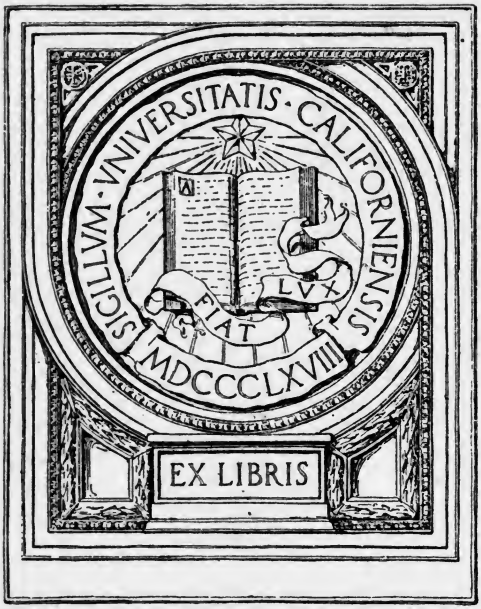
Clinical Laboratory Tech for Nurses

Gibson


SIGILLVM · VNIVERSITATIS · CALIFORNIENSIS
LVX
FIAT
MDCCCLXVIII
EX LIBRIS

CLINICAL LABORATORY TECHNIC FOR NURSES

BY

ANNA L. GIBSON, R.N.

Matron of the Boston City Hospital Relief Station, East Boston, Mass., Instructor of Laboratory Technic to Graduate Nurses, and Assistant Matron Superintendent of the Huntington Hospital, Harvard Medical School, Boston, Mass.

WHITCOMB & BARROWS

BOSTON, 1916

Thomas Todd Co., Printers
14 Beacon Street, Boston, Massachusetts

TO

EMMA M. NICHOLS, R.N.

Superintendent of Nurses, The Boston City Hospital, Boston, Mass.

IN APPRECIATION OF THE
INSPIRATION, KNOWLEDGE, AND TRAINING
WHICH I RECEIVED
FROM ASSOCIATION WITH HER

PREFACE

THIS book owes its existence to the frequent request on the part of graduate nurses, whom it has been my pleasure to instruct in laboratory technic during the past three years, for a simple, comprehensive text-book, that the nurses might be able to grasp the principles of clinical laboratory technic.

This book was originally compiled as a handbook for practical clinical laboratory work, since no single text-book covered the work.

The arrangement of the several chapters has worked itself out from a series of lessons which give simple and reliable methods. By these methods information may be obtained without unnecessary detail which requires a considerable knowledge of general chemistry and elaborate apparatus.

Standard works on Bacteriology, Chemistry, Hematology, Histology, and Parasitology have been consulted freely, and references are given at the end of each chapter from which more detailed information may be obtained.

A great deal of the material and the drawings have been taken from my notebook.

Grateful acknowledgment is made to Dr. Thomas Ordway, with whom I have had the privilege of working the past three years at the Huntington Hospital; to Dr. Ellis Kellert, Director of the Bender Laboratory, Albany, New York, who gave me valuable assistance the

two years we were associated at the Huntington Hospital; and to Dr. Ernest Tyzzer, Assistant Professor of Pathology, Harvard Medical School, and Director of Huntington Hospital, Harvard Medical School.

ANNA L. GIBSON, R. N.

BOSTON, MASSACHUSETTS.
September, 1915.

CONTENTS

CHAPTER I

LABORATORY EQUIPMENT

Glassware

It is very important that all glassware used in the laboratory should be made of Bohemian or Jena glass. Many test tubes are made of the ordinary glass, which is silicate of calcium and sodium, and sometimes lead oxide is used instead of calcium carbonate.

This glass is easily acted upon by chemical substances, and should not be subjected to heat.

Tubes, flasks, and beakers should be made of potassium carbonate glass—Bohemian glass—as this glass is characterized by its great hardness, difficult fusibility, and by its resistance to the action of chemical substances, sudden changes of temperature, and high steam pressure.

The best glass tubing and rods are made of Jena glass, as it is chemically superior to the Bohemian glass, more resistant to acidulous fluids and sudden changes of temperature.

Care of Glassware

All glassware used in the laboratory work must be thoroughly clean before using. New glassware should be placed in 0.5% solution of nitric acid to remove the alkali frequently present; thoroughly rinse in running water.

Glass slides are cleaned by immersing in cleaning solution, then washing in water; dry with a towel and flare

both sides over a Bunsen flame. Oil which has dried on slides can be removed with xylol.

Old test tubes containing culture media should be re-sterilized for one hour, or boiled for one hour in a 5% solution of soda; this destroys the bacteria and loosens the material in the tubes.

Test tubes and flasks are dried in the autoclave, then plugged with non-absorbent cotton and sterilized one-half hour at 15 pounds pressure.

A good cleaning fluid is made as follows:

Potassium bichromate	60 c.c.
Concentrated sulphuric acid	300 c.c.
Water	400 c.c.

Dissolve the potassium bichromate in water with heat. Cool, then add slowly the sulphuric acid.

Glass Droppers and Capillary Pipettes

Take a piece of tubing and heat it in the middle of a Bunsen flame, revolving the tubing while heating; and when it becomes soft in the center, remove from the flame and with a steady pull separate the ends. Cool, file, and break off. Flare the rough ends in the flame.

Glass Stirring Rods

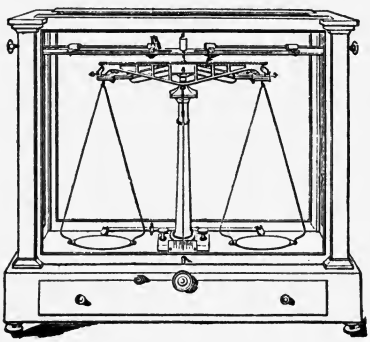
Take a piece of glass rod, file off the desired length, then round off the rough ends in the flame by constant rotation.

Weights and Measures

The Analytical Balance. The poise in the ordinary balance is not disturbed by slight variations of weight, but in chemical analysis a more sensitive instrument is

necessary. The beam is made as light as possible, the bearings sharp and hard, the adjustments capable of being brought to the last degree of refinement and provided with appliances for arresting its action at will. It is inclosed in a glass case for protection against dust, moisture, and currents of air.

The beam is divided by notches into tenths, and carries weights shaped as riders, and these riders lessen in value as they are moved towards the center.



Analytical Balance

A rider weighing .01 gram in the pan weighs .09 gram at first notch from the pan, .08 gram at the second.

Large brass weights equal grams; large platinum weights, 0.5 gram; small weights, .05 gram; and the rider, .01 gram.

The gram is the unit of weight and equals the weight of 1 c.c. of distilled water at 4° C.

1 kilogram = 1,000 grams = 100,000 centigrams = 1,000,000 milligrams.

1 kilogram = 2.20462 pounds = 35.2739 ounces = 15,432.35 grains.

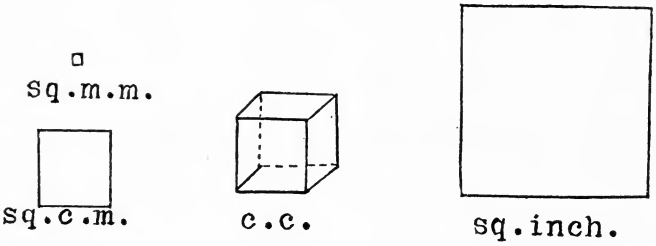
Always lift the weights with the forceps provided for that purpose.

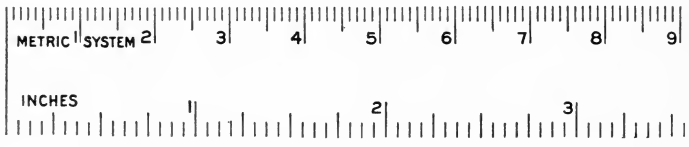
A watch glass is used as a receptacle for reagents weighed. First ascertain the weight of the glass and add the amount to the required amount of the reagent.

A meter equals 39.37 inches.

A cubic meter is the unit of space for the number of organisms in air. It contains 1,000 liters. It is equal to 1.308 cubic yards or 35.316 cubic feet. 1,000 cubic feet, the unit of space in disinfection, is equal to 28.3 plus cubic meters.

A cubic centimeter is the unit of space for organisms





Comparison of Inches and Centimeters

in water, milk, vaccines, etc. There are approximately 16 drops in 1 c.c.

Cubic millimeter is the unit of space for blood cells. There are 1,000 cubic millimeters in 1 cubic centimeter, and 1,000,000 cubic millimeters in 1 liter.

A liter is the unit of space for volumetric solutions. It contains 1,000 cubic centimeters, and is equal to 1.0567 quarts or 33.8 ounces. A liter of distilled water equals 1 kilogram.

The arc is the unit of surface and is the square of ten meters.

Decimal Table

	Length	*Weight*	*Capacity*
1,000	kilometer	kilogram	kiloliter
100	hectometer	hectogram	hectoliter
10	decameter	decagram	decaliter
1	Meter	Gram	Liter
0.1	decimeter	decigram	deciliter
0.01	centimeter	centigram	centiliter
0.001	millimeter	milligram	milliliter or cubic centimeter

Reagents

The reagent bottles should be made of Jena glass, which is free from lead and other impurities, and these bottles should be fitted with ground glass stoppers. All reagents should be chemically pure (C. P.).

Liquid Reagents

Nitric acid, C. P. (HNO_3); acetic acid, ($HC_2H_3O_2$); sulphuric acid, C. P. (H_2SO_4); hydrochloric acid, C. P. (HCl); ammonic hydrate, (NH_4OH); sodic hydrate, (NaOH), U. S. P.

Solid Reagents

Cupric sulphate, caustic soda, sodium chloride, potassium iodide, potassium chromate, ammonium sulphate, magnesium sulphate, ammonium chloride, sodium acetate, potassium ferrocyanide, potassium acetate, guaiac, benzidin, potassium chlorate, picric acid, citric acid, lead acetate, sulphanilic acid, sodium nitrite, sodium carbonate, mercuric chloride, potassium bromide, sodium nitroprusside, alphol naphthol, phenylhydrazin hydrochlorate,

di-methyl-amino-azo-benzine, di-methyl-paraphenylene-diamine, Eosin Gruber, w. g.

The different alcohols used in making up the various reagents are: absolute alcohol, which contains not less than 99% by weight of pure ethyl alcohol, C_2H_5OH; alcohol of a stated percentage, *e. g.*, 50%, means a mixture with water which contains the stated percentage, *e. g.*, 50%, by volume of pure ethyl alcohol; methyl alcohol, a pure substance, CH_3OH, prepared by the purification of commercial wood spirit; and methylated alcohol or methylated spirit, which may be used instead of pure ethyl alcohol in preparing solutions of various percentages of alcohol. Methyl alcohol is a mixture of 19 parts of ethyl alcohol and 1 part commercial methyl alcohol. Commercial methyl alcohol is impure and must not be used in making Eosin-Azur, Louis Jenner's, Leishman's, Wright's, or Romanowsky stains.

The amounts of distilled water and absolute alcohol required to produce saturated solutions of dyes in common use are indicated in the following table:

	Dye	*Water*	*Alcohol (c.c.)*
Bismarck Brown	1	7	7
Fuchsin (Basic)	1	10	2.5
Gentian Violet	1	7	7
Hematoxylin	1	2	1
Methyl Violet	1	5	1
Methylene Blue	1	7	7
Thionin Blue	1	5	10

Table of Equivalents

Liquids		*Approximate*		*Accurate*
1 minim	=	0.06 c.c.		0.061 c.c.
16 "	=	1. c.c.		
1 fl. dram	=	4. c.c.		3.697 c.c.
1 fl. ounce	=	30. c.c.		29.574 c.c.
1 pint	=	500. c.c.		473.197 c.c.
1 gallon	=	4,000. c.c.		3,785. c.c.
Solids				
1 kilogram	=	2.2 lbs. av.		
1 gram	=	15. grains		15.432 grains
1 milligram	=	$\frac{1}{65}$ grain		0.0154 grain

Solutions

Approximately correct solutions are made by using the following method:

For 1–1,000 ($\frac{1}{10}$%) use 15 grains to a liter.

For 1–100 (1%) use 5 grains to the ounce.

EXAMPLES. 1. Make 2,500 c.c. of a 1–500 solution of potassium permanganate. 15 grains, or 1 c.c., to 500 c.c. is a 1–500 solution. If 15 grains, or 1 c.c., is used to every 500 c.c., for 2,500 c.c. we would use as many c.c. as there are 500 in 2,500, or 5 c.c. (75 grains).

2. Make 500 c.c. of 1–10,000 solution caustic potash. If it takes 15 grains to 1,000 c.c. to make a 1–1,000 solution, to make a 1–10,000 solution, which is 10 times weaker, take $\frac{1}{10}$ of 15 grains, or 1.5 grains, for 1,000 c.c. of a 1–10,000 solution, and for 500 c.c. of a 1–10,000 solution take $\frac{1}{20}$ of 15 grains, or 0.75 grain.

3. Make 75 c.c. of a $\frac{1}{3}$% solution of acetic acid. $\frac{1}{3}$% equals 1–300. 75 c.c. ÷ 300 c.c. = .25 c.c. If 1 c.c. is used to every 300 c.c., to 75 c.c. we would use $\frac{1}{4}$ as much; therefore with a pipette measure 0.25 c.c.

If there is any difficulty in removing the glass stoppers

from the reagent bottles, they are easily loosened by gently tapping the neck of the bottle with another piece of *glass*.

Common Elements. (J. Am. Chem. Soc., 1908)

	Symbols	*Atomic Weights*
Aluminum	Al	27.1
Antimony	Sb	120.2
Arsenic	As	75.
Barium	Ba	137.4
Bismuth	Bi	208.
Boron	B	11
Bromine	Br	79.96
Cadmium	Cd	112.4
Calcium	Ca	40
Carbon	C	12.
Chlorine	Cl	35.18
Chromium	Cr	52.1
Cobalt	Co	59.
Copper	Cu	63.6
Fluorine	F	19.
Gold	Au	197.2
Hydrogen	H	1.008
Iodine	I	126.97
Iron	Fe	56.
Lead	Pb	206.9
Magnesium	Mg	24.36
Manganese	Mn	55.
Mercury	Hg	200.
Nickel	Ni	58.7
Nitrogen	N	14.01
Oxygen	O	16.
Phosphorus	P	31
Platinum	Pt	194.8
Potassium	K	39.15
Silicon	Si	28.4
Silver	Ag	107.93
Sodium	Na	23.05
Sulphur	S	32.06
Tin	Sn	119.
Zinc	Zn	64.9

The plus sign is used to express the admixture of elements.

EXAMPLE. $H_2 + O$ means that 2 units of hydrogen are mixed with 1 of oxygen.

$2H_2O$ signifies that two parts of the compound formed when hydrogen 2 units and oxygen 1 unit united by chemical attraction.

Solutions

A standard solution can be made of any strength, but a certain strength is the convenient standard for ordinary work; this is the normal solution.

Normal Solutions

A normal volumetric solution is made by dissolving the hydrogen equivalent or atomic weight divided by its valence in distilled water and making the volume up to one liter.

The molecular weight in grams of a base, salt or acid, is divided by the valence, and the valence of a base is the number of hydroxyls combined with it; the valence of an acid is the number of replaceable hydrogen atoms which it contains.

If the molecule of the element is univalent, one liter will contain the weight in grams equal to the molecular weight of the element; if bivalent, a weight in grams equal to one-half its molecular weight; if trivalent, a weight equal to one-third its molecular weight.

EXAMPLE. HCl is univalent (having a valence of 1). H = 1.008. Cl = 35.18. The sum of these atomic weights equals 36.188. Therefore dissolve 36.188 grams of absolute HCl in sufficient distilled water to make one liter.

NaOH is univalent. Na = 23, O = 16, H = 1.008. The sum of these atomic weights equals 40.008. Dissolve 40.008 grams of NaOH in distilled water and make up to one liter.

H_2SO_4 is bivalent. H_2 = 2.016, S = 31.83, O_4 = 63.52. The sum of these atomic weights equals 97.366. As it has two replaceable H atoms, it would contain in one liter of water one-half this number, or 48.683 grams of absolute H_2SO_4.

Solutions of this strength are designated by the capital letter N; of twice this strength, by 2N; one-half or one-tenth, by N/2 or N/10. Equal volumes of normal solutions react together completely. One liter of normal NaOH or KOH will neutralize one liter of normal H_2SO_4 or HNO_3.

Standard Solutions

Standard solutions are of accurately known value, and titration is the process whereby a standard solution is brought into reaction.

Indicators are substances which furnish an accurate indication of the point at which the desired reaction is exactly completed, and the indicator should show the end point of the titration.

To obtain a standard solution, use a solution of sodium hydroxide of known strength. Units of this solution will be equivalent to definite amounts of acid in the solution neutralized.

Oxalic acid, being a crystalline solid of constant composition, is used as the acid reagent, and an N/10 solution is made which if accurately measured should give N/10 solution in which the error is less than 1%.

METHOD. Clean volumetric flasks and beakers with cleaning solution; rinse thoroughly in running water, twice with distilled water, and then with a small portion of the solution they are to contain.

Pick out pure crystals of oxalic acid, place in a watch glass, and weigh with analytical balances, the weight of the glass having been previously ascertained.

The molecular weight of oxalic acid is 126.048. As it is diabasic, divide by two. 63.024 grams are necessary to make one liter of N solution. To make N/10, take 6.3024 grams.

Pour the crystals into a dry beaker, rinsing off the watch glass with distilled water; stir with a glass rod until dissolved.

Place the glass rod in the neck of a liter volumetric flask and carefully pour the oxalic solution from the beaker. Rinse the beaker several times with distilled water, then wash off the rod and fill the flask from a wash bottle of distilled water up to the mark; the last few drops can be blown from the wash bottle.

As the inside neck of the flask is wet from the addition of water, a filter paper which has been rolled up is inserted and rotated until the neck is dry, being careful not to touch the fluid.

Stopper and thoroughly mix.

Having N/10 oxalic acid, N/10 sodium hydroxide is prepared as follows: Sodium hydroxide is very hygroscopic, therefore a normal solution cannot be accurately prepared by weight. Weigh out 5 grams and dissolve in 1,100 c.c. of water. Titrate with the oxalic solution.

Titration

A definite amount of oxalic acid solution is measured with a graduated pipette and placed in a flask. Add 50 c.c. of distilled water, then 3 drops of alizarin or phenolphthalein, as an indicator that the point of neutralization or end of the reaction can be accurately determined. Phenolphthalein is used when neither ammonia nor bicarbonates are titrated. In titrating acid and alkali, always run the alkali into the acid.

EXAMPLE. Pipette 10 c.c. of the oxalic acid solution into an Erlenmeyer flask; do not blow out the amount left in the end of the pipette. Pour 50 c.c. of distilled water into the flask and add 3 drops of phenolphthalein. Rinse out a burette with distilled water and with the sodium hydroxide solution, then fill with the solution until it rises above the zero mark. A few c.c. are run out until the mark is reached. Place the flask of oxalic solution on a filter paper for a white background and run in the sodium hydroxide solution, a few c.c. at a time, shaking the flask gently. Add the sodium solution until there is a distinct pinkish color, which is the end of the reaction.

If 9.8 c.c. of the sodium hydroxide solution were required to produce a distinct pink color, it is stronger than the N/10 oxalic acid solution, as only 10 c.c. would have been necessary if the sodium solution had been N/10. Therefore 9.8 c.c. of the sodium solution are equivalent to 10 c.c. of the N/10 oxalic solution. The sodium hydroxide must be diluted in the proportion of 9.8 to 10. Measure exactly 1,000 c.c. of the too concentrated sodium hydroxide solution and add 20 c.c. of distilled water.

Equivalent Fahrenheit and Centigrade Tables

AUTOCLAVE TEMPERATURE

Sterile dressings, media, disinfection of spore-bearing bacterial contamination

C.	F.
125	258
120	250
115	240
110	230
105	220
100	212

FREEZING TEMPERATURE

Preserving biological products; post-mortem material

C.	F.
5	
	40
4	
3	
2	
1	
0	32

BODY TEMPERATURE

Growth of important pathogenic organisms

C.	F.
41	
40	
	104
39	
38	
37	98.2

ROOM TEMPERATURE

Culturing gelatin (melting point, 25° C.) as in water work

C.	F.
14	75
23	
22	
21	69.8
20	68

PARAFFIN AND PASTEURIZING

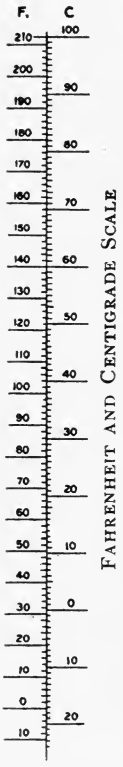
Bacterial vaccines and paraffin bath

C.	F.
80	176
75	
70	158
65	
60	140

Fahrenheit scale is divided into equal divisions or degrees, the lowest of which is a mixture of equal parts of sal-ammoniac and snow, and the highest, the boiling

point of pure water; the freezing point of water on this scale is 32°.

Centigrade scale is divided into 100 equal parts, or degrees, the space of expansion from the freezing point to the boiling point of pure water. The number of degrees between the boiling point and freezing point in Centigrade is 100, and in Fahrenheit it is 212—32 or 180. EXAMPLE. 100:180::degree to be converted:X. By division with 20, 5:9:: degree to be converted:X; *i. e.*, the degree to be converted is multiplied with 9, the result divided with 5, and 32 added to the result.



FAHRENHEIT AND CENTIGRADE SCALE

$$\frac{\text{Centigrade} \times 9 \text{ plus } 32}{5} = \text{Fahrenheit.}$$

$$\frac{\text{Fahrenheit} - 32 \times 5}{9} = \text{Centigrade.}$$

Laboratory Rules

1. All possible cleanliness should be observed in the care of apparatus.

2. The hands should be washed with a disinfectant after working with pathogenic bacteria, and then with soap and water.

3. Pencils and labels should never be moistened with the lips.

4. Discarded cultures should be covered with a disinfectant, then resterilized.

5. Culture media should be put in receptacles provided for that purpose, and not in the sink.

6. Pipettes which have been used to handle infectious material should be placed in a glass receptacle containing cleaning fluid.

7. Any infectious material dropped on the table or floor should be immediately wiped with a disinfectant.

8. All bottles should be plainly labeled.

9. All cultures and tissue specimens should be labeled with the patient's name, source, and date.

10. Sterilize the platinum loop before and after use.

11. When using the autoclave, see that there is sufficient water before turning on the pressure.

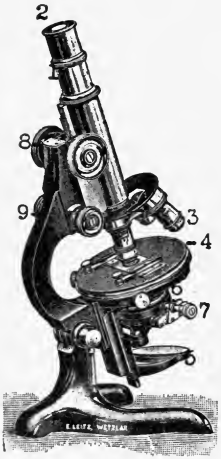
12. Whenever material is placed in the centrifuge tubes, see that they balance evenly.

CHAPTER II

THE MICROSCOPE

One of the most important pieces of apparatus used in the laboratory is the microscope.

1. **The microscope** consists of a tube 160 millimeters (6.4 in.) long, having two systems of lenses, which conduct the rays as they pass from the objective to the oculars.



MICROSCOPE

2. The Ocular, or eyepiece, is at the upper end of the tube. There are various oculars and they are numbered from one to ten, the magnifying power of the ocular increasing as the number advances.

3. The Objective is a system of converging lenses at the lower end of the tube, which forms a magnified inverted image of the object.

4. The Stage, with clips to hold a slide in position during examination.

5. The Reflector or small mirror has two sides, a concave and a plane mirror. The reflector directs the rays of light upward through the object in the optical axis of the microscope.

6. The Sub-Stage Condenser is a system of lenses between the stage and the mirror. These lenses collect and condense the rays coming from the reflector so that

they are focused upon the object, thus giving a brilliant illumination.

7. The Iris Diaphragm controls the intensity of the illumination and is just below the sub-stage condenser. The gradations of light are obtained by means of a small lever.

8. The Coarse Adjustment is a rack and pinion mechanism which rapidly raises and lowers the barrel and its attachments.

9. The Fine Adjustment is just below the coarse adjustment. This very gradually raises and lowers the barrel in order to obtain exact focus.

Illumination

Direct sunlight should be avoided. North light is to be preferred, as it is the most uniform and steady. The character and color of artificial light is much improved by inserting a piece of blue glass between the concave reflector and the object.

Focusing

Focus the body tube down until the objective touches the cover glass, then with the eye to the eyepiece focus up carefully.

Often one acquires the habit of using only the right or left eye for microscopic work, but it is better to learn to use both. Both eyes should be open when using the microscope.

Always examine a specimen first with a low power and then with a high power objective.

When the oil-immersion objective is used, a drop of cedar oil is placed between the slide and the end of the

objective, and the objective is brought into contact with this and the observation made through the oil. The oil-immersion objective is so constructed that when in use the pencil of light passing through the object to the objective traverses only media of the same refractive index, and cedar oil has the same refractive index as glass.

The oil acts as a third lens and increases the magnifying power of the microscope.

A fine hair cemented into one of the oculars serves as a pointer and is of great aid in singling out some special object of interest.

Objects are often accidentally present in microscopical preparations, such as air bubbles. If small, they may look entirely dark; if large, they are clear in the middle, with a broad, dark border, due to refraction of the light.

Linen fibers, well-defined, rounded, slightly twisted.

Cotton fibers, longer, broader, but thinner and more twisted than linen.

Woolen fibers and hairs have the same structure, although the wool is finer and is curled.

Dust of the room, showing groups of black particles of carbon (soot), and shed epithelium-cells derived from the epidermis.

Molds, with long, branching filaments (hyphæ), and the torula-like particles (spores) from which hyphæ may in some instances be seen sprouting.

Yeast particles or torulæ: each torula contains a clear vacuole and has a well-defined outline, due to a membrane.

Starch granules: fine concentric lines are seen in the granules, arranged around a minute spot which is placed near the smaller end of the granule.

Lens paper or fine linen moistened with xylol should

be used to wipe the objectives, and this must be applied carefully, as the lenses are mounted in balsam.

The microscope should be lifted by some one of its solid parts, and never by any part above the stage.

The glass surfaces should never be touched with the fingers, and the metal parts should be kept free from liquids, especially acids and alkalies.

CHAPTER III

URINE

The urine is the yellowish liquid solution of organic and inorganic substances excreted and secreted by the kidneys, and is a solution of the waste products of the body.

The condition of the kidneys and their capability for work are determined by accurate chemic and microscopic examination, and such information is of great importance in judging the diagnosis and prognosis of disease.

Anuria is the suppression of the secretion in the kidneys or obstruction in the urinary tract.

Polyuria is the increase in the elimination of urine as a whole, both fluid and solid.

Hydruria is the increase in the total twenty-four hour amount out of proportion to the solids.

Oliguria is the diminution in the total excretion of the urine.

Consistence

Clear and liquid, frequently turbid and viscid (ropy). In normal urine this turbidity is caused by precipitated phosphates or urates; in abnormal urine it results from casts, cells, etc. The slight cloud-nubecula which urine develops on standing is formed by bacteria, desquamated cells, and traces of mucus.

Odor

Aromatic, due to volatile acids. The odor of ammonia is important only in fresh urine. If free ammonia is

present, it can be detected by holding red litmus paper over the mouth of a tube within which is boiled some perfectly fresh urine. If present, the litmus paper will turn blue. Acetone odor indicates diabetes mellitus; putrid odor denotes pus.

Collection for Analysis

A single specimen may be collected for qualitative examination, and as there is a marked variation in the urine at different times a day, a specimen should be taken several hours after a meal, when it contains the greatest amount of abnormal elements.

At a convenient hour in the morning the bladder is emptied and the urine thrown away; then all the urine voided in the following twenty-four hours, including the urine in the bladder the same hour the next day, will represent the total amount for twenty-four hours. Collect this twenty-four hour amount of urine in a two and a half liter bottle, which should be perfectly clean, well corked, and kept in a cool place.[1] Place 5 c.c. of chloroform or formalin in the bottle, as urine begins to decompose within a few hours after it has been voided. Dust, feces, and expectoration interfere with analysis, and care should be taken that these particles do not enter the bottle.

Color

Normal urine varies from straw to amber color, and this color is derived from urochrome and several other pigments.

[1] Chloral and chloroform reduce Fehling's solution and should not be used if the urine is to be tested for glucose.

Formaldehyde coagulates albumin and reduces Fehling's.

Thymol is used for saccharine urine, as it has no reducing action (1 gr. to 1 fl. oz. of urine). Boric acid may be used, 5 grs. to 4 fl. oz. of urine.

