Introduction to chemical methods of clinical diagnosis / by H. Tappeiner.

INTRODUCTION TO
CHEMICAL METHODS
OF
CLINICAL DIAGNOSIS

BY

DR. H. TAPPEINER

TRANSLATED FROM THE GERMAN EDITION

WITH AN APPENDIX ON

MICRO-BIOLOGICAL METHODS OF DIAGNOSIS

BY

EDMOND J. McWEENEY

Edw. N. Gregg.
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BY

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TRANSLATED FROM THE SIXTH GERMAN EDITION

WITH AN APPENDIX ON

MICRO-BIOLOGICAL METHODS OF DIAGNOSIS

BY

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TRANSLATOR'S PREFACE

THE object of this little book is to provide the student with an inexpensive and reliable pocket-guide to the simpler chemical and micro-biological manipulations required in modern diagnosis. Completeness has not been so much aimed at as thoroughness of description, and only methods of proved efficiency have been selected.

My original idea was to translate and edit Professor Tappeiner's excellent little work, which gives, as I think, a more thorough and up-to-date account of the chemical side of clinical diagnosis than can be found elsewhere in the same compass. The Appendix is the outcome of a request by Messrs. Longmans that I should increase the book's sphere of utility by writing an account of such microscopical and biological procedures as are most frequently called for in the diagnosis of disease. In complying with this request I have kept steadily in view the points upon which, according to my experience, the student stands most in need of definite information and skilled guidance—

points not to be found in the text-books commonly read, or information on which is scattered or inadequate. Thus, for example, the Blood has always appeared to me to be a subject upon which accurate, clearly expressed modern information is unusually difficult of access to the student whose reading is confined to the ordinary text-books, and in view of its rapidly growing importance I have endeavoured to treat it with some minuteness of detail. And here let me acknowledge my indebtedness to Dr. R. C. Cabot's work on 'The Clinical Examination of the Blood' (London : Longmans, 1897), as well as to Dr. Stengel's article on 'The Blood,' in Stedman's 'Twentieth Century Practice,' vol. vii. (London : Sampson Low, 1897). I have made free use of both of these excellent sources of information, and have not hesitated to adopt certain passages *verbatim et literatim*. My thanks are also due, and are hereby tendered, to Messrs. Wm. Wood and Co. of New York, and to Mr. Young J. Pentland, of Edinburgh and London, for allowing me to make use of illustrations from books published by them. I am also under a great obligation to my friend Dr. J. B. Coleman, physician to the House of Industry Hospitals, for reading the proofs, as well as for many valuable suggestions.

To some it will seem, no doubt, that I have described certain operations—notably the examination of the sputum for tubercle bacilli—with wearisome profusion of petty detail. My reply is that I hope to

place the student or practitioner in a position to carry out this and other important procedures for himself without other guide save this little book, and have, therefore, striven to foresee and provide against all the little pitfalls and errors of *technique*, the accumulation of which leads to the failure of the operation and—which is a more serious matter—the discouragement of the operator.

In conclusion let me point out that this does not purport to be a mere urine-testing book, nor, on the other hand, does it pretend to be a text-book of clinical diagnosis. The little book is an endeavour to give a succinct account of some of the more useful modern diagnostic procedures, clinical and biological, and is intended for use in the ward, and in the clinical laboratory, as well as in the study by candidates preparing for examination. Into its pages I have striven to compress an amount of practical information not hitherto presented, so far as I am aware, to the British student in so small a compass. Whether I have succeeded is another matter.

E. J. McWEENEY.

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A. REAGENTS IN GENERAL USE

These are to be used, unless otherwise directed, in the strength here given. In the case of officinal substances the pharmacopœal strength (B.P.) is selected.

Reagents directed to be used in dilute solution are to be treated with water in the proportion of 1 part reagent to about 10 parts of water.

1. Strong Acetic Acid
   96 per cent.; specific gravity 1·064. (The glacial acid with about 3 per cent. of added water.)
2. Strong Hydrochloric Acid
   Specific gravity 1·16.
3. Strong Nitric Acid
   In order to render it suitable for all purposes it should be exposed to the light till it assumes a somewhat yellow colour.
4. Caustic Soda Solution
   Should contain 15 per cent. of NaOH and have a specific gravity of 1·159–1·163. [*Liq. sodæ* (B.P.) is too weak.—*Transl.*]
5. Saturated Solution of Common Salt
   26 per cent.; specific gravity 1·201.
6. Half-saturated Solution of Chlorinated Lime
   Prepared by diluting a saturated solution of Calx Chlorinata (B.P.) with an equal volume of water; it must be freshly made from time to time.
7. Perchloride of Iron (Liq. Ferri Perchlor. B.P.)
   Specific gravity 1·11.
   Always has an acid reaction, but must not contain any free acid, and when treated with ammonia must give a precipitate of iron hydroxide which persists on shaking.

8. Potassium Ferrocyanide (Yellow Prussiate of Potash)
   10 per cent. solution.
9. Copper Sulphate
   10 per cent. solution.
10. Nylander's Reagent (Prep. § 20, 3)
    To be kept in a black bottle or in the dark
11. Rectified Spirit
12. Chloroform

B. REAGENTS IN OCCASIONAL USE.

1. Glacial Acetic Acid
2. Strong Sulphuric Acid
   98 per cent.; specific gravity 1·843.
3. Caustic Potash
4. Ammonia (Liq. Ammoniæ, B.P.)
   10 per cent. NH_3; specific gravity 0·959.
5. Milk of Lime
6. Calcined Magnesia
7. Common Salt
8. Ammonium Sulphate
9. Magnesium Sulphate
10. Calcium Chloride (Solid)
    To be kept in a tightly stoppered bottle.
11. Zinc Chloride (Liq. Zinci Chlor. B.P.)
12. Zinc Acetate
13. Watery Solution of Iodine in Potassium Iodide (Lugol's Sol.)
14. Sodium Nitroprusside (Solid)
15. Millon's Reagent
    Mercury is dissolved by the aid of gentle heat in its own weight of strong nitric acid. Twice the volume of water is added, and after a few hours the reagent is freed by decantation from any crystalline precipitate that may have formed.
16. Potassio-Mercuric Iodide
    Mercuric iodide is dissolved in hot potassium iodide solution to saturation, and solution diluted with several volumes of water.
17. Lead Acetate (Liq. Plumbi Subacetat. B.P.)

18. Silver Nitrate

    5 per cent. solution. To be kept in the dark or in a black bottle

19. Ether

20. Methyl Violet

21. Congo-Paper

22. Tropæolin OO

23. Vanillin

24. Phloroglucin

25. Phenylhydrazin

26. Esbach's Reagent (Prep. § 9)

27. Starch

28. Tincture of Guaiacum

29. Turpentine (Ol. Tereb. B.P.)

30. Liquefied Carbolic Acid B.P. (Acid Carbol. Liq.)

31. Grape Sugar

C. APPARATUS.

One Dozen Test-tubes 6 in. to 8 in. long by about $\frac{5}{8}$ in. wide

In doing the tests they are filled one-quarter to one-third full of urine, *i.e.* so as to contain 5 to 7 c.cm.

Small Filters, Watch-glasses, Small Porcelain Capsules (3 in. to 4 in. wide), Platinum Foil, Glass Rods, Urinometer, &c.

D. INSTRUMENTS, REAGENTS, AND APPARATUS NECESSARY FOR MICRO-BIOLOGICAL METHODS OF DIAGNOSIS.

1. Microscope with three Objectives

   (Zeiss A. D. and homog. imm., Leitz 3, 7, and homog. imm. or $\frac{1}{2}$ in., $\frac{1}{6}$ in., and oil imm. $\frac{1}{12}$ in. of English manufacturers)

2. Slips of best Crown-glass with Ground Edges

3. Cover-glasses $\frac{5}{8}$ in. or $\frac{3}{4}$ in. No. 1 Squares

4. Bunsen or Spirit Lamp

5. Forceps (Cornet's and Ordinary)

6. Scalpels, Scissors, &c.
7. Filter-paper and Gummed Labels
8. Glassware

   Comprising watch glasses, or glass pots for stains, beakers, flasks, Petri's dishes, funnels, glass tubing (especially 'quill' tubing for making capillary pipettes), glass rod, &c.
9. A Piece of Thick Crown Glass about 18 in. by 8 in.

   The back of which is enamelled white at one end and black at the other, is very useful for working on.
10. Bacteriological Apparatus

    Inoculation-needles, sterilisers, substrata, incubators, thermometers, heat-regulators, &c.
11. A Centrifugal Apparatus (Daland's Hæmatocrit)
12. Thoma-Zeiss Hæmacytometer
13. Gowers' Hæmoglobinometer
14. Stains

    Comprising Ehrlich's or Delafield's hæmatoxylene; methylene-blue, basic fuchsin, gentian violet (best kept in saturated alcoholic solution); eosin (watery and alcoholic); acid fuchsin; Bismarck brown; the Biondi Heidenhain triple stain (best obtained in powder from Dr. G. Grübler & Co., Bayer'sche Strasse, Leipzig); carbol-fuchsin (made by adding to 5 per cent. carbolic solution one-tenth of its volume of saturated alcoholic solution of basic fuchsin).
15. Clearing and Mounting Materials

    Aniline oil; Xylol; Canada balsam dissolved in xylol.
16. Absolute Alcohol: Non-mineralised Methylated Spirit
17. 'Formaline'

    (40 per cent. aqueous solution of formic aldehyde.)

INTRODUCTION TO CHEMICAL METHODS

OF

CLINICAL DIAGNOSIS

I. GENERAL CHARACTERS OF THE URINE

§ 1. *Changes undergone by the Urine after Evacuation*

Freshly passed normal urine is clear. After it has stood for some time, a *cloud of mucus* (*Nubecula*) gradually forms which may contain a few mucus-corpuscles and epithelial cells from the urinary passages. After standing for a longer period (over twenty-four hours) it deposits a slight sediment, consisting of *uric acid, urates, and oxalate of lime*, if alkaline fermentation has not meanwhile set in.

Sooner or later, as a rule not before the end of twenty-four hours, *alkaline fermentation* comes on—most rapidly in the case of dilute, faintly acid urine, containing albumen, blood, or mucus, and in specimens that have been collected in vessels soiled from previous use. The urine becomes turbid and alkaline owing to the development of bacteria, which change the urea into carbonate of ammonia. As a result of this,

sediments are formed, consisting of earthy phosphates, ammonio-magnesian phosphate, and, later on, of ammonium urate, whilst the odour becomes unpleasant (evolution of ammonium carbonate).

§ 2. *Reaction of the Urine*

1. *Acid.*—It turns blue or violet litmus-paper red—the normal reaction of human urine—and is due, not to the presence of free acid, but to that of acid salts, chiefly monosodium phosphate NaH_2PO_4 (acid phosphate of soda).

2. *Neutral or Amphoteric.*—It turns blue litmus-paper red, and red litmus-paper blue. This reaction is chiefly caused by the presence of both mono- and di-sodium phosphate, NaH_2PO_4 and Na_2HPO_4, of which the former has an acid and the latter an alkaline reaction.

3. *Alkaline.*—It turns red or violet litmus-paper blue, and yellow turmeric-paper brown. This reaction may be due to:

(*a*) *So-called fixed alkali, i.e.* the non-volatile alkaline phosphates and carbonates, *e.g.* di-sodium phosphate, Na_2HPO_4, tri-sodium phosphate, Na_3PO_4, and, more rarely, sodium carbonate, Na_2CO_3.

Such urine is clear or turbid when freshly passed, but after standing a short time is always turbid, owing to the separation out of earthy phosphates, $Ca_3(PO_4)_2$ and $Mg_3(PO_4)_2$, with which a few crystals of ammonio-magnesian phosphate *may* be mingled.

The alkaline reaction of the test-paper does not disappear on drying the paper in the air.

(*b*) *Ammonium Carbonate.*—Such urine was already in a state of alkaline fermentation whilst still in the bladder, and presents the same characters as urine in which the change has set in through keeping.

It is cloudy from bacteria and suspended earthy phosphates, *with which numerous crystals of ammonio-magnesian phosphates are invariably mingled.*

The alkaline reaction of the test-paper disappears on drying (dissipation of the ammonium carbonate).

Urine in the incipient stage of alkaline fermentation may also give an amphoteric reaction.

Causes of Alteration of Reaction in normal and pathological Urines

(*a*) *Acidity is increased by*:

1. Greater concentration of the urine due to increased excretion of water through other channels.
2. Increased albuminous metabolism (meat diet, febrile wasting).

(*b*) *Acidity is diminished or removed by*:

1. Increased ingestion of water.
2. Diminished metabolism (anæmic and cachectic states).
3. Consumption of carbonates and salts of vegetable acids. The latter are oxidised in the system and converted into carbonates (vegetable diet, acid wines, drugs, mineral waters).
4. Removal of acid from the blood during secretion of the gastric juice, temporarily during meals, permanently in habitual vomiting.
5. Rapid absorption of alkaline transudations and exudations.
6. Presence of alkaline fluids in the urinary passages (vesical catarrh, gonorrhœa, rupture of abscesses, &c.)
7. Alkaline fermentation.

§ 3. *Colour of the Urine*

We may distinguish:

1. Colorations due to *normal urinary pigments*:
   Pale urines.—Colourless to straw-yellow.
   Normal urines.—Golden to amber-yellow.
   High-coloured urines.—Reddish-yellow to red.

The urinary pigments, especially urobilin or its chromogen, are increased: Relatively, by increased concentration of the urine. *Absolutely*, by increased metabolism, fever, and hæmorrhages into the tissues when the hæmoglobin is there converted into urobilin.

The urinary pigments are diminished: in dilution of the urine and anæmic states.

Pale urine, without increase in quantity, excludes acute febrile conditions.

2. Colorations due to *abnormal pigments*, which are:

(*a*) Formed in the system and present, under pathological circumstances, in the urine.

(1) *Hæmoglobin.*—Bright red to brownish black.

(2) *Bile-pigment.*—Yellowish green to brownish.

(3) *Melanin.*—Brown to black.

[(4) *Indoxyl-sulphates.*—Brown.—*Transl.*]

Melanin urines are rare. When freshly passed they mostly contain melanogen, which is converted into the pigment, *gradually*, by standing exposed to the air; *quickly*, by addition of oxidising agents.

(*b*) Introduced into the system with Food or Drugs.

(1) After ingestion of *Carbolic Acid*, *Salol*, *Tar*, *Fol. Uvæ Ursi*, and similar bodies, compounds of sulphuric acid and phenols pass over into the urine (conjugate sulphates, pyrocatechin-sulphuric acid, hydroquinone-sulphuric acid). These bodies are colourless in themselves, but readily undergo decomposition and are oxidised, when the reaction is alkaline, into greenish-black products — humous substances (carbolic-acid urine).

(2) After ingestion of *Rhubarb*, *Senna*, and *Chrysarobin*, Chrysophanic acid passes over into the urine. So long as the reaction remains acid no abnormal coloration appears, but when it becomes alkaline the urine assumes a brownish to a blood-red tinge. On addition of acid the colour disappears again (dist. from hæmoglobin).

(3) After ingestion of *Santonin* the urine assumes a saffron-yellow to a greenish tinge, like that of icteric urine. On addition of liq. sodæ it becomes red, as in the case of rhubarb or senna (dist. from bile-pigment).

§ 4. *Specific Gravity*

The specific gravity of a 24-hours sample of normal urine averages 1·017 to 1·020, water being 1·000.

Pathological variations extend from 1·002 to 1·040. The determination of the specific gravity of a given quantity of urine affords a relative measure of its concentration. From the specific gravity of a mixed 24-hours sample the quantity of solid constituents excreted in that period may be approximately calculated. The last two decimals are multiplied by 2 (Trapp's coefficient) or 2·33 (Häser's coefficient), and the figure so obtained expresses the weight in grams of the solids contained in 1000 ccm. of urine. From this the quantity contained in the 24-hours urine may be readily calculated.

Determination of the Specific Gravity.—This is done with the hydrometer (urinometer) in the following way: The urine (filtered if necessary [1]) is poured into a suitable glass cylinder until the latter is three-quarters full. The cylinder should be held obliquely to avoid frothing. Any foam that may have formed is removed with a scrap of blotting-paper. The urinometer, which must be clean and dry,[2] is then *slowly* allowed to sink, until it floats quite freely. The reading is then obtained by placing the eye on a level with the lower margin of the surface of the fluid,[3] and determining the point at which it cuts the scale. A sensitive urinometer should allow half degrees (differences of 0·0005 in the density) to be accurately read off, and possess a scale graduated from 1000, the density of distilled water, to 1050, the highest density of human urine. (This scale may be conveniently divided between two hydrometers.) Urinometers give quite accurate results only for the temperature (usually 15° C.) for which they are adjusted. The

[1] A specimen containing particles of suspended matter gives too high a gravity.

[2] Should the spindle be wet or dirty, it will show too high a gravity.

[3] In other words, when one just loses sight of the posterior margin of the fluid-surface.

urine ought, therefore, strictly speaking, to be at the temperature in question. It is, however, sufficient for practical purposes if the temperature approximates to that required; in other words, if the urine is at room temperature. When the specimen is too warm the specific gravity comes out too low, and *vice versâ*. A difference of 3° C. roughly corresponds to one degree of the urinometer.

§ 5. *Quantity of the Urine*

The amount of urine passed by normal adults in twenty-four hours averages 1400 to 2000 ccm., and 1500 may be taken as the mean. Pathologically the amount may sink to *nil* (Anuria) or may rise to somewhere about 10,000 ccm. (Polyuria).

Generally speaking, the quantity passed is inversely proportional to the specific gravity, the depth of colour, and the amount of acidity. High-coloured, strongly acid urines have a high specific gravity, and the quantity is diminished; pale, faintly acid urines have a low specific gravity, and the quantity is increased. A pale urine with increase of specific gravity and quantity passed points to Diabetes Mellitus.

II. ALBUMEN

§ 6. *Classification of the most important Proteids*

I. Genuine (native) Proteids

To this category belong the proteids which occur in the system; they are soluble in dilute saline solutions, and coagulate when the solutions are boiled.

1. *Globulins*	2. *Albumins*
Precipitable from their solutions by addition of an equal volume of saturated solution of ammonium sulphate.	Precipitable only by saturating the liquid with solid ammonium sulphate.
Insoluble in water:	Soluble in water:
Serum-globulin (Paraglobulin)	Serum-albumin
Fibrinogen	Egg-albumin
Myosin	Muscle-albumin.
Vitellin	

II. Acid-albumins and Alkali-albuminate (Protein)

These are derived from native albumins through the action of acids or alkalis. They are insoluble in water and neutral salt solutions; soluble in dilute acids and alkalis. Such solutions do not coagulate when boiled; the proteid separates out on neutralising the solution; acid solutions are precipitated by concentrated solution of common salt.

III. Coagulated Proteids

Are soluble in concentrated, insoluble in dilute, acids and alkalis ; also insoluble in water and saline solutions.

§ 7. *General Reactions of Proteids*

Solutions containing proteid give the following reactions :

1. *Concentrated mineral acids in moderate quantities (not too small)* : precipitates, which are redissolved on further addition of the reagent.

Nitric acid is the quickest of all to precipitate, and the slowest to redissolve, the albumen. The precipitate turns yellow (xantho-proteic reaction), slowly in the cold, more rapidly when heated. The turbidity is still visible in 20,000-fold dilution, *i.e.* in a solution which contains one part of albumen to 20,000 of water.

2. *A few drops of acetic or hydrochloric acid + tannic acid solution* : a flocculent precipitate.

3. *Solutions of salts of heavy metals* : precipitates of metallic albuminates, which are for the most part redissolved in excess of the reagent.

4. *A few drops of acetic or hydrochloric acid* + 1 *to* 3 *drops of potassium ferrocyanide solution* : flocculent precipitate still quite recognisable in 50,000-fold dilution.

5. *Acetic or hydrochloric acid + solution of potassio-mercuric iodide* : white precipitate still visible as a turbidity in 20,000-fold dilution.

6. *Hydrochloric or acetic acid added till the reaction is strongly acid, then half a volume of saturated NaCl solution added and the mixture boiled* : a white flocculent precipitate still visible in 20,000-fold dilution.

7. Three volumes of alcohol : white flocculent precipitate.

8. *Millon's Reagent* (*solution of mercuric nitrate containing some nitrous acid*): white precipitate which becomes a beautiful red on heating: limit of delicacy, 20,000-fold dilution.

Tyrosin, aromatic oxyacids, and phenols give the same reaction.

9. *Liquor Sodæ + a drop of very dilute copper sulphate solution*: blue precipitate which, on shaking, dissolves with a pink tinge; on further addition of copper sulphate the solution becomes violet and at last blue (biuret reaction). Limit of delicacy, 2000-fold dilution.

10. *Heating a small quantity with a mixture of one volume concentrated sulphuric acid and two volumes glacial acetic acid*: beautiful reddish violet coloration. This reaction, which is due to Adamkiewicz, depends on the formation of furfurol, which yields bright-coloured products when brought in contact with certain decomposition products of albumen. Other acids give similar results (cf. also § 35, 6b).

§ 8. *Demonstration of Albumen in Urine*

Albuminuria may be produced by admixture of albuminous fluids, such as blood, pus, chyle, with urine, which, when secreted, was free from albumen. In these cases there are present, as a rule, only small quantities of albumen, but there is invariably a sediment consisting of the formed elements (blood-corpuscles, pus-cells, &c.) characteristic of these fluids.

These *accidental* albuminurias are to be distinguished from those of renal origin. The latter are produced by parenchymatous changes in the kidney or by circulatory disturbances, and are generally accompanied by an organised sediment (tube-casts, renal-epithelium, blood-corpuscles, &c.) in greater or lesser amount.

The amount of the albumen contained in urine generally varies between 0·05 and 1 per cent., or from 0·5 to 15 grams per day. Larger quantities, up to 30 grams, are unusual. Traces, however slight, are of diagnostic significance. As regards their influence on nutrition and the composition of the blood, quantities under 2 grams per day (= 0·1 per cent.) are looked upon as *small*; up to 8 grams (= 0·5 per cent.) as *moderate*; from 8 to 15 grams per day (= 0·5 to 1 per cent.) as *large*.

