General hæmatology : with special reference to the child in health and disease / by W.M. Feldman.

POCKET-MONOGRAPHS
ON PRACTICAL MEDICINE

General and Child Hæmatology

W. M. FELDMAN

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GENERAL HÆMATOLOGY

With Special Reference to the Child in Health and Disease

BY

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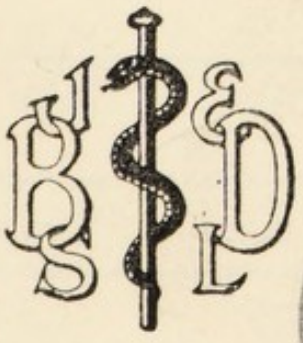

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To the Memory of my Mother
Pessel
Who died at the age of 82 years
On Friday, the 18th of March, 1932.

CONTENTS.

PREFACE.

In this account of the various general and pædiatric aspects of hæmatology I have attempted not only to deal with the subject comprehensively, but to combine economy of space with clearness of exposition. To accomplish such a task I have—without omitting anything of practical importance or of academic interest—refrained from giving such details as can only be appreciated by the expert hæmatologist, but which would only bewilder the practical physician for whom the book is meant. I have also omitted all references to literature, as the inclusion of even a moderately complete bibliography—especially in connection with blood-groups—would easily have doubled the size of the book. For the benefit of those whom this little book may stimulate to go farther afield in search of information, I append on next page a short list of books in English which the reader will find helpful, and some of which contain excellent bibliographies.

W. M. Feldman.

150, *Harley Street*, W.1.

Books for further Reading.

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General and Child Hæmatology.

CHAPTER I.

Introductory Survey.

Congenital and acquired properties of the blood—Blood in diagnosis—Volume of blood and of corpuscles in the body under different conditions—Physical and biochemical properties of whole blood : refractive index, coagulation, reaction, osmotic pressure, cataphoresis, viscosity, etc., and the influence of age and environmental pathological factors upon them—Coagulation time, bleeding time, prothrombin time, calcium time—Capillary resistance test —Acidosis and alkalosis, their diagnosis and treatment— The use of hypertonic saline, or glucose—The suggested value of cataphoresis for clinical purposes.

THE ideal specialist has been defined as "one who knows everything about something and something about everything." The ideal pædiatrician should, therefore, be a physician who knows all about child medicine and something about everything which is in some way connected with the anatomy, physiology, psychology, hygiene (physical and mental), pathology, biology and sociology, and one might even add the anthropology and ethnology of child life. Hæmatology touches the subject of pædiatrics at a number of these points. Those properties of the blood which are affected by environment, or which are acquired as the result of disease, are of course the immediate concern of the pædiatrician, inasmuch as a considerable part of the diagnosis, prognosis, as well as of the treatment of disease in children depends upon the qualitative

and quantitative alterations in the character of the various parts of the blood. The blood also possesses certain properties which are inborn and fixed for any particular individual, are unaltered by the vicissitudes of life, and in respect of hereditary transmissibility behave as Mendelian characters. These constitutional peculiarities concern the pædiatrician not only from the point of view of therapeutics—when the question of blood-transfusion arises—but also in connection with several sociological, scientific and medico-legal problems in which the child plays an important role.

Blood in Diagnosis.—Blood consists, of course, of plasma—which in turn consists of fibrinogen and serum—corpuscles (red and various forms of white), and platelets. The biochemical and biophysical characters of the serum ; the morphological, physical, and biochemical characters of the blood-cells, as well as their absolute and relative numbers, have important bearings in the diagnosis of disease as well as in treatment.

Volume of Blood in the Body.—A knowledge of the total amount of blood in the body at any time is of importance not only from the point of view of assessing the significance of a cell-count, inasmuch as a different count—either in the same child at different times, or in different children at the same time—might be more apparent than real, and be due to a difference in the plasma content on the two occasions, but also from the standpoint of transfusion when it is necessary to ascertain the amount of blood to be given to any particular child. Too small an injection would fail in its purpose, while too large a one might overfill the heart and cause death from acute cardiac dilatation. The volume of blood in the living body is estimated by injecting intravenously a known quantity of an innocuous dye which is not too quickly excreted, and after allowing enough time for it to become thoroughly mixed with the blood, estimating its percentage

concentration in a measured sample of blood. Thus if 0·5 grm. of the dye (vital red) were injected, and its concentration in a sample withdrawn is found to be 1 per 10,000, then the total volume of the blood in the body is obviously 10,000 times the amount of dye injected, i.e., 5,000 c.c., or 5 litres, which is about the amount in a 70-kilo adult. It has been shown that under normal conditions the blood-volume is at all ages a function of the body surface, and as a child has a larger surface per unit body-weight than has an adult, it must also have a comparatively greater blood-volume. Hence, while the full adult transfusion dose is 500-600 c.c., i.e., about 3 c.c. per lb., a young child needs at least 7 c.c. per lb., and may have 10 c.c. as a maximum. See further, Chapter VII.

Total Volume of Corpuscles.—A knowledge of the volume occupied by all the cells in the blood is important, and is ascertained by centrifuging a small quantity of oxalated (i.e., non-coagulable) blood in a graduated tube (hæmatocrit) and reading off the volume of the packed cells at the bottom of the tube. Normally, it is found to be about 43 per cent. of the total blood-volume in the adult and about 55 per cent. in the newborn infant. If the average size of the cells is bigger, or if they are present in larger numbers, the total cell volume will obviously be greater.

Properties of Blood.

Refractive Index of the Blood.—The fluctuation of the water content of the blood, and therefore also of the water metabolism in general, has also been studied by means of the variations in the refractive index of the serum. This index depends upon the protein concentration, and therefore the less water a blood contains the greater will be its refractive index. It has been found that the refraction curve

of a newborn baby's blood is a true mirror image of the weight curve : as the weight begins to fall immediately after birth, the refraction curve rises ; when the weight reaches its minimum, the refraction reaches its maximum ; with increase in weight, the refraction curve falls. Hence the initial loss of weight by the newborn baby is, in part at any rate, due to loss of

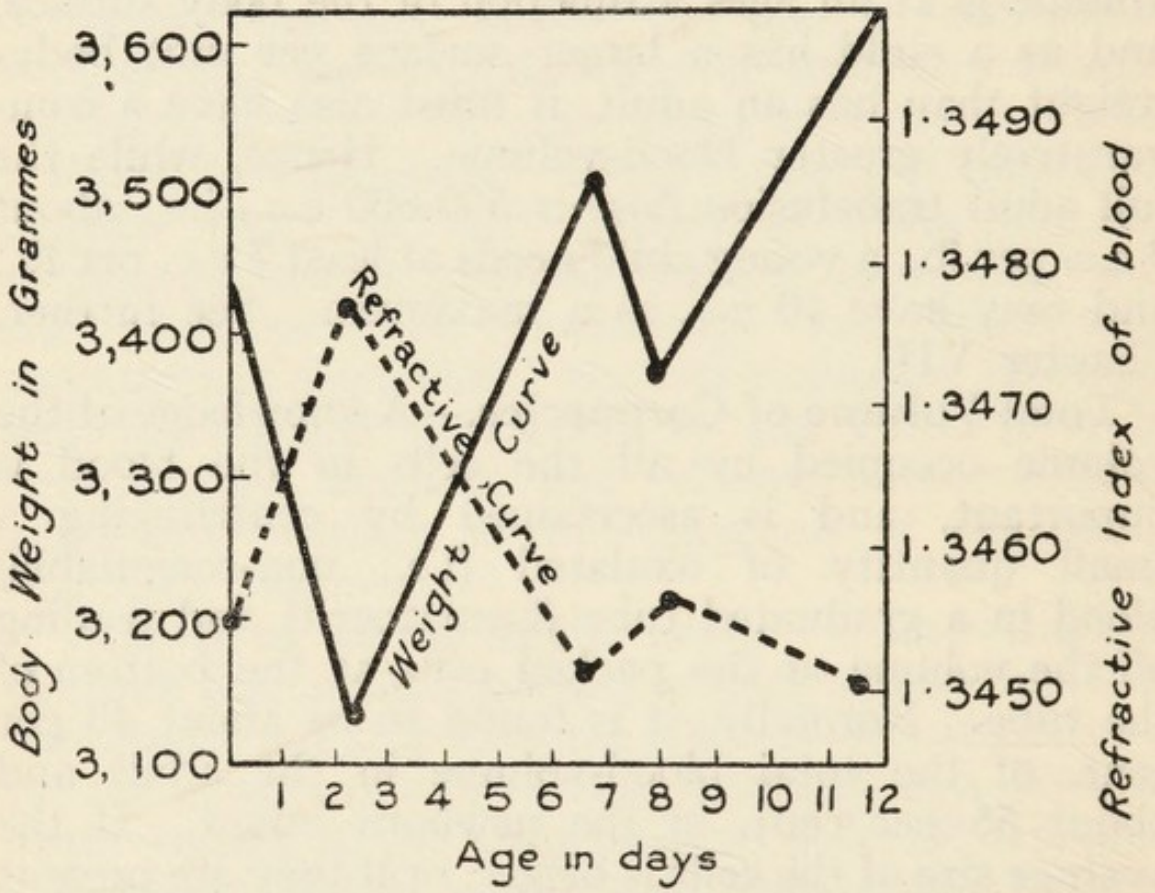

FIG. 1.—Relation between weight of body and refractive index of blood in a newborn baby.

water by the tissues which they make up by absorbing water from the blood (see fig. 1).

Similar studies, as well as the actual estimation of the blood-volume by means of the vital red method, have confirmed the theoretical expectation that changes in blood-volume occur whenever there is lack of balance between the amount of water taken into the body and that eliminated from it. When the balance is positive, i.e., on the side of the intake,

e.g., in cases of heart and kidney disease with diminished secretion of urine, the blood becomes more watery **(hydræmia)**, with increase in the total volume, relative diminution in the cell-count, etc. A similar condition prevails, very temporarily, immediately after the introduction of extra fluid by transfusion of saline. When the balance is negative, i.e., when more water is eliminated than is taken in, there results a water deficit in the blood **(anhydræmia),** with diminution in the total volume, relative concentration of the various metabolites in the plasma, as well as of the corpuscles, giving a high cell-count. This condition occurs chiefly in the severe diarrhœa and vomiting of infants (summer diarrhœa), but may also occur after prolonged and profuse sweating, unless a sufficiency of water is given to counterbalance the loss. The consequences of anhydræmia, and its treatment, will be dealt with later (see p. 36).

Clotting.—Normally, when blood is shed it clots very soon, and the clot forming at the wound tends to stop further bleeding. In certain cases, however, not only will bleeding continue abnormally—and even dangerously—long, but will occur on the slightest provocation, e.g., in hæmophilia, purpura, leukæmia, jaundice, etc. Moreover, in normal individuals, blood remains fluid so long as it is inside the blood-vessels, but in certain cases intravascular clotting takes place, e.g., venous thrombosis after typhoid, or influenza, or, in adults, arterial clotting in cases of coronary thrombosis. To understand these phenomena, and what is more important, to be able to deal with the abnormal cases intelligently, it is necessary to have a clear idea of **the nature of coagulation.**

Clotting is brought about as the result of interaction, in the presence of calcium ions, between certain enzymes (present in the serum and the blood-cells), and a substance called prothrombin (produced by

the platelets), giving rise to the formation of thrombin. This thrombin then combines with the fibrinogen to form fibrin—which is the clot. The reason that circulating blood does not clot intravascularly is that such blood contains antithrombin, which holds the prothrombin in combination, thus hindering its conversion into thrombin. In shed blood, however, the prothrombin is liberated from its combination with the antithrombin by the action of a substance (thromboplastin) derived from the disintegration of the platelets, leucocytes and tissue cells. These processes may be summarised as follows :—

(i) Thromboplastin + antithrombin = liberation of prothrombin.

(ii) Prothrombin + calcium ions = thrombin.

(iii) Thrombin + fibrinogen = fibrin = clot.

Lengthening of coagulation time therefore occurs under one or more of the following conditions :—

(1) *Deficiency of Prothrombin.*—This may be due to a paucity of platelets, or to a poor quality of platelets which may be present in normal numbers. The amount of prothrombin in any given sample of blood may be tested by the *prothrombin time,* i.e., by the time it takes the given blood to clot, in the presence of a known concentration of calcium, as compared with the time it takes a normal blood to clot under equal calcium concentration. Plasma from which all calcium has been removed by means of sodium oxalate, is mixed with a *known* amount of $CaCl_2$, and the time is noted when coagulation occurs. An equal quantity of normal oxalate plasma is treated with the same amount of $CaCl_2$ and the time it takes for the blood to clot is noted. This prothrombin time of the given sample will be greater or less than that of the normal sample, according as it contains less or more than the normal amount of prothrombin. **The normal prothrombin time is about ten minutes.** If it is much longer than fifteen minutes, there is a

deficiency of prothrombin. If in such a case the platelet count is normal, the inference is that the quality of the platelets is poor as regards its prothrombin content—in the same way as that of a red corpuscle may be poor in respect of its hæmoglobin content (see "Colour Index," p. 43). In hæmophilia, for instance, the prothrombin time may be as long as five hours, although the platelet count is normal. On the other hand, in certain hæmorrhagic diseases of the newborn, e.g., melæna neonatorum, the prothrombin time is prolonged because the platelet count is low.

(2) *Deficiency of Calcium.*—This may be tested by the *calcium time*, i.e., the time it takes for a measured quantity of non-oxalated plasma of the given sample to clot after mixing with a known amount of $CaCl_2$. A blood containing a normal amount of calcium ions will not have its coagulation time altered by the extra addition of the $CaCl_2$, but a blood whose coagulation time is prolonged on account of calcium deficiency will have the coagulation time approach the normal value after such addition of extra calcium. This occurs in obstructive jaundice when the calcium is held in combination by the bile. In such cases administration of calcium is indicated, prophylactically, before operations.

(3) *Deficiency of Fibrinogen.*—The amount of fibrinogen in a sample of blood can be estimated from the amount of fibrin formed. Fibrinogen is manufactured by the liver and therefore deficiency of that substance occurs in cases of liver atrophy, or syphilitic cirrhosis. Normally, blood contains 0·3 grm. of fibrinogen per 100 c.c. of plasma, hence delay of coagulation time accompanied by diminution of the amount of fibrinogen is one of the tests of hepatic inefficiency.

(4) *Increase of Antithrombin.*—The amount of antithrombin in a given sample of blood can be

estimated by the amount of thromboplastin (derived from tissue extracts and platelets) needed to bring the amount of liberated prothrombin up to the normal standard—as shown by the prothrombin time.

Antithrombin is increased in certain cases of sepsis and purpura. The frequency of fatal suprarenal hæmorrhage in newborn infants of pre-eclamptic mothers, is probably due to the transmission of maternal toxin to the fœtus, resulting in the formation of antithrombin in a blood which is already deficient in platelets and prothrombin—as the blood of every newborn infant is. Similarly, it has been shown experimentally in animals that chloroform intoxication of pregnant females results in hæmorrhages in the young. From this one learns that operations on very young infants in whom the platelet count is very small, should preferably be done without chloroform anæsthesia.

Accelerated coagulation time—which of course is due to any one or more of the opposite conditions—is of interest in cases of intravascular clotting or thrombosis, e.g., sinus thrombosis in cases of suppurative otitis media, or venous thrombosis after typhoid, etc. These conditions, however, are not common, and in any case the coagulation time is of no diagnostic, or differential diagnostic, significance.

The normal coagulation time varies in length with the method employed for its determination, of which several are available. Hence any laboratory report regarding the coagulation time of a given sample of blood should state the method used, and what is the normal time given by that method.

Characters of the Clot.—The *consistency* and *contractility* of a clot are important. Normally, a clot is firm, and difficult to break, and thus effectively arrests hæmorrhage. In purpura hæmorrhagica and certain other conditions the clot is soft and friable and easily yields to the force of the blood behind it.

Also normally, when blood is collected in an unoxalated test-tube, the clot after a time begins to retract and express serum. In the hæmorrhagic diathesis retraction is absent. These phenomena cannot be associated with the number of platelets, since in some conditions in which the platelet count is normal, the consistency and contractility of the clot may be abnormal. It is possibly associated with low fibrinogen content.

Bleeding time, as measured by the duration of bleeding from a sharp prick, after removing successive drops of blood by means of blotting paper, does not depend upon the factors influencing coagulation, and is therefore independent of coagulation time. It depends upon, and varies with, the degree of permeability of the capillaries as measured by the *capillary resistance test,* which consists in compressing the upper arm with the armlet of a sphygmomanometer until the pulse at the wrist is obliterated for two minutes. The test is positive if purpuric spots appear on the forearm, and the degree of permeability is to some extent proportional to the number of such spots. **The normal bleeding time is two and a half minutes,** but in certain hæmorrhagic conditions it may be as long as an hour—although coagulation time and platelet number are normal.

Reaction.—The reaction of any solution depends on the relative numbers of free H and OH ions present in it. When there is perfect balance between them the solution is neutral—as is the case with pure water, in which the H_2O is split up into equal numbers of H and OH ions. In that case it has been found that the H-ion concentration (expressed as cH), is about 10^{-7}, i.e., $1/10^7$ grm. of hydrogen per litre. This is expressed as pH = 7. If the H-ion concentration exceeds that of the OH, e.g., when it is $1/10^{6\cdot5}$, i.e., when pH = 6·5, the solution is acid. On the other hand, when the H-ion concentration

is below that of the OH ion, e.g., when $cH = 1/10^{7\cdot5}$, i.e., when pH = 7·5, the solution is alkaline. Hence a solution is neutral, acid, or alkaline, according as its pH is equal to, less, or greater than 7. Normally blood is slightly alkaline, with a pH = 7·4. In premature babies pH = 6·5, which means that the blood is considerably acid. As susceptibility to infection varies with the degree of blood acidity, it follows that the premature infant is more liable to infection and sepsis.

Maintenance of pH Equilibrium. It is essential for health that the blood should be always slightly alkaline, but in the course of ordinary metabolism, there is a considerable ebb and flow of strong acids and alkalis in the blood, and yet its reaction keeps constant at about pH = 7·4. How is this pH equilibrium maintained? The answer is that the acid-base balance is preserved by several different mechanisms, as follows :—

(1) The presence within it of such substances as bicarbonates and carbonic acid which can combine with considerable quantities of strong acids or alkalis respectively without appreciably affecting the reaction. These substances, which in virtue of their peculiar property are called "buffer" substances, act as follows :—

(i) $NaHCO_3 + HCl = NaCl + H_2CO_3$. But carbonic acid is an extremely weak acid, because of its very feeble dissociation into free H— and HCO_3- ions, so that its presence in a solution does not materially increase the H-ion concentration (or diminish the pH). The bicarbonates constitute the "alkaline reserve" of the blood, because so long as they are present in excess there can be no perceptible lowering of the pH.

