. med. Wochenschrift*, Nos. 40, 41, 1888.
LANGHANS.—*Virch. Archiv*, 1870.
LAZARUS.—*Die Anaemie*, p. 15, 1900.
LEBER.—*Fortschritte d. Med.*, 1888.
LEISHMAN.—*Brit. Med. Journ.*, January 1902.
LOCKE.—*Boston Med. Journ.*, September 1902.
LOEPER.—*Soc. Anat.*, May 1901.
KERR.—*Philad. Med. Journ.*, Aug. 1900.
LABBE and LORTET.—*Compt. rendus*, 1902.
LÖWIT, M.—*Studien zur Phys. u. Path. des Blutes u. der Lymph*, Jena, 1892.
MONTEL.—Thèse de Bordeaux, 1900.
MUIR, R.—*Journ. of Path. and Bact.*, 1901.
PFEFFER, W.—*Untersuchungen aus d. bot. Anst. z. Tübingen*, 1884; *Berichte der deutscher bot. Gesellsch.*, 1883.
POEHL.—*Arch. f. exp. Path. v. Pharmak.*, xxv., 1889.
PORTIER.—*Compt. rend. soc. de Biologie*, 1898.
RICHTER and SPIRO.—*Archiv f. exp. Path. u. Pharmak.*, xxxiv., 1894.
RIEDER.—*Beiträge zur Kentniss der Leucocytose*, Leipzig, 1892.
ROGER.—*Les maladies infectieuses*, Paris, 1902.
ROGERS, L.—*Brit. Med. Journ.*, 1904.
ROMER.—*Virch. Archiv*, 1892.
SABRAZÈS and MURATET.—*Acad. des Sciences*, 1903.
SALMON.—"Glycogène et Leucocytes," Thèse, Paris, 1899.
SAMOILOFF.—*Arbeit. d. pharmak. Inst. z. Dorpat*, 1893.
SHAW, BATTY.—*Journ. of Path. and Bact.*, viii., 1903
STAHL.—*Bot. Zeitung*, 1884.
STASSANO.—*Compt. rend.*, p. 680, 1898.
STEPHENS and CHRISTOPHERS.—*The Study of Malaria*, 1905.
SMITH, LORRAIN.—*Journ. of Path.*, London, 1900.
SPRONCKE.—*Centralblatt f. klin. Medizin*, viii., p. 469.
WALDSTEIN.—*Berl. klin. Wochenschr.*, No. 17, 1895.
WASSERMANN.—*Münch. med. Wochenschrift*, April, May, 1902.
WOLFF, M.—*Zeits. f. klin. Med.*, li., 1903.

WRIGHT, A. E.—"On the Inoculation Treatment of Tuberculosis," *Clinical Journal*, Nov. 9, 1904. (The other papers of this author are given here.)

WRIGHT and STEWART DOUGLAS.—*Proc. Roy. Soc.*, 1904.

ZOLLIKOFER.—"Zur Jodreaktion d. Leucocyten," Inaug. Dissert., Berne, 1899.

LECTURE VI

ACQUISTO.—*Moleschott's Untersuch.*, xv., 1893.

ARGUTINSKY.—"Zur Kentniss der Blutplättchen," *Anat. Anzeiger*, xix., 1901.

ARNOLD, FR.—*Handbuch der Anat. des Menschen*, Freiburg, 1845.

ARNOLD, J.—"Zur Morphologie und Biologie der rothen Blutkörpen," *Virch. Archiv*, cxlv., 1896.

ASCHOFF.—(Thrombi and Platelets), *Virch. Arch.*, cxxx., 1892.

BIZZOZERO.—*Virch. Archiv*, xc., 1882; *Gazetta degli Ospitali*, p. 57, 1884; "Festschrift Rudolf Virchow," *Internat. Beitr. z. Wissensch. Medicin*, Bd. i., 1891.

BRODIE and RUSSELL.—*Journ. of Physiol.*, xxi., 1897.

BÜRKER.—*Pflüger's Archiv*, cii., 1904.