Urinary albumen is commonly a *mixture of albumen and globulin*. *Demonstration of the globulin* or its separation from the albumen may be effected by means of the different behaviour of the two bodies towards salts, *e.g.* ammonium sulphate. A determined volume of urine, having been rendered alkaline with ammonia, the phosphates filtered off if necessary, is treated with an equal volume of cold saturated solution of ammonium sulphate. The globulin is thus precipitated, whilst the albumen, which is only precipitable by several volumes of the saline solution, remains dissolved. The possibility of mistaking acid urate of ammonium for the globulin is prevented by the fact that the former separates out more slowly, and by the colour of the deposit.

The possibility of *simulated albuminuria* through contamination of the urine with albuminous substances (menstrual blood, fæces, semen, sputum, &c.) must be kept in view.

Albumen may be suspected when the urine *froths* much on shaking. Before undertaking the chemical demonstration, it is necessary to get rid of suspended sediment or bacteria by *filtration*. If this does not succeed at once, the urine should be shaken up with calcined magnesia, or a few drops of liquor sodæ are added, whereupon the earthy phosphates are precipitated and bring the cause of the turbidity down with them.

For preliminary examinations in practice, the result may be achieved, when the turbidity is slight, by pouring urine into two test-tubes of equal calibre, in one of which the urine is tested, whilst the other is kept for comparison.

1. COLD NITRIC ACID TEST (HELLER'S RING-TEST)

A few cubic centimetres of concentrated nitric acid are poured into a test-tube which is then held in as slanting a position as possible, and about the same amount of urine allowed to flow slowly down on to the nitric acid so that the two fluids do not mix: if there is formed, either at once or after a few minutes, a sharply defined ring-like haze at the junction of the two fluids, albumen is present.

The test depends on the formation of acid-albumen (§7, 1); it is made more sensitive by the superposition of the fluids.

The superposition may be very evenly effected by filtering the urine, and allowing it to drop from the filter down the side of the test-tube.

Possible sources of error:

(*a*) *Precipitation of nitrate of urea* (large crystals). This only takes place with highly concentrated urines and after some time. It may be completely prevented by previous dilution of the sample of urine.

(*b*) *Precipitation of uric acid* (ring-like haze somewhat above the junction of the two fluids, or, if present in large quantity, haziness of the whole fluid). It only occurs with concentrated urines; the uric acid is liberated from its salts by the nitric acid, and partly separates out because of its slight solubility in cold water. This may be prevented by diluting the urine with one or two volumes of water before the test.

(*c*) *Precipitation of resinous acids* (uniform cloudiness). These appear as salts, in the urine, after abundant ingestion of balsam of copaiba, styrax, turpentine, and, being insoluble in water, are precipitated by the nitric acid. They may be distinguished from albumen by test 2 or 3.

(*d*) Precipitation of albumose (a rare occurrence, cf. § 12).

(*e*) *Coloured rings*. The urinary pigments are oxidised by the nitric acid. This produces in every

specimen, but especially in highly coloured ones, a brownish-red ring at the junction of urine and nitric acid ; in presence of much indican or urorosein the ring is violet or pink. When bile pigment is present, the coloured zones of Gmelin's reaction appear. All these rings are easy to distinguish from albumen because turbidity is absent.

2. BOILING WITH ADDITION OF NITRIC ACID

The urine is brought to boiling-point in a test-tube, and, whether a precipitate has already formed or not, strong nitric acid is added till the reaction is strongly acid (5–10 *drops*). If the precipitate does not dissolve, or only appears on addition of the acid, then albumen is present.

Estimation of the quantity : With very small quantities of albumen there is slight haziness ; with about 0·1 per cent. there is a flocculent precipitate, which, on settling, almost fills up the concave bottom of the tube ; with 1 per cent. about one-half the column of urine is occupied ; finally, when very large amounts (3 per cent.) of albumen are present, the whole fluid forms a compact coagulum.

When only very little nitric acid has been added, albumen may (especially in alkaline urines) remain in solution, and, conversely, when a great deal of acid has been added, the albumen may become redissolved, especially if the specimen is boiled again after adding the acid, and, above all, if the acid has been added before boiling-point is reached.

The colour of the coagulum is commonly white ; a greenish hue points to the presence of bile pigment ; a brownish tinge indicates hæmoglobin.

The invariable *darkening of the urine* is due to the oxidation of the urinary pigment by the nitric acid.

Effervescence (CO_2) is often observed as the result of decomposition of carbonates in alkaline urine.

Urea, uric acid, and albumose being readily soluble when heated, *misinterpretations* are here confined to the *precipitation of resinous acids*. These are readily soluble in alcohol. If, therefore, their presence is to be reckoned with, the sample is treated with 2 volumes of rectified spirit, whereupon the resinous acids are dissolved, but albumen remains unchanged.

Alcohol should not, however, be added until the sample has become quite cold, nor unless the prescribed quantity of nitric acid has not been exceeded, lest the alcohol be oxidised with violent evolution of gas.

Reasons for adding the Nitric Acid:

(*a*) *The urine may contain albumen without coagulating when boiled.* This often happens with urines of alkaline reaction and moderate albumen-content. The albumen is in such cases converted into alkali-albuminate, which remains dissolved on boiling, but is precipitated by mineral acids (nitric).

(*b*) *The urine may give a precipitate on boiling even in the absence of albumen*—owing to the presence of earthy phosphates or mucous substances. The former are soluble in any acid, the latter only in mineral acids, not in acetic.

In very faintly acid or faintly alkaline urines the calcium and magnesium phosphates are present in solution as diphosphates which decompose on boiling into soluble monophosphates, and insoluble triphosphates which form a flocculent precipitate exactly like that of albumen, thus:

$4CaHPO_4$ Dicalcium phosphate (neutral phosphate of lime)	=	$Ca(H_2PO_4)_2$ Monocalcium phosphate (acid phosphate of lime)	+	$Ca_3(PO_4)_2$ Tricalcium phosphate (basic phosphate of lime)
$4MgHPO_4$ Dimagnesium phosphate	=	$Mg(H_2PO_4)_2$ monomagnesium phosphate	+	$Mg_3(PO_4)_2$ Trimagnesium phosphate

These precipitates are readily soluble in acids. They disappear, therefore, on addition of nitric acid, and cannot be mistaken for albumen.

A similar precipitation occurs when the urine contains acid calcium and magnesium carbonates $Ca(CO_3H)_2$ $Mg(CO_3H)_2$ in solution (which regularly happens with vegetarians). These salts are converted by boiling into insoluble neutral carbonates, $CaCO_3$ $MgCO_3$, carbonic acid being evolved. These precipitates are also readily soluble in acids.

3. BOILING AFTER ADDITION OF COMMON SALT AND ACETIC ACID

The urine is treated with acetic acid until the reaction is strongly acid (five drops), at least $\frac{1}{6}$ volume of saturated

NaCl-solution is added, and the mixture boiled without having regard to any precipitate that may meanwhile have formed: should the precipitate already formed (if any) not dissolve, or should it appear for the first time on boiling, albumen is present.

This test has the advantage of avoiding discoloration of the urine, and of causing the albumen to come out in large flocculi, giving a clear filtrate, which may be subjected to further tests, *e.g.* for sugar. In urgent cases it may be applied without chemicals or apparatus by using vinegar (which contains up to 6 per cent. of acid), and kitchen salt in an iron spoon or the like.

Resinous acids may possibly be precipitated by the acidulation of the specimen and become a source of error, which, however, can readily be avoided by addition of alcohol after the liquid has cooled.

Other precipitates which are likewise formed before heating (albumoses) dissolve again on boiling. This is also the case with uric acid, which, however, in the presence of so weak an acid as acetic, forms so slowly as hardly to become noticeable.

It must further be noted that *when considerably more than* $\frac{1}{6}$ *volume of NaCl solution has been added, the albumen is precipitated before the application of heat.* This is owing to the fact that native proteids in presence of concentrated NaCl solution are rapidly converted by acetic acid into acid albumins, which are precipitated by acetic acid + NaCl, according to § 6.

4. ACETIC ACID AND FERROCYANIDE TEST

The urine is made strongly acid with acetic acid (five drops) and one to three drops of potassium ferrocyanide-sol. added: cloudiness or a flocculent precipitate indicates albumen.

This is the most sensitive test of all, especially when carried out in the same way as Test 1, viz. by mixing a few ccm. of dilute acetic acid with a few drops of the ferrocyanide solution, and causing the urine to form a layer on top; a ring-like turbidity ensues even with the faintest traces of albumen.

Mistakes from separation out of resinous acids are avoided by subsequent treatment with alcohol. *Uric acid* comes out very slowly. The turbidity may be due to *albumose* or *mucin-like substances*, but then the other tests fail.

When the amount of albumen is very small, the turbidity takes some minutes to form.

Very concentrated urines may have to be diluted with an equal volume of water, in order to produce the precipitate, as it is somewhat soluble in strongly acid solutions.

5. PORTABLE REAGENTS FOR ALBUMEN

The four tests just described are one and all both sensitive and reliable when properly applied. It is nevertheless advisable not to rely on one alone, but to perform at least two—*e.g.* 1 and 3, or 2 and 4.

The following tests have been recommended on account of the ease with which they may be used at the bedside, being solid and not requiring to be boiled :

Metaphosphoric Acid

A small piece of metaphosphoric acid (vitreous phosphoric acid, HPO_3) is thrown into the urine, or the latter is stirred in a watch-glass with a little stick of the acid, which may be put up like a lunar caustic pencil. Any albumen present will come out in the form of a coagulum, soluble again in excess of the acid (according to general reaction No. 1 *supra*). This test does not allow of the recognition of much less than 0·1 per cent. of albumen. Errors due to uric or resinous acids may occur with this as with the other tests; but suitable dilution with water will prevent mistake in the case of the former substance. The acid must be kept in well-closed bottles because it readily attracts water, and becomes converted into ordinary phosphoric acid which does not precipitate albumen.

Geissler's Test-Paper

This consists of strips of strong filter-paper, some of which are soaked with concentrated citric acid solution, others with potassio-mercuric iodide, and dried. The urine is poured into a small capsule or glass, and a strip of the acid paper immersed. When enough citric acid is dissolved (which is ascertained by dipping in a blue litmus strip and observing when it is strongly reddened), the acid paper is withdrawn and replaced by a mercuric paper. Turbidity, or a flocculent precipitate, speedily indicates the presence of albumen. This test depends on general albumen reaction No. 5 *supra*; it is very sensitive, but not characteristic of albumen *alone*. In addition to the substances which have already been repeatedly alluded to as causing turbidity in presence of acid, *peptones* and *alkaloids* also give rise to precipitates. All these may of course be distinguished from albumen by further procedures, *e.g.* warming, addition of alcohol, &c., but the test loses thereby its vaunted simplicity, and has then no advantage over the others.

Stütz's Reagent

Sodio-mercuric chloride $HgCl_2NaCl$ precipitates albumen in presence of common salt and an acid (citric). Gelatine capsules are

filled with the dry reagents, and the test is performed by opening a capsule and shaking the contents out into the urine. In presence of albumen a flocculent precipitate is at once formed. Concentrated urines are previously diluted with half their bulk of water to avoid separation out of uric acid. Alkaloids are not precipitated.

§ 9. *Quantitative Estimation of Urinary Albumen*

Accurate determinations are obtained by weighing the albuminous coagulum or by measuring the lævo-rotatory effect of albuminous urine on polarised light. The last named method, however, yields accurate results only where the quantity of albumen exceeds 0·5 per cent. and is hard to carry out when the sample is cloudy or high-coloured.

Methods yielding approximate results suffice for the practitioner.

1. *Roberts's and Stolnikow's method as modified by Brandberg.*

This is founded on the observation that in albumen test, No. 1 *supra* (Heller's ring), the more albumen there is present the sooner the ring makes its appearance. When there is 1 part of albumen in 30,000 of fluid the ring appears in from 2 to 3 minutes. If, therefore, a urine containing an unknown amount of albumen be diluted with known volumes of water until the reaction is delayed for the above-mentioned period, the urine thus diluted contains 0·0033 per cent. of albumen, and the amount originally present in the undiluted urine may readily be calculated.

Mode of Performance :

1. A series of dilutions (see the list, *infra*) are made with the urine.

2. A few cubic centimetres of nitric acid are placed in each of a series of test-tubes, care being taken not to wet their sides with acid.

3. With a finely drawn-out piece of glass tubing (pipette) a certain quantity (the same in each case) of one of the dilutions is introduced into each of the test-tubes so as to form a layer over the acid, and the time noted which elapses before a bluish-white ring just begins to be visible. The sample which yields the result in from 2 to 3 minutes forms the basis of calculation according to the following table :

Dilution of the urine	How prepared	Percentage of albumen in the undiluted urine when ring appears in from 2 to 3 mins.
*10-fold	Urine 1 pt., water 9 pts. [' One-tenth urine ' used in preparing further dilutions]	0·033
20 ,,	'One-tenth urine' 1 pt., water 1 pt.	0·067
*30 ,,	,, ,, 1 ,, ,, 2 pts.	0·100
50 ,,	,, ,, 1 ,, ,, 4 ,,	0·167
80 ,,	,, ,, 1 ,, ,, 7 ,,	0·267
100 ,,	,, ,, 1 ,, ,, 9 ,,	0·333
*150 ,,	,, ,, 1 ,, ,, 14 ,,	0·500
200 ,,	,, ,, 1 ,, ,, 19 ,,	0·667
*300 ,,	,, ,, 1 ,, ,, 29 ,,	1·000
400 ,,	,, ,, 1 ,, ,, 39 ,,	1·333
500 ,,	,, ,, 1 ,, ,, 49 ,,	1·666

It is not advisable to make all the dilutions at once, but to experiment at first only with those marked *. Thus we learn whether the amount is higher or lower than $\frac{1}{10}$, $\frac{1}{2}$, or 1 per cent., and need then only make up the intervening dilutions for accurate determinations.

The dilutions are made as follows :

1. The ' one-tenth urine : ' 5 c.cm. of urine are measured off into a beaker glass with a graduated pipette and 45 c.cm. water run in from a burette.
2. The further dilutions: 1 c.cm. of ' one-tenth urine ' is accurately measured off into a beaker glass with a graduated pipette and the requisite number of cubic centimetres of water added from the burette.

2. *Esbach's Method*

Principle.—The height of the column of precipitated albumen is measured in a tube resembling an ordinary test-tube, but provided with graduations (albuminometer).

Test-solution.—A solution of 10 grams picric acid and 20 grams of dry citric acid in 1 litre of water. With the exception of some very rare cases, as yet unexplained, this reagent precipitates all albumen, but also creatinin, uric acid, peptones, and resinous acids, as well as alkaloids and other organic bases—a fact to be borne in mind when the patient is taking large doses of antipyretics, piperazin, &c.

Mode of Performance.—The albuminometer is filled with the urine (acidified with acetic acid if necessary) up to the mark U, and the test-solution added up to the mark R. The stopper is then put in, and the fluids caused to mingle by inverting the tube a few times slowly so as to avoid frothing. The instrument is then placed upright, whereupon the coagulated albumen sinks slowly to the bottom.

After twenty-four hours the height of the column of albumen is read off by the graduations on the tube. These run from 1 to 7 and give the amount of albumen in grams per litre of urine. Should the urine contain more than 0·7 per cent. it is previously diluted with an equal volume of water, and the result multiplied by 2. In my own modification of the instrument the space up to the mark U is divided into two equal parts by a mark $\frac{U}{2}$. When the urine requires dilution it is poured in up to the latter mark, filled up to U with water, the fluids mingled, and reagent then added as usual. The simplest way of making the dilution is to fill the tube first with urine and then with water up to U, mix the fluids (in another vessel), and then fill up to the mark with the mixture.

As the height of the column depends on several variable factors (density of the urine, temperature, amount of albumen) Esbach's method yields only approximate results sufficient for moderate practical requirements.

§ 10. *Fibrin*

The urine occasionally contains fibrin in cases of chyluria, hæmorrhages into, and inflammation of, the urinary tract, as occurs, for instance, after the application of fly-blisters. The fibrin is either already coagulated when passed or separates out only after standing, and then forms a flocculent sediment, or a jelly filling the vessel (coagulable urine).

Fibrin behaves like a coagulated proteid. It is insoluble in water and saline solutions as well as in dilute acids and alkalis. The latter cause it to become gelatinous in the cold, and the jelly is slowly dissolved on prolonged boiling. The solution gives the general proteid reactions.

§ 11. *Mucin*

The mucous substances contained in urine comprise true *mucin* on the one hand, and a *nucleo-albumin* resembling mucin in its reactions, on the other. They are *products of the urinary mucous membrane*, and traces are therefore to be found in almost every normal urine. In *inflammatory and irritated conditions of the kidney, bladder, and urethra* these substances appear in relatively larger quantities, so that their presence is of diagnostic value as giving information about the state of the passages. The test is based upon the fact that acetic acid precipitates them, and that they are but slightly soluble in excess, especially in presence of but small quantities of salts. *The urine is therefore diluted with one or two volumes of water and treated with acetic acid*, whereupon it becomes more or less distinctly turbid in presence of mucous substances. These cannot be confounded with albumen if tests 1 and 2 are employed, for mucous substances are soluble in mineral acids.

§ 12. *Albumose*

Albumoses are intermediate stages formed during the conversion of albumen into peptone. They have been repeatedly observed in the urine during various morbid conditions (large quantities, for example, in multiple myeloma) [tumour of bone medulla, soft lympho-sarcoma, *Transl.*]. Albumose is also found in urine that contains semen, being a constituent of that secretion.

Urines containing albumose give the following reactions:

1. The specimen, acidified, if necessary, with acetic acid, *becomes cloudy on warming when the temperature reaches* 60° *C. or thereabouts*, and clears up again perfectly on boiling, only to become turbid again on cooling.
2. *By treating the boiling urine with a little nitric acid* (as in albumen test 2), a precipitate does not appear until the specimen has cooled. This precipitate becomes redissolved on heating (often with a bright yellow coloration), and reappears on cooling.
3. *Addition of a moderate quantity of acetic acid and a few drops of potassium ferrocyanide solution* (albumen test 4) produces a turbidity which differs from that due to albumen by dissolving when warmed and reappearing when cooled. It should be borne in mind that even very dilute mixtures of potassium ferrocyanide and acid are decomposed on boiling, with evolution of hydrocyanic acid and formation of a white precipitate which quickly turns blue.
4. *Addition of at least* $\frac{1}{6}$ *volume* [$\frac{1}{6}$ of the volume of the urine actually submitted to the test, *Transl.*] *of saturated NaCl solution and a little acetic acid* (albumen test 3), produces a precipitate which is redissolved on boiling. If ordinary albumen is also present, the precipitate persists on boiling, or only forms at that stage. The turbid liquid is filtered boiling, and allowed to cool. If the filtrate then becomes cloudy, the presence of albumose is indicated.

§ 13. *Peptones*

Small quantities of peptones often occur during the rapid breakdown of normal or pathological tissues, *e.g.* the involution of the puerperal uterus, acute yellow atrophy of the liver and phosphorus poisoning, after hæmorrhages, in carcinoma, phthisis, croupous pneumonia and extensive richly cellular exudates and abscesses.

Peptones are readily soluble in water, do not coagulate when their solutions are boiled, and in presence of most of the ordinary tests for albumen, *e.g.* nitric acid, acetic acid + NaCl, acetic acid + potassium ferrocyanide, yield *no* precipitate.

Peptones in Kühne's sense are only precipitated by alcohol, tannic and phosphotungstic acids, whereas *peptones in Brücke's sense* are

precipitated—in addition to the above reagents—by potassio-mercuric iodide + acetic acid, metaphosphoric acid, and saturation with ammonium sulphate. Millon's reagent and liquor sodæ + copper sulphate produce the colorations described under the general proteid reaction. They are, however, not available for the direct demonstration of urinary peptone, because they are either not characteristic or not sufficiently delicate. The peptone must first be isolated from the urine by precipitation with phosphotungstic acid. [Devoto's method consists in saturating the acidified and boiling urine with solid ammonium sulphate. Any proteid demonstrable (by the biuret reaction, for instance) in the cold filtrate is looked upon as peptone.—*Transl.*]

§ 14. *Removal of the Albumen*

Several of the procedures yet to be described require the removal of any albumen that may be present in the urine. All, save minute insignificant traces, may be got rid of by boiling the specimen after suitable acidulation and then filtering.

The urine, if already acid, is heated until it just begins to boil and then withdrawn from the flame. If neutral, or alkaline, it must first be *slightly* acidified with dilute acetic acid. In case the albumen does not come out at once in large flocculi, and the boiling fluid shows only a diffuse turbidity (as generally happens), a few drops of dilute acetic acid must be carefully added until the desired result is achieved. The sample is then quickly boiled up for a moment and thrown at once on the filter. The coagulation is successfully accomplished if the albumen is suspended in the form of coarse flocculi and the supernatant liquid is quite clear and runs quickly through the filter. Should cloudiness still persist, there has been either too much or too little acetic acid added. In the first eventuality it is easy to add more; in the latter case the excess may be neutralised by careful addition of dilute soda solution. But it is simpler to try again with a fresh sample of urine.

When a great deal of albumen is present in urine

or other fluid, the following modified procedure will be found more successful: 20 to 40 c.cm. of water are brought to the boil in a porcelain capsule, and half as much or an equal volume of urine allowed to run in slowly with continued stirring, so as not to stop the boiling. The reaction is tested, acetic acid added, if necessary, so as to produce slight acidity and formation of large albuminous flocculi, and the liquid is then filtered.

Precipitation by acetic acid alone is often utilised as a test for albumen. It is, however, hardly suitable for the purpose, because of the care necessary in adding the acid. Thus, if too much be used, considerable quantities of albumen may remain in solution—in fact *all*, when traces only are present.

