a delicate hyaline peripheral zone can be distinguished. In ordinary blood-smears they are usually clumped in masses. A single platelet lying upon a red corpuscle may easily be mistaken for a malarial parasite (Plate VI and Fig. 95).

Blood-platelets are being much studied at present, but, aside from the facts mentioned under their enumeration (p. 213), little of clinical value has been learned. They have been variously regarded as very young red corpuscles (the "hematoblasts" of Hayem), as disintegration products of leukocytes, as remnants of extruded nuclei of erythrocytes, and as independent nucleated bodies. The most probable explanation of their origin seems to be that of J. H. Wright, who, from his recent studies, regards them as detached portions of the cytoplasm of certain giant-cells of the bone-marrow and spleen.

VIII. BLOOD PARASITES

A. Bacteria

Bacteriologic study of the blood is useful in many conditions, but in general, the elaborate technic involved takes it out of the reach of the clinician. As applied to the diagnosis of typhoid fever, however, the technic of blood-cultures has been so simplified that it can be carried through by any one who is competent to do the simplest cultural work.

Typhoid bacilli can be detected in the blood in practically every case of typhoid fever in the first week of the disease; in about 80 to 85 per cent. of cases in the second week; and in decreasing percentages in the later weeks. The blood-culture, therefore, offers the most certain means

of early diagnosis. It is in a sense complementary to the Widal reaction, the former decreasing and the latter increasing in reliability as the disease progresses. The blood-culture gives best results before the Widal appears, as one would expect from the fact that the Widal test depends upon the presence of antibodies which destroy, or, at least injure, the bacilli. The two methods together will establish the diagnosis in practically every case at any stage. Bacilli disappear from the blood in convalescence and reappear in a relapse.

Technic of Blood-cultures in Typhoid Fever.—The blood may be obtained in one of two ways:

(*a*) With a spring-lancet make a deep puncture in the edge (not the side) of the lobe of the ear, as for a blood-count. Allow the blood to drop directly into a short culture-tube containing the bile medium. By gentle milking, 20 to 40 drops can usually be obtained. This simple method of obtaining blood is especially applicable during the first week of the disease when bacilli are abundant. Contamination with skin cocci is possible, but does not usually interfere when the bile medium is used.

(*b*) In the later weeks of the disease a larger quantity of blood is needed. Prepare the skin on the front of the elbow, as for a minor operation, or simply rub well with alcohol. Tie a bandage tightly around the upper arm, have the patient open and close the fist a few times, and when the veins are sufficiently distended insert a hypodermic needle attached to a syringe into any vein that is prominent. The needle should go through the skin about $\frac{1}{4}$ inch from the vein with the bevel at its tip uppermost, and should enter the vein from the side in a direction opposite to the blood-current (Fig. 96). Unless too small a needle is used, blood will begin to rise in the syringe as soon as the needle has entered the vein.

Suction is not necessary. When sufficient blood is obtained, the bandage is first removed, the needle is withdrawn, and the blood is allowed to run into a tube of culture-medium. It is usually easy to secure 5 to 10 c.c. of blood. The procedure causes the patient surprisingly little inconvenience, seldom more than does an ordinary hypodermic injection. There is rarely any difficulty in entering the vein except in children, and in adults when the arm is fat and the veins are small. If desired, one of the veins about the ankle can be used. Instead of a syringe one can use a large glass tube

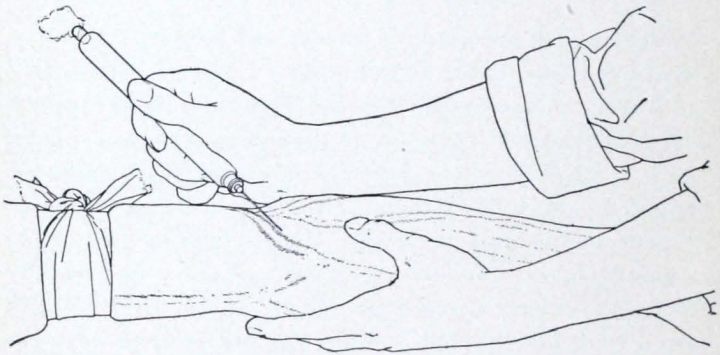


Fig. 96.—Method of obtaining blood for a blood-culture.

which has been drawn out at the ends and one end ground to fit a " slip-on " needle. Either a large hypodermic needle or a small antitoxin needle may be used. These little instruments (Fig. 96) can be made by any glass-blower at a cost of about fifty cents, and several of them can be kept on hand in test-tubes sterilized ready for use.

As special culture-medium, ox-bile is generally used. It favors the growth of the typhoid bacillus and retards the growth of other organisms. A good formula is given on p. 405.

As soon as convenient after the blood is added, place the tubes in the incubator. After about twelve hours examine

for motile bacilli. If none are found, transfer a few drops to tubes of bouillon or solidified blood-serum and incubate for twelve hours longer. If motile, Gram-negative bacilli are found; they are almost certainly typhoid bacilli. Further study is not necessary in practice, although desirable from a scientific point of view. The only bacilli which might cause confusion are the paratyphoid and colon bacilli, which can be distinguished by gas production in glucose media, indol production, and their effect upon litmus milk. The agglutination test for the identity of the bacillus is not available clinically, since freshly isolated bacilli do not agglutinate well.

B. Animal Parasites

Of the animal parasites which have been found in the blood, five are interesting clinically: the spirochæta of relapsing fever; trypanosomes; malarial parasites; filarial embryos; and the embryos of *Trichinella spiralis*.

1. Spirochæta recurrentis is described on p. 330.

2. Trypanosoma Gambiense.—Various trypanosomes are common in the blood of fishes, amphibians, birds, and mammals (Fig. 113). They live in the blood-plasma and do not attack the corpuscles. In some animals they are apparently harmless; in others they are an important cause of disease. They are discussed more fully on p. 333.

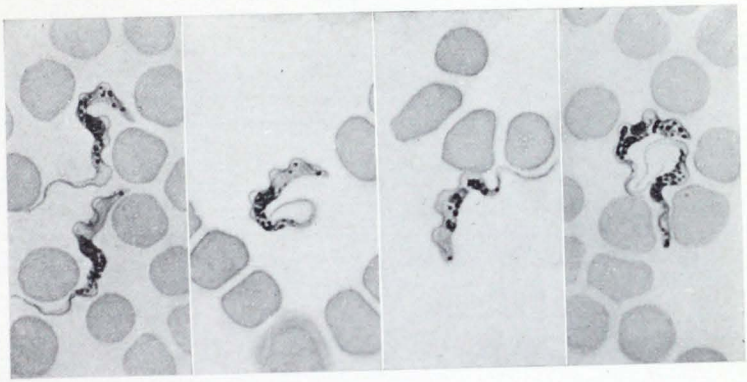
The trypanosome of human blood, *Trypanosoma gambiense* (Plate VII), is an actively motile, spindle-shaped organism, two or three times the diameter of a red corpuscle in length, with an undulating membrane which terminates at the anterior end in a long flagellum. It can be seen with medium power objectives in fresh blood, but is best studied with an oil-immersion lens in preparations stained as for the malarial parasite. Human trypano-

somiasis is common in Africa. As a rule, it is a very chronic disease. "Sleeping sickness" is a late stage when the organisms have invaded the cerebrospinal fluid. Infection is carried by the tsetse fly, *Glossina palpalis*.

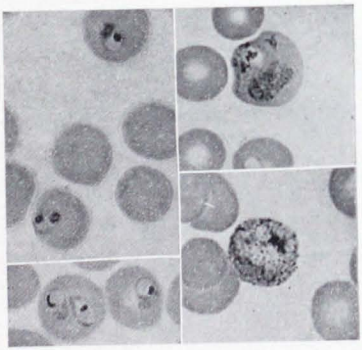
3. The Malarial Parasites.—These protozoa belong to the Sporozoa (p. 338), order Hemosporidia, the members of which are parasites in the blood of a great variety of vertebrates. Three species, constituting the genus *Plasmodium*, are associated with malarial fever in man: *Plasmodium vivax*, *P. malariæ*, and *P. falciparum*, the parasites respectively of the tertian, quartan, and estivo-autumnal types of malaria. The life histories of the three are so similar that they may well be described together.

(1) **Life Histories.**—There are two cycles of development: one, the *asexual*, in the blood of man; and the other, the *sexual*, in the intestinal tract of a particular genus of mosquito, *Anopheles*.

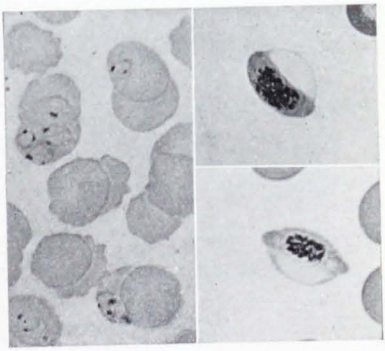
(*a*) *Asexual Cycle.*—The young organism enters the blood through the bite of the mosquito. It makes its way into a red corpuscle, where it appears as a small, pale "hyaline" body. This body exhibits ameboid movement and increases in size. Soon, dark-brown granules derived from the hemoglobin of the corpuscle make their appearance within it. When it has reached its full size—filling and distending the corpuscle in the case of the tertian parasite, smaller in the others—the pigment granules gather at the center or at one side; the organism divides into a number of small hyaline bodies, the spores or merozoites; and the red corpuscle bursts, setting spores and pigment free in the blood-plasma. This is called segmentation. It coincides with, and by liberation of



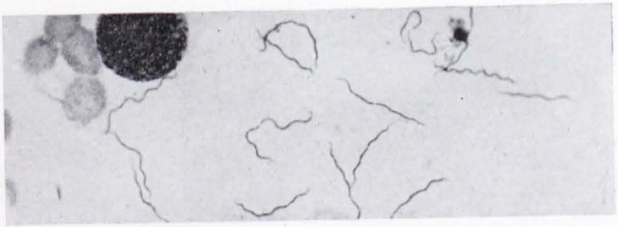
Trypanosoma gambiense (slide presented by Professor F. G. Novy).



Tertian malarial parasites, one red cell showing malarial stippling.



Estivo-autumnal malarial parasites, small ring forms and crescents.



Spirochæta novyi.

Animal parasites of the blood; × 1000 (photographs by the author).

toxins causes, the paroxysm of the disease. A considerable number of the spores are destroyed by leukocytes or other agencies; the remainder enter other corpuscles and repeat the cycle. Many of the pigment granules are taken up by leukocytes. In estivo-autumnal fever segmentation occurs in the internal organs and the segmenting and larger pigmented forms are not seen in the peripheral blood.

The asexual cycle of the tertian organism occupies forty-eight hours; of the quartan, seventy-two hours; of the estivo-autumnal, an indefinite time—usually twenty-four to forty-eight hours.

The parasites are thus present in the blood in great groups, all the individuals of which reach maturity and segment at approximately the same time. This explains the regular recurrence of the paroxysms at intervals corresponding to the time occupied by the asexual cycle of the parasite. Not infrequently there is multiple infection, one group reaching maturity while the others are still young; but the presence of two groups which segment upon the same day is extremely rare. Fevers of longer intervals—six, eight, ten days—are probably due to the ability of the body, sometimes of itself, sometimes by aid of quinin, to resist the parasites, so that numbers sufficient to cause a paroxysm do not accumulate in the blood until after several repetitions of the asexual cycle. In estivo-autumnal fever the regular grouping, while usually present at first, is soon lost, thus causing "irregular malaria."

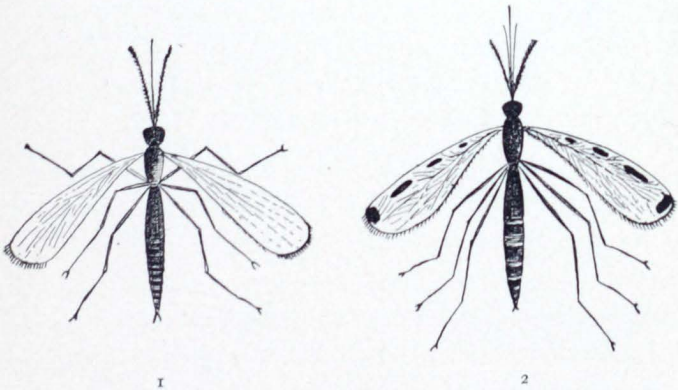
(*b*) *Sexual Cycle.*—Besides the ameboid individuals which pass through the asexual cycle, there are present with them in the blood many individuals with sexual

properties. These are called *gametes*. They do not undergo segmentation, but grow to adult size and remain inactive in the blood until taken up by a mosquito. Many of them are apparently extracellular, but stained preparations usually show them to be surrounded by the remains of a corpuscle. In tertian and quartan malaria they cannot easily be distinguished from the asexual individuals until a variable time after the blood leaves the body, when the male gamete sends out one or more flagella. In estivo-autumnal malaria the gametes take distinctive ovoid and crescentic forms, and are not difficult to recognize. They are very resistant to quinin and often persist in the blood long after the ameboid forms have been destroyed, but are probably incapable of continuing the disease until they have passed through the cycle in the mosquito.

When a malarious person is bitten by a mosquito, the gametes are taken with the blood into its stomach. Here a flagellum from the male unites with the female, which soon thereafter becomes encysted in the wall of the intestine. After a time it ruptures, liberating many minute rods, or sporozoites, which have formed within it. These migrate to the salivary glands, and are carried into the blood of the person whom the mosquito bites. Here they enter red corpuscles as young malarial parasites, and the majority pass through the asexual cycle just described.

The sexual cycle can take place only within the body of one genus of mosquito, *Anopheles*. Absence of this mosquito from certain districts explains the absence of malaria. It is distinguished from our common house-mosquito, *Culex*, by the relative lengths of proboscis and palpi (Fig. 97), which can be seen with a hand-lens, by

its attitude when resting, and by its dappled wing (Fig. 98). Anopheles is strictly nocturnal in its habits; it usually flies low, and rarely travels more than a few hundred yards from its breeding-place, although it may be carried by winds. These facts explain certain peculiarities in malarial infection; thus, infection occurs practically only at night; it is most common near stagnant water, especially upon the side toward which the prevailing winds blow; and the danger is greater when per-



1 2

Fig. 97.—Mosquitoes—*Culex* (1) and *Anopheles* (2) (Bergey).

sons sleep upon or near the ground than in upper stories of buildings. The insects frequently hibernate in warmed houses, and may bite during the winter. A mosquito becomes dangerous in eight to fourteen days after it bites a malarious person, and remains so throughout its life.

(2) **Detection.**—Search for the malarial parasite may be made in either fresh blood or stained films. If possible, the blood should be obtained a few hours before the chill —never during it nor within a few hours afterward, since

at that time (in single infections) only the very young, unpigmented forms are present, and these are the most difficult to find and recognize. Sometimes many parasites are found in a microscopic field; sometimes, especi-

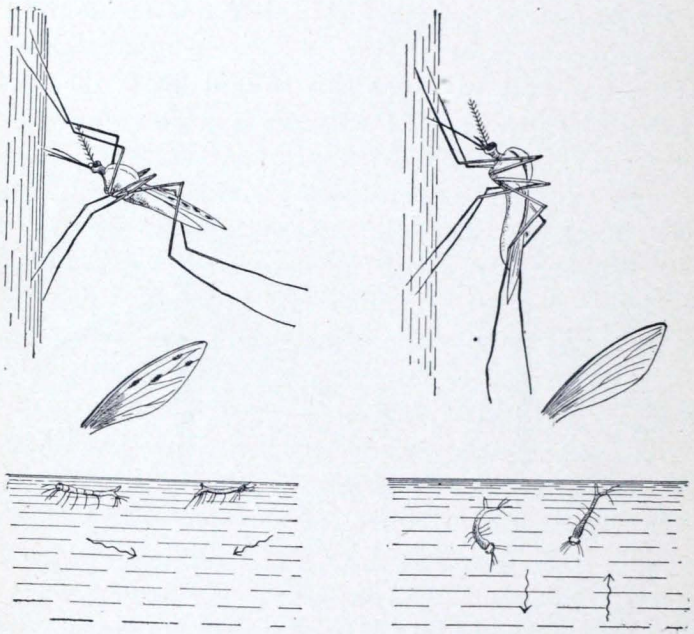


Fig. 98.—Showing, on the left, *Anopheles* in resting position, its dappled wing, and the position of its larvæ in water; on the right, *Culex* in resting position, its plain wing, and the position of its larvæ in water. The arrows indicate the directions taken by the larvæ when the water is disturbed (Abbott).

ally in estivo-autumnal infection, owing to accumulation in internal organs, careful search is required to find any, despite very severe symptoms. Quinin causes them rapidly to disappear from the peripheral blood, and few or none may be found after its administration. In the

absence of organisms, the presence of pigment granules within leukocytes—polymorphonuclears and large mononuclears—may be taken as presumptive evidence of malaria. Pigmented leukocytes (Plate VI) are most numerous after a paroxysm.