DEETJEN, H.—"Eine Methode zur Fixinung der Bewegungs zustände von Leukocyten und Blutplättchen," *Münch. med. Wochenschr*, xliv., 1897; "Untersuchungen über die Blutplättchen," *Virch. Archiv*, clxiv., p. 239, 1901.

DEKHUYZEN.—"Über die Thrombocyten (Blutplättchen)," *Anat. Anzeiger*, xix., 1901.

DRUEBIN.—*Arch. f. Anat. u. Physiol.*, 1893.

EBERTH and SCHIMMELBUSCH.—*Die Thromböse nach Versuchen und Leichenbefunden*, Stuttgart, 1888.

EHRLICH and LAZARUS.—*Die Anämie*, 1900.

EISEN.—*Journ. of Morph.*, xv., Boston, 1899.

VAN EMDEN.—*Bijdragen tot de Kenniss von het Bloed*, Leiden, 1896.

ENGEL, C. S.—*Leitfaden zur klin. Untersuch. des Blutes*, Berlin, 1895; *Arch. f. mikr. Anat.*, xxiii., 1893.

FREUND.—*Jahrb. d. k. k. Gesellsch. der Aerzte*, 1886.

HAYCRAFT.—*Journ. of Anat. and Phys.*, xxii., 1888.

HAYCRAFT and CARLIER.—*Brit. Med. Journ.*, p. 229, 1888.

HAYEM.—"Recherches sur l'evolution des l'hématies," *Arch. f. Physiol.*, v., 1878.

HIRSCHFELD.—*Virch. Archiv*, clxvi., 1901.

KEMP.—*American Journ. of Physiol.*, vi., p. 11, 1902.

KEMP and CALHOUN.—*American Journ. of Physiol.*, 1901.

KLEMENSIEWICZ.—*Ziegler's Beiträge*, 1903.

KOPSCH.—*Anat. Anzeiger*, xix., 1901.
LAKER.—*Virch. Archiv*, cxvi., 1889.
LAZARUS.—*Die Anämie*, 1900.
v. LIMBECK.—*Grundriss einer klin. Path. des Blutes*, Jena, 1896.
LÖWIT, M.—"Du Blutplättchen," *Ergeb. der All. Path.*, 1895; *Centralblatt f. Allgem. Path.*, iii., 1891; *Virch. Arch.*, cxvii.
MANN, G.—*Methods and Theory of Physiological Histology*, Oxford, 1903.
MAXIMOW.—*Arch. f. Anat. u. Physiol.*, 1899.
MORAWITZ.—*Deutsches Arch. f. klin. Med.*, lxxix., 1904; *Ergeb. d. Physiol.*, 1905.
MOSEN.—*Archiv f. Anat. u. Physiol.*, 1893.
NEUMANN.—*Virch. Archiv*, 1896.
v. NOORDEN.—*Die Bleisucht*, 1897.
OSLER.—*Proc. Roy. Soc.*, xxii., 1874.
OSLER and SCHÄFER.—*Centralblatt f. der med. Wissensch.*, p. 576, 1873.
PAKES.—*Trans. Path. Soc., London*, 1900.
PAPPENHEIM, A.—*Münch. med. Wochensch.*, p. 989, 1901.
PETRONE.—*Arch. ital. de Biologie*, 1901.
PREISICH and HEIM.—*Virchow's Archiv*, clxxviii., 1904.
PUCHBERGER.—*Virch. Archiv*, 1903.
RANVIER.—*Gaz. med. de Paris*, 1873.
SACERDOTTI.—*Anat. Anzeiger*, xvii., 1900.
SCHIEFFERDECKER.—*Gewebe Uhre*, p. 370, 1891.
SCHULTZE, MAX.—*Arch. f. mikros. Anat.*, i., 1865.
SCHWALBE, E.—*Virch. Archiv*, 1899 and 1902; *Wiener med. Kundshaw*, No. 10, 1903; *Untersuchungen z. Blutgerinnung*, Braunschweig, 1901; and *Ergebnisse d. Allgem. Path.*, xiii., 1902 (full literature).
SHERRINGTON.—*Proc. Roy. Soc.*, p. 279, 1900.
SCHNEIDER.—*Virch. Archiv*, clxxiv., 1903 (literature).
WRIGHT, A. E.—*Trans. of Path. Soc. of London*, p. 302, 1900.
WLASSOW.—*Ziegler's Beiträge*, xv., 1894.
WOOLDRIDGE.—Collected Papers; and *Die Gerinnung des Blutes*, Leipzig, 1891.

