Appendix

Appendix

A. Apparatus

The Microscope

The most important piece of apparatus for the laboratory worker is a good compound microscope. It should be equipped with a triple or quadruple nosepiece and a removable mechanical stage. A monocular is adequate for almost all purposes but if long hours are to be spent using the microscope a binocular soon pays back its extra expense in saving to the eyes. Binoculars are almost a necessity in busy hematology, parasitology, and pathology laboratories. A stereoscopic (dissection) microscope is essential in the examination and dissection of entomologic and helminthologic specimens. A simple microscope (magnifying glass, hand lens) is only rarely needed, and an ocular may be substituted when such low magnification is required.

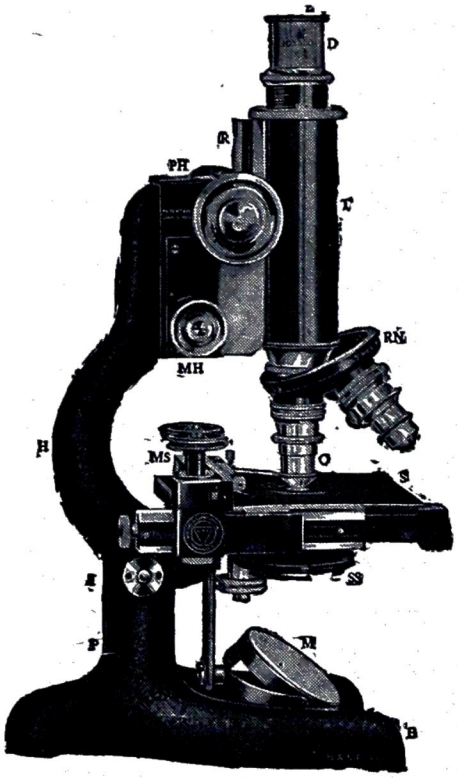
Objectives. These are the most important lenses of the microscope and should be selected with great care. They vary chiefly in color correction, numerical aperture, equivalent focus, and magnification.

Color Correction. Three general types of objectives are manufactured: (1) the achromatic, corrected for two colors and spherically for one; (2) the apochromatic, corrected for three colors and spherically for two; (3) the semiapochromatic (fluorite), whose color correction is better than that of the achromatic but not as good as the apochromatic. Achromatic objectives are adequate for all general work and are much cheaper than the other types. The better lenses are valuable in hematology, bacteriology, malariology, and photomicrography.

Numerical Aperture. Most objectives have the letters N.A. and a figure marked upon them expressing their numerical aperture. From a practical standpoint this gives the relative proportion of the rays which proceeding from an object can enter the lens of the objective and form the image. Of course, the greater the number of rays, the greater the N.A., the better the definition, and consequently the better the objective. The *resolving power* of a lens, i.e., the shortest distance between two lines or points at which they can be distinctly seen as two instead of one indistinct point or line, is closely related to the N.A. The wave length of light divided by the N.A., or twice this figure if a condenser is used, will equal the theoretical resolving power of the correctly employed lens. For example: Using green light (wave length about 0.55μ) and an oil-immersion objective (N.A. 1.25) with a condenser, then $0.55 \div 2(1.25) = 0.22\mu$ or the resolving power of the objective.

EQUIVALENT FOCUS. An objective is usually designated by its equivalent focal i.e., the focus of a simple theoretical lens which has the same power and characte the combination of lenses in the given objective. It does not represent the wor tance of an objective, by which is meant the distance from the upper surface coverglass to the lower surface of the objective. The usual objectives are the 16-mm. dry), the 4-mm. (high dry), and the 2-mm. (oil immersion).

MAGNIFICATION. Modern objectives are usually marked with their magnifying This figure multiplied by the magnification of the ocular used with the lens will the total magnification of the objective-ocular bination at the eye point. For clinical micros objectives of three powers usually suffice, a power (10×), a medium power (40×), and a power (90×). 3×, 20× and 60× lenses may prove valuable.



Parts of microscope. (E) Eye-piece. (D) Drawtube. (R) Rack (coarse adjustment). (PH) Pinion head. (T) Body tube. (MH) Micrometer head (fine adjustment). (RN) Revolving nose-piece. (O) Objectives. (MS) Mechanical stage. (S) Stage. (H) Handle, a part of the arm. (SS) Substage. (I) Inclination joint. (P) Pillar. (M) Mirror. (B) Base.

Most individuals are able to use a magnific of between 800 and 1000 times the numerical a ture of the objective. This depends upon the r ing power of their eyes. Magnification beyond resolving power is "empty," i.e., no more d are seen if the magnification is increased by use of a higher ocular.

IMMERSION OBJECTIVES. Although most objec are constructed to work in air certain ones of magnification require the immersion of the fr lens in a fluid, usually oil. Several kinds of mersion oil are used, cedarwood oil probably gi the best optical conditions but being sticky gummy. For general laboratory use mineral to be preferred. A drop of oil should also be pl between the upper surface of the condenser the lower surface of the slide if maximum defini is to be obtained with an oil-immersion objec In the clinical laboratory, however, this is se required.

Oculars. The ocular in common use is known a negative or Huygenian lens, i.e., an ocula which the lower lens (collective) assists in for the real inverted image which is focused at level of the diaphragm within the ocular. negative ocular must be reversed in order to as a simple magnifier. The second type of eye the positive ocular, acts as a simple microscope, magnifying the image formed en by the objective and located below the ocular. In order to get full advantage apochromatic objectives, especially in photomicrography, it is necessary to em with them so-called compensating oculars which are designed to compensate for correct the residual color defects in the extra-axial portion of the visual field. oculars may also be used with the ordinary achromatic objectives.

By fixing one end of a hair on the rim of the diaphragm inside the ocular with a drop of balsam one has a satisfactory pointer to locate any particular cell in the scopic field.

Use of the Microscope. If the microscope is to be used practically and inte the following rule must be kept constantly in mind: *The higher the magnificatio smaller the field of view, the shorter the working distance, and the less the de*

. Always use the lowest power practicable as the field is larger and the image er. This applies to the ocular as well as to the objective.

All preparations should be examined first with the low-power objective to select suitable areas for further examination and to get all possible details. With tissue sections a preliminary study with a magnifying glass or even the unaided eye may give a surprising amount of information.

Position. Although some workers prefer to use the microscope with the body tube inclined by the inclination joint, yet one gets just as good results by keeping the tube perpendicular and it is better to become accustomed to such a position since it is necessitated when working with fluid mounts.

The Eye. It is advisable to cultivate the use of both eyes in doing microscopic work. When using a monocular the unemployed eye should be kept open with accommodation relaxed, since squinting causes fatigue. A strip of cardboard 4 or 5 inches long, with an opening to fit over the tube of the microscope, leaving the other end to block the vision of the unused eye, will prevent the strain.

Illumination. Proper illumination is very important in microscopic work; unless the light is utilized to the best advantage the best results cannot be obtained. A north light, or, south of the equator, a southern light is desirable. Direct sunlight or an excessively bright light is to be avoided if possible or reduced by white shades or curtains. Artificial light is more practical because the intensity remains constant. A ground glass or blue filter should be used between the light source and the mirror, depending upon the type of light desired. A binocular microscope requires more light than a monocular; good results are unobtainable without sufficient light. However, too much light is as bad as not enough.

Correct Use of the Mirror. Use the flat side of the mirror for almost all purposes if there is a condenser on the microscope. In cases where the condenser is lacking, or a contour image is desired, as, for example, in hanging-drop preparations and fresh smears of intestinal protozoa or blood, the concave mirror is preferable. The mirror must be centered so that it is filled by the source of light and the maximum intensity of illumination is secured. The quality of the image is reduced if the mirror is used in any other position.

Correct Use of the Condenser. The condenser should be kept at almost its highest elevation. Only when contour images are desired should it be lowered. With high powers the iris diaphragm is used to cut down the light, never the condenser. The sharpest image is obtained when the aperture is the smallest, but never close the iris diaphragm to a point where the cone of light it delivers has a lesser optical angle than that of the objective used or the value of N.A. will be lowered.

Focusing. When using the high dry or oil-immersion objectives it is very important to focus on the preparation in the following way. Lower the objective with the coarse adjustment until it is almost in contact with the coverglass or slide, controlling it with the eye on a level with the stage. Then, looking through the eyepiece, elevate the objective until the object comes into view, and focus sharply with the fine adjustment. Never use the fine adjustment to lower the objective. Contact cannot be felt and the coverglass is apt to break. This ruins the preparation and may injure the lens. Particular care is necessary in focusing hanging-drop preparations since the coverglass is easily broken and there is the risk of infection if virulent organisms are present. Very few microscopes are parfocal. Use caution in shifting from one objective to another.

Care of the Microscope. The following precautions should be observed in order to prevent injury to the microscope:

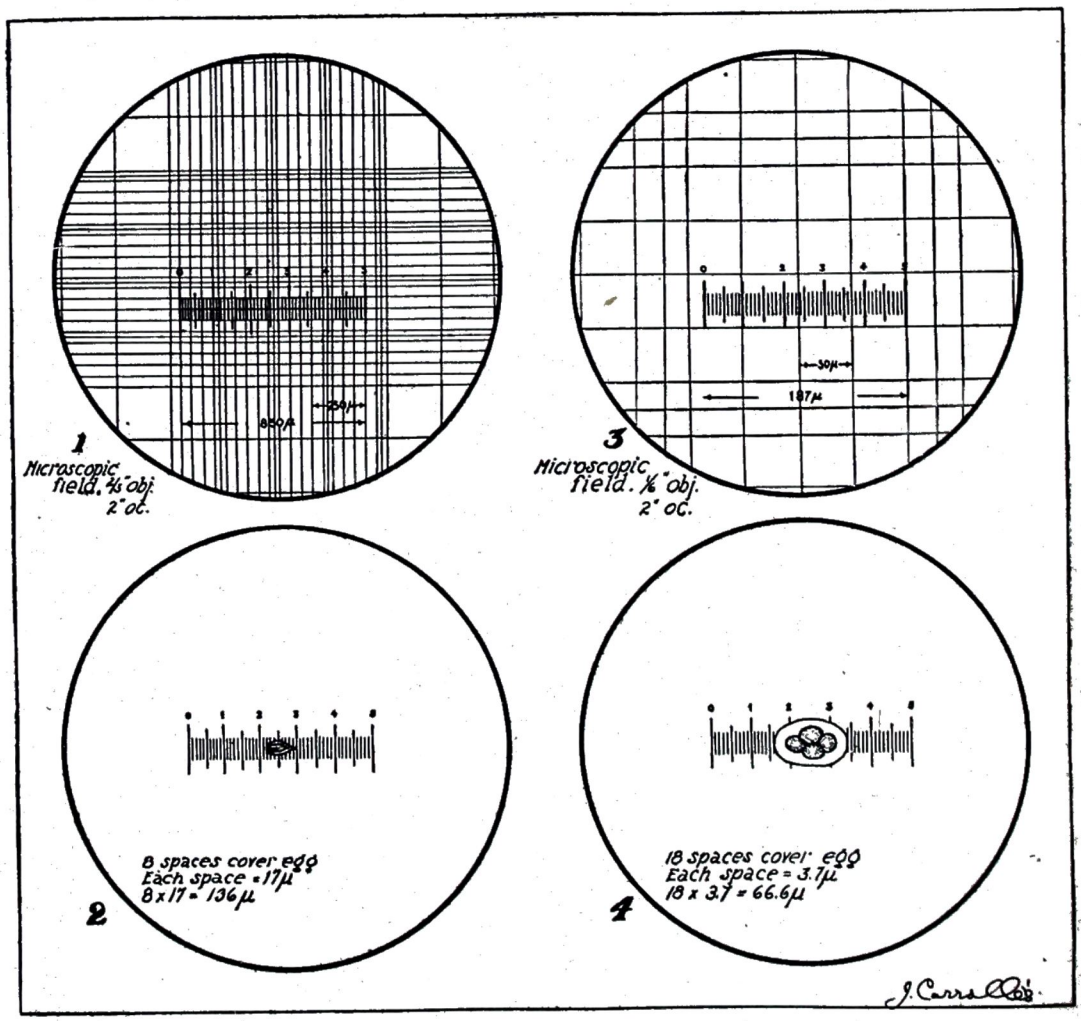
1. If the fine adjustment works through the arm of the microscope, always grasp the instrument by the pillar which supports the stage. In those microscopes, however, which are not constructed in this way the arm has a handle portion made to serve in lifting the instrument.

e not, it should be raised. The ocular micrometer is us th 50 or lines or spaces, separated by longer lines into groups of 5 and 10.

Calibration. All micrometers have an arbitrary length and the apparent length dep on the magnification. Consequently each one must be calibrated for use with *each c bination of objective and eyepiece.* A stage micrometer or hemacytometer is used this standardization. A stage micrometer has ruled lines separated from each other ⅒ mm. (100μ). Some of these ⅒-mm. spaces are again ruled with 10 lines gi spaces which are only ⅒₀ mm. (10μ) apart. If one does not have such a scale, howe a hemacytometer makes a very satisfactory substitute. In any system of ruling a hemacytometer, whether it be Thoma-Zeiss, Türck, or Neubauer type, the sm squares in the central ruling are used for counting red cells. These are in groups 16, and each one is ½₀ mm. or 50μ square. It is these 50μ spaces which are used in bration.

Procedure for Calibration with a Hemacytometer. The principle is exactly the s as with a stage micrometer.

1. Focus on area of the hemacytometer with the smallest squares (50μ), noting there are triple or double lines and single lines.
2. Place a single line of the hemacytometer so that it corresponds exactly with the z line of the ocular micrometer.
3. Being careful not to move the hemacytometer or ocular look to the apparent r



(*1*) Fifty lines of ocular micrometer covering 17 small square spaces (50μ each) of h tometer. Each ocular micrometer space = 17μ. (*2*) Schistosome egg fills 8 spaces of o micrometer. Egg is 136μ long. (*3*) Fifty lines of ocular micrometer cover 3.75 small square Each space = 3.7μ. (*4*) Hookworm egg fills 18 ocular micrometer spaces. Egg is 66.6μ long.

and note a second place where a line of the hemacytometer exactly coincides with one of the micrometer.

4. Note the number of lines on the micrometer (*a*) and the number of spaces on the hemacytometer (*b*) which thus are equal in distance.

5. Since each space on the hemacytometer is equal to 50μ multiply *b* by 50 and divide by *a*. This gives the value of each individual space on the micrometer *with the ocular, objective, and tube length employed in the calibration*. It must be recalibrated for every eyepiece-objective combination. *Note:* With the higher powers often there is no line to the apparent right on the micrometer which exactly coincides with one of the hemacytometer. In this case interpolation is required.

Example: If 20 lines of the ocular micrometer cover 4 spaces of the hemacytometer, then $4 \times 50\mu \div 20 = 10\mu$ = length of one space of the micrometer.

To measure the egg of an intestinal parasite, for example, simply focus on the egg and note the number of spaces covering it. Multiply this number by the value in microns of the space for the objective-eyepiece combination used.

Filters

There are several types of filters in common use for removal of bacteria from liquids.

Berkefeld Filters. These are made of diatomaceous earth, pressed into the shape of a hollow candle and cemented into a metal base which is drawn out into a tube. This is inserted in a glass mantle which holds the fluid to be filtered, and is attached by means of a rubber stopper to an ordinary suction flask or to some special device. There are three grades of porosity: V (viel), coarse (pores 8 to 12μ), is for clearing solutions and does not retain all bacteria. N, normal (pores 5 to 7μ), retains ordinary bacteria. W (wenig), fine (pores 3 to 4μ), retains bacteria and some viruses.

Mandler Filters. These filters, which are much used in the United States, are similar in construction and use to the Berkefeld filters. They are made of diatomaceous earth, asbestos, and plaster of Paris. There are three grades: "preliminary," "regular," and "fine," corresponding approximately to V, N, and W Berkefelds.

Chamberland Filters. These are made of unglazed porcelain (kaolin with a little sand). They are pressed into candles open at one end, into which the stem of a funnel can be fitted by means of a rubber stopper. The fluid filters through the candle from within outwards. There are nine grades: L 1 (coarse, like Berkefeld V, not retaining bacteria), L 1 bis, L 2, L 3 (like Berkefeld N, pores 2.7μ, retaining most bacteria), L 5, L 7, L 9, L 11, and L 13 (finest, retaining some viruses).

All of these filters are obtainable in various sizes.

Seitz Filters. These consist of a special asbestos pad which fits in a metal holder. The grade EK ("germicidal") removes ordinary bacteria. The discs are discarded when exhausted. These pads liberate alkali in the solutions, particularly the older types.

Ultrafiltration through specially prepared collodion membranes is much used in the study of viruses. The technic is complicated. (See Elford: *J. Path. & Bact.*, 34:505, 1931.)

Testing Filters. Gross defects (cracks, leaking joints) may be detected by immersing the candle in water with the closed end up and attempting to blow air through it. If it seems intact, assemble the filter, sterilize in the autoclave, and filter a liquid to which has been added sufficient of a 24-hour broth culture of prodigiosus (*S. marcescens*) or some similar organism to give about 100,000,000 organisms per ml. Culture liberal amounts of the filtrate. If no growth appears within 48 hours, the filter may be regarded as intact.

Cleaning Candles (Rivers' Method). If infectious material has been filtered, soak in some disinfectant such as cresol which does not coagulate protein. Scrub the surface with a brush, and force through the filter from within outward water (or salt solution if the fluid filtered contained globulin) until clean. Boil half an hour in 2 per cent sodium carbonate, and then in several changes of distilled water. Force water through the candle until it is clean and all alkali has been removed. If clogged with organic

material, Chamberland candles may be dried in a warm oven and gradually heate a muffle furnace to a dull red heat, and then slowly cooled. Berkefeld filters often c if so heated.