Halliburton's Table

Color	*Cause of Color*	*Pathologic Conditions*
Nearly colorless.	Dilution or diminution of normal pigment.	Various nervous conditions, hydruria, diabetes insipidus, granular kidney.
Dark yellow to brown-red.	Increase of normal or occurrence of pathologic pigment.	Acute febrile diseases.
Milky.	Fat globules. Pus corpuscles.	Chyluria. Purulent disease in urinary tract.
Orange.	Excreted drugs, *e. g.*,	Santonin, chrysophanic acid.
Red or reddish.	Unchanged hemoglobin. Pigment in food (logwood, madder, bilberries, fuchsin).	Hemorrhage or hemoglobinuria.
Brown to brown-black.	Hematin. Methemoglobin. Melanin. Hydrochinone and catechol.	Small hemorrhages. Methemoglobinuria. Melanotic sarcoma. Carbolic acid poisoning.
Greenish yellow, brown approaching black.	Bile pigment.	Jaundice.
Dirty green or blue.	A dark blue scum on surface, with a blue deposit, due to an excess of indigo-forming substances.	Cholera, typhus; seen especially when the urine is putrefying.
Brown-yellow to red-brown, becoming blood-red upon adding alkalies.	Substances contained in senna, rhubarb, and chelidonium, which are introduced into the system.	

Urine may darken on exposure to the air, owing to the presence of alkapton, a decomposition product of the proteins found as a crystalline body in the urine.

Reaction

In the majority of cases the mixed twenty-four hour amount is acid to litmus. The reaction may undergo marked changes in both physiological and pathological conditions.

Animal food produces acid urine, and a vegetable diet may give a neutral or alkaline urine.

The reaction varies according to the time of day the urine is voided. It may be neutral for some time after a meal, or alkaline to litmus, as at that time the secretion of hydrochloric acid into the stomach during the process of digestion reduces the store of acids in the body. This change is known as the "alkaline" tide.

Urine may become alkaline owing to the conversion of urea into ammonium carbonate by micro-organisms.

Microscopically such a urine shows amorphous phosphates, ammonium magnesium crystals, and ammonium urate crystals.

If a urine is alkaline immediately after voiding, it may be due to a fixed alkali or ammonia, and ammoniacal fermentation is ordinarily due to cystitis.

Acid fermentation occurs in urine having a normal acidity when voided, which upon standing becomes strongly acid. The sediment of such urine shows acid urates, uric acid, calcium oxalate crystals, and fungi.

Blue litmus paper is changed red by acid urine. Red litmus paper is changed blue by alkaline urine.

When urine changes blue litmus paper red and red

litmus paper blue, it has an amphoteric reaction, due to the simultaneous presence in the urine of acid and alkali salts.

Specific Gravity

The specific gravity of a body is its weight, as compared with the weight of an equal bulk of a standard body taken as a unity.

The specific gravity of normal urine is 1,021. This means that, taking distilled water at 77° F. as 1, each cubic centimeter of urine weighs 1,021 grams. The urine should be at the same temperature. If it is not, to every 7° above normal add one unit of the last order to the last reading; to every 7° below normal subtract one unit of the last order.

EXAMPLE. The urinometer is graduated for 77° F. and reads 1,018 at 63° F.; then the specific gravity at 77° F. would be 1,018 — 0.002, or 1,016. 77° — 63° = 14°, and one unit is subtracted to every 7° below normal; 14° divided by 7° = 2°, therefore 2° × 0.001 (one unit of the last order) = 0.002. 1,018 — 0.002 = 1,016.

The relative proportion of solid matter in the urine is shown by the specific gravity, and by knowing the total amount in twenty-four hours an approximate idea of the absolute solids is obtained by multiplying the last two figures of the specific gravity by Long's coefficient, *i. e.*, 2.6. The solid content in 1,000 c.c. is obtained by multiplying the last two figures of the specific gravity observed at 25° C. by 2.6.

EXAMPLE. Sp. gr. = 1,020. 20 × 2.6 = 52 grams, the amount of solids in 1,000 c.c. of urine. The total

amount in twenty-four hours is 1,500 c.c.; therefore it will contain 1,000 : 1,500 :: 52 : x, or 78 grams.

Meat, drugs (potassium acetate), albumin, and diabetes mellitus raise the specific gravity.

There is a decrease in chronic interstitial nephritis and diabetes insipidus.

METHOD. The urinometer glass is filled three-fourths full of urine; then introduce the urinometer and wait until it finds the correct level before reading the scale. The last mark seen below the surface (the meniscus) is the correct reading of the scale, and this should be read through the fluid from below upward.

When the amount of urine is so small that it is necessary to dilute to get sufficient to take the specific gravity, add four times as much water as urine, making five volumes.

EXAMPLE. To one volume of urine add four volumes of water, making five volumes. If the specific gravity of this mixed fluid reads 1,005, then that of the urine will be 1,000 plus (5 × 5), or 1,025.

Albumin

Albuminuria is a condition in which serum albumin or serum globulin appears in the urine, and these two protein substances are of the greatest pathologic import.

In the accidental albuminuria the albumin is not excreted by the kidneys, but arises from the blood, lymph, or some albumin containing exudate coming in contact with the urine at some point below the kidneys.

The renal type is the more serious, as the albumin is secreted by the kidneys.

Nitric Acid Test. (Heller's)

Place 10 c.c. of filtered urine in a wine glass, incline the glass, and pour HNO_3 slowly down the side. If albumin is present, a white zone of precipitated albumin will form at the zone of contact.

Various colored zones, due to the presence of indican, bile pigment, or to the oxidation of other organic urinary elements, may form, but these should never be mistaken for the white zone which alone indicates serum albumin, serum globulin, albuminose, and peptone.

Roberts' Ring Test

Place 5 c.c. of Roberts' reagent in a test tube, and by means of a pipette allow the urine to flow slowly down the side. A white zone of precipitated albumin forms at the point of juncture if albumin is present.

Albumose

To 10 c.c. of urine add 5 drops of acetic acid; boil, then filter. On the surface of 5 c.c. of nitric acid in a test tube gently pour some of the cooled filtrate. A white ring at the junction of the fluids indicates the presence of albumose, and this will disappear on warming, to reappear again on cooling.

Coagulation or Boiling Test

Fill the test tube half full of filtered urine and gently heat the upper half to boiling, being careful that this half of the fluid does not mix with the lower half. A turbidity indicates serum albumin, serum globulin, or phosphates. Acidify the urine with 5 drops of acetic acid; the turbidity, if due to phosphates, will disappear. If albumin is present, a white cloud appears on heating, disappears on boiling, reappears on cooling.

Quantitative Test for Albumin. (Esbach's Method)

This test is made by means of a standard graduated glass tube or albuminometer. Fill the tube with urine to the letter U, then add Esbach's reagent to R; close the tube with a stopper and invert several times. Let the tube stand 24 hours, then read off the number of grams of albumin to the liter, as will be indicated by the number on the side of the tube on a level where the albumin settles. If the urine is alkaline, add a few drops of acetic acid. Urine heavy with albumin should be diluted with one or two volumes of water.

Kwilecki's Modification

Add 10 drops of a 10% solution of $FeCl_3$ to the urine before introducing the Esbach's reagent; warm the tube.

EXAMPLE. The protein precipitate is level with the figure 2 of the graduated scale, therefore the urine contains 2 grams of the protein to the liter, or .2% (the amount of protein in per cent).

Magnesium Mixture for Turbid Urine

Filter 10 c.c. of urine; add sufficient magnesium mixture to make the urine alkaline. After testing with litmus paper, add a few drops of acetic acid. Filter, and test for albumin with nitric acid.

Test for "Bence-Jones" Bodies

Mix 15 c.c. of urine in a test tube with an equal amount of saturated solution of NaCl. Add 2 c.c. of a 40% NaOH solution; shake thoroughly. Heat the upper contents of the tube to boiling and add lead acetate (10%) solution, drop by drop, continuing the heating. A brown or black precipitate (sulphur) shows this form of albumin.

Albumin. (Goodman and Stearn's Method)

To 5 c.c. of phosphotungstic acid solution add 2 c.c. of filtered urine with a pipette graduated in tenths. Shake after addition of each tenth; add urine until a whitish cloud appears. The number of tenths is read off and expressed in terms of 100 c.c. EXAMPLE. If it takes 1 c.c. of diluted urine (1–10) there is 0.0001 gram albumin, or 1 gram in 100 c.c.; if 0.7 c.c. of diluted urine, then 0.07 c.c. of undiluted urine equals 0.0001 gram albumin. 7 c.c. = .01 gram albumin, 700 c.c. = 1 gram. 700:1.0 ::100:x, or .142%.

Sugar

Very small amounts of glucose probably occur in every normal urine. In diabetes mellitus there is a permanent or persistent glucose excretion. Glucosuria is a transient type and accompanies various disorders, particularly cerebral digestive affection, certain forms of poisoning, morphine, carbon monoxid, chloral hydrate, oil of turpentine, corrosive sublimate, amyl nitrite, and prolonged hunger. Sometimes perfectly healthy people excite glucose in urine after too abundant ingestion of sugar.

Urine containing much glucose presents a light or pale color and has a high specific gravity.

The most common sugar occurring in urine is glucose. Levulose is sometimes present with glucose. Lactose is occasionally found in the urine of nursing women; laiose, maltose, and pentose are rare. If albumin is present, it should be removed before testing for sugar. This is done by boiling and adding a few drops of acetic acid, and filtering.

Fehling's Test responds to all the carbohydrates, and

every urine should be tested for sugar by this reduction test.

Fermentation Test responds only to glucose, levulose, and maltose.

Phenylhydrazin Test responds to glucose, levulose, maltose, lactose, and pentose. This is a very delicate and reliable test.

Polarimetric Test: glucose, lactose, and maltose, and to a slight extent pentose, rotate the ray of polarized light to the right; while levulose, β-oxybutyric acid, and glycuronates rotate polarized light to the left. Normal urine is often slightly dextro-rotary.

Fehling's Test

Mix equally 1 c.c. of Fehling's A and B, add 3 c.c. of water, and boil. This is done to determine whether the solution will of itself cause the formation of a precipitate of brownish-red cuprous oxide. If no precipitate forms, add 10 drops of urine.

A red precipitate indicates that one or more of the reducing sugars are present. If the solution remains clear, heat again, and if no precipitate now appears, sugar is not present. If a precipitate appears on this second heating, it may be due to sugar or to conjugate glycuronates, uric acid, nucleoprotein, or other substances. Cupric hydroxide may also be reduced to cuprous oxide, and this in turn be dissolved by creatinine, a normal urinary constituent, and this will give the urine a greenish tinge and may obscure the sugar reaction. The test is repeated, omitting the second heating, and the tube allowed to stand 24 hours. If the test is doubtful because the color reaction is yellow or green instead of red, the urine may

be fermented and again tested, after 24 hours. If there is no reaction, the first test was due to sugar.

Benedict's Modification of Fehling's Test

The following mixed solution does not deteriorate upon long standing:

Copper sulphate	8.65	grams
Sodium citrate	86.5	grams
Sodium carbonate (anhydrous)	50.	grams
Distilled water to	500.	c.c.

Dissolve the sodium citrate and carbonate in 300 c.c. of distilled water with the aid of heat, filter, and make up to 425 c.c.

Dissolve the copper in 50 c.c. of hot distilled water, then make up to 75 c.c. Add this to the carbonate-citrate solution slowly, and with constant stirring.

Method. Add 10 drops of urine to 5 c.c. of the reagent; boil for a few seconds. The solution will remain clear if no sugar is present. A red or yellow precipitate indicates the presence of sugar.

Fehling's Quantitative Test

Dilute 10 c.c. of urine with distilled water up to 100 c.c. Mix, then transfer to a burette.

Pipette 10 c.c. each of Fehling's solution A and B in a flask and dilute with 60 c.c. of distilled water; boil. Allow 0.5 to 1 c.c. of diluted urine to flow into the boiling Fehling's solution. Make a reading of the burette, and boil after each addition of urine. Continue running urine from the burette until Fehling's solution is completely reduced and there is absolute disappearance of all blue

color from the meniscus,[1] when viewed by transmitted light. Note the number of c.c. of diluted urine used. Fehling's solution is a standard solution of copper sulphate, and when accurately prepared 20 c.c. are reduced by 0.05 gram glucose.

EXAMPLE. If 8 c.c. of diluted urine were used in reducing 20 c.c. of Fehling's solution, then 0.8 c.c. of undiluted urine was required, and this amount contained 0.05 gram glucose. The percentage in the sample would be calculated thus: 0.8:0.05::100:x, which equals 6.25%.

1,400 c.c. of urine were voided in 24 hours; then 0.8:0.05::1,400:x, or 87.50 grams glucose.

Fermentation Test

This test is important because the fermentable sugars are the pathological ones. Carbon dioxide and alcohol are formed in the fermentation of sugar, and the production of alcohol and the disappearance of sugar lower the specific gravity.

The urine should be sterilized by boiling, and if not already acid, acidify with hydrochloric or tartaric acid. Cool, and determine the specific gravity. Add a small piece of yeast about the size of a bean. Place 200 c.c. of this urine in a flask and stopper sufficiently close to prevent the escape of alcohol. Let the flask of urine stand in a warm room 24 hours, then test with Fehling's solution. If sugar is still present, let it stand for another 24 hours. If the reducing substance is still present, it is not fermentable sugar. Record the specific gravity.

It has been found that a decrease of 0.001 (a fall of

[1]The meniscus is seen as a clear line just beneath the surface of the fluid (blue at first and later colorless).

one point) in the specific gravity corresponds to 0.23% of sugar in the urine tested.

EXAMPLE. The specific gravity of the urine before fermentation is 1,030. After the fermentation is completed, the specific gravity is 1,008, a decrease of 22 points; and this multiplied by 0.23 gives the amount, 5.06%, of sugar in the urine tested.

Polarimetric Method

The optical activity of sugars are estimated by means of the polarimeter, and this method is necessary in order to distinguish glucose from levulose, and to identify β-oxybutyric acid. Glucose is called dextrose because of its ability to turn the ray of polarized light to the right. The degree of dextro-rotation is read on a graduated scale and calculated as percentages or grams of glucose. The urine must be free from albumin, and is clarified by adding 2 c.c. of lead acetate or charcoal. Filter, and fill the tube of the polariscope with the urine, avoiding bubbles, and measure the angle of rotation of polarized light to the right or left.

EXAMPLE. Every interval on the scale corresponds to $\frac{1}{2}$%, and between these intervals are lines which are equivalent to $\frac{1}{10}$%. The number of scale intervals passed is 7. The zero point stands between 7 and 8 at the mark corresponding to 0.3 per cent; therefore 7 half per cent equal 3.5 plus 0.3, or 3.8 per cent.

Phenylhydrazin Test

To 10 c.c. of urine add 1 gram of phenylhydrazin and 1 gram of sodium or potassium acetate; heat gently for 2 minutes.

A yellowish deposit forms at the bottom of the tube if sugar is present, and on microscopical examination needle-shaped crystals of bright sulphur-yellow are found. If no sugar is present, only brownish amorphous globules and yellow scales will be found.

Glucose crystallizes in fine, yellow needles, which are arranged singly and in groups.

Lactose forms in burr-like clusters.

Maltose occurs in the form of rosettes.

If the urine contains 0.2% of sugar, the precipitate appears in a few minutes. This test is sensitive for 0.03% of sugar.

Gerhardt's Test for Acetone

To 10 c.c. of urine in a test tube add 10 drops of acetic acid, then add 10 drops of freshly prepared sodium nitroprusside solution and mix; carefully overlay with 2 c.c. of concentrated ammonia.

If acetone be present, a violet-red ring will develop at the point of contact. The amount of acetone will be increased in fever, with starvation, with purely meat diet, in diabetes mellitus, in certain forms of digestive disturbance, and in some cases of carcinoma.

Acetone is a physiological as well as a pathological constituent of the urine.

Guaiac Test for Blood

To 5 c.c. of urine add 10 drops of freshly prepared alcoholic solution of guaiac, or an alcoholic solution of guaiaconic acid. Shake gently and add 20 drops of old turpentine or hydrogen peroxide, drop by drop, until a blue color is obtained, which indicates the presence of blood.

Benzidin Test for Occult Blood

Add 2 c.c. of a saturated solution of benzidin, in alcohol or acetic acid, to 2 c.c. of 3% hydrogen peroxide and 1 c.c. of urine. Blood is indicated by the appearance of a green or blue color.

Arnold-Lipliawsky Test for Diacetic Acid

This test is negative toward acetone, β-oxybutyric acid, and various drugs, as antipyrin, phenacetin, salicylates, etc. It detects diacetic acid when present in the proportion of 1:25,000.

The reagent consists of 1% aqueous solution of potassium nitrite, 1 c.c. of which is mixed with 2 c.c. of a 1% solution of ρ-amino-acetophenon (1 gram of ρ-amino-acetophenon dissolved in 100 c.c. of distilled water), and hydrochloric acid added, drop by drop, until the solution becomes colorless.

Method. Place 5 c.c. of urine and an equal volume of the reagent in a test tube, add a few drops of concentrated ammonia, and shake the tube. To 2 c.c. of this colored solution add 10 c.c. of hydrochloric acid, 2 c.c. of chloroform, and 3 drops of ferric chloride solution, and carefully mix the fluids. The chloroform will assume a violet or blue color if diacetic acid is present, otherwise the color may be yellow or light red.

Uric Acid

To 30 c.c. of urine add 2 c.c. HCl; let it stand 24 hours. Crystals of uric acid, if present, will separate out.

Murexid Test

Place 2 c.c. of urine in a porcelain dish, add 1 drop of nitric acid, and evaporate to dryness. Cool and add 1 drop

of ammonia. A blue or violet color indicates the presence of uric acid or urates.

Urea

Place a drop of urine on a slide, add a drop of nitric acid, and partially evaporate by warming gently. Crystals of urea nitrate will show if urea is present.

Weyl's Test for Creatinine

To 5 c.c. of urine add 10 drops of sodium nitro-prusside (saturated solution). Mix and add 10 drops of potassium hydroxide (20%). A ruby-red color results which soon turns yellow.

Salkowski Test

To the yellow solution obtained in Weyl's test above add an excess of acetic acid and apply heat. A green color results and is in turn displaced by a blue color. If creatinine is present, a precipitate of Prussian blue will form.

Test for Indican or Potassium Indoxyl Sulphate

The test for indican is based upon the fact that an excess of HCl will liberate the indoxyl, and by the addition of an oxidizing agent this is converted into indigo blue, and finally this can be recognized in small amounts by extraction from the bulk of urine with chloroform. The presence of more than a trace indicates the existence of undesirable decomposition in the intestinal tract.

METHOD. Add 10 c.c. of urine to the same amount of Chemically Pure concentrated HCl. Mix and add 3 drops of freshly prepared $\frac{1}{2}$% solution of potassium permanganate. If indican is present, a purplish cloud will form. Then add a few drops of chloroform and then a few more drops of permanganate solution. Shake vigorously and

the color will change to a deep blue, due to the precipitation of indican by the chloroform.

Test for Melanin

In cases of melanotic sarcoma, the urine treated with iron chlorid assumes a deep black color.

Urochromogen and Diazo Tests in Tuberculosis

(Journal A. M. A., October 10, 1914)

Place 1 c.c. of limpid urine diluted with 3 c.c. of water in two test tubes. To the first tube add 3 drops of 1–1,000 solution of potassium permanganate; the other tube use as a control. A distinct canary yellow indicates a positive reaction.

Iodine Test

To 10 c.c. of urine in a test tube add 2 c.c. of tincture of iodine (dilute 1–10 in alcohol). The presence of bilirubin is indicated by a distinct emerald green ring at the point of contact.

Urobilin Test

Fill a test tube with 12 c.c. of urine, add 1 drop of concentrated hydrochloric acid, shake gently, and allow to separate by standing. Pour off the supernatant fluid and add three times its volume of alcohol. To this alcoholic extract add 1 drop of a 5% solution of zinc chloride and 1 drop of ammonium hydroxide. Zinc hydrate will be precipitated and should be filtered off. A green fluorescence indicates the presence of urobilin.

Urobilin is an abnormal product and is increased in carcinoma, appendicitis, Addison's disease, acute infectious diseases, and pancreatic disease.

Urophæin

To 10 c.c. of urine add 2 c.c. of H_2SO_4. A brownish red color indicates the presence of urophæin.

Urochrome

Add 10 c.c. of HCl to 10 c.c. of urine and heat the mixture. The presence of urochrome is indicated by a bright red color.

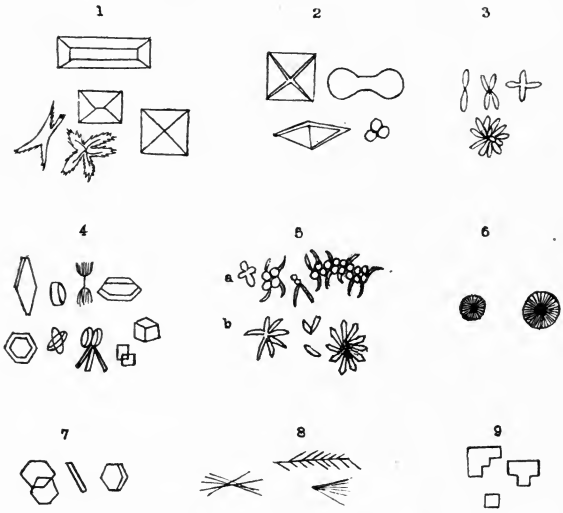
β-oxybutyric Acid.—Black's Test

Concentrate 10 c.c. of urine to one-fourth its volume in an evaporating dish at a gentle heat. The residue is acidified with a few drops of concentrated HCl. Add plaster of Paris to make a thick paste; allow the mixture to stand until it begins to "set." Then break up with a stirring rod and extract twice with ether by stirring and decantation. Pour off ether and evaporate spontaneously or over a water bath. Any β-oxybutyric acid present will be extracted by the ether. Dissolve the residue in water and neutralize it with barium carbonate. To 5 c.c. of this neutral fluid add 1 to 2 drops of commercial acid hydrogen peroxide. Mix and add a few drops of Black's reagent (5 grams of ferric chloride and 0.4 gram ferrous chloride in 100 c.c. of water).

The presence of β-oxybutyric acid is indicated by a gradual development of a rose color, which increases to its maximum intensity and then gradually fades. The disappearance of the color is due to the oxidation of the diacetic acid. Care should be taken that the solution is cold, neutral, and that an excess of hydrogen peroxide and Black's reagent are not added. One part of β-oxybutyric acid in 10,000 parts of solution may be detected by this test.

Urinary Sediments

The urinary sediment is the deposit which is found in the urine after standing. This sediment is classified as



URINE CRYSTALS — *Gibson*

1. Triple Phosphate. 2. Calcium Oxalate. 3. Calcium Carbonate. 4. Uric Acid. 5. (*a*) Ammonium Urate; (*b*) Sodium Urate. 6. Leucin. 7. Cystin. 8. Tyrosin. 9. Cholesterin.

non-organized, or chemic, and organized, or anatomic deposits.

The sediment examination is useful in showing the presence of abnormal elements excreted by the kidneys.

When the concentrated sediment has been obtained, either by centrifugalization or sedimentation, a drop is taken from the tube with a pipette. Place the index finger on the upper opening of the pipette and insert it into the tube, carrying it down into the fluid to the sediment: then

release the pressure of the finger and allow a few drops to enter the pipette, maintaining a firm pressure of the finger on the upper end while withdrawing the pipette. Place a drop on a slide and examine with a low power lens and subdued light, then with high power and slightly more light.

The non-organized sediments are usually crystalline, although a few are amorphous. The crystalline deposits are important only when found in freshly voided urine; they may be precipitated in any urine which stands and undergoes fermentation.

The organic sediments consist of various cells, casts, yeast fungi, spermatozoa, and bacteria.

Non-organized Sediments Occurring in Normal Urine

Uric Acid Crystals: Rhombic prisms, rosettes, and hexagonal plates, usually yellow—may be colorless. Soluble when heated with NaOH.

Calcium Oxalate Crystals: Colorless, envelope and dumb-bell shaped. They are derived from various foods.

Calcium Carbonate Crystals: These crystals are found in alkaline urine, sometimes in slightly acid urine. It crystallizes in the form of granules, spicules, and dumb-bells.

Ammonium Magnesium Phosphates: "Triple Phosphates"; these occur in sediment in two forms, the prismatic form of crystals and the amorphous, feathery type. In faintly acid urine they have the coffin-lid appearance, but in alkaline urine they take a variety of shapes. These are pathological when found in fresh urine.

Amorphous Phosphates of Ca and Mg are commonly

found in alkaline urine. They appear as bulky, opaque, white deposits, and this whitish, flocculent deposit is precipitated by heat and dissolved by acetic acid. These earthy phosphates are the only salts of phosphoric acid that are found in the urinary sediment.

Ammonium Urates: This salt of uric acid is formed during ammoniacal fermentation of the urine, and is only abnormal if the urine is fresh; any urine upon standing for several hours is apt to deposit crystals of uric acid. Acid ammonium urate occurs as yellowish red or dark brown spherical bodies, radially striated and studded with fine, prismatic spicules—"thorn apple crystals."

Amorphous Urates are of little importance. They have a pinkish or brick-dust appearance, due to uroerythrin, a coloring matter found in the urine of acute rheumatism. They also occur as rods and spikes; are dissolved by NaOH, but not by acetic acid.

Non-organized Sediments Occurring in Abnormal Urine

Cystin Crystals: Colorless hexagonal plates or quadrilateral prisms; soluble in HCl and ammonia, insoluble in acetic acid, water, alcohol, and ether.

Leucin Crystals are highly refractive, yellow spheres, resembling fat globules; insoluble in ether.

Tyrosin Crystals appear as fine, radiating needles. They are colorless. Tyrosin and leucin crystals are found together in nephritis, gout, cirrhosis, and carcinoma of the liver.

Bilirubin and Hematoidin Crystals appear as red granules, needles, and rhombic plates. They have no clinical significance.

Crystals of Uric Acid may be suggestive of gravel or calculus if blood and other symptoms are present.

The presence of an excessive number of calcium oxalate crystals may signify oxaluria.

Ammonium–magnesium Phosphate Crystals, when formed in fresh ammoniacal urine, are suggestive of infection of the urinary passages and calculus.

Cholesterin Crystals are large, regular, and irregular plates. These crystals are found in the sediment in cystitis, nephritis, pyelitis, and chyluria.

Organized Sediment

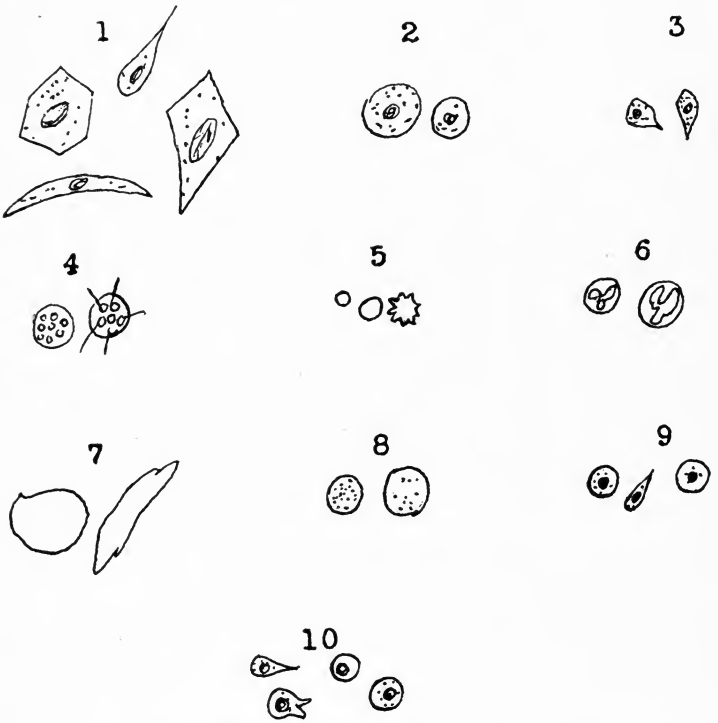
Various forms of epithelia are found in nearly all urines. They are the formed elements coming from the different parts of the genito-urinary tract, and are supplied by the progressive desquamation of the mucous surfaces. An increase in number indicates inflammatory process in the part producing them. They are more or less granular and possess one or more nuclei. The size, shape, and condition of their protoplasm and nuclei should be carefully observed. If the sediment is of such volume and density as to obscure blood cells, casts, and pus, dilute with a drop of clear urine.

Large, round, epithelial cells, with dense refractive protoplasm, come from the neck of the bladder; other large, round cells may come from the membranous and prostatic urethra.

Epithelium from the Urinary Tract

Small, round, epithelial cells may come from the renal tubules, the pelvis of the kidney, or the urethra. They are mononuclear and slightly larger than the leucocytes.

