MANUAL
OF
LABORATORY DIAGNOSIS

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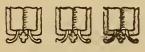

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This book is compiled
from lectures given by the
following instructors
at the Johns Hopkins
Medical School.

S. R. Miller, M. D., Lectures on Urine, and Blood.

Marjorie D. Batchelor, M. D., Lectures on Stomach Analysis.

F. A. Evans. M. D., Lectures on Sputum and Stools.

W. A. Baetjer, M. D., and S. R. Miller, M. D., Lectures on Blood.

C. G. Guthrie, M. D., Lectures on Parasites.

PREFACE.

Requests have come from time to time for outlines of the general course in Clinical Microscopy, as given at the Johns Hopkins Medical School, or for summaries of the individual subjects covered. Such outlines have not any particular merit other than that they serve as guides for teaching, or compends, useful for the quick reviewing of any particular topic. It has always seemed a wiser policy to encourage each student, carefully to take his own notes and prepare for himself such a working outline as best suited his own needs. Condensed notes and compends in general are too prone to stimulate superficiality to warrant their unqualified recommendation. Moreover the practice here has been to have each instructor cover a different subject each year, thereby widening the scope of each man's knowledge and interests; each improving on his predecessor's lectures, if possible. Consequently, no set plan is followed and stereotyped lectures have been consistently avoided.

The present summary of the lectures given in the past year, is published chiefly as a result of the enthusiasm and painstaking work of one of the students. It is not intended that these notes shall be comprehensive: the interpretation of tests, etc., has not been given much space, and, by reason of the universal "war-time lack of time," careful correction has not been possible. The notes are presented merely as representing the essential subject matter covered in the lectures on urine analysis, examination of the gastric contents, sputum, feces, parasitology and hematology. The publication of these notes had the sanction of the late Dr. Theo. Janeway, whose interest in the application of clinical labora-

tory methods to the diagnosis of disease was always keen and enthusiastic. It had been his intention to write this preface as evidence of his approval of the project. By reason of their absence in France, Drs. W. A. Baetjer, C. G. Guthrie and Frank Evans have been unable to review the sections previously covered by them. Knowing full well their great interest in the subject, it has seemed wise for me to stand sponsor for them all, and to permit publication, but only with a clear understanding of the limits of the book, the constant changing of methods and interpretations, and the lack of balance unavoidable in a work of this kind.

Sydney R. Miller, M.D.

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ALBUMINURIA

Definition: The accumulation of a coagulable protein in the urine. The protein has, in true cases, escaped through the renal parenchyma.

Two groups:

1. True condition in which the renal parenchyma is faulty.

2. False. Condition in which the protein has been added to the urine during its passage through the G. U. tract.

Origin:

1. Blood protein.
2. Secretion or degeneration of renal cells.
3. Substances added along G. U. tract.

No chemical method has been devised to differentiate these different forms. Most of protein is of blood origin all agree.

Serum albumin. Greatest in frequency and amount. 5 to 40 grams per day. 20 to 100 mg. occurs normally, but is not detectable. Albumin is only clinically present when it can be detected by the ordinary laboratory means. This is set as a standard. Serum albumin is soluble in water, coagulable by heat, precipitated by alcohol and salts of the heavy metals, and by saturation with magnesium sulphate, and is Levo-rotatory 62 degrees.

Serum globulin. This is always present with serum albumin. It represents from 10 to 75 per cent of total protein. It is insoluble in water and dilute acetic acid in the cold. It is precipitated by saturation with magnesium sulphate and half saturation with ammonium sulphate and partially precipitated by sodium chloride saturation.

Pseudo-globulin, euglobulin, and *fibrinogen.* These belong to the globulin group. Fibrinogen is practi-

cally never encountered and when it is is perfectly obvious, for the urine undergoes spontaneous clotting. Euglobulin and pseudo-globulin are probably the same as nucleo-albumin. They are of no practical importance.

Nucleo-histones are of no practical significance.

Bence-Jones protein is protein sui generis.

Albumoses are characterized by a tendency to be soluble upon heating and insoluble upon cooling. They are increased in acute types of nephritis and in the absorption of exudates.

OCCURRENCE OF ALBUMINURIA.

1. With renal lesion.
2. Without renal lesion.

Occurrence of albuminuria without definite renal lesion.

1. Severe muscular exercise.
2. Severe emotional upsets.
3. After cold baths.
4. Alimentary ingestion of excessive amounts of proteins.
5. New-born, first 8 to 10 days of life.
6. Pregnancy, toward the end or after parturiton.
7. Adolescence, 12 to 16 years of age.
8. Hypostatic, associated with large spleens, noticeable after the patient has lain down for some time.
9. Cyclic, orthostatic, or lordotic. This type occurs in young adults, 12 to 20 years of age. Albumin appears only after the patient has been up and about for some time. Early morning urine is albumin free. These patients usually have abnormal lordosis, and when corrected the albuminuria clears up. According to Jehle there is a deficiency of renal function while the patient is standing, which

clears up when he lies down. The condition does not predispose one to nephritis, but tends to clear up as the patient grows older.

Salient features of functional disturbance.

1. Albumin slight.
2. Occurs chiefly in young people.
3. Transient or intermittent.
4. Occurs without other evidences of renal disease.
5. No renal disease etiology.
6. Patients tend to be a bit peaked and have a neurotic tendency, with vasomotor instability, dermatographia, etc.

Albuminuria in those with transient renal injury.

1. Fevers (with casts). Disappears with the disappearance of the fever and patient does not suffer inconvenience during rest of life.

2. Toxic. Caused by drugs and secondary to other states such as pyorrhea, genito-urinary infection, etc. Dental treatment, etc., may clear up condition.

3. Hematogenous. Caused by altered condition of blood as in leukaemia, pernicious anaemia, purpura, scurvy, beriberi, and jaundice.

4. Traumatic. Caused by injury in the vicinity of the kidney or in remote parts of the body. Occurs in epilepsy, brain tumors, and sometimes after bimanual palpation of the kidney as well as other abdominal manipulations.

5. Intermittent, characterized by periodic occurrence, indicative of:

(a) Insidious development of nephritis.
(b) Stasis.

Summary.

Regard faint traces of albumin in people under 20 years of age in good condition as functional. Ex-

amine last voiding at night and first in the morning to determine whether it is orthostatic, or hypostatic. Use care to exclude extra-renal sources of albumin, bladder infection, vaginal discharge, etc. Regard albuminuria in febrile and toxic conditions as nephritis, at that time at least. One cannot judge the extent or the type of nephritis by the frequency of albuminuria. The absence of albumin and casts does not exclude nephritis.

Detection of proteins in the urine: Serum albumin, serum globulin, nucleo-albumin, and albumoses.

In order to detect these substances the urine must be:

1. Absolutely clear, especially if small traces are to be detected. Make urine clear by filtration or by the use of kieselguhr or animal charcoal. When the urine comes through the filter paper turbid add about 1/10 volume of kieselguhr and refilter.
2. Urine should be faintly acid.
3. Urine should not be too concentrated or too dilute. In cases where it is very dilute 1005 to 1006 specific gravity, it should be made more concentrated by the addition of about 1/10 volume of sodium chloride.
4. In doubtful cases don't rely on one test alone.

Heat and acetic acid test.

After the urine has been made clear by the above methods take a test tube about 2/3 full of the clear urine and heat the upper third in a bunsen flame.

If no cloud appears the urine is probably normal.

If a marked cloud appears it can be:

1. Calcium phosphate.
2. Calcium carbonate.
3. Albumin.

In order to differentiate these add 5 per cent acetic

acid drop by drop. If cloud is due to calcium phosphate it disappears; if it is due to calcium carbonate it disappears with the evolution of gas; if it is due to albumin it persists, becomes greater or becomes slightly less, in accordance with the amount of the former substances present. It must be remembered that these protein substances are soluble in an excess of acid or alkali. To detect very faint traces use a dark background.

Sources of error: (a) Technique. (b) Other precipitable substances.

1. Nucleo-albumin. This is precipitated with 5 per cent acetic in the cold. Albumin is only precipitated after heating.

2. Resinous acids such as cubebs, guiac, etc., give a precipitate with heat and acetic acid. These if shaken with alcohol or ether are dissolved.

3. Albumoses. The precipitate of these comes down after cooling, and reheating gives re-solution.

Heat and nitric acid test.

Heat urine to boiling and add 20 per cent HNO_3 drop by drop. A cloud is fairly indicative of albumin. It has the same sources of error as the heat and acetic acid test.

These two tests are sensitive to .005 grams in 100 cc.

Heller's test.

Urine and concentrated HNO_3 are layered in equal parts, the urine above and the acid below.

A cloud at the line exactly can be:

1. If white, albumin. Its breadth depends upon the quantity of albumin present. After a time it tends to diffuse upwards.

2. Thymol. This has a grayish or brownish color. There is also a zone of greenish color below and a

reddish one above the line of contact. Confirm by shaking with petroleum ether.

3. Albumoses. The cloud given by these is identical with that of albumin. By heating this cloud disappears.

4. Urea nitrate. This cloud is yellowish brown and is made up of crystals which give a shimmer.

A cloud above the line of contact may be:

1. A broad, yellowish band about 1½ cm. above line of contact due to urates in concentrated urines. They are dissolved by heating.

2. Nucleo-albumin gives a cloud ¼ to ¾ cm. above the line of contact. It tends to disappear by dilution. By diluting the urine to 1005 to 1008 nucleo-albumin and albumoses can be eliminated.

This test is sensitive from .002 to .007 grams per 100 cc.

Potassium ferrocyanide and acetic acid test.

From 5 to 10 cc. of urine in a test tube to which is added acetic acid. Do not heat. Filter and to the filtrate add 10 per cent potassium ferrocyanide, drop by drop. Albumin gives a whitish precipitate.

Sources of error:

1. Iron present in large amounts. Color different.
2. Boiling urine in a glass container.
3. Reagent itself.
4. Albumoses which upon heating disappear.
5. Nucleo-albumin same as albumoses.

QUANTITATIVE TESTS FOR ALBUMIN.

The most common and easily performed is the employment of the Esbach tube. It differs in accuracy from the Kjeldahl method by .3 gm. Urine is added to the mark U and reagent to the mark R. The tube is inverted ten or twelve times to insure thorough mixing of the contents and is then allowed to

stand for about 24 hours and read. The reading below gives the number of grams per liter.

*Reagents used*s

1. *Picric acid.* This is merely mentioned to be condemned. It not only precipitates albumin but also albumoses, uric acid, creatinin, resinous acids, etc.

2. *Tsuchiya's reagent.*

Phosphotungstic acid..... 1.5 grams
Concentrated HCl..... 5.0 cc.
95 per cent alcohol g. s. ad....100.0 cc.

The advantage of this reagent is that it eliminates the above error.

The disadvantages of the test are that it is inaccurate in urines containing over 4 per cent albumin; and in some cases the precipitate sticks to the sides, may float or may settle unevenly.

GLOBULINS:

Purdy's test.

Globulins are soluble in salt solution, but insoluble in distilled water. Dilute the urine and get a cloud, or, better still, drop the urine in a test tube half full of distilled water and watch for a cloud against a black background.

Ammonium sulphate method.

Make the urine alkaline with ammonium hydroxide and filter. Layer equal amounts of the filtrate and saturated ammonium sulphate. A cloud at the line of contact is fairly indicative of globulin. For more accurate determination do following test: Urine plus equal volume of saturated ammonium sulphate solution. Let stand an hour and filter. Wash precipitate with half saturated amonium sulphate until filtrate is albumin free. To further differentiate it, dissolve precipitate in water and heat on water

bath to coagulate the proteins. Filter and wash the precipitate with water. To the precipitate add 1 per cent solution of sodium carbonate and heat on the water bath. Filter and neutralize with acetic acid. A precipitate signifies globulin (Webster).

BENCE-JONES PROTEIN.

(S. R. Miller and Walter A. Baetjer, J. A. M. A., 1-19-18, vol. 70, pg. 137-139.)

This is the most infrequent type of protein found in the urine. In 1846 Bence-Jones described the characteristics of the protein, which were the formation of a gelatinous precipitate at a low temperature which disappeared upon boiling and reappeared upon cooling. In 1876 Rustizky described multiple myeloma. In 1889 Kahler described a case of multiple myeloma with Bence-Jones protein in the urine. The association of multiple myeloma with Bence-Jones protein is almost constant but not specific, for it does occur in other conditions. In no case, however, where Bence-Jones protein has been found has the bone-marrow been found to be normal. Multiple myeloma are bone tumors similar to sarcoma, occurring usually in people beyond forty years of age, and in men more than in women. The condition is one of a deep-seated bone lesion associated with pain, emaciation, cachexia, etc.; it is confined to the marrow and lives at the expense of the cancellous tissue; it is limited to the long bones, the sternum and ribs, rarely involving the skull.

Bence-Jones protein has been demonstrated in five types of cases:

1. Multiple myeloma composed of (a) myeloblasts, (b) myelocytes, (c) erythroblasts.

2. Chronic leukaemias (a) lymphatic, (b) myelocytic.

3. Metastasis to bone from cancer elsewhere.

4. Other bone diseases present.

5. Cases without any lesions present.

Usually not over two years elapse from the discovery of Bence-Jones protein to the death of the individual. In two cases there was reported a high blood pressure.

Nature of Bence-Jones protein.

It gives rise to amino acid partition and is therefore a real protein, but it differs from any other known protein and is never found to be a part of the metabolism of the individual. It contains a carbohydrate radicle, but no phosphorous, and is therefore not a nucleo-protein. It also contains from 1 to 2 per cent of sulphur. It has been injected subcutaneously, intravenously and intrarectally, but is always excreted as such.

Theories regarding Bence-Jones protein.

1. A substance derived from the secretory activity or degeneration of tumor masses themselves.

2. The tumor itself secretes an enzyme which can influence protein metabolism in such a manner that this protein is formed.

3. Individuals with Bence-Jones protein have anomalies in urine excretion and metabolism similar to those excreting alkapton and cystin substances. It is probable that these individuals on account of their perverted metabolism are more prone to tumors.

Character of the urine.

The amount varies from 1200 to 2000 cc. per day with a normal specific gravity. It has a pale smoky color but is clear; it is acid in reaction, rarely neu-

tral or alkaline; it has a tendency to foam on shaking, which foam is more abundant and more persistent than normal. There may be as much as 70 grams of the protein present in the 24 hour specimen, and there is no other type of protein nor any casts present unless there is some nephritic condition superimposed.

Characteristics of the urine in this disease.

1. Continuous excretion regardless of everything.
2. Intermittent type, in which days or months may elapse without its excretion.
3. The appearance of the Bence-Jones protein sometimes antedates any demonstrable lesion.
4. The largest amounts are excreted in multiple myeloma which may run as high as 50 to 75 gm. per day. In other cases, such as metastatic cancer or sarcoma, from 20 to 35 gm. are excreted.
5. There is also a decreased chloride content.
6. A spontaneous precipitate may occur in the urine after standing for some time or it may occur in the bladder and give rise to a urine resembling chyle.

Tests.

1. Heat and acetic acid. The urine clouds at about 55 degrees. It is coagulated and precipitated from 10 to 20 degrees lower than any blood protein (40 to 60 deg.) It dissolves upon boiling and reappears upon cooling. Coagulation depends upon the acid and salt concentration of the urine.
2. Urine treated with 25 per cent HNO_3 in the cold gives a cloud which redissolves at first until excessive acid is added. This precipitate is soluble at 100 deg. and insoluble at 60 deg. Any dilute mineral acid will do the same.
3. Urine plus 2 volumes of saturated ammonium

sulphate gives a complete precipitation at room temperature. This precipitate is readily soluble in water and does not pass through a dialysing membrane, differing from albumoses and pentoses.

4. Urine plus 2 volumes of a saturated solution of sodium chloride gives no precipitate unless the urine is previously acidified with acetic acid. The precipitate is not soluble in water.

5. Urine plus 2 volumes of 95 per cent alcohol gives complete precipitation. Immediately after precipitation, the precipitate is soluble in water, but if kept in contact with the alcohol for some time, it becomes insoluble in water, but is soluble in a dilute solution of ammonia.

6. It gives a positive biuret reaction.

SEDIMENTS OF THE URINE.

Inorganic.

1. Acid urine
   - *a* Uric acid.
   - *b* Amorphous urates.
   - *c* Calcium oxalate and sulphate.
   - *d* Xanthine.
   - *e* Cystin.
   - *f* Leucin.
   - *g* Tyrosin.
   - *h* Hippuric acid.
   - *i* Bilirubin.
   - *j* Cholesterin.
2. Alkaline urine.
   - *a* Amorphous phosphates and carbonates.
   - *b* Magnesium phosphate.
   - *c* Triple phosphates.
   - *d* $CaCO_3$.
   - *e* Ammonium biurate.

One may find any kind of acid crystals in an

alkaline urine for they may persist after the urine has become alkaline.

Uric acid crystals.

There are three conditions necessary for their formation:

1. Concentrated urine; 2. High acidity; 3. Cold.

They form crystals brick red in color and have a tendency to cling to the sides of the container. They mean nothing concerning purine metabolism. They take a variety of shapes, rhomboid, prisms, etc. When made artificially they are colorless. They are soluble in HCl and insoluble in acetic acid.

Urate sediments.

These also occur when the urine is: 1. highly acid; 2. concentrated, and 3. cold. They take the urinary pigments with them, urochrome and uroerythrin. Their shape is amphorous, sometimes resembling needles. They are soluble by heating to 50 to 60 degrees, and with acetic and mineral acids.

Calcium oxalate crystals.

These are formed mostly from vegetables such as, rhubarb, celery, spinach, peas and beans, and also from green fruits. A small portion is formed from the body (nucleins—uric acid—oxalic acid). In oxaluria as much as 20 to 30 grams may be excreted in a day on a mixed diet. Neurasthenia is often associated with its excretion but not the cause. Its excretion is sometimes increased in jaundice, and hepatic disease, and definitely increased in gout

when it is most likely formed from uric acid. In this condition determine whether it is excreted frequently and whether it is associated with uric acid perversion and calculi formation. They occur as four-sided prisms with a square base and also in dumbbell crystals.

They are perfectly white in color, are insoluble in acetic acid, but soluble in HCl.

Calcium sulphate.

These are perfectly white in color and rare in occurrence. Their shape is long and oblong.

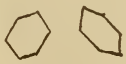
Xanthin, white and somewhat egg shaped.

(After Hawk)

Tyrosin, colorless and in needles arranged in sheathes and rosettes.

Leucin, never a spontaneous sediment.

Cystin, colorless, four-sided or prism shaped crystals. Insoluble in acetic acid and soluble in HCl.



Hippuric acid. Occur rarely. They are irregular, six-sided crystals.

Bilirubin crystals, yellow or reddish brown in color, occur rarely in hemorrhagic nephritis, after transfusion, jaundice, and acute yellow atrophy. Occur in needles and rhomboid shaped crystals.

Cholesterin crystals, white, and in the form of steps, one upon the other. Soluble in chloroform.

Amorphous phosphates and carbonates.

These constitute the chief sediment of alkaline urine. Both are soluble in acetic acid, the carbonates in addition give off gas.

Magnesium phosphate.

These are generally amorphous but occasionally resemble triple phosphates.

Triple phosphates. ($MgNH_4PO_4$)

These are one of the commonest forms of xls found in standing urine, and are characteristic of ammoniacal urine. These are coffin-lid, or when eroded, fern-leaf in appearance. They are colorless and soluble in acetic acid.

Calcium carbonate crystals.

These may be amorphous or crystalline and are generally found with amorphous phosphates. In shape they are either dumb-bell or spheroidal with concentric radiations. They are colorless and soluble in acetic acid with evolution of gas.

Ammonium biurate crystals.

These occur in the shape of thorn-apples and are dark brown in color. They dissolve in acetic acid and give uric acid. They are of no significance.

SCHEME FOR RUNNING DOWN INORGANIC SEDIMENTS.

1. Warm sediment.
   a Urates readily soluble.
   b Calcium sulphate soluble with difficulty.
2. If not soluble by (1) add 3 to 5 drops of glacial acetic acid.

Solution indicates:

   a Amorphous phosphates and carbonates.
   b Triple phosphates.
   c Calcium carbonate.

Non-solution indicates:

   a Uric acid.
   b Calcium oxalate.
   c Calcium sulphate.
   d Organized sediments.
   e Tyrosin.
   f Cystin.
   g Leucin.

3. Add from 3 to 5 drops of concentrated HCl to insoluble sediment.

Solution indicates:

a Calcium sulphate.
b Cystin.
c Tyrocin.
d Leucin.
e Xanthin.

These are also soluble in NH_4OH.

4. Uric acid is insoluble in acids and ammonia, but is soluble in KOH.

5. Cholesterin is soluble in chloroform.

6. Hippuric acid is soluble in alcohol.

ORGANIZED SEDIMENTS.

Mucus threads.

Poorly refractile, interlacing, fibres found normally and of no significance. Responsible for the nubecula formation.

Epithelial cells.

These may arise from any part of the genito-urinary tract, viz.: pelvis of kidney, bladder. urethra, prostate. Irregular cells occur in the bladder and vaginal tract. Sheets of cells are more frequent from the vagina. Renal cells tend to be round or cuboidal, a little larger than a pus cell, and have a large vesicular nucleus. They may occur in any type of nephritis, but are more apt to occur in larger numbers in acute nephritis. Similar cells arise from the ureter and prostate, those from the latter being about twice as large. One cannot tell the type, location or extent of the lesion by the type of cell found, although renal epithelium does not occur normally. Tailed cells were formerly thought to arise from the pelvis of the kidney, but they are also found in the deeper layers of the bladder and vagina.

Pus cells.

A few may be encountered in normal specimens, especially in women. Large numbers indicate patho-

logical conditions either of the G. U. tract or outside. The number of pus cells occurring in diseases of the cortex of the kidney is few; the number occurring in pyelitis, pyelonephritis, and kidney abscess is many. Cystitis is the most frequent cause of pus in the urine. They are better identified in acid urine, so accordingly, if the urine be alkaline as it usually is in cystitis, it will aid in identification by adding a few drops of acetic acid under the cover slip, which will bring out the nuclei.

Tripperfaden.

Are shreadlike bits which float about. Microscopically they are masses of pus cells in the meshes of mucus. They occur in chronic urethritis.

Red blood cells.

These never occur normally except in women during menstruation. They disintegrate very rapidly, tend to be shrunken and crenated in concentrated urine, laked in urine of low specific gravity, and are often difficult to recognize. When they arise from the kidney they tend to be mixed with the urine, when from the bladder they tend to occur in clumps. In alkaline urine they tend to go to pieces very quickly.

Spermatozoa.

Disintegrate very rapidly. Found occasionally in adult males.

Tissue fragments.

Renal or bladder new growths.

Casts. (Cylindruria).

Descending tubular elements from the kidney.

Theories of origin:

1. Casts represent visible coagulated albumins which have escaped from the kidney.

2. Irritated kidney cells excrete a colloidal substance which is coagulated in the tubes and may en-

gulf any cellular substance present there at the time.

They indicate a condition of renal irritation and are more indicative of it than albumin.

Types of casts.

1. Cellular.
2. Granular.
3. Amorphous.

Cellular casts.

a Epithelial.
b Pus or W B C.
c Red blood cell.

Granular casts.

a Fine and coarsely granular.
b Fatty.
c Urate.
d Bacterial.

Amorphous casts.

a Hyaline.
b Waxy.

Theory.

Hyaline casts are the fore-runners of all the others, and are the matrix upon which the others are built. They have straight sides, rounded ends, are uniform caliber throughout, narrow, may be short or long, and are poorly refractile. One can build all the other forms around them. Any cast containing even but one cell is called a cellular cast and designated by the type of cell it contains. They are encountered in a number of conditions, acute and chronic nephritis, etc. Cellular casts change into granular casts, going through a stage of fatty degeneration. Coarsely granular casts are yellowish in color and finely granular casts are colorless. Granular casts can finally revert to the hyaline form.

Waxy casts differ from hyaline casts in being

highly refractile. They seem brittle, have irregular ends, and may be wavy in outline. They tend to be broader and have transverse lines of fission. They stain with Lugal's solution but are not necessarily limited to amyloid disease. They are a fairly good indication of tubular stasis and probably represent metamorphosed hyaline casts.

Occurrence of casts.

Casts are occasionally encountered in normal individuals, but whenever they are found they should be regarded as evidence of renal irritation, whether slight, transitory, and of no importance or persistent and of serious import. They occur after strenuous exercise and in febrile states. Neither the type nor the number allows one to judge the extent of the renal injury, for the more normal the kidney the greater is the response to irritation. Casts tend to appear and run parallel with albuminuria but are more sensitive. The occurrence of albuminuria without casts usually indicates that one has not looked long enough or that the urine has become alkaline and they have disintegrated.

Albumin without casts does not predispose to the development of nephritis. It gives some evidence of tuberculosis and other diseases higher up.

Albumin with few casts represents in general the same thing as above. There is, however, a higher mortality incidence.

Albumin with granular casts indicating cardio-renal disease, has in ten years a much higher mortality incidence than the two previous.

Casts tend to be more cellular the more acute the condition. Waxy casts are indicative of tubal stasis. It is not so much the type nor the number of casts, but it is their relation to other clinical evidence that is of importance. (Read in Osler's book

of reprints "On the Advantage of a Trace of Albumin and a Few Tube Casts in the Urine of Certain Men Above Fifty Years of Age.")

Cylindroids.

These may possess any and all the characteristics of casts. They are usually straight and hyaline and rarely waxy.

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BACTERIOLOGY OF THE URINE.

Bacilluria.

Indicates bacilli in the urine irrespective of their source.

Types found:

1. Tubercle bacilli. They may be excreted through the kidney and not represent a lesion of the G. U. tract. When associated with r. b. c. and w. b. c., their local source is more likely. Don't confuse with Smegma bacilli. To obtain a more certain dagnosis inoculate a guinea pig intraperitoneally, keep 3 or 4 weeks, kill, and look for typical findings in the retro-peritoneal lymph glands, spleen or liver. Bladder tbc. is often associated with an acid urine and sterile pus.

2. Gonococci. Gram negative and intracellular. Difficult to find them in Neisser cystitis.

3. Typhoid bacilli. Found during the disease and often for weeks or months after recovery. May be due to a bacillaemia or due to local lesion.

4. Colon bacillus. Common invader and cause of pyelitis and cystitis.

5. Paratyphoid. Infrequent cause of pyelitis.

6. Cocci often found with Neisser infection.

7. Streptococci often occur from systemic infection, nephritis, etc.

NEPHRITIC CONDITIONS

PATH	AMOUNT	COLOR	SP. GR.	ALBUMEN	SEDIMENTS	ETIOLOGY	DURATION	RESULTS
Stasis	Oliguria Never Anuria	Dark	High	Slight	Rich in Urates, Gran & Cellular Casts	Cardiac Decompensation	As Long as Stasis Lasts	Improves as Heart May Get Infarction
Acute Nephritis	Oliguria to Anuria	Dark Smoky May Contain Blood	Normal	Large Amt. Sometimes Only Albumoses	All Types Casts, Rbc, Wbc & Epith Free Rbc	Acute Infection or Exposure	Few Days to Months	B. P. Normal Oedema (Face 1st) Uraemia
Chr. Parenchymatous Large Pale	Oliguria Rarely Anuria	Dark	High	Large Amounts	Cellular Detritus of All Kinds Fattycasts	MayFollow Acute Neph or Pus Elsewhere	2 Months to 1-2 Years	Cure Rare Oedema Anasarca Uraemia Cardiac Fail
Chr. Contract d	Polyuria	Pale	Low	Trace Intermittent	Absent or Few Numbers Hyaline or Gran Casts	Second to Arterio Sclerosis	Years	B. P. Incr. Dropsy Rare. Car. Hyper Apoplexy
Amyloid	Normal or Plus	Pale Clear	Normal or Below	Large Amounts	Hyaline Waxy and Gran Casts	Lues or Other Superimposed Conditions	Depends on Associ. cted Conditions	B.P. Norm or Below Oedema Com. Ureamia Rare
Pyelitis	Normal or Plus	Turbid	Normal or Plus	Slight	Pus Cells	Tb. or other Organisms	Few Weeks to Months	Associated With Fever & Cachexia May Lead Amyloidosis, Septicaemia or Peritonitis From Perforation

8. Any organism in the blood may be swept through the kidney. This may be the cause of much nephritis due to kidney injury. The general methylene blue stain detects all the bacteria except two:

1. Acid fast bacilli. Use carbo-fuchsin.

2. Gonococci. Use gram stain.

Animal parasites in the urine.

1. Amoeba.

2. Echinococcus (look for hooks and laminated membranes).

3. Filarial larvae.

4. Eggs of Schistosoma hematobium (human blood fluke) or Bilharziasis (Egyptian hematuria).

5. Oxyuris (pin worms, occasionally found in young girls. Wanders through urethra into bladder).

6. Trichomonas vaginalis (of no importance).

GENERAL CONSIDERATIONS.

Urine examined for the following considerations:

1. Renal and G. U. conditions.

2. General metabolism of the body.

3. Food metabolism.

Urine examination criteria.

1. Single voidings are of no practical importance.

2. Should be carried out on 24-hour specimens and in some instances the day and night specimens should be kept separate.

3. Fluid intake and general diet should be taken into consideration.

4. Correlate urinary and clinical findings.

5. Abnormalities should be confirmed at subsequent examinations.

Single voidings examined for:

1. Albumin. If but a faint trace is present, examine two specimens, one at night and one in the morning, to see whether it is orthostatic.

2. Gross abnormalities, blood, pus, etc.

3. Microscopical elements, such as blood, casts, and various forms of crystals, pathological cells, and parasites.

4. Constituents requiring chemical analysis, such as bile, urobilin, blood, indican, acetone, diacetic acid, sugar, etc.

5. Specific gravity determination.

6. Reaction, acid, alkaline, or amphoteric.

Collections of 24-hour specimens:

1. Establish hours between which the specimen is to run. If the hours determined are between 7 A. M. and 7 P. M., have the patient void at 7 A. M. and throw the specimen away. Collect all voidings, including the one at 7 A. M., the following morning.

2. Containing vessel should be clean at the start, cool, corked and clearly labeled with:

Name of patient,
Date,
Hours of collection,
Total intake,
Preservative used.

3. Day and night specimens should be kept separate in some cases.

Preservatives used for urine:

Type.	Amount.	Advantage.	Disadvantage.
Chloroform	2 to 3 drams per liter.	No bulk, easily removed, crystals preserved.	Not good for casts. Reducing agent.
Formalin.	½ to 2% by volume or 5 to 8 drops per L.	Preserves casts.	Adds bulk, reducing agent, error in sugar test, forms crystals with urea, interferes with urobilin and indican tests.
Thymol.	A few crystals.	Preserves sediments	False tests for bile and Heller's albumin.
Toluol	A thin layer.	No volume, urine can be secured below.	Expensive and inflammatory.

Common preservatives in use are chloroform and toluol.

Physical properties of urine, normal and abnormal.

1. Normally it is a clear, tranparent shade of yellow. After standing 15 to 45 minutes a "nubecula," or fog, settles to the bottom, which is composed of mucus and epithelial cells.

2. Urine cloudy when voided.
   (a) Frequently voided after heavy meals of vegetables. Due to excess of phosphates. Not abnormal.
   (b) Presence of some kind of organized sediment, blood, desquamated epithelial cells, pus, bacteria.
   (c) Cloudy, with the smell of ammonia, indicates cystitis.

3. Urine becomes cloudy on standing.
   (a) Development of bacteria.
   (b) Precipitates of calcium, phosphorus, and carbonates. Formation of ammonium carbonate by bacteria.

(c) Concentrated urine, becoming quickly cooled, deposits amorphous urates.

Odor of urine.

The normal urinary odor is due to aromatic substances. The urinous odor is due to the action of bacteria. A fruity odor occurs in diabetes, due to the excretion of acetone. A foul odor occurs in urines containing large amounts of albumin, as in cancerous and inflammatory conditions in the lower G. U. tract. The ingestion of asparagus gives a peculiar odor, due to methyl mercapton. Drugs, such as asafedita and valerian, give their characteristic odor. Turpentine gives the odor of violets; menthol that of pepperment; cubebs, copaiba, tolu and saffran that of spices.

Amount of urine excreted depends upon:

1. Individual himself.
   (a) Sex, males more than females.
   (b) Children void proportionately more, viz: adults 1 cc per kilo body wt., children 4 cc per kilo.
   (c) Weight of individual.
2. Fluid intake.
3. Pressure and velocity of blood current.
4. Condition of renal parenchyma.
5. Loss of fluid by other means: perspiration, respiration, intestines, formation of transudates and exudates.
6. Vasomotor phenomena.

The normal excretion varies between 900 and 1200 cc per day. Functional capacity is from 20 to 25 liters. Often on forced water 9 to 10 liters are excreted in typhoid without damage to the kidney. Normally at night less is excreted than during the day, the ratio being 100 parts by day to 60—80 parts by night. In some forms of nephritis, hepatic

insufficiency and cardiac disease, this ratio is reversed.

Nycturia signifies the excretion of more urine at night than during the day. It occurs characteristically in chronic diffuse nephritis.

Polyuria signifies the excretion of 3000 cc or more urine per day.

Causes:

1. Increased fluid intake.
2. Diuretics.
3. Nervous disorders. (a) organic; (b) functional.
4. Diabetes mellitus and insipidus.
5. Chronic nephritis.
6. Absorption of exudates.
7. Epicritical (end states of acute febrile disorders).
8. Sometimes in ureteral stricture.

Oliguria signifies the excretion of 800 cc or less in 24 hours.

Causes:

1. Decreased fluid intake.
2. Loss by other means.
   (a) physiological
   (b) pathological
      1. Formation of exudates and transudates.
      2. Acute febrile states.
      3. Acute nephritis.
      4. End state of chronic nephritis.
      5. Chronic diarrhoea.
      6. Vomiting.

Anuria signifies no urine.

Causes:

1. Obstruction to urinary passages.
2. Reflex (functional neuroses, Dietl's crisis).

3. Renal, acute nephritis, or end stage of chronic nephritis.

4. Prerenal conditions. Poisons: bichloride, arsenic, anaesthetics. Occasionally after veronal medication (S. R. Miller).

Pollakiuria signifies unduly frequent passage of urine.

Occurrence:

1. Polyuria of nephritis.
2. Prostratic disease.
3. Bladder disease.
4. Ureteral stricture.

Specific gravity.

Methods of determination:

1. Balance picnometer.

2. Urinometer which is calibrated at 15 deg. C. The reading should be taken at the junction of the lower meniscus and fluid. The Sp. gr. depends upon the amount of urine excreted and the amount of solids therein. Sodium chloride and urea are the chief sources of high Sp. gr. in normal urine. Normal urine has a Sp. gr. between 1015 and 1020 for 1200 to 1500 cc per day.

Clinical value of specific gravity:

1. Estimation of total solids of urine. Haeser's coefficient equals 2.33 times the last two figures of the reading and gives the weight of solids per 1000 cc. Normally the total solids average from 60 to 70 grams per day on the basis of 1500 cc output.

2. Estimation of urea (Webster).

3. In polyurias, with low specific gravity, it points to chronic diffuse nephritis, and with high specific gravity to diabetes mellitus.

4. Oliguria, with low specific gravity, gives bad prognosis in nephritis.

5. Extensive oedema, with low Sp. gr., points to renal trouble.

6. Normally urines collected at intervals during the day show a variation in the Sp. gr. of 10 points. In chronic nephritis there is a tendency to the fixation of the reading at about 1010 (Hyposthenuria).

Reaction of the urine.

Normal urine is faintly acid, partly from the acid sodium phosphate and partly from free acids, such as sulphuric, oxalic and hippuric, which occur when more animal than vegetable diet is taken. More acid occurs in the morning, and less after heavy meals.

Variations in the total acids:

1. Use of drugs, sodium bicarbonate and acid sodium phosphate.

2. Hyperacidity increases; hypoacidity decreases acid.

3. Less after intestinal hemorrhage and after oedema due to nephritis.

Classification of reactions:

1. Acid.

2. Neutral.

3. Amphoteric, effecting both red and blue litmus.

4. Alkaline.

Alkalinity:

1. Fixed.

2. Volatile. Hold wet litmus in the fumes of the urine and the ammonia there present turns litmus blue. This condition leads one to suspect inflammation of the bladder.

Acidity of urine is due to acid sodium phosphate (NaH_2PO_4)' and free organic acids.

Quantitative determination of acids:

Folin's method:

Urine	25 cc.
Powdered Potassium oxalate (Prevents dissolution of salts of calcium.)	5 to 20 grams
1% solution phenolphthalein	2 drops

Titrate to the end point (a faint pink) with N/10 NaOH.

The acidity is expressed in terms of:

1. Total no. of cc. necessary to neutralize total 24-hour output, 617 is about normal.

2. Percentage, number of cc. necessary to neutralize 100 cc. of urine, 35 to 40 per cent normal.

COLORING MATTER OF URINE

The normal color of urine is a shade of yellow or amber. Ordinarily the color varies directly with the specific gravity, but here are two exceptions: (1) Diabetes, which is associated with a faint yellowish green color and may have a Sp. gr. of 1040 or over. (2) Chlorosis, which may also be associated with a pale urine and high Sp. gr.

Normal pigments of urine.

I. Urochrome, which is responsible for most of the yellow color and which is of little importance clinically. Also urochromogen, precurser of urochrome.

II. Uroerythrin, which is responsible for the salmon-red color. It is a pigment increased on excessive meat diet and in fevers. When extracted with

amyl alcohol it gives characteristic spectroscopic bands.

III. Urobilin, which is a complicated group of pigments, possessing a pyrrol nucleus. Normal urine contains 30 to 100 mg. per 24 hours.

IV. Urobilinogen, which is unstable and by sunlight is changed into urobilin.

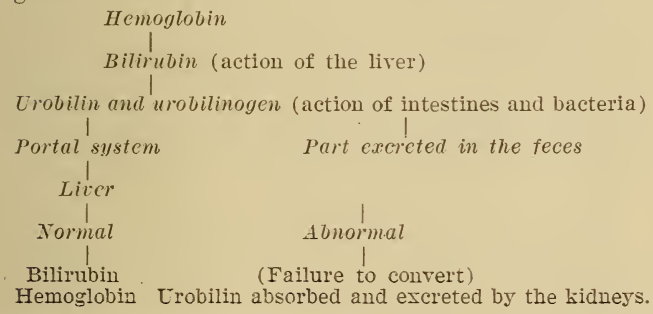
Origin of urobilin:

1. Hematogenous theory, that urobilin may be formed directly from the blood without the intervention of the liver.

2. Hepatic origin. Liver entirely responsible.

3. Nephrogenous theory, that under certain conditions the epithelium of the kidney changes bilirubin into urobilin.

4. Enterogeneous theory, indicated by the diagram.



Significance of urobilinuria:

1. Bile is entering the intestines.
2. Extensive blood destruction.
3. Hepatic insufficiency.
   (a) Physiological.
   (b) Actual hepatic disease.
4. A certain amount of renal efficiency.
5. Absence of urobilin means total obstruction of the bile duct.

Occurrence of urobilinuria:

1. Common in hepatic cirrhosis.

2. Chronic passive congestion (prognostic evidence).

3. Hemolytic anaemia.

4. Malaria.

5. Pneumonia. Appearing at the time of the crisis is a favorable sign, especially if the patient is jaundiced before. If in the serum of such a patient the prognosis is usually hopeless.

6. Measles and scarlet fever and all affections which lead to liver damage. *Appearance of the urine in urobilinuria.* The urine takes on a dark yellowish color. Output is irregular and single voidings show great variations in the amount excreted. Tests for urobilin:

Schlessinger's.

To about 5 cc. of urine add a few drops of Lugal's solution, 1 to 2 cc. of NH_4OH, and an equal volume of 10% alcoholic solution of zinc acetate. Filter and examine filtrate for greenish fluorescence.

Spectroscopic examination:

Extract with amyl alcohol.

Alkaline solution gives broad band between E and F.

Acid solution gives intensified bands in same place and the E—b space is filled.

Ehrlich's benzaldehyde test.

Use a 2% solution in concentrated HCl. Use 3 drops of this reagent to 3 to 5 cc. of urine. The presence of a pyrrol derivative gives a cherry-red color. Heating before adding reagent gives intensification of same.

V. Indican (Indoxyl sulphate) 5 to 45 mg. per day. Animal proteins alone give it.

Tryptophane = { indol, skatol } absorbed = { indoxyl, skatoxyl }

These are conjugated with sulphuric acid and excreted as sodium or potassium salts. Indicanuria is increased upon a meat diet and is not excreted on a non-protein diet. Urine is generally normal color when voided, but on standing becomes dark.

Formation:

1. Extra-intestinal, due to protein decomposition in the body (bronchitis, abscesses and empyemias).
2. People with inborn errors of metabolism.
3. Gastro-intestinal tract diseases or disorders:
   (a) Transient phenomena. (b) Constant occurrence. (c) Recurrent type due: 1, to pathological conditions of intestinal tract. 2. Perversion of intestinal tract secretion.

Indicanuria is not necessarily associated with constipation, but is due to pathological conditions in the lining of the intestinal tract or of its secretions. Subacidity may give it.

Tests for indican (Obermayer's).

Equal parts of urine and reagent (0.2% solution of ferric chloride in fuming HCl). It is best to filter out bile pigments with $PbSO_4$ before adding reagent. To the urine and reagent add 2 cc of chloroform and shake 12 times. Chloroform extracts indican (blue), which sinks to the bottom of the test tube. KI gives a deep cherry red color with the same test. Albumin, unless present in large amounts, does not interfere with the reaction. Thymol gives violet red shade, which is obviated with sodium thiosulphate.

VI. Hematoporphyrin. Occurs in urine in such small amounts that it is normally difficult to detect. It is an iron-free derivative of hemoglobin.

Increased amounts occur in:

1. Certain diseases, rheumatism, phthisis, Addison's disease, paroxysmal hemoglobinuria, exophthalmic goiter, lead poisoning, syphilis and other diseases.

2. Use of hypnotics such as trional, veronal, sulpronal and tetronal.

Test for Hematoporphyrin.

Strictly spectoscopic. Take 5 cc of urine and add 10 cc of 10% NaOH. Filter and to the precipitate add 5 to 10 drops of dilute HCl and 15 cc of alcohol. Filter and examine the filtrate spectoscopically.

Abnormal pigments of the urine.

A. Blood pigments: Hemoglobin, methemoglobin, hematin and hematoporphyrin.

Occurrence:

I. *Hematuria.* A condition in which blood as such is present in the urine, and is visible with the naked eye or with the microscope. The urine is turbid, red-tinged, smoky, and sometimes clots are seen.

Occurrence of hematuria:

1. General diseases. Yellow fever, typhoid fever, smallpox, leukemia, and purpura.

2. Renal origin: (a) Acute congestion or inflammation, (b) acute congestion following poisons, (c) renal infarction, (d) stone in kidney, (e) tbc. of kidney, (f) tumors of kidney, (g) parasites, such as filaria and bilharzia.

3. Genito-urinary tract conditions: Passage of stone, Dietl's crisis, stone in bladder, tumors of bladder, and urethral conditions similar.

4. Traumatism, operative or accidental.

5. Renal epistaxis. In this condition sudden

unexplained hemorrhages occur with no pain. It occurs in one or the other kidney and does not lead to more serious conditions later on.

II. *Hemoglobinuria.* In this condition the formed elements of the blood are absent and merely the pigments are present. The urine is usually clear and dark brown.

Occurrence:

1. Toxic conditions, following severe burns, exposure to cold, poisons and fevers.

2. Essential or paroxysmal type characterized clinically by:

(a) Pronounced hemoglobinuria.
(b) Aching in the lumbar region.
(c) Chills, fever and headache.

This type often follows exposure to cold. In this condition the patient's own blood has amboceptor, which is capable of uniting with patient's own corpuscles. Cause not known. Majority are spyhilitics, acquired or congenitally so.

Tests for blood and hemoglobinuria:

Chemical tests.

1. Heat and acetic acid test. Brown coagulum forms, which tends to float. Decolorized with acid alcohol.

2. Heller's test. Make urine alkaline with sodium or ammonium hydroxide, gently warm. A precipitate of phosphates and carbonates forms, which turns brown.

3. Teichmann's hemin test. To urine add NaOH. Filter and wash with water. Dry by pressing between filter papers. Fragments of ppt. are placed upon glass slide, to which is added a crystal of NaCl. Add three drops of glacial acetic acid and place cover slip over mixture. Heat gently, do not

boil, and as acid evaporates replace it. As soon as the material becomes brown, allow to cool slowly. A positive test shows, microscopically, crystals rhomboid in shape and in sheathes. These crystals are hyprochlorate of hematin.