Filtration. This is better carried out by suction than by pressure. Filtration should rapid, but the negative pressure should not exceed 35 to 50 cm. Hg. The liquid to filtered should be cleared of detritus by preliminary centrifugation and filtration thro paper, cotton, or a coarse filter.

The filtrability of a particle depends only in part upon the relative size of the parti and the pores of the filter. The composition and reaction of the liquid medium is equ important (see p. 189). If the medium is acid, the filters will usually retain small teria and some viruses which will pass through if it is slightly alkaline. Filtration viruses is facilitated by suspending them in meat infusion broth or in 10 per cent se rather than in salt solution, or by first drawing some sterile broth through the filter.

Capillary Pipets

With the possible exception of the platinum loop, there is no piece of apparatus s generally useful as the capillary pipet. It is made from a piece of ¼-inch soft-glass tub about 6 inches long. Held by the ends and constantly revolved, this is heated in a B sen flame, preferably fitted with a fish-tail tip, until it becomes soft in the center. It then removed from the flame and, with steady traction, drawn out so that there int venes a capillary portion 18 to 20 inches long. When cool, it is filed and this capill portion broken off in the middle. One then has two capillary pipets. By using a rub bulb, such as comes on medicine droppers, one has a means of sucking up and for out fluids. The bulb should be pushed on about ½ to ¾ inch; this gives a firmer s to control the pressure on the bulb.

Bacteriologic Pipet. A bacteriologic pipet is made from a piece of tubing 9 inches lo The tubing is heated successively in the flame points 3 inches from each end; in e instance the tube is drawn out just sufficiently to make a constriction. Then, follow the procedure described in the preceding paragraph and heating the middle of the tu two capillary pipets are obtained. A piece of cotton is lightly pushed into the larger en

Wright's Tube. This tube, with a hooked end which permits hanging the crook the centrifuge guard, is the best known apparatus for securing small quantities of blo for serum tests. By filing and breaking the thicker part of the tube, the serum is ma directly accessible to a capillary rubber-bulb pipet, or to the tip of a hemacytometer pipe thereby facilitating dilution of the serum.

Lyon's Blood Tube. To make this tube, a 5- or 6-inch section of ½-inch tubing heated in the center and drawn out as for making two bacteriologic pipets. This divided, and the large end sealed off in the flame. Next the capillary end is sealed Then a very small flame is applied to a point on the large end just before it begins taper to the capillary part. The heat causes the air sealed off inside to force out a bl hole. To use, the sealed capillary end is broken off and allowed to suck up blood fr a drop just as with the Wright tube. I consider this tube superior to that of Wright.

Although these pipets may be sterilized during the flaming, and used immedia afterward, it is better to keep on hand a supply, suitably wrapped and autoclaved use on occasion.

Apparatus and Technics in Anaerobic Methods

The following methods and equipment are best adapted to small diagnostic labo tories.

When organisms can be grown in liquid matrix and surface colonies are not necess use deep tubes of Brewer's medium, semisolid agar, or cooked meat. Anaerobiosis can assured for the last two if the tubes are heated to drive off oxygen prior to inocul and sealed with paraffin oil during incubation. By making the semisolid agar fairly

it is possible to obtain isolated colonies in the depth of the medium. These can be reached by warming the tube gently and sliding the entire mass out into a Petri plate. Then with a sterile scalpel the mass can be sectioned at the desired level.

If it is desirable to obtain surface colonies on solid media it is best to use a system containing pyrogallic acid. Take two evaporating dishes at least 1 inch deep and have one at least an inch greater in diameter than the other. Pour the desired medium into the smaller of these exactly as is done with a Petri dish. After inoculating the surface, place about 1 Gm. of pyrogallic acid in the large dish and invert the smaller over it. Now run in a few milliliters of 5 per cent sodium hydroxide and immediately seal the space between the two dishes with paraffin oil. The same technic can be used for slants by cutting the plugs off flush with the tube, pushing them down, adding pyrogallic acid and hydroxide solution, and putting in a tight stopper. Incubate in an inverted position.

When it is necessary to provide an anaerobic atmosphere for a large number of cultures use a jar or can which can be tightly sealed. In the bottom of this place 1.5 Gm. powdered chromium and 0.5 Gm. sodium carbonate, per liter capacity of the jar; use a small beaker to contain the reagents. Into this powder pipet 15 ml. of 15 per cent (by volume) sulfuric acid. Place the cover on loosely until effervescence is about completed and then tighten it down and seal with Scotch tape or paraffin. Hydrogen gas is given off during the reaction and remains during the period of anaerobiosis; consequently, keep the container away from flames. An indicator which will demonstrate loss of anaerobic condition can be made by adding 2 drops of Loeffler's alkaline methylene blue to a tube of glucose broth and heating until the oxygen is driven out. Return of the blue color indicates the presence of oxygen.

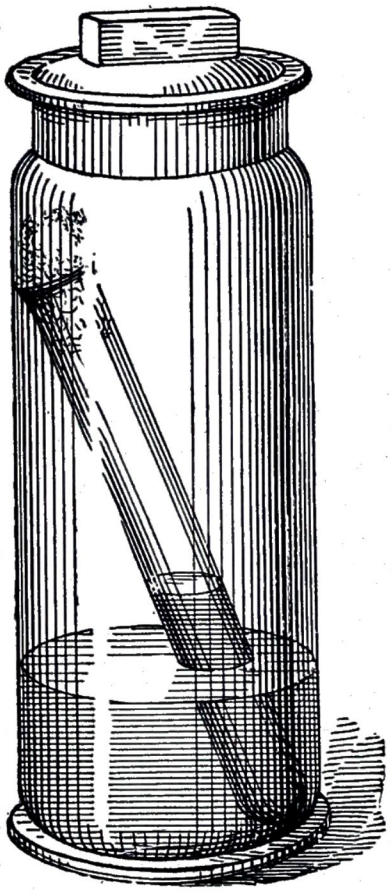
Novy Jar. This is one of the most convenient and satisfactory devices for securing strict anaerobic conditions. Any one of several methods described may be used for abstraction of oxygen. A good substitute may be improvised from an ordinary museum jar, the top of which is perforated to admit a rubber stopper carrying one or two glass tubes through which air can be withdrawn and hydrogen introduced. (A glass stopper is better.)

Method of McIntosh and Fildes. This method or one of its modifications is now regarded as giving the most satisfactory anaerobic conditions obtainable. It depends upon the oxidation of hydrogen by the oxygen in the jar due to the catalytic action of platinized, or preferably palladinized, asbestos wool. The culture tubes or plates are put in the jar. The asbestos wool, protected by a small cage of copper gauze, is heated red hot and suspended in the jar from the lid. The jar is quickly closed tightly, and sealed with plasticine or some substitute. A stream of hydrogen from a cylinder provided with a good reducing valve is then introduced so that it passes over the asbestos wool *very slowly* to avoid an explosion. This is continued until the oxygen is exhausted, and the inlet tubes are then closed. As an indication that an adequate degree of anaerobiosis has been secured McIntosh and Fildes suggested using a tube of sterile 2 per cent dextrose alkaline broth tinged with methylene blue. This is decolorized (reduced) when the oxygen is abstracted, and regains the blue color when oxygen is admitted.

Simple *replacement of air by hydrogen* from a cylinder or from a Kipp generator serves fairly well if palladinized asbestos is not available. It is more effective alternately to exhaust the air from the jar with an ordinary suction pump, and replace with hydrogen several times.

Buchner's Method. In this method 1 Gm. each of pyrogallic acid and caustic potash or soda for every 100 ml. of space in the vessel containing the culture is used to absorb the oxygen. It is convenient to drop in the pyrogallic acid; then to put in place the inoculated tubes or plates; then quickly to pour in the amount of caustic soda, in a 10 per cent aqueous solution, and immediately to close the containing vessel. A large test tube in which a smaller one containing the inoculated medium is placed, and which may be

closed by a rubber stopper, is very convenient. A good rubber-band fruit jar is s factory. A desiccator may be used for plates.



Arrangement of tubes for cultivation of anaerobes by Buchner's method. (MacNeal.)

Tarozzi's Method. In this method, pieces of fresh ste organs are added to broth. Pieces of kidney, liver, spleen are best suited. After adding the tissue the me may be heated to 80° C. for a few minutes without int fering with the anaerobic-producing properties of fresh tissues. This is also a feature of Noguchi's meth of culturing *Treponema pallidum*.

To get effective anaerobiosis it is necessary to put pieces of tissue in the bottom of deep tubes of plain or glucose agar or broth, and to pour over the top sterile paraffin oil, or preferably melted petroleum jelly or paraffin. Liquid oil retards but does not prevent diffusion of oxygen into the medium. Such tubes can be put in a Novy jar and the oxygen largely exhausted by replacement with hydrogen or by the pyrogallic acid method.

Increased Carbon Dioxide Tension. Many organisms appear to grow better when the surrounding atmosphere contains more carbon dioxide and water vapor than is ordinarily present. The candle jar satisfies these conditions most easily. Plates and flasks are placed in wide-mouthed jars or cans equipped with tight-fitting tops. A small piece of candle is placed in with them and lighted. The top secured tightly while the candle is still burning. When candles are not available a pledget of cotton soaked in alcohol will serve the purpose. Single culture tubes can be provided with increased carbon dioxide by cutting plugs off flush with the tube and pushing them down int the lumen. A few drops of alcohol are then placed on the plug, ignited, and a rubber stopper inserted.

Partial Oxygen Tension. By incubating deep tubes glucose agar which have been melted and cooled just before inoculation, without seali with oil or paraffin, gradations of partial oxygen tension are obtained from aerobic co tions at the surface to practically complete anaerobiosis at the bottom. Some strains streptococci, for example, grow only in a restricted zone below the surface.

B. Preparation of Tissues for Examination in Microscopic Sections

The most important step in the preparation of sections of tissues for histologic examination is proper and immediate fixation. This step in the technic is often in the hands of the surgeon at the time of the operation or the physician at autopsy, and it should understood by them that a satisfactory diagnosis can be made only when the pieces o tissue are *at once dropped into a fixative.* Various protozoa, as amebae, disintegrate one or two hours unless properly fixed, and body cells show degeneration after tissues have been left without fixation for a few hours, which changes may be in preted as pathologic.

Drop into the solution slices of tissue, not more than ¼ inch thick, *as soon as cut.* Leave in the fixative for 24 hours or longer when the specimen is to be sent away a laboratory for diagnosis. *The pathologist will attend to the other steps.*

We use two fixation solutions in routine work, one of 10 per cent formalin and of Zenker's solution. This latter requires prolonged washing of tissues following fixa and has little advantage over formalin for ordinary purposes.

Fixation. The piece of tissue to be fixed must not be too large. Using a sharp sca

or preferably a razor, a section of tissue about ½ inch square and not more than ⅛ inch thick should be dropped into the bottle containing the fixative. The bottom of this bottle should have a thin layer of cotton with a piece of filter paper covering it. There should be at least twenty times as great a volume of fixing fluid as of tissue to be fixed. Delicate tissues, as pieces of gut, should be attached to pieces of glass, wood, cardboard, or blotting paper before being placed in the fixative. In fixing certain specimens of tissue, especially pieces of slit intestine, it is a good plan to lay the specimen, while wet, peritoneal side downward, on a piece of thick dry filter paper or lintless blotting paper; this prevents the specimen from being curled up. The whole is put into the fixing fluid, and the paper removed after fixation. The number or name of the specimen may also be written on the paper.

Formalin. The most convenient fixative for routine purposes is a 10 per cent solution of ordinary commercial formalin (4 per cent of formic aldehyde gas), either in water or, preferably, in 0.85 per cent salt solution. Fixation is complete in from 12 to 24 hours. By placing in the incubator at 37° C., 2 to 12 hours in the formalin solution suffices. If fixed in the paraffin oven (56° C.), fixation is accomplished in about one-half hour. Formalin once used for fixation must be thrown away.

Zenker's Fluid. Zenker's fluid probably gives the best histologic pictures and is the most satisfactory fixative for hematoxylin staining. It is a modification of Müller's fluid. The formula is:

Potassium bichromate	2.5 Gm.
Mercuric chloride	5.0 Gm.
Water	100.0 ml.

Just before use add glacial acetic acid 5 ml.

Zenker's fluid fixes in about 24 hours. After all corrosive sublimate fixatives one should wash the tissues in running water for 12 to 24 hours. The precipitate of mercury in the tissues is best removed by treating the section on the slide with Lugol's solution, rather than the tissue in bulk with iodine alcohol. A saturated corrosive-sublimate solution in salt solution with the additional 5 per cent glacial acetic acid may be used as a substitute for Zenker's fluid.

Alcohol. Where the tissue is to be examined chiefly for bacteria absolute alcohol is the best fixative. The piece of tissue should be small, not over ⅛ inch thick, and is to be suspended by a string to the cork so as not to lie on the bottom where the alcoholic strength tends to become weaker. Better histologic details are secured by fixing for two hours with 80 per cent alcohol and then transferring to absolute for 12 to 24 hours.

Note: Most laboratories prefer to receive tissue that has been fixed in formalin. Alcohol interferes with frozen sectioning and *postal regulations forbid alcohol in mail.*

Dehydration. After washing for 12 to 24 hours in running water following corrosive sublimate fixation, or simply washing for a few minutes after formalin, the tissues should be placed in 70 per cent alcohol in which they may be kept indefinitely.

C. Equivalent-normal Solutions

An equivalent-normal solution contains the hydrogen equivalent of a substance, expressed in grams, dissolved in sufficient distilled water to make 1 liter. The hydrogen equivalent is the number of grams that will unite with 1 Gm. of hydrogen or its equivalent. For an acid, the hydrogen equivalent would be the molecular weight divided by the number of *replaceable* hydrogen atoms that it contains. For a base, it would be the molecular weight divided by the number of hydroxyl (OH) groups.

To make a normal (indicated by N) solution, dissolve in distilled water the proper amount of the substance, and make up the volume to exactly 1000 ml. Thus sodium hydroxide has one hydrogen equivalent: Na = 23, O = 16, and H = 1, so one dissolves 40 Gm. sodium hydroxide in distilled water, and makes the volume up to exactly 1 liter.

Again, oxalic acid has the formula $(COOH)_2.2H_2O$, with a molecular weight of 1 containing two carboxyl groups, it has two hydrogen equivalents and it is necessary divide by 2. So we dissolve 63 Gm. in water, and make up to 1 liter.

Preparation. If a chemical laboratory is not accessible, one may prepare such solutio with an error so slight as to be unimportant in clinical work in the following manner Select perfect crystals of oxalic acid, such as can be obtained in a drug store, weigh out, on the most accurate apothecary scales available, 6.3 Gm. of the mo perfect crystals in the bottle. Put these preferably in a volumetric flask, and make u with distilled water to 1 liter. The use of a measuring cylinder is less accurate. Wi care, this method should give N/10 oxalic acid in which the error is less than 1 per cent.

Sodium hydroxide being very hygroscopic, it is impossible accurately to prepare a normal solution directly by weighing the solid. Having N/10 acid at hand, an N/10 NaOH solution may be prepared by weighing an excess of the substance, about 5 Gm. stick caustic soda, and dissolving it in about 1100 ml. distilled water. By means of a pipet, place 10 ml. in a beaker, and add 6 drops phenolphthalein solution (1 per cent in 95 per cent alcohol). Fill a buret with N/10 oxalic acid, and run it into the NaOH solution until the violet-pink color is just discharged. It is well to repeat the titration, and use an average. Reading the number of milliliters of N/10 acid used, we can calculate the strength of the NaOH solution. If 10.5 ml. of oxalic acid solution were required, it would show that the NaOH solution was stronger than N/10, as only 1 ml. would have been necessary if it had been of N/10 strength. It is, therefore, nece sary to dilute in the proportions of 10 : 10.5. To do this, take exactly 1000 ml. of the too concentrated NaOH solution, add 50 ml. distilled water to it, mix thoroughly, and there is then 1050 ml. of N/10 NaOH. Calculation: $10 : 10.5 = 1000 : x$.

It is sometimes desirable to use *carbonate-free NaOH,* and this may easily be pr pared by dissolving 100 Gm. C.P. NaOH in 100 ml. distilled water. The NaO dissolves completely, but any Na_2CO_3 present is insoluble. Let it settle, or it may b centrifuged. Of the clear supernatant solution obtained after sedimentation, 55 ml. dis solved in CO_2-free water sufficient to make 1 liter will give approximately N/1 NaOH Standardize in the usual way. Such a solution must be protected from the CO_2 of the by storing in a paraffin-lined bottle, the two-holed rubber stopper of which bears guar tubes, and a glass tube for siphonage, the latter being lined and coated with para The guard tubes comprise one containing soda lime (or NaOH solution) and one wi H_2O, and are arranged in series so that, when siphonage is established, the soda lime r moves CO_2 from the incoming air, and then the H_2O prevents the carrying of any al into the bottle.

Water can be freed from CO_2 by vigorous boiling for 15 to 20 minutes (not longer or by aeration for several hours, the air sucked through having been passed throu NaOH solution (or soda lime tubes) and water. If the water is exposed to the CO_2 will again be absorbed, so, if stored, it should be protected by the method describe for the NaOH solution above, although the paraffin lining of tube and bottle is n essential.

Since Acidum Hydrochloricum, U.S.P., is about two-thirds water (68.1 per cent), order to make N/10 HCl, which would require 3.65 Gm. absolute acid per liter, it necessary to take about three times this amount of U.S.P. acid. Take 12 ml., add distilled water to make 1100 ml. Place 10 ml. in a beaker, add phenolphthal solution, and titrate. If 11 ml. of N/10 NaOH were required, it would be necessary add 100 ml. water to 1 liter of the diluted acid. Calculation: $10 : 11 = 1000 : x$. acid and alkali solutions can be made in the same way as are those described.