III. HÆMOGLOBIN

§ 15. *Characters*

1. VARIETIES OF HÆMOGLOBIN

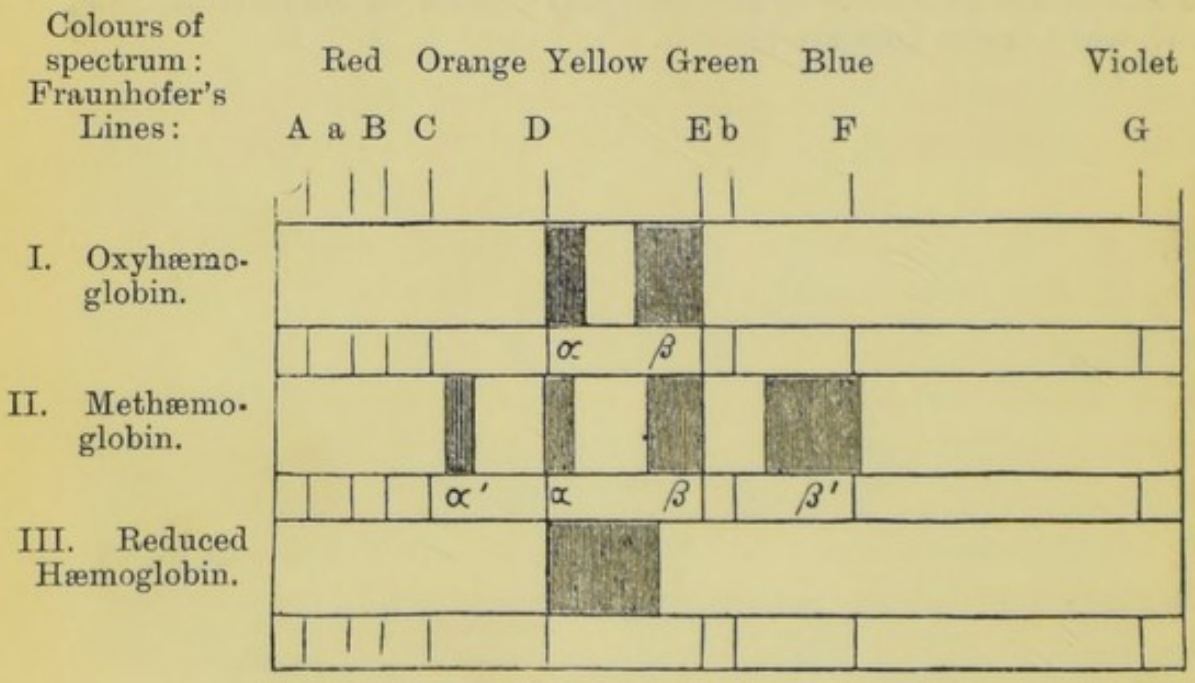

FIG. 1

Oxyhæmoglobin.—Its watery solutions are characterised by their brilliant red hue, and by two absorption bands in the spectrum. With a good pocket spectroscope and bright daylight these can be made out (fig. 1, I. *a* and *β*) from a stratum of liquid 1–2 cm. thick, even when the solution is very dilute (down to 0·01 per cent.)

Methæmoglobin contains as much oxygen as oxyhæmoglobin, but more closely combined. It is pro-

duced from the latter on addition of a crystal of ferricyanide of potash, as well as through the action of acids and acid salts (hence its presence in urine). In acid or neutral solution it is characterised by its brown colour and the presence of two additional absorption bands, a' and β', in the spectrum. All the four bands (fig. 1, II., a' a β β') are, however, visible only when the solutions contain considerable quantities of methæmoglobin; when more dilute, the only band that appears is a' in the red.

Reduced Hæmoglobin is formed from the two just mentioned by reduction, for example, by means of a few drops of sulphide of ammonium or ammoniacal solution of ferrous tartrate. Moderately dilute solutions have a greenish brown-red tinge and give a broad ill-defined absorption band (fig. 1, III.) On shaking up with air it is converted into oxyhæmoglobin.

Carboxy-hæmoglobin yields two absorption bands in nearly the same position as those of oxyhæmoglobin. They do not, however, disappear on addition of reducing-agents.

A sample of blood, containing both carboxy- and common hæmoglobin, will, therefore, after a certain amount of dilution, display the bands α and β and the band of reduced hæmoglobin; after further dilution it will still show the bands α and β.

Other tests for carbonic oxide are based upon the production of carboxy-hæmatin, which, in contrast to ordinary hæmatin, possesses a fine red colour. All reagents which decompose hæmoglobin with splitting off of hæmatin are more or less adapted for this purpose. Thus, *if a sample of blood* (10 *c.cm.*) *is treated, for example, with a mixture of* 15 *c.cm. of a* 20 *per cent. potassium ferrocyanide solution and* 2 *c.cm. of* 50 *per cent. acetic acid*, precipitation of albumen and hæmatin takes place in a short time. With ordinary blood the material is dirty brown, with CO-blood a beautiful red. The distinction of colour is quite recognisable, even when the blood is only one-tenth saturated with CO. After standing some time the CO-hæmatin passes over, as a result of dissociation, into oxyhæmatin.

Hydrocyanic-acid methæmoglobin is formed on addition of even extremely dilute hydrocyanic acid to methæmoglobin. It possesses a brilliant red hue, and in concentrated solutions shows a faint absorption band like that of reduced hæmoglobin. In order to test for hydrocyanic acid by this means, a solution of methæmoglobin is first prepared by mixing 1–2 c.cm. of blood with 100 c.cm. of water, and adding a small crystal of potassium ferricyanide. Some of this

solution is then run off into two test-tubes of equal bore, and the fluid to be tested for hydrocyanic acid is added to one of them. Even with very dilute solution of HCN the liquid turns a beautiful bright red, whilst the other sample only shows a yellowish-brown hue.

2. DECOMPOSITION OF HÆMOGLOBIN

Boiling, acids, and alkalis split up hæmoglobin into hæmatin and albumen.

Hæmatin is amorphous reddish-brown, in thick layers brownish-black, insoluble in water and acids, readily soluble in alkalis and precipitable, as the corresponding compound, from these solutions, on addition of alkaline earths (calcium, magnesium). It is also somewhat soluble in hot acetic acid, and in presence of a little NaCl crystallises out as hydrochlorate of hæmatin (hæmin), in characteristic rhombic tables (fig. 2).

FIG. 2

The *Albumen* is different according to the reagent used. Boiling yields coagulated albumen, acids produce acid-albumin, and alkalis, alkali-albuminate.

A solution of hæmoglobin therefore yields:

(*a*) On heating alone, or with addition of acid: a brown coagulum of albumen and hæmatin.

(*b*) On heating with addition of liq. sodæ: a dark brown fluid containing dissolved alkali-albuminate and hæmatin.

On adding salts of alkaline earths to this solution—for instance, by adding half its volume of normal urine—the hæmatin is precipitated as red flocculi. Heller's test for hæmoglobin in urine is based on this reaction.

§ 16. *Demonstration of Blood or Hæmoglobin in Urine*

Colour of the Urine: reddish-yellow, red, brown to brownish-black ['smoky,' 'porter-coloured,' *Transl.*].

The hæmoglobin is present either as *oxyhæmoglobin* (red urine) or as *methæmoglobin* (brown or 'smoky'

urine), and may be either (*a*) *dissolved in the urine* (Hæmoglobinuria), or (*b*) *contained in the blood-corpuscles* (Hæmaturia). Which form is present is decided by the microscopic examination for red blood-corpuscles.

Freshly passed samples contain the blood-pigment as oxyhæmoglobin in cases of abundant vesical hæmorrhage, as methæmoglobin in all cases of hæmoglobinuria, in many cases of renal hæmorrhage, and in some of slight vesical hæmorrhage. Oxyhæmoglobin when present in any quantity is readily detected by the red colour of the urine. Methæmoglobin, on the other hand, owing to its brown tinge and slight colouring power, may be present in considerable amount without producing any striking alteration of the colour of the urine.

After the urine has been kept some time the methæmoglobin passes over into oxyhæmoglobin or is present as reduced hæmoglobin.

In addition to the spectroscopic examination, the following reactions serve as *tests for hæmoglobin* :

1. *Boiling Test.—Heat the urine till it boils* : brown coagulum (not very delicate).

With alkaline samples one sometimes obtains only a brown coloration, and the coagulum only appears on acidulation with acetic acid (*cf.* characters of hæmoglobin 2*b*).

2. *Heller's Test.—Make the urine alkaline with liq. sodæ* (5 drops) *and heat till it boils* : a flocculent precipitate of a beautiful blood-red colour, which, in presence of very small quantities of hæmoglobin, may not be visible till it sinks to the bottom.

The reaction depends on the formation of hæmatin, which is taken up by the earthy phosphates that are precipitated by the alkali. One c.cm. of blood in a litre of urine [one part per thousand, *Transl.*] is thus readily demonstrable. When there is no hæmoglobin in the sample the phosphatic precipitate is white. *Rust-brown flocculi* are obtained when either too much or too little alkali has been added, or when the heat has been too strong ; they are not so distinctly visible as the blood-red ones, but are equally characteristic. *Dark-coloured samples* often do not allow of the ready recognition of the colour of the flocculi, which must then be separated by filtration.

Alkaline urines sometimes yield no precipitate at all, especially when all the calcium phosphate has already come out as sediment. The test must then be repeated on another sample to which a little of some calcium salt has been added—an equal volume of normal urine serves very well.

After ingestion of chrysarobin, rhubarb, and senna, a yellow pigment (chrysophanic acid) passes into the urine. It turns red with alkalis, and on heating is precipitated along with the phosphates in red flocculi. It is easy, however, to distinguish this from a precipitate of hæmatin, which remains undissolved as red-brown flocculi on addition of acetic acid, whereas the compound of phosphates and chrysophanic acid is completely dissolved. In the latter case, tests 1, 3, and 4 yield negative results. Considerable quantities of chrysophanic acid always attract attention by the red tinge they impart to the urine (especially the foam) on addition of caustic alkali.

After ingestion of santonin the urine gives the same reaction. For the mode of distinguishing between the two pigments see § 28, 8.

3. *Schönbein-Almén's [Guaiacum] Test. — An emulsion of equal parts of tincture of guaiacum and ozonised turpentine (i.e. turpentine that has stood a long time exposed to the air) is caused to form a layer on top of the sample of urine.* At the junction of the two fluids a white ring forms through precipitation of the resin, and this ring at once becomes a beautiful blue should hæmoglobin be present. [Ozonised ether may be conveniently substituted for the turpentine. *Transl.*]

The test depends on the transference, by hæmoglobin, of ozone from the turpentine to the guaiacum. The guaiacum is consequently oxidised (turned blue). This is almost more sensitive than the spectral test. It must, however, be remembered that *guaiacum resin is also turned blue by pus and oxidising substances*, *e.g.* ferric chloride. The change then takes place without addition of turpentine.

When the urine is alkaline the test is less sensitive. It is therefore well to acidulate with acetic acid.

The most sensitive tincture is that made up of 1 part guaiacum resin to 18 parts of spirit. It must be *freshly prepared*, or at any rate kept in a bottle of dark glass. The turpentine, on the other hand, is kept in a half-filled vessel exposed to the light. Occasional testing of these reagents, by means of urine to which blood has been designedly added, is very desirable.

4. *Hæmin Test.*—The precipitate obtained by test 1 or 2 is collected on a small filter, washed, and dried at a gentle heat.

A small fragment is then laid on a glass slide, a minute crystal of common salt added, a cover-glass applied, and the space between the two glasses filled

up with glacial acetic acid. The preparation is then heated for about a minute over a small flame, so that the fluid does not boil strongly, but only gives off minute bubbles. The acid lost by evaporation is replaced by occasional addition of a drop. In presence of hæmatin the fluid becomes gradually brownish-red. When this has occurred the preparation is allowed to cool down to about 45° C. by removing it to a suitable distance from the flame, and kept at this temperature till all the acetic acid has evaporated. The hæmin crystals are then sought for by focussing the microscope on the brownish patches in the specimen. Filling up the space beneath the cover-glass with glycerine brings out the crystals still more clearly.

Fine type specimens of hæmin crystals are obtained by allowing a minute droplet of blood to dry on a slide, adding a trace of salt, covering, and proceeding as above.

Hæmato-porphyrin (iron-free hæmatin) appears in the urine in various diseases, most characteristically in chronic sulphonal-poisoning. The brownish-red urine either gives directly—when suitably diluted—the characteristic absorption spectrum (in acid solution a band before D and a second wider one between D and E, in alkaline solution 4 bands distributed over the whole spectrum), *or* it must be first precipitated with alkaline solution of barium chloride, and the hæmato-porphyrin then extracted from the precipitate with warm alcohol containing a little sulphuric acid.

IV. PIGMENTS

§ 17. *Indican* (*Indoxylsulphuric acid* $C_8H_6N.O.SO_2OH$)

Origin.—A product of proteid decomposition in the bowel, hence, in small traces, a normal constituent of the urine. Larger quantities appear in digestive disturbances, ileus, cancer of stomach and liver, typhoid, intestinal tuberculosis, peritonitis, and also in putrid decompositions taking place within the body.

Jaffé's Test.—A test-tube is nearly half filled with urine and about the same amount of strong hydrochloric acid added; 2 or 3 c.cm. of chloroform and 1 drop of a half-saturated solution of chlorinated lime are then introduced, and the mixture extracted with the chloroform by repeated inversion of the test-tube, the open end of which is closed with the thumb. In presence of indican the chloroform which sinks to the bottom is of a blue colour.

The reaction depends on the splitting-up by the HCl of the indican into indoxyl and sulphuric acid, and the oxidation of the former to indigo-blue by the chlorinated lime. The indigo-blue being insoluble in water separates out as a finely divided blue precipitate and is taken up by the chloroform. If the addition of the chlorinated lime is proceeded with drop by drop, the blue coloration disappears when the urine is poor in indican through oxidation of the indican to isatin, which is yellow. On the other hand, if the sample is rich in indican, the blue coloration at first increases and only gives place to the yellow tinge after addition of a good many drops. A quantitative estimation of the indican may readily be based on this reaction. If the sample is heated with the chlorinated lime or other oxidising agents, such as nitric acid, red colorations are produced, because under these circumstances indigo-red is chiefly formed instead of indigo-blue.

It is important to avoid too vigorous agitation with chloroform, which then separates with difficulty out of the emulsion so formed.

Albuminous urine must previously be coagulated.

The chlorinated lime may conveniently be replaced by one or more drops of ferric chloride.

[According to the translator's continued experience nitric acid acts quite as well as chlorinated lime, and has the additional advantages of being found on every urine-testing table and of keeping well, which is not the case with the chlorinated lime. About 2 fluid drams each of urine and strong hydrochloric acid, together with 1 or 2 drops (*not more*) of strong nitric acid, are the quantities he finds most convenient. The application of a gentle heat—far short of boiling—will be found to increase greatly the yield of indigo-blue, but care must, of course, be taken to cool the sample by immersing the test-tube in cold water before adding the chloroform.]

§ 18. *Bile-pigments*

1. *Properties.*—The colouring matters of the bile are bilirubin and biliverdin. Both are insoluble in water and acids, soluble in alkalis, and precipitable from solution by calcium salts in the form of reddish-brown or green flocculi on account of their forming with calcium combinations that are insoluble in water. It is in this combination that they are present in gall-stones.

Bilirubin is insoluble in alcohol, soluble in chloroform which is coloured yellow; out of this solution it crystallises in brownish-red prisms and tables.

Biliverdin, on the other hand, is insoluble in chloroform, soluble in alcohol, from which it crystallises in indeterminate forms.

2. *Tests.*—Icteric urine is yellowish-green to a porter-like brown in colour; its foam is yellow. Any sediments that may be present (oxalate of lime, tube-casts, epithelial cells) are often stained a vivid yellow.

Gmelin's Test.—The urine is carefully poured on to concentrated, somewhat yellowish, nitric acid, so as to form a supernatant layer. In presence of bile-pigment several superposed coloured rings make their

appearance at the junction of the two fluids—a green ring on top, and then from above downwards blue, violet, red, and yellow zones.

A similar play of colour, in the shape of concentric rings, is obtained by filtering the urine and placing a drop of nitric acid on the inner side of the still moist filter-paper. The delicacy of this modification (Rosenbach's) increases with the quantity of urine that has run through, which should not be less than 50 c.cm. The reaction depends on the successive oxidation of bilirubin to other pigments, of which the first to be formed is biliverdin, and does not succeed unless the nitric acid is somewhat yellow, *i.e.* unless it contains some peroxide of nitrogen. This body is developed when the acid has stood some time exposed to the light or, more quickly, by warming it with a shaving of wood or a piece of sugar.

The presence of albumen—save in very large amount—does not interfere with Gmelin's reaction, as the green comes out distinctly in the white albumen ring.

Other oxidising agents, *e.g.* dilute tincture of iodine (Smith's test), likewise give a biliverdin-ring by superposition.

Remarks: 1. Gmelin's reaction is not demonstrative unless the *green ring* is distinctly visible.

2. Many samples become green on addition of any mineral acid. These appear to contain preformed biliverdin (biliprasin).
3. The urine may contain bile-pigment without giving the reaction, when, for instance, other pigments (urobilin) obscure the play of colours, or when there is not enough unaltered bile-pigment present. The colour of the urine does not by any means always correspond to the intensity of Gmelin's reactions. It may become necessary to isolate the bilirubin out of the urine either by acidulation and extraction with chloroform, or by precipitation with alkaline earths by Huppert's method, which admits of the recognition of 2 per cent. of bile, whilst Gmelin's test only gives a positive result with 5 per cent.

The urine is accordingly treated with milk of lime or chloride of barium solution + liq. sodæ. The precipitate from icteric urine is coloured yellow and contains the bile-pigments in combination with calcium (or barium). If the precipitate is filtered off and boiled with alcohol, to which a few drops of dilute sulphuric acid have been added, a beautiful green solution of biliverdin is obtained.

The *bile-acids* cannot be directly demonstrated in urine. Pettenkofer's test with sugar and sulphuric acid is interfered with by the presence of substances which give similar reddish-violet colorations with the sulphuric acid, so that unequivocal results are only obtained when the urine contains 0·5 per cent. of bile-acids—a quantity probably in excess of what is reached in jaundice. The bile-acids must, therefore, be first isolated from the urine (§ **35**, 6).

§ 19. *Urobilin*

This substance arises through decomposition of blood-pigment and through reduction of bilirubin, and is therefore called also hydrobilirubin. It is, however, questionable whether the pigments developed in each case are quite identical.

It is an amorphous reddish-brown substance soluble with difficulty in water, readily soluble in alcohol and chloroform, and forming with alkalis salts that are readily soluble, whilst with earths and heavy metals its salts are insoluble.

Urobilin does not react to Gmelin's test for bile-pigment.

It is present, even in normal urine, in minute quantities, as a chromogen, which is very easily converted into urobilin, especially in presence of acids. Larger amounts are always to be found whenever the red corpuscles are destroyed in considerable numbers —for instance, in fevers, and after blood extravasations (cerebral hæmorrhages, infarctions, retro-uterine hæmatocele, extra-uterine pregnancy, scurvy, &c.). In such cases the urine is always deep reddish-brown, and sometimes gives the same yellow foam as in jaundice.

TESTS

1. *Spectroscopic.*—Urine containing much urobilin shows an absorption band between the green and the blue, roughly corresponding to the position of the fourth methæmoglobin band (fig. 1, β').

The band is plainer with acid than with alkaline samples, and in the latter is somewhat displaced towards the left; it is also very distinct after the following test has been applied.

2. *Fluorescence.—The urine is treated with* 5 *drops of* 10 *per cent. chloride of zinc solution, and ammonia added, until the precipitate at first formed redissolves on shaking.* In presence of urobilin in not too small

quantity the liquid, after subsidence of the phosphates in suspension, shows green fluorescence when viewed against a dark background.

In this reaction the urobilin is first precipitated as the zinc salt and then dissolved in the ammonia. All solutions of urobilin fluoresce, and urines that contain much of it often fluoresce before any treatment has been resorted to; solutions of the zinc salt, however, possess the property in the highest degree.

Very small quantities of urobilin are hardly demonstrable in urine. It must first be isolated by shaking up the acidulated urine with chloroform, and then removed from the latter by agitation with very dilute liq. sodæ.

Urorosein is a red, very unstable pigment, perhaps related to urobilin. It may occur in diabetes, chlorosis, ulceration of the stomach, cancer of the œsophagus, typhoid, perityphlitis, and nephritis. Such urine displays, on addition of about $\frac{1}{10}$ per cent. by volume of a strong mineral acid, a pink or rosy tinge. Addition of an alkaline carbonate causes it to disappear (distinction from indigo-red). The reddish tinge assumed by normal urine on treatment with an equal volume of concentrated hydrochloric acid seems also to depend on the formation of urorosein.

[§ 19 *bis*. Ehrlich's 'Diazo' reaction. This is a colour-reaction given by the urine of typhoid fever when brought in contact with diazo-benzene-sulphonic acid and ammonia. Two solutions are required in addition to the ammonia:

1. A 5 per cent. solution of hydrochloric acid saturated with sulphanilic acid.

2. A 0·5 per cent. solution of sodium nitrite.

These are kept in separate well-stoppered bottles, and mixed at the time of use in the proportion of 40 of the first to 1 of the second, and the mixture well shaken. Diazo-benzene sulphonic acid is thus formed. Equal parts of urine and reagent are shaken up in a test-tube, and a few cc. of ammonia carefully poured down the side so as to form a supernatant layer. A red or carmine ring formed at the junction of the liquids indicates a positive reaction. 'If the mixture be then poured into a porcelain basin containing water, a salmon-red colour will be obtained if the reaction be positive, while a yellow or orange colour is obtained when negative' (Simon). The important point is the red or pink colour. Normal urines yield a yellow or orange hue. The reaction is by no means characteristic of typhoid fever, being encountered in many other febrile, as well as in some afebrile, conditions. It occurs, however, with greater regularity in typhoid than in any other disease. Some clinicians (v. Jaksch) regard it as indicative of acetone, but others (Simon) deny this. Sero-diagnostic methods have deprived the diazo reaction of any importance it may ever have possessed.—*Transl.*]

V. GRAPE-SUGAR (DEXTROSE)

§ 20. *Tests for Sugar in the Urine*

1. MOORE-HELLER'S TEST

The urine is treated with one-fourth to one-third of its volume of liq. sodæ [*potassæ equally good—Transl.*] *and boiled for* 2 *to* 3 *minutes.* In presence of sugar it assumes a dark yellow to a dark brown hue, according to the amount present.