Errors: 1. Heating too much.
2. Too rapid cooling.
3. Excess of NaCl.

Sensitive in dilution 1 to 100,000 parts.

Guiac Test: For blood—

1. Make fresh tincture of guiac with alcohol; should be shade of light yellow; mix with equal volume of ozonized oil of turpentine, or H_2O_2.

2. Four or five cc urine, to 6 to 8 drops glacial acetic acid; allow to stand 8 minutes; extract with ether.

Pour solution 2 upon 1 in such manner as to form layer; a deep blue ring will form if blood is present.

Benzidine Test for Blood—

1. Two per cent. alcoholic solution is taken and mixed with equal volume ozonized oil of turpentine, or H_2O_2.

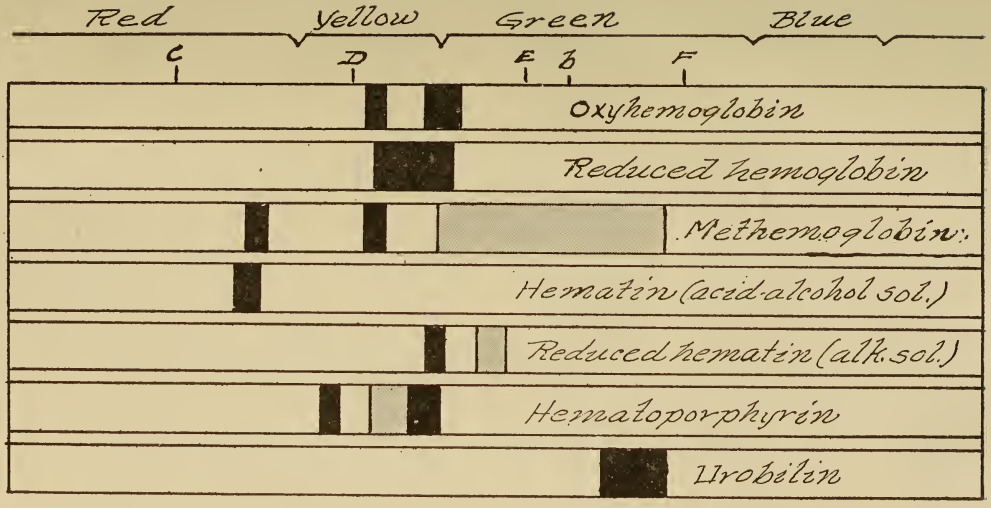
2. Four to five cc urine plus 6 to 8 drops glacial acetic acid; let stand 8 minutes and extract with ether.

Stratify 2 upon 1; a greenish ring will form at line of contact if blood is present.

III. *Methemoglobin.* Occurs in spontaneous decomposition of blood; also following poisoning by the chlorates, nitrates, arsenic, acetanilid, antipyrene. sulphonal, turpentine.

IV. *Hematoporphyrin.* See previous discussion.

B. Bile pigments. Bilirubin is the most frequent.
Sources of choluria (a) Hepatic origin.
(b) Hematogenous origin.


Red
Yellow
Green
Blue
C
D
E
b
F
Oxyhemoglobin
Reduced hemoglobin
Methemoglobin
Hematin (acid-alcohol sol.)
Reduced hematin (alk. sol.)
Hematoporphyrin
Urobilin

Hepatic origin occurs:

1. Cirrhosis of the liver.
2. Cancer of the liver.
3. Obstruction to the biliary passages.

Hematogenous origin occurs:

1. Where blood pigments are present in excess of the mobilizing power of the liver, as in pernicious anaemia, malaria, pneumonia, yellow fever.

(All jaundice is essentially obstructive in origin.)

Appearance of the urine in choluria: greenish yellow, brown, pure green.

Tests for bile:

1. Foam test. Shake a specimen of urine which produces foam. If bile is present the foam persists and is of yellowish color. Normal urine does not produce much foam, and the foam which is produced is white in color.

2. Gmelin's test.

Regent: Strong HNO_3 plus HNO_2. This can be made by boiling con. HNO_3, in which is placed a match stick, until it takes on a yellowish color.

Method: The urine is layered upon the reagent thus made. A positive test shows a green ring at the line of contact, and a yellow ring slightly above. Below the line of contact appear a series of colors ranging from blue to red from above downward.

Errors: 1. Too much NHO_2.
2. Too much albumin.
3. Urine containing too much indican.
4. Urine too concentrated.

5. Urine containing antipyrene or thymol. These drugs can be ruled out satisfactorily.

Rosenbach's modification of Gmelin's test.

Acidify urine with HCl and filter 4 or 5 times through same filter paper. Let paper dry. Touch a single drop of HNO_3 plus HNO_2 solution to the paper. Concentric rings will appear, green on the outside.

Smith's test.

Make urine acid with 5% acetic acid. Layer upon urine 1% alcoholic solution of iodin. Bile pigments give emerald green at line of contact some time after, which tends to diffuse upward. (Rather indefinite test.)

Nakayama's test.

To 5 cc. of urine add from 5 to 10 cc. of 10% solution of barium chloride. Remove ppt. by decantation. Treat the ppt. with the following reagent:

95% alcohol	99 cc.
Con. HCl	1 cc.
Ferric chloride	.4 gm.

Bring slowly to a boil. Bile pigments give emerald green. If urine changes to green, add HNO_3 and get red color. (Indefinite test.)

C. *Melanin.* Normal pigment of hair and choroid coat of the eye. Pathological increase due to over-activity of the cells which form it.

Occurrence: Melanotic sarcoma, Addison's disease, ochronosis and sometimes in malaria. Urine is colorless when excreted, but turns dark either upon standing or the addition of alkaline or oxidizing agent.

Tests: All three of the following must be positive:

1. Ferric chloride gives black ppt.
2. Precipitate soluble in sodium carbonate (black).
3. Mineral acids plus sodium carbonate solution give black ppt.

D. *Alkapton bodies.* When in urine designated alkaptonuria. Occurs in people who have an inborn hereditary error in metabolism. It is characterized by the inability of the body to break up the benzene ring. Tyrosin and phenylalanin are changed to uroleucin and homogenstic acid. Urine is normal in color when voided, but becomes dark on standing. Occurs in children whose parents are first cousins. It is a life-long condition, but not dangerous. Sometimes victims develop ochronosis.

E. *Phenol derivatives.* These substances consist of sulphuric acid in conjugation with phenol para cresol, pyrocatechin, and hydroquinone. They are excreted mostly in conditions associated with putrefaction in the intestinal tract or elsewhere in the body.

Sources: (a) Administration of drugs. (b) Protein metabolism.

F. *Diazo compounds,* due to alloxyproteic acid.

Tests: Ehrlich's Diazo Reaction.

Reagent: Solution a. Aqueous sol. sodium nitrite 0.5 %

Solution b. Sulphanilic acid 5 grams
Concentrated HCl 50 cc.

Water q. s. ad 1000 grams

1 part of sol. (a) plus 50 parts of sol. (b) plus equal vol. of urine. Shake quickly. Add .1 volume of

NH_4OH and shake quickly. A positive test is a deep-red color throughout the urine and a red foam. Red foam is the most important. After 12 hours a granular greenish precipitate occurs. When doubtful, wait for this test. Naphthalene, opium, chrysarobin and other drugs may give color quite similar, but the foam is not red and no greenish ppt. is obtained.

Occurrence: The diazo reaction is never found in health. It does occur in typhoid fever, where it finds its most useful application. It is said to occur in the first or second week of the disease in 80% of the cases. In case of relapse the test again becomes positive. The reaction also occurs in pneumonia, scarlet fever, very frequently in measles before the rash, and sometimes in tuberculosis. It never occurs in rheumatic fever or meningitis. If the test is persistently positive in tuberculosis, it indicates a progressive lesion.

Ehrlich's egg yellow test: This is due to the presence of urobilinogen. This test is the same as the former, except that after adding the NH_4OH the urine takes on a yellow color.

Occurrence: Just prior to the crisis in pneumonia.

Russo's test: 5 cc. of urine plus 5 drops of 1-1000 methylene blue. Positive reaction gives emerald green color. Blue color is negative.

Occurrence: It is said to be negative in miliary tbc and positive in typhoid (Dr. Evans did not find it satisfactory).

Positive reaction said to occur in measles, smallpox and chronic and suppurative tbc.

The reaction is said to be negative in varioloid, varicella, scarlet fever, miliary tbc, appendicitis and malaria.

G. *Medicines.* Phenolphthalein, eosin, salol, methylene blue, etc.

H. *Chyluria,* characterized by the excretion of emulsified fat. Urine varies from cloudy yellow to milky appearance. The surface is covered with a layer of free fat.

Causes: 1. Parasitic. Observed in the Far East, due to infection by one form of filaria. Elephantiasis is often associated with it.

2. Non-parasitic. Etiology unknown. Occurs spontaneously and intermittently in people otherwise well. Sometimes due to rupture or obstruction of cysterna chyli.

I. *Lipuria.* Characterized by the excretion of fat in microscopical quantities.

Causes: 1. Large ingestion of fats.
2. Extensive bone injuries.
3. In diabetes.
4. Frequent in tbc.
5. After catheterization when oil is used.

TABLE OF COLOR OF URINE AND ITS CAUSE.

Yellow and Orange	*Pink and Red*
Normal	Hematuria
Urobilin	Hemoglobinuria
Choluria	Hematoporphyrin
Medicinal	#when alkaline
Rhubarb#	Pyramidon
Senna#	Trional
Santonin#	Sulphonal
Phenolphthalein#	Eosin
Chrysophanic ac#	Rosanilic acid
Cascara#	

Brown and Black	*Blue and Green*	*Milky or Whitish*
Choluria	Methylene blue	Lipuria
Melanuria	Urobilin	Chyluria
Alkaptonuria		Phosphates
Phenol derv's.		Bacterial infect.
"Black Water Fever"		Dirty container.
Indicanuria		
Medicinal		
Salicylic ac.		
Salol		
Resorcin		

Optical activity of urine: Normally levo-rotatory from .01 to .18 deg.

Increased: 1. In excretion of levulose.
2. Glycuronic acid excretion.
3. Albumin (½ gm. per 1000 cc.).
4. Diabetes (B-Oxybutyric acid).

INORGANIC URINARY CONSTITUENTS.

The composition of the urine depends upon the diet of the individual, both in quality and quantity. Normally 1200 to 1500 cc. are excreted daily with 60 to 70 gms. of solids, organic and inorganic.

Inorganic	25	to	30	gms.	*Organic*	35	to 40	gms.
NaCl	10	to	15	gms.	Urea	15	to 40	gms.
P2O5	1	to	5	gms.	Ammonia	.7	to 4.27	gms.
SO3	1	to	3½	gms.	Creatinin	1.0	to 1.5	gms.
CO3	?	to	?	gms.	Uric acid	.5	to 1.25	gms.
Na2O	4	to	7½	gms.	Hippuric acid	.7		gm.
K2O	2	to	4	gms.	Other			
CaO	.1	to	.3	gm.	constituents	1.5	to 2.3	gms.
MgO	.1½	to	.4	gm.				
Fe	1	to	11	mg.				

Chlorides. The excretion of chlorides depends upon the intake in health.

Source: (a) Vegetable foods in combination with K and Ca.
(b) NaCl seasoning most important source.

Chlorides are taken in order to prevent the accumlation of K salts in the blood. There is a chloride balance as characteristic as the N balance. Increased ingestion of NaCl is followed by increased excretion. Chloride poor or free diet gives a drop in the excretion to a certain extent, but no further. The proportion in the blood is a constant, as well as that in the tissues.

Retention occurs:

1. Active stage of all fevers. In pneumonia there may be a suppression till the crisis. A chloride crisis also occurs.
2. Formation of transudates and exudates.
3. Profound and continuous vomiting and diarrhoea.
4. Hyperacidity.
5. Oedema (myocardial or nephritic).

Theories of retention.

1. Histo-retention theory (Strauss and Loeb): Excess of salts in the tissues requires water to maintain osmotic pressure.
2. Colloidal (Fisher): In cases of oedema the colloids in the tissues have increased hydrating capacity.

Salt excretion:

1. Following febrile states.
2. Absorption of transudates and exudates.
3. Polyuria.
4. Pneumonic crisis.
5. Following oedema from salt retention.
6. After chloroform anaesthesia.

Quantitative tests:

1. Volhard's method (1874): Precipitation of chlorides in a known volume of urine by an excess of standardized solution of $AgNO_3$ in HNO_3, and then the titration of the excess of $AgNO_3$ with NH_4SCN, using iron ammonia alum as indicator. (Objection to method is that it requires 4 solutions.)
2. Arnold's method, a modification of the former (1885). Differs from the former in the strength of the standard solution and filtration after addition of $AgNO_3$.

Solutions: 1. $AgNO_3$ solution such that 1 cc. equals .01 gm. NaCl or 29.075 gm. $AgNO_3$ in 1000 cc. H_2O.

2. NH_4SCN solution such that 2 cc. equals 1 cc. $AgNO_3$ sol.

3. Saturated solution of iron ammonia alum.

4. Pure HNO_3.

Procedure:

Urine	10 cc.	Place in a 100 cc. volumetric flask for 10 minutes, agitate from time to time. Overcome coloring by 3 to 5 drops of 10% sol. of potassium permangnate. Add H_2O up to 100 cc. and filter.
HNO_3	5 cc.	
Std. $AgNO_3$	20 cc.	
H_2O	50 cc.	

To 50 cc. of the filtrate add 5 cc. of the iron ammonia alum sol. for indicator and titrate with NH_4SCN to end reaction, which is red-brown color.

3. Lutke Martius method (1892):

Solution: A. AgNO. 17.5 gms.
25% HNO_3 900.0 cc.
{ 25% HNO_3 equals 225 cc. con. NHO_3 in 675 cc. of distilled H_2O }
10% iron ammonia alum 50.0 cc.
Water q. s. ad. 1000.0 cc.

B. N/10 NH_4SCN

Titrate solution A against a N/10 HCl solution.

In making solution B dissolve 7.6 gm. of NH_4SCN in less than 1000 cc. of water. Add water till 1 cc. of it equals 1 cc. of A, which is determined by titration.

Method of performing determination:

10 cc. of urine plus 25 cc. of N/10 $AgNO_3$ plus water q. s. ad 100 cc. Add a few drops of potassium permangnate to decolorize urine if necessary. Allow to stand and filter or draw off 50 cc. Titrate the filtrate with N/10 NH_4SCN to the end point, using iron ammonia alum as indicator.

Calculation: Example 6.2 cc. N/10 NH_4SCN to produce end reaction.

2 times 6.2 equals 12.4 cc. $AgNO_3$ are uncombined in 10 cc. of urine.

25 minus 12.4 equals 12.6 cc. N/10 $AgNO_3$ are combined as AgCl in 10 cc.

1 cc. N/10 $AgNO_3$ equals .00585 gm. NaCl.

12.6 times .00585 equals gms. NaCl per 10 cc. (10 to 15 gms. per 24 hrs. normal).

Principle: When N/10 NH_4SCN is added it combines with the unused N/10 silver nitrate as long as there is any present. When it is all in combination with NH_4SCN it begins to combine with the iron ammonia alum, which gives a brown color and is the end point in the reaction.

Purdy's centrifuge method:

10 cc. of urine, 1 cc. of NHO_3 and 4 cc. of a 5% solution of $AgNO_3$ are placed in a special centrifuge tube. Agitate and centrifuge at 1200 revolutions for three minutes. Read the number of grams per 1000 on the scale.

Bayne-Jones method:

Either use a special flask or an ordinary 25 cc. graduate. In case the special flask is used, the urine is added to the mark U. Titrate with N/20 NH_4SCN solution to the end point and read grams per liter on the scale (iron ammonia alum indicator).

In case the 25 cc. graduate is used, add 5 cc. of urine and 10 cc. of N/20 $AgNO_3$. Titrate as before with N/20 NH_4SCN, and note the reading.

Calculation:

V equals total volume at end of titration.
V-15 equals number cc. of N/20 NH_4SCN

$\frac{V-15}{2}$ equals number cc. N/10 $AgNO_3$ uncombined

$10 - \frac{V-15}{2}$ equals number cc. N/10 $AgNO_3$ combined as AgCl

1 cc. N/10 AgCl equals .00585 gm. NaCl

$10 - \frac{V-15}{2}$ X .00585 equals gm. NaCl in 5 cc. urine

$10 - \frac{V-15}{2}$ X .00585 X 200 equals gm. NaCl in 1000 cc.

20.475 minus .585 V equals gms. per 1000 cc.
(.1 to .3 gm. error)

Phosphates. Excreted as sodium, potassium, calcium and magnesium salts of H_3PO_4 as well as with glycerin from the breaking down of lecithin.

Source: 1. Food ingested.
2. Breaking down of proteins, ingested or endogenous. Nucleo-proteins most abundant source.

Amount: 1 to 5 gm. per day expressed in terms of P_2O_5

Excretion: Depends upon amount taken. Greater on alkaline diet. Majority of earthy phosphates are excreted in the feces (Ca and Mg). Phosphate metabolism is still in a state of uncertainty, for it is not known where P_2O_5 is stored. Bone takes up a great amount, but does not account for amount ingested. Ratio between N excretion and P_2O_5 is P:N::1:7.

Increased:
1. Animal diet.
2. Physical exercise.
3. Starvation.
4. Conditions where protein disintegration is going on.

5. Phosphatic diabetes, where urine has characteristics of diabetes, but no sugar. There is associated with it dryness of the skin and excessive thirst. The c-h metabolism is not abnormal.
6. Phosphorous poisoning.
7. Meningitis and tuberculosis.
8. Emotional states.
9. Mental work.

Decreased:

1. Vegetable diet.
2. Certain diseases:
   Addison's disease,
   Hepatic cirrhosis,
   Certain types of nephritis,
   Acute yellow atrophy,
   Chronic lead poisoning,
   Certain types of bone disease.
3. Pregnancy.
4. Runs parallel with uric acid excretion in gout.

Phosphaturia is characterized by the frequent and constant excretion of cloudy urine at the time of voiding. It is noticed mostly in nervous, sexual neurasthenics of the male sex. Noticed also in cyclic vomiting of children and hypoacidity. This condition represents not a disease, but a condition in which the amount of acidity of urine is diminished, now called alkalinuria.

Sulphates. Excreted in three forms:

1. Preformed or neutral.
2. Conjugated or ethereal.
3. Neutral, unoxidized or organic.

Total excretion about 2½ gm. in 24 hours on a mixed diet, expressed in terms of H_2SO_4.

Source: Amount in diet relatively small. Most

of it arises in the destruction of protein. Amount excreted increases with increase in protein catabolism.

Ethereal sulphates result from the conjugation of indol, saktol and phenol with sulphuric acid, and can be taken as a fair estimate of protein decomposition.

Occurrence:

1. Excessive intestinal putrefaction.
2. Rich protein diet.
3. Hypoacidity.
4. Massive pus formation anywhere in body.

Neutral sulphates: increased in jaundice and cystinuria.

Carbonates: Vary with the amount of carbonate forming material in the food. Vegetables contain organic acids which are easily converted into carbonates. Generally sedimented as $CaCO_3$.

Sodium and potassium: Excreted as salts, amount determined as Na_2O (4—7½) and K_2O (2—4).

Sources:

1. NaCl in the food.
2. Potassium in vegetable foods.

The excretion is of little significance clinically.

Calcium and magnesium: Excreted generally as phosphates (CaO .1—.3) (MgO .1½—.4).

Source: Food, most of it is lost in the feces; bone destruction, diabetes; excretion is little understood; its significance is increasing.

Iron: Always present in small amounts. Increased in blood destruction, such as fever, malaria, pernicious anameia. In diabetes it runs parallel with the sugar output, 2½ mg. of iron to 100 grams sugar.

Heavy metals: Mercury, lead and arsenic found pathologically.

NITROGENOUS BODIES OF URINE.

Amount normally excreted varies between 10 and 16 grams a day. Amount excreted depends upon:

1. Amount of food ingested.
2. Tissue metabolism.

In health there is a nitrogen equilibrium, i. e., the N. excreted equals the N ingested. In starvation a level is reached on the fourth or fifth day, which will last three or four weeks. When food is again taken the amount of excreted N is less than the ingested N. People feel best on from 4 to 6 grams of N excretion.

To determine the N excretion the following factors must be taken into consideration:

1. Total N of food.
2. Character of food in terms of ability to produce alkaline or acid urine.
3. Age of patient.
4. Previous state of nutrition.
5. Previous diet. Previously there should be seven days of diet with regulation of:
   a. Diet,
   b. Exercise,
   c. Amount of food intake.
   d. The condition of the intestines.

Nitrogen Partition:

	Mixed D.	N-free.	Mixed.	N-free.
Total N.....	16 gms.	3.6 gms.	100%	100%
Urea	13.9	2.2	86.87	61.7
NH_3.....	.7	.42	4.37	11.3
Uric Acid	.12	.09	.75	2.5
Creatinin	.58	.6	3.63	17.2
Undetermined..	.7	.29	4.37	7.3

Urea N represents great bulk of total N output in 24 hours. In N free diet, urea N decreases; ammonia and creatinin are increased, at expense of urea.

Nitrogen excretion:

Physiological—Increased:

1. On heavy protein intake.
2. Four to 6 days after birth.
3. Excessive intake of water.
4. Exercise.

Decreased on diets rich in fats and carbohydrates.

Pathological—Increased:

1. In acute febrile conditions with high fevers. There is no relation between the height of temperature and the amount of N excreted. N balance can be maintained by proper diet.
2. Chills.
3. Increased respirations. Urea is relatively decreased, while ammonia salts, organic acids and uric acid are increased.
4. Toxic conditions, such as cancer, leukemia, exophthalmic goiter.
5. Diabetes mellitus and insipidus.
6. Absorption of exudates.
7. Nephritis, especially in albuminuria of marked degree.

Decreased:

1. Convalescence after fever.
2. Dysentery.
3. Formation of exudates.

Methods of N determination.

Kjeldahl:

The principle of this method is the oxidization of the organic products with the production of sulphates. NH_3 plus H_2SO_4 equals $(NH_4)_2SO_4$. The NH_3 is liberated by the addition of strong NaOH and is received in a known amount of standard acid.

Procedure:

Into a pyrex kjeldahl flask place

Urine	10 cc.
K_2SO_4	10 grams or a teaspoonful (oxidizer).
$CuSO_4$	1 medium-size crystal (catylitic action).
Con. H_2SO_4	15 cc.

Heat gradually to the boiling point and continue to boil till the solution takes on an absolutely green color, and boil five minutes longer. Allow the solution to cool and then add the following:

Water	250 cc.
Talcum	2 tablespoonsful.
40% NaOH	120 cc. This should be added last and in such a manner that it does not mix with the solution. Pour on side of tilted flask and it will go to bottom.

Connect the flask with a distilling apparatus and mix the contents by shaking. Either distill 30 minutes or distill over 150 cc. This distillate is received in 25 cc. of N/4 H_2SO_4 and the excess of acid is titrated with N/10 NaOH, using phenolphthalein as an indicator.

1 cc. N/10 H_2SO_4 equals .0017 grams NH_3.

1 cc. N/4 H_2SO_4 equals .00425 grams NH_3.

N equals .823$\times NH_3$ by weight.

.00425$\times$.823 equals .003497 grams N for each cc. of N/4 H_2SO_4.

.0017$\times$.823 equals, .0014 grams N for each cc. of N/10 H_2SO_4.

Folin's method:

This is a colorimetric test. 1 cc. of urine is put through the oxidizing process and received in

N/HCl. 5 cc. of Nessler's reagent:

HgI	10 grams
KI	5
NaOH	20
H_2O	100 cc.

are added. The color of this is compared with a standard color made up of 5 cc. of the reagent plus 1 mg. of N.

Urea.

About 80 to 90% of the total N in a mixed diet is excreted in the form of urea. 60 to 65% of the total N is excreted in the form of urea in an N-free diet. From 15 to 60 grams, or an average of 30 grams, are excreted in a mixed diet per day.

Modes of formation:

1. NH_3 salts. (Protein—hydrolysis—amino acids —NH_3.) Action of bacteria and enzyme action of tissues.
2. Deamidization method. (Protein—amino acid —blood—tissues—protein.) When amino acids are in excess NH_3 is split off in the liver and oxy-organic acids are formed. The liver protects the body from NH_3 poisoning.
3. Arginin plus arginase gives urea and ornithin.

Variations in excretion.

1. Physiological } Increased and decreased same
2. Pathological } as total N.
3. Relation of urea to liver function.
4. Relation of urea to renal function. One can't determine the renal function by the estimation of the urea alone, but one must take

into consideration the amount of urea in the blood.

5. Relation of urea to acidosis. Normal reaction of blood alkaline. The fixed alkalinity of the blood can't be reduced beyond a certain point without certain things happening. As an excess of acids is formed, an excessive amount of NH_3 salts is formed. Urea decreases as output of NH_3 increases.

Qualitative tests for urea. (Not used clinically.)

1. Biuret. Crystals of urea heated to boiling give cyanuric acid and biuret. Add $CuSO_4$ and NH_4OH and get blue color.
2. Furfurol. 1 to 2 drops of furfurol plus 1 to 2 drops con. HCl, plus 1 to 2 drops urine. Positive test gives dark blue to black color.

Quantitative estimation of urea:

Knop-Hufner method.

This test relies upon the decomposition of urea with sodium hypobromide and the measurement of the N evolved. The reaction takes place according to this equation: $CO(NH_2)_2 + 3NaOBr = 3NaBr + 2N + CO_2 + 2H_2O$. The CO_2 is absorbed in an excess of alkali and the amount of N gas measured.

Doremus Ureometer.

The principle of the Knop-Hupfer method is used and a graduated ureometer measures the amount of gas.

Solutions:

1. 20% NaOH.
2. Bromine.

When ready to use, add 1 cc. of the bromine to 40 cc. of the NaOH solution. Fill the ureometer tube and add by means of a curved pipet 1 cc. of urine to the upright part of the tube. Read grams off on the scale. Method unsatisfactory.

Folin method.

The principle of this method is that crystalized magnesium chloride boils in its own water of crystalization at 160 degrees centigrade. If urea be present it is decomposed into ammonia and carbon dioxide. If acid be present the ammonia formed will combine with the acid and can be titrated.

Urease method (Marshall).

The principle of this method is this: The soy bean extract contains an enzyme capable of splitting urea into ammonium carbonate.

Characteristics of the enzyme:

1. It is soluble in water.
2. It reacts quantitatively with urea. It will split off a certain amount in a given time and no more.
3. It does not form NH_3 from any other source than urea, and will form NH_3 in the presence of any other substance, except (5).
4. Its optimum activity is 55 deg. C.
5. Its activity is destroyed by acids, heavy metals and alcohol above 20%.
6. It has a self-retarding effect. After a certain amount of $(NH_4)_2CO_3$ is formed its action becomes inhibited.

Direct method of performing test:

Test tube A. 10 cc. of urine.

Test tube B. 10 cc. of urine.

1—2 urease tablets dissolved in water.

Allow these to stand:

1. At room temperature over night, or
2. 45 to 50 deg. C for 1 hour, or
3. 37 deg. C. for 3 hours.

Titrate both tubes for alkalinity, using methyl orange as an indicator.

Subtract the no. of cc. used in titrating A from the no. used in titrating B. 1 cc. of N/10 HCl equals .003 grams of urea or .0014 grams N.

The disadvantage of the test is that the whole solution becomes clouded and the end reaction is an obscure one.

Indirect method.

Test tube A. 5 cc. of urine.
Aqueous solution of 1 or 2 urease tablets.
A layer of kerosene to prevent foaming.
Stopper and allow to stand as before.

Test tube B. 25 cc. of N/10 HCl.
2 drops of 1% sol. alizarin.
A layer of kerosene.

After tube A has stood its proper length of time, connect it up to a suction apparatus in such a way that the indrawn air first passes through a solution of H_2SO_4. Allow air to be drawn through for about a minute in order to remove any free ammonia that may be present. Now, connect tubes A and B in such a way that the air current passes from A into B. When this has been done, add a teaspoonful of dry potassium carbonate to tube A, quickly cork, shake and start suction apparatus. Allow air current to pass from A into B for about 30 minutes. The ammonia in A is freed and caught in the N/10 HCl in B. Titrate the excess of HCl in B with N/10 NaOH.

Subtract the number of cc.'s necessary to bring about the end reaction from 25 cc., the amount of N/10 HCl originally added. Repeat this process, using 5 cc. of urine to which no urease solution is added. This gives the preformed ammonia. This value subtracted from the former gives the amount of NH_3 formed from urea.

Make the calculation of the amount of urea on

the basis of: 1 cc. of N/10 HCl equals .003 grams of urea or .0014 grams N.

Uric acid.

Amount:

.2 to 2.0 grams per day.

Mixed diet .37 gm. or .8% total N.

N-free diet .09 gm. or 2.5% total N.

Origin.

Uric acid is not a product of protein decomposition.

Nucleo-protein plus pepsin, trypsin, etc., gives nucleic acid and protein.

Nucleic acid plus tetranuclease gives purin and pyrimidin dinucleotide.

Purin nucleotide plus phospho-nuclease and purin nuclease give: the former, adenosin and guanosin; the latter, adenin and guanin.

Adenin plus adenase gives hypoxanthin.

Guanine plus guanase gives xanthin.

Adenosin plus adenosin deaminase and guanosin plus guanosin deaminase give xanthin and inosin, which when the c-h radical is split off give xanthin and hypoxanthin.

Xanthin and hypoxanthin plus xanthin oxidase (liver) gives uric acid.

Sources of uric acid:

1. Exogenous. The greater portion of nucleic acid comes from the diet: sweet bread, liver, caffein and theobromin.
2. Endogenous.
   a. Breaking down of tissue nuclei.
   b. Purin bases free in the body.
3. Synthesis. There is no evidence that this occurs in man, but it does occur in birds.

Uric acid destruction in the body.

In birds it is destroyed by ferments and allantoin is the excretory product.

The following facts are known:

1. Uric acid fed can be recovered quantitatively in the urine.
2. Endogenous purine metabolism in persons on an N-free diet is constant.
3. Uric acid can be broken down by other routes than allantoin.
    a. Glycocoll.
    b. Oxalic acid-urea.
    c. Persons fed on purin bases excrete half in the form of urea. Purin bodies can be excreted in forms not going through the uric acid stage.
4. When uric acid reaches the system it is excreted as such.

Characteristic properties of uric acid.

In the cold it is sparingly soluble in H_2O (1—40,000). It is fairly soluble in blood serum (1—1,000). Its best solvent is a solution of urea. It is insoluble in alcohol, ether, chloroform and acetic acid. It is somewhat soluble in HCl, H_2SO_4 and weak alkalines. Solutions of uric acid in water are neutral to litmus. Uric acid in sufficient concentration reduces Fehling's solution, but not Nylander's. Uric acid is kept in solution under the following conditions:

1. With neutral phosphates.
2. When pigments are present.
3. As neutral urates.
4. As monosodium or biurate—

    Ammonium biurate (urinary type of sediment).

    Sodium biurate (gout).

Excretion of uric acid.

Increased:

1. Purin or meat rich diets.
2. Increased protein decomposition.
3. When leucocytosis is predominant feature in leucaemia, especially when treated with X-ray. From 12 to 15 gms. per day is sometimes excreted.
4. Pernicious anaemia. (In secondary anaemia less than normal.)
5. Articular rheumatism and after medication with salicylates, atophan, colchicum and urotropin.

Decreased:

1. Vegetable diet.
2. Certain types of chronic nephritis.
3. Chronic lead poisoning (diagnostic).
4. Quinine and opium.

Uric acid in relation to gout: All theories have fared badly.

1. In cases of chronic gout the excretion of uric acid upon a given diet will be the same as in normal individuals on the same diet in most cases.
2. The excretion of uric acid in chronic gouty individuals persists in being lower than normal.
3. Gouty patients fed with purin bases will show a lower curve of uric acid excretion than normal.
4. On a purin free diet gouty patients will show a lower endogenous excretion than normal.
5. The blood of gouty patients contains more uric acid than normal, 4 to 5 mg. instead of 1 mg.
6. The elimination of uric acid is less than normal from 1 to 3 days preceding an acute gouty attack.
7. With the start of the acute symptoms there is a rise of the excretion above normal which

lasts for three or four days, and then is followed by a much lower output than normal.

8. The relation between an excess of uric acid in the blood and in the urine in acute gouty manifestations is not known.
9. Gouty individuals show utter abnormal conditions of metabolism.
10. Probable explanation of gout is that gouty individuals do not possess necessary uric acid elimination and certain tissues seem to possess an affinity for uric acid.

Factors modifying uric acid excretion.

1. Diet.
2. Tissue metabolism.
3. Indeterminate amount of free purin bases.
4. Amount of conversion of uric acid into urea.
5. Amount of uric acid destruction in the body.
6. How much synthesis occurs.
7. Varying degree of blood retention.
8. Renal capacity of eliminating uric acid.
9. Amount of urinary phosphates.
10. Degree of urinary concentration.
11. Akalinity of urine.

Quantitative uric acid determination.

Folin method:

Urine	300 cc.
Folin's reagent	75 cc.

(gives precititate of phosphates)

Folin's reagent:

Am. Sulph.	500 gm.
Uranium acetate	5 gm.
10% acetic	60 cc.
H_2O q. s. ad.	1,000 cc.

Wait 15 minutes and filter. Place 125 cc. of the filtrate (which equals 100 cc. of urine) in an Ehrlemeyer flask. Add 5 cc. of concentrated NH_4OH, let

stand 24 hours and filter. Collect the precipitate of ammonium urate, wash it with ammonium sulphate until the filtrate no longer shows a reaction with $AgNO_3$, pierce the filter paper and wash the precipitate into a breaker with 100 cc. of water. Add 15 cc. of con. H_2SO_4, heat over a flame, and while still hot titrate with N/20 potassium permanganate (1.567 gm. in 100 cc.) to end point, which is a reddish blush for from 15 to 30 seconds.

Calculation: 1 cc. of N/20 potassium permanganate equals 3.75 mg. of uric acid. (Uric acid has the property of reducing potassium permanganate.)

Correction: For each 100 cc. of urine add 3mg. of uric acid, for a certain amount of urate is soluble and does not change into uric acid.

Ammonia: Depends upon protein intake and runs parallel with N excretion. On a mixed diet about .7 gms. are excreted in 24 hours or 4.37% of the total N, and upon an N-free diet about .42 gm. or 11.3% of the total N is excreted.

Increased:

1. Decreased oxidation.
2. Acute febrile conditions.
3. Liver diseases.
4. Uraemia.
5. Toxic vomiting of pregnancy.
6. Diabetes, characteristically abnormal.
7. Starvation.
8. Chloroform poisoning.

Decreased:

1. Nephritis.
2. Most akaline therapy.
3. Conditions with low HCl.

NH_3 elimination is associated with acidosis. In this condition one finds: 1. Increase of acids with formation of neutral salts. 2. Lowered blood and

tissue alkalinity. 3. Carrying capacity of the blood for CO_2 lowered and CO_2 accumulates in the tissues (tissue asphyxia). 4. Increase in the H concentration of the blood stimulates the respiratory center and air-hunger results.

Occurrence of acidosis:

1. Diseases with clinical symptoms.
   Diabetic acidosis.
   Acute nephritis and acute diseases associated with nephritis.
   In late stages of primary and secondary contracted kindey.
   Food intoxication of children.
   Atrophic cirrhosis.
2. Diseases without clinical symptoms.
   Acute febrile conditions.
   Advanced cachexia.
   Severe anaemia.
   After general anaesthesia.
   Cardiac conditions.

Quantitative ammonia determination.

Schlosing method:

The objection to this method is that the results are too high.

Folin's method:

10 to 20 cc. of urine are introduced into an aerating cylinder and a layer of kerosene added to reduce the tendency to foam. The apparatus is connected with a flask containing a known quantity of N/10 H_2SO_4 (20 cc.) and sufficient water to well cover the absorption tube. About one gram of sodium carbonate is added to the urine and the ammonia liberated is aspirated by means of a suction pump into the known acid solution. The current of air should first pass through a dilute H_2SO_4 solution in order to absorb any ammonia that may be present

in the indrawn air. Aerate for one hour and titrate the excess of acid with N/10 NaOH, using methyl orange or alizarin as indicator..

1 cc. of N/10 H_2SO_4 equals .0017 grams of NH_3.
or .0014 grams of N.

Creatinin: About .56 gm., or 3.6% of the total N, is excreted upon a mixed diet, and about .6 gm., or 17.2% of the total N, is excreted on an N-free diet. It is an anhydride of creatin, but the relationship between the two is not clear. Creatin probably comes from arginin, and creatinin is formed by the action of anhydrating ferments. There is a remarkably constant excretion of creatin irrespective of the N intake. Folin considers creatinin a valuable index of protein metabolism, while Shaffer thinks it an index of some special process of normal metabolism occurring mostly in muscles.

Exogenous elimination depends upon the ingestion of meats.

Endogenous elimination has the following characteristics:

1. Constant on N-free diet.
2. Varies with different individuals.
3. Apparently independent of total N metabolism.
4. Depends upon weight of individual.

A normal individual excretes from 7 to 12 mg. per kilo in 24 hours.

Source: Largely due to muscle metabolism of the body. It is, however, formed in other than muscle tissue. There is a diminished excretion in anaemia, marasmus, myositis ossificans, chlorosis, phthisis, chr. diffuse nephritis, progressive muscular atrophy, and pseudohypertrophic paralysis. It is said to be increased in the acute stages of some fevers and exophthalmic goiter. The N excretion runs more or less parallel with protein decomposition, but creati-

nin does not. The liver is one main source of formation.

Qualitative tests for creatinin.

Jaffe's test:

To urine add picric acid and dilute NaOH (10%), which turns a brilliant shade of red and which becomes darker on standing. Upon the addition of acetic acid it changes to an ochre color.

Weyl's test:

To urine add nitroprussid of soda and a few drops of 10% NaOH. A deep red orange or ruby color appears, which, upon the addition of a little glacial acetic acid, turns to green and upon heating to Berlin blue.

Quantitative determination.

Folin method (colorimetric):

A standard solution is made up by the following method: One gram of pure creatinin is dissolved in 1000 cc. of water. 1 cc. of this plus 20 cc. picric acid plus 1.5 cc. of 10% NaOH plus enough water to make 100 cc. gives the standard color.

One cubic centimeter is treated in the same manner, and the resulting color is compared with the standard solution by means of a colorimeter.

Undetermined Nitrogen.

Mixed diet .7 grams or 4.37%
N-free diet .29 grams or 7.3 %

This is determined by subtracting the sum of the other forms of N from the total N.

Source:

1. Amino acids which are known to be excreted. Tyrosin, leucin, glycocoll (1—2 grams per day). In cystinuria there is a perverted metabolism to amino acid; 181 such cases have been reported. These patients do not have the power to reduce the amino acids

further than cystin. Cystin calculi frequently form. A marked hereditary tendency prevails in this disease in which males are more frequently effected than females. In diaminouria putrescin and cadaverine are excreted.

2. Hippuric acid. 0.1 to 1 gram per day. Benzoic acid foods, such as fruit, berries, prunes, etc., combine with glycocoll.
3. Oxyproteic acid } These are responsible for
   Alloxyproteic acid } Ehrlich's diazo reaction. Their excretion is probably increased in cancer. They contain an S mollecule.
4. Allantoin. This is normally present only in traces. It exists normally and abnormally in conditions little understood.

REDUCING BODIES OF THE URINE.

Subjects to be considered:

1. General review of carbohydrates.
2. Qualitative determination of glucose.
3. Sugars and reducing bodies other than glucose.
4. Quantitative determination of sugar.
5. Scheme for running down reducing substances in urine.
6. Acid bodies.
7. Diabetes.

Carbohydrates.

The available carbohydrates of the body exist as glycogen, which is stored in the liver and muscle tissues until called upon by the tissues for energy, heat and tissue formation. Sugar is constantly being converted by the liver into glycogen and back again into sugar in accordance to the body needs. The balance between these two in health is such that the percentage in the blood is from .1 to .15%.

Origin of carbohydrates:

1. Sugars and starches.
2. Proteins and amine acid bodies.
3. Fats.

Function of carbohydrates:

1. Energy.
2. Heat.
3. Protecting agency for protein.
4. When in excess forms fats.
5. Actual synthesis of tissues (nucleic acid).

Regulation:

1. Supply (glycogenetic function).
2. Storage (glycogenesis).
3. Tissue consumption (glycolysis).

When any one of these breaks down glycosuria may result, also in cases of renal permeability for carbohydrates (renal diabetes and phloridzin diabetes).

The adrenal and pancreas are antagonistic in function. In health the two are in perfect equilibrium, but in diabetes the restraint is removed from the liver. The pancreas also seems to give off something to the tissues which enables them to burn carbohydrates to CO_2 and H_2O. In diabetes this falls short and sugar is eliminated through the kidneys, due to its accumulation in the blood.

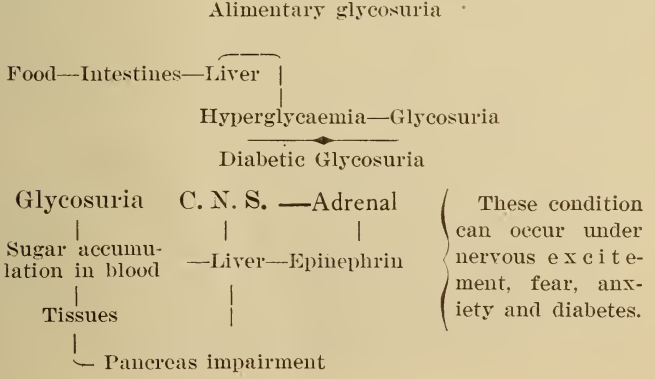
Normal urine contains a small amount of glucose (animal gum or isomaltose). It amounts to about .3 to .6 grams in 24 hours. Glycuronic acid occurs in amounts of about .004 grams in 100 cc. Traces of pentose occur occasionally after the ingestion of fruits. Lactose and galactose occur frequently in sucklings.

Assimulation limit for carbohyrates (two kinds):

1. Individuals who show sugar after the ingestion of large amounts of starch (glycosuria ex amylo).
2. Individuals excreting sugar following sugar intake (glycosuria e saccharo). A normal individual can take as much as 150 to 200 grams of glucose on an empty stomach without showing glucose in the urine. Persons with nephritis have a lower sugar threshold. The liver has the greatest tolerance for glucose and least tolerance for lactose and galactose. Maltose, levulose and saccharose come in the intermediate group.

Hamman's method of making threshold test:

From 150 to 200 grams of dextrose are dissolved in 150 cc. of water and flavored with lemon or orange juice. The mixture is made ice-cold with cracked ice and the volume made up to 300 cc. This is taken slowly on a fasting stomach and the blood and urine sugar are followed together, the blood being withdrawn at 1, 2, 3, 4 and 5 hours after taking and the urine being examined at the same time.



Qualitative test for glucose.

Trommer's test:

To 2 parts of urine add 1 part of 10% NaOH. To this add 10% solution of CuSO4 till a bluish precipitate results and remains after shaking (add the $CuSO_4$ drop by drop). Warm the solution (do not boil), and in the presence of sugar a reddish or yellowish precipitate will form, spreading gradually downward and leaving a decolorized supernatent liquid.

Reaction:

$H_2O + NaOH + 1$ drop $CuSO_4 = Cu(OH)_2$
$CuSO_4 + 2NaOH = Na_2SO_4 + Cu(OH)_2$
$Cu(OH)_2 +$ heat $= CuO$ (black) $+ H_2O$
$2CuO +$ sugar $+$ heat $=$ reduction
Yellow $= Cu_2OH$ (alkalinity low)
Red $= Cu_2O$ (alkalinity high)

The color depends upon the degree of alkalinity. Uric acid, creatinin glycuronic acid, albumin, allantoin, mucin, lactose, maltose and alkapton have the property of holding $Cu(OH)_2$ in solution and reduce it to lower salts by heating. A false reduction by these substances can be avoided by warming and not boiling, for these substances reduce only upon boiling, and it tends to occur on cooling. They give a dirty yellowish green color to the previous blue solution. Dilute urine for more accurate determination.

Requirements necessary for positive reaction:

1. Prompt formation of a precipitate on warming, settling to the bottom and leaving a clear solution above, before cooling.
2. Avoid adding $CuSO_4$ to excess, which gives black precipitate and masks reaction.

3. Any discoloration after prolonged boiling should be neglected.

4. Albumin should be removed before performing test if it is present in large amounts.

Qualitative tests for sugar.