LECTURE VII

GUAIACUM TEST

BATELLI, F., and Mdlle. STERN.—"Recherches sur la Catalase," *Archivio di Fisiologia*, Maggio, 1905.
BETHE, M.—*Morph. Arbeiten*, ed. by G. Schwalbe, p. 207, 1892.
BERTRAND, and BERTRAND and BOURQUELOT.—*Compt. rend. Soc. de Biol.*, 1894, 1895, 1896, 1897.
BRANDENBERG.—*Münch. med. Wochenschr.*, No. 6, 1900.

BRUNTON, LAUDER, and CASH.—*St Bartholomew's Hospital Reports*, 1882.
CATHCART.—*Journ. of Physiol.*, xxi., 1904.
COTTON.—*Bulletin de Soc. Chem.*, 1902.
DEUTSCH.—*Centralbl. f. Bakt.*, xxix., 1901.
DOEBNER (OSCAR) and LÜCKER.—*Archiv f. Pharmak.*, pp. 234, 590, ccxxxiv., 1896.
DURHAM.—*Proc. Royal Soc.*, lxxiv., 1905.
GEPPERT.—"Über das Wesen der CHN-Vergiftung," *Zeits. f. klin. Med.*, xv., 1889.
GESSARD.—*Comptes rendus Soc. de Biologie*, p. 1304, 1902, and p. 285, 1905.
GONNERMANN.—*Pflüger's Archiv f. Physiol.*, lxxxii., 1900, and lxxxix., 1902.
HEDIN, S. G.—*Journal of Physiol.*, xxx., 1903.
HENOCQUE.—*Arch. d'anat. microscopique*, 1899.
HIRSCHFELD.—*Virch. Arch.*, cxlix., 1897.
HOPPE.—*Virch. Arch.*, xxiii., 1862.
KLEIN.—*Fol. Haematologica*, 1905.
KOBERT and KROHL.—*Arbeiten des pharmakol. Inst. z. Dorpat*, vii., 1891.
LANSTEINER.—*Centralblatt f. Bakt.*, xxvii., 1900.
LOEW, O.—*U.S. Dept. Agricult. Reports*, No. 68, 1901.
MAHOMED.—*Medico-Chirurg. Trans.*, London, lvii., 1874.
MOSER.—*Vierteljahrsschrift f. gericht. med.*, p. 44, 1901.
MEYER, E.—*Münch. med. Wochenschrift*, 1905.
NENCKI and SIEBER.—*Arch. f. exp. Path. u. Pharmak.*, xx., 1886; and *Zeits. f. physiol. Chemie.*, xxx.
POZZI-ESCOT.—"The Reducing Enzymes," *Amer. Chem. Journal*, 1903.
RAUDNITZ.—*Ergeb. d. Physiol.*, ii., 1903.
ROSELL.—Inaug. Dissert., Strassburg, 1901.
SCHALFIJEW.—Quoted from vol i., *Text-book of Physiology*, ed. by Schäfer.
SCHMIDT, A.—*Arch. f. path. Anat. u. Phys.*, xlii.
SCHÖNBEIN.—*Journ. f. pract. Chemie*, lxxxix.
SCHUNCK (E.) and MARCHLEWSKI.—*Proc. Royal Soc.*, 1896.
SENTER.—*Zeits. f. physikalische Chemie*, xliv., 1903.
SPITZER.—*Zeits. f. phys. Chem.*, No. 66, 1897.
VITALI.—*Chem. Centralbl.*, p. 1528, 1897.