Large, polygonal, squamous cells come from the bladder, but they may also enter the urine from the prepuce of the male or vulva of the vagina.



EPITHELIUM FROM THE URINARY TRACT — *Gibson*

1. Squamous epithelium from the bladder. 2. Cells from the neck of the bladder. 3. Epithelium from the prostatic urethra. 4. Cells from the seminal passages. 5. Red blood cells. 6. Leucocytes. 7. Scaly epithelium. 8. Compound granule cell. 9. Urethral cells. 10. Pelvic cells.

Cylindrical and caudate cells may come from the neck of the bladder or the pelvis of the kidney.

Leucocytes are present in small numbers in normal urine. An increase in number denotes an inflammatory condition of some portion of the urinary tract. They are recognized by their polymorphic nuclei. In acid urine they are often shrunken, and in alkaline urine they are often degenerated and swollen.

Pus

The difference between a pus cell and a leucocyte is only quantitative. When many leucocytes are present, the term pus is used; when few, they are called leucocytes.

The origin of pus is important. Pus from the kidney is usually mixed with casts and small, round cells; if with squamous cells, it is probably from the bladder. A catheterized specimen is necessary in order to rule out the vagina as a source.

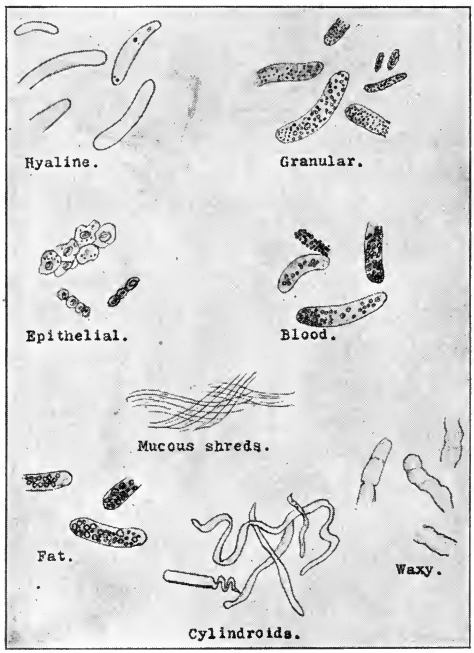
Red blood cells are pale, non-granular, non-nucleated discs. In concentrated urine they may present a crenated appearance, and in alkaline urine they are often destroyed, forming masses of brown granules. Normal blood indicates a hemorrhage in the pelvis or bladder; abnormal blood signifies that it is higher up in the tubules.

Casts

Casts are cylindrical formations which originate in the uriniferous tubules; they generally indicate some kidney disorder. They have uniform marginal outlines with well-defined borders, and show the molded effect of the kidney tubules.

Hyaline Casts: Transparent, their shape and character may be determined by staining with iodine solution, gentian violet, or fuchsin. Generally albumin is present with this type of cast.

Granular Cast: The basic substance of this cast is hyaline, and the granules consist of albumin, degenerated epithelial cells, erythrocytes, and leucocytes. They are



Casts — *Gibson*

finely granular and coarsely granular, according to the nature and size of the granules.

Epithelial Casts: Hyaline casts covered with epithelial cells from the lining of the uriniferous tubules. These are particularly abundant in acute nephritis.

Blood Casts: Hyaline basis, covered with erythrocytes. They are characteristic of acute diffuse nephritis and

acute congestion of the kidney, and they denote renal hemorrhage.

Fatty Casts: Fat globules and fatty acid crystals are deposited upon hyaline or granular casts. They indicate fatty degeneration of the kidney, and are found in subacute and chronic inflammation of the kidney.

Waxy Casts: Similar to the hyaline form, but are somewhat larger and appear more solid, having a sharper outline and a light yellow color.

Cylindroids: Flat in structure, with smaller diameter than casts, having branching ends. These "false casts" may become coated with granules and appear granular in structure.

Mucous Shreds: These shreds of mucus are long, wavy, transparent bodies, which are much thinner than casts or cylindroids.

Tubercle Bacilli in Urine. (J. A. M. A., March, 1915)

Acidify the urine with 30% acetic acid, 2% of its volume with a 5% solution of tannic acid. Place this in the ice chest 24 hours. Centrifugalize the precipitate, then redissolve with a dilute acetic acid solution; centrifugalize again, and smear the sediment on a slide and stain. The precipitate may be treated with normal sodium hydroxide solution, then cultivated.

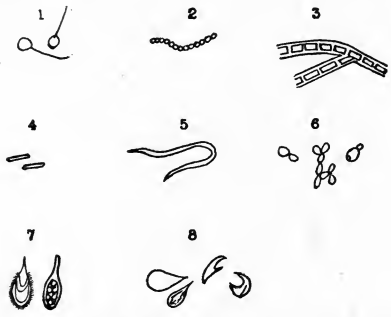
Other Cellular Elements

Spermatozoa, cells from neoplasms, micrococcus urea, streptococcus, staphylococcus, colon, typhoid, and tubercle bacilli may find entrance and grow in the urinary tract. To be of diagnostic value, care should be taken to prevent their entering from other sources.

Examination of bacteria should be upon fresh urine obtained by catheterization.

Molds and yeasts are sometimes found in diabetic urine.

Animal parasites, hooklets, and daughter cysts of echinococcus and bilharzia hematobia occasionally find their way into the urine.



OTHER CELLULAR ELEMENTS OF URINE — *Gibson*

1. Spermatozoa. 2. Micrococcus urea. 3. Molds. 4. Bacilli. 5. Vinegar eel. 6. Yeast fungi. 7. Bilharzia hematobia eggs. 8. Echinococcus eggs and hooklets.

Formaldehyde

The excretion of formaldehyde by the kidneys of patients taking urotropin varies, as the kidneys of some patients do not decompose the drug, and urotropin depends for its action on formaldehyde, into which it is decomposed in the kidney.

Burnham's Test for Formaldehyde

To 10 c.c. of urine in a test tube, slightly warmed, add 3 drops of phenylhydrazine hydrochlorate solution (0.5% aqueous or alcoholic solution).[1] Overlay with a few drops of a saturated solution of NaOH. Positive reaction will

[1] Add 3 gtts. of nitro-prusside solution.

become deep purplish black, changing quickly to dark green, gradually getting lighter.

Systematic Scheme in Charting

The following terms are commonly used in qualitative reactions:

MACROSCOPICAL—Slightest possible trace; faint trace; trace; small amount; moderate amount; large amount; very large amount.

MICROSCOPICAL—An occasional; a few; a moderate number; many; very many.

Quantitative Estimation of Phosphates

Place 50 c.c. of urine in a flask and add 5 c.c. of sodium acetate solution. Heat the mixture to boiling point. A standard solution of uranium nitrate is run into the hot mixture by means of a burette, until a precipitate ceases to form and a drop of the mixture assumes a brownish red color when brought in contact with a drop of potassium ferrocyanide solution in a porcelain dish.

This is the end point of the precipitation.

The number of c.c. of uranium solution used is read off from the scale on the burette.

1 c.c. of the standard uranium solution is equivalent to 0.005 gram of P_2O_5 (phosphoric acid). Therefore the number of grams of P_2O_5 in 50 c.c. of urine is estimated by multiplying by 0.005.

EXAMPLE. 10 c.c. of uranium solution were used. Then in 50 c.c. of urine there are 10×0.005, or 0.05; and in 100 c.c. of urine there are $10 \times 0.005 \times 2$, or 0.1% P_2O_5.

Normally, 3.5 grams per day.

Quantitative Estimation of Chlorides. (Volhard-Harvey Method)

Pipette 5 c.c. of urine into a porcelain dish and dilute with 20 c.c. of distilled water.

Precipitate the chlorides with exactly 10 c.c. of standard silver nitrate solution; add 2 c.c. of ferric ammonium sulphate (indicator).

A solution of standard ammonium sulphocyanate is run in from a burette until a yellowish color appears in the mixture.

Subtract the number of c.c. of sulphocyanate solution used from 10 c.c., the quantity of silver nitrate solution taken, and this will give the number of c.c. of the silver nitrate solution actually used in the precipitation of the chlorides.

1 c.c. of silver nitrate solution is equivalent to 0.01 gram of sodium chloride, and the number of c.c. of silver nitrate solution used multiplied by 0.01 gram will give the weight of sodium chloride in 5 c.c. of urine. Calculate from this the weight of sodium chloride in 24-hour amount of urine.

The weight of chlorine may be estimated by multiplying by the factor 0.006.

Volhard Method

To 10 c.c. of urine in a 100 c.c. flask add 5 c.c. of pure nitric acid and an excess of standard silver nitrate solution (25 c.c. measured from a burette). Dilute with water to 100 c.c.

Filter the precipitated chloride. Pipette 50 c.c. of filtrate into a beaker; add 5 c.c. of iron alum solution. Run in standard potassium sulphocyanide (KCNS) solution from a burette.

The appearance of a permanent red color, due to the formation of sulphocyanide of iron when all the silver has been precipitated, is the end reaction.

EXAMPLE. 6 c.c. of KCNS solution were used. 2 c.c. of KCNS solution corresponds to 1 c.c. of silver solution, therefore 6 c.c. of the KCNS used corresponds to 3 c.c. of silver solution (excess in the 50 c.c. of filtrate).

In 100 c.c. there were 6 c.c. of silver solution in excess, but in the 100 c.c. there were 10 c.c. of urine; therefore, of the 25 c.c. of silver solution added to the 10 c.c. of urine, 6 c.c. were in excess. And to precipitate the chlorides in 10 c.c. of urine, 25 — 6 c.c. silver solution are required, = 19 c.c.

As 1 c.c. silver solution corresponds to 0.01 gram of sodium chloride, 10 c.c. of urine would contain 19 × 0.01, and 100 c.c. of urine would contain 19 × 0.01 × 10, or 1.9%.

Normally, 15 grams of sodium chloride are excreted per day.

Quantitative Estimation of Ammonia. (Malfatti's Method)

Most methods of estimating ammonia are too elaborate for any but special laboratories. The following method is one of the simplest, and this method depends upon the fact that when a solution of an ammonium salt is treated with formaldehyde, urotropin is formed according to the following equation:

$$_4NH_4Cl + 6CH_2O = N_4(CH_2)_6 + {}_4HCl + 6H_2O.$$

The acid liberated is determined by titration. Pipette 25 c.c. of urine into a 250 c.c. flask. Add 50 c.c. of ammonia-free distilled water; then add 4 drops of 1%

alcoholic solution of phenolphthalein as indicator. Run in N/10 NaOH from a burette until a definite pink color is obtained in the flask. Add 5 c.c. of commercial formalin that has been neutralized. The acids combined with the ammonia are now liberated; the pink color disappears. Add more N/10 NaOH until the pink color returns.

Each c.c. of additional N/10 NaOH required for this equals 0.0017 gram NH_3.

According to Mathison, this method is made more accurate by adding 15 grams of pulverized neutral potassium oxalate to the urine before titration, as this precipitates the calcium.

Renal Functional Test. (Rowntree and Geraghty, Journal A. M. A., 1911)

Method. The bladder should be emptied, then give the patient 500 c.c. of water.

Exactly 1 c.c. of the phenolsulphonephthalein solution from an ampoule, which contains more than 1 c.c., is injected into the lumbar muscles. In 10 minutes obtain a specimen and add a few drops of alkali; then at the end of an hour from the time the specimen, rendered alkaline, first shows coloration, the entire contents of the bladder should be carefully collected and the amount of drug excreted accurately estimated. When used as a differential test, the secretions of the two kidneys should be separately collected by ureter catheterization for 1 hour from the time the urine from either side first shows coloration.

To open the ampoule, file the neck between the small bulb and the body of the ampoule.

Fill the wedge-shaped cell of the colorimeter with a

standard solution made by diluting exactly 1 c.c. of phenolsulphonephthalein solution from an ampoule with 200 c.c. of water, adding 10 c.c. of a 5% solution of sodium hydroxide, and then sufficient water to make 1 liter.

Dilute the specimen of urine with 200 c.c. of water and render alkaline with 10 c.c. of 5% solution of sodium hydroxide, then further dilute to make 1 liter. Fill the rectangular cup to the mark. The cup is then placed in the apparatus and the latter manipulated until the colors, as seen through the prism, are identical, when the percentage of excretion will be indicated on the scale. If the coloration is slight, showing small excretion of the phthalein, then the dilution should be carried only to 250 or 500 c.c. and the readings on the scale divided by 4 or 2.

Cryoscopy

Cryoscopy is a method of determining the freezing point of urine. It tells somewhat concerning the functional capacity of the kidneys in relation to the excretions of solids. The test should be made upon the mixed excretion for 24 hours. Toluol or thymol may be used as preservatives.

Urine may be disinfected with carbolic acid (5%), formalin (10%), or chloride of lime.

References: Physiological Chemistry, Hammarsten. Text-Book of Physiology, Howell. Clinical Examination of Urine, Ogden. Physiological Chemistry, Hawk.

Acute Nephritic Diet

		Calories
Breakfast	Milk, 6 oz. Cream (top cream), 3 oz. Zwieback (one slice, 3 x 2 x ½) 1 cubic inch butter	
10.00 A.M.	Milk, 6 oz. Cream, 2 oz. Sugar, 1 oz.	
11.30 A.M.	Milk, 6 oz. Cream, 2 oz. Zwieback (1½ slices) Butter (1 cubic inch)	
2.00 P.M.	Milk, 6 oz. Cream, 2 oz. Sugar, 1 dr.	
4.30 P.M.	Milk, 6 oz. Cream, 2 oz. Rice (3 tb.) Sugar, 4 dr. Zwieback (1 slice) Butter	
8 to 10 P.M.	Milk, 6 oz. Cream, 2 oz.	
Once at night, if awake	Milk, 6 oz. Cream, 2 oz.	2,900

Courtesy of The Boston City Hospital.

Low Salt Diet ("Salt Free")

One liter—32 oz. of milk in 24 hours.

Salt Free Bread } *ad lib.*
Salt Free Butter }

Sugar, rice, potatoes, stewed fruit, fresh green vegetables, chocolate, coffee, tea, cereals, salad without salt.

Small portions of meat.

One or two eggs daily.

Add no salt to food

Diet in Nephritis

Breakfast. (Any one food from each group may be given)

1. Cereals:	
Cooked	4 tb.
Cornflakes	8 tb.
Shredded wheat	1 biscuit
2. Meat:	
Lamb chop	1
Pork chop	1
Mutton chop	1
Fish	2 tb.
Oysters	8
3. Eggs	1
4. Bread	1 slice
Muffins	1
Rolls	1

Supper. (Any one food from each group may be given)

1. Cereals:	
Cornflakes	8 tb.
Cooked	4 tb.
2. Vegetables:	
Green corn	3 tb.
Peas	2 tb.
Prunes	8 tb.
3. Oysters	8
4. Eggs	1
5. Bread	1 slice
6. Coffee or tea, with milk and sugar.	

Dinner. (Any one food from groups 1, 2, 4, 5, 6; any two from group 3)

1. Soup	3½ oz.
2. Meats:	
Steak (2 x 1 x 1 in.)	
Chops (lean)	1
Fish	2 tb.
3. Vegetables. (Any two of these may be given)	
Potato	2 tb.
Mashed	4 tb.
Parsnips	4 tb.
Carrots	4 tb.
Squash	4 tb.
Turnips	4 tb.
Onions	4 tb.
Butter beans	2 tb.
Lima beans	2 tb.
4. Macaroni	4 tb.
Vermicelli	4 tb.
5. Puddings:	
Rice	4 tb.
Tapioca	2 tb.
Bread	2 tb.
Cornstarch	2 tb.
Ice cream	2 tb.
6. Bread	1 slice
Rolls	1
Muffins	1

The following may be given *ad lib.*: Butter, olive oil, olives, tomatoes, lettuce, celery, sweet fruits of any kind, sugar.

Courtesy of The Boston City Hospital.

Strict Diabetic Diet

Meats.
Beef, mutton, ham, bacon, poultry, shrimp, bologna, sausage, lamb, pork chops, steak, tongue, pigs' feet, brains, bone marrow, smoked or pickled meats, scraped or corned beef.

Fish.
All kinds. No dressing containing flour. Crabs, lobsters, sardines, etc.

Soups.
Clear (not containing a farinaceous substance). Beef juice.

Gelatin.

Eggs.
Prepared any way, with large amounts of butter.

Butter, olive oil: large amounts.
Cheese.
French dressing (olive oil, vinegar, etc.).
Coffee, tea, without sugar.
Akoll and Alpha biscuits.

Vegetables, etc., with 5% or less.
Lettuce, spinach, string beans, celery, asparagus, cucumbers, Brussels sprouts, unspiced pickles, olives, grape fruit, cauliflower, tomatoes, rhubarb, clams, scallops.

Foods, 6% or less.
Cabbage, radishes, pumpkin, oysters, liver.

Foods, 10% or less.
Onions, squash, turnip, carrots, beets, lemons, oranges, cranberries, peaches.

100 G. Carbohydrate Diet

Strict diet	0.
Vegetables (5%, 6%, 10% groups)	10.
Cream, ½ pt.	6.
Oatmeal, 1 gill (dry)	24.
Bread, 1 oz.	30.
Potato, 1 medium, baked or mashed; 2 tb.	15.
Orange or grape fruit, 1	15.
	100 gms.

Courtesy of The Boston City Hospital.

Urine

Name.....Age.....Sex.....Date.....

Color.
Odor.
Sediment.
Specific Gravity.
Reaction.
Albumin.
Sugar.
Bile.
Indican.

Microscopical Examination.—

Special Chemical Examination

24° amount.
Blood.
Acetone.
Diacetic acid.
β-oxybutyric acid.
Sugar—quantitative.
Albumin—quantitative.
Total solids.
Chlorides.
Total nitrogen.
Ammonia.
Creatinine.
Urea.
Uric acid.
Phosphates.

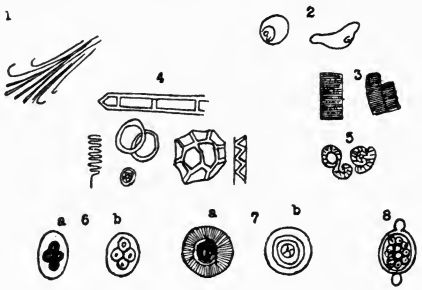
Microscopical Examination.—

Signed.....

CHAPTER IV

FECES

The Feces are the residue which remains after complete digestion and absorption in the intestines, and the residue is different qualitatively and quantitatively according to the variety and quantity of food. The normal stool varies in quantity from 250 to 500 grams.



FECES

1. Connective Tissue. 2. Ameba. 3. Meat Fibers. 4. Various Vegetable Cells. 5. "Soaps." OVA: 6. Hookworm, (*a*) Necator Americanus; (*b*) Ankylostoma Duodenale. 7. Tenia, (*a*) Tenia Solium; (*b*) Tenia Saginata. 8. Trichocephalus.

Color

The color of normal feces is due to hydrobilirubin, also called stercobilin, and it originates from the bilirubin which is secreted into the intestine in the bile, being formed by the reducing activity of certain bacteria.

A mixed diet produces a stool which varies in color

from light to dark brown; meat diet, a dark stool; milk diet, a light stool.

Calomel colors the stool green; bismuth subnitrate, black; senna and rhubarb, yellow; iron gives a gray or black stool.

Blood will color the stool black if the source is in the alimentary canal. Greenish yellow, liquid stools are characteristic of typhoid. Sprue stools are whitish, putty colored, filled with air bubbles.

Consistency

Normally, the consistency may vary from a well-formed stool to a pasty discharge.

The feces are soft when the absorption is prevented, intestinal secretion increased, as in cholera, and where there is an increase of fat. In steatorrhea the stools are pale and greasy.

Constipation gives a hard, compacted stool, also scybalous masses. When there is a stricture of the lower bowel, the feces occur in slender, cylindrical masses.

Odor

Due to skatole and indole, two gaseous products of proteid decomposition, and also to hydrogen sulphide.

Reaction

Normally alkaline to slightly acid.

Micro-chemical Examination of Feces

Thoroughly mix feces, transfer a portion as large as a walnut to a mortar, and grind with water to a thin mush. Place 2 drops on two separate slides.

The first drop is simply covered with a cover glass and examined for the following: muscle fibers, connective tissue, fatty acids, soaps, leucocytes, erythrocytes, vegetable cells, micro-organisms.

The second drop is mixed with a drop of 36% acetic acid and heated until bubbles appear. Cover with a glass and examine for fatty acid flakes. This also differentiates between connective tissue and mucus, the former being rendered transparent.

Add 2 drops of Sudan III or Scharlach R. to the third drop. This will stain the fat globules red.

A drop of Lugol's solution is added to the fourth drop, as this will stain starch granules, fungi, yeasts, etc., blue or violet.

Food

Shreds of undigested food may be due to improper mastication.

In a meat diet the muscle fibers show their characteristic striations.

Fat and Fatty Acid

When fat is taken too freely into the diet, and in diseases of the pancreas, fat appears in the stools as highly refractive globules. It is soluble in ether.

Soaps

Irregular yellow masses of fat.

Mucus

Mucus gives the stool a glairy, slimy appearance. If mixed with fecal material, it is from the small intestine; otherwise it is from the colon.

Carbohydrates

These appear in single starch granules and in masses of granules in cellulose envelopes.

Intestinal Parasites.—Ameba.—Entameba

An ameba consists of a single cell without a protective covering, and without organs of locomotion; it moves and ingests food by means of pseudopodia (temporary protrusion of the outer layer), and is constantly changing its shape. Amebæ are parasitic and may occur in any part of the alimentary canal.

AMEBA COLI: A harmless parasite, small, distinct nucleus, sluggish movements.

AMEBA HISTOLYTICA: Pathogenic, finger-shaped, indistinct nucleus, active movements.

AMEBA TETRAGENA: Pathogenic, lobose-shaped, distinct nucleus, active movements.

The amebæ of dysentery are found in fresh stools. Examine in warm NaCl. Fix in solution of absolute alcohol 25 c.c., ether 25 c.c., and 5 c.c. of —20% corrosive sublimate solution (2 grams in 10 c.c. absolute alcohol). Stain with carbol fuchsin.

Examination of Feces for Eggs. (Journal A. M. A., February, 1915)

1. Thoroughly mix 2 grams of feces with 5 c.c. of a 2% solution of cresol in a centrifuge tube.

2. Centrifugalize at high speed 1 minute, then decant and add fresh cresol solution, mix, and centrifugalize again. Repeat the process three times.

3. Remove a small portion of the bottom sediment with a clean pipette and place on a slide; add a drop of

anilin gentian violet, mix, and place a cover glass over it. The entire slide, with the exception of the real eggs, is stained violet.

Various vegetable detritus may be mistaken for intestinal parasites, as banana, which simulates tapeworm segment; and celery fibers, oxyuria. The oxyuris vermicularis (pinworm) is often found in the stools of children.

Micro-organisms

Various species of micro-organisms are found in the feces. Streptococci fecalis are found in normal stools—a non-virulent type. About one-third of the dry substance of stools of a normal adult consists of bacteria. B. coli, B. lactis aërogenes, and B. Welchii are the gas-forming organisms. The fecal output of bacteria decreases under the influence of water-drinking with meals.

Tubercle Bacilli

In intestinal tuberculosis the bacilli are found imbedded in the mucus. They may have their origin from sputum which has been swallowed.

Take a small amount of the stool and mix with water; centrifugalize. Place a drop of the scum on a slide, dry, stain with carbol-fuchsin stain (Ziehl-Neelsen).

Rapid Method for Cultures. (The Journal of Experimental Medicine)

Collect feces in wide-mouthed jars, dilute with three volumes of water, mix well. Filter through several thicknesses of gauze to remove solid food particles.

Saturate filtrate with sodium chloride. Let stand one-half hour. At the end of time all bacteria will be found

floating. Collect this floating film in a wide-mouthed bottle, add equal volume of normal sodium hydrate. Shake well; leave for digestion in the incubator at 37° C. for 3 hours, shaking every half hour.

Neutralize to sterile litmus paper with normal hydrochloric acid. Centrifugalize, and inoculate sediment into special media. Growth will appear in two or three weeks.

Pus

It is difficult to detect pus in the feces unless there is a large amount. Mix a small amount of feces with normal salt solution, which is isotonic, and make a smear. Stain with Wright's stain.

Guaiac Test for Blood

To 10 c.c. of fecal suspension add 5 drops of acetic acid; shake. Extract with 10 c.c. of ether and decant 5 c.c. Add 12 drops of freshly prepared gum guaiac solution (take a piece the size of a pea and dissolve in 70% alcohol), then add 20 to 30 drops of hydrogen peroxide. A bluish color will result if blood is present.

Dry Test for Occult Blood. (Journal A. M. A., January 30, 1915)

Place a clean slide on a sheet of white paper and smear with feces. Cover with benzidin reagent. Blue or green color indicates blood.

Benzidin Reagent

Benzidin	1 gram
Glacial acetic acid	2 c.c.
H_2O_2 (3%)	20 drops

Benzidin Test for Occult Blood

Make a thin fecal suspension with distilled water, and heat to boiling to render the oxidizing enzymes inactive. To 2 c.c. of saturated solution of benzidin in glacial acetic acid add 3 c.c. of 3% hydrogen peroxide and 2 to 3 drops of the cooled fecal suspension.

A clear green or blue color appears within 1 to 2 minutes in the presence of blood.

If the mixture is not shaken, a ring of color will form at the top. This test is of value only in cases of organic disease, and has for its condition a diet without meat.

Phenolphthalein Test

To 4 c.c. of thin fecal suspension add 2 c.c. of the phenolphthalein reagent (1 to 2 grams of phenolphthalein and 25 grams of KOH in 100 c.c. of distilled water. Add 10 grams of powdered zinc and heat gently until the solution is decolorized). Add a few drops of hydrogen peroxide. A pink or red color promptly forms in the presence of blood.

Hydrobilirubin

Mix a small amount of feces with a few c.c. of concentrated mercuric chloride ($HgCl_2$), and allow to stand 6 to 24 hours. If hydrobilirubin is present, it will be indicated by a deep red color, and this is due to the formation of hydrobilirubin mercury. If unaltered bilirubin is present in any portion of the feces, that portion will be green in color, due to the oxidation of bilirubin to biliverdin.

Bilirubin

This is normally reduced to hydrobilirubin, and is not found in the feces, as a rule, unless this process is interfered with, as by too rapid passage of the intestinal contents through the canal. The absence of both bilirubin and hydrobilirubin has an important bearing upon the diseases of the bile passages.

Gmelin's Test

Place a few drops of concentrated nitric acid in an evaporating dish, and allow a few drops of feces and water to mix with it. The usual play of colors—green, blue, violet, red, and yellow—is produced.

Fermentation Test. (Steele)

The apparatus for this test is easily made by taking a 2-ounce, wide-mouthed bottle, fitted with a perforated rubber cork, through which run a glass tube to a test tube, also fitted with a rubber cork with two perforations. This test tube is connected with a second test tube of the same size by means of a bent tube. A piece of glass tubing, which extends more than half the length of the test tube, is inserted in the second perforation of the tube to allow for the escape of the air.

Method. Take 4 grams of solid feces, add 2 c.c. of distilled water, stir until thoroughly mixed. Place this in the bottle. Fill with sterile water. The first tube is filled nearly full of water and fitted into place. The second tube is fitted on empty. Note the reaction. Incubate 24 hours.

When the gas forms by fermentation in the bottle, it will rise into the first test tube, and the amount will be shown by the water forced into the second tube.

Normally, there is practically no gas shown in this test and the reaction remains about the same.

It is pathologic when more than one-third of the second tube is filled with the water.

In carbohydrate fermentation the reaction becomes markedly acid after 24 hours; in fermentation of albumins it becomes alkaline, with a foul odor.

Test Diet in Intestinal Diseases. (Schmidt's)

Milk	1 liter
Zwieback	100 grams
Eggs	2
Butter	50 grams
Beef	125 grams
Potatoes	190 grams
Oatmeal gruel	80 grams

This diet contains about 102 grams albumin,
111 " fat,
191 " carbohydrates,
or a total of 2,234 calories.

8 A.M. 0.5 liter of milk (or 0.5 liter of cocoa prepared from 20 grams of cocoa powder, 10 grams of sugar, 400 grams of water, and 100 grams of milk) and 50 grams of zwieback.

10 A.M. 0.5 liter of oatmeal gruel (made from 40 grams of oatmeal, 10 grams of butter, 200 c.c. of milk, 300 c.c. of water, 1 egg). This should be strained.

12 M. 125 grams of chopped beef (raw weight) broiled rare with 20 grams of butter, so that the interior will remain rare, and 250 grams of potato broth (made from 190 grams of mashed potatoes, 100 c.c. of milk, and 10 grams of butter).

