alternate freezing and thawing, by the serum from the blood of another animal, electricity, and numerous other methods.

Isotonic Solutions, as applied to the blood, refer to liquids containing soluble substances with an attraction for water, in quantities sufficient to prevent the imbibition of water by the red corpuscles. The liquid must be concentrated enough to prevent the absorption of water by the corpuscle. A certain amount of water exists normally in the corpuscle. If the concentration of the surrounding liquid is diminished this equilibrium is disturbed and water passes into the corpuscle; enough water may pass into it to cause swelling and ultimately the discharge of the hæmoglobin, thus producing laky blood.

A solution containing a lesser percentage of salt or other soluble substance than suffices to keep the hæmoglobin is known as "hypisotonic"; while a solution containing a greater percentage of salt than will preserve the equilibrium is known as "hyperisotonic." The essential fact is an alteration of the permeability of the corpuscle either by the solution or mechanically, so that the hæmoglobin, or one or more of its constituents, diffuses itself into the surrounding fluid.

In isotonic solutions the concentration varies according to the substance used. Thus a solution of sodium chloride from 0.64 to 0.9 per cent., or of sugar 5.5 per cent., or of potassium nitrate 1.09 per cent., is isotonic to the corpuscles, or, at least, the latter do not imbibe water sufficient to cause them to discharge their hæmoglobin. The isotonic relations of certain substances have been quite well worked out for the red blood corpuscles; but it seems reasonable that the other cells of the body have likewise an isotonic relationship with fluids coming in contact with them; and speaking generally, it may be said that normal blood and lymph are isotonic to the various tissue elements, and must be kept so in order to avoid pathological conditions.

White Blood Corpuscles or Leucocytes.—These elements were discovered by Hewson in 1770. They are uncolored bodies possessing the power of amœboid movement, are often of a spherical form, and are slightly larger but much less numerous than the red corpuscles. The investigations of Ehrlich, Schultze, and others have shown that there are at least three different forms of leucocytes present in the blood, corresponding, perhaps, to three stages in their development: (1) A small spherical cell, with scanty protoplasm and a relatively large nucleus, amœboid movement very doubtful. They are called lymphocytes because they resemble the leucocytes found in lymph glands and are supposed to be brought into the blood through the lymph. (2) Mononucleated leucocytes. They possess a greater amount of cytoplasm, are of a finely granular character, and have some power of amœboid movement. (3) Polynucleated or polymorphous leucocytes. They are large, irregular in form, finely granular, and possess a lobulated nucleus or as many as four separate nuclei. They have very pronounced amœboid movements, and form about 70 to 75 per cent. of the white corpuscles.


Fig. 526.—Ehrlich's Granules in Leucocytes. × 1,200 times. α, acidophile granules, relatively large and regularly arranged; ε, neutrophile granules; β, amphophile granules, not numerous and irregularly arranged; γ, mastzellen with granulations of unequal size; δ, basophile granulations. α, δ, and ε, from normal blood; γ, from leukæmic blood of man; β, from the blood of the guinea-pig. (From Boehm and von Davidoff, after H. F. Müller.)

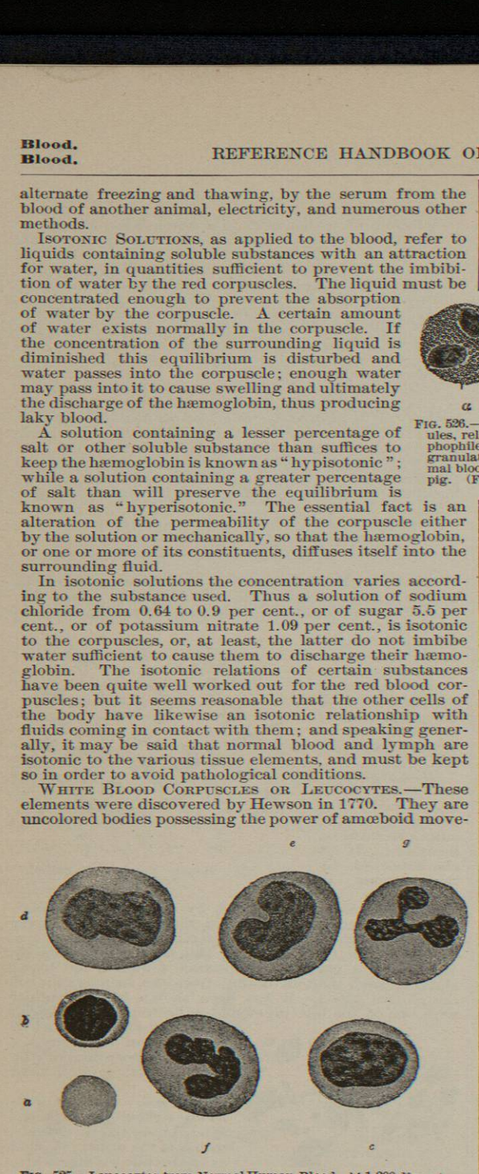


Fig. 525.—Leucocytes from Normal Human Blood. × 1,200 diameters. (From Boehm and von Davidoff, after H. F. Müller.) *a*, Red blood disc; *b*, small mononuclear leucocyte; *c*, large mononuclear leucocyte; *g*, leucocyte with a nucleus of very irregular shape; *d*, *e*, *f*, leucocytes representing transitional forms between *c* and *g*.

Other classifications have been made, based upon microscopic structure and reaction to stains. Ehrlich divides the leucocytes into three groups, according to the size and the staining of the granules found in the cytoplasm. The *oxyphiles* or *eosinophiles* or acidophiles are those leucocytes in which the granules stain only with acid aniline dyes—*i.e.*, where the acid portion of the dye acts as the stain (eosin, picric acid, aurantia). The *basophiles* are those in which the granules stain only with basic dyes (dahlia, acetate of rosanilin). The *neutrophiles* are those in which the granules stain only with neutral dyes (picrate of rosanilin). A later paper by Kanthack and Hardy states that the neutrophile granules of Ehrlich are in reality oxyphile granules. In human blood they find the finely granular oxyphile cells (neutrophile and amphophile cells of Ehrlich) make up 60 to 80 per cent. of the whole number of leucocytes, the lymphocytes (and hyaline cells) (basophile granules) 20 to 30 per cent., and the coarsely granular oxyphile cells (eosinophile cells of Ehrlich) less than 5 per cent., but these proportions are far from constant.