(*a*) *In Fresh Unstained Blood* (Plate VIII).—Obtain a small drop of blood from the finger or lobe of the ear. Touch the center of a cover-glass to the top of the drop and quickly place it, blood side down, upon a slide. If the slide and cover be perfectly clean and the drop not too large, the blood will spread out so as to present only one layer of corpuscles. Search with an oil-immersion objective, using very subdued light.

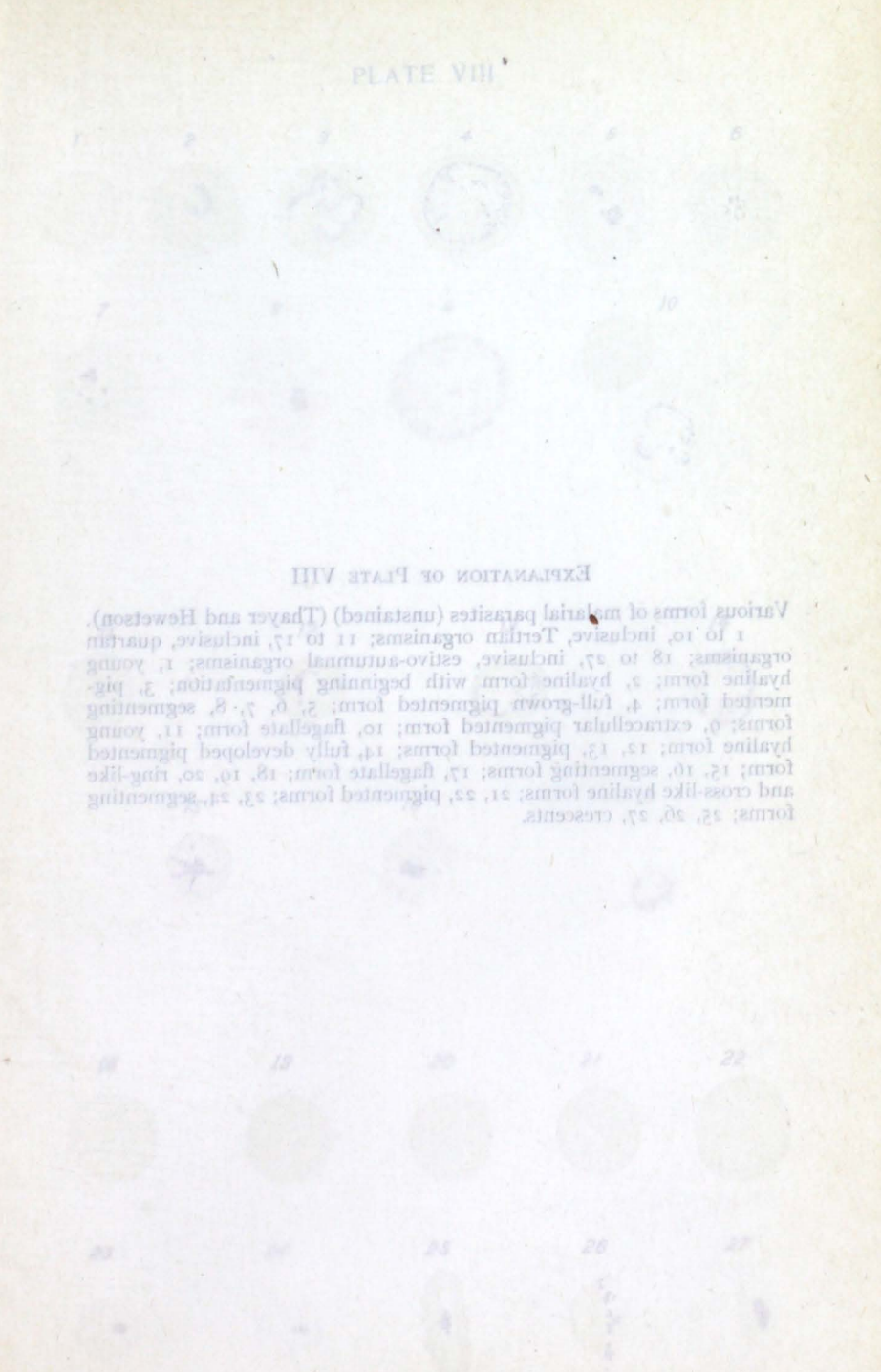
The young organisms appear as small, round, ring-like or irregular, colorless bodies within red corpuscles. The light spots caused by crenation and other changes in the corpuscles are frequently mistaken for them, but are generally more refractive or have more sharply defined edges. The older forms are larger colorless bodies containing granules of brown pigment. In the case of the tertian parasite, these granules have active vibratory motion, which renders them conspicuous; and as the parasite itself is very pale, one may see only a large pale corpuscle in which fine pigment granules are dancing. Segmenting organisms, when typic, appear as rosets, often compared to daisies, the petals of which represent the segments, while the central brown portion represents the pigment. Tertian segmenting forms are less frequently typic than quartan. Flagellated forms are not seen until ten to twenty minutes after the blood has left the vessels. As Cabot suggests, one should, while searching, keep a sharp lookout for unusually large or pale cor-

puscles, and for anything which is brown or black or in motion.

(*b*) *In Stained Films* (Plates VI and VII).—Recognition of the parasite, especially the young forms, is much easier in films stained by Wright's or some similar stain than in fresh blood. When very scarce, they may sometimes be found, although their structure is not well shown by the method of Ruge. This consists in spreading a very thick layer of blood, drying, placing for a few minutes in a fluid containing 5 per cent. formalin and 1 per cent. acetic acid, which removes the hemoglobin and fixes the smear, rinsing, drying, and finally staining. Carbol-thionin is very useful for this purpose. If Wright's stain be used in this method, it is recommended that the preparation be subsequently stained for a half-minute with borax-methylene-blue (borax, 5; methylene-blue, 2; water, 100).

In films which are properly stained with Wright's fluid the young organisms are small, round, ring-like or irregular, sky-blue bodies, each with a very small, sharply defined, reddish-purple chromatin mass. Many structures —deposits of stain, dirt, blood-plaques lying upon red cells (Fig. 95), etc.—may simulate them, but should not deceive one who looks carefully for both the blue cytoplasm and the reddish-purple chromatin. A plaque upon a red corpuscle is surrounded by a colorless zone rather than by a distinct blue body. Young estivo-autumnal parasites commonly take a "ring" form (the chromatin mass representing the jewel), which is infrequently assumed by the other varieties. The older tertian and quartan organisms show larger sky-blue bodies with more reticular chromatin, and contain brown granules of pigment, which,

PLATE VIII



Explanation of Plate VIII

Various forms of malarial parasites (unstained) (Thayer and Hewetson). 1 to 10, inclusive, Tertian organisms; 11 to 17, inclusive, quartan organisms; 18 to 27, inclusive, estivo-autumnal organisms; 1, young hyaline form; 2, hyaline form with beginning pigmentation; 3, pigmented form; 4, full-grown pigmented form; 5, 6, 7, 8, segmenting forms; 9, extracellular pigmented form; 10, flagellate form; 11, young hyaline form; 12, 13, pigmented forms; 14, fully developed pigmented form; 15, 16, segmenting forms; 17, flagellate form; 18, 19, 20, ring-like and cross-like hyaline forms; 21, 22, pigmented forms; 23, 24, segmenting forms; 25, 26, 27, crescents.

PLATE VIII

however, is less evident than in the living parasite. The chromatin is often scattered through the cytoplasm or apparently outside of it, and is sometimes difficult to see clearly. Typical "segmenters" present a ring of rounded segments or spores, each with a small, dot-like chromatin mass. With the tertian parasite, the segments more frequently form an irregular cluster. The pigment is collected near the center or scattered among the segments. In estivo-autumnal fever usually only the small "ring bodies" and the crescentic and ovoid gametes are seen

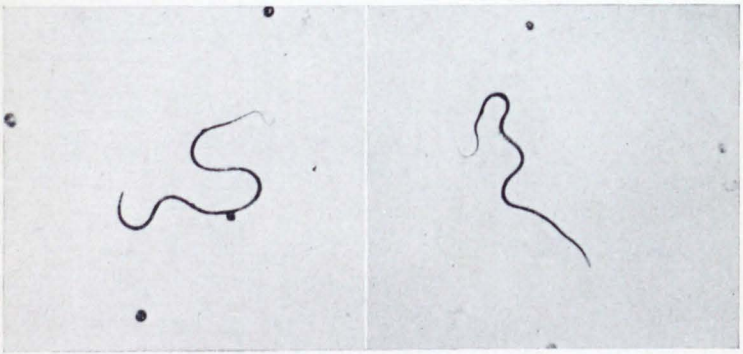


Fig. 99.—Filarial embryos in blood. Stained. Red corpuscles decolorized; a few leukocytes remain (× 200) (photographs by the author).

in the blood. The gametes are easily recognized. Their length is somewhat greater than the diameter of a red corpuscle. Their chromatin is usually centrally placed, and they contain more or less coarse pigment. The remains of the red cell often form a narrow rim around them or fill the concavity of the crescent.

While the parasites are more easily found in stained preparations, the varieties are more easily differentiated in fresh blood. The chief distinguishing points are included in the table on page 256.

VARIETIES OF THE MALARIAL ORGANISM

TERTIAN.	QUARTAN.	ESTIVO-AUTUMNAL.
Asexual cycle, forty-eight hours.	Seventy-two hours.	Usually twenty-four to forty-eight hours.
Substance pale, transparent, comparable to hyaline tube-cast.	Highly refractive, comparable to waxy tube-cast.	Highly refractive.
Outline indistinct.	Distinct.	Distinct.
Ameboid motion active.	Sluggish.	Active.
Mature asexual form large; fills and often distends corpuscle.	Smaller.	Young forms, only, in peripheral blood.
Pigment-granules fine, brown, scattered throughout. Very active dancing motion.	Much coarser, darker in color, peripherally arranged. Motion slight.	Very few, minute, inactive. Distinctly pigmented forms seldom seen.
Segmenting body rarely assumes typical "daisy" form. 15 to 20 segments.	Usually typical "daisy." 6 to 12 segments.	Not seen in peripheral blood.
Gametes resemble asexual forms.	Same as tertian.	Appear in blood as distinctive ovoids and crescents.
Red corpuscles pale and swollen.	Generally darker than normal.	Dark, often bronzed.

4. Filarial Embryos.—A description of the filariæ whose embryos appear in the blood will be found on p. 356.

The embryos can be seen in stained preparations, (Fig. 99), but are best found in fresh unstained blood. A rather large drop is taken upon a slide, covered, and examined with a low power. The embryo can be located by the commotion which its active motion produces

among the corpuscles. This motion consists almost wholly in apparently purposeless lashing and coiling movements, and continues for many hours.

5. Embryos of Trichinella Spiralis.—The worm and its life-history are described on page 363. It has recently been shown that diagnosis of trichiniasis can frequently be made by detection of the embryos in the blood during their migration to the muscles. Of eleven such examinations which have been reported within the past two and a half years, six were positive. The earliest time at which the embryos were found was the sixth day after the onset of symptoms; the latest, the twenty-second day.

The method is very simple. One to 10 c.c. of blood are obtained from the ear or a vein, as described on page 245, and mixed with ten times its volume of 3 per cent. acetic acid. The mixture is centrifugalized, and large drops of the sediment are placed on slides, covered, and searched with a low-power objective. The embryos are not difficult to recognize. They are about 125 μ long and 6 μ broad.

IX. SERUM REACTIONS

1. Agglutination.—In the blood-serum of persons suffering from certain infectious diseases there exist soluble bodies, called agglutinins, which have the property of rendering non-motile and clumping the specific microorganism of the disease, and have little or no influence upon other bacteria. This "agglutination" takes place even when the blood is greatly diluted. Undiluted normal blood can agglutinate most bacteria, but loses this power when diluted to any considerable degree. These

facts are taken advantage of in the diagnosis of several diseases.

When applied to the diagnosis of typhoid fever, the phenomenon is known as the *Widal reaction*. As yet, it is the only agglutination reaction which has any practical value for the practitioner.

Either blood-serum or the whole blood may be used. Serum is the better. To obtain it, it is convenient to use little vials, such as can be made by breaking off the lower

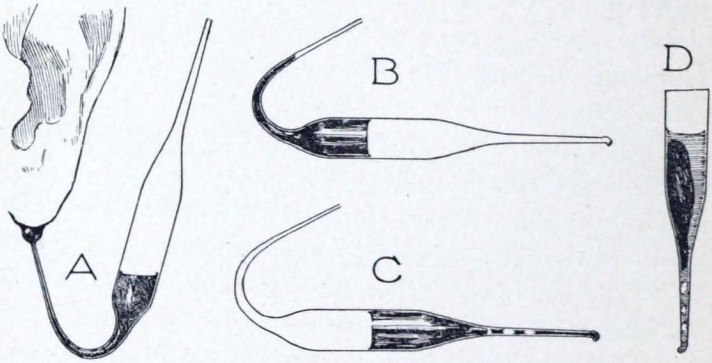


Fig. 100.—Method of obtaining blood in a Wright capsule: A, Filling the capsule; B, the bulb has been warmed and the capillary end sealed in a flame; C, cooling of the capsule has drawn the blood to the sealed end; D, the serum has separated, and the top of the capsule has been broken off.

half-inch of the tubes which have contained peptonizing powder. They must, of course, be well cleaned. One of these is filled to a depth of about $\frac{1}{4}$ inch from a puncture in the finger or the ear, and is set aside for a few hours. When the clot has separated, it is picked out with a needle, leaving the serum. It is, however, more satisfactory to obtain the blood in a Wright capsule (Fig. 100). This capsule is easily made from a piece of glass-tubing as indicated in Fig. 161.

One drop of the serum is then added to nine drops of normal salt solution, making a dilution of 1 : 10. Distilled water may be used for dilution, but is more liable to cause error. The dilution can be more accurately made in the leukocyte pipet of the Thoma-Zeiss instrument. When the whole blood is used, it can be secured in this pipet and at once diluted with the salt solution. When it must be transported a considerable distance, dried blood is most convenient. A large drop is allowed to dry upon a clean slide or unglazed paper. It will keep for months without losing its agglutinating power. When ready to make the test, the dried stain is dissolved in ten drops of normal salt solution, care being taken that the drops are about the same size as the original drop of blood.

The reaction can be detected either microscopically or macroscopically:

Microscopic Method.—(1) The blood or serum having been obtained and diluted 1 : 10 as just described, mix it with a bouillon culture of the typhoid bacillus to any desired dilution. One drop of each makes a blood-dilution of 1 : 20, etc. The culture should be between eighteen and twenty-four hours old, and the bacilli must be actively motile. A stock agar culture should be kept at room temperature, and bouillon tubes inoculated the day before the examination is to be made. Agar cultures can be purchased from dealers in biologic products. They must be renewed monthly.

Instead of the bouillon culture, McFarland recommends the use of a suspension made by removing some of the growth from the surface of a fresh agar culture and mixing it well with a little sterile water. It is then necessary to examine the suspension microscopically to make sure that there are no natural clumps.

(2) Place a few drops of the mixture of blood and culture upon a perfectly clean slide and apply a cover-glass. The cover may be ringed with vaselin to prevent evaporation, but this is not usually necessary.

(3) Examine at intervals with a high dry lens—a 4 mm. will answer very well. The light must be very subdued. At first the bacilli should be actively moving about. If the blood be from a case of typhoid, they will gradually lose their motion and gather together in clumps (Fig. 101). The clumps should be large, and the few bacilli remaining isolated should

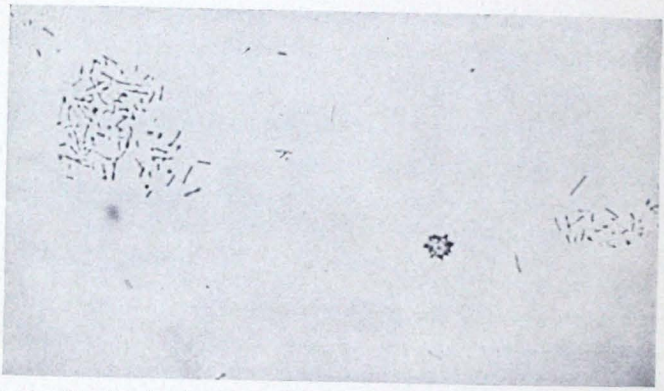


Fig. 101.—Showing clumping of typhoid bacilli in the Widal reaction. At one point a crenated red blood-corpuscle is seen (Wright and Brown).

be motionless. Pseudoreactions, in which there are a few small clumps of bacilli whose motion is not entirely lost, together with many freely moving bacilli scattered throughout the field, should not mislead. As a control, a drop of the culture should always be examined before making the test.