(ii) The carbonic acid reacts with strong alkalies as follows :—

$$H_2CO_3 + NaOH = NaHCO_3 + H_2O.$$

Like carbonic acid, the bicarbonate dissociates very feebly into Na and HCO_3 ions. There are, therefore, very few Na-ions to form NaOH through reacting with water ($Na + H_2O = NaOH + H$), and therefore there is no appreciable increase in the alkalinity, i.e., no increase in pH.

There are several other such buffer bodies in the blood, e.g., phosphates, proteins, etc., but the bicarbonates and carbonic acid are the most important. Normally, the ratio $NaHCO_3/H_2CO_3$ in the blood is 20/1, but it is clear that while the buffer mechanism can avoid gross changes in the H-ion concentration or pH, it cannot prevent considerable fluctuations in the value of this ratio. Such fluctuations are, however, prevented by

(2) Respiratory activity, and

(3) Renal activity.

Respiratory activity prevents large accumulation of H_2CO_3, because this acid splits up into CO_2 and water, and the CO_2 stimulates the respiratory centre to increased pulmonary ventilation, resulting in a more rapid loss of CO_2 through the breath. The kidneys supplement the other two defence mechanisms (which would soon be overtaxed in the presence of the continued influx of acids and alkalis into the blood), by removing excess of acid as well as of alkali by means of the urine.

Acidosis and alkalosis are the states in which, as the result of a breakdown in the defence mechanism, there is an alteration in the amount of alkaline reserve in the direction, respectively, of a diminution of pH below 7·4, or of an increase to above that figure. The reader will note that acidosis does not necessarily mean an acid condition of the blood, but one in which the normal alkalinity is diminished. **Acidosis** in children may occur in consequence of increased loss of $NaHCO_3$ from the body due to renal inefficiency (such as occurs in uræmic states, etc.), or

increased accumulation of acids in the blood. The latter may occur under any of the following conditions, viz. :—

(i) Impaired pulmonary activity, occurring in pulmonary atelectasis, cardiac decompensation, etc., when H_2CO_3 fails to be adequately eliminated.

(ii) Endogenous production of acid bodies, such as occurs in disturbed carbohydrate metabolism in cases of starvation or of diabetes, when the combustion of either stored or ingested fat—instead of proceeding to the end-products of CO_2 and water—stops short at the stage of fatty acid and ketone bodies. The acidosis so produced is generally spoken of as **ketosis.**

(iii) Exogenous accumulation of acid radicles, as the result of ingestion of excessive amounts of acids—such as HCl—or of NaCl, NH_4Cl, or $CaCl_2$.

Alkalosis in children occurs when there is an increased loss of CO_2 through the lungs (e.g., in cases of hyperpnœa due to pyrexia) ; increased loss of HCl, such as occurs in vomiting of pyloric stenosis, when the HCl of the gastric juice which would normally be reabsorbed through the intestine, is lost in the vomit ; or when there is an increased accumulation of $NaHCO_3$ or other alkali, due to overdosage, or to defective excretion through the kidneys.

The **diagnosis** of these two states is of very great importance. In cases of pyloric stenosis, for instance, one is apt to associate the state of starvation with a condition of acidosis and ketosis as the result of imperfect oxidation of body fat. If an erroneous diagnosis be made in such a case and treatment directed to acidosis rather than alkalosis—either before or after operation—it would practically certainly make a difference between the baby's life and death. The diagnosis is made in several ways, as follows :—

(i) Estimation of alkali reserve, by finding the amount of combined CO_2 in the plasma. In normal

infants this varies from 50 to 60 volumes per cent. An amount of less than 40 volumes per cent. indicates acidosis, and the exact percentage of the CO_2 volume determines its degree.

(ii) Determination of the CO_2 content of the alveolar air. Normally, alveolar air contains about 4·5 per cent. to 6·5 per cent. of CO_2. In acidosis less CO_2 is eliminated by the lungs, and therefore the CO_2 content in the alveloar air is less.

(iii) Determination of the pH of the blood.

If the acidosis is due to impaired carbohydrate metabolism, the ketone bodies may be detected in the urine, by means of the $FeCl_3$ or the sodium nitroprusside reactions, but these reactions will not detect acidosis in which there is no formation of ketone bodies. As to the significance of the reaction of the urine to litmus, one may say that it is only helpful if the urine is alkaline or neutral, when freshly passed, to exclude acidosis, but neither does an acid reaction signify acidosis, nor does an alkaline reaction indicate alkalosis.

The **treatment** of acidosis depends upon its cause, but it consists mainly in the administration of alkalis at the rate of 0·5 grm. of $NaHCO_3$ per kilo body-weight (corresponding roughly to about one quarter the normal $NaHCO_3$ content of the body), except when the acidosis is due to damaged kidneys, in which case, owing to the defective renal excretion, excessive doses of alkali might convert an acidosis into an alkalosis. In addition, administration of glucose either orally, rectally or intravenously, in doses of $\frac{1}{2}$ grm. per kilo body-weight, accompanied in the case of ketosis by $\frac{1}{2}$ unit of insulin per kilo body-weight, is of very great value, and in case of diabetic coma indispensable. In these two cases alkalies are of questionable value. Alkalosis is treated by the intraperitoneal, or subcutaneous administration of a pint of normal saline.

Osmotic Pressure of the Blood.—Blood-plasma contains many substances in solution—some of crystalloid, and others of colloid nature. The former give rise to osmotic pressure, and the total concentration of all the salts is equal to that of a 0·9 per cent. solution of common salt. Hence, 0·9 per cent. saline, called normal saline, has the same osmotic pressure as blood-plasma, and is said to be isotonic with it. A solution of salt weaker than 0·9 per cent. is hypotonic, while one stronger than 0·9 per cent. is hypertonic. As water will pass by osmosis from a solution of lower to one of higher concentration, it follows that if the blood is made of higher concentration than the other body fluids, water will flow from those fluids into the blood until the concentration is equalised. This fact is utilised therapeutically in cases of increased intracranial pressure due to excess of cerebrospinal fluid, e.g., hydrocephalus and cerebral tumour, etc. By raising the concentration of the plasma from 0·9 per cent. to say 1·1 per cent. it is possible to determine a flow of about a litre of fluid from the other fluids into the blood, because as the concentration is thus raised by about 20 per cent. there will have to be an increase in volume of blood by the same amount for the concentration to return to the original 0·9 per cent. As the total volume of blood in the adult is 5 litres, it means that about a litre of water will thus reach it by osmosis. In order to raise the concentration to 1·1 per cent., one must obviously introduce the salt in as high a concentration as conveniently possible, so as to introduce the minimum amount of water from the outside; 1 c.c. saline per kilo is a conveniently small quantity to give. As the total amount of salts per 100 c.c. of blood is equivalent to 0·9 grm. NaCl, therefore the whole 5,000 c.c. of blood contains the equivalent of 45 grm. NaCl. Raising the concentration by 20 per cent. means

raising the amount of NaCl by 20 per cent. of 45, i.e., by 9 grm., and this is to be introduced in a bulk of 1 c.c. per kilo, i.e., in a total bulk of 70 c.c. Therefore the strength of the saline needed is about 13 per cent. In practice one gives to an adult 70-100 c.c. of a 15-30 per cent. solution—according to requirements ; a child needs about 2 c.c. of such solutions per kilo body-weight, because of its relatively greater blood-content (see p. 3). The indications for such treatment are :—

(i) In cases of intracranial tumour, for the purpose of relieving headache and other symptoms of intracranial pressure until it is convenient to operate ; to facilitate intracranial operations ; to obtain temporary consciousness in cases of coma so as to be able to get the patient's co-operation in the examination of the fundi, etc., as well as in obtaining a history for the purpose of arriving at a correct diagnosis in an emergency.

(ii) In cases of meningitis, or hydrocephalus, when it is not possible to obtain cerebrospinal fluid either by the lumbar or by any other route, such frequently repeated procedures might be utilised for therapeutic purposes instead.

Fifty per cent. glucose in *normal* saline may be used instead of the hypertonic saline, and has certain advantages ; its effect is more permanent, it is useful in ketosis, and it is also nutritive. It is obvious that when such treatment is applied the patient must not take any water to drink. The hypertonic solution is best given intravenously, but may also be given per rectum, intraperitoneally, or—in the case of young infants—orally. In older persons such oral administration may cause nausea.

It is to be noted that although, as stated above, 13 per cent. saline given in doses of 1 c.c. per kilo should determine a flow of 1 litre of water into the blood by osmosis, the actual flow is considerably

less, because the equalisation of concentration is effected not by the flow of water alone, but by the passage of salt in the opposite direction also, and the actual amount of water flow depends on the relative amounts of blood and other body fluids.

Cataphoresis of the Blood.—The colloidal particles of the blood-serum protein are charged with electricity, and will therefore travel to the cathode or anode of an electric battery with which the serum is in circuit, according as they carry a positive or negative charge. This phenomenon is called *cataphoresis*. It is easy to measure the rate of migration of these particles, which is proportional to the difference of potential between the surface of the particles and the surrounding liquid (fig. 2). This potential difference will obviously vary with the H-ion concentration of the blood, i.e., with the nature of the electrolytes contained in it, since the H and OH ions themselves carry +ve and −ve charges respectively. Bendien has made use of this phenomenon to study the behaviour of the blood serum-protein under various pathological conditions, and he believes that he can diagnose early cancer or sarcoma in this way, since he is of opinion that when the potential difference between the serum particles and the local tissues is of a certain magnitude, the state is favourable for the development of cancer—if there is an exciting cause such as local irritation. Bendien has also found that when the potential difference is of that particular magnitude, the serum will flocculate with a mixture containing a given amount of sodium vanadate, Na_3VO_4, and acetic acid. He thus uses this mixture for the early diagnosis of malignant disease. The value of this test is, however, being disputed.

There are several other physical properties of the blood that have been studied by physiologists, e.g., specific gravity, viscosity, electrical conductivity,

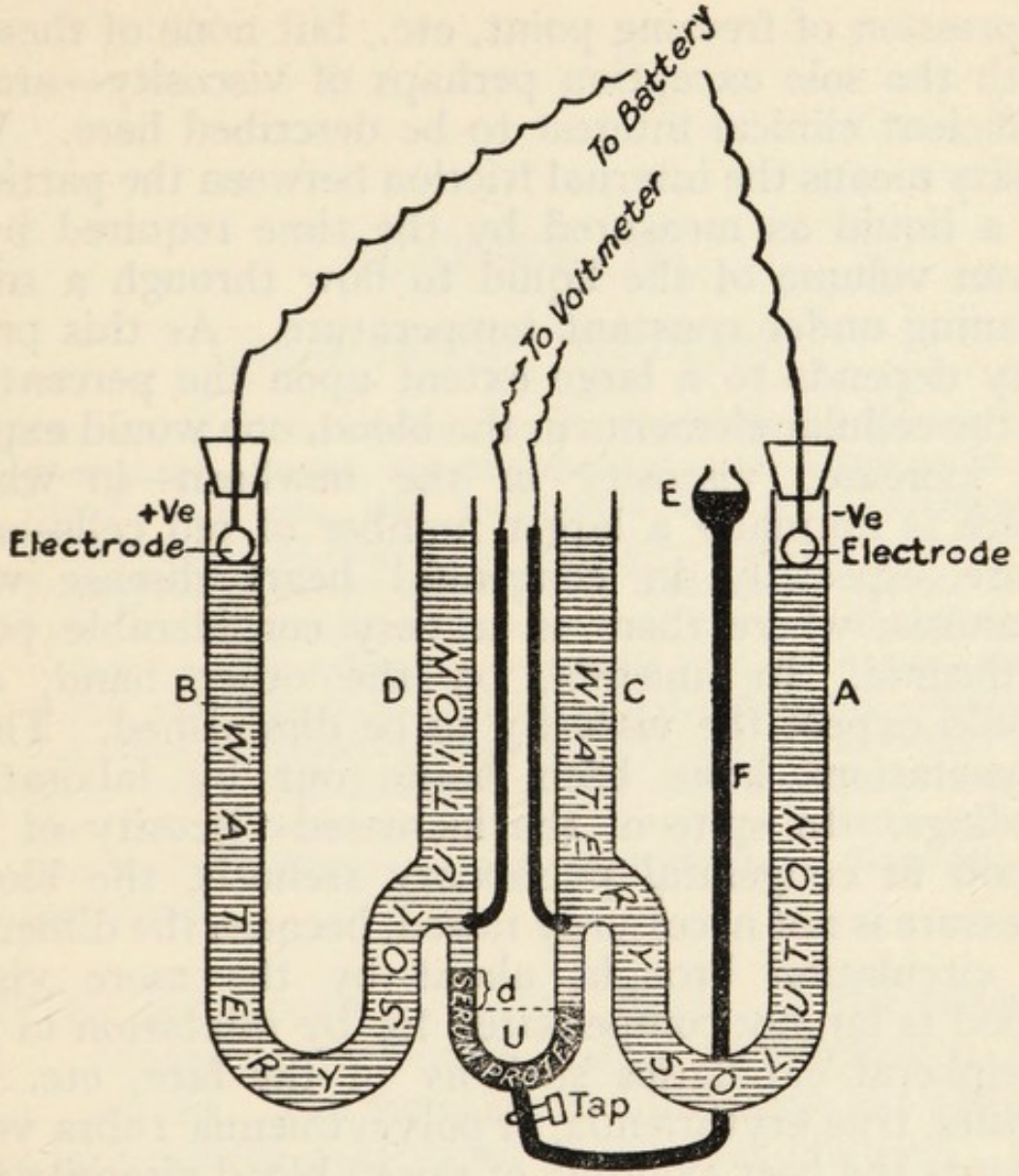

FIG. 2.—Cataphoresis apparatus (after Bendien and Janssen). Tubes A, B, C and D contain a watery solution of a given electrolyte. The thistle funnel E and Tube F up to the tap are filled with a solution of serum protein containing the same concentration of the same electrolyte. On opening the tap the protein solution gradually rises into the U-tube, displacing the aqueous solution, and settles in both limbs of the U-tube at the same level. When the U-tube is half filled with the protein solution, the tap is closed, and the apparatus is connected with a battery, and the current is so regulated that the potential difference between the two limbs of the U-tube is 10 volts, as measured by the voltmeter. Under the influence of the current the colloidal serum particles begin to move towards the +ve or −ve electrode of the battery, according as they carry −ve or +ve charges. The direction and amount of displacement (d) of the serum protein in the U-tube after two hours measures the direction and velocity of the colloidal particles.

depression of freezing point, etc., but none of these—with the sole exception perhaps of viscosity—are of sufficient clinical interest to be described here. Viscosity means the internal friction between the particles of a liquid as measured by the time required by a given volume of the liquid to flow through a small opening under constant temperature. As this property depends to a large extent upon the percentage of the cellular elements in the blood, one would expect an increased viscosity in the newborn—in whom there is normally a larger number of red cells—and more especially in congenital heart disease with cyanosis, where there is a very considerable polycythæmia. In anæmia, on the other hand, one would expect the viscosity to be diminished. These expectations have been borne out by laboratory findings. In spite of the increased viscosity of the blood in congenital pulmonary stenosis, the blood-pressure is not necessarily raised, because the difficulty in circulation brought about by the more viscid blood is largely compensated for by dilatation of the peripheral capillaries such as of the face, etc. In adults, true erythræmia, or polycythæmia rubra vera, affords the best example of raised blood viscosity (see p. 63).

CHAPTER II.

Chemistry of the Serum.

Importance of blood chemistry in diagnosis, prognosis, and treatment—Sugar, its metabolism and function in the blood; acidosis, and lactic-acid acidosis—Glycolysis—Maintenance of sugar equilibrium—Hyperglycæmia and hypoglycæmia: symptoms and treatment—Sugar tolerance test—Lævulose tolerance test for hepatic efficiency—Non-protein nitrogen, urea, uric acid, creatinin, etc.—Addis' ratio, Ambard's coefficient—Cholesterol—Minerals: chlorides, calcium, phosphorus, bicarbonates—Bile pigment—van den Bergh test for differentiating between obstructive and hæmolytic jaundice—Icterus index—Antibodies—Water content—Anhydræmia: diagnosis and treatment.

THE fluid portion of the blood, or the plasma, consists, as already stated, of fibrinogen and serum. The function of fibrinogen has already been sufficiently dealt with in the first chapter; this chapter will be devoted to the serum. As the serum carries the products of body metabolism, a knowledge of its qualitative composition, as well as of the physiological range in variation of the percentage contents of the constituents normally present in it, is of very considerable value to the pædiatrician in the diagnosis, prognosis, and treatment of disease—more particularly as regards disturbances of carbohydrate metabolism, renal inefficiency, rickets, spasmophilia, disturbance of acid-base equilibrium, etc.

Sugar.—The normal range of variation in the sugar content of an adult's blood, several hours after a meal containing carbohydrate, is 0·08-0·12 per cent., i.e., 0·08-0·12 grm. per 100 c.c., or 80-120 mgm. per

100 c.c. At birth it may be as low as 0·05 per cent., but it gradually rises until it reaches the level of about 0·09 per cent. at two weeks—after which the range becomes the same as in the adult.

The problem of carbohydrate metabolism—i.e., the series of transformations which ingested carbohydrates undergo resulting in the circulation of sugar (glucose) in the blood, the purpose that the blood-sugar serves, the mechanism by which the sugar level is kept constant at about 0·1 per cent. despite the variable amounts ingested—is both academically interesting and practically important. Very little, if any, of the sugar normally found in the blood is of endogenous origin, i.e., the result of the conversion of protein or fat into carbohydrate, but is practically entirely derived exogenously, i.e., from ingested carbohydrate. Any carbohydrate taken into the body, whether it be a monosaccharide ($C_6H_{12}O_6$)—such as dextro-rotatory glucose (dextrose), or lævo-rotatory fructose (lævulose)—which gets absorbed into the blood at once ; or a disaccharide ($C_{12}H_{22}O_{11}$) such as cane sugar, lactose, or maltose, and polysaccharide $(C_6H_{10}O_5)_n$ like starch, which under the action of appropriate enzymes become hydrolysed into monosaccharides before absorption into the blood,[1] ultimately reaches the blood in the form of monosaccharide. Indeed, under normal conditions it is only one particular kind of monosaccharide, viz., dextrose, or glucose, which actually circulates in the blood-stream. If lævulose is either taken by the mouth, or formed out of polysaccharides by hydrolysis, it is removed from the blood-stream by the liver (see " Lævulose Test for Hepatic Efficiency," p. 26). Under abnormal conditions, such as carbohydrate starvation, or diabetes, it is possible for sugar to be formed in the body out of protein.