Fehling's test:

Solution A.	$CuSO_4$	34.65 gm.
	H_2O qs. ad.	1000 cc.
Solution B.	Rochelle salts	173 gm.
	NaOH	50
	H_2O qs. ad.	1000 cc.

Equal parts of solution A and B are mixed, which gives a clear blue solution. Boil mixture in a test tube and add urine not over 1/2 by volume. Sugar gives reduction in from 5 to 10 seconds. If no reduction occurs, bring the solution back to boil. Prolonged boiling gives misleading results. Sensitive to .08%.

Errors: Normal Constituents—Uric acid, creatinin, blood pigments, glycuronic acid and phenol derivatives; also reduced by chloroform and formaldehyde. Drugs—Trional, sulphonal, camphor, morphia, salicylates, benzoic acid, antipyrene and phenacetine.

Benedict's test.

Benedict's solution:

$CuSO_4$	17.3 gm.	
Sodium citrate	173.0 gm.	
Sodium carbonate	200.0 gm.	(crystals)
H_2O qs. ad.	1000.0 cc.	

Method of preparing: Sodium citrate and sodium carbonate crystals are dissolved in 700 cc. of hot water. Filter while hot and add a proper amount of solution of $CuSO_4$ to make 1000 cc. This solution is permanently stable, lasting 5 to 10 years,

and the test is more delicate than Fehling's. Sensitive to .02—.05%.

Technique of the test: To 5 cc. of the reagent add 5 drops of urine. Boil vigorously for 2 or 3 minutes and let cool spontaneously. A positive reaction is indicated by a reddish or reddish yellow precipitate. If less than .3% is present a positive reaction is likely to occur only as the solution cools. In the absence of sugar the solution remains clear or slightly turbid. It is not reduced by normal urinary constituents.

Haine's test. Same as former, except that sodium carbonate is replaced by glycerin. Not as sensitive as Benedict's.

Nylander's test. Copper is replaced by bismuth subnitrate.

Solution:

Bismuth subnitrate	2 gm.
10% NaOH	100 cc
Rochelle salts	4 gm.

Keep solution in a brown bottle and don't expose to light.

Technique of test: To 9 parts of urine add 1 part of reagent. Put in water bath for 5 minutes; boiling is permissible. A positive test is indicated by a black precipitate of bismuth. In urine with only a small percentage of sugar the precipitate takes on more of a brownish color somewhat darker at the bottom.

Advantages of the test: It is not reduced by normal urinary constituents or alkapton.

Disadvantages of test: It is reduced by hexoses, pentose, glycuronic acid, marked indicanuria and urobilinuria. It is also reduced by sulphonal, trional, rhubarb, senna, cascara and large doses of urotropin and quinine.

Galactose.

Conditions which show galactose in the urine:

1. Infants nursing at breast and having gastro-intestinal disturbance.
2. Used in test for hepatic function (formerly).

Disaccharides:

1. Lactose, which splits up into } Glucose / Galactose
2. Maltose, which splits up into } Glucose / Glucose
3. Saccharose, which splits up into } Levulose / Glucose

Lactose:

Physiologically it occurs during the puerperium and sometimes throughout the period of nursing. It also occurs in persons on an exclusive milk diet. The tolerance for it is low, 80 to 100 grams. It never occurs in diabetes. It does not ferment with yeast unless it is first broken down to glucose and galactose by ferments or bacteria, the former of which is fermentable by yeast. To obtain osazones the urine should be evaporated down in order to make it more concentrated, extracted with an alcohol, evaporate extract, take up in a small amount of water and apply phenylhydrazine test. Their melting point is 200 degrees.

Rubner's test for lactose: To from 5 to 8 cc. of urine add a large excess of basic lead acetate, boil several minutes and filter. To the filtrate add NH_4OH till a precipitate forms, redissolves and a permanent precipitate recurs, which is a brick red color. The filtrate also takes on a reddish color. A red solution with a yellow precipitate indicates glucose. Lactose is to be expected in urines giving slow reduction and D rotatory.

Maltose.

There are only a few cases on record of its occurrence in the urine. It is associated with interstitial lesions in the pancreas. It should be suspected in urine when rotation is greatly in excess of the amount of sugar present, which is determined by titration. To determine its presence, hydrolyze the urine for one hour with dilute acid. Neutralize and test by titration and with polarimeter.

Saccharose.

This sugar is not known in pathological conditions. Chronic maligners often put this sugar in the urine in order to get into a hospital for the winter. The urine usually has a high specific gravity, and is of a syrupy nature. Hydrolyze for an hour with weak acid. Neutralize with Na2CO3, titrate and polarize.

Fermentation:

It is said that only those sugars which have 3 or a multiple of 3 carbon atoms will ferment, which fact does not hold good, only 2 fermentable sugars occurs in urine—glucose most frequent, levulose next.

Technique:

Controls.

1. Normal urine + yeast = O
2. Normal urine + yeast + dextrose = positive
3. Specimen of urine alone = O

Test.

4. Specimen of urine + yeast = + according to presence of sugar.

Do not mix by violent shaking when adding yeast, and thus admit small bubbles. No. 3 can be omitted by first boiling No. 4. Allow to stand for from 3 to 4 hours at 37½ degrees. Sensitive from .1 to .05%.

Phenylhydrazine test:

Given by all hexoses, pentoses, glucose, levulose

and mannose, but the melting point of the crystals is different.

Technique:

Phenylhydrazine	5 drops
Urine	4 cc.
Glacial acetic	0.5 cc.

Boil and shake 2 minutes, and while still warm add 4 to 6 drops of 20% NaOH. Do not add enough to make alkaline. Boil from 30 seconds to one minute longer. Cool spontaneously. If sugar be present the solution will become turbid and deposit a yellow precitate which indicates crystals of osazone. This test is extremely sensitive and is the court of last appeal for the determination of a sugar. The crystals have a characteristic appearance arranged in sheathes. To purify the crystals the filter paper is punctured and the precipitate washed into a beaker containing 60% alcohol. Heat and dissolve the crystals; evaporate down and crystals reappear. Repeat several times. The crystals are insoluble in chloroform, water and ether. They are slightly soluble in absolute alcohol, fairly soluble in hot glacial acetic acid, but best solvent in 60% alcohol. Uric acid, glycuronic acid, oxalic acid and acetone may give positive tests. This test should not be done on a urine containing albumin.

Dextrosazone

Monosaccharides.

Glucose, levulose and galactose.

Levulosuria.

There are 3 clinical groups.

1. Alimentary. 100 grams is the normal limit of tolerance. Churchman (See J. H. H. Bul.) found that 26% of normal persons show levulosuria in 100 grams of the sugar, while some patients with actual hepatic disease do not. Churchman's conclusions on the Strause levulose liver test are:
    1. The test is modified by extrahepatic factors of sugar metabolism (renal, adrenal and pancreatic complications).
    2. There are difficulties in the test (nausea and vomiting).
    3. The clinical value of the test is insufficient to warrant its continuance.
2. Diabetic. A good many diabetics have a tolerance for levulose. The occurrence of levulosuria constantly with glucose indicates a rather serious prognosis. It also gives an error in calculating the glucose excretion.
3. Idiopathic. There are only 8 of these cases reported in the literature. Those patients who show continual levulosuria generally have some complication in the glands of internal secretion. Three of the cases reported had hypophyseal trouble and others had trouble with gonads.

Seliwanoff's test for levulose:

Urine	10 cc.
Concentrated HC1	5 cc.
Resorcin	few X1s.

Bring the solution to a boil or put in boiling water

for 30 seconds. First a reddish blush appears, and upon standing and allowed to cool a granular reddish precipitate forms. Mannose, maltose and glucose in large amounts will give the test, also.

Pentosuria. Kinds:

1. Alimentary. Follows heavy ingestion of pentose containing food, such as apples, plums, cherries, etc. It probably occurs more frequently than recognized. It is characterized by optically active xylose and arabinose.
2. Diabetic. Frequent in severe types. It is characterized by L xylose, which probably comes from broken-down pancreatic nucleoprotein.
3. Essential or ideopathic (Dr. Janeway, Am. J. Med, Sci., Sept., 1906).

Features:

1. Constant excretion irrespective of the diet.
2. Hereditary tendency.
3. Patients excrete it for long periods without evil effect.
4. Sugar excreted in this disease optically inactive.
5. Amount excreted is usually small (.2 to .6%). One case with 1% reported.
6. Specific gravity somewhat increased.
7. Amount of urine not excessive.
8. Acidity high.
9. Pentoses fed come through in the urine as such.
10. Pentose may be formed from glucose, but not determined.
11. Glycuronic acid excretion continues as normal.

Tests for pentose.

Phloroglucin (Tollen's). To urine add a small amount of HC1 saturated with phroglucin. Heat in water bath. A deep-red color indicates a positive reaction. Watch for the initial shade of red to appear and then cease heating. When extracted with amyl alcohol and examined spectroscopically a band is seen between D and E.

Errors: Test given also by lactose, galactose and glycuronic acid.

Orcin. To urine add HC1 and a few crystals of orcin. Heat in water bath. The development of a green color and the formation of a greenish precipitate indicates a positive test. Extracted with amyl alcohol gives a band between C and D.

Bial's method:

Solution:	30% HC1	300 cc.
	10% Ferric chloride	30 drops
	Orcin	1 gm.

About 4 or 5 cc. of this solution are heated to boiling, and urine is added drop by drop (not over 1 cc.). A positive reaction is indicated by a clear emerald green color. This test is best of the three.

Glycuronic acid.

Glycuronic acid is an oxidation product of sugar metabolism and is not concerned with protein metabolism. It is not a forerunner of diabetes and does not represent anything but detoxication. Its osazone has the lowest melting point of any met with. It has an aldehyde group, which accounts for its reducing properties. Pure glycuronic acid is dextro-rotatory.

Test for glycuronic acid (Tollen's):

To 5 cc. of urine add ½ cc. of 1% alcoholic solution of naphthoresorcin and 5 cc. of con. HC1. Put in water bath and allow to stand 15 minutes. Allow

to cool at room temperature for 4 minutes, and then cool under tap. Add ether, which extracts a blue-violet color, indicating a positive test. Examined spectroscopically, an absorption band occurs at b, but is not specific.

Clinical significance:

1. Glycuronic acid combines with toxic substances and appears in the urine as glycuronates. Glycuronic acid combines readily with the coal tar products, morphin, cocain, etc.; therefore, after such medication look for glycuronates in the urine.
2. Copper and bismuth reduction absent or atypical.
3. No fermentation occurs.
4. Glycuronates in fresh urine are levo-rotatory, and osazones are formed with difficulty (m.p = 114 to 115 deg.).
5. They are easily confused with pentoses because reactions are same, but can be differentiated by optical activity, glycuronates being levo-rotatory and after hydrolysis become dextro-rotatory.

SCHEME FOR DETECTION OF AN UNKNOWN REDUCING BODY IN THE URINE.

I. Fehling's test:

A. Negative, no sugar present.

B. Positive, proceed with

II. Nylander's test:

A. Negative, eliminates confusing substances in I.

B. Positive, sugar is probably present, so proceed with

III. Fermentation test:

A. Positive, sugar present may be glucose or levulose.

B. Negative, reducing substance may be: Lactose, maltose, saccharose, pentose, glycuronic acid. These become positive with hydrolysis.

IV. Polariscopic examination:

A. No. I, II and III positive:

Urine D-rotatory: indicates glucose.

Urine L-rotatory: indicates levulose.

B. No. 1, II positive and III negative:

Urine D-rotatory indicates: Lactose maltose, saccharose, alimentary pentosuria.

Urine inactive indicates pentose.

Urine L-rotatory indicates glycuronic acid.

V. Phenylhydrazine:

A. Osazones easily obtained with dextrose, levulose, pentose, maltose.

B. Osazones obtained only by special procedure with lactose.

C. Osazones obtained after hydrolysis with glycuronic acid or saccharose.

Glycuronic acid osazones have melting point of 114 to 115 degrees.

Pentose osazones have melting point of 156 to 160 degrees.

Remaining osazones have melting point of 200 to 205 degrees.

QUANTITATIVE DETERMINATION OF SUGAR.

Urinary output and specific gravity. (Naunyn.)

This method is inaccurate, but in the absence of other means gives some information as to the amount present.

Amount of Urine.	Specific Gravity.	% of Sugar.
2000 cc.	1028 to 1030	2 to 3%
3000 cc.	1028 to 1032	3 to 5%
5000 cc.	1030 to 1035	5 to 7%
6 to 10.000	1030 to 1042	6 to 10%

Specific gravity-fermentation method.

This method is based upon the principle that fermentation by yeast results in lowering the specific gravity. A small amount of albumin can be neglected and the urine should be acid in reaction; if not so, make acid with acetic.

Technique: 50 cc. of a 24-hour specimen of urine are taken and the specific gravity is determined. Add 2 cc. of a thick yeast emulsion and allow to stand from 24 to 48 hours at 37 deg. C., until the reduction test is negative. Again make specific gravity determination. The difference between the first and second determination multiplied by 234 coefficient equals the per cent of glucose. This method is accurate to .1%, but sugar present should not be less than .5%.

Fermentation method.

A. Einhorn's method. Mix a sufficient quantity of urine to fill the special fermentation tube with a piece of yeast the size of a pea. Incubate for 24 hours at room temperature or 6 hours at body temperature. Read the amount of gas given off and its equivalent percentage of sugar on the scale. Run controls: Activity of yeast, fermentable substance in yeast, and urine alone without yeast.

B. Lohnstein's method. This is the most accurate. To ½ cc. of urine add .2 cc. of yeast solution (1

part yeast to 3 parts water) and layer this solution on the mercury in a special fermentation apparatus. Tilt the apparatus until the mercury in the upright column lies even with the zero point on the scale. Turn the cork so as to shut off the air and incubate from 5 to 6 hours and read.

Titration method.

1. Benedict's method.

Advantages: 1. Single solution. 2. Permanent. 3. Sharp end point. 4. Accurate to a marked degree.

Solution:

1. Pure $CuSO_4$	200 gm.	
2. $NaCO_3$ crystaline	18 gm.	(accurate)
3. Na or K citrate	200 gm.	
4. KSCN	125 gm.	
5. 5% solution KSCN	5 cc.	
6. H_2O qs. ad.	1000 cc.	

Dissolve Nos. 2, 3 and 4 in about 800 cc. of boiling H_2O and filter. To filtrate add No. 1 dissolved in hot H_2O. Allow to cool and add No. 5 and No. 6.

Technique: 25 cc. of the reagent are placed in a porcelain dish and 10 to 20 gm. of Na_2CO_3 added to make the end reaction more clear. Run the urine diluted 1 to 10 in from a buret, rapidly at first, which gives a white precipitate, and then 2 or 3 drops at a time, boiling 30 seconds between each addition. Add distilled water if the solution becomes low. The end reaction is the permanent disapperance of the blue color.

Calculation: 25 cc. of the reagent equals .05 gm. of glucose or .053 gm. of levulose. x cc. = .05 gm.,

$$1 \text{ cc.} = \frac{.05}{x} \text{ gm.} \quad 1500 \text{ cc.} = \frac{.05}{x} \text{ gm.} \quad \times 10 \times 1500.$$

2. Bang titration method.

Urine is boiled in a known excess of alkaline cop-

TABLE OF REDUCING BODIES

Reducing Body	Cu Sol.	Nylander's	Fermentation	Polarization	Pheylhydrazine	M. P. & Rot. of Osazone	Special Tests
Glucose	Positive	Positive	Positive	D. 52.7°	Positive	205° L. 1° 32''	
Levulose	Positive	Positive	Positive	L. 92°	Positive	205° L. 1° 30''	Seliwanoff's Phenylmethylhydrazine
Lactose	Slow Positive	Slow Positive	Negative	D. 52.5°	Negative In Urine	200° Inactive	Phenylhydrazine after concentration and extraction
Pentose	Slow Positive	Slow Positive	Negative	Essential or Ideopathic inact. Al. & Diabetic slight D	Positive	156° —160° Inactive	Phloroglucin Orcine
Galactose	Slow Positive	Slow Positive	Slow Positive	D. 81°	Positive	188°—193° D. 0.48° in Neuberg's pyridin alcohol mixture	

Continued on Page 87

Maltose	Positive	Postiive	Negative	D. 138°		206° D. 1' 30''	Ferments After Hydrolysis
Conjugated Glycuronic acid	Slow Positive	Slow Positive	Negative	D. Rot L after Hydrolysis	After Hydrolysis	115° ?	Phloroglucin Orcine After Hydrolysis
Saccharose	Positive or Negative	Positive or Negative	Negative	D. Rot.	After Hydrolysis	See Glucose & Levulose	
Homogentisic acid of Alkaptonuria	Positive	Negative	Negative	Negative	Negative	Negative	
Chloroform	Atypical Positive	Negative	Negative	Negative	Negative	Negative	Heat Urine First and It Does Not Reduce

Continued From Page 86

per, and the copper remaining is titrated with a standard solution.

Solution: A.

1.	Potassium bicarbonate	100 gm.
2.	Potassium carbonate	500 gm.
3.	KSCN	400 gm.
4.	CuSO4	25 gm.
5.	H_2O qs. ad.	2000 cc.

Dissolve 1, 2 and 3 in 1300 cc. of hot H_2O in the order named. Dissolve No. 4 in 200 cc. of H_2O separately. Mix while hot. Cool at room temperature, add No. 5.

Solution B.

KSCN	200 gm.	
Hydroxylamine sulphate	6.25 gm.	(accurate)
H_2O qs. ad.	2000 cc.	

Technique: Urine is diluted to not over .6% of sugar. 10 cc. of the diluted urine are placed in an Erlemeyer flask and 50 cc. of solution A are added and the contents boiled 3 minutes by the watch. All the sugar present is used up, but not all the copper present is reduced. Titrate the copper remaining with solution B until colorless.

Calculation: 1 cc. of solution B equals 59.4 mg. of glucose.

Summary of quantitative determination methods: Specific gravity method should be discarded where other instruments are available. Specific gravity-fermentation method is not very accurate unless one has a delicate urinometer. Fermentation methods are all right if sufficient controls are run. Lohnstein method good. Titration method best of all.

Estimation of sugar with the polariscope.

Principles:

Light rays vibrate in all directions. Some sub-

stances have the power of double refraction, Iceland spar for instance. Light passing through this is resolved into two sets of rays, one of which vibrates in all directions, the other vibrates in but one plane. In the polariscope the entering rays are polarized and the ray vibrating in all directions is deflected by means of a Nichol prism. Another Nichol prism is used near the eye-piece as an analyzer. A quartz plate obscures half the visual field. Some substances have the power of turning this polarized ray to the right or left. The substance whose specific rotation is to be determined is put in a tube of a known length, 1 decimeter, and the polarized ray passed through it. After determining the zero point of the instrument the unknown substance is put in the pathway of the polarized light and the analysing prism turned until both sides of the field are of equal illumination. The degree of rotation is read on the scale.

Specific rotation.

This is the amount of rotation of 1 gram of a substance per cubic centimeter of water in a tube 1 decimeter in length.

Formula:

$$P = \frac{A \times 100}{\text{Sp. rt.} \times LD}$$

A = reading in degrees.
Sp. rt. = specific rotation.
LD = length of tube in decimeters.
P = percentage.
P = A when the tube is of the proper length, viz: 188.6 m m for glucose.

Technique: A mixed 24-hour specimen of urine is

made free from albumin and absolutely clear. To do this the following may be used:

1. Heat and acetic acid and filter.
2. Filter after adding kieselguhr.
3. Normal lead acetate, not basic, filter.
4. 25% HCl and animal charcoal, filter.

The zero point of the instrument is determined by taking the average of 5 readings, using a sodium flame. This point is indicated by both sides being equally illuminated. Carefully fill the tube so as to leave no bubble within, place in the instrument and take the average of 5 readings. Where a 188.6 m m tube is used each degree of rotation equals 1% glucose.

Essential urinary findings in diabetes.

There is generally a polyuria caused by the hyperglycaemia. Sugar in the blood, not in a colloidal state as normally, acts as a diuretic. The amount of urine excreted depends upon the amount of hyperglycaemia. From 5 to 8 liters are excreted per day. Polyuria with low specific gravity would indicate a possible development of both diabetes mellitus and insipidus. When polyuria of severe cases diminishes on restriction of diet it indicates a good prognosis. When low polyuria and high specific gravity occur together the prognosis is more favorable than great polyuria and low specific gravity.

Variations in sugar excretion.

By securing specimens every 2 hours it is found that the maximum output of sugar occurs at noon and late afternoon. The minimum output is in the early morning. If one should examine a 24-hour mixed specimen, the concentration of sugar may be so low that it may be missed; hence, keep 2-hour specimens separate in doubtful cases and examine.

Amount of sugar excreted.

The average percentage of sugar in the urine in diabetes is from 2 to 3%. 3% at 3 liters a day would give 90 grams. Maximum output 1500 gm. 6 to 8% is considered high. More is excreted on a hot than a cold day. In febrile conditions there is a tendency for glycosuria to disappear. The amount of sugar is increased on a carbohydrate rich diet. Sometimes lactose or levulose are tolerated well. The amount of sugar excreted usually falls during diabetic coma.

Specific gravity.

The specific gravity is usually high, varying from 1025 to 1040. 1074 has been reached. In urines with 1060 readings and over, look out for frauds. Sugar may occur in urines with a specific gravity as low as 1007 to 1016.

Color of urine.

The color is usually a pale greenish yellow. A pale color with a high specific gravity is characteristic.

Acidosis.

The characteristics of the urine are:

1. Acid bodies are present, B oxybutyric acid acetone and diacetic acid.
2. The reaction is characteristically acid, rarely neutral or alkaline. By the Folin titration method there is increased alkali tolerance. Normally an individual will excrete alkaline urine upon the ingestion of from 5 to 10 grams of sodium bicarbonate; in diabetes it will require from 100 to 250 grams a day to make the urine alkaline.

Nitrogen elimination in diabetes.

Normally 15 gm. are eliminated in 24 hours. In diabetes 20 to 30 grams are excreted. The nitrogen

partition is unchanged until acidosis appears, when the NH_3 increases. Severe symptoms are indicated when the NH_3 output reaches 2 or more grams per day.

Amino acid nitrogen in diabetes.

Normally .1 gm. per day is excreted. In diabetes this is increased to about .9 gm.

Albuminuria and casts in diabetes.

Albumin and casts are seen in patients passed middle life in whom there is arterial or nephritic change. Kolz's sign is showers of hyaline casts preceding acidosis or coma.

Acid bodies in the urine.

These include 3 substances:

B oxybutyric acid.		Diacetic acid.		Acetone.
CH_3		CH_3		CH_3
$CHOH$	$-H_2=$	CO	$-CO_2=$	CO
CH_2		CH_2		CH_3
$COOH$		$COOH$		

Although these bodies occur in diabetes, they also occur in other conditions. They may arise from carbohydrates, fats or proteins. From a carbohydrate source there is no evidence, but there is a possibility of their coming from the tyrosin group of protein. There is no parallelism, however, between their excretion and destruction of body protein as there is between their excretion and fat destruction.

Tests for acetone:

Legal's. To about 8 cc. of urine add a few crystals of sodium nitroprussid and a few drops of NaOH or KOH, which gives a red color and which fades to a yellow both in normal and diabetic urine, except that the transition is slower in the latter. While still red add a few drops of glacial acetic acid, and if the urine changes to a purple or deep red color it denotes the presence of acetone. If it changes

to a green color it shows the presence of creatinin.

Le Noble's. This test is better than Legal's, for it eliminates aldehyde bodies and creatinin. To about 8 cc. of urine add a few crystals of sodium nitroprussid and a few drops of NH_4OH. Before the red color fades to a yellow add a few drops of glacial acetic acid as before. A deep red or purple color indicates the presence of acetone.

Lieben's. Depends upon the formation of iodoform crystals. To about 5 cc. of urine add a small quantity of Lugal's solution or tincture of iodine and a few drops of NaOH. Warm slightly and examine the precipitate for iodoform crystals.

Gunning's modification of Lieben's is a more specific test. To a small quantity of urine add about 5 cc. of either Lugal's solution or tincture of iodine, and then NH_4OH till a permanent precipitate forms. Upon standing this turns to a yellow or yellowish brown color, and upon microscopical examination hexagonal crystals of iodoform are found. It is preferable to set the solution aside for 24 hours before examination.

Frommer's. This test is the most specific, being sensitive in dilution 1—1,000,000. The reagent used is a 10% alcoholic solution of salicylaldehyde. To a small quantity of urine add a few drops of NaOH or KOH and 10 to 12 drops of the reagent. Keep in water bath at 72 degrees for 3 or 4 minutes. A purple or dark red color indicates a positive reaction.

Le Noble's modification of Legal's test and Gunning's test are best clinically.

Diacetic acid.

Gerhardt's test. Reagent: 10% ferric chloride solution.

Add reagent to urine in slight excess, whereupon a precipitate of phosphates forms. Either filter at

this point or continue adding the reagent without filtering. It is better to filter. Upon the addition of more reagent the previous reddish-brown color is changed to a Burgundy red. This test is also given by salicylates, conjugated glycuronates, after taking phenacetine, antipyrene, acetates and trionate medication. These can be differentiated, however, by first heating. Diacetic acid is broken up and color disappears; drugs, on the other hand, continue to give the reaction.

B oxybutyric acid.

There is no satisfactory test for this. The urine shows a greater titration determination than is indicated by the polaroscope. It is L-rotatory, specific rotation being —24.12 degrees. Urine after fermentation still L-rotatory also points to B-oxybutyric acid.

Autoketonogenic function of carbohydrates.

1. Administration of carbohydrates diminishes ketones.
2. Lipaemia is associated with maximum excretion of acetone bodies.
3. Administration of fatty acids is followed by excretion of acetone bodies in animals. This occurs also in people on an insufficient diet or who have an error in metabolism where carbohydrates do not spare fats.

This condition is characterized by the following:

A. 1. Large amounts of acetone bodies

2. Increased NH_3.

3. No characteristic anatomical lesion

This condition is found in diabetes, starvation and cachexia.

2. Excess of NH_3.
3. Large amounts of unoxidized N and lactic acid

This condition is found in phos. poisoning, chloroform poisoning, toxaemia or pregnancy and cyclic vomiting.

Coma supervenes when acetone bodies are in the greatest concentration, in both the blood and the urine. Coma does not occur in their absence.

Acidosis is further proved to be the cause of coma:

1. There is decreased alkali in the blood.
2. Concentration of CO_2 in the blood reduced from 36 m m to as low as 3.2 m m tension.
3. There is a greatly increased alkali tolerance.
4. Administration of alkali results in improvement.

Allen's theory of diabetes.

1. Pancreas is the seat of the trouble.
2. Diabetes is a specific disease.
3. It is explained by a lack of amboceptor, which is necessary to produce blood colloidal sugar. Sugar free in the blood and not in a colloidal state acts as a diuretic.

Treatment.

1. Supply deficient amboceptor.
2. Protection. Restrict carbohydrates up to a point of no sugar in the urine and blood sugar not greater than .17% and no acetone bodies in the urine. By restricting the carbohydrates you attack acidosis. There is no explanation for this except that by starvation you make the body learn to metabolize sugar properly.

Diabetes insipidus.

This is a condition characterized by the excretion of large amounts of urine with a low specific gravity

without any demonstrable kidney lesion. The condition frequently shows a hereditary disposition.

Symptomatic group.

1. Polydypsia (excessive fluid intake).
2. Central nervous system injuries. Lues often found at autopsy.

Ideopathic group.

Lately some internal glandular pathology has been determined. Sometimes the posterior lobe of the hypophysis has been found diseased, especially in distrophia adiposa genetalia. The inability of the kidney to concentrate urine is the chief symptom. When salt is given to a normal individual he excretes it with a rise in the specific gravity of the urine. In this condition the concentration in the urine remains unchanged, but a greater quantity of fluid is excreted. The total solids are the same, but more fluids are required in the latter condition.

Characteristics of the disease.

1. Polyuria with 5 to 49 liters a day.
2. Excessive thirst.
3. Urine:
    a. Low specific gravity.
    b. Practically colorless.
    c. Weakly acid.
    d. Hypotonic (blood cells rapidly disintegrate).
    e. Polyuria greater at night.
    f. Free from albumin and sugar.

Ferments in the urine.

Pepsin. Normal in small amounts and easily detected. It is absent in gastric carcinoma, subacidity and occasionally in achylia gastrica. It is increased in pneumonia and is of some prognostic value.

Test for pepsin.

Fibrin is washed and then soaked in the urine

for from 5 to 6 hours. It is then placed in a weak solution of HC1 and incubated. If pepsin is present digestion will take place.

Lipase. Normally present, but in smaller amounts than pepsin. It is increased in jaundice, diabetes mellitus and hemorrhagic pancreatitis.

Test for lipase (Castle and Loevenhart).

Flash A	Flash B	Flash C
Urine 5 cc.	Boiled urine 5 c c.	Urine 5 cc.
Phenolphthalein	x cc. N/10 NaOH	x cc. N/10 NaOH
N/10 NaOH to a	.25 cc. ethylbutyrate	.25 cc. ethylbutyrate
pink color— x cc.	1 cc. toluol	1 cc. toluol

Incubate flasks B and C for 24 hours at 37½ degrees. At the end of this time add ½ cc. more of N/10 HC1 than N/10 NaOH, extract with 25 cc. of alcohol and 50 cc. of ether. Titrate to end point, using phenolphthalein as indicator.

1 cc N/10 NaOH equals .0088 grams butyric acid.

Diastase. Origin not known. It may have its origin from polymorphoneutrophils of the blood, the pancreas, or the liver. It is formed from great measure from the pancreas, for removal of this organ causes a great diminution in the amount of diastase.

Wohlgemuth's test.

Reagent: 1% starch solution free from precipitate.

Dilute 2 cc. of urine with 6 cc. of distilled water.

Into each of 9 test tubes add 5 cc. of the 1% starch solution and then the following amounts of the diluted urine: Tube 1, .88 cc.; tube 2, .72 cc.; tube 3, .56 cc.; tube 4, .40 cc.; tube, 5, .32 cc.; tube 6, .24 cc.; tube 7, .16 cc.; tube 8, .08 cc., and tube 9, .00 cc. Add sufficient distilled water to each tube to make the volume up to 6 cc., and a small amount of toluol to prevent bacterial action. Cork tubes and incubate at 37½ degrees for 24 hours. At the end of this time check action of ferments by chilling in ice water. Fill tubes nearly to top with cold water,

add 3 drops of N/10 iodine solution and shake. Determine the highest dilution which shows no blue color, and the amounts of starch and urine therein. For instance, if tube 5, with .32 cc. of diluted urine, should be the first tube not to show the reaction, it would mean that .08 cc. of undiluted urine were capable of completely splitting 5 cc. of 1% starch. Formula: .08 : 5 :: 1.0 : x or x = 62.5

STOMACH ANALYSIS.

The study of gastric analysis divides itself into four subdivisions, dependent upon the four chief functions of the stomach:

1. That of a mixing chamber in which the food is prepared for the later intestinal digestion—hence a study of *Secretion.*

2. That of digestion—hence a study of *Ferments.*

3. That of a reservoir from which food passes further along, requiring a study of *Motility.*

4. That of a place of absorption, requiring a study of *Absorption.*

We shall limit ourselves to the knowledge obtainable from a study of the gastric contents and leave to other departments the additional important information to be gained by direct abdominal examination and X-ray.

The condition of the gastric contents may be studied from the vomitus or by emptying the stomach by means of the stomach tube. By this means we may study the activity of the stomach—as to its powers of secretion, ferment formation, motility, and absorption.

I. Secretions.

A. Vomitus. Vomitus consists of stomach contents plus mucus and saliva. It represents no definite phase in gastric digestion; it is, therefore, of much less value along certain important lines than the gastric contents artificially removed. Much valuable information can be gained from its study, however, especially in the following particulars: 1‘ Time of vomiting. 2. Amount. 3. Reaction. 4. Odor. 5. Color. 6. Macroscopical contents. 7. Microscopical examination.

Types of vomiting.

1. Cerebral.
   a. Organic brain changes (no nausea, projectile).
   b. Drugs. Apomorphine.
2. Systematic.
   a. Local. Drugs, emetics, acute gastritis.
   b. Psychic—unpleasant sights or smell.
   c. Toxic. Uraemia, pregnancy, ethyl, alcohol.
   d. Reflex. Gastric crises, acute inflammation of gastro-intestinal tract, renal colic.
   e. Obstructions.

Points of special interest.

1. Time.
   Morning (alcoholism, pregnancy, hypersecretion).
   After meals (ulcer, esophageal obstruction, pyloric obstruction).
   Definite time (neurotic).
2. Amount (relation to food and water intake).
   Large (stasis-atony or obstruction—shows food remains).
   Hypersecretion has little or no food present.
3. Reaction. The reaction is acid, with the foling exceptions:
   1. Esophageal obstruction.
   2. Presence of intestinal contents (bad retching).
   3. Occasionally in achylia.
   4. Alkali poisoning, or therapy.
4. Odor.
   1. Fecal (obstruction, paralytic ileus, peritonitis with complete motor insufficiency).
   2. Almonds (hydrocyanic poisoning).
   3. Garlic (arsenated hydrogen).
   4. Sour (uraemic, acetone), etc.).

5. Color. Varies with presence of: 1, food; 2, bile; 3, blood; 4, poisons; 5, fungi.

6. Macroscopic appearance.

1. Bile (retching).
2. Food (retention).
3. Blood (dark red or coffee ground).
4. Fecal.
5. Cancer tissue.
6. Mucus.

7. Microscopical examination. (see under Fasting stomach).

8. Chemical analysis (see under Test meals).

B. Stomach tube. It is better taken if ice cold and lubricated with oil. Some cases warrant cocainizing the throat.

1. Factors regarding patient. He should be sitting upright or lying on side. A rubber apron should be tied around neck so as to prevent soiling clothes. False teeth should be removed and other foreign bodies. He should be instructed to breath normally.

2. Passage of tube. Hold as a pen, avoid pressing on tongue, and push back to posterior wall and then down. A swallowing movement at this point helps. Resistance is encountered on the floor of the stomach, 40 cm. or 18 in. in adults, 9 in. in child). Remove tube if there is great pallor or cyanosis.

3. Emptying stomach. Have patient cough, or bear down with abdominal muscles or use Politzer or Boas bags.

Indications for the passage of the stomach tube.

1. Emptying the stomach in poisoning, distention or stasis.
2. Lavage in stasis, fermentation, mucus, hiccoughs, post-operative vomiting or chronic ulcer with high acidity. A pint of warm water is usually safe for an adult with

sodium bicarbonate or HCl, as the case demands. Beware of habit formation.

3. Gavage. Coma, forced feeding, local mouth conditions, pyloric stenosis (infancy).
4. Medication. Castor oil, salts, emetics, silver nitrate, etc.

Contra-indications for the passage of the stomach tube.

1. All general conditions in which excitement and retching are associated with harmful results to the patient.
2. Local conditions in which trauma associated with its passage might be dangerous as aneurism, esophageal vacicosities, bleeding ulcer, corrosive poisons, etc.

C. Fasting stomach examination.

The patient should be instructed to eat a supper in which rice or some other easily recognized food is included, such as spinach, raisins, prunes, etc. After this meal nothing should be eaten until after the passage of the stomach tube the following morning.

1. Normal findings.
   a. Macroscopic. 10—100 cc. fluid, often bile tinged from retching. There may be a few streaks of blood from injury to the mucus membrane. No food remains should be present.
   b. Microscopic. An occasional starch granule and a few bacteria. No yeasts, no Oppler-Boas bacilli, no sarcinae, no food remains.
   c. Chemical. The gastric contents should be slightly acid (usually test for free HCl). No lactic acid should be present and is not tested for as a routine unless suspected. Guiac test should be negative and is not

tested for as a routine. Housemann's test should be negative. This test is used to determine retention and is carried out in the following manner:

Allow the gastric contents to stand in a conical container for about 30 minutes; pour off the supernatent fluid, add 10—15 drops of Lugal's solution or iodine to the sediment and water till transparent. Starch granules show up blue and are indicative of retention.

2. Abnormal findings.

a. Macroscopic. Food remains, large amount of fluid (hypersecretion), body tissues (cancer fragments, blood, pus, mucus), foreign bodies (rarely), parasites.

b. Microscopic. Oppler-Boas bacilli, B. Bulgaricus, sarcinae, yeasts, infusoria, protozoa (amoeba, flagellates), eggs and worms. Food remains (meat fibers, starch, fats). Crystals, fatty acid, triple phosphates, calcium oxalate, bile crystals (leucin, tyrosin, cholesterin). Body cells r.b.c., w.b.c., cancer cells).

D. Test meals. The advantage of giving test meals and then obtaining the gastric contents is that a study can be made of the effects of various meals on normal individuals and a standard can be obtained by which we can judge deviations in the abnormal conditions. Test meals should be of such a character as to (1) produce a normal, psychic and chemical flow of gastric juice; (2) be easily taken by even ill people; (3) be easily recovered (not obstructing stomach tube).

1. Ewald test meal.
   40 grams of bread (without crust because of color) well masticated.
   400 cc. of water. (Tea as a substitute not advisable on account of interference with guiac test.)
   Remove in one hour.
   Normal findings:
      30—50 cc. fluid with residual bread.
      Free HC1 20—40%.
      Total acidity 40—60%.
   Disadvantages:
      a. Little psychical stimulation.
      b. Some lactic acid introduced.
   Dock modification.
      1 shreaded wheat biscuit in place of bread. (More palatable.)
2. Boas test meal.
   Oatmeal (1 liter of water, 1 tablespoonful oatmeal boiled to 500 cc.)
   This modification eliminates the introduction of lactic acid.
   Remove in one hour.
   Normal findings:
      Very little residual material, acidity?
3. Riegel test meal.
   Mid-day heavy meal:
      400 cc. soup,
      200 gm. beefsteak,
      150 gm. potato,
         1 glass of water.
      Remove in three to four hours.
   Advantages:
      a. Good psychicial stimulation of gastric juice.
      b. Longer period before removal, hence more information.

Disadvantages:

a. Too heavy for many conditions.

b. Blocks tube.

4. Fisher test meal.

Bread and tea (modified Ewald).

¼ pound chopped, seasoned, lean beef.

Remove in 3 hours.

Advantages same as Riegel.

Disadvantages:

Too heavy for many cases.

5. Salzer test meal.

Double meal.

Breakfast: Meat, milk, rice and egg.

Four hours later:

Stale bread and water.

Remove one hour after second meal.

Normal findings:

No remains of first meal.

Advantages:

Especially adapted for study of motility.

6. Sahli test meal.

A fat savory soup of which 250 cc. are given and 50 cc. kept for comparison.

Remove gastric contents 1 hour after taking in 2 portions:

a. As much as can be obtained directly.

b. With a known amount of water wash out the remaining.

By reckoning the per cent of fat content (method of Mathieu and Remond-Webster, pg. 58) of the various specimens it becomes possible to determine quantitatively the amount of gastric contents in the stomach at the time of its removal; then by reckoning the acidity per cent it becomes possible to tell the actual amount of acid in the stomach and not merely its per cent.

E. Examination of recovered gastric contents.

1. Amount. Ewald 30—50 cc. More in stasis or hypersecretion.

2. Reaction. Acid, rarely alkaline. Duodenal regurgitation and a few achylias may produce an alkaline reaction. Generally sufficient organic acid is present to cause acidity.

3. Odor. Same as for vomitus. (Uraemic, rancid, acetone, fecal, etc.)

4. Color. Limited also by previous emptying to bile and blood, fresh or coffee-ground.

5. Macroscopic. Layer formation.

Normal. Fluid and food residue.

Abnormal. *Retained* food (not removed by previous emptying). *Blood*, fresh or old. Consider possibility of trauma from stomach tube, also rule out blood from nose, mouth, throat and lungs. *Pus*. Ulcerated carcinoma, ruptured abscesses, pyorrhea. *Tissue*. Especially in carcinoma. *Mucus*. Gastritis.

6. Microscopic. (See Fasting stomach.)

7. Chemical analysis.

a. Routine.

1. Presence or absence of HCl.
2. Amount of HCl present.
3. Amount of combined acid.
4. Organic acids, especially lactic.
5. Occult blood.

b. When indicated.

1. Ferments.
2. Special tests (especially for cancer, which are taken up under that subject).

For the acid determination the necessity of devising methods for the differentiation of organic and inorganic acids is obvious. When we speak of a test as specific for HCl it really means for inorganic acids in general, but acids other than HCl are elimi-

nated from consideration. Most of the tests we use as specific for HCl will, unfortunately, give suspiciously positive results with organic acids if they are in high enough concentration.

1. Qualitative tests for HCl.
    1. Litmus. Turns red from any acid.
    2. Congo red paper (filter paper dipped in acids it gives an orange to a pinkish congo red, alcohol solution, and dried). It is supposed to differentiate organic from inorganic acids; deep blue with HCl, bluish red with organic acids. With sufficient concentration organic acids will also give a definite blue.
    3. Topfer's (dimethyl amido azo benzol, 5% alcoholic solution). This is the most generally used reagent. It gives a yellow color in alkaline solutions, and with HCl it gives a deep red to a pinkish orange, depending upon the concentration. With organic acids it gives an orange to a deep pinkish orange, depending upon the concentration. It is sensitive to .02 parts of HCl in 1000 cc. It gives suggestive positive reactions with organic acids in concentrations above 5%.
    4. Gunzberg's (2 gm. phloroglucin, 1 gm. vanillin in 30 cc. of absolute alcohol or in 100 cc. 80%). Keep in dark bottle. Reacts with no organic acids nor acid salts regardless of concentration.

    Sensitive to .05 parts of HCl in 1000 cc.

    Method of performing test:

    1 drop of reagent is allowed to dry in a porcelain dish. (Heating is permissible without burning.)

    1 drop of gastric contents is placed over the drop of the dried reagent and also dried with great care.

HCl gives a purple red color. (Mintz modification of Gunzberg.

5. Boas.

5 gm. resorcin,
3 gm. cane sugar,
100 cc. 95% alcohol.

More stable than Gunzberg's, with similar delicacy and same color reactions.

6. Tropeolin.

Saturated alcoholic solution Tropeolin.

Use as Gunzberg's. Less delicate and color difference between organic and inorganic is too close to be of practical value.

II. Quantitative Tests for Free HCl.

1. 10 cc. of gastric contents well mixed and unfiltered. (Filtering causes some H_2O loss and, therefore, HCl concentration.) If much mucus is present, filtering will be necessary regardless of slight error associated with it.

Avoid including food in the 10 cc.

Technique of performing test.

To 10 cc. of gastric juice add a drop of Topfer's reagent. If a deep pink color results, proceed with quantitative determination. If a pinkish orange color results, stop at once and do a Gunzberg test to determine the presence of any HCl.

A. Should the resulting color be a deep pink or red color, add from a buret, drop by drop, with constant stirring, enough N/10 NaOH to change the color to a definite *lemon* color.

Calculation: No. cc N/10 NaOH $\times$ 10 = acidity per cent.

B. Should the resulting color with the Topfer's be a pink orange and although only one drop were used, a fresh 10 cc. of gastric contents will be necessary, for the presence of Topfer's reagent gives a false positive with Gunzberg's reagent. If the qual-

itative test with Gunzberg's shows no HCl present, it will be unwise and impossible to carry out the test. If the fresh gastric contents gives a positive Gunzberg test, then from a buret add, very carefully, N/10 NaOH to 10 cc. of the gastric contents and test with Gunzberg's reagent after the addition of each 1—2 drops, for there is not much free HCl present and the end point must not be passed for accurate determination.

Calculation: Same as with Topfer's.

Note: Normal acidity is .2 to .3%, probably as high as .5%, when just out of the gland, but quickly falls to .2 to .3% from contact with (1) mucus of gastric glands, (2) protein combination, (3) alkaline duodenal regurgitation.

Expression of acidity:

1. In terms of acidity per cent, which is the no. of cc. of N/10 NaOH necessary to neutralize 100 cc. of gastric contents.
2. Actual amount of HCl present based upon the fact that 1 cc. of N/10 NaOH is equal to .00365 gm. of free HCl. Determine 100 cc. .2 to .3% equals 20 to 40 acidity per cent.

Definition of terms:

Euchlorhydria, when free HCl is .2 to .3% or 20—40 acidity per cent.

Hypochlorhydria, when free HCl is decreased .1%. [Chronic gastritis (subacute), incipient cancer, fevers, severe anaemias, many mental diseases, c.p.c. chronic nephritis, etc.]