Ehrlich's classification is perhaps more suitable for pathological than for normal conditions of the blood.

It is said that the eosinophiles and neutrophiles are relatively few in normal blood. Eosinophile granules occur in the polynucleated cells. These cells are greatly increased in leukæmia. The basophile granules occur also in connective-tissue corpuscles, especially in the neighborhood of epithelium; they are always greatly increased where chronic inflammation occurs. The large polynucleated amœboid cells found outside the vessels in inflammations exhibit a neutrophile reaction. Neutrophile granules are not found in the lymphocytes nor in the mononuclear cells. It is questionable if such a classification even for pathological conditions is wholly satisfactory, since no definite function for the granules has yet been established, and it is still undetermined whether the specific granules are permanent or temporary structures.

It is indeed a question if the different forms of leucocytes are distinct histological elements having independent origins and different functions, or whether they do not, after all, represent different stages in the development of a single kind of cell, the lymphocytes representing an early and the polynucleated leucocytes the last stage.

*Specific Gravity.*—Leucocytes have a lighter specific gravity than the red corpuscles, as is shown when the blood clots slowly, as it does normally in some animals, *e.g.*, the horse. The red corpuscles, on account of their greater density, sink to the bottom, while the leucocytes, being much lighter, form the upper portion of the clot.

*Number.*—The leucocytes average about 1 for every 500 of the red corpuscles, or 10,000 per cubic millimetre in man and 9,000 per cubic millimetre in woman. The number will vary under different conditions. They are increased after digestion, hemorrhages, pregnancy, in diseases in which suppuration occurs, and in leucocythæmia, in which disease they may equal even the red corpuscles.

Conditions which diminish the number of leucocytes are fasting, old age, and the action of certain medicines.

Leucocytes are more numerous in the capillaries and veins of the spleen, liver, glands, and intestinal mucosa than in the corresponding vessels of the skin, muscles, and general cellular tissue. Leucocytes are also more numerous in the blood of the newly born—about 19,000 per cubic millimetre (Hayem).

The chemical constituents of leucocytes are variable and difficult to analyze with accuracy. Lilienfeld, who has carried on investigations along this line, gives the following quantitative composition:

| | |
|---|---|
| Water | 88.51 |
| Solids | 11.49 |
| Proteid | 1.76 |
| Nuclein | 68.78 |
| Histon (proteid part of nucleo-proteid) | 8.67 |
| Lecithin | 7.51 |
| Fat | 4.02 |
| Cholesterin | 4.40 |
| Glycogen | .80 |

Pus cells are leucocytes which show a considerable amount of fatty degeneration and are generally dead.

*Functions.*—The functions of leucocytes are various; some theories formerly accepted are now advanced with doubt, among which may be mentioned the assistance supposed to be rendered in the absorption of peptones and fats. Recent and careful experiments point toward the intestinal epithelial cells as the important factor in this process. Peptones appear to be converted into coagulable albumin during their passage through the epithelium, as practically no peptone is found in the blood or chyle. It has likewise been quite satisfactorily demonstrated that by far the greater amount of fat is likewise taken up by the epithelium and but little, if any, by the leucocytes.

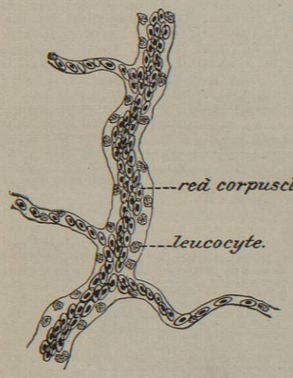


Fig. 527.—A Vein (Frog), As It Appears When the Current Begins to Slow. The red corpuscles remain in the central portion of the blood stream, while the leucocytes creep slowly along the wall of the vessel, sticking here and there. (After Craig.)

Leucocytes, or their products, are an undoubted factor in the coagulation of blood. Further details will be discussed under Coagulation.

One of the most peculiar properties of leucocytes is their power of independent movement, in virtue of which they are often called "wandering" cells. A leucocyte may be considered in some ways as a typical cell formed of unmodified protoplasm, wholly unspecialized and potentially equivalent to a unicellular animal, *e.g.*, an amœba. Movement is accomplished by the pushing out of a portion of the cell substance to form a pseudopodium; this may be retracted, and other pseudopodia formed, and in this way the corpuscle may move or "flow" from place to place, and envelop or ingest such particles as come in its way. This form of motion was first noticed in the amœba, and is therefore called amœboid in the leucocyte. In a certain sense *body* corpuscles would be a more appropriate term than leucocytes; for they or similar cells are found in many locations and wander everywhere in the spaces of the connective tissue. They pass into the blood-vessels with the lymph, and pass out of them again in virtue of their power of amœboid movement. This process of migration occurs normally, but is greatly increased under certain pathological conditions. This phenomenon of migration is known as diapedesis (discovered in 1846 by Waller), and is especially prominent when a vascular part is irritated, causing dilatation and congestion of the vessel. The leucocytes then begin to adhere to the wall; they accumulate and finally pass through, probably *between* the endothelial cells of the capillaries. After this emigration they may wander away, or become organized and form new tissue (Waller), or they may accumulate, degenerate, and form pus. Red corpuscles are also sometimes seen to pass through the walls. Leucocytes may, however, play another part, namely, the repair of injured tissues. A clean healthy wound is soon covered with a layer of lymph, which is largely composed of white corpuscles. If repair progresses in a healthy manner the leucocytes may become organized and assist in the development of connective tissue, forming the cicatrix by which the wound is closed.

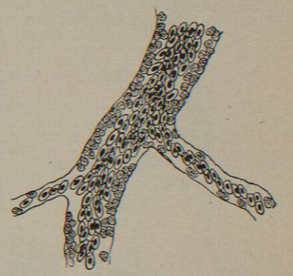

Fig. 528.—Showing a Vessel in Which the Current has Nearly Come to a Standstill. The leucocytes are arranged in rows along its wall. (After Craig.)

Certain amœboid cells of the blood, lymph, and splenic pulp are able to ingest or "eat up" foreign particles with which they may come in contact. This process is known as phagocytosis, and the cells are therefore known as phagocytes. This term, however, does not include all leucocytes nor does it exclude all other cells, as some fixed cells—*e.g.*, the endothelial cells of blood-vessels—show phagocytic properties by being able to send out protoplasmic processes, while, on the other hand, the small immobile lymphocyte is not a phagocyte. Although the phenomenon of phagocytosis has not as yet as exact a physiological value as some other processes, there is little doubt but that it plays an important rôle in the defence of the organism from outside invaders, as, for example, pathogenic bacteria, etc. This action of the phagocytes is of very great interest and importance. Metschnikoff, from his investigation upon the lower as well as the higher organisms, has added much useful information to science. He found that in some instances the leucocytes were victorious over the invaders, the latter being ingested and destroyed. In other cases the invaders were successful, by poisoning, weakening, overpowering by larger numbers and ultimately destroying the leucocytes. Metschnikoff supposed that the immunity to certain diseases, possessed normally by some animals and conferred on others by vaccination with certain protective substances, is due largely to the success of the phagocytes in the fight with the bacteria. More recent investigations show that Metschnikoff's phagocytic theory of immunity requires some modification, at least for man and the higher animals, although some of the observations upon which his theory was built still retain their value. He concluded that in order to confer immunity, the leucocytes under-

went certain changes and acquired a kind of "education" which enabled them to fight bacteria against which they were previously helpless. It seems in the light of the

FIG. 529.

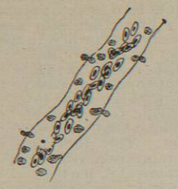

FIG. 530.

FIG. 531.

FIG. 532.

FIGS. 529 TO 532.—FIG. 529.—Leucocytes sending forth processes which penetrate the wall of the vessel. (After Craig.) FIG. 530.—Leucocytes partly through vessel wall, showing constriction in centre. (After Craig.) FIG. 531.—Leucocytes after penetrating wall regain former shape. (After Craig.) FIG. 532.—Appearance of vessel and surrounding tissue after diapedesis has gone on for some time. (After Craig.)

more recent investigations that the substances which can confer immunity may be present not only in the leucocytes, but in other cells as well, and even in the serum. Such substances have the power to destroy, or at least inhibit, the growth of bacteria.

Correlated with the question of phagocytosis is the question of the ultimate fate of the particles ingested by the leucocytes. This matter has been investigated by Miss E. J. Claypole in amphibia (*Necturus* and *Cryptobranchus*) and by Berry in some of the mammals (rat, rabbit, and dog), the latter corroborating in all important respects the statements of the former. A further important function of the leucocytes, as scavengers of the body, is suggested. Miss Claypole says: "Considering the leucocytes as scavengers of the animal body in addition to their other functions, if their individual fate is inseparable from that of the waste material which they contain, they themselves must become waste material when their active functions cease, and must ultimately disappear from the organism. In the first place, they wander from the circulation through the tissues to the epidermal and mucous surfaces (amphibia), and are carried away with the other waste products of the body; in the second place, they are destroyed in the spleen by the splenic cells. The large numbers found in various stages in the first condition and the relatively large number of ingested spleen cells prove that the destruction of leucocytes is by no means insignificant."

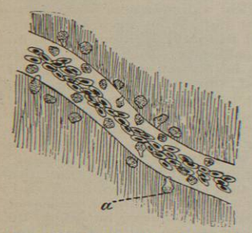

FIG. 533.—Small Vein of Mesentery. Showing the arrangement of the granules during and after penetration of wall by the leucocyte. (After Craig.)

Maurel takes the view that the different forms of the leucocytes are merely the intervening stages in their development. He does not accept the view that leucocytes multiply in the blood. He has seen what he terms apparent division of the corpuscle, one portion budding off from the parent substance but still connected by an almost invisible strand, and later a complete reunion of the two portions. According to his classification there are three main types: 1. A small, colorless and immobile leucocyte, evidently corresponding to the lymphocyte of other writers. 2. The second type consists of five forms of leucocytes, all of which are characterized by the power of displacing a larger or smaller amount of their substance and possess a greater or less degree of mobility. They differ in respect to their granularity. 3. Two forms, one very granular, immobile, and deformed; the other showing beginning signs of disintegration.

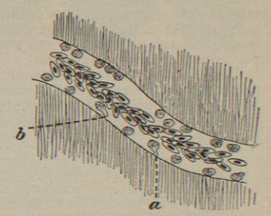

FIG. 534.—Small Vein in Mesentery. Leucocytes sending off a process clear of granules (*a* and *b*). (After Craig.)

The above types represent the periods of development and decay; the second type representing the active life of the corpuscle.

In determining the effect of temperature upon the leucocytes of the human blood, Maurel found that at about 25° C. (77° F.) movements as evidenced by displacement of their cellular substance were generally manifested. Between 25° and 16° C. these displacements were very feeble, and at 16° they ceased. If this temperature, however, was of short duration and gradually raised the movements would return. At 14° C. (57° F.) life disappeared from the corpuscles completely. He concludes that a temperature of at least 25° is required to cause satisfactory movements in the cells and that the above temperature is essential for the occurrence of diapedesis. Between 25° and 32° C. the displacement of substance is very slight. From 32° to 39° C. the movements are very marked. From 39° C. (102° F.) to 43° C. (109° F.) the maximum degree of activity occurs. Above 44° C. (111° F.) the life of the corpuscle is very quickly menaced, and at 45° C. (113° F.) the leucocyte takes on a spherical form and dies in a short time. The temperature as usually taken (axilla, etc.) is supposed to be one to two degrees lower than the internal temperature (liver, etc.), so that from the above figures, the greatest activity of the leucocytes in the human body takes place at the normal and during a febrile state of medium intensity. In animals of different species in which the temperature is normally different from that of man, the variations in the activity of the leucocytes are correlated with the temperature of that species of animal.

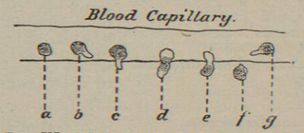


FIG. 535.—Diagrammatic Representation of the Manner in Which a Leucocyte Traverses the Wall of a Capillary Blood-Vessel. (After Craig.) *a*, Leucocyte before penetrating; *b*, leucocyte sending off process and granules beginning to withdraw to farther end of cell; *c*, leucocyte partly through wall, granules at upper end of cell; *d* granules passing through the wall; *e*, granules arranged in the portion of the leucocyte farthest from vessel; *f*, leucocyte, after penetration, resuming its original condition; *g*, leucocyte swept from wall, showing retention of the clear penetrating process.

In addition to studying the power of resistance exhibited by the leucocytes with respect to extremes of temperature, Maurel has also investigated their power of resistance against certain physical and chemical agents and drugs. As with temperature, the leucocytes of different species will react differently toward the agents just mentioned. He found that while human leucocytes are sensitive to cocaine and very much so to atropine, those of the rabbit were only one-third as sensitive to cocaine and not at all to atropine. The leucocytes of the frog will absorb certain pathogenic bacteria with impunity, while those of the rabbit and man will not absorb them, but are rapidly killed.

Horbaczewski has called attention to the fact that uric acid originates chiefly from the nuclein of disintegrated leucocytes, and the greater the number of leucocytes in the blood the greater is the destruction of the same, and hence the elimination of uric acid is correspondingly increased. Observations on the elimination of uric acid harmonize with this theory. In leukæmia, where there is an abnormally large number of leucocytes, the elimination of uric acid is greatly increased. Those drugs which increase the number of leucocytes also increase, in general, the elimination of uric acid.

*Origin of Leucocytes.*—It is evident that leucocytes are formed largely in lymph glands, from the fact that the lymph leaving such glands is much richer in corpuscles than the lymph coming to the glands. The fact that lymph coming to the glands contains leucocytes indicates that, although some of them may get into the lymph by diapedesis, other parts are also concerned in their production, *e.g.*, diffuse adenoid tissue, or special collections of it such as the tonsils, Peyer's patches, and the solitary follicles in the intestine, and the splenic corpuscles. To a small extent the white corpuscles may multiply in the blood by karyokinesis. The latter method may perhaps occur more generally in the so-called cold-blooded animals than in mammals.

*The fate of the leucocytes* is much less easy to ascertain than that of the red corpuscles from the fact that the former contain no such pigment as hæmoglobin or other substance that would assist in following the path of their destruction. It is not improbable that some of the leucocytes undergo disintegration in the blood itself and that their constituents aid in maintaining a proper proteid equilibrium. It has been shown by the work of Miss Claypole and of Berry that many of the leucocytes are undoubtedly destroyed in the spleen, and it is not at all unlikely that other localities are also involved in this process. That they are constantly breaking down is certain, for they are constantly being produced.

BLOOD PLATELETS OR PLAQUES.—The *Blutplättchen* (Bizzozero) or hæmatoblasts (Hayem) are small, circular, sometimes irregular bodies appearing nearly homogeneous in structure and varying in size (0.5 to 5.5 microns). They average about 3 microns in diameter and are always smaller than the red corpuscles. Hayem's view that they are hæmatoblasts or precursors of the red corpuscles is now considered erroneous. Their number in the blood has been variously estimated from 180,000 to over 600,000 per cubic millimetre. Taking 400,000 as the average number, they would be forty times as numerous as the leucocytes and about one-twelfth as numerous as the red corpuscles. Although there has been a great amount of histological research upon the platelets, very little is known of their function or chemical composition. It is generally believed that they are not independent cells. In drawn blood they disintegrate almost immediately; this fact prevented their discovery for a long time after the blood had been studied microscopically. To study them, the blood must be drawn at once into some fixing solution (osmic acid, etc.). They can be seen, however, in capillary blood-vessels which have just been removed from animals, in which the blood is still fluid and the constituents therefore still alive. They have not been found in lymph. According to Löwit they consist chiefly of a globulin and play an important part in fibrin formation (coagulation). Lilienfeld from a chemical standpoint considers that they consist of nucleo-proteid, a substance like that obtained from the nuclei of leucocytes. From this ground, some take the view that the platelets are derived from the nuclei of the leucocytes, and that when the multinucleated forms go to pieces the fragments of the nuclei persist for a longer or shorter time in the blood as platelets and are eventually dissolved in the plasma. The special function of the platelets, beyond that of assisting in coagulation, still remains to be discovered.

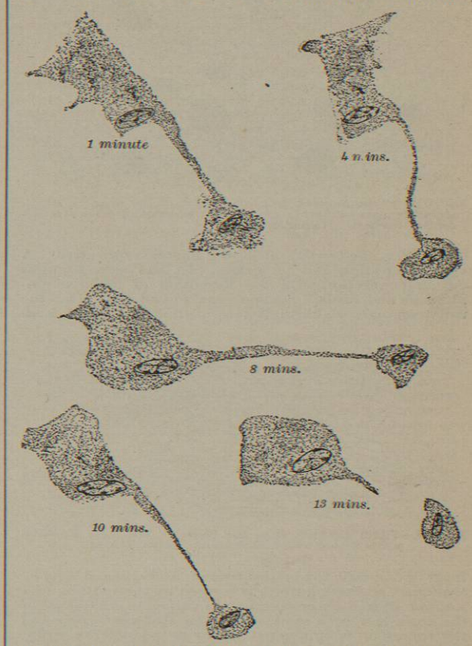


FIG. 536.—Figures of a Leucocyte Undergoing Division (Amphibia). (After Claypole.)

FIBRIN.—When blood coagulates there separates from it an albuminous substance, which is nearly insoluble. This substance is fibrin, and remains in the clot. If coagulation is prevented by whipping the blood, the fibrin adheres to the rods of the whip in the form of elastic threads or fibrin masses. These may be washed free from the adhering corpuscles by using a stream of water. While wet the fibrin has a white, stringy appearance, later drying into an irregular mass. The threads which compose fibrin, as seen in a microscopic preparation of blood, interlace with one another and form a fine network, which entangles the blood corpuscles in its meshes. These have a strong tendency to retract, and explain why a clot shrinks and squeezes out the serum from its interior.

Fibrin exists in the lymph as well as in the blood.

The blood of mammals and of amphibia, as shown by Gage, exhibit an interesting difference in regard to the fineness of the constituent threads. In the frog (*Necturus* and *Cryptobranchus*) the threads are relatively very much finer than those in mammals, requiring careful observa-

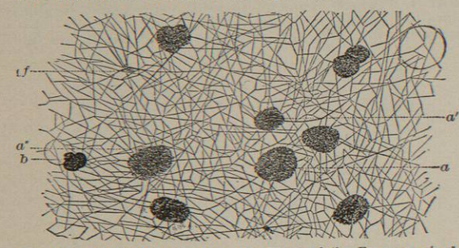


FIG. 537.—Fibrin Network from the Blood of the Frog. *a*, *a'*, *a''*, Granular mass of fibrin; *gf*, isolated granules of fibrin; *b*, deformed leucocyte. (After Renaut.)

tion with a one-twelfth-inch oil-immersion objective. A very interesting and important fact has been brought out in connection with the formation of fibrin in amphibians and birds. Semmer has shown that after the usual amount of fibrin has formed in the blood from

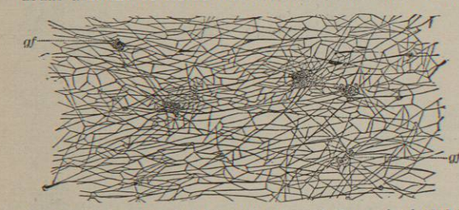


FIG. 538.—Fibrin Network from the Blood of Man. *gf*, *gf*, Nodal points formed by grains of fibrin. (After Renaut.)

frogs or domestic fowls (also true of *Necturus* and *Cryptobranchus*), additional fibrin is formed by the addition of a solution of sodium or magnesium sulphate. After all of the fibrin had been removed in the customary way and the source of it eliminated as far as possible by whipping the blood and pouring off the serum and white corpuscles, when the salt solution was added additional fibrin appeared, which seemed to differ in no way from the original fibrin. Experiments carried on in exactly the same way upon the blood of mammals failed in every instance to develop any extra fibrin, from which it is concluded that the additional fibrin, in the cases cited, was due to certain substances contained in the nucleated red corpuscles, these substances having been liberated by the solvent action of the salt. A probable explanation of this phenomenon would seem to be that in all nucleated red corpuscles nucleo-proteid material is present; in non-nucleated corpuscles this material is absent or very slight in amount. As the nucleo-proteids are believed to be essential in causing the blood to clot (see under *Coagulation*), we can readily understand that the additional supply of nucleo-proteid set free from the disintegrated nucleated red corpuscles could assist in the formation of more fibrin. Since the non-nucleated corpuscles contain none or an insufficient amount of the nucleo-proteid material, no additional clotting or fibrin was formed. It would seem that this question of the presence or absence of nucleo-proteids in the red corpuscles is an important one in explaining some of the differences that exist in coagulation and fibrin formation in the mammals on the one side and the birds and amphibia on the other.

Fibrin is insoluble in water, alcohol, and ether. It expands in 0.1 per cent. hydrochloric acid, also in 0.1 per cent. caustic potash or soda, to a gelatinous mass, which after several days will dissolve at the ordinary temperature; it will dissolve more readily but still slowly at body temperature. Fibrin is also slowly soluble in five to ten per-cent. solutions of certain salts, such as sodium chloride, sodium sulphate, potassium nitrate, magnesium sulphate, ammonium sulphate, also in iodides and in solutions of urea. It is said that the fibrin obtained from venous blood is slightly more soluble in salt solutions than that obtained from arterial blood.

Blood yields from 0.2 to 0.4 per cent. of its weight of dry fibrin (Schäfer).

For the elementary composition of fibrin, Hammarsten gives the following:

| | |
|---|---|
| C | 52.68 |
| H | 6.83 |
| N | 16.91 |
| S | 1.10 |
| O | 22.48 |

The way in which fibrin is formed and certain other phenomena will be discussed under *Coagulation*.

PLASMA AND SERUM.—It is convenient to describe these two components of the blood together, for, with the exception of certain proteids or fibrin factors present in the plasma, the remaining constituents are identical with those in serum. The relationship of the different parts of the blood to each other may be conveniently shown as follows:

Living blood = plasma + corpuscles.
Dead blood = clot + serum.
clot = corpuscles + fibrin.
serum = plasma − fibrin(ogen).
plasma = serum + fibrin(ogen).

Plasma contains about ten per cent. of solids, the red corpuscles about forty per cent.

Plasma may be separated from the corpuscles in various ways. A common method is to collect the blood in a receptacle surrounded by ice. Cold retards coagulation so that the corpuscles may have time to settle at the bottom of the jar, leaving the clear plasma entirely separated. Another method is by the use of the hæmatocrit, centrifugalizing, etc.; the corpuscles accumulate in an almost solid mass and the volume can be read directly. The average percentage of corpuscles in human blood, as obtained by various methods, is 48 per cent. for males, very nearly one-half of the entire amount of blood; in females it is 43.3 per cent., and in children from six to thirteen years it is about 45 per cent.

*Salted Plasma.*—Coagulation may be delayed by the addition of certain substances to the blood, among which

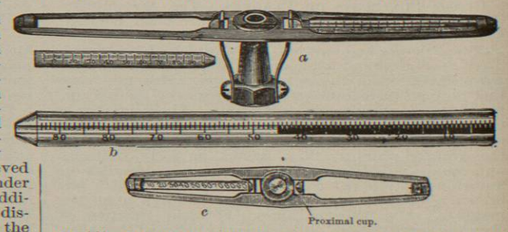


FIG. 539.—Daland's Hæmatocrit. *a*, Tubes have lens fronts magnifying the column of blood. (About half actual size.) *b*, Percentage tube. (Nearly twice actual size.) *c*, Top view of hæmatocrit. (One third actual size.)

are neutral salts. If the blood flowing from a cut artery is allowed to mix with an equal volume of a saturated solution of sodium sulphate, or with a ten-per-cent. solution of sodium chloride, or with one-third of its bulk of a saturated solution of magnesium sulphate, coagulation will be prevented and the corpuscles will subside, leaving the plasma diluted with the salt solution (salted plasma) clear. If, however, the above solutions are diluted with a sufficient amount of water, coagulation will usually occur.

*Oxalate plasma* is obtained by the addition of potassium or sodium oxalate to the blood; these salts combine with the calcium of the blood forming the insoluble calcium oxalate. The corpuscles and plasma separate as previously described and coagulation is prevented. If, however, more calcium be added to the mixture, coagulation occurs. The importance of the presence of calcium salts in the process of coagulation will be referred to later on.

*Gases.*—The gases present in the plasma have not been altogether satisfactorily investigated. They are probably not very different from those in the serum. According to Bunge, in the dog they consist of from 43 to 57 volumes of carbonic anhydride, 2.25 of nitrogen, and 0.25 of oxygen. The oxygen and nitrogen are probably simply in solution in the plasma, the carbonic anhydride for the most part being in chemical combination with sodium as the carbonate and bicarbonate. The alkaline phosphates also exist in combination. In the corpuscles, where they are present in considerable quantity, they may play an important part in fixing the $CO_2$.

*Chemical Analysis of Plasma.*—The average of three analyses of the plasma from the horse, made by Hammarsten, is given below, 1,000 parts having been used. An analysis by Hoppe-Seyler, also of the horse, is given for comparison.

| | Hoppe-Seyler. | Hammarsten. |
|---|---|---|
| Water | 908.4 | 917.6 |
| Solids | 91.6 | 82.4 |
| Total proteids | 77.6 | 69.5 |
| Fibrin | 10.1 | 6.5 |
| Globulin | .... | 38.4 |
| Serum albumin | .... | 24.6 |
| Fat | 1.2 | |
| Extractive substances | 4.0 | 12.9 |
| Soluble salts | 6.4 | |
| Insoluble salts | 1.7 | |

*Inorganic Substances in Plasma.*—Plasma consists of about ninety per cent. of water, the inorganic salts occurring to the extent of about 0.8 per cent. The principal salt is the chloride of sodium. Carbonate of sodium is probably the next most abundant salt, and it is to this salt chiefly that the plasma owes its alkalinity and its power of absorbing carbonic acid. In addition to these, small amounts of the following salts appear to be present: chloride of potassium, sulphate of potassium, phosphate of calcium, phosphate of sodium, and phosphate of magnesium, and probably chloride of calcium.

*Organic Constituents.*—For convenience these may be divided into proteids (serum albumin, etc.) and non-proteids, and the latter again divided into nitrogenous (kreatin, etc.) and non-nitrogenous (dextrose, etc.).

*Proteids.*—The proteids of plasma are: one or more closely allied albumins (serum albumins); two globulins, namely, serum globulin and fibrinogen; a nucleo-proteid or nucleo-proteids. Blood normally contains neither albumose nor peptones. The *albumins* of plasma are also found in the serum after the blood has coagulated, and from this fact they have doubtless received the name of serum albumins. According to its susceptibility to heat coagulation, Halliburton has shown that there are in reality three kinds of albumin: $\alpha$-albumin coagulating at 72 to 75° C.; $\beta$-albumin at 77 to 78° C.; and $\gamma$-albumin at 83 to 86° C. In the plasma of horse, ox, and sheep blood, the $\alpha$-albumin is absent, the other two are present; in man and all other mammals and birds investigated by Halliburton, all three were found present. In the reptiles, amphibia, and fishes examined only the $\alpha$-albumin was found.

The *globulins* of plasma consist of serum globulin and fibrinogen. Serum globulin (paraglobulin, Kühne; fibrino-plastic substance, A Schmidt) coagulates at a temperature of 75° C. This degree of coagulation is almost constant for the animals examined. According to Hammarsten the percentage of the globulins in the plasma of man is 3.10 per cent.; in the horse 4.56 per cent.; in the ox 4.17 per cent.

The *fibrinogen* is the substance which is responsible for the so-called spontaneous coagulability of the plasma. It is precipitated from plasma along with serum globulin by saturation with magnesium sulphate or sodium chloride. This precipitate forms the plasmine of Denis, and on being dissolved in a more dilute solution of salt causes coagulation. As a result of numerous researches, it seems likely that fibrinogen is not a simple substance, but is probably either a mixture or a loose combination of three substances: (1) fibrinogen proper, coagulating at 56° C; (2) fibrino-globulin (Hammarsten), coagulating at 65° C.; (3) a nucleo-proteid.

Mathews,* in a recent article on the origin of fibrinogen, concurs with Hoffmeister in the view that leucocytes are concerned in proteid digestion, basing the evidence chiefly on the fact that the blood proteids remain practically constant, even during fasting. Under the latter condition the proteid material is derived from the wasting tissues and the leucocytes are considered as carriers of the proteid to the blood, in a way analogous to that seen in the phagocytes during the removal and assimilation of the wasting tissues of the tadpole's tail, etc.

His experiments also confirm those of Bizzozero and Dastre, that by successive bleedings, defibrinations, and reinjections the fibrinogen may be completely removed from the blood of dogs and cats. The lack of fibrinogen appeared to cause no serious or characteristic symptoms. After the withdrawal of the fibrinogen, in the manner above described, it redeveloped in the body with considerable rapidity, reaching its normal amount in two or three days; it sometimes developed to more than the normal amount. The redevelopment of the fibrinogen took place normally in the absence of the spleen, pancreas, kidneys, and reproductive organs. Experiments also showed that the muscles apparently had no influence upon the production of fibrinogen. On the other hand, the fibrinogen is not redeveloped (or at a greatly reduced rate) if the small and large intestines be removed. The conclusion is that the intestinal area more than any other part of the body is concerned in the formation of fibrinogen. The blood of the mesenteric vein is constantly richer in fibrinogen than arterial blood, while other venous blood is poorer in fibrinogen than arterial. Beyond the fact that *nucleo-proteid* is present in the plasma and that it seems to be an important factor in the development of fibrin, very little is known of it. It is supposed to be confined to the leucocytes and platelets, and possibly there is a trace of it in the red corpuscles (mammals), which escapes when the blood is shed. The principal reasons for this belief are that there is known to be a considerable amount of nucleo-proteid in leucocytes and similar cells (lymph, thymus, etc.). In plasma where the corpuscles have been precipitated, more nucleo-proteid is obtained from the lower layers of the plasma where the leucocytes are most abundant, and least from the upper layers where the leucocytes are scarce. Again, the pericardial, hydrocele, and ascitic fluids frequently contain no leucocytes. When this is the case no nucleo-proteid is present, and there is no coagulation although the fluids contain fibrinogen.

*Nitrogenous Substances Not Proteids.*—The most important of these are urea (0.02 to 0.05 per cent.), kreatin, kreatinin, and uric acid. According to Grehant and Quinquand, the quantity of urea in the blood taken from the splenic, portal, and hepatic veins is slightly greater than that taken from the carotid artery. Lecithin is also said to occur in plasma. Jecorin is said to be a constant constituent of the plasma. It is a substance which reduces Fehling's solution and is soluble in ether,

* American Journal of Physiology, vol. iii., 1899.

only as may be employed at the bedside, the reader is referred for full information upon the more complicated tests and apparatus for detecting blood stains to the article *Blood Stains*.]

VI. *Dried Specimen of Blood.*
1. Apparatus and technique employed in taking dried specimen of blood.
2. Apparatus and technique employed in fixing dried specimen of blood.
3. The dyes employed for staining the blood.
4. The technique employed in staining the blood.
5. Formulæ of blood stains.
6. Table of all blood cells and their staining peculiarities.

VII. *Table Showing All the Information Obtained by the Use of the Instruments Described in this Article.*

VIII. *A Chart for Making Full Report upon the Blood.*

[NOTE.—The tables which conclude this article are not intended to be complete explanations of the diseases therein mentioned. They are, however, accurate for reference for all blood changes in those diseases. For full instruction upon the following not included in this article the reader is referred to other articles in this HANDBOOK, such as those on *Anæmia*, *Leucocytosis*, *Blood Stains* (*Medico-Legal Consideration*), *Histological Technique*, *Malaria*, *Typhoid Fever*, etc.]

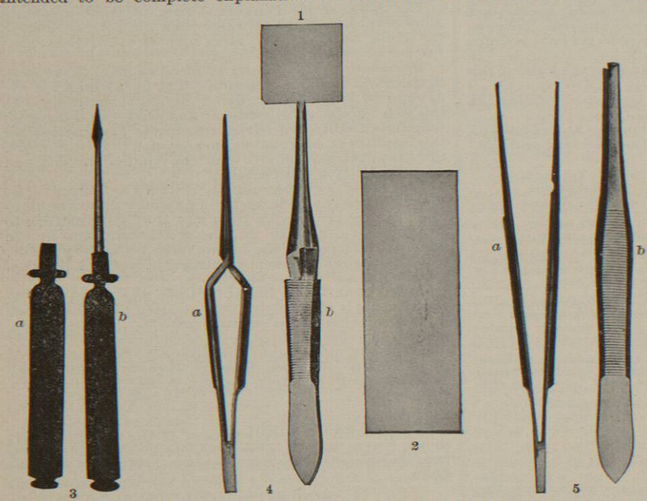


FIG. 540.—1, Bausch and Lomb, ⅝ inch, No. 1 cover slip; 2, Bausch and Lomb, extra thin slide; 3, blood sticker, two views—*a*, point in sheath for carrying instrument in pocket; *b*, point in place ready for use; 4, clamp forceps, two views—side view, showing close apposition of tip of limbs; front view, showing proper extent of grasp of cover slip (1); 5, open forceps, two views—side view, front view. (Note: Instruments made by F. Arnold & Son, instrument makers, Baltimore, Md.)

## I. FRESH-BLOOD SPECIMEN.

In preparing the blood for microscopic examination, however so careful the technique employed, the operator is subjecting one of the most delicate tissues of the body to very rough and unnatural surroundings. When one realizes the exquisite smoothness of the intima of the blood-vessels, the wonderfully regulated temperature of the body, the delicate structure of the red cell, the even more delicate structure of the plate which without most careful preservation is entirely destroyed a few seconds after exposure to the air—when one realizes all these factors against observation of the blood as it appears in the circulation, it is a surprise that any technique is delicate enough. This realization at the same time emphasizes the importance of observing every precaution which experience has shown to be necessary in the preparation of the blood specimen which shall be, outside of the body, as nearly as possible representative of the blood as it appears in the circulation.

The object then of apparatus and technique is to produce surroundings as nearly like those normal to the blood in circulation as possible. To accomplish this we must observe the following: (1) Absolute cleanliness and freedom from moisture; (2) avoid chilling the blood; (3) avoid exposure to air; (4) avoid rough treatment of the drops and consequently of the constituents thereof—the corpuscles.

*The Cover Slip.—Absolute Cleanliness.* The cover slip should be the thinnest made and square in shape (Bausch and Lomb, No. 1, ⅝ in. (see Fig. 540, 1). The thicker cover slips are often too thick for the focus of the one-twelfth immersion lens, and not infrequently specimens from interesting cases at a distance from the laboratory and received by mail cannot be examined accurately owing to this annoying condition. It is a good rule for those engaged in blood work not to have the thicker cover slips under any circumstance.

*The Slide.*—For the same reason the *thin* slide should be employed (see Fig. 540, 2). Two jars, one containing thick slides for urine work, and thin for blood work, will be found a convenient arrangement. These cover slips and slides should be kept in fifty per cent. alcohol. When to be used they should be rubbed with a bit of clean silk, or some fabric free from lint specks. They should be rubbed quite free of any cloudiness or dust and then dried. This last can be done either by pouring a little ether over the glass and rubbing again or by heating gently over a flame. The cleaned slip and slide should now be laid upon a sheet of clean paper. The following instruments, as first recommended by W. S. Thayer of the Johns Hopkins University, will be found essential:

*The clamp forceps* are so made that the extreme tips of the limbs meet before any other portion, and this enables one to take a small but firm grasp of the cover slip (see Fig. 540, 4, *b*). The clamp, moreover, enables the operator to give his whole attention to following the technique, as given below, without being diverted by keeping a grasp on the cover slips. By placing the cover slip in the clamp forceps it can be kept free from dust and moisture from the fingers and will be ready for use.

The lobe of the ear is by far the best locality for puncture. For the following reasons: (1) Less sensitive; (2) the act of puncture and the blood are not seen by the patient, important with nervous people and children; (3) pressure, if necessary, is more readily exerted and produces better results; (4) the skin in this locality is softer and cleaner; (5) there is less likelihood of subsequent infection.

The lobe of the ear is then gently wiped (not rubbed, as this produces hyperæmia) with a silk rag moistened in alcohol and the skin is then dried with ether. If ether is not to be had, it is better either to dilute the alcohol three or four times or to use distilled water. Alcohol if left on the skin hardens the blood and so injures the specimen.

*The Sticker.*—The sticker is now employed (see Fig. 540, 3). This is extremely sharp and is diamond shaped on cross section; the object being to produce a similarly shaped wound, and therefore one which will the more readily bleed. A surgical needle may be used, but an ordinary needle or pin is very unsatisfactory. If, however, one or the other of the latter is all that can be obtained, it should be boiled. When cleanliness is observed, it is only necessary to dip the end of the sticker into alcohol before and after making the puncture.

The most dependent portion of the lobe of the ear is now punctured by a stabbing motion, the sticker being held as in illustration (Fig. 541). The first drop is wiped away, the second allowed to flow, if possible, without pressure being made upon the skin. If pressure be necessary it should be exerted far away from point of puncture, so that this artificial means shall not alter the character of the drop taken for examination.

*Avoid Chilling the Blood.* It must be remembered that the blood is being submitted to a lower temperature than normal in this proceeding, and in order to *avoid chilling the blood* it is a good plan to warm the slide and cover slip over a spirit lamp, or to have another person briskly rub the slide with a bit of silk.

The clamp forceps holding the cover slip (see Fig. 540, 4) are now brought toward the ear, and the under surface of the cover slip—and therefore that free from dust—is made to touch the *apex of the drop* of blood, *not the skin of the ear* (Fig. 542). The cover slip is at once carried to the warmed slide and gently lowered.

FIG. 541.—Method of Making Puncture for Fresh and Dried Specimens of Blood. Shows position of hands and wrist just as puncture is about to be made.

*Avoid Rough Treatment.* The cover slip should not be dropped on the slide. One side of the cover slip should be rested on the slide and gently lowered until the limb of the forceps touches the slide. The forceps are then opened and by a quick jerk drawn away (see Fig. 543).

*Avoid Exposure to Air.* It must be remembered also that the air is a destructive medium to the blood, and the time occupied for these steps must be as brief as possible.

The cover settles and the good blood specimen spreads *equally in all directions*, being for the most part circular with fine spicules at the periphery of the circle. The drop should not be so large as to reach the margin of the cover slip when on the slide.

This technique may be summarized as follows:

1. Apparatus. Thin slide, square thin cover slip, sticker and clamp forceps. Alcohol, ether, spirit lamp, silk cloth.
2. Absolutely clean point of puncture and apparatus.

FIG. 542.—Method of Taking Drop of Blood for Fresh and Dried Specimens of Blood. Shows how to hold forceps and ear, how to steady hands, and the extent of grasp of cover slip by clamp forceps.

3. Seize cover slip in clamp forceps and place near at hand.
4. Light puncture of the most dependent portion of lobe of ear.
5. Warm slide and cover slip gently over spirit lamp; or have another person rub slide briskly with silk cloth.
6. Wipe away drop from point of puncture. (If pressure is required, exert it far away from point of puncture.)
7. Touch apex of drop with under surface and centre of cover slip.
8. Place cover slip promptly but gently on slide.

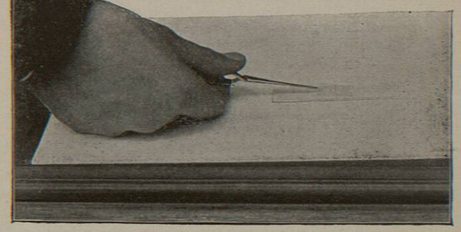

FIG. 543.—Correct Position of Hand in Holding Clamp Forceps. Cover slip resting on slide. The illustration represents the position just before the forceps are opened and jerked away.

*Characteristics of a Good Fresh-Blood Specimen.—Gross:*
1. Blood does not reach margin of cover slip. 2. Is of