The **function** of the blood-sugar is twofold. In

[1] $C_{12}H_{22}O_{11} + H_2O = 2C_6H_{12}O_6$; $C_6H_{10}O_5 + H_2O = C_6H_{12}O_6$.

the first instance it is passed on to the voluntary muscles, where in the presence of insulin, derived from the pancreas, it is completely burned up into CO_2 and H_2O, producing 4 calories per gramme, thus supplying the body with muscular energy. Secondly, it helps the ingested or stored-up fat to be completely burned up to CO_2 and H_2O. As Naunyn has well put it: Fats can only burn completely in a flame of carbohydrate. When such complete combustion of fat occurs, 9 calories per gramme are produced—thus supplying a very large amount of body heat. If, however, there is a sugar deficiency in the blood, fat combustion is incomplete: it stops short at the stage of acetone, and beta-oxybutyric and diacetic acids. These " ketone bodies " produce the symptom complex characteristic of endogenous acidosis, or " ketosis " (see p. 12).

Lactic Acid Acidosis.—Another kind of endogenous acidosis is that produced as the result of incomplete combustion of carbohydrate itself, with the formation of the intermediate product lactic acid. Normal blood contains a glycolytic ferment which breaks down some of the sugar to the stage of lactic acid, so that in normal children the blood contains 10-30 mgm. of lactic acid per 100 c.c. When a muscle contracts lactic acid is formed out of the glucose. During recovery from contraction some of the lactic acid gets completely oxidised to CO_2 and H_2O, and the rest is reconverted into glucose or fat. This is done at once in the presence of a normal supply of oxygen, but if there is insufficient aeration as the result of pulmonary disease, cardiac decompensation, or deficiency of hæmoglobin, or of oxidation in cases of prolonged and severe muscular exercise, an excessive amount of sugar is broken up incompletely and lactic acid accumulates in sufficient amounts to lower the alkali reserve of the blood and cause acidosis. The same condition may occur in

cases of very feeble circulation due to cardiac debility or to very low blood-pressure, or anhydræmia in infants when the circulation is necessarily very slow. In some of these cases the lactic-acid content of the blood has been found to be as high as 260 mgm. per cent., with the amount of combined CO_2—as estimated by titration with N/100 HCl—as low as 15 per cent., instead of the normal 50-60 per cent. (see p. 13).

It is owing to this glycolytic production of lactic acid, even in normal blood, that blood-sugar determinations, as well as estimations of alkali reserve, should be done on bloods as soon as possible after collection ; otherwise each of these estimations will be found to be low, because of the destruction of some of the sugar, and the fixation of the alkali by the lactic acid so formed. The addition of sodium fluoride will, however, delay glycolysis.

Maintenance of Sugar Equilibrium.—The sugar in the blood, under the action of insulin from the pancreas, is utilised by the muscles. When more sugar reaches the blood than the muscles can burn up, its excessive accumulation in the blood-stream is prevented by its diversion into the liver, where—after dehydration and polymerisation—it is stored in the form of insoluble glycogen $(C_6H_{10}O_5)n$, thus keeping the sugar level uniform at about 0·1 per cent. When there is more glucose than the liver can cope with, the percentage rises in the blood until it reaches a level of about 0·16-0·18 per cent., when the excess is excreted by the kidneys, with the production of a temporary glycosuria (alimentary glycosuria). One therefore speaks of 0·16-0·18 per cent. as the " renal threshold " for blood-sugar. The threshold value may, however, vary under different pathological conditions. The kidneys, for instance, may be particularly permeable to sugar, when glycosuria will appear when the blood-sugar level is as low as 0·8 per

cent. or even less. Such a low sugar threshold giving rise to glycosuria is therefore called renal glycosuria, and needs no treatment; indeed restriction of carbohydrate or administration of insulin in such cases is positively harmful because of the possibility of causing dangerous hypoglycæmia. In cases of chronic diabetes, on the other hand, the threshold value may be high, so that glycosuria may be absent even when the blood-sugar level is considerably above 0·2 per cent. It is obvious, therefore, that neither does the presence of glycosuria necessarily denote diabetes, nor does its absence definitely exclude it; it is only a proper blood analysis which makes the diagnosis certain. The presence of a glycolytic enzyme in the blood also helps to break up some of the glucose with the production of lactic acid (see p. 21).

Hyperglycæmia and hypoglycæmia are the states in which, as the result of a breakdown in the adjustment mechanism, there is an excess or diminution respectively in the percentage content of sugar in the blood. *Hyperglycæmia* (when the fasting blood, i.e., that collected in the morning before breakfast, or not less than four hours after a meal, contains more than 0·12 per cent. of sugar), and *hypoglycæmia* (when the blood-sugar level in the same post-absorptive state is less than 0·08 per cent.), may thus occur as follows :—

Hyperglycæmia	Hypoglycæmia
Hyposecretion of insulin, with consequent slow combustion of sugar, as in true diabetes.	Excess of glycopyretic substances in the blood, e.g., overdose of insulin, or synthalin (a preparation of guanidin), or possibly the endogenous proteolytic formation of histamin (a ptomaine) out of the amino-acid histidin.
	Hypersecretion of insulin occurs in cases of tumours of the islets of Langerhans.

Hyperglycæmia	Hypoglycæmia
Excessive ingestion of carbohydrate (= alimentary hyperglycæmia)—a purely temporary phenomenon.	Defective absorption of sugar, e.g., in starvation, marasmus, etc., or when food passes too rapidly through bowel, in diarrhœa, or excessive purgation by aperients.
Hyperfunction of adrenal, as in strong emotion, when the excess of adrenalin mobilises the glycogen from the liver and pours it into the blood in the form of glucose ; hypersecretion of posterior pituitary lobe—in cases of tumour ; hyperthyroidism.	Hypofunction of adrenals (? in Addison's disease), of posterior pituitary lobe (Fröhlich's disease), of thyroid (cretinism, etc.).
Defective amount of glycolytic enzyme in the blood, said by some to be the case in diabetes, but denied by others.	Excess of glycolytic enzyme in the blood, said by some to be the case in leukæmia, but denied by others.
Infection, especially in those hereditarily predisposed to diabetes.	In certain cases of asthma.
Lack of muscular exercise (= defective combustion of sugar).	Prolonged and severe muscular exercise, e.g., in Marathon runners (= excessive combustion of sugar).
High renal threshold, = high blood-sugar without glycosuria.	Low renal threshold, e.g., in renal glycosuria = glycosuria with low blood-sugar.

It is to be noted that unless the sugar determination is made on freshly withdrawn blood, there may be an apparent hypoglycæmia due to glycolysis (see p. 22).

Symptoms of Hyperglycæmia.—When the hyperglycæmia is considerable, as in true diabetes, the osmotic pressure of the blood is raised, water passes from the tissues into the blood by osmosis, and the patient feels thirsty in order to replenish his de-

hydrated tissues. The excessive drinking produces polyuria, and the inability to utilise the ingested carbohydrate produces wasting. In addition, the incomplete combustion of fat (in the absence of burning sugar) may result in ketosis (see p. 21).

Symptoms of Hypoglycæmia.—These are apprehension, tremors, sweating, difficulty of articulation, convulsions and coma. Their onset does not, however, depend upon the degree of the hypoglycæmia ; in some patients they may occur with a blood-sugar of 0·08 per cent., while in others they fail to show themselves, even when the sugar level is no more than 0·04 per cent.

Treatment.—The treatment of *hyper*glycæmia when due to diabetes, is dietetic, with or without the administration of insulin, or synthalin.[1] For details the reader must refer to the ordinary textbooks. The treatment of *hypo*glycæmia is most important, as the patient's life depends upon the emergency treatment applied. In the early stages it may be sufficient for the patient to swallow a few lumps of cane sugar. In severe cases, however, e.g., coma or convulsions, glucose at the rate of about 1 grm. per kilo body-weight, should be given intravenously or per rectum, in 10 per cent. solution, together with 10-15 minims of 1/1,000 adrenalin intramuscularly, or hypodermically. *It is important not to mistake the coma of hypoglycæmia with that of hyperglycæmia and ketosis*, as the treatment of the one is definitely contra-indicated in the other. *Diabetic coma* must be treated by large doses of insulin about ½ unit per kilo combined with glucose, as well as the giving of alkalies. A quick diagnosis is made in an emergency by the smell of acetone in the breath, the presence of ketone bodies in the urine (see p. 13), and possibly the presence of sugar in the urine, in cases

[1] Synthalin reduces the amount of sugar in the blood but is said to cause hepatic degeneration.

of ketosis, and by the absence of glycosuria, and especially of ketone bodies in cases of hypoglycæmia.

Glucose Tolerance Test.—This aims at assessing the capacity of the individual to keep the sugar balance in a state of equilibrium. After a sample of blood has been taken, the child is given 1½ grm. of glucose per kilo body-weight (dissolved in 3 c.c. of water flavoured with lemon juice), and specimens of blood are taken every half-hour for the next two to two and a half hours. In the adult a dose of glucose half that size is enough. The percentage of sugar in each sample is plotted as the ordinate with time as abscissa. The nature of the curve provides the necessary information (see fig. 3):—

In *normal adults* the sugar level begins to rise immediately after the ingestion of the glucose, reaches a maximum of about 0·15-0·17 per cent. after one hour, and returns to normal within about two hours. *Normal infants* can tolerate glucose better than adults, so that the maximum level reached after an hour is less, viz., about 0·13-0·15 per cent.

In *diabetes* the sugar rises to a higher maximum (0·2-0·3 per cent. or more) and, what is more important, does not return to the fasting level within two to two and a half hours.

In *renal glycosuria* the fasting level is considerably below normal, and does not rise much after the glucose ingestion.

In *cœliac disease* the sugar tolerance is so good that the sugar level does not rise much—if at all—after ingestion of glucose.

Lævulose Tolerance Test for Hepatic Efficiency.—If lævulose be substituted for the glucose in the tolerance test, hyperglycæmia does not occur, because it is removed from the blood by the liver. Hence it is suggested that a rise of blood-sugar, after lævulose ingestion, is evidence of hepatic inefficiency. The test is still *sub judice*.

In most cases it is sufficient to determine the amount of sugar in the fasting blood, and two hours after glucose. In normal individuals the two figures are identical, and the line joining them is horizontal. In diabetics, the second figure is higher, and the joining line shows a rise.

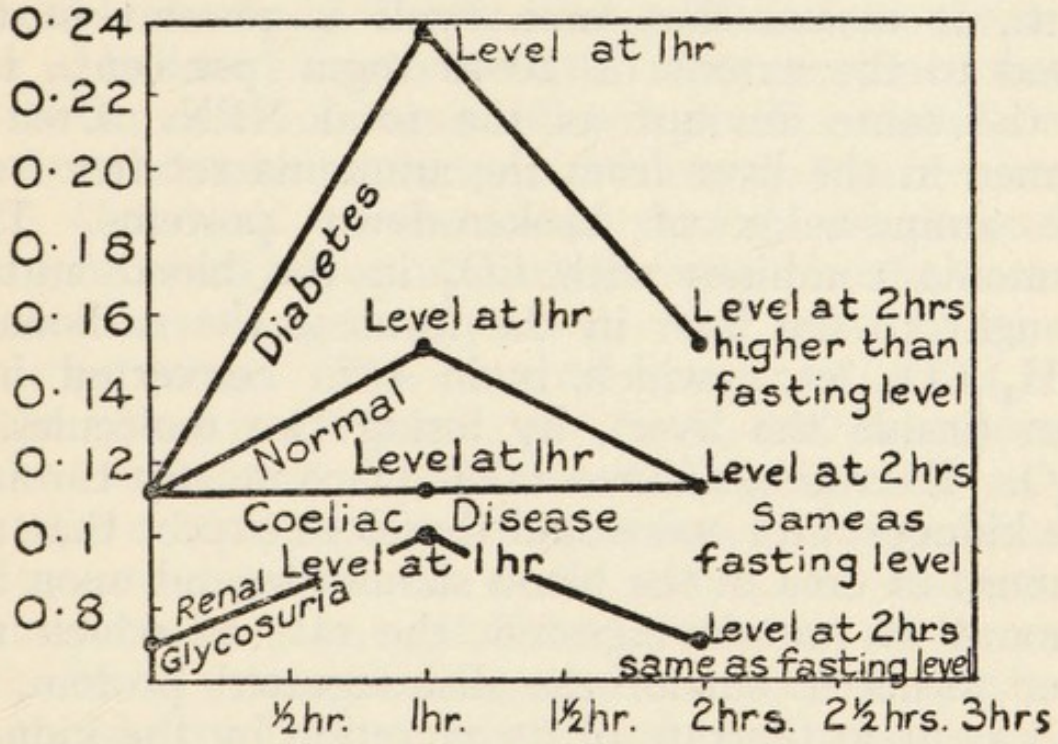

FIG. 3.—Blood sugar curves.

Non-protein nitrogen (NPN) consists of the nitrogen remaining in the blood after precipitation of the protein, and represents that present in urea, uric acid, creatinin, etc., formed as the result of the combustion of exogenous (i.e., ingested) and endogenous (i.e., tissue) protein. The total NPN in the blood ranges between about 20 and 40 mgm. per cent., of which about one half comes out of urea. These figures are practically identical for adults and children, although a figure higher than 30 mgm. per cent. in a child under 10 years is considered abnormal. Increased NPN indicates nitrogen retention due to renal inefficiency, and its prognostic significance is worse in chronic than in acute nephritis. Thus

100 mgm. per cent. is of serious omen in chronic, but not in acute, nephritis, and while 300 mgm. per cent. is almost invariably fatal in a chronic case, it is not so hopeless in an acute case.

(1) *Urea*, $CO(NH_2)_2$, as seen from its formula, contains about half its weight of nitrogen. As urea nitrogen is present to the extent of 10-20 mgm. per cent., it means that urea itself is present in the blood to the extent of 20-40 mgm. per cent., i.e., in the same amount as the total NPN. Urea is formed in the liver from the ammonia set free from the amino-acids of broken-down proteins. This ammonia combines with CO_2 in the blood and is brought to the liver in the form of the carbonate, $(NH_4)_2CO_3$, etc., which is in turn converted into urea (inside the liver), by losing two molecules of H_2O. Excretion of urea takes place mostly through the kidneys, and one would therefore expect that the amount of urea in the blood should depend upon the amount of protein ingested, the rate at which the liver forms it out of the disintegrated protein, as well as upon the rate of its excretion by the kidney. In practice, increase of blood-urea or of NPN means impaired renal excretion, with urea or NPN retention. It has, however, been suggested that by estimating the amount of blood-urea in the post-absorbtive state, and also four hours after eating 1 grm. of protein per kilo body-weight, the efficiency of the liver can be assessed. With a normally functioning liver the second sample of blood should contain about 60 per cent. more urea than the fasting specimen; in cases of liver injury the increase (as found experimentally in animals) is less. This *protein test for hepatic function* is still on its trial.

Addis' ratio, viz., $\frac{\text{Urea in 1 hour's urine,}}{\text{Urea in 100 c.c. of blood}}$, which in the normal adult $= \frac{1{\cdot}25 \text{ grm.}}{0{\cdot}025 \text{ grm.}} = 50$, furnishes a

better index of renal efficiency. When there is urea retention due to renal inefficiency and not to excessive intake of protein, the numerator is less and the denominator more, so that the ratio becomes less and less than 50 as the efficiency of the kidney diminishes.

Ambard's coefficient is a very complicated ratio in which the body-weight is also taken into account, and is believed to give a still more accurate index of the amount of renal efficiency. For details the reader must refer to the special books on biochemistry.

(2) *Uric acid* in the blood comes endogenously, from the breaking-up of nuclear matter in the tissues, as well as exogenously from the metabolism of nucleoproteins. The normal range of uric acid in the blood is 1-3 mgm. per cent., and is increased in leukæmia and febrile conditions, as well as in gout (in adults), owing to excessive disintegration of endogenous (nuclei of white cells), or exogenous nucleoproteins. In nephritis there is also an increase in the blood uric acid owing to diminished elimination. As the destruction of uric acid in animals seems to be effected by the liver, its accumulation in the blood in persons on high purin diets should serve as an index of hepatic function. It is questionable, however, if it does.

(3) *Creatinin* is derived entirely endogenously, mostly from muscle, and not from ingested protein. Its accumulation in the blood therefore affords a good indication of the excretory inefficiency of the kidneys. Its normal range is 1-2 mgm. per cent. An amount higher than 5 mgm. per cent. is of very grave prognostic significance in chronic, though less serious in acute nephritis.

Proteins—These consist of serum albumen, serum globulin, and fibrinogen. The total amount ranges between 7 and 8 per cent., of which fibrinogen constitutes about 0·3 per cent., and the globulin/albumen

ratio is about 1 : 2. While the total protein is of interest in cases of acidosis and dehydration, in each of which conditions refractometric determinations have shown a rise, the globulin/albumen ratio shows greater variations in physiological and pathological conditions. Thus in infancy the ratio is less, and in cases of infection it is increased. The information on the subject is, however, not sufficient to be utilised for diagnostic purposes.

Cholesterol, which is an unsaturated secondary alcohol, exists in the blood in two forms : free, and combined in the form of esters (i.e., salts of fatty acids). In the red cells it is free, but in the plasma it is present in both forms—especially the ester. The free form is constant in amount both in health and disease, but the combined form in the plasma, whose normal range is 150-180 mgm. per cent., varies under different pathological conditions. It is increased in renal but not in cardiac œdema (thus forming a useful diagnostic test), in *obstructive* jaundice (e.g., congenital obliteration of the bile-ducts, impaction of gall-stone, etc.), and in pregnancy. In lipæmia of diabetes it may rise to 800 mgm. per cent. Decreased cholesterol is found in epilepsy, febrile diseases and in wasting conditions. The normal amount also varies with age : 90 mgm. per cent. at birth and 136 mgm. per cent. at one year.