Anachlorhydria, absence of free HCl. (Cancer, pernicious anaemia, neurasthenia, etc.)

Achylia, absence of HCl and ferments.

Hyperchlorhydria, free HCl from .2 to .9%. (Ulcers, chlorosis, reflex, migraine.) (Surmont-Dahon, defective NaCl output, compensation.)

Estimation of HCl deficit.

To 10 cc. of gastric contents add 1 drop of Topfer's reagent, which should not give positive reaction. Confirm absence of free HCl with Gunzberg's test. Into the 10 cc., with Topfer's reagent as an indicator, add, drop by drop, N/10 HCl, stirring constantly, until a definite positive reaction is obtained. For more accurate determination control end point with Gunzberg's reagent.

Calculation: No. cc. of N/10 HCl required $\times$ 10 = acidity per cent.

III. Combined Acid.

In order to calculate the entire HCl acidity per cent, one must determine, in addition to the free HCl, the HCl combined with foods, especially the proteins. The amount of combined HCl can be determined by one of two methods:

1. Einhorn method. This method is simple, quick and sufficiently accurate for clinical work. Method of performing test:

To 10 cc. of gastric contents add a drop of Topfer's reagent. Titrate with N/10 NaOH and determine the free HCl. When the end point of the above determination is reached add a drop of phenolphthalein and continue to add N/10 NaOH until a definite pink color is obtained lasting at least 30 seconds.

Calculation: No. cc. N/10 NaOH required to bring about reaction after the free HCl has been neutralized $\times$ 10 = acidity per cent for combined acid.

In cases where no free HCl exists, i. e., negative Topfer's and Gunzberg's reaction, the HCl deficit and combined HCl should be determined, which will require two 10 cc. portions. Determine the HCl deficit as above. The combined HCl is determined by adding the phenolphthalein at once and titrating with the N/10 NaOH. The fallacies of this method

are obvious, for such a determination of combined acids includes: 1, combined HCl; 2, acid salts; 3, organic acids. When the free HCl is high and there is no evidence of stasis, the bulk of combined acid determined will be represented by combined HCl, but where the free HCl is low or absent, especially when associated with stasis, acid salts and organic acids will often comprise the greater part of this acid calculation.

2. Topfer's method. This method is of value in differentiating between the constituents above enumerated. Procedure:

A. Determine total acidity, including free, combined, and salts, using phenolphthalein as indicator. (10 cc. gastric contents plus 1 drop of indicator plus N/10 NaOH till end point). Calculate acidity per cent.

B. Determine free acid, both HCl and organic, and salts as follows:

To 10 cc. of gastric contents add 2—3 drops of a 1% aqueous solution of alizarin monosulphonate of sodium, which turns yellow with acid and violet with alkali. This indicator reacts with free acids, organic and HCl, and salts, but does not react with organically bound HCl.

Titrate with N/10 NaOH till a pure violet color not growing darker with the addition of more alkali. Calculate acidity per cent.

C. Determine free HCl with Topfer's or Gunzberg's reagent. Calculate acidity per cent.

Explanatory example:

A = 80 acidity % (includes all acids, free, salts, and combined).

B = 22 acidity % (includes all acids and salts not organically bound).

C = 20 acidity % (includes only free HCl).

A—B = 58 acidity % = combined HCl (also combined organic acids if HCl is insufficient for lactic

acid being weaker will not combine as long as HCl is present to do so).

B—C = 2 acidity % = organic acids and salts.

(A—B) plus C = total HCl, or 78 acidity %.

In cases where there is a free HCl deficit and organic acids are present, the combined acids may be part HCl and part organic, or all organic. Where delicate discrimination is needed and it is necessary to know if any HCl is excreted by the gastric glands, the following method should be adopted:

3. Incineration with $BaCO_3$.

If any HCl is present it will combine with $BaCO_3$ and form the soluble $BaCl_2$. The addition of H_2SO_4 will precipitate insoluble $BaSO_4$. The appearance of a precipitate after this procedure signifies the presence of HCl. Filtration, incineration, weighing and calculation will give amount of HCl.

IV. Organic Acids.

Practically all foods contain some fatty acids. Where rancid butter, vinegar, sour milk, etc., are included in the diet, the amount may be considerable. Carbohydrates, contained in milk, bread, meat, etc., always contain some lactic acid, and the finding of an excess amount in the gastric contents after the ingestion of such foods points to a fermentative process at work in the stomach, due possibly to a combination of decreased HCl and decreased motility. Boas thinks that with a normal diet there should be no appreciable formation of organic acids in the stomach during normal digestion. Bacterial decomposition, in the absence of HCl, plays an important source of their production. The presence of a fat-splitting ferment would also produce them.

In fermentation processes lactic acid, butyric acid and acetic acid are generally found. Lactic acid is used as an index of organic acid production, because (1) it is produced in greater amounts than

the others and is thus more easily detected; (2) qualitative tests for it are quick and simple.

Quantitative tests for organic acids are not used clinically. No clinical information is obtained by quantitative data because there exists no correlation between the amount of organic acid present and the severity of the lesion.

A. Qualitative consideration of lactic acid.

With the Ewald meal in normal digestion, as a rule, no lactic acid is found in the gastric contents; but there is a slight abnormality, even within normal limits, in which it may be found. In such borderline cases the use of the Boas meal is advisable.

Normally the small amount of lactic acid in the Ewald meal is either (1) absorbed or (2) cannot be detected on account of the presence of the normal amount of HCl, which interferes with its tests.

Pathologically, lactic acid appears in conditions of stagnation and low HCl output. Such conditions are found in (1) carcinoma, with obstructive features; (2) hypochlorhydria or achlorhydria, with sluggish motility; (3) benign stenosis, with low HCl output.

Tests for lactic acid:

1. Uffelman's.

Reagent: 1% phenol, 20 cc.; dil. ferric chloride, 1 drop; dist. water q.s. to make a delicate amethyst color.

Procedure: Divide amount of reagent between two test tubes, and into one add 5—8 drops of clear gastric juice (filtered, if necessary), and into the other add 5—8 drops of distilled water for a control.

A positive test is indicated by the formation of a canary yellow color in the tube containing the gastric juice. If the reagent is not sufficiently diluted and is of too dark a color, a small amount of lactic acid will not give the characteristic lemon color, but

will simply decrease the intensity of the purple color.

Sources of error: 1, yellow gastric juice; 2, acid sodium phosphate; 3, cane sugar; 4, glucose; 5, alcohol; 6, other organic acids.

Modification: If doubt exists, make an ether extract of the gastric juice, evaporate, take up the residue in distilled water and apply the test.

2. Kelling modification of Uffelmann's test.

Reagent: Distilled water, 20 cc.; 10% ferric chloride, 1 or 2 drops.

Procedure: Divide between two test tubes. Into one put a few drops of clear gastric juice, and into the other a few drops of distilled water for a control. A positive test, as before, is the formation of a canary yellow color.

Sources of error: Same as for Uffelmann's, except that acetic acid will not give yellow color.

3. Strauss test.

This is the best clinical test, for it detects lactic acid when in pathological amounts.

Procedure: Into a Strauss funnel put 5 cc. of gastric juice and a drop of HCl to free any organically-bound lactic acid. Upon this pour 20 cc. of alcohol-free ether. Invert several times, avoiding the formation of an emulsion. Allow the gastric juice to escape through the stop-cock below and replace it with an equal quantity of distilled water. Shake several times in order that the lactic acid extracted with the ether can be taken up by the water and add a few drops of a 10% solution of ferric chloride.

An intense green color will appear if more than .1% lactic acid is present, paler shades of green if less is present.

B. Qualitative consideration of butyric acid.

Physiologically, none is present in the stomach during digestion unless (1) heavy carbohydrate meal has been taken, (2) it is taken directly in the

form of rancid butter, etc., (3) it finds it way into the gastric juice from the mouth. Fugge has shown that it may be formed from lactic acid.

Method of detection:

1. Odor specific.

2. Shake material with ether, evaporate ether extract, take up with water and a small amount of $CaCl_2$, and upon warming butyric acid will settle out, recognized by (1) droplets, (2) odor of rancid butter.

C. Qualitative consideration of acetic acid.

Acetic acid is frequently found in large amounts when taken in the form of vinegar. Pathologically, it is found in conditions of stasis with yeasts and fungi. The stomach in this condition is usually dilated and atonic. Yeast action on carbohydrates yields alcohol, which, in turn, when acted upon by yeasts and bacteria, yields acetic acid.

Method of detection: Shake the material with ether and extract with water. Neutralize with $NaCO_3$, carefully. Acid prevents the necessary reaction and alkali causes formation of ferric hydroxide when ferric chloride is added. Add a few drops of ferric chloride and look for appearance of deep red color. Upon boiling a reddish precipitate of basic ferric acetate will also form when acetic acid is present.

D. Detection of blood.

The guiac and benzidine tests are carried out in the same manner as for similar tests in the urine.

II. Consideration of Ferments.

A. *Pepsin* is the chief ferment and is secreted by the peptic glands.

B. *Lipase,* which is slight in amount and of questionable origin.

Possible origin:

1. Regurgitated duodenal contents, pancreatic origin.
2. Derived from secretion of Brunner's glands in intestine.
3. Dual origin from both.

C. *Diastase*, a small amount of which is of gastric origin, but the greater amount of which is due to swallowed saliva.

A. *Pepsin*. This ferment is secreted as propepsin, is activated by HCl, and destroyed by alkali. Other acids will activate it, but in higher concentration, viz: HCl, .2—.4%, lactic 1—1 plus %. The action of pepsin is continuous, and a great amount of work is accomplished by a small amount of pepsin, provided that the products of its digestion are removed. Stasis hinders its action.

Decrease in amount of pepsin. Due to disease of peptic glands. Pepsin is generally present when the HCl is high, low or even absent. Pepsin may be absent in the following conditions: 1. Carcinoma, especially the liniis plastica type. 2. Atrophic gastritis. 3. Occasionally in pernicious anaemia. The output of pepsin has no relation to the amount of HCl present.

Qualitative tests for pepsin:

These are based upon the digestion of protein in the form of :1. Fibrin, which is obtained by whipping ox blood and is then washed, kept in alcohol for two or three days, then carmine for two or three days, and finally preserved in glycerin till used. 2. Albumin, which is made by cutting the whites of hard-boiled eggs into small squares and preserved in glycerin.

Procedure: 1. Into 25 cc. of gastric juice with free HCl place either a number of pieces of fibrin or albumin prepared as above. Incubate for 15 minutes to an hour. The fibrin swells up in 15 to 30

minutes and liberates the carmine. It is all gone normally in 1 to 1½ hours. The albumin swells up in 30 to 60 minutes and is well gone in three hours.

Quantitative tests for pepsin.

1. Hammerschlag's.

1% solution of egg albumin in 0.4% HCl (egg albumin 1 part, 0.4% HCl 13 parts)	10 cc.
Gastric juice	5 cc.

Incubate at 37 deg. for one hour and run a control tube with 5 cc. of distilled water instead of gastric juice. At the end of an hour run an Esbach determination on both the tubes, also on the original 1% solution. Normally 90% of the albumin is digested in the hour.

Sources of error: Albuminoses are thrown down with albumin.

2. Mett's method. (The one of choice.)

Preparation of tubes: Take several whites of eggs, cut them, filter through gauze and use the liquid portion to fill capillary tubes 10 to 30 cm. long by 1 to 2 mm. bore. Fill tubes by suction, seal with bread crumbs, drop into boiling water (95 to 100 deg.) for 5 minutes, and then seal ends with paraffin to prevent drying. When ready to use cut tubes into 2 cm. lengths.

Sources of error to be avoided in preparation of gastric juice:

1. If too concentrated, especially after meals, there may be present substances which inhabit digestion, i. e., NaCl and carbohydrates in solution.

2. Schultz's law holds with digestion up to 3.6 mm. The length of the cylinder of albumin digested by any gastric juice is proportional to the duration of digestion and is independent of the diameter of the capillary tube, provided that the length of the

digested column does not exceed 7 mm. For this reason the gastric juice is diluted.

3. Result of peptic digestion hinders further digestion.

4. Pepsin is activated by HCl and its presence is essential.

Procedure: Into a small dish place

Filtered gastric juice	1 cc.
.18% HCl	16 cc.
2 cm. Mett's tubes	2

Incubate 24 hours. Read four ends, take average and square.

Note: It is often desirable to set up two additional dilutions of gastric juice, using 32 cc. and 64 cc. of .18% HCl to 1 cc. of gastric juice.

Unit of digestion equals the amount of pepsin necessary to digest 1 mm. length of albumin in 24 hours in the presence of .18% HCl.

Calculation. This is based upon Schultz's law, which may be stated as follows: Relative amounts of pepsin, in constant acid solutions and time of action, are proportional to the square of the length of the column of digested albumin, or the square of the length of the digested cylinder of albumin is proportional to the pepsin concentration, provided the length of the digested column is less than 3.6 mm.

Example: Average length 3 mm.

$3^2 \times 16$ (dilution) $= 144$ units.

Significance:

0 to 70 units	—low.
70 to 150 units	—average.
150 to 250 units	—high.

B. Rennin (chymosin).

Pepsin and rennin run parallel in most cases, but marked variations may occur. One or the other may be suppressed or follow different curves of secretion. Some have tried to show that pepsin

and rennin are identical, but this is probably not true, for there are some differences between these two ferments, viz: pepsin acts only in acid media, while rennin acts in acid, neutral, or slightly alkaline media. Its action is to make insoluble casein from caseinogen.

Qualitative test for rennin:

Neutral gastric juice	5 cc.
Fresh milk	5 to 10 cc.

Incubate for 15 minutes. Coagulation shows the presence of rennin.

Quantitative test (Boas).

In a rack place 6 test tubes. In the first tube place 1 cc. of gastric juice and 9 cc. of distilled water, which gives a dilution of 1 to 10. Into the remaining tubes place 5 cc. of distilled water. With a 5 cc. pipette mix the contents of the first tube by drawing the solution up and down three or four times, then transfer 5 cc. of it to the second tube, mix as before, transfer 5 cc. from the second to the third tube and so on till the last tube, when 5 cc. are discarded after mixing. The dilutions are as follows:

1. 1—10. 2. 1—20. 3. 1—40. 4. 1—80.
5. 1—160. 6. 1—320.

After these dilutions have been made add to each tube 5 cc. of fresh or, preferably, boiled milk, and 2½ cc. of 1% CaC12.

Place in a thermostat for 30 minutes and read. In abnormal conditions the coagulation is low, if only in the first tube, interference due probably to acid salts. Normally, there should be coagulation up to 1—160.

C. Lipase.

This ferment does occasionally occur, but its action is very slight. Gastric digestion is not con-

cerned with the splitting of fats. In testing for this ferment be sure to use a fat-free meal.

Qualitative test for lipase:

1. Wash stomach out thoroughly.
2. Give fat-free test meal. Remove.
3. Incubate, adding some neutral butter.

Positive results show presence of fatty acids.

Examination of the duodenal contents for ferments.

Method of obtaining duodenal contents:

Give pt. 200 c. of olive oil by mouth.

Half hour later pass stomach tube and obtain regurgitated duodenal contents.

Set up for examination of duodenal contents for ferments.

Tubes (2 duplicate sets, A & B)	Gastric contents diluted 1-100	Distilled water	Casein 1% in tubes A.	Kahlbaum solution starch 1% in tubes B.	Monobutyrin 1% in tube C.
I	2 cc	0	2 cc	2 cc	2 cc
II	1 cc	1 cc	2 cc	2 cc	2 cc
III	0.7 cc	1.3 cc	2 cc	2 cc	2 cc
IV	0.4 cc	1.6 cc	2 cc	2 cc	2 cc
V	0.2 cc	1.8 cc	2 cc	2 cc	2 cc
VI	0.1 cc	1.9 cc	2 cc	2 cc	2 cc
VII	0.0 cc Control	2.0 cc	2 cc	2 cc	2 cc

Incubate tubes at 38 deg. C for 30 min. in water bath, cool rapidly and read at once.

Titrate tubes A with a saturated solution of NaCl, which gives white ppt. Add Lugal's solution to tubes B and obtain blue color if starch is present. Titrate tube C with N/10 NaOH, using phenolphthalein as indicator.

Method of preparing casein solution:

N/10 NaCl	5 cc.
Casein	0.1 gm.
Dist. water	45 cc.

Boil, with constant watching and stirring, for from 3 to 5 minutes. Allow to cool, make neutral with HCl or N/10 NaOH (phenolphthalein as indicator) and bring volume up to 100 cc. with dist. water.

Method of preparing starch solution:

Starch	1 gm.
Dist. water	100 cc.

Boil from 3 to 5 minutes, allow to cool, and make up volume to 100 cc.

Method of preparing Monobutyrin solution:

Monobutyrin	1 cc.
Dist. water	99 cc.

Normally, tube No. VI. shows digestion of casein and starch.

The lipase test is practically never done because of numerous sources of error and uncertain end point.

In diastase test the last positive tube is the tube just before the one which shows blue, probably brownish, due to presence of erythrodextrin.

Gastric digestion.

A. Protein. Pepsin and HCl change albumin into acid albumin, then into albumoses (four in number), then into peptones. Gastric digestion is not carried beyond the peptones or the slightly lower polypeptid stage. When lower products of digestion are found their presence is due to the action of other ferments, either intestinal or those excreted by malignant growths.

B. Carbohydrates. Some inversion of sugar occurs in stomach, due to the acid there present. By the action of the salivary ferment starch is changed into soluble starch, erythrodextrin, achrodextrin and, finally, into maltose. Ptyalin does not act in the presence of free HCl, but its action is so rapid that from 50 to 75% of starch is converted into the

soluble form. If the HCl is kept low, starch digestion will continue much farther than when it is present in higher concentrations.

C. Fat. Practically none is digested normally as lipase is present only in a trace.

Motility.

Disturbance in this function of the stomach is of more importance than its secretory disorders. Food can pass into the intestine and be digested there irrespective of gastric secretion. If motility is impaired, however, food stays and stagnates in the stomach, causing more or less disturbance.

Motor disturbances:

1. Vomiting, already considered, important because of loss of food.
2. Hypermotility, in which condition food is rushed through, and often associated with hyperacidity. Consider spasm also in these conditions.)
3. Hypomotility, which is important and serious.

Results of motor disturbances: 1. Disorders of secretion. 2. Disorders of digestion. 3. Disorders of absorption.

Causes of stasis:

1. Cancer with obstruction, associated generally with hypochlorhydria, achlorhydria or achylia.
2. Ulcer with obstruction, associated generally with hyperchlorhydria.
3. Spasm, generally associated with hyperchlorhydria.
4. Atony, with or without hypersecretion.
5. Ptosis, which interferes with mechanical passage of food.

Study of Motility.

1. Not by acid content.

2. Not by the size of the stomach, for a large stomach may have normal motility.

Terms: Ectasia (or ectasis) signifies dilation with motor insufficiency. Atonic gastric ectasia signifies weakness of muscles of stomach. Hypertonic gastric ectasia signifies pyloric stenosis.

Food should be out of the stomach in from 7 to 8 hours, no matter how heavy or large the meal.

Tests which give information of gastric motility.

a. Leube. A Riegel meal is washed out in 6 hours with 1 liter of water. Normal equals slight trace of food.

b. Boas. A simple evening meal of meat, bread and butter is washed out the next day. If any food remains there is considerable motor insufficiency.

c. Ewald-Siever's method: 1 gram of salol is given after a meal and the urine is collected every 15 minutes for 2 hours. Test with ferric chloride for phenol, which gives violet color. Salol is broken down in the alkaline media of the small intestine into phenol and salicylates and is excreted in the urine.

Difficulties of test:

1. The gastric juice acts slightly upon salol in about 15 minutes, breaking it up.
2. Small amounts of salicylic acid and phenol may be absorbed from the stomach.

Normally, phenol appears in the urine in from 45 to 75 minutes and is all excreted in 24 to 27 hours.

Abnormally, if not detected before 75 minutes indicates motor insufficiency; if not detected before 24 hours indicates stenosis. If detected in 15 minutes an error is indicated.

d. Winternitz. The method is the same as the preceding, except that iodipin is used in place of salol. Iodipin is not touched by the gastric juice, but requires pancreatic secretions and bile to free

iodin. The saliva is tested for iodin with starch paste. Normally, it appears in from 15 to 45 minutes.

e. Sahli test meal (fat soup). (see text books for detail.) The method is incorrect if lipase is present in the stomach.

f. An evening meal of substances easily recognized, such as raisins, rice, spinach, etc. Recovery of any of the meal (macroscopically or microscopically) the next morning shows impaired motility of a degree depending upon the findings.

g. Direct fluoroscopic examination with barium, which is the best method when available.

Consideration of absorption. Not of much importance clinically.

Penzoldt-Faber test:

Two to 3 grains of KI are given in a capsule after a meal, which is rapidly absorbed by the mucus membrane and appears in the saliva, normally, in 30 to 40 minutes. Test the saliva every few minutes with a few drops of HNO_3 and a small amount of starch paste, when a positive reaction is indicated by a blue color.

Indirect examinations.

Where it is not possible to remove contents of stomach, indirect methods may be used. They are not very accurate, but may give much help at times.

a. Gunzberg's method: 0.2 gm. of KI are placed in the thinnest possible strongly vulcanized rubber tubing about 2.5 cm. long, which is then tied with three threads of fibrin hardened in alcohol. Test by placing in water for 1 hour to be sure of no leak. The patient is allowed to swallow the bag three-quarters of an hour after an Ewald meal. Test saliva as in Penzoldt test. The HCl and pepsin digest the fibrin threads and free the KI which is

absorbed. It should appear in the saliva in three-quarters of an hour.

b. Sahli's Desmoid bag. A bag of ordinary rubber-dam, containing a 0.05 gm. pill of methylene blue and 0.1 gm. of iodoform, tied with dried but chemically-untreated catgut, which is digested by the gastric ferments, but not the pancreatic. The bag is swallowed after the noon meal. The methylene blue appears in the urine, normally, in 6 hours, coloring it green; iodin in the saliva in 2 hours.

Special test for carcinoma.

Wolff Junghan's test. This is a soluble albumin test for carcinoma, very delicate, and applicable only to stomach contents. There should be no free HCl present and no occult blood for the successful application of the test, and it should be read within half hour after it has been set up. Positive results occurring after that time should be ignored.

Reagent:	Phosphotungstic acid	0.3 gm.
	HCl (concentrated)	1.0 cc.
	96% alcohol	20.0 cc.
	Distilled water qs. ad.	200.0 cc.

Procedure: Six carefully cleaned test tubes (cleaned with soap and water, alcohol and ether) are put in a rack and to them are added the following:

Tubes.....	I	II	III	IV	V	VI
Filtered gastric contents.....	1 cc	0.5 cc	0.2 cc	0.1 cc	0.05 cc	0.025 cc
Dist. water.....	9 cc	9.5 cc	9.8 cc	9.9 cc	9.95 cc	9.975 cc
Dilution equals ..	1-10	1-20	1-50	1-100	1-200	1-400

After these dilutions have been made apply a clean rubber stopper to each tube and invert several times to mix. Do not use fingers as stoppers on account of errors in test.

1 cc. of the reagent is now carefully layered on

each of the tubes, which layering must be perfect.

The presence of a white ring in the first three tubes is normal. A ring in the fourth is non-committal. A ring in the fifth or sixth speaks for carcinoma and is considered a positive test.

Several points of clinical value:

1. High free HCl with low combined suggests hyperchlorhydria with hypermotility.

2. High (or normal) free HCl with high combined suggests hyperchlorhydria with stasis, probably spasm or stenosis.

3. Low free HCl with moderate combined suggests hypomotility with perhaps normal acid output.

4. HCl deficit with high combined suggests stasis with organic acid production.

5. HCl and mucus vary inversely as to their amounts. Mucus is never found with a high HCl. In gastritis, where mucus is characteristically present, HCl is low or absent.

6. Organic acids do not form if HCl is present in normal or increased amounts.

SPUTUM.

Definition: Sputum includes all the secretions which come from the respiratory passages.

Collection of sputum. Avoid contamination from the mouth especially when making cultures. In children this can be done by stretching a piece of gauze over the finger and tickling the throat. The child will cough and raise sputum, which will adhere to the gauze. When a quantitative examination is to be made, collect sputum in conical glass containers and look for stratification. Sometimes the gastric contents are examined in patients who swallow their sputum.

Color of sputum:

Gray—mucus.

Yellow—pus (purulent).

Yellowish-gray—pus and mucus (muco-purulent).

Red—blood usually. When the lung is the source, it is frothy; when from the mouth, it its not frothy; when from the stomach, it is dark red or coffee-ground in appearance, and is never frothy.

Occurrence of blood in the sputum (hemoptysis).

1. Pneumonia.
2. Infraction of lung.
3. Weeping aneurism.
4. Tuberculosis.
5. Tumor metastasis to lung.
6. Blastomycosis.
7. Echinococcus cysts.
8. Paragonimus westermani (lung fluke).

Green—resolving pneumonia, pyocyaneus infection, rupture of subdiaphragmatic abscess.

Black—coal and iron workers.

White—starch granules in sputum of bakers.

Various colors—dye workers.

Amount of sputum. This varies within a wide range. Large amounts are met with in:

1. Bronchial affections with much secretion.
2. Bronchiectatic and tubercular cavities.
3. Pulmonary edema.
4. Perforating empyemas.

Odor of sputum. Normally the sputum should have no odor.

Sweetish odor—pulmonary abscess, occasionally in tbc. and oral sepsis.

Foul—putrid bronchitis, gangrene, bronchiectasis.

Cheese-like—perforating empyema.

Putrefactive—stagnation in lungs or receiving cup.

Macroscopic appearance.

Layer formation. Top, frothy; bottom, granular; serous fluid between; used to be considered diagnostic of bronchiestasis, but it may occur whenever there are large amounts of sputum.

Curshmann's spirals. These consist of a central core around which are wound strands of threads. The core is highly refractile and may be fibrin. Microscopical examination is necessary in order to make out the structure Charcot-Leyden crystals and eosinophiles are usually found with them.

Dittrich's plugs. These are cheese-like masses usually about the size of a mustard seed, yellowish-white to gray in color, sometimes forming casts of the bronchi or bronchioles from which they come. They are also of frequent occurrence in the crypts of tonsils of otherwise normal individuals. Upon crushing them they give a disagreeable odor. Microscopically, they show large numbers of bacteria,

fatty acid crystals, fat globules and cellular detritus.

Cheesy masses (rice bodies). These are small, yellowish masses varying in size from that of a pin-point to a pea. They may be pigmented by decomposition products of hemoglobin. Upon crushing they do not give a disagreeable odor. They occur most frequently in tuberculosis, also in abscess and gangrene.

Fibrinous casts. In as much as not all the material making up these casts is fibrin, they are more properly called "bronchial casts." Many are branching and the size of the bronchus from which they came. When composed of fibrin, they stain beautifully with Weigert's fibrin stain. With acetic acid they are not precipitated, and are friable in consistency. They occur in pneumonia and fibrinous bronchitis.

Bronchioliths. These are calcified collections of debris and secretions.

Pneumoliths. The majority of these are tuberculous in origin, formed by the calcification of caseous areas. They have a chalky or calcarious consistency.

Elastic tissue. This appears as grayish, opaque flakes, which are most readily recognized by putting the sputum between two glass plates and looking toward the light. The other structures can be dissolved away by boiling the sputum in 10% NaOH, equal parts of each, after which the sputum is diluted and the sediment examined. The fibrils of elastic tissue are not as wavy as those of fibrous tissue and are not dissolved by NaOH.

Origin of elastic tissue:

1. Walls of arteries. Appear in sheets.

2. Bronchi. Have branches.
3. Alveoli. Appear in squares.

When elastic tissue is found it means lung destruction.

Echinococcus membranes. These may be expectorated in rather large masses, which are tough, thick and porcelain-like in color. Microscopically, they show laminated structure.

Microscopical examination.

White blood cells. The ordinary polymorphonuclear neutrophil is of little significance and is almost invariably found. The eosinophiles are found in asthma associated with Charcot-Leyden crystals.

Red blood cells. These, also, are of little importance.

Epithelial cells.

1. Pavement epithelium coming from mouth, pharynx and upper larynx.
2. Cylindrical epithelium coming from nose and bronchi may be ciliated.
3. Alveolar epithelium coming from alveoli often contain coal pigment (phthisis melanotica) and are found in normal sputum, especially in the morning. They are increased in any irritation of the respiratory tract. In chronic passive congestion of the lungs they often contain hemotoidin or hemosiderin granules, and are then known as "heart-failure cells."

Crystals.

Fatty-acid. These needle-shaped crystals occur singly or in groups. Heat changes them into fat droplets. They are soluble in ether and alkali. They occur in gangrene, putrid bronchitis, and chronic tuberculosis.

Cholesterin. These crystals resemble steps superimposed upon one another. They occur frequently in association with fatty-acid crystals in empyema, chronic lung abcesses and chronic tuberculosis.

Hematoidin. These crystals are rhomboid or needle-shaped, and ruby-red in color. They occur rarely in lung abscesses, empyema, perforating liver abscesses and old hemorrhages.

Tyrosin and leucin. These crystals are of rare occurrence and are formed only by the decomposition of protein material. They occur in empyema, lung abscess or perforating liver abscess.

Triple phosphates. These occur in the same conditions as leucin and tyrosin.

Calcium oxalate. These occur in conditions associated with decomposition.

Charcot-Leyden. These are found in association with eosinophiles and are probably derived from them. They occur most frequently in asthma, and less frequently in fibrinous bronchitis and hay fever.

Animal parasites.

1. Amoeba buccalis (A. gingivalis and A. dentalis probably the same).
2. Amoeba histolytica. This parasite may occur in rupture of amoebic abscess through diaphragm. See section on parasites.
3. Echinococcus granulosus and cysticus. The hooklets, scolices, or portions of the cyst wall will diagnosticate the condition. These should be looked for in unexplained hemorrhages from the lungs.

4. Pargonimus westermani (lung fluke). Look for the eggs of this parasite, which are operculated on blunt end. They measure .1 mm. x .05 mm. This parasite is a common cause of hemoptysis in Japan.

Fungi.

1. Streptothrix actinomyces. The sputum with this infection is glairy and mucilaginous or purulent and contains yellowish granules about the size of a pin head, the so-called "sulphur granules." If one of these granules is placed upon a slide and crushed with a cover-slip and examined, threads are found centrally and club-shaped processes are seen peripherally. They can be stained with Lugal's solution.
2. Aspergillus. Look for doubly-contoured threads with brownish pigmented spores. They are best seen after the sputum has been treated with 10% KOH. They occur occasionally in bronchiectasis and tuberculosis.
3. Blastomyces. Treat the sputum with dilute KOH and look for yeast-like, doubly-contoured, refractile bodies. They occur occasionally in systematic blastomycosis.

Bacteria.

I. Tubercle bacillus. Pick out grayish opaque flakes and stain with Ziehl-Neelsen stain:

5% carbolic acid water	90 parts
Concentrated alcoholic fuchsin	10 parts

Stain for three minutes, during which time gently heat over a bunsen flame and replace the stain as it evaporates. Wash the excess of stain off with water and decolorize with a 2% solution of HCl in 80% alcohol. When no more stain comes off, counterstain with 1% methylene blue. The tubercle bacilli

retain the red fushsin stain while the other elements are decolorized and take the blue stain. It is of importance that not all acid-fast organisms are alcohol acid-fast as is the tubercle bacillus.

When a more careful search is desired, the antiformin method should be used. A good modification is that of Loeffler's: 10 to 20 cc. of sputum are mixed with an equal quantity of

Sodium hypochlorite	10 grams
Sodium hydroxide	10 grams
Water	100 cc.

Boil for not longer than 15 minutes and centrifugalize at high speed for from 1 to 2 hours. Make smears of the sediment and stain as above.

Guinea pig inoculation should also be resorted to when in doubt. The sputum is injected intraperitoneally. If the animal is first given a strong radiation with the X-ray it will succumb much more quickly than otherwise.

Cultivaton of the tubercle bacillus.

Petroff egg media:

Meat infusion glycerin (500 grams of meat infused with 500 cc. of a 15% solution of glycerin in water for 24 hours)	1 part
Beaten eggs (sterilize shells by pouring boiling water over them and break into sterile beaker and beat thoroughly. Filter through sterile gauze into sterile graduate)	2 parts
1% alcoholic solution of gentian violet to make a dilution of	1-10,000

Tube in sterile test-tubes and inspissate for three consecutive days, the first at 85 deg. C till solidified and on the two remaining at 75 deg. C for one hour.

An equal quantity of sputum and 3% NaOH are

mixed and incubated for 30 minutes to digest the former. Neutralize with N/HCl and centrifugalize at high speed for 10 minutes. Inoculate the sediment on to a few Petroff tubes and seal with paraffin. Seven to ten days are required for growth. The gentian violet inhibits the growth of other organisms.

(Method of isolating the organism from the blood. Dr. Mildred C. Clough, J. H. H. Bul., Dec., 1917, page 363.)

10 to 20 cc. of the patient's blood are hemolysed in sterile distilled water and centrifugalized at high speed for 1½ hours. Three-fourths of the sediment is planted upon a blood agar slant, which is then sealed with paraffin to prevent drying. A growth appears after incubation of 7 to 20 days. The other fourth is inoculated into a guinea pig.

II. Pneumococcus. Pneumococci are divided into four groups, in accordance with their serological reactions. See Monograph of the Rockefeller Institute for Medical Research No. 7, October 16, 1917.

Type I. Causes about 33.3% of the cases of lobar pneumonia. The disease runs a severe source. The organism is practically never found in normal sputum.

Type II. Causes about 13% of the cases of lobar pneumonia. The disease runs a severe course. The organism is practically never found in normal sputum.

Type III. Causes about 13% of the cases of lobar pneumonia. The disease runs a most severe course. The organism is found in about 28.1% of normal persons.

Type IV. Causes about 20.3% of the cases of lobar pneumonia. The disease usually runs a mild course. The organisms are found in about 18.2% of normal persons.

Types 11-a, 11-b and 11-x are found in about 18.2% of normal persons.

Isolation of the pneumococcus and determination of group.

1. Mouse inoculation. Obtain a specimen of sputum as free from saliva as possible; wash in six changes of sterile salt; grind a piece the size of a bean in a sterile mortar, add sterile salt solution drop by drop till a homogeneous solution is formed that will readily pass through the needle of a small syringe. Inject 0.5 to 1.0 cc. of this emulsion intraperitoneally into a mouse. Either wait until the mouse is dead or test by peritoneal puncture for the presence of pus, and kill if pus is found. In either case wash the peritoneal cavity out with 4 to 5 cc. of sterile salt solution after making preliminary smears and cultures on blood agar plates. Make a homogeneous suspension of the peritoneal washings and set up tubes as follows for agglutination:

Tube	1	2	3	4	5
	Put 0.5 cc of suspension into each of 5 tubes,				
	G. No. 1 serum 1-20, 0.5 cc	G. No. 2 serum undiluted 0.5 cc	G. No. 2 serum 1-20, 0.5 cc	G. No. 3 serum 1-5, 0.5 cc	Bile
Type I....	++	—	—	—	Dissolved
Type II...	—	++	++	—	Dissolved
IIa, IIb IIx	—	+	—	—	Dissolved
Type III..	—	—	—	+	Dissolved
Type IV...	—	—	—	—	Dissolved

(Incubate for 1 hour at 37 deg. C.)

Precipitin test. For this test the peritoneal washings are centrifugalized until the supernatent fluid is water clear. The clear fluid is pippetted off and the following set-up made:

Tube	1	2	3	4
	Put 0.5 cc of supernatent fluid in each tube.			
	Serum No. 1 1-10, 0.5 cc	Serum No. 2 undiluted 0.5 cc	Serum No. 2 1-10, 0.5 cc	Serum No. 3 1-5, 0.5 cc
Type I....	—	—	—	—
Type II...	++	++	++	—
IIa, IIb IIx	—	+	—	—
Type III..	—	—	—	+
Type IV...	—	—	—	—

(The precipitin reaction usually occurs at once. If it does not, incubate.)

2. Sputum cultures. Obtain a specimen of sputum as free from saliva as possible and wash through six solutions of sterile salt solution. A blood-tinged flake is the one of choice. After washing the flake place a small portion of it upon a blood agar plate and break it up with a platinum wire bent at a right angle. Streak three agar plates with the wire without reinoculation. Incubate for 24 hours and then look for small colonies with a green zone about them. Pick colonies, inoculate broth tubes, and carry out agglutination test as given.

3. Precipitin test in the urine. This test is often

positive within 12 hours after the initial chill and remains positive for some days. When it is positive it furnishes a rapid and accurate method of determining the group of the organism. For this test 0.5 cc. of urine are placed in each of three test tubes. To the first tube is added 0.5 cc. of Group No. 1 serum undiluted; to the second, 0.5 cc. of Group No. 2 serum undiluted, and to the third, 0.5 cc. of Group No. 3 serum undiluted. If a precipitate does not come down immediately, incubate at 37 deg. C. for one hour. The presence of the precipitin reaction in the urine indicates a severe infection and is of bad omen, especially if it increases from day to day.

4. Avery's method. (Jour. A. M. A., Vol. 70, No. 1, Jan. 5, 1918, page 17.) This method is based upon the fact that carbohydrates and blood proteins when added to suitable media accelerates the growth of the pneumococcus, and also that bile dissolves the organism.

Special media for the test:

Meat infusion broth, 0.3 to 0.5 acid to phenolphthalein (sterilized in arnold sterilizer on three consecutive days)	100 cc.
20% glucose (sterile)	5 cc.
Defibrinated rabbit's blood (sterile)	5 cc.

Tube media in small tubes about 4 cc. to the tube.

A kernel of sputum the size of a bean is selected and washed in six changes of sterile salt solution, emulsified in broth and inoculated into one of the tubes of special media. Incubate at 37 deg. C. for five hours and streak a blood-agar plate for the isolation of the organism and subsequent confirmation of type. Remove the blood cells from the special media by slow centrifugalization. Remove 3 cc. of the bacterial emulsion to another centrifuge tube and add to it 1 cc. of sterile ox bile. Incubate till

the solution of the pneumococci has taken place and perform the precipitin test, using 0.5 cc. of serum and bile solution. If ox bile is not obtainable, perform the agglutination test after the cells have been removed.

5. Krumwiede and Valentine's method. (Jour. A. M. A., Vol. 70, No. 8, Feb. 23, 1918, page 513.)

This method is based upon the fact that many sputums are comparatively rich in soluble antigens, and these antigens are not destroyed by heating to boiling.

From 3 to 10 cc. of sputum are placed into a test tube, which is then placed into boiling water till the albumins are coagulated; that is, if the specimen is a suitable one. Break up the coagulum with a platinum wire and add just enough N/NaCl to carry out the test after it has been centrifuged. In some instances no saline is necessary, for sufficient fluid separates to carry out the test. After the coagulum has been broken up agitate it in the saline and place again into boiling water for a few minutes to extract the antigen, shaking gently. Centrifugalize and use the supernatent fluid for the test.

Place 0.2 cc. of the undiluted anti-pneumococcus serum in narrow tubes and upon each layer an equal quantity of the supernatent fluid. Place in a water bath at 55 to 60 deg. C. and observe in several minutes. If a great quantity of the antigen is present in the sputum, a ring will be observed in a short time, but if smaller quantities are present, longer incubation will be necessary. The advantage of this test and of the other tests is to determine the group of the organism and the employment of serum in the treatment should it be Group No. 1.

III. Meningococcus. See monograph by Simon Flexner on "Mode of Infection, Means of Preven-

tion, and Specific Treatment of Epidemic Meningitis," the Rockefeller Institute for Medical Research, 1917. "The meningococcus enters and leaves the body by way of the nasopharyngeal membrane."

Mode of identification. The West tube is used to swab the nasopharynx. This tube consists of a glass tube about 7 mm. inside diameter and bent at nearly a right angle at one end. A copper wire carrying a cotton swab at one end and a loop on the other is inserted into the tube, which is then plugged at both ends and sterilized. The nasopharynx is swabbed by inserting the glass tube up behind the soft palate, then pushing the cotton swab out against the pharyngeal wall and swabbing it, and finally pulling the cotton into the tube again and withdrawing the whole thing. The object is to prevent saliva from getting on the swab, which is destructive to the organism. The inoculated swab is then run over a series of three plates of sheep serum agar, which should be made as follows:

Melted meat infusion agar with a plus 0.4 to phenolphthalein and cooled to 50 deg. C.	100 cc.
Dextrose	1 %
Sheep serum 1 part, distilled water 3 parts (sterilized at 15 lbs. for 40 minutes)	20 cc.

Avoid chilling the plates and incubate at 37 deg. C. for from 16 to 20 hours.

A medium more favorable for growth is made as follows:

Nutrient agar melted and cooled to 50 deg. C.	100 cc.
Sterile rabbit's blood 5cc laked in sterile distilled water 40 cc.	20 cc.

The colonies of the meningococcus are small, delicate and their outlines fade away into the medium.

Make smears and stains of suspicious colonies

and transfer to laked-blood or sheep serum agar slants. The meningococcus together with M. catarrhalis, flavus and pharyngis-siccus are gram negative. Incubate the slant inoculations for 24 hours, and then emulsify in salt solution and subject to the following agglutination:

Polyvalent	1-50	1-100	1-200	1-500	1-1000	1-2000
Serum	0.8 cc.	0.8 cc.	0.8 cc.	0.8 cc.	0.8 cc.	0.8 cc.
Emulsion	0.2 cc.	0.2 cc.	0.2 cc.	0.2 cc.	0.2 cc.	0.2 cc.

Incubate at 55 deg. C. for 16 hours and read. Any culture which is agglutinated in a dilution of 1-200 and ferments glucose and maltose with acid production, but does not ferment saccharose, is considered provisionally as meningococcus and the person from whom it was isolated is regarded as a carrier.

Some differential points between meningococcus and other gram negative organisms of throat:

Micrococcus flavus: Colonies yellow and opaque; indiscriminate agglutination, agglutinated by normal horse serum in a dilution of 1-50 and by polyvalent serum in a dilution of 1-100 or slightly higher.

Micrococcus catarrhalis: Exerts no action on glucose, and no agglutination in higher dilutions of specific sera.

Other indefinite gram negative micrococci: Ferment saccharose.

Olitsky method for the identification and isolation of the meningococcus. (Jour. A. M. A., Jan. 19, 1918, Vol. 70, No. 3, page 153.)

This method takes advantage of a fluid medium which serves to eliminate other organisms resembling the meningococcus and reduces the time necessary for the identification to about 12 hours.

Medium: Glucose broth, 1% (made from veal infusion and having acidity of from plus 0.5 to 0.7 phenolphthalein) 100 cc.
Sterile, clear, unheated, normal horse serum 5 cc.

Tube this medium in 8 or 10 mm. tubes, about 1 cc. to the tube. Suspicious colonies are fished from a plate inoculated with the nasopharyngeal secretion of a suspected carrier and are seeded into these tubes, a colony to a tube. The tubes are then incubated 12 hours, and at the end of this time a great many negatives can be eliminated.

Organisms which must be eliminated:

1. Micrococcus flavus, crassus, pharyngis-siccus and unclassified gram positive bacilli will show firm agglutination below and slight turbidity above.
2. Bacillus influenzae will not grow for the want of hemoglobin.
3. Micrococcus catarrhalis grows with a dense turbidity, and often shows a pellicle on the surface.
4. The gram positive staphylococci grow with dense turbidity, show agglutinated masses in the sediment, and often have a pellicle.
5. Streptococci grow with a clear or turbid supernatent fluid, but show an agglutinated sediment.

The meningococci give a faint turbidity and a slight sediment forms which *emulsifies uniformly* when the tube is shaken. The suggestive positive tubes are separated from the definitely negative ones, and to the former is added 0.1 cc. of a 1-10 dilution in 0.85 saline of a high titer polyvalent antimeningococcus serum. Incubate the tubes at 37 deg. C. for two hours in the water-bath and the tubes containing meningococci will show definite agglutination, and those which do not will remain

unchanged. From the positive tubes cultures can be obtained for further identification.

IV. Diphtheria bacillus. It is important to remember that there are organisms in the throat of healthy persons which have the morphological characteristics of the diphtheria bacillus, but which produce no toxin, these being the diphtheroids; also, that one examination of a diphtheretic throat may give negative results: hence, upon the ability to produce toxin should rest the final diagnosis of the organism and one examination should not suffice in suspicious cases.