D. Determination of Hydrogen-ion Concentration

The significance of the hydrogen-ion concentration and the nature of buffer soluti have already been discussed in the section on Acid-base Equilibrium (p. 798).

hydrogen-ion concentration, abbreviated as $[H^+]$, or C_{H+}, is a measure of the intensity of the acidity of a solution, as contrasted with the amount of acid present as determined by titration. It is commonly expressed as a fractional part of a normal solution, which contains 1 Gm. of H per liter. Thus the $[H^+]$ of a 0.01 N solution of a strong acid, since most of the H is dissociated, is nearly 0.01. Since the $[H^+]$ of the body fluids and secretions is low, it is convenient and customary to substitute for these small fractions the logarithm of the reciprocal of the fraction; i.e., the pH.

Since appreciable although minute amounts of H are dissociated even in strong alkaline solutions (the amount diminishing as the alkalinity increases), the reaction of such solutions may also be expressed in terms of their pH. The stronger the acid the lower the pH. Thus the pH of a tenth normal solution of a strong acid would be approximately 1.0, while that of a tenth normal solution of a strong alkali would be nearly 14.

To measure the pH directly requires electrometric determinations which are impracticable for routine purposes. Instead it is customary to estimate it colorimetrically, by adding a suitable indicator to the solution and comparing the color obtained with that yielded by the same indicator in buffered solutions of varying but known pH.

Indicators. The color change of indicators is dependent upon the $[H^+]$, or its complement, the $[OH^-]$ of the solution, and for each indicator there is a definite range of pH through which there is manifested a gradual change from the full alkaline to the full acid tint. Table 83 gives this range for several indicators, those italicized being the ones especially recommended for this work by Clark and Lubs.

Table 83

COLOR CHANGE AND pH RANGE OF INDICATORS

Indicator	*pH Range*	*Color Change*	
		Acid	*Alkaline*
Thymol blue	1.2– 2.8	Red	Yellow
Töpfer's reagent (dimethyl-amino-azobenzol)	2.9– 4.0	Red	Yellow
Bromphenol blue	3.0– 4.6	Yellow	Blue
Congo red	3.0– 5.0	Blue	Red
Methyl orange	3.1– 4.4	Red	Yellow
Bromcresol green	4.0– 5.8	Yellow	Blue
Methyl red	4.4– 6.0	Red	Yellow
Litmus (azolitmin)	4.5– 8.3	Red	Blue
Cochineal	4.8– 6.2	Yellow	Lilac
Bromcresol purple	5.2– 6.8	Yellow	Purple
Alizarin	5.5– 6.8	Yellow	Blue
Bromthymol blue	6.0– 7.6	Yellow	Blue
Neutral red	6.8– 8.0	Red	Orange
Phenol red (phenolsulfonphthalein)	6.8– 8.4	Yellow	Red
Cresol red	7.2– 8.8	Yellow	Red
Thymol blue	8.0– 9.6	Yellow	Blue
Cresol phthalein	8.2– 9.8	Colorless	Red
Phenolphthalein	8.3–10.0	Colorless	Red
Thymol phthalein	9.3–10.5	Colorless	Blue

Buffers. By mixing solutions of proper buffer substances in suitable proportions, mixtures of any desired pH values may be obtained. A suitable indicator is chosen from the list, and a small amount is added to each of the mixtures and to the unknown. The result is a series of graded standard tints with one of which the tint of the unknown is matched. A rough estimate of the pH of the unknown can be obtained by systematically

testing it with different indicators, since reference to the list will show the pH at their full acid or alkaline color may be expected.

Table 84 gives the proportions in which buffer solutions must be mixed in order produce desired pH values. The citric acid-phosphate series was proposed by McIlvain but we have slightly varied the proportions in order to secure colorimetric correspondence with the buffer mixtures of Sørensen and of Clark and Lubs. The boric acid, KCl-NaO series is that of Clark and Lubs, and the resultant mixtures in it are to be diluted to ml. before use. Use $Na_2HPO_4.2H_2O$ (Sørensen's phosphate) in M/5 strength; M/1 citric acid; a solution containing 12.4048 Gm. boric acid and 14.912 Gm. KCl p liter; and M/5 NaOH.

The chemicals employed in the preparation of such solutions must be specially purified—a task probably not within the ability of the usual clinical laboratory. The NaOH solution must be prepared and stored as indicated on p. 922. We will not give the details of this purification inasmuch as the laboratory with the equipment and experience necessary for this will have access to the literature. The buffer solutions and indicator solutions are readily purchasable, and, for the usual laboratory, we would advise that they be so obtained.

Sørensen's M/15 phosphate mixtures are also much used for pH range from 5.8 to 8.2.

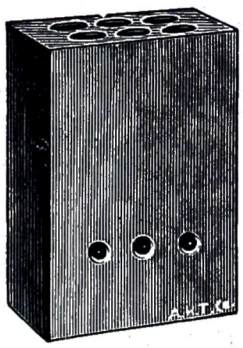
Dissolve 9.08 Gm. of KH_2PO_4 in 1 liter of water.

Dissolve 9.47 Gm. anhydrous Na_2HPO_4 in 1 liter of water.

Prepare mixtures according to Table 85.

Procedure. Select test tubes of clear resistant glass, uniform in diameter, by introducing exactly 10 ml. water into each, and selecting those in which it rises to the same height. It is well to make permanent marks on these tubes at 5 ml. and 10 ml. Into a series of these tubes introduce 10 ml. (or 5 ml.) of standard buffer solutions selected from the range of the pH anticipated in the solution to be tested. In three additional tubes put an equal volume of this solution. It should be clear, and diluted up to five or ten times with distilled water if highly colored. To each standard tube and to one tube of the unknown add, with a pipet or medicine dropper held vertically, equal volumes of a suitable indicator solution and mix.

For the indicators that they recommend Clark and Lubs suggest 5 drops for 10 ml. of solution. The concentration of the indicator solution should be 0.02 per cent for cresol red, phenol red, methyl red, and cresol phthalein; for the others, 0.04 per cent. The solutions are prepared by grinding 0.1 Gm. of the dry dye in a mortar with N/10 NaOH, using the following volumes of alkali: For cresol red, 2.88 ml.; phenol red, 3.1; methyl red, 4.07; bromphenol blue, 1.64; bromcresol purple, 2.78; and thymol blue, 2.38. These are diluted with distilled water to 500 ml. to make a 0.02 per cent solution; to 250 ml. if a 0.04 per cent solution is desired.



Block comparator. (Courtesy, A. H. Thomas Co.)

Precise determinations require many precautions as regards background, source of light, exclusion of adventitious light, etc. It very advantageous to use a wooden comparator block, as illustrated. We prefer to use diffuse daylight if possible and to back the comparator block with ground glass plate or with thin, plain unglazed white paper. In colorimetric comparisons the unknown and standar solutions must be balanced as regards any factors that would affec the shades of color compared. These are particularly intrinsic color or turbidity in the unknown solution and the thickness of the wat columns. To avoid these errors, arrange the tubes in the block thus: In the two late spaces next to the observer put the tubes containing the unknown solution without indicator; in the middle space, a tube of water. In the spaces next to the light put, in the middle space the unknown solution containing indicator; in the lateral spaces standard tubes, changing the latter until the one is found which most nearly matches the color

Table 84

PROPORTIONS OF BUFFER SOLUTIONS PRODUCING DESIRED pH

pH	*M/5 Phosphate, ml.*	*M/10 Citric Acid, ml.*	*pH*	*Boric acid-KCl, ml.*	*M/5 NaOH, ml.*
2.2	0.40	19.60	7.8	50.0	2.61
2.4	1.24	18.76	8.0	50.0	3.97
2.6	2.18	17.82	8.2	50.0	5.90
2.8	3.17	16.83	8.4	50.0	8.50
3.0	4.11	15.89	8.6	50.0	12.00
3.2	4.94	15.06	8.8	50.0	16.30
3.4	5.70	14.30	9.0	50.0	21.30
3.6	6.23	13.77	9.2	50.0	26.70
3.8	6.77	13.23	9.4	50.0	32.00
4.0	7.40	12.60	9.6	50.0	36.85
4.2	7.99	12.01	9.8	50.0	40.80
4.4	8.42	11.58	10.0	50.0	43.90
4.6	8.82	11.18			
4.8	9.50	10.50			
5.0	9.86	10.14			
5.2	10.52	9.48			
5.4	10.94	9.06			
5.6	11.37	8.63			
5.8	11.85	8.15			
6.0	12.43	7.57			
6.2	12.82	7.18			
6.4	13.22	6.78			
6.6	14.50	5.50			
6.8	15.40	4.60			
7.0	16.47	3.53			
7.2	17.12	2.88			
7.4	17.78	2.22			
7.6	18.45	1.55			
7.8	18.95	1.05			
8.0	19.15	0.85			

Dilute each mixture to 200 ml.

Table 85

SØRENSEN'S M/15 PHOSPHATE MIXTURES IN pH RANGE FROM 5.8 TO 8.1

pH 20° C.	*M/15 Na_2HPO_4 ml.*	*M/15 KH_2PO_4 ml.*	*pH 20° C.*	*M/15 Na_2HPO_4 ml.*	*M/15 KH_2PO_4 ml.*
5.8	8.0	92.0	7.0	61.1	38.9
5.9	9.0	91.0	7.1	66.6	33.4
6.0	12.2	87.8	7.2	72.0	28.0
6.1	15.3	84.7	7.3	76.8	23.2
6.2	18.6	81.4	7.4	80.8	19.2
6.3	22.4	77.6	7.5	84.1	15.9
6.4	26.7	73.3	7.6	87.0	13.0
6.5	31.8	68.2	7.7	89.4	10.6
6.6	37.5	62.5	7.8	91.5	8.5
6.7	43.5	56.5	7.9	93.2	6.8
6.8	49.6	50.4	8.0	94.7	5.3
6.9	55.4	44.6	8.1	95.8	4.2

the unknown. Interpolate if necessary. One must not rely on a color match with the e tube of a standard series. The result must be checked by using another series so selecte that the pH of the unknown falls well within its range.

It is imperative that the glassware be chemically clean, that the chemicals used be the purest obtainable, and that the distilled water be neutral and free from CO_2. If the standard solutions are sealed in ampules of resistant glass having exactly the same diameter as the tubes used for the unknown solutions, and if they are kept in a dark cool place when not in use, they may usually be used for several months or more, but the colors should be checked occasionally.

Examination of Urine. The pH of the urine changes very rapidly on exposure to the air, because of the escape of CO_2. To get really accurate determinations it must be preserved (and if possible be collected) under oil, and examined under oil. In a tube put 8 ml. distilled water, add the indicator solution, and cover with oil. With a pipet introduce 2 ml. of urine, keeping the tip of the pipet under the surface of the water. Then proceed as above. The usual pH is about 6, the range being from about 4.8 to 7.5. Bromcresol green or purple may be used for acid specimens, bromphenol blue for average acidities, and phenol red for alkaline specimens.

Bicolor Method. The bicolor method of determining the pH has many advantages over the use of buffer solutions. The standard solutions are more permanent, are less affected by variations of temperature, and errors due to impurities in the buffers are eliminated, although pure indicator solutions are essential. The method has been elaborated by Gillespie and by Hastings et al. (1925) and applied to determinations of the pH of the urine and blood plasma, and it yields accurate results. (For details consult original article or Peters and Van Slyke: *Quantitative Clinical Chemistry.*)

Table 86

DETERMINATIONS OF pH VALUES WITH BICOLOR METHOD

Drop Ratio (Front: Back)	*pH Values with*			
	Methyl Red (0.008%)	*Bromcresol Purple (0.012%)*	*Phenol Red (0.004%)*	*Thymol Blue (0.008%)*
1 : 9	4.05	5.3	6.75	7.85
2 : 8	4.4	5.7	7.1	8.2
3 : 7	4.6	5.9	7.3	8.4
4 : 6	4.8	6.1	7.5	8.6
5 : 5	5.0	6.3	7.7	8.8
6 : 4	5.2	6.5	7.9	9.0
7 : 3	5.4	6.7	8.1	9.2
8 : 2	5.6	6.9	8.3	9.4
9 : 1	5.95	7.2	8.65	9.75

Barnett and Chapman introduced a relatively simple procedure which illustrates t principles of the method and is sufficiently accurate for most practical purposes.

Place 18 test tubes in rack in 2 rows of 9 each. Beginning at the left in the fro row, place 1 drop of indicator in the first tube, 2 in the second, and increase by 1 dro in each succeeding tube as one passes to the right. Treat the rear row similarly, but be at the right and pass to the left. To each tube in the front row, add 1 drop (2 to drops for the thymol-blue series) N/20 NaOH; to each tube in the rear row, add 1 dro N/20 HCl (use 1 drop 2 per cent KH_2PO_4 for thymol-blue series instead of HCl). Fill tubes to 5-ml. level with water. This standard series is viewed from the front in suc manner that the line of vision traverses two tubes—one in the front row and its

ner in the rear row—the total amount of indicator in each pair being 10 drops. The composite colors form a graded series, and the preceding table shows the pH values represented by each pair. The methyl red solution is prepared by grinding in acid-free alcohol until dissolved, and then diluting 3 volumes with 2 volumes of H_2O. The preparation of the other indicators is given above.

In a tube containing 10 drops of indicator solution put sufficient of the unknown solution to bring the volume to 5 ml. and mix. Put in a comparator block and back with two tubes of water. Also put a pair of standard tubes in the block and back with a tube containing the unknown solution without indicator. Change the pairs of standard tubes until a pair is found which matches the color of the unknown. Since this procedure necessitates looking through three tubes in series, the openings for inspecting the tubes must be drilled lengthwise through the comparator block.

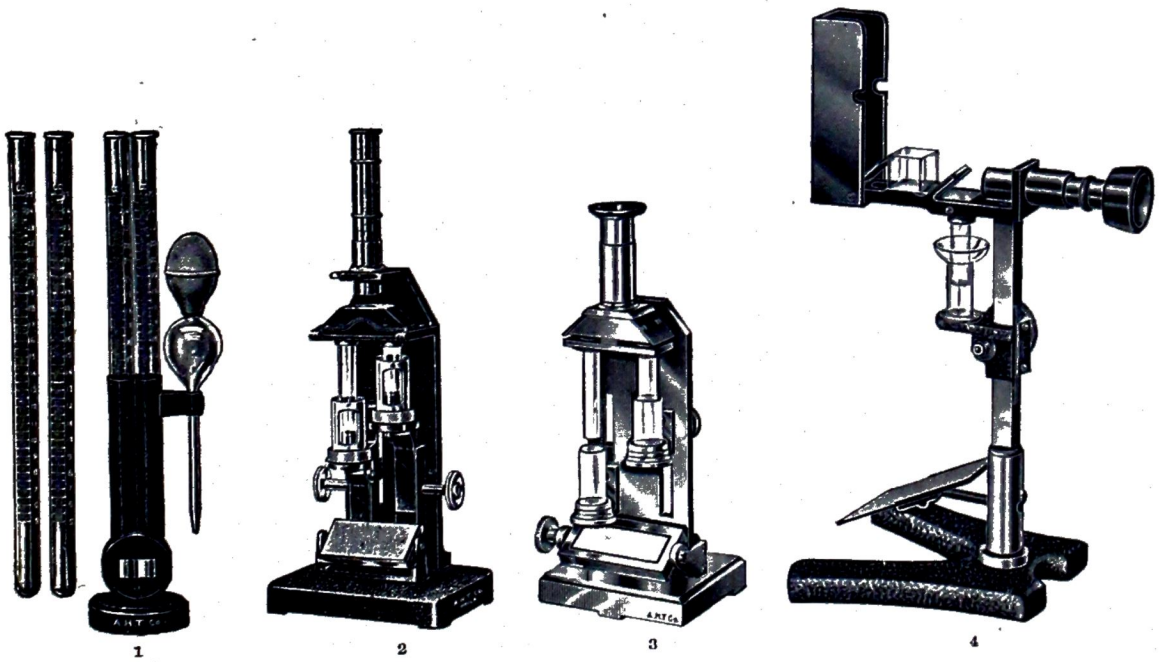
E. Colorimetric Determinations

There are two types of visual colorimeters in general use; in one, the plunger type, the intensity of the colors of the two solutions is matched by varying the depths of the solutions; in the other this is accomplished by diluting one of the solutions, the depth of the fluid traversed by the line of vision remaining constant.

With the plunger type the relative concentrations of the substance will vary inversely as the readings (depths), and the calculation follows the general formula:

$$\frac{S}{U} \times F = X$$

in which S and U are, respectively, the readings of the standard and unknown solutions when colors are matched, F is a factor, and X is the result sought. F is constant under the conditions of each determination, and its value is determined by three considerations, viz.: (1) The actual amount (in terms of X) of substance (nitrogen, uric acid, sugar, etc.) used in preparing the standard mixture, (2) the relative volumes to which unknown and standard have been diluted, and (3) the relation of the *actual* amount of substance (blood, urine, etc.) used in preparing the unknown to the terms in which we desire to express X. For example, take the calculation for nonprotein nitrogen of blood

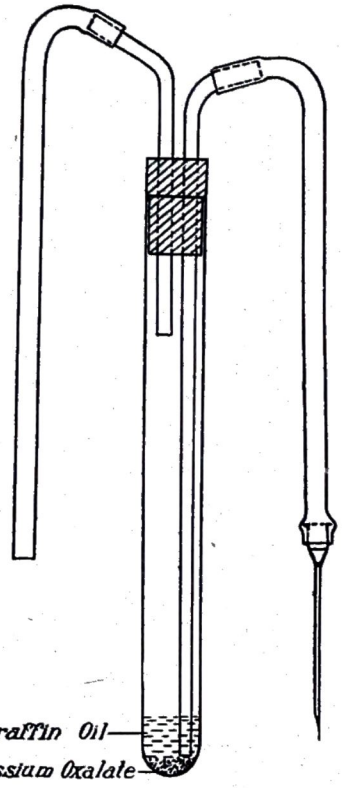


Types of colorimeter: (1) Myers. (2) Duboscq. (3) Duboscq (Pellin). (4) Bock-Benedict. (Courtesy, A. H. Thomas Co.)