Mineral Constituents.—Those of importance are:—

Chlorides.—According to French observers there are two types of nephritis, viz., the *azotæmic*, marked by nitrogen retention, and the *hydropigenous*, characterised by œdema and chloride retention. On this theory, salt-free diets are indicated in nephritis œdema. The theory is, however, not universally accepted, and some believe that such œdema, though possibly in some way connected with chloride retention, is due to protein loss from the plasma as the

result of excessive albuminuria. The normal chloride content of the plasma is 500 mgm. per cent., and this helps to keep the blood's H-ion concentration at the normal level. A fall of blood-chlorides occurs in excessive vomiting (see " Alkalosis "), in diabetes and in pneumonia. The threshold value of blood-chloride, i.e., the concentration below which no chlorides appear in the urine, is about 530 mgm. per cent.

Calcium and Phosphorus.—Calcium is necessary for the growth of bone, coagulation of the blood, and for its sedative action on the nervous system—in which latter respect it has an antagonistic action to that of sodium and potassium ions. The normal K/Ca ratio is about 2:1. When the ratio rises the excitability of the nervous system is increased. This has been demonstrated both clinically and experimentally. Phosphorus is used by the body for the purpose of bone growth as well as of maintenance of pH equilibrium—the phosphates in the blood serving as buffer bodies. The normal Ca variation is 9-11 mgm. per cent., and that of P is 5-7 mgm. per cent. In rickets the Ca content may be normal, but the P content is low, viz., 1-2 mgm. per cent. After treatment with cod-liver oil, ultra-violet rays, or irradiated ergosterol, the P content rises, and the ricket heals, as shown by the X-rays, so that the progress of the disease can be gauged by the P content of the blood. In cases of spasmophilia, especially in well-marked tetany, there is hypocalcæmia—the Ca content falling as low as 5 mgm. per cent., although the P content is normal. It is to be noted, however, that spasmophilia occurs only when there is a deficiency of *ionised* Ca in the blood—even though the total Ca content may be normal, as in the case of alkalosis where the excess of alkali decreases the ionisation of the Ca. In *cœliac rickets*, owing to Ca loss in the stools in the form of insoluble Ca soaps,

the Ca level is low, and that of P is high. In certain bone diseases in adults there is a hypercalcæmia (e.g., osteitis fibrosa which is associated with non-palpable tumours of the parathyroids) with a Ca content of 15 mgm. per cent. or more. Removal of the affected parathyroid cures the disease.

Bicarbonates.—The amount of bicarbonates in the blood-plasma is a measure of the alkaline reserve of the blood—upon which depends the condition of acidosis or alkalosis (p. 11).

Bile Pigment.—The normal variation of bilirubin in the blood is 0·2-0·5 units, or 1-2·5 parts in a million —a unit being 1/200,000 bilirubin. The normal renal threshold value for bilirubin, i.e., the blood-level at which the pigment passes from the blood into the urine, is 4 units, or 1/50,000 (= 2 mgm. per cent.), but in hæmolytic jaundice—such as acholuric family jaundice, and the jaundice of pernicious anæmia—there is no bilirubin even when the blood contains 18 units of bilirubin. It is now known that bile is not formed by the true parenchymatous liver-cells, but by the reticulo-endothelial system of cells which line the capillaries of the bone-marrow, spleen, and other connective tissue. The Kupffer cells, i.e., the endothelial cells lining the vascular capillaries inside the liver, belong to that system. The bilirubin formed in those cells out of the hæmoglobin of the broken-down red blood-corpuscles is brought to the liver by the portal vein, where it is absorbed by the polygonal parenchymatous liver-cells, which in turn excrete it into the bile canaliculi. Now chemical tests have shown that the bilirubin in the portal capillaries is not identical with that in the bile canaliculi. Hence the polygonal liver-cells modify the bilirubin brought to them by the portal vein. The *van den Bergh test* enables one not only to estimate the number of bilirubin units, but also to discriminate between the two kinds of bilirubin : that which has,

and that which has not, passed through the parenchymatous liver-cells. The van den Bergh reagent is the same as the Ehrlich diazo reagent, and gives an immediate bluish violet colour when mixed with bilirubin from the bile canaliculi. This is the *immediate direct reaction.* If, however, it is mixed with bilirubin from the portal capillaries, i.e., before its passage through the true liver-cells, no change occurs in the colour—unless the mixture is allowed to stand for a few minutes (the direct delayed reaction), or unless the bilirubin has previously been treated with alcohol, when an immediate change in colour takes place. This constitutes the *indirect reaction,* i.e., the immediate change in colour with the diazo reagent after addition of alcohol. It is believed that the direct reaction given by bilirubin which has passed through the true liver-cells is due to the fact that it is free bilirubin, but that the bilirubin in the portal capillaries is combined with a plasma protein radicle, and therefore does not give a reaction until it has been liberated from such combination either by standing for some time, or by means of alcohol. Hence it is believed that bilirubin formed by the reticulo-endothelial cells is combined with protein, and the action of the true liver-cells is to liberate the bilirubin from this combination.

Although the threshold value for bilirubin between the blood and the kidneys is 4 units, that between the blood and the parenchymatous liver-cells is very low—about 0·3 units. Therefore, if the bilirubin content of the portal capillary blood is higher than the normally functioning liver-cells can absorb, or if the liver-cells are not functioning properly to deal with a normal supply of bilirubin, a portion of the pigment remains unabsorbed and finds its way into the general circulation, giving rise to jaundice. Such jaundice is called "retention" or "hæmolytic" jaundice, and the serum examined by the van den

Bergh test gives the delayed or indirect reaction characteristic of combined or unmodified bilirubin. This type of jaundice occurs in icterus neonatorum, acholuric family jaundice and, in adults, in pernicious anæmia, in all of which there is excessive destruction of red corpuscles. It also occurs in functional derangement of the parenchymatous liver-cells, in cases of catarrhal jaundice. If the bilirubin succeeds in being absorbed by the parenchymatous liver-cells and being passed on to the bile canaliculi, but as the result of some obstruction in the bile-ducts outside the liver, fails to reach the duodenum, there is a regurgitation of the bile from the overfilled canaliculi into the general circulation, with the production of jaundice. Such jaundice is called "regurgitation" or "obstructive" jaundice, and the serum gives the immediate direct van den Bergh reaction characteristic of free or modified bilirubin. The jaundice, in cases of congenital obliteration of the bile-ducts in infants, or stone in the common bile-duct, etc. in adults, belongs to this type. In the writer's experience, the van den Bergh test has not proved so decisive, as a case of congenital obliteration of the bile-ducts gave an indirect reaction, and one of non-obstructive jaundice gave a direct reaction. It is possible that the indirect reaction in the obstructive case may have been due to the fact that the serum on which the test was carried out may have been allowed to stand a little too long before examination, so that the bilirubin may have been freed from its combination, but such an explanation will not of course account for the direct reaction in the non-obstructive case. Nevertheless, in spite of occasional inexplicable anomalous results, the reaction is, in general, of very great diagnostic value.

A third type of reaction—the *biphasic*—in which a deep red colour occurs at once, followed some time later by a change to violet (i.e., immediate and

delayed reactions), indicates the presence of both types of bilirubin—which is the case when both regurgitation and retention exist together, e.g., in late cases of catarrhal jaundice, in cirrhosis of the liver, etc.

It is to be noted that the test being quantitative (by comparing the colour with a standard solution of bilirubin), can be utilised to watch the progress of a case of jaundice.

The *icterus index* is another measure of the amount of bilirubinæmia. The depth of colour of 1/10,000 solution of $K_2Cr_2O_7$ is taken as the unit, and a serum whose colour corresponds with this has an index of 1. A colour corresponding to a concentration twice as strong has an index of 2, and so on. This has the advantage over the van den Bergh method in that the standard solution used in the latter does not keep its colour constant. Its disadvantage, however, is that it does not differentiate between the two kinds of bilirubin, and therefore also between the different types of jaundice. The normal index is about 5, but clinical icterus does not show itself with an index less than 15. The method may therefore be used to determine the pre-icteric stage in which there is merely hyperbilirubinæmia without jaundice, in the same way as the estimation of sugar in the blood can detect hyperglycæmia in the absence of glycosuria.

Antibodies.—Normal serum also contains various antibodies, such as agglutinins, lysins, opsonins, precipitins, etc., upon which depend the agglutination tests for typhoid (Widal's test), the complement-fixation test for syphilis (Wassermann's reaction), the precipitin tests for distinguishing human from other blood, etc. The opsonic index, which was very popular when first introduced by Almroth Wright, is not used much at the present day (see p. 48). The presence of these bodies or of immunising substances

in the maternal blood and milk provides the fœtus with a post-natal immunity against certain infectious diseases from which the mother suffered in her childhood, lasting a few weeks or months, and the breast-fed baby for another term of immunity.

For the use of immunised serum for the prophylaxis and treatment of disease see p. 73, and for the determination of a child's susceptibility to disease from an estimation of the amount of antibodies in its serum see Schick and Dick tests, p. 74.

The Water Content of the Blood.—This varies with the amount of water taken into the body and that eliminated from it. When the balance is positive, i.e., on the side of intake, the blood becomes more watery (**hydræmia**), with increase in total volume (as estimated by means of vital red, or by the refractive index), relative diminution in the cell-count protein and other plasma constituents, etc. This condition occurs temporarily after transfusion of intravenous saline; in cases of cardiac and renal disease in which there is a diminished secretion of urine, or diminished excretion of NaCl; or in diabetes when there is water retention due to high sugar concentration. When the balance is negative, i.e., when more water is eliminated than is taken in, there results a water deficit in the blood (**anhydræmia**), with diminution in total volume, and relative increase of the various plasma metabolites, as well as of the corpuscles, giving a relatively high cell-count. This condition occurs chiefly in severe summer diarrhœa, but may also occur after prolonged and profuse sweating, unless sufficient water is given to counterbalance the loss.

The symptoms of anhydræmia are: (1) Marked and rapid loss of weight—as much as half a pound or more a day in a small infant—due mainly to the loss of water. This is accompanied by dryness and loss of elasticity of the skin, pinched appearance of

the face, depression of the fontanelle, small volume of pulse, etc. (2) Increased concentration of all the plasma constituents, as pointed out above, resulting in a relatively high-cell count, and in a high NPN level. (3) Increased viscosity, with consequent diminished blood-flow through the various organs including the kidneys, resulting in suppression of urine with uræmia—which is one of the causes of death in the acute gastro-enteritis of infancy. (4) Owing to the diminished urinary excretion, there is a retention of chlorides with the production of acidosis—a condition aided by the accompanying starvation with consequent incomplete combustion of body fat (see " Ketosis "). (5) Some degree of fever—**dehydration fever**—due either to infection or to disturbance of the heat-regulating mechanism, the consequence of desiccation of the body tissues.

The treatment of anhydræmia logically consists in checking the water loss, i.e., the diarrhœa, by means of opium in doses appropriate to the age of the child, supplying fluid, by the mouth—if there is no vomiting—or subcutaneously, intraperitoneally, or intravenously. The amount to be given is a pint, repeated if necessary in five or six hours. The fluid is to be given in the form of 10-15 per cent. solution of glucose. The glucose acts as a food as well as a diuretic, and counterbalances the ketosis, if any. In addition, if acidosis is very marked, the amount of alkali reserve must be made up by means of intravenous or intraperitoneal $NaHCO_3$ at the rate of 0·5 grm. per kilo body-weight—which is equivalent to about one-quarter the normal bicarbonate content of the plasma. If the diarrhœa has ceased, it is dangerous to give more alkali, for fear of inducing alkalosis.

CHAPTER III.

The Corpuscles.

The numbers and characters of the red and white cells in adults and children and their variations under pathological conditions—Price-Jones curve, volume index, halometry, colour index, saturation index, fragility of red cells, rate of sedimentation—Types of leucocytes—Arneth count—Opsonic index.

THE corpuscles differ in number and characters in early life from what is normal in the adult—a fact which is important to remember when assessing the significance of a cell-count. The total volume of the cells as found by centrifuging some blood in a small graduated tube (hæmatocrit), until they become closely packed at the bottom of the tube, is 43 per cent. of the volume of the whole blood in the adult, and 45-65 per cent. with an average of 54 per cent. in infants under one month old. At birth, also, the number of erythrocytes is high: 6,000,000 or more per c.mm.—the accepted figure in adults being 5,000,000—and some of them are nucleated and remain so for a few days. Their number is higher in premature infants. During infancy and early childhood the number of red cells is normally somewhat low, so that it is not safe to diagnose anæmia in a young child on the sole basis of a somewhat low blood-count. The number of white cells is high during the first three years (16,000 at birth, and 11,000 at 3 years—the normal for the adult being 7,000 per c.mm.), so that one must beware of diagnosing leucocytosis in such a young child when the number of leucocytes is no more than

12,000 per c.mm. The excess of white cells is mostly due to the large number of lymphocytes. A white count of more than 15,000 usually indicates infection when the excess is on the side of the polymorphs. In the leukæmias there is, of course, a very high lymphocytosis in the case of lymphatic leukæmia, and a myelocytosis in the case of the myelogenous type ; the latter is, however, exceedingly rare in early life. Leucopenia exists in certain stages of typhoid, measles, etc., while in eczema, intestinal parasites, etc., there is eosinophilia. In congenital heart disease with cyanosis, there is persistent polycythæmia (6,000,000 or more), and in premature babies there is considerable anæmia.

The physical characters of the corpuscles that are of interest to the clinician and pædiatrician are : size, shape, hæmoglobin content, fragility, and rate of sedimentation in the case of the red cells, and the character of the nucleus in the case of the polymorphonuclear variety of the white cells. The affinity for acid and basic dyes is a *chemical character* which is of great importance in the case of both red and white cells. Each of these characters varies under different pathological conditions, but the standard of normality also varies with age.

Red Cells : *Size.*—The average diameter of a red cell is 7·3 μ in the adult. In the newborn it is much larger—8·5 μ. Hence the average volume of an individual cell, as well as the total volume of all the red cells, as given by the hæmatocrit, is greater in the young infant than in the adult (see Chap. I, p. 3). In acholuric jaundice, owing to the excess of microcytes (40-50 per cent. instead of the normal $\frac{1}{2}$-2 per cent.), the average size of a corpuscle is less than normal, and so also is the total volume of the corpuscles. In the case of pernicious anæmia, on the other hand, owing to the excess of macrocytes and megalocytes, the average size as well as the total

volume is greater than normal. This state of microcytosis or macrocytosis can be detected by actual measurement, which entails the tedious labour of measuring some 1,000 cells, and either taking the average, or plotting a Price-Jones curve—which is a frequency curve in which the cell diameters are the abscissæ and their respective percentage frequencies are the ordinates. In normal persons the resulting curve corresponds in shape to a normal

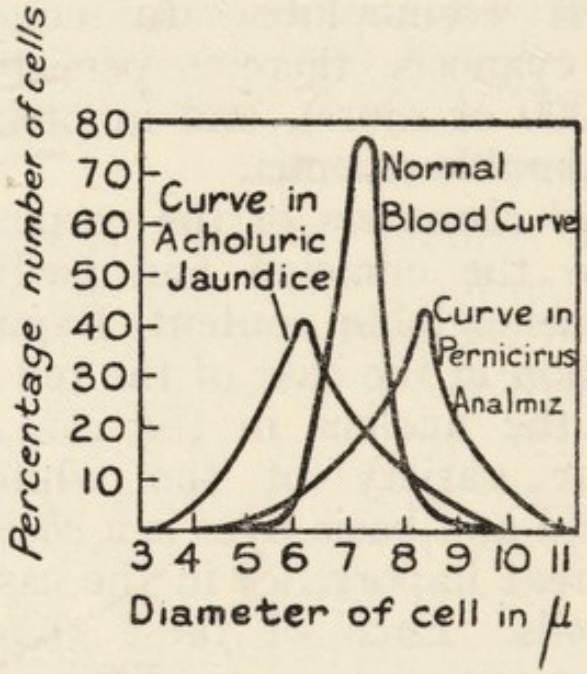

FIG. 4.—Price-Jones curves in various blood conditions. Abscissa gives the diameter of a cell in μ, and the ordinate at any point gives the percentage number of cells of that particular diameter.

frequency curve which is symmetrical because the frequency of any cell below the average size is equal to that of a cell correspondingly above the average. When there is a preponderance of macrocytes (newborn, and pernicious anæmia), the average size is larger and the curve is asymmetrical—its peak being shifted to the right. In acholuric jaundice, there is a microcytosis with a shift of the Price-Jones curve to the left (fig. 4).

A much simpler way of finding the size of an

average cell is to find the total volume of the cells from the hæmatocrit reading, and divide this by the total number of cells as found by the hæmocytometer. When both are normal, this ratio $\frac{\text{total volume}}{\text{total count}}$ which is called the *volume index* (V.I.), i.e., the average volume of a cell, is 1. If the increase in total volume is greater than the increase in the number of cells, V.I. is greater than 1, i.e., the average size of the cell is greater than normal (e.g., in the newborn and in pernicious anæmia). In acholuric jaundice the total volume is diminished to a greater extent than the cell-count, making V.I. less than 1, and the average size of a cell less than normal. Pernicious anæmia does not exist in children, but a similar blood-picture is found in cases of helminthiasis (dibothryocephalus latus). In these conditions the hæmatocrit reading is increased and the cell-count is diminished, showing that the average cell is larger than normal. Thus the average hæmatocrit reading in a number of newborn infants was found to be 54 per cent. (= 126 per cent. of the adult reading, 43), and the average cell-count was 3,960,000 (= 79 per cent. of the adult 5,000,000). Therefore, V.I. = 126/79 = 1·59, which means that the average volume of the infant's red cell is 1·59 times that of the adult's. As volume varies with the cube of the diameter, therefore the diameter of the infant's cell is $\sqrt[3]{1{\cdot}59}$ = 1·17 times the diameter of an adult's cell = 1·17 × 7·3 = 8·5 μ—which agrees with that found by actual measurement (see p. 39).

A much more expeditious and extremely neat way of finding the average size of the corpuscles is the *diffraction method*, which depends upon the fact that when a beam of light passes through a blood-film, it is broken up by the cells into a number of circular colour halos, from which the mean diameter of the cells can be determined in a moment by means

of a special apparatus called a *halometer*. The larger the halos the smaller the average size of the cells.

In the writer's opinion it is almost possible to diagnose pernicious anæmia and acholuric family jaundice by means of the halometer alone. A halometer reading of less than 6·4 μ is very suspicious of acholuric jaundice, and one greater than 8·4 is diagnostic of pernicious anæmia. With readings less than these extremes (e.g., 6·8 μ or 7·8 μ) other tests have to be applied.

Shape.—The normal red cell is round, but under pathological conditions it may become pear-shaped (poikilocytosis), as seen in pernicious and other forms of anæmia, or sickle-shaped (sicklæmia, or meniscocytosis), a condition first described in negroes but recently found in the white races, and believed to be genetically transmissible in accordance with the Mendelian laws of inheritance. The condition may be symptomless, or may be accompanied by weakness, malaise, and other evidence of severe anæmia.