2.30 P.M. The same as at 8 A.M.

5.00 P.M. The same as at 10 A.M.

Starting the Experiment

Starve the patient from noon of day before experiment until the next morning.

One hour before breakfast on day of experiment give charcoal mixture (10 grams of charcoal, 10 grams of acuiem, 60c.c. of peppermint water, well shaken together), or 5 grains of carmine may be given.

These solutions color the first part of the stool belonging to the metabolism experiment.

The urine is collected from 8 o'clock in the morning until 8 o'clock the following morning.

Separation of different days' urine is desirable in order to study daily variation.

The urine is made up each day to a definite value and aliquot parts taken for analysis. Empty the bladder just before stool. After the stool is washed and the filter paper used is added to the food used for analysis (in order to offset impurities in paper mixed with feces), the feces are dried, ground up, and well mixed, then analyzed.

Vomitus

If the patient vomits, the amount should be analyzed separately and the result subtracted from the food.

McCrudden's Method

Feed the patient on weighed amounts of food. Determine the per cent of nitrogen and other constituents to be studied in each amount of food, then estimate the total amount. These elements are determined in the feces and urine, and the amounts ingested are compared with those excreted.

Foods taken for analysis are all mixed together in same

proportion that they are given to the patient, and the mixture analyzed as a whole.

At each meal a certain volume or weight of each food, well mixed, is given to the patient and same amounts saved for analysis. At the end of the experiment all food taken for analysis is well mixed, and after the addition of a few drops of HCl (to retain the nitrogen) as much water as possible is evaporated in a steam water bath.

Ending the Experiment

Starve the patient from noon on last day of experiment until the following morning. One-half hour before the first meal is given, after the experiment is over, the charcoal mixture is given. The feces are saved until they become blackened.

Test for Diastase

In each tube of a series of six place 2 c.c. of a $\frac{1}{10}$ % solution of soluble starch. (Keep stock in ice chest.) As a control for reagent add iodine solution to tube 6, to see if the starch is all right.

Make a thin fecal suspension in sodium carbonate 1–10,000 (1 gram of feces to 100 c.c. of solution).

To tube number 1 add 1 c.c. of the fecal suspension.
" " " 2 " .75 " " " " "
" " " 3 " 0.5 " " " " "
" " " 4 " .25 " " " " "
" " " 5 " 0.1 " " " " "
" " " 6 " 0.0 " " " " "
(control).

Incubate 30 minutes at 40° C., then add to each tube a

few drops of iodine or Lugol's solution. If the starch is undigested the reaction is a deep blue color, showing pancreatic insufficiency.

Test for Trypsin

Make a casein solution by dissolving 0.5 gram of casein (which must be chemically pure: white, not yellowish) in 500 c.c. of a 1–1,000 solution of sodium carbonate. Test this solution by adding 1% acetic acid. If a precipitate forms, the solution is all right.

In a series of six tubes place 5 c.c. of the casein solution.

Make a fecal suspension by dissolving 1 gram of feces in 100 c.c. of a 1–1,000 sodium carbonate solution. Clear by filtering.

To tube number 1 add 1 c.c. of the fecal suspension.
" " " 2 " .75 " " " " " "
" " " 3 " 0.5 " " " " " "
" " " 4 " .25 " " " " " "
" " " 5 " 0.1 " " " " " "
" " " 6 " 0.0 " " " " " "
(control).

Add a few drops of toluol to each tube, shake, let stand at 40° C. for 24 hours. Then add to each tube a few drops of acetic acid (1%) solution. If casein is present, there is a precipitate. If it has been digested (by trypsin), there is no precipitate.

References: Parasitologie Animale, Neveu-Lemaire. Physiological Chemistry, Hammarsten.

Fat Free Diet

				Calories	
Breakfast.					
Skimmed milk	1 glass (6 oz.)			84	
Toasted bread	2 slices			120	
Boiled egg	1			80	284
Dinner.					
I. Meats	2 chops (lamb or pork). *Must be lean.*				
or	Steak (2 x 2 x 2)			200	
	Chicken				
	Roasts (beef, lamb), lean				
II. Fish	1 portion				
III. Fresh vegetables	Lettuce				
	Spinach			50	
	Peas				
	String beans				
IV. Toasted bread	2 slices			120	
V. Skimmed milk	1 glass (6 oz.)	80			450
or					or
VI. Light soups	(without vegetables) 6 oz.	20			390
Supper.					
Cooked fruits	Prunes or apricots			80	
1 boiled egg				80	
2 slices toast				120	
1 glass skimmed milk				84	364

Give no fats, greasy foods, sugar, butter, nor whole milk.

Courtesy of The Boston City Hospital.

Feces

Name..... Spec.....

Date.....

Quantity
Character
Macroscopic Exam.
Mucus
Parasites
Microscopic Exam.
Pus
Blood
Parasites
Blood (guaiac test)
Hydrobilirubin

Test Diet Feces
Quantity
Character
Macroscopic Exam.

Microscopic Exam.
Proteid
Carbohydrates
Fats
Fermentation test

REMARKS:

Signed.....

CHAPTER V

GASTRIC CONTENTS

Vomitus

All vomitus should be carefully examined and the amount, color, odor, and consistency noted. Tests for free hydrochloric acid may be made, but it is seldom used for chemical analysis. Microscopic examination should be made for necrotic shreds, red blood cells, and sarcinæ.

Gastric Contents

The contents should be obtained in the morning and about 12 hours after the last meal. Ewald's test breakfast of one slice of bread and 400 c.c. of water acts as a stimulant to acid production, and is given to test the absorptive and motor activity of the stomach.

One hour after the test meal the contents are obtained by means of the stomach tube, and this tube will pass more easily if it has previously been chilled in a receptacle filled with cracked ice.

Over 50 to 60 c.c. contents indicates some abnormality, as stasis or hypersecretion.

Character

Normal contents are thin and watery, sometimes bile-colored, frothy, and may contain mucus and cells.

Abnormal contents may be red from blood. Changed blood has the appearance of "coffee grounds." It may be cloudy from food and pus, and thick and ropy from mucin.

A few starch granules or fat droplets may be found in normal contents, but more than this indicates stasis.

Under normal conditions the gastric contents become acid 15 minutes after the ingestion of food, and this is due to the presence of free acid or acid salts, lactic acid, and traces of hydrochloric acid. Within an hour hydrochloric acid predominates and no lactic acid is found. The contents should be examined as soon as possible, as lactic acid readily develops if the contents are left in a warm place.

Hyper-acidity is an excessive degree of acidity.

Hypo-acidity is a deficiency of acid.

Hyper-chlorhydria is a large amount of hydrochloric acid secreted by the stomach cells.

Hypo-chlorhydria is a small amount or absence of hydrochloric acid.

Achlorhydria is the absence of combined acids.

Achylia Gastrica is the absence of hydrochloric acid and acid ferments.

Litmus indicates the presence or absence of acid elements.

The presence of free hydrochloric acid shows that the acid secreting power of the stomach is not destroyed. Its absence in fasting contents has no significance.

Congo Red Paper Test

Moisten the end of the congo red paper with the gastric contents, and if hydrochloric acid is present the red is changed to blue.

Topfer's Test

(Topfer's reagent is a 0.5% alcoholic solution of dimethyl-amido-azo-benzene.)

To 10 c.c. of gastric contents add 2 drops of Topfer's reagent. A carmine color indicates the presence of a free mineral acid.

Mucin

Filter 5 c.c. of contents, add a few drops of acetic acid. A white precipitate indicates mucin.

Gastric Contents

Congo Red Test for HCl

1 c.c. of gastric contents, 10 c.c. of water, and 2 drops of a concentrated solution of congo red in 50% alcohol. The presence of acid is indicated by a blue color.

Resorcin Test

Dissolve 3 grams of cane sugar and 5 grams of resorcin in 100 c.c. of alcohol.

Equal drops of this reagent and gastric contents are slowly evaporated to dryness in a porcelain dish. A rose red color indicates the presence of HCl.

Uffelmann's Test for Lactic Acid

Place 2 drops of carbolic acid and 6 drops of ferric chloride solution (U. S. P.) in a test tube and add water until the solution is a deep amethyst blue. Add 5 drops of gastric contents. The presence of lactic acid will be indicated by a lemon-yellow color.

Gunzburg's Test for Organic Acids

Place a drop of the contents in a porcelain dish and gently warm. As soon as it begins to evaporate add a drop of Gunzburg's reagent so it will just touch the other

drop; heat without burning. Red crystals appear at the zone of contact if free mineral acid is present.

Ferric Chloride Test for Lactic Acid

To 10 c.c. of gastric contents add 10 c.c. of ether, shake, then decant the ether extract.

Dilute the ferric chloride solution until the yellow color is almost lost, and to this add the ether extract. The zone of contact becomes yellow if lactic acid is present.

Tests for Food

Filter 10 c.c. of contents and add 1 drop of osmic acid. Fat droplets will stain black; a drop of Sudan III will stain the droplets red.

Add 1 drop of tincture of iodine to 10 c.c. of filtered contents. Starch granules, if present, will appear blue.

Test for Pepsin

Add sufficient HCl to give the test for free HCl. Boil an egg 5 minutes and place the coagulated white in a tube. Place the tube in the gastric contents and incubate ½ to 4 hours. The albumin will show signs of digestion if pepsin is present.

Test for Rennin

Neutralize 10 c.c. of gastric contents with NaOH, add 10 c.c. of milk, and incubate 10 to 15 minutes. The milk will coagulate if rennin is present.

Guaiac Test for Blood

To 15 c.c. of gastric contents add 5 drops of glacial acetic acid, and shake. Extract with 10 c.c. of ether and decant 5 c.c. Add 12 drops of gum guaiac solution (a

piece of guaiac the size of a pea dissolved in 70% alcohol), freshly prepared. Add 20 to 30 drops of hydrogen peroxide. A bluish color will result if blood is present.

Benzidin Test for Occult Blood

Boil the gastric contents, then cool. Add 2 c.c. of a saturated solution of benzidin in acetic acid to 10 c.c. of the cooled gastric contents. Shake, and add 30 drops of hydrogen peroxide. A bluish green color is positive.

When there is but small amount of material to test for acidity, use

$\frac{N}{1}$ NaOH for every 100 c.c. of material.

$\frac{N}{10}$ NaOH " " 10 c.c. " "

$\frac{N}{20}$ NaOH " " 5 c.c. " "

Tests for Common Poisons

Reinsch's Test for Arsenic

Acidify the vomitus or stomach contents with HCl, and allow to stand 1 hour. Filter through several layers of gauze. Immerse a brightly polished strip of copper foil in the filtrate and boil. A gray coating will appear on the copper if arsenic is present. Dissolve this in nitric acid and evaporate to dryness. Add a few drops of silver nitrate to the residue. A red precipitate indicates the presence of arsenic of silver.

Carbolic Acid

Acidify the gastric contents with HCl, filter, and add a few drops of bromine water. Carbolic acid gives a yellow precipitate.

Chloral

To 10 c.c. of gastric contents add 10 c.c. of ether. Shake, and decant the ether; add 1 c.c. of silver nitrate and 1 c.c. of ammonia. Boil 3 minutes. In the presence of chloral a mirror of metallic silver should form.

Corrosive Sublimate

Add 10 c.c. of ether to 10 c.c. of gastric contents; mix thoroughly. Decant the ether and concentrate it by evaporation. Add potassium iodide. The presence of mercury is indicated by a precipitate of iodide of mercury, and this is soluble in potassium iodide.

Hydrochloric Acid

To 10 c.c. of filtered gastric contents add a few drops of nitric acid and 2 c.c. of silver nitrate solution. Silver chloride appears as a white precipitate. As normal gastric contents contains HCl, make a control with 0.2% HCl solution and compare the density of the two precipitates.

Nitric Acid

The contents are neutralized with carbonate of potash. Filter and concentrate the filtrate by evaporation. Mix with an equal amount of sulphuric acid. Cool, then add a solution of ferrous sulphate. Nitric acid is indicated by the appearance of a black ring at the junction of the two fluids.

Oxalic Acid

Add caustic potash to the contents and boil. Potassium oxalate will form even if lime has been given to the patient. Filter and add calcium sulphate solution. A white precipitate indicates oxalate, and this is soluble in HCl, insoluble in acetic acid.

Phosphorus

To 10 c.c. of contents add 10 c.c. of water; acidify with sulphuric acid. Place this in a glass retort with a long condensing tube and distil in the dark. The condensing vapors are made luminous by the traces of phosphorus.

Prussic Acid

Neutralize the contents and distil slowly on a water bath. Add silver nitrate. Prussic acid gives a white precipitate, soluble in hot nitric acid, insoluble in cold. Prussian blue will form by adding liquor potassæ, hydrochloric acid, and a few drops of sulphate of iron.

Sulphuric Acid

Add a few drops of barium chloride to the filtered contents. A white precipitate indicates sulphuric acid, which is insoluble in HCl or HNO_3.

Alkaloids.—Otto's Method

Acidify the contents with dilute sulphuric acid to convert the alkaloid into sulphate. Filter through several layers of gauze. Mix with ether to remove fat. Pour off the ether and add small amounts of potassium carbonate to liberate the alkaloid. Mix with ether again, pour off the ether, and test as follows:

Belladonna

To 5 c.c. of the ethereal solution add a few drops of nitric acid and evaporate upon a water bath. Cool and add 1 drop of potash dissolved in absolute alcohol. The presence of atropin is indicated by the appearance of a violet color changing to red.

Opium

Dissolve 5 c.c. of the ethereal solution which has been evaporated to dryness with cold sulphuric acid. Add 1 drop of 20% bichromate of potash. A bright green color indicates the presence of morphine.

Strychnine

Add 2 drops of sulphuric acid to the ethereal solution which has been evaporated to dryness, and 2 drops of 20% bichromate of potash. Strychnine will give a purple blue color changing to red.

Examination of Fasting Contents. (12 hours' fast)

Amount.—Normally, 10 to 12 c.c.

Character.—Clear, sediment, sirupy, ropy.

Food Residue.—Macroscopic: Normal or excess of food, sediment. Microscopic: Epithelial cells, blood, bacteria, sarcinæ (indicative of stasis).

Blood.—Guaiac Test.

Reaction.—Litmus, phenolphthalein.

Free HCl.—Topfer's reagent.

Lactic Acid.—Fe_2Cl_6, ethereal extract.

Mucin.—Acetic acid.

Ewald's Test Meal

Wheat bread, 35 to 70 grams.

Water, 300 to 400 c.c.

Given when the stomach is empty, *i. e.*, as a breakfast.

Examination of contents.

Quantity.—50 to 150 c.c. 200 c.c. or more is abnormal, indicating hypersecretion or stasis. 500 or more suggests dilation of stomach, benign or malignant stenosis of the

pylorus. If well digested, there should be a layer of finely divided bread residue on the bottom of the glass containing the stomach contents, and over this a layer of semi-transparent gastric juice. If poorly digested, the contents will consist of fluid and many coarse lumps of bread.

Character.—The proportion of solids to liquids is 1 to 3. The proportion of liquids is much higher in hypersecretion.

Color.—Blood or bile.

Odor.—Normal (faintly sour) or fetid (rancid).

Reaction.—Litmus. HCl is decreased in many chronic cases: carcinoma of stomach and advanced gastritis; it is often absent in neurasthenic and hysterical individuals. The absence of HCl affords favorable opportunity for bacterial action in the stomach, and lactic acid is a very common product of this bacterial fermentation. Large amounts of lactic acid are suggestive of carcinoma, and is generally accompanied by the Boas-Oppler bacillus, which plays an important part in the lactic fermentation. This organism, which is identical with the Bulgarian bacillus, is found in 75 to 85% of the cases of carcinoma of the stomach.

Enzymes.—Pepsin, rennin. The absence of pepsin and rennin is less common than that of HCl. The estimation of pepsin is chiefly of value in cases suggesting an advanced lesion of gastric mucosa, and in which free HCl has been known to be absent.

Quantitative Analysis.—Method No. 1

Add 3 drops of Topfer's reagent to 10 c.c. of unfiltered contents. A bright red color indicates the presence of free HCl.

Titrate with N/10 NaOH until the red color disappears.

The number of grams of HCl in 1 liter of normal solution (=36.5 grams HCl) divided by 1,000 equals the number of grams of HCl in 1 c.c. normal solution.

This amount divided by 10 equals the amount of HCl in 1 c.c. of N/10 HCl.

Therefore, 36.5 divided by 10,000 equals 0.00365 gram of HCl in 1 c.c. of N/10 HCl.

0.00365 multiplied by the number of c.c. of N/10 NaOH used in titrating equals the amount of HCl in 10 c.c. of contents used. Normally, 0.01 to 0.14%. The amount of HCl is expressed in terms of N/10 NaOH solution which is necessary to neutralize 100 c.c. of gastric contents (normally, 30 to 40 c.c.). This number multiplied by 0.00365 equals the percentage of free HCl (normally, .01 to .14%).

Total Acidity

To the same 10 c.c. of gastric contents in which free HCl has been neutralized 3 drops of phenolphthalein are added.

Titrate with N/10 NaOH until a permanent red appears after stirring. The number of c.c. of N/10 NaOH which would neutralize 100 c.c. of gastric contents, including both the Topfer's and phenolphthalein titration (30 to 40 c.c. N/10 NaOH).

Multiply this by 0.00365 to find percentage of total acidity in terms of HCl (normally, 15 to 30%).

Tryptophane Test. (A. M. A., LV, 1910)

Four or five hours after a regular meal some contents are secured and filtered. Mix 5 c.c. of the juice with

sterile 2% Witte's peptone, add 1 c.c. of toluene, shake, and incubate at body temperature 24 hours. At the end of that time 3 or 4 c.c. of the mixture are taken and, if not acid, treated with a few drops of 3% acetic acid. Add a bromine water (saturated solution), drop by drop, until in case the reaction is positive, a reddish violet color appears.

This test is based on the fact that carcinomatous tissue contains an enzyme of stronger proteolytic power than pepsin, causing the appearance of amino acids, including tryptophane.

Blood and trypsin must not be present. (The latter may be regarded as absent if bile has not been detected.)

Quantitative Analysis of Gastric Contents.—Second Method

Topfer's Method for determining total acidity, combined acidity, and free acidity, also acidity due to organic acids and acid salts.

Total Acidity

Filter 10 c.c. of gastric contents and add 3 drops of 1% alcoholic solution of phenolphthalein (1 gram of phenolphthalein in 100 c.c. 95% alcohol).

Fill the burette with N/10 NaOH and take a reading. Titrate until a pink color appears which does not disappear on stirring and is not intensified by further addition of a drop. The free HCl is then neutralized in the contents used.

Take a reading of the burette. The number of c.c. used in titrating multiplied by 0.00365 equals the acidity of the 10 c.c. of the gastric contents in terms of HCl

(1 c.c. of N/10 HCl acid contains 0.00365 gram HCl acid).

EXAMPLE. 10 c.c. of the filtered gastric contents are neutralized by 8.1 c.c. of N/10 NaOH solution. As 1 c.c. of N/10 NaOH neutralizes 0.00365 gram of HCl, 8.1 c.c. will neutralize 0.00365 × 8.1 c.c., or .0295 gram of HCl, the amount in the 10 c.c. of gastric contents; and in 100 c.c. it would take ten times as much, or 0.295 gram HCl or per cent.[1]

Normally, the percentage is 0.07 to 0.18% (or 20 to 50 c.c. N/10 NaOH to 100 c.c. gastric contents).

Combined Acidity

Add 3 drops of 1% of an aqueous solution of sodium alizarin sulphonate to 10 c.c. of gastric contents. Take a reading of the burette. Titrate with N/10 NaOH until a violet color appears. Take a reading. This indicator used reacts to all acidities; therefore, in order to determine the amount of N/10 NaOH necessary to neutralize the combined acidity of 10 c.c. of gastric contents, subtract the burette reading from the reading obtained in the determination of the total acidity.

Free Acidity

Filter 10 c.c. of gastric contents and add 4 drops of di-methyl-amino-azobenzol. Take a reading of the burette. Titrate with N/10 NaOH until the red color is replaced by lemon-yellow. If the lemon-yellow appears as soon as the indicator is added, free acid is absent. This indicator reacts only to free acid, therefore the amount

[1] Calculated in per cent, *i. e.*, the amount in 100 c.c. = 0.295 per cent.

of N/10 NaOH used indicates the cubic centimeters necessary to neutralize 10 c.c. of gastric contents.

Acidity Due to Organic Acids and Acid Salts

Subtract the number of c.c. of N/10 NaOH used to neutralize the free acid in 10 c.c. of gastric contents from the number of c.c. used in neutralizing the combined acids. The remainder indicates the number of c.c. of N/10 NaOH necessary to neutralize the organic acids and acid salts in 10 c.c. of gastric contents.

Tryptophane Test.—Second Method

1. Test meal—water sweetened and bread. Remove in about 1 hour.
2. Incubate part, or let stand at room temperature 24 to 48 hours.
3. Filter contents—6 to 7 c.c. in a test tube.
4. Add a few drops of 3% acetic acid.
5. Add saturated aqueous solution of bromine, drop by drop, up to 4 drops from a pipette.

Result. Reddish violet or rose color shows tryptophane. If the color does not come, let stand 15 to 20 minutes. If negative, add more bromine solution, drop by drop, until mixture is yellowish or rose red. Let stand and reddish color grows deeper. Shake after each additional bromine.

References: Hawk's Physiological Chemistry; Hammarsten's Physiological Chemistry; Journal of the A. M. A., LV, 1910.

Stomach Contents

Name..... Spec.....

Date .....

Fasting Contents	Test Meal Contents
Quantity	Quantity
Character	Character
Food Residue	Reaction
Sediment	Free HCl
Mucin	Total Free HCl
Blood	Total Acidity
Reaction	Combined HCl
Free HCl	Pepsin
Lactic Acid	Rennin

Microscopical Examination:

Signed.....

CHAPTER VI

SPUTUM

The Sputum consists of saliva, buccal mucus, and the secretions from the respiratory passages.

Quantity

The amount expectorated in twenty-four hours varies. It is very large in advanced tuberculosis with cavity formation, and scanty in phthisis and bronchitis.

Consistency

Ordinarily, sputum is sticky, viscous fluid. It may, however, be purulent, serous, or bloody.

Muco-purulent is the most common and is often seen in pneumonia, bronchitis, and phthisis. It is not characteristic of any particular condition.

The sputum in phthisis, abscess of lung, or empyema is purulent, thick, green, or yellow.

Coin-like masses of pus, which sink in water, occur in the sputum in tuberculosis.

Dittrich's plugs are gray or yellowish masses, foul smelling, and are characteristic of septic bronchitis and gangrene of the lung.

Curschmann's spirals are yellowish coiled masses found in the sputum of bronchial asthma.

Color

Black or gray sputum is from carbon inhaled or due to food.

Rusty-colored sputum is common in pneumonia.

Greenish discoloration of the sputum is due to the activity of certain chromogenic bacteria.

Sputum discolored with streaks of blood may be due to coughing or vomiting.

Odor

Sputum is nearly odorless. If it has stagnated in the body it may have a putrid odor. On standing it may acquire a foul odor from decomposition. In tuberculosis it has a sweet, penetrating odor.

Reaction

Fresh sputum is generally alkaline; on standing it may become acid.

Microscopic Examination

The unimportant constituents are various kinds of epithelial cells, leucocytes, sometimes red blood cells, particles of food, and fungi.

The important elements are pus, blood, elastic tissue, animal parasites, and plant parasites.

A specimen should be stained and examined for bacteria associated with special diseases, as tuberculosis, pneumonia, etc.

Bacteria in Sputum. (Smith's Modification of Wright's Stain)

1. Make a smear, dry, and fix by passing through a flame. Cover with aniline-gentian violet 10 seconds, gently steaming. Wash in water.

2. Gram's iodine solution 30 seconds. Wash with 95% alcohol until color ceases to come out.

3. Wash with ether (to remove fat). Absolute alcohol 5 seconds.

4. Saturated aqueous solution of eosin 2 minutes.

5. Wash with Löffler's methylene blue, then after the excess of eosin has been removed, steam with methylene blue 5 seconds.

6. Wash with water, rinse with alcohol, and clear with xylol.

The pneumococcus is stained blue-black, while the capsule is stained pink. Influenza bacilli and other bacteria which do not stain by Gram's method are clearly brought out, also eosinophilic leucocytes.

The tubercle bacilli are slender, non-motile rods, occurring singly and in pairs, usually slightly curved. Branching and club-shaped forms are sometimes observed, also spherical granules. The bacilli have a thin capsule which contains the greater portion of the waxlike substance peculiar to the bacillus. Spread the material in a thin layer, dry, and fix by passing the slide through a flame.

Ziehl-Neelsen Method of Staining Tubercle Bacilli

1. Cover surface of the slide with carbol-fuchsin solution, steaming for 3 minutes over a Bunsen flame.

2. Wash in water.

3. Decolorize with 20% sulphuric acid until the red color disappears.

4. Wash thoroughly in water.

5. Wash in 95% alcohol for 30 seconds.

6. Wash in water.

7. Stain with Löffler's or Gabbet's stain. The tubercle bacilli are stained red.

There are many other acid-fast bacilli which have the

same staining reactions as the tubercle bacilli, but they differ in having no pathogenic power for guinea pigs and in growing readily on any media.

The tubercle bacillus is pathogenic, not only to man, but also to animals.

The guinea pigs are very susceptible and are much used for the detection of tubercle bacilli in suspected material. An intraperitoneal injection of a large dose will cause death in 10 to 20 days.

Antiformin Method of Making Cultures of Suspected Tubercle Bacilli.—Antiformin Mixture

Sodium carbonate	600 c.c.
Chloride of lime	400 c.c.
Distilled water	4,000 c.c.

Dissolve the sodium carbonate in 1,000 c.c. of distilled water, triturate the chloride of lime in the remainder of the water, filter, mix, and filter.

To make a 20% solution, take equal parts of the above mixture and caustic soda (15%).

To 10 c.c. of sputum add 250 c.c. antiformin mixture. Place in a sterile centrifuge tube, mix well, cork, stand at room temperature 24 hours, then centrifuge, washing five times with salt solution.

Pipette off the supernatant fluid.

Culture in egg media and glycerine agar.

Rapid Method. (Journal of Experimental Medicine, Vol. XXI, No. 1)

Fresh sputum is advisable. To 5 c.c. of sputum add 3% sodium hydrate, shake vigorously, and incubate 20 to

30 minutes until sputum is well digested. Neutralize to sterile litmus paper with normal hydrochloric acid; centrifugalize. Inoculate special media with the sediment. Growth obtained in one week.

NOTE.—It is very difficult to disinfect sputum, because the bacteria present are surrounded by a dense envelope of mucus. A 5% solution of carbolic acid is the most effective disinfectant.

Sputum

Name..... Date.....

Macroscopic Examination:

- Color
- Odor
- Consistence
- Blood
- Character—
  - Mucous
  - Muco-purulent
  - Purulent
  - Serous

Microscopic Examination:

- Blood Cells
- Pus Cells
- Casts
- Elastic Tissue
- Parasites
- Tubercle Bacilli
- Other Bacteria

REMARKS:

Signed.....

CHAPTER VII

THE BLOOD

The Blood is an opaque, viscous fluid, and the viscosity or measure of stickiness of the blood as it comes in contact with the vessel walls is increased by a meat diet and decreased by the abundant use of fluids. It consists of a colorless liquid, the plasma, containing erythrocytes, leucocytes, blood-plates, and blood-dust.

Color

Dark red, due to the presence of red blood corpuscles. The redder it is, the richer it is in oxyhemoglobin; the darker, the greater amount of reduced hemoglobin.

Pathogenic Changes in Color

Cherry red in coal-gas poisoning.

Brownish red or chocolate color in poisoning from aniline, potassium chlorate, hydrocyanic acid, and nitrobenzol.

Odor

The odor is characteristic and is due to volatile and fatty acids.

Taste

Salty and at the same time insipid.

Specific Gravity

This varies with the amount of hemoglobin and is influenced by age and sex, digestion, exercise, pregnancy, etc.

Average in adults is 1.058 to 1.062.

Amount

The total amount of blood in the normal adult is said to amount to $\frac{1}{12}$ or $\frac{1}{14}$ of the body weight.

Reaction

Alkaline, due to mono-sodium carbonate and di-sodium phosphate in solution in the blood.

Laked Blood

Laked blood or hemolysis is the dissolution of the blood corpuscles. The red blood cells are separated from the hemoglobin and rendered transparent by the action of certain substances, such as chloroform, ether, or water.