(see p. 766). S and U are determined, and we are to express X as milligrams per 100 m blood.

$$\frac{S}{U} \times 0.3 \times \frac{50}{100} \times \frac{100}{0.5} = \frac{S}{U} \times 30 = \text{milligrams nonprotein nitrogen per 100 ml. blood.}$$

(1) We have used 0.3 mg. nitrogen (not ammonium sulfate); (2) standard is diluted to 100 ml. and unknown to 50 ml.; therefore multiply by $^{50}/_{100}$ (or divide by 2); (3) 5 ml. filtrate represents 0.5 ml. whole blood, and X is to be expressed in terms of 100 ml.



Tube used in collecting blood. (*J. Biol. Chem.*, 30:289, 1917.)

With the dilution type of colorimeter, the same reasoning applies, but we have here a direct proportion, and the general formula for the calculation becomes $U \times F = X$, if, as is usually the case, it is the unknown solution which is diluted.

It has been advised usually to set the standard solution at a fixed point and vary the depth of the unknown to match it. The calculation may be simplified by setting the unknown at some appropriate height and varying the depth of the standard. In the example above, e.g., if the unknown is set at 30, the reading of the standard gives the value of X directly.

Permanent standards of tinted glass or of stable solutions for practically all the examinations can be purchased, but very few are satisfactory. It is usually preferable to prepare the standard at the time of the test, using the same reagents as for the unknown. Standard and unknown solutions should be handled in the same way and finished practically simultaneously. In using a colorimeter take precautions against retinal fatigue and adventitious light. Adjust the scales necessary so that they read zero when the cups ar elevated so that they just touch the plungers, or determine a correction factor. Make sure that the fields equally illuminated and check by reading the standard against itself. If the field i divided by a line, this line must be sharply focused and the halves of the field mad equal in area.

If a regular colorimeter is not available, Nessler tubes or even graduated cylinders test tubes can be used quite satisfactorily for approximate results provided their internal diameter is identical, the length of the column of fluid being measured by a ruler.

Photoelectric Colorimeter. The principle on which the use of this type of instrument based depends upon the fact that when a photoelectric cell is exposed to light a feebl electric current is generated which is proportional to the intensity of the light and c be measured with a sensitive galvanometer. If a tube containing a colored solution interposed between the cell and the light the intensity of the light reaching the cell is duced, and there is a corresponding reduction in the strength of the current. denser the color, the less the light reaching the cell, and the more the current is reduc It is thus possible to compare the color of an "unknown" solution with that of standard solution containing a known amount of the substance to be measured. T comparative density of the colors is expressed by a logarithmic and not a simple ari metic ratio between the strengths of the currents.

Owing to the technical difficulties and expense involved in constructing a galvanome sufficiently sensitive and accurate for this purpose, many of the instruments now in

are provided with two identical photoelectric cells so arranged in series with one another (and with a galvanometer and a calibrated variable resistance) that when the cells are equally illuminated and the instrument is properly adjusted, the currents produced by the two cells balance each other and there is no flow through the galvanometer. If a tube containing a colored solution is interposed between the light and one of the cells the current produced by this cell is reduced, the balance is upset, and a current will flow through the galvanometer. By adjusting the variable resistance the balance is restored and the galvanometer reading is brought to zero. The instrument is provided with a scale indicating the resistance in the circuit, and if the calibrations on the scale are spaced logarithmically, as in the Klett-Summerson colorimeter, and proper precautions are observed, the density of the colors is directly proportional to the scale readings, and no calibrated curves or charting on special semi-logarithmic paper are necessary. We have found this instrument simple, convenient, and adequate for the usual routine laboratory procedures.

In order to secure the necessary sensitiveness and accuracy, it is essential to insert a suitable light filter of colored glass between the light and the photoelectric cells. As a rule this should be one which removes most of the light except that fraction which is specifically absorbed by the solution being measured. Thus, if a red solution is being examined, which absorbs blue light but lets red come through, a blue filter should ordinarily be used to absorb the light in the lower (red) end of the spectrum. This increases markedly the difference in the scale readings obtained with two similar red solutions of different density and increases the sensitiveness of the instrument correspondingly. A similar advantage may be obtained by using such filters with visual colorimeters, but this is not ordinarily done in routine work. The proper filter varies with the solution to be tested and must be ascertained, either from the directions for use issued with the instrument employed, or elsewhere. If this is not certain, a test should be made with a number of different dilutions of known strength, and each read with several different filters. That filter should be chosen which gives readings which are most nearly proportional to the known quantities of material present. For most ordinary determinations with the Klett-Summerson colorimeter, three filters suffice: a blue filter with a special range of about 400 to 465mμ; a green filter with a range of 500 to 570mμ; and a red filter with a range of 640 to 700mμ. For certain determinations a special filter with a different or more restricted range may be necessary. Readings high on the scale (above 400 to 500) are not accurate, and should be lowered either by using smaller quantities of the material to be measured or by a different filter. Certain precautions must be observed in adjusting and using the instrument, which vary with the type of instrument and should be ascertained from the directions issued with it.

It is possible to construct a calibration curve, using several different dilutions of the standard of known strength and charting the results obtained. Subsequent tests with unknown solutions can be read directly from such charts without using a standard solution for each test. This is dependable only if the technic is so standardized by long practice that it is precisely repeated in every essential detail in every test. In the average laboratory where routine tests are made this is usually impracticable. It is far safer to set up a standard solution with each test, as minor differences in technic will ordinarily affect the standard and unknown solutions proportionately. The same objection applies to the use of colored-glass standards or similar makeshifts. If such curves are used for special reasons, the technician must practice with standard solutions until essentially identical scale readings can regularly be reproduced. New charts must be prepared with each new batch of reagents or other apparently minor change in procedure.

The chief advantages of this type of colorimeter are that readings can be made much more quickly and that accurate readings can be made with solutions too pale to read with a visual colorimeter. Determinations can therefore be made with smaller amounts of material. The photoelectric colorimeter also obviates errors due to retinal fatigue

or difficulties some individuals have in matching colors closely. The other poten errors inherent in colorimetric procedures remain, and results obtained are not nec sarily more precise or dependable than those obtained with a visual colorimeter. If solutions are in the slightest degree turbid, opalescent, or "off color," the error be as great or often greater than with a visual colorimeter.

F. Anatomic and Physiologic Normals

In examinations in the pathologic or chemical laboratory the following may be co sidered approximately as normal findings:

I. Anatomic Normals. Averages.

ADRENALS. Length, 2.4 to 2.8 inches (6 to 7 cm.). Breadth, 1.2 to 1.4 inches (3 to 3. cm.). Weight, 0.17 to 0.21 ounce (5 to 6 Gm.) each. Left usually larger.

AORTA. Length, varies, 17 to 20 inches (42.5 to 50 cm.). Thickness of wall, 0.06 to 0.08 inch (1.5 to 2 mm.). Diameter, 0.75 to 1.25 inches (1.7 to 3 cm.). Weight, 1.2 to 1. ounces (35 to 45 Gm.).

APPENDIX. Length, quite variable, 3.5 to 4 inches (9 to 10 cm.). Diameter 0.25 inch (6 mm.). Weight, 0.25 to 0.5 ounce (7 to 14 Gm.).

BLADDER. Capacity, 16 ounces (500 ml.) when normally distended. Thickness of wall, 0.1 inch (2.5 mm.). Weight, 1 to 2.1 ounces (30 to 60 Gm.).

BRAIN. Weight, female 44 to 45 ounces (1250 to 1275 Gm.); male 48 to 51 ounces (1365 to 1450 Gm.). Length, 6.5 inches (16.5 cm.). Transverse diameter, 5.5 inches (1 cm.). Vertical diameter, 5 inches (12.7 cm.). Dimensions in female are 0.4 inch (1 cm.) less.

ESOPHAGUS. Length, 10 to 12 inches (25 to 30 cm.). Diameter of lumen, 1.25 inche (3 cm.). Thickness of wall, 0.3 inch (8 mm.). Weight, 1.4 ounces (40 Gm.).

FALLOPIAN TUBES. Length, 3 to 5 inches (7.6 to 12.6 cm.). The right usually the longe Diameter of lumen averages 0.1 inch (2.5 mm.).

GALL-BLADDER. Length, 3 to 4 inches (7.5 to 10 cm.). Diameter, 1 to 1.25 inches (2.5 to 3 cm.). Thickness of wall, 0.04 to 0.07 inch (1 to 2 mm.). Capacity, 1 to 1.5 ounces (30 to 45 ml.).

HEART. Weight, female 8.8 to 9.8 ounces (250 to 280 Gm.), male 9.5 to 12.7 oun (270 to 360 Gm.). Length, 4.5 to 5.5 inches (11.5 to 14 cm.). Breadth, 3 to 4 inch (7.5 to 10 cm.). Thickness, 2 to 3.1 inches (5 to 8 cm.). Thickness, wall left ventricl 0.35 to 0.47 inch (9 to 12 mm.), right ventricle, 0.1 to 0.12 inch (2.5 to 3 mm.). C cumference, mitral orifice, 4.1 to 4.3 inches (10.4 to 10.9 cm.). Circumference, tricusp orifice, 4.7 to 5 inches (12 to 12.7 cm.). Circumference, aortic orifice, 3 to 3.2 inches (7 to 8 cm.). Circumference, pulmonary orifice, 3.4 to 3.6 inches (8.5 to 9 cm.).

INTESTINES. Small intestine, length, 22.5 ft. (6.75 meters); ⅖ jejunum and ⅗ ile Diameter from 1.85 inches (47 mm.) in duodenum to 1.06 inches (27 mm.) at the en of ileum. Large intestine, length, 70.9 to 76.8 inches (180 to 195 cm.). Duoden length, 10.2 to 11.2 inches (26 to 28.5 cm.).

KIDNEYS. Weight, left, 5.3 ounces (150 Gm.), right, 5 ounces (140 Gm.). Thickness cortex, 0.4 inch (1 cm.). Length, 4.5 inches (11.5 cm.). Breadth, 2.50 inches (6 cm.). Thickness, 1.25 inches (3.2 cm.). The left longer and the right thicker.

LIVER. Weight 50 to 60 ounces (1440 to 1680 Gm.). Greatest transverse diameter, to 9.5 inches (20 to 24 cm.). Greatest anteroposterior diameter, 3.9 to 5.9 inches (10 to 1 cm.). Vertical diameter, 5 to 6 inches (12.7 to 15 cm.).

LUNGS. Weight, combined, 36 to 45 ounces (1020 to 1290 Gm.). Weight, male, right lung, 24 ounces (680 Gm.), left lung, 21 ounces (600 Gm.). Weight, female, right lun 17 ounces (480 Gm.), left lung, 14.8 ounces (420 Gm.). Length, 10 to 12 inches (26 30 cm.). Anteroposterior diameter at base, 7 to 8 inches (17.5 to 20 cm.). Transvers diameter at base 4 to 5 inches (10 to 12.7 cm.). The right lung is shorter, broader thicker than the left. Dimensions in the female average 1 inch (2.5 cm.) less.

MAMMARY GLAND. Weight in adult, 5.25 to 7 ounces (150 to 200 Gm.). Weight during lactation, 14 to 31.75 ounces (400 to 900 Gm.).

OVARIES. Weight (each), 0.12 to 0.25 ounce (4 to 8 Gm.). Length, 1.5 inches (3.8 cm.). Breadth, 0.75 inch (1.9 cm.). Thickness, 0.5 inch (1.2 cm.).

PANCREAS. Weight, quite variable, 2.1 to 4.8 ounces (60 to 135 Gm.). Length varies, average 6 to 8 inches (15 to 20 cm.).

PARATHYROIDS. Length, 0.2 to 0.25 inch (6 to 7 mm.). Breadth, 0.15 to 0.17 inch (3 to 4 mm.). Thickness, 0.05 to 0.075 inch (1.5 to 2 mm.).

PINEAL GLAND. Length, 0.4 inch (1 cm.). Breadth, 0.2 inch (5 mm.). Thickness, 0.2 inch (5 mm.). Weight, 3 gr. (0.2 Gm.).

PITUITARY BODY. Length, 0.3 inch (8 mm.). Breadth, 0.5 inch (1.2 cm.). Weight, 5 to 10 gr. (0.3 to 0.6 Gm.).

PROSTATE. Weight, 0.8 ounce (22 Gm.). Length, 1.25 to 1.5 inches (3.1 to 3.8 cm.). Breadth, 1.5 to 1.75 inches (3.8 to 4.5 cm.). Thickness, 1 inch (2.5 cm.).

SALIVARY GLANDS. Parotid, weight, 0.8 to 1 ounce (25 to 30 Gm.). Sublingual, weight, 0.06 to 0.09 ounce (2 to 3 Gm.). Submaxillary, weight, 0.25 to 0.3 ounce (8 to 9 Gm.).

SEMINAL VESICLES. Length, 2 inches (5 cm.).

SPINAL CORD. Length, 18 inches (45 cm.). Weight, 0.9 to 1 ounce (27 to 30 Gm.). Transverse diameter averages 0.5 inch (1.2 cm.). Anteroposterior diameter averages 0.4 inch (9 mm.).

SPLEEN. Weight, 5.5 to 6.9 ounces (155 to 195 Gm.). Length, 4 to 5 inches (10 to 12.5 cm.). Breadth, 3 inches (7.7 cm.). Thickness, 1 to 1.5 inches (2.5 to 3.7 cm.).

STOMACH. Capacity, 1 to 2 quarts (1 to 2 liters). Thickness of wall, 0.25 inch (6 mm.). Weight, 4.5 to 6.2 ounces (125 to 175 Gm.).

TESTES. Weight, 0.65 to 0.8 ounce (20 to 25 Gm.) each. Length, 1.5 inches (3.8 cm.). Breadth, 1 inch (2.5 cm.). Thickness, 0.8 inch (2 cm.).

THORACIC DUCT. Length, 15 to 18 inches (37.5 to 45 cm.).

THYMUS GLAND. Weight at birth, 0.5 ounce (13.7 Gm.) and increases to 0.9 ounce (26.2 Gm.) at end of second year when it gradually decreases until gland disappears. Dimensions at birth, length, 2.4 inches (6 cm.), breadth, 1.5 inches (3.7 cm.) and thickness 0.25 inch (6 mm.).

THYROID. Transverse diameter, 2.4 to 2.8 inches (6 to 7 cm.). Height, 1.2 inches (3 cm.). Weight, 1 to 1.4 ounces (30 to 40 Gm.).

URETERS. Length, 11.2 to 12 inches (28 to 30 cm.). Slightly longer on left side and longer in male. Diameter of lumen varies, averages 0.1 inch (2.5 mm.).

URETHRA. Male. Length, 6.4 to 8.25 inches (16 to 20.6 cm.). Prostatic, 1 to 1.25 inches (2.5 to 3.1 cm.); membranous, 0.6 to 1 inch (1.5 to 2.5 cm.) and the anterior 4.75 to 6 inches (12 to 15 cm.). Female. Length, 1.5 inches (3.8 cm.). Diameter of lumen averages 0.25 to 0.4 inch (7 to 10 mm.).

UTERUS. (Virginal) length, 2.8 inches (7 cm.). Breadth, 1.6 inches (4 cm.). Thickness, 1 inch (2.5 cm.). Weight, 1.4 to 1.8 ounces (40 to 50 Gm.). The dimensions of a multiparous uterus are each increased 1 cm. or more and the weight is increased 0.7 ounce (20 Gm.). Length of cavity in virgin, 2 inches (5 cm.), in multiparae, 2.25 inches (5.7 cm.).

VAGINA. Length, 3 to 3.5 inches (7.6 to 8.9 cm.). The posterior wall is slightly longer than the anterior.

II. Physiologic Normals (Adult).

BLOOD: (Values are in mg. per 100 ml. whole blood unless otherwise noted.)

Specific gravity	1.041 to 1.067 (1.026 to 1.032 for serum)
Reaction (see p. 798)	pH 7.3 to 7.5
Total solids	19 to 23%
Hemoglobin	15.6% (by weight) (females, 14.2%)

Serum albumin	3.8 to 5.2%
Serum globulin	2.0 to 3.5%
Fibrin	0.2%
Total nitrogen	2.6 to 3.5% (plasma, 0.6 to 1.1%)
Nonprotein nitrogen	25 to 39 (plasma, 20 to 30)
Urea nitrogen	12 to 18
Amino-acid nitrogen	6 to 8 (plasma, 4 to 7)
Ammonia nitrogen	about 0.1
Uric acid (Folin-Wu method)	2 to 3 (extremes, 1 to 4)
"Creatinine"	1 to 2
Creatine	3 to 5 (plasma, 0 to 3.8)
Sugar (Folin-Wu method)	70 to 120 (60–100 true)
Chlorides (as NaCl)	450–500 (plasma, 580 to 620)
Fat (Bloor's fat method)	about 600
Cholesterol (Bloor's method)	140–210
Lecithin (Bloor's "lecithin")	30 (plasma, 22)
Acetone bodies	0 to 4
Bicarbonate (plasma)	53 to 75 vol. % CO_2
Oxygen capacity	20.9 vol. %
CO_2 tension (arterial)	about 40 mm. Hg
Calcium	5.3 to 6.8 (serum or plasma, 9.0 to 11.0)
Magnesium	2.3 to 4 (serum or plasma, 1.6 to 3.0)
Sodium	170 to 225 (serum or plasma, 310)
Potassium	153 to 240 (serum or plasma, 18–21)
Phosphorus, total (as H_3PO_4)	about 120 (plasma, 35 to 40)
Phosphates, inorganic (as P)	(serum) 3.2 to 4.3
Sulfates (as S)	0.5 to 1.0
Diastase (see p. 797)	8 to 64 units

CEREBROSPINAL FLUID (see p. 749): (Values are in mg. per 100 ml. unless other noted.)

Specific gravity	1.001 to 1.008
Pressure	5–12 mm. Hg or 70–160 mm. H_2O
Serum albumin	about 6
Serum globulin	20–30
Nonprotein nitrogen	15–35
Urea nitrogen	10–15
Creatinine	1–2
Sugar	50–80
Chlorides	720–750

STOMACH CONTENTS (see normal acidity curve, p. 864):

One hour after Ewald test meal:

Reaction	pH 0.9–1.6
Quantity	40 to 50 ml.
Total acidity	40 to 80
Free hydrochloric acid	25 to 50
Pepsin	64 to 256 Mett units

Residuum (fasting):

Quantity	20 to 100 ml. (rarely 150)
Total acidity	10 to 50
Free acidity	0 to 30
Pepsin	3 (Mett)

Trypsin	7 (Spencer)
Bile	present in about 60% of cases
Gastric mucus	traces

DUODENAL CONTENTS (see pp. 867–868).

URINE (24-hour specimen; values in Gm. unless otherwise specified):

Quantity	25 ml. per kg. body weight
Specific gravity	1.015 to 1.025
Reaction	acid to litmus (pH 4.8 to 8.0, average about 6.0)
Total solids	55 to 70
Total nitrogen	10 to 16
Urea	10 to 40 (supplies about 85% of total N)
Uric acid	0.2 to 2
Creatinine	1 to 1.5
Ammonia	0.5 to 1.2 (see NH_3 quotient, p. 839)
Hippuric acid	0.6 to 1
Albumin (see p. 828)	
Chlorides (as NaCl)	10 to 15
Sulfur (as H_2SO_4)	2 to 2.5
Phosphorus (as P_2O_5)	1 to 5 (about 90% of phosphates are acid phosphates)
Calcium (as CaO)	0.1 to 0.6
Magnesium (as MgO)	0.2 to 0.6
Acetone bodies	about 10 mg.
Sugar (see p. 833)	

FECES:

Average daily output, moist feces (Hawk)	110 Gm.
24-hour amount on ordinary mixed diet	110 to 170 Gm. (25 to 45 Gm. solids)
24-hour amount on vegetable diet	to 350 Gm. (about 75 Gm. solids)

Fatty substances (averages expressed as per 1 Gm. dried feces):

Total fat	150–250 mg.
Total fatty acid	90–130 mg.
Total soap	50–100 mg.
Total neutral fat	10– 20 mg.

RESPIRATION:

Alveolar air:

Oxygen	14.5%
Carbon dioxide	about 5.5% (35 to 40 mm. CO_2 tension)
Nitrogen	80%

Air hunger in diabetes or chronic nephritis begins only when CO_2 tension has fallen to 20 or 25 mm.

G. Important Diseases and Injuries Due to Toxic Plants

Ackee (Akee) Poisoning (Vomiting Sickness of Jamaica). This disease is caused by eating the unripe fruit ("akee") of the tree *Blighia sapida*. This tree is common in the West Indies, although it is believed to have been imported from West Africa. The ripe, naturally opened fruit is harmless and is valued as a food. According to Scott (1939) poisoning is caused by eating fruit which is not ripe, which has been picked from a dead branch or injured tree, or which has lain upon the ground. Illness begins acutely about two hours after ingestion with abdominal pain and vomiting. After a remission of a few hours there is a recurrence of vomiting and in the severe cases

convulsions, coma, and usually death. The poison is in the arillus (outer coat) fruit and according to Jordan and Burrows (1937) in the seeds. Evans and (1938) obtained from unripe arilli a saponin which was hemolytic, and highly for kittens and guinea pigs in which it produced hemorrhages and fatty deg changes, particularly in the liver and kidneys. Arilli from fully opened fruit were toxic for these animals and nonhemolytic. The toxic effect is cumulative when suble amounts are taken at daily intervals. In the West Indies many deaths have be tributed to akee poisoning, but it seems evident that similar symptoms may fo the ingestion of other irritating or poisonous foods.

Arrow Poisons. Many primitive people add poison to the physical injury prod by their arrows. Although animal secretions, particularly snake venoms, have been they are inferior in effect to the vegetable poisons. In some arrow poisons snake veno combined with the plant extract, but as the venom is often treated in such a way to destroy the toxins, the effect must be due largely or entirely to the vegetable poi Material of bacterial nature is also used, particularly the tetanus bacillus, but most of potent arrow poisons are vegetal.

ACOCANTHERA. This genus of the Apocynaceae includes several species which a powerful poison widely used by the African natives. A decoction is prepared the wood or roots, evaporated over a fire to a syrupy consistency, and painted over heads of arrows. The active principle is ouabain, a glucoside related to those in d talis, which speedily causes death from cardiac failure after a brief period of ra irregular pulse, dyspnea, and often convulsions.

STROPHANTHUS. Several species of this genus, also of the Apocynaceae, have be used by the African natives to produce arrow poisons. At least two species, *S. sarm tosus* and *S. gratus,* are found in Liberia, growing as bushes with striking p pentacle-like flowers. The seeds are cooked in water, the extract evaporated to syrup, and often mixed with the heads of snakes and sometimes with a little veget resin. The active principle, strophanthin, is a glucoside closely related to ouabain, causes death from injury to the heart muscle. Both drugs have been used in s doses as cardiac stimulants as a substitute for digitalis.

STRYCHNOS. The principal arrow poison of the Amazon river tribes, curare, is tained by extracting the bark of various species of *Strychnos.* According to A. H ton Rice, the sheath or rind of a vine, called Itary-cipo (*S. toxifera,* etc.), is mace and triturated, boiled with a little water, and put into a tipiti press made from the jaci palm (*Desmoncus macroacanthus*). This is allowed to exude slowly, then boiled to consistency of an unguent and stored in little pots. Into this curare are dipped tips of the slender darts made from the footstalks of the pataua palm (*Oenoca bataua*). These are used in blow pipes made from the paxiuba palm (*Iriartea setige* Curare contains two alkaloids: curarine, which paralyzes voluntary muscles by in rupting connection with the peripheral nerves at the motor end plates so that the mal lies helpless on the ground; and curine, which paralyzes the heart.

Certain Malay tribes use poisons from this genus which seem to contain strychni and brucine as well as curarine, and may cause convulsions as well as paralysis. O poisons used by certain Himalayan tribes have aconite as a base. *Hyoscyamus fale* (Solanaceae) has been employed by some of the natives in the interior of Africa by the Tuaregs of the Sudan. Like ouabain it acts as a cardiac poison, although contains hyoscyamine and scopolamine.

The sap and seeds of other species such as *Cerbera odollam* and *Thevetia nerifo* and in India the sap of *Antiaris toxicaria* have been used as arrow poisons, acting powerful cardiac poisons.

Atriplicism. In North China poisoning has followed the ingestion of a weed, *A littoralis,* which grows in gardens around Pekin and which is sometimes eaten by very poor in times of famine. It has been claimed that the poisoning is caused by a

insect often present on the weed. About 15 hours after ingestion there appears itching of the fingers, quickly followed by swelling and discoloration. This swelling extends up the backs of the hands and outer surface of the forearms. The face also becomes swollen so that the eyelids may be closed, and the nose becomes cyanosed and cold. The swollen parts may ultimately develop blisters and ulcers. Later the finger tips may become gangrenous, as in Raynaud's disease. According to Yang (1940) symptoms do not appear unless the individual is exposed to bright sunlight, and as the lesions are limited to exposed areas, he and also Uyky regard the cutaneous lesions as a photosensitive dermatitis.

Cannabis Indica. This plant, Indian hemp, is the source of a serious drug addiction in Central Asia. In India the forms of the drug generally used are: "gangah," the dried flowering tops, which are smoked mixed with tobacco; "bhang," a mixture of the dried leaves and capsule, which is made into decoctions and is the cheapest form of the drug; and "charas," the resinous exudate obtained from the cut female heads of the plants. This latter is the most expensive and concentrated of the preparations. In Arabia a confection is made from charas and is known as "hashish." It is sometimes mixed with extracts of different Solanaceae such as datura and nux vomica, and in this form is said to be taken daily by millions of the inhabitants of Asia and Africa, although stringent regulations against its sale have been made in some localities. The drug gives a feeling of well-being, followed by hallucinations of sight and hearing, often of a sensual character. This is followed by dimness of vision, drowsiness, and stupor. Addicts may become insane.

Cannabis Sativa. The name "marihuana" has been given in Mexico and the United States to the flowering tops of this hemp plant, a member of the flax family which is widely cultivated in the United States and elsewhere for its fiber. It also grows wild as a weed. The flowering tops, especially those from the pistillate (female) plant, are smoked in India under the name "gangah." In some instances an extract is made from the tops and used to impregnate tobacco cigarettes. In the United States hemp cigarettes bear various other names, such as reefers, muggles, the weed, etc.

The intoxication, which may arise from smoking a single cigarette and may come on within about an hour, is characterized by excitement, mental confusion, talkativeness, and often spells of "hysterical" laughter. Visual hallucinations and sexual illusions are also features of the intoxication. Although euphoria is experienced, at the same time there is anxiety which may lead to ideas of suicide. Many of the patients treated in hospitals show psychoneurotic traits and the type of personality in which use of a drug for the pleasurable phases of its action is likely to be manifest. True addiction apparently does not occur, since neither an increase in tolerance nor withdrawal symptoms have been clearly demonstrated. In some cases, however, opium derivatives have been added in order to create an opium addiction.

There is general agreement that marihuana cigarette smoking brings about weakening of restraint and impairment of judgment, and it would seem that the excitation of sexual illusions might well lead to sex crimes or reckless indulgences. Many instances have been cited of homicidal attacks by persons when under the influence of marihuana. Bromberg (1939), however, in reporting his study of this addiction at Bellevue Hospital, questions whether it predisposes to crime, stating that no cases of murder or sexual crime were established as due to marihuana in 67 trials in the U. S. County Court of General Sessions.

The use of marihuana seems frequent in underworld resorts and has been reported among the better social classes. The dangers attending the use of the drug, especially by young people in the public schools, were first emphasized in New Orleans about 1926, and a federal law making the use of cannabis illegal was passed in 1937. In his book "Marihuana" (1938), Walton states: "The situation is of the utmost gravity and is one which calls for drastic measures of eradication."

Dermatitis Venenata. There are a great many plants in various parts of which cause various types of dermatitis—erythematous, vesicular, or urticari best known of these plants belong to the Rhus family. In the United States by most common cause of plant dermatitis is poison ivy, *Rhus toxicodendron,* and the related variety called "poison oak." The poison sumac, *Rhus vernix,* in the central and northeastern states, and poison wood, *Metopium toxiferum,* in the southeast, produce a similar, often more severe dermatitis. For poisoning to occur, must be direct contact with the plant—the idea that a volatile poison is given erroneous. The plants seem more irritating when wet than dry, and a moist skin is susceptible than a dry one.

The exciting agent is an oil, toxicodendrol, and in susceptible persons a amount (1/1000 mg.) can produce dermatitis. There are marked individual differen reaction to these plants, and susceptibility is generally believed to be due to a f allergic hypersensitiveness. Repeated attacks do not seem to confer immunity. S clinical improvement and diminished susceptibilty have been reported following ment with a series of injections of Rhus extracts. The extracts are solutions vegetable oil of substances extracted from the fresh leaves of the plants.

Following exposure the skin should be scrubbed with soap and water. Alcoholic or solutions spread the inflammation.

In Japan the lacquer from *Rhus vernicifera* causes a cutaneous edema affecting face and extremities, which is followed by a papular eruption.

In the United States there are many other plants capable of causing derma Among these are the parsnip (*Pastinaca sativa*), the lady slipper (Cypripedium), sp (Euphorbia) and the primrose (Primula), particularly when in blossom. Lily rash dermatitis caused by handling various bulbs or stalks. Those handling the vanilla may suffer from an itching dermatitis. The rue group of plants is often responsible skin irritation. Certain individuals may become specifically hypersensitive and violently to plants which are harmless to the great majority of individuals.

Mangoes. Kirby Smith (1938) described a dermatitis that may follow eating m Burning and itching may appear six to eight hours after ingestion, affecting parti the hands, neck, face and lips. The irritating resin is present in the skin or rind of fruit and in the stem or sap, but not in the edible portion of the fruit.

Dhobie-mark Dermatitis. Livingood, Rogers, and Fitz-Hugh (1943) described cases of dermatitis in American soldiers in India, which was caused by wearing cl which had been marked with the juice of the marking nut from the *ral* or *bela* tree. The dermatitis occurred only in the exact spots touched by the dhobie mark. dermatitis recurred if marked clothes were again worn. Reaction to patch tests with material were positive. Symptoms appeared within 8 to 24 hours and varied fr moderate erythema and edema to vesicular oozing and crusting lesions. (This mu distinguished from the "dhobie itch," frequently observed in the Philippine Isl which is due to a fungus [trichophyton] infection.)

Goldsmith (1943) described a dermatitis resembling ivy poisoning in mail c and clerks in Washington, which was caused by handling mail which had been taminated in the mail pouch with "bhilawanol oil" from the "Indian marking *Semecarpus anacardium*. This plant, like Rhus and the mango, is a member of the Anacardiaceae. Of about 50 individuals exposed, 16 were attacked. Undoubtedly o species of this family are capable of causing a similar dermatitis.

Favism. This is a disease characterized by an acute hemolytic anemia and caused inhaling pollen from the flowers of the bean plant (*Vicia faba*) or by eating the b It is most frequent in Italy and especially in southern Sicily and Sardinia, w morbidity rate as high as 5.17 per cent has been reported. Heredity seems to part, some families giving a history of favism over many generations. Ingestion of beans is more apt to cause it than eating cooked beans. This in connection with

hypersensitiveness to the bloom indicates some type of allergic reaction. McCrae and Ullery (1933) reported a case in an Italian in Philadelphia who gave a positive cutaneous allergic reaction to an extract of the bean. In Italy, the attack comes on shortly after exposure with irregular fever and hemoglobinuria, and later pallor and jaundice. The abrupt and profound fall in red blood cells and hemoglobin (together probably with the associated renal injury) may cause death within a very short time. The mortality is about 8 per cent. Recovery, if it occurs, is complete.

Robinson (1941) reported six typical cases in Palestine following the eating of broad beans.

Ginger Paralysis. In 1930 an extensive outbreak of paralysis occurred in the south-central and southwestern portion of the United States, particularly in Tennessee and Cincinnati. It was largely restricted to adult males, all of whom had drunk Jamaica ginger from one-half to three weeks before the attack. It was characterized by a flaccid paralysis of the distal muscles of the legs which was preceded for three or four days by numbness and aching of the calves. In some of those afflicted the arms were subsequently involved. In some, death occurred, attributed to respiratory paralysis. In these cases examination showed degeneration of the myelin sheaths and axis cylinders of the radial, ulnar, sciatic, external popliteal, anterior and posterior tibial nerves. Carillo also reported degenerations in the spinal cord. The mortality was low, but disability in some cases persisted for two years and more. It has been estimated that from 10,000 to 15,000 cases occurred. It was later shown that the poisoning was due to contamination of the Jamaica ginger with about 2 per cent of triorthocresyl phosphate.

Similar poisoning has followed the use of apiol containing from 28 to 50 per cent of triorthocresyl phosphoric acid. Apiol is an alcoholic extract of the fruit of the common parsley (*Carum petroselinum* or *Apium sativum*). It has been used as an abortifacient, in menstrual disturbances and in malaria.

Hemlock. Poisoning from species of *Cicuta* (water hemlock, of the Parsley family) is not uncommon in man and cattle. The poison, cicutoxin, is a resinous substance found chiefly in the roots and root stalks. The leaves and fruit apparently may be eaten by animals without harm. Poisoning has occurred chiefly as a result of confusing the plant with parsnips or other edible roots. The symptoms are pain in the stomach, nausea, vomiting, diarrhea, dilated pupils, labored breathing, sometimes pulmonary edema, a feeble rapid pulse, and violent convulsions. Death may occur from respiratory failure.

The poison hemlock (*Conium maculatum*) is one of the most widely known poisonous plants because of its reputed use by the Greeks for the execution of State prisoners, notably Socrates. In recent times poisoning has occurred as a result of mistaking the seeds, leaves, or root for those of edible plants (anise, parsley, parsnips). It causes gradual progressive paralysis of the muscles, often loss of vision, and death from respiratory paralysis, without convulsions.

Jengkol Poisoning. In the Dutch East Indies poisoning has followed the ingestion of the fruit pods or beans of *Pithecolobium geminum*, a species of Mimosaceae. The fruit, which is said to have a high content of vitamin B, is eaten avidly by the natives in spite of its foul odor. It is sometimes put through an appetizing process (which increases its toxicity) by burying it in the ground for 10 days. When the beans begin to sprout they are taken up, ground, roasted. They contain ethereal oils which exert a powerful irritating action. If considerable is eaten, there may be severe colicky pains in the abdomen and flanks, as in renal colic, and vomiting, constipation, flatulence, and vesical tenesmus. The urine contains large amounts of blood and many small sharp crystals (jengcolic acid) which some believe injure the kidneys mechanically. There may be anuria, and death may follow, but recovery is the rule. The diagnosis is easily made from the characteristic disgusting odor of the breath and urine.