HAEMIN TEST

BIFALVI.—*Centralbl. f. d. med. Wissenschaft*, No. 17, 1886.
KÜSTER.—*Zeits. f. physiol. Chemie*, xl., 1900.
LAIDLAW.—*Journ. of Physiol.*, xxi., 1904.
LEWIN and ROSENSTEIN.—*Virch. Archiv*, cxlii., 1895.
SCHALFIJEW.—Quoted from Gamgee's article, "Haemoglobin," in vol. i., *Text-book of Phys.*, ed. by E. A. Schäfer.
TEICHMANN.—*Zeits. f. rat. Med.*, iii., 1853.
VIRCHOW.—*Virch. Archiv*, xii., 1857.

PRECIPITIN TEST

ASCOLI.—*Münch. med. Wochenschrift*, March 1902.
BASHFORD.—*Journ. of Path. and Bact.*, viii., 1902.
BORDET.—*Ann. de l'Inst. Pasteur*, 1899.
BRIEGER and EHRLICH.—*Zeitschrift f. Hygiene*, xiii., 1893.
CASTELLANI.—*Lancet*, June 1902.
D'ESTE EMERY.—*St Bartholomew's Hospital Journal*, 1902.
GRAHAM-SMITH and SANGER.—*Journ. of Hygiene*, 1903.
GRUBER.—*Wiener klin. Wochenschrift*, 1896.
HUNTER, A.—*Proc. Physiol. Soc.*, xxx., p. 9, 1903.
KRAUS.—*Wiener klin. Wochenschrift*, Aug. 1897.
MERKEL.—*Münch. med. Wochenschrift*, 1904.
MEYER.—*Münch. med. Wochenschrift*, No. 15, 1904
MEYER (F.) and ASCHOFF.—*Berl. klin. Wochenschrift*, July 1902.
MYERS, W.—*Lancet*, vol. ii., 1900.
NUTTALL.—*Brit. Med. Journ.*, Nov. 1901 and April 1902; also *Blood Immunity and Relationship*, Cambridge, 1904 (full literature).
NUTTALL and DINKESPIEL.—*Brit. Med. Journ.*, May 1901.
SCHIROKICH.—*Wratsch.*, No. 29, 1901.
TSCHISTOWITCH.—*Ann. de l'Inst. Pasteur*, xiii., 1899.
UHLENHUTH.—(1) *Deutsche med. Wochenschrift*, Feb. 1901; (2) *Deutsche med. Wochenschrift*, Sept. 1902 (precipitin test for blood); (3) *Deutsche med. Wochenschrift*, Sept. 1901 (precipitin test for meats).
UHLENHUTH.—Das biolog. Verfahren zur Erkennung und Unterscheidung v. Menschen u. Tierblut, sorvie anderer Eiweissubstanzen u. s. Andwendung in d. forens. Praxis, Jena, 1905.
WASSERMANN.—*Deutsche med. Wochenschrift*, No. 12, 1904.
WASSERMANN and SCHÜTZE.—*Berl. klin. Wochenschrift*, Feb. 1901.
WRIGHT, A. E.—*Brit. Med. Journ.*, p. 1069, 1902.
WRIGHT, A. E., and STEWART DOUGLAS.—*Proc. Roy. Soc.*, lxxiii., 1904.
ZIEMKE, A. E.—*Deutsche med. Wochenschrift*, Jan. and Oct. 1901.

LECTURE VIII

BULLOCH, W.—*Brit. Med. Journ.*, p. 563, Sept. 1904.
WRIGHT, A. E., and STEWART DOUGLAS.—*Proc. Roy. Soc.*, lxxii., 1903, lxxiii., 1904.

ACUTE POST-HAEMORRHAGIC ANAEMIA

HERZ.—*Virch. Arch.*, cxxxiii.
LAACHE.—*Die Anaemie*, Christiana, 1883.
LAZARUS.—*Die Anaemie*, 1900.

SECONDARY ANAEMIA

BOYCOTT and HALDANE.—*Journ. of Hygiene*, iii., iv., 1903, 1904.
v. JAKSCH.—*Wiener klin. Wochenschrift*, Nos. 22, 23, 1889.
v. LIMBECK.—*Grund. d. klin. Path. des Blutes*, p. 335, Jena, 1896.