The use of a 0.3% acetic acid solution as a diluting fluid for the purpose of counting the white corpuscles lakes the red corpuscles, and it not only preserves the white cells, but it also makes their nuclei more distinct.

Erythrocytes

The number of erythrocytes or red blood corpuscles present in the fluid obtained from well-developed males in good physical condition is 5,500,000 per cubic millimeter, and the normal content of the blood of females is from 4,000,000 to 4,500,000 per cubic millimeter.

Their source is in the bone marrow. They are $\frac{1}{3240}$ of an inch in diameter. Microscopically they appear as non-nucleated biconcave discs of a pale hue.

Function

1. To carry oxygen from the lungs to the tissues.
2. To carry carbon dioxide from the tissues to the lungs.

The erythrocytes may be increased after transfusion, by residing in a high altitude, as a result of strenuous exercise, in starvation, after meals, after hot and cold baths, after the administration of certain drugs and radium, and accompanying certain diseases, such as cholera, diarrhea, yellow atrophy of the liver.

A decrease occurs in the different forms of anæmia (oligocythemia).

Polycythemia—an increase in the number of red blood cells.

Leucocytes

The leucocytes, or white blood corpuscles, differ from the erythrocytes in being larger and containing a nucleus. The normal number in the human blood varies between 5,000 and 10,000 per cubic millimeter. Microscopically they appear as white, spheric, ameboidal masses of protoplasm.

Function

1. To protect the body against pathogenic bacteria.
2. To aid in the absorption of the fats and peptones from the intestines.
3. To take part in the process of blood coagulation.
4. To help maintain the normal composition of the blood plasma as to proteids.

A leucocytosis is an increase in leucocytes. Physiological leucocytoces accompany pregnancy, parturition, and digestion, as well as those due to thermal influences.

Pathological leucocytoces occur in inflammatory, infectious, post-hemorrhagic, and toxic conditions.

Leucopenia

A diminution in the number of leucocytes, as in typhoid fever.

Blood Platelets

These are circular or oval discs, 2 to 3 microns in diameter. The average number is about 500,000 per cubic millimeter.

The origin and function of the blood platelets are unknown. There is some evidence that in shed blood they take part in the process of coagulation. Some authorities claim that they are independent cells, others that they are fragments of disintegrated cells.

Blood-dust

Hemokonia, or blood-dust, is one of the form elements held in suspension in the liquid called blood-plasma, and appears as small hyaline, refractive bodies.

Bacteriology of the Blood

Pyogenic and other organisms are often found by direct inoculation of ordinary culture media with blood.

Blood cultures are valuable in the following diseases: typhoid fever, pneumonia, pelvic diseases, endocarditis, local diseases of the throat, and malaria.

A vein in the arm is punctured under strict aseptic precautions. Scrub the arm 2 inches above and 2 inches below the elbow with soap and water, then alcohol. Insert a sterile needle in median or basilic vein. Use sterile test tube for receptacle.

Plant in fluid media or agar plates, or take 2½ c.c. of blood and add to 5 c.c. of sterile ox bile, and place this in a thermostat at 120° F. Then take 2 loopfuls of the blood ox bile and inoculate a tube of bouillon, and incubate over night.

If a motile bacillus develops, it is probably typhoid, and can be determined by a Widal test.

The Opsonic Index

Opsonin is the substance developed in the blood when the body is invaded by bacteria, and this substance increases the phagocytic activity of the leucocytes for this particular organism.

The opsonic index is the measure of this activity.

Blood Plasma

The intercellular substance of the blood.

Blood Serum

Blood serum is the light yellow fluid which exudes after the blood coagulates and the clot forms. It is the plasma deprived of its fibrin.

Coagulation

Normal coagulation occurs in about 3 to 5 minutes. The clotting of blood depends upon the conversion of fibrinogen into fibrin.

Method of Determining the Rate of Coagulation

Puncture the lobe of the ear, then take a piece of capillary glass tubing and holding it downward from the puncture let it fill for 3 or 4 inches. At intervals of

30 seconds scratch the capillary tubing at short distances and break off between the fingers.

When coagulation has taken place, a long, worm-like coagulum is formed.

Color Index

In normal blood this is approximately 1. To obtain the color index divide the percentage of the hemoglobin by the percentage of red blood cells, 5,000,000 red cells being considered as 100%. To obtain the percentage of red cells, multiply the two extreme figures to the left by 2. EXAMPLE. A count shows the presence of 2,400,000 red cells: the percentage would be 48 (24×2). The hemoglobin percentage is 72; then the color index would be 72 divided by 48, or 1.5.

Sahli's Hemoglobinometer for Obtaining the Percentage of Hemoglobin in the Blood.—Method

First wash off the lobe of the ear with alcohol, then take hold of the lower end with the thumb, first and second fingers. Insert a glover's needle quickly. Wipe off the first drop of blood. The second drop is drawn up by suction into the pipette to the 20 cmm. mark.

Pour N/10 HCl into the graduated tube to the mark 10 on the scale of the tube, then add to this the 20 cmm. in the pipette. When the mixture assumes a clear, bright color, add distilled water, drop by drop, until the color matches the color in the standard tube.

Talquist's Hemoglobin Scale

This is a book of specially prepared filter paper, with a scale of ten shades of blood colors. These are so tinted

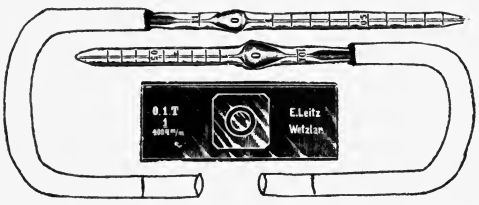
as to match blood taken up on a piece of filter paper, and are graded from 10 to 100. The comparison should be made as soon as the blood on the filter paper has lost its humid gloss.

Oligochromemia

A diminution in the normal amount of hemoglobin.

Hemacytometers or Blood Counters

These instruments are used for determining the numerical ratio between the red and white corpuscles contained



HEMACYTOMETERS

in the human blood. Undiluted blood, owing to the excessively large numbers of blood discs contained in a single cubic millimeter, is unsuitable for counting, and it is necessary to dilute the blood in certain proportions. A definitely determinable dilution is obtained with the aid of the mixing pipettes.

The melanger or large pipette is used for counting the red corpuscles, and gives a dilution of 1:200.

Method

Cleanse the lobe of the ear with alcohol and insert a sterile glover's needle or a straight Hagedorn needle by a rapid puncture. The first drop of blood should be wiped

away, and as soon as the blood is flowing freely, put the point of the pipette into the drop as it emerges from the ear; by sucking gently on the rubber attached to the other end, draw up the blood to the mark 0.5 on the pipette. Wipe the end of the pipette, then plunge the point into Gower's solution and fill the bulb up to the mark 101. Thoroughly mix the blood by rolling the pipette between the palms.

The small pipette is used for counting the white corpuscles and gives a dilution of 1:20. The bore of this tube being large, it fills and empties more readily, therefore it should be kept in a horizontal position. Draw the blood up to the mark 0.5 on the pipette, wipe the end of the pipette, then plunge the point into 0.3% acetic acid, and fill the bulb up to the mark 11.

When the white count is very high, the red counter may be used, diluting the blood 200 times instead of 20, and this must be remembered when determining the final count.

Clean the pipettes with water, then alcohol and ether. The mouthpieces attached to the rubber tubing should be boiled often and kept in 95% alcohol.

The Counting Chamber

The number of corpuscles contained in a cubic millimeter of the dilute solution of blood is determined by counting in a cell of known depth and area. The counting chamber consists of a plane glass plate with a circular hole, which being cemented to a plane slide of stout glass forms a well. The bottom of the latter is formed by a disc, the thickness of which is exactly 0.1 mm. less than that of the perforated glass.

The counting chamber consists of 16 squares of $\frac{1}{4}$ mm. side, each of which is again divided into 16 small squares having sides $\frac{1}{20}$ mm. The area of each of the small squares is $\frac{1}{400}$ square millimeter, and the distance between the floor and the lower surface of the cover is $\frac{1}{10}$ mm.; therefore, each square represents $\frac{1}{4000}$ cubic millimeter.

Adjusting a Drop of Blood

1. Place the cover glass (an optically plane glass) in position; then by slightly pressing with the rubber end of a pencil, concentric rainbow rings will be seen. These are known as Newton's rings, and must remain after pressure is removed, otherwise there is dust under the glass.

2. Blow out 3 drops of the diluted solution in the pipette.

3. Place a drop upon the surface of the counting chamber of such a size that when the cover glass is let down over it the whole of the disc is covered without any being spilled into the moat around it.

4. Place the cover glass over the drop before the corpuscles have time to settle.

5. After waiting a minute for the corpuscles to settle, if the distribution seems uniform the counting is begun.

Register for Recording the Number of Blood Cells Counted

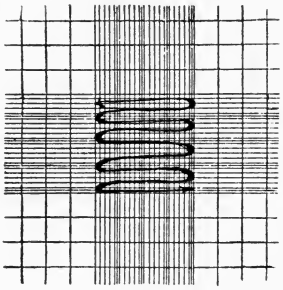
Place the index finger of the left hand through the ring, holding the Register in position so as to bring the pusher directly under the thumb. In commencing to count, only the 0's should appear; thus, 000. The Register will count to 999; the next count causes the 000's to appear, making

1,000. To set the Register at 0, press the pusher down the required number of times until the 0 appears at the right, and then change the other figures at the left by turning the thumb buttons on back of the Register till the 0's appear, always turning the thumb button of middle dial first.

To clean the counting chamber, rinse in cold water and dry carefully.

Counting the White Blood Corpuscles

Count all the corpuscles in the 400 small squares, ruling



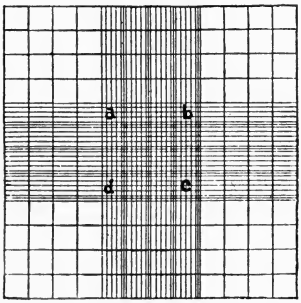
out those touching the outside lines. Count four separate drops, then divide by four to get an average; multiply the result by the dilution ($\frac{1}{20}$), then by 4,000 (each square is equivalent to $\frac{1}{4000}$ of a cubic millimeter), and divide by 400 (the number of squares counted). The result will be the number of corpuscles per cubic millimeter.

EXAMPLE. Average number of corpuscles in four drops is 45.

$$\frac{45 \times 20 \times 4{,}000}{400} = 9{,}000$$

Counting the Red Corpuscles

Count all the corpuscles in the 25 small squares, beginning at the upper left-hand corner (*a*), counting the 25 small squares, then the upper right-hand corner (*b*), the



lower right-hand corner (*c*), and the lower left-hand corner (*d*), making a total count of 100 small squares.

The sum of all the corpuscles in the 100 small squares is multiplied by the dilution ($\frac{1}{200}$), then by 4,000, and divided by 100 (the number of squares counted).

EXAMPLE. The number of corpuscles counted in 100 squares is 685.

$$\frac{685 \times 200 \times 4{,}000}{100} = 5{,}480{,}000$$

Vital Staining of Blood. (Journal A. M. A., March, 1915)

Stain fresh without fixation. The stains may be dissolved in physiological salt solution, Ringer-Locke solution, or even directly in the blood itself. The blood is mixed with the stain and examined immediately, preferably on a warm stage.

The staining solution may be placed on the glass, the

margin being sealed with paraffin or balsam, to prevent evaporation.

In typical vital staining the nucleus is not stained, only certain granules and fibers in the cytoplasm taking the stain. As the cell begins to die, "post-vital staining of whole or parts of the cells may occur. Lymphocytes are recognized by the size and shape of the nuclei; neutrophile leucocytes, by their fine granulations; eosinophiles, by their coarser granules.

Among the stains used in vital staining are methyl violet, neutral red fuchsin, toluidine blue, thionine, and Nile blue; 0.1 c.c. of any one of these stains to 250 c.c. of water or salt solution.

Blood Parasites

(*a*) Trichinella, found in cases of trichinosis.

(*b*) Filaria bancrofti.

(*c*) Filaria sanguinis hominis. This parasite is found in the blood during the night, and causes dilatation of the lymphatics, chyluria, abscesses, and elephantiasis.

(*d*) Trypanosomes, found in cases of sleeping sickness.

Modification of Ringer-Locke Solution

Sodium chloride	6. c.c.
Calcium chloride	0.2 c.c.
Potassium chloride	0.4 c.c.
Sodium carbonate	0.2 c.c.
Grape sugar	1.0 c.c.
Distilled water	1,000. c.c.

In studying mitochondria, or small, rod-shaped granules in the cytoplasm of leucocytes, a $\frac{1}{10000}$ solution of Janus green is used. The Janus green (diethylsafraninazo-dimethylanilin) is dissolved in 0.85% salt solution.

Mechanical Stage

This stage is a great aid to exact work in differential counting. It is attached to the stage of the microscope by two set screws. By rack and pinion giving equal speed in both movements, right and left scale reads 60 mm.; backward and forward, 110 mm. It is provided with one adjustable and one fixed stop, which is actuated by a spring to hold the slide in place.

Erythrocytes.—Wright's Stain

a. Normal red blood cell.

b. Microcyte, a small red blood corpuscle.

c. Crenated cells. If the density of the plasma is increased in any way, as by evaporation, many of the red cells become shrunken and crenated by the passage of water out of the corpuscle.

d. Macrocyte, a large red blood corpuscle.

e. Poikilocyte, a malformed, pear-shaped red cell.

f. Red cells showing degenerative changes.

g. Normoblast, a nucleated red cell of the ordinary size.

h. Megaloblast, a large nucleated red cell.

i. Amitosis, or direct cell division.

j. Free nucleus.

k. Polychromatophilia, various stains or tints.

l. Achromia, absence of color.

m. Stippled cells, characteristic of lead poisoning.

n. Blood platelets.

o. Karyokinesis, mitotic or indirect cell division.

p. Malarial organisms.

Leucocytes.—Wright's Stain

a. Lymphocyte.

b. Large mononuclears.

c. Polymorphonuclear neutrophiles.

d. Eosinophiles.

e. Mast cells.

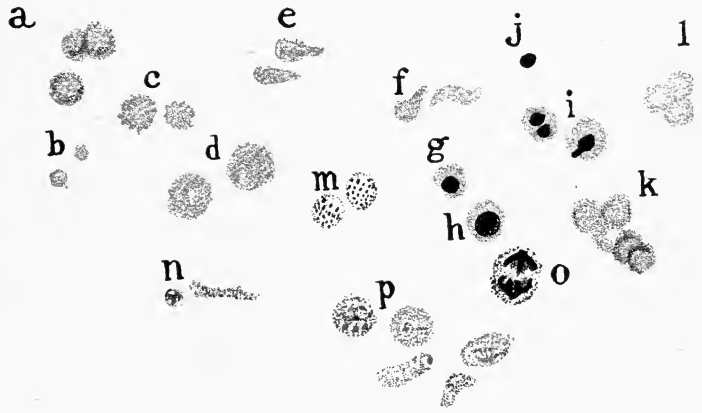
f. Myelocytes, neutrophilic.

g. " eosinophilic.

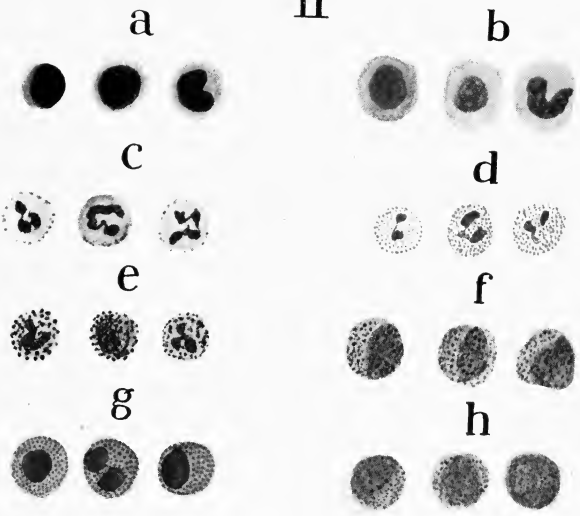
h. " basophilic.

NOTE.—Neutrophilic granules are purple; basophilic, dark blue; acidophilic, red.

I



II



Leucocytes.—Wright's Stain

Lymphocytes

These cells are about the size of a red blood cell, and are derived from the lymph glands. They have a large nucleus, with small margin of protoplasm. The nucleus stains intensely, while the protoplasm is light blue and free from granules.

Large Mononuclear.—Endothelial Cell.—Transitional Cell

Authorities disagree in regard to the classification of this cell. It is the parent of the polymorphonuclear neutrophile and derived from the bone marrow. It has a large amount of protoplasm free from granules, and usually has a large nucleus.

Polymorphonuclear Neutrophiles

These cells have a multiple, irregular-shaped nucleus. The nucleus stains deeply and the protoplasm is dotted with neutrophilic granules. They are derived from the neutrophilic myelocytes of the bone marrow.

Eosinophiles

These cells have an irregular-shaped nucleus, and the protoplasm is covered with coarse acidophilic granules which are highly refractive. They are formed from the acidophilic myelocytes of the bone marrow.

Mast Cell

The nucleus of this cell is multiple and irregular, and the protoplasm is covered with large basophilic granules. They are formed from the basophilic myelocytes.

Pathogenic Leucocytes

Myelocytes

NEUTROPHILIC.—The nucleus is large and irregular, and the protoplasm is covered with neutrophilic granules.

EOSINOPHILIC.—This cell has a large oval nucleus, and the protoplasm is covered with eosinophilic granules.

BASOPHILIC.—The nucleus of this cell is large and the protoplasm is covered with small basophilic granules.

Leucocytosis

Increase of leucocytes in the blood. It occurs normally during digestion and in pregnancy; pathologically, in fever, traumatic anemia, and in conditions of inflammation.

Lymphocytosis

Abnormal number of lymphocytes. This condition occurs in diseases of infancy, hereditary syphilis, splenic tumors, and pertussis.

Anemia

A condition in which the quality and quantity of the blood is deficient.

Myelogenous Leukemia

A disease of the bone marrow. Marked increase in myelocytes and enlargement of the spleen.

Lymphatic Leukemia

A disease of the lymphatic organs, with a marked increase of lymphocytes.

Smears for Differential Count

Cleanse the ear lobe with alcohol, and after puncturing it with a sterile needle wipe off the first drop. A clean slide is held without touching its surface, and touched to the summit of the drop as soon as it emerges. This drop is then drawn out smoothly over the glass with the edge of another slide.

The slide should not come in contact with the skin while it is being charged with the blood, and no pressure should be used in spreading the drop.

Dry quickly by waving the slide in the air.

Cover the smear with Wright's stain for 1 minute.

Add same amount of water to the stain, let this remain 3 minutes, then wash the slide with water.

A differential count of 400 cells should be counted to secure reliable results. The percentage value of each variety is then figured.

Normal varieties and figures in 1 cmm. of blood:

Polymorphonuclear neutrophiles	65–70%
Lymphocytes	20–35%
Transitionals—large mononuclears	3– 5%
Eosinophiles	2– 4%
Mast cells	½%

While making the differential count, the variations in size of the red blood cells, achromia, and polychromatophilia, and the number of blood platelets should be noted.

Oxydase Reaction in Myelogenous Leukemia

(J. of Pathology and Bacteriology, Cambridge, Eng., Vol. XV, 1911)

This test depends on the production by active oxygen

of an insoluble dye from a combination of two soluble substances.

The two reagents used are 1% solution of alpha-naphthol and 1% solution of dimethyl-para-phenylene-diamine.

These solutions, when brought together in aqueous solution in presence of an alkali, become slowly oxidized even in air with the production of a blue dye, indophenol blue; which, being insoluble in water, precipitates.

The addition to the mixture of an oxidizing agent greatly accelerates the dye formation, and the oxidizing ferment of leucocytes brings it about with great rapidity.

A brief fixation in 1% osmic acid for 5 minutes, or alcohol for 10 minutes, gives a more satisfactory result.

Method

Equal parts of 1% aqueous solution of the two reagents are mixed and the film is immersed in the mixture for 10 to 20 minutes (face downward).

If the film is then washed in water and examined in water or glycerin, the granular leucocytes are seen stained a dark opaque blue, from precipitation of indophenol in their protoplasm, while the nuclei remain unstained.

Red corpuscles and lymphocytes show no reaction.

Blood with a leucocytosis of over 20,000 per cubic mm. reacts positively.

This property, which seems to belong only to large mononuclears and granular leucocytes, is used to differentiate myelogenous from lymphatic leukemia.

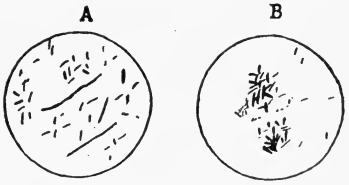
Blood Platelets.—Counting. (Journal A. M. A., May 20, 1911)

The blood is diluted 1 : 100 in the pipette used for counting red blood corpuscles. The diluting solution consists of 2 parts of an aqueous solution of brilliant cresyl blue (1 : 300) and 3 parts of aqueous solution of potassium cyanide (1 : 1400), and these solutions are kept in separate bottles and mixed immediately before using. The cresyl blue solution should be kept on ice and the cyanide solution should be made up every ten days; the mixed solution should be filtered before using.

The ordinary blood-counting chamber is used; count with the high dry objective. After the chamber is filled, it is left at rest for 10 minutes, in order that the platelets may settle.

Agglutination Test

One of the properties of the blood is the power of agglutinating, or clumping and rendering immobile the bacteria which it may be called upon to attack. This property also, though present at all times, is capable of development and increase, as is seen in the course of various diseases; as, for example, in typhoid fever, where the specific agglutinins of the patient's blood are so developed as to show a clumping of typhoid bacteria when the patient's serum and a culture of typhoid bacteria are brought together.



WIDAL REACTION

A—B. typhosus before adding typhoid blood. *B*—A typical reaction; the bacilli collect in clumps, *i. e.*, become "agglutinated."

First cleanse the lobe of the patient's ear with alcohol, then make a small incision with a needle or stilette, letting the blood flow into a capillary pipette. When this clots, the serum is blown out on the loop of a sterile platinum wire and added to the fluid culture. To obtain a fluid culture of typhoid bacilli, make a transplant from a culture on agar-agar to bouillon, and place in the incubator over night.

Paratyphoid Bacilli

These bacilli differ from typhoid bacilli by producing gas in glucose media and by showing different agglutination reactions. The two types generally recognized are known as "A" and "B."

Type "A," in all other respects, is like the typhoid bacillus.

Type "B" makes milk alkaline, but does not coagulate it, and after eight or ten days it becomes translucent.

Bacillus Coli Communis

This bacillus differs from typhoid as follows:

1. It is not motile.
2. Produces gas in media containing glucose.
3. Changes the blue color of litmus milk media to a pink color, and usually coagulates the milk.
4. Does not show the clump reaction.

Agglutination Test.—Widal Reaction

Take 9 drops of the fluid culture with a sterile platinum loop and place at separate points on a glass slide. Add a loopful of patient's serum and mix all together. Cover with a cover glass and note the time (this makes a dilu-

tion of $\frac{1}{10}$). Then take 1 loopful of the $\frac{1}{10}$ and add to the 4 drops which have been placed on the opposite end of the slide, and mix. This makes a dilution of $\frac{1}{50}$. Label and note the time.

If before the end of 1 hour the great majority of the bacilli are both clumped and motionless in the $\frac{1}{10}$ dilution, the reaction is only suggestive; but if in the $\frac{1}{50}$ dilution

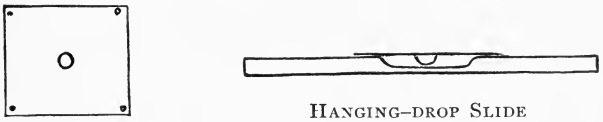
A—9 drops of bouillon culture 12–48 hours old.

B—4 drops from the same bouillon.

agglutination occurs, it is practically conclusive evidence of typhoid infection. It takes from 4 to 6 hours for the complete change to occur, and no less than six bacteria must become agglutinated to constitute a positive reaction.

Hanging-drop Slide

Place drops of oil at the four corners of the cover glass. Take 1 loopful of the $\frac{1}{10}$ or $\frac{1}{50}$ dilution, place in center



HANGING–DROP SLIDE

of the cover glass, and then place the glass drop-side down over the depression of the hanging-drop slide.

As a control, take 3 drops of the bouillon culture and place on a slide free from serum, keeping it under observation during the experiment. The bacilli must not clump and must remain motile.

Detecting B. Typhoid in Small Amounts of Blood.—Liebermann's Method. (Journal A. M. A., March, 1915)

Draw 2 drops of blood into 1 c.c. of distilled water. One drop represents 0.05 c.c. of serum and the dilution is equal to $\frac{1}{20}$.

To this add 1 c.c. of saline and continue diluting with different amounts of saline, making up to 1 c.c. each time.

Koenigsfeld's Method. (J. A. M. A., March, 1915)

Two kinds of medium are used, Endo's and Drigalski-Conradi's; mannite is used in the latter instead of lactose. Bile is added to each tube, and this, on top of the slanting solid medium, is inoculated with 3 to 4 drops of blood.

The bile has a tendency to check the development of all except typhoid bacilli. The colonies develop in 10 to 14 hours.

The colon bacillus turns both tubes red; acid-forming cocci turn the Endo media red, but leaves the mannite tube colorless.

B. typhosus develops rapidly and permits an early agglutination test.

Wassermann Reaction.—Obtaining the Blood

The arm is scrubbed with alcohol 2 inches above and 2 inches below the elbow. Apply the tourniquet, but do not obliterate the pulse. Insert the needle in the basilic vein and withdraw 10 c.c. of blood. Place this in the ice chest to coagulate.

Pipette off 2 c.c. of the clear serum and incubate at 56° C. for 1 hour.

It has been found that if the blood serum of a case of

syphilis is mixed in the presence of complement with extracts of syphilitic liver or alcoholic extracts of normal organs, the complement will be fixed and prevented from taking part in the subsequent hemolitic reaction.

Three distinct substances are required to form a complete reaction:

1. A cell to be destroyed or a poison to be neutralized.
2. A substance capable of destroying the cell or neutralizing the poison (amboceptor or antibody).
3. A completing substance or the complement, without which the cell cannot be destroyed or the poison neutralized.

Wassermann Test.—(Noguchi's Serum Diagnosis of Syphilis.)—Apparatus Needed

6 pipettes graduated 0.1 c.c.
2 pipettes, 10 c.c.; graduated 0.1 c.c.
6 pipettes, 1 c.c.; graduated 0.01 c.c.
24 small test tubes, 10 x 1 cm.
2 flasks (100 c.c.).
Test tube racks with parallel rows of holes.
Glass tubing, $\frac{3}{8}$-inch bore.

Method. Place 8 c.c. of normal salt solution in a flask; allow 2 drops of blood from the ear or finger of a normal person to drop into the flask (1 drop of blood to every 4 c.c. of salt solution). Place in the refrigerator over night. The cells gravitate to the bottom and are washed with an excess of salt solution. The supernatant fluid is poured off and replaced with fresh salt solution. Centrifuge 4 times, shaking the tube after each addition of fresh salt solution. A 1% suspension of washed corpuscles is used.

The Test. Place 1 tube for each test in the front row and 1 tube for its control in the rear row.

Add 1 drop of serum to be tested (0.02 c.c.) from a capillary pipette. 0.1 c.c. of 40% fresh Guinea-pig serum, made by adding 1 part of complement to 1½ parts of normal salt solution, is added to each tube. Where fresh complement cannot be obtained, dried slips of paper, each containing 2 units of complement, may be substituted. To the first tube add the slip bearing the antigen.

1 c.c. of a 1% suspension of human corpuscles is added to both tubes. Shake thoroughly at intervals.

Control. To each tube of the positive control add 1 capillary drop of a syphilitic serum known to give a positive reaction.

To each tube of the negative control add 1 capillary drop of normal serum known to give a negative reaction.

Add complement to each tube.

Antigen only is placed in the tubes of the front row.

1 c.c. of the corpuscle suspension is now added to each tube and the rack is placed in the incubator.

An hour is allowed for the antibody to combine with the antigen and for the complement to be fixed.

When the dried paper is used, a longer period of incubation is necessary.

On the opposite page the plate shows the different stages of the test.

The top rack shows:

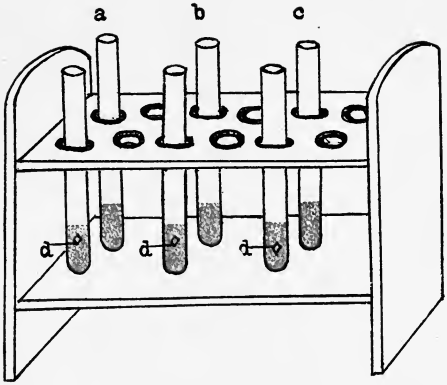
a. Two tubes for diagnosis.