Kava or Yangona. An intoxicating nonalcoholic drink is made from the roots or leaves of the pepper plant, *Piper methysticum*, and is used as a ceremonial beverage in

many of the islands of the South Pacific. The parts of the plant are first chewed girls who have good teeth and good health, and the masticated material is p bowl and treated with cocoanut milk. A sort of quiet, drowsy intoxication with of the legs results in those not habituated. In the chronic intoxication a con debility results, and a marked roughness of the skin is said to develop.

Lathyrism. This disease is characterized by the insidious onset of pains in th and muscles and weakness of the legs, which gradually progresses to the develo of a spastic paraplegia. There is wasting of the muscles and exaggeration of flexes, incontinence of urine and loss of sexual power. The arms are rarely inv There are no sensory disturbances, and no mental or cardiac disturbances. C lathyrism is practically indistinguishable from primary lateral sclerosis. It is rarely but runs a protracted course.

It is common in India, Abyssinia, and Algeria. Chopra (1940) emphasizes its freq in India and states that examples of lathyrism in man in the form of spastic par are commonly seen every day in the streets of Calcutta.

The disease has been attributed to eating bread made from the flour of the pea, *Lathyrus sativus,* or related vetches. In most of India this plant, know Khesari dal, is an important article of food for both man and animals. Mo amounts may be eaten with impunity, symptoms appearing only when large am are taken, especially to the exclusion of other foods. It has therefore been sug that the syndrome is due to a deficiency of vitamin A, or possibly of tryptophane is lacking in the Lathyrus seed proteins. There appears to be no direct evidence toxic substance in the peas. Acton and Chopra concluded that the disease is not to Lathyrus but to a contaminating weed, *Vivia sativa,* from which a poisonous stance, divicine, has been obtained.

Minchin (1940) has reported similar cases from southern India where *Lathyrus sa* is not eaten, but where the diets are grossly deficient in vitamins and proteins. He gested that the syndrome is due to a deficiency of tryptophane, and therefore directly dependent upon ingestion of this vetch. Although the question is manifestly settled, the theory of a dietary deficiency—probably a complex one—has great appeal seems to conform best to the facts now known. There is abundant experimental dence that degenerations of the nervous system can be produced in animals suitably deficient diet.

Manchineel Poisoning. The manchineel tree (*Hippomane mancinella,* of the E biaceae) has long been known as a source of poisoning in northern South Am Central America, the West Indies, and Florida. It is a large, handsome tree with like or laurel-like leaves. The fruit, which resembles crabapples, has a pungent r disagreeable taste but has sometimes been eaten with serious results. Hypersensitiv dividuals who handle the fruit may suffer from a dermatitis characterized at erythema and later by the formation of vesicles and bullae. The mucous membr will become acutely inflamed if contaminated by the fibers. If the fruit is eaten, may be swelling of the lips, blisters, and erosions of the buccal mucosa, dysp nausea, vomiting, and diarrhea with blood in the stools. More rarely there profound collapse and death.

All parts of the tree appear to be toxic. There is a legend that death may from sleeping under the shade of the tree. Raindrops falling on the skin of a sheltered under it may cause irritation. The latex contains a greenish resin which toxic principle, and this has been used by the natives as a liniment. Smoke from the ing wood is said to cause severe conjunctivitis, and the sawdust may cause rhinitis, laryngitis, conjunctivitis, and lacrimation. The dried fruit has been used diuretic, and the seeds contain a purgative oil.

Milk Sickness. Cattle eating richweed (white snakeroot, *Eupatorium ageratoi Urticae folium*) in the eastern United States or rayless goldenrod (*Aplopappus*

phyllus) in Texas acquire a serious disease called "trembles" or in some cases "alkali disease" or "milk sickness." In man, after ingestion of milk or butter from such a cow, there may appear anorexia, nausea, and vomiting which prevent the taking of food and water, and soon bring about an acidosis with a high mortality. The late symptoms are a subnormal temperature, an extremely low blood pressure, the presence of diacetic acid in the urine, and the odor of acetone in the breath and urine. The blood shows a marked ketosis, a lipemia, and a hypoglycemia. The poisonous principle, trematol, one of the higher alcohols, is found in the leaves and stems.

Mushroom Poisoning. The best known edible mushrooms are (in England and the United States) the meadow mushroom, *Agaricus campester,* which grows only in open pastures, and (in France and Italy) the champignon, *Marasmius oreades.* There are no criteria by means of which edible mushrooms can be distinguished from poisonous varieties. There is a widespread entirely erroneous belief that if a silver coin is put in the dish in which mushrooms are cooked, it will be tarnished if poisonous varieties are present. Poisonous mushrooms cannot be recognized by the taste—the most poisonous species are said to have a very agreeable flavor. In Washington an Italian officer who was regarded as an expert in the recognition of edible species purchased in a market some mushrooms which had been collected in nearby Virginia. He breakfasted on these mushrooms and spoke of their fine flavor. In about 15 minutes he became acutely ill, developed blindness, dysphagia, and convulsions and died within 24 hours. If one has not the expert knowledge required to identify the species with certainty, one should eat only mushrooms which have been passed upon by a competent (preferably official) inspection. Even in cultivated beds poisonous species occasionally develop.

There is marked individual variation in susceptibility. In the case of a family of six poisoned by *Amanita nappa,* reported by Bentkowski, four died (one of whom had eaten only a mouthful); one became ill but recovered, and the sixth who had eaten heartily suffered no ill effects.

In the United States most cases of poisoning are due either to *Amanita muscaria,* the fly amanita, or to *A. phalloides,* the death cup. Both species are very common and widely distributed. One feature which helps to identify this genus is the persistence of a portion of the veil encircling the stem a little below the cap.

The poison in *A. muscaria* is muscarin, an alkaloid related to pilocarpin. This type is distinguished by the early appearance of symptoms (within three hours), and death in the fatal cases occurs within 24 hours. There are nausea, vomiting, diarrhea, severe abdominal pain, sweating, salivation, lacrimation, miosis, often a slow irregular pulse, and in fatal cases convulsions and coma. Atropin is an efficient antidote, and although the symptoms are violent, the mortality is low in properly treated cases (about 10 per cent).

The poison in *A. phalloides* is a toxin. In poisoning of this type the symptoms are late in appearing (6 to 18 hours), and although the mortality is from 50 to 70 per cent, death usually occurs only after five to eight days. In addition to the gastrointestinal symptoms noted above, there may be great thirst, anuria, jaundice (after two or three days), cyanosis, drowsiness, delirium, or coma. There is degeneration of the renal tubular epithelium and liver necrosis. Extensive degeneration of the ganglion cells of the cerebral cortex, basal ganglia, cerebellum, and brain stem have been described (Vander Veer and Farley, 1935). Atropin has no effect. An antitoxin has been used in Europe with alleged good results.

Another rarer type of mushroom poisoning characterized by an acute hemolytic anemia, hemoglobinuria, and jaundice has been described. Recovery is the rule.

Mustard-oil Poisoning (Epidemic Dropsy). This disease resembles beriberi in that it is characterized by dropsy associated with myocardial disturbances without anesthesia or marked paralysis. The disease has been recognized in India (Calcutta) since 1877. Outbreaks have recurred at intervals since 1913, one of the worst in 1926, when over

2000 cases were observed. In an outbreak in Mauritius in 1879 one-tenth of the were attacked and a large number died. In 1926 in Fiji an outbreak occurred was confined entirely to the Indian population. This suggested that the poisoning caused by mustard oil used in preparing curries. In subsequent experiments on h volunteers in the Calcutta jail, characteristic symptoms were produced by feeding suspected oil. Chopra and Badhwar (1940) pointed out that in some of the epid the mustard oil was adulterated with katakar oil from the seeds of *Argemone mexi* the Mexican poppy or shialkata, which superficially resemble mustard seeds. In fur experiments in human beings they produced typical disease symptoms by administ food cooked in oil containing controlled amounts of Argemone oil. Lal et al. (1 reported the isolation from the Argemone oil of a white crystalline basic subs which seems to be the toxic principle. Rats fed the purified mustard oil suffered no effects. The exact nature of the substance was not reported.

Opium. The use of opium is common among the inhabitants of India and the Ori and there are many addicts in Europe and America. In India, opium is almost invaria taken in pill form, and when used in this way the ill effects are much reduced. mental, moral, and physical deterioration so common in those who smoke opium, as China and Persia, or in those who use morphine or other alkaloids hypodermically, is n so marked. There is also less tendency to increase the dose. Every medical man sh read De Quincey's "Confessions of an English Opium Eater" to appreciate the slight eff this habit had on the author during the first few years of his addiction. He took drug as did the native of India. In the Far East, native mothers and wet nurses som times smear the nipples of the breast with the drug, and it has been a not uncom custom for the native nurses or ayahs to soothe babies to sleep by dipping their fin in opium and allowing the babies to suck upon these fingers. Such treatment is viously highly deleterious to the child. The minute contracted pupils may suggest form of intoxication in cases of obscure illness.

Miscelianeous Sources of Plant Poisoning. In addition to the diseases describ numerous other instances of poisoning by plants are known, some accidental in ori and some intentional. Chestnut (1938) listed only about 30 species of plants associat with accidental poisoning in man, usually the result of confusing poisonous and h less species. Muenscher (1939), however, listed 400 species of plants in the Uni States which have caused poisoning either in man or animals. The families whi contain the largest number of poisonous species are the Liliaceae, Ranunculac Leguminosae, Euphorbiaceae, Umbelliferae, Solanaceae, and Compositae.

Prussic acid occurs in many valuable foods, and if not removed may cause serio poisoning and even death. The linseed (flax) plant, *Linum usitatissimum,* contains cyanogenetic glucoside which occurs early in the development of the plant and persis in the seeds.

Mandioca Poisoning. The roots of the plants *Manihot aipi,* the sweet cassava, *Manihot utilissima,* the bitter cassava, constitute one of the most important articles of d in many parts of Africa, South America, and the West Indies. The roots are gene dried and ground into a powder and used as a flour for making cassava cakes. In m civilized areas they are used to produce starch and tapioca. Bitter cassava contains glucoside which liberates hydrocyanic acid in the presence of water and causes serio poisoning if eaten without preparation. To avoid poisoning, the tuber must be scra grated, squeezed free of its milky juice, and thoroughly washed. It is then often d in the sun.

According to Holland (1938) the ripe fruit of the cultivated tree *Pangium edule,* New Guinea, is wholesome. The unripe fruit, however, and the fruit of the wild at all stages are highly poisonous and cause serious illness and even death. The kern contain a large amount of hydrocyanic acid, probably in combination as a glucosi The scraped kernels are said to have a sweet cocoanut-like taste and cannot be dete

when added to other food. They have been often used for homicidal purposes. The chief use of the nuts in some villages has been in stealing fowls. The scraped kernels are thrown to fowls which die quickly after eating them. If the crop is then removed, the fowl may be eaten.

Oxalic acid is contained in variable quantity in many edible plants, as sour grass and rhubarb, and may cause poisoning if ingested in sufficient quantity.

Various species of *Jatropha,* physic nuts, found in India and the West Indies, cause severe diarrhea and gastrointestinal irritation similar to that caused by croton oil. A similar poisoning in Tanganyika has been reported as due to eating the nuts of the coral plant.

The consumption of unripe persimmons, usually by children, has resulted in the accumulation of a mass of pulp in the stomach, forming a bezoar similar to the well-known hair balls. These may become large and require operation for removal.

Plants Used Especially for Homicidal or Suicidal Purposes. The root of *Gloriosa superba,* sometimes called wild aconite, a plant widely distributed in tropical Asia and Africa, has been used as a poison. The toxic effects which are due to the active principle, "superbine," resemble those of aconite poisoning—tingling and numbness of the lips and pharynx, nausea, vomiting, abdominal pain, cardiac depression, and collapse with terminal convulsions. Consciousness is usually retained.

The roots of various species of *Aconitum* have been used for the same purpose, death often taking place in from three to four hours.

Various plants belonging to the order Solanaceae are used in many parts of the Tropics and by criminals in temperate climates to produce unconsciousness. Chopra has reported fatal cases of poisoning from *Jatropha* in India, in which the symptoms were dryness of the mouth and throat, dilatation of the pupils, and delirium.

The seeds of *Datura fastuosa* are used by the Thugs in India, and various other plants whose alkaloids have an action similar to belladonna are used by the natives of many parts of the Tropics. The seeds of datura have only a slight taste, and consequently are easily introduced into food. *D. sanguinea* has been employed in Peru and Colombia, and *D. ferox* and *D. arborea* in Brazil. The characteristic seeds are sometimes found in the feces or in fatal cases in the intestine.

The dried leaves of *Hyoscyamus niger,* henbane, are the basis of some of the "knock-out" drops used by the underworld. In poisoning from these plants the face is flushed, the pupils widely dilated, the eyes bright and shining. The throat is very dry. There is marked disturbance of vision. At first the victim is very talkative and soon becomes violent, but later there is drowsiness followed by coma.

The yellow oleander, *Cerbera thevetia,* which is found in India, contains highly poisonous glucosides, nereoside, and oleandroside, in the milky latex. Similar substances from *Urechites suberecta,* urechitin and urechitoxin, possess a cumulative action, and sudden death may occur before suspicion of poisoning is aroused.

In New Guinea, Holland (1928) reported that eating the roots of wild species of *Derris* is the commonest means of suicide among the natives. The root is known as "bun," and it was identified as Derris root only after the plant became of value as an insecticide. The poisonous substance of *Derris eliptica* is rotenone. Other toxic resins, such as derride, are also present. The conditions Holland found at autopsy are indicative of acute congestive heart failure. As an antidote the natives use the sap expressed from the roots of the banana, which is mucilaginous and acts as an emetic.

The juice of a species of *Asclepias* (Milkweed family) has been used in India as an infanticide. The symptoms of poisoning are salivation, vomiting, cramps, and final collapse. Common native poisons employed in Brazil are prepared from *Paullinia pinnata* which contains an alkaloid timboin, and from the fruit of *Thevetia ahonai* which contains the poison thevetosin. Both of these cause vomiting and acute respiratory failure. In the Dutch East Indies the common poison is extracted from the roots of species of

Ascaris Infection. Examine feces for characteristic ova. Worms occasion in feces or vomitus.

Ascites. Culture fluid on blood agar. Determine whether transudate or exudat p. 760.) If tuberculosis is suspected digest sediment with alkali, and examine cen for acid-fast bacilli by staining, special cultures, and guinea-pig inoculation. See cirrhosis of the liver, edema, nephritis.

Asthma. Examine sputum for eosinophils, Charcot-Leyden crystals, Curschmann's s Make culture from sputum for possible causative organism in cases due to resp infection. Eosinophilia. Make cutaneous allergic tests. Severe cases with cyanosis s decreased O-saturation in arterial blood. May show acidosis (CO_2 excess) with plasma bicarbonate.

Bacteremia. Make blood culture. Usually neutrophilic leukocytosis with shift to the

Bacteriuria. See Urinary-tract infections.

Balantidium Infection. Look for large motile ciliates in feces.

Banti's Disease. Moderate to severe anemia, usually hypochromic in type. Leukop Test fragility of the red cells to exclude hemolytic jaundice. Make van den Bergh and determine icterus index. Test liver function. Examine feces for occult blood.

Blackwater Fever. Hemoglobinuria; pink foam to urine; test filtrate for hemoglo spectroscopically and by benzidine or orthotolidine test. Malarial parasites found in blood films in rare cases. Leukopenia. Monocytosis. Examine blood serum for he globin and bilirubin (van den Bergh test). Reaction to Donath-Landsteiner test nega

Blastomycosis. Examine sputum, pus, or scrapings from margins of ulcers in 10 cent KOH for spherical, budding yeast cells with highly refractile, double-contoured Make culture on glucose agar plates.

Botulism. Inject an infusion of the suspected food into a guinea pig (see p. 72); stools and blood of patient. Culture anaerobically on glucose agar. Culture may be in a dark place at room temperature.

Bronchiectasis. Sputum abundant, purulent, often foul, tends to separate into layers on standing. Small hemoptyses common.

Bronchitis. Culture sputum on blood agar plates. Stain film by Gram's method. special cases inoculate a mouse.

Bronchopneumonia. Culture sputum on blood agar. Make blood culture.

Brucellosis. Make blood culture at onset of febrile paroxysm; incubate in atmosph of 10 per cent CO_2. Make cultures from urine, feces, and local foci in special cas Guinea-pig inoculation sometimes successful. After fifth day make agglutination t Intradermal tests, an opsonic index. Relative lymphocytosis, often leukopenia. Differ tiate from typhoid fever, tuberculosis, malaria, kala-azar.

Carbon-monoxide Poisoning. Test blood for CO spectroscopically and by Sayers-Y method. Secure blood at earliest possible moment and protect from air.

Cerebrospinal Fever. See Meningitis, meningococcus.

Cestode Infections. Examine feces for ova, which are not always present. If a segm is obtained, press between two glass slides and examine the branchings of the ute

Chancroid—Ducrey's Bacillus. Examine smears for short, Gram-negative coccoba occurring in chains. Culture material aspirated from bubo in sterile clotted human rabbit blood which has been inactivated at 56° C. for 30 minutes. Skin test with vac Syringe and media must be warm.

Chlorosis. Hypochromic microcytic anemia, sometimes severe, in adolescent girls. Lo saturation index. Gastric acidity normal. Differentiate from chronic posthemorr anemia.

Cholecystitis. May be moderate leukocytosis. Fluid obtained by duodenal drain may show bile-stained pus cells and other abnormalities. (See p. 868.) Culture on bl agar plates and enteric media. Van den Bergh test.

Cholelithiasis. Examine blood and urine for bilirubin (van den Bergh test). Bile obtained by duodenal drainage often shows increase in cholesterin crystals and calcium bilirubinate precipitate, and evidences of cholecystitis (q.v.). Blood cholesterol increased, with obstructive jaundice. Roentgenogram (Graham test).