Hæmoglobin Content.—As hæmoglobin carries oxygen to, and CO_2 away from, the tissues, and thus regulates tissue nutrition as well as the pH of the blood, its concentration in the blood is of very great importance. Normally, the O_2 capacity of the blood, i.e., the amount of O_2 that can be carried by all the red cells when fully saturated with Hb., is 200 c.c. of oxygen per litre. This corresponds to 138 grm. of Hb. per litre, and *accurately standardised hæmoglobinometers* should register 100 per cent. with blood of that concentration. As a litre of blood contains normally 5×10^{12} red cells, such concentration means $200/5 \times 10^{12} = 4 \times 10^{-11}$ c.c. of oxygen per cell. Now, each corpuscle will be fully saturated with Hb. either when the amount of Hb. and the number of corpuscles are each 100 per cent. normal, or when each is reduced

to the same extent. In each case the *colour index* (C.I.), i.e., $\frac{\text{Hb. (per cent. of normal),}}{\text{Red cell-count (per cent. of normal),}}$ will be 1. On the other hand, if the cell-count is reduced by less than the Hb. reduction, e.g., when cell-count is 4,000,000 (= 80 per cent. normal), and Hb. is only 60 per cent. normal, then C.I. = 60/80 = 0·75, i.e., less than 1 (fig. 5).

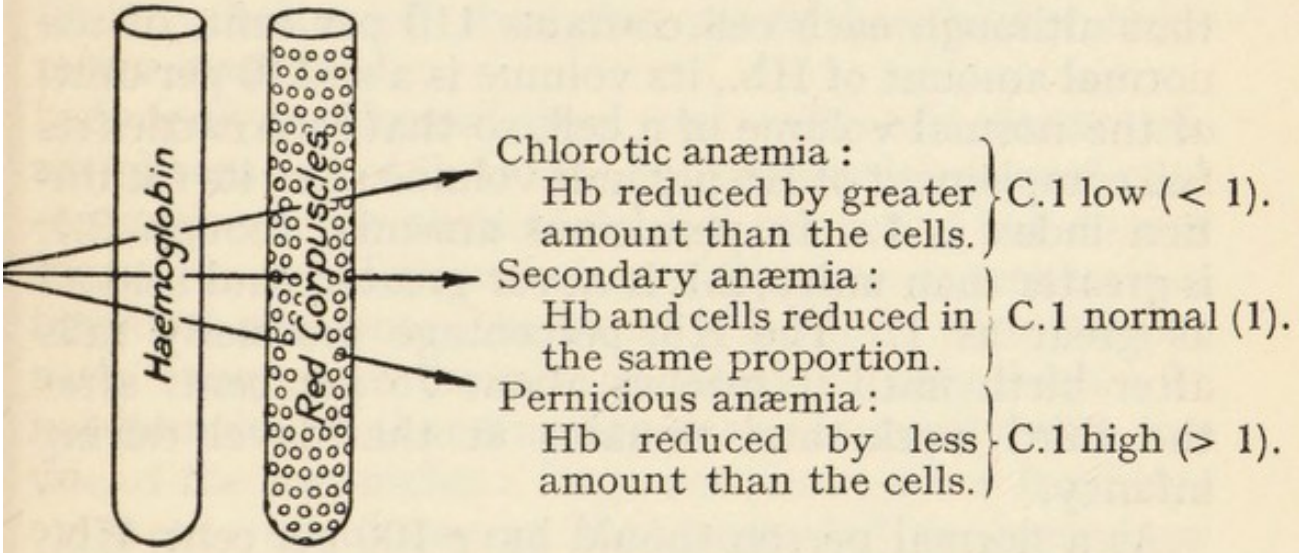

FIG. 5.—Relation between Hb, red cell count and colour index in different types of anæmia.

It would at first seem that as the Hb. concentration of a cell cannot exceed 100 per cent., no blood can have a C.I. greater than 1, and yet we know that this is actually the case in the newborn (in whom it may be 140 per cent.), and in pernicious anæmia. The explanation of this apparent paradox is that in each of these cases the Hb. concentration of the cell is really not more than 100 per cent., but it contains more than the normal amount of Hb. because its average volume is bigger than normal—as shown by the halometer and hæmatocrit readings as well as by the shifting of the Price-Jones curve to the right. The actual concentration of Hb in the cell i.e., the amount of Hb. per unit volume of cell, is called the *saturation index* (S.I.), and is measured

by the amount of Hb. per cell, as given by the C.I., divided by the average volume of a cell as given by the V.I.; i.e., S.I. = C.I./V.I. This is never greater than 1. Thus in a newborn baby the red cell-count was found to be 5,500,000 (= 110 per cent. normal) and the hæmatocrit reading was 52 (= 121 per cent. the adult's normal of 43), making V.I. = 121/110 = 1·1. The Hb. was 120 per cent., making C.I. = 120/110 = 1·1, i.e., the same as the V.I., showing that although each cell contains 110 per cent. of the normal amount of Hb., its volume is also 110 per cent. of the normal volume of a cell, so that each carries its full complement of Hb per unit volume; i.e., its saturation index is 1. In pernicious anæmia, though C.I. is greater than unity, S.I. is never greater, and seldom as great as 1. The Hb. percentage gradually falls after birth until it reaches about 75 per cent. after the third week, and remains at that level during infancy.

As a normal person should have 100 per cent. Hb., and 100 per cent. normal cell-count, it follows that a normal C.I. should be 1. Actually the average C.I. is no more than about 0·8, an anomaly which is accounted for by the fact that most of the hæmoglobinometers in use are calibrated on too high a standard. Thus, what is actually 100 per cent. Hb. viz., 138 grm. Hb. per litre, though marked accurately on Haldane's apparatus as 100 per cent., is given on other apparatus as anything between 80 and 90 per cent., so that a normal sample of blood would, on these apparatus, give a C.I. of 0·8 to 0·9.

An interesting point is the *relation between the volume of a cell and its surface area.* The volume of a spherical cell of diameter 1 mm. is 0·52 c.mm., and its surface area is 3·14 sq. cm., so that it has 6 units of area per unit volume. But a cell of 2 mm. diameter has a volume of 4·16 c.mm. and a surface area of 12·56 sq. cm., so that it has 3 units of area per unit

of volume.[1] In other words, the small cell has a larger surface area per unit volume than has the large one. As substances enter and leave the cell through the surface, it follows that the functional activities of a red corpuscle per unit weight of contained Hb. are better in the case of a small than in the case of a large corpuscle. Hence, if two persons have the same hæmoglobinometer and hæmatocrit readings, the one who has a lower halometer reading is functionally better off than the one with a higher halometer reading.

Fragility.—Normal-sized red cells are destroyed, with liberation of their Hb., by hypotonic saline of concentration lower than about 0·4 per cent., but do not hæmolyse in solutions of much higher concentration than 0·4 per cent. As blood is isotonic with 0·9 per cent. saline, the margin of safety is very high. It has been found that fragility varies inversely with the size of the corpuscles : large ones have a low fragility, or great resistance, so that they fail to hæmolyse even with saline of lower concentration than 0·4 per cent., while small cells have a higher fragility, or lower resistance, so that saline stronger than 0·4 per cent. succeeds in hæmolysing them. Hence, in any condition of macrocytosis, e.g., at birth, and in pernicious anæmia, the fragility is diminished, while in microcytosis, e.g., acholuric family jaundice, fragility is increased, hæmolysis taking place even with 0·6-0·8 per cent. saline. Hence it is clear that the theory of icterus neonatorum being due to increased vulnerability of the red cells is untenable—because the red cells of the newborn are, owing to their large size, actually less fragile than normally.

It is possible that the increased fragility of microcytes is due to their senility—young cells being

[1] The volume of a sphere of radius r is $\frac{4}{3} \pi r^3$ and its surface is $4 \pi r^2$.

large and resistant, and old cells being small and fragile. The fragility test is of particular importance in the diagnosis of acholuric family jaundice in children, this being the only condition in which hæmolysis occurs even with 0·6-0·8 per cent. saline.

The *rate of sedimentation* of red cells in citrated blood (i.e., non-coagulable blood), collected and allowed to stand in a narrow vertical tube, is another interesting character which has been utilised in clinical medicine. The normal rate in adults is 3 mm. per hour in men and 7 mm. in women—in whom it is increased still more during menstruation and pregnancy. It is increased in children, being 7 mm. at about two years, and 6 mm. at about six years. The rate is proportional to the amount of fibrinogen, and to a less extent to that of globulin in the blood. It is claimed that in certain cases of infection, such as tuberculosis and rheumatism in the active stage, the sedimentation rate (S.R.) is increased so that the test can be utilised to ascertain, for instance, whether a child with a bruit of rheumatic origin may or may not get up. The value of the test, however, is disputed.

For the significance of abnormal red cells, such as normoblasts, reticulocytes, etc., see next chapter.

White Cells.—These cells, whose number in the blood has already been mentioned on p. 38, may be divided according to the presence or absence of granules in the protoplasm, the affinity of the granules to dyes, and the characters of their nuclei into :—

(*a*) GRANULAR. (1) *Neutrophile.*—The granules staining with neutral dyes. Owing to the lobulated shape of their nuclei, they are also called polymorphonuclear leucocytes. They are phagocytic, and are the most numerous in the normal blood, viz., about 65 per cent. of total leucocytes in the adult.

(2) *Eosinophile.*—The granules stain red with acid

dyes. They constitute 2-4 per cent. of the total whites.

(3) *Basophile*, or mast cells, the granules staining purple with basic dyes (methylene blue). They form about 0·5 per cent. of the total whites.

(*b*) NON-GRANULAR (1) *Lymphocytes*.—Small (25 per cent.) and large (about 2 per cent.). Their nuclei stain deeply with basic dyes.

(2) *Transitional*, or *Endotheliocytes*.—The largest cells in the blood.

The Arneth Count.—Apart from the well-known value of a differential count of the different kinds of whites, in various pathological conditions, Arneth suggested that a differential count of the different kinds of the polymorphonuclear neutrophile cells is of value for estimating the resistance of an individual to infection. The mature multinucleated cells which prevail in the blood are derived from large non-granular mononuclear cells, and hence in a state of imminent or actual infection there is an accelerated production of these phagocytic, and therefore protective, cells with the result that cells in an immature state are thrown into the blood-stream—in the same way as immature macrocytes are thrown into the circulation when there is excessive destruction of red cells. Normally, the numbers of different polymorphs, in respect of the number of lobes in their nuclei, are in round numbers, as follows :—

Class I.	One-lobed nucleus (most immature) ..	10%
Class II.	Two-lobed nucleus (maturer than I but still immature)	25%
Class III.	Three-lobed nucleus (normal maturity) ..	45%
Class IV.	Four-lobed nucleus (senile stage) ..	15%
Class V.	Five-lobed nucleus (decrepit and disintegrating)	5%

Plotting the class of cell as an abscissa and its frequency as ordinate, a frequency curve of these various polymorphs—an Arneth curve—is obtained (fig. 6). In cases of infection, even before a leuco-

cytosis shows itself, there is said to be a considerable increase in the percentages of Classes I and II, which have been hastily thrown into the circulation for defensive purposes, so that the curve shows a shift to the left. There is a similar shift to the left in normal early infancy (i.e., during the first week of life).

The Opsonic Index.—The function of the polymorphs is phagocytic, i.e., they ingest and destroy any invading microbes. Phagocytosis, however, can

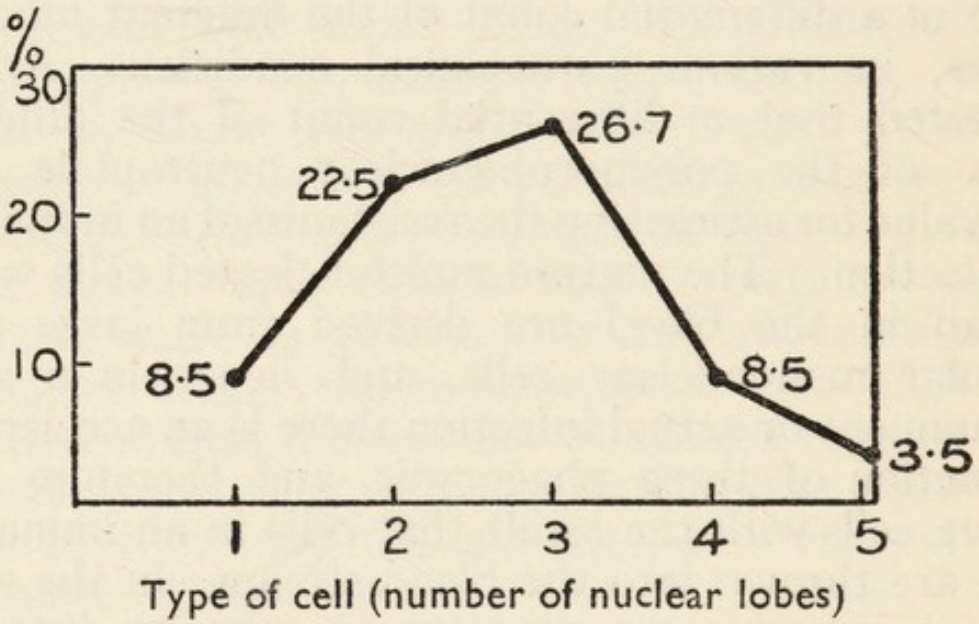

FIG. 6.—Normal Arneth curve.

only occur if the microbes have previously been acted upon by an antibody in the serum called opsonin. The greater the amount of opsonin in a serum the more the number of microbes bathed by it that can be destroyed by an average leucocyte. If a certain volume of a suspension of a culture of any particular microbe be mixed with equal volumes of the patient's serum and emulsion of human leucocytes washed free from plasma, in a capillary tube, and after incubation at 37° C. for 15 minutes be made into films and examined microscopically, the average number of microbes ingested by a leucocyte can be

counted. If this is compared with the number ingested by the same leucocytes when the same bacteria were bathed with serum from a normal person, one obtains the *opsonic index* of the patient's serum for that particular organism. If the index is much below the normal, which is 1 (say when it is 0·5 or 0·6), then it is inferred that his resistance is low. This test, which was very popular when it was first introduced by Almroth Wright, has now been given up, because of the amount of time and labour occupied in carrying it out, and the great technical skill required to obtain accurate results. It is interesting to note, however, that the phagocytic power of the two-lobed leucocyte has been found to be the highest, and that of the one-lobed and five-lobed leucocytes lowest—affording evidence of the relative ages of these cells.

For the *staining reactions of the red cells* see next chapter, pp. 51 and 52.

CHAPTER IV.

Maintenance of Corpuscular Equilibrium.

Number of corpuscles destroyed daily—Average life of a corpuscle, and of platelet—Balance between destruction and regeneration of cells—Balance tests—Origin of different blood-cells and platelets.

BLOOD-CELLS are not immortal: they have a limited span of life, on reaching which they die and disintegrate in the reticulo-endothelial system—chiefly the spleen—liberating their hæmoglobin out of which the iron-free bile-pigments are formed, the iron being stored in the liver for future use. As 1 grm. of bilirubin comes from 25 grm. of hæmoglobin, the daily amount of Hb., and therefore also the daily number of red cells destroyed, can be estimated from the amount of bilirubin discharged with the bile through a biliary fistula. The latter has been found to be 2 grm., corresponding to 50 grm. Hb. As the total Hb. in all the corpuscles is about 700 grm., we see that *the total number of corpuscles that perish daily is* 7 *per cent.* (= 50/700), which makes the *average life of a red cell* 14 *days* (= 100/7). More accurate determinations have been made by noting how many days after a transfusion the corpuscles in specimens of blood from the recipient will fail to be agglutinated by the serum of a person belonging to a group incompatible with that of the recipient. The moment this happens marks the time of disappearance of all the donor's red cells (see pp. 81 & 82). Such estimations make the average life of a donor's erythrocytes 4 weeks, or 28 days, so that their death-rate is 100/28

$= 3\frac{1}{2}$ per cent. Hence the *red cells have an average death-rate of* $3\frac{1}{2}$-7 *per cent. per day, and an average life of two to four weeks.* The number of leucocytes or platelets destroyed daily cannot be easily ascertained (see however p. 53), but it is believed that the *average life of a platelet is four days.*

Normally, the number of new cells and platelets minted by the reticulo-endothelial system (different parts of the red bone-marrow in the case of the reds, polymorphonuclears of the whites, and the platelets respectively, and the spleen and lymphatic gland in the case of the lymphocytes), and daily thrown into circulation, are just enough to make up for those that died. When the balance between their death- and birth-rates is disturbed, different blood-diseases with different blood-pictures result, according as to which process is relatively in excess : destruction or regeneration (see Chap. VI.). To be able to form an opinion regarding the relative rates at which these processes are taking place at any moment, it is necessary to be familiar with the morphological characters of the red and white cells at different stages of their lives from their embryonic, through their mature to their senile stages—as the appearance of immature forms in the blood-stream is a sign of rapid regeneration, while that of senile cells is indicative of degeneration and rapid destruction.

The **red cells** are elaborated in the red bone-marrow from large nucleated cells called megaloblasts, which go through stages of evolution during which they diminish in size, lose their nuclei, as well as their excessive affinity for basic dyes. These stages are : normoblasts (= nucleated but normal in size) ; macrocytes (large size but non-nucleated) ; reticulocytes (normal-sized and non-nucleated but containing in their cytoplasm fine threads or dots as shown when fresh blood-smears are stained with brilliant cresyl blue). The megaloblasts, therefore, are the

most immature, and the reticulocytes are the least immature of the red cells. The reticulocytes, on entering the circulation, lose their threads and become normal erythrocytes. The affinity of the immature cells for both the acid and the basic portions of the ordinary blood-stains is called *polychromatophilia*, or *polychromasia*, and shows itself by the assumption of a grey colour which is a mixture of the red produced by the acid (eosin), and the blue due to the basic portion (methylene blue). This property is lost when the cell becomes mature; they then become acidophilic alone and only take up the red eosin stain —being unaffected by the basic part. Inside the blood-stream the cells, after a time, begin to show signs of senility and decay, and go through stages of involution during which they diminish in size (microcyte stage), crenation, fragmentation, poikilocytosis, and *achromasia* (loss of affinity for both the acid and basic parts of the stain). When they become very old they are broken up by the spleen and other parts of the reticulo-endothelial system with the formation of bilirubin out of the hæmoglobin. Normally, the immature cells cannot pass the endothelial lining of the capillaries separating the marrow from the blood-stream, but when the demand for cells is great, these immature forms overcome the barrier and reach the circulation to make up for those that perished. The greater the casualty rate among the corpuscles the greater the effort on the part of the red bone-marrow to fill the gap, and the greater, therefore, the degree of immaturity of the recruits that are sent to the front.