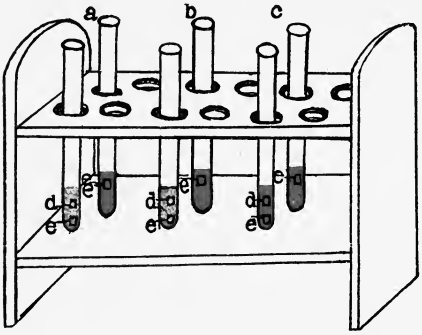
b. Two tubes for positive control.

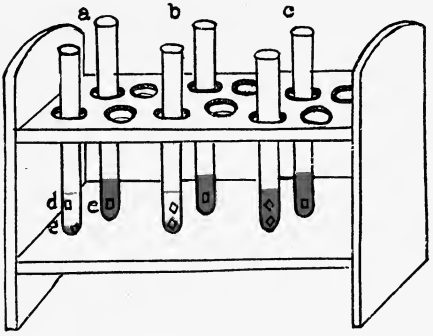
c. Two tubes for negative control.

d. Antigen.

The contents of the tubes are as follows:


a
b
c
d
d
d


a
b
c
d
e
d
e
d
e


a
b
c
d
e

Front: Test serum plus complement (2 units) plus antigen plus corpuscle suspension (1 c.c.).

Rear: Test serum plus complement (2 units) plus 0 plus corpuscles (1 c.c.).

Appearance of the tubes after the first incubation (1 hour at 37° C.).

The middle rack shows:

d. Antigen.

e. Amboceptor.

The two pieces of paper in the front tube represent antigen and amboceptor. In the rear tube the one piece represents amboceptor.

Appearance of the tubes after the second incubation, with the addition of the anti-human amboceptor slips.

Hemolysis occurred in both tubes of the negative set. In the positive control tubes it took place in the rear tube only, the same as in the two tubes for diagnosis; therefore the reaction is positive and the serum is syphilitic.

The bottom rack shows:

The appearance of the tubes after several hours.

The absence of hemolysis in the front tubes for diagnosis and positive control means positive reaction.

Antigen

Mash a liver, heart, or kidney of man, ox, guinea pig, rabbit, or dog, and extract with 10 parts of absolute alcohol at 37° C. for several days. Filter through paper and collect filtrate.

Dry by evaporation with an electric fan. Take up the residue with a small quantity of ether and add 5 volumes of acetone. After the precipitate settles, decant off the supernatant fluid.

The dark brown insoluble residue contains antigen lipoids, and the strength of this is estimated by titration.

METHOD. Dissolve 0.2 gram of the residue in 5 c.c. of ether, then make an emulsion by adding gradually 100 c.c. of physiological salt solution. Filter, if necessary, to remove any precipitate.

Front row of tubes: 0.04 c.c. complement plus 1 c.c. blood suspension plus 1 drop positive syphilitic serum.

Rear row of tubes: 0.04 c.c. complement plus 1 c.c. blood suspension plus 1 drop of normal serum.

Add decreasing doses of antigen emulsion to be tested in both sets.

Incubate at 37° C. for 1 hour, then add 2 units of amboceptor. Incubate 2 hours.

EXAMPLE:

Amount of antigen emulsion in c.c.	*Front row* *Inhibition of hemolysis through fixation phenomenon*	*Rear row* *Inhibition of hemolysis due to the binding property of antigen alone*
0.4	Complete inhibition.	Slight inhibition.
0.3	" "	Much "
0.2	" "	Partial "
0.1	" "	None }
0.07	" "	None } Complete hemolysis.
0.05	" "	None }

The quantity which has given complete inhibition with the syphilitic serum, but no inhibition with the normal serum, is the one to use in the following reactions.

Dilute the antigen in salt solution so that 1 c.c. shall contain the amount required.

EXAMPLE. 0.05 c.c. is wanted: then mix 0.5 c.c. with 9.5 c.c. of salt solution. Add 1 c.c. of this to each tube in the test.

Preparation of Complement Slips

Serum is obtained from large guinea pigs. Cut the carotid artery and collect the blood in a large, flat dish. Cover this and leave at room temperature for 4 hours, then place in the refrigerator. Place squares of thick blotting paper in a sterile, flat dish, and pour the serum over it until the paper is soaked and an excess remains.

Remove the paper to another dish and quickly dry in a current of air at a temperature not above 10° C.

Standardize in the following manner: In a series of tubes, each containing 1 c.c. of erythrocyte suspension and 1 unit of amboceptor, add bits of complement paper (5 mm. width strips) of increasing length, 2, 3, 5, 7, 10, and 15 mm.

Incubate at 37° C. for 2 hours.

The tube in which hemolysis is just complete contains 1 unit of complement.

The fixation test requires 2 units, therefore the remaining paper is measured off into squares having twice the dimension of that bit found for 1 unit.

These slips should not be used if it is possible to obtain the fresh guinea-pig serum; and if the fresh serum is used, titrate the complement, using for the test double the quantity necessary to produce complete hemolysis in 1 c.c. of a 5% emulsion of blood cells in the presence of 2 units of amboceptor.

Amboceptor

This is made by immunizing rabbits against human blood cells. The human blood corpuscles are collected in a sterile centrifuge tube. Fill the tube two-thirds full of 0.85% salt solution. Centrifugalize 3 minutes, then decant

the salt solution; add more, and repeat the process 3 times. Increasing amounts are injected in large rabbits intraperitoneally in the following manner:

1st	injection	5 c.c.	washed	human	corpuscles.
2d	"	8 cc.	"	"	"
3d	"	12 c.c.	"	"	"
4th	"	15 c.c.	"	"	"
5th	"	20 c.c.	"	"	"

Injections are made at 4 or 5-day intervals. Nine or 10 days after last injection bleed the rabbit from the carotid artery. Place the blood collected at room temperature for 4 hours. Collect the clear serum by decantation and leave the clot for another 24 hours. Repeat this for 3 days, or until no more serum is given out by the clot. Mix the portions of serum collected. 0.001 c.c. of serum or less, which will cause complete hemolysis of 1 c.c. of 1% suspension of human erythrocytes plus 0.02 c.c. of guinea-pig serum, will equal 1 unit.

Amboceptor Slips

Cut thin filter paper (Schleicher & Schull, No. 590–597) into squares, 10 x 10 cm., and soak in the serum; absorb the excess with another sheet of paper. Dry at room temperature (place the squares on a sheet of unbleached muslin).

When thoroughly dry, cut into 5 mm. widths and standardize.

Method. Take 6 tubes and add 1 c.c. of the erythrocyte suspension, 0.02 c.c. complement, and then add increasing lengths of the amboceptor strip, *i. e.*, 1 mm., 2 mm., 3 mm., 4 mm., 5 mm., 6 mm. Incubate 2 hours.

The smallest strip which causes complete hemolysis at the end of the 2 hours contains 1 amboceptor unit.

Mark the strip in sections of twice this length and cut off when doing the test. Keep the strips dry and in a sealed receptacle.

Definition of Terms

Agglutinins

When bacteria or foreign blood corpuscles are injected into an animal, a new property is developed in the serum of that animal; and this serum, when deprived of its own complement, by inactivation or dilution, is capable of clumping.

Amboceptor

One of two active principles necessary to cause hemolysis, bacteriolysis, or any cytolysis caused by serum, the other active principle being complement.

Antibodies

Immune body is a synonym of antibody. Antibodies possess specific affinity for the antigens which are used for their production. A group of antibodies is capable of producing antibodies when injected into another animal, thus forming anti-antibodies.

Anti-complementary Action

Substances capable of reducing or removing the action of the complement.

Antigen

Substances which have the power of producing specific antibodies, as bacteria and blood corpuscles, which are

antigens because they produce specific antibodies called amboceptor and agglutinins. Diphtheria toxin is an antigen, and injections of it are followed by a specific antitoxin.

Complement

One of the two active principles necessary for hemolysis. The other principle is called amboceptor, and is unable to cause a dissolution of cells without the first; hence the term complement.

Cytolysis

Dissolution of cells by specific amboceptors and complement.

Hemolysis

Dissolution of blood corpuscles by various forces, setting the hemoglobin free into the medium in which corpuscles are suspended. Distilled water will cause hemolysis, also acid and alkalies. Hemolysis by serum is somewhat different, and is caused by two distinct groups of substances, complement and amboceptor, both contained in the serum, and the one is inactive without the other.

Immune Bodies

Synonymous with antibodies.

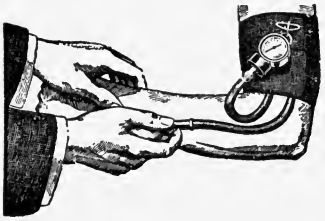
Inactivation

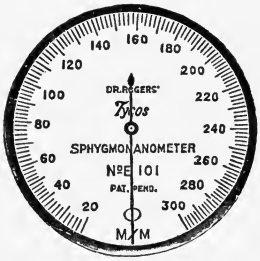
When fresh serum, which contains both amboceptor and complement, is heated at 55° for ½ hour, it becomes inactive, because of the destruction of the complement. The amboceptor is not affected materially by the process.

Blood Pressure

Pressure is generally applied to the brachial artery by means of the arm band.

The blood pressure is variable, especially that in the arteries. This pressure is expressed as being equal to so





"Tycos" Blood Pressure Apparatus

The gauge measures pressure from 0 to 300 millimeters of mercury and the dial is movable. The needle should be adjusted at the 0 mark before taking the pressure. The dial is graduated to read in millimeters and fifths of millimeters.

many millimeters of mercury, and by this expression is meant that the pressure within the artery is able to support a column of mercury that many millimeters in height.

Systolic Pressure

The systolic pressure is the maximum pressure caused by the systole of the heart, or the apex of the pulse wave.

Diastolic Pressure

This is the minimum pressure in the artery—the pressure at the end of the diastole of the heart or at the bottom of the pulse wave.

Under normal conditions the systolic pressure in the adult, expressed in terms of a mercury column, equal

110–115 millimeters, while the diastolic pressure is only 65–75 millimeters. Maximum pressure during the first years of life varies from 75–90 mm. Hg. The pulse pressure is the difference between the systolic and diastolic pressure. The pulse pressure in the artery averages 45 millimeters of mercury.

The mean pressure is obtained by dividing the sum of the systolic and diastolic pressure by 2, or by adding half of the pulse pressure to the diastolic pressure. Pulse pressure is the difference between the systolic and diastolic pressure.

Palpatory Method

Find the pulse at the wrist of the arm to which the arm band has been applied, and while the pulse is under observation raise the pressure with the hand bellows or pump until the pressure within the constricting band is sufficient to prevent the im-pulse from reaching the wrist. The position of the arrow on the face of the dial at the instant when the pulse passes the compressing band will represent the systolic pressure. Readings should be made at the return of the full pulse.

Auscultatory Method

This method is used where there is no diastolic fluctuations. Instead of feeling the pulse, a pulse tone caused by pressure of the constricting cuff is listened to through the stethoscope. Raise the pressure to the obliteration of the pulse, then place the stethoscope over the brachial artery below the cuff. The pressure is gradually allowed to fall and a pulse tone is heard as the circulation commences, and this tone undergoes a number of changes

until it becomes very faint and almost disappears. The reading of the sphygmomanometer at this moment represents the diastolic pressure.

Factors Influencing Blood Pressure

Posture: Standing Sitting Supine Rt. Lateral L. Lateral
132 134 152 155 110

Age: During the first years it varies: 75–90.
15 years to 21: 100–115.
Adults: 120–140.

Sex: Female sex is lower as a rule.

Time of day: In the early hours of sleep there is a decided fall, which gradually rises towards morning. Minimum—A.M. Maximum—P.M.

Exercise: Raises pressure, also emotion and excitement.

Clinical value: In the treatment of children, as a guide to stimulation and other treatment; in obstetrics; in diagnosis and treatment of toxemias; also in myocarditis, nephritis, typhoid fever, pneumonia, and arterio-sclerosis.

REFERENCES: Hammarsten's Physiological Chemistry. Mallory's Principles of Pathologic Histology. Schleip's Atlas of Hematology.

Blood

Name..... Date.....

Specimen taken from.....

Hemoglobin:

Examination of Stained Specimen.
Red Corpuscles .....
Volume Index .....
Variation in size .....
Poikilocytosis .....
Achromia .....
Polychromatophilia .....
Stippling .....
Nucleated Corpuscles .....
Normoblasts .....
Megaloblasts .....

White Corpuscles: Differential Count. Cells Counted:

Polymorphonuclear Neutrophiles .....%
Lymphocytes .....%
Large Mononuclears .....%
Eosinophiles .....%
Mast Cells .....%
Myelocytes, Neutrophilic .....%
Eosinophilic .....%
Basophilic .....%

%

Number of Red Corpuscles .....per cmm.
" " White " ..... " "
Parasites.
Color Index.
Blood Plates.

REMARKS:

Signed.....

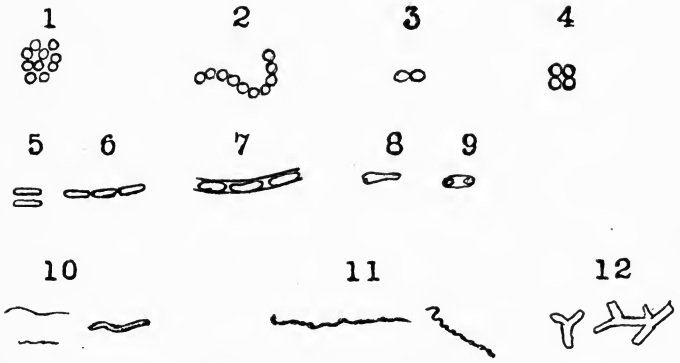
CHAPTER VIII

BACTERIA

Form

The form of bacteria is very simple. They are either spheres, straight rods, or bent rods (spiral).

They are known as cocci or micrococci in spherical form; the straight rods are called bacilli; and the bent rods, spirilla.



VARIOUS FORMS OF BACTERIA

1. Staphylococci. 2. Streptococci. 3. Diplococci. 4. Tetracocci. 5. Bacilli. 6. Bacilli in chains. 7. Bacilli forming spores. 8. Bacilli forming drumsticks. 9. Bacilli forming clostridia. 10. Spirilla. 11. Spirochetæ. 12. Involution forms (degenerate).

Chemical Composition

The composition varies with the species and nature of the culture media. They consist mostly of water, 80 to 88%, and varying amounts of salts, fats, and other albuminous substances.

Size

The unit of measurement in microscopy is the micron (μ), or micromillimeter. This is .001 of a millimeter, or approximately $\frac{1}{25000}$ of an inch.

Micrococci, bacilli, and spirilla average about 1 micron.

Color

Brilliant coloring is displayed by many bacterial growths.

Rose-red, Orange-yellow } Sarcinæ.

Red: B. Prodigiosus deposits a moist red material on bread and other articles of food.

Green, Blue } B. Pyocyaneus: Green fluorescence; gives color to pus.

Phosphorescence: Bacteria in the ocean and fish.

Ferments

Fermentation is the formation of useful products by the action of bacteria. Ferments dissolve protein substances, form sugar from starch, change cane sugar into glucose, decompose fat into fatty acids and glycerin, and convert ammonia into nitrous acid, which forms nitrites; these nitrites are changed to nitric acid, then to nitrates.

Acid

Lactic, butyric, and formic acid are produced by bacteria.

Gas

The putrefactive action of bacteria forms hydrogen sulphide, nitrogen, carbon dioxide, and other gases. Fill

the fermentation tube with glucose bouillon, sterilize, then inoculate.

Bacteria can be studied microscopically, in the living and unstained, by means of the "hanging drop" preparation.

METHOD. Transfer a drop of fluid media in which bacteria have been growing to a slide; or if the bacteria have been growing in solid media, make an emulsion with 1 drop of bouillon or distilled water. Smear the four sides of the cover glass with balsam or vaseline, and invert over the slide so that the drop hangs freely within the hollow space. The movements, development, and multiplication of the bacteria may be easily observed.

Nakanishi's Method

Cover a slide with an aqueous solution of methylene blue. Dry, then place the emulsified bacteria on a cover slip, and drop face downward upon the blue background of the slide. Cytoplasm is stained blue and nuclear material is stained a reddish color.

Movement

Progressive (in some cases the movement is vibratory). Bacteria move back and forth, but do not change their relative positions, and this is known as the Brownian movement.

Only a few of the micrococci are motile. Many of the bacilli and spirilla are motile, and this movement is a change of position caused by certain protoplasmic processes which these bacteria possess, known as cilia or flagella. The cilium has a simple curve, while the flagellum has a compound curve.

Spores

Bacilli and spirilla form spores within the cell, and these are called endospores.

Examination by Cultures

The material to be examined is obtained by means of "swabs," and these swabs are made with a piece of rattan or wire about 15 cm. in length, on one end of which is firmly twisted a small pledget of absorbent cotton. Place this in a test tube, cotton end first; stopper the tube with cotton and sterilize ½ hour at 180° C.

Method. Remove the stopper, taking care to keep the end which enters the tube sterile. Bring the cotton end of the swab in contact with the material to be examined; avoid touching anything else. Replace the swab in the tube and insert the stopper. Take the tube of medium which is to be inoculated and pass the stopper through the flame. Hold the two tubes in the left hand, between the thumb and first finger, in a slanting position. Remove the stoppers and hold between the fingers of the right hand, and inoculate the surface of the medium by gently rubbing the swab over it. Flame the end of the tube of medium, replace the stopper, flame again, incubate 12 to 24 hours. Smear and stain.

The majority of bacteria require oxygen for their growth, but some fail to grow unless it is excluded.

Aërobes

"Obligatory aërobes" requiring oxygenated environment as a necessary condition for growth are: gonococcus, bacillus influenza, bacillus pestis.

Anaërobes

"Obligatory anaërobes" which develop only in an environment from which free oxygen has been excluded are the bacillus tetanus, bacillus malignant edema, bacillus anthrax, bacillus aërogenes capsulatus, and bacillus botulinus.

"Faculative anaërobes" are numerous parasitic and saprophytic bacteria which develop and multiply both under aërobic and anaërobic conditions.

Pasteur's Method for Cultivating Anaërobes

Add 2 c.c. of sterile albolene, oil, or paraffin to the test tube of media. Cool the lower portion of the media to 40° C. while leaving the paraffin fluid, and inoculate the media.

Wright's Method

1. Inoculate culture media in test tube.
2. Place the absorbent cotton stopper in until it lies 15 mm. below the mouth of the tube.
3. Fill space above with dry pyrogallic acid.
4. Pour sufficient strong solution of sodium hydrate (10%) to dissolve the pyrogallic acid. This must be done quickly. Insert rubber stopper in tube so as to close it tightly.

Vaccines

1. Add 1 or 2 c.c. of sterile salt solution or special vaccine solution to the culture media, and gently rub off the growth with a sterile platinum loop.
2. Pour this emulsion in a sterile test tube and repeat the process with 4 other tubes of inoculated media.

3. Heat the end of the test tube containing the emulsion, and also a piece of glass tubing, then join these two ends and draw out the test tube about 1 inch from the mouth in a blowpipe flame. Draw this out, cool, file the constricted portion, and seal in the flame.

4. Shake vigorously.

5. Place the tube in a water bath at 60° C. Heat 1 hour.

6. Shake, then break open, and take a few drops for cultures and standardization.

Special Solution for Vaccines

Normal NaCl	179 c.c.
Glycerin	20 c.c.
Phenol	1 c.c.

Methods of Standardization.—No. 1

1. Draw up vaccine to 0.5 mark with red blood cell counter.

2. Dilute carbol fuchsin ($\frac{1}{10}$) up to the mark 101.

3. Count as in making a red blood cell count.

Method No. 2. (Journal A. M. A., March, 1915)

1. Draw up the bacterial suspension to the 0.5 mark in a leucocyte pipette, then Callison's fluid to the 11 mark. Shake thoroughly and allow to remain in the pipette 10 to 15 minutes.

2. Shake again and place a small drop on the center of a 0.02 mm. counting chamber. When properly filled, place the chamber on the stage of the microscope, which has been previously leveled.

3. After 15 minutes begin the count.

EXAMPLE.

$$\frac{\text{Number of bacteria counted} \times \text{the dilution} \times 20{,}000}{100 \text{ (number of squares counted)}} =$$

number of bacteria in 1 cubic millimeter. Multiply the result by 1,000 to obtain the number of bacteria in 1 cubic centimeter.

Callison's Diluting Fluid

Hydrochloric acid	2 c.c.
Mercuric chloride ($\frac{1}{500}$)	100 c.c.

Add acid fuchsin, 1% aqueous solution, until the solution is a cherry red.

A dilute vaccine is prepared for injection. The ordinary doses are: Gonococci, streptococci, pneumococci, and colon vaccines, 5,000,000; staphylococci, 200,000,000 to 1,000,000,000.

The Plate Method of Petri

The Petri plates consist of two circular glass dishes; the larger is used as a cover. Before using, these plates are wrapped in paper and sterilized $\frac{1}{2}$ hour at 180° C.

A pure culture or one variety of bacteria is obtained in this way, as each organism develops from a colony of its own kind, in an area somewhat isolated.

Melt 2 or 4 tubes of agar-agar or gelatin, cool to 40° to 42° C., then with a sterile platinum loop introduce a loopful of infected material into the first tube, and mix thoroughly. Four loopfuls of this are taken and mixed with the second tube, etc. Pour the contents of the 2 or

4 tubes on separate Petri dishes, cover, label the dilution, and incubate.

When agar-agar is used, incubate 24 hours at 37° C.; but when gelatin is used, it should be set in a cool place to solidify, and then in a place free from dust, at room temperature, for several days.

Bacteria of Pathogenic Significance Commonly Encountered in Pathological Processes in Man

1. Staphylococci Pyogenes Aureus: Small cocci arranged in masses or clumps. Stained dark blue by Gram's method. The colonies on culture media are golden yellow in color.

Staphylococcus albus and staphylococcus pyogenes citreus differ from staphylococcus pyogenes aureus in color of their colonies, and they are much less pathogenic.

2. Streptococci Pyogenes: Small cocci arranged in chains. The growth on culture media occurs in minute, grayish, translucent colonies. Stained dark blue by Gram's method.

3. Pneumococci: Oval, lancet-shaped organisms arranged in pairs. In pus and blood the organism is invested with a hyaline zone called the capsule. Stained dark blue by Gram's method. The colonies appear minute and colorless on culture media, resembling drops of dew.

4. Bacilli Coli Communis: Medium-sized bacillus with rounded ends, sometimes short and coccus-like. Decolorized by Gram's method. Growth on culture media appears as rounded, grayish, viscid-looking colonies.

5. Bacillus Typhosus: Medium-sized bacillus with rounded ends, generally short, but sometimes long or thread-like. Decolorized by Gram's method. Growth on

culture media is similar but slower than that of coli communis.

6. Bacillus Tuberculosis: Slender rods which occur singly and in pairs, usually slightly curved. Branching and club-shaped forms are sometimes observed, also spherical granules. Stained by Gram's method. Stained red by carbol fuchsin stain. Growth on special media appears as dry, cream-colored, granular patches.

7. Bacillus Diphtheria: These bacilli vary in size and shape. They occur in irregular forms, often club-like in shape, with a constriction in the middle. They show great variability in the staining of different parts of the protoplasm. Stained by Gram's method. Löffler's and Neisser's stains are generally used. Growth on culture media appears as round, elevated, smooth colonies.

Bacteria of Pathogenic Significance Not Commonly Encountered in Pathological Processes in Man

1. Gonococci: Medium-sized cocci composed usually of two hemispheres separated by a narrow, unstained interval. Decolorized by Gram's method, *i. e.*, they are stained a bright red. Special media is required, and the colonies appear as minute, grayish, translucent points.

2. Aërogenes Capsulatus: Bacilli varying in length, ends rounded or square-cut; occur singly or in pairs and in clumps. Stained by Gram's method. This bacillus will not grow in the presence of oxygen. Colonies in anaërobic cultures are oval, grayish to brownish white.

3. Bacillus Influenza: Small bacilli with rounded ends of variable length. Sometimes occur in pairs and resemble pairs of cocci. Growth on special media appears as small, glassy, transparent points. Decolorized by

Gram's method. The end may be more deeply stained than central portions.

4. Bacillus Acne: Short, broad bacillus, often showing beaded appearance. Stained by Gram's method. It is anaërobic; small colonies.

5. Bacillus Anthracis: The organism grows in long, segmented threads, varying in length. The segment represents the bacillus. Growth on media appears as small, white colonies.

Bacillus Pyocyaneus (Bacilli of Green Pus): Small bacilli with rounded ends. Decolorized by Gram's method. Appearance on media is slightly elevated, viscid layer of greenish color.

Lesions Caused by Bacteria

Staphylococcus pyogenes aureus.	Furuncle, Carbuncle, Broncho-pneumonia, Abscess of lung, Infection of wounds.
Streptococcus pyogenes	Erysipelas, Tonsilitis, Septicemia, Endocarditis, Broncho-pneumonia.
Pneumococcus ..... (Diplococcus Lanceolatus)	Broncho-pneumonia, Lobar pneumonia, Meningitis, Endocarditis, Septicemia.

Gonococcus (Diplococcus Gonorrhea) ..... { Salpingitis, Vaginitis, Peritonitis, Endocervicitis, Metritis, Prostatitis, Ophthalmia.

M. Catarrhalis ..... Catarrh.

B. influenzæ ..... Influenza.

B. coli communis ..... { Cystitis, Cholecystitis.

B. tuberculosis ..... { Tuberculosis. { Spine (Pott's Disease), Lung, Skin (Lupus), Lymph-nodes (Scrofula).

B. diphtheriæ ..... Diphtheria.

Treponema pallidum ..... Syphilis.

B. typhosus ..... Typhoid fever.

B. aërogenes capsulatus ..... { Necrosis of wounds, Emphysematous gangrene, Gas cysts.

B. anthrax ..... Anthrax.

B. pyocyaneus ..... Pus.

B. acne ..... Acne.

Staining Methods

The glass surface of the slide should be absolutely clean, and this can be done by burning the surface for a moment in a Bunsen or alcohol flame. Then place a loopful of distilled water on the slide, touch this with the hot platinum loop, resterilize the loop, and transfer a colony from the culture into the loop of water. Stir with a circular motion. Fluid cultures do not need dilution.

When the smear is dry, pass the slide three times through the flame, film side up, to fix the preparation.

The three most important routine stains are Gram's stain, Löffler's methylene blue, and carbol fuchsin (Ziehl-Neelsen).

Gram's Stain

1. Cover smear with gentian violet 1 minute.
2. Wash in tap water.
3. Lugol's solution 1 minute.
4. Wash in tap water.
5. Decolorize with 95% alcohol until no more violet color runs off.
6. Wash.
7. Counterstain with dilute carbol fuchsin or Bismarck brown.

Smith's Simplification of the Gram Stain

Six wide-mouthed, 4-ounce bottles are needed to hold the following solutions:

Bottle 1 contains alcohol-saturated gentian violet, with distilled water in the proportion of 1 part stain to 3 parts water.

Bottle 2 contains plain distilled water.

Bottle 3 contains Gram's iodine solution.

Bottle 4 contains absolute methyl alcohol.

Bottle 5 contains plain distilled water.

Bottle 6 contains Ziehl-Neelsen carbol fuchsin, $\frac{1}{10}$.

Method. Dip the slide into Bottle 1 from 5 to 10 seconds, with constant stirring. Transfer to Bottle 2 for a few seconds, shake off the excess of water, dip into Bottle 3 for 5 seconds, with constant agitation of the slide.

Transfer to Bottle 4, where it takes but a few seconds to decolorize, then wash in Bottle 5 and lastly in Bottle 6 for 2 seconds. Wash in tap water.

The Gram-positive bacteria are stained a deep violet, but it must be kept in mind that old, degenerate, and dead cultures do not stain characteristically. The pathogenic cocci are nearly all Gram-positive, with a few exceptions, and the pathogenic bacilli are Gram-negative, except the acid-fast ones, diphtheria, and acne organisms.

Gram-positive	*Gram-negative*
B. pyogenes aureus.	Meningococcus.
B. pyogenes albus.	M. melitensis.
M. tetragenus.	B. coli communis.
Pneumococcus.	Sp. choleræ asiaticæ.
Anthrax bacillus.	B. mallei.
Tubercle bacillus.	B. proteus.
Lepra bacillus.	B. of bubonic plague.
Tetanus bacillus.	B. of Koch-Weeks.
Diphtheria.	M. catarrhalis.
Diphtheroids.	B. typhosus.
B. aërogenes capsulatus.	B. dysenteriæ (Shiga).
Oïdium albicans.	B. pyocyaneus.
Mycelium of actinomyces.	B. pneumonia (Friedländer).
Saccharomyces.	B. of influenza.
Hoffmann's bacillus.	B. of chancroid.
B. xerosis.	Gonococcus.
B. acne.	