Cholera. Smears from flecks in rice-water stools show many vibrios with "fish-in-stream" arrangement. Culture on Dieudonné plates. If sparse, use enrichment method. Identify organism with cholera immune serum. After fourth day test serum for agglutinins. Intense dehydration with high blood counts, high plasma proteins, and high specific gravity of blood. Anuria with high nonprotein blood nitrogen. Acidosis from loss of base. Depletion of chlorides. Differentiate from food poisonings, arsenic or antimony poisoning, bacillary dysentery, algid pernicious malaria.

Chyluria. Centrifuge urine and examine for microfilariae. Examine blood for microfilariae (not always present). Urine contains many highly refractile fat globules soluble in ether.

Cirrhosis of Liver. Do Kolmer or Kahn test. Icterus index and van den Bergh test. Test urine for bilirubin and urobilin. Make tests of liver function (p. 874) or determine A/G ratio. Examine feces for occult blood. Late cases may show macrocytic anemia. (See also Ascites; Banti's disease; Liver, necrosis of.)

Coccidioidomycosis. Examine fresh moist specimen of sputum, cleared with 10 per cent KOH for spherical yeastlike cells which may contain endospores. Culture and inoculate animals if necessary. Make intracutaneous tests, and complement-fixation test if possible. If ulcers are present, examine scrapings in a similar way. Biopsy if necessary. Make blood culture. Exclude tuberculosis by repeated stains, cultures and guinea pig inoculation. (See p. 235.)

Colitis, Chronic Ulcerative. Examine feces or preferably scrapings from ulcers (proctoscope) for pus, blood, mucus. Exclude amebic and bacillary dysentery by fresh warm-stage preparations, cultures, and agglutination tests. Neutrophilic leukocytosis, often secondary anemia, increased sedimentation rate. Roentgenograms.

Colitis, Mucous ("Spastic Colitis"). Examine feces for mucus in large masses, containing epithelial cells, often eosinophils, no pus cells, no blood. No leukocytosis, normal sedimentation rate. Roentgenograms.

Coma. Examine urine for sugar, ketone bodies, albumin, casts, blood. Examine blood for sugar, CO_2 combining power, nonprotein nitrogen or urea, and in special cases for alcohol and CO. Consider possibility of other poisons. Examine spinal fluid (caution, see p. 746), especially for pressure, presence of red cells and xanthochromia (subarachnoid hemorrhage). Make leukocyte count and blood culture if febrile.

Conjunctivitis. Stain smear by Gram's method and with dilute carbolfuchsin. Culture secretion on blood agar and plain agar. (See p. 731.)

Coronary Thrombosis. Neutrophilic leukocytosis. Accelerated sedimentation rate.

Cyst. Examine fluid for echinococcus hooklets and scolices. Test for pancreatic ferments and for urea.

Cystitis. See Urinary tract infections.

Dehydration. Examine blood for increase in hemoglobin concentration and in plasma protein (the best gauge). Specific gravity is increased.

Dengue. Neutrophilic leukopenia.

Dermatophytoses. Examine scrapings from skin in 10 per cent KOH for fungi. Culture on Sabouraud agar.

Diabetes Insipidus. No albuminuria or glycosuria. No nitrogen retention. Ability to concentrate urine lost, restored temporarily by pituitrin injections.

Diabetes Mellitus. Examine urine for sugar and ketone bodies. Examine blood (fasting) for sugar, cholesterol. In doubtful cases do glucose tolerance test. Make tests for acidosis (q.v.). In severe cases test for decrease in blood bases (or chlorides) and for dehydration.

Diphtheria. Make smears and cultures on L s or whole-egg by Gram's and Loeffler's or Neisser's method. Look for parallel rods con granules. In special cases isolate on tellurite blood agar and inject guinea pig w culture filtrate as test for virulence. Make Schick test on contacts. Differe streptococcus and Vincent's infections.

Diphyllobothrium Infection. Operculated ova in feces. If segments are obtaine one between two glass slides and look for characteristic rosette-shaped uterus. M anemia occurs in very rare instances.

Dracunculus Infection. Moisten blister or ulcer with a few ml. of water. fluid excreted by worm for striated larvae.

Duodenal Ulcer. Make gastric analysis (for hyperacidity, blood, hypermotili amine feces for blood. Roentgenograms.

Dysentery, Amebic. Examine mucus from fresh warm stool (warm stage) for actively putting forth bladelike pseudopodia. If necessary pass rectal tube, give purge, or scrape base of ulcer through a proctoscope. Pathogenic amebae often c red cells. Examine feces for cysts. Smear of feces shows granular detritus, often Leyden crystals, few pus cells. Monocytosis. No eosinophilia.

Dysentery, Bacillary. The sanguinolent mucus contains many pus cells phagocytic endothelial cells. Emulsify mucus in sterile broth or salt solution and a S. S. and MacConkey media. Identify organisms isolated by agglutination with i sera. Neutrophilic leukocytosis. After 7 to 10 days make agglutination tests.

Echinococcus Disease. Examine fluid from cyst for hooklets. Eosinophilia. Mak plement-fixation, precipitin, or cutaneous tests with special antigen (see p. 649). genograms.

Edema. Examine urine for evidence of nephritis. Test renal function. Determine p proteins, A/G ratio, blood chlorides. (See Nephrosis.)

Elephantiasis. See Filariasis.

Empyema. Neutrophilic leukocytosis. Make Gram's stain of smear from pus. on blood agar plates. Roentgenogram.

Encephalitis, Epidemic. Examine spinal fluid. (See p. 201.) Make cultures and st sediment to exclude ordinary types of meningitis.

Endocarditis, Subacute Bacterial. Make blood culture (repeatedly if negative). usually show *S. viridans,* rarely pneumococcus, gonococcus, or Pfeiffer bacillus.) ate neutrophilic leukocytosis (inconstant), often increase in macrophages. Progr anemia. Increased sedimentation rate. Examine urine for albumin, casts, blood (r emboli).

Enteritis, Tuberculous. Stain smear from feces for acid-fast bacilli. Inoculate guine There are often present in feces acid-fast, sporelike bodies which should not be for tubercle bacilli. Tubercle bacilli may be present in feces when there is no ment of intestine (swallowed sputum, particularly in children).

Eosinophilia. Look for ova of intestinal parasites in feces. Look for eviden trichinosis, hydatid disease, filariasis, various skin diseases, asthma and other aller actions, and lesions of the bone marrow.

Erythremia. See Polycythemia.

Espundia (Mucocutaneous Leishmaniasis). Examine scrapings from base of ulc leishmanial forms. Stain with Wright or Giemsa. Preferably obtain material by as by puncture near edge of ulcer. If bacterial contamination can be avoided, cul special media. Obtain material from adjacent lymph nodes by puncture.

Exophthalmic Goiter. High basal metabolic rate. Low blood cholesterol, high Glucose tolerance curve shows high peak, often prolonged. Glycosuria common, lymphocytosis.

Favus. Place hair or portion of favus cup on a slide and examine in 10 per ce for mycelium and spores which are very irregular.

Filariasis. Examine blood (day and night) for microfilariae, either in fresh moist preparation or better in thick films stained by Giemsa. (In elephantiasis microfilariae are often absent from blood.) Aspirate lymph varix or hydrocele and examine sediment for microfilariae. Examine sediment of chylous urine or ascitic fluid. Often eosinophilia. Complement-fixation test positive with special antigen.

Flagellates, Intestinal. Examine feces emulsified in salt solution for motile flagellates. Emulsify another portion in Gram's solution to study flagella. Stain smear with iron hematoxylin for encysted forms. (See pp. 541 and 548.)

Furunculosis. Examine stained film and culture on blood agar. Determine blood sugar, or preferably make glucose-tolerance test.

Fusospirochetosis (Vincent's Angina and Stomatitis). Stain films of material from the depths of the lesion with dilute carbolfuchsin or Fontana's stain. (The spirochetes and fusiform bacilli are present in large numbers; a few may be present in normal mouths.) Make cultures on Loeffler's serum and blood agar to exclude diphtheria and streptococcal infection. Examine blood to exclude leukemia and agranulocytic angina. There may be a marked lymphocytosis. Exclude syphilis by Kolmer or Kahn test. In pulmonary infections examine *perfectly fresh* sputum in stained films and darkfield preparations. (See Abscess of lung.)

Gas Gangrene. (Infections are usually mixed; important organisms are *Cl. perfringens, Cl. oedematiens* and *Cl. oedematis-maligni*. Precise identification difficult but important because of serum treatment.) Make hanging drop and stain smears from exudate by Gram's method and for capsules. (All are Gram-positive; *Cl. perfringens* is encapsulated and nonmotile; the other two are motile and nonencapsulated.) Make anaerobic cultures in milk, glucose agar, and meat tubes. (*Cl. perfringens* causes gas formation and disruption of the tube overnight.) Blood cultures (anaerobic) may be positive. (For isolation and identification by inoculation into immunized animals see p. 67.)

Gastric Cancer. Examine fasting stomach contents for retention of food, lactic acid, Boas-Oppler bacilli, occult blood. Test meal shows achlorhydria and delayed motility. Occult blood in feces. Usually anemia, hypochromic microcytic; often neutrophilic leukocytosis. Increased sedimentation rate. Roentgenogram.

Gastric Ulcer. Examine fasting stomach contents for free HCl, food retention, sarcinae, blood. Test meal shows normal or high acid, often delayed emptying. Occult blood in feces, often intermittent. Roentgenogram.

General Paresis. Examine spinal fluid: shows increased cell count, positive globulin test, paretic colloidal gold curve, positive reactions to Kolmer and Kahn tests. Blood shows positive reactions to Kolmer and Kahn tests.

Glanders. Smears from pus show characteristic Gram-negative bacilli, parallel beaded rods. Culture on acid glycerin agar and potato. Inoculate male guinea pig intraperitoneally (see p. 121). Complement-fixation and agglutination reactions may be positive. (Mallein tests in animals.)

Glycosuria. Examine urine for sugar and apply special tests for glucose. Fasting blood sugar and glucose-tolerance test. Consider renal diabetes and other causes for glycosuria. (See pp. 774 and 779.)

Gonococcus Infection. Gram's stain of smear from urethra, cervical canal, or eye shows intracellular, Gram-negative diplococci. Make culture. (See p. 49.) Complement-fixation test useful in chronic infections.

Gout. Uric acid in blood often increased. Examine serum or plasma. Examine crystals curetted from tophus, if present, by murexide test.

Granuloma Inguinale. Stain scrapings from ulcers for Donovan bodies in large mononuclear cells (Wright or Giemsa).

Hematuria. Examine sediment for red cells. Apply orthotolidine test. (For causes see p. 82.)

Hematuria, Egyptian. See Schistosomiasis.

Hemochromatosis. Examine urine for sugar and for hemosiderin granules. Fasting blood sugar or glucose-tolerance test if in doubt. Make van den Test liver function. Examine piece of excised skin for hemosiderin.

Hemoglobinuria. Examine centrifuged sediment for intact red cells. "Sha may be found with much debris. Test filtered urine for hemoglobin spectr and by benzidine or orthotolidine test.

Hemophilia. Prolonged coagulation time. Normal bleeding time, normal count, normal clot retraction. Negative reaction to tourniquet test. May hemorrhagic anemia. (A congenital abnormality limited to males.) Roentgen joints in selected cases.

Hepatitis. See Jaundice, catarrhal.

Hodgkin's Disease. Examine section of excised lymph gland. The blood moderate leukocytosis, monocytosis (10 per cent to 15 per cent), increased count, occasionally eosinophilia (no constant changes). In late stages, a severe Biopsy of marrow in special cases. Roentgenogram of chest.

Hookworm Infection. See Ancylostomiasis.

Hypersensitiveness. Test for hypersensitiveness to pollens, animal hair, foo by cutaneous tests or intracutaneous injections of suitable extracts, or in spe by patch tests. To determine if a patient is sensitized to a serum, inject intrad 0.1 ml. of a 1 : 10 dilution of the serum. If he is sensitized, an urticarial wheal velop within 10 or 15 minutes. A drop of serum in 1 : 10 dilution may be instilled in conjunctiva (see p. 295).

Hypertension, Essential. Examine urine and test renal function, especially by c tration tests, to determine extent to which kidney is involved.

Infectious Mononucleosis. Leukocytosis with high lymphocytosis (up to 90 per Many pathologic lymphocytes. (May not appear until several days after onset persist for months.) After 5 to 10 days test serum for "heterophil" agglutinins for red cells.

Influenza. Leukopenia and granulocytopenia. Frequent secondary infections, ticularly of the respiratory tract.

Intestinal Parasites. Examine feces for ova.

Jaundice, Catarrhal (Infectious Hepatitis). Test urine for bile. High icterus. Positive van den Bergh reaction; type variable. (See p. 872.) Feces show dimin absent bile pigments, increased fat. Test liver function. Differentiate from Weil's d arsenic and phosphorus poisoning, types of obstructive jaundice.

Jaundice, Familial Hemolytic. Usual features of hemolytic anemia marked. of red cells in hypotonic salt solution increased. Reticulocytes much increased. R have normal volume but diameter is small (more globular than normal). Look dences of cholelithiasis.

Jaundice, Infectious (Weil's Disease). Examine blood (first three days) for lep in stained films or darkfield preparations (sparse). Preferably inoculate a guin with 1 to 5 ml. of blood intraperitoneally and at autopsy examine the liver for lepto Blood cultures may be made in Fletcher's medium and incubated at 25° to 30° C. the twelfth day examine urine sediment for leptospira by smear and guinea-pig in tion. If stock cultures are available, after 10 to 14 days patient's serum may give a tive adhesion phenomenon or agglutination or protection test. Leukocytosis.

Jaundice, Obstructive. Blood shows high icterus index and positive direct v Bergh tests, increase in cholesterol and often in diastase. Prothrombin time in Urine shows bilirubin, no urobilin. Feces show increased fat and (if obstruction i plete) no bilirubin or urobilin. Fragility of red cells normal or diminished. Liver f may be reduced in chronic cases.

Kala-azar. Culture blood. Search for *Leishmania* within the leukocytes and in blood films, either directly or from the buffy coat in which the leukocytes

centrated, after centrifugation of citrated blood. If not found, examine material obtained splenic puncture or more safely by puncture of the liver or sternal bone marrow. (They are found occasionally in excised lymph glands or aspirated gland juice.) Blood globulin markedly increased. Make formol-gel and antimony tests. Marked leukopenia with relative monocytosis. Differentiate from brucellosis, typhoid and paratyphoid fever, Banti's disease, chronic malaria, leukemia.

Ketosis. Examine urine for ketone bodies. Test pH and titratable acidity of urine. Test for acidosis (q.v.).

Lead Poisoning. If exposure is recent, examine urine for lead. Blood shows early increase in reticulocytes and many stippled cells; later anemia, in acute cases severe and hemolytic in type. Fragility of red cells decreased. Often neutrophilic leukocytosis. Examine urine for albumin and casts. In late cases test renal function.

Leishmaniasis. See Kala-azar, Oriental sore, and Espundia.

Leprosy. Diagnosis depends upon demonstrating leprosy bacilli in smears stained by Ziehl-Neelsen method. Decolorize lightly with 20 per cent aqueous H_2SO_4. Morphology characteristic. Usually abundant in material from granulomas or scrapings from ulcers, especially from nose. If no lesions are evident, examine scrapings from nasal mucous membrane or skin clips from ear lobe. Examine blood during febrile periods; make thick films, dehemoglobinize and stain as above, or stain films from sediment after digestion with alkali. Cultures and animal inoculation useless except to exclude tuberculosis. Serologic reaction for syphilis in the blood positive in about 60 per cent of the cases.

Leukemia, Acute. Total leukocyte count ranges from normal to 150,000, occasionally much reduced. Diagnosis depends upon presence of many primitive leukocytes with nongranular basophilic cytoplasm and nuclei showing fine chromatin network and nucleoli. Progressive anemia with normoblasts. Reduced platelet count. Prolonged bleeding time. Increased basal metabolic rate. Marrow shows marked increase in primitive type of leukocyte.

Leukemia, Chronic Lymphatic. Leukocyte count usually from 20,000 to 100,000, with 75 to 99 per cent small lymphocytes, a few lymphoblasts and Rieder cells. May be a few myelocytes. Progressive anemia. Normoblasts rare. Basal metabolic rate increased. Blood uric acid high. Marrow (biopsy or sternal puncture) shows increase in lymphocytes.

Leukemia, Chronic Myelogenous. Total leukocyte count usually from 200,000 to 500,000, with a large proportion of myelocytes. In terminal stage many myeloblasts. Progressive anemia. Normoblasts nearly always present, often numerous. Platelets increased. Basal metabolic rate increased. Blood uric acid high. Examine sternal marrow.

Leukemia, Monocytic. Leukocyte count ranges from normal to 400,000 with 20 to 90 per cent monocytes and monoblasts. Examine fresh supravitally stained films as well as fixed films. Myelocytes usually present, may be numerous. Usually marked anemia and reduced platelets. Examine sternal marrow.

Liver, Necrosis of. Test liver function (see p. 873). Bilirubin in blood increased. Urobilinuria. In advanced stages shows: Increase in ratio of NH_3/urea in urine, with leucin and tyrosin crystals, usually only after concentration. High nonprotein nitrogen with low urea in blood. Hypoglycemia. Decreased fibrinogen. Prolonged prothrombin time. Decreased sedimentation rate. Acidosis may occur.

Lymphogranuloma Inguinale. Frei test.

Madura Foot. Discharge may contain fish-roe granules which show mycelium and peripheral clublike structures. Culture on Sabouraud's agar.