The **white cells** have different origins depending upon the kind to which they belong. The *polymorphs* are derived from large non-granular mononuclear cells, called myeloblasts, in the red bone-marrow, but in a part different from that concerned with the minting of red cells, so that there is no necessary

relationship between the appearance of immature reds and whites in the circulation. The myeloblasts pass through several stages in which they diminish in size, and their nuclei, instead of being unilobular become more irregular (two-lobed, three-lobed, four-lobed, and five-lobed). The three-lobed cell is probably the mature one, and the more multi-lobed ones are those past their prime. As they get older still they begin to disintegrate as shown by vacuolation of their cytoplasm, fragmentation, and dissolution with the formation of uric acid out of the nucleo-proteins of their nuclei.

The *lymphocytes* originate from the adenoid tissue in the spleen and lymphatic glands, and—when in great demand—possibly also from the red bone-marrow, though from a different part from those out of which the reds and polymorphs are derived. Their fate is unknown ; although they vanish under administration of benzol, radium, and deep X-rays, (compare the treatment of leukæmia), their end-products have not as yet been traced.

Cannula lymph from the thoracic duct of a dog gave an hourly flow of 25 c.c. of lymph with a lymphocyte content of 11,000 per c.mm. implying a daily output of $24 \times 25 \times 11{,}000{,}000 = 6{,}600{,}000{,}000$ lymphocytes. As the total number of lymphocytes in the circulation at any moment is about 2,000,000,000, it follows that these cells are renewed three times a day. (See Yoffey, *B.M.J.*, December 10, 1932, p. 1053.)

The *platelets* come from the red bone-marrow, but from a part other than those which mint the red and white cells.

When the demand upon these hæmatopoietic organs (red marrow, spleen and lymphatic glands), is great, they increase in extent, and if the demand is excessive or long-continued the bone marrow may finally become exhausted. As, however, the red marrow in adults

occupies the ends of long bones only, there is plenty of room for it to extend into the yellow marrow of the shafts when need demands, but in young children red marrow occupies the whole of the long bones, so that their reserve power is limited. Children, therefore, cannot stand hæmorrhage or blood-destruction as well as adults.

Assessment of the Economic Condition of the Blood.—Whenever there is a disturbance of corpuscular balance it is important from the points of view of diagnosis, prognosis, and treatment, to estimate as far as possible the relative rates of corpuscular destruction and regeneration. This can be moderately well done by applying the facts given in the foregoing paragraphs :—

The *rate of degeneration of red cells* is estimated by the number of fragmented and crenated cells, the amount of achromasia, and the number of microcytes as shown by the halometer, the hæmatocrit, or the Price-Jones curve which is shifted to the left. As microcytosis is correlated with fragility, the greater the fragility the greater the degree of degeneration.

Rate of Destruction of Hb.—This does not necessarily depend upon the degree of degeneration of the cells. Hæmolytic anæmia may occur either when the red cells are senile and fragile, as in the case of acholuric family jaundice, or when there is a toxin in the blood which destroys even normal cells, as in the case of pernicious anæmia—which occurs in adults only. The rate of hæmolysis is estimated by the number of van den Bergh units of bilirubin in the blood, as well as the amount of urobilin in the urine and fæces. The presence of more than a half unit of bilirubin in the blood means excessive hæmolysis. Urobilin is normally removed from the blood by the liver, but when there is excess the liver cannot cope with it, and some of it appears in the urine. When, however,

liver function is low, urobilin may appear in the urine even when the amount of bilirubin in the blood is normal. *These estimations may therefore be used also as a test for liver function.* If the rate of erythrocyte destruction is so great that the liver cannot convert the liberated Hb. into bilirubin, there is an accumulation of Hb. in the plasma (hæmoglobinæmia), accompanied by hæmoglobinuria—or rather methæmoglobinuria. This condition occurs in cases of poisoning by phosphorus, chlorates, carbolic acid, or other benzole derivatives (p. 63) after transfusion of hypotonic solutions or of incompatible bloods (see p. 69), in severe infections, in malaria (blackwater fever), in hæmoglobinuria of the newborn (Winckel's disease), after exposure to cold (paroxysmal hæmoglobinuria), etc.

The rate of regeneration of red cells is estimated by the amount of polychromasia and by the type of immature cell present : the more immature the cell the more desperate the effort at regeneration, and the graver therefore the outlook ; the greater the number of the less immature cells such as reticulocytes, the better the prognosis—because it shows that the bone-marrow has a plentiful reserve of nearly mature cells upon which to draw. The normal number of reticulocytes in the blood is about 1 per cent., but in favourable cases of hæmolytic anæmia the number may reach 50-60 per cent.

The rate of polymorph degeneration is shown by the number of vacuolated cells, the degree of Arneth shift to the right, and the amount of uric acid in the blood.

The rate of polymorph regeneration is given by the type of immature cell thrown into the blood. A marked Arneth shift to the left is a favourable sign, but the appearance of myelocytes and especially of myeloblasts shows that the bone-marrow is mobilising its last reserve forces.

For lymphocytes and platelets there are no balance tests.

The Significance of Immature Corpuscles in the Child's Circulation.—It must be remembered that not only are immature red and white cells, viz., reticulocytes, normoblasts, one- and two-lobed polymorphs, etc., common findings in blood-films of normal newborn infants, but that during infancy—and to a less degree during early childhood—the hæmatopoietic system is very susceptible to pathological stimuli. Hence the appearance of immature corpuscles in early life has not the same significance as in the adult. This is so because during early life the blood-forming organs are much more active, and the endothelial barrier preventing the entrance of immature forms into the circulation is feebler, so that a relatively unimportant blood-destruction, or infection, will produce a blood-picture which in the adult would indicate affection of much severer degree.

The Significance of Microcytosis and Macrocytosis—While extreme microcytosis and macrocytosis are characteristic of acholuric family jaundice and pernicious anæmia respectively (p. 42), milder degrees of either condition are found in anæmias due to other causes. Thus a halometer reading of less than $7{\cdot}3\,\mu$ is found in hyperthyroidism, chlorosis, anæmia due to hæmorrhage and in carcinoma, which are therefore examples of *Micro*cytic anæmia. *Macro*cytic anæmia (not so marked as pernicious anæmia) is found in von Jaksch's anæmia, jaundice, sprue, *Dibothryocephalus latus* infection, poisoning by benzine and its derivatives, which destroy the smaller sized cells, and in hypothyroidism.

CHAPTER V.

The Platelets.

The number of platelets in adults and children ; their origin, average duration of life and their function in the arrest of hæmorrhage.

THE normal number of platelets in the adult is about 350,000 per c.c. In the newborn they are considerably fewer, and if their number is less than about 50,000 it is not safe to operate—especially under an anæsthetic—owing to the risk of hæmorrhage. They are manufactured in the red bone-marrow, but their place of destruction is not known ; it may possibly be the spleen. Their average duration of life is about four days, so that any transfusion given in the case of a diminished number of platelets must—until the regenerative power of the red marrow is restored or the rate of platelet destruction is decreased—be repeated every three or four days. The view regarding the role of platelets in connection with hæmorrhage has undergone modification in recent years. It was originally believed that in purpura hæmorrhagica the essential cause of the hæmorrhage was a thrombopenia, i.e., a poverty of platelets, and the disease was therefore called *essential thrombocytopenic purpura hæmorrhagica* (Werlhoff's disease). It is now recognised, however, that platelets may be entirely absent without there necessarily being any hæmorrhage ; and vice-versa, there may be the hæmorrhagic diathesis without a diminution in the platelet-count, e.g., in Henoch's

purpura. The cause of the bleeding in such a condition seems to be increased permeability of the capillary walls, as shown by the capillary resistance test, and the function of the platelets seems to be to form masses of thrombi in the neighbourhood of the injured capillary wall—thus helping to stop the bleeding. By promoting clotting they supplement the work of the thrombi. The increased permeability is due to a toxin coming from the spleen, and hence splenectomy cures the hæmorrhagic diathesis. In those cases in which bleeding persists after splenectomy the function of the spleen may have been taken up by other parts of the reticulo-endothelial system.

In acholuric family jaundice the spleen destroys the decrepit, excessively fragile red cells, and splenectomy therefore prolongs their life and delays their hæmolysis, thus helping the slow regeneration to keep pace with the destruction of the cells. The excessive fragility, however, may still remain after operation, because though given an extra lease of life, the old cells have not become rejuvenated by the splenectomy. Hence, although the operation improves the general health in such cases, microcytosis, as shown by the halometer, still remains, and the fragility is still increased—though possibly not to the same extent.

CHAPTER VI.

Blood Diseases.

Classification of Blood Diseases, Diagnosis and Treatment of the various types.

IN the chapter on "The Chemistry of the Serum" an account was given of the alterations in the concentrations of the various ingredients of that fluid brought about by diseases in other parts of the body, and it was shown how an analysis of the blood was of help in the diagnosis, prognosis and treatment of those diseases. This chapter will deal briefly with diseases in which the abnormal character of the blood or of the hæmatopoietic organs is the main pathological condition, and the changes in the other parts of the body are secondary to the abnormal state of the blood. *Blood diseases may be classified into*: (*a*) *abnormal conditions of the formed elements*—red and white corpuscles, and platelets; (*b*) *abnormal conditions of the fluid portion*—which may, or may not, depend upon the qualitative or quantitative alteration of the formed elements.

(*a*) **The abnormal conditions of the formed elements may be quantitative** when the normal balance between destruction and regeneration is upset as the result of hypo- or hyperfunction of one or other of the blood-forming or blood-destroying organs; or *qualitative* when there is dysfunction of those organs.

A. Quantitative alteration in the formed elements due to disturbance of equilibrium between destruction and regeneration.

1. *Destruction rate normal, but regeneration rate subnormal.*

(i) Hypofunction of red bone-marrow, which may be complete or incomplete. If complete—as in true idiopathic aplastic anæmia, where the red marrow is entirely replaced by yellow—there is no regeneration at all of any of the formed elements. A blood-film may be almost acellular, but the cells found are either normal or senile (fragmentation, achromasia, etc.), none being of the immature type—because there is no regeneration. Hb. is correspondingly very low. The patient, therefore, suffers from increasing pallor and dyspnœa, the result of anoxæmia, or want of oxygen, as well as general weakness. The almost entire absence of platelets renders him particularly liable to hæmorrhage, which may be fatal. A typical blood-count is: Reds, 1,200,000; total whites, 800 (polymorphs 10 per cent., lymphocytes 60 per cent., the relative lymphocytosis being due to the fact that the other parts of the reticulo-endothelial system are not affected); platelets, 30,000. Bone puncture fails to reveal any signs of regeneration of cells. Transfusion is only of temporary help, and splenectomy is definitely contra-indicated, since sections of the organ reveal normoblasts and myeloblasts, showing that it is attempting to take on some of the functions of the bone-marrow. The disease is necessarily fatal.

In the incomplete variety of aplastic anæmia which may follow acute infections, benzole or TNT[1] poisoning, or prolonged exposure to radium or X-rays, the blood-picture varies with the portion of the marrow affected whether, that concerned with the production of red (erythropenia) or white cells (true granulopenia or agranulocytosis) as contrasted with leucopenia (which is a secondary condition) or with the minting of platelets, thrombopenia or thrombocytopenia (see

[1] T.N.T. = Trinitrotoluene.

" Purpura Hæmorrhagica ") ; but generally it is of a composite, but partial, nature. The disease when of the incomplete type is curable by repeated small transfusions which stimulate the bone marrow to activity. The other type is generally fatal. The *bioscopic* examination of a bone by means of a puncture, as well as the absence of a granulocytocidal toxin in the blood, as shown experimentally, proves that the disease is a hypofunction of the bone-marrow.

(ii) Deficiency of Hb. due to lack of iron, so that the healthy marrow has no " straw " to make " bricks." Congenital anæmia (in twins and prematures), and alimentary anæmia (malnutrition, prolonged feeding of babies on milk alone which contains no iron), come within this category ; so does the now rare disease chlorosis, although the exact cause of Hb. poverty in this condition is unknown, and it is for this reason called a " primary " anæmia, as contrasted with the others, which are " secondary." The blood-picture shows no evidence of either degeneration or regeneration, but the red count is low and the Hb. is lower still, making the C.I. considerably less than 1 (0·5-0·6).

Treatment consists in removal of the cause, administration of iron, heliotherapy, fresh air, etc.

2. *Destruction rate excessive, but regeneration rate is either normal or not so rapid as the destruction rate.*

(i) Anæmia due to Hæmorrhage. A sudden loss of more than one-third of the blood, which is not immediately replaced, is fatal—because the body cannot accommodate itself suddenly to carry on its respiratory activities with only two-thirds of the amount of Hb. But in gradual bleeding, a loss of even two-thirds in 24 hours is not necessarily fatal. The blood-picture varies with the interval after the hæmorrhage. Immediately after, the total blood-volume is of course reduced, but the blood-count and Hb. percentage are normal. A little later, owing to absorption

of water to make up the volume, the cell-count and Hb. percentage fall. Later still, there is evidence of regeneration in proportion to the amount of blood lost.

(ii) Hyperfunction of the spleen, e.g., splenic anæmia of infancy (von Jaksch's pseudo-leukæmia of infants), splenic anæmia of childhood (Banti's disease), Gaucher's disease, etc.: The nature of these diseases is not quite understood, but the blood-picture shows signs of degeneration and regeneration. In von Jacksch's disease, which is generally accompanied by rickets, the spleen is large, the red count is 1,000,000 to 2,000,000, the whites are 20,000 to 40,000, with about 60 per cent. lymphocytes, and some myelocytes —but much fewer than in leukæmia. Hb. is low, and C.I. is about 0·7. Immature forms of the cells formed by the bone-marrow and lymphoid tissue are found. In Banti's disease, the liver as well as the spleen is enlarged, and in Gaucher's disease puncture of the enlarged spleen reveals typical Gaucher cells which are large (20-80 μ), pale, and monoclear.

It is to be noted that the term " splenic anæmia " does not express any clinical entity. Rather does it apply to those cases in which there is anæmia together with splenomegaly, but which do not fit in with any of the clinical entities—such as leukæmia, hæmolytic jaundice, malaria, etc., in which these two symptoms are also prominent. Hence the diagnosis of splenic anæmia should only be made when all possible causes have been eliminated by blood-count, fragility test, Wassermann reaction, malaria, tuberculin test, absence of symptoms and signs of rickets, absence of Gaucher cells on splenic puncture, etc.

Treatment.—For von Jaksch's disease, the indications are: Treatment of rickets by cod-liver oil, ultra-violet light, irradiated ergosterol, etc., as well as frequent small transfusions. Banti's disease, if

recognised early, is benefited by splenectomy. Gaucher's disease, being a generalised disease rather than a primary endothelioma of the spleen as it was originally supposed, splenectomy or any other treatment is useless.

(iii) Toxin which destroys the red cells, e.g., pernicious anæmia (in adults only).

(iv) Premature senility of the red cells, e.g., acholuric family jaundice. Both (iii) and (iv) belong to the type of hæmolytic anæmia, the one of the macrocytic and the other of the microcytic type, and their blood characters have already been sufficiently dealt with. The treatment of (iii) is by liver feeding, and that of (iv) is splenectomy.

3. *Destruction rate normal, regeneration rate excessive.*

(i) Hyperfunction of red marrow. When this is primary, there results the true *idiopathic erythræmia* (very rare in early life), which is analogous to leukæmia. If secondary, i.e., compensatory, due to cyanosis, the result of insufficient oxygenation of red cells (e.g., in congenital pulmonary stenosis, or at high altitudes), the condition is a *polycythæmia*, or *erythrocytosis*, analogous to leucocytosis. In addition to an increased red count, a blood-film shows immature red cells (macro- and normo-cytes, etc.) ; leucocytosis, with increase of polymorphs, and relative diminution of lymphocytes ; immature polymorphs (myelocytes and myeloblasts). The blood-volume is also increased to reduce the high viscosity due to the large cell-count, which would unduly impede the circulation. A *relative polycythæmia* occurs in cases of anhydræmia (see p. 36). The *treatment* that may be tried for the idiopathic variety is : (1) X-rays or radium to the epiphyses; (2) bleeding; (3) oxygen inhalation; (4) benzole or phenyl hydrazine (gr. ii) once a week, to be controlled by frequent blood-counts as it rapidly

destroys red cells, and may lead to fatal results. The disease is, however, incurable.

(ii) Hyperfunction of the red marrow portion which mints the granular leucocytes. When this is primary it gives rise to *myelogenous leukæmia* (acute or chronic —each of which is extremely rare in early life), characterised by debility, great splenic enlargement, little, if any, lymphatic enlargement, very high white count (about 300,000), of which about 50 per cent. are myelocytes or myeloblasts, and lymphocytes are no more than about 10 per cent. (Contrast von Jaksch's anæmia, in which there is also splenomegaly and a high white count, but in which the whites do not exceed about 40,000, the myelocytes are very few and the lymphocytes form about 60 per cent.)

When the increase in polymorphs is secondary to some inflammatory or septic focus, the condition is called *polymorphonuclear leucocytosis*, or preferably *polynucleosis*. In this condition immature cells are absent. Eosinophilic leucocytosis, or *eosinophilia*, is present in cases of helminthiasis, hydatid disease, bronchial asthma, certain skin diseases, and a few other conditions.

(iii) Hyperfunction of the adenoid tissue of the lymph glands and spleen. When primary it gives rise to *lymphatic leukæmia*, of which the acute type is more common in children than the chronic, characterised by lymphatic enlargement, debility, whiteness of skin and blood, a white count of 50,000-500,000, of which lymphocytes form 90-100 per cent. Myelocytes are few or absent, and immature lymphocytes are abundant. The distinction between these and immature granulocytes is made by the *oxidase stain*, which is taken up by the latter only. A condition of "Aleukæmic leukæmia" may occur in the late stages of leukæmia in which there is a qualitative change in the white cells without any increase in their number. Secondary *lymphocytosis*

occurs in a variety of conditions, such as pertussis, congenital syphilis, tuberculosis, &c., and in infectious mononucleosis, or glandular fever, The latter generally accompanies acute infection of the upper respiratory tract, such as tonsillitis, &c., and is characterised by a clinical picture not unlike that of leukæmia: general glandular enlargement, and a large increase of lymphocytes. The total white count is, however, generally below 50,000, and the lymphocytes are rarely over 80%. The condition is transient, and disappears in a few days to a few weeks.