Löffler's Stain for Diphtheria

1. Cover smear with Löffler's alkaline methylene blue for 2 minutes.
2. Wash in tap water; dry.

Neisser's Method

This method accentuates the deeply stained granules in the bodies of the individual bacilli.

1. Cover with Neisser's solution No. 1 for 5 seconds.
2. Wash in tap water.
3. Bismarck brown, 3 to 5 seconds.
4. Wash in tap water; dry.

Ziehl-Neelsen Stain for Tubercle Bacilli

1. Cover smear with carbol fuchsin, steaming 1 to 5 minutes.
2. Sulphuric acid (20%), 20 seconds.
3. Alcohol (95%) until no more color will come out.
4. Wash in tap water.
5. Cover with Löffler's methylene blue 10 seconds.
6. Wash in tap water; dry.

Gabbet's Method.—Tubercle Bacilli

1. Carbol fuchsin, steaming 1 to 5 minutes.
2. Wash in tap water.
3. Cover with Gabbet's solution 1 minute.
4. Wash in water; dry.

The tubercle bacillus may be confounded with the bacillus of leprosy and the smegma bacillus. It may be differentiated from the smegma bacillus by the fact that it is not decolorized by alcohol after treatment with acid.

The bacillus of leprosy stains more easily and loses its color more quickly than the tubercle bacillus. They occur in greater numbers and are frequently beady. They do not produce lesions when injected into guinea pigs.

Moeller's Spore Stain

1. Chloroform (in covered dish), 2 minutes.
2. Chromic acid (5%), 1 minute.
3. Wash thoroughly in water.
4. Carbol fuchsin, 1 to 3 minutes' steaming.
5. Decolorize with sulphuric acid (1%) until only a light pink color remains.
6. Wash in water.
7. Saturated aqueous methylene blue, 1 minute.
8. Wash, dry, and mount.

The bodies of the bacteria are stained by the methylene blue, and the spores are stained by the fuchsin and appear red.

Welch's Method for Capsules

1. Cover with glacial acetic acid for a few seconds.
2. Drain with filter paper and place in a dish of aniline water gentian violet for a few seconds.
3. Wash in solution of sodium chloride (2%).
4. Examine in this same solution.

Bacteria stain a dark violet, and the capsules a pale violet.

Löffler's Method for Staining Flagella

1. Cover with freshly filtered mordant solution consisting of:

Aqueous solution of tannic acid (20 grams tannic acid to 100 c.c. water)	10 c.c.
Cold saturated solution of ferrous sulphate	5 c.c.
Saturated aqueous or alcoholic solution of gentian violet or fuchsin	1 c.c.

2. Heat gently for 1 minute.

3. Wash in water.

4. Cover with freshly prepared and filtered solution of aniline gentian violet or aniline fuchsin, and heat gently for 1 minute.

5. Wash in water.

Stain for Spirochæte

Draw off 2 c.c. of blood. Centrifugalize with 5 drops of acetic acid, or enough to hemolize. The whites and spirochæte are precipitated to the bottom of the tube. A small amount of this precipitate is taken up with a capillary pipette and smeared on a slide.

1. Dry, and cover with equal parts of 5% formalin and 5% acetic acid for 3 minutes.

2. Cover with 5% acetic acid for 3 minutes.

3. Cover with concentrated ammonium hydroxide for 3 minutes.

4. Then with 3 or 4% silver nitrate solution for 2 or 3 minutes.

Syphilis

Cleanse the lesions from any adherent exudate, and make a smear preparation from the juice of the tissue obtained by pressure and scraping. Dry and stain. The smear may be fixed and stained as a blood smear for malarial parasites by Wright's stain.

Giemsa's Method

1. Fix the smear by drawing three times through the flame, or placing in absolute alcohol 15 minutes.

2. Dilute 10 drops of Giemsa's stain with 10 c.c. of

distilled water and cover the smear. This process is repeated four or five times at 15-second intervals. The parasites take an intense dark red stain.

Another Method.—Mallory and Wright

1. After fixing smear with heat, cover with 10 c.c. of a 5% solution of glycerin mixed with 13 drops of Giemsa's stain, which has been heated to 60° C.

2. Allow this hot solution to remain on the smear 15 to 30 minutes. Wash in water.

If intense staining is desired, add to the distilled water before mixing it with the stain 1 or 2 drops of 1% potassium carbonate solution to 10 c.c. of water.

India Ink Stain

Place 5 loopfuls of Chin-Chin India Ink near the end of a slide; add 5 loopfuls of distilled water and 1 loopful of material to be examined. Mix, then place the top of another slide on half of the first slide, press together, and pull apart sideways. Dry and examine.

Label all smears with the patient's name, date, source, and hospital number.

Bacteria

Source.....Date of Isolation.....Name.....

Detailed Features.

I. Morphology.

1. Vegetative Cells. Medium used.....temperature..... age....days.

Form: round, short rods, long rods, short chains, long chains, filaments, commas, short spirals, long spirals, clostridium, cuneate, clavate, curved.

Limits of size.....

Size of majority.....
Ends: round, truncate, concave.

2. Endospores.
Form: round, elliptical, elongated.
Limits of size.....
Size of majority.....
Wall: thick, thin.
Sporangium wall: adherent, not adherent.
Germination: equatorial, oblique, polar, bi-polar, by stretching.

3. Flagella No.....Attachment: polar, bi-polar, peritrichic.
How stained.....

4. Capsules, present on.....

5. Zoöglœæ Pseudozoöglœæ .....

6. Involution Forms, on.....in.....days at .....° C.

7. Staining Reactions:
Löffler's Alkaline Methylene Blue.....Special Stain.....
Gram .....Carbol Fuchsin.....Neisser.....
India Ink.....

II. Cultural Features.

1. Medium used.....
Growth: invisible, scanty, moderate, abundant.
Form of growth: filiform, echinulate, beaded, spreading, plumose, arborescent, rhizoid.
Elevation of growth: flat, effuse, raised, convex.
Luster: glistening, dull, cretaceous.
Topography: smooth, contoured, rugose, verrucose.
Optical characters: opaque, translucent, opalescent, iridescent.
Chromogenesis.....
Odor: absent, decided, fetid.
Consistency: slimy, butyrous, viscid, membranous, coriaceous, brittle.
Medium grayed, browned, reddened, blued, greened.

References: Pathogenic Bacteria and Protozoa, Park. Pathological Technique, Mallory and Wright.

Descriptive Chart. Society of American Bacteriologists

Glossary of Terms

AGAR HANGING BLOCK: A small block of nutrient agar cut from a poured plate and placed on a cover glass, the surface next the glass having been first touched with a loop from a young fluid culture or with a dilution from the same. It is examined upside down, the same as a hanging drop.

AMEBOID: Assuming various shapes like an ameba.

AMORPHOUS: Without visible differentiation in structure.

ARBORESCENT: A branched, tree-like growth.

BEADED: Stab or stroke, disjointed or semi-confluent colonies along the line of inoculation.

BRIEF: A few days, a week.

BRITTLE: Growth dry, friable under platinum needle.

BULLATE: Growth rising in convex prominences, like a blistered surface.

BUTYROUS: Growth of a butter-like consistency.

CHAINS: Short chains, composed of 2 to 8 elements. Long chains, composed of more than 8 elements.

CILIATE: Having fine, hair-like extensions like cilia.

CLOUDY: Fluid cultures which do not contain pseudo-zoöglœæ.

COAGULATION: The separation of casein from whey in milk. This may take place quickly or slowly, and as the result either of the formation of an acid or of a lab ferment.

CONTOURED: An irregular, smoothly undulating surface, like that of a relief map.

CONVEX: Surface the segment of a circle, but flattened.

COPROPHYL: Dung bacteria.

CORIACEOUS: Growth tough, leathery, not yielding to the platinum needle.

CRATERIFORM: Round, depressed, due to the liquefaction of the medium.

CRETACEOUS: Growth opaque and white, chalky.

CURLED: Composed of parallel chains in wavy strands, as in anthrax colonies.

DIASTASIC ACTION: Same as Diastatic; conversion of starch into water-soluble substances by diastase.

ECHINULATE: In agar stroke, a growth along line of inoculation, with toothed or pointed margins; in stab cultures, growth beset with pointed outgrowths.

EFFUSE: Growth thin, veily, unusually spreading.

ENTIRE: Smooth, having a margin destitute of teeth or notches.

EROSE: Border irregularly toothed.

FILAMENTOUS: Growth composed of long, irregularly placed, or interwoven filaments.

FILIFORM: In stroke or stab cultures, a uniform growth along line of inoculation.

FIMBRIATE: Border fringed with slender processes, larger than filaments.

FLOCCOSE: Growth composed of short, curved chains, variously oriented.

FLOCCULENT: Fluids which contain pseudozoöglœæ, *i. e.*, small, adherent masses of bacteria of various shapes, floating in the culture fluid.

GRAM'S STAIN: A method of differential bleaching after gentian violet, etc.

GRUMOSE: Clotted.

INFUNDIBULIFORM: Form of a funnel or inverted cone.

IRIDESCENT: Like mother-of-pearl. The effect of very thin films.

LACERATE: Having the margin cut into irregular segments, as if torn.

LOBATE: Border deeply undulate, producing lobes.

LONG: Many weeks or months.

MAXIMUM TEMPERATURE: Temperature above which growth does not take place.

MEDIUM: Several weeks.

MEMBRANOUS: Growth thin, coherent, like a membrane.

MINIMUM TEMPERATURE: Temperature below which growth does not take place.

MYCELIOID: Colonies having the radiately filamentous appearance of mold colonies.

NAPIFORM: Liquefaction with the form of a turnip.

NITROGEN REQUIREMENTS: The necessary nitrogenous food. This is determined by adding to nitrogen-free media the nitrogen compound to be tested.

OPALESCENT: Resembling the color of an opal.

OPTIMUM TEMPERATURE: Temperature at which growth is most rapid.

PELLICLE: In fluid bacterial growth, either forming a continuous or an interrupted sheet over the fluid.

PEPTONIZED: Curds dissolved by trypsin.

PERSISTENT: Many weeks or months.

PLUMOSE: A fleecy or feathery growth.

PSEUDOZOÖGLŒÆ: Clumps of bacteria, not dissolving readily in water, arising from imperfect separation or more or less fusion of the components, but not having the degree of compactness and gelatinization seen in zoöglœæ.

PULVINATE: The form of a cushion, decidedly convex.

PUNCTIFORM: Very minute colonies, at the limit of natural vision.

RAISED: Growth thick, with abrupt or terraced edges.

RAPID: Developing in 24 to 48 hours.

REPAND: Wrinkled.

RHIZOID: Growth of an irregular branched or root-like character, as in B. mycoides.

RING: Same as Rim; growth at the upper margin of a liquid culture, adhering more or less closely to the glass.

SACCATE: Liquefaction the shape of an elongated sack, tubular, cylindrical.

SCUM: Floating islands of bacteria, an interrupted pellicle, or bacterial membrane.

SLOW: Requiring 5 or 6 days or more for development.

SPORANGIA: Cells containing endospores.

SPREADING: Growth extending beyond the line of inoculation.

STRATIFORM: Liquefying to the walls of the tube at the top and then proceeding downward horizontally.

THERMAL DEATH-POINT: The degree of heat required to kill young fluid cultures of an organism exposed for 10 minutes (in thin-walled test tubes of a diameter not exceeding 20 mm.) in a thermal water bath. The water must be kept agitated so that the temperature shall be uniform during the exposure.

TRANSIENT: A few days.

TURBID: Cloudy, with flocculent particles; cloudy plus flocculence.

UMBONATE: Having a button-like, raised center.

UNDULATE: Border wavy, with shallow sinuses.

VERRUCOSE: Growth wart-like, with wart-like prominences.

VERMIFORM–CONTOURED: Growth like a mass of worms or intestinal coils.

VILLOUS: Growth beset with hair-like extensions.

VISCID: Growth follows the needle when touched and withdrawn; sediment on shaking rises as a coherent swirl.

ZOÖGLŒÆ: Firm, gelatinous masses of bacteria, one of the most typical examples of which is the Streptococcus mesenterioides of sugar vats (Leuconostoc mesenterioides), the bacterial chains being surrounded by an enormously thickened, firm covering, inside of which there may be one or many groups of the bacteria.

CHAPTER IX

CULTURE MEDIA

Culture media consist of various liquid and solid nutritive substances in or upon which bacteria will grow, and the media must be adjusted to the peculiarities of the individual bacteria.

Sterile test tubes are used as containers, stoppered with cotton which has been dipped in melted paraffin, then inserted into the tube. This prevents evaporation and the invasion of molds.

The most common media have for their basis an extract or infusion of meat, to which peptone and sodium chloride are added.

If fresh meat is used, take 500 grams (1 pound) of lean meat, finely chopped. Cover with 1,000 c.c. of water.[1] Place in the ice chest over night. The following day skim, strain, and make the infusion up to 1,000 c.c. Then add 10 grams of peptone and 5 grams of sodium chloride which have been dissolved in a few c.c. of the infusion.

Boil 10 minutes, then place on the scales and balance with its counterpoise and 1 kilo weight on the other side. Add sufficient hot water to the media to make up to 1,000 c.c. Boil 5 minutes; test for acidity.

The reaction may be tested with litmus paper and dilute HCl added when the media becomes too alkaline. This

[1] The meat may be boiled for ½ hour; filter and make up to 1,000 c.c.

method is sufficient for the cultivation of bacteria for ordinary purposes.

Bacteria are susceptible to slight variations in the acidity and alkalinity of media, and the reaction has a marked effect upon their morphology and mode of growth. In the cultivation of bacteria of the air, soil, and water, a more exact adjustment of the reaction is made by titrating, using phenolphthalein as an indicator.

The neutral point of litmus corresponds with a reaction of +1.5 to phenolphthalein.

Titration

[1]Media in the process of preparation is usually acid; sometimes 5 c.c. of $\frac{N}{1}$ HCl are added.

Fill the burette with $\frac{N}{20}$ NaOH, make a reading, and record.

5 c.c. of the medium to be tested are measured in a pipette and transferred into a small porcelain dish. Add 45 c.c. of hot water (cold water contains carbon dioxide and might give alkalinity to the media) and 5 drops of phenolphthalein (0.5% in 50% alcohol).

Place this small dish under the burette and carefully run in the $\frac{N}{20}$ NaOH until a distinct pink color remains in the fluid after stirring.

[1]To make more acid: Add to each 100 c.c. of the medium 1 c.c. of normal HCl for each degree that the reaction is to be changed. EXAMPLE. 1 liter of medium with a reaction of .8 degree is to be made 1; then 0.2 c.c. of normal HCl should be added to each 100 c.c., or 2 c.c. for the liter. To make more alkaline, add normal NaOH.

EXAMPLE. 0.4% acidity is desired (standard required by the American Public Health Association; *i. e.*, 1.5% normal NaOH added to medium makes it neutral to phenolphthalein). Adjustment of the reaction to this standard is made by adding to the medium

$$\frac{N}{1}\ \text{NaOH or}\ \frac{N}{1}\ \text{HCl}$$

The first reading of the burette is 25, and after adding to the medium and obtaining the pink color, the burette reads 25.8. The titration shows that 5 c.c. of the medium requires 0.8 c.c. $\frac{N}{20}$ NaOH to make it neutral to phenolphthalein, therefore the total amount will require 0.8%, or 8 c.c., for a liter. $25.8 - 25 = 0.8$. The reaction required is such that 0.4% of the $\frac{N}{20}$ NaOH should be used to make the medium neutral. Subtracting 0.4 from 0.8, we have as a result 0.4 excess acidity. Therefore to every 5 c.c. in the 1,000 c.c. of medium, 0.4 c.c. of the $\frac{N}{20}$ NaOH is added to neutralize the acidity, or the $\frac{N}{1}$ NaOH may be used. $1{,}000 \div 5 \times 0.4 = 80$ c.c. $\frac{N}{20}$ NaOH, or 4 c.c. $\frac{N}{1}$ NaOH.

When the titration is finished, cool the medium to 60° C.; add 3 eggs which have been beaten lightly with 200 c.c. of cold water. Boil 20 minutes, stirring occasionally. Filter, and run 10 c.c. in sterile test tubes. Sterilize ½ hour at 120° C. on 3 successive days.

Bouillon

Formula for 1,000 c.c.:

Beef extract (Liebig's)	3 grams
Or lean beef	500 "
Peptone (Witte's)	10 "
Sodium chloride	5 "
Water	1,000 c.c.

Glucose Bouillon

Add 10 grams of glucose to the preceding bouillon medium.

Agar-agar (plain)

Formula for 1,000 c.c.:

Agar-agar	15 grams
Beef extract	3 "
Or lean beef	500 "
Peptone	10 "
Sodium chloride	5 "
Water	1,000 c.c.

Filter into a 1,000 c.c. flask, sterilize ½ hour, filter again to remove the precipitates of phosphates, and run 10 c.c. in sterile test tubes. Slant tubes while cooling after the third sterilization.

Glucose Agar-agar

Add 10 grams of glucose to the agar-agar medium.

Glycerin Agar-agar

Add 60 c.c. of glycerin to the plain agar-agar medium after its filtration.

Gelatin (plain)

Formula for 1,000 c.c.:

Dissolve 100 grams of *golden seal* French gelatin in 1,000 c.c. of bouillon. Boil 5 minutes. Neutralize the acidity of the gelatin with a 10% solution of caustic soda to a faint alkalinity. Cool to 60° C. and beat an egg into it, then boil 10 minutes. Filter and run into test tubes, 10 c.c. for "slant" and 15 c.c. for "stab" cultures.

Fill the chamber of the sterilizer with steam before putting in the media. Sterilize 15 minutes, 3 successive days, at 10 pounds pressure. Plunge tubes in cold water after each sterilization.

Sugar-free Bouillon.—Method No. 1

Formula for 1,000 c.c.:

Chopped lean beef	500 grams
Water	1,000 c.c.

Place in the ice chest 12 to 24 hours. Filter, and add a 24-hour bouillon culture of colon bacillus. Neutralize, then incubate 12 hours. Add the 10 grams of peptone, 5 grams of sodium chloride, and heat gently until the peptone dissolves, then boil 30 minutes. Balance and add water to make up for loss by evaporation. Titrate, filter, and store in tubes or flasks. Sterilize.

Blood Serum.—Löffler's

Formula:

Glucose bouillon	1 part (300 c.c.)
Beef blood serum	3 parts (900 c.c.)

The blood serum is collected at the abattoir in thoroughly clean glass jars. Place in a cool place 24 to 48

hours, to allow it to clot and the serum to separate. Remove the serum with a sterile pipette.

Slant tubes and solidify by heating 3 hours at 80° to 90° C.

Sterilize in Arnold sterilizer 20 to 30 minutes on 3 successive days.

Dextrose bouillon may be used instead of glucose, and this is made by adding 1% dextrose to the plain bouillon. B. diphtheria is cultivated on this media.

Litmus Milk

Formula:

Fresh milk	300 c.c.
Litmus solution	50 c.c.

Take fresh milk and place in the autoclave for 1 hour at 60° C., then skim. Boil 8 litmus cubes in 100 c.c. of water, filter, and add sufficient to give the milk a pale blue color. Sterilize 30 minutes at 4 or 5 pounds pressure on 3 successive days.

This media is used for determining certain of the physiological properties of bacteria—whether they produce in their growth acid or alkali. Colon bacilli turn litmus milk pink. Typhoid and colon bacilli are sometimes confused; they can be differentiated with litmus milk.

Egg Medium.—Dorset

Formula:

Fresh eggs	4
Sterile distilled water	25 c.c.

Fresh eggs are broken under aseptic conditions into a sterile graduate. To every 4 eggs add 25 c.c. of sterile

distilled water, mixing the whites and yolks thoroughly with a sterile glass rod. Strain the mixture through a sterile cloth, then run 10 c.c. into sterile tubes, and slowly harden in form of "slants" in the autoclave 4 hours at 73° to 76° C. on 3 successive days. This medium is used for the cultivation of tubercle bacilli. Just before inoculating the medium, add 1 c.c. of sterile distilled water to each tube, to supply the moisture required for the development of the bacilli, and incubate over night at 38° C. Colonies should become visible after 7 days.

Modification of Endo's Medium

Formula:

Cold water	1,000 c.c.
Powdered agar	15 grams
Peptone (Witte's)	10 "
Beef extract (Liebig's)	3 "

Cook 1 hour in a double boiler, then add enough of a 10% solution of sodium carbonate to make slightly alkaline to litmus. Filter, and sterilize 1 hour at 15 pounds pressure. To 1 c.c. of 10% solution of fuchsin (made up with 96% alcohol) add 10 c.c. of freshly prepared 10% solution of sodium sulphite (anhydrous). Arnoldize 20 minutes, then add 1% of this solution and 1% of lactose (C. P.) to the agar medium. Sterilize 30 minutes at 15 pounds.

When used, the medium is melted and poured into sterile Petri dishes, and left uncovered until the agar is solid; then inoculate with a suspension of feces in sugar-free broth. Incubate 1 hour.

Rub the infected sugar-free broth over the surface of the Petri dish with a sterile glass rod. Incubate 18 hours.

B. coli neutralize the action of the sodium sulphite by forming acid from the lactose, and the colonies are red. Typhoid, dysentery, and paratyphoid bacilli remain colorless and do not form colonies.

Jackson's Lactose Bile Media

To 10 c.c. of ox bile from the abattoir, or from human bile from cases of gall bladder drainage which has been sterilized, add 1% of peptone and 1% lactose. Tube in fermentation tubes.

B. typhosus and B. coli will outgrow all other microorganisms on this media. Arnoldize ½ hour on 3 successive days at 73° to 76°.

Special Media for Gonococcus

No. 1		*No. 2*	
Agar-agar (2%)	2 parts	Agar-agar	2 parts
Hydrocele fluid	1 part	Blood (human)	1 part

Melt the agar-agar and bring to a temperature of 40° C. in a water bath. Add the sterile hydrocele fluid or blood.

The tubes may then be infected and their contents poured into sterilized Petri dishes.

A Substitute for Ordinary Blood Serum.—Stitt's

Glucose bouillon (1%)	10 to 15 c.c.
Eggs	1

Make a smooth mixture in a mortar and tube. Inspissate and sterilize as for ordinary serum slants.

Gibson's Modification

Formula for 500 c.c.:

Glucose bouillon (1%)	120 c.c.
Eggs	8
Glycerin	20 c.c.

Wash eggs with water, then with 70% alcohol. The eggs are then broken under aseptic precautions into a wide-mouthed receptacle (sterile), and mixed thoroughly with the glucose bouillon and glycerin. Strain through a sterile cloth and run 10 c.c. of the mixture into sterile test tubes, and slowly harden in the form of "slants" in the autoclave at 3 or 4 pounds pressure for 2 hours on 3 successive days. The door of the autoclave should be left partly open.

Sugar-free Bouillon.—Method No. 2

1. Bouillon, 1,000 c.c.

2. Cool in a flask and add 10 c.c. of a 24-hour culture of B. coli communis.

3. Stopper with cotton; incubate 18 hours. (The bacteria ferment and destroy any sugar present, and render the broth sugar-free and acid.)

4. Heat thoroughly, to kill the colon bacilli.

5. Place 15 grams of purified talcum (U. S. P.) in a mortar; add the bouillon culture, stirring constantly. Filter until the bouillon is perfectly clear.

Sugar Bouillons

In special work the following formula is used:

Sugar-free bouillon	1,000 c.c.
Glucose, or other pure sugars	10 c.c.

Dissolve and tube in Durham's or ordinary fermentation tubes.

Sterilize several times at 5 pounds pressure, never heating over 15 minutes at a time, as heat changes the sugars.

The amount of sugar in bouillon made from Liebig's meat extract is so small that for ordinary purposes the sugar is added directly to the bouillon.

Special Media for Ameba.—Musgrave and Clegg

Agar-agar	20 grams
Sodium chloride	3 grams
Beef extract	3 grams
Water	1,000 c.c.

The agar should be 1% alkaline to phenolphthalein.

Place the material containing the ameba in a sterile flask; add 1 c.c. of alkaline bouillon to each 100 c.c. of material. Set aside 24 to 48 hours. Inoculate Petri plates containing the nutrient agar with a loopful of material from the surface of the flask. Incubate at 37° C.

Differential Food Media.—Hiss. (Mt. Prospect Laboratory, Brooklyn, N. Y.)

Agar	15 grams
Gelatin	15 "
Liebig's extract	5 "
Sodium chloride	5 "
Dextrose	10 "
Distilled water	1,000 c.c.

Reaction, 1.0% normal acid.

This medium is semi-fluid at 37° C., and B. typhosus

has a tendency to swim out from the colonies, forming branch-like processes which distinguish it from B. coli.

Hesse's Media for Typhoid. (Mt. Prospect Laboratory, Brooklyn, N. Y.)

Agar	5 grams
(4.5 grams absolutely dry)	
Peptone (Witte)	10 grams
Liebig's extract of beef	5 "
Salt	8.5 "
Distilled water	1,000 c.c.

4.5 grams of agar are dissolved in 500 c.c. of distilled water, making up the loss in weight by evaporation. Dissolve 10 grams of peptone, 5 grams of meat extract, and 8.5 grams of salt in 500 c.c. of distilled water; the loss in weight by evaporation is made up by adding distilled water. Add the two solutions together, boil 30 minutes, make up loss in weight, then filter through absorbent cotton held in the funnel by cotton flannel. Filter until perfectly clear. Test the reaction and adjust to 1.0% normal acid. Sterilize 20 minutes at 15 pounds pressure. *Cool with running tap water and store in ice chest.*

In cases of infected water and milk the preliminary cultivation in bile is necessary, in order to increase the typhoid germs in numbers over the various other species always present in contaminated supplies.

Method. Place 8 tubes containing 9 c.c. of sterile distilled water in a rack, together with 8 sterile Petri dishes. Number the tubes and plates from 1 to 8.

Into tube No. 1 place 1 c.c. of feces, water, or milk to be tested, and which has been previously inoculated and

incubated at least 24 hours. Mix thoroughly with 9 c.c. of distilled water, then place 1 c.c. of this mixture in plate No. 1 and 1 c.c. into tube No. 2.

After mixing thoroughly with the 9 c.c. of water in tube 2, place 1 c.c. of the mixture in plate No. 2 and 1 c.c. into tube 3. Proceed in this manner through the series of dishes. Add to each Petri dish 10 c.c. of the liquefied Hesse agar, cooled to 40° C., and mix thoroughly. Cool in the ice chest to set the medium, and incubate 24 hours at 37° C.

B. typhosus is characteristic on Hesse agar only when the dilution is sufficiently high to produce but a few bacteria on the plate. It is distinguished from B. coli by the formation of colonies of much larger size, and consisting of a broad, translucent, or scarcely turbid zone between the white opaque center, or nucleus, and the circular, narrow, white seam, or edge.

Potato Slants

Clean Irish potatoes thoroughly with a stiff brush. Pare off the outer portion and cut out cylinders with a cork borer, ½ inch in diameter, then divide the cylinders diagonally.

Wash in running water several hours.

Place a small piece of glass tubing in the bottom of the test tube, then drop in the potato, base downward. Sterilize at 15 pounds pressure for 20 minutes.

Glycerin Potato

Soak the potato slant in 6% glycerin for 1 hour. Proceed as previously directed.

Special Media for Rapid Culture of Tubercle Bacilli
(Journal of Experimental Medicine, Vol. XXI, No. 1)

Two parts of egg (white and yolk).
One part of meat juice.
Gentian violet sufficient to the proportion of 1 to 10,000.

MEAT JUICE: 500 grams of beef or veal are infused in 500 c.c. of a 15% solution of glycerin in water. Twenty-four hours later the meat is squeezed through a sterile meat press and collected in a sterile beaker.

EGGS: Sterilize the shells of the eggs by immersion for 10 minutes in 70% alcohol, or by pouring hot water upon them. Break the eggs into a sterile beaker, mix well, and filter through sterile gauze. Add one part by volume of meat juice.

GENTIAN VIOLET. Add sufficient 1% alcoholic gentian violet to make a dilution of $\frac{1}{10000}$.

Tube 3 c.c. in sterile test tubes and inspissate for 3 successive days. On the first day at 85° C., until all medium is solidified, changing the places of the tubes if necessary; on the second and third days for not more than 1 hour at 75° C.

Differential Medium for Acid-producing Bacteria
(Journal A. M. A., Vol. LXIV, No. 6)

Agar medium	100 c.c.
Milk sugar	1.5 grams
Congo red (aqueous solution, 1%)	30 c.c.

Typhoid colonies grown on this media are red and generally transparent.

Colon colonies develop black, with a light halo.

REFERENCES: Mallory and Wright's Pathological Technique. Hiss and Zinsser's Bacteriology. Stitt's Practical Bacteriology and Parasitology.

CHAPTER X

BODY FLUIDS

Examination of body fluids is useful for determining the diagnosis of meningitis and the differentiation between inflammatory fluid and hypostatic fluid in cases of pleural or peritoneal effusion; *i. e.*, between pleurisy and hydrothorax, peritonitis and ascites.

Albumin is determined by the methods used in the examination of urine.

Transudates and Exudates

A transudate is similar to lymph and collects in a body cavity on account of various circulatory conditions.

An exudate is a heavier, more cloudy liquid, which is poured out upon a serous or other surface as the result of inflammation.

	Transudate	Exudate
1.	Appearance: Clear.	Clear to thick and creamy.
2.	Clotting: Almost never spontaneously.	Usually.
3.	Sp. gr.: Usually less than 1,020.	Usually more than 1,020.
4.	Albumin: 1% or less.	3% or over.
5.	Nucleo-protoplasm: Little.	Much more.
6.	Sediment: Slight, endothelial cells, few leucocytes.	Considerable; many leucocytes.

Cerebrospinal Fluid

1. Amount: 5 to 10 c.c. normally.
2. Clear.
3. Slightly alkaline.
4. Specific gravity: 1,005 to 1,010.
5. Protein, nucleo-albumin, etc.: Considerable amount.
6. Clotting: It may on standing.
7. Sediment: Slight; epithelial or endothelial cells.

Pericardial Fluid

1. Amount: Small.
2. Clear: Normally.
3. Alkaline.
4. Protein: High per cent, and contains more fibrin than any other physiological fluid.

Synovial Fluid

1. Color: Yellow.
2. Appearance: Thick, viscid, and sticky; contains synovin, a mucin-like body.
3. Protein: Much.

Pleural Fluid

NON–INFLAMMATORY TRANSUDATE: Light yellow, clear, few formed elements. Specific gravity, 1,015 or below.

INFLAMMATORY EXUDATE: Yellow, clear or turbid from fibrin, and formed elements present. Specific gravity, 1,020.

Purulent Pleurisy

Citron color, turbid. Specific gravity, 1,020.

Albumin: Large amount.

Cholesterin, uric acid, bile pigment, and sugar often present.

Smears

Fill centrifuge tube with 3 c.c. of a 2% sodium citrate and salt solution; add 9 c.c. of fluid. Centrifugalize 3 minutes, then pour off the supernatant fluid and add 9 c.c. of a 1% aqueous solution of formalin. Mix; centrifugalize for 5 minutes. Make smears from the sediment.

Lymphocytosis indicates a tuberculous process.

A large number of polymorphonuclear and eosinophilic leucocytes indicates meningococcic or pneumococcic infection.

Large epithelial cells are found in hydrothorax.

Moritz Test

Fill a 50 c.c. cylinder with the fluid; add 2 drops of a 5% solution of acetic acid. A heavy, cloud-like precipitate shows the fluid to be an exudate.

Rivalta's Test

Fill a 100 c.c. cylinder with distilled water, add 2 drops of acetic acid, then add 1 drop of the fluid. A nebulous cloud as the drop of fluid sinks indicates an exudate.

Peritoneal Fluid

Normal fluid is a clear, pale straw color. Specific gravity, 1,005 to 1,015.

Slightly albuminous.

Microscopically it shows very few formed elements.

Ascitic Fluid

Clear, yellow, albuminous; coagulates on standing.

In ascites adiposus the fluid has a milky appearance, due to the presence of cells that have undergone a fatty degeneration.

In ascites chylosus the fluid contains chyle, and in pseudochylous ascites the fluid resembles chyle but does not contain fatty matter.

Specific gravity is about 1,015.

Albumin, 2%.

Microscopically it shows few leucocytes, usually fatty and rarely desquamated epithelial cells.

Hydrocele	Spermatocele
1. Appearance: Dark, but clear.	Colorless, watery, or slightly milky.
2. Sp. gr.: 1,014 to 1,026.	1,006 to 1,010.
3. Sometimes coagulates spontaneously.	Contains cell detritus, fat globules, and spermatozoa.

The fluid should be collected in sterile receptacles under aseptic precautions.

Stain smears with Löffler's methylene blue carbol fuchsin (Ziehl-Neelsen) and Gram's stain.

Cytodiagnosis

The examination of cellular elements of fluid with reference to the variety of cell which predominates.

Smears are made from the sediment and stained with Wright's stain.

The infecting bacteria are generally diplococcus intracellularis or pneumococcus.

Cerebrospinal Fluid

The cell count should be done as soon as possible. Normally there are 3 to 9 cells per cubic millimeter. In general paresis and tabes there are 40 to 100 cells per cubic millimeter.

An excess of polymorphonuclear and eosinophilic leucocytes is indicative of meningococcic or pneumococcic infection, and a lymphocytosis indicates a tuberculous process.

Counting the Cells

Draw up Grubler's polychrome methylene blue in a white blood cell counter to the mark 0.2, then spinal fluid up to 11. Stain 3 minutes. Counting chamber should be 0.2 mm. deep.

Staining

Centrifugalize, and drop the sediment with a fine capillary tube at intervals on the slide; dry and fix in the flame. Stain with Wright's or Gram's stain.

Butyric Acid Test

Draw up .2 c.c. of fluid in a sterile graduated pipette; add .5 c.c. of a 10% solution of butyric acid. Boil 1 minute, then add .1 c.c. of N/1 NaOH and boil 1 minute.

A precipitate indicates a lesion.

The Lange Colloidal Gold Test

Clean all apparatus with cleaning solution, then with distilled water and doubly distilled water; sterilize. All solutions are made up with doubly distilled water.

1. Place 10 tubes in a rack.
2. To the first tube add 1.8 c.c. of a 0.4% salt solution from a burette.

3. To each of the following tubes add 1 c.c. of the 0.4% salt solution.

4. Add to the first test tube 0.2 c.c. of the spinal fluid, making a dilution of $\frac{1}{10}$, using a sterile pipette, 1 c.c. graduated $\frac{1}{100}$ to the tip.

5. Pipette 1 c.c. from this tube into the second tube, mix by blowing, and so on to each tube, the extra c.c. from the last tube being thrown away. This gives dilutions of the spinal fluid of $\frac{1}{10}$, $\frac{1}{20}$, $\frac{1}{40}$, $\frac{1}{80}$, $\frac{1}{160}$, and so on to $\frac{1}{5120}$.

6. Add 5 c.c. of gold reagent to each tube from the burette, without shaking.

The results are read in 12 to 24 hours.

When there is a positive reaction the gold is flaked out, and this causes a color change which varies with the intensity of the reaction. These changes from the original red through red blue, blue red, violet or dark blue, light blue to clear are represented by 1 to 5 pluses.

Minute quantities of blood do not affect the reaction, but large quantities produce unreliable results.

Albumin Test

1. 1 c.c. of spinal fluid.

2. 3 drops of trichloride acetic acid ($33\frac{1}{3}\%$), and let stand until precipitate settles.

3. Measure with a millimeter rule.

3	millimeters	equals	normal.
3 to 4	"	"	plus.
4 to 5	"	"	2 pluses.
5 to 7	"	"	3 "
7 to 10	"	"	4 "
10 to 15	"	"	plus 5.
15 plus	"	"	" 6.

Nonne's Globulin Test

1. 1 c.c. of spinal fluid.
2. 1 c.c. of saturated $(NH_4)_2SO_4$.

Pour the $(NH_4)_2SO_4$ under the spinal fluid and examine for white ring at junction of fluids. Faintest possible trace equals plus.

Trace with black background equals 2 pluses.

Trace without black background equals 3 pluses.

Flocculent precipitate equals 4 pluses.

If blood is present, a pink layer usually appears in upper stratum of globulin test.

Gold Solution

1 liter of doubly distilled water; heat on slow flame to 60° C. Keep thermometer in the flask. When the temperature is reached, add 10 c.c. of a 1% aqueous solution of gold chloride (Merk's 15 grains ampules to 100 c.c. of water; keep in a brown bottle).

10 c.c. of a 2% aqueous solution of K_2CO_3.

Add solutions simultaneously. Heat rapidly to just under boiling. Remove from flame and add immediately 10 c.c. of a 1% aqueous solution of 40% formalin. Shake, and continue to shake until proper color develops. If it does not develop, throw away and start over again. Solution should be red, perfectly clear by transmitted light, and opalescent by reflected light.

References: Boston Medical Journal, December 10, 1914. Journal A. M. A., September 6, 1913. Mallory's and Wright's Pathological Technic.

CHAPTER XI

MILK

Composition

Human Milk		Cow's Milk	
Fat	3 to 4%	Fat	3.5%
Lactose	5 to 8%	Lactose	4.5%
Protein	1 to 2%	Protein	3.9%
Ash	0.2 to 0.4%	Ash	0.7%
Water	87 to 88%	Water	87.4%

Reaction

Human Milk	Cow's Milk
Amphoteric.	Slightly alkaline.

Specific Gravity

Human Milk	Cow's Milk
1,028 to 1,032.	1,031.

Definition of Terms

Separated Milk: Milk from which fat has been partially or wholly removed, either by centrifuge or gravity.

Fat Free: Separated milk which contains no fat, or at least a fractional per cent such as may be obtained in the lowest quarter of a quart of milk which has been setting 6 or more hours.

Cream: That which remains after separated milk has been removed from whole milk. It contains most of the

fat of whole milk and certain percentages of all the other elements of whole milk.

WHOLE MILK: This is considered 4% cream.

WHEY: Contains all the sugar and water of milk, while most of the fat and all caseinogen have been removed.

CASEINOGEN: Mother substance, from which casein is obtained by precipitation or coagulation.

Fermentation

Place milk in a warm room and it becomes acid. This is due to the action of bacterium lactis, which changes the milk-sugar (lactose) into lactic acid and precipitates the casein.

Bacillus aërogenes capsulatus and bacillus odematis maligni produce butyric acid fermentation.

Yeasts produce alcoholic fermentation.

The chromogenic bacteria, B. cyanogenus, B. prodigiosus, and others, produce the blue, green, and yellow changes which sometimes occur in milk.

Strepto-lacticus is found in sour milk.

Milk containing over 1,000,000 bacteria to the c.c. is considered harmful to the infant.

Methods of Preservation.—Refrigeration

Milk kept at or below 10° C. (50° F.) will remain sweet and uncurdled for several days; but cold will not preserve milk indefinitely, nor will it kill bacteria.

Sterilization

If fresh milk is boiled 15 minutes, a scum is formed, due to the coagulation of lactalbumin and globulin. This kills the bacteria which cause lactic fermentation, and

the milk will remain sweet for several days if the receptacle is stoppered with cotton. Boiled milk is less digestible and nourishing as the fats, sugars, casein, and albumin are altered. Milk is rendered germless when heated at a temperature of 68° to 75° C. (154° to 167° F.), and the taste and digestibility are not materially altered. A temperature of 68° C. is sufficient for the destruction of tubercle bacilli; 60° C. for typhoid; 58° C. for diphtheria; and most saprophytes will be killed at a temperature of 75° C.

Pasteurization

This method is preferred to complete sterilization, as it does not affect materially the nutritive value of milk. Pathogenic bacteria and non-pathogenic micro-organisms that cause the souring of milk are destroyed. Spores are not destroyed by this process. Place the fresh milk in sterile glass bottles, stopper with cotton and stand in a vessel of water (a double boiler), and heat the water to 70° C. for ½ hour. The milk will remain sweet 24 hours.

Test for Pasteurized or Sterilized Milk

1. Place 10 c.c. of milk in a test tube and heat to 70° C.

2. Place 10 c.c. of milk in a second test tube and heat to 80° C.

3. Cool, and add to each 1 c.c. of paraphenylene diamine solution; add a few drops of hydrogen peroxide. There is an instantaneous change to deep blue color, caused by the unchanged enzymes in the first tube; while the overheated milk in the second tube does not change to the blue color for some time.

Other preservatives used in connection with milk are

boric acid, borates, formaldehyde, hydrogen peroxide, salicylic acid, and salicylates.

Test for Boric Acid and Borates

Place 2 c.c. of the milk in a porcelain dish and expel the water by heating over a water bath. Then heat the solids over a low flame until a white or light gray ash is obtained. Add 2 drops of dilute HCl acid in 1 c.c. of water. Soak a piece of turmeric paper in a dish, then remove and dry in the air. Boric acid is indicated by a deep red color, which changes to green or blue upon adding dilute alkali.

Formaldehyde.—Leach's Test

Mix 10 c.c. of the milk and 10 c.c. of concentrated HCl containing 0.002 gram ferric chloride in a porcelain dish, and gradually raise the temperature of the mixture on a water bath nearly to boiling, with occasional stirring. A violet color is produced if formaldehyde is present.

Hydrogen Peroxide

To 10 to 15 c.c. of milk add 3 drops of a 2% aqueous solution of paraphenylenediamine hydrochloride. Shake. A blue color appears immediately in the presence of hydrogen peroxide.

Salicylic.—Remont's Method

Acidify 20 c.c. of milk with sulphuric acid. Shake vigorously to break up the curd. Add 25 c.c. of ether, mix, and decant 5 c.c. of the ethereal extract; evaporate to dryness.

Boil the residue with 10 c.c. 40% alcohol. Cool, and

make up the volume to 10 c.c. Filter, and to 5 c.c. of filtrate, which represents 2 c.c. of milk, add 2 c.c. of a 2% solution of ferric chloride. A purple or violet color indicates the presence of salicylates.

Fat

To 5 c.c. of milk in a small Babcock tube add an equal volume of sulphuric acid (sp. gr., 1.83) and enough of a mixture of concentrated HCl and amyl alcohol to fill the neck of the tube. Centrifugalize 5 minutes. The per cent of fat is read off directly on the tube, and is accurate to within 0.5%. If the top of the fat column is not at zero, add water and centrifugalize 1 minute.

Counting Bacteria

Mix the contents of the can, as the cream contains more bacteria than the milk. Place the specimen in a sterile test tube. With a sterile pipette dilute the milk in sterile broth or salt solution. If an initial dilution of $\frac{1}{100}$ is made, quantities ranging from 1 c.c. to 0.1 c.c. of this will furnish 0.01 c.c. to 0.001 c.c. of the milk. Inoculate cooled tubes of melted agar with varying amounts of these dilutions and pour on sterile Petri plates. Incubate 12 to 24 hours, then count the colonies.

Infected Milk

If typhoid is suspected, use same media and method (Hesse) as in detecting typhoid in feces.

References: Medical Chemistry and Toxicology, Holland. Physiological Chemistry, Hawk.

CHAPTER XII

PREPARATION OF TISSUE

The specimen should not be larger than ½ inch square, and this should be placed in fixing solution as soon as possible. Zenker's fluid is considered the best general fixative for histological study, as the bacteria, nuclear figures, and fibrils of all kinds are better preserved. Tissues fixed in Zenker's fluid and corrosive sublimate must be transferred after thorough washing in water to 70% alcohol, then 80% alcohol.

Tissues hardened in alcohol and formaldehyde may remain indefinitely in those fluids; and tissue fixed by alcohol, or any other reagent except formaldehyde, must be washed in running water several hours before freezing.

Tissue fixed in formaldehyde, wash 20 to 30 minutes.

Freezing Method for Rush Diagnosis

The microtome is fastened firmly in position and the wire attached. Connect the other end of the wire to the tube of carbon dioxide.

Place a piece of the tissue on the freezing box of the microtome, with a few drops of water beneath it. Turn the gas on slowly at first, then rapidly.

Hold the handle of the knife so that the thumb presses against the end of the wooden part, and apply the edge, bevel side downward, at an angle of 45°.

Turn the wheel of the microtome screw with the other

hand, and cut several sections without changing the position of the hand or the angle of the knife. Place the cut sections in a small pan of water. Clean a slide by rubbing it with alcohol, then spread a thin layer of albumin mixture on the surface with a fine brush, and rub this in thoroughly with the finger, having previously cleaned the finger by wiping it with alcohol.

Dip the slide into the dish under the section so that it can be floated on and spread out evenly on its surface.

Drain and blot with a *smooth* blotting paper. Cover with 95% alcohol, drain, and cover with absolute alcohol. Drain and cover with a thin solution of collodion. Drain, and if the tissue is to be examined for rush diagnosis, cover with Löffler's methylene blue (dilute $\frac{1}{3}$) for 15 seconds.

Delafield's Alum-Hematoxylin Method

After fixing the tissue on the slide in the manner described above, cover with alum-hematoxylin stain 3 to 5 minutes. Wash in several changes of water.

Cover with contrast stain of eosin (aqueous solution of $\frac{1}{10}$ to $\frac{1}{2}$%) 2 to 5 minutes.

Remove the excess of stain by three changes of 95% alcohol.

Clear in oleum origani cretici. Mount in xylol balsam.

If the oleum origani is not used, remove the excess of stain with absolute alcohol, then clear with xylol, and mount in xylol balsam.

Satisfactory sections are obtained from fresh tissue frozen in this manner, but the formation of ice crystals frequently causes tearing of delicate tissue, like the brain, spinal cord, and retina; so that it is better to infiltrate

such tissues with a mass that does not crystallize in the freezing mixture, but becomes hard and tough.

The delicate tissue is soaked in a sirup made by dissolving 1 pound of loaf sugar in 1 pint of water and boiling.

Remove the sirup from the outside of the tissue with a cloth and put it into ordinary gum mucilage for 1 hour, then place on the microtome and freeze in the usual manner.

Mayer's Albumin Solution

White of 1 egg	25 c.c.
Glycerin	25 c.c.
Salicylate of soda	0.5 gram
Water	4 c.c.

Dissolve the salicylate in the water, then add the white of egg and beat thoroughly. Add the glycerin and filter.

Collodion Mixture

Flexible collodion	1 c.c.
Absolute alcohol	15 c.c.
Ether	15 c.c.

It is necessary to imbed the sections in celloidin or paraffin for finer histological work, and for the highest class of work in tissue cutting it is necessary to have a microtome in which the knife is a fixture. This process is a much longer one than the freezing method, and takes from 24 to 36 hours.

Fresh tissue may also be examined in teased preparations made by cutting a very small section and dividing it by means of two sharp needles. Place on a slide in a drop of normal salt solution. Cells may be easily obtained.

if the tissue is soft, by scraping the cut surface with the edge of a knife.

Decalcification

Place a small piece of bone in formalin (10%) for 24 hours, then transfer to concentrated sulphuric acid for 24 hours. Wash thoroughly in alkaline water and then in tap water. Proceed as with other frozen sections.

Nicolle's Method

1. Löffler's methylene blue, 10 minutes.
2. Acetic acid (1–500), 10 seconds.
3. Tannin (1%), 5 seconds.
4. Wash in water, 95% alcohol, absolute alcohol, and xylol. Mount in balsam.

Van Gieson's Stain

1. Stain deeply in alum-hematoxylin.
2. Wash in water.
3. Stain in Van Gieson's solution 3 to 5 minutes.
4. Wash in water.
5. Dehydrate in 95% alcohol.
6. Cover with oleum origani cretici.

Zenker's Fluid

Bichromate of potassium	12.5 grams
Corrosive sublimate	25 grams
Water	500 c.c.

Dissolve the corrosive sublimate and bichromate in water with the aid of heat. To every 50 c.c. of Zenker's solution, which is sufficient for one specimen, add 2.5 c.c. of glacial acetic acid before dropping in the tissue. Fixation is accomplished in 24 hours.

Mounting

Xylol balsam is the best reagent used for permanent mounts. It has a high index of refraction, and tissues mounted in it become very transparent.

To clean the microtome wipe with alcohol, then ether, and lastly wipe with an oily cloth.

REFERENCES: Mallory and Wright's Pathological Technic. Stohr's Textbook of Histology. Lee's The Microtomist's Vade-Mecum.

APPENDIX

Reagents for Blood

Gower's Diluting Solution for Counting Red Blood Cells

Sodium sulphate	7.5 grams
Acetic acid	20.0 c.c.
Aqua	128 c.c.

Haymen's Solution for Counting Red Blood Cells

Mercuric bichloride	0.25 gram
Sodium sulphate	2.5 grams
Sodium chloride	0.5 gram
Distilled water	100 c.c.

Diluting Solution for Counting White Blood Cells

Acetic acid	1 c.c.
Distilled water	300 c.c.

Toisson's Solution for Simultaneously Counting Red and White Cells

Methyl violet	0.05 gram
Neutral glycerin	30 c.c.
Distilled water	80 c.c.

Mix and add:

Sodium chloride	1.00 gram
Sodium sulphate	8.00 gram
Distilled water	80 c.c.

Filter. 12 minutes required to stain white blood cells.

Wright's Stain

Sodium bicarbonate (C. P.)	0.5 gram
Distilled water	100 c.c.
Methylene blue (B. X.)	1 gram

Sterilize 1 hour at 15 pounds pressure. When cold add 500 c.c. of $\frac{1}{1000}$ solution Eosin Gruber w. g., or enough to make the mixture purple with a metallic scum on the surface. The precipitate is then collected on a filter and allowed to dry. When thoroughly dry, dissolve this precipitate, 0.5 gram in methyl alcohol, Merck reagent, 100 c.c., which makes the stain.

Reagents for Urine

Nitric acid, (HNO_3).
Acetic acid, ($HC_2H_3O_2$).
Sulphuric acid, C. P. (H_2SO_4).
Hydrochloric acid, (HCl) C. P.
Ammonic hydrate, (NH_4OH).
Sodic hydrate, (NaOH), U. S. P.

Esbach's Reagent

Picric acid	10 grams
Citric acid	20 grams
Water	1,000 c.c.

Fehling's Reagent

Solution "A":

Copper sulphate	34.65 grams
Distilled water	500 c.c.

Solution "B":

Sodium potassium tartrate	173 grams
Sodium hydroxide	125 grams
Water	500 c.c.

Magnesia Mixture

Ammonium chloride	1 part
Magnesium sulphate	1 part
Ammonia water	1 part
Water	8 parts

Dissolve the salts in the water, then add the ammonia water.

Nylander's Reagent

Bismuth subnitrate	2 grams
Rochelle salt	4 grams
Sodium hydroxide (8%)	100 c.c.

Phosphotungstic Solution

Phosphotungstic	1.5 grams
Hydrochloric acid	5 c.c.
Alcohol	100 c.c.

Standard Potassium Sulphocyanide (KCNS) Solution

Potassium sulphocyanide	8.29 grams
Distilled water to	1,000 c.c.

2 c.c. corresponds to 1 c.c. of standard silver nitrate solution.

Sodium Acetate Solution

Sodium acetate	100 grams
Distilled water	800 c.c.

Dissolve this and add 100 c.c. of 30% acetic acid. Make up to 1 liter.

Uranium Acetate Solution

Uranium acetate	34 grams
Distilled water	1,000 c.c.

1 c.c. equals 0.005 gram P_2O_5, phosphoric anhydride.

To Standardize: To 50 c.c. of a standard solution of disodium hydrogen phosphate, of such strength that 50 c.c. contains 0.1 gram P_2O_5, add 5 c.c. of sodium acetate solution and titrate with the uranium solution to the correct end reaction.

1 c.c. of uranium solution should precipitate 0.005 gram of P_2O_5.

20 c.c. of uranium solution should be required to precipitate 50 c.c. of the standard phosphate solution.

Standard Ammonium Thiocyanate Solution

(1 c.c. equals 1 c.c. standard silver nitrate)

Ammonium thiocyanate	12.9 grams
Distilled water	800 c.c.

Place 20 c.c. of standard silver nitrate solution in a flask, 5 c.c. of a cold saturated solution of ferric alum, and 4 c.c. of nitric acid (sp. gr., 1.2). Add water to 100 c.c. Mix. Fill burette with the ammonium thiocyanate solution, run in until a permanent red-brown color is obtained, which is the end point and indicates that the last trace of silver nitrate has been precipitated. Make a reading and calculate the amount of water necessary to use in diluting the ammonium thiocyanate so that 10 c.c. may equal 10 c.c. of the silver nitrate. Make the dilution and titrate again.

Standard Silver Solution

Silver nitrate	29.042 grams
Distilled water to make	1,000 c.c.

1 c.c. equals 0.01 gram of sodium chloride, or 0.006 gram chlorine.

Indicator:

Ferric ammonium sulphate crystals	100 grams
Dissolved in 25% nitric acid	100 c.c.

Standard Solution Uranium Nitrate

Uranium nitrate	44.8 grams
Distilled water to make	900 c.c.

Titrate the solution with a standard phosphate solution; the amount of water to be added to the remainder of the uranium solution, so that 1 c.c. will be equivalent to the 0.005 gram of P_2O_5, can be calculated.

Ammonium Sulphocyanate Solution

Ammonium sulphocyanate	13 grams
Distilled water	800 c.c.

Titrate this solution against the standard nitrate and estimate how much water should be added to the remainder to make it exactly equivalent to the standard silver solution.

Roberts' Reagent

Concentrated HNO_3	1 c.c.
Saturated solution of $MgSO_4$	5 c.c.

Reagents for Gastric Analysis

Congo Red Solution

Congo red	0.5 gram
Alcohol (95%)	10 c.c.
Water	90 c.c.

Dissolve the dye in the water and add the alcohol.

Resorcin Solution

Resorcin	5 grams
Cane sugar	3 grams
Alcohol (95%)	100 c.c.

Uffelmann's Reagent

Carbolic acid (4%)	10 c.c.
Water	20 c.c.
Ferric chloride solution (U. S. P.)	1 drop

(This solution should be prepared fresh for use.)

Gunzburg's Reagent

Phloroglucin	2 grams
Vanillin	1 gram
Alcohol	30 c.c.

(Keep in colored bottle.)

Lugol's Iodine Solution

Iodine	1 part
Potassium iodide	2 parts
Water	50 parts

Reagents for Stains

Gram's Stain.—No. 1

Gentian violet	2 grams
Aniline oil	9 c.c.
Alcohol (95%)	33 c.c.

No. 2

Gentian violet	2 grams
Distilled water	100 c.c.

Mix 1 c.c. of No. 1 with 9 c.c. of No. 2; filter. This solution will not keep longer than three weeks.

Gram's Iodine Solution

Iodine	1 gram
Potassium iodide	2 grams
Distilled water	300 c.c.

Löffler's Methylene Blue

Saturated alcoholic methylene blue	30 c.c.
Solution KOH, $\frac{1}{10000}$	100 c.c.

The dilute KOH solution may be made by adding 1 c.c. of a 1% solution to 99 c.c. of water.

Neisser's Stain.—No. 1

Methylene blue	0.1 gram
Alcohol (95%)	2 c.c.
Glacial acetic acid	5 c.c.
Distilled water	95 c.c.

Dissolve the methylene blue in the alcohol, then add the acetic acid and water, and filter.

No. 2

Bismarck brown	0.2 gram
Water (boiling)	100 c.c.

Dissolve the dye in the boiling water and filter.

Carbol Fuchsin

Basic fuchsin	1 gram
Alcohol	10 c.c.
Carbolic acid (1–20)	100 c.c.

Gabbet's Stain

Methylene blue	2 grams
Sulphuric acid	25 c.c.
Water	75 c.c.

Giemsa's Stain

Azur II eosin	0.3 gram
Azur II	0.08 gram
Glycerin, C. P.	25 c.c.
Methyl alcohol	25 c.c.

Dissolve the dye in the glycerin at 60° C., then add the methyl alcohol at the same temperature.

Aqueous Alum-Hematoxylin Stain

Hematoxylin crystals	1 gram
Saturated aqueous solution of ammonia alum	100 c.c.
Water	300 c.c.
Thymol	1 crystal

Dissolve the hematoxylin crystals in water with heat. Expose to the light in a bottle lightly stoppered. After 10 days keep in a tightly stoppered bottle.

Delafield's Hematoxylin

Hematoxylin crystals	4 grams
Alcohol (95%)	25 c.c.
Saturated aqueous solution of ammonia alum	400 c.c.

The hematoxylin is dissolved in the alcohol and added to the alum solution. Expose the mixture in an unstoppered bottle for 4 days. Filter and add:

Glycerin	100 c.c.
Alcohol (95%)	100 c.c.

Keep in the light until the color is dark, then filter and keep in a tightly stoppered bottle. Dilute 10 to 15 times when staining.

Polychrome Methylene Blue

This stain is an old alkaline solution of methylene blue and carbonate of potassium in which, as a result of oxidation, methyl violet and methylene red form.

The process of oxidation requires months. A ripened solution may be obtained from Grubler.

INDEX

Clinical laboratory technic for nurses.