Malaria. Examine thick and thin films stained by Giemsa's or Wright's stain. To identify an object as a malarial parasite in stained films, one should be able to make out at least two of three characters: (1) chromatin; (2) bluish or greenish cytoplasm; and (3) pigment. Crescents are diagnostic for malignant tertian, equatorial banding for quartan. Marked irregularity of outline of parasite and the presence of Schüffner's (reddish) dots in cytoplasm of red cells indicates benign tertian. Leukopenia with mono-

cytosis. Leukocytosis during the paroxysms. Anemia of hemolytic type. Plasma increased. Almost all show positive reaction to serologic test for syphilis dur periods. May try provocative procedures. In special cases make sternal (or puncture.

Malignant Neutropenia. Leukopenia, becoming extreme, with disappear granulocytes. Red cells, platelets, coagulation factors usually normal. Granul marrow often greatly reduced. Make blood culture to exclude sepsis. Stain films Vincent's organisms. Differentiate from sepsis, acute leukopenic leukemia, and a anemia. Examine sternal marrow.

Measles. Blood shows neutrophilic leukopenia. Diazo reaction in urine usually cedes eruption and is not found in German measles. (For use of convalescent s see p. 212.)

Melanosarcoma. Urine usually turns black after exposure to the air because of formation of melanin. (This occurs, rarely, in other conditions, e.g., alkapto Does not reduce Benedict's solution.

Meningitis, Lymphocytic Chorio. Spinal fluid shows increased pressure, slightly creased globulin, 50 to 2000 lymphocytes, meningitic colloidal gold curve, sterile c Inoculate guinea pig subcutaneously, or mice intracerebrally. Virus-neutralizing bodies in blood after sixth week. Differentiate from encephalitis, poliomyelitis, tube lous meningitis, neurosyphilis.

Meningitis, Meningococcus. Blood cultures often positive in early cases and in meningococcemia. Spinal fluid purulent, under high pressure. Culture *immediately* warm blood agar. Examine stained film for Gram-negative intracellular or extrac diplococci. Smooth virulent strains show capsular swelling with specific antis Neutrophilic leukocytosis. Differentiate from septic meningitis, tuberculous men poliomyelitis, encephalitis, benign lymphocytic choriomeningitis. Detect carriers by tures from posterior nasopharynx.

Meningitis, Tuberculous. Spinal fluid clear or opalescent, shows increased pres increased globulin, lymphocytic pleocytosis, decreased sugar, markedly decreased rides. Stain for tubercle bacilli in fibrin web which forms on standing or centr sediment after alum flocculation (p. 755). Make cultures or inoculate guinea pig. ferentiate from benign lymphocytic choriomeningitis, encephalitis, poliomyelitis.

Mercury Poisoning. Examine urine or gastric contents for mercury. Examine u for volume (oliguria), albumin, casts, renal epithelium. Determine blood nonpr nitrogen or urea, and creatinine. If severe, test for acidosis. Renal function tests sh impairment.

Myeloma, Multiple. Bence-Jones protein usually found in urine. High plasma gl lin (up to 9 per cent), even without Bence-Jones proteinuria. May show high calcium and low phosphorus. Anemia, occasionally erythroblastic, with leukocytosis myelocytes. Roentgenogram.

Myiases. To identify fly larvae, make rearings to adults, or examine breathing slits posterior stigmata of larvae.

Myxedema. Basal metabolic rate retarded (to —40 per cent). Blood shows cholesterol, low glucose. Low flat glucose-tolerance curve. Anemia, either macrocytic hypochromic microcytic in type. Often lymphocytosis.

Nephritis, Acute and Chronic. Examine urine, especially for albumin, casts, b and pus cells. The presence of blood and red cell casts indicates an acute pr In acute cases look for streptococcal throat infections. (See p. 37.) Test renal fun Determine blood nonprotein nitrogen or urea, and if high, creatinine or phosphorus. impaired, determine blood chloride or bases, and test for acidosis. Anemia comm advanced stages. (See "Nephrosis.")

Nephrolithiasis. Examine urine for blood and pus. The presence in *freshly* v urine of clumped calcium oxalate or uric acid crystals is suggestive. Examine

for cystin crystals. Make culture to determine whether pyelitis is present. Roentgenogram. Determine Ca and P in blood to exclude hyperparathyroidism.

"Nephrosis." "Nephrotic" Stage of Glomerular Nephritis. Examine urine. Oliguria, high fixed specific gravity, marked albuminuria, many casts, epithelial cells with doubly refractile fat droplets, pus cells, but few or no red cells. Chloride excretion reduced. Phthalein excretion normal. Blood cholesterol very high, plasma albumin reduced, A/G ratio inverted. Chlorides may be high. No nitrogen retention. Reaction to Congo red test positive. Sedimentation rate much accelerated. Basal metabolic rate retarded. Anemia common. Look for evidence of intercurrent infections.

Ochronosis (Alkaptonuria). Urine contains homogentisic acid, which turns black on standing exposed to the air (especially if alkaline). Reduces Benedict's solution but not bismuth salts, and is not fermented by yeast. Rare anomaly of metabolism.

Onchocerciasis. Aspirate fluid from a nodule and look for motile microfilariae in fresh preparation, or stain by Giemsa. If negative, excise a nodule and look for adult worm or (very numerous) microfilariae. Clip off a bit of skin or conjunctiva, shake up in a few drops of salt solution and look for microfilariae. (Microfilariae are not usually present in the blood.) Reaction to complement-fixation test positive with special antigens.

Ophthalmia Neonatorum. See Gonococcus infection.

Oriental Sore. Examine scrapings from base of ulcer for leishmanial bodies. Stain with Wright's or Giemsa's stain. Preferably obtain material by aspiration by puncture near edge of ulcer. If bacterial contamination can be avoided, culture on special medium.

Oroya Fever. Acute, rapidly developing hemolytic anemia. Rodlike organism, *Bartonella bacilliformis,* in red cells.

Osteomalacia. Either blood calcium or phosphorus (or both) usually decreased. Blood phosphatase increased. Roentgenogram.

Otitis Media. Neutrophilic leukocytosis. Make smears from discharge and culture on blood agar. Make blood culture.

Pancreatic Disorders. Examine feces for undigested starch, muscle fibers, and abnormal amounts of total fat and neutral fat. Examine duodenal contents, especially for enzymes. (See p. 867.) Test urine for sugar. Determine blood sugar and diastase. Increased diastase in urine. Make glucose-tolerance test.

Paragonimiasis. Examine fresh sputum for light-yellow, operculated ova, averaging 90 by 65μ. Also for pus, blood, elastic fibers. Exclude tuberculosis. Ova in feces in about 40 per cent of cases.

Paratyphoid Fever. Examine as for typhoid fever.

Pertussis. In early stages, examine stained smears from sputum for small, oval, Gram-negative, bipolar-staining bacilli. Culture sputum (or use "cough plate" method) on Bordet-Gengou medium. Usually a very marked lymphocytosis.

Piedra. Examine hairs for small gritty masses which consist of spores arranged like mosaics about hairs.

Pituitary Disorders. In hyperpituitarism (acromegaly) there may be polyuria and glycosuria associated with hyperglycemia and reduced glucose tolerance. In hypopituitarism (dystrophia adiposogenitalis, Fröhlich's syndrome) blood sugar is low, glucose tolerance increased. Basal metabolic rate slightly retarded (but normal if calculated on basis of the ideal weight of the individual).

Plague, Bubonic Type. Examine material obtained by gland puncture for *P. pestis.* Stain smears, culture, and inoculate a mouse or guinea pig to identify. *Pneumonic type:* Examine the thin, watery, blood-tinged sputum in the same way. To obtain pure culture, inoculate on unbroken skin or nasal mucosa of animal. *Septicemic type:* Make blood culture. *P. pestis* may be sufficiently numerous to be found in blood films. Leukocytosis. Differentiate from tularemia.

Pleural Fluid. Determine specific gravity, albumin content, total cell count. Centrifuge a small portion collected in citrated salt solution, treat sediment with 1 per cent

formalin, centrifuge again, and stain sediment by Gram's and Ziehl-Neelsen bacteria and by Wright's stain for differential count. Culture on blood agar late a guinea pig. In tuberculosis the fluid usually shows a lymphocytosis.

Pneumonia, Acute Lobar. Isolate pneumococcus from sputum (or ma lung puncture) by culture and mouse inoculation. Determine type directly from by Neufeld's "Quellung" reaction; confirm with culture later. Make blood culture positive early; late in disease indicates a bad prognosis). Marked neutrophilic l with shift to the left, and reduction in eosinophils. Low count indicates poor Urine chlorides much reduced. Blood chlorides reduced, nonprotein nitrogen oliguria is marked.

Pneumonia, Atypical. Make culture and stain film of sputum to exclude bac fection. Leukocyte count not increased. Examine serum at intervals for "cold" agg

Poliomyelitis. Examine spinal fluid. (See p. 205.) Culture on blood agar to ordinary forms of meningitis. Moderate neutrophilic leukocytosis at onset.

Polycythemia. High red cell count (over six million) with increased blood vol Increased viscosity. Evidences of active red cell regeneration, neutrophilic leukoc with often a few myelocytes, and increased platelets. High blood calcium. metabolic rate often accelerated. In selected cases test renal function and liver func

Pregnancy. Increased sedimentation rate. Reaction to Friedman or Aschheim-Z test positive. (See p. 881.)

Pregnancy, Toxemia of. Examine urine for evidences of nephritis. Test for k bodies, ratio of NH_3/urea and other evidences of acidosis. Tests for renal function liver function.

Prostatic Hypertrophy. Examine urine for albumin, casts, and evidences of infec Make culture. Test nonprotein nitrogen or urea and creatinine in blood. Usual tests renal function not applicable except with catheterization.

Purpura Haemorrhagica (Thrombocytopenic). Platelet count reduced. Bleeding prolonged. Clot retraction impaired. Reaction to tourniquet test positive. Coagulati time usually normal. May show secondary posthemorrhagic anemia. Leukocyte c variable.

Pyelitis. See Urinary tract infections.

Pyloric Obstruction. Examine fasting stomach contents for evidences of retenti Roentgenogram. Oliguria. Dehydration, with high red cell volume, high plasma pr Low blood chlorides and bases, and high nonprotein nitrogen and urea (n creatinine), falling rapidly if diuresis is established. If HCl is vomited, alkalosis high plasma bicarbonate and tetany. (See Gastric ulcer, Gastric cancer.)

Rabies. Keep dog, which has bitten patient, alive to observe symptoms. If dog been killed, make smears from cornu Ammonis and stain by Giemsa's or Mann's stain Negri bodies. (See p. 207.)

Relapsing Fever. Examine blood for spirochetes with darkfield or India-ink metho or in thick smears stained by Wright's or Giemsa's stain. (They may be absent fr peripheral blood during afebrile period.) If not found, inoculate a mouse and ex its blood after 24 and 48 hours. Neutrophilic leukocytosis in acute cases. Differentiate fr malaria, yellow fever, Weil's disease.

Rickets. Blood phosphorus usually low, calcium normal. In some cases calcium is lo and phosphorus normal. Product of Ca $\times$ P is below 40 and often below 30. Bl phosphatase increased. Examine blood for anemia. Roentgenogram.

Rickettsial Infections. Inoculate nearly grown male guinea pig intraperitoneally 1 ml. blood obtained during febrile period. Take temperature of pig regularly watch for febrile reaction after 5 to 12 days. Watch for swelling of scrotum, and amine scrapings from tunica vaginalis for rickettsiae (Mooser bodies). Examine sections for small proliferative nodules and perivascular infiltrations. (See Ta

) Test serum of patient after seven days for agglutinins for Proteus OX_{19} (Weil-). Moderate neutrophilic leukocytosis.

Rocky Mountain Spotted Fever. See Rickettsial infections.

Scabies. With the aid of a hand lens examine the infected skin for a black line which marks the tunnel for the parasite. The female can be found at the end of the tunnel and removed. Look for ova or mites in any stage of development in scrapings from skin.

Scarlet Fever. Neutrophilic leukocytosis of 12,000 to 15,000 with early increase in eosinophils. Make culture from throat on blood agar for hemolytic streptococci. Test for Schultz-Charlton reaction. Watch urine for evidences of nephritis. Make Dick tests and throat cultures on contacts.

Schistosomiasis. Examine urine (*S. haematobium*) and feces (*S. japonicum* and *S. mansoni*) for ova, particularly in masses of blood-tinged mucus. Blood in urine. Examine blood for anemia and eosinophilia. In late stages test liver function. Complement-fixation reaction positive with special antigen.

Scurvy. Reaction to tourniquet test of capillary resistance positive (as in purpura). Reduced excretion of cevitamic acid in the urine. May cause hypochromic anemia.

Septicemia. Make blood culture. Neutrophilic leukocytosis with shift to the left.

Smallpox. Initial leukopenia followed by neutrophilic leukocytosis in pustular stage. Monocytes increased. Try Paul's or McKinnon's inoculation tests. (See p. 195.)

Sporotrichosis. Culture on Sabouraud's agar or potato for eight days or more. Direct smears usually do not show organisms.

Sprue. Examine the frothy, pultaceous stools for undigested food and excess fat (25 to 40 per cent), chiefly fatty acids. Make gastric analysis (occasionally an achlorhydria). Examine blood for anemia, usually macrocytic, like pernicious anemia; occasionally hypochromic. Blood calcium reduced. Glucose tolerance curve has flat peak.

Syphilis. *Primary stage:* Look for *T. pallidum* in serous exudate from chancre in dark-field preparations (or stained films). If negative, and ulcer is healing, examine juice aspirated from regional bubo.

Secondary, tertiary and *latent* cases: Make complement-fixation or flocculation tests. Examine serous fluid expressed from secondary lesions by darkfield. Examine spinal fluid if clinical evidence of disease of the nervous system is present; and in all cases before treatment is stopped.

Tetanus. Inoculate white mouse or guinea pig and make anaerobic cultures from curettings from the wound. (See p. 70.) Rarely found in smears. Inject filtrate from culture into two guinea pigs, one of which should be protected by injection of anti-tetanic serum.

Thrombocytopenia. See Purpura haemorrhagica.

Thrush. Make scrapings from lesions and examine in 10 per cent KOH solution. The organism, *Candida albicans,* may be cultivated on Sabouraud's medium. It slowly liquefies gelatin and blood serum and acidifies and clots milk. In cultures there are budding yeastlike forms and mycelial threads.

Thyroid Disorders. See Exophthalmic goiter and Myxedema.

Transfusion. To select donor, secure individuals of the same blood group (or group O), and match the serum of the recipient with the cells of the donor and vice versa. Test for Rh factor in all women, and in men receiving repeated transfusions. Exclude syphilis by Kolmer or Kahn test and by physical examination. Exclude malaria by history and stained thick blood films.

Trichiniasis. Usually high leukocytosis and eosinophilia. Secure suspected meat, examine for encysted larvae in press preparations, or digest in artificial gastric juice, and collect larvae in Baermann apparatus (see p. 648). May feed meat to rat or mouse and examine muscles similarly after 10 days. During second or third week take 5 to 10 ml. blood in dilute acetic acid and examine sediment for larvae. After second week excise a

bit of muscle from deltoid or pectoralis near insertion and examine. Make test with Bachman antigen.

Trypanosomiasis. Examine blood or gland juice for trypanosomes in fresh prep or stained thick films. When sparse, concentrate in blood by centrifugation and films from leukocyte layer (see p. 522). If not found, inoculate a rat or guinea intraperitoneally with blood, gland juice, or emulsion of excised gland and blood at intervals. Reaction to formol-gel test often positive. In lethargic stage e spinal fluid for parasites. Cell count and globulin increased. Differentiate from kala malaria, syphilis.

Tuberculosis. Make acid-fast stain of smears from sputum, fasting stomach c feces, or urinary sediment. If necessary, first concentrate by digesting in alkali or formin and centrifuging. Culture on special egg-yolk media, or blood agar to few drops of glycerin have been added. Inoculate guinea pig. Blood cultures may positive in miliary tuberculosis. High monocyte-lymphocyte ratio indicates progres lesion. Positive diazo reaction in urine an unfavorable sign. In special cases make cutaneous tuberculin tests. Sedimentation rate increased in proportion to activity disease.

Tularemia. In the early stages inoculate mouse or guinea pig with material local lesion or regional glands, or with blood. At autopsy look for characteristic (small caseous foci in organs) and make cultures from blood and organs on gl cystin blood agar. After the first week test blood for agglutinins. Differentiate Brucellosis, plague.

Typhoid Fever. Neutrophilic leukopenia; relative lymphocytosis; eosinophils redu or absent. Blood culture usually positive during the first week, later less frequ obtained. Culture urine and feces on MacConkey, S. S., and bismuth sulfite me Make agglutination test after 7 to 10 days. In suspected carriers culture urine feces or duodenal contents. Differentiate from paratyphoid fever, brucellosis, ma typhus, miliary tuberculosis, liver abscess, kala-azar.

Typhus Fever. See Rickettsial infections.

Undulant Fever. See Brucellosis.

Urinary-tract Infections. Examine sediment immediately in hanging drop and films stained by Gram's method. Collect specimen with sterile precautions or by cath and culture on MacConkey and blood agar plates. In special cases search for tuber bacilli by stain and culture and confirm by guinea-pig inoculation. Digest sediment alkali if abundant or contaminated.

Yellow Fever. Early neutrophilic leukocytosis which in a few days falls to nor or below. Increasing albuminuria with granular and epithelial casts from the first or ond day. Oliguria or anuria in fatal cases. Bile pigments are present in blood and ur in increasing amounts from the second or third day. Inject blood of patient (during the first three days intracerebrally into mice. Serum of cases after recovery shows life long protective power. Differentiate from severe malaria, blackwater fever, infectio jaundice, relapsing fever, dengue, influenza.

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Illustrations are indicated by **bold-faced** figures.