The reaction depends on oxidation of the grape-sugar, and in pure sugar solutions is very sensitive, but is only demonstrative of sugar in urine when the *brown* coloration is well marked, in other words, when there is a good deal of sugar present [at least 0·5 per cent., *Transl.*] ; for dark *yellow* colorations may be produced even in sugar-free urine by boiling with liq. sodæ.

The flocculent precipitate which is generally formed during this test consists of the earthy phosphates which are precipitated by the soda in the cold, and become agglomerated into great flocculi when heated. It is a perfectly normal occurrence.

2. TROMMER'S TEST

The urine is treated with one-fourth of its volume of liq. sodæ, and solution of copper sulphate is added drop by drop with vigorous shaking, until a small quantity of copper hydroxide remains undissolved. If the urine dissolves much copper hydroxide and assumes meanwhile a beautiful blue colour, the presence of sugar is probable.

The mixture is now warmed just to boiling-point,

preferably selecting one spot near the surface of the fluid. If orange-red streaks or clouds (consisting of precipitated copper suboxide) appear, forming a sharp contrast with the blue surrounding fluid—throughout the whole of which they gradually extend—the presence of sugar is proved.

Trommer's test produces the typical result just described only in pronouncedly diabetic urines. In many other cases the urine dissolves an abundance of copper hydroxide, and the blue solution changes colour on boiling, becoming yellow or brown owing to reduction; but a distinct precipitation of copper suboxide either does not occur at all (the precipitated phosphates may be tinged brown owing to a little entangled copper hydroxide), or it only takes place while the liquid is cooling.

In a number of such cases precipitation of the suboxide while the heat is being applied may be obtained by very accurate saturation with copper sulphate. As the precipitate of earthy phosphates caused by the alkali often makes it hard to decide when the copper hydroxide begins to remain undissolved, the following method may, with advantage, be resorted to.

Urine enough for about 4 tests is treated as above described with liq. sodæ and copper sulphate until saturation point is nearly reached: a quarter of the mixture is then poured over into another test-tube and heated.

If no precipitate is obtained, but only a yellow coloration, some more copper sulphate is added to what remains, and then another portion run off and tested, and so on until either a distinct precipitate of suboxide forms, or the persistence of the green colour after heating shows that an excess of copper hydroxide is present. In the first case the presence of sugar is proved; in the second, Trommer's test is indecisive. The reduction *may* be due to sugar, but it may also be caused by other reducing substances. This point can only be certainly determined by test 3, 4, or 5.

The answer to the questions, why the suboxide is not always precipitated, and why precipitation, and not mere decolorisation (reduction), must decide the presence of sugar, is based upon a knowledge of the principle of the reaction and its relation to normal urine.

During the explanation it will become apparent how necessary it is to adhere rigorously during the performance of the test to the very letter of the directions.

Reaction between liq. sodæ and copper sulphate.

1. If to pure water some liquor sodæ and copper sulphate solution be added, a bulky blue precipitate of copper hydroxide is formed—$CuSo_4 + 2NaOH = Na_2SO_4 + Cu(OH)_2$—which does not dissolve in the liq. sodæ, and when boiled changes into brownish-black copper oxide, CuO, or, more accurately, $Cu(OH)_2 + 2CuO$.

2. If, however, the water contains certain substances like glycerine, tartrates, ammonia, albumen, or grape-sugar, the precipitated copper hydroxide becomes dissolved on shaking, and forms a blue fluid. The power of dissolving copper hydroxide is thus by no means characteristic of sugar alone.

Substances that dissolve copper hydroxide.

3. This blue solution when brought to boiling point behaves variously.

Behaviour on heating.

If glycerine, tartaric acid, &c. are present, there is no change; but if sugar is present, a yellow or red precipitate of copper suboxide is at once formed, and the fluid is decolorised.

The cause of this phenomenon is oxidation of the sugar which in hot alkaline solution withdraws from the copper hydroxide a portion of its oxygen, and converts it into red copper suboxide Cu_2O or yellow hydrated copper suboxide $Cu_2(OH)_2$.

This process is spoken of as reduction.

The capacity for reducing copper hydroxide in alkaline solution is not, however, peculiar to grape-sugar alone, but is shared by many organic bodies. These are called 'reducing substances.'

Reducing substances.

Some of these substances occur in the urine, as may readily be shown by performing Trommer's test with a normal specimen. Another important property of the urine thus comes to light.

4. If to normal urine some liq. sodæ is added, a precipitate of earthy phosphates is obtained, which, at first hardly perceptible, gradually sinks in colourless flocculi to the bottom. If now a solution of copper sulphate be added drop by drop, every drop produces a bulky blue precipitate of copper hydroxide, which dissolves again on vigorous shaking. If the addition be continued till a small trace of copper hydroxide remains undissolved, which is generally the case after 2 or 3 drops, and the bluish green liquid is heated to boiling, it loses this colour, and becomes deep yellow by transmitted, reddish yellow by reflected, light; the copper hydroxide has therefore been reduced. A *precipitation*, however, does not take place, for the fluid remains perfectly clear, the sediment of phosphates remaining distinct at the bottom, and coloured reddish brown by traces of copper suboxide entangled in it. Only very concentrated (febrile) urines sometimes give a dirty greenish yellow precipitate. From this behaviour it follows:

Power of normal urine to dissolve and reduce copper hydroxide and to keep copper suboxide in solution.

(*a*) That normal urine contains substances which are able to dissolve copper hydroxide (uric acid, creatinin, salts of ammonia).

(*b*) That it contains substances which reduce copper hydroxide (uric acid, glycuronic acid, carbohydrates, pyrocatechin, &c.)

(*c*) That normal urine contains substances which keep copper suboxide in solution (uric acid, creatinin, salts of ammonia, &c.)

The capacity of normal urine to dissolve and reduce copper hydroxide is not great, 3 to 5 drops of copper sulphate being about the limit for about 10 c.cm. of urine. Its power of dissolving copper suboxide is, however, much greater, as the following experiment shows.

5. If normal urine be treated with grape-sugar until a solution of about 0·5 per cent. strength results, and this be tested as above, the same phenomena occur to a more pronounced extent: considerable amount of copper hydroxide dissolved, strongly marked yellow coloration on heating, but no precipitation of copper suboxide, at least not while heat is being applied nor immediately afterwards. The cause of this behaviour is the great power which normal urine possesses, especially when heated, of dissolving copper suboxide. Hence, not only the suboxide formed by the reducing substances, but also that due to the oxidation of the sugar, is kept in solution. It is only when the presence of still greater quantities of sugar has given rise to an excess of copper suboxide that all does not remain in solution, but separates out as a precipitate.

6. Normal urine containing up to 0·5 per cent. of sugar behaves like the above-described pathological urine with incomplete Trommer's Reaction (absence of precipitation of copper suboxide). Such urines *may* contain sugar, but the *proof* is wanting, because the strong reduction may be caused by other reducing substances, which have already been shown to be present in every urine to a greater or lesser extent.

Precipitation of copper suboxide essential to prove presence of sugar.

Only when, in addition to reduction, *precipitation* of the suboxide takes place is sugar proved to be present; for experience teaches that other reducing substances do not occur in urine in quantities large enough to provoke precipitation of suboxide.

7. The test for sugar based upon precipitation of copper suboxide is, however, only reliable if the temperature be kept under boiling-point. The heating must therefore be stopped just short of boiling. Ebullition causes many normal urines to give a precipitate of suboxide, which, however, does not usually come out during but after ebullition, whilst the specimen is cooling.

Necessity of keeping the heat below boiling-point.

8. A practical conclusion from the presence in urine of solvents for copper suboxide is that care must be taken, when dealing with small amounts of sugar and concentrated urines, to obtain a maximal formation of copper suboxide so as to increase the likelihood of some of it being precipitated.

Necessity of saturation with copper.

Sugar reduces under the influence of heat rather more copper hydroxide than it can dissolve in the cold. Hence the maximal formation of copper suboxide is to be obtained by originally adding just so much copper sulphate that the hydroxide precipitated is not completely redissolved, but a little remains over in suspension, so that the urine is to a certain extent supersaturated with copper hydroxide.

The directions given for Trommer's test are based upon these considerations. How necessary it is to adhere to them rigidly may readily be shown by treating a somewhat concentrated normal urine with 0·5 per cent. of sugar, and subjecting three samples of it to Trommer's test, with insufficient, correct, and excessive addition of

copper sulphate, respectively. In the first case, the bluish green solution of copper hydroxide will only turn yellow or brownish red on boiling, on account of the oxide being reduced but not precipitated; whilst the excess of grape-sugar yields Moore's test. In the second case there ensues a typical precipitation of the suboxide, whereas in the third case only a dirty green turbidity is produced, because the great excess of undissolved and unreduced hydroxide is capable of preventing, or, at any rate, of concealing, the precipitation of the suboxide.

9. Normal urines to which sugar has been intentionally added, as well as many pathological samples, may (v. § 5, p. 36) contain sugar up to 0·5 per cent. without giving, by Trommer's test, a precipitate of copper suboxide, which (v. § 6, p. 36) is alone conclusive of sugar. In typical diabetic urines, on the other hand, precipitation occurs with a sugar-content of as little as 0·2 per cent. Smaller amounts of sugar are accordingly detectible in diabetic than in normal urine.

Distinction between diabetic and transitory glycosuria.

This remarkable fact is explained by the diminution undergone by the copper-suboxide-dissolving substances in diabetic urine as a result of the polyuria. Accordingly, in cases where Trommer's test fails to give a typical suboxide precipitate, it is often possible to obtain one by repeating the test with the urine diluted fivefold (artificial polyuria).

The uncertainty in which Trommer's test often leaves the observer is perhaps calculated to induce him to give up using it as a test for urinary sugar, and to resort instead to one or other of the sensitive tests immediately to be described. This would be going too far. *Trommer's test maintains its value for the physician because it supplies a criterion of the amount of sugar present, and therefore of the severity of the case.* Should it readily succeed, even with insufficient hydroxide saturation, the amount of sugar is large and the case, generally speaking, one of pronounced diabetes. Should, on the other hand, suboxide precipitation fail altogether, or occur only on very accurate saturation with hydroxide, whilst the more sensitive tests give a positive result, then the sugar-content is not more than 0·2 to 0·4 per cent., and there is a possibility that the case may be only one of transitory glycosuria (§ 22).

(*a*) Albuminous urine dissolves but does not reduce copper hydroxide. As, however, the precipitation of the suboxide may be hindered thereby, it is better on the first appearance indicative of albumen, viz. when the first drops of copper sulphate added to the alkalised urine dissolve with a violet hue (Biuret reaction, § 7), to get rid of the albumen by treating another sample with acetic acid + NaCl (§ 8, 3) and repeat the sugar-test on the filtrate.

Presence of albumen and ammonia.

(*b*) If a urine contains ammonium carbonate (alkaline fermentation) it dissolves considerable quantities of copper hydroxide, forming a blue fluid, which does not at first alter on heating, but on prolonged boiling may yield a black precipitate of copper oxide.

(*c*) If a urine contains both ammonium carbonate and sugar it may dissolve and reduce a good deal of copper hydroxide, and yet yield no precipitate of suboxide, because the ammonia may keep the latter in solution.

3. NYLANDER'S TEST WITH ALKALINE BISMUTH SOLUTION

(Böttger's test modified by Almén and Nylander)

Principle of the Reaction.—If a watery solution of grape-sugar be treated with liq. sodæ and a knife-point-full of bismuth subnitrate, $NO_3Bi(OH)_2$, and boiled for a minute or two, the bismuth salt turns black on account of the sugar withdrawing oxygen from it and converting it into black oxide or metallic bismuth. This reaction, discovered by Böttger, is in this form not conclusive of sugar alone, but, if performed as modified by Almén and Nylander, it succeeds with hardly any other urinary constituent, and is therefore conclusive of sugar.

Preparation of the Reagent: 4 grams of Rochelle-salt (sodium-potassium tartrate) are dissolved in 100 c.cm. of warm liq. sodæ, which must contain 8 per cent. of Na_2O = 10 per cent. NaOH, and possess a specific gravity of 1·115 at 15° C.; 2 grams of bismuth subnitrate are then added, and the mixture shaken. The bismuth salt is converted by the soda into the hydroxide, $Bi(OH)_3$, which is kept in solution by the tartrate. After cooling, the reagent is freed by decantation from any undissolved bismuth, or filtered through glass-wool. If kept in the dark it remains good a long time.

Performance of the Test.—The urine is treated with one-tenth of its volume of the reagent and boiled for 1 *to* 3 *minutes.* In presence of grape-sugar the bismuth hydroxide is reduced and a black, finely divided precipitate of bismuth suboxide formed, which remains long in suspension. With a sugar percentage of 0·2 or more the blackening comes on in a minute or two, and is so deep that the sample becomes quite opaque. With a smaller percentage of sugar the blackening only comes on distinctly after boiling for a longer time (3 minutes), and traces of sugar down to ·025 per cent. are not generally perceptible during the heating, but become evident by the greyish black hue which they give to the earthy phosphates precipitated by the soda. The phosphatic precipitate

from sugar-free urine is pure white. Samples very rich in sugar first give Moore-Heller's test.

Out of 100 samples of normal urine 14 reacted slightly to this test, and ceased to do so after prolonged exposure to the action of yeast, thus showing that no urinary constituent save sugar reacts to this test. This only holds good, however, when the solution is accurately made up, especially when the caustic soda solution is not stronger than that prescribed.

After ingestion of rhubarb and senna the urine gives Nylander's test even in the absence of sugar, but not Trommer's. The precipitated bismuth falls with surprising rapidity to the bottom. The presence of considerable quantities of rhubarb and senna is at once revealed on addition of the reagent by the red coloration; smaller quantities are to be tested for with Heller's hæmoglobin test.

After ingestion of salol, antipyrin, and turpentine reduction also occurs; this is probably also the case with many other medicaments which are excreted in the form of glycuronic acid, a point which must be carefully borne in mind.

Albuminous urine turns reddish brown on boiling with Nylander's reagent, owing to decomposition of the albumen with formation of sulphide of bismuth, which forms a precipitate on standing. This is reddish brown when albumen does not exceed 0·2 per cent., and is easily distinguishable from bismuth reduced by sugar; but when the percentage of albumen is larger the precipitate is brownish black, and might therefore give rise to mistakes. Cases also occur when, despite the presence of sugar, no reduction whatever occurs, owing, no doubt, to the albumen having combined with all the available reagent. It is therefore better to previously precipitate the albumen according to § 8, 3 or § 14.

Presence of much ammonium carbonate (alkaline fermentation) may hinder the reaction, because the sodium hydrate of the reagent combines with this salt and forms carbonate of soda and ammonia. The latter at once escapes on boiling, and is therefore unable to keep the solution sufficiently alkaline for reduction to take place.

4. WORM-MÜLLER'S TEST WITH COPPER SULPHATE AND ALKALINE ROCHELLE-SALT SOLUTION [Fehling's Sol.]

Necessary reagents.—1. Watery solution of copper sulphate (blue vitriol $SO_4Cu + 5H_2O$), strength 2·5 per cent. 2. Solution of 10 grams pure sodium-potassium tartrate (Rochelle-salt) in 100 c.cm. normal liq. sodæ (4 per cent. NaOH).

Performance:—5 c.cm. urine are heated in a test-tube to boiling-point; the same is done with a mixture of 2·5 c.cm. alkaline tartrate solution, and 1 or 2 c.cm. copper sulphate solution. As a rule

1·5 c.cm. $CuSO_4$ are used. When, however, the specific gravity is lower than 1·018, it appears advisable to begin with 1 c.cm., and if higher than 1·025 2 c.cm. may, as a rule, be added.

The boiling of the two fluids is simultaneously interrupted, and after 20 to 25 seconds they are mixed and allowed to stand without shaking. Immediately after mixture the fluid generally appears bluish green but alters, if sugar is present, through precipitation of hydrated copper suboxide, and the more sugar is present the sooner does the change take place. With a percentage of 0·1 of sugar the precipitation generally occurs after 4 or 5 minutes as a finely divided sediment, which is visible in reflected light as a dirty yellowish-green cloudiness.

As, in presence of small quantities of sugar, the reaction only takes place with certain very definite quantities of copper, if no precipitate be at first obtained, the test must be repeated with increasing dosage of $CuSO_4$ (2·5, 3, 3·5, 4 c.cm.) until it either occurs or the fluid is no longer decolorised, *i.e.* until it remains green, showing that an excess of $CuSO_4$ is now present. With this test as little as 0·025 per cent. of grape-sugar or 0·05 milk-sugar can be demonstrated in urine.

Normal urines often (18 times out of 100 samples) give a reaction which corresponds to 0·025 to 0·05 per cent. of grape-sugar, and disappears after the action of yeast. This can hardly be due to any other substance than grape-sugar.

The reason why the ordinary reducing substances of urine do not react to this modification is partly the presence of the alkaline tartrate, but chiefly the maintenance of a temperature below the boiling-point (60° to 70° C.). It is in order to make sure of this that the fluids after being separately boiled are only mixed after cooling for the specified time. Grape-sugar still reduces very actively at this temperature, whilst the other substances no longer do so.

5. FERMENTATION TEST

Principle.—Saccharine urine is fermented by yeast, the most important products being alcohol and carbonic acid, according to the equation : $C_6H_{12}O_6 = 2C_2H_6O + 2CO_2$. The alcohol remains in solution and the carbonic acid is evolved as gas, but may be collected and so rendered perceptible.

As no other urinary constituent, normal or pathological, gives a similar reaction with yeast, the test is a very reliable one, and sufficiently delicate (down to 0·1 per cent. sugar). The fact, however, that commercial yeast may yield some (preformed) CO_2, and further that it is not always active, necessitates two control tests ; and moreover, one must await the result. This method will therefore be resorted to only when the others leave one in doubt. Its value chiefly lies in the fact that it depends upon a principle essentially different from that of the reduction tests.

Performance.—Some urine is poured into a test-tube, a piece of fresh brewer's yeast as big as a pea is shaken up with it, the tube filled up with urine and closed air-tight with a rubber stopper, through which passes a short bent piece of glass tube.

A second test-tube of equal size is filled in the same way with normal urine and yeast, and a third with normal urine, yeast, and a knife-point-full of powdered grape- or cane-sugar. All then are placed bottom upwards in beaker glasses and kept in a warm place (25° to 30° C). If after 12 to 24 hours an abundance of gas has formed in tube 3, the yeast used was active. If gas is also present in tube 1 and absent from tube 2, or, at any rate, present in very much smaller amount, the presence of urinary sugar is proved.

For the sake of further control some caustic alkali may be introduced with a curved pipette. The gas will disappear if it consists of CO_2.

Faintly acid or alkaline urines require previous treatment with a few drops of a 10-per-cent. tartaric acid solution, in order to suppress the putrefactive processes inimical to alcoholic fermentation.

With 0·1 per cent. of sugar the gas about fills the concave bottom of the test-tube; with 0·2 per cent. there is distinctly more because the urine is already saturated with CO_2, and therefore does not absorb any more. From this point on the measure of gas is about proportional to that of sugar.

Special fermentation tubes are very convenient. [Einhorn's saccharimeter is a good form, and Southall's ureameter-tubes work very well, *Transl.*] They are U-shaped glass tubes provided with a foot. The longer limb is closed above, the shorter one is provided with a pear-shaped dilatation and open. The former is filled completely with the mixture of urine and yeast, and the gas accumulates in its upper (closed) end, whilst the displaced fluid accumulates in the dilated part of the short limb. [Loss of gas may be completely prevented by pouring into the dilated part sufficient mercury to seal the closed limb, *Transl.*]

6. PHENYLHYDRAZIN TEST

The urine (10 *c.cm.*) *is heated with* 0·5 *gram hydrochlorate of phenylhydrazin and sodium acetate for* 1 *hour on the water bath*:— In presence of sugar there appears, either immediately on cooling, or

some hours afterwards, a precipitate of slender yellow acicular crystals, a compound of phenylhydrazin with grape-sugar (phenylglucosazon). The precipitate must be abundant (filling the bottom of the test-tube or forming thick clouds in the liquid) and must consist either macro- or, at any rate, microscopically, entirely or nearly so of yellow needles arranged in fasciculate or radiating groups.

Amorphous precipitates in the form of brownish yellow scales occur in specimens which are free from sugar, and are due to the glycuronic acid always present in urine, and the phenylhydrazin compound of which appears as an amorphous precipitate after a little heating.

7. INDIGO TEST

If a watery solution of grape-sugar, which has been rendered strongly alkaline with carbonate of soda, is treated with a solution of indigo-carmine (sulphindigotic acid) until the colour is distinctly blue, and boiled, it becomes green, red, yellow, and when shaken up with air the series is repeated in the inverse order until the colour is once more blue. Sugar reduces the indigo-blue to indigo-white, which is then oxidised by the air back again to indigo-blue.

This test, as applied to the urine, is neither particularly sensitive nor characteristic of grape-sugar. The commercial test-papers for urinary sugar are based upon it. These are strips of paper of which some are soaked in indigo solution, others in soda solution, and dried. A strip of indigo-paper is soaked in water, and the urine treated with the blue solution until it assumes a faint blue colour; a large piece of soda-paper is then added and the mixture boiled: in presence of sugar the above-mentioned play of colours is produced.

§ 21. *Quantitative Estimation of Urinary Sugar*

The amount of sugar present in the urine of Diabetes Mellitus does not usually exceed 4 per cent.—in severe cases 5 to 6 per cent.; still larger quantities, up to 10 per cent. (500 grams per diem), are rare.

Accurate estimations of the sugar are obtained by titration with Fehling's solution or by measurement of the rotation to the right which polarised light undergoes in its passage through the saccharine urine.

The polarimeter gives accurate results when more than 0·4 per cent. of sugar is present, provided that the urine does not contain lævo-rotatory substances, such as albumen or oxybutyric acid (in severe diabetes): in such cases the values obtained are too low. High-coloured urines must first be decolorised with animal charcoal, or, still better (because the charcoal stops some of the sugar), with solution of acetate of lead (10 c.cm. to 90 c.cm. urine).

Approximate estimations of clinical value are obtainable by the two following methods:

I. DETERMINATION OF SPECIFIC GRAVITY BEFORE AND AFTER ALCOHOLIC FERMENTATION (ROBERTS'S METHOD)

Performance.—The specific gravity of the urine is determined, as usual, with a delicate urinometer, and the temperature noted.

A piece[1] of fresh commercial pressed yeast is then added, distributed by shaking the urine cylinder, covered with a sheet of filter-paper to prevent evaporation, and a beaker glass inverted over it.

The fermentation is complete in from 24 to 28 hours at room-temperature (20° to 24° C.), as can be recognised by the fact that the urine which was cloudy now becomes much clearer, that no more gas is developed and frothing ceases, whilst the yeast has fallen as a powdery sediment to the bottom—and still more certainly by the failure of a sample, removed by means of a pipette, to react to tests for sugar. The urinometer is now dropped in very carefully so as not to stir up the yeast at the bottom, the gravity read off, and the temperature ascertained. Should the latter be higher than before fermentation, the figure 0·0003 ($\frac{1}{3}$ of a urinometer degree) must be added for every degree C. in the difference; if lower, then the same figure is subtracted. *The required estimation is now obtained by subtracting the specific gravity so corrected from the specific gravity of the urine before fermentation, and multiplying the difference by* 230. [Every degree of sp. gr. lost = 1 grain of sugar per fl. oz., *Transl.*]

Comparative sugar estimations by fermentation and the other quantitative tests have shown that a difference of 0·001 (1 urinometer degree) in the gravity corresponds to 0·230 gram of sugar. Therefore the sugar percentage X of a urine, the difference of the specific gravity of which before and after fermentation = D, may be expressed as follows: $X = D \frac{0{\cdot}230}{0{\cdot}001}$.

With urines containing more than 0·2 per cent. of sugar and careful urinometer readings—down to $\frac{1}{2}$ degree—this method yields accurate estimation down to 0·1 per cent.

II. MEASUREMENT OF THE VOLUME OF CO_2 DEVELOPED DURING FERMENTATION, OR OF THE URINE WHICH IT DISPLACES

Apparatus for this purpose is recommended by Eichhorn and Fiebig (cf. § 20, 5, remark).

[In view of the fact that good active yeast is not always immediately within reach, and takes a day to give its result, the translator considers it advisable to add a simple method of titration which has yielded him accurate results.]

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[1] For 150 c.cm. about the size of a hazel-nut. For very accurate work $\frac{1}{2}$ to 1 gram of yeast purified by sedimentation in water.

III. TITRATION WITH FEHLING'S SOLUTION.

Reagent.—This is made as follows: 34·64 grams of crystallised copper sulphate is carefully weighed out and dissolved in warm distilled water, brought up to 500 c.c. and placed in a clean stoppered bottle labelled A; 125 grams of potassium hydrate and 173 grams of sodium-potassium tartrate (Rochelle salt) are likewise dissolved in distilled water, the solution carefully brought up to 500 c.c., and kept in another stoppered bottle marked B. From each bottle 5 c.c. is carefully measured out and mixed in a test-tube. This constitutes Fehling's solution. Some of the urine is then diluted with four times its bulk of water in a 100 c.c. measure glass, care being taken to ensure complete admixture. A small measure glass, graduated to $\frac{1}{10}$ of a c.c., is then filled with this dilute urine up to the 10 c.c. mark, and a perfectly clean, dry pipette introduced. The 10 c.c. of Fehling's solution is now brought to boiling-point, and the dilute urine added drop by drop (taking care not to lose or spill any of the drops) until reduction, as evidenced by disappearance of the blue colour, appears to be complete. After each addition of urine the liquid must be boiled for an instant and shaken. A point is soon arrived at when it becomes hard to determine whether any blue colour remains. A little of the boiling solution should now be poured on to a small moist filter; if the clear filtrate still shows the blue colour it must be poured back, and more urine must be cautiously added from the pipette, and so on until the filtrate becomes colourless. When this is the case acidulate the filtrate with acetic acid, and add a drop of potassium-ferrocyanide solution. A brown coloration indicates that some unreduced copper is still present, and that another drop of the dilute urine is needed. To determine the end of the reaction is the chief difficulty of the process. The pipette is then carefully withdrawn from the measure glass (in which it has, of course, been carefully replaced after each addition of urine), any urine contained in it is carefully blown out into the glass, the amount of dilute urine used determined, and divided by 5 to give the amount of undilute urine which it contained. Ten c.c. of Fehling's solution, prepared as above, is completely reduced by 0·05 gram of sugar, which is therefore the amount contained in the urine used. A simple calculation gives the quantity in grams per litre or parts per thousand, or, if preferred, the result may be expressed as grains per fluid ounce by bearing in mind that a gram = 15·5 grains avoirdupois approximately, and that a fluid ounce is about = to 28·4 c.c.

[Suppose, for example, 8·8 c.c. of the dilute urine was needed to reduce all the copper in the 10 c.c. of Fehling's solution, then $\frac{8 \cdot 8}{5} = 1 \cdot 76$ c.c. of the original urine contained 0·05 gram sugar, and $\frac{\cdot 05 \times 1000}{1 \cdot 76} = 28 \cdot 40$ grams per litre, or 2·84 per cent., or, roughly, 12·5 grains per fluid ounce.—*Transl.*]

§ 22. *Chief Points to be borne in Mind when examining Urine for Sugar*

1. Determine the specific gravity, if possible, in a mixed 24-hour sample.

If it is above 1020 and the quantity passed daily is larger than normal: diabetes probable.

2. Perform Trommer's test in the usual way.

(*a*) The urine dissolves much copper hydroxide, reduces, and yields a precipitate of suboxide:

More than 0·2 per cent. is present.

(Pass on to No. 4.)

(*b*) The urine dissolves much copper hydroxide, and reduces, but gives no precipitate of suboxide.

A reducing substance is present in increased quantity—perhaps sugar.

(Pass on to No. 3.)

3. Perform Nylander's test.

(*a*) It gives a very slight or negative result:

The urine is free from sugar or contains only the traces which occasionally occur in normal specimens.

(*b*) It gives a decided result, *i.e.* the sample becomes black and opaque after boiling 2 or 3 minutes.

Sugar is present in abnormal amount.

(Pass on to No. 4.)

4. The increased sugar excretion (glycosuria, mellituria) demonstrated may be due to elimination of milk-sugar from stagnation of milk in pregnancy or lactation (lactosuria), to excessive ingestion of sugar (physiological glycosuria), to various intoxications and other maladies (infectious diseases, heart and lung troubles, cirrhosis of the liver, nervous disturbances, &c.).

After ingestion of certain drugs, such as camphor, turpentine, kairin, nitrotoluol, chloral, chloroform, there appear in the urine bodies from which a sugar-

like reducing substance, glycuronic acid $C_6H_{11}O_7$, is split off.

In all these cases the increased sugar elimination is a *temporary* symptom—transitory glycosuria.

5. The increased sugar-elimination is not attributable to any of the four causes enumerated; with a mixed diet the symptom is permanent; it diminishes or disappears on meat diet and increases when the food is rich in carbohydrates: *diabetes mellitus.*

6. The positive result of Trommer's and Nylander's test was stated above to prove the presence of grape-sugar in urine; as already mentioned this is founded upon the facts that in such specimens the reaction ceases after the action of yeast, and that the only reducing substance known to occur in the urine and susceptible of being fermented by yeast is grape-sugar.

It is advisable in all cases that are not quite clear, despite a positive result by the two reduction-tests above mentioned, to make certain of the presence of grape-sugar by a fermentation experiment. This may be done either as laid down in § 20, 5, or, in accordance with § 21, a larger amount of urine may be subjected to fermentation and the reduction test repeated at its conclusion. Should it still give a positive result, the reduction cannot be caused by sugar.

In cases of *feigned diabetes* where *cane-sugar* has been added to the urine the specific gravity is high, and the plane of polarisation is rotated to the right, but reduction only occurs after boiling with dilute mineral acids.

Addition of commercial grape-sugar which always contains dextrine is recognised by the rotatory power of the urine being greater than corresponds to the sugar-content obtained by titration.

Phloridzin diabetes disappears on stoppage of the drug, and starchy diet does not cause its recurrence.

VI. ACETONE AND DIACETIC ACID, LEUCIN AND TYROSIN

§ 23. *Acetone* CH_3COCH_3

A volatile fluid smelling of fruit; contained in the urine mostly in increased albuminous metabolism (diabetes, fever; meat diet and starvation in the healthy).

Demonstration by Legal's Test. The urine is treated with 2 *or* 3 *drops of a concentrated freshly prepared solution of sodium nitro-prusside and a few drops of liq. sodæ.* This causes it to assume a red colour, which in a few minutes passes over into yellow. *If now, without waiting for this to take place, an excess of acetic acid be added,* a beautiful crimson red colour is struck, whereas urine free from acetone turns yellow.

The characteristic point is *not* the red coloration in alkaline solution, for this is given by creatin, a normal urinary constituent, but its persistence after addition of acetic acid.

The green and blue colours which appear in the fluid after it has stood some time are due to the formation of Berlin blue.

The test is not particularly sensitive, less than 0·8 mg. of acetone not being demonstrable. If the amount present be smaller, the urine must be acidified and distilled, and the distillate tested by means of the sensitive *iodoform reaction of Lieben*; of the distillate 3 or 4 c.cm. are treated with a few drops of iodo-potassic iodide [Lugol's solution] and liq. sodæ. In presence of acetone the liquid at once, or after a few minutes, becomes cloudy from precipitation of iodoform, which is readily recognisable by its smell and the shape of its crystals (six-sided tables or stellate figures). In presence of alcohol, which gives the same reaction, Gunning's modification, which reacts only to

acetone, must be resorted to : tincture of iodine + ammonia, or solution of iodine in iodide of ammonium, is added to the distillate. In addition to iodoform, however, there appears a black precipitate of iodide of nitrogen, which slowly disappears and allows the iodoform to be recognised.

§ 24. *Diacetic Acid* ($CH_3CO\text{-}CH_2COOH$)

Has a great tendency to split up into acetone and carbonic acid, and behaves to Legal's test like the former substances. With ferric chloride it gives a bordeaux-red colour, which disappears again in a short time owing to decomposition of the acid (Gerhardt's Reaction).

It is contained, whatever the food, in the urine of most severe cases of diabetes (especially in diabetic coma) ; in some cases, however, only when the patient is on meat diet, very rarely in high fever and nervous disturbances of adults, oftener in children.

Test for its presence in urine. Treat with a drop or two of liq. ferri perchlor., filter off the yellowish white precipitate of iron phosphate, and add a little more perchloride. In presence of diacetic acid there arises a violet or bordeaux-red coloration. As, however, sulphocyanic acid, formic acid, acetic acid, salicylic acid, and derivatives of thallin, antipyrin, &c., yield iron compounds of the same hue, the two following control tests must be performed with fresh samples in order to make the result certain.

The first sample is boiled, and, after cooling, tested with ferric chloride as above. The red colour should no longer appear, as the diacetic acid is decomposed by boiling.

The second sample is acidified with dilute sulphuric acid and extracted with ether, which is then separated off and shaken up with very dilute watery solution of ferric chloride. In presence of diacetic acid the watery stratum turns bordeaux-red. The colour disappears on heating or on standing some time.

In this test the diacetic acid is liberated from its salts by the sulphuric acid, and passes over into the ether.

§ 25. *Leucin and Tyrosin*

These are frequent in acute yellow atrophy of the liver and phosphorus poisoning; rare in severe typhoid, smallpox, and pernicious anæmia.

Leucin is moderately soluble in water, and crystallises from impure solutions in dull-coloured spheres and nodules in which one can often make out from the delicate radial streaking or concentric rings that they are made up of thin superposed layers. They are distinguished from fat-droplets by their slight refractive power and their insolubility in ether, and from ammonium urate by their behaviour to acids.

Tyrosin is soluble with difficulty in cold water, more readily in acids and alkalies. It crystallises in delicate needle-shaped crystals massed together in bundles or sheaves, or in spheres the structure of which shows them to be made up likewise of needles. Warmed with Millon's reagent it gives a beautiful red colour.

Leucin is probably never present as a urinary sediment, tyrosin rarely. But both may generally be caused to separate out by evaporating the urine down, or precipitating it with acetate of lead, freeing the filtrate from lead with sulphuretted hydrogen, and concentrating it. The precipitate is then subjected to chemical and microscopical examination.

VII. INORGANIC AND ACCIDENTAL CONSTITUENTS OF URINE

§ 26. *Sulphuretted Hydrogen*

The usual cause of hydrothionuria is decomposition of sulphur-containing urinary constituents within the urinary passages by certain bacteria, *e.g. B. coli communis* (cases of bacteriuria, cystitis, pyelitis). Only when the freshly passed urine shows no trace of decomposition or of bacterial turbidity is a remote origin (from collections of gas or pus, communication with the bowel) to be suspected.

None but perfectly fresh samples are suitable for this examination, as sulphuretted hydrogen may develop by fermentation in any urine that has stood some time, whereas, on the other hand, that produced by the other causes above mentioned may soon disappear by oxidation.

Formation of sulphuretted hydrogen may be experimentally produced in any urine by adding 2 per cent. peptone or 0·5 per cent. sodium sulphate and inoculating with *Bacterium coli.*

Test.—The urine is placed in a flask, and a strip of paper moistened with lead acetate and ammonia is wedged in the neck with a stopper. The specimen is repeatedly shaken and allowed to stand some time. In presence of sulphuretted hydrogen the paper becomes brown or black, at the edges at least.

The coloration depends on the formation of lead sulphide. *When the amount of H_2S present is small* a stream of air should be blown against the paper by means of an arrangement like an inverted wash-bottle; in presence of H_2S the point impinged upon is turned brown in a few minutes.

Strips of filter paper soaked with lead acetate may be dried and kept in stock. They are wetted with dilute ammonia before being used.

Pneumaturia, a condition in which gas escapes during micturition with noise and interruption of the stream, is usually due to other gases (CO_2, H, CH_4) and not to H_2S. The symptom is most frequently observed in diabetics who have contracted a cystitis due to *Bacillus coli*.

§ 27. *Chlorides*

The determination of the pathological variations in the excretion of normal urinary constituents is a complex procedure, and is only of value when undertaken on the 24-hour sample, and when at the same time the quantity and composition of the food together with its utilisation in the system are being accurately ascertained. The chlorides (NaCl, KCl) alone form an exception to this rule, inasmuch as in all acute febrile diseases they undergo so striking a diminution—amounting, during the height of an attack of croupous pneumonia, to almost complete disappearance—that it may be at once recognised by the aid of a simple test.

Test.—The urine is made strongly acid with nitric acid and a few drops of silver nitrate solution added. In presence of an abundance of chlorides a thick, cheesy precipitate of silver chloride is obtained, whilst if chlorides are low, only a milky turbidity is produced.

The other silver salts, especially the phosphate, are kept in solution by the nitric acid.

§ 28. *Accidental Constituents*

1. MERCURY.

The urine (250 c.cm.) is treated with 5 c.cm. dilute hydrochloric acid and some brass-foil added and digested for an hour at 60° to 80° C. The turnings are then washed with water, alcohol, and ether, dried and heated in a long, narrow, dry test-tube to a dull red heat. The mercury, which was deposited as amalgam on the brass, volatilises and sublimes on the cold parts of the tube in droplets of microscopical size. After cooling, if a fragment of iodine is thrown into the tube and volatilised by gentle heat, the little

globules become visible as a red scum (iodide of mercury).

This will demonstrate 0·0001 gram Hg.

2. CHLORATE OF POTASH

The urine is heated with a quarter of its volume of concentrated hydrochloric acid. It first becomes reddish or violet owing to the decomposition of indican by the HCl, and then, in presence of chloric acid, becomes yellow or quite colourless.

As little as 0·01 per cent. of potassium chlorate is demonstrated by this reaction. The chloric acid is set free by the HCl, and, being decomposed, yields chlorine which destroys the urinary pigments, and escapes as a gas which, if present in any quantity, is easily known by its smell, and the bleaching of a moistened strip of litmus-paper held in the mouth of the test-tube.

Bromides give a similar reaction.

3. IODIDES OF THE ALKALIES

(*a*) *The urine is treated with a few drops of starch solution,*[1] *and carefully poured on to the surface of some concentrated yellowish nitric acid.* In presence of iodine a deep blue fugitive ring appears at the point of contact.

As little as 0·001 per cent. of potassium iodide is thus demonstrable. The addition of small quantities (0·1–0·2 gram.) of this salt to medicine thus affords a ready means of finding out whether it has been taken.

(*b*) *Treat the urine with* 5 *to* 10 *drops of concentrated yellowish nitric acid, add* 1 *or* 2 *c.cm. chloroform, and invert the tube a few times, keeping it closed with the thumb.* In presence of iodine the chloroform which sinks to the bottom acquires a beautiful violet tinge.

This is somewhat less sensitive than the previous test. In both, the iodine is liberated from its salts by the NO_2 contained in the yellowish

[1] Made by boiling 1 part starch with 30 to 50 of water.

nitric acid, and passes over into the chloroform (or combines with the starch). Instead of the nitric acid other oxidising agents, *e.g.* chlorine water, chloride of lime, might be used.

4. BROMIDES OF THE ALKALIES

The urine is treated with some chlorinated lime (calcium hypochlorite) solution and hydrochloric acid, or with chlorine water; 1 *c.cm. chloroform is added and the mixture treated as in* 3 (*b*). In presence of bromine the chloroform becomes yellow.

Not less than 0·1 per cent. of potash, soda, or ammonium bromide is so demonstrable.

5. PHENOL (CARBOLIC ACID)

Urine containing phenol gives with *ferric chloride* a bluish violet, and on heating with *Millon's reagent* a purple-red coloration; this latter is much the more sensitive reaction, but neither is conclusive of phenol alone (cf. this §, 6 and 7, and § 7, 8).

6. SALICYLIC ACID, SALOL, SALICYLURIC ACID

Treat with a few drops of ferric chloride. In normal urine a mere yellowish cloudy precipitate of iron phosphate is obtained, but in presence of salicylic acid an intense violet coloration in addition.

When minute traces only are suspected (under 0·008 per cent.) the urine is to be acidulated with HCl, shaken up with ether, and the test applied to the latter after addition of a little water.

This substance, like iodide of potash, on account of its easy demonstration and relative harmlessness, *is suitable for adding to medicines in order to test whether they are being taken.*

The reaction only succeeds when *the ferric chloride is as nearly as possible neutral*, because free acids decompose the salicylate of iron. Phenol behaves in precisely the same way.

For other substances which yield coloured iron salts, cf. § 24, diacetic acid.

7. TANNIN (DIGALLIC ACID)

Appears in the urine partly as gallic acid. Both acids are coloured dark blue to blue-black by *ferric chloride*, whilst *alkalies* cause them to turn gradually brown, owing to absorption of oxygen.

8. CHRYSOPHANIC ACID (DIOXYMETHYLANTHRACHINON)

Contained in the urine after exhibition of chrysarobin, rhubarb, and senna. It is coloured red by *alkalies*, and yields red precipitates with *lime-salts*.

After taking santonin a substance which is likewise turned red by alkalies appears in the urine. Chrysophanic acid differs from this substance by being soluble in ether, and giving the red colour on addition of *liq. sodæ* to the ethereal solution.

9. BALSAM OF COPAIBA

After ingestion of this substance the urine becomes lævo-rotatory and *reduces copper oxide vigorously, but not bismuth*. On treatment with a mineral acid (HCl) it becomes turbid from precipitation of resinous acids and assumes a purplish to a violet tinge. Heat and oxidising agents, *e.g.* chlorinated lime, facilitate the reaction.

10. ALKALOIDS

These give with *acetic acid* and *potassio-mercuric iodide* precipitates which, unlike those given by albumen, peptone, and mucin, are soluble in alcohol.

The procedures requisite in testing for the individual alkaloids are too complicated for description in a work like the present.

VIII. SEDIMENTS

§ 29. *Unorganised Sediments*

A. FROM ACID URINE

1. *Uric Acid.—Uric acid generally occurs in large crystals, not unfrequently recognisable with the naked eye*, of a reddish-yellow or reddish-brown hue (owing to entangled urinary pigment) and of divers shapes. The fundamental form is the four-sided rhombic table (fig. 3, 1). Cutting off the acute angles gives

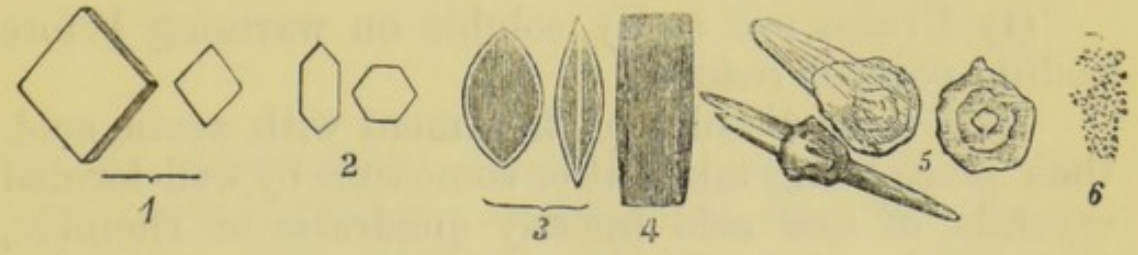

Fig. 3

rise to *six-sided tables* (fig. 3, 2), while *elliptic tables* (whetstone-form, fig. 3, 3) are produced by rounding off the obtuse angles. Crystals lying on the curved sides look like rectangular prisms (fig. 3, 4); two or more often lie crosswise or grow through each other (*rosette-form*), or lie with their broadest sides in contact and the largest crystal in the middle (*barrel-form*). *Quadratic tables* and aggregates of spheres

(dumb-bells), as well as structures of very irregular shape, often furnished with pointed processes, also occur. These latter are formed when the precipitation of uric acid takes place very quickly, or when the urine has a great tendency to deposit it, and are thus indicative of calculous diathesis (Ultzmann).

2. *Urates.*—Form a clay-yellow to a bright red powder (lateritious sediment) often adhering to the vessels, and consisting of acid salts of uric acid (especially sodium urate $C_5H_3N_4O_3Na$), usually together with some of the acid itself. The colour is caused by entangled urinary pigment.

Urates form fine granules which lie in irregular heaps or in moss-like branching rows, the colour of which cannot be distinguished under the microscope (fig. 3, 6).

In the uric acid infarct of the newly-born and in gouty concretions the urate of soda occurs as masses of delicate needles or prisms.

Uric acid usually occurs crystallised in whetstone-form.

Chemical character of Uric Acid and Urates:

(1) Urates are easily soluble on warming before boiling-point is reached.

(2) Urates dissolve on treatment with acetic acid, their place being taken after some time by well-formed crystals of uric acid (mostly quadratic or rhombic, four- or six-sided tables).

(3) Uric acid dissolves slowly in hot water, quickly in the cold on addition of alkali (liq. sodæ).

(4) Urates and Uric acid give the murexide test:

A sample of the sediment is placed in a small porcelain capsule, a few drops of concentrated nitric acid are poured on it and gently heated over a small flame. It dissolves with effervescence. If now the solution be cautiously evaporated just to dryness, a yellow or reddish stain is left, which, when moistened with a trace of ammonia, becomes purple-red, whilst with liq. potassæ or sodæ a beautiful violet or blue is struck. The colour disappears on heating—which serves as a distinction from Xanthin and Guanin.

3. *Calcium oxalate*, oxalate of lime (CaC_2O_4), owing to its lightness often sinks very slowly, and is thus readily overlooked.

MICROSCOPIC APPEARANCE

Calcium oxalate occurs by itself or mixed with other sediments in two forms:

1. *In shining octahedra, often very minute* (envelope-form, fig. 4, 1), more rarely in shorter or longer prisms with *pyramidal ends* (fig. 4, 2—combination of the octahedron with the prism). Well-formed octahedra are characteristic of oxalate of lime, but the other forms may be confounded with ammonio-magnesian phosphate.

2. In *round or oval discs* with delicate radial striation and a central depression or hourglass-

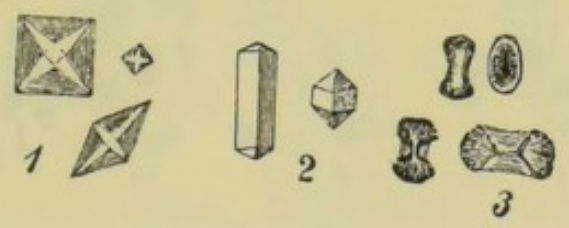

FIG. 4

shaped constriction (dumb-bell form, fig. 4, 3). These rarer forms are not characteristic, and may be confounded with calcium carbonate, uric acid, and ammonium urate.

Chemical characters.—Calcium oxalate is insoluble in acetic acid, soluble in mineral acids (hydrochloric).

B. FROM FAINTLY ACID (AMPHOTERIC) URINE

1. *Dicalcium Phosphate* (neutral phosphate of lime, $CaHPO_4 + 2H_2O$).

Wedge-shaped crystals agglomerated in bundles or rosettes (fig. 5, 1); they are often very small and so

closely packed that the structure is recognisable with difficulty. One of the rarer sediments.

Readily soluble in acetic acid.

2. *Ammonio-magnesian Phosphate* (*vide* c 2).

C. FROM ALKALINE URINE

1. *Earthy Phosphates.* — *Tri-calcium and Tri-magnesium Phosphate* (basic phosphate of lime and magnesia $Ca_3(PO_4)_2$ and $Mg_3(PO_4)_2$.

Small amorphous granules either lying singly or grouped together in scales of indeterminate shape or cell-like globules (fig. 5, 2).

Readily soluble in acetic acid.

2. *Ammonio-magnesian Phosphate*—Triple Phosphate ($NH_4MgPO_4 + 6H_2O$).

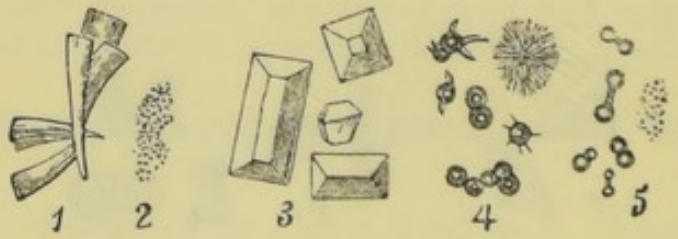

Fig. 5

Large, three-, four-, or six-sided prisms with oblique terminal surfaces (*coffin-lids*, fig. 5, 3).

The usual sediment of alkaline fermentation.

Readily soluble in acetic acid.

3. *Ammonium Urate.—Large globules and aggregates of globules, mostly dark yellow in colour, and often coated with minute pointed crystals* ('hedgehog' crystals) ; more rarely in well-formed needles united in globular masses (fig. 5, 4).

A frequent sediment of alkaline fermentation.

Soluble in acetic acid and replaced later on by crystals of uric acid ; very slightly soluble in water.

4. *Oxalate of Lime* (*vide* A 3).

Other sediments (uric acid, urates) of acid urine may also be present if the reaction has not become alkaline until they have fallen to the bottom. Such deposits often persist for a considerable time, especially if enclosed in mucus, which greatly delays the diffusion of the (alkaline) reaction.

D. RARE SEDIMENTS

1. *Calcium sulphate* $CaSO_4 + 2H_2O$.

Long prisms or elongated tables mostly cut off abruptly at the ends, occurring either singly or in packets.

Insoluble in acetic acid, soluble with difficulty in mineral acids and in water.

2. *Calcium Carbonate* $CaCO_3$.

Amorphous or crystalline, in globules or masses of globules (dumb-bells), or as iridescent membranes floating on the surface of the urine. Readily soluble in acetic acid with formation of carbon dioxide (effervescence). A normal sediment of vegetarian urine.

3. *Crystalline Tri-magnesium Phosphate* $Mg_3(PO_4)_2 + 22H_2O$.

Large, smooth, strongly refractive, lengthily rhombic crystals. Readily soluble in acetic acid.

4. *Cystin.*

Colourless six-sided tables. Insoluble in acetic acid and acid carbonate of ammonium, soluble in mineral acids and alkalies, also in ammonia, and by this feature easily distinguishable from uric acid. During alkaline fermentation it decomposes with evolution of H_2S. It gives a crystalline compound with benzoyl-chloride.

To test chemically for cystin a small quantity of sediment is dissolved in liq. potassæ with the aid of a gentle heat, diluted with water, and some solution of nitroprusside of sodium added: a violet hue betrays the presence of alkaline sulphides, *i.e.* of cystin.

5. *Tyrosin.*

Delicate needles united into globules or bundles (cf. § 25)

6. *Hippuric acid.*

Needles or rhombic prisms and columns. Might be confounded with ammonio-magnesian phosphate or uric acid, but is distinguished from the former by its insolubility in acetic acid, and from the latter by the absence of the murexide reaction.

7. *Bilirubin.*

Amorphous yellow granules or yellow needles and leaflets. Has also been found enclosed in pus-corpuscles and fat-droplets.

8. *Hæmoglobin.*

Amorphous or crystalline enclosed in tube-casts.

9. *Fat.*

Highly refractive globular droplets, soluble in ether. Smells of acrolein when heated on platinum foil.

In *Lipuria* fat alone is present, in *Chyluria* fat with albumen, which affords a ready distinction.

10. *Indigo-blue.*

Blue platelets or stellately arranged convoluted needles.

§ 30. *Organised Sediments*

1. *Mucus* is present in urine partly dissolved (more accurately, no doubt, much swollen-up), and partly as a cloudy turbidity which sinks as sediment to the bottom.

Under the microscope mucus usually appears quite transparent, and is only rendered visible by the extraneous elements embedded in it (sediments, epithelial cells, leucocytes, &c.); when such mucoid coagula are ribbon-like in shape they often display a distant resemblance to tube-casts. The addition of a little dilute acetic acid makes the mucous substance much more distinct.

Agglutination of pus-cells by a mucoid material gives rise to thread-like structures (Gonorrhœa-threads).

2. *Leucocytes* in small numbers *exist normally in mucus*. Under the microscope they may be mistaken for epithelial cells, but are distinguishable from the latter by means of the glycogen-reaction, *i.e.* the mahogany-brown tinge they strike with solution of iodine in potassium iodide. They are present in larger numbers as a *constituent of the pus* in purulent cystitis, pyelitis, and urethritis, when they may form a yellow sediment. In acid or neutral urine their

form is well preserved, but in alkaline urine (rendered alkaline by fermentation), they become swollen up by the ammonia into a shapeless slimy mass (often while still within the bladder), the microscopic examination of which reveals only detritus and free nuclei. On inverting the vessel the whole of the pus often falls out as a tenacious slimy mass.

Donné's test for pus depends on this behaviour. The urine is poured away as completely as possible, a scrap of caustic potash thrown in amongst the sediment and stirred up with it. The pus-corpuscles are changed into the above-mentioned tough, slimy mass.

Vitali's test for pus is based on the behaviour of guaiacum resin, already described on p. 26. A few c.cm of sediment, acidified if necessary, are covered with a stratum of guaiacum tincture; after a short time the well-known blue colour makes its appearance. The examination for albumen enables one to decide the question whether the leucocytes are derived from pus or from mucus. *Urine that contains pus always contains albumen also.*

3. *Red blood-corpuscles,* generally swollen-up and pale.

4. *Epithelial cells:*

Roundish . . .	From the urinary tubules.
Large, flat, polygonal .	From the superficial layers of the urinary passages.
Roundish, often furnished with processes . .	From the deeper layers of the urinary passages.

5. *Tube-casts:*

Epithelial casts . .	Composed of the desquamated epithelial lining of the urinary tubules.

Blood casts . . .	Red and white corpuscles cemented together with fibrin, and present as the result of a previous hæmorrhage into the tubules.
Granular *Hyaline* } *casts* . . *Waxy*	Coagulated products of exudation into the urinary tubules, and altered blood and epithelial casts.

6. *Spermatozoa.*

7. *Fragments of neoplastic tissue.*

8. *Micro-organisms* (bacteria, yeasts and moulds, parasites).

§ 31. *Examination of Sediments*

1. *Removal of Samples.*—A pipette or piece of drawn-out glass tube is closed above with the finger and the point thrust down into the sediment. The finger is then raised a moment, and when enough sediment has entered, the pipette is again closed and removed from the liquid. The urine which has adhered to the outside of the tube is allowed to trickle off, and the finger is then again raised to allow a suitable quantity of the sediment to run into the test-tube or on to the slide.

If there is but little sediment in the urine glass, the supernatant fluid should be poured off and the remainder allowed to re-deposit its sediment in a conical glass or test tube.

A small centrifugal machine, *e.g.* one constructed like a top and made to spin round by pulling a string, is very useful for this purpose. [Daland's 'Hæmatocrit' is a very good form. It is primarily intended for

blood, but possesses an attachment for urinary work. —*Transl.*]

Samples should always be taken from different parts of the sediments.

It is also desirable for certain diagnostic purposes to examine the urine both at once after evacuation and after standing some time, and also, when urethral disease is suspected, to collect and examine the portion of the urine that is first passed (a few teaspoonfuls) apart from the remainder of the secretion.

2. *Chemical examination.*—A general idea of the principal constituents of a sediment may be quickly obtained by means of a few tests performed in a test-tube, or, if there is but little material, in a watch-glass, with addition of some water, if necessary.

Heat the sediment
- Readily soluble . . Urates
- Insoluble
  Add a few drops of strong acetic acid
  - Soluble
    - Earthy phosphates
    - Earthy carbonates
    - Triple phosphate
    - Ammonium urate (slowly soluble)
  - Insoluble
    - Uric acid
    - Calcium oxalate
    - Organised sediments

More accurate information is obtained by the

3. *Micro-chemical Examination.*—Most of the objects encountered in the microscopical examination will be recognisable by careful inspection ; for the sake of greater certainty, as well as to clear up the nature of doubtful forms, the aid of chemical tests may be invoked. A small drop of the reagent is placed at the margin of the cover-glass, and the results produced by its gradual diffusion into the preparation observed.

Penetration is accelerated by placing a scrap of blotting-paper at the opposite border of the cover-glass.

1. A drop of strong acetic acid dissolves the following structures :

(*a*) Crystalline . .	Triple phosphate. Ammonium urate (slowly, with subsequent precipitation of uric acid).
(*b*) Amorphous . .	Earthy phosphates Earthy carbonates (with effervescence). Urates (with subsequent deposition of uric acid).

2. A drop of hydrochloric acid added to the same or a new preparation dissolves (besides the foregoing) . . . Calcium oxalate.

3. Undissolved by acetic and hydrochloric acid . . . . Uric acid.
The majority of the rarer and of the organised sediments.

IX. URINARY CALCULI

§ 32. *Classification by Chemical Composition*

1. *Cystin calculi* . .	Smooth, dull yellow, not very hard, rare.
2. *Oxalate calculi* . .	Consist of calcium oxalate; the smaller ones are smooth and pale brown (hemp-seed calculi); the large ones are nodular or tuberculated, and often dark brown, owing to included hæmoglobin (mulberry calculi). They are very hard.
3. *Uratic calculi* . .	Consist of uric acid, either alone or accompanied by urates; they are hard, mostly smooth, and yellow to reddish brown in hue. Calculi consisting exclusively of ammonium urate (in children) are small, soft, and bright yellow.

4. *Phosphatic calculi* .	Consist of earthy phosphates and triple phosphate; they are gritty, greyish white, and their outer layers readily shell off.
5. *Mixed calculi* . .	Consist of strata of different composition, generally of a nucleus of uric acid or calcium oxalate (primary calculus) with peripheral layers of earthy and triple phosphate (secondary calculus).

§ 33. *Examination of Calculi*

The calculus is reduced to powder; if large, it is sawn through and specimens of the nuclear and peripheral substance taken for separate examination.

1. A sample of the powder is heated on platinum foil until the latter begins to glow.

(*a*) The material burns and leaves little or no residue . . .	Uric acid Cystin (burns with a blue-green flame, and a sharp smell like hydrocyanic acid). Ammonium urate Other urates (leave some residue).

(*b*) The material burns either not at all or only imperfectly (turns black and leaves a considerable residue which is insoluble in water) .	Calcium oxalate (residue effervesces on application of acid). Earthy phosphate. Triple phosphate.

2. A second sample is gently heated for some time with dilute hydrochloric acid, and cooled to allow of the deposition of any dissolved uric acid.

(*a*) Undissolved . .	Uric acid (to be subjected to the murexide test). Organic ground-substance.
(*b*) Dissolved . . .	Calcium carbonate (with evolution of gas). Calcium oxalate. Earthy phosphates. Triple phosphate. Cystin.

In order to further differentiate the dissolved substances, the solution is filtered, strongly diluted with water, and super-saturated with ammonia.

No precipitate . .	Calcium carbonate (a specimen tested with oxalate of ammonia yields a precipitate of calcium oxalate). Magnesium carbonate (a specimen tested with sodium-phosphate yields a precipitate of ammonia-magnesium phosphate). Cystin (a specimen made faintly acid with acetic acid gives gradual separation of six-sided tables).
A precipitate . . .	Earthy phosphates. Triple phosphate. Calcium oxalate.

Treat with acetic acid until the reaction is just faintly acid.

Dissolved . . .	Earthy phosphates. Triple phosphate.
Undissolved, or separate out slowly	Calcium oxalate (in octahedra, prismatic rods, or spheroids). Cystin (six-sided tables soluble in mineral acids and ammonia).

X. DIGESTIVE TRACT

§ 34. *Saliva*

The normal specific gravity of the saliva is 1.002 to 1·006.

Its normal reaction is alkaline, but it may become acid in *diabetes mellitus*, fever, and digestive disturbances.

The saliva normally contains mere traces of coagulable albumen; in salivation due to iodine or mercury the amount of albumen increases.

On allowing saliva to trickle into water containing some acetic acid a precipitate is obtained which dissolves in mineral acids—mucin. Further, the saliva *frequently*, but by no means *constantly*, contains potassium sulphocyanide, and when this is the case it turns reddish to blood-red on addition of a few drops of hydrochloric acid and very dilute solution of ferric chloride, owing to the formation of sulphocyanide of iron.

$$6KSCN + Fe_2Cl_6 = Fe_2(SCN)_6 + 6KCl.$$

On shaking up the red fluid with ether the latter becomes red whilst the saliva is decolorised. Use may be made of this reaction, taken in connection with the low specific gravity and the poverty in albumen, to distinguish saliva which has been first

swallowed and subsequently vomited (watery vomit) from other kinds of vomited matter.

To demonstrate the presence of *ptyalin*, equal parts of saliva and starch paste are mingled, and after standing a few minutes are examined by Trommer's and Nylander's tests. As a control, the saliva itself and the starch paste are subjected to the same tests.

Salivary calculi and dental concretions consist of calcium carbonate together with some calcium phosphate. They dissolve with effervescence in acetic or hydrochloric acid.

§ 35. *Gastric Contents*

1. EXAMINATION FOR HYDROCHLORIC ACID

Litmus-paper is unsuitable for this purpose, because it reacts to acid salts and organic acids; but there are a number of colouring matters which are not changed at all by acid salts, and are only altered by organic acids in much higher concentration than would be required in the case of mineral acid.

Organic acids (lactic, acetic, and butyric) react on an average only in strengths above 0·5 per cent. which do not occur in the stomach, because the fermentation by which they are produced ceases when the acid formed amounts to 0·5 per cent. Hydrochloric acid, on the contrary, reacts when present in the proportion of a few hundredths per cent. The hydrochloric acid secreted during digestion is, however, at once fixed by the proteids of the food and by the albumoses and peptones formed during the process, as well as in rare cases by tyrosin, leucin, and other putrefaction bases, chlorides being formed. It is not until these substances have been saturated with the acid that we find *free* HCl *demonstrable by the above-mentioned colouring matters*. This point is reached about three-quarters of an hour after a *test-meal* (Leube), or half to one hour after a *test-breakfast* (Ewald); thereafter the gastric contents give the reaction for free HCl. Departures from this rule point to disturbance of the gastric function. Thus the tests fail, for example, very often in carcinoma of the stomach, and appear earlier and more strongly in gastric ulcer.

The amount of acid secreted may be estimated by titrating the filtered contents with deci-normal caustic soda, 1 c.cm. of which = 0·00365 HCl. If litmus be used as indicator the entire amount of HCl secreted is obtained (both the free acid and that combined with proteid, &c.); by employing one of the following reagents (preferably phloroglucin vanillin) only that which is free is indicated.

Minute quantities of organic acids may be neglected because their equivalent is but small compared with that of the HCl, but larger ones must be previously removed by repeated shaking up with excess of ether (200 c.cm. each time). In order to effect speedy separation of the two liquids it is well to heighten the specific gravity of the sample of gastric contents (20 to 40 c.cm.) by evaporating in a flask down to one-half (which causes no loss of HCl), and adding about 1 gram of perfectly neutral sodium sulphate.

(*a*) Methylanilineviolet Test

A watery solution of this pigment, diluted until the colour is a moderately deep violet, is turned blue by 0·03 per cent. solution of hydrochloric acid, becomes green with acid of over ½ per cent. strength, whilst 5 per cent. decolorises it altogether.

In the case of organic acids, on the other hand, stronger solutions are required in order to effect a change of colour—of lactic, for instance, 0·5 per cent.; of acetic, 2·5 per cent. would be necessary.

Application: 5 *to* 10 *c.cm. of water are treated with* 2 *to* 3 *drops of a concentrated watery solution of methyl violet so as to cause the water to assume a distinct violet tinge. An equal quantity of filtered gastric contents is then treated in the same way, and the two samples compared.* If the mixture of stain and gastric contents has taken on a distinct blue tinge, hydrochloric acid is present in quantity above 0·03 per cent.

(*b*) Congo-red Paper Test

Congo-red is turned blue by free acid, but not by salts of acid reaction. Hydrochloric acid in strength above 0·05 per cent. turns it dark blue, weaker solutions give pale blue or violet colorations.

Organic acids of strength below 0·5 per cent. give either no change at all or (lactic) only a weak violet tinge, and a dark blue colour is only yielded by more concentrated solutions than ever occur in the stomach. The most convenient way of applying the test is by means of papers impregnated with the stain. Such are to be had in commerce under the above name.

Application: a drop of the gastric contents is let fall on the paper. If the entire area on which it falls becomes of an intense dark-blue colour, hydrochloric acid to the amount of at least 0·05 per cent. is present. If, on the other hand, only a washed-out blue spot is

obtained, or if only the periphery of the moistened area becomes dark blue (blue ring), free acid is present, but one is left in doubt as to whether this acid is hydrochloric or organic, or a mixture of the two.

On drying the colour gets paler if due to lactic acid, but persists, on the other hand, or becomes even more distinct, if due to hydrochloric acid.

(*c*) Tropæolin Test

A watery solution of Tropæolin, OO (oxynaphthylazophenylsulphonic acid), normally yellow or yellowish red in hue, on addition of 0·02 per cent. hydrochloric acid becomes pink to brownish red (according to the conditions under which the reaction takes place). Organic acids in strength below 0·5 per cent. merely give a yellow coloration.

The test is most simply performed by means of strips of filter-paper which have been dipped a short time in a concentrated alcoholic solution of the pigment, and then dried.

Leaving them in too long diminishes their sensitiveness. Care must be taken to procure a reliable sample of the drug—such as may be had, for instance, from Th. Schuchardt in Görlitz.

Application: a drop of the gastric contents is let fall on the paper. If the place at once becomes dark-brownish red, turning lilac on drying over a small flame, hydrochloric acid of at least 0·05 strength is present. Organic acids in stronger solution yield only a faint brown coloration and no lilac at all.

(*d*) Phloroglucin-vanillin Test

A solution of 2 *grams of phloroglucin and* 1 *gram vanillin in* 30 *grams of alcohol is prepared, and a few drops of this are mingled with a similar amount of filtered gastric contents and cautiously evaporated in a porcelain capsule over a small flame,* so as not to reach boiling-point. In presence of as little as 0·005 to 0·01 per cent. of hydrochloric acid a beautiful red deposit is obtained. Organic acids even in much higher strength give no reaction.

[(*e*) DIMETHYL-AMIDO-AZO-BENZOLE TEST

This test, lately introduced by Töpfer, is probably the most delicate of the whole series of aniline colour-reactions for free mineral acids. It will indicate as little as 0·002 per cent. of hydrochloric acid in watery solution. It is applied by adding one or two drops of a half-per-cent. alcoholic solution of the reagent to about 10 c.cm. of the filtered gastric contents contained in a small beaker or test-tube. The presence of even minute traces of free hydrochloric acid is indicated by a bright pink or cherry-red colour, whereas with pure water or vomited matter devoid of free mineral acid a fluorescent yellow coloration is obtained. Organic acids under 0·5 per cent. do not give the red reaction, and even higher percentages fail to yield it in presence of albumin, peptones, and mucin, such as are usually found in vomited matter. The reaction likewise fails with acid salts and loosely combined hydrochloric acid, and is therefore a delicate and conclusive test for the free mineral acid.—*Transl.*]

2. TESTS FOR LACTIC ACID AND ITS SALTS

(*a*) *Ferric Chloride Test.*—One drop of liq. ferri sesquichlor. in 50 c.cm. water, when viewed in a thin stratum, yields a hardly perceptible yellow hue; but the fluid becomes at once distinctly yellow when 1 c.cm. of it is mixed with the same amount of a lactic acid solution of at least 0·01 per cent. strength. Acetic and butyric acids, on the other hand, leave the colour unchanged, as is also the case with hydrochloric acid in solutions of less than 0·3 per cent. strength. Proteids, salts, peptone, and the like exercise hardly any disturbing effect on the reaction.

This test is, therefore, highly suitable for the demonstration of lactic acid in the contents of the

stomach, and is applied by simply mixing equal parts of the reagent and filtered gastric fluid.

(*b*) *Ferric Chloride and Carbolic Acid Test* [Uffelmann's—*Transl.*]. A mixture of 1 drop liq. ferri sesquichlor., 10 c.cm. 4 per cent. carbolic acid and 20 c.cm. water has an amethyst-blue colour when freshly prepared, but at once assumes a pure yellow to a greenish-yellow tinge when treated with an equal volume of 0·01 per cent. lactic acid solution. The other acids that occur in vomited matter (hydrochloric, acetic, butyric) produce in strengths inferior to 0·3 per cent. only a pale yellow to a pale grey coloration.

This reaction is in reality the same as the preceding one. It is, however, more striking because the carbolic acid reaction (blue tinge) must first disappear before the characteristic canary-yellow of the lactic acid reaction can become visible.

3. TESTS FOR ACETIC AND BUTYRIC ACIDS

A pretty large quantity of the filtered gastric contents is repeatedly shaken up with several changes of ether. This is then allowed to evaporate, whereupon a fluid residue of acid reaction remains behind which, if it contain volatile organic acids, emits a characteristic odour, and may be subjected to the following special tests.

(*a*) *Test for Acetic Acid.—A portion of the residue is taken up with water, accurately neutralised, and treated with a drop of ferric chloride.* In presence of acetic acid the fluid becomes blood-red, and on boiling deposits a brownish-red precipitate of basic acetate of iron.

Formic acid gives the same reaction. The diagnostic significance of the test is, however, in no wise altered by the fact, for even though formic acid were present in the stomach—which is not hitherto known to be the case—it could only be there, like acetic and butyric acids, as the result of an acid fermentation.

(*b*) *Test for Butyric Acid.—The remainder of the residue is dissolved in a drop or two of water, and a very small fragment of calcium chloride added.* Butyric acid being insoluble in saline solution separates out in small oily droplets floating on the surface.

4. DIGESTION TEST

Free hydrochloric acid (*i.e.* demonstrable by the above-mentioned tests) and pepsin are essential for the peptonisation of albumen. According to existing observations the incapacity of a sample of gastric secretion to digest is owing to want of acid. Complete absence of pepsin (atrophy of gastric mucous membrane) has been very rarely observed.

The digestion test is, therefore, as a rule only a test for free hydrochloric acid carried out in another way. It is more complicated, but, on the other hand, the rapidity of the process allows of a more accurate quantitative estimate than can be gained from the intensity of the colour reactions.

The mutual agreement of the results obtained by testing for free HCl with colouring matters, and by digestion, is only absent in one particular case : the presence of lactic acid in a perfectly free state, not combined even with albumen. In contrast to other organic acids this works nearly as well as hydrochloric. A sample of gastric secretion, therefore, containing pepsin and lactic acid, but no free hydrochloric (cases of gastric cancer), is, therefore, quite capable of digesting, but is nevertheless abnormal.

(*a*) Carmine-fibrin Test

The fibrin obtained by whipping blood is washed with water until white, and put for 24 hours into a 1-per-cent. ammoniacal solution of carmine. After removal from this it is repeatedly washed until it ceases to give off colouring matter, and the shreds which are still dark red put into glycerine, where they keep good for years. Before use they are washed out in water. The colouring matter adheres so closely to the fibrin that it cannot be withdrawn from it by any solvent—not even by dilute hydrochloric acid. It does not go into solution until the fibrin itself has been dissolved, and this will be indicated by the liquid becoming red. Carmine-fibrin is, therefore, a very delicate reagent for testing digestive power.

Performance.—A few shreds of carmine fibrin are washed out and placed in 0·25 per cent. hydrochloric acid (obtained by diluting officinal hydrochloric

acid one hundredfold with water), until they are swollen up to a jelly. The unabsorbed hydrochloric acid is then pressed out with the fingers, and the shreds thrown into a test-tube. In another test-tube are placed a like number of shreds that have been washed out but not treated with hydrochloric acid. Five to 10 c.cm. of the filtered gastric contents are then poured into each tube. If the gastric secretion contains sufficient quantities of hydrochloric acid and pepsin, the solution of the fibrin will be indicated in five minutes, even at room-temperature, by the red coloration of the fluid, and if digestion is vigorous all the fibrin will actually be dissolved at the end of that time. If solution only occurs in the case of the fibrin that has been treated with hydrochloric acid, there is deficiency of acid ; if even this fails to dissolve, there is also deficiency of pepsin.

(*b*) Test with Discs of White of Egg

Discs, 1 mm. thick and 1 cm. in diameter, are cut with a double knife and cork-borer from the white of hard-boiled eggs and kept, like the fibrin shreds, in glycerine. They are much more difficult of solution by the gastric juice than fibrin, and even with a very vigorous secretion and a temperature of 38° C. the digestion lasts from half to one hour. The time taken up by this process is thus sufficiently long to permit more accurate comparison of the digestive power of different samples.

Method.—Two discs, after being washed with water, are placed in test-tubes and 10 c.cm. of filtered gastric secretion poured over each. To one of the samples one or two drops of concentrated (25 per cent.) hydrochloric acid are added, and both are placed in the incubator at 38° C. If a sufficient amount of pepsin be present the disc ought to be dissolved—at any rate, that to which the HCl was added—in one to two hours.

5. TEST FOR RENNET-FERMENT

10 c.cm. neutral or amphoteric milk are treated with 2 c.cm. of neutralised and filtered gastric contents and put in the incubator. In presence of rennet ferment coagulation of the casein occurs in from 10 to 30 minutes. The reaction must then be again tested, lest the casein might have been coagulated by acid formed subsequently to the admixture.

6. DEMONSTRATION OF THE DIGESTIVE PRODUCTS FORMED BY PEPSIN IN CONJUNCTION WITH HYDROCHLORIC ACID

The filtered gastric contents are accurately neutralised with dilute caustic potash : *syntonin is precipitated.*

The filtrate is treated with an equal volume of saturated common salt solution, a few drops of acetic acid added and boiled : *coagulation of the albumen that was merely in solution.*

The filtrate gives the *albumose reactions* : (1) nitric acid, (2) acetic acid + potassium ferrocyanide, (3) acetic acid + common salt, produce precipitates which dissolve on heating and reappear on cooling.

By saturation of the filtrate with powdered ammonium sulphate the albumoses are almost completely removed, leaving the *peptones* in solution and demonstrable by the biuret-reaction. In performing this last-named test, at least a double volume of *liq. sodæ* must be added so as to convert all the ammonium sulphate into sodium sulphate (which is partly precipitated) and leave an excess of sodium hydrate.

7. TESTS FOR BILE

(*a*) In acid vomited matter *bile-pigment* is partly changed into biliverdin, as indicated by the green colour of biliary vomit; for the most part it is undissolved, having been precipitated by the acid. The material is therefore filtered, and Rosenbach's modification of Gmelin's test performed with the filter-paper, or the residue is extracted with hot alcohol containing sulphuric acid, whereby a beautiful green or bluish-green solution of biliverdin is obtained.

(*b*) *Bile-acids* are demonstrable by Pettenkofer's reaction. The fluid is treated in a small porcelain capsule with 1 to 3 drops of dilute sulphuric acid (1 part concentrated acid to 5 of water), and a trace of grape-sugar, and the mixture evaporated over a small flame at a temperature of from 60° to 80° C., the dish being tilted to and fro all the while. In presence of more than 0·05 per cent. of bile-acids a magnificent purple-red colour is obtained either at once or during the evaporation when the fluid is nearly dried up. Excess of sugar must be avoided, and over-heating guarded against by moving the capsule to and fro; otherwise black products are obtained which conceal the colour.

Proteids, peptones, and many other organic bodies give a similar coloration; when such are present the direct application of Pettenkofer's test is therefore no longer to be relied on, but the bile-acids must first be isolated, which may be most conveniently accomplished by Neukomm's process, based upon the preparation of such of the lead-salts of the bile-acids as are soluble in alcohol.

8. TESTS FOR HÆMOGLOBIN OR BLOOD

Unaltered blood is only to be found in the gastric contents after considerable hæmorrhage quickly followed by vomiting; in most cases the blood is

changed into material resembling coffee-grounds, the colour of which is due to methæmoglobin and hæmatin.

(*a*) *Hæmin Test.*—A droplet of the substance is dried with gentle heat on the slide and treated as described in § 16, 4.

(*b*) *Heller's Test.*—The gastric contents are rendered faintly alkaline with liq. sodæ and filtered. To the filtrate is added an equal volume of normal urine (for the sake of the earthy phosphates), and the mixture heated to boiling-point. In presence of hæmatin the flocculent precipitate of phosphates assumes a beautiful ruby-red hue.

(*c*) *Guaiacum Test* (see § 16, 3).—The reagent is poured on to the surface of the gastric contents, which have been previously acidified, if necessary, with acetic acid. The test is not always reliable, as many oxidising agents which may be accidentally present in the stomach are capable of making the guaiacum turn blue, and this, unlike hæmoglobin, without addition of oil of turpentine or ozonic ether.

9. TEST FOR CARBONATE OF AMMONIA

In uræmia and cholera this salt is found in the stomach as the result of decomposition of urea. On addition of a little liq. sodæ to such specimens the ammonia is liberated, and may be recognised by the sharp smell and the white fumes that are formed on the approach of a glass rod moistened with acetic or hydrochloric acid. The fumes are due to the combination of the acid vapours with those of ammonia forming acetate or chloride of ammonium, as the case may be.

Mere traces of ammonia may be recognised by sticking a scrap of red litmus or turmeric paper to the convex side of a watch-glass with a drop of water, treating some of the vomited matter with liq. sodæ in

a wide test-tube or beaker, and covering with the watch-glass. The vapours of ammonia evolved on addition of the alkali are detected by the change of colour of the test-paper.

§ 36. *Fæces*

Results of diagnostic value are yielded by the physical and microscopical characters of the fæces rather than by chemical investigations which are for the most part too complicated.

1. EXAMINATION FOR BILE-PIGMENTS

Normal fæces do not contain any unaltered bile-pigment, but chiefly hydrobilirubin (urobilin), which does not react to Gmelin's test; in catarrh of the small intestine, on the other hand, they contain bilirubin and biliverdin, and are therefore frequently green in colour. According as the fæces are acid or alkaline, the pigment is suspended or in solution, and Gmelin's test is responded to either by the residue or the filtrate. The fæces may also be extracted on the water-bath with alcohol together with a few drops of acetic acid, and the faintly reddish-brown solution yields the characteristic absorption bands of urobilin. On adding a couple of drops of tincture of iodine and some chloride of zinc and subsequent excess of ammonia, magnificent fluorescence is obtained together with the absorption bands of urobilin and cholecyanin.

2. EXAMINATION FOR ALBUMEN

The fæces are diluted or stirred up with 1-per-cent. solution of common salt (obtained by diluting the saturated solution with twenty volumes of water),

filtered, and the filtrate subjected to the proteid reactions. The possible presence of mucin should be kept in view.

3. EXAMINATION FOR BLOOD

Blood derived from the stomach or upper part of the intestine is always converted into methæmoglobin and hæmatin, and gives the fæces a brownish-red to a brownish-black hue. It is only in rectal hæmorrhage and profuse extravasations from higher up the bowel that unaltered hæmoglobin is found.

The same methods are employed for the demonstration of blood in the fæces as in the vomited matter.

(*a*) The hæmin test.
(*b*) Heller's test.
(*c*) The guaiacum test.

4. CRYSTALS

(*a*) *Ammonio - magnesian phosphate.* — Occurs normally and in various pathological conditions.

Microscopic and chemical characters given in § 29, C.

(*b*) *Dicalcium phosphate* (*vide supra*, § 29, B).—This and other calcium salts are often tinged of an intense yellow with bile-pigment.

(*c*) *Calcium oxalate* (envelope form).—Derived from the food (vegetables) (*vide* § 29, A).

(*d*) *Calcium lactate.*—As tufts of delicate needles in catarrhal states of the bowel, especially in children.

(*e*) *Lime and magnesia soaps of the higher fatty acids.*—Needle-shaped crystals aggregated in tufts and packets ; abundantly present in acholia.

(*f*) *Cholesterine.* — Readily recognisable by its crystalline form, solubility in ether, and colour-reaction with sulphuric acid and iodine (cf. § 38).

Like the foregoing, often found in sputum, old exudations, contents of cysts, &c.

(*g*) *Hæmatoidin.*—Reddish-yellow needles. After intestinal hæmorrhages.

(*h*) *Charcot's crystals.*—A phosphoric acid compound of an organic base of the composition C_2H_5N. Colourless double pyramids with a dull lustre, often with arched surfaces (fig. 6, together with the *corpora amylacea*, so frequent in the prostatic secretion). Insoluble in cold water, alcohol, ether, and chloroform; readily soluble in acids, alkalies, and alkaline carbonates.

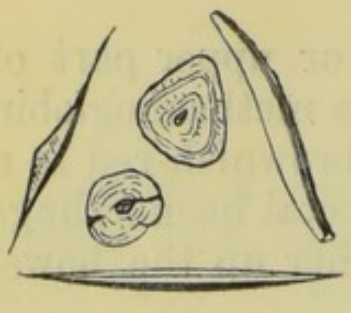

Fig. 6

Unlike the foregoing substances, these crystals are found only in pathological conditions: anchylostoma-anæmia, dysentery, typhoid, catarrh of the small intestines, &c.

They are also contained in the sputum, especially in *bronchial asthma*; also in the blood, spleen, and bone-marrow in *leukæmia*; and, finally, in a state of solution form an integral part of the *prostatic secretion.*

If a drop of this secretion be mingled on the slide with a drop of ammonium phosphate solution, Charcot's crystals soon separate out—usually in about an hour—forming characteristic objects. Their demonstration is useful for the differential diagnosis between prostatic inflammation (prostatorrhœa) and gonorrhœa.

§ 37. *Concretions*

In order to find these the fæces are to be stirred up with water and strained through a sieve.

1. BILIARY CALCULI

These consist chiefly of layers of cholesterin and bilirubin + lime salts in various proportions; the nucleus is richest in the last-named component. They are whitish yellow, more rarely brownish red in

colour, have a greasy feel, glistening fracture-surfaces, and a much lower specific gravity than other calculi.

A fragment heated on platinum foil melts, catches fire, and burns away, leaving a little ash.

The more accurate chemical examination of these calculi is performed by pulverising, boiling with water (to remove traces of bile-acids and other substances), and extracting the residue with a warm mixture of equal parts of alcohol and ether. Cholesterin passes into solution, whilst the residue contains the compounds of calcium with bile-pigment (chiefly bilirubin-calcium).

On evaporation of the solution the cholesterin crystallises out generally in the form of large, very thin, rhombic tables (fig. 7), more rarely forming needles with a silky lustre. If strong sulphuric acid diluted with one-fifth of its volume of water be allowed to come in contact with cholesterin on the slide, the tables melt at the edges and become crimson; if iodine dissolved in iodide of potassium solution is now added, they turn violet, blue, green, and red. A trace of cholesterin or cholesterin-ester (Lanolin) dissolved in anhydrous acetic acid, and treated with a few drops of concentrated sulphuric acid, gives a violet coloration quickly passing over into deep green. The presence of water must be absolutely excluded (Liebermann's reaction).

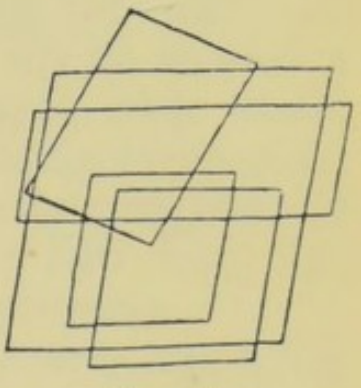

Fig. 7

Bilirubin-calcium is decomposed by digestion with dilute hydrochloric acid, and the bilirubin extracted with chloroform. The extract may then be either allowed to crystallise or subjected to Gmelin's test.

2. FÆCAL CALCULI

Consist mostly of an organic nucleus (fruit-stones, blood-clots, inspissated fæcal masses, &c.) upon which layers of earthy phosphates or triple phosphate have been deposited.

They may be examined by sawing them across and testing a fragment on platinum foil. It will turn black and prove only imperfectly combustible, leaving a considerable residue. Another fragment may be digested with very dilute hydrochloric acid and gentle heat. The phosphates pass into solution, whilst the nuclear substance remains behind and may be subjected to microscopic examination.

XI. PATHOLOGICAL FLUIDS

§ 38. *Transudates and Exudates*

1. *Transudates* are transparent yellow or greenish-yellow fluids with a few cellular elements (leucocytes, endothelial cells). Generally speaking, they either *do not coagulate spontaneously at all*, or only do so after a long interval; but if they are mingled with blood, either intentionally or accidentally during evacuation, they yield a gelatinous or membranous coagulum of fibrin. Old transudates that have lain long unabsorbed in serous cavities lose this property.

Transudates have a *low specific gravity* and a *low percentage of albumen* (fibrinogen, serum-globulin, serum-albumin). The amount changes with the seat of origin; hydrocele, pleura, peritoneum, subcutaneous tissue, and cerebral ventricles follow each other in the descending scale.

The amount of other solids is small, and characteristic substances are absent; succinic acid was found in a case of hydrocele.

2. *Exudates.*—Of the different varieties only the *serous* calls for description here, as the purulent and hæmorrhagic are recognisable by their mere appearance. Serous exudates are like transudates in colour, but are richer in cellular elements, and therefore turbid. *Almost without exception they coagulate spontaneously*, either immediately after evacuation or

in twenty-four hours at latest. Exudates have a *higher specific gravity* and a higher *percentage of albumen* than transudates, and the figures do not change with the seat of origin.

These distinctions are not sufficient, however, to enable a hard and fast line to be drawn between the two kinds of fluids. Intermediate cases occur, *i.e.* transudates which have a higher albumen percentage than the lowest of the exudates, and *vice versâ*. This does not, however, prevent certain limits being established—for exudates, a minimal albumen percentage and specific gravity, and for transudates corresponding maximal limits, which are found by experience not to be overstepped. These limits are, according to Reus:

—	Percentage of albumen		Specific gravity	
	Exudates exceed	Transudates fall below	Exudates exceed	Transudates fall below
Pleura . . .	4·0	2·5	1,018	1,015
Peritoneum .	4·0–4·5	1·5–2·0	1,018	1,012
Skin . . .	4·0	1·0–1·5	1,018	1,010
Meninges . .	—	0·5–1	1,018	1,009

The determination of the percentage of albumen, or more simply of the specific gravity,[1] supplies us, therefore, in the absence of other indications, with a means of distinguishing transudates from exudates. Of course this only holds good for unmixed and uncomplicated processes. Œdematous fluids containing less than 0·1 per cent. of albumen point to a severe kidney trouble with amyloid degeneration.

[1] The parallelism of specific gravity and albumen percentage is explained by the fact that albumen is the only solid constituent of these fluids, the quantity of which is subject to alterations within the specified limits. The only exceptions to this rule would be cases in which considerable quantities of sugar (diabetes), of urinary constituents (uræmia), or of chyle (obstruction in the thoracic duct) pass out into the serous cavities.

The most accurate way of determining the albumen percentage is by coagulation and weighing; but recourse may also be had to the approximate methods employed for the estimation of urinary albumen.

The specific gravity is determined as laid down in § 4. Turbid specimens must be given time to deposit their sediment or be filtered. It is well, however, to allow even clear samples to stand twelve hours in the interest of accurate readings, for these fluids contain gases which must be first evolved, otherwise too low a gravity will be indicated.

§ 39. *Contents of Ovarian Cysts*

The contents of ovarian cysts are very variable in character. Between a bright yellow, limpid fluid as clear as water, on the one hand, and a tough, adhesive, whitish, dirty brown, or greenish-yellow mucoid mass on the other, all intermediate stages occur. Accordingly the specific gravity is also very variable, between 1·002 and 1·055; 1·010 to 1·024 is the usual range.

Ovarian fluids contain albumen (serum-globulin and serum-albumin) in various proportions, as well as a characteristic constituent which is never wanting, pseudomucin (Hammarsten),[1] a body allied to mucin. It is this material which gives rise to the peculiar characters of ovarian fluids, when it is present in considerable quantity. Its watery solutions are mucoid, hard to filter, and behave as follows:

REACTIONS OF PSEUDOMUCIN

1. *Boiling*: no alteration.

2. *Addition of acetic acid*: no precipitate (distinction from mucin).

[1] Pseudomucin is identical with Scherer's metalbumin. Scherer's paralbumin is a mixture of pseudomucin and albumin.

3. *General tests for albumen*: nitric acid, acetic acid + NaCl, acetic acid + potassium ferrocyanide; no precipitate, the fluid only becomes thicker.

4. *Boiling with Millon's reagent*: brownish-red coloration.

5. *Addition of alcohol*: thread-like precipitate resembling fibrin and retaining its solubility in water even after standing under alcohol several days.

6. *Boiling with dilute mineral acids*: splitting-off of a copper-reducing substance.

Ovarian fluids containing considerable quantities of pseudomucin possess a tough slimy consistency, and on treatment with alcohol give a shreddy precipitate which at once stamps them as ovarian. When, however, the pseudomucin is only present in small quantities, these characters are not well marked. In such cases and, generally speaking, whenever a certain proof of the presence of pseudomucin is required, the following tests must be applied.

TESTS FOR PSEUDOMUCIN

(*a*) *Boiling test.*—The fluid, after being quite faintly acidulated with acetic acid, is heated to boiling-point and a few drops of acetic acid carefully added till the albumen separates out in the form of flocculi—the method described in § 14 for the separation of the albumen. If the filtrate is quite limpid there is no pseudomucin in the fluid; a whitish opalescent filtrate, on the other hand, indicates the presence of pseudomucin. As, however, similar filtrates may be obtained from pure albumen solutions through imperfect *technique* in removing the albumen, one ought not to rest contented with a single test, but several ought to be done, varying the amount of acetic acid each time. Only when all the tests give the same result—viz. the cloudy opalescent character of the filtrate, is the

presence of pseudomucin to be looked upon as highly probable. One should never omit, however, to subject the filtrate to the following test, more especially as it is more sensitive.

(*b*) *Reduction test.*—The filtrate is concentrated on the water bath and precipitated with alcohol. The flocculent precipitate is squeezed out and thrown into water. In presence of pseudomucin it dissolves, forming an opalescent fluid. Part of this is subjected to Trommer's test for sugar or other reducing substance. A negative result will be obtained, as the substances in question, if present at all, would have remained behind dissolved in the alcohol. The remainder of the fluid is treated with excess of acetic acid, filtered free from any precipitate (mucin ?) thereby produced, and then treated with hydrochloric acid until the sample contains about 5 per cent. of the latter. It is now heated in a test-tube until it becomes dark yellow or brown. On cooling it is neutralised with concentrated liq. sodæ and again subjected to Trommer's test. If pseudomucin were present in the original fluid, an undoubted precipitate of copper suboxide is now obtained (Hammarsten).

Nylander's test now also gives a positive result.

With typical fluids it suffices to work with as little as 10 c.cm., but when, on the other hand, the sample is thin and watery, several hundred c.cm. must be used in order to obtain the reduction test.

§ 40. *Hydronephrosis Fluid*

The fluid evacuated from a hydronephrosis is usually limpid, possesses a specific gravity of 1·008 to 1·020, and in composition closely resembles a very dilute urine. Traces of albumen are often demonstrable. It may undergo secondary alterations of various kinds ; thus admixture of mucus or pus may render it turbid

and highly albuminous; complete cessation of secretion may produce disappearance of the specific urinary constituents, &c.

A certain diagnosis is attainable by chemical methods only when the fluid contains the urinary constituents (urea and uric acid) in considerable quantity. Small amounts of these substances have been detected in other fluids (thus, urea in hydatids, uric acid in ascitic fluids from cases of gout).

1. EXAMINATION

The fluid is neutralised and concentrated on the water bath till it becomes like a very thin syrup when cold. A drop of this is placed on a glass slide, a cover-glass put on, and a drop of strong nitric acid run in. In presence of urea a characteristic crystalline precipitate of nitrate of urea forms at the junction of the fluids, either at once or after some little time. The fundamental form is a rhombic table, the narrower angles of which measure 82°. By rounding-off of the obtuse angles six-sided tables are formed which often lie on one another in an imbricated manner. This is the commonest shape (fig. 8).

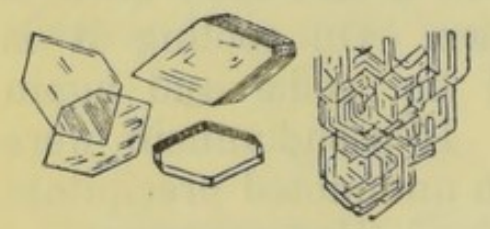

FIG. 8

This simple test, however, succeeds only with fluids which are relatively rich in urea and do not contain certain other substances, especially albumen and similar bodies. In such cases the fluid must be evaporated to a thick syrup and extracted with alcohol. The extract is itself concentrated to a syrup. This will sometimes stiffen into a solid crystalline mass of urea (long, thin, four-sided prisms). The residue or the above-mentioned crystals, pressed in blotting-paper

till dry, are dissolved in a little water and tested as above described for formation of nitrate of urea. If no characteristic crystallisation now occurs, it may be concluded that the fluid does not contain urea in quantity sufficient to establish the diagnosis of hydronephrosis.

2. EXAMINATION FOR URIC ACID

Albumen if present must first be got rid of according to § 12. The filtrate is then concentrated to a small bulk, treated after cooling with a few drops of strong hydrochloric acid, and laid aside in a cool place. In presence of uric acid in not too minute quantity, crystals separate out, which may be recognised as such by their form and the murexide test (§ 29).

§ 41. *Contents of Hydatid Cysts*

Such fluid is generally limpid, neutral or alkaline in reaction, of low specific gravity (1·007 to 1·015), and rich in common salt, which crystallises out when the fluid evaporates. Albumen is absent, or is present only in traces ; larger amounts are rare and mostly occur only after repeated tapping. Succinic acid or its salts have been found in small quantities as constant constituents A simple way of demonstrating them is to evaporate the fluid to syrupy consistency, acidulate it with hydrochloric acid, and extract with ether. After evaporation of the ether the succinic acid remains behind as an impure crystalline mass (monoclinic prisms or six-sided tables). Its watery solution gives with ferric chloride a rusty-coloured flocculent or gelatinous precipitate of succinate of iron.

APPENDIX

ON MICRO-BIOLOGICAL METHODS OF DIAGNOSIS

BY THE TRANSLATOR

I. THE BLOOD

A. NORMAL CHARACTERS AND CONSTITUENTS

Reaction, alkaline.

Specific gravity, about 1·059.

Composition, plasma, in which float (1) Red discs (erythrocytes), (2) white corpuscles (leucocytes), (3) blood platelets.

(1) RED DISCS: **Number**—$4\frac{1}{2}$ to $5\frac{1}{2}$ millions per c.mm.

Shape.—Circular, biconcave discs.

Colour.—Pale yellow or amber.

Size.—7·5 μ in diameter.

Isotonicity.—Equivalent to 0·46 per cent. salt solution.

Nucleus.—Absent.

Staining Reactions.—Whilst still living, *achromatophil, i.e.* insusceptible of being stained; after fixation *monochromatophil, i.e.* selecting a single stain out of a mixture of acid and basic stains. An *acid* stain is normally selected.

(2) LEUCOCYTES.—Almost spherical, nucleated, often amœboid, often granular, colourless; generally

present in the proportion of 1 or 2 per thousand red corpuscles: average number per cubic millimetre of blood 7,500.

The following varieties are distinguished:

(*a*) *Small mononuclear or 'lymphocytes.'*—*Size* about the same as that of a red disc, or but little larger —7–10μ. Nucleus round, nearly filling the cell and staining deeply with basic stains. Protoplasm non-granular, forming a narrow ring round the nucleus.

(*b*) *Large mononuclear.*—Larger than the preceding; diameter, 13–15μ. Nucleus round, larger than that of the lymphocytes and staining more faintly. Protoplasm non-granular.

(*c*) *Transitional Forms.*—Resemble the large mononuclear variety, but nucleus is irregular or indented.

(*d*) *Polymorpho-nuclear.*—Somewhat smaller than (*b*) and (*c*); nucleus polymorphous, twisted, seldom truly broken-up, staining rather deeply. Protoplasm abundant, studded with neutrophil granules (ε-granules of Ehrlich).

(*e*) *Eosinophiles.*—Usually polymorpho-nuclear, occasionally mononuclear or transitional. The protoplasm thickly studded with large, spherical, oxyphil granules (*a*-granules of Ehrlich). Cohesion of cell less than that of other leucocytes, hence they often break in preparation.

(*f*) *'Mast' cells* [German Mastzellen = stuffed cells] are leucocytes with basophil granules. They rarely occur in normal blood.

Normal Percentage of each Variety.

	Per cent.
Small lymphocytes . . .	20–30
Large ,, ...	4–8
Polymorpho-nuclear neutrophiles .	62–70
Eosinophiles	$\frac{1}{2}$–4
Basophiles	$\frac{1}{4}$–$\frac{1}{2}$

Classification of Stains.—Some of the aniline (coal-tar) stains are termed basic, *e.g.* methylene blue, fuchsin, methyl green, gentian violet, dahlia, safranin. Others are called 'acid,' *e.g.* acid fuchsin, eosin, picric acid, aurantia, orange G. The 'basic' anilines and hæmatoxylene have a powerful affinity for certain constituents (chromatic substance) of nuclei. The 'acid' anilines stain protoplasm. 'Neutral' stains in Ehrlich's sense are made by mixing 'basic' with 'acid' stains.

Ehrlich's Classification of the Granules that occur in Leucocytes.—*a*, or eosinophile, are the largest of all, take up 'acid' stains, are highly refractive, and occur in various kinds of leucocytes, which are then called 'eosinophile cells.'

β, or amphophile, are distinguishable from the *a*-granules chiefly by their preference for *indulin* (a greyish-black acid stain), as well as by the fact that they take up both acid and basic stains. They are unimportant.

γ, or 'mast'-cell granulations, take up basic dyes. They are coarse, not highly refractive, and occur chiefly in leukæmia.

δ-granules are minute basophile, and occur in large mononuclear cells. Whether they are entitled to a separate position is a matter of doubt.

ε, or neutrophile. These are extremely fine uniform granules staining with 'neutral' dyes, *e.g.* methylene blue + acid fuchsin, or the Biondi-Heidenhain mixture (of methyl green, acid fuchsin, and orange). All polymorphonuclear leucocytes are normally crammed with them. They also occur in *myelocytes*, but are seldom or never found in ordinary mononuclear forms. An oil-immersion lens is requisite for their detection.

(3) Blood-platelets.— Small (diameter, 2–3*μ*) colourless discs, very adhesive, forming shapeless

masses in the fresh preparation; number about 300,000 per c.mm.

B. VARIATIONS OF THE BLOOD

The blood may deviate from the above standard both physiologically and pathologically.

The Reaction undoubtedly varies, but is not at present susceptible of very accurate measurement.

The Specific Gravity may vary in both directions. It varies with the percentage of hæmoglobin, and may therefore serve as a means of estimating the amount of that substance, *unless the patient is dropsical.*

The red discs may vary as regards—

(*a*) *Number*: increased in plethora (unimportant), diminished in anæmia (oligocythæmia).

(*b*) *Shape*: irregular forms (poikilocytes), often pear-shaped, occur in anæmia.

(*c*) *Colour*: this depends on the amount of hæmoglobin; depth of tinge occasionally *increased* in pernicious anæmia; always diminished in chlorosis.

(*d*) *Size*: small (2 to 4 or 5μ) forms, called *microcytes*, and large (9 to 12 or 14μ) forms, called macrocytes or megalocytes, occur in anæmias; the latter variety, often with pale discoplasm and devoid of biconcavity, is prone to occur in pernicious anæmia.

(*e*) *Isotonicity.*—Variations of this character are as yet clinically unimportant.

(*f*) *Nucleus.*—All red discs originally possess a nucleus, but lose it either by solution or extrusion before entering the general circulation. Nucleated red discs occur in severe anæmic and leukæmic states, and are of three kinds: *microblasts*, *normoblasts*, and *megaloblasts*, according to their size. Normoblasts are young red discs. Their nucleus stains very deeply, and is often seen to be undergoing extrusion. They are characteristic of *regenerative* processes.

Megaloblasts have a large, faintly staining nucleus, and are regarded as indicative of degeneration, Microblasts have very little protoplasm round the nucleus. They are likewise degenerative in character.

(*g*) *Staining Reaction.*—Normal red discs take up acid stains; in disease they sometimes take up mixtures of basic with acid stains, either diffusely or in patches—*polychromatophilia.*

The leucocytes may vary—

(*a*) *In total number*, which may be *increased*, temporarily and physiologically (*leucocytosis*); permanently and pathologically (*leukæmia*), or *diminished* (*leucopenia*) in malnutrition, after hot baths, and in infectious diseases which are not associated with leucocytosis—*e.g.* influenza, measles, miliary tuberculosis, malaria, and especially typhoid.

(*b*) *In proportionate representation*: in *leucocytosis* the number of *polymorpho-nuclear neutrophiles* is increased; in *leukæmia* the increase is mainly confined to the *mononuclear* variety. Eosinophile cells are sometimes increased in leukæmia, but by no means always; often increased in asthma, emphysema, certain skin diseases, and, according to Neusser, in several other affections. They are generally decreased in croupous pneumonia and in certain cases of tuberculosis.

(*c*) *In the occurrence of abnormal varieties*: the *myelocyte*, or marrow-cell, is the most important of these. It is a large spherical cell (average diameter about 15 μ) with a large, faintly staining, undivided nucleus excentric in position; the protoplasm is filled with neutrophile granules. It differs from the mononuclear leucocyte in possessing neutrophile granules, from the polymorpho-nuclear in the shape of the nucleus, and from both in being usually larger. It is devoid of amœboid motility, and its usual abode is the bone-marrow. These cells are abundant in, but not absolutely characteristic of, spleno-medullary

leukæmia. Cells otherwise identical often occur with eosinophile granules, and are called eosinophile myelocytes.

C. CHARACTERS OF THE BLOOD IN VARIOUS ABNORMAL CONDITIONS

I. Leucocytosis—leucocytes increased

The most important point is that the *increase in the total number of leucocytes never involves a diminution in that of the polymorpho-nuclear variety.*

- Leucocytosis
  - A. Physiological (relative proportions of the several varieties unchanged).
    1. Of the newly-born
    2. Of digestion
    3. Of pregnancy
    4. Of puerperium
    5. Of violent exercise, massage, and cold baths
    6. Of the moribund.
  - B. Pathological (increase chiefly confined to polymorpho-nuclear variety).
    1. Post-hæmorrhagic
    2. Inflammatory
    3. Toxic
    4. In malignant disease
    5. Due to therapeutic and experimental influences.

Inflammatory or infective leucocytosis has the greatest practical importance. Its intensity varies directly with the severity of the exciting cause (infection), the power of resistance possessed by the patient, and the development of the local reaction. Thus it is pronounced in pneumonia, abscess, carbuncle, and totally absent in uncomplicated cases of typhoid,

malaria, influenza, and tuberculosis. Present in septic (suppurative) forms of meningitis, it is usually absent in the tubercular variety, and this seems to hold good of the other serous membranes.

II. Eosinophilia

'An increase in the number of eosinophiles, with or without an increase in the total leucocyte count,' occurs in some forms of leukæmia, but is *not* diagnostic. Physiologically in infancy, and according to Neusser in—

A. Bone affections (sarcoma, leukæmia, osteomalacia).
B. Skin diseases (pemphigus, pellagra, urticaria, exanthema of scarlet fever and syphilis).
C. Diseases of genitals (especially ovaries).
D. Disturbance of sympathetic nervous system.

A case of *trichinosis* studied by Thayer at the Johns Hopkins Hospital yielded 64 per cent. of eosinophiles.

III. Lymphocytosis

'Lymphocytosis is a relative increase in the lymphocytes or young cells in the blood, with or without an increase of the total leucocyte count' (Cabot). It occurs physiologically in infancy, and is prolonged beyond the age at which such an increase in the lymphocytes is normal, by rickets, hereditary syphilis, and scurvy. In adults 'it is most marked in chlorosis, pernicious anæmia, and the anæmia secondary to syphilis, in the later weeks of typhoid, and in lactation' (Cabot). *An absolute increase of lymphocytes, associated with glandular tumours, is diagnostic of lymphatic leukæmia.*

IV. Pernicious Anæmia

A. *Naked-eye Changes*: blood pale, watery, fluid; slow to coagulate.

B. *Fresh Specimen*: no rouleaux; great diminution of corpuscles; macrocytes and poikilocytes numerous.

C. *Enumeration*: red discs reduced to about one-fifth of normal, averaging in Cabot's 52 cases, 1,200,000 per c.mm.; white cells diminished also in uncomplicated cases.

D. *Hæmoglobin*: absolutely much diminished, but relatively increased, *i.e.* percentage generally too high for the number of red discs present—*e.g.* 1,000,000 red discs and 35 per cent. of hæmoglobin (high colour-index).

E. *Stained Films*: macrocytes, some microcytes, poikilocytes, 'Maragliano's changes' (*i.e.* white spots or streaks in red corpuscles; they may be reduced to a ring, the centre remaining unstained; absence of central biconcavity), polychromatophilia. Nucleated red discs present, especially megaloblasts. Lymphocytosis present.

V. Chlorosis

A. *Naked-eye Changes*: blood pale, fluid; coagulates quickly.

B. *Fresh Specimens*: Red discs very pale, small-sized; blood-platelets increased.

C. *Enumeration*: red discs diminished, but not excessively; 4,000,000 is the average on first examination, according to Cabot. White cells may be normal or diminished in number. Lymphocytosis usual.

D. *Hæmoglobin*: percentage much diminished, averaging one-half normal. (Specific gravity always diminished in proportion; may touch 1·030.)

E. *Stained Films*: diameter of many red cells diminished; poikilocytes present in severe cases; nucleated red corpuscles rare, and when present usually normoblasts.

VI. Secondary Anæmia

The condition of the blood in pernicious anæmia, chlorosis, and secondary anæmia is well contrasted in the following table, slightly modified from Cabot:

—	Pernicious anæmia	Chlorosis	Secondary anæmia
Red discs	About 1,000,000	Rarely under 2,000,000	May be 1,000,000 or less
White cells	Usually decreased	Usually normal	Usually increased
Hæmoglobin	Often relatively high	Always relatively low	Relatively low
Megaloblasts	Often constitute majority of nucleated red cells	Rare	Rare; never exceed normoblasts
Normoblasts	Less numerous than megaloblasts	Occasional; always more numerous than megaloblasts	Common in severe cases
Size of red discs	Increased	Diminished	Various, but not increased
Lymphocytes	Increased	Increased	Usually diminished
Polymorpho-nuclear neutrophiles	Decreased	Decreased	Usually increased
Myelocytes	Common	Rare	Rare
Rapidity of coagulation	Decreased	Increased	Usually increased

VII. Leukæmia

A. **Spleno-medullary Form.**—*Red Discs*: moderately diminished, about 3,000,000 per c.mm.

Normoblasts: extremely numerous; nuclei often fragmented or undergoing extrusion; mitoses occasionally met with.

Megaloblasts: rather rare.

Leucocytes in general: enormously increased; about 450,000 per c.mm. When case is first observed, average ratio to red discs 1 : 10.

Lymphocytes: absolutely increased, relatively much diminished (from 20 or 30 per cent. of all leucocytes down to about 7 per cent.) No excess of large forms.

Polymorpho-nuclear Neutrophiles: absolutely much increased; relatively diminished (to about 50 per cent. of all leucocytes); increased variability in size and staining reactions.

Eosinophiles: absolutely much increased; relatively but slightly so (average about 4·4 per cent.); many are myelocytes.

Myelocytes: enormously increased, both absolutely and relatively (average about 37 per cent. of all leucocytes); many have eosinophile granules. This increase of myelocytes (and not the increase of eosinophiles) is the characteristic and diagnostic feature of the blood of spleno-medullary leukæmia.

B. **Lymphatic Form.**—*Red Discs*: diminished, rather more so than in the spleno-medullary form, average 2,730,000 per c.mm.

Normoblasts: much rarer than in the spleno-medullary form.

Megaloblasts: much rarer than in the spleno-medullary form.

Leucocytes in general: increase not so marked as in the spleno-medullary form; average 141,000 per c.mm.

Lymphocytes: enormously increased, both relatively and absolutely (over 90 per cent. of all leucocytes present). Large or small forms may predominate.

All other forms of leucocyte: very scanty.

VIII. Hodgkin's Disease (Lymphadenoma or Pseudo-leukæmia)

Cabot [1] sums up the blood conditions thus:

'Normal blood in early stages; later often marked anæmia; sometimes leucocytosis.'

D. EXAMINATION OF THE BLOOD

1. Fresh Preparations.—*Mode of Procedure.* A perfectly clean (v. *infra*, p. 105) