the wisdom of employing it in the treatment of fevers and inflammatory diseases—a practice which was so much in vogue at the time when venesection was used empirically. This proposition, however, does not meet with favor. It is maintained that the relief afforded is but temporary, and that the tendency to fatty changes, present in pyrexia, is heightened by the repeated withdrawals of blood. The symptoms undoubtedly show marked improvement immediately after the operation, but the greater debility which results and the prolonged convalescence prevent its general acceptance. The only acute disease in which venesection receives much attention is pneumonia. In addition to the benefits derived from the lessening of the plethora and the easing of the overworked heart, the lessening of the inflammatory process and the probable limiting of the extent of the consolidation must also be considered as results of some value. At all events numerous cases have been reported in which venesection has proved serviceable, and the subject is therefore worthy of every consideration. It is evident, however, that if any benefit is to be derived from this method of treatment it must be begun early before the pathological processes have made much advance. When it is resorted to at the outset, in a plethoric patient with sthenic symptoms, a full pulse, difficult respiration, pain, and fever, the operation acts most favorably, and there is every reason to believe that it renders the attack less severe. Frequently the venesection is resorted to too late in the disease as a last resort, and although the distressing symptoms may be allayed for the moment, the ultimate result is rarely favorable.

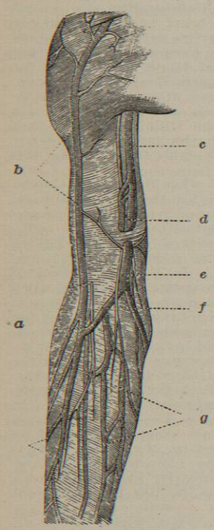

FIG. 601.—The Superficial Veins of the Forearm; Skin Removed. *a*, Median cephalic; *b*, cephalic; *c*, brachial; *e*, basilic; *f*, median basilic; *g*, anterior ulna; *d*, median nerve.

METHOD OF PROCEDURE.—Formerly, blood was withdrawn from the veins of the arm, the external jugular, the veins beneath the tongue, the scrotal veins, and the veins of the leg; but at the present time a vein on the anterior surface of the forearm is the one chosen, unless some particular reason exists why another should be selected. The veins in this region that become most prominent, when the circulation in them is arrested, are the median cephalic and the median basilic. The former is the proper one to select, as the median basilic is in close relation to the artery. The median cephalic is external to the tendon of the biceps and away from any region of danger. It will be found, however, that the size and prominence of the veins in front of the elbow vary greatly, and generally the most marked one is selected regardless of its name or position.

In performing the operation strict antiseptic precautions should be observed in the preparation of the site and in the selection of the knife and dressings. The arm is allowed to hang suspended and a firm bandage is applied above the elbow, sufficient force being employed to compress the superficial veins, and yet not enough to intercept the arterial flow. The vein is opened either longitudinally or in a direction slightly oblique to the axis of the vessel, the point of the blade being inserted directly into the vessel and withdrawn with a downward cutting motion. Before one makes the incision he should place the thumb of the left hand below the point of opening, in order to secure the vessel firmly. Care must also be observed that the skin is tense and in its proper relation to the vessel, otherwise the incision through the skin may not coincide with the opening into the vein, and instead of a free flow of blood there will be only an exudation of blood into the cellular tissue. As soon as the blood flows freely, the pulse of the other arm and the patient's face should be kept under observation to determine the effect on the circulation and to detect any symptoms of syncope. Twenty, thirty, or even forty ounces should be removed, according to the condition and temperament of the patient. The quantity taken should always be sufficient to make a decided impression upon the circulation. Frequently the amount withdrawn does not exceed a few ounces. This is too small a quantity to afford any benefit to the patient, and as a further result venesection is discredited. When sufficient blood has been removed the flow is readily checked by applying a compress over the incision, removing the constricting band, and bandaging the arm, which bandage may be removed in twenty-four hours.

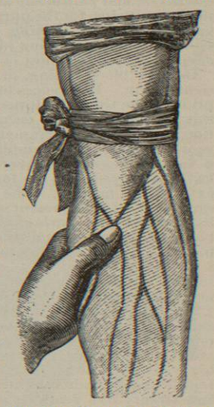
FIG. 602.—Forearm with Ligature Applied. Finger at junction of median basilic and median cephalic veins.

It is often desirable to abstract blood from some prominent part for the purpose of relieving a local hyperæmia or congestion, as in many bruises and ecchymoses, in disease about the eye, nose, ear, gums, etc., and in some forms of cellulitis. Such local blood-letting is also occasionally employed to lessen the congestion of deep organs by withdrawing blood from the surface of the body near to the affected part. In all cases the blood flows from the capillaries or superficial vessels, and the quantity is very slight in comparison with that which is abstracted in general blood-letting. To accomplish this end, leeches, scarification, and wet-cupping are the means employed.


FIG. 603.—Glass Leech Tube.

LEECHING.—This means of locally abstracting blood is still frequently employed, but not to such an extent as formerly. When any large amount of blood is required, a number of leeches must be used. A single leech will absorb from two to three drachms of blood, and this may be increased as much more by warm fomentations applied after the leech has dropped off. The leeches may be placed in a wine glass which should then be inverted over the desired spot. If the area is very limited, or if the blood is to be withdrawn from the nasal cavity, gums, or any cavity, a leech glass should always be utilized. The leech will bite more freely if removed from the water an hour or more before needed, and for the same reason the part should be thoroughly cleansed. When a leech shows no inclination to bite, rubbing the skin with sweetened water will sometimes induce it to fasten upon the spot. The peculiar bite of the leech always leaves a permanent scar, and care must be observed in selecting a site which is not conspicuous.

SCARIFICATION AND CUPPING.—By these means blood is abstracted through small superficial incisions into the skin, the flow being augmented by the cupping glass when that instrument is employed. The incisions may be made with an ordinary knife or lancet, or by means of a scarificator. In the latter instrument the blades may be adjusted to any desired length so that the cutis vera of the part may be incised without penetrating to the deeper tissues. The cupping glass may be one of those specially constructed for this purpose, or an ordinary wine glass may be made to serve the same end. The cavity of the glass is heated over a spirit lamp, or by burning a small quantity of spirit in it, and then the glass is quickly inverted over the desired part and placed in such a way that the air will not enter. As the heated air cools its density is increased and suction force is exercised upon the incised surface. The glass should be removed as soon as it ceases to act and a fresh one applied. A cupping glass with a rubber bulb attached, on the principle of the ordinary breast pump, is also utilized.

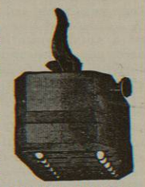
FIG. 604.—The Scarificator.

DRY CUPPING.—Very frequently cupping glasses are applied without an incision being made for the removal of blood. This is known as "dry cupping." The application of the glasses produces a rapid flow of blood to the part, and when repeated rapidly over a small area, the withdrawal of blood from organs lying below the surface is sufficient to relieve them if inflamed and congested. A single glass may be applied and quickly reapplied, but it is more satisfactory to employ a number of glasses and apply them at the same time. They are applied after the same manner as described for wet cupping. Dry cupping has the advantage of not making any incision and not causing any scar. The therapeutic action is somewhat different from that of wet cupping, as the counter-irritation is much greater and more prolonged. Junod's boot is an instrument prepared on the same principle as that of the cupping-glass, its purpose being to withdraw blood from the body into one of the limbs, in order that relief may be afforded to congested organs without the permanent loss of any blood. It consists of a metallic vessel into which a limb may be placed and which closes so tightly that air cannot enter or escape, except by means of an exhausting syringe. As the air is withdrawn from the cavity, the blood flows into the vessels of the limb which becomes greatly swollen and congested. This appliance has never met with much favor, as the constitutional effect is not very satisfactory and the local action is often severe.

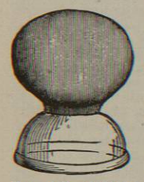
FIG. 605.—Cupping Glass, with Elastic Rubber Cap or Bulb.

Beaumont Small.

BLOODROOT.—SANGUINARIA. *Red Puccoon.* "The rhizome of *Sanguinaria Canadensis* L. (fam. *Papaveraceæ*), collected in the autumn" (U. S. P.). It is doubtful if this definition can be complied with, as the aerial portions die in early summer, and before fall all traces which would indicate the presence of the rhizome have disappeared. The plant is a low perennial, with a thick, fleshy, horizontal root-stock, from which one or two leaves and a single handsome white flower appear early in the spring, enclosed at the base by several sheathing scales. The leaves are kidney-shaped, variously lobed, and grow much larger and broader as the season goes on. The flower is about 3 cm. across (one and one-fourth inches), regular, perfect, spreading; sepals two, falling early; petals from six to twelve, rather narrow; stamens numerous, ovary and capsule one-celled, with two placentæ; ovules (and seeds) numerous, with prominent caruncles. An opaque, orange-colored juice is found in all parts of the plant, especially in the rhizome, where it is very abundant and dark. Bloodroot is a native of North America, and is occasionally cultivated as an ornamental plant both here and in Europe. The dried rhizome is about 5 cm. (two inches) long, and 1 cm. in diameter, slightly flattened, indistinctly annulated, and evidently shrunken and wrinkled. It is reddish brown externally, variously bent and twisted, and now and then branched. It breaks with a short fracture, and displays a pink surface, finely dotted with dull red points; this surface becomes dark by exposure, and finally is uniformly brownish red. Odor slight, disagreeable. Taste bitter, acrid, nauseous, and persistent. Powder sternutatory.


FIG. 606.—Bloodroot.

The principal constituent is the alkaloid *sanguinarine* ($C_{20}H_{13}NO_4$), discovered and named by Dana in 1829. When pure it is in white crystalline needles or tufts, insoluble in water, but easily dissolved by alcohol, ether, oils, etc.; it forms with the principal acids beautiful salts of brilliant orange or red color. The powder excites violent sneezing. Its taste, when dissolved, is that of the rhizome intensified. Chelerythrine ($C_{21}H_{17}NO_4$) exists in smaller amounts and yields yellow salts. Small amounts of at least two other alkaloids exist, with irritant resin, starch, citric and malic acids.

Action.—The several alkaloids of bloodroot have very dissimilar actions when used separately, but that of sanguinarine is overpowering and determines that of the drug. It is a most powerful irritant, locally and systemically. It was formerly used as a caustic for morbid growths, and has had many uses as a counter-irritant. It is powerfully irritant to the mucous membranes, and sialagogue. It is a powerful and even fatal emetic and cathartic. Systemically, it irritates both the spinal and cerebral centres, producing tetanic convulsions and intoxication or violent delirium. It depresses muscular fibre, and this at length greatly depresses both the circulation and the respiration.

These properties can be utilized, by small doses, in improving both appetite and digestion, and in producing expectorant effects, the latter either by internal administration, or by inhalation of very weak preparations, or by application to the throat. It was formerly a much-used emetic in doses of gr. xv. to lx., but this use is now considered barbarous. It is very little used at the present time, and then chiefly as an expectorant, in doses of 0.2 to 0.5 gm. (gr. iij. to viij). Its excretion is accompanied by stimulation of intestinal and renal secretion and of peristalsis. It is also a stimulating emmenagogue. A fluid extract and a fifteen per cent. tincture, each containing a little acetic acid, are official. *H. H. Rusby.*

BLOOD STAINS.—In criminal trials the medical witness is often called to determine whether stains found on weapons—as knives, clubs, or daggers—or upon the

clothing of a suspected person, or upon the floor, walls, or ground where a homicide is supposed to have been committed, were caused by blood or by some other coloring matter. So also it is often equally important to determine whether stains acknowledged to be blood are the blood of a human being or that of one of the lower animals. The object of this article is to show how, and to what extent, these important questions may be answered. Such investigations have often served to convict the guilty, and in other cases triumphantly to acquit the innocent.

The examination of blood stains calls for the consideration of: (1) physical characteristics; (2) chemical reactions; (3) crystalline properties; (4) optical properties; and (5) microscopical appearances of blood corpuscles and other constituents of blood.

1. Physical Appearance of Blood Stains.—The color of blood stains varies with the amount of serum and the absorbent properties of the object upon which the stain dries. Generally the more permeable the tissue or object stained, the brighter is the color after blood has dried upon it.

Upon polished steel or other metal, blood dries in dark brown, shiny scales, however thin the blood may be. Upon silk or glass it assumes about the same color as upon polished metal. Upon varnished or very hard wood, blood stains have also a dark shining surface. Put upon soft porous wood, or any soft tissue, as cotton cloth, the blood displays a dull brown color or a rose tint, yet even on cloth a thick clot, when dry, presents a brown but glistening surface.

It sometimes happens that upon tissues or objects of a brown, maroon, or dark blue color, blood stains are quite invisible by full daylight, but they become conspicuous by artificial light, especially if examined by light obliquely reflected. This is especially the case with dark-colored furniture, wallpaper, or any dark paint, on which blood stains easily seen by the light of a candle are quite invisible by daylight.

Stains upon steel or other metallic instruments, if quickly dried by exposure to the air, are cracked and of a fine red color; but stains on similar instruments, kept in a damp situation, are of a dirty brown, tending toward a yellow rusty color, surrounded with an ochrey areola, yielding no color or albumen in water; even a solution of potash extracts only a small quantity of albumen.

Upon glass, marble, plaster, flint, sandstone, and earth, blood stains preserve their ordinary characteristics; but upon wood containing tannin they form with the tannin an insoluble compound, and water in which such stains are macerated fails to yield characteristics of blood. In such cases the surface can sometimes be scraped off and tested free from the tannin.

On felt and some kinds of cloth, blood forms shiny spots appearing like mucilage. In examining garments, searching for blood stains, a small magnifying glass is of great service in distinguishing small specks or drops of blood.

2. Chemical Reactions.—(*a*) *Guaiacum Test for Blood.*—If any red substance is suspected to be blood, place a drop on white paper; or if dry, moisten with water and then place it on the paper. Let fall upon the stain thus formed a drop of tincture of guaiacum. If the stain turns blue with no other treatment it is not blood; or if it contains blood it also contains starch, a salt of iron, or some other foreign substance. If it does not turn blue by the action of the guaiacum, drop upon it a watery solution of hydrogen dioxide, when, if it is blood, it will quickly assume a beautiful sapphire blue color. A particle of blood scarcely visible to the naked eye may be detected by this test. If the blue color does not quickly appear by this test it is not blood. If the stain gives the blue color by this test it may be blood; and other tests may be applied to determine with certainty that it is. The white paper should be tested with guaiacum and dioxide before the stain is applied.

(*b*) *Action of Solvents and Other Reagents.*—Stains upon cloth may be cut out and suspended in a test tube containing distilled water. The bottom of the cloth should dip into the water, but the stain should not touch the sides of the tube. Under these circumstances the coloring matter of blood will usually detach itself from the tissue and fall in reddish striæ to the bottom of the tube.

When a solution of blood is heated the color disappears, but vegetable colors in general are unchanged by heat. A solution of blood is made a little darker by the addition of a small quantity of ammonia, but the color is not destroyed. The red, pink, or scarlet infusion of fruit or flowers or roots and the juices of fruits are changed to green or violet by ammonia, and cochineal is changed to crimson. Hypochlorous acid quickly destroys all organic coloring matter except blood, which withstands the acid much longer. Two minutes are sufficient to destroy most colors except that of blood. Stains of colcothar and grease and those of rust resist for a long time the action of hypochlorous acid, but disappear instantly on contact with chloride of tin, which does not act upon the coloring matter of blood. Carbon mixed with the colored fluid cannot be readily discolored by any reagent.

If the stains are upon weapons, from which it is impossible to detach a superficial layer, they may be moistened with drops of water. If they are upon wood, plastered wall, or stone, we may scrape the surface and test the material removed. If upon the point of a dagger or other narrow blade, this material may be placed in a tall, narrow vessel. In general, if the material of the stain can be scraped off, the fine powder thus obtained may be treated with a minute portion of fluid in a test tube, watch glass, or on a concave slide or cover glass, such as is used with the microscope. The method of dealing with minute specks, when these are all that can be obtained from a stain, will be described further on. If the powder obtained is abundant and contains foreign matter, it may be placed in a small gauze bag and suspended in a test tube as described above. If the stains are spread in streaks on the surface of the instrument, a plate of glass may be adjusted on a perfect level, and a few drops of distilled water placed on the glass; then the instrument should be so arranged that the stain touches the water while the instrument does not touch the glass. After one or two hours the water will be colored by the stain; but whatever process we employ, it is important to avoid contact of the water with the steel or iron so as to form rust. In all cases only a small quantity of water should be used, and if the liquid contains much foreign matter it should be filtered before using reagents.

3. Crystalline Properties.—Crystals characteristic of blood were discovered by Teichmann, and great importance is attached to this test. To obtain hæmin crystals, digest the stained tissues, or the powder obtained from the stain, in a one-fourth-per-cent. solution of common salt for twenty-four hours, next allow the fluid to dry on a glass slide at a temperature of 80° or 100° F., and then apply a drop of glacial acetic acid, heating it over the flame of a lamp. As the fluid evaporates a great number of crystals appear, in the form of rhomboidal plates with angles of 55° and 125°, of yellow, red, or brown color, depending on the thickness of the crystalline plates. In the dog and in man they are long parallelograms. In the squirrel they are hexagonal plates; and in the guinea-pig they are in the form of tetrahedral crystals. The length of the crystals from human blood is from one and one-half to three times the breadth. They are generally very small, and if obtained from minute stains their obtuse angles are somewhat rounded, as are the crystals of uric acid found in the brick-dust sediments of urine.

The microchemical examination of stains to produce *hæmic crystals*, or crystals of hæmatin, or Teichmann crystals, when only minute stains are to be examined, is so important that we deem it desirable to give the following directions, which we have translated from "Précis de médecine légale," by Ch. Vibbert:

"The Teichmann crystals are so characteristic that when once seen they are ever afterward easily recognized. The operations for obtaining them are exceedingly simple, but require great care and patience. To avoid failure,

persons but little accustomed to these minute investigations should follow carefully the methods here described, especially if, as often happens, only minute portions of the suspected material are available for these researches.

"(*a*) *Solution of the Stain.*—If the stain is small and no speck or clot that may be removed can be seen, cut out the stain, following the edges of it, with a pair of scissors; place the stained piece of cloth on a glass slide, and put

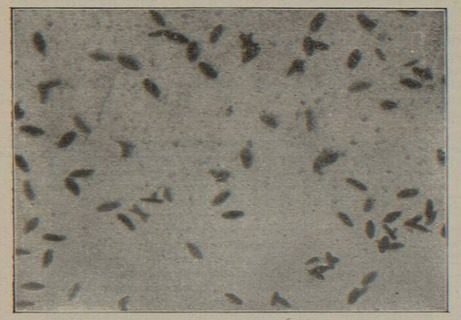

Fig. 607.—Hæmin Crystals.

on it a few drops of water—just enough to moisten it, for it is better to get a solution somewhat concentrated. After macerating till the liquid is considerably colored, press out the liquid with a scalpel or needle, and remove the cloth, leaving the colored fluid on the slide. Avoid spreading the liquid, but dry it slowly, so that it shall make a small, deeply colored spot on the glass: not entirely opaque but somewhat transparent, so that the preparation can be examined with the microscope.

"If the stains are very small, several may be taken and macerated together to obtain sufficient coloring matter for the subsequent manipulations. If the stain is on wood, a thin layer of the wood may be removed by a sharp knife or lancet, and treated as directed for a fragment of cloth; only the maceration should be continued much longer.

"If it is not possible to lift the stain from the object, a ring of wax may be formed around it, making an inclosure that will hold a little water, which is then applied to absorb the stain. When the water has absorbed the coloring matter, it may be transferred to the slide by means of a pipette.

"(*b*) *Evaporation of the Liquid.*—We may allow the liquid to evaporate spontaneously, but the evaporation can be hastened by the employment of heat, it being necessary, however, to heat the liquid very moderately, not exceeding a temperature of 60° C. (140° F.); for if the heat is sufficient to coagulate albumen, it prevents the extraction of the coloring matter, and of course no crystals will be obtained. We generally warm the slide over the flame of an alcohol lamp, being careful that the temperature does not exceed that which is not uncomfortable when the slide is laid upon the back of the hand. It is important to heat the liquid around the borders and not in the centre, so as to prevent its spreading over the slide, which would much interfere with subsequent operations.

"(*c*) *Action of Reagents.*—To the evaporated residue a little common salt should be added. Too much is injurious. It is better to make a solution of 1 part of chloride of sodium to 500 or 1,000 parts of distilled water, and add one or two drops of this solution. *It is more convenient to use this solution in the first place for dissolving the stain*, instead of using simple distilled water. It saves time and nothing is changed. Sometimes crystals can be obtained by treatment with acetic acid without the addition of salt, there being sufficient chloride of sodium in the blood; but it is better to add the salt than risk a failure in an important case.

"At whatever stage the process the chloride of sodium has been added, the preparation must be thoroughly dried afterward before the acetic acid is applied. This acetic acid monohydrate is called glacial or crystallizable. It solidifies at the freezing point, or zero Centigrade, and does not again liquefy until the temperature is raised to 17° C. The addition of a small quantity of water transforms it into acid hydrate, which interferes with this reaction.

"Place a drop of acetic acid monohydrate on the preparation and evaporate with heat, which may be increased and continued, though the evaporation will be more successful if the heat does not quite reach the boiling point. When there is only a small quantity of the suspected material, it is especially important to use all these precautions. The acid should be taken up with a tube drawn out to a fine point, so that only a very small drop may be added at a time; place the droplet in the centre of the red deposit on the slide, and allow it to spread a little, but not to spread beyond the limit of the stain; for this purpose the heat of the lamp should be applied around the borders at various points, tipping the slide as may be necessary to retain the fluid on the red deposit only; a red line is thus formed about the borders when the coloring matter becomes thickened, and the acid remains on the stain till evaporation is completed. It is on this border that the crystals are commonly formed, and they are to be looked for by aid of the microscope.

"It is not common to find crystals after adding only a single drop of acid, but it is generally necessary to add drop after drop, evaporating each with the greatest care, examining the specimen from time to time where the red lines are formed one after the other as it is repeatedly treated with acid and dried. When this experiment is performed under favorable conditions, a multitude of distinct crystals are found. Often, however, we find only irregular masses of brown or dark coloring matter, and the remainder of the preparation filled with coagulated albumen and foreign bodies which have become mixed with the stain. If we have added solid particles of chloride of sodium to the coloring matter, we are apt to find crystals of this salt formed in cubes, in stars, or in small, colorless globules, and even lance-shaped crystals of acetate of sodium may be formed. All of these crystals, arranged together in beautiful forms, may often be found.

"In such cases, we select a point where the coloring matter is collected in considerable quantity, and there apply another small drop of acid and let it evaporate as before. By repeating this operation we sometimes obtain a large collection of very small crystals mingled with other matter; but when we find crystals in the form of a cross or a star, it is very certain that they are chlorohydrate of hæmatin, and we may perfect the preparation by the addition of small drops of acetic acid.

"If doubt still remains in regard to the nature of the crystals, the specimen should be examined by the aid of polarized light. The albuminous or saline substances allow the field to remain dark, but the crystals of hæmatin appear bright on a dark field.

"By carefully following the precautions stated above, one can almost always obtain crystals of hæmatin, even with a very minute quantity of blood. The reaction is successful with very old stains. Many experts have obtained crystals of hæmatin from stains of blood ten, fifteen, and even forty years old. It sometimes happens, however, that it is impossible to obtain crystals when the stains are only a few months old. This is especially the case when the blood has been allowed to putrefy before drying. Contact with perspiration, grease, or tannin also seems to interfere with the formation of crystals.

"Two sources of error are to be noticed in connection with this test for blood. Crystals of murexide (purpurate of ammonia) have a form very similar to that of crystals

of hæmatin, but they are of a bright red, and they acquire a violet color by contact with a solution of potash; again, it is very difficult to imagine how it can be possible to obtain crystals of murexide by treating a stain with common salt and acetic acid. A mistake may easily occur with crystals formed with indigo. Cloth colored with this material will sometimes furnish a deposit of crystals which will not dissolve in acetic acid and which have a form very similar to that of crystals of hæmatin. Their color is blue, it is true, but when the color is very deep it can scarcely be distinguished from brown. Descoust, by simply washing with water a piece of flannel of blue violet color, obtained crystals having almost exactly the form and reddish-yellow color of crystals of hæmatin. This is a more serious source of error than is generally admitted in books on legal medicine.

"When a stain to be examined is found on clothing colored with indigo, it is important before examining the stain itself to examine pieces of the garment which are not stained, to see whether they will deposit crystals of indigo. The comparison between these crystals and those obtained from the stain may remove all uncertainty. if, for example, the crystals obtained from the stain are very numerous, while those obtained from the unstained cloth are very few and of a blue color. If the similarity of the two species of crystals is very close we may try the guaiacum test. The crystals of hæmatin will color the guaiacum blue, while those of the indigo will have no effect upon it.

"When the crystals of hæmatin have been obtained they may be indefinitely preserved by adding a little glycerin and covering the preparation with thin glass. The expert should carefully preserve the preparation, to serve as a proof of the correctness of his conclusion."

4. Spectrum Analysis of Blood Stains.—One of the most important methods of distinguishing solutions of blood stains from those of other colored fluids is by the use of the spectroscope. The peculiar spectra produced by the passage of light through solutions of blood were noticed by Hoppe in 1862, and were suggested by him as a means of medico-legal research. Stokes, in 1864, and Sorby, Lethby, MacMunn, and others have added largely to our knowledge of this subject.

The coloring matter of fresh blood is known as hæmoglobin, and, according to Preyer, it contains nearly all the iron of the blood. By the action of reagents, such as acetic, tartaric, and citric acids, the *bright red* of fresh blood becomes changed to *brownish red*, known as hæmatin. This is a permanent chemical change. The same change of hæmoglobin into hæmatin takes place when blood has been kept for a long time. The fresh blood stain is bright red (hæmoglobin); the old stain is brown (hæmatin). Hæmoglobin and hæmatin are each capable of existing in two states of oxidation, each state producing in the spectrum its own characteristic absorption bands. If a blood stain is kept in a damp place the hæmoglobin is rapidly changed into hæmatin, or both hæmoglobin and hæmatin may be decomposed. But if the stain is kept dry, it becomes in time of a brown color. This change is hastened if the stain is exposed to a strong light. This brown coloring matter is methæmoglobin, which Sorby regards as peroxidized hæmoglobin. This alteration is much more rapid in an atmosphere containing coal gas, or a trace of sulphurous or any other weak acid. The change is also very rapid when a stained garment is worn next the skin, as the acid perspiration hastens the change. If the color of a blood stain be a bright red it is evident that it is recent, but if it be brown it is not necessarily an old stain. When fresh blood, or a solution from a recent stain, is placed before the slit of a spectroscope, two dark absorption bands are seen between the Fraunhofer lines D and E, the line near D being darker, narrower, and more sharply defined than the other (Sp. 1–1, Fig. 609). If the strength of the solution is increased the bands grow wider and the orange and blue are gradually obliterated. If the strength of the solution is diminished the line near E first fades away and then the line near D also soon disappears. If the stain has been changed to brown by the action of the air the coloring matter becomes peroxidized, and the solution then gives a single band between the positions of the two above described (Sp. 2–2, Fig. 609). The coloring matter giving the single band is known as methæmoglobin.

Alkaline Hæmatin.—Make a saturated solution of carbonate of potash in alcohol and pour a few drops of blood or solution from a recent stain into the solution. The color immediately changes, and when examined in the spectroscope a broad, lightly shaded band is seen covering Fraunhofer's line D. Caustic potash and caustic soda in alcohol when added to blood give the same spectrum, but different from that produced by aqua ammoniæ or by carbonate of potash. It consists of three bands, one in the red, the other two like the bands produced by fresh blood. In the addition of a reducing agent the spectrum of reduced hæmatin appears, consisting of two bands similar to the bands of fresh blood, but removed farther from D toward the blue part of the spectrum.

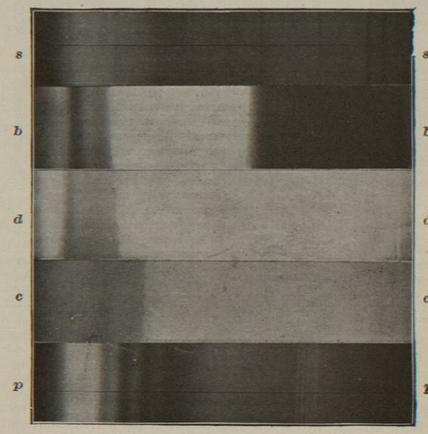

Fig. 608.—*s s*, Solar spectrum; *b b*, human blood spectrum; *d d*, didymium spectrum; *c c*, carmine spectrum; *p p*, potassium permanganate.

In Fig. 608 are shown at *s–s* the Fraunhofer lines of the solar spectrum. Below are shown the spectra of different colored fluids: *b–b*, human blood; *d–d*, nitrate of didymium, of a faint pink color, in solution with lines similar to those of blood but easily distinguished from them; *c–c*, carmine solution, and *p–p*, the spectrum of a solution of permanganate of potash with three dense lines and one very faint in the red. All the spectra were photographed on the same scale. All the fluids were illuminated by the electric light, and the right half of the spectra shows carbon and other lines produced by the flame of the carbon electrodes. Many more might be presented, but these specimens are given to illustrate the marked distinction, as shown by the spectroscope, between blood and other colored fluids.

Instruments for Spectrum Analysis of Blood Stains.—As the absorption bands produced by colored fluids are broad and not very sharply defined, an instrument of much less dispersive power than that used for colored flames is required. The microspectroscope made by R. and J. Beck, to be attached to the microscope instead of the objective, is very useful when the quantity of colored matter is considerable, as it allows the use of the micrometers in the eyepiece of the microscope to mark the

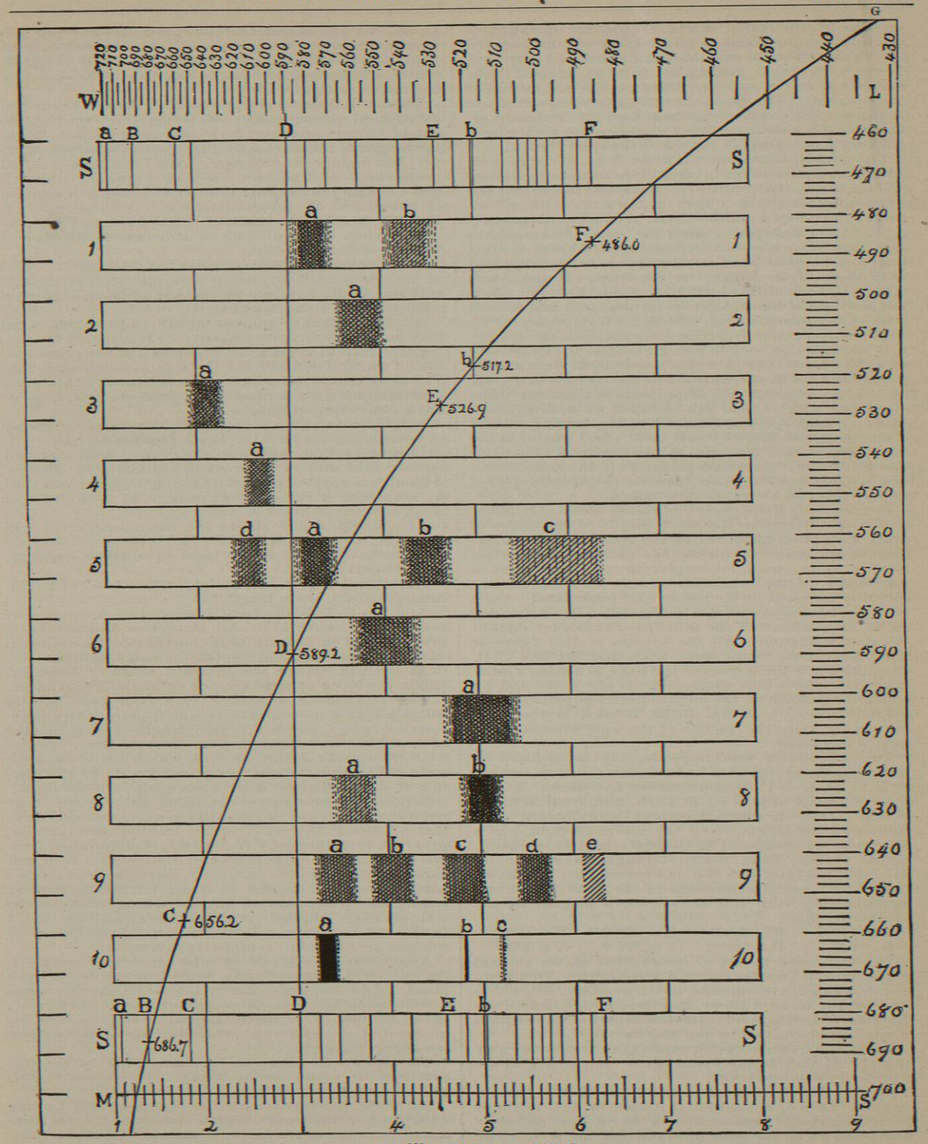

Fig. 609.—Spectometry of Blood.

position of absorption bands. When the quantity of coloring matter is small, or a mere speck of clot moistened on the slide is to be examined and tested, the objective, as low a power as practicable, is to be retained in the microscope and a microspectroscope inserted instead of the eyepiece. The Sorby-Browning microspectroscope in place of the eyepiece has been used for this purpose, but with this instrument it is very difficult to get the light from the clot centred in the slit of the spectroscope.

The microspectroscope made by Zeiss, of Jena, is inserted in the microscope in place of the ordinary eyepiece, and the direct vision prism can be turned to one side and by opening the slit the object on the stage of the microscope can be found and focussed; then when the prism is replaced the absorption bands are seen. It has also a side stage where a comparison specimen of blood or other fluid can be placed and its absorption bands compared with those of the object on the stage. It also has a micrometer scale illuminated by a side light by which the positions of the bands can be measured and records made, as illustrated in Fig. 609, M, S. With such a microspectroscope, under favorable circumstances, the absorption bands can be seen in a single corpuscle of perfectly fresh blood, but not in a single corpuscle from a dried stain. But the bands can often be clearly seen in a speck of clot $\frac{1}{100}$ or even $\frac{1}{1000}$ of an inch in diameter when moistened with normal salt solution or with glycerin water. In the spectrum analysis of blood stains, careful comparison with known solutions of blood treated in the same manner may be made by placing the known solution before the comparison prism, as in the Zeiss instrument, when any variation between the spectra placed side by side can be readily determined.