Diphtheroid

The throat is swabbed with a small piece of cotton and the surface of a serum agar plate is streaked with it. Incubate from six to eight hours when the diphtheria colonies will be visible and contaminating organisms will not. Make smears from colonies and stain with Loffler's methylene blue. Stained specimens of the cultivated bacillus show the typical beaded appearance with clubbed ends. For more careful study use Neisser's stain.

V. Bacillus mucosus capsulatus. This gram negative non motile bacillus varies from coccoid forms to longer bacilli. They are surrounded by a large capsule which is easily demonstrable. They grow easily on plain agar, which growth is mucoid and sticky. They often cause a very fatal broncho or lobar pneumonia, the sputum of which is slimy and sticky.

VI. Influenza bacillus. This is a very short, moderately thick, gram negative bacillus which grows singly in pairs or sometimes forms threads. It has 2 polar bodies which make it resemble a gram neg. diploccus. It is a frequent secondary invader in respiratory and pulmonary infections, such as chronic bronchitis, bronchiectasis and tuberculosis. The orbanisms grow best on blood agar, upon which the colonies appear as small, colorless dewdrops. It has not yet been proved to be the cause of influenza epidemics.

Avery's influenza media:

Defibrinated blood, centrifugalized and cells resuspended in amount of broth equal to original volume of blood	1 cc.
Agar (sterile)	95 cc.
Sodium oleate 2% (neutralized and autoclaved)	5 cc.

This media is said to inhibit other organisms of the throat and to enhance the growth of the influenza bacillus.

VII. Spirochaeta pallida. This organism is sometimes found in the mouth in association with syphilitic lesions there. It must be differentiated from S. dentalis, S. buccalis and S. refringens. In general it may be said that under the dark field illuminator these other spirochaetes have a much livelier movement than the pallida, which hardly moves out of the field. It has a lashing motion, a spinning motion about its long axis and a slight backward and forward motion. It is usually easy to identify the pallida from these characteristics.

VIII. Spironema vincenti. This organism is found in Vincent's angina in association with a cigar-shaped bacillus. They are both readily stained with dilute carbo-fuchsin and gentian violet.

Sputum in various diseases.

1. Lobar pneumonia due to the pneumococcus. The course of the disease can be followed by the type of sputum. It is blood tinged for the first three or four days and mucoid in consistency. After this blood cells disappear and it assumes a rusty appearance from altered blood pigments. This lasts until after the crisis, when it becomes muco-purulent, and later serous. Occasionally one sees pneumonia without sputum at all. In some cases a green color is noted in the later stages of the disease, due to altered blood pigments. In pneumonia due to the B. mucosus capsulatus the sputum is slimy and sticky and very tenacious.

2. Tuberculosis. This disease may have almost any kind of sputum. As a rule, in the early stages there may be little or no sputum; later it may become mucoid or muco-purulent and blood streaked. On microscopical examination one may find the acid-fast organisms and elastic tissue.

In chronic tuberculosis the amount of sputum varies from little to tremendous amounts. It may be bloody, muco-purulent, or purulent.

In ulcerative tuberculosis one finds sputum of a sweetish odor, blood clots, a great deal of elastic tissue, caseous lumps which do not give a bad odor on crushing, and on microscopical examination one may find all kinds of contaminating organisms.

In fibroid tuberculosis one may have no sputum, but it may be mucoid or muco-purulent.

3. Abscesses. The sputum is abundant, cheesy, purulent, and masses of blood may be present. Occasionally one finds masses of lung tissue and elastic tissue.

4. Gangrene. The sputum is the same as abscess, except that the odor is characteristically very foul and penetrating.

5. Infarction. Immediately after the accident the sputum is stringy and mucoid and blood streaked, or there may be a marked hemoptysis. The sputum soon becomes rusty and "prune juice" in character, which change comes on sooner than in pneumonia.

6. Chronic passive congestion. In this condition the sputum is thin and abundant. It may be slightly rusty. On microscopical examination one finds "heart-failure cells," i. e., alveolar epithelium containing blood pigments.

7. Asthma. There is no sputum in the early stages of the attack, but when the attack breaks pearls of Launec (mucoid globules) make their appearance. On careful examination one finds, also, Curshmann's spirals, Charcot-Leyden crystals and eosinophiles.

8. Bronchitis. There may be no sputum or there may be present pearls of Launec and Charcot-Leyden crystals.

Fibrinous bronchitis. In this type of bronchitis there may be fibrin casts of the bronchi.

Purulent bronchitis. The sputum has a mucoid base with a yellowish appearance from the presence of pus cells.

Ulcerative bronchitis. Epithelial cells remain unchanged. Goblet and ciliated cells appear occasionally. Tissue fragments and blood may be present.

Chronic bronchitis. The sputum is usually thin, may be tinged with blood, and may contain Dittrich's plugs. In the later stages of the disease the sputum may become foul, abundant and muco-purulent.

9. Bronchiectasis. The sputum usually occurs periodically in large amounts and has a fetid odor. In the early stages it is thin and watery, but later

(Continued on Page 146)

it may resemble the sputum in abscess, except that there is not so much pus. Cartilage, elastic tissue, clots of blood and tissue masses may be present. Its separation into three layers, viz., top, brownish froth; middle, clear and mucoid, and bottom, granular; is not characteristic for this disease, but occurs whenever there are large amounts of sputum.

10. Pulmonary oedema. This is usually a terminal event, but frequently occurs after too vigorous thoracentesis. It may start during the tapping and may last from 5 minutes to 24 hours. Huge amounts of fluid are given off. It is a safe rule not to draw off more than 1500 cc. at one tapping.

STOOLS.

Constituents of stools.

I. Food remnants. These are undigestible or unabsorbed. Normally, there are some and it is difficult to draw a line between the pathological and the normal under various conditions. When an excess of meat fibres occur, the condition is called *creatorrhea*; and when an excess of fat occurs, the condition is called *steatorrhea*. It is important to become familiar with vegetable cells in order not to confuse them with animal parasites or their ova.

Fat. Fat occurs as soaps and fatty acids and sometimes as neutral fat. If it occurs as neutral fat, it is yellow and clear; while as fatty acid, it is white and glistening. Soaps usually occur as the insoluble calcium and magnesium soaps, but in rapid peristalsis the soluble sodium and potassium soaps may occur. Neutral fats indicate the absence of lipase. Fatty acid increase indicates an excess of fat in the diet over that which can be absorbed or hyperperistalsis. In the decreased secretion of bile an excess of fats occur also.

DIFFERENTIATION OF FATS, FATTY ACIDS & SOAPS.

Test.	Neutral fat.	Fatty acid.	Soap.
Heat	Melted	Melted	0
Ether	Dissolved	Dissolved	0
Sudan III	Stained	Crystals 0 Globules +	0
Osmic acid Scharlack R.	Stained	Stained	0
H_2O	0	0	Na. & K. dissolved Ca. & Mg. undissolved
KOH	0	+	0
Carbol fuchsin	0	+	0

Soaps can be broken up with acetic acid and then stained with Sudan III. Some fatty acids with a low melting point appear in globular form and stain with Sudan III.

Sugar and starch. These are not usual. Occasionally they come through in a cellular envelope. Test with iodin which turns starch granules blue, and Benedict's solution which is reduced by sugar.

II. Intestinal tract secretions.

Bile. Occult bile never seen in the adult. To determine the presence of bilirubin or hydrobilirubin emulsify about 2 or 3 cc. of the stool in a concentrated solution of mercuric chloride. Allow to stand 24 hours and examine microscopically. Green signifies the presence of bilirubin; red indicates hydrobilirubin.

Urobilin. This is normal in certain amounts, but large amounts are pathological. To determine its presence use Schlessinger's test as follows: Make an acid-alcohol extract of the stool, add a few drops of Lugal's solution, 1 or 2 cc. of NH_4OH, and an equal volume of an alcoholic solution of zinc acetate. Filter and examine the filtrate for a greenish fluorescence. Spectroscopic examination of the intestinal fluid normally is positive in dilutions up to 1 to 9,000. In higher dilutions than this it is pathological.

Mucus. When mucus is abundant it means catarrhal inflammation. In a disease resembling asthma one may get complete casts of the colon, which are usually passed between stools. To test for mucus is usually unnecessary.

Ferments. These are usually tested for in order to determine whether the pancreas is functioning normally or whether the pancreatic secretion is entering the intestine. Since trypsin and lipase are more easily destroyed by bacteria and each requires activation to show its maximum efficiency, and also since proteolytic action of the intestinal bacteria may complicate the findings, the study of the diastase of the stools is probably the best means of obtaining this information.

Method of performing test:

1. The patient is given a light meal and a high enema the night before.
2. At 7 A. M. the next day, 750 cc. of milk are given.
3. At 7.30 and 8.00, half an ounce of Epsom salts.
4. At 8.30, a glass of water containing a teaspoonful of bicarbonate of soda.

Save all stools up to 2 P. M. in a vessel containing 2 ounces of toluol and keep in the ice-chest or cool room. If less than 400 grams of cc. are obtained, an enema of a pint of water is given. From 400 to 1,100 cc. are to be expected.

Titration of the diastase: Dilute the stool with enough water to make 3,000 cc.. Dilute 2 cc. of this with 48 cc. of isotonic salt, which gives a dilution of 1 to 25, and then centrifugalize to clear. Set up tubes as follows:

1	2	3	4	5	6
1 cc stool	1 cc stool	1 cc stool	1 cc stool	1 cc stool	1 cc stool
1-25	1-50	1-100	1-200	1-400	1-800

Add 2 cc of 0.1% starch solution, after dilutions

are made, to each. Incubate at 37 deg. C. for half hour and add a few drops of a 1% solution of iodin to each tube. Blue shows the presence of undigested starch.

Calculation:

½ cc. of 1-25 diluted stool digests 2 cc. of 0.1% starch.

1 cc. of 1-25 diluted stool digests 4 cc. of 0.1% starch, or 0.4 cc. of a 1.0% starch solution.

1 cc. of undiluted stool digests 10 cc. of a 1% starch solution. The unit is the digestion of 1 cc. of a 1% starch solution by 1 cc. of undiluted stool. The minimum of normal is 600 units.

(See article by Dr. Brown in Boston Med. and Surg. Jour., Nov. 30, 1916, Vol. CLXXV, pages 775 to 784.)

Schmidt and Strasburger diet for pancreatic efficiency:

1. Morning: 500 cc. of milk and 50 grams of zweibach.
2. Forenoon: 500 cc. of oatmeal gruel composed of:

Oatmeal	40 gm.
Butter	10 gm.
Milk	200 cc.
Water	300 cc.

3. Noon: 125 gm. (raw weight) of chopped beef, broiled rare and 20 gm. of butter.

250 gm. of potato broth composed of:

Mashed potato	190 gm.
Milk	100 cc.
Butter	10 gm.

4. Afternoon: Give same as for forenoon.
5. Evening: Give same as for dinner.

This diet should be given three days or longer in order to obtain stools in which it is present. In

pathological conditions there will be food remnants of all kinds.

III. Decomposition products.

Gasses: H_2S, CH_4, N. H, and CO_2, which are the products of fermentation and putrefaction.

Crystalline bodies: Indol and skatol. These give the feces their characteristic odor.

IV. Bacteria. These make up about one-third by weight of the stool.

A. Those which normally occur: The majority of these belong to the colon group, among which the more common ones are: B. coli, including B. fecalis alkaligenes, and B. acidi lactici; B. lactis aerogenes, B. subtilis, B. proteus vulgaris, etc. A few streptococci and staphylococci are sometimes found.

B. Those which occur under pathological conditions.

1. Bacillus typhosus. This organism occurs in the stools of typhoid patients and of typhoid carriers. One of the best differential culture media for identification is Endo's media, which is made, according to Robinson and Rettger's modification, as follows:

Agar	25 grams
Meat extract	5 grams
Pepton	10 grams
Water	1000 cc.

Dissolve the agar, pepton and meat extract, and autoclave at 15 pounds pressure for 30 minutes. Filter through cheese cloth and cotton, add 10 cc. of 10% sodium carbonate, adjust reaction to # plus 0.1 phenolphthalein, put in 100 cc. containers and autoclave at 10 pounds for 10 minutes.

When ready to use, add to each 100 cc. of agar:

20% lactose solution (Arnoldized)	5 cc.
10% freshly prepared anhydrous sodium sulphite sol. (sterile)	1 cc.
Saturated alcoholic fuchsin (basic)	0.5 cc.

Pour plates and allow to harden without the covers upon them.

Plus 0.1 phenolphthalein means the addition of 0.1 cc. of N/HCl to 1000 cc. of the medium after the neutral point of the solution has been determined with phenolphthalein as indicator.

The basic fuchsin is colorless in an alkaline medium, but turns red in acid solution. Since the typhoid bacillus does not ferment lactose, the typhoid colonies will remain colorless, while the colon colonies will turn red because the colon bacillus ferments lactose.

An emulsion of the stool is made in isotonic salt solution and a loopful of the emulsion is transferred to an Endo plate. With a bent wire the surface of the plate is streaked, and, without flaming, a second plate is treated likewise. Incubate 24 hours and pick colonies. In order to be relatively sure that a colony picked is a typhoid one, microscopic agglutination can be done in the following way: A low dilution of anti-typhoid serum is made (1-100), and one drop of this is placed upon a clean slide. A drop of salt solution is placed upon the same slide near the drop of serum, and in it an emulsion of one of the suspicious colonies is made. The two drops are now coalesced, and within 2 or 3 minutes, if the colony picked is one of typhoid, definite clumps can be made out. One must be sure in this procedure to have the drops large enough to prevent drying, which would give false readings. If the colony picked gives agglutination, the remainder is inoculated upon agar for further identification. If

one should titrate specific sera with typhoid, paratyphoid (a) and paratyphoid (b), one could determine the dilution of each, which would agglutinate the organism for which it was specific and not those which it agglutinates in lower dilutions. By this rapid slide agglutination one can, by properly diluting the sera, sometimes determine which of the typhoid group of organisms he is dealing with.

2. Dysentery bacilli. These are a group of bacilli which are responsible for some forms of dysentery and which are differentiated by sugar reactions and agglutination tests. Their isolation can be accomplished by the use of Endo's media or brilliant green media, for which see Park and Williams Bacteriology. These are short gram negative rods, with little or no motility. The colonies are not as large nor as opaque as the typhoid or colon and most of the groups are colorless on Endo's media. They also appear later than the foregoing. By use of the polyvalent serum they can be detected by macroscopic slide agglutination, and then transferred to other media for more careful study. In making cultures it is desirable to pick the bloody flakes of mucus.

3. Tubercle bacillus. These are occasionally swallowed and must be excluded. The smegma bacillus must also be differentiated, which is not alcohol acid-fast. Particles of blood-stained mucus are most likely to show the organisms. The antiformin method is not very satisfactory unless the organisms are present in large numbers.

4. Cholera spirillum. This organism can usually be diagnosed from morphology and serum reactions. It can grow in a media too alkaline for the growth of other organisms. In fluid media it seeks the surface where oxygen is most abundant. It liquifies gelatin slowly, unlike S. metchnikovi.

V. Animal parasites. See section on parasitology.

VI. Foreign bodies.

1. Gall stones. These may be large or small. Stones as large as the gall bladder itself may ulcerate into the intestine and cause obstruction. Smaller stones must be differentiated from cartilage, insoluble soaps, enteroliths and fecal masses, which can be done by crushing, dissolving in ether and allowing to crystallize out. In the case of gall stones, one will find cholesterin crystals.

2. Intestinal sand. This is mucus impregnated with calcium salts. These smaller particles may become matted together into larger enteroliths.

3. Objects swallowed. Coins, buttons, safety pins, hair, etc.

4. Objects left in the abdominal cavity during operation. Clamps, sponges, knives, etc., may ulcerate through the bowel and be passed per rectum.

VII. Tumor fragments. If these come from any distance they are digested beyond recognition. Occasionally they aid in the diagnosis of tumor.

VIII. Pus cells. A few occur normally. When in very great numbers they may arise from ruptured abscess. When seen in clumps they may indicate some ulcerative process, such as dysentery, ulcerative colitis, syphilis, carcinoma, tuberculosis or typhoid.

IX. Blood. That coming from the mouth must be excluded. If the blood is at the bottom of the stool its source must be low down in the intestinal tract; if on top, its source is higher up. If blood comes from high up in the intestinal canal, it will be dark and give the stool a characteristic tarry appearance, although its source may be high up and appear dark red if there be rapid peristalsis. Blood from the stomach gives a tarry black appearance.

Blood occurs in any ulcerative condition of the

intestinal tract, such as typhoid, dysentery, carcinoma, ulcer, etc. It also occurs as a result of rupture of blood vessels into the stomach or intestine, as in Banti's disease, aneurism or hemorrhoids.

Tests for blood. The best is the guiac test. Make a watery emulsion of the stool, add a few drops of glacial acetic acid, extract with ether and perform in the same manner as with the urine. The benzidine test can be carried out in the same manner.

A good preliminary test is the following:

Make a smear of the stool upon a glass slide, and before it dries add a solution of benzidine in glacial acetic acid plus an equal volume of hydrogen-peroxide. The absence of a green color shows that no blood is present. If the material turns a green color, a more accurate test, such as the above, should be performed.

X. Crystals.

1. Fatty acid. Long or short needles singly or in groups.
2. Calcium phosphate. Wedge shaped in rosettes, or singly.
3. Magnesium phosphate. Rhombic plates.
4. Triple phosphates. Coffin-lid crystals.
5. Calcium carbonate, sulphate and oxalate. (See urine.)
6. Calcium lactate. Radiating needles in sheet-like masses. Found in children on milk diet.
7. Cholesterin. Usually occur as steps, one superimposed upon the other. In doubtful cases test with concentrated sulphuric acid. The color changes from yellow to blood red, violet, green and, finally, blue.
8. Charcot-Leyden crystals. These are colorless, double-pyramid crystals.

9. Hematoidin crystals. These are reddish yellow, rhombic plates, groups of needles or amorphous masses.

Color of stool. The normal color is brownish, due to hydrobilirubin. Bilirubin occurs in children, but never in adults. The color also depends upon exposure to the air and the character of the diet as well as medication. Milk gives a light-brown color; meat, dark brown; bismuth, black; senna and rhubarb, yellow, and calomel, a green, which is due to bilirubin not changing into hydrobilirubin. Blood gives all shades from bright red to black. Clay-colored stools occur in pancreatic disease due to excessive amount of fat, the action of bacteria reducing bile pigments, or the absence of bile due to the occlusion of the bile duct. Yellowish-green stools often occur in hypermotility of the intestine.

Odor of stool. Normally it depends upon the presence of indol, skatol, methane and hydrogen sulphide gas. The odor is most marked on a heavy meat diet and less marked on a vegetable or milk diet. It is almost lost during fasting. An excess of fermentation over putrefaction will give a sour odor. In some cases of severe dysentery and carcinoma of the large bowel the stool has an intensely disagreeable odor.

Number and amount. There are great individual variations. There may be a normal number of stools and yet be constipation. Normally from 120 to 250 grams are excreted a day.

STOOLS IN DISEASE.

There are no characteristic stools in disease, but the stools of some diseases are of some diagnostic help.

1. Obstructive jaundice. (Due to stone in the common or hepatic duct, carcinoma at the head of the pancreas, or duodenitis with temporary closure of the common duct.) The stool is strictly acholic and clay-colored if the obstruction is complete and fat globules and crystals are seen. Clay-colored stools resembling this are sometimes found where no obstruction to the bile excretion exists, but where bilirubin appears in the form of a leukobase and can be demonstrated by the bilirubin test. Clay-colored stools associated with nausea and vomiting and jaundice give a complete picture. If there is a history of colics, search, at least for three days, for stone. If one is found, confirm by crushing, extract with ether, evaporate and examine for cholesterin crystals.

2. Pancreatic disease. (May be associated with stone shutting off the pancreatic duct.) The stools are very large and have an abundance of neutral fat. They may resemble vaseline or freshly-fallen snow. They may be odorless or sour. Besides the great quantity of neutral fat, fatty acids are found and great quantities of undigested meat fibres.

3. Carcinoma of the stomach. There is usually associated hyperperistalsis, but occasionally anti-peristalsis. In the cases with hyperperistalsis there occurs a copious diarrhea. If no blood is present, they are pale or yellow in color, but if blood is present, they are brown to black. The odor is usually foul. On microscopical examination one finds undigested meat fibres more frequently in this disease than any other.

4. Carcinoma of the rectum. The stool may be

normal in size, shape and consistency, or, if the growth has caused a stricture, they may be ribbon-like. They may show a coating of blood if ulceration has taken place, and mucus. Occasionally tissue fragments are found.

5. Annular carcinoma of the sigmoid. The growth may constrict the lumen and cause obstruction. There is usually constipation, distension, much gas, and there may be hyperperistalsis. Metastasis occurs late and the prognosis is usually good.

6. Amyloid disease. Abundant and frequent stools with little odor. No tenesmus, pain, blood nor mucus. Diarrhea continuous.

7. Hypoacidity. Foul-smelling, abundant diarrhea, with meat particles, much mucus, and blood if associated with carcinoma. Often a little hydrochloric acid will relieve condition.

8. Mucus colitis. The cause may be due to a foreign protein, much like asthma. Charcot-Leyden crystals and eosinophiles are found.

9. Sprue. There is irritation of the whole gastro-intestinal tract. There is stubborn diarrhea and pain.

10. Metallic poisoning. (Arsenic and mercury.) These drugs cause a gastro-enteritis. Mercury is excreted in the colon and causes an intense irritation and watery stool.

11. Stricture of the rectum. This may be due to any of a number of causes: syphilis, carcinoma, and sometimes a congenital malformation. Ribbon stools are characteristic. If there is ulceration, as in carcinoma and lues, there will be an excess of mucus and pus. Ribbon stools always indicate rectal examination.

12. Constipation. There is a diminution in the number of stools, large appetite, a feeling of depression, and usually headache. This is more common

and chronic in women. A decrease in the number of stools may be due to impaction of fecal masses in the rectum. In many cases these can reached with the finger and dislodged or broken up.

13. Megalo-colon. (Hirschsprung's disease.) There is a congenital weakness of the wall of the colon. The bowels may move once a week, once a month, and one case is reported of once a year. These patients present all the signs of constipation.

14. Typhoid. This is associated frequently with constipation, especially in the early stages. During the stages of ulceration profuse watery and foul-smelling stools occur. They may be yellowish-green in color and give rise to the term "pea soup stools." Hemorrhages are frequent which are usually preceded by oozing and the appearance of occult blood. Clinically, this indicates the cessation of hydrotherapy and feeding. Typhoid cultures are easily obtained.

15. Cholera. The stools are usually profuse with an abundant watery discharge, although moderate cases show only a moderate amount of diarrhea. Flakes in mucus, rice-like in appearance, give the term "rice-water stools."

16. Amoebic dysentery. This sometimes runs an intermittent course. There are periods of intense diarrhea, followed by periods of normal stools and even constipation. Amoebae are found in the patches of bloody mucus.

17. Tuberculosis. There is a great variation in the stools, which depends upon the site of the lesion. In tabes mesenterica there is an interference with absorption, and one finds an excess of fatty acids and soaps in the stools. This is unlike pancreatic disease, where an excess of neutral fat and fatty acid occur. Dietary indiscretions lead to great diarrhea.

A. OUTLINE OF ROUTINE FOR MAKING QUALITATIVE EXAMINATIONS OF BLOOD.

I. Cleaning glass ware (slides and cover slips).

a. Gross cleaning—several methods.

(1) Wash with soap and water, then with much clean, hot water (never allow the water to cool till all the soap is removed), followed by distilled water, finally put in 95% alcohol (may be put into ether or chloroform after alcohol).

(2) Soak in acid mixture (conc. nitric acid or a mixture of sulphuric acid and potassium bichromate) for 6 to 24 hours, then wash thoroughly in clean water, then distilled water. Finally run through two thorough washings with 95% alcohol and put in ether or chloroform (ether may be omitted—keep in alcohol—closed vessel).

b. Fine cleaning.

(1) Keep in alcohol (ether or chloroform), and when needed polish with a clean cloth (old linen preferable) absolutely free from grease and dust. Ex. unfold an old but clean and ironed handkerchief—use the inside surface which no finger has touched.

(2) Take from alcohol (ether or chloroform) and polish, keeping them in a clean, dry, well-closed receptacle until used. Ex. A closed glass dish. (Avoid wooden or pasteboard boxes, as fragments fall from such materials.)

(3) Never hold with fingers, always use *clean* forceps to handle glass ware.

(4) Dust off with clean camel's hair brush before using.

(5) It is often advantageous to blow across glass ware just before placing blood on it—care must be exercised that no particles of saliva fall on glass.

II. Making smears.

a. Preliminary details.

(1) Use glass ware cleaned in manner suggested under I. Cover glasses must be square (round will not do); cover glasses must not be too thick, as oil immersion cannot be focused upon preparation; size 22 mm. preferable, thickness No. 1 essential.

(2) Glass ware must be flat—avoid concave surfaces—blood will not spread.

(3) Dip blood sticker in alcohol before using (never flame sticker—dulls point).

(4) Wash skin with alcohol and dry before sticking. Alcohol frees skin from grease and is antiseptic; drying prevents blood drop from spreading over skin.

(5) Avoid cyanosed areas and avoid cyanosis by squeezing area which is to be stuck, in order to make the blood flow. Blood examinations from such areas are useless for many purposes.

(6) Stick deep enough to bring from 2 to 4 drops of blood—preferably without the assistance of any pressure. While any pressure is undesirable, a slight amount is sometimes necessary.

(7) Wipe off the first drop—use the second, which is more representative of actual blood picture.

b. Actual smearing.

(1) Cover slip method.

(a) With instruments.

1. Hold cover slip No. 1 in cross bill forceps.
2. Hold cover slip No. 2 in clean, plain forceps.
3. Brush the cover slip with a clean camel's hair brush to remove any lint or dust.
4. Place drop on cover slip No. 2. Drop should be size of small black-headed pin. Let it reach the cover slip by capillary attraction. Never touch skin with cover slip.
5. With drop on cover slip No. 2, place cover slip No. 2 on cover slip No. 1 in such a maner that the drop is spread without causing bubbles. (Ex. Let cover slip No. 2 come down so that cover slip No. 1 touches edge of drop first and gradually touches whole drop—or let the drop reach the first cover slip by capillary attraction).
6. Let drop spread until it has *almost* stopped spreading—experience is needed for this.
7. Pull cover slip in an absolutely horizontal manner. Any tendency to a vertical pull will ruin the preparation. (Holes in the smear will result.)
8. Place preparations (smear side up) on a clean paper and allow them to dry in the air.
9. Protect smear from insects, ants and flies especially, as they will quickly ruin smear.

(b) Without instruments.

Cover slips may be handled by the fingers instead of with forceps in making smears provided the following precautions are observed:

1. Use forceps to take cover slips from the clean container.
2. Hold cover slip between fingers so that only the edges are touched and never the surface of the glass.
3. Cover slip No. 1 is held in left hand, usually between thumb and forefinger. Place drop on cover slip No. 2, which is held in right hand, and then proceed to make the smear as directed above, with the exception that the pulling is done by holding the cover slip with the fingers rather than with forceps.

(2) Slide method (use of slides instead of cover slips).

A drop, larger than the one used for cover slip method, is placed on a slide and drawn across it so as to make a smear. Various methods for spreading are suggested.

(a) By means of a second slide, preferably with a beveled edge and of a width less than the slide on which the smear is to be made (i. e., break off a corner of a slide to make it narrower)..

(b) A small glass rod (width less than slide), to which another glass rod has been fused, to serve as handle, in such a way that the combination

looks like a T. The cross-beam of the T is used as the spreader.

(c) Cigarette paper, hat pins, long needles, or plain glass rods may also be used as the spreading agent.

(d) The spreader (whichever method suggested being used) may be placed to the left of the drop, and the blood in this way pulled across to the right, or

(e) The spreader may be made to touch the drop from the right and the drop pulled across the slide to the right. (This method is to be preferred.)

(f) The thickness of the spread can be varied by changing the degree of angulation of the slide and spreader.

(N.B.—The drop should be placed well to the left of the slide, so that a long spread may be pulled to the right).

c. Criteria for good smears.

(1) Smooth, even spreads, with R. B. C. lying flat (never in rouleaux)). Their edges may touch, but there must be no overlapping.

(2) At least 8 such areas (low power) without holes and without streaks are required to constitute the minimal requirement for a good smear.

d. Relative merits of cover slip and slide methods.

(1) The cover slip method gives a much better distribution of W. B. C. and is the method necessary to use in making differential W. B. C. counts.

(2) The slide method gives a good distribution of R. B. C. The large W. B. C. (polymorphonuclear and large mononuclear) are pushed to the edges and the lyphocytes remain scattered through the spread. This makes satisfactory differential counting impossible. When R. B. C. are to be examined for parasites, the slide method is superior to the cover slip method, inasmuch as more fields are available for study and the R. B. C. distribution is as good as with the cover-slip method.

III. Making stained preparations.

a. *With Wilson stain.*

(1) The stain. A modified Romanowski stain—a polychrome methylene blue-eosin stain. (For details of making it see Emerson, Webster, etc.)

(2) Technique of applying stain.

(a) Use freshly made smear for staining; smears 2 to 4 days old do not stain so well as those stained very soon after making.

(b) Place preparation with smeared surface up (smeared surface has not the gloss of the clean glass surface) in staining forceps on the edge of a table or on a cork for support in such a manner that it may easily be taken up with a pair of forceps.

(c) With a dropper drop 6 to 8 drops of Wilson stain on the smear, being careful to avoid shaking the bottle of stain before using it in order to keep stain free from any precipitate which may be present. Let the stain

remain on the smear 60 sec. (using second hand of watch for accurate calculation) ; then

(d) Add the same number of drops of distilled water, and let the preparation stand an addition 4 min. (by the watch) ; then

(e) Taking the cover slip with forceps (preferably staining forceps to prevent possibility of dropping cover glass) and holding it carefully in a perfectly horizontal manner so that the scum which has formed on the surface may not touch the glass, float off rather than wash off the scum with a stream of water (preferably distilled), which is at first run very slowly, and then more briskly so as to free the smear from all traces of excess stain. All this time the cover slip is held absolutely horizontally. Washing should take from 5 to 8 seconds.

(N. B.—1. For staining, do not hold smear with any forceps used for pulling smears—forceps used for pulling smears must be kept absolutely clean.

2. Each fresh supply of stain requires a new formula for stain and water combination. In general equal numbers of drops of stain and water are used.)

(f) After washing is completed, the cover slip, still held in the staining forceps, is tilted and the lower edge touched to a blotter, so that the

excess of water is quickly drained off.

(N. B.—If the cover slip be held with any forceps other than staining forceps, great care must be exercised to prevent fluid collected at the forceps' tip from running over the preparation and streaking it. If any such forceps be used, it is best to tilt the cover slip immediately after washing, in such a manner that the excess of water on the cover slip runs *toward* the forceps. Remove the cover slip from the forceps and holding it in the hand, drain off the excess of water by touching an edge to a blotter. (Preferably that edge held previously by the forceps.)

(g) After washing, the smears are dried in one of the following ways:

1. By air drying: a. Generally by placing the smear against some support and letting one edge rest on a blotter. b. By waving it gently in the air, holding it with either forceps or finger (touching only the edge of the cover slip if the finger method is employed).

2. By blotting: Place preparation between layers of fine blotting paper (it must be absolutely free from dust). Apply light pressure to the upper layers of the blotting paper to facilitate drying. Then pick up the preparation and remove it to a dry place, and again apply light

pressure. Be careful not to push the smear along the blotter nor to press too heavily, for holes and streaks in the preparation will be the result.

(N. B.—The staining is thought to be better if the blotting method is employed, but many good smears have been ruined by slight negligence in the manner of blotting.)

(h) After drying the preparation (if it be a cover slip), mount it in Canada balsam (*acid free*) in one of the following ways:

1. Preferably smear side down as the preparation is then protected from insects and any acid or alkali in the air.

2. Smear side up if the cover slip is too thick to permit focusing the oil immersion when mounted smear side down.

(N. B.—If slides are used instead of cover slips, the technique is identical except that the preparation is not mounted, and for examination oil immersion is applied directly to the surface of the smear.)

(3) Criteria for a good stain.

R. B. C.—Are of a buff color, neither lemon nor red.

Platelets—Are well stained—nuclear purple blue stain, with the architecture plainly visible.

W. B. C.—Are stained as follows:

Polymorphonuclears.

P. M. N.—Nucleus—Deep purple, retic-

ular, chromatin pronounced, more polymorphous than polynuclear. Granules—May or may not be seen; vary in size; pink or violet. Protoplasm—Faint pink.

P. M. E.—Nucleus—Larger than P. M. N., fewer lobes; takes lighter purple color; reticulated. Granules — Large, round or oval; bright red; tend not to overlie nucleus. Protoplasm—Faint pink.

P. M. B.—Nucleus—Chromatin scanty, stains light purple. Granules—Large, vary in size; purple to black; generally some overlying nucleus. Protoplasm—Faint pink.

Lymphocytes.—Nucleus—Large, round or oval; slightly notched, chromatin pronounced, deep purple; clear zone outside. Granules—Normally none; old cells (?) show azure granules; red violet; vary in size; few to a cell. Protoplasm—Scanty, crescentic ring; homogeneous sky to deep blue; slightly reticulated.

Large Mononuclears. — Nucleus — Large, oval, indented, horse shoe, kidney or very irregular shape; chromatin poor; light blue or purple color; generally eccentric. Protoplasm—Abundant, often irregular, clear, reticulated, pale blue (reticulated nodosities give granular appearance). Granules—None (azurophilogranules frequent).

Malarial parasites are beautifully stained with Wilson stain. The areas between the cells must be clear and free from all suggestion of stain. The cells must stand out with distinctness with no suggestion of hazy edges. There must

be no precipitate present.

(4) Common pitfalls in staining with Wilson stain.

(a) Precipitate on the preparation.

1. Due to: a. Faulty washing, by not holding preparation horizontal and floating off scum, thus permitting the scum, which always forms, to touch the smear. b. Permitting dust to settle on the smear.

2. Prevented by: a. Holding the preparation horizontal all during the washing and learning to play the stream of water in washing to the best advantage. b. Keeping the smears clean during the interval which elapses between pulling and staining.

(b) An indefinite serum-like stain between the cells, due to insufficient washing.

(c) Tearing preparation by improper blotting.

(d) Deterioration of stain supply due to:

1. Acids kept in the same locker.

2. Water from mixing pipettes or putting stain in bottle washed with water.

b. *With Jenner stain.*

(1) The stain. A simple methylene blue-eosin, alcoholic stain. (For details see Emerson, Webster, etc.)

(2) Technique of applying stain.

(a) Freshly made smears stain better than those 24 to 48 hours old.

(b) Staining, washing, blotting and

mounting are carried out in a manner like that described under Wilson stain with the following differences:

1. Place 6 to 8 drops of stain on the smear and leave for 2 min. (use watch for timing), then add the same number of drops of water and leave for an additional 2 min. (use watch), wash and dry.

(N. B.—With each fresh supply of stain a new formula for the stain and water combination, as well as for the time relation, is necessary).

(3) Criteria for a good stain.

R. B. C.—Are of a darker color than the R. B. C. with Wilson stain, pink rather than buff, although it is possible and advisable to have them look as much like the R. B. C. of the Wilson stain as possible.

Platelets—Stain rather poorly—a pale blue.

W. B. C. are stained as follows: The nuclei are not so well stained as with Wilson stain, but granules stand out well.

Polymorphonuclear—Nuclei blue, but distinctly paler than with the Wilson stain. Neutrophile granules a deep pink. Eosinophile granules a very deep pink. Basophile granules purple blue.

Lymphocytes—Nuclei a moderately pale blue, protoplasm tinged with blue.

Mononuclears—Nuclei paler blue than those of the lymphocytes, protoplasm a faint blue. Azurophilic

granules show less prominently than with the Wilson stain.

Malarial parasites are *not* well stained.

The intercellular areas are clear and show no stain.

(4) Pitfalls in using Jenner stain.

These are almost absent. The chief difficulty is in giving the nuclei a deep enough blue stain. Improper stains show the nucleus to have a faded-out bluish tinge. By changing the water and stain combination and by washing more quickly the nuclei can be made to take a better stain.

c. *With Ehrlich stain.*

(1) The stain—a complex triacid aqueous-alcohol stain. Do not shake the bottle before using.

(2) Preliminary details:

(a) Old smears stain better than fresh. Ex. smears 5 to 10 days old are better than those 5 to 10 hours old. It is advisable to have 15 to 25 smears of the same age when an Ehrlich is to be stained.

(b) Before staining, smears must be fixed by heat in the following manner:

1. Heat a copper bar for 30 to 40 min. in a place free from draughts, so that the flame will not be shifting. (N. B.—Place flame under point of bar.)

2. At the end of that time, with a dropper having a small bored point, drop a few small drops of

water (3 to 4) on the bar in an effort to find the spheroidal point. (Spheroidal point equals point on bar where water rolls off in globules.) Let at least 5 min. elapse between trials, so that the heat lost by the application of the water will be surely regained.

3. When the spheroidal point has been determined by repeated tests, draw a chalk line across the bar at that place.

(N.B.—Inaccurate determination of the spheroidal point is to blame for many poor Ehrlichs.)

4. Place preparation, smear side up, outside the spheroidal point, with the edge closest to the flame exactly on the line. The method is carried out as follows:

Fix a smear 20 sec., another one 30 sec., still another 40 sec., on up at intervals of 10 sec., until 90 to 150 sec. (Devise a method for carefully differentiating the variously heated smears so that in staining no confusion will arise as to the fixation time of any particular smear, for ex. 20 sec. or 90 sec.)

5. In general, the older the smears the shorter the required time of heating. Ex. smear 10 days old may be well fixed at 30 sec.; smear 1 day old may be well fixed at 150 sec. or more.

(3) Actual staining.

(a) Place the stain on the smear and let it remain 5 min. At the end of

that time *pour the stain back into the bottle,* and wash off the adhering amount of stain in the sink. Dry and blot as directed under the other staining methods.

(Ehrlich stains are not easily ruined in the staining and washing, but are generally spoiled by poor fixing.)

(4) Criteria for a good Ehrlich.

(a) The cells should stand out clearly with no intercellular stain.

(b) The R. B. C. should be buff, without the slightest suggestion of any red color, and without being a lemon yellow.

(N. B.—Smears which are underfixed or markedly over-fixed have a reddish color in the R. B. C. Smears which are moderately over-fixed have lemon color in the R. B. C.)

(c) The W. B. C. stain as follows:

P. M. N.—Nucleus—Blue green or robin's egg blue; no structure visible. Protoplasm—Faint pink. Granules—Lilac, if correctly fixed; red from short fixation.

P. M. E.—Nucleus—Light green color. Protoplasm—Generally none seen. Granules—Dark red or crimson.

P. M. B.—Nucleus—Light green, reticular. Protoplasm—None seen. Granules—No stain, often seen as colorless vacuoles.

Lymphocytes. — Nucleus — Light blue green. Protoplasm—Light pink

or violet, often very faint. Granules —None seen.

Large Mononuclears.—Nucleus—Faint blue or green. Protoplasm—Pink or light violet. Granules—None seen.

(d) Platelets are not stained.

(e) Malarial parasites are not stained.

(5) General technique used in staining with Ehrlich.

(a) From 15 to 25 smears; select 3 or 4 of the best and lay them aside.

(b) Take the less good smears and run through the various times for fixing already suggested.

(c) Stain these, and after mounting examine them carefully with oil immersion, and note the following points:

1. Under-fixed smears will have reddish staining R. B. C., often with stained intercellular spaces. The W. B. C. will have a dirty appearance with granules not standing out distinctly.

2. Well fixed smears will have the R. B. C. of a buff color (neither yellow nor red). The W. B. C. will stand out distinctly with neutrophilic and eosinophilic granules well stained and standing out sharply against a *clear unstained protoplasm.*

3. Over-fixed smears will have the R. B. C. lemon color. The granules in the polymorphonuclear white cells will not be clear cut, and if

stained will be seen lying in a stained protoplasm. There is a smudgy look to the polymorphonuclear cells. The mononuclears are often so faintly stained as to be missed.

4. If still further over-fixed, the R. B. C. lose their lemon color, and again take on a reddish hue.

(d) Having discovered a well-stained smear, note the heating time for it. and then fix any number of good smears (of the same age) for a similar time.

(N. B.—The need of again determining accurately the spheroidal point is evident since by placing these goods smears at a point which is no longer the spheroidal point, they will be ruined.)

d. General points with regard to stained blood preparations.

(1) Be careful to mount cover slips in a *neutral balsam,* if any acidity is present (as frequently occurs) the color will fade at a rate proportional to the degree of the acidity, the nuclear element, particularly, being attacked.

(2) Keep the preparations away from bright sunlight, from acid or alkaline fumes.

(3) After using oil, remove it with xylol. (N. B.—Never attempt this if the smear has been freshly mounted.)

IV. Differential Counting.

The cover slip method must be used for this as follows:

a. With a mechanical stage count across and

up and down over the good areas of the spread. Be careful to use such a technique that the same areas will not be counted twice.

b. 300 W. B. C. must be counted. In order to get the true proportion it is necessary to include in this number broken cells and undertermined cells as well as those which can be classified.

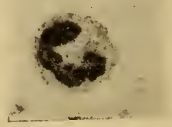
c. A W. B. C. count should always be made at the time a differential is counted. It is only by this means that relative or absolute changes in the number of cells is determined.

d. The classification of cells is as follows:

(1) Polymorphonuclear neutrophiles (P. M. N.).

The nuclei—irregular, 2 to 3 lobes, often appearing actually polynuclear. The granules—pink or violet (Wilson), pinker (Jenner), lilac (Ehrlich).

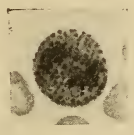
(N. B.—With Wilson the granules may not stand out distinctly and one sees only the more or less homogenous pink protoplasm.)



(2) Polymorphonuclear eosinophiles (P. M. E.).

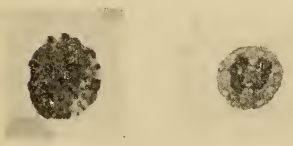
The nucleus is irregular, generally bilobed and may give the appearance of being actually polynuclear. The granules are large as compared with the neutrophilic granules and are all of the

same size. They stand out very distinctly and stain a deep pink to crimson with all stains. These cells tend to be a trifle larger than the neutrophiles and are rather fragile. For the latter reason they can often be found broken with their granules scattering. Eosinophiles are in general recognized by the large, constant size of their granules rather than by the depth of the granular stain.



(3) Polymorphonuclear basophiles (P. M. B.).

The nucleus is of the size and shape of the eosinophilic nucleus, but sometimes is entirely hidden by the deeply staining basophilic (blue) granules which completely fill the cell. These granules are not stained by the Ehrlich stain.

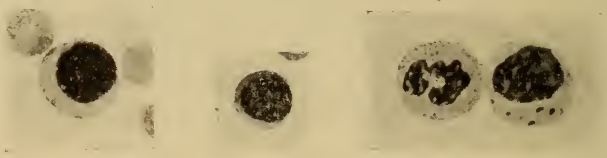


Wilson Stain Ehrlich Stain

(4) Lymphocytes.

Small and large types with the size of the P. M. N. taken as the arbitrary

dividing line: lymphocytes smaller than a P. M. N. are considered small lymphocytes (S. L.). Those as large as or larger than the P. M. N. being considered as large lymphocytes (L. L.).



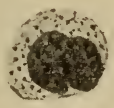
L. L. S. L. P. M. N. L. L.

The nucleus is round and takes a deep blue stain. The protoplasm stains a pale blue (darker than the protoplasm of the large mononuclear) and gives an impression of thickness. It is sometimes described as waxy (in contradistinction to the thin tissue paper thickness of the protoplasm of the large monos.). The cell is fairly regular in outline and is round in shape.

(5) Large mononuclears are large cells with a single nucleus, which is round or kidney shaped. The nuclear stain is lighter than that of the lymphocyte and the nucleus appears less dense. In the protoplasm, which is stained a pale blue, can be seen the reticular nodosities, which may be so marked as to give a granular appearance. No true granules are ever present, although the reddish azurophilic granules are often found. This cell is proportionally large, with a relatively

large amount of protoplasm. It gives the impression of great thinness.

(The term transitional is given to those cells when the nucleus is kidney shaped. It probably represents an older type of cell.)



Transitional

V. Fresh blood examinations.

a. Technique.

(1) Place a drop of blood the size of a pin-head on a cover slip (procured as for making smear).

(2) Place the cover slip on the convex side of a slide in such a manner that the drop is spread without bubble formation. Let the cover slip remain on the slide.

(3) Study first with the low power, then with high, dry and oil immersion.

b. Criteria for a good preparation.