Normal blood may produce clumping if time enough be allowed. The diagnostic value of a positive reaction is, therefore, impaired unless clumping takes place within a limited time. With dilution of 1 : 40 the time limit should not exceed

forty-five minutes; with 1 : 80, one and one-half hours. Tests based upon lower dilution than 1 : 40 are probably not reliable.

Macroscopic Method.—The principle is the same as that of the microscopic method. Clumping of the bacilli causes a flocculent precipitate, which can be seen with the naked eye. A dead culture gives the same results as a living one. This method is as reliable as the microscopic and is more convenient for the practitioner, although it requires more time.

Dead cultures, together with apparatus for diluting the blood, are put up at slight cost by various firms, under the names of typhoid diagnosticum, typhoid agglutometer, etc. Full directions accompany these outfits and need not be repeated here.

Recently, Bass and Watkins have described a modification of the macroscopic method (using very concentrated suspensions of the bacilli) by which the test can be applied at the bedside. Clumping occurs within two minutes. The apparatus has been put upon the market by Parke, Davis & Co.

The Widal reaction is positive in over 95 per cent. of all cases of typhoid fever. It may, rarely, be positive in other conditions, owing, sometimes at least, to faulty technic. It seldom appears before the fifth or sixth day; usually during the second week, but sometimes not until convalescence. It is, therefore, of less value in early diagnosis than is the blood-culture (p. 244). When it once appears it remains during the whole course of the disease, and frequently persists for years.

2. Opsonins.—That phagocytosis plays an important part in the body's resistance to bacterial invasion has long been recognized. According to Metchnikoff, this

property of leukocytes resides entirely within themselves, depending upon their own vital activity. The studies of Wright and Douglas, upon the contrary, indicate that the leukocytes are impotent in themselves, and can ingest bacteria only in the presence of certain substances which exist in the blood-plasma. These substances have been named *opsonins*. Their nature is undetermined. They probably act by uniting with the bacteria, thus preparing them for ingestion by the leukocytes; but they do not cause death of the bacteria, nor produce any appreciable morphologic change. They appear to be more or less specific, a separate opsonin being necessary for phagocytosis of each species of bacteria. There are, moreover, opsonins for other formed elements—red blood-corpuscles, for example. It has been shown that the quantity of opsonins in the blood can be greatly increased by inoculation with dead bacteria.

To measure the amount of any particular opsonin in the blood Wright has devised a method which involves many ingenious and delicate technical procedures. Much skill, such as is attained only after considerable training in laboratory technic, is requisite, and there are many sources of error. It is, therefore, beyond the province of this work to recount the method in detail. In a general way it consists in: (*a*) Preparing a mixture of equal parts of the patient's blood-serum, an emulsion of the specific micro-organism, and a suspension of washed leukocytes; (*b*) preparing a similar mixture, using serum of a normal person; (*c*) incubating both mixtures for a definite length of time; and (*d*) making smears from each, staining, and examining with an oil-immersion objective. The number of bacteria which have been taken up by a definite

number of leukocytes is counted, and the average number of bacteria per leukocyte is calculated; this gives the "phagocytic index." The phagocytic index of the blood under investigation, divided by that of the normal blood, gives the *opsonic index* of the former, the opsonic index of the normal blood being taken as 1. Simon regards the percentage of leukocytes which have ingested bacteria as a more accurate measurement of the amount of opsonins than the number of bacteria ingested, because the bacteria are apt to adhere and be taken in in clumps.

Because of its simplicity the clinical laboratory worker will prefer some modification of the Leishman method, which uses the patient's own leukocytes. It is, perhaps, as accurate as the original method of Wright, although variations in the leukocyte count have been shown to affect the result. Two pipets like those shown in Fig. 164 are used.

(1) Make a suspension of the specific organism by mixing a loopful of a young agar culture with 1 c.c. of a solution containing 1 per cent. sodium citrate and 0.85 per cent. sodium chlorid. Thoroughly break up all clumps by sucking the fluid in and forcing it out of one of the capillary pipets held vertically against the bottom of the watch-glass.

(2) Puncture the patient's ear, wipe off the first drop of blood, and from the second draw blood into the other pipet to the grease pencil mark, let in a bubble of air, and draw in the same amount of bacterial suspension.

(3) Mix upon a slide by drawing in and forcing out of the pipet.

(4) Draw the mixture high up in the pipet, seal the tip in the flame, and place in the incubator for fifteen minutes.

(5) Repeat steps 2, 3, and 4 with the blood of a normal person.

(6) After incubation, break off the tip of the pipet, mix the blood-bacteria mixture, and spread films on slides.

(7) Stain with Wright's or Harlow's blood-stain.

(8) With an oil-immersion lens count the bacteria which have been taken in by 100 leukocytes, and calculate the average number per leukocyte. Divide the average for the patient by the average for the normal person. This gives the opsonic index. If in the patient's blood there was an average of 4 bacteria per leukocyte, and in the normal blood 5 bacteria per leukocyte, the opsonic index would be $\frac{4}{5}$ or 0.8.

Wright and his followers regarded the opsonic index as an index of the power of the body to combat bacterial invasion. They claimed very great practical importance for it as an aid to diagnosis and as a guide to treatment by the vaccine method. This method of treatment consists in increasing the amount of protective substances in the blood by injections of normal salt suspensions of dead bacteria of the same species as that which has caused and is maintaining the morbid process, these bacterial suspensions being called "vaccines." Vaccine Therapy (Chapter IX) has taken a permanent place among our methods of treatment of bacterial infections, particularly of those which are strictly local, but the opsonic index is now little used either as a measure of resisting power or as an aid to diagnosis and guide to treatment.

3. Wassermann Reaction.[1]—The Wassermann test for syphilis, like the Widal test for typhoid fever, de-

[1] By Clough T. Burnett, Professor of Bacteriology, University of Colorado.

pends upon the detection in the patient's blood-serum of specific antibodies, *agglutinins* in the case of typhoid, *immune bodies* or *amboceptors* in the case of syphilis. These antibodies have been produced by the tissues in response to the entrance of the invading organism. If they are present, it is assumed that the patient has or has had syphilis. The Wassermann test is, however, much more complicated than the Widal test, and can be properly performed only by a trained laboratory worker. It is the aim here to explain only the general principles of the method, together with its clinical significance. For a proper understanding of the test the principles of bacteriolysis and hemolysis must first be presented.

Bacteriolysis and Hemolysis.—In 1894 Pfeiffer, working with guinea-pigs immunized to cholera, found that when living cholera germs were introduced into the peritoneal cavity of an immune animal they lost their motility within a few minutes, and very shortly were seen to disintegrate and go into complete solution. This has been known as Pfeiffer's phenomenon, or *bacteriolysis*. It was later demonstrated that this reaction could take place outside the animal body if the bacteria were mixed in the test-tube with the blood-serum or peritoneal fluid of a cholera immune animal. Subsequent researches showed that while an old or heated immune serum failed to cause this solution of the bacteria, upon the addition of a normal fresh serum this property returned. This addition of a normal serum to a serum which has lost its solvent action is called reactivation of the serum. These changes may best be demonstrated by the following chart:

BACTERIOLYSIS.

Immune serum,	fresh	+ bacteria		= *solution.*
Normal "	"	+ "		= no solution.
Immune "	heated	+ "		= no solution.
Immune "	"	+ normal serum	+ bacteria	= *solution.*

From the chart it is clear that there are two substances concerned in bacteriolysis, one of which is found in any fresh serum, but is easily destroyed, and is called the *complement.* The other substance is found only in the immune serum, is relatively stable, and is known as the *immune body* or *amboceptor.*

In hemolysis we find an analogy to bacteriolysis. Let a rabbit be immunized to sheep's blood-corpuscles. Now, if washed sheep's blood-corpuscles be subjected to the action of fresh serum from this rabbit, a speedy solution of the red cells ensues. If this serum is allowed to stand for several days, or is heated one-half hour to 56° C., it will completely lose its solvent power. Now, the addition of a fresh normal serum, even of another species, will reactivate the heated or old serum. The following chart will indicate these reactions:

HEMOLYSIS.

Rabbit serum, immune		+ corpuscles (sheep's)		= *solution.*
Rabbit "	heated	+ "	"	= no solution.
Normal "		+ "	"	= no solution.
Rabbit "	heated	+ normal serum	+ corpuscles (sheep's)	= *solution.*

In hemolysis, as in bacteriolysis, besides the antigen (substance giving rise to amboceptors or antibodies) there are two substances. One of these is specific, *i. e.*, only found in immune serum, and reacting only with the substance used in producing the immune serum. This sub-

stance is relatively stable, and is known as the amboceptor. The other substance is found in any serum, is absolutely non-specific, is easily destroyed, and is called the complement. In neither case will the amboceptor nor the complement acting alone cause a solution of the antigen.

There are three substances necessary to bacteriolysis and hemolysis. To produce bacteriolysis there must be the specific antigen (as cholera vibrio in Pfeiffer's phenomenon), the immune serum containing the amboceptor, and a complement. These three substances comprise the bacteriolytic system. Likewise, in hemolysis there is the red blood-cell, the amboceptor, and a complement, which comprise the hemolytic system.

It will be noted that there is one factor common to both systems, viz., the complement. It will be evident that if we place in a test-tube a complete bacteriolytic system, with just enough complement to cause solution of the bacteria, and place this for a sufficient time in the optimum temperature for bacteriolysis, and then add two elements of the hemolytic system (amboceptor and blood-cells), no hemolysis will ensue, because all of the complement was used by the bacteriolytic system.

Bordet and Gengou in 1901 showed that it was possible to utilize this fact in the diagnosis of certain bacterial infections. For instance, the heated serum of a suspected typhoid case plus typhoid bacilli is added to a serum containing complement (fresh guinea-pig serum) and incubated one hour. If this suspected serum contains typhoid amboceptors, there will have been a combination effected between the three elements of the bacteriolytic system, so that there will be no free complement left. If no amboceptor is present, all of

the complement will remain unattached. Now, in order to show whether this complement has been fixed or deviated, two elements of a hemolytic system are added—namely, amboceptor and red corpuscles, and if the complement is fixed, no hemolysis can ensue.

This is easily understood from a study of the swinging pendulum diagram, in which the complement is represented in the pendulum.

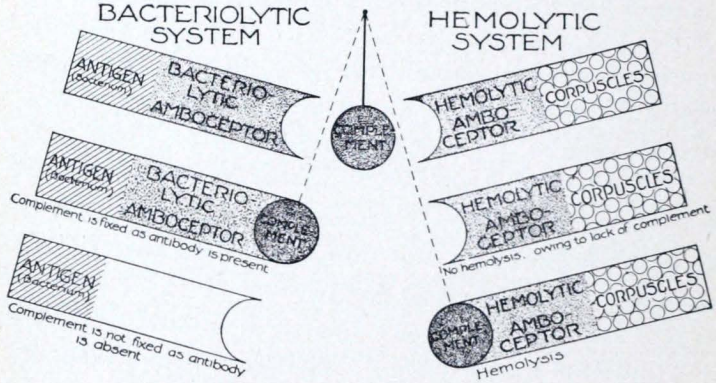


Fig. 102.—Pendulum diagram illustrating hemolysis.[1]

This principle of "complement deviation" having been utilized in the diagnosis of infectious diseases, it occurred to Wassermann in 1906 to apply it to the diagnosis of syphilis. The antigen first used was the extract of a fetal syphilitic liver, but subsequent work has shown that the same reaction may be obtained with normal liver or spleen tissue, or with certain lipoid substances, and in this sense is not a true antigen-antibody reaction. The antibodies in the syphilitic blood, however, are specific. These are analogous to the bacteriolytic amboceptor of the pendulum diagram.

[1] Not original, but unable to place credit where due.

Technic of the Wassermann Test.—The following reagents are necessary:

Antigen.—Extract of fetal syphilitic or normal liver, diluted 1 : 10.

Antibodies (Analogous to Bacteriolytic Antibodies).—The serum or spinal fluid of the suspected patient. As controls, the serum of a syphilitic known to contain antibodies and the serum of a normal person known not to contain antibodies.

Complement.—Fresh guinea-pig serum. Other fresh normal sera may be used. This is diluted 1 : 10.

Hemolytic Amboceptor.—The serum of a rabbit which has been immunized to sheep's red blood-corpuscles. This serum is inactivated before use by heating to 56° C. one-half hour, and diluted 1 : 1000 before using.

Corpuscle Suspension.—Sheep's blood is defibrinated, washed three times with normal salt solution, and then diluted with normal salt solution to make a 5 per cent. suspension.

In using these various reagents it is necessary to know that they are potent and of the proper strength, that is, to establish the titre of the reagent. This being determined, we are now ready for the Wassermann test, as carried out in the table on page 270.

In the luetic control tube there will occur a combination between the antibodies in the serum and the antigen, which together will cause a fixation of the complement, so that when later the two elements of the hemolytic system are added, no hemolysis will occur. This inhibition of hemolysis indicates a positive syphilitic reaction.

Control tube No. 3 is used to show that there is nothing in a normal serum which can effect this combination and deviation. Tube No. 4 shows that the patient's serum alone is not anticomplementary. Tube No. 5 shows that the hemolytic system is effective. Tube No. 6 shows that the

WASSERMANN REACTION

Tube I.	Tube II.	Tube III.	Tube IV.	Tube V.	Tube VI.	Tube VII.
Suspected.	Luetic Control.	Normal Control.	Control.	Control.	Control.	Control.
Patient's serum, c.c. 0.2	c.c. Luetic serum, 0.2	Normal serum, c.c. 0.2	Patient's serum, c.c. 0.2	c.c.	c.c.	c.c. .....
Complement,[1] 0.4	Complement, 0.4	Complement, 0.4	Complement, 0.4	Complement, 0.4	Complement, 0.4	.....
Antigen,[2] 0.4	Antigen, 0.4	Antigen, 0.4	.....	.....	Antigen, 0.4	Antigen, 0.4
Salt solution, 2.0	Salt solution, 2.0	Salt solution, 2.4	Salt solution, 2.0	Salt solution, 2.6	Salt solution, 2.2	Salt solution, 2.6
Incubate one hour at 37° C.						
Amboceptor,[3] 0.2	Amboceptor, 0.2	Amboceptor, 0.2	Amboceptor, 0.2	Amboceptor, 0.2	Amboceptor, 0.2	.....
Corpuscles,[4] 1.0	Corpuscles, 1.0	Corpuscles, 1.0	Corpuscles, 1.0	Corpuscles, 1.0	Corpuscles, 1.0	Corpuscles, 1.0
Salt solution, 0.8	Salt solution, 0.8	Salt solution, 0.8	Salt solution, 0.8	Salt solution, 0.8	Salt solution, 0.8	Salt solution, 1.0
Incubate two hours at 37° C.						
Make observations first; then set aside in cold for eighteen to twenty-four hours and take final reading.						
No hemolysis if syphilitic.	No hemolysis.	Hemolysis.	Hemolysis.	Hemolysis.	Hemolysis.	No hemolysis.

[1] The complement is a 1:10 dilution of guinea-pig serum.
[2] This is the antigen diluted 1:10.
[3] The serum of rabbit which has been immunized to sheep's blood-corpuscles is diluted 1:1000 and constitutes the amboceptor.
[4] A 5 per cent. suspension of washed lamb's corpuscles.

antigen alone is not anticomplementary. Tube No. 7 is introduced to show that hemolysis will not occur in the absence of the complement.

Modifications.—Certain modifications of this test have been suggested, chief of which is the **Noguchi test.** This differs from the Wassermann mainly in that an antihuman hemolytic system is used instead of an antisheep, because, according to the author, there is an appreciable error in the Wassermann in that there is present "in human serum a variable amount of natural antisheep amboceptor" capable of so changing the results that with sera containing only a small amount of syphilitic antibody the result will be negative.

The following reagents are used:

1. Antihuman hemolytic amboceptor prepared by repeated injections of a rabbit with washed human blood-corpuscles.
2. Complement. Fresh guinea-pig serum.
3. Antigen. Organ extracts or a solution of lecithin.
4. 1 per cent. suspension of human blood-corpuscles.
5. The suspected serum.
6. A known syphilitic serum.
7. A serum known not to contain syphilitic antibodies.

With these reagents the procedure is very little different from that outlined for the Wassermann test.

Noguchi has further simplified it for the small laboratory by drying the amboceptor serum on slips of filter-paper, which can be kept a considerable time. The same procedure can be carried out with the antigen. While at first similar complement slips were prepared, it is now known that fresh complement is indispensable.

Value of Wassermann Test.—The reaction is positive in 95 to 98 per cent. of all cases with syphilitic manifestations. In the late cases only a very slight inhibition of hemolysis may be noted. This has given rise to considerable difficulty in the interpretation of results. Kaplan states that the report should read "negative" or "positive," with no report of the degree of inhibition.