CHLOROSIS

LORRAIN SMITH.—*Trans. of Path. Soc., London*, li., 1900.
PAPPENHEIM, A.—*Zeits. f. klin. Med.*, xliii., 1901.

PROGRESSIVE PERNICIOUS ANAEMIA

BABES.—*Virch. Archiv*, cxli., 1895.
BIERMER —*Correspondenzblatt f. schweizer Aerzte*, No. 1, 1872.
COHNHEIM.—*Virch. Arch.*, lxviii., 1876.
EICHHORST.—*Die prog. pern. Anämie*, Leipzig, 1878.
GEELMUYDEN.—*Virch. Arch.*, cv., 1886.
GULLAND and GOODALL.—*Journ. of Path. and Bact.*, x., 1905.
HUNTER, W.—*Pernicious Anaemia*, London, 1901.
HAYEM.—*Du Sang*, Paris, 1889.
LAACHE.—*Die Anaemie*, Christiana, 1883.
LORRAIN SMITH.—*Trans. of Path. Soc., London*, li., 1900.
MÜLLER, H. F.—*Deutsches Arch. f. klin. Med.*, li., 1893.
QUINCKE.—*Deutsches Arch. f. klin. Med.*, lxxv., 1880.
SCHAPIRO.—*Zeits. f. klin. Med.*, xiii., 1888.
SCHAUMAN and TALLQVIST.—*Deutsche med. Wochenschrift*, No. 20, 1898.
WILTSCHUR.—*Deutsche med. Wochenschr.*, 1893.

LEUKAEMIA

BENNETT, HUGHES.—*Edinburgh Med. and Surg. Journ.*, lxiv., 1845.
FULLER.—Quoted from Aitken's *Science and Practice of Medicine*, 1868.
JÖHNE.—*Virch. Arch.*, 1903.
NEUMANN, E.—*Arch. f. Heilkunde*, x., 1869.
REID, J.—*Edinburgh Med. and Surg. Journ.*, 1841.
VIRCHOW.—"Weisses Blut," *Froriep's Journ.*, Nov. 1845.
WÖLFF.—*Berl. med. Wochenschr.*, 1892

ACUTE LYMPHATIC LEUKAEMIA

BRADFORD and SHAW.—*Medico-Chirurgical Trans.*, xxiv., 1902.
PHEAR.—*Medico-Chirurgical Trans.*, lxxxiv., 1901.

CHLOROMA

DOCK.—*Amer. Journ. of Med. Sciences*, Aug. 1893.
DOCK and WARTHIN.—"A new case of Chloroma, with a study of cases since 1893," *Trans. of the Assoc. of Amer. Physicians*, xix., 1904.

GÜMBEL.—*Virch. Arch.*, clxxi., Hft. 3, p. 514.
NAEGELI.—*Deutsche med. Wochenschr.*, p. 287, 1900.
TÜRK.—*Vorlesung. ü. klin. Haemat.*, Bd. i., 1904.

MYELOGENOUS LEUKAEMIA

BILLINGS and CAPPS.—*Amer. Journ. of Med. Sciences*, Sept. 1903.
WALZ.—*Centralbl. f. Pathol.*, xii., 1901 (literature).
WESTPHAL.—"Über das Vorkommen der Charcot-Leydenschen Krystalle beim Lebenden," *Deutsches Arch. f. klin. Med.*, lvii., 1896.

PSEUDO-LEUKAEMIA

COHNHEIM.—*Virch. Arch.*, xxxiii., 1865.
PINKUS.—"Pseudo-leukaemie," in Bd. viii., *Nothnagel's Specielle Path. u. Therapie.*

PROTOZOA IN BLOOD

CASTELLANI.—*Brit. Med. Journ.*, Sept. 1904.
CUNNINGHAM, D. D.—*Scientific Memoirs of the Medical Officers of the Army in India*, 1886.
DONOVAN.—*Indian Med. Gazette*, 1903.
KOCH, R.—*Deutsche med. Wochenschr.*, Nov. 1905.
LEISHMAN.—*Brit. Med. Journ.*, i., p. 1254, 1903.
ROGERS, L.—*Brit. Med. Journ.*, Sept. 17, 1904.

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