(1) The spread must be thin, with the cells lying flat and not overlapping.

c. Common difficulties in making a good, fresh blood preparation.

(1) R. B. C. overlap or lie in rouleaux, due to

(a) Unclean glass ware.

(b) Too large a blood drop.

(2) Uneven spread, due to

(a) Dirty glass ware.

(b) Careless spreading of the drop.

d. Findings in a fresh blood preparation.

(1) R. B. C.

(a) Color—Greenish yellow with the intensity depending upon the amount of hemoglobin present.

(b) Shape—Round (if lying free without touching other cells), with a biconcavity, the apparent degree of which depends upon the amount of hemoglobin content. Abnormalities in the shape of the cell as a whole constitutes a condition known as *poikilocytosis*.

(c) Size—Diameter of 7.5 mikra; normally there is only the slightest variation in size. With noticeable variation in size the condition is spoken of as *anisocytosis*.

Large cells = *macrocytes* (approximately above 9 mikra).

Small cells = *microcytes* (approximately below 6 mikra).

(d) Degenerations—Due to trauma associated with the spreading and to the drying of the preparation, depending, of course, primarily upon the inherent fragility of the cells.

1. Total degeneration.

The cell shrinks and becomes darker in color; the margin becomes irregular: varying numbers of prickly points appear, giving the picture of a thorn apple, *crenation*. These points when appearing in profile are readily recognized, but when looked straight down upon may give the impression of cellular inclusions.

2. Partial degeneration.
   a. Maragliano bodies—an endoglobular degeneration, generally in the center of the cell, but frequently at the periphery, occurring as a single or multiple degeneration in a cell. The body is round or elliptical and looks like a vacuole. Its size and position in the cell often change. Confusion arises with nucleated R. B. C. and the early stage of malarial parasites.
   b. Bacilliary degenerations—rod-like hyaline areas in the cells, with a vibratory motion so that the "area" may move through the whole substance of the cell. Often confused with bacilli.
   c. Ehrlich's hemoglobinemic degeneration —cells appearing with a dark center and a light periphery often giving the appearance of a small cell superimposed upon a larger one. The condition probably is due to areas of condensed protoplasm with the hemoglobin separated from the stroma.

Pessary Form

3. Differentiation of degenerations from actual inclusions in the R. B. C., such as nuclei or malarial parasites.
   a. Changing the focus upon a degeneration results in a variation in the apparent size of the degeneration greater than that of the containing R. B. C.

b. Changing the focus upon a nucleus or malarial parasite results in no such proportional change in size, the nucleus or parasite merely becomes more or less distinct as the focus is changed.

(2) W. B. C.

Colorless round bodies, varying from 8 to 17 mikra in diameter. The shape is irregular, and amoeboid motion may occasionally be seen. In the polymorphonuclear cell the granules may be easily distinguished, and eosinophiles and basophiles can be differentiated from the neutrophiles by the larger size of the granules of the former. The nuclei appear as hyaline areas within the cells. The mononuclear cells are recognized with more difficulty, inasmuch as there are no granules. The nucleus appears as a round hyaline area. The protoplasm is clear. Unless the light is well cut down, such cells are easily overlooked.

(3) Platelets.

They appear as small granular masses lying singly or in groups. Because of their sticky nature, they are never seen floating, but adhere to the glass or to corpuscles.

(4) Blood dust.

Dancing particles which give the impression of cocci. They probably represent extruded granules from polymorphonuclear cells.

B. OUTLINE OF TECHNIQUE OF QUANTITATIVE BLOOD EXAMINATIONS.

I. Blood counting.

a. Apparatus and use of solutions. (For description see Emerson, Ed. IV, page 459.)

(1) Counting chamber.

(a) Never wash with alcohol or ether as the cement is in this manner dissolved. Clean with a soft cloth and cold or luke-warm water (never hot).

(N. B.—The glass platforms on the counting chamber are mounted in balsam as a cement, hence the need of avoiding alcohol, ether, xylol, and heat in any form in cleaning.)

(b) Before using have the counting chamber and cover slip so clean that Newton's rings appear when the cover slip is placed on the slide. Any grease, dust or lint will preventthe rings from appearing.

(2) Blood pipettes.

(a) Care of the pipettes.

1. Cleaning.

a. Fill the bulb three times with distilled water, shaking each time, emptying and refilling.

b. Repeat the above procedure by filling with 95% alcohol three times.

c. After using alcohol, fill the bulb three times with ether. In this way the pipette will be thoroughly dried and cleaned. If the above procedure has been prop-

erly carried out, the glass bead will not adhere to the side of the bulb and there will be no discoloration anywhere in the pipette.

d. Never let saliva be drawn into the pipettes. Prevent this by either using a force pump for cleaning pipettes or at times removing the rubber tubing and blowing out the accumulated saliva.

2. Take all precautions against breaking the point of the pipette. The slightest nick which enters the bore renders the pipette useless because of the resulting inaccuracy in dilutions.

(b) Use of the pipettes.

1. R. B. C.

a. Blood is drawn to point 0.5 with great accuracy.

Hayem's Solution
(for diluting r. b. c.)

Mercuric chloride	0.500	gm.
Sodium chloride	1.00	gm.
Sodium sulphate	5.00	gm.
Distilled water	200.00	cc.

b. Diluting fluid (Hayem's solution) is drawn to point 101 with equal accuracy (resulting dilution in the bulb of the pipette is 1 : 200).

c. With a finger placed over each end of the pipette, shake the pipette for 5 min. with a transverse rather than a longitudinal

motion. (Shake the bulb to and fro *across* the bulb—a longitudinal motion results in an uneven dilution by shaking cells into capillary tube of pipette.)

d. After sufficient shaking expel 2 or 3 drops from the pipette (representing the fluid which remained in the bore and was not mixed in the bulb) and use the third or fourth drop to place in the counting chamber.

2. W. B. C.

a. Blood is drawn to point 0.5 with great accuracy.

Turk's Solution

(for diluting w. b. c.)

1% Aqueous gentian violet	1 cc.
Glacial acetic acid	1 cc.
Distilled water	100 cc.

b. Diluting fluid (Turk's solution) is drawn to point 11 with equal accuracy (resulting dilution in the bulb of the pipette is 1 : 20).

c. Shake and place drop in counting chamber as described above.

3. If a second preparation is to be made from a pipette and during the interval the pipette has been laid aside, it is necessary to repeat the 5 min. of shaking, inasmuch as the cells will have settled and no accurate count could be made without another thorough shaking.

b. Technique of placing the drop in the counting chamber.

(1) Single counting chamber.

From a pipette place a drop in the manner described above on the central raised ring of glass. This drop must be of such a size as to cover the entire ring after the cover slip is placed over it, but must not be large enough to cover the moat and spread over the surrounding raised glass platform. It is permissible, but undesirable, to have a little fluid enter the moat. With the drop placed, lower the cover slip over it in such a manner that no bubbles are formed. If bubbles occur, wash the counting chamber and repeat the attempt. Newton's rings.

(N. B.—Bubbles cause an uneven spread of cells.)

(2) The Burker Double Counting Chamber (with two central raised glass platforms, making it possible to make two preparations simultaneously). Place the cover slip on the chamber and see that Newton's rings are present—do not remove the cover slip. From the pipette let a drop run under the cover glass over each central glass platform. The size of the drop must be gauged by practice. It must be sufficiently large to cover the whole platform and yet not large enough to cover the moat. No bubbles are permissible. If they occur, a fresh preparation in the counting chamber must be made.

c. Counting cells in the counting chamber. (Refer to Emerson, Webster, etc.)

(1) R. B. C.

(a) With high-power focus on the central portion of fine ruling.

(b) Some cells will be found lying outside the specified area, but touching the lines of division—count such cells when they lie on dividing lines to the left or above the section being counted. Omit those lying on the right or below.

(c) Begin counting at the extreme left —move to the right over the upper row of 5 small squares, then pass to the next lower row and move to the left, then descend another row and pass to the right, and proceed in this fashion for each of the 5 rows in each of the 4 corner units.

(d) Count the 4 corner squares on two preparations.

(e) Calculation.

1. The area of the fine ruling represents 1 square mm. of 1/10 mm. depth.
2. This area is broken up into 16 subunits, of which number the 4 corner ones in each of two preparations have been counted; therefore, 1/2 cu. mm. of diluted blood has been counted.
3. Let x = the sum of cells counted in the 8 corner units; therefore, x = number of cells in 1/20 cu. mm. of diluted blood.
4. In the red pipette the blood was

diluted 200 times; therefore, 200 x = number of cells in 1/20 cu. mm. of undiluted blood, or 4000 x = number of cells in 1 cu. mm. of blood. Therefore, when the 4 corners in 2 preparations have been counted the number found is multiplied by 4000. The re-resulting figure represents the R. B. C. count or the number of R. B. C. found in 1 cu. mm. of blood.

(2) W. B. C.

(a) With the low-power focus on the ruled portion of the counting chamber and pick out the four large corner areas of coarse ruling (each such area is the size of the central finely ruled area and represents 1 sq. mm.).

(b) With low-power count the 4 corner areas and also the whole central finely ruled area—thus counting 5 sq. mm. on each of 2 preparations, or 10 sq. mm. in all.

(c) Use the same method of including those cells which touch dividing lines to the left and above and omit those lying outside the specified area, but touching dividing lines to the right and below.

(d) Begin at the extreme left and work to and fro in the manner suggested for R. B. C. counting.

(e) The cells counted in the 10 areas (represented by x) will represent the number found spread over a

surface of 10 sq. mm. with a 1/10 mm. depth; therefore, x = number of cells in 1 cu. mm. of diluted blood.

(f) The dilution in the white pipette was 20; therefore, 20x = number of cells in 1 cu. mm. of whole blood. Thus when the 10 squares have been counted, the number of cells found multiplied by 20 = the W. B. C. count or the number of W. B. C. found in 1 cu. mm. of blood.

d. Requirements for satisfactory counts.

(1) R. B. C.

(a) A satisfactory count requires that the difference in the number of cells counted in each of the 4 corner squares in 2 preparations shall not not exceed 25.

(b) Such satisfactory counts shall be made on two successive days, with not more of a variation between the total counts than 200,000 cells.

(2) W. B. C.

(a) A satisfactory count requires that the difference in the number of cells counted in each of the 5 sq. mm. areas in 2 preparations shall not exceed 8.

(b) Such satisfatcory counts shall be made on two successive days, with not more of a variation between the total counts than 200.

Because of the W. B. C. of an individual vary with bathing, eating, exercise, etc., it is advisable to make the counts on the successive days under conditions as nearly constant as possible.

Ex. of satisfactory counts.

May 4th	W.B.C.	May 5th	W.B.C.	May 10th	R.B.C.	May 11th	R.B.C.
Prep. 1	42	Prep. 1	32	Prep. 1	149	Prep. 1	153
	34		40		150		148
	41		40		135		130
	38		40		149		128
	40		40				
Prep. 2	42	Prep. 2	40	Prep. 2	150	Prep. 1	155
	36		40		148		135
	35		40		145		150
	38		39		127		131
	21		33				
	387		384		1153		1130
	387 X 20 = 7,740				1153 X 4,000 = 4,612,000		
	384 X 20 = 7,680				1130 X 4,000 = 4,520,000		

II. Haemoglobin determinations.

a. Talquist's method (see Emerson, Ed. IV, page 525, for full explanation).

(1) Technique.

With a drop of blood on a piece of provided filter paper match its color in a scale of standard colors which is to be found in the book.

(2) Difficulties.

The scale of standard colors does not hold its registration of color values accurately; age and exposure to light tend to fade them.

(N. B.—If this method is to be used, the individual color scale should be standardized from time to time by running parallel determinations with it and a Sahli or Miescher instrument.)

b. Sahli method (see Emerson for diagram and full description).

(1) Apparatus and its use.

(a) In order to insure a homogeneous shade, the sealed standard tube (filled with a standard acid hematin susnension) should be inverted sev-

eral times before a determination of Hb. is to be made.

(b) The pipette is filled with blood up to the 20 cu. mm. mark (the only mark on the pipette). The filling must be done accurately and all blood adhering to the outside of the pipette must be carefully removed before the pipette is emptied into the calibrated tube.

(c) The calibrated tube, which must be clean and dry, is first filled with a stock solution of N/10 HCl exactly to the 10 mark. (This is used to lake the blood which is introduced into the tube and to transform the liberated haemoglobin into acid hematin.) Into this solution the pipette containing the blood is carefully introduced and the contents of the pipette expelled by means of careful blowing. The acid solution is cautiously drawn into the pipette and repeatedly expelled in order to remove thoroughly all blood from the pipette. The blood is allowed to remain in the acid for exactly one minute by the watch, and then distilled water is added until the shade agrees with that in the standard tube.

(N. B.—In mixing the contents of the calibrated tube the following points are of value:

1. If during the addition of fluids the upper part of the tube has been kept dry, it is possible, by

careful tilting, to form a meniscus across the upper portion of the tube. When this has been accomplished the tube may be safely inverted and the fluids thoroughly mixed.

2. The fluid in the tube should never be permitted to touch finger, as a loss of fluid occurs which amounts approximately to 1 degree on the scale, with a resulting inaccuracy in the ultimate reading.

(2) Method of taking the readings.

(a) When a reading is to be made, the tube should always be placed in the stand with its ground glass back.

(b) Let the light come through the back of the stand.

(c) Readings are best made by daylight. Artificial light makes the differentiation of shades difficult.

c. Miescher method (see Emerson for diagram and full description).

(1) Consideration of points not sufficiently emphasized.

(a) The cells must be thoroughly dry and clean before the bottoms are screwed in. The groove in the glass bottom and top should be oiled each time before placing them in position. The floor must be screwed in *tightly,* so that there may be no interchange of fluids between the two sides of the cell. Place distilled water in one side of each cell and let the preparation stand

for several minutes to be sure the compartment is water tight before adding the blood preparation to the other side.

(b) For normal blood a dilution of 300 is satisfactory. Draw blood to the 2/3 mark on the pipette, and then fill with 0.1% Na_2CO_3.

(*N.* B.—Any trace of $NaHCO_3$ in the diluting fluid must be avoided, as it will cloud the preparation, making a satisfactory reading impossible.)

(c) Shake the pipette thoroughly until the solution becomes perfectly clear and the shade homogeneous.

(d) Fill the empty side (which has been proven to be water tight) of both the 12 and 15 mm. cells at the same time and make readings *at once* on first one and then the other preparation. (The pipette contains enough of the preparation to permit of the filling of both the 12 and 15 mm. chambers at the same time.)

(e) The readings are made in a dark room, prepared for the purpose. A candle flame affords the necessary light for illumination of the preparation, and the scale may be seen by using a flash light, or turning on the electric light momentarily. Ten successive readings should be made on both the 12 and 15 cu. mm. cells. The scale should be turned alternately too high and too low and the color matched by running

through the higher shades down to the same shade and again by running through the paler shades up to the same shade. The average of the 10 readings is the one taken as final.

(f) The tables of calculations which accompany the Mieschers are *not* interchangeable. A preparation on a Miescher must be calculated according to the table made for that special instrument.

d. Requirements in Hb. work.

On the day on which the R. B. C. count is passed (which is the day of the second successful R. B. C. count) a Sahli determination shall be made and repeated again the following day. A difference of 5 is permitted in the readings on the two days. A Miescher reading shall be made on two successive days, the first Miescher shall be made on the day of the second Sahli determination. Readings with the two cells, both calculated for the 15 mm. cells, shall not vary over 2 points.

C. ADDITIONAL BLOOD EXAMINATIONS.

I. Coagulation time. (Use of Bogg's coagulometer. See Emerson for full explanations and diagram.)

a. The following points require emphasis:

(1) Consider the time from the moment the drop appears on the skin until the radial motion in the drop is seen under the microscope.

(2) Focus with the low power of the microscope on that part of the drop just opposite the blowing tube.

(3) Blow as lightly as possible on the drop, moving only 6 to 10 corpuscles.

(4) Blow not oftener than at 30 sec. intervals—more frequently blowing produces a vicious cycle and the radial motion may never appear.

b. Requirements.

(1) Make three determinations in succession on the same day. The difference in coagulation time should be less than 45 sec.

II. Bleeding time.

a. Make a finger incision sufficiently deep so that a drop of blood 2 mm. in diameter will appear spontaneously without any squeezing.

b. Take a piece of smooth filter paper and blot off the drop every 30 sec. (no manipulation of the part is permitted).

c. Divide the number of blots by 2 and the resulting figure represents the bleeding time in terms of minutes.

d. Requirements: Carry out this procedure once.

III. Fibrinolysis test. (Goodpasture, J. H. H. Bulletin, November, 1914.)

a. Test based on the ability of the blood in certain pathological conditions to digest its own clot within a few hours at body temperature.

b. Technique.

Draw 3-5 cc. of blood into a small, clean test tube. Incubate 12 hours at body temperature.

(1) Positive test.

Complete digestion will have occurred within 12 hours.

(2) Negative test.

Partial digestion or none at all within 12 hours.

c. Action probably due to the activity of normal proteolytic ferments of the blood, operating by virtue of a diminution or absence of normal anti-proteolytic ferments.

d. Fibrinolysis has been found associated with chronic liver injury with severely impaired hepatic function where a hemorrhagic tendency exists.

DIFFERENTIAL W. B. C. COUNT.

Dec. 10, 1918. Wilson stain

W. B. C.—8600 300 Cells Counted. Ehrich or Jenner

		Cells Counted.	Percent.	Absolute Number.
1.	P. M. N.....	204	68	5848
2.	P. M. E.....	6	2	172
3.	P. M. B.....	3	1	86
4.	Lymbho's small.	60	20	1720
5.	Lymbho's large..	3	1	86
6.	Large mono's...	15	5	430
7.	Transitionals...	9	3	258
8.	Unclassified.....	...	..	....
		300	100	8600

CHART FOR SPECIAL BLOOD EXAMINATION.

Patient's Name. Stain Used.
Ward or Hospital. Date of Preparation.
Doctor's Name. Date of Examination.

I. General Comment on Preparation, Stain, etc.
II. R. B. C.
1. Size.
2. Shape.
3. Color.
4. Regeneration forms:
a. Nucleated R. B. C.; type; numerous?
b. Basophilia; punctate or diffuse?
c. Nuclear particles.
d. Cabot rings.
5. Additional Remarks.
III. W. B. C.
1. Apparent number (relative to R. B. C.).
2. Apparent differential.
3. Presence of abnormal forms:
a. Myelocytes; type?
b. Myeloblasts.
c. Irritation forms.
d. Fragile W. B. C. ("smudges")
e. Pigmented W. B. C.
4. Additional Remarks.
IV. Platelets.
Apparent number (relative to R. B. C.).
Normal; increased; decreased.
V. Parasites.
VI. Additional Remarks, General.
VII. Impression.
1. Summary of Important Evidence.
2. Probable Diagnosis.

Blood Report Number. Name of Examiner.

HEMATOLOGY.

NOTE: The following notes on blood are rather sketchy in character and are, therefore, of more value to those who attended the lectures covering this subject than to those who did not. It is hoped, however, that even to the latter they may prove to be of some value.

The formed elements of the blood.

I. Red blood cells (erythrocytes). Normal consideration.

1. Embryological origin of the red cells. There is a great discussion and difference of opinion.

(a) Primary blood islands: 1. Hemoblasts. 2. Bildungszellen of Koelliker.

From the outer cells arise endothelial cells. From the inner ones the primary *erythroblasts* arise, which contain no hemoglobin and are colorless and nucleated. All cells are originally of intravascular origin. All are nucleated up until the fourth week. By the third month only one-eighth are nucleated, and at birth none are nucleated.

Extravascular formation soon commences, and is especially active in the liver, spleen and marrow, the latter becoming more and more the main site. Hemoglobin-free cells disappear quite early.

(b) Primary erythroblasts, essentially identical with megaloblasts of Ehrlich; hence the term "return to embryonal form" in anaemias.

(c) Discussion of the Monistic and Dualistic theory of origin: the discussion centers around the point as to whether

the red and white cells have a common origin.

Dualists,	Monists,
Naegeli,	Shridde,
Ehrlich,	Maximow,
Howell,	Pappenheim,
et al.	et al.

The main mass of evidence tends to establish these points:

1. Erythroblasts occur at a time much earlier than any leucocytes.
2. Primary cells of Koelliker do not form white blood cells, otherwise it is impossible to understand why later on in the vessels one finds only erythroblasts and neither Bildungs cells nor white cells.
3. Primary erythroblasts and endothelial cells are sisters.
4. No direct evidence of transition stages of red blood cell into white blood cell or the reverse.

Schridde and Maximow maintain this idea:

Bildungzellen are in reality "large lymphocytes. From these there arise both white blood cells and red blood cells. This is said to be the "primary erythroblast" and may be quite identical with the Myeloblast of Naegeli.

2. Post foetal formation of erythrocytes.

Normally from marrow only. (1868 Neumann.) The marrow contains many ripe and unripe cells, most of which are nucleated and from 6-12 microns in size.

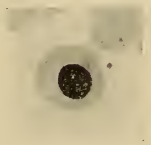
Chief variety:

(a) Megaloblasts — Definition used here: nucleated cell, densely staining, with nucleus showing radial arrangement of chromatin and as large or larger than a normal erythrocyte.

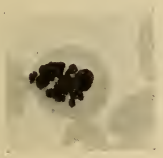
(b) Intermediates — Nucleated, larger than

normoblasts. Clinically not important.

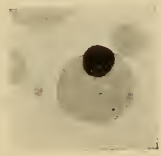
(c) Normoblasts—Nuclei: 1. Dense and pycnotic—old (mature—Howell). 2. Clear and radial—young (immature—Howell).



Bunting's view: Erythrocytes enter blood stream without nuclei. Loss due to Karyorrhexis (a) Karyolysis, (b) Extrusion.



Karyolysis



Extrusion

Although extrusion actually occurs, it is thought that this is not the normal mode.

3. Consideration of normal erythrocytes.

(a) Number. The average count stays constantly throughout adult life. It varies from 4,500,000 to 6,000,000. A deviation of half a million is of no pathological significance, unless associated with a change in the color index. At birth the count is about 6,000,000. After the first few weeks it falls to 4,000,000. After this at about the tenth year, or at puberty, it reaches 5,000,000. After 35 the count decreases, and at 60 it is about 4,500,000. In robust plethoric individ-

uals the count may be around 6,000,000 and mean nothing.

(b) Functions.

1. Internal respiration.
2. Lipoid carriers.
3. Bile pigment (iron).

(c) Life history.

There exists a balance between destruction and formation. The normal demand upon the marrow causes the outpouring of no abnormal forms.

4. Pathological considerations.

Pathological changes of very much importance concern chiefly:

1. Variations in the number.
2. Variations in the hemoglobin content.
3. Abnormalities in size and form.
4. Abnormal red cells in the peripheral blood.
5. Abnormal staining reactions.
6. Vital staining reactions.
7. Altered resistance of erythrocytes.

(1) Variations in the number of R. B. C.

(a) Increased—Polycythaemia,
Erythremia.

Physiological.

1. Early infancy.
2. Following vigorous exercise.
3. After hot or cold baths.
4. High altitudes.

Pathological.

1. Concentration of the blood-excessive fluid output, or decreased fluid intake.
2. Prolonged vasoconstriction.
3. Conditions of chronic dyspnoea.
4. True polychthaemia (excessive forma-

tion). Resistance of cells not increased by test-tube method.

(b) Decreased counts. *Oligocythaemia.*

1. Insufficient formation—aplasia, toxic states, etc.
2. Excessive destruction.
3. Hydraemia.
4. Actual blood loss.

Upper and lower limits of counts.

Higher limits—up to 9,000,000 (Osler found 11,000,0000).

Average from 4,500,000 to 5,000,000.

Lower limits — 5,000,000 to 100,000 (Osler).

Average for pernicious anaemia—1,000,000 to 2,000,000.

Average for secondary anaemia—well above 2,000,000.

(2) Variations in the Hemoglobin content.

1. Reduction proportionate to R. B. C.
2. Good R. B. C. count, but tremendous hemoglobin reduction.
   a. Chlorosis,
   b. Many chronic anaemias.

Bone marrow insufficiency.

Further discussion of the "Color Index":

It is important to remember that the color index is wholly independent of variations in blood volume, vasomotor effects and the like. It really gives a clear insight into conditions of the bone marrow.

Types:

(a) Normal—.8 to 1.

(b) Lower than 1—Chlorosis, cancer, nephritis, hemorrhages.

(c) Higher than 1—Pernicious anaemia, anaemias of childhood, hemolytic anaemias with jaundice.

(3) Abnormalities in shape and size.

1. Anisocytosis—Variation in size. Not a normal event.

Terminology:

a. Microcytes, 1-6 microns.

b. Normocytes, 6-9 microns. (Secondary anaemia.)

c. Macrocytes, 9-12 microns.

d. Megalocytes, 12-16 microns.

e. Gigantocytes, 16-20 microns. (Pernicious anaemia.)

(a) Microcytes—Expression of budding process, poor ancestry, small marrow cells. Not an expression of cellular youth.

(b) Normal erythrocytes.

(c) Macrocytes—Swollen cells, never terribly large.

(d) Megalocytes — Characterized by: 1. Size. 2. Rich hemoglobin content. 3. Essentially absent delle. These are really embryonic cells. They occur mainly in Pernicious Anaemia and anaemias of childhood. They appear when demands upon the bone marrow are too great. Their appearance depends not upon the severity of the anaemia, but upon the nature and the type of the anaemia.

(e) Gigantocytes.

2. Poikilocytosis—Variation in shape.

Poikilocytes may be artificially made by

pressure or heat. They occur in benign forms of secondary anaemia, but in much larger proportions in Pernicious Anaemia.

(4) Abnormal red cells in the circulation.

1. Microblasts.
2. Normoblasts.
3. Intermediates.
4. Megaloblasts.

The appearance of these cells denotes severe irritation of the bone marrow. They are found in: 1. Pernicious anaemia. In remissions normoblasts predominate. 2. Oftenest in myeloid leukaemia. 3. Children's blood in certain obscure anaemias. Embryonic blood building is reverted to easier in children.

(5) Abnormalities in staining.

1. Anisochromia: Various forms including hemoglobinaemic degeneration of Ehrlich.
2. Polychromasia: a. Anaemic degeneration of Ehrlich. b. Polychromatophilic degeneration of Ehrlich (Gabritschewsky).

Common occurrence: Common in blood-forming organs, embryonic. Occasionally seen in normals.

Considered as a coagulation necrosis by Ehrlich and a degeneration. The common view held is that they are youthful cells and their presence shows that regeneration is taking place. Their presence may also mean a degenerative process in a sense that the marrow cannot hold these cells back. They occur in cells subjected to stasis. Their appearance does not necessarily run parallel with the severity of anaemia nor

are they necessarily poor in hemoglobin for they occur in cases with a high color index.

3. Basophilic reacting substances in R. B. C.

Origin:

Nuclear remains.

Nuclear substance not chromatin.

Protoplasmic changes.

(a) Nuclear particles.

(b) Howell Jolly Bodies.

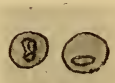
One or two in a cell; round, peripheral, nuclear stains, common in orthochromatic cells.

(c) Chromatin staubschen.

End stage of nuclear decay. They are bright red, peripheral, minute dots.

(d) Cabot ring bodies.

J. Med. Res. 1903. They are bright red or bluish with Ehrlich. Appear in a variety of shapes. Invariably in polychromatic cells. Occur in any anaemia, especially Pernicious Anaemia, Leukaemias and Lead Poisoning. Never found in blood-forming organs,



therefore a pathological change. Represents especially resistant nuclear remains (nuclear membrane). Supports theory of karyolysis.

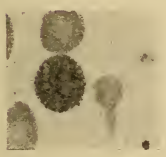
(e) Red basophilia with Giemsa stain.

(f) Schuffner's granules.

These can be seen in fresh R. B. C. Occur in Malaria. It is claimed that they are not identical with true basophilia.

(g) Punctate Basophilia, common form.

Appear as round or angular specks. They are absent in Ehrlich stain. Chief interest resides in their meaning: Regeneration (?), degeneration (?), youth (?). They are not seen in fresh blood.



Punetate Basophilia

Diffuse Basophilia

Occurrence: Anaemias and illnesses with anaemia. They are absent in some anaemias, hence not due to anaemia per se.

Uncommon in chlorosis.

Uncommon in adult bone marrow.

In P. A. not a striking feature.

Absent in aplastic blood, but present in marrow.

Commonly associated with other R. B. C. changes.

Tuberculosis, nephritis, gout.

They are an essentially regenerative phenomena.

Designated here: Diffuse or Punctate polychromasia.

(6) Vital staining of erythrocytes. Really a post vital method.

Methods:

1. Dry method (not used much).
2. Widal-Abrami and Brule.

Solution and stain:

Unna's Polychrom. stain	10 cc
NaCl .8%	10 cc
Sod. Oxalate 2%	10 cc

Draw blood to 1 in the white pipette and solution to 11. Mix and study.

Blood may be collected in a centrifuge tube and then a film be made and fixed with heat.

Another method using a pipette is diluting the blood with ½% Brilliant Cresyl Blue in .8% NaCl. Examine wet.

3. Method of Vaughn. Place a drop of stain upon the ear, make puncture through it and examine wet.

Appearance of cells.

Occurrence: 1-2% of cells in normal blood. Bone marrow, all anaemias. 30-40% of cells in the new-born without polychromasia. Evidence of youth and regeneration. Quite different from basophilia according to most.

(7) Resistance of the red blood cells (Hamburger) 1902.

Method of making hypotonic solutions:
Burette method, using H_2O and 1% NaCl.

% Strength Desired.	1% NaCl.	H_2O.	Volume.
.75 %	7.5 cc.	2.5 cc.	10 cc.
.7	7.0	3.0	10
.65	6.5	3.5	10
.6	6.0	4.0	10
.55	5.5	4.5	10
.5	5.0	5.0	10
.45	4.5	5.5	10
.4	4.0	6.0	10
.35	3.5	6.5	10
.3	3.0	7.0	10
.25	2.5	7.5	10
.2	2.0	8.0	10

Use .1 to .3 cc. of washed R. B. C. in each dilution. Normal minimal resistance is .47. Maximal resistance is .3.

Variations in resistance:

1. Decreased in hemolytic anaemias.
2. Increased resistance in anaemias with blood poisons.
3. Increased resistance under iron and arsenic therapy.
4. Results in anaemias in general not conclusive. Increased in P. A. and carcinoma.

Consideration of the White Blood Cells.

Classification of Normal White Blood Cells.

A. Polymorphonuclear neutrophiles, P. M. N.
B. Polymorphonuclear eosinophiles, P. M. E.
C. Polymorphonuclear basophiles, P. M. B.
D. Lymphocytes.
E. Large mononuclears,
F. Transitionals

One and the same.

A. Polymorphonuclear neutrophiles.

I. Description.

1. Size and shape:

10-12 microns, but as high as 15 microns, because they are so easily flattened out.

2. Nucleus.

As many as five, never less than two lobes.

They are joined by fine strands of chromatin.

3. Protoplasm.

Granular, takes a slightly acid stain.

4. Granules.

Many granules, sometimes overlapping nucleus. In the same cell the granules are apt to be of the same size.

5. Function.

Active scavengers of the body.

II. Origin of cell.

Strictly from the bone marrow and in adults from the flat bones only, although in disease the long bones may also take part.

III. Normal numbers and variations.

65-68% of the cells of the blood (in some books per cent given higher). 4,500 per cubic millimeter.

IV. Abnormal.

Increased—in any acute infection, particularly in pus formation. Confusing cell-metamyelocyte.

B. Polymorphonuclear eosinophile.

I. Description.

1. Size. Larger than P. M. N. (12-14 microns). Variations in size, both large and small.

2. Nucleus. Less multiple division of nucleus.

3. Nucleoli absent.
4. Protoplasm slightly acid, full of large granules (1 micron) which do not overlap nucleus.
5. Function not known.
6. Confusing cell-metamyelocyte.

II. Origin of cell.

Bone marrow.

III. Normal numbers.

2-4% (average 2½%).

IV. Abnormal.

Diagnostic in several conditions.

1. Skin diseases.
2. Parasitic infections (especially intestinal).
3. Blood diseases:
   a. Leukaemias—enormous numbers.
   b. Increase in other anaemias (good omen).
4. Anaphylactic phenomena in general.
5. Scarlet fever.

Decreased:

1. Frank leucocytosis, "*septic factor.*"
2. Poor bone marrow regeneration (aplastic anaemias).
3. Conditions with lymphocytic increase.

C. Polymorphonuclear basophiles.

I. Description of cell.

About the same as P. M. E., except that the granules take basic stain, scattered throughout the cell and vary in size within the same cell.

Soluble in water.

Function—nothing known.

II. Origin—bone marrow.

III. Number—½ to 1%.

IV. No increase except in myeloid leukaemias.

D. Lymphocytes.

I. Description of cell.

Size and shape: 7 to 10 microns (large in children).

Nucleus—picnotic.

Nucleoli—1 or 2, never more. Characteristically possessing good surrounding membrane.

Protoplasm—scant, in fresh looks granular. With Romanowski stain has greenish blue tint and edge looks thick.

Few granules varying in size and shape, grouped toward the periphery, in about ⅓ of cells.

Basic stain.

Never amoeboid. Function little known. (Do not give indol blue reaction.) Hinted that they play important immunity role. (Experiments in cancer and tuberculosis.)

II. Origin of cell.

From lymphatic glands, spleen, etc. Always of lymphatic origin, never bone marrow.

III. Normal numbers.

18 to 30% (average 25%).

IV. Abnormal.

Increased:

1. Lymphoid leucaemia.
2. Typhoid, malaria, pertussis, tuberculosis, syphilis.
3. Marked vagus stimulation.
4. Disturbance of endocrine glands.

Decreased:

1. Pronounced leucocytosis.
2. Extreme disease of lymphoid system.

E. Large mononuclears,

F. Transitionals,

One and the same cell.

The origin and the nature of the granulation of these cells are the cause of much discussion. Classed as non-granular cell they are really granular.

I. Description.

1. Size—largest cells, 12 to 25 microns.
2. Nuclei—pale, compact, leptochromatic. No clearly defined membrane.
3. Protoplasm—basic, well-defined reticulation.
4. Nucleoli—without staining no nucleoli are seen, but with vital staining as many as three may be seen.
5. Granulation — peculiar characteristic type. No azure or fuchsin granules.

II. Origin.

Probably myeloid. There are several points in favor of this view:

(a) They give oxidase reaction.

(b) They tend to increase in conditions where bone-marrow is stimulated.

III. Normal numbers and variations.

6-8% when taken together (1-3% large mononuclears, 3-4% transitionals).

Increase:

In any case of myeloid stimulation. (Drug intoxication, especially salvarsan.)

Three common views as to origin:

1. Young lymphocytes.
2. Endothelial cells.
3. Myeloid.

Function—little known; increased especially in malaria.

Abnormal White Blood Cells. (Never in peripheral blood normally.)

A. Myeloblast—parent cell of all myeloid white blood cells.

Size—12-15 microns (great variation).

Nucleus—large, lepto chromatic.

Stain—cytoplasm stains a purple or light blue; nucleus stains a lighter blue.

Protoplasm—plentiful, often thicker on one side of the cell than on the other side.

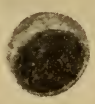
Nucleoli—numerous (3-6), closely grouped, easily seen, but with no clearly defined nucleolar membrane.

Granules—non-granular cell.

Function—gives rise to other cells.

Differentiation from lymphocytes:

1. Usually larger in size.
2. Nucleus doesn't stain as intense a blue, i. e., not picnotic.
3. Protoplasm has a reddish tinge. Lymphocyte, greenish with clear perinuclear zone.
4. Has three or more nucleoli.
5. They give oxidase reaction.
6. Where myeloblasts occur, various stages to myelocytes usually occur also.



B. Myelocytes—three varieties.

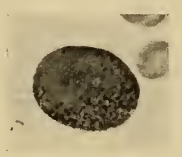
a. Neutrophilic myelocyte.

b. Basophilic myelocyte.

c. Eosinophilic myelocyte.

Myelocytes are the same in size, shape and staining reactions as the myeloblasts. They differ in having distinct types of granulation. In neutrophilic and eosinophilic myelocytes,

the youth of the cell is shown by the differences in size and staining reaction.



Occurrence of Myelocytes:

1. Never normally.
2. Myeloid leukemia (more acute the disease, more numerous the myeloblasts—showing higher origin).
3. Marked hyper-leukocytosis.
4. Bone marrow exhaustion (long standing anaemias).
5. Pseudo-leukaemia of infants.

In general, when there is a marked number of myelocytes it may be regarded as myeloid leukaemia until proved to the contrary.

Differentiated from:

1. Lymphocytes:
   a. Character of nuclei.
   b. Perinuclear zone (clear zone in lymphocyte).
   c. Number of nucleoli.
   d. Protoplasm.
2. Large Mononuclears and Transitionals.
   a. Protoplasm more reticulated in L. M.
   b. Difference in granulation. (Granules of L. M. do not stain with Ehrlich.)

Function—give rise to P. M. N., P. M. E. and P. M. B. in the peripheral blood.

Promyelocytes are the intermediate cells between the myeloblast and the myelocytes.

Metamyelocytes are intermediate cells be-

tween the myelocytes and the normal P. M. N., P. M. E. and P. M. B.

C. Abnormal myeloblasts — "irritation cells of Turk."

Size—large.

Protoplasm—very basic. 3-10 vacuoles.

3-5 nucleoli.

Multilobular nucleus.

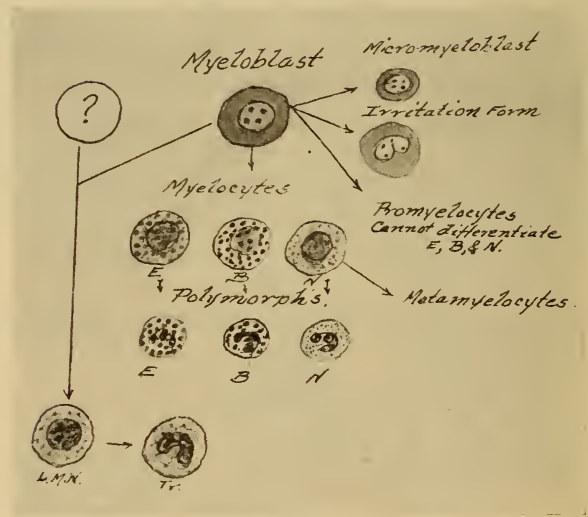
Stain as do myeloblasts.

Non-granular.

No perinuclear zone.

No characteristic presence or absence in any disease.

Genesis of W. B. C.



Other Abnormal Cells.

1. Pathological lymphocyte—*Rieder cells.*
2. Irritation forms—Turk cells,
   Pathological mononuclears.
3. Plasma cells.
4. Megakaryocytes.

1. Pathological lymphocytes—Rieder cells.

Large cells—often resemble myeloblasts.

Entirely different from normally occurring lymphocytes.

Nucleus trachychromatic.

Characteristics are:

Abnormal lobing, nuclei as a rule stain poorly.

Usually lymphocytic in type with Giemsa and Romanowsky.

Protoplasm sparse and may seem absent; often broad azure granules and shows basic staining. Well defined, clear perinuclear zone and vacuoles. Reticulated.

Cells give no guiac, nor indol blue reaction.

One sees numerous transitional forms to normal, large and small lymphocytes, but never to transitionals or large mononuclears.

Occurrence—Acute leukemia and in the aleukemic state. Chronic leukemia, uncommon. Basedows; occasionally in infectious diseases.

Cells are easily broken up and, therefore, give rise to smudges.

2. Irritation forms of Turk—Pathological myeloblasts. (See above.)

Large cells with round or oval nucleus, leptochromatic.

Protoplasm abundant, basic, vacuolated (3-8-10, more than lymphocyte).

Azure granules absent, protoplasm is red-brown with Ehrlich.

No perinuclear zone.

Indol blue reaction etc.

Occurrence: Leucocytosis: fat necrosis. Acute myeloid leukemia. Lead poisoning—not uncommon. Never normally.

3. Plasma cells.

Occur in the blood very rarely, best dismissed.

Cells of lymphatic nature, large, eccentric nuclei with spoke-like structure and unusually thick strands of chromatin.

Protoplasm intensely basic and perinuclear zone marked.

4. Megakaryocyte.

Giant cell of the blood.

Occur very seldom: large, bizzare-shaped nucleus, many nucleoli.

Inner granular cell surrounding nucleus and outer basic zone.

Bone marrow — myeloblast — megakaryocyte.

Give rise to platelets.

Evidences of age and youth in W. B. C.

1. Evidence of youth is basic reticulated protoplasm. (Seen best in myeloblasts, also in young P. M. N.'s.)
2. Presence of basophilic granules along with neutro or eosinophilic. All granules in early age possess a basic component. Ehrlich first called attention to this. (Larger the granules the younger the cell.)
3. Young cells usually have easily demonstrable nucleoli. They are often not visible with ordinary staining methods. (The younger the cell the easier it is to see nucleoli.)
4. Young cells have characteristic round nuclei. (They are seen in myeloblasts and young lymphocytes.)

Oval nucleus—sign cell is older.

Nuclear polymorphism is sure evidence of age.

Open network in young cells.

5. The size of the cell is not necessarily an evidence of youth, though it is often so.

6. Staining characteristics of the nucleus:
   Young cells have paler nuclei (?).
   Young cells show network structure.
   Older cells show picnosis.

Review of main differences between myeloid and lymphoid cells:

(a) Histological differences.
(b) Morphological differences.
(c) Chemical differences.
(d) Biological differences.
(e) Embryological differences.
(f) Comparative anatomy differences.
(g) Pathological differences.

Remarks on Nomenclature—confusing terms.

(a) Acidophile cells—eosinophiles.
(b) Basophile cells—two varieties meant:
   1. Cells with basic protoplasm.
   2. Cells with basic granulations: a. old, b. new.
(c) Hematoblast has two meanings:
   1. Blood platelet—Hayem.
   2. Megakaryocytes.
(d) Leukoblast (Pappenheim)—Promyelocyte.
(e) Lymphoblast is used in two ways:
   1. Parent cells of lymphocyte.
   2. Large cells of germ centers.
(f) Lymphoid cells—a bad term.
(g) Marrow cells—white bone marrow cells, granular and non-granular.
(h) Mast cells—basophilic leucocytes.
(i) Myeloblasts referred to as:
   1. Lymphoid bone marrow cells.
   2. Bone marrow lymphocytes.
(j) Rieder cells.

Remarks on the varieties of bone marrow.

Occurrence of each:

(a) Erythroblastic marrow—red.

(b) Myelocytic marrow—grayish.

(c) Myeloblastic marrow—gray.

(d) Lymphatic marrow—grayish.

(e) Aplastic or regenerative—yellow.

Remarks on differential blood counts.

Normal cells:

P. M. N.

P. M. B.

P. M. E.

Lymphocytes—small and large (small mononuclears).

Transitionals.

Large mononuclears.

Pathological cells:

Myelocytes—neutrophilic.

Myelocytes—basophilic.

Myelocytes—eosinophilic.

Pathological lymphocytes.

Myeloblasts.

Always note abnormal red blood cells and platelets.

Possible changes in W. B. C. count:

1. Unchanged.
2. Decreased—leucopaenia.
3. Increased—leucocytosis.

Leucocytosis mild—mild infection—good resistance.

Leucocytosis moderate—severe infection—poor resistance.

Leucocytosis high—severe infection—good resistance.

Leucocytosis low—severe infection—poor resistance.

Occurrence of Leucocytosis:

1. Physiological.
   (a) New-born (12 to 20,000).
   (b) Digestion.
   (c) Pregnancy.
   (d) Cold and exercise.
2. Pathological.
   (a) Post hemorrhagic.
   (b) Inflammatory.
   (c) Toxic—acidosis, gout, lead poisoning.
   (d) Many inectious diseases.

eucopenia occurs in:

   (a) Emaciation.
   (b) Infectious diseases, malaria, influenza, measles, pertussis, typhoid.
   (c) Chronic lues, tuberculosis.

Differential blood pictures:

1. W. B. C. increased—formula normal or increased in any cell type:
   (a) Leucocytosis with normal balance—in infectious diseases not associated with pus (mumps).
   (b) Leucocytosis with P. M. N. increase—pus.
   (c) Leucocytosis with P. M. E. increase—parasitic, skin infections, anaphylactic.
   (d) Leucocytosis with mononuclear increase—infection of lymphatic system.
2. W. B. C. Normal—with cells of one or other variety increased.
   (a) Leucocytosis—poor resistance.
   (b) P. M. E.—parasites.
   (c) Lymphocytes—in children, lues., tbc., etc.
3. W. B. C. Decreased—formula relatively normal, one or another cell increased.
   (a) Leucopaenia with normal P. M. N. count in overwhelming infection (high % P. M. N.)

(b) Increased P. M. E.—particularly in severe anaemias starting to improve.

(c) Lymphocytosis — pertussis, typhoid, malaria, tbc., P. A.

(d) Large Mononuclears and Transitionals—typhoid and leukaemias.

A Consideration of Blood Platelets.

Blood platelets originate from Megakaryocytes. They disintegrate rapidly. In stained specimens there is a membrane with purple staining chromatin inside the cell. They have amoeboid activity, which accounts for their bizarre shapes.

Function of platelets: To form thrombin and neutralize antithrombin. They are also thought to play same role in immunity.

They can be studied:

1. In good smears.
2. Indirectly—drop into normal metaphosphate and count platelets and R. B. C. Calculate number of platelets by proportion.
3. Aqueous solution of cresyl violet 1-300.
   Aqueous solution of KCN 1-1400.
   Mix before use in proportion of two parts of the first to three of second. Keep iced before hand. Wait from 15 to 20 minutes, and then platelets are plainly seen.
   Normal count is 250,000 to 300,000.

Increased in:

Toxic or infectious states if mild. Stronger ones decrease them. (Increase is good omen.)

Chronic diseases.

In secondary anaemia unless there is bone marrow aplasia.

In myeloid leukemia.

Critically after hemorrhage.

After splenectomy in two to four days.

Decreased in:

Overwhelming infection.
Lymphoid leukamia.
Pernicious anaemia.
True aplastic anaemias.
Purpura hemorrhagica.
Hemophilia.
After benzol.

Considerable emphasis laid upon the studies of Duke and Minot.

The Anaemias or Erythropaenias.

1. General definition of anaemias and other terms.
   Oligaemia—oligocythaemia.
   Oligochromaemia—hydraemia.
2. General symptomatology of anaemic states:
   (a) Color—is only judged in terms of mucus membranes. The color of the skin in S. A. tends to be doughy white, while in P. A., with the same or lower per cent of Hb, the skin tends to be a lemon color.
   (b) Eyes—eye grounds with loss of vision and floating spots from hemorrhages in the retina.
   (c) Dyspnoea—weakness.
   (d) Gastro-intestinal symptoms—loss of appetite, sore tongue or mouth, dermatitis from underlying anaemia or achylia. (Flatulence—especially in people over 35.) Jaundice.
   (e) Oedema—swelling in feet and ankles. Swelling of feet and ankles not always orthostatic.
   (f) Neurasthenia.
3. Classifications of anaemias.
   (a) Etiological:
   Defective formation—hypoplastic.
   Increased destruction—consumptive.

(b) Haematological:
Chlorotic (low index).
Pernicious (high index).

(c) Histological:
Primary myelogenous — primary — embryonic erythropoesis.
Secondary myelogenous—secondary — post embryonic erythropoesis.

(d) Clinical grouping:

Primary.	Secondary.
Chlorosis	Acute hemorrhagic
Pernicious	Chronic hemorrhagic
Primary aplasia	Chronic secondary
Leukanaemia	Secondary aplastic
Leukaemia	Hemolytic
Anaemias of children.	

Blood picture in stages of secondary anaemia.

1. Oligaemia without anaemia—proportional decrease (C. I. normal)—inhibition on the part of the bone marrow.
2. Hydraemia—with thirst—reduced cell count with low C. I. characteristic.
3. Low C. I.—regeneration—metaplasia—immature cells—normoblasts, macrocytes, intense marrow activity. Megaloblasts occur infrequently in true secondary anaemia. Red cell formation exceeds hemoglobin.
4. Normal—formation of red blood cells stops—restitution of Hb. Hemoglobin may exceed red cell formation—index plus.

Causes of S. A.

1. Loss of blood.
2. Infections—chronic.
3. Malignant tumors.
4. Chemical—paroxysmal hemoglobinuria.
5. Parasites.
6. Secondary purpura.

Leucocytes—no characteristic changes, usually normal and normal differential.

Platelets—constantly increased.

Treatment: Much iron given. (Some anaemic people called chlorotic, but this is really a misnomer.)

Pernicious Anaemia.

There is a limitation of the term. Pernicious anaemia is best given up and the term chronic hemlytic anaemia used or "Addison-Biermer type of anaemia." Other terms are Addison anaemia, Addison-Ehrlich and Biermer-Ehrlich anaemia. Biermer gave the first classical description in 1868. Ehrlich showed that there is a reversion to embryological type. Pernicious anaemia is not a disease entity, but a similar picture can be caused by conditions not necessarily fatal. The typical case shows a typical blood picture, certain symptoms, etc., with a chronic, progressive, hemolytic anaemia of unknown cause. The group is activity increasing. In horses an analagous anaemia of infectious origin occurs which can be transmitted.

Conditions which require exclusion:

1. Worms—mostly of the flat-worm variety. (Fatty acids given off by the segments can cause anaemia in other animals.)
2. Malignant tumors.
3. Syphilis (clears up under therapy).
4. Severe chronic types of malaria.
5. Toxin given off during puerperium.
6. Toxins due to metals—lead, etc.

The cause of the disease is unknown, but there are various theories:

1. Toxins arising from gastro-intestinal tract. This is a favorite theory, emphasized by Sir William Hunter. Bad teeth and mouths responsible.
2. Achylia gastrica—not true gastric atrophy.
3. Primary cause in spleen. (Comparatively re-

Enumeration of the distinguishing features:

1. Usually the history is helpful.

   Never omit careful gastric, stool, sputum, urine examinations for occult blood.

2. R. B. C. not usually so low as in P. A. Stained smears show characteristic degree of pallor, shadows of cells. (Bone marrow may throw out deep staining cells.)

   Smaller R. B. C.—degree of anisocytosis and poikilocytosis less.

   Normoblasts rather than megaloblasts (therefore C. I. very generally definitely below 1—watch C. I. carefully).

3. Platelets increased, or, at least, more than in P. A.

4. W. B. C. either slightly increased (early stages), normal, or decreased (later stages), but not as much as in P. A. and less reduction of P. M. N. Seems a sliding down of whole picture rather than repression of leucocytes.

III. Secondary Aplastic Group — Aregenerative Anaemias.

Enumeration of the causes—bone marrow not changed to red.

1. Drugs, benzol, lead, arsenic, etc.
2. Long hemorrhagic state.
3. Severe septic conditions.

Chief interest resides in the ability to diagnose cases from aplastic forms of P. A.

History very important.

Blood picture, as a rule, shows:

1. Parallel reduction of R. B. C. and hemoglobin.
2. Color index nearly normal (perhaps a little under).

3. Anisocytosis and poikilocytosis usually slight.
4. Absence of myeloid elements with leukopaenia.
5. Few or no nucleated R. B. C. Platelets gone.

IV. Hemolytic Anaemias or Hemolytic Icterus.

Described by French: congenital family disease. Clinically a disease often present from early life with periods of fever, icteric tint, weakness, anaemia, urobilinuria and splenomegaly.

A. Chronic icterus.
   1. Congenital.
   2. Acquired.
B. Acute forms—new-born, infections, hepatic diseases.

Blood findings:

1. Anaemia is variable; usually moderate (rarely below 2,500,000).
2. Usually many regenerative evidences, especially basophilic cells.
3. Large numbers of vitally staining R. B. C.
4. C. I. around normal—sometimes greater than 1.
5. Characteristic microcytosis.
6. Punctate basophilia—uncommon.
7. Usually W. B. C. slightly plus.
8. Platelets increased.
9. Diminished resistance of R. B. C.

Good prognosis.

Secondary anaemia occurs often in people who live under poor hygienic conditions. Marked improvement with fresh air, good food, etc.

Secondary anaemia develops rapidly in patients stricken with acute infections. (Typhoid, pneumonia, rheumatic fever, etc.)

1. Patient's history—can't pin onset to any definite time.
2. Physical examination.
   (a) Characteristically well nourished.
   (b) Lemon or straw color.
   (c) Complete achylia gastrica.
   (d) Urobilinuria—constant.
   (e) Frequent retinal hemorrhages.
3. Blood side.
   (a) R. B. C. reduction out of proportion to hemoglobin.
   (b) Anisocytosis and poikilocytosis, with prevalence of macrocytes.
   (c) High C. I.
   (d) Leukopaenia.
   (e) Diminution in blood pressure.

Differential diagnosis:

I. Anaemias of Childhood.
   1. Occur in young children.
   2. Tendency to revert to embryological blood picture (megaloblasts predominate).
   3. R. B. C. count reduced markedly and hemoglobin reduced proportionately. Low C. I.
      Poililocytosis and anisocytosis marked.
      Polychromatic cells.
   4. W. B. C.
      Leucocytosis (20,000).
      Predominant cell—P. M. N.
      Some abnormal W. B. C.'s.
   5. Platelets about normal.

   Clinically:

      No characteristic lemon color to the skin—rather pasty.
      No urobilinuria.
      Rapid febrile course.
      Enlargement of liver and spleen.

II. Aleukemic states of leukemia.

7. Cachexia with poor hygiene.

I. Acute Hemorrhagic Anaemias.

Enumeration of common causes.

(a) Severe hemorrhage.

(a) G. I. tract. (Eoophageal, pharyngeal—acute haem.—gastric ulcer.)

(c) Respiratory (tbc., erosions of aneurisms, parasitic).

(d) G. U. (renal epistaxis, renal neoplasm t b, bladder conditions).

Regeneration quickest in men (25 to 40). Slowest in children. Minimum C. I. occurs around ninth day: nucleated R. B. C. 7-8 days.

Rapidity of regeneration after hemorrhage.

Blood loss—4.5% body weight—entirely reformed within 8 days.

Blood loss 2% body weight—within 8 days.

II. Chronic Hemorrhagic Anaemia.

Succession of hemorrhages without recovery between.

Enumeration of common causes:

(a) Mild hemoptysis.

(b) Tbc.

(c) Hemorrhoids.

(d) Extreme and too frequent menstruation.

(e) Nose irritation, etc.

Blood findings:

R. B. C. down to 1,000,000, usually small and pale.

Nucleated R. B. C. not abundant.

Platelets increased or not.

Leukopaenia common.

Picture varies with duration of anaemia; long-continued insults, bone marrow exhaustion and aregeneration becomes poor.

Ultimately blood picture may resemble P. A.

cent theory.) There is said to be much fatty acid in the circulation, which disappears after spleenectomy.

No theory holds good for all anaemias. It is a toxic anaemia, probably the expression of absorption or the action of one or several toxins of varied origin (probably related to the fatty acid or lipoid group), causing morphological and functional changes in the bone marrow and constant reversion to embryonic type.

The disease occurs in people of middle years (35-40), but can occur in people much older (in the 70s) and has been known in children. It is no respector of persons, occurring among the rich as well as the poor, but probably more in the male sex. It sometimes has a family tendency.

Clinical symptoms and picture.

1. Gastro-intestinal symptoms:
   (a) Sore tongue.
   (b) Active stomatitis.
   (c) Achlorhydria.
   (d) Loss of appetite, indigestion, weight in stomach after eating.
   (e) Frequently diarrhea.
2. Cardio—respiratory system.
   (a) Dyspnoea.
   (b) Swelling of ankles.
3. Nervous system.
   (a) Numbness or tingling of hands or feet. (Exclude syphilis.)
   (b) Depressed, easily fatigued.
   (c) Changes in the cord (unsteadiness of gait, cushion under feet, etc.)
4. Urine.
   (a) Slight albuminuria.
   (b) Urobilinuria, prolonged and persistent.
5. Slight irregular fever.

6. Other findings:

(a) Yellow tint to the skin and conjunctiva.
(b) Petechiae, not characteristic.
(c) Liver and spleen may be slightly enlarged.
(d) Good nutrition.
(e) Patients don't come to doctor till blood is way down.

Blood Picture.

Total volume reduced. Hydraemia, pale, coagulation often delayed. Spreads quickly, corpuscles seem to settle quickly. Clot small (Brilliant yellowish green tint to serum-urobilin).

Formed elements.

Red blood cells reduced to 1,500,000. (As low as 113,000, with recovery.) Striking anisocytosis—general tendency to predominance of deeply staining macrocytes. High percentage of poikilocytes. Moderate basophilia. Nucleated reds in small numbers, megaloblasts predominating. Platelets decreased. Hemoglobin per cell high. C. I. .95 to 1.5. W. B. C. decreased per cent of P. M. N., P. M. E., P. M. B., L. M., and transitionals while the per cent of Lymphocytes is increased. During a remission the eosinophilic cells are increased together with other cells of myeloid origin, while the lymphocytes fall.

Clinical course of disease:

The onset is usually gradual and progressive. The disease may last from 1 to 10 years. It is characterized by a feeling of well-being till the R. B. C. count drops markedly low. Wave-like remissions occur, which may last from 1 to 6 years. An occasional cure is reported. The end usually comes from an intercurrent infection or from an acute exacerbation. A gradual mental stupidity and toxaemia comes near the end and the patients die similar to those with hepatic disease.

Valuable points in diagnosis and prognosis.

Primary Anaemia. An anaemia for which there is no ascertainable cause.

Chlorosis. A very rare disease of unknown etiology. Scarcely ever found in the United States, sometimes in Germany. Limited to female sex and usually appears at onset of adolescence (14 to 20), but has been known to occur up to 35. Distinct family and recurrent tendency, especially if individual ever has anaemia. Numerous theories as to cause—"love-sick anaemia"—also some think due to constitutional deficiency of the bone marrow to synthesize hemoglobin.

No typical pathological findings.

Chlorosis is a distinct disease and anaemia is only a symptom of it.

No actual destruction of R. B. C.

Symptoms: Weakness, fatigue, fainting periods, dizziness, G. I. symptoms (perverted taste, fondness for pickles, etc.), menstrual function is disturbed.

Chlorosis is possibly an internal secretion disturbance.

Hysteria is common.

Characteristic green (sea-sick green) color to patient's face. (It was once thought to be du to poor nutrition, but this theory is probably wrong.)

Blood findings: R. B. C. decreased; however, this decrease is relatively slight and wholly out of proportion with the great decrease in Hb. (Here Dr. Thayer gives the R. B. C. as 4,000,000 and Hb. as 42%.) This consequently results in a phenomenally low C. I. (below 0.5). Pale R. B. C. Anisocytosis and poikilocytosis slight and often absent. If there is anisocytosis, the common cells are microcytes. Nucleated R. B. C. are few, and those that occur are of the late normoblastic type. The occurrence of a megaloblast is extremely rare.

ble, not caseous, and often have multiple hemorrhages in the acute forms. They rarely give rise to obstruction, as there is no tissue reaction around them.

Spleen: The form is smooth, with the notch preserved. Rarely reaches to the umbilicus, and rarely produces abdominal discomfort.

Bones: Pain present in the late stages.

Skin: There may be present multiple lymphomata, especially on the face and upper throat. They are small, painless, and never break down. Lymphodermia perniciosa, a disease with great itching, may be the first symptom.

Mouth: Affections of the mouth are relatively uncommon. Mikulicz's disease is symmetrical tumor formation of lacrimal and salivary glands.

Heart and lungs: Negative.

Urine: Bence-Jones proteinuria and uric acid notable.

Temperature: Fever not predominant.

Duration: 3-5 years before use of X-ray. There is a tendency to intercurrent infections, which obliterate picture.

Blood findings:

Gross appearance—creamy, almost purulent.

R. B. C. normal or slightly reduced.

C. I. about normal.

Slight anisocytosis and poikilosytosis with polychromasia—normoblasts.

W. B. C. leucocytosis may reach 600,000. Lymphocytes, 90-96%.

Reider cells may be present. Azure granules are absent. Otherwise cells look like normal, except that they stain with a "Heller Farburg."

The nucleus is less picnotic, therefore the nucleoli can be seen.

Many of the cells crush indicating youth. The

Hemoglobin low. C. I. .6 to .7.

Platelets gone.

Pronounced leukopaenia (1,200 to 2,000).

At autopsy one finds metaplasia of bone marrow. No islands of actively functioning red or white cells.

(Musser):

1. Coagulation time distinctly delayed.
2. No reticulated blood cells ever found.
3. Minimal resistance unchanged.
   Maximal resistance increased.

Etiological factors in aplastic anaemia:

Three groups:

1. So-called aplastic anaemia from chronic repeated hemorrhages, characterized chiefly by history.
2. Destructive property of some toxin, hemolytic in origin. They include all the chronic hemolytic anaemias.
   The clinical history is very important.
3. Aplastic P. A. In the aplastic stages of P. A. there is a constantly dropping W. B. C. count and no evidences of R. B. C. regeneration.

Naegeli says a better name for aplastic anaemia is *aregenerative* anaemia.

Important points in all cases:

1. Clinical history.
2. Rarely reduction of R. B. C. is as great as in P. A.
3. Age—usually under 30 or over 60.

V. Carcinoma of the stomach.

Possible existence of pain.

Patient's history—same general symptoms, but usually loss of weight.

G. I. findings may or may not have free HCl. (Pyloric end do not.)

. Occult blood apt to be absent in P. A.

Stomach hypermotile in P. A.

Blood:

Allow to clot—likely to look normal in carcinoma.

In P. A. serum has high color, but not same proportion between clot and serum.

R. B. C. rarely below 2,000,000.

Poikilocytosis and anisocytosis less marked as a rule.

C. I. hovers around 1, varies more than in P. A.

Platelets usually plentiful.

W. B. C. usually normal or above. P. M. N. increase.

X-ray does not always show distinction.

Leukemias.

A leukemia is a disease characterized by an increase in the white cells of the blood usually abnormal, and by a general hyperplasia of the leukopoietic organs of the body. The blood condition is a symptom of this hyperplasia. There may be a digression between hyperplasia and leucocytosis. Leukemias may have a count as low as 50,000, and a leucocytosis may reach 100,000. Leukemias were recognized by Virchow in 1845 and clinically in 1850. The classification at that time was:

1. Splenic, 2. Lymphatic; depending upon which was enlarged.

There are no true transitional stages between the lymphatic and the myeloid which speaks for the dual origin of these cells. In lymphoid leukemia the bone marrow is involved very late.

Possible sources of origin:

1. Parasitic—amoeba.
2. Cancer.

lymph glands, exophthalmos, periosteal infiltrations, etc.

Myeloses.

Myeloid leukemias are general hyperplasia of the myeloid tissues. They have the same general grouping as the lymphatic leukemias.

1. Chronic myeloid leukemia.
2. Acute myeloid leukemia.
3. Chloromyelosis (myeloid chloroleukemia).
4. Aleukemic myelosis.

1. Chronic Myeloid Leukemia.

Occurs chiefly in people in middle life, more in men than in women, rarely in children. It is characterized by slow development with gastric symptoms, weakness, poor sleep, often marked sweating. (Often tuberculosis is suspected.)

Special symptomatology.

(a) Enormous enlargement of spleen, with smooth surface, hard, and notch preserved. Usually sensitive.

(b) Liver enlarged, smooth, hard, keeps normal shape. Rarely jaundice.

(c) Lymph glands not enlarged and inconspicuous.

(d) Bones frequently tender and patient often has bone aches.

(e) Ascites.

(f) No enlargement of tonsils (except late).

(g) Irregular fever.

(h) Hemorrhagic diathesis rare, however, tendency to skin eruptions.

(i) Priapism.

(j) Amenorrhea.

Blood findings:

Gross color unchanged at first.

Blood thick, coagulation time increased. Oxidase reaction marked.

Remission stage—decrease in W. B. C.

Usually well marked anaemia, but never so severe as P. A.

Characteristics of Secondary Anaemia. C. I. low, cells pale, if nucleated tend to be normoblasts. Nucleated forms tend to be present more than they ever are in P. A.

Differential count shows greater peculiarities than P. A.

III. Leukanaemia.

Larger number of megaloblasts.

Severe anaemia with leucocytosis.

Probably atypical forms of Von Jaksch's (may occur in older people).

Rapid course—usually die within 2 months.

IV. Aplastic anaemias.

An anaemia different from any pernicious or secondary anaemia, characterized by retrogressive changes in the bone marrow. (At autopsy one finds all the bone marrow replaced by fat.)

1. Primarily of young people. (May be seen from 5 to 60.) Well under 30 as a rule.

2. Clinical course different. Rapid and progressively fatal. (Two months and as low as 6 days.)

3. From the start tendency to subcutaneous and mucus hemorrhages.

4. High and persistent fever.

5. Blood picture:

R. B. C.—severe and intense anaemia. (1,000,000.)

No poikilocytosis or anisosytosis.

May be slight general increase in size.

Absence of polychromasia of any type. No nucleated reds.

protoplasm is basic and seems almost absent in many.

Blood platelets—diminished.

Diagnosis —blood picture.

Differential diagnosis: Lymphosarcoma, tbc., and myeloid leukemia.

Prognosis: Fatal after remissions.

2. Acute Lymphatic Leukemia.

1. Fever, with prostration.
2. Gangrene and ulcer in G. I. tract.
3. Hemorrhagic tendency.
4. Rapid course of disease. Anaemia, cachexia, death in one to two months. (Minimum one week.)
5. More acutely fatal and common in children.
6. There is high irregular fever.
7. Progressive splenic and lymphatic enlargement.

Diagnosis: Ulcerative tonsilitis and stomatitis. Prodromal period, with occasional mild attacks of fever and general malaise, followed in a few weeks with cervical glandular enlargement, then with a flare up and extensive hemorrhage and the picture above given.

Blood picture—abounds in lymphoid cells.

Other groups:

1. Like acute rheumatic fever—polyarticular, usually painless involvement.
2. Like typhoid—diarrhea and abdominal pain.
3. Like pleurisy.
4. Like meningitis.

3. Chlorlymphadenoses. In this condition there are tumors in various parts of the body with characteristic greenish tinge. They are divided into groups according to location.

1. Tumors in or on the skull. Symptoms—exophthalmos, generally symmetrical, with swelling of the occipital and temporal regions.

2. Subperiosteal infiltration of the spinal column, ribs and pelvic bones.

3. Lymphatic leukemia with tumors, but only at autopsy does the green color of the tumors become apparent.

In all the cases the blood picture is that of chronic and acute lymphatic leukemia, and the tumors show chronicity, which runs with leukemia. Most cases are rapidly fatal and show no remissions. Over 50 per cent of the cases are in children.

4. Aleukemic Lymphadenoses.

This is a leukemic state with low W. B. C. count.

Clinical differences—lymph glands not as swollen, course of disease is quite chronic and may develop into chronic lymphatic leukemia.

Blood picture is practically the same, qualitatively, as lymphatic leukemia, but absolute numbers are lower.

Naegeli groups into six types:

I. General lymphatic hyperplasia (aleukemic blood picture).

II. Lymph hyperplasia more localized—glands adhere to one another and the underlying tissues so that the diagnosis of lymphosarcoma is sometimes made.

III. Very large splenic tumor with only slight lymphatic hyperplasia. Spleen shows uniform enlargement and keeps normal form.

IV. Localized lymphatic infiltrations in pharynx, larynx, eyelids and cheeks. Spleen may be large or small.

V. Slight anaemia and low fever, aleukemic state. Suddenly acute leukemia, severe hemorrhagic diathesis and death in a few hours. Actual bleeding from skin.

VI. Chloroma in skull with aleukemic blood picture. Enlargement of spleen, liver and

3. Infectious—present view. Facts which support view:
   (a) Stormy, rapidly progressive, febrile condition.
   (b) Apparently follows septic conditions.
   (c) Apparently contageous in a few cases at least. Similar condition can be transmitted in animals.

Lymphadenoses.

These are conditions associated with lymphatic hyperplasia and with characteristic blood picture.

Kinds:

1. Chronic lymphatic leukemia.
2. Acute lymphatic leukemia.
3. Aleukemic lymphadenoses.
4. Chlorlymphadenoses.

1. Chronic Lymphatic Leukemia.

Begins in childhood or in young adults with slow onset and probably aleukemic states, with only qualitative blood picture changes during period of hyperplasia. Sexes evenly distributed.

Symptoms:

1. Marked enlargement of tonsils.
2. General symmetrical enlargement in neck and axilla.
3. Splenic enlargement with perisplenitis.
4. Hemorrhagic diathesis (bleeding from any mucus membrane).
5. Severe headache and visual disturbances.
6. Usually anaemic symptoms (languor, slight loss of weight, etc.).
7. Stitch in side and signs of pleurisy.

Glands: There are generally enlargements somewhere, sometimes ahead of the other symptoms. Sometimes only the retroperitoneal glands are involved. They are usually soft, isolated, not adherent, rarely matted together. They are grayish, fria-

R. B. C. 3,500,000 to 4,000,000. Polychromasia, large numbers of nucleated reds. Hb. reduced proportionately—therefore about normal C. I.

Platelets markedly increased.

W. B. C.

Increased and may go up to 1,000,000.

Large myelocytes abundant. Larger types predominate with basic protoplasm, irregular in size and staining reactions. Nuclei show a fine network structure. Some myeloblasts. Typical P. M. N. is predominating cell. Eosinophiles increased in absolute count. Mast cells in no other cases seen so frequently. L. M. and Transitionals increased in actual numbers. Lymphocytes only in small numbers (absolute count little changed). Frequently find true megakaryocytes, but so fragile as to be only recognized by the nucleus.

There are three changes of interest:

1. Changes under X-ray and arsenic treatment. Cell count may drop to normal or may be same general count, with immature cells almost disappearing.
2. Changes during infectious diseases—may loose all abnormal cells.
3. Appearance of myeloblasts. Increase markedly as disease grows worse. Increased enormously in cases treated by X-ray and influenced unfavorably. In agonal stage may rise to 20-50%.

Prognosis.

There are stages of remission. An apparently slight case, perhaps lasting for years, may suddenly become acute, and some slight cause may bring about a change for the worse. Etiology unknown.

Treatment:

Arsenic.

Benzol—great care should be exercised. Give small doses in a bland oil and stop when the count reaches

20,000 to 30,000. Wait till the count stops falling before giving again.

X-ray and radium—in vogue.

2. Acute Myeloid Leukemia.

Runs a course like acute lymphatic leukemia. Has a clinical picture of lymphatic. Distinguished only by blood picture. The more acute the disease the less typical the characteristics.

The course of the disease is steadily progressive and fatal.

3. and 4. Same thing is true as in other two groups.

It is to be remembered that any leukemia may undergo an aleukemic stage either normally or induced.

Distinguish chronic myeloid leukemia from:

1. Hyperleucocytosis due to infection—usually in children.
2. Severe anaemias may show high leucocytosis—rarely hyperleucocytosis.
3. Metastasis of malignant tumors in bone marrow. Picture more distorted.

Blood findings in infectious diseases:

1. Leucocytosis with absence of eosinophiles points to pyogenic infection, the so-called "septic factor." The reappearance of eosinophiles is a favorable sign.
2. Lymphocytosis occurs in pertussis, rickets, congenital lues, tbc., measles, typhoid, influenza, diseases of endocrine glands.

1. Pertussis—hyperleucocytosis, lymphocytosis, B. Pertussis in the sputum, R. B. C. unchanged. W. B. C. 22,000 to 25,000. 40 to 60% small lymphocytes.
2. Poliomyelitis—leucocytosis, 20,000 to 50,000 W. B. C. count with 80% P. M. N. Spinal fluid with cell increase, especially small lymphocytes. Absence of "septic factor."

3. Epidemic meningitis — leucocytosis, constant septic factor, bacteraemia, spinal fluid shows specific organism with purulent fluid. R. B. C. unchanged, W. B. C. 12,000 to 55,000. P. M. N. 85 to 90%. Eosinophiles diminished. Spinal fluid cell count shows almost exclusively pus cells.

4. Malaria—leucopaenia, absence of septic factor. Increase in L. M. Pigmented leucocytes, and presence of organism. R. B. C. show anaemia usually, C. I. constantly low. Usually signs of regeneration. W. B. C. increased before paroxysm, after chill rapidly developing leucopaenia (2,000 to 3,000). P. M. N. decreased. Phagocytes of L. M., with marked amoeboid activity.

Thayer's classification of blood findings in malaria:

(a) Ordinary S. A., with abundant regeneration.
(b) Blood picture essentially P. A.
(c) Rapidly fulminating.
(d) Severe, chronic, aregenerative S. A.

5. Measles—leucopaenia, diazo reaction in urine. Moderate pre-eruptive leucocytosis. Platelets decreased or absent.

6. Diphtheria—leucocytosis, septic factor, bacilli from throat, albuminuria, high R. B. C. at first, later mild anaemia. W. B. C. 14,000 to 30,000, present from early stage, and falls slowly. Eosinophiles absent. P. M. N. 90%. Leucocytes should fall rapidly after antitoxin.

7. Scarlet fever—leucocytosis, absence of septic factor, eosinophiles increased. Diazo reaction. Streptococci in smears or blood cultures. R. B. C. unchanged. W. B. C. show a consistent increase especially before the rash, normal at three weeks. P. M. N. 90 to 95%. Glandular infections (P. M. N. go up). If leucocytes do

not respond it means severe infection. Platelets increased. Dahl's leucocytic inclusions may be present.

8. Typhoid fever—S. A., leucopaenia, increase in lymphocytes, blood culture, Widal about tenth day, late diazo, no septic factor. R. B. C. 5,500,000 to 4,500,000. Hb. reduced more than R. B. C. always. Nucleated forms rarely. W. B. C.—the greater the leucopaenia the more severe the case. P. M. N. 50%, eosinophiles absent in three-quarters of the disease. Mono increase. Favorable findings are moderate leucopaenia, with a return of eosinophiles in three weeks. Hemorrhage and perforation: 1. Liver dullness. 2. W. B. C. going up from the depths. The count may go down. It may remain stationary with alteration in liver dullness. Hemorrhage—W. B. C. increase and then leucopaenia.
9. Pneumonia—hyperleucocytosis, septic factor, fibrin increase. Rusty sputum, chloride reaction, pneumococci in the blood and sputum. R. B. C. 4,000,000. W. B. C. increased 15,000 to 25,000. The count may diminish at first. P. M. N. 80 to 85%. Septic factor marked.

Hodgkin's Disease.

Hodgkin in 1832 described this disease very imperfectly.

Three theories as to origin:

1. Cohnheim and Sternberg. A tbc. origin based on (a) acid fast organism. (b) Tbc. elsewhere. (c) Perhaps human reaction to bovine. Still an open question. Probably coincidental rather than true etiological fact.
2. True tumor. Described as pseudo-leukemia. Against it: (a) Histology. (b) Attempts to transplant a failure.
3. True infectious granuloma. Bunting and

Yates) Diphtheroid organism and vaccine treatment. Now stands as follows:

(a) The diphtheroid can be cultivated from glands.

(b) The diphtheroid can be cultivated elsewhere.

(c) Glands in general have a normal flora (Bloomfield).

Blood picture (Bunting and Yates).

1. Relative or complete absence of anaemia.
2. Relative and positive increase in L. M.
3. Platelets increased notoriously.
4. Eosinophilia.
5. Leucocytosis with P. M. N. increase.

Many observers fail to get this picture. They get a slight tendency to this with many variations. There is no consensus of opinion as to etiology or blood picture. Only positive diagnosis is removal and examination of gland.

Conditions with anaemia and splenomegaly:

1. Banti's disease—splenic enlargement, tendency to hemorrhage, occurs after 25 to 30. Blood picture is not characteristic.
2. Splenic anaemia—see above.
3. Gaucher's disease—in young children, progressive splenic enlargement, anaemia, no jaundice, no blood destruction, no characteristic blood picture, familial disease.
4. Hemolytic anaemia, acquired or inherited. True bone marrow dystrophy, hemolytic function of the spleen. Icterus, constant urobilin in acholuric urine. Moderate secondary anaemia, decreased resistance of R. B. C. Reticulated, vitally staining R. B. C. Splenectomy curative.
5. Pernicious Anaemia with splenic enlargement.

Paroxysmal Hemoglobinuria.

The patient either has hemorrhage after exposure

to cold or has secondary anaemia from unexplained hemorrhage. 95% have lues, and it responds to specific therapy. The diagnosis is often made by subjecting patient to cold.

Experiment: Use patient's serum and his washed corpuscles.

Patient's serum plus R. B. C. at 37 deg. C. gives no hemolysis.

Patient's serum plus R. B. C. at 0 deg. C. gives no hemolysis.

Patient's serum plus R. B. C. at 37 deg. C., after chilling at 0 deg. C., gives hemolysis.

Purpura and Pathological Hemorrhages.

1. Definition of purpura: A tendency to spontaneous hemorrhages developing in and beneath the skin and mucus membrane in any part of the body. The size varies from pin point to several cm. in size.
   (a) Pin point, small areas—petechiae. Chiefly on extremities, especially legs.
   (b) Lines or streaks—vibices.
   (c) Large areas—ecchymoses.
   (d) Extensive areas — suggilation (covering whole thigh, etc.).

When recent they are usually bright red, but later become livid and purple. No remnants in time. They are generally not elevated, no induration, cannot bepressed out, differing from erythremia in this respect. Gangrene and ulceration common.

They frequently come in crops, and are commonest on the legs and arms, and occur mostly on the extensor surfaces.

2. Occurrence:

1. Symptomatic—secondary.

2. Idiopathic—primary blood trouble.

1. Symptomatic purpura.

(a) Acute infectious diseases. It may occur in any infectious disease, especially where there is bacteraemia: 1. Typhus. 2. Smallpox. 3. Epidemic meningitis. 4. Endocarditis, where it is especially valuable in connection with the clinical history.

(b) Chronic malnutrition—cachexia and malignant states in general (especially of the bone).

(c) Conditions associated with jaundice. Connection not known, but may be due to some impaired function of the liver.

(d) Blood diseases.

(e) Chronic nephritis—rare.

(f) Drugs—rare. (Iodin, mercury, antipyrene, aromatic drugs as copaiba, turpentine, etc.).

(g) Mechanical injuries. Trauma and also due to the bite of parasites, etc.

(h) Nervous forms—with hysterical seizures or along nerve trunks in neuritis.

Only when all these causes are excluded can one go to the "Primary purpuras" in which the fundamental cause is probably found in disturbances in the coagulation factors of the blood.

2. Idiopathic Purpura.

It was formerly thought that:

1. Purpura simplex or Werlhoff's Disease,

2. Purpura rheumatica or Schonlein's Disease,

3. Henoch's Purpura, and

4. Peliosis hemorrhagica

were all seperate diseases, which view has been

largely abandoned. There is great confusion in the literature, and it is probably necessary to retain the terms until more precise knowledge is at hand concerning the idiopathic purpura (Morbus maculosus).

Occurrence of True Purpura.

There were 41 cases in 18,000 admissions in J. H. H. They occur more in the fall and winter months. More in males than in females—young rather than old. There is a clear-cut heredity role.

Causes: 1. Vascular injury. 2. Infection with bacteria. 3. Pathological condition of the blood, particularly coagulation factors.

In any case of pathological hemorrhage certain blood studies are of prime importance, viz:

1. Estimation and enumeration of platelets. Below 60,000, bleeding apt to occur; below 20,000, bleeding certain.

   Best method is to use fresh blood.

   Minot—Prepare slide with film of cresyl blue (1-300) dried. This will keep a long time. Secure drop of blood upon a cover-slip invert upon the prepared slide. Platelets and reticulated forms show well. The platelets stain a characteristic blue.

2. Determine the bleeding time.

   Vena puncture (Dr. Howell). Be sure to hit the vein on the first try. 1 cc. of blood in a clean tube (8 mm. in diameter). Normal blood can be inverted in 7-10 minutes. Also note when the first signs of coagulation begin (slight concavity). The length of the coagulation time does not run parallel with the tendency to hemorrhages.

3. Retractility and Firmness of the Clot.

   In hemorrhagic states either failure to retract, or soft, flabby clot occurs. Flabby clot

indicates decreased thrombin or fibrinogen.

Non-retractility indicates decreased platelets.

Sometimes "reclotting" occurs. Remove clot and serum again clots.

This is associated with lack of retractility.

4. Other studies of much value when possible are:
   1. Effect of addition of calcium on the coagulation time.
   2. Determination of the pro thrombin time.
   3. Determination of the anti thrombin and fibrinogen.
   4. Determination of fibrinolytic ferments.

Purpura Simplex.

This is a disease with skin symptoms exclusively and slight pains. There may be a few eruptions coming out in crops. Duration 6 to 8 weeks.

Purpura Rheumatica or Schönlein's Disease, Peliosis Rheumatica.

The term has been horribly misused. It is applied to any condition of arthritis with purpuric, erythematous, or urticarial lesions. It is best to adopt the term "Simple purpura with arthritis."

Clinically:

1. Young adult males.
2. Average duration, five weeks.
3. Recurrent arthritic signs, polyarticular rheumatism, especially of the knees and ankles.
4. Simple purpura.
5. Fever slight or absent.
6. Never fatal.
7. History of previous rheumatic fever, rare.
8. Salicylates not helpful.
9. Purpuras rare in true rheumatic fever.
10. Association of urticaria, erythema, and angio neurotic oedema.

Henoch's Purpura.

1. Recurrent purpura.

2. Abdominal crises, melena, hematemesis.
3. Arthritis common.
4. Nephritis common and severe.

Occurs in males in early life. Recurrent attacks. Beware of early operations.

Blood picture. Purpura simplex, Purpura rheumatica, and Henoch's Purpura differ only in degree, frequency and extensiveness of the purpura. S. A. picture, platelets normal or increased. No coagulation disturbances evident.

Purpura Hemorrhagica—Aplastic Anaemia.

The condition is idiopathic or acquired, acute, subacute, or chronic. Tendency to hemorrhages under the skin and mucus membrane anywhere. Skin eruptions, diminished blood platelets. Delayed bleeding time. Non-retractility and often flabby clot. Often true hereditary tendency.

Aplastic Anaemia.

This is a true disease entity. It is acute with progressively downward course (3 to 6 weeks). Occurs in young males. Rarely associated with arthritis. Clinically there is no evidence of blood destruction. Some unknown toxic substance inhibits all the myeloid elements. C. I. below 1. (.8 to .9.) Platelets absent, or, if not absent, curiously large. Leucopaenia, lymphocytes increased relatively. Tendency to purpura, but not to bleeding. In Purpura hemorrhagica only one myeloid element is inhibited, i. e., platelets. The white count is increased with true P. M. N. increase. Other myeloid elements show increase. Signifies intravascular destruction. Both diseases, Purpura Hemorrhagica and Aplastic Anaemia, occur in same people. Some cases of the former turn into the latter.

1. Aplastic Anaemia—complete, rapid myeloid destruction.
2. Purpura hemorrhagica:

(a) Continuous: 1. Acute (3 days). Always find platelets below normal. 2. Chronic.

(b) Intermittent. Clears up and then returns. Platelets low only during attack.

3. Intermediate Group.

Intermittent type. There is a tendency for the platelets to decrease. It does not go onto true aplastic anaemia.

Other Diseases.

Hemophilia. A blood deficient in pro thrombin and a true or relative excess of antithrombin. Platelets normal. Mendelean heredity.

Scurvy—unbalanced diet, bleeding tendency, loose gums. Often cured by introducing suitable food. No disturbed coagulation picture.

Barlow's Disease—Infantile Scurvy. This is a periosteal affair, occurring in the lower extremities. It is characterized by pain in the bone. Look for it in children who stop walking.

Essential hematuria. See notes on urine.

MATCHING OF BLOOD FOR TRANSFUSION

Hemolysins:

1. Traumatic.
2. Toxic.

Other classification:

1. Heterologous. Exemplified by rabbit and sheep.
2. Autohemolysis — rarely autoagglutination — noted in R. B. C. counting.
3. Isohemolysis—90% possess isoagglutinin; 25% isohemolysis.

Grouping is established by the time the individual is two years old and remains unchanged throughout life.

Red Blood Cells of	I.	II.	III.	IV.
Serum I. (10%)	0	0	0	0
Serum II. (40%)	+	0	+	0
Serum III. (7%)	+	+	0	0
Serum IV. (43%)	+	+	+	0

Even by gross methods one can tell whether agglutination takes place or not.

Method of matching blood.

1. Cross agglutination. Obtain serum from each patient; also corpuscles washed twice with isotonic salt solution and resuspended in salt solution to make a faint pink color. Place a drop of the receiver's serum upon a cover-slip and near it a drop of the donor's r. b. c. Also place a drop of the patient's r. b. c. upon a cover-slip and near it place a drop of the donor's serum. With a small glass rod mix the first two drops, and with another glass rod mix the second two drops. Invert the cover-slips and place over hollow-ground slides well sealed with vaseline. Examine immediately microscopically, and again in an hour. Exclude any donor whose cells are agglutinated by the receiver's serum or whose serum agglutinates the receiver's blood. Usually members of the same group match, but this method is the safest because, sometimes, groups do not match, especially in severe anaemias.

2. Using known sera or r. b. c from groups II. and III.

Corpuscles.

	Group II.	Group III.	Group of pt.
Serum of pt.	0	0	I.
" "	0	Agg.	II.
" "	Agg.	0	III.
" "	Agg.	Agg.	IV.

Agglutination

No Agglutination

Serum.

	Group II.	Group III.	Group of pt.
R. B. C. of pt.	Agg.	Agg.	I.
" "	0	Agg.	II.
" "	Agg.	0	III.
" "	0	0	IV.

After finding a donor of the same group as the patient, match their corpuscles and serum as in proceedure 1.

The serum may be preserved at least a year if kept on ice.

Members of Group I. may be universal recipients.

Members of Group IV. may be universal donors.

Members of Group IV. should only get Group IV. blood.

Group II. and III. very serious.

The amount given at a transfusion varies from 500 cc. to 3 liters.

CEREBROSPINAL FLUID

Up until 1891 no particular importance was placed in spinal fluid examinations but now very important

clinical evidence can be obtained thereby. The existence of a tumor at the base of the skull is only real contraindication advanced against lumbar puncture.

Posture—lying down.

Site of puncture—3-4 or 4-5 lumbar spines.

Two methods of entrance—mid-line or to right or left of mid-line.

Only discomfort is going through skin.

There are about 40 to 70 cc. in the average individual. 5 cc. is ample for an examination, 2-3 cc. are enough.

Avoid lumbar puncture reaction, which consists of severe headache, head pulling backward—relieved by lying down. Often dizziness and nausea follow.

Good routine: 1. Have patient plan to stay in bed for 24 hours following, with only one pillow. 2. For first four hours a glass of water every hour. 3. 1 cc. of pituitary extract immediately after puncture. 4. Patient should sit quietly two or three hours after the 24, then move about the house, and, upon the slightest evidence of headache, go back to bed.

Normal Spinal Fluid.

Clear, colorless, sparkling, resembles distilled water. Normal pressure varies with posture, but markedly increased in certain diseases. 120-140 mm. H_2O, 6-10 mm. Hg.

Increased:

1. Acute meningitis.
2. Poliomyelitis and tbc. meningitis.
3. Intracranial pressure.

In some cases absolutely diagnostic, but in most only confirmatory.

Inorganic constituents:

Chlorides—.5 to .6 gm. per 1000 cc.

Organic constituents:

Dextrose—.4 to .6 gm. per 1000 cc.

Complete absence in C. S. meningitis—of value.

Notable increase in diabetes.

Protein—probably serum globulin, no readily demonstrable amount of protein.

Reactions:

1. Noguchi—butyric acid.
2. Ross-Jones—saturated ammonium sulphate, faint rings between substances.
3. Pandy's—aqueous saturated carbolic. Normal spinal fluid shows no cloud with 1 drop, almost imperceptible with 10. Abnormal fluid shows milky ppt. Exclude blood.

Increase in protein abnormal, but not specific for any condition. In acute inflammatory conditions of central nervous system, severity usually runs parallel with protein increase. Depends upon location. In syphilis there is increase in globulin.

Cytology:

4-5 cells per c. mm. usually of the S. M. type. 8-12 cells, probably an increase. Above 12 cells distinctly abnormal.

Method of counting: Cell counts should be made as soon as possible, for they settle quickly and degenerate. The white pipette is used and is filled up to 1 with stain. Giemsa is good as well as .2% methyl violet in water. The uncentrifugalized fluid is then drawn up to 11. Shake thoroughly and count on Fuchs-Rosenthal counting chamber. The chamber contains 3.2 c. mm. of fluid. Count all white cells in entire ruled area, multiply by 11 and divide by 32. An ordinary counting chamber can be used with the same method of staining. In this case an entire large square is counted and the result multiplied by 10, or all of the 9 large squares can be

counted, an average taken, and the result multiplied by 10.