Butler obtains the following results:

	No. of cases.	Per cent. positive.
Controls, non-syphilitic	53	0
Primary syphilitic	10	100
Secondary syphilitic	36	95
Tertiary syphiltic	31	94
Latent cases	16	56
Parasyphilis and visceral syphilis	55	76
Total cases	201	

Kaplan, in a study of diseases of the nervous system, obtained the following results: In 249 cases of quiescent tabes the Wassermann reaction was positive in 44 per cent.; in 57 cases of active tabes, 88 per cent., and in 64 cases of general paresis, 88 per cent.

By the Noguchi method about 7 per cent. of non-syphilitic sera will cause inhibition of hemolysis, while with the Wassermann, in about 9 per cent. of known syphilitic sera, hemolysis will occur. For this reason in doubtful cases it is well to apply both methods.

It is probable that a positive reaction almost always means active syphilis even without manifestations, but it is not absolutely specific for syphilis, for the reaction has been obtained in leprosy. Kaplan states that "old leprosy cases present a much more definitely positive reaction than cases of old syphilis." Many workers believe that a positive reaction in a late case may only

indicate that the patient has once had syphilis. Against this view stands the fact that in other infectious diseases antibodies diminish or entirely disappear a few months after the active infection, and that in latent cases the reaction may disappear under treatment. On the other hand, there are certain cases which are considered clinically as cured, and have remained so for years, that will continue to give the positive reaction in spite of any treatment.

In the application of the test to the diagnosis of diseases of the nervous system and viscera one should always bear in mind the possibility of a dual pathologic process, and a positive test should not be allowed to entirely overshadow the clinical findings.

Effect of Treatment.—The positive reaction frequently disappears after a short course of treatment with mercury. This may be permanent, or, after a variable length of time, the reaction may return. Some cases thoroughly treated persist in giving a positive reaction. In hereditary syphilis it is often impossible to get rid of the reaction. Because the reaction may return, it is always safer to make several tests before deciding that further treatment is not indicated. This suggests that while a positive reaction may be accepted as an indication of syphilis, a negative reaction obtained after treatment may not exclude syphilis.

Following treatment with salvarsan ("606"), Noguchi, in a total of 102 cases, finds that the positive reaction becomes negative in 33.7 per cent.

X. TESTS FOR RECOGNITION OF BLOOD

1. Guaiac Test.—The technic of this test has been given (p. 125). It may be applied directly to a suspected fluid or, better, to the ethereal extract. Add a few cubic centimeters of glacial acetic acid to about 10 c.c. of the fluid; shake thoroughly with an equal volume of ether; decant, and apply the test to the ether. In case of dried stains upon cloth, wood, etc., dissolve the stain in distilled water and test the water, or press a piece of moist blotting-paper against the stain, and touch the paper with drops of the guaiac and the turpentine successively.

2. Teichmann's Test.—This depends upon the production of characteristic crystals of *hemin*. It is a sensi-

Fig. 103.—Teichmann's hemin crystals (Jakob).

tive test and, when positive, is absolute proof of the presence of blood. A number of substances—lime, fine sand, iron rust—interfere with production of the crystals; hence negative results are not always conclusive. Dissolve the suspected stain in a few drops of normal

salt solution upon a slide. If a liquid is to be tested, evaporate some of it upon a slide and dissolve the residue in a few drops of the salt solution. Let dry, apply a cover-glass, and run glacial acetic acid underneath it. Heat *very gently* until bubbles begin to form, replacing the acid as it evaporates. Allow to cool slowly. When cool, replace the acid with water, and examine for hemin crystals with 16 mm. and 4 mm. objectives. The crystals are dark-brown rhombic plates, lying singly or in crosses, and easily recognized (Fig. 103). Failure to obtain them may be due to too great heat or too rapid cooling. If not obtained at first let the slide stand in a warm place, as upon a hot-water radiator, for an hour.

XI. SPECIAL BLOOD PATHOLOGY

The more conspicuous characteristics of the blood in various diseases have been mentioned in previous sections. Although the great majority of blood changes are secondary, there are a few blood conditions in which the changes are so prominent, or the etiology so obscure, that they are commonly regarded as blood diseases. These will receive brief consideration here.

A. Anemia

This is a deficiency of hemoglobin, or red corpuscles, or both. It is either primary or secondary. The distinction is based chiefly upon etiology, although each type presents a more or less distinctive blood-picture. Secondary anemia is that which is symptomatic of some other pathologic condition. Primary anemia is that which progresses without apparent cause.

1. Secondary Anemia.—The more important conditions which produce secondary or symptomatic anemia are:

(*a*) *Poor nutrition*, which usually accompanies unsanitary conditions, poor and insufficient food, etc.

(*b*) *Acute infectious diseases*, especially rheumatism and typhoid fever. The anemia is more conspicuous during convalescence.

(*c*) *Chronic Infectious Diseases.*—Tuberculosis, malaria, syphilis, leprosy.

(*d*) *Chronic exhausting diseases*, as heart disease, chronic nephritis, cirrhosis of the liver, and gastro-intestinal diseases, especially when associated with atrophy of gastric and duodenal glands. The last may give an extreme anemia, indistinguishable from pernicious anemia.

(*e*) *Chronic poisoning*, as from lead, arsenic, and phosphorus.

(*f*) *Hemorrhage.*—Either repeated small hemorrhages, as from gastric cancer and ulcer, uterine fibroids, etc., or a single large one.

(*g*) *Malignant Tumors.*—These affect the blood partly through repeated small hemorrhages, partly through toxic products, and partly through interference with nutrition.

(*h*) *Animal Parasites.*—Some cause no appreciable change in the blood; others, like the hookworm and *Dibothriocephalus latus*, may produce a very severe anemia, almost identical with pernicious anemia. Anemia in these cases is probably due both to toxins and to abstraction of blood.

The blood-picture varies with the grade of anemia.

Diminution of hemoglobin is the most characteristic feature. In mild cases it is slight, and is the only blood change to be noted. In very severe cases hemoglobin may fall to 15 per cent. Red corpuscles are diminished in all but very mild cases, while in the severest cases the red corpuscle count is sometimes below 2,000,000. The color-index is usually decreased.

Although the number of leukocytes bears no relation to the anemia, leukocytosis is common, being due to the same cause.

Stained films show no changes in very mild cases. In moderate cases variations in size and shape of the red cells and polychromatophilia occur. Very severe cases show the same changes to greater degree, with addition of basophilic degeneration and the presence of normoblasts in small or moderate numbers. Megaloblasts in very small numbers have been encountered in extremely severe cases. They are especially abundant and may even predominate over the normoblasts in dibothriocephalus infection. Blood-plaques are usually increased.

2. Primary Anemia.—The commonly described varieties of primary anemia are pernicious anemia and chlorosis, but splenic anemia may also be mentioned under this head.

(1) **Progressive Pernicious Anemia.**—It is frequently impossible to diagnose this disease from the blood examination alone. Severe secondary anemia sometimes gives an identical picture. Remissions, in which the blood approaches the normal, are common. All the clinical data must, therefore, be considered.

Hemoglobin and red corpuscles are always greatly

diminished. In none of Cabot's 139 cases did the count exceed 2,500,000, the average being about 1,200,000. In more than two-thirds of the cases hemoglobin was reduced to less extent than the red corpuscles; the color-index was, therefore, high. A low color-index probably indicates a mild type of the disease.

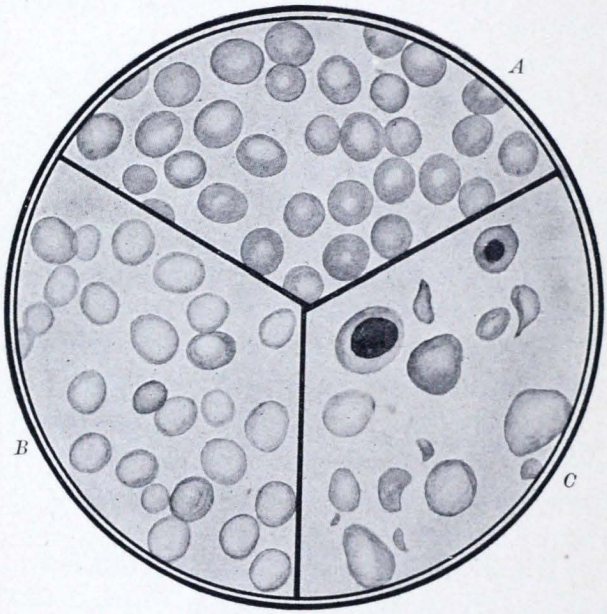


Fig. 104.—*A*, Normal blood; *B*, chlorosis; *C*, pernicious anemia. The plate shows the sharp contrast between cells rich in hemoglobin and the pale cells of chlorosis, and also the poikilocytes and marked variations in size noted in pernicious anemia. A normoblast and megaloblast also appear. Stained smears (from Greene's "Medical Diagnosis").

The leukocyte count may be normal, but is commonly diminished to about 3000. The decrease affects chiefly the polymorphonuclear cells, so that the lymphocytes are relatively increased. In some cases a decided absolute increase of lymphocytes occurs. Polymorphonuclear leukocytosis, when present, is due to some complication.

Explanation of Plate IX

Fig. 1.—Preparation from an advanced case of progressive pernicious anæmia from unknown cause: *a*, Megaloblasts or gigantoblasts; the protoplasm shows marked polychromasia; *b*, stained granules in erythrocytes with normally stained protoplasm; *c* and *d*, polychromatophilic degeneration; *e*, megalocytes; *f*, normocytes.

Fig. 2.—Preparation from the same case taken some time later while the patient was subjectively and objectively in perfect health: *a*, Punctate erythrocytes with normal and anæmic degenerated protoplasm; *b*, polynuclear leukocyte; *c*, normal red blood-corpuscles; *d*, somewhat enlarged erythrocytes.

Fig. 3.—Series of cells from a case of severe progressive pernicious anæmia of unknown etiology; preparation made two days antemortem: *a*, Nucleated red blood-corpuscles characterized as normoblasts by the intense staining of the nuclei; *a'* and *a''*, karyokinetic figures in erythrocytes; the protoplasm finely punctate; *b*, beginning karyolysis in a megaloblast; *c*, erythroblasts with coarse granulation of the protoplasm; *d*, nuclear remains (?) and fine granulation of the protoplasm; *e* and *f*, finely punctate red blood-corpuscles; *g*, megalocyte with two blue nuclei; nuclear remains (?) in the polychromatophilic protoplasm.

(Nothnagel-Lazarus.)

Explanation of Plate IX

Fig. 1.—Preparation from an advanced case of progressive pernicious anemia from unknown cause: *a*, Megaloblasts or gigantoblasts; the protoplasm shows marked polychromasia; *b*, stained granules in erythrocytes with normally stained protoplasm; *c* and *d*, polychromatophilic degeneration; *e*, megalocytes; *f*, normocytes.

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(Nothnagel-Lazarus.)

PLATE IX

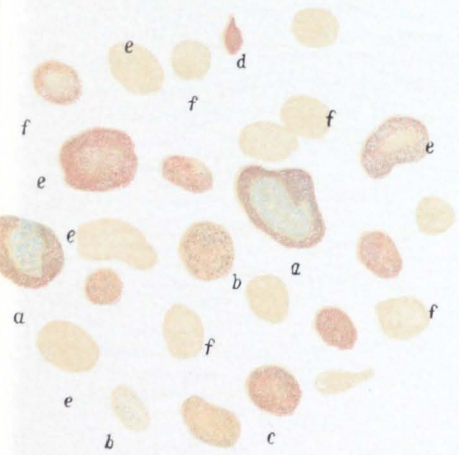


Fig. 1.

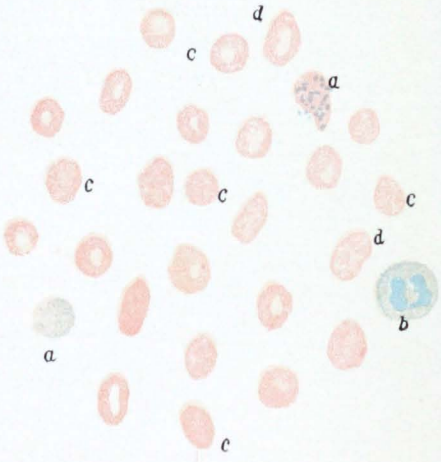


Fig. 2.

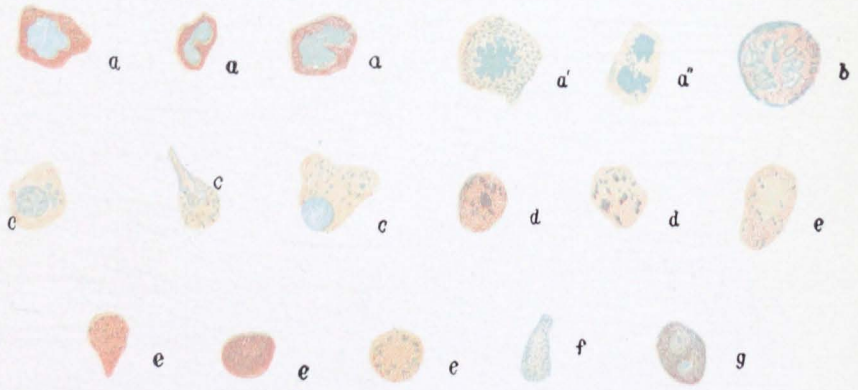


Fig. 3.

The red corpuscles show marked variation in size and shape (Plate IX and Fig. 104). There is a decided tendency to large oval forms, and despite the abundance of microcytes, the average size of the corpuscles is generally strikingly increased. Polychromatophilia and basophilic degeneration are common. Nucleated red cells are always present, although *in many instances careful search is required to find them.* In the great majority of cases megaloblasts exceed normoblasts in number. This ratio constitutes one of the most important points in diagnosis, since it is practically unknown in other diseases. Blood-plaques are diminished.

The rare and rapidly fatal anemia which has been described under the name of **aplastic anemia** is probably a variety of pernicious anemia. Absence of any attempt at blood regeneration explains the marked difference in the blood-picture. Red corpuscles and hemoglobin are rapidly diminished to an extreme degree. The color-index is normal or low. The leukocyte count is normal or low, with relative increase of lymphocytes. Stained smears show only slight variations in size, shape, and staining properties of the red cells. There are no megaloblasts and few or no normoblasts.

(2) **Chlorosis.**—The clinical symptoms furnish the most important data for diagnosis. The blood resembles that of secondary anemia in many respects.

The most conspicuous feature is a decided decrease of hemoglobin (down to 30 or 40 per cent. in marked cases), accompanied by a slight decrease in number of red corpuscles. The color-index is thus almost invariably low, the average being about 0.5.

As in pernicious anemia, the leukocytes are normal or

decreased in number, with a relative increase of lymphocytes.

In contrast to pernicious anemia (and in some degree also to secondary anemia) the red cells are of nearly uniform size, are uniformly pale (Fig. 104), and their average diameter is somewhat less than normal. Changes in size, shape, and staining reactions occur only in severe cases. Erythroblasts are rarely present. The number of plaques is generally decreased.

(3) **Splenic Anemia.**—This is an obscure form of anemia associated with great enlargement of the spleen. It is probably a distinct entity. There is decided decrease of hemoglobin and red corpuscles, with moderate leukopenia and relative lymphocytosis. Osler's 15 cases averaged 47 per cent. hemoglobin and 3,336,357 red cells. Stained films show notable irregularities in size, shape, and staining properties only in advanced cases. Erythroblasts are uncommon.

B. Leukemia

Except in rare instances, diagnosis is easily made from the blood alone. Two types of the disease are commonly distinguished: the *myelogenous* and the *lymphatic*. Atypical and intermediate forms are not uncommon. Pseudoleukemia, because of its clinical similarity to lymphatic leukemia, is generally described along with leukemia.

1. Myelogenous Leukemia (Plate X).—This is usually a chronic disease, although acute cases have been described.

Hemoglobin and red corpuscles show decided decrease. The color-index is moderately low.

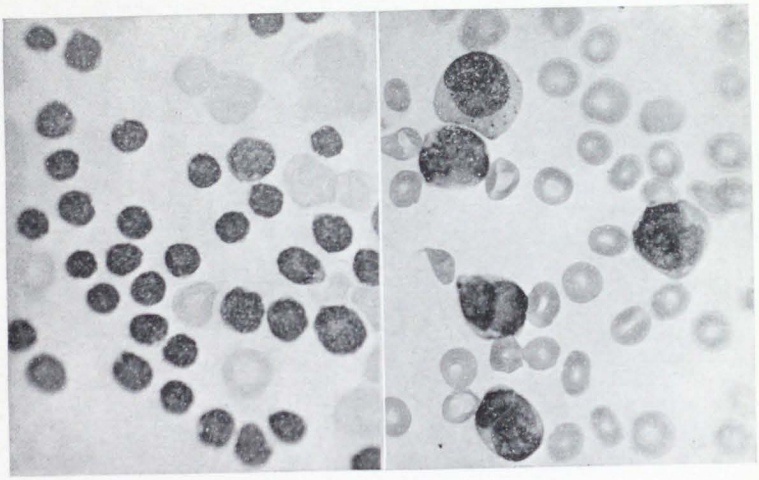


Fig. 1.—Blood in lymphatic leukemia; × 700. On the left, chronic form of the disease; on the right, acute form (courtesy of Dr. W. P. Harlow).

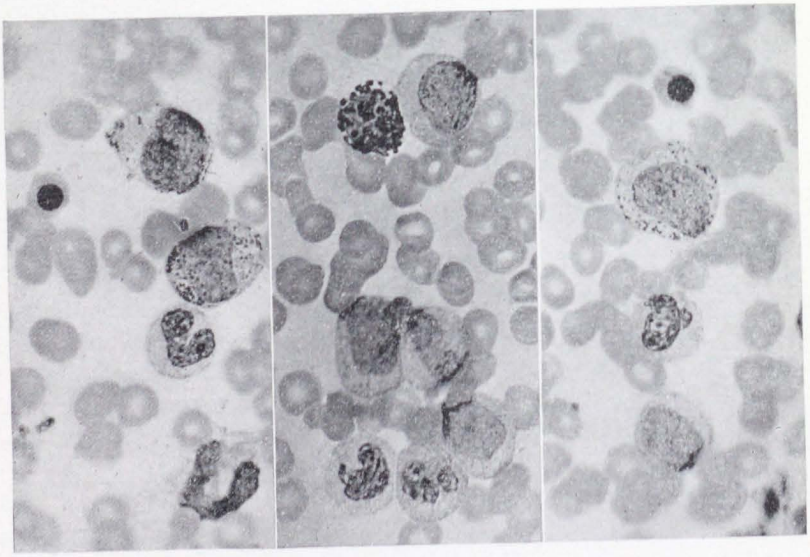


Fig. 2.—Blood in splenomyelogenous leukemia. Wright's stain. × 700 (photographs by the author).

Most striking is the immense increase in number of leukocytes. The count in ordinary cases varies between 100,000 and 300,000. Counts over 1,000,000 have been met. During remissions, the leukocyte count may fall to normal.

While these enormous leukocyte counts are equaled in no other disease, and approached only in lymphatic leukemia and extremely high-grade leukocytosis, the diagnosis, particularly during remissions, depends more upon qualitative than quantitative changes. Although all varieties are increased, the characteristic and conspicuous cell is the myelocyte. This cell never appears in normal blood; extremely rarely in leukocytosis; and never abundantly in lymphatic leukemia. In myelogenous leukemia myelocytes usually constitute more than 20 per cent. of all leukocytes. Da Costa's lowest case gave 7 per cent. The neutrophilic form is generally much more abundant than the eosinophilic. Both show considerable variations in size. Very constant also is a marked absolute, and often a relative, increase of eosinophiles and basophiles. Polymorphonuclear neutrophiles and lymphocytes are relatively decreased.

The red cells show the changes characteristic of a severe secondary anemia, except that nucleated reds are commonly abundant; in fact, no other disease gives so many. They are chiefly of the normoblastic type. Megaloblasts are uncommon. Blood-plaques are generally increased.

2. Lymphatic Leukemia (Plate X).—This form may be either acute or chronic. There is marked loss of hemoglobin and red corpuscles. The color-index is usually moderately low.

The leukocyte count is high, but lower than in the myelogenous type. Counts of 100,000 are about the average, but in many cases are much lower. This high count is referable almost wholly to increase of lymphocytes. They generally exceed 90 per cent. of the total number. In chronic cases they are chiefly of the small variety; in acute cases, of the large form. Myelocytes are rare.

The red corpuscles show the changes usual in severe secondary anemia. Erythroblasts are seldom abundant. Blood-plaques are decreased.

3. Pseudoleukemia (**Hodgkin's disease**) resembles lymphatic leukemia in that there is marked and progressive enlargement of the lymph-nodes. There is, however, no distinctive blood-picture. The changes in hemoglobin and red cells resemble those of a moderate symptomatic anemia, with rather low color-index. The leukocytes are commonly normal in number and relative proportions.

4. Anæmia Infantum Pseudoleukæmica.—Under this name von Jaksch described a rare disease of infancy, the proper classification of which is uncertain. There is enlargement of liver and spleen, and sometimes of lymph-nodes, together with the following blood changes: grave anemia with deformed and degenerated red cells and many erythroblasts of both normoblastic and megaloblastic types; great increase in number of leukocytes (20,000 to 100,000) and great variations in size, shape, and staining of leukocytes, with many atypic forms, and a few myelocytes.

The table on the following page contrasts the distinctive blood-changes in the more common conditions.

DIFFERENTIAL DIAGNOSIS OF BLOOD DISEASES

	Hemoglobin.	Red Corpuscle Count.	Color-index.	Leukocyte Count.	Stained Films. Red Corpuscles.	Stained Films. Leukocytes.
Secondary anemia.	Diminished according to degree of anemia.	Normal in mild cases; diminished in all others.	Normal or slightly diminished.	Not necessarily affected; leukocytosis common.	Variations in size and shape in moderate cases; variations in staining reactions and normoblasts in severe cases.	Normal proportions or increase of polynuclears.
Pernicious anemia.	Diminished	Greatly diminished.	High.	Normal or diminished.	Marked variations in size, shape, and staining reactions. Average size increased. Tendency to large oval forms. Erythroblasts always present; megaloblasts exceed normoblasts.	Lymphocytes relatively, sometimes absolutely, increased.
Chlorosis.	Greatly diminished.	Slightly diminished.	Low.	Normal or diminished.	Nearly uniform size and shape; average size decreased; pale centers. Erythroblasts very rare.	Lymphocytes apt to be relatively increased.
Myelogenous leukemia.	Decidedly diminished.	Decidedly diminished.	Usually slightly diminished.	Extremely high.	Similar to secondary anemia, except normoblasts generally very numerous.	Large numbers of myelocytes (average, 20 per cent.). Absolute increase of eosinophiles and basophiles. Relative decrease of polynuclears and lymphocytes.
Lymphatic leukemia.	Markedly diminished.	Markedly diminished.	Usually slightly diminished.	Very high.	Similar to secondary anemia. Erythroblasts not numerous.	Lymphocytes exceed 90 per cent. Other varieties relatively decreased.

CHAPTER IV

THE STOMACH

LABORATORY methods may be applied to the diagnosis of stomach disorders in: I. Examination of the gastric contents removed with the stomach-tube. II. Certain other examinations which give information as to the condition of the stomach.

I. EXAMINATION OF THE GASTRIC CONTENTS

Stomach digestion consists mainly in the action of pepsin upon proteins in the presence of hydrochloric acid and in the curdling of milk by rennin. The fat-splitting ferment, lipase, of the gastric juice has very little activity under normal conditions of acidity.

Pepsin and rennin are secreted by the gastric glands as zymogens—pepsinogen and renninogen respectively—which are converted into pepsin and rennin by hydrochloric acid. Hydrochloric acid is secreted by certain cells of the fundus glands. It at once combines loosely with the proteins of the food, forming acid-metaprotein, the first step in protein digestion. Hydrochloric acid, which is thus loosely combined with proteins, is called "combined" hydrochloric acid. The acid which is secreted after the proteins present have all been converted into acid-metaprotein remains as "free" hydrochloric acid, and, together with pepsin, continues the process of digestion.

At the height of digestion the stomach-contents consist essentially of: (1) Water; (2) free hydrochloric acid; (3) combined hydrochloric acid; (4) pepsin; (5) rennin; (6) mineral salts, chiefly acid phosphates, of no clinical importance; (7) particles of undigested and partly digested food; (8) various products of digestion in solution. In pathologic conditions there may be present, in addition, various microscopic structures and certain organic acids, of which lactic acid is most important.

A **routine examination** is conveniently carried out in the following order:

(1) Give the patient a test-meal upon an empty stomach, washing the stomach previously if necessary.

(2) At the height of digestion, usually in one hour, remove the contents of the stomach with a stomach-tube.

(3) Measure and examine macroscopically.

(4) Filter. A suction filter is desirable, and may be necessary when much mucus is present.

(5) During filtration, examine microscopically and make qualitative tests for—(*a*) free acids; (*b*) free hydrochloric acid; (*c*) lactic acid.

(6) When sufficient filtrate is obtained, make quantitative estimations of—(*a*) total acidity; (*b*) free hydrochloric acid; (*c*) combined hydrochloric acid (if necessary).

(7) Make whatever additional tests seem desirable, as for blood, pepsin, or rennin.

A. Obtaining the Contents

Gastric juice is secreted continuously, but quantities sufficiently large for examination are not usually obtainable from the fasting stomach. In clinical work, therefore, it is desirable to stimulate secretion with food—

which is the natural and most efficient stimulus—before attempting to collect the gastric fluid. Different foods stimulate secretion to different degrees, hence for the sake of uniform results certain standard "test-meals" have been adopted. Those mentioned here give practically the same results.

1. Test-meals.—It is customary to give the test-meal in the morning, since the stomach is most apt to be empty at that time. If it be suspected that the stomach will not be empty, it should be washed out with water the evening before.

(1) **Ewald's test-breakfast** consists of a roll (or two slices of bread), without butter, and two small cups (300 to 400 c.c.) of water, or weak tea, without cream or sugar. It should be well masticated. The contents of the stomach are to be removed one hour afterward, counting from the beginning, not the end of the meal. This test-meal has long been used for routine examinations. Its disadvantage is that it introduces, with the bread, a variable amount of lactic acid and numerous yeast-cells. This source of error may be eliminated by substituting a shredded whole-wheat biscuit for the roll. The shredded wheat test-meal is now widely used and is probably the most satisfactory for general purposes.

(2) **Boas' test-breakfast** consists of a tablespoonful of rolled oats in a quart of water, boiled to one pint, with a pinch of salt added. It should be withdrawn in forty-five minutes to one hour. This meal does not contain lactic acid, and is usually given when the detection of lactic acid is important, as in suspected gastric cancer. The stomach should always be washed with water the evening previous.

2. Withdrawal of the Contents.—The Boas stomach-tube, with bulb, is probably the most satisfactory form. It should be of rather large caliber, and have an opening in the tip and one or two in the side near the tip. When not in use it should be kept in a vessel of borax solution, and should be well washed in hot water both before and after using.

It is important confidently to assure the patient that introduction of the tube cannot possibly harm him; and that, if he can control the spasm of his throat, he will experience very little choking sensation. When patients are very nervous it is well to spray the throat with cocain solution.

The tube should be dipped in warm water just before using: the use of glycerin or other lubricant is undesirable. With the patient seated upon a chair, his clothing protected by towels or a large apron, and his head tilted forward, the tip of the tube, held as one would a pen, is introduced far back into the pharynx. He is then urged to swallow, and the tube is pushed boldly into the esophagus until the ring upon it reaches the incisor teeth, thus indicating that the tip is in the stomach. If, now, the patient cough or strain as if at stool, the contents of the stomach will usually be forced out through the tube. Should it fail, the fluid can generally be pumped out by alternate compression of the tube and the bulb. If unsuccessful at first, the attempts should be repeated with the tube pushed a little further in, or withdrawn a few inches, since the distance to the stomach is not the same in all cases. The tube may become clogged with pieces of food, in which case it must be withdrawn, cleaned, and reintroduced. If, after all efforts, no fluid

is obtained, another test-meal should be given and withdrawn in forty-five minutes.

As the tube is removed, it should be pinched between the fingers so as to save any fluid that may be in it.

The stomach-tube must be used with great care, or not at all, in cases of gastric ulcer, aneurysm, uncompensated heart disease, and marked arteriosclerosis. Except in gastric ulcer, the danger lies in the retching produced, and the tube can safely be used if the patient takes it easily.

B. Physical Examination

Under normal conditions 30 to 50 c.c. of fluid can be obtained one hour after administering Ewald's breakfast. More than 60 c.c. points to motor insufficiency; less than 20 c.c., to too rapid emptying of the stomach, or else to incomplete removal. Upon standing, it separates into two layers, the lower consisting of particles of food, the upper of an almost clear, faintly yellow fluid. The extent to which digestion has taken place can be roughly judged from the appearance of the food-particles.

The *reaction* is frankly acid in health and in nearly all pathologic conditions. It may be neutral or slightly alkaline in some cases of gastric cancer and marked chronic gastritis, or when contaminated by a considerable amount of saliva.

A small amount of *mucus* is present normally. Large amounts, when the gastric contents are obtained with the tube and not vomited, point to chronic gastritis. Mucus is recognized from its characteristic slimy appearance when the fluid is poured from one vessel into another. It is more frequently seen in stomach washings than in the fluid removed after a test-meal.

A trace of *bile* may be present as a result of excessive straining while the tube is in the stomach. Large amounts are very rarely found, and generally point to obstruction in the duodenum. Bile produces a yellowish or greenish discoloration of the fluid.

Blood is often recognized by simple inspection, but more frequently requires a chemic test. It is bright red when very fresh, and dark, resembling coffee-grounds, when older. Vomiting of blood, or *hematemesis*, may be mistaken for pulmonary hemorrhage, or *hemoptysis*. In the former the fluid is acid in reaction and usually dark red or brown in color and clotted, while in hemoptysis it is brighter red, frothy, alkaline, and usually mixed with a variable amount of mucus.

Particles of food eaten hours or even days previously may be found, and indicate deficient motor power.

Search should always be made for *bits of tissue* from the gastric mucous membrane or new growths. These, when examined by a pathologist, will sometimes render the diagnosis clear.

C. Chemic Examination

A routine chemic examination of the gastric contents involves qualitative tests for free acids, free hydrochloric acid, and organic acids, and quantitative estimations of total acidity, free hydrochloric acid, and sometimes combined hydrochloric acid. Other tests are applied when indicated.

1. Qualitative Tests.—(1) **Free Acids.**—The presence or absence of free acids, without reference to the kind, is easily determined by means of Congo-red, although the test is not much used in practice.

Congo-red Test.—Take a few drops of a strong alcoholic solution of Congo-red in a test-tube, dilute with water to a strong red color, and add a few cubic centimeters of filtered gastric juice. The appearance of a *blue color* shows the presence of some free acid (Plate XI, B, B′). Since the test is more sensitive to mineral than to organic acids, a marked reaction points to the presence of free hydrochloric acid.

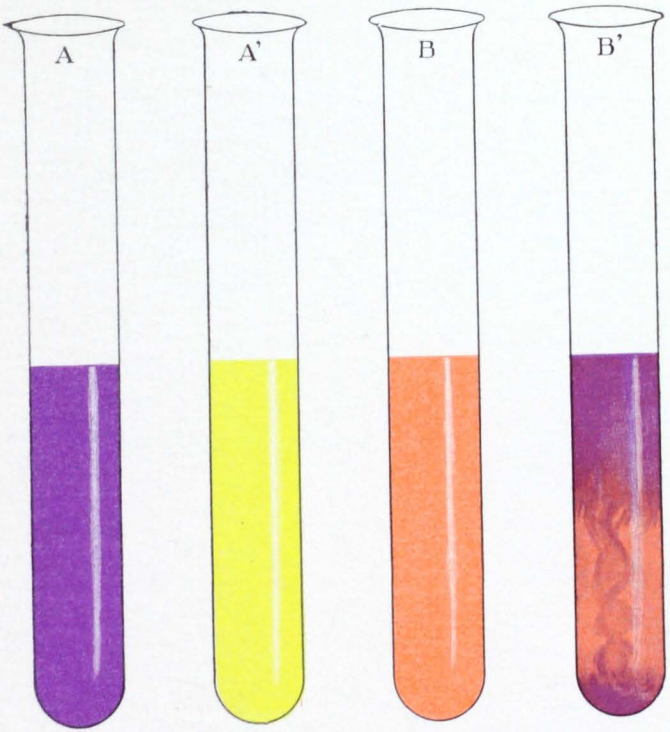
Thick filter-paper soaked in Congo-red solution, dried, and cut into strips may be used, but the test is much less delicate when thus applied.

(2) **Free Hydrochloric Acid.**—In addition to its digestive function, free hydrochloric acid is an efficient antiseptic. It prevents or retards fermentation and lactic-acid formation, and is an important means of protection against the entrance of pathogenic organisms into the body. It is never absent in health.

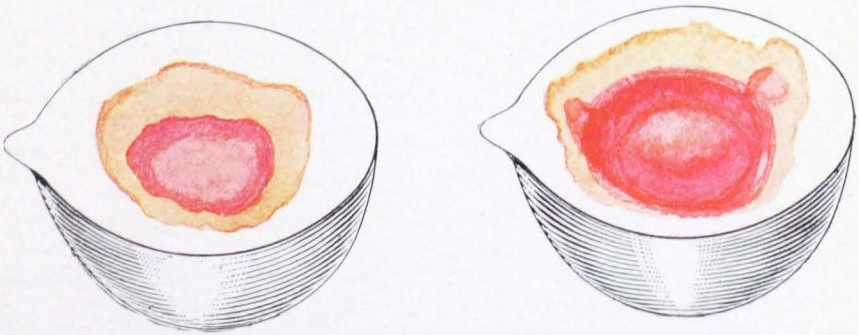
Amidobenzol Test.—To a little of the filtered gastric juice in a test-tube, or to several drops in a porcelain dish, add a drop of 0.5 per cent. alcoholic solution of dimethylamido-azobenzol. In the presence of free hydrochloric acid there will at once appear a *cherry-red color*, varying in intensity with the amount of acid (Plate XII, C). This test is very delicate; but, unfortunately, organic acids, when present in large amounts (above 0.5 per cent.), give a similar reaction.

Boas' Test.—This test is less delicate than the preceding, but is more reliable, since it reacts only to free hydrochloric acid.

In a porcelain dish mix a few drops of the gastric juice and the reagent, and slowly evaporate to dryness over a flame, *taking care not to scorch.* The appearance of a *rose-red color*, which fades upon cooling, shows the presence of free hydrochloric acid (Plate XI, 1).



A, Uffelmann's reagent; A', A after the addition of gastric fluid containing lactic acid; B, water to which three drops of Congo-red solution have been added; B', change induced in B when gastric fluid containing free hydrochloric acid is added (Boston).



1, Resorcin-test for free hydrochloric acid; 2, Günzburg's test for hydrochloric acid (Boston).

Boas' reagent consists of 5 gm. resublimed resorcinol, and 3 gm. cane-sugar, in 100 c.c. alcohol. The solution keeps well, which, from the practitioner's view-point, makes it preferable to Günzburg's phloroglucin-vanillin reagent (phloroglucin, 2 gm.; vanillin, 1 gm.; absolute alcohol, 30 c.c.). The latter is just as delicate, is applied in the same way, and gives a sharper reaction (Plate XI, 2), but is unstable.

(3) **Organic Acids.**—Lactic acid is the most common, and is taken as the type of the organic acids which appear in the stomach-contents. It is a product of bacterial activity. Acetic and butyric acids are sometimes present. Their formation is closely connected with that of lactic acid, and they are rarely tested for. When abundant, they may be recognized by their odor upon heating.

Lactic acid is never present at the height of digestion in health. Although usually present early in digestion, it disappears when free hydrochloric acid begins to appear. Small amounts may be introduced with the food. Pathologically, small amounts may be present whenever there is stagnation of the gastric contents with deficient hydrochloric acid, as in many cases of dilatation of the stomach and chronic gastritis. The presence of notable amounts of lactic acid (more than 0.1 per cent. by Strauss' test) is strongly suggestive of gastric cancer, and is probably the most valuable single symptom of the disease.

As already stated, the Ewald test-breakfast introduces a small amount of lactic acid, but rarely enough to respond to the tests given here. In every case, however, in which its detection is important, the shredded-wheat biscuit or Boas' test-breakfast should be given, the

stomach having been thoroughly washed the evening before.

Uffelmann's Test for Lactic Acid.—Thoroughly shake up 5 c.c. of filtered stomach fluid with 50 c.c. of ether for at least ten minutes. Collect the ether and evaporate over a water-bath. Dissolve the residue in 5 c.c. water and test with Uffelmann's reagent as follows:

In a test-tube mix 3 drops concentrated solution of phenol and 3 drops saturated aqueous solution of ferric chlorid. Add water until the mixture assumes an amethyst-blue color. To this add the solution to be tested. The appearance of a *canary-yellow color* indicates the presence of lactic acid (Plate XI, A, A′).

Uffelmann's test may be applied directly to the stomach-contents without extracting with ether, but is then neither sensitive nor reliable, because of the phosphates, sugars, and other interfering substances which may be present.

Kelling's Test (*Simon's Modification*).—This is much more satisfactory than Uffelmann's. To a test-tube of distilled water add sufficient ferric chlorid solution to give a faint yellowish tinge. Pour half of this into a second test-tube to serve as a control. To the other add a small amount of the gastric juice. Lactic acid gives a distinct yellow color which is readily recognized by comparison with the control.

Strauss' Test for Lactic Acid.—This is a good test for clinical work, since it gives a rough idea of the quantity present and is not sufficiently sensitive to respond to the traces of lactic acid which some test-meals introduce. Strauss' instrument (Fig. 105) is essentially a separating funnel with a mark at 5 c.c. and one at 25 c.c. Fill to the 5 c.c. mark with filtered stomach fluid, and to the 25 c.c. mark with ether. Shake thoroughly for ten or fifteen minutes, let stand until the ether separates, and then, by opening the stop-cock, allow the liquid to run out to the 5 c.c. mark. Fill to the 25 c.c. mark

with water, and add two drops of tincture of ferric chlorid diluted 1 : 10. Shake gently. If 0.1 per cent. or more lactic acid be present, the water will assume a strong greenish-yellow color. A slight tinge will appear with 0.05 per cent.

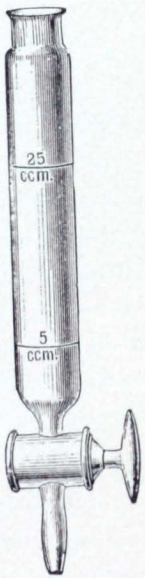


Fig. 105.—Separatory funnel for Strauss' lactic acid test (Sahli).

(4) **Pepsin and Pepsinogen.**—Pepsinogen itself has no digestive power. It is secreted by the gastric glands, and is transformed into pepsin by the action of a free acid. Although pepsin digests proteins best in the presence of free hydrochloric acid, it has a slight digestive activity in the presence of organic or combined hydrochloric acids.

The amount is not influenced by neuroses or circulatory disturbances. Absence or marked diminution, therefore, indicates organic disease of the stomach. It is an important point in diagnosis between functional and organic conditions. Pepsin is rarely or never absent in the presence of free hydrochloric acid.

Test for Pepsin and Pepsinogen.—With a cork-borer cut small cylinders from the coagulated white of an egg, and cut these into discs of uniform size. The egg should be cooked very slowly, preferably over a water-bath, so that the white may be readily digestible. The discs may be preserved in glycerin, but must be washed in water before using.

Place a disc in each of three test-tubes.

Into tube No. 1 put 10 c.c. distilled water, 5 grains pepsin, U. S. P., and 3 drops of the official dilute hydrochloric acid.

Into tube No. 2 put 10 c.c. filtered gastric juice.

Into tube No. 3 put 10 c.c. filtered gastric juice and 3 drops dilute hydrochloric acid.

Place the tubes in an incubator or warm water for three hours or longer. At intervals, observe the extent to which the egg-albumen has been digested. This is recognized by the depth to which the disc has become translucent.

Tube No. 1 is used for comparison, and should show the effect of normal gastric juice.

Digestion of the egg in tube No. 2 indicates the presence of both pepsin and free hydrochloric acid.

When digestion fails in tube No. 2 and occurs in No. 3, pepsinogen is present, having been transformed into pepsin by the hydrochloric acid added. Should digestion fail in this tube, both pepsin and pepsinogen are absent.

(5) **Rennin and Renninogen.**—Rennin is the milk-curdling ferment of the gastric juice. It is derived from renninogen through the action of hydrochloric acid. Lime salts also possess the power of transforming renninogen into the active ferment.

Deficiency of rennin has the same significance as deficiency of pepsin, and is more easily recognized. Since the two enzymes are almost invariably present or absent together, the test for rennin serves also as a test for pepsin.

Test for Rennin.—Neutralize 5 c.c. filtered gastric juice with very dilute sodium hydroxid solution; add 5 c.c. fresh milk, and place in an incubator or in a vessel of water at about 104° F. Coagulation of the milk in ten to fifteen minutes shows a normal amount of rennin. Delayed coagulation denotes a less amount.

Test for Renninogen.—To 5 c.c. neutralized gastric juice add 2 c.c. of 1 per cent. calcium chlorid solution and 5 c.c. fresh milk, and place in an incubator. If coagulation occurs, renninogen is present.

(6) **Blood.**—Blood is present in the vomitus in a great variety of conditions. When found in the fluid removed after a test-meal, it commonly points toward ulcer or carcinoma. Blood can be detected in nearly one-half of the cases of gastric cancer. The presence of swallowed blood must be excluded.

Test for Blood in Stomach-contents.—To 10 c.c. of the fluid add a few cubic centimeters of glacial acetic acid and shake the mixture thoroughly with an equal volume of ether. Separate the ether and apply to it the guaiac test (p. 125); or evaporate and apply the hemin test (p. 274) to the residue. When brown particles are present in the fluid, the hemin test should be applied directly to them.

2. Quantitative Tests.—(1) **Total Acidity.**—The acid-reacting substances which contribute to the total acidity are free hydrochloric acid, combined hydrochloric acid, acid salts, mostly phosphates, and, in some pathologic conditions, the organic acids. The total acidity is normally about 50 to 75 *degrees* (see method below) or, when estimated as hydrochloric acid, about 0.2 to 0.3 *per cent.*

Töpfer's Method for Total Acidity.—In an evaporating dish or small beaker (an " after-dinner " coffee-cup is a very convenient substitute) take 10 c.c. filtered stomach-contents and add three or four drops of the indicator, a 1 per cent. alcoholic solution of phenolphthalein. When the quantity of

stomach fluid is small, 5 c.c. may be used, but results are less accurate than with a larger amount. Add decinormal solution of sodium hydroxid drop by drop from a buret, until the fluid assumes a rose-red color which does not become deeper upon addition of another drop (Plate XII, A, A′). When this point is reached, all the acid has been neutralized. The end reaction will be sharper if the fluid be saturated with sodium chlorid. A sheet of white paper beneath the beaker facilitates recognition of the color change.

In clinical work the amount of acidity is expressed by the number of cubic centimeters of the decinormal sodium hydroxid solution which would be required to neutralize 100 c.c. of the gastric juice, each cubic centimeter representing one *degree* of acidity. Hence multiply the number of cubic centimeters of decinormal solution required to neutralize the 10 c.c. of stomach fluid by ten. This gives the number of degrees of acidity. The amount may be expressed in terms of hydrochloric acid, if one remember that each degree is equivalent to 0.00365 per cent. hydrochloric acid. Some one suggests that this is the number of days in the year, the last figure, 5, indicating the number of decimal places.

Example.—Suppose that 7 c.c. of decinormal solution were required to bring about the end reaction in 10 c.c. gastric juice; then $7 \times 10 = 70$ *degrees* of acidity; and, expressed in terms of hydrochloric acid, $70 \times 0.00365 = 0.255$ *per cent.*

Preparation of decinormal solution is described in textbooks on chemistry. The practitioner will find it best to have them made by a chemist, or to purchase from a chemic supply house. Preparation of an approximately decinormal solution is described on page 436.

(2) **Hydrochloric Acid.**—After the Ewald and Boas test-breakfasts the amount of free hydrochloric acid varies normally between 25 and 50 degrees, or about 0.1

to 0.2 per cent. In disease it may go considerably higher or may be absent altogether.

When the amount of free hydrochloric acid is normal, organic disease of the stomach probably does not exist.

Increase of free hydrochloric acid above 50 degrees (*hyperchlorhydria*) generally indicates a neurosis, but also occurs in most cases of gastric ulcer and beginning chronic gastritis.

Decrease of free hydrochloric acid below 25 degrees (*hypochlorhydria*) occurs in some neuroses, chronic gastritis, early carcinoma, and most conditions associated with general systemic depression. Marked variation in the amount at successive examinations strongly suggests a neurosis. Too low values are often obtained at the first examination, the patient's dread of the introduction of the tube probably inhibiting secretion.

Absence of free hydrochloric acid (*achlorhydria*) occurs in most cases of gastric cancer and far-advanced chronic gastritis, in many cases of pernicious anemia, and sometimes in hysteria and pulmonary tuberculosis.

The presence of free hydrochloric acid presupposes a normal amount of combined hydrochloric acid, hence the combined need not be estimated when the free acid has been found. When, however, free hydrochloric acid is absent, it is important to know whether any acid is secreted, and an estimation of the combined acid then becomes of great value. The normal average after an Ewald breakfast is about 10 to 15 degrees, the quantity depending upon the amount of protein in the test-meal.

Töpfer's Method for Free Hydrochloric Acid.—In a beaker take 10 c.c. filtered stomach fluid and add 4 drops

of the indicator, a 0.5 per cent. alcoholic solution of dimethyl-amido-azobenzol. A red color instantly appears if free hydrochloric acid be present. Add decinormal sodium hydroxid solution, drop by drop from a buret, until the last trace of red just disappears, and a canary-yellow color takes its place (Plate XII, C, C′). Read off the number of cubic centimeters of decinormal solution added, and calculate the degrees, or percentage of free hydrochloric acid, as in Töpfer's method for total acidity.

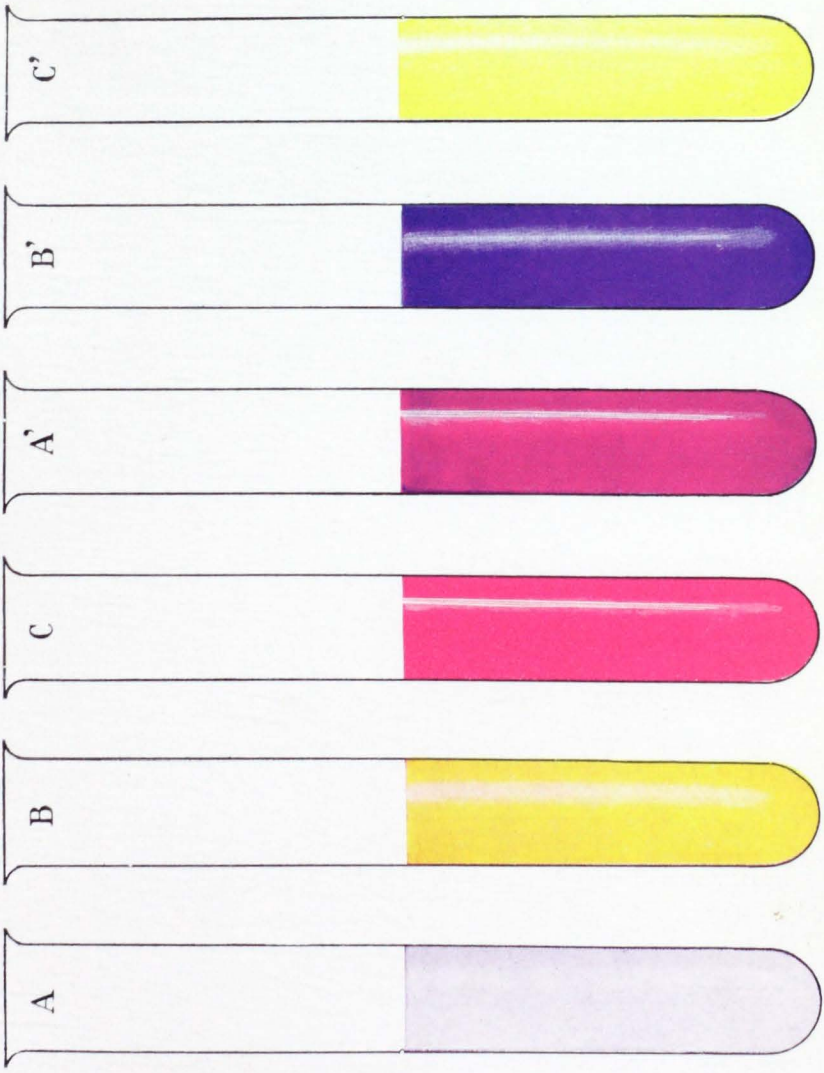
When it is impossible to obtain sufficient fluid for all the tests, it will be found convenient to estimate the free hydrochloric acid and total acidity in the same portion. After finding the free hydrochloric acid as just described, add 4 drops phenolphthalein solution, and continue the titration. The amount of decinormal solution used in both titrations indicates the total acidity.

Töpfer's Method for Combined Hydrochloric Acid.—In a beaker take 10 c.c. filtered gastric juice and add 4 drops of the indicator, a 1 per cent. aqueous solution of sodium alizarin sulphonate. Titrate with decinormal sodium hydroxid until the appearance of a bluish-violet color which does not become deeper upon addition of another drop (Plate XII, B, B′). It is difficult, without practice, to determine when the right color has been reached. A reddish-violet appears first. The shade which denotes the end reaction can be produced by adding 2 or 3 drops of the indicator to 5 c.c. of 1 per cent. sodium carbonate solution.

Calculate the number of cubic centimeters of decinormal solution which would be required for 100 c.c. of stomach fluid. This gives, in degrees, *all the acidity except the combined hydrochloric acid*. The combined hydrochloric acid is then found by deducting this amount from the total acidity, which has been previously determined.

Example.—Suppose that 5 c.c. of decinormal solution were required to produce the purple color in 10 c.c. gastric juice;

PLATE XII



A, Gastric fluid to which a 1 per cent. solution of phenolphthalein has been added; B, gastric fluid to which a 1 per cent. solution of alizarin has been added; C, gastric fluid to which a 0.5 per cent. solution of dimethylamido-azobenzol has been added; A', A after titration with a decinormal solution of sodium hydroxid; B', B after titration with a decinormal solution of sodium hydroxid; C', C after titration with a decinormal solution of sodium hydroxid (Boston).

then $5 \times 10 = 50$ = *all the acidity except combined hydrochloric acid.* Suppose, now, that the total acidity has already been found to be 70 degrees; then $70 - 50 = 20$ *degrees* of combined hydrochloric acid; and $20 \times 0.00365 = 0.073$ *per cent.*

When free hydrochloric acid is absent, it is probably more helpful to estimate the **acid deficit** than the combined hydrochloric acid. The acid deficit shows how far the acid secreted by the stomach falls short of saturating the protein (and bases) of the meal. It represents the amount of hydrochloric acid which must be added to the fluid before a test for free hydrochloric acid can be obtained. It is determined by titrating with $\frac{n}{10}$ hydrochloric acid, using dimethyl-amido-azobenzol as indicator, until the fluid assumes a red color. The amount of deficit is expressed by the number of cubic centimeters of the decinormal solution required for 100 c.c. of the stomach fluid.

(3) **Organic Acids.**—There is no simple direct quantitative method. After the total acidity has been determined, organic acids may be removed from another portion of the gastric filtrate by shaking thoroughly with an equal volume of neutral ether, allowing the fluids to separate, and repeating this process until the gastric fluid has been extracted with eight or ten times its volume of ether. The total acidity is then determined, and the difference between the two determinations indicates the amount of organic acids.

(4) **Pepsin.**—No direct method is avaliable. The following is sufficient for clinical purposes:

Hammerschlag's Method.—To the white of an egg add twelve times its volume of 0.4 per cent. hydrochloric acid

(dilute hydrochloric acid, U. S. P., 4 c.c.; water, 96 c.c.), mix well, and filter. This gives a 1 per cent. egg-albumen solution. Take 10 c.c. of this solution in each of three tubes or beakers. To *A* add 5 c.c. gastric juice; to *B*, 5 c.c. water with 0.5 gm. pepsin; to *C*, 5 c.c. water only. Place in an incubator for an hour and then determine the amount of albumin in each mixture by Esbach's method. Tube *C* shows the amount of albumin in the test-solution. The difference between *C* and *B* indicates the amount of albumin which would be digested by normal gastric juice. The difference between *C* and *A* gives the albumin which is digested by the fluid under examination. Schütz has shown that the amounts of pepsin in two fluids are proportionate to the squares of the products of digestion. Thus, if the amounts of albumin digested in tubes *A* and *B* are to each other as 2 is to 4, the amounts of pepsin are to each other as 4 is to 16.

Certain sources of error can be eliminated by diluting the gastric juice several times before testing. The most important of these are that the law of Schütz holds good only for comparatively dilute solutions, and that the products of peptic activity inhibit digestion.

Mett's method is generally preferred to the preceding. Put three or four Mett's tubes about 2 cm. long into a small beaker with diluted gastric juice (1 c.c. of the filtrate plus 15 c.c. twentieth-normal hydrochloric acid). Place in an incubator for twenty-four hours, and then measure as accurately as possible the column which has been digested, using a millimeter scale and a hand lens or, better, a low power of the microscope and an eye-piece micrometer. Square the average length of this column (law of Schütz) and multiply by the degree of dilution, 16. The maximum figure obtained in this way is 256, representing a digested column of 4 mm.

Prepare Mett's tubes as follows:

Beat up slightly the whites of one or two eggs and filter. Pour into a wide test-tube and stand in this a number of

capillary glass tubes, 1 to 2 mm. in diameter. When the tubes are filled, plug their ends with bread crumbs, and coagulate the albumin by heating in water just short of boiling. Dip the ends of the tube in melted paraffin and preserve until needed. Bubbles, if present, will probably disappear in a few days. When wanted for use, cut the tubes into lengths of about 2 cm. Discard any in which the albumin has separated from the wall.

D. Microscopic Examination

A drop of unfiltered stomach-contents is placed upon a slide, covered with a cover-glass, and examined with the 16 mm. and 4 mm. objectives. A drop of Lugol's

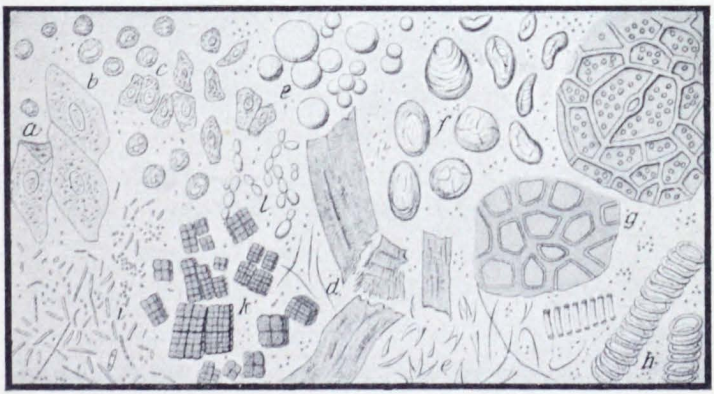


Fig. 106.—General view of the gastric contents: *a*, Squamous epithelial cells from esophagus and mouth; *b*, leukocytes; *c*, cylindric epithelial cells; *d*, muscle-fibers; *e*, fat-droplets and fat-crystals; *f*, starch-granules; *g*, chlorophyl-containing vegetable matters; *h*, vegetable spirals; *i*, bacteria; *k*, sarcinæ; *l*, budding (yeast) fungi (Jakob).

solution allowed to run under the cover will aid in distinguishing the various structures.

Under normal conditions little is to be seen except great numbers of starch-granules, with an occasional

epithelial cell, yeast-cell, or bacterium. Starch-granules are recognized by their concentric striations and the fact that they stain blue with iodin solutions when undigested, and reddish, due to erythrodextrin, when partially digested.

Pathologically, remnants of food from previous meals, red blood-corpuscles, pus-cells, sarcinæ, and excessive numbers of yeast-cells and bacteria may be encountered (Fig. 106).

Remnants of food from previous meals indicate deficient gastric motility.

Red Blood-corpuscles.—Blood is best recognized by the chemic tests already given. The corpuscles sometimes retain a fairly normal appearance, but are generally so degenerated that only granular pigment is left. When only a few fresh looking corpuscles are present, they usually come from irritation of the mucous membrane by the tube.

Pus-cells.—Pus is rarely encountered in the fluid removed after a test-meal. Considerable numbers of pus-corpuscles have been found in some cases of gastric cancer. Swallowed sputum must always be considered.

Sarcinæ.—These are small spheres arranged in cuboid groups, often compared to bales of cotton. They frequently form large clumps and are easily recognized. They stain brown with iodin solution. They signify fermentation. Their presence is strong evidence against the existence of gastric cancer, in which disease they rarely occur.

Yeast-cells.—As already stated, a few yeast-cells may be found under normal conditions. The presence of considerable numbers is evidence of fermentation.

Their appearance has been described (p. 171). They stain brown with iodin solution.

Bacteria.—Numerous bacteria may be encountered, especially in the absence of free hydrochloric acid. The *Boas-Oppler bacillus* is the only one of special significance. It occurs in the majority of cases of cancer, and is rarely found in other conditions. Carcinoma probably furnishes a favorable medium for its growth.

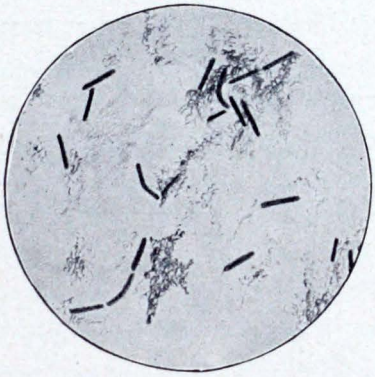


Fig. 107.—Boas-Oppler bacillus from case of gastric cancer (Boston).

These bacilli (Fig. 107) are large (5 to 10 μ long), non-motile, and usually arranged in clumps or end to end in zig-zag chains. They stain brown with iodin solution, which distinguishes them from *Leptothrix buccalis* (p. 377), which is not infrequently found in stomach fluid. They also stain by Gram's method. They are easily seen with the 4 mm. objective in unstained preparations, but are best recognized with the oil lens, after drying some of the fluid upon a cover-glass, fixing, and staining with a simple bacterial stain or by Gram's method.

A few large non-motile bacilli are frequently seen; they cannot be called Boas-Oppler bacilli unless they are numerous and show something of the typical arrangement.

E. The Gastric Contents in Disease

In the diagnosis of stomach disorders the practitioner must be cautioned against relying too much upon examinations of the stomach-contents. A first examination is especially unreliable. Even when repeated examinations are made, the laboratory findings must never be considered apart from the clinical signs.

The more characteristic findings in certain disorders are suggested here.

1. Dilatation of the Stomach.—Evidences of retention and fermentation are the chief characteristics of this condition. Hydrochloric acid is commonly diminished. Pepsin may be normal or slightly diminished. Lactic acid may be detected in small amounts, but is usually absent when the stomach has been washed before giving the test-meal. Both motility and absorptive power are deficient. The microscope commonly shows sarcinæ, bacteria, and great numbers of yeast-cells. Remnants of food from previous meals can be detected with the naked eye or microscopically.

2. Gastric Neuroses.—The findings are variable. Successive examinations may show normal, increased, or diminished hydrochloric acid, or even entire absence of the free acid. Pepsin is usually normal.

In the neurosis characterized by continuous hypersecretion (gastrosuccorrhea), 40 c.c. or more of gastric juice can be obtained from the fasting stomach. Should

the fluid contain food-particles, it is probably the result of retention, not hypersecretion.

3. Chronic Gastritis.—Free hydrochloric acid may be increased in early cases. It is generally diminished in well-marked cases, and is often absent in advanced cases. Lactic acid is often present in traces, rarely in notable amount. Secretion of pepsin and rennin is always diminished in marked cases. Mucus is frequently present, and is very significant of the disease. Motility and absorption are generally deficient. Small fragments of mucous membrane may be found, and when examined by a pathologist, may occasionally establish the diagnosis.

4. Achylia Gastrica (Atrophic Gastritis).—This condition may be a terminal stage of chronic gastritis. It is sometimes associated with the blood-picture of pernicious anemia. It gives a great decrease, and sometimes entire absence of hydrochloric acid and ferments. The total acidity may be as low as 1 or 2 degrees. Small amounts of lactic acid may be present. Absorption and motility are usually not affected greatly.

5. Gastric Carcinoma.—As far as the laboratory examination goes, the cardinal signs of this disease are absence of free hydrochloric acid and presence of lactic acid and of the Boas-Oppler bacillus. These findings are, however, by no means constant.

It is probable that some substance is produced by the cancer which neutralizes the free hydrochloric acid, and thus causes it to disappear earlier than in other organic diseases of the stomach.

The presence of lactic acid is the most suggestive single symptom of gastric cancer. In the great majority of cases its presence in notable amount (0.1 per cent. by

Strauss' method) after Boas' breakfast, the stomach having been washed the evening before, warrants a tentative diagnosis of malignancy.

Carcinoma seems to furnish an especially favorable medium for the growth of the Boas-Oppler bacillus, hence this micro-organism is frequently present.

Blood can be detected in the stomach fluid by the chemic tests in nearly one-half of the cases, and is more common when the new growth is situated at the pylorus. Blood is present in the stool in nearly every case.

Evidences of retention and fermentation are the rule in pyloric cancer. Tumor particles are sometimes found late in the disease.

6. Gastric Ulcer.—There is excess of free hydrochloric acid in about one-half of the cases. In other cases the acid is normal or diminished. Blood is often present. The diagnosis must be based largely upon the clinical symptoms, and where ulcer is strongly suspected, it is probably unwise to use the stomach-tube.

II. ADDITIONAL EXAMINATIONS WHICH GIVE INFORMATION AS TO THE CONDITION OF THE STOMACH

1. Absorptive Power of the Stomach.—This is a very unimportant function, only a few substances being absorbed in the stomach. It is delayed in most organic diseases of the stomach, especially in dilatation and carcinoma, but not in neuroses. The test has little practical value.

Give the patient, upon an empty stomach, a 3-grain capsule of potassium iodid with a glass of water, taking care that none of the drug adheres to the outside of the capsule.

At intervals test the saliva for iodids by moistening starch-paper with it and touching with yellow nitric acid. A blue color shows the presence of an iodid, and appears normally in ten to fifteen minutes after ingestion of the capsule. A longer time denotes delayed absorption.

Starch paper is prepared by soaking filter-paper in boiled starch and drying.

2. Motor Power of the Stomach.—This refers to the rapidity with which the stomach passes its contents on into the intestines. It is very important: intestinal digestion can compensate for insufficient or absent stomach digestion only so long as the motor power is good.

Motility is impaired to some extent in chronic gastritis. It is especially deficient in dilatation of the stomach due to atony of the gastric wall or to pyloric obstruction, either benign or malignant. It is increased in most conditions with hyperchlorhydria.

The best evidence of deficient motor power is the detection of food in the stomach at a time when it should be empty, *e. g.*, before breakfast in the morning. When more than 60 c.c. of fluid are obtained with the tube one hour after a Ewald breakfast, deficient motility may be inferred.

Ewald's salol test is scarcely so reliable as the above. It depends upon the fact that salol is not absorbed until it reaches the intestines and is decomposed by the alkaline intestinal juices.

The patient is given 15 grains of salol with a test-breakfast, and the urine, passed at intervals thereafter, is tested for salicyluric acid. A few drops of 10 per cent. ferric chlorid solution are added to a small quantity of the urine. A violet

color denotes the presence of salicyluric acid. It appears normally in sixty to seventy-five minutes after ingestion of the salol. A longer time indicates impaired motor power.

3. To Determine Size and Position of Stomach.—After removing the test-meal, while the tube is still in place, force quick puffs of air into the stomach by compression of the bulb. The puffs can be clearly heard with a stethoscope over the region of the stomach, and nowhere else.

If desired, the patient may be given a dram of sodium bicarbonate in solution, followed immediately by the same amount of tartaric acid, also in solution; or he may take the two parts of a seidlitz powder separately. The carbon dioxid evolved distends the stomach, and its outline can easily be determined by percussion.

4. Sahli's Desmoid Test of Gastric Digestion.—Two pills, one containing 0.1 gram iodoform, the other 0.05 gram methylene-blue, are wrapped in little bags made of thin sheets of rubber and tied with a string of catgut. The bags must be carefully folded and tied. For detailed directions the reader is referred to Sahli's book, Diagnostic Methods.

The patient swallows the two bags with the aid of a little water during the noon meal, and the urine is tested at intervals thereafter. According to Sahli, the catgut is digested by gastric juice and not by pancreatic or intestinal juices. If gastric digestion is normal, iodin and methylene-blue can be detected in the urine in the afternoon or evening of the same day. The reaction may occur when digestion is very poor, provided gastric motility is diminished, but it is then delayed. If the

reaction does not appear, gastric digestion has not occurred.

Methylene-blue is recognized in the urine by the green or blue color which it imparts. It is sometimes eliminated as a chromogen; and a little of the urine must be acidified with acetic acid and boiled to bring out the color.

To detect the iodin, some of the urine is decolorized by gently heating and filtering through animal charcoal. To 10 c.c. are then added 1 c.c. dilute sulphuric acid, and 0.5 c.c. of a 1 per cent. solution of sodium nitrite and 2 c.c. of chloroform. Upon shaking, a rose color will be imparted to the chloroform if iodin be present.

CHAPTER V

THE FECES

As commonly practised, an examination of the feces is limited to a search for intestinal parasites or their ova. Much of value can, however, be learned from other simple examinations, particularly a careful inspection. Anything approaching a complete analysis is, on the other hand, a waste of time for the clinician.

The normal stool is a mixture of—(*a*) water; (*b*) undigested and indigestible remnants of food, as starch-granules, particles of meat, plant-cells and fibers, etc.; (*c*) digested foods, carried out before absorption could take place; (*d*) products of the digestive tract, as altered bile-pigments, mucus, etc.; (*e*) products of decomposition, as indol, skatol, fatty acids, and various gases; (*f*) epithelial cells shed from the wall of the intestinal canal; (*g*) harmless bacteria, which are always present in enormous numbers.

Pathologically, we may find abnormal amounts of normal constituents, blood, pathogenic bacteria, animal parasites and their ova, and biliary and intestinal concretions.

The stool to be examined should be passed into a clean vessel, without admixture of urine. The offensive odor can be partially overcome with turpentine or 5 per cent. phenol. When search for amebae is to be made, the vessel must be warm, and the stool kept warm until examined; naturally, no disinfectant can be used.

I. MACROSCOPIC EXAMINATION

1. Quantity.—The amount varies greatly with diet and other factors. The average is about 100 to 150 gm. in twenty-four hours.

2. Frequency.—One or two stools in twenty-four hours may be considered normal, yet one in three or four days is not uncommon with healthy persons. The individual habit should be considered in every case.

3. Form and Consistence.—Soft, mushy, or liquid stools follow cathartics and accompany diarrhea. Copious, purely serous discharges without fecal matter are significant of Asiatic cholera, although sometimes observed in other conditions. Hard stools accompany constipation. Rounded scybalous masses are common in habitual constipation, and indicate atony of the muscular coat of the intestines. Flattened, ribbon-like stools result from some obstruction in the rectum, generally a tumor or stricture from a healed ulcer, most commonly syphilitic. When bleeding piles are absent, blood-streaks upon such a stool point to carcinoma.

4. Color.—The normal light or dark-brown color is due chiefly to hydrobilirubin, which is formed from bilirubin by reducing processes in the intestines, largely the result of bacterial activity. The stools of infants are yellow, owing partly to their milk diet and partly to the presence of unchanged bilirubin.

Diet and drugs cause marked changes: milk, a light yellow color; cocoa and chocolate, dark gray; various fruits, reddish or black; iron and bismuth, dark brown or black; hematoxylin, red, etc.

Pathologically, the color is important. A golden yellow

is generally due to unchanged bilirubin. Green stools are not uncommon, especially in diarrheas of childhood. The color is due to biliverdin or, sometimes, to chromogenic bacteria. Putty-colored or "acholic" stools occur when bile is deficient, either from obstruction to outflow or from deficient secretion. The color is due less to absence of bile-pigments than to presence of fat. Similar stools are common in conditions like tuberculous peritonitis, which interfere with absorption of fats, and in pancreatic disease.

Notable amounts of blood produce tarry black stools when the source of the hemorrhage is the stomach or upper intestine, and a dark brown or bright red as the source is nearer the rectum. When diarrhea exists the color may be red, even if the source of the blood is high up. Red streaks of blood upon the outside of the stool are due to lesions of rectum or anus.

5. Odor.—Products of decomposition, chiefly indol and skatol, are responsible for the normal offensive odor. A sour odor is normal for nursing infants, and is noted in mild diarrheas of older children. In the severe diarrheas of childhood a putrid odor is common. In adults, stools emitting a very foul stench are suggestive of malignant or syphilitic ulceration of the rectum or gangrenous dysentery.

6. Mucus.—Excessive quantities of mucus are easily detected with the naked eye, and signify irritation or inflammation. When the mucus is small in amount and intimately mixed with the stool, the trouble is probably in the small intestine. Larger amounts, not well mixed with fecal matter, indicate inflammation of the large intestine. Stools composed almost wholly of mucus and

streaked with blood are the rule in dysentery, ileocolitis, and intussusception.

In the so-called mucous colic or membranous enteritis shreds and ribbons of altered mucus, sometimes representing complete casts of portions of the bowel, are passed. The mucus sometimes takes the form of frog-spawn-like masses. In some cases it is passed at variable intervals, with colic; in others, with every stool, with only vague pains and discomfort. It is distinguished from inflammatory mucus by absence of pus-corpuscles. The condition is not uncommon and should be more frequently recognized. It is probably a secretory neurosis, hence the name "membranous enteritis" is inappropriate.

7. Concretions.—Gall-stones are probably more common than is generally supposed, and should be searched for in every case of obscure colicky abdominal pain. Intestinal concretions (enteroliths) are rare. Intestinal sand, consisting of sand-like grains, is especially common in neurotic conditions, such as mucous colitis.

Concretions can be found by breaking up the fecal matter in a sieve (which may be improvised from gauze) while pouring water over it. It must be remembered that gall-stones, if soft, may go to pieces in the bowel.

8. Animal Parasites.—Segments of tapeworms and the adults and larvæ of other parasites are often found in the stool. They are best searched for in the manner described for concretions. The search should be preceded by a vermicide and a brisk purge. Patients frequently mistake vegetable tissue (long fibers from poorly masticated celery or "greens," cells from oranges, etc.) for intestinal parasites, and the writer has known physicians to make similar mistakes. Even slight famil-

iarity with the microscopic structure of vegetable tissue will prevent the chagrin of such errors.

9. Curds.—The stools of nursing infants frequently contain whitish curd-like masses, due either to imperfect digestion of fat or casein or to excess of these in the diet. When composed of fat, the masses are soluble in ether, and give the Sudan III test. If composed of casein, they will become tough and fibrous-like when placed in formalin (10 per cent.) for twenty-four hours.

II. CHEMIC EXAMINATION

Complicated chemic examinations are of little value to the clinician. Certain tests are, however, important.

1. Blood.—When present in large amount blood produces such changes in the appearance of the stool that it is not likely to be overlooked. Traces of blood (occult hemorrhage) can be detected only by special tests. Recognition of occult hemorrhage has its greatest value in diagnosis of gastric cancer and ulcer. It is constantly present in practically every case of gastric cancer, and is always present, although usually intermittently, in ulcer. Traces of blood also accompany malignant disease of the bowel, the presence of certain intestinal parasites, and other conditions.

Detection of Occult Hemorrhage.—Soften a portion of the stool with water, shake with an equal volume of ether to remove fat, and discard the ether. Treat the remaining material with about one-third its volume of glacial acetic acid and extract with ether. Should the ether not separate well, add a little alcohol. Apply the guaiac test to the ether as already described (p. 125).

In every case iron-containing medicines must be stopped, and blood-pigment must be excluded from the food by giving an appropriate diet, *e. g.*, bread, milk, eggs, and fruit. At the beginning of the restricted diet give a dram of powdered charcoal, or 7 grains of carmin, so as to mark the corresponding stool.

2. Bile.—Normally, unaltered bile-pigment is never present in the feces of adults. In catarrhal conditions of the small intestine bilirubin may be carried through unchanged. It may be demonstrated by filtering (after mixing with water if the stool be solid) and testing the filtrate by Gmelin's method, as described under The Urine.

Hydrobilirubin will give a red color if a little of the stool be rubbed up with saturated mercuric chlorid solution and allowed to stand twenty-four hours. The red color is likewise imparted to microscopic structures which are stained with hydrobilirubin. A green color in this test shows the presence of unchanged bilirubin.

III. MICROSCOPIC EXAMINATION

Care must be exercised in selection of portions for examination. A random search will often reveal nothing of interest. A small bit of the stool, or any suspicious-looking particle, is placed upon a slide, softened with water if necessary, and pressed out into a thin layer with a cover-glass. A large slide—about 2 by 3 inches—with a correspondingly large cover will be found convenient. Most of the structures which it is desired to see can be found with a 16 mm. objective. Details of structure must be studied with a higher power.

The bulk of the stool consists of granular débris. Among the recognizable structures met in normal and pathologic conditions are: Remnants of food, epithelial cells, pus-corpuscles, red blood-corpuscles, crystals, bacteria, and ova of animal parasites (Fig. 108).

1. Remnants of Food.—These include a great variety of structures which are very confusing to the student. Considerable study of normal feces is necessary for their recognition.

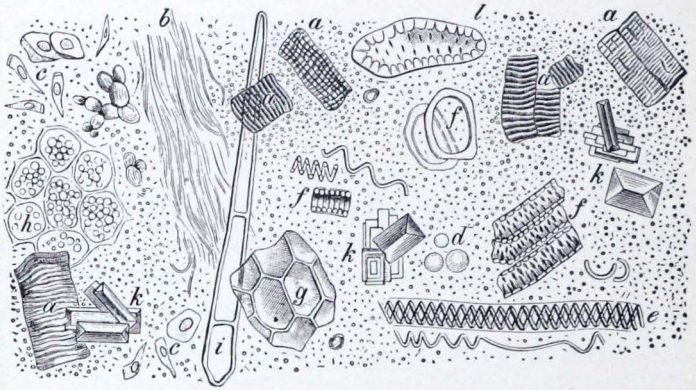


Fig. 108.—Microscopic elements of normal feces: *a*, Muscle-fibers; *b*, connective tissue; *c*, epithelial cells; *d*, white blood-corpuscles; *e*, spiral vessels of plants; *f–h*, vegetable cells; *i*, plant hairs; *k*, triple phosphate crystals; *l*, stone cells. Scattered among these elements are micro-organisms and débris (after v. Jaksch).

Vegetable fibers are generally recognized from their spiral structure or their pits, dots, or reticulate markings; *vegetable cells*, from their double contour and the chlorophyl bodies which many of them contain. These cells are apt to be mistaken for the ova of parasites. *Starch-granules* sometimes retain their original form, but are ordinarily not to be recognized except by their staining reaction. They strike a blue color with Lugol's solu-

tion when undigested; a red color, when slightly digested. *Muscle-fibers* are yellow, and when poorly digested appear as short, transversely striated cylinders with rather squarely broken ends (Fig. 109). Generally, the ends are rounded and the striations faint, or only irregularly round or oval yellow masses are found. *Curds of milk* are especially important in the stools of children. They must be distinguished from small masses of *fat* (p. 314).

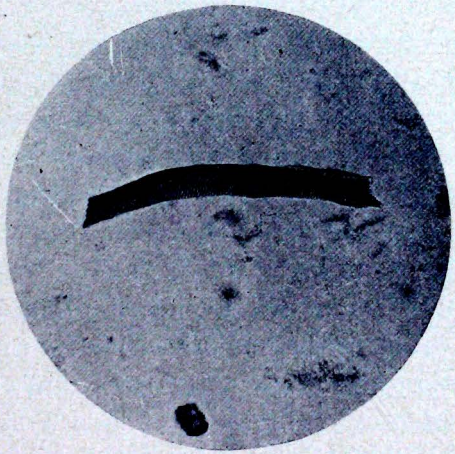


Fig. 109.—Poorly digested muscle-fiber in feces showing striations (× 200) (photograph by the author).

Excess of any of these structures may result from excessive ingestion or deficient intestinal digestion.

2. Epithelial Cells.—A few cells derived from the wall of the alimentary canal are a constant finding. They show all stages of degeneration, and are often unrecognizable. A marked excess has its origin in a catarrhal condition of some part of the bowel. Squamous cells come from the anal orifice; otherwise the form of the cells gives no clue to the location of the lesion.

3. Pus.—Amounts of pus sufficient to be recognized with the eye alone indicate rupture of an abscess into the bowel. If well mixed with the stool, the source is high up, but in such cases the pus is apt to be more or less completely digested, and hence unrecognizable. Small amounts, detected only by the microscope, are present in catarrhal and ulcerative conditions of the intestine, the number of pus-cells corresponding to the severity and extent of the process.

4. Blood-corpuscles.—Unaltered red corpuscles are rarely found unless their source is near the anus. Ordinarily, only masses of blood-pigment can be seen. Blood is best recognized by the chemic tests (p. 274).

5. Bacteria.—In health, bacteria constitute about one-third of the weight of the dried stool. They are beneficial to the organism, although not actually necessary to its existence. It is both difficult and unprofitable to identify them. The great majority belong to the colon bacillus group, and are negative to Gram's method of staining.

In some pathologic conditions the character of the intestinal flora changes, so that Gram-staining bacteria very greatly predominate. As shown by R. Schmidt, of Neusser's clinic in Vienna, this change is most constant and most striking in cancer of the stomach, owing to large numbers of Boas-Oppler bacilli, and is of considerable value in diagnosis. He believes that a diagnosis of gastric carcinoma should be very unwillingly made with an exclusively "Gram-negative" stool, while a "Gram-positive" stool, due to bacilli (which should also stain brown with Lugol's solution), may be taken as very strong evidence of cancer. A Gram-positive stool due

to cocci is suggestive of intestinal ulceration. The technic is the same as when Gram's method is applied to other material (p. 409), except that the smear is fixed by immersion in methyl-alcohol for five minutes instead of by heat. Fuchsin is the best counterstain. The deep purple Gram-staining bacteria stand out much more prominently than the pale-red Gram-negative organisms, and one may be misled into thinking them more numerous even in cases in which they are much in the minority. The number of Boas-Oppler bacilli can be increased by administering a few ounces of sugar of milk the day before the examination. The bacteria can be obtained comparatively free from food remnants by mixing a little of the feces with water, allowing to settle for a short time, and making smears from the supernatant fluid.

Owing to the difficulty of excluding swallowed sputum, the presence of the tubercle bacillus is less significant in the feces than in other material. It may, however, be taken as evidence of intestinal tuberculosis when clinical signs indicate an intestinal lesion and reasonable care is exercised in regard to the sputum. Success in the search will depend largely upon careful selection of the portion examined. A random search will almost surely fail. Whitish or grayish flakes of mucus or blood-stained or purulent particles should be spread upon slides or covers and stained by the method given upon p. 168. In the case of rectal ulcers, swabs can be made directly from the ulcerated surface.

6. Crystals.—Various crystals may be found, but few have any significance. Slender, needle-like crystals of fatty acids and soaps (Fig. 36) and triple phosphate

crystals (Fig. 108) are common. Characteristic octahedral crystals of calcium oxalate (Fig. 51) appear after ingestion of certain vegetables. Charcot-Leyden crystals (Fig. 9) are not infrequently encountered, and strongly suggest the presence of intestinal parasites. Yellowish or brown, needle-like or rhombic crystals of hematoidin (Fig. 36) may be seen after hemorrhage into the bowel.

7. Parasites and Ova.—The stool should be well mixed with water and allowed to settle. The ova will be found in the upper or middle portions of the sediment. The flagellates are best found in the liquid stool after a dose of salts. Descriptions will be found in the following chapter.

IV. FUNCTIONAL TESTS

1. Schmidt's Test Diet.—Much can be learned of the various digestive functions from a microscopic study of the feces, especially when the patient is upon a known diet. For this purpose the standard diet of Schmidt is generally adopted. This consists of:

Morning.....0.5 liter milk and 50 gm. toast.
Forenoon.....0.5 liter porridge, made as follows: 40 gm. oatmeal, 10 gm. butter, 200 c.c. milk, 300 c.c. water, and one egg.
Midday.....125 gm. hashed meat, with 20 gm. butter, fried so that the interior is quite rare; 250 gm. potato, made by cooking 190 gm. potato with 100 c.c. milk and 10 gm. butter, the whole boiled down to 250 c.c.
Afternoon.....Same as morning.
Evening.....Same as forenoon.

At the beginning of the diet, the stool should be marked off with carmin or charcoal. One should famil-

iarize himself with the microscopic appearance of the feces of normal persons upon this diet.

Deficiency of starch digestion is recognized by the number of starch-granules which strike a blue color with iodin. With exception of those inclosed in plant cells none are present normally.

The degree of protein digestion is ascertained by the appearance of the muscle-fibers. Striations are clearly visible only when digestion is imperfect (Fig. 109). According to Schmidt, the presence of nuclei in muscle-fibers denotes complete absence of pancreatic function. The presence of connective-tissue shreds indicates deficient gastric digestion, since raw connective tissue is digested only in the stomach. These shreds can be recognized macroscopically by examining in a thin layer against a black background, and microscopically by their fibrous structure and the fact that they clear up when treated with acetic acid.

Digestion of fats is checked up by the amount of neutral fat.

2. Sahli's Glutoid Test.—The Schmidt test diet involves some inconvenience for the patient, and interpretation of results requires much experience upon the part of the physician. A number of other methods of testing the digestive functions have been proposed. The glutoid test of Sahli is one of the most satisfactory. This is similar to his desmoid test of gastric digestion described on page 308. A glutoid capsule containing 0.15 gram iodoform is taken with an Ewald breakfast. The capsule is not digested by the stomach fluid, but is readily digested by pancreatic juice. Appearance of iodin in the saliva and urine within four to six hours

indicates normal gastric motility, normal intestinal digestion, and normal absorption. Instead of iodoform, 0.5 gram salol may be used, salicyluric acid appearing in the urine in about the same time. For tests for iodin and salicyluric acid, see pages 307 and 309.

The glutoid capsules are prepared by soaking gelatin capsules in formalin. Sahli states that filled capsules can be purchased of A. G. Haussmann, in St. Gall, Switzerland.

3. Müller's Test for Trypsin.—A calomel purge is given two hours after a meal. Particles of the feces are placed upon solidified blood-serum. This is incubated at a temperature of 55° to 60° C. to prevent action of bacteria. Digestion of the serum—indicated by a translucent, roughened, depressed surface—presumably shows the presence of trypsin, and indicates pancreatic sufficiency. Trypsin can seldom be detected without the preceding purge.