For recording observations and results obtained it is important to determine the wave length of the centre of every absorption band, noting the conditions and treatment of the stain by which it was obtained. To obtain wave lengths, each microspectroscope must have a micrometer of its own, with a scale of wave lengths carefully adjusted to the prisms and lenses used. Such tables and scales are shown in Fig. 609. Spectrum S–S shows the positions of the principal Fraunhofer lines of the solar spectrum. All the spectra in this figure or plate are drawn to the same scale, and the line D–D, from the top to the bottom of the page, is used as a starting point from which measurements are made and by which the position of the micrometer is adjusted. The didymium line, *b*, in Sp. 10–10, is used in the same manner for fixing the position of the scale when artificial light is employed.

The micrometer measurements may be reduced to wave lengths by means of an interpolation curve, shown at B, C, D, E, *b*, F, G, constructed as follows: At the right hand is a scale of equal parts, numbered from 460 to 700. These numbers represent in millionths of a millimetre the length of all the waves of light which require to be considered in the analysis of blood stains. Across vertical lines, drawn through the Fraunhofer lines in the spectra S S, S S, horizontal lines are drawn from the scale at the right side of the plate at positions corresponding with the known wave lengths of the Fraunhofer lines.

a = 718.5	C = 656.2	E = 526.9	F = 486.0
B = 686.7	D = 589.2	*b* = 517.2	G = 430.7

Through these points of intersection of the vertical and horizontal lines the curve B F is drawn. Then from points in this curve opposite to the numbers 460, 470 to 700, vertical lines are drawn, and these enable us to draw the scale W L, which serves to determine the wave length of any band measured by the micrometer M S.

The position of absorption bands thus determined may be recorded, and at any future time compared with the records of other observers.

In Fig. 609, Sp. 1–1, at *a* and *b*, are shown the absorption bands of normal blood drawn in their relations to the Fraunhofer lines as seen in the Sp. S–S, and their true positions on the scale of wave lengths, W L. The band *b* is seen to be broader and paler than the band *a*. In Sp. 8–8 are shown the absorption bands of an ammoniacal solution of carmine, where *a* is shown to be paler than *b*, and also that *b* has a wave length 517 at its centre, while the band *b* in the spectrum of blood 1–1, *b*, has the wave length 538.*

The Sp. 9–9 shows the positions of the five bands in a solution of permanganate of potassa. In Sp. 5–5 the bands *a b c* are produced by a solution of alkanet, where the band *a* is almost exactly like *a* in tae blood spectrum 1–1, but *b* and *c* are different. If a little alum is added to the solution of alkanet, the band *d* appears, while *a b* and *c* remain unchanged; if alum is added to a solution of blood, both the bands disappear. At *a*, in Sp. 2–2, Fig. 609, is shown the band of reduced hæmoglobin produced when hydrosulphate of ammonia is added to a solution of normal blood.

Other reducing agents produce the same effect. One of the best is made by dissolving equal parts of tartaric acid and double sulphate of iron and ammonia, and then adding a little aqua ammoniæ.

An ammoniacal solution of tartrate of protoxide of tin may be used as a reducing agent. It is prepared by adding tartaric acid to an aqueous solution of the protochloride of tin, and neutralizing the solution with ammonia. The acid should be added in such quantity that after over-saturation by ammonia no precipitate is formed, but a clear solution remains.

Sulphide of sodium is highly recommended by Preyer as a reducing agent. Dr. Beale recommends, as a deoxidizing solution, protosulphate of iron, with sufficient tartaric acid added to prevent precipitation by alkalies. A small quantity of this solution, made slightly alkaline by ammonia or carbonate of soda, is to be added to a weak solution of blood in water.

At *a*, in Sp. 3–3, is shown the band peculiar to acid hæmatin, wave length 640.

At *a*, in Sp. 4–4, is the band of alkaline hæmatin, wave length 605.

At *a*, Sp. 6–6, is shown the absorption band of a solution of fuchsin, wave length 548.

At *a*, Sp. 7–7, is the band produced by a solution of eosin, wave length 517. All absorption bands vary in intensity and in breadth as the strength of the solution varies, but the centre of the band remains stationary.

At *a b c*, in Sp. 10, are shown a series of beautiful bands produced by a solution of sulphate of didymium.

This remarkable substance, of a faint pink hue, in solution almost as colorless as water, gives a broad absorption band *a*, central at a wave length of 575 millionths of a millimetre, extending from 571.5 to 578. The line *b* is very sharply defined at wave length 523 of the same scale. This substance serves as an index to fix the position of the micrometer scale when using artificial light.

Besides the substances mentioned above, some other red solutions give absorption bands somewhat similar to that of blood, but with careful manipulation and appropriate tests they may be certainly distinguished from blood. The coloring matter of the petals of the red variety of *Cineraria*, a variety of chlorophyll, gives two bands somewhat like that of blood, but the micrometer readily shows that they occupy different positions on the scale of wave lengths. Add ammonia, and the blood bands remain unchanged, while those of the *Cineraria* solution are altered or completely destroyed.

A solution of *cochineal in alum* gives the bands *a b*, Sp. 8–8, Fig. 609, differing little from those of fresh blood. Add ammonia, and in both solutions the bands become more intense. Now add excess of boric acid; in the solution of cochineal the bands shift toward the blue end of the spectrum, while in that of blood they remain unchanged.

The other reds likely to be confounded by the unpractised eye with blood are *lac-dye, alkanet, madder red,* and *munjeet,* dissolved in each case *in alum.*

But if the spectra be carefully examined side by side with blood, or their wave lengths determined on the scale

*The scale of wave lengths are in millionths of a millimetre.

by their positions seen on the micrometer, it will be apparent that the bands produced by these bodies are not the same, either in position or in character. Further, none of them will stand the action of ammonia, while they are all bleached with sulphite of potash, which has no action on blood. In the examination of *a mixture of magenta and blood*, the magenta bands may entirely mask those produced by blood. The various blood spectra may, however, be easily obtained unmixed by adding to the solution a trace of sulphite of soda, which completely removes the magenta coloring matter, but leaves the blood untouched.

All the supposed fallacies disappear if we successively obtain the various blood spectra with the reagents already described.

Examination of a Recent, or Comparatively Recent, Blood Stain on a White Fabric.—(1) If you can detach a portion of the blood, do so; but if this is impossible, cut out a small piece of the stained fabric, and soak it for about ten minutes in a few drops of cold distilled water in a watch glass. Then squeeze the colored fluid out and set it aside for a short time, so that any insoluble matters may be deposited. Provide a glass cell about one-eighth of an inch in diameter and half an inch high, which may be made of a section of barometer tubing cemented to a slide with gutta-percha, with a diaphragm of tin foil between the slide and the end of the tube. With a fine drawn pipette introduce the red fluid into this cell, and place it on the stage of the microscope with an objective of low power, and illuminate with sunlight or other strong light, when, with the microspectroscope in place of the eyepiece, if the solution is blood the characteristic absorption bands will readily be seen. If there is a sufficient quantity of the solution, several tubes may be filled. If the tube contains any sediment, it may be laid to one side until the solution is clear. The objective should be focussed near the top of the tube, a little below the surface of the fluid. If the blood is tolerably recent, the spectrum of oxidized hæmoglobin with its two well-defined absorption bands in the green will be apparent, as shown in Fig. 609, Sp. 1–1. If such a spectrum is obtained, it is evident that it has been very little changed by exposure, and that it is probably of recent origin. If the spectrum contains two faint bands in the green, and an extra band in the red, it indicates that the stain has undergone a change. Now add a trace of ammonia to the solution in the tube, stirring it with a platinum wire, when you will obtain two bands in the green and none in the red.

(2) To a second tube of the suspected solution add, first, a very little ammonia, and then a small quantity of Rochelle salt. With this no change will be produced in the spectrum, the ordinary bands being visible as before. Now add to the liquid in the cell a piece (about one-fortieth of an inch in diameter) of the double *sulphate of protoxide of iron and ammonia*; stir the solution with the platinum wire, with as little exposure to the air as possible. Cover the cell with a piece of thin glass. The two bands seen previously will have disappeared, and will be replaced by a single intermediate band, fainter but broader than either of the other two, Sp. 2–2, Fig. 609. This is the spectrum of reduced hæmoglobin. The same effect will be produced by a trace of solution of sulphide of ammonium.

The specimen of hæmoglobin thus reduced may be again and again oxidized by exposure to the air and vigorous stirring with the platinum wire. It can be again deoxidized by further addition of the iron salt, or by sulphide of ammonium, if that was previously employed for deoxidation.

This deoxidation and reoxidation of the hæmoglobin is a very characteristic reaction, and serves to distinguish blood from most other substances. If the solution of blood is merely covered with thin glass and kept for some time in the cell, the reduction of the hæmoglobin will be effected spontaneously, and without the addition of any reagents.

A question may arise here, whether this spectrum can possibly be due to iron. The question is at once answered by the fact that hydrosulphide of ammonium produces the same result, as also does the tin solution described on page 78, only that it acts more slowly than the iron salt.

(3) Add to another portion of the suspected solution, in a cell, a minute fragment of *citric acid*, stirring it thoroughly with a platinum wire. The acid will change the hæmoglobin into hæmatin. If previous to the addition of citric acid the two bands of oxidized hæmoglobin were visible in the green, they will disappear, and if the solution is tolerably strong a faint band will be visible in the red (Sp. 3–3, Fig. 609). Add now an excess of ammonia, by dipping the plantinum wire into the ammonia solution and stirring the moistened wire immediately into the liquid in the cell. The band in the red, if present, will now disappear, the original bands either not being restored at all or restored only to a slight extent. This is a most important change to note, since it shows that the acid has effected a permanent change in the original coloring matter of the blood. Add now to the solution in the cell a very small particle of the double sulphate of iron and ammonia, and cover the liquid over immediately with thin glass. Remove the excess of liquid with blotting paper, and in order to exclude air it is advisable to fix the glass cover on the cell with gold size. Turn the cell over and over again, for a few minutes. In cold weather the process of oxidation is slow, and even a quarter of an hour may elapse before it is complete. By this means the hæmatin will be reduced, and two well-marked bands will be seen, the one nearest the red being the first to appear, and both being a little more distant from the red than the bands of fresh blood. If the solution be very turbid, the precipitate may be allowed to collect on the side by keeping the tube for a short time in a horizontal position. Preserve and mark the specimen for further examination if necessary.

By exposing this solution of deoxidized hæmatin to the air, assisted by vigorous stirring, we may often succeed in bringing back again not only the oxidized hæmatin band, but also the bands of oxidized hæmoglobin.

(4) Lastly, add to some of the liquid under examination a small quantity of *boric acid*. If the solution be blood, no immediate change will be observed in the position of the bands. The above-mentioned method of examining blood stains of recent origin requires some modifications in special cases.

Old Blood Stains or Blood Stains on Colored Fabrics.—If blood stains are old, the coloring matter will probably be found to be scarcely at all acted on by cold water. Either citric acid or ammonia must then be used for dissolving it. If the fabric be white, ammonia should be employed in preference to citric acid, but if it be colored, test, first of all, which of these two reagents has the less action on the dye-coloring matter, and then use that one, for the purpose of dissolving the blood, which acts less on the color of the cloth. To determine this, place a little of the fabric in two watch glasses and apply a solution of ammonia to one and a solution of citric acid to the other. In general, ammonia should be used in preference to citric acid, but in the case of red fabrics ammonia will generally dissolve so much of the dye stuff that subsequent investigations with the colored solution are rendered much more troublesome and complicated. Hence, if the stain be found on scarlet cloth or other red material, citric acid should be used as the solvent. If the stain be found insoluble both in ammonia and in citric acid, then it should first of all be acted on with ammonia solution and a moderate heat afterward applied.

Proceed then with the solutions in the manner already described, examining them with the spectroscope both before and after deoxidation with the iron salt. The age of a stain does not in general interfere with the spectroscope test, as Mr. Sorby has been able to discover hæmatin with the spectroscope after forty-four years, and others have done the same after thirty years.