In both the lymphatic and myelogenous leukæmia, the red cells are diminished and the platelet-count is low ; hence the tendency to hæmorrhages.

Not only do the two forms of leukæmia frequently overlap, but erythræmia and leukæmia may be present together, due to general hyperfunction. When leukæmia is accompanied by periosteal tumours the condition is called *chloroma*. But the hæmatopoietic organs may be overgrown without overfunctioning; one then gets lymphadenoma (Hodgkin's), lymphosarcoma, myeloma, etc., in which the blood-pictures are merely those of secondary anæmia. The *treatment* of leukæmia, which is merely palliative, is the application of X-rays or radium to the spleen and lymphatic glands; this is frequently followed by a great drop in the white count—which, however, is temporary. Also transfusion succeeds in stopping otherwise intractable bleeding.

B. DYSFUNCTION OF THE HÆMATOPOIETIC SYSTEM producing defective corpuscles (sickle-cell anæmia), or platelets deficient in prothrombin (hæmophilia, hæmorrhagic diseases of the newborn, etc.).

(*b*) **The abnormal conditions of the plasma** are those in which there is a tendency to extravasation of blood which may be either spontaneous due to defective capillary resistance—with or without a thrombopenia—e.g., true purpura hæmorrhagica, and

Henoch's purpura respectively ; or the result of slight trauma, when due to defective amount of prothrombin although the platelet-count is normal, e.g., hæmophilia, hæmorrhagic diseases of the newborn, etc. (see also under " Coagulation Time," pp. 6-8).

True purpura hæmorrhagica is characterised by thrombocytopenia, lengthened bleeding-time, and diminished capillary resistance. Its cause is unknown, and it is differentiated from hæmorrhage due to aplastic anæmia or leukæmia in which there is also a thrombocytopenia, by the blood-pictures—inasmuch as in purpura the blood-film shows nothing abnormal apart from the paucity of platelets and the characters of a secondary anæmia. It is also distinguished from hæmophilia by the soft and non-retractile clot and by the fact that in the latter there is no thrombopenia, but the coagulation and prothrombin times are very greatly prolonged, the bleeding time is normal, the bleeding is not spontaneous but the result of slight injury, and is of the nature of hæmatoma rather than purpura. Also in hæmophilia there is a history of hereditary transmission by Nasse's law : through normal females to males.

Scurvy may also have to be diagnosed from the various hæmorrhagic conditions. The blood-picture in scurvy is that of secondary anæmia (due to the hæmorrhages), but the coagulation, bleeding and prothrombin times, as well as capillary resilience, are normal. The other clinical features of scurvy are also well marked.

The *treatment of bleeding in the hæmorrhagic diseases* consists of blood injections. In the case of purpura hæmorrhagica or hæmophilia it is given by intravenous transfusion from a compatible donor (see p. 70). and in hæmorrhagic disease of the newborn when transfusion may be difficult, it is sometimes sufficient to inject the donor's blood—20-30 c.c. intramuscularly, repeated if necessary in a few hours. Such a method

does not involve previous blood-grouping. The introduction of the blood supplies every one of the ingredients necessary for coagulation, in the case of hæmophilia, etc., or for counteracting the tendency to spontaneous hæmorrhage by supplying a sufficiency of platelets necessary for the protection of the permeable capillary wall. For the frequently recurring bleeding of purpura hæmorrhagica, splenectomy should be done after a preliminary transfusion. In cases where splenectomy is difficult on account of adhesions, it is sufficient to tie the splenic vessels; this arrests the circulation of the toxin manufactured by the spleen.

Synopsis of Classification of Blood Diseases.

(*a*) *Abnormal conditions of formed elements.*

A. Quantitative.

1. Destruction rate normal, regeneration rate subnormal.
   - (i) Hypofunction of bone-marrow.
   - (ii) Deficiency of Hb, due to defective iron metabolism.
2. Destruction rate excessive, regeneration rate normal.
   - (i) Hæmorrhage.
   - (ii) Hyperfunction of spleen.
   - (iii) Erythrocytocidal toxin.
   - (iv) Premature senility of red cells.
3. Destruction rate normal, regeneration rate excessive.
   - (i) Hyperfunction of bone-marrow.
   - (ii) Hyperfunction of adenoid tissue.

B. Qualitative = Dysfunction.

(*b*) *Abnormal conditions of plasma.*

CHAPTER VII.

Blood in Treatment.

Transfusion: indications for; amount of blood used; blood grouping; symptoms of incompatibility. Autohæmotherapy; serotherapy.

THERAPEUTICALLY, blood is utilised in two main ways:—

(1) Whole blood—as such, or after immunization—either intravenously (transfusion), or intramuscularly.

(2) Blood-serum.

1 (*a*). Transfusion is indicated in cases of severe anæmia (whether acute, the result of sudden profuse hæmorrhage, or chronic)—to supply hæmoglobin for respiratory purposes, as well as to stimulate the hæmatopoietic organs; in uncontrollable hæmorrhage (e.g., in purpura hæmorrhagica, hæmophilia, leukæmia, hæmorrhagic diseases of the newborn, &c.), where by supplying platelets or other substances necessary for coagulation, it usually acts like magic in arresting the bleeding; in cases of CO poisoning—to supply active hæmoglobin; and in certain septic and toxic conditions, such as septicæmia, pyæmia, &c., with the object of not only diluting the poison, but also of supplying antibodies and phagocytes. In cases of infective endocarditis, transfusion has not yet succeeded in saving a life—even if the donor has been previously immunized against the causative organism.

When blood is used for transfusion, the amount needed is about 3 c.c. per lb. in an adult, and 7-10 c.c. per lb. in the child. It is not, however, the

blood of every individual that is suitable for transfusion into every child. In the early days of transfusion fatal results were not at all rare—due, as was subsequently shown, to the clumping, or hæmolysis, of the donor's corpuscles inside the blood-vessels of the recipient. This clumping is called *isoagglutination*, to distinguish it from the agglutination produced by mixing bloods from two different species, and has been shown by Landsteiner and his disciples to be due to the interaction of a substance called *isoagglutinogen* of which there may be two kinds, called A and B, present singly or together, in the red cells of one blood, with a corresponding substance—a or b respectively—called *isoagglutinin*, present either singly or together in the serum of the other. A acted upon by a, or B acted upon by b, causes agglutination, but the interaction of A and b, or of B and a, does not cause it. It stands to reason, therefore, that no one blood can, under ordinary conditions, contain A and a together, or B and b together—or else that person's own blood-cells would be agglutinated inside his vessels. A kind of autoagglutination or hæmolysis may, however, take place at cold temperatures (e.g., paroxysmal hæmoglobinuria), or in certain pathological conditions, such as hæmolytic jaundice. Theoretically, the following types of blood are possible :—

Type	Corpuscles (agglutinogen)	Serum (agglutinin)	Formula	Frequency in population
1	O (none)	o (none)	O(o)	
2	O	a	O(a)	
3	O	b	O(b)	
4	O	ab (i.e., a + b)	O(ab)	45%
5	A	o		
6	A	b	A(b)	40%
7	B	o	B(o)	
8	B	a	B(a)	10%
9	AB (i.e., A + B)	o	AB(o)	5%

Actually, only four main groups are met with, viz., O(ab), A(b), B(a), and AB(o). They are designated groups O, A, B, and AB, and their frequencies in the European population are in the same order—as shown in the table. The older classification into Groups I, II, III and IV, should be given up because what Jansky calls Group I is called by Moss Group IV, and vice-versa (fig. 7), so that a good deal of confusion is caused by such nomenclatures. Moreover, the names O, A, B, and AB at once give information of the characters of the blood and its behaviour on mixing with blood of another group.

As the danger of transfusion is the possible agglutination, etc., of the corpuscles of one blood by the serum of another, and as the donor's serum becomes so diluted inside the recipient that its capacity for agglutinating the recipient's corpuscles is very slight in most cases, it is necessary to ensure before a transfusion that the donor's corpuscles and the recipient's serum are compatible. **Group O individuals are universal donors** because their corpuscles being devoid of any agglutinogen cannot be agglutinated by the serum of any recipient. The names and addresses of such persons who are healthy in every respect (Wassermann reaction —ve, blood-count and Hb. content normal, absence of malaria, T.B., asthma, etc.), are kept by the British Red Cross Society, and they may be called upon for any emergency transfusion. On the other hand, **people belonging to Group AB are universal recipients,** because their serum containing no agglutinin, will not agglutinate the cells of any donor. The inter-relation between the different groups is shown in the diagram (fig. 7).

When a universal donor is not immediately available, the patient's blood must be matched with that of any donor before a transfusion. This can be done by mixing a drop of blood from each on a slide in the form of a hanging-drop preparation, and

examining for clumping under the microscope. This must be carried out by an expert who will not mistake rouleaux formation for true agglutination, or vice versa. The fact that the donor is a near relative does not dispense with the need of such matching (see p. 77).

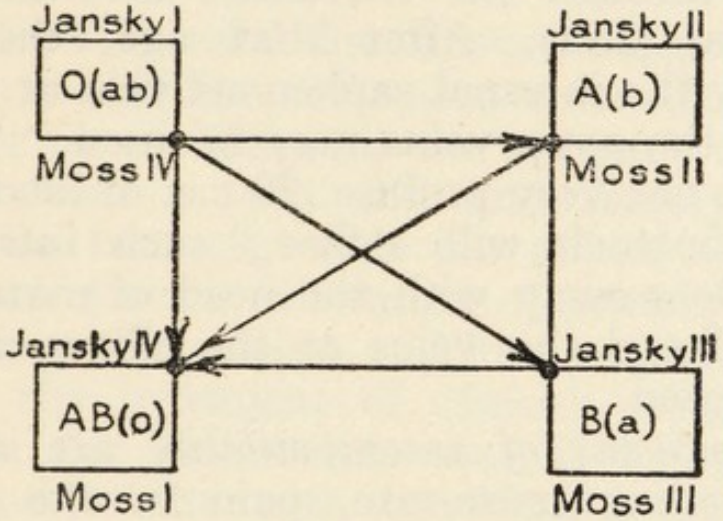

FIG. 7.—Diagram to show the compatibility and incompatibility of the different groups among one another. The arrows show with which groups, in addition to its own, any particular donor's blood is compatible. Thus group O(ab) is shown by the arrows to be compatible with the other three groups; groups A (b) and B (a) to be compatible with their own respective groups, as well as with AB (o); whilst group AB (o) is incompatible with any group other than its own. Universal donors (O) cannot receive the blood of any group other than its own, and universal recipients (AB) cannot give blood to any group other than its own.

Transfusion in Early Infancy.—Although in the case of groups other than O, the corpuscles of the newborn contain agglutinogen, its serum—no matter to what group it belongs—has been alleged to be free from agglutinin, which is said to develop in later infancy. If that were true it would follow that all newborn babies are universal recipients, and may, in cases of severe umbilical hæmorrhage, melæna neonatorum, uncontrollable bleeding after circumcision, &c., receive blood intravenously from any

individual (e.g., the father), without previous matching. This is true to some extent, but not universally, for in some cases powerful agglutinins derived from the mother are present at birth. Hence the same precautions must be taken for an infant as for an adult. In infants under 1 year old, transfusion can be given through the fontanelle into the superior longitudinal sinus. After that age, the external jugular, or the internal saphenous vein at the ankle, or one of the scalp veins may be used. When the bleeding is not very profuse, 20 c.c. of blood injected into the buttock will suffice; such intramuscular injection does away with the need of matching. In later childhood the veins at the elbow can be conveniently used.

The *symptoms of incompatibility* are respiratory distress, falling pulse-rate, pain in the back and hæmaturia. As soon as these are noticed the operation must be stopped, and 15 minims of 1/1,000 adrenalin together with 1/100 grain of atropine injected subcutaneously. These symptoms are due to hæmolysis and precipitation of hæmoglobin in the renal tubules. The intrarenal obstruction thus produced leads to uræmia which kills the patient. As the precipitation only occurs in the tubules, where the urine is acid, but not in the glomeruli, where the urine is alkaline, it has been suggested that alkalinisation of the urine before a transfusion might be a useful safeguard.

Other dangers from a transfusion—even when universal donors are used—are: too large or too rapid an injection causing overfilling of the heart; anaphylaxis due to products of digestion contained in the donor's blood to which the recipient is susceptible—a danger which can be avoided by letting the donor fast before a transfusion; and, in cases of infective endocarditis, embolism due to detachment of a vegetation from the valves. There are several others which are enumerated in books devoted to

transfusion, but one of interest from the point of view of incompatibility is high agglutinin content of the donor's blood which might cause excessive agglutination of the *recipient's* corpuscles. The blood-matching method described above should detect agglutination or hæmolysis of the cells of each specimen.

1 (*b*). Blood is used for intramuscular injection in two ways: (i) That of a donor for the arrest of hæmorrhage (see p. 72); (ii) the patient's own blood withdrawn from a vein and immediately injected into his own buttock, with the object of creating protein shock (due to liberation of protein from the disintegrating corpuscles). This *autohæmotherapy* is used in the treatment of diseases of an allergic nature, and the author has used it for asthma in children and adults with considerable success. It may also be used for obstinate allergic eruptions. The initial dose for an adult is 5 c.c., which is gradually increased to 20 c.c. or more. In the case of a young child it is advisable to start with 2 c.c. of either its own or the parent's blood, and rapidly increase the dose. Injections are given two to three times a week for about three months. The method can be modified by mixing the blood in the syringe with water before injecting into the muscle. The water helps the disintegration of the corpuscles. Autohæmotherapy may also be used in cases of septicæmia for the purpose of producing a "fixation abscess" (i.e., a local abscess into which the bacteria from the blood tend to collect).

(2) The use of serum containing immune bodies for therapeutic purposes (*serotherapy*) is, of course, well known to every medical practitioner. Suffice it therefore to mention here the use of diphtheria antitoxin for prophylactic and curative purposes, that of antitetanic serum for prophylactic purposes, and that of anti-streptococcic serum in cases of

septicæmia. In these cases the serum is derived from immunised animals. In recent years the use of serum of people who have recently passed through an attack of some infectious disease has been introduced to protect a person who has been in contact with the same disease. The method has been tried in a number of diseases, including anterior poliomyelitis, chicken-pox and mumps, but the most striking success has been obtained in the case of measles. The blood from a convalescent case is much more potent than that of a person who has had the disease many years ago ; and the earlier in the incubation period it is given the more certain the protection. The donor must of course be free from any other infectious disease, including T.B. and syphilis. 5 c.c. is given intramuscularly for a child under 3 years, and twice as many c.c. as the child's age for older children. If given before the fifth day of incubation there is complete immunity conferred which lasts about three weeks—i.e., long enough to protect against infection from the disease which the child may have been incubating, but further injections would be needed in the case of subsequent exposures. If given between the sixth and ninth days the child goes through a very attenuated attack which affords prolonged (? permanent) immunity.

The use of serological products for diagnosis and immunisation is very interesting and important, but consideration of the subject would take us too far afield. It will suffice to mention merely the use of diphtheria toxin, or of toxin-antitoxin in the Schick test for the susceptibility to diphtheria ; the use of serum from a scarlet fever convalescent for intra-dermal injection into a patient suspected of suffering from that disease—when blanching of the rash over the point of injection indicates that the case is scarlet fever ; and the use of scarlet fever antitoxin for immunisation of contacts.

CHAPTER VIII.

Bacteriology and Parasitology of Blood.

Indications for blood cultures and their significance. Serological reactions. Parasites found in the blood in tropical diseases.

BLOOD-CULTURES are indicated in cases of septicæmia to ascertain the nature of the organism; in cases of suspected septicæmia or infective endocarditis, to confirm the diagnosis as well as to identify the responsible organism; and also in cases of bacteræmia—such as typhoid fever, in which organisms can be grown in some 90 per cent. of the cases during the first week of the disease. It should be remembered that while a positive culture in which the possibility of contamination (especially in the case of organisms commonly found in the skin) can be eliminated, is absolute evidence of the presence and character of a bacteræmia, a negative culture does not exclude it. If, in suspected cases of septicæmia, staphylococci are found, the possibility of contamination from the skin should be excluded by means of a second culture—especially if the number of bacteria found is very small. Also, the rapidity with which growth occurs in cultures, and the number of colonies grown per 1 c.c. of blood may be taken as an index of the severity of the bacteræmia.

In cases of sub-acute or chronic bacterial endocarditis (endocarditis lenta) the number of bacteria may be so small that no growth may occur for many days or weeks. The culture should be made from a specimen withdrawn when the temperature is its

highest. It may be mentioned that this disease is very rare in early childhood.

The bacteriological examination of blood-serum for agglutinins (e.g., Widal's test in typhoid), hæmolysins and complement fixation (Wassermann's reaction in syphilis), etc., has already been referred to.

The parasitology of the blood is of very little practical importance in this country. In tropical climates, the parasites often found in the blood are the *Plasmodium malariæ* (within the red corpuscles) ; filaria, mostly found in evening specimens of blood ; the spirillum of relapsing fever ; and the various trypanosomes.

CHAPTER IX.

Blood in its Biological and Sociological Aspects.

Mendelian transmission of group factors: Its application to sociological, criminological and scientific problems. Blood groups in relation to pathology, anthropology and ethnology.

EXTENSIVE statistical studies throughout the world of the blood-groups of parents and children, for a number of generations, have shown conclusively that the three group factors, A, B and O, are transmitted from parent to child in a Mendelian manner, and that A and B behave as dominants, while O behaves as a recessive. As recessive characters can only show themselves when transmitted from *both* parents, while a dominant character will show itself either when transmitted from both (= pure dominant, or homozygote), or when one of the parents has transmitted the dominant and the other the recessive factor (= impure dominant, or heterozygote), it follows that the genetic constitutions of individuals of the various groups are :—

Group O = OO; Group A = AA (= homozygote), or AO (= heterozygote).
Group B = BB, or BO (for similar reasons); and
Group AB = AB.

Applying the Mendelian laws it follows that with parents belonging to certain groups, the groups to which the children can, or cannot belong, can be foretold with certainty, so that if a child belongs to the impossible group it can be asserted with certainty

that one or other of the putative parents is not the real parent. The following is such a table :—

Parents' groups	Possible groups of the children	Impossible groups of the children
O × O	O	A, B, or AB
O × A	O or A	B or AB
O × B	O or B	A or AB
O × AB	A or B	O or AB
A × A	O or A	B or AB
B × B	O or B	A or AB
A × B	O, A, B, or AB	None impossible
A, B, AB × AB	A, B, or AB	Group O

Practical Application of the above Facts.