Increased in:

1. Infectious meningitis conditions. Usually P. M. N. increase. Fluid cloudy.
2. Tbc. meningitis. Fluid clear. S. M. increase.
3. Syphilis. S. M. increase. Cell increase means irritated surface.
4. Tabes. Normal or increased.
5. Paresis. S. M. increase.

In tbc. meningitis it is usually possible to find the organism. If the fluid is allowed to stand over night in an ice-chest the organisms will often be found in the pellicle which forms.

In syphilis of the C. N. S. the Treponema pallidum has been found repeatedly, with the dark field illumination.

In infective meningitis it is the rule to find the causative organisms in stained smears.

Much of the interest in the spinal fluid examination centers around the Wassermann action. The disease may become localized in the C. N. S. and the blood give a negative reaction while the spinal fluid gives a positive. A positive Wassermann is never found in a S. F. which is globulin free. The test is positive in about 100% of paretic types, 60% of tabetic, and 80 to 85% of all C. N. S. lues. After therapy there is at first abundant protein, which tends to decrease. A positive Wassermann may remain in spite of protein decrease; this is called "Wassermann fast." Therapy should be continued till the S. F. returns to normal.

Xanthochromia. This is a rare finding where the S. F. is a yellow color, a marked cell increase, and the fluid undergoes spontaneous coagulation. The cause is a tumor in the spinal cord.

Colloidal Gold Reaction.

This reaction depends upon the globulin and albumin content of the spinal fluid in certain quantitative proportions. It is not specific for syphilis, but is of clinical value for its diagnosis nevertheless.

Method of preparing the gold solution (L. D. Felton, J. A. M. A. 1917, pg. 73-92.) The glassware should be thoroughly cleaned as follows:

1. Boiled in an ivory soap solution for half an hour.

2. Brushed, rinsed and filled with sulphuric acid-bichromate mixture.

3. Just before use the beaker is emptied and rinsed for two minutes with tap water and five times with singly distilled water. Glassware so cleaned can be used for colloidal gold work as long as a month if it is used for nothing else.

Preparation of Distilled Water:

One cc. of a 10 per cent solution of potassium permanganate and 1 cc. of a saturated solution of barium hydroxide are added to two liters of the water to be distilled. The first quarter of the distillate as well as the last are thrown away. Water thus prepared can be stored away in hard glass containers for an indefinite time.

Reagents:

Chemically pure potassium carbonate, 2 gm. to 100 cc.

Chemically pure gold chloride, 1 gm. to 100 cc.

Chemically pure formaldehyde, 1:40.

Technic for 1000 cc.:

Gold chloride, 100 cc.

Potassium carbonate, 8 cc.

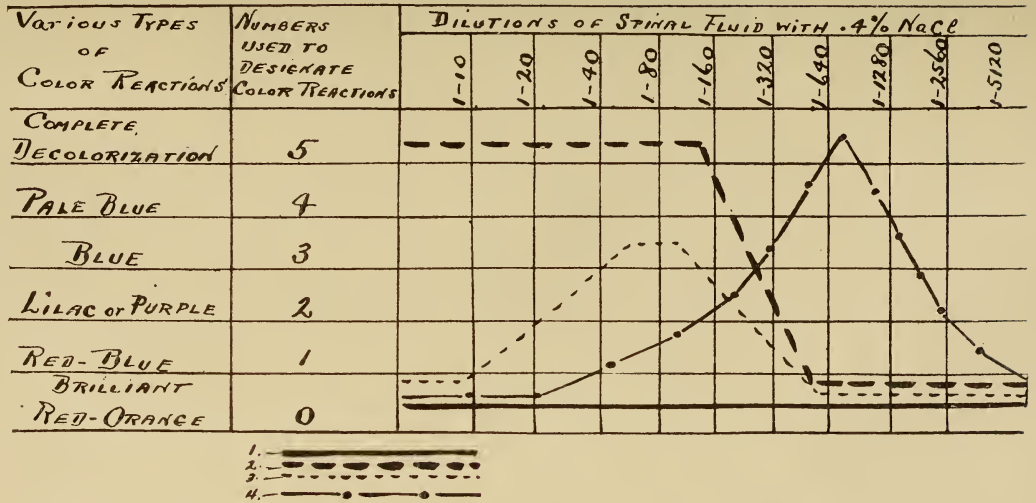
Formaldehyde, 6 cc.

The potassium carbonate and gold chloride are added to the beaker of cold water. The contents of the beaker are then heated until they boil briskly and then the formaldehyde is added drop by drop until the development is almost complete, when the remainder of the formaldehyde is thrown in. Stirring is unnecessary. Should the solution become very alkaline after long standing it should be corrected according to Miller and his associates (Bull Johns Hopkins Hos. 1915, 26, 391.)

The Colloidal Gold Test:

"Into the first of 11 clean test tubes, reserved especially for that purpose, put 1.8 cc. of fresh, sterile 0.4 per cent NaCl solution. Into each of the remaining 10 tubes put 1 cc. of salt solution of the same strength. Now add to the first tube, by means of a clean, dry, certified 1 cc. pipette, 0.2 cc. of the spinal fluid to be tested. Mix well. Transfer 1 cc. of the resultant 1 to 10 dilution of spinal fluid to the second tube, and again mix thoroughly and transfer 1 cc. of this dilution to the third tube. Proceed in this manner up to and including the tenth tube. By this method a series of dilutions of the spinal fluid is secured, in geometrical progression, ranging from 1 to 10 to 1 to 5120. Now add to each of the 11 tubes 5 cc. of a suitably prepared and standardized colloidal gold solution, shake each tube thoroughly and set the series of tubes aside for subsequent observations. It will be noted that the eleventh tube serves as a salt control, since it contains no cerebro-spinal fluid." The series can be read fairly accurately within an hour, as little change occurs after that time.

Showing the four common reaction types with their maximum color changes in different dilutions of the spinal fluid.



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TERMS, ETC., USED IN THE CLASSIFICATION OF PARASITES.

1. *Kingdom*—as Animal, Vegetable.

2. *Phylum* or *Branch*—a primary division of the animal or vegetable *Kingdom*.

Ex.: *Protozoa*, *Arthropoda* or *Vertebrata*.

3. *Class*—the division next below the *Phylum*.

Ex.: *Insecta* or *Flagellata*.

4. *Order*—the division next below the *Class*, and made up of a group of *Families* agreeing closely in some striking characteristic.

(There is no fixed, systematic termination for the divisions above that of the *Superfamily*, but, as seen from the examples given, many of them end in *a*.

Ex.: *Haemosporidia* or *Diptera*.

5. *Superfamily*—the division next below the *Order*. Always terminates in *oidea*.

Ex.: *Ixodoidea* (— *Ixodidae* (family) plus *Argasidae* (family).

6. *Family*—the division next below the *Superfamily*. The name is formed by adding the ending *idae* to the root of the name of the type genus. In printed matter the family name should appear in Roman type.

Ex.: Strongylidae or Culicdae.

7. *Subfamily*—the division next below the *Family*. The name is formed by adding the ending *inae* to the root of the name of the type genus.

Note—Not all *Families* are divided into *Subfamilies*, or grouped into *Superfamilies*.

Ex.: Strongylidae or Culicrae.

8. Genus—the division next below the *Subfamily*. Separate species of animals which agree in the main characteristics of size, proportion of parts and general structure are placed in the same genus. Each genus usually contains several species, but may have only one. The generic name should be Greek or

Latin, preferably a noun, in the nominative, singular: it should be invariably spelled with a capital letter.

Ex.: Ascaris or Culex.

9. Species—the division next below the Genus. It consists of animals in all respects alike or which differ in only minor details. (The male and female of a species may be unlike, but in mating they produce young having characteristics similar to those of the parents.) The specific name should be:

1. A noun in the genitive, or
2. An adjective, which should agree in gender, case and number with the generic name. (Exception—may have a masculine noun as the specific with a feminine generic name.)
3. A modern patronymic—
   = Complete name plus *i* for a man.
   = Complete name plus *ae* for a woman.
4. Always spelled with a small letter.

In naming a species, the name of the genus is always written first, with a capital letter, followed by the specific or descriptive term, with a small letter. When *printed,* both should be in *italics;* when *written,* both are underscored—

Ex.: *Ascaris lumbricoides* or *Musca domestica.*

Type Species—the species of a genus always referred to as representing the genus. Other species may, for good reasons, be transferred to another genus, but the type species is permanently in the genus. Many favor reduplication in the naming of the type species, as

Heterophyes heterophyes.

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CLASSIFICATION OF THE MORE IMPORTANT PARASITES OF MAN.

Group I.—Protozoa (Phylum).

Class A.—Rhizopoda or Sarcodina.

A class of Protozoa whose functions of locomotion and prehension are performed by temporary protrusions of protoplasm called pseudopodia.

A. Order—Gymnamoeba.

Genus—*Endamoeba.*

Species:

1. *E. coli*—non-pathogenic.
2. *E. histolytica* — (Includes *E. tetragena*) — Amoebic dysentery; Liver abscess.

Class B.—Flagellata or Mastigophora (to wear a whip).

A class of Protozoa in which flagella or undulating membranes serve the functions of locomotion and prehension.

(1). Genus—*Spirochaeta* or *Spiroschaudinnia.*

Species:

1. *S. recurrentic*—(S. obermeieri)—European relapsing fever.
2. *S. duttoni*—African relapsing fever.
3. *S. carteri*—Asiatic relapsing fever.
4. *S. novyi*—American relapsing fever.
5. *S. refringens.*
6. *S. vincenti*—Vincent's angina.

(2) Genus—*Treponema.*

Species:

1. *T. pallidum*—Syphilis.
2. *T. pertenue*—Yaws.

(3) Genus—*Trypanosoma.*

Species:

1. *T. gambiense.*
2. *T. rhodesiense* } Sleeping sickness.

(4) Genus—*Schizotrypanum.*

Species:

1. *S. cruzi*—Brazilian trypanosomiasis.

(5) Genus—*Leishmania.*

Species:

1. *L. donovani*—Kala-azar.
2. *L. tropica—Oriental sore.*
3. *L. infantum*—Infantile splenomegaly.

(6) Genus—*Babesia.*

Species:

1. *B. bigemina* — (*Piroplasma bigeminum*)— Texas fever of cattle or Red water fever.

(7) Genus—*Trichomonas.*

Species:

1. *T. vaginalis.*
2. *T. intestinals.*

Flagellate diarrhoea.

(8) Genus—*Lamblia.*

Species:

1. *L. intestinalis.*

Flagellate diarrhoea.

(9) Genus—*Cercomonas.*

Species:

1. *C. hominis.*

Flagellate diarrhoea.

Class C.—Infusoria or *Ciliata.*

A class possessing contratile vacuoles and numerous fine cilia which are shorter than flagella and have a sweeping stroke.

A. Order—Heterotricha.

Genus—*Balantidium.*

Species:

1. *B. coli.*

Class D—Sporozoa.

These possess no motile organs. They live as parasites in tissues and cells of other animals. Reproduction by spores.

A. Order—Coccidaria.

(1) Genus—*Eimeria.*

Species:

1. *E. stiedae.*

(2) Genus—*Isospora—Luci.*

Species:

1. *I. bigemina.*

B. Order—Haemosporidia.

Genus—*Plasmodium.*

Species:

1. *P. vivax*—Terian malaria.
2. *P. malariae*—Quartan malaria.
3. *P. falciparum* — (Laverania malariae) — Aestivo-autumnal malaria.

C. Order—Sarcosporidia.

Genus—*Sarcocystis*—*Stuntu etaoin*

Species:

1. S.—?.

Group II.—Vermes (Phylum) worms.

Class A.—*Platyhelminthes* or *Platodes.*

Flat worms. Divided into two main orders.

A. Order—Trematoda or Trematodes.

Flukes—possess intestine, but no anus; one or two suckers present; body never segmented nor ciliated. Order divided into three main families.

1. *Fasciolidae* (family).

Two suckers, one terminal, the other adjacent to it; situated ventrally; hermaphroditic.

(1) Genus—*Fasciola.*

Species:

1. *F. hepatica*—Liver fluke disease—Hepatic distomiasis.

(2) Genus—*Fasciolopsis.*

Species:

1. *F. buskii*—Intestinal distomiasis.

(3) Genus—*Dicrocoelium.*

Species:

1. *D. lanceatum*—Hepatic distomiasis.

(4) Genus—*Paragonimus.*

Species:

1. *P. westermanii*—Pulmonary distomiasis —Parasitic haemoptysis.

(5) Genus—*Clonorchis.*

Species:

1. *C. sinensis*—Japanese liver fluke disease.

(6) Genus—*Opisthorchis.*

Species:

1. *O. felineus*—Siberian liver fluke disease.

(7) Genus—*Heterophyes.*

Species:

1. *H. heterophyes*—Intestinal distomiasis.

2. *Paramphistomidae* (family).

Two suckers, one at either extremity; hermaphroditic.

(1) Genus—*Gastrodiscus.*

Species:

1. *G. hominis*—Intestinal distomiasis.

3. *Schistosomidae* (family).

Leaf-like male which by a folding-in of its sides makes a channel for the thread-like female. Sexes separate.

(1) Genus—*Schistosoma.*

Species:

1. *S. haematobium*—(African blood fluke).
2. *S. japonicum*—(Asiatic blood fluke).
3. *S. mansoni*—(West Indian blood fluke).

B. Order—Cestoda.

Tapeworms; intestine absent; 2 to 4 suckers on head; adult body segmented and parasitic in intestine; larvae are parasitic elsewhere. Two main families.

1. *Taeniidae* (family).

Head with four cup-like suckers; genital pores lateral.

(1) Genus—*Taenia.*

Species:

1. *T. solium*—(Pork or armed tapeworm).
2. *T. saginata*—(Beef or unarmed tapeworm).
3. *T. echinococcus*—Hydatid disease.

(2) Genus—*Dipylidium.*

Species:

1. *D. caninum* — (*Tænia cucumerina;* the double pored or dog tapeworm).

(3) Genus—*Hymenolepis.*

Species:

1. *H. nana*—(*Tænia nana;* the dwarf tapeworm).
2. *H. diminuta* —(*Tænia diminuta; Hymenolepis flavopunctata;* the rat tapeworm).

2. *Dibothriocephalidæ* (family).

Head with two elongated slit-like suckers; median genital pores; ventral, median, rosette uterus; single set of genital organs in each segment.

Genus—*Dibothriocephalus.*

Species:

1. *D. latus*—(the broad or fish tapeworm).

Class B.—Nemathelminthes.

Round worms, covered with a cuticle which is often ringed; well developed alimentary canal; sexes are usually separated; males smaller in size, posterior end curved or curled, sometimes showing umbrella-like swelling, the copulatory bursa; genital opening in the male near anus, in female midway; develop as a rule in damp earth from the eggs as rhabditiform larvæ; a few are viviparous. Families important to man are:

A. Order—Nematoda or Nematodes.

1. *Filariidæ* (family).

(1) Genus—*Filaria.*

Species:

1. *F. bancrofti* — (*F. sanguinis hominis; F. nocturna*) — Filariasis; Elephantiasis (?).
2. *F. loa.*
3. *F. perstans.*

(2) Genus—*Dracunculus.*

Species:

1. *D. medinensis* — Guinea worm; "Fiery serpent" (?).

2. *Angiostomidae* (family).

Genus—*Strongyloides.*

Species:

1. *S. stercoralis*—Cochin China diarrhea.

3. *Trichinellidae* (family).

(1) Genus—*Trichinella.*

Species:

1. *T. spiralis*—(*Trichina spiralis*)—Trichiniasis.

(2) Genus—*Trichiuris.*

Species:

1. *T. trichiura* — (*Trichocephalus dispar;* whipworm).

4. *Strongylidae* (family).

(1) Genus—*Ancylostoma.*

Species:

1. *A. duodenale*—Old World hookworm). Uncinariasis or Hookworm disease.

(2) Genus—*Necator.*

Species:

1. *Necator americanus*—(New World hookworm). Uncinariasis or Hookworm disease.

(3) Genus—*Trichostrongylus.*

Species:

1. *T. instabilis* — Parasite of sheep and goats; accidental, but harmless parasite of man; eggs may be mistaken for hookworm ova.

(4) Genus—*Dioctophyme.*

Species:

1. *D. renale*—(*Eustrongylus gigas;* the dog kidney worm).

5. *Ascaridae* (family).

No intermediate host necessary.

(1) Genus—*Ascaris.*

Species:

1. *A. lumbricoides*— Eel worm)— Ascariasis.

(2) Genus—*Toxocara.*

Species:

1. *T. canis*—(*Ascaris mystax; Toxascaris mystax; Belascaris mystax;* the dog ascaris or eel worm).

(3) Genus—*Oxyuris* s. 1. or *Enterobius.*

Species:

1. *O. vermicularis* — *E. vermicularis;* pinworm or seatworm.

GROUP III.—ARTHROPODA (PHYLUM)—(jointed-limbed invertebrates).

Class A.—Arachnoidea.

(Resembling a spider.) These have the head and thorax fused together; possess four pairs of ambulatory appendages; never have compound eyes; chitinous exoskeleton; no antennae; body is often segmented; respiration is by tracheal tubes. Those of chief interest belong to the general order *Acarina,* which includes chiefly the *mites* and *ticks.*

(1) Genus—*Sarcoptes.*

Species:

1. *S. scabiei*—(Itch mite)—Scabies or "the itch."

(2) Genus—*Demodex.*

Species:

1. *D. folliculorum.*

(3) Genus—*Leptus.*

Species:

1. *L. autumnale* — (Harvest bug or "chigger").

Class B.—Insecta.

Possess one pair of antennae, three pairs of mouth parts and three pairs of legs. The body is divided into three parts—head, thorax and abdomen. Usually have two pairs of wings. Most insects show metamorphosis; ova develop into voracious, worm-like *larvae;* then follows the encased *pupa* stage, and this finally turns into an *imago* or fully developed insect. The following are important in their relation to man:

A. Order—Siphunculata

(Flat; wingless; no metamorphosis.)

1. *Pediculidae* (family).
   (1) Genus—*Pediculus.*
   Species:
   1. *P. capitis*—(Head louse).
   2. *P. vestimenti*—(Body louse). Pediculosis
   (2) Genus—*Phthirius.*
   Species:
   1. *P. pubis*—(*Phthirius inguinalis; Pediculus pubis;* Crab louse). Pediculosis.

B. Order—Hemiptera or Rhynchota.

Insects possessing a sucking beak; metamorphosis not marked.

1. *Cimicidae* (family).
   Genus—*Cimex.*
   Species:
   1. *C. lectularius*—(Acanthia lectularia; the bed-bug).

C. Order—Siphonaptera

Laterally flattened, wingless insects which undergo complete metamorphosis.

1. *Pulicidae* (family).
   (1) Genus—*Pulex.*
   Species:
   1. *P. irritans*—(European human flea).
   (2) Genus—*Ctenocephalus.*

Species:

1. *C. canis*—(*C. serraticeps;* American dog and human flea).

(3) Genus—*Sarcopsylla.*

Species:

1. *S. penetrans* — (Pulex penetrans; Tropical sand flea; "chigger").

(4) Genus—*Xenopsylla* or *Laemopsylla.*

Species:

1. *X. cheopis*—(Laemopsylla cheopis; rat flea of India; plague flea).

(5) Genus—*Ceratophyllus.*

Species:

1. *C. fasciatus*—(Rat flea.)

D. Order—Diptera.

Have distinct mouth parts—for biting or sucking; undergo complete metamorphosis; possess one pair of wings; other pair usually rudimentary.

1. *Culicidae* (family).

(1) Genus—*Culex.*

Species:

1. *C. quinquefasciatus*—(*C. fatigans;* House mosquito).

Transmits:

1. Dengue fever.
2. *Filaria bancrofti.*
3. Proteosoma infection of birds.

(2) *Aedes* or *Stegomyia.*

Species:

1. *A. calopus*—(*Stegomyia calo pus; S. fasciataQ*—Transmits fellow fever.

(3) *Anopheles.*

Species:

1. *A. maculipennis*—Transmits malaria.

2. *Muscidae* (family).

(1) Genus—*Glossina.*

Species:

1. *G. palpalis*—Transmits sleeping sickness *Trypanosoma gambiense.*
2. *G. morsitans* — Transmits *Trypanosoma rhodesiense* and *Trypanesoma brucei.*

(2) Genus—*Stomoxys.*

Species:

1. *S. calcitrans*—(Stable fly).

(3) Genus—*Musca.*

Species:

1. *M. domestica*—(Common house fly).

Endamoeba histolytica.

Two forms of the amoeba occur in the stools, the mobile and the encysted. In examining the feces for this organism it is best to pick out a bloody particle of mucus if such is present, make an emulsion with a small amount of salt solution upon a glass slide, place a cover-slip over it and examine with low power for small refractile bodies. If such are found then examine with the high power for confirmation and details.

Walker's table for differentiating E. Coli and E. Histolytica. (Barker Vol. II. page 421.)

MOBILE STAGE.

E. histolytica.

1. Appearance hyaline.
2. Refractiveness more feeble.
3. Movements active in fresh stool.
4. Nucleus more or less indistinct.
5. Chromatin of nucleus scanty.

E. coli.

1. Appearance porcelaneous.
2. Refractiveness pronounced.
3. Movements sluggish.
4. Nucleus distinct.
5. Chromatin of nucleus abundant.

Encysted Stage.

E. histolytica.

1. Cyst smaller.
2. Cyst less refractive.
3. Usually contains elongated refraction bodies known as "chromidial bodies."
4. Nuclei never more than four.
5. Cyst wall thinner.

E. coli.

1. Cyst larger.
2. Cyst more refractive.
3. Cysts do not contain "chromidial bodies."
4. Nuclei 8, occasionally more.
5. Cyst wall thicker.

In general it may be said that when one finds an amoeba with phagocyted r. b. c. one should suspect a pathogenic form. This does not always hold true but is fairly reliable. Coli may contain one or two, while histolytica usually contains a great many.

Pathology—The amoeba attack most frequently the upper part of the large intestine, where they enter the crypts of the mucosa and produce small necrotic areas. From here they invade the submucosa and undermine smaller or larger patches. The organism frequently find their way into the liver and produce abscesses there.

Diagnosis—The finding the amoeba in the stools.

TRYPANOSOMA GAMBIENSE.

Size—18 to 25 microns by 2 to 2½ microns.

Pathology—The organism enters the circulating blood and at first causes a varying febrile condition. Later the patient goes into the sleeping-sickness proper, which condition is hopeless.

Diagnosis—Make stained preparations of the blood. If the organisms are few a method of concentrating the organisms can be carried out as follows: Make a vena puncture and draw off about 15 cc. of blood into 3 cc. of a 2.5 per cent solution of sodium citrate in N/NaCl, which will prevent it from clotting. Centrifugalize and draw off cellular elements, make smears and stain, as follows: Add acid alcohol to fix smear and remove hemoglobin al-

lowing to stand 30 minutes to 2 hours. Wash in running water for half to two hours to remove all acid. Dry in the air and stain with Romanowsky stain. A surer method is to inject rats with a small amount of blood and examine rat's blood for the parasites.

Lumbar Puncture—The organisms are present in the second stage of the disease. Draw off fluid, centrifugalize and examine sediment.

Gland Puncture—Aspirate a suspected gland with a hypodermic needle. A surer method is to remove a gland and examine or inject preparations of it into rats.

Mode of Infection—Through the common fly, *Glossina palpalis*. There are two methods of transmission.

1. Immediate or direct.
2. Indirect. A sexual type of development takes place which requires an average of 24 days for the fly to become infective. This can be varied by change in temperature. The fly is infective as long as he lives.

TRICHOMONAS INTESTINALIS.

This organism is probably the same as *Trichomonas vaginalis*. It is found in the vagina, urethra, large and small intestine, stomach and sputum.

Size—20 to 25 microns by 8 to 12 microns.

Chart of Malaria Parasites

Fresh Blood	Plasmodium vivax (Cycle—48 hours) Tertian	Plasmodium malariae (Cycle—72 hours) (Quartan)	Plasmodium falciparum (Cycle—24-72 hours) Aestivo-Autumnal (E.-A.)
Infected R. B. C.	Not modified by hyalines; later are much swollen, paler (after 18 hours).	Not modified at first. After 24 hrs. become smaller, crenated, dark, brassy color.	Shrunken, shriveled, brassy, even from early hyaline forms. Uninfected cells often show same changes.
Hyalines	Large, disk shaped, ring forms, or irregular in outline. Multiple infection occurs, not common; more irregular form than Q. or E.-A. About 1/3 di. of R. B. C.	Smaller than tertian forms. Very hard to distinguish from tertian, but are slightly more refractile. Multiple infection uncommon. Usually ring forms, occasionally irregular.	Smallest forms; 1/6 di. R. B. C.; "signet ring" form very common and maintained for a long time. Multiple infection common, 2-7 in one cell. Position toward centre of cell.
Amoebold activity	Marked at first, ceases later. Pseudopodla look llke isolated granules.	Definitely amoeboid, but not as active as in tertian. Motions slow	Usually not very amoeboid. May become so.
Refractility	Slight; hard to see hyaline forms, or trace pseudopodia.	Increase with growth. Protoplasm looks waxy. Pseudopodia can be traced.	Most refractile form of malarial parasite.

Stained Blood	Tertain	(Quartan)	Aestivo-Autumnal
Infected R. B. C.	Enlargement noticeable, often distorted. Stains poorly. Red stippling (Schuffner's granules) esp. common, may occur in early forms.	Normal or smaller. Take a dark reddish stain: Schuffner's granules extremely uncommon.	Cells of small size, darker staining. Schuffner's granules extremely common. Maurer's spots—coarse, sparse, cleft-like, reddish dots, may be rarely seen.
Hyalines	Ring of blue proto., round chromatin mass, red-violet, with achromatic zone. Whole encloses a stained area of the R. B. C.—the nutritive vacuole. Chromatin at thin part of ring.	Same generally, but chromatin is not dense, rather an irregular clump of red-violet granules. Proto. darker blue. Smaller than tertian. Chromatin nearer the centre. Nutritive vacuole.	Proto. scanty, stains faint blue. Chomatin in one to 3 masses or filaments. Often projects beyond the ring. Nutritive vacuole.
Pigment	Fine, irregularly scattered, appears a greenish brown. In masses at periphery in the pro-segmenters; single central mass in segmenter.	Little influenced by stain; size and distribution as in the fresh. Color, a dark greenish brown or blue-black.	Amount and distribution as in fresh forms. Appears deep brown.
Chromatin	Becomes more irregular with growth. Full grown breaks up into 15-20 clusters, in achromatic zones. Stains a deep red-violet.	Less dense in hyalines, in older forms is a cluster of fine granules with achromatic zone. Stains a more intense red than tertain.	Often in several masses in "ring forms." Compact—stains a deep red.

Pigment	Comes at 10-12 hours. Fine, diffuse, golden brown, actively dancing, up to 36 hrs., then motility gradually ceases. Runs into pseudopodia.	Appears in 20-24 hours. Coarse, black, tends to be peripheral. Motility up to 24-36th hr., then ceases.	Early forms, 2-3 granules, generally motionless, peripheral, later forms (rarely seen), pigment then is coarse, black and central.	Protoplasm	Stains a definite blue. Thickest opposite chromatin in hyalines. Irregular in outline. With growth stains more deeply.	Throughout stains more intensely, and is generally regular in outline, circular.	Scantier throughout than in others—tends to stain a delicate blue in hyalines, not very dark in mature forms.
Segmenters	R. B. C. not visible. Margins crenated. Divide into "Mulberry" form, 15-20 segments, each with a central refractile dot. Not commonly seen.	Parasites very waxy. Divide into "Daisy" form, 6-12 segments Refractile dots on periphery of segments. Commonly found.	Very rarely seen. Simulate closely the tertian forms with 8-18 segments. (32 in cultures).	Macrogametes	Intense blue protoplasm. Chrom. scanty, tends to be peripheral, stains brilliant red, compact. Pigment blue-black rods, perpheral or as a "mid-central wreath."	In general, similar to tertian. These forms show more intense staining reactions, they are apt to be smaller and show, as in the fresh, coarser, blacker pigment.	Long, slender. Chromatin compact, central. Protoplasm deep blue with polar intensification. Pigment near center, in masses or chromatin surrounding wreath, coarse, black.
Macrogametes	Adults, 9-12 mu. Round. Protoplasm gran. Pigment coarse, rods or wreaths, diffuse, inactive, dark. Very refractile.	In general, similar to tertian. Both forms smaller than tertian; whereas the pigment is somewhat coarser. They are more rarely seen.	Crescentic forms 11-15 mu. long. Protoplasm opague and gran. Pigment coarse, rod-like, black, central. Change to ovoids or round forms prior to fertilization.	Microgametocytes	Proto. faint blue. Chromatin abundant, looser, intense red, central, has an achromatic zone. Pigment greenish-blue, fine, diffuse. Chrom. in 4-8 peripheral masses prior to flaggellation.		Kidney shaped. Proto. stains less intensely; chromatin not so brilliant, loose network, often scattered throughout. Pigment finer, diffuso, greenish brown.
Microgametocytes	Adults, 8-10 mu. Less granular. Round or oval. Pigment more abundant, active, diffuse. Tends to become central prior to flaggellation.		Crescents, 7-10 mu.; more kidney shaped, less granular. Pigment finer, diffuse, or in polar clumps, sluggishly motile. Become ovoid or round prior to flaggellation.	Remarks	Schuffner's granules suggest tropical infection. Parthenogenesis commonest in this type.	Band forms very common on 2nd day. Not so often seen in other 2 to 20 meters by	Male and female ovoids and round forms show the same essential differences.

LAMBLIA INTESTINALIS.

Other names—Megastoma entericum, Hexamitus duodenalis, Dimorphus muris, Megastoma intestinale.

Size—15 to 16.5 microns by 10 to 12 microns.

Pathology—Non-pathogenic. Large numbers often occur in the feces.

Balantidium coli—(Paramoecium coli).

Size—70 to 100 microns by 50 to 70 microns.

Pathology—Often found in connection with various types of diarrheal affection but may be found in persons without intestinal symptoms. It is capable of setting up a severe ulcerative colitis not unlike amoebic dysentery (Strong and Bowman) and severe anaemia (Barker).

FASCIOLA HEPATICA.

Other names—Distomum hepaticum, Distomum cavi, Fasciola humana, Cladocaelium hepaticum, common liver fluke.

Size—20 to 50 by 8 to 13 millimeters.

Size of egg—130 to 145 microns by 70 to 90 microns.

Pathology—Usual habitat is the gall ducts but is frequently found in the gall bladder, intestines, portal system, and subcutaneous cysts. Infection is rare in man (32 cases recorded).

Mode of infection—The intermediate host is the snail of the genus Limnaea. Infection occurs in swallowing the encysted cercaria deposited on plants in marshes.

Method of diagnosis—Finding ova in the feces.

SCHISTOSOMUM HEMATOBIUM.

(Infection called Distomiasis and Bilharziasis)

Other names—Bilharzia hematobia, gynecophorus, Distomum hematobium, Distoma capense, Thecosoma.

Size of male—12 to 15 mm. by 1 mm.

Size of female—20 mm. by 0.25 mm.

Size of eggs—0.16 mm. by 0.05 mm.

Pathology—The parasites inhabit the blood of the portal vein, and the vessels of the pelvis, rectum and bladder.

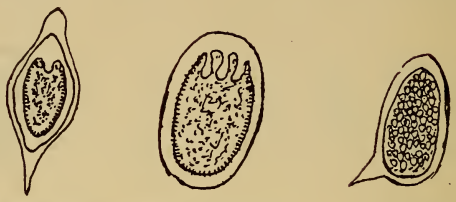
Mode of infection—Through drinking water and occasionally through the skin with a cycle much like that of the hook-worm. Egg cysts form in the mu-

Table of Tape-worms

Name	T. soleum	T. Saginata	T. echinococcus	Dipylidium caninum	Hymenolepis nana	Hymenolepis diminuta	Dibothriocephalus latus.
Other name	Pork tape-worm. Armed tape-worm. Pork measley t-w.	Beef tape-worm. Unarmed tape-w. Fat tape-worm.	Hydaid tape-worm	T. cucumerina T. canina T. moniliformis T. elliptica Dipylidium caninum Dipylidium cucumerinum	T. aegyptica. T. nana. Hymenolepsis murina. Diplacanthus nana.	Hymenolepis diminuta. Hymenolepis flavopunctata. T. leptocephala. T. flavopunctata. T. minima. T. varerina.	Bothriocephalus latus. T. lata. Dibothrium latum. Bothriocephalus latissimus. Fish tape-worm.
Host of larval stage	Hog and man.	Cattle.	Sheep, hogs, cattle (20 mammals) Reported in man.	Dog, flea, human flea, dog louse	Rat, mouse, man.	Moths, beetles, etc.	Fresh water fish (pike, perch, salmon family).
Host of adult stage	Man.	Man	Dog, wolf, hyena, jackal, cat.	Dog, cat, man (25 cases)	Rat, mouse, man.	Rat, mouse, man (12 cases).	Dog, cat, man.
Color	Grayish white.	Grayish white.	Grayish white.	Often reddish brown	Grayish white.	Grayish white.	Grayish yellow to brown.
Length	2 to 10 meters.	4 to 10 meters.	2.5 to 5 millimeters.	15 to 35 cm. by 1.5 to 3 mm.	5 to 45 mm. by 0.5 to 0.9 mm.	10 to 60 mm. by 3.5 mm.	2 o 20 meters by 20 mm.
Segment picture							

Segment number	800 to 900.	1000 plus.	3 to 4.	80 to 150	100 to 200.	60 to 1300.	3000 to 4200.
Segment size	10-12x5-6 mm.	16-25x4-7 mm.	Terminal, 2 mm. long.	8-11x1.5-3 mm.	0.4-0.9 x 0.014-0.03 mm.		2-4 x 10-20 mm.
Segment shape	Oblong, longer than broad.	Oblong, longer than broad.	Oblong, longer than broad.	Swollen in center, looks like chain of beads	Broader than long.	Broader than long.	Anterior 2/3 broader than long. Post. 1/3 quadrate or broader than long.
Head picture							
Head: No. hooklets	Double row 22 to 32.	none.	28 to 50.	3 to 4 crowns 48 to 60 in all	Single row. 24 to 30.	none.	none.
Head: No. suckers	4	4	4	4	4	4	2 grooves.
Eggs: Picture							
Eggs: Size	31 to 36 microns.	20 to 30 microns.	30 to 36 x 25-30 microns.	43 to 50 microns	30 to 38 x 42 to 56 microns.	28 to 36 microns.	68 to 71 x 44 to 45 microns.

cosa of the bowel and bladder, causing hemorrhages from either.



S. hematobium S. joponicnm S. manisoni

PARAGONIMUS WESTERMANII.

This organism is a very frequent cause of hemoptysis in Japan. The eggs can be found in the unstained sputum which are yellow, operculated, and 77 to 102 by 40 to 75 microns in size.

FILARIA BANCROFTI.

Size—270 to 340 microns long by 7 to 11 microns broad.

Pathology—These parasites and their ova cause obstruction of the lymphatics. They reach the general circulation by way of the thoracic duct. Chyluria is a very frequent occurrence due to the rupture of the varicose lymph vessels of the bladder. Elephantiasis develops in the extremities due to lymph obstruction.

Method of diagnosis—There is usually pain and fever with lymph tumor, elephantiasis, hematochyluria, enlarged spleen. Examine the blood during the night for the parasites, for they appear in the circulation only at this time.

STRONGYLOIDES STERCORALIS.

Other names—Anguillula intestinalis et stercor-

alis, Leptodera, intestinalis et stercoralis, Pseudorhabditis stercoralis, Rhadonema strongyloides, Rhadonema intestinalis, Strongyloides intestinalis.

Size of egg—70x45 microns.

Forms:

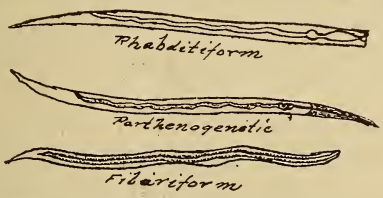
1. Parasitic adults. Parthogenetic females which live in human intestines. 2.2 to 3 mm in size. Eggs indistinguishable from those of hookworm, but only the embryos are passed in the feces. Eggs develop into

2. Rhaditiform embryos, 200 to 400 microns in length, and in a few days these develop into

3. Free living adult males and females. Male .7 mm. and females 1 mm. in size. The females produce from 30 to 40 eggs, which develop into

4. Free living rhabitiform embryos, 220 microns long. When they attain the size of 550 microns they moult and change into

5. Filariform embryos having elongated cylindrical esophagus. This is the infecting stage for man which occurs in much the same way as with the hook-worm, *i e*, through the skin or the mouth direct. In temperate zones cycles 3 and 4 may not occur.



Method of diagnosis:

1. Finding the rhabitiform embryo (2) in the fresh feces.

2. Occasionally the eggs, strung together end to end and surrounded by a delicate tube are also found in the feces.

Pathology—They live in the upper intestinal tract. Large numbers cause anaemia and diarrhea.

TRICHINELLA SPIRALIS.

Size of male—1.4 to 1.6 mm x 40 microns.

Size of female—3 to 4 mm x 60 microns.

Pathology—A disease especially of slaughter-house rats, but also of dogs, cats, hogs and man, an accidental host. There are three stages of the infection. In the first the cysts present in the contaminated digested meat are digested and males and females develop. The females burrough into the mucosa and proliferate rapidly. In the second stage they enter the lymphatics and gain access to the circulating blood. In the third stage they stop for the most part in striated muscle in which they become encysted. A myositis develops.

Symptoms of infection—

1. Intestinal disturbance. In animals this is very severe. There is profound intoxication, a drop in the w.b.c. count with an associated drop in eosinophiles.

2. Tremendous inflammation of the muscles associated with fever, enlargement of the spleen, and an increase in the polymorphonuclear neutrophile and eosinophile count. Sometimes there occur as many as 80 per cent. eosinophiles. There is pain on muscular effort. The muscles of the eye ball, the calves and the diaphragm are all tender. After the first acute symptoms the patient doesn't suffer, but he is doomed to harbor the parasites the rest of his life.

Diagnosis—Never made by finding the parasite in the stools. They can be found in the stomach and

intestines. In the dog the parasites can be found in the blood within 5 days after the infection, and this is the most satisfactory method in man in the early stages of the disease. A syringe full of blood is removed from a vein and is laked by the addition of 2-3 per cent of acetic acid. The laked blood is centrifugalized and the supernatent fluid is decanted. The sediment is then washed if necessary and again centrifugalized. Preparations are made from the sediment and are examined under low power. In the later stages of the disease the diagnosis is made by excising a piece of muscle, which is then embedded, sectioned and stained.

Mode of infection—Eating improperly cooked pork contaminated with the parasite.

Trichiuris trichiura.

Other names—Ascaris trichiura, Trichocephalus trichiurus, Trichocephalus hominis, Trichocephalus dispar, Trichocephalus mastigodes, whip-worm.

Size of male—40 to 45 microns.

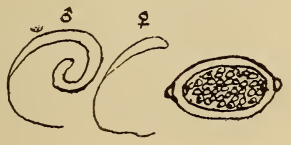
Size of female—45 to 50 microns.

Size of eggs—54 by 33 microns.

Pathology—They live in the colon especially in the caecum and rarely in the appendix. They are an infrequent cause of anaemia and diarrhea. Enormous numbers may be present without symptoms.

Mode of infection—Eating food contaminated with the embryos. The eggs passed in the feces require considerable time for development. They resist freezing for many months.

Method of diagnosis—Examine the feces for the characteristic eggs.



ANCYLOSTOMA DUODENALE.

Other names—Old World hook-worm, *Uncinaria duodenalis, Strongylus quadridentatus, Dochmius ancylostomum, Sclerostoma duodenale, Strongylus duodenalis, Dochmius duodenalis*, European hook-worm.

Size of male—8 to 10 mm long.

Size of female—12 to 18 mm long.

Characteristics of head—Two pairs of strong curved ventral teeth. One pair of knob-like dorsal teeth. One pair of ventral lancets.

Size of eggs—52 to 61 by 32 to 38 microns.

Pathology—They inhabit the duodenum, jejunum and upper ilium and cause severe anaemia through loss of blood and toxin formation. Ground itch is the local lesion of the larva.

Mode of infection—

(a) Ingestion of unclean vegetables or contaminated water.

(b) Through the skin. The larva bores through the skin of the feet and enters the lymph and blood stream. They reach the lungs and bore their way into the bronchi whence they are raised with the bronchial secretion and swallowed.

NECATOR AMERICANUS.

Other names—New World hook-worm, *Unicaria americana, American* hook-worm.

Size of male—6 to 9 mm long.

Size of female—8 to 15 mm long.

Characteristics of head—A dorsal and a ventral pair of lips at mouth. Prominent dorso-median buccal teeth. Four buccal lancets.

Size of eggs—64 to 72 by 36 to 40 microns.

Mode of infection and pathology—Same as for the Old World form.

Method of diagnosis—

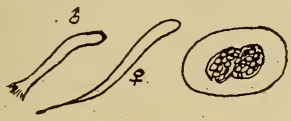
Look for larva in the feces:

1. By direct examination of the feces (40 per cent. yield).

2. By adding water to the stool and passing it through a series of sieves and then allowing it to settle and examining the sediment.

3. Increase specific gravity of the stool and centrifugalize. They are relatively light and come to the top where they can be secured. (55 per cent. yield.)

4. Cultural method. Put stool in petrie dish and enough water to keep moist. Make a well in the center filled with water. Examine the water in the well from day to day. (99 per cent. yield.)



Parasites having eggs resembling hook-worm eggs:

1. *Strongyloides stercoralis.* Eggs infrequently passed in the feces.

2. *Trichostrongylus instabilis* (See Jour. A. M. A. December 23, 1916, Pg. 1908).

The eggs of this worm are larger and have a tendency to point at one end and be flattened at the other. There are from 4 to 32 segments within the egg.

3. *Haemonchus contortus.*

4. *Strongylus contortus.*

ASCARIS LUMBRICOIDES.

Size of male: 15 to 25 cm.
Size of female: 20 to 40 cm.
Characteristics of head: It has 3 conical lips.
Color: Grayish to reddish yellow.
Size of eggs: 50 to 75 microns by 36 to 55 microns.

Fertilized Unfertilized

Pathology: They inhabit the small intestine, usually one or two being present. Occasionally large numbers are found. While remaining in the intestine they rarely cause trouble, but when they wander up the common bile duct they often set up a pancreatitis and a hepatitis with liver abscesses. During anaesthesia they may be regurgitated, as well as at other times, and find their way into the air passages. Anaemia and intestinal obstruction are also caused at times by this infection.

Symptoms of infection: There are many. Itching at the anus, irritability, picking at the nose, restlessness, convulsions in children. A few worms may cause no symptoms.

Mode of infection: The eggs are expelled in the feces and develop into embryos. These, when swallowed with contaminated drinking water or fruits, cause the disease. Eggs discharged in the feces are not immediately infective, but are only so after the development of the embryo.

Method of diagnosis: Look for the eggs and worms in the stools. There is usually an associated eosinophilia.

Toxicora canis.

Other names: *Ascaris canis, Ascaris lumbricus canis, Ascaris teres, Ascaris caniculae, Ascaris cati, Ascaris tricuspidata, Ascaris felis, Ascaris werneri, Ascaris marginata, Ascaris alata, Fusaria mystax.*

Size of male: 40 to 60 mm. long by 1 mm. thick.

Size of female: 120 to 180 mm. long by 1 mm. thick.

Size of egg: 68 to 72 microns.

Pathology: This worm is not uncommon and may be found accidentally in man.

OXYURIS VERMICULARIS.

Other names: *Ascaris vermicularis, Fusaria vermicularis, Ascaris graecorum,* pin-worm, thread-worm, seat-worm.

Size of male: 3 to 5 mm. by 0.3 to 0.4 mm.

Size of female: 10 mm. by 0.6 mm.

Characteristics of head: Male has 6 pairs of papillae; female has 3 small nodular lips.

Size of eggs: 50 by 16 to 20 microns.

Pathology: The early stages live in the small intestine, where they copulate. The males soon die and the females wander to the large bowel as far as the anus. They migrate from here and deposit their eggs in the skin about the anus and cause intense itching. The eggs lodge under the finger nails upon scratching, and often find their way into the mouth, causing auto-infection. The females may wander up the vagina, uterus, tubes or into the urethra and bladder.

Method of diagnosis: Not readily made from the examination of the stools, but from the symptoms and the examination of the scrapings of the skin in the anal region, where the eggs are likely to be found.

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