A. Sociology.

(1) *Cases of Disputed Paternity*.—The table shows that if a child shows in its blood the presence of an agglutinogen—whether A or B—then at least one of its parents must possess the same factor in his or her blood. If absent in the mother it must be necessarily found in the father, and therefore its absence from the blood of the putative father conclusively excludes him from being the real father. Such a table is therefore of value in the case of a husband repudiating the legitimacy of any particular child, or to exonerate a man when an affiliation order is sought for a child born out of wedlock. It will of course be realised that the finding of the particular agglutinogen in the man's blood does not necessarily prove him to be the father—except when the question of paternity lies between that man and another whose blood does not contain that agglutinogen. If the test is carried out by an expert who is aware of, and takes care to eliminate, all the sources of error, the result is absolutely reliable, and indeed is now accepted as evidence by the legal tribunals of many countries in the world. Even within the British Isles it was accepted for the first time early

in 1932, by a Dublin Court, to absolve a man in an affiliation case. In England such evidence has as yet not been used for medico-legal purposes, although the fact that not one out of nearly 13,000 children belonging to over 10,000 mothers has been found to belong to a group contrary to that given in the above table, should make the test as admissible as the finger-print test. In a *cause célèbre* tried in London a few years ago, when a husband repudiated the paternity of his wife's child, such iso-agglutination evidence might have been invaluable.

(2) *Case of an Impostor claiming to be the "Long-lost Child."*—Here again if his blood-group is one of the impossible ones, his imposture is at once exposed.

(3) *Identification of a Baby.*—Although in most cases it is the identity of the father that is at issue, cases sometimes arise in which the mother's identity has to be decided. It sometimes happens that two babies are accidentally interchanged in a maternity hospital and sent home with the wrong mothers. In a case that occurred in a maternity hospital in London a few years ago, a Gentile baby was circumcised in the Jewish ritual manner because it was mistaken as belonging to a Jewish mother. In doubtful cases of this nature, the grouping of the children's and parents' bloods would solve the problem. In the case of such an interchange of two illegitimate children, however, where the identities of the fathers are unknown, such as occurred in the famous "Judgment of Solomon" case, a "Solomon" would still be needed to adjudicate.

(4) *Adopted versus Real Child.*—It has happened that a childless couple have for fraudulent purposes connected with legacies surreptitiously adopted a baby which they attempted to pass off as their own. Here the blood-grouping test would afford invaluable help—unless the couple in question were so clever as to choose a baby that is within the possible groups.

(5) *Diagnosis of Identical Twins.*—It is sometimes necessary to decide, for scientific purposes, whether two infants belonging to the same sex are uni- or bin-ovular. If the groups to which they belong are different then the twins could not have come from the splitting of an ovum that was fertilised by the same spermatozoon. On the other hand, the fact that they do belong to the same group does not necessarily prove them to be uni-ovular, although it affords good presumptive evidence in its favour; and the rarer the incidence of the group in question in the population (see table on p. 69), the stronger the evidence.

(6) *To Decide the Possibility of Superfœtation.*—Several cases have been published within the last few years of twins or triplets where one (or two) of the children belonged to one or other of the possible groups as given in the above table, while the other belonged to one of the impossible groups. It is obvious of course that the husband cannot have been the father of both (or of the three). Sabolotny, in 1928, for instance, recorded in a Russian medical journal a case of triplets belonging respectively to Groups A, A and B, although the mother belonged to Group O, and her husband belonged to Group A. As child B cannot obviously belong to the husband, the case has been put forward as one of superfœtation. Another and more probable solution is that all the three children belong to one illegitimate father of Group AB.

B. Blood Groups in Criminology.—The precipitin test distinguishes between human and other mammalian blood, but the iso-agglutination test, which can be applied to old blood-stains, narrows down the issue by finding the groups to which belong the bloods of the stain, the victim and the accused on whom the stain was found. If the victim's blood is of a different group from that of the stain, the case

for the Crown would practically collapse. If in addition the accused pleaded that the blood-stain is due to an attack of hæmoptysis, or epistaxis, and the stain is found to belong to the same group as himself, the confirmation should be quite sufficient to acquit him. On the other hand, if the blood of the stain belongs to the same group as the victim (but not as the accused), and the accused cannot name a person from whom it came and who belongs to the same group, the verdict of "guilty" is practically inevitable.

On the assumption that the serum of a newborn contains no iso-agglutinins, it has been suggested that it is possible to differentiate between the blood of an adult and that of a newborn baby. This assumption, however, is not correct, as we have seen before (see p. 72).

In the present state of knowledge it is as yet impossible to assign a given blood to a given individual—except as we have seen by exclusion. Recent work, however, suggests that such individualisation may become possible in the not very remote future. At the same time it may be pointed out that the probability of two people belonging to the same group diminishes from Group O to Group AB. Thus, the percentage frequency of Group O being 45 per cent., the probability of two given individuals being of that group is $0{\cdot}45 \times 0{\cdot}45 = 0{\cdot}2025$, i.e., one-fifth; while the probability of two given people (chosen at random and unrelated) belonging to Group AB is $0{\cdot}05 \times 0{\cdot}05 = 0{\cdot}0025$, i.e., $\frac{1}{400}$. Hence, in an affiliation case, for instance, where the real father must belong to Group AB (see table, p. 78), and the accused is found to belong to this group, the chances are 400 to 1 that he is the actual father.

C. Blood-groups in Pathology.—(1) The iso-agglutination test has been used to study the duration

of life of a red corpuscle when infused into a recipient's circulation (see p. 50).

(2) *Relation between Blood-groups and Susceptibility to Disease.*—It has been stated by some investigators that people belonging to a particular blood-group are, in respect of certain diseases, more susceptible than those belonging to another group. Thus, tuberculosis is claimed to be particularly severe and to run a more rapid course in Groups A and AB, i.e., in those possessing factor A, than in Group O, and the same has been alleged to be the case in respect of the various child ailments such as scarlet fever, diphtheria, whooping-cough, etc. The results of different observers, however, are so contradictory as to cancel themselves out in the aggregate. Indeed, if one takes all the published figures one finds that the percentage distribution of the different groups among people suffering from any particular disease is not significantly different from that among the ordinary population in the same locality.

It has also been said that certain pathological conditions either in the newborn, such as icterus neonatorum, or in the expectant mother, such as toxæmia of pregnancy, are due to incompatibility between the maternal and fœtal bloods. This statement, also, has not been substantiated. It will be realised of course that for pregnancy toxæmia to be related to blood-group incompatibility, it would be necessary to assume that fœtal agglutinins had broken through the placental barrier, and for icterus neonatorum the maternal agglutinins or lysins must have reached the fœtus. Theoretically such a thing is not impossible, but statistics show that not only may incompatibility be present without either of these conditions, and vice versa, but that the frequency of such incompatibility is the same in normal and toxæmic mothers, as well as in normal and icteric babies. It has also been asserted that

group incompatibility, which is a property of all cells of the body including the leucocytes (a fact which explains the ill-effects occasionally encountered in the course of a transfusion with an otherwise compatible blood), may account for certain cases of sterility or abortion ; the incompatibility in such cases being between the husband's spermatozoa and the wife's ova. This again needs further investigation and substantiation. Hæmophilia is another condition which was alleged to be linked with a group factor. This, however, cannot be the case because hæmophilia is a sex-linked character but the group factor is not. The only condition which seems to be definitely associated with a blood-group is that of criminality and moral imbecility, which is particularly high in Group B. This may, however, be due to the fact that in Europe Group B contains a large number of families that came from Asia, who were unable to adjust themselves to European environment and would thus be more likely to commit anti-social acts.

D. BLOOD-GROUPS IN ANTHROPOLOGY AND ETHNOLOGY.—Investigations all over the world have revealed the following facts : (i) The percentage distribution of the different groups is different among different peoples. (ii) Those primitive races who have remained entirely free from admixture with other races (e.g., the Indians of Peru) consist entirely of Group O, to the utter exclusion of the factors A and B. (iii) With increase of admixture, there is a corresponding decrease of the percentage frequency of Group O, accompanied by the appearance of the other groups, e.g., the Red Indians consist of 91 per cent. Group O, 8 per cent. Group A, and 1 per cent. Group B. Group AB is entirely absent among them. It is therefore believed that originally mankind consisted entirely of Group O ; that the characters A and B appeared as mutations of the O character

at some remote period, in western and eastern parts of the world respectively; and that the different blood-groups in the diverse peoples were evolved from the gradual fusion of the various bloods. Hence the percentage distribution of the various groups among any particular set of people gives information of that people's racial impurity. Moreover, as it is believed that factor A originated in the West (Europe), and factor B in the East (Asia), the relative prevalence of the A or B factor is believed to afford an indication of the degree of admixture with European and Asiatic bloods respectively.

The estimation of this relative frequency of the A and B factors has been attempted in a number of different ways. It is obvious that mere estimation of the frequencies of Groups A and B is not enough, because each of the factors of these groups is also found in Group AB. L. and H. Hirszfeld, who are pioneers in this field of work, introduced what they named the Racial Biochemical Index (R.B.I.), viz., $\frac{A + AB}{B + AB}$, where A, B and AB stand for the respective frequencies of these groups. For the numbers given in the table on p. 69, R.B.I. $= \frac{40 + 5}{10 + 5} = \frac{45}{15} = 3$. An index greater than 3 is found in Western peoples, such as Americans, English, French, etc., while an index less than 1 is characteristic of the East, such as the Indians. This index, however, in the absence of other data, is of no great scientific value because it gives no information of the frequency of the O factor in the particular people under investigation. Several other indices have therefore been proposed, but the formula which supplies the most scientific and accurate index for the study of racial relationships is Bernstein's Racial Biological Index, which has been derived mathematically from genetic principles.[1]

[1] See Appendix.

The index is $\frac{p}{q}$, where p stands for $1 - \sqrt{O + B}$, and q stands for $1 - \sqrt{O + A}$ (the letters O, A and B under the root signs denoting the decimal frequencies of these respective groups). Thus, in the case of the figures in the table on p. 69, we have O = 45 per cent. = 0·45, A = 0·4, and B = 0·1, so that $p = 1 - \sqrt{0{\cdot}45 + 0{\cdot}1} = 1 - \sqrt{0{\cdot}55} = 1 - 0{\cdot}743 = 0{\cdot}257$, and $q = 1 - \sqrt{0{\cdot}45 + 0{\cdot}40} = 1 - 0{\cdot}971 = 0{\cdot}029$. Therefore $\frac{p}{q} = \frac{0{\cdot}257}{0{\cdot}029} = 8{\cdot}9$.

If in any group of people the ratio $\frac{p}{q}$ is different from that in another group known to be racially related to it, the inference is that one of these groups contains a greater (or less) admixture of foreign blood than the other.

APPENDIX.

Mathematical Derivation of Bernstein's Racial Biological Index.

LET p, q, and r represent the respective decimal frequencies of the *factors* A, B, and O in the population. Then, as the total of all the frequencies must be unity (i.e., 100 per cent.) we have $p + q + r = 1$. Now, as the various blood-groups are the results of the various combinations of the three group factors, and as the different combinations of such factors are algebraically represented by the various terms of the expression $(p + q + r)^2$, viz., $p^2 + q^2 + r^2 + 2pq + 2pr + 2qr$, we get the following expressions for the frequencies of the various *groups* in terms of the frequencies of the group *factors* :—

Frequency of Group O = r^2

,, ,, A = $p^2 + 2pr$ (p^2 representing the homozygotes AA, and 2pr the heterozygotes AO)

,, ,, B = $q^2 + 2qr$ (q^2 = homozygotes, and 2qr = heterozygotes)

and ,, ,, AB = 2pq.

These equations enable one to calculate the values of p, q, and r from a knowledge of the frequencies of the various blood-groups O, A, B, and AB in the population. If we designate by O, A, B, and AB the frequencies of these respective groups in the population, we get from the above :—

$O + A = r^2 + 2pr + p^2$, i.e., $= (r + p)^2$, whence $r + p = \sqrt{O + A}$.

$\therefore p = \sqrt{O + A} - r, = \sqrt{O + A} - (1 - p - q)$, since $p + q + r = 1$.

Whence, $q = 1 - \sqrt{O + A}$
Similarly, $p = 1 - \sqrt{O + B}$ } (see p. 85).

Further, from $O = r^2$, we get $r = \sqrt{O}$.

The formulæ also enable us to compute the theoretical frequencies of the two other groups, if those of Group O and either of Group A or of Group B are known. Thus, using the figures on p. 69, for Groups O and A, viz., 0·45 and 0·40 respectively, we get $r = \sqrt{0{\cdot}45} = 0{\cdot}67$. But $q = 1 - \sqrt{O + A} = 1 - \sqrt{0{\cdot}85} = 1 - 0{\cdot}92 = 0{\cdot}08$. But $p = 1 - (q + r)$, $\therefore p = 1 - (0{\cdot}08 + 0{\cdot}67) = 0{\cdot}25$.

Hence frequency of Group B which $= q^2 + 2qr = 0{\cdot}0064 + 2 \times 0{\cdot}08 \times 0{\cdot}67 = 0{\cdot}11$, i.e., 11 per cent., which agrees well with the 10 per cent. given in the table on p. 69.

Also $AB = 2pq = 2 \times 0{\cdot}25 \times 0{\cdot}8 = 0{\cdot}4$, i.e., 4 per cent., which agrees equally well with the 5 per cent. given in the table. Indeed, the figures in the table only represent the actual observed values in round numbers. In actual fact the observed frequencies found from an examination of 20,000 cases are : Group O = 45 per cent, Group A = 41 per cent ., Group B = 10 per cent., Group AB = 4 per cent.

Hence, these formulæ, in addition to enabling us to calculate group frequencies, afford the strongest possible evidence not only of the hereditability of the group factors, but also of the particular manner in which they are transmitted. Indeed, von Dungern and Hirszfeld first suggested that the number of transmissible group factors were not three (viz., A, B, and O), but two (viz., A and B), which were dominant to the characters a and b. On the basis of such an hypothesis we get the following calculation :—

If p and q are the respective frequencies of the factors A and B, and r and s those of a and b, then

$(p + q) = 1$, and $(r + s) = 1$, so that the possible combinations of these various factors are given by the various terms of the expression $(p + q)^2 (r + s)^2$, i.e.,

$$p^2r^2 + r^2q^2 + q^2s^2 + s^2p^2 + 2pqr^2 + 2pqs^2 + 2rsp^2 + 2rsq^2 + 4pqrs.$$

Hence, the various *group* frequencies in the population are :—

Group O $= q^2s^2$,
Group A $= p^2s^2 + 2pqs^2$, (p^2s^2 representing the homozygotes AAbb, and $2pqs^2$ the heterozygotes Aabb) $= s^2(1 - q^2)$,
Group B $= q^2(1 - s^2)$, in a similar manner, and
Group AB $= p^2r^2 + 2pqr^2 + 2rsp^2 + 4pqrs = (1 - q^2)(1 - s^2)$.

Now from equation $O = q^2s^2$, we get $s^2 = \frac{O}{q^2}$, and from equation $B = q^2(1 - s^2)$, we get $q^2 = B + q^2s^2 = B + O$.

$\therefore$ s^2 which $= \frac{O}{q^2} = \frac{O}{O + B}$,

Similarly, $q^2 = \frac{O}{O + A}$

Hence, frequency of Group AB, which is given by $(1 - q^2)(1 - r^2)$, is

$$(1 - \frac{O}{O + A})(1 - \frac{O}{O + B}) = \frac{A \times B}{(O + A)(O + B)}$$

Substituting now the values for O, A and B, as given in the table on p. 69, we get

$$AB = \frac{0{\cdot}40 \times 0{\cdot}10}{(0{\cdot}45 + 0{\cdot}40)(0{\cdot}45 + 0{\cdot}10)} = 9 \text{ per cent.}$$

instead of the actual 5 per cent.

Similarly, with the distribution of the various groups in different races, we find that the theoretical frequency of AB as obtained by means of Bernstein's formula agrees very closely with the observed frequency ; but when calculated by means of the Hirszfeld formula the result is greatly in excess of the observed

figure. Hence, we are entitled to say that *on mathematical grounds the theory of Bernstein, i.e., of triple allelomorphs, takes precedence over the double allelomorph theory of v. Dungern and Hirszfeld. Indeed, there is every reason to believe that Bernstein's theory represents truly the actual mode in which the transmission of the group factors from parents to children is effected.*

Were the matter one of purely academic interest only, a discussion of the relative merits of the two theories would be entirely out of place in a book of this size, whose aim is mainly practical. But the matter has an important practical bearing, inasmuch as the children of matings of parents belonging to certain different groups can on the Hirszfeld theory belong to certain groups from which they would theoretically be excluded on Bernstein's theory. Thus, although when neither parent belongs to Group AB, the possible and impossible groups to which the children can belong are the same on either theory, this is not the case when one or other parent does belong to that group. In that case the children can, on Bernstein's theory, belong to either A or B only (in equal numbers in the case of combination O × AB), or to groups A, B and AB in the case of the other combinations (see table, p. 78), they can, on the Hirszfeld theory, belong in all such cases to *all* groups. The validity of one or other theory is therefore of medico-legal importance in some cases of disputed paternity or cognate problems mentioned in the last chapter. From what has just been said it must be concluded that the possible and impossible groups to which the children of different matings can belong are those given in the table on p. 78. Indeed, actual observations by a number of observers on 214 families of the O × AB combination, with 747 children, have shown that there were 298 children of Group A, and 293 children of Group B, which not only accounts for nearly 80 per cent. of the children, but also

satisfies the 1 : 1 ratio demanded by Bernstein's theory. In addition, three of the remaining children have since been definitely discovered to be illegitimate, leaving 153 children of other groups whose existence is to be accounted for on Bernstein's theory. The majority of the children can be accounted for on the basis of illegitimacy ; for although there is no definite evidence of such a supposition, nevertheless the fact is remarkable that the greater number of Group O children came from families in which the mother rather than the putative father belonged to Group O, while the greatest number of the Group AB children came from families in which the mother rather than the putative father belonged to Group AB. Such children can therefore be attributed to extramarital unions in each case. In the few remaining cases which cannot be explained in this manner, the possibility of a faulty technique in the grouping may provide the explanation ; and here again it is a remarkable fact that such discrepancies mostly occurred in the earlier researches, i.e., before the year 1925, when some of the pitfalls in the technique of blood-grouping were not yet fully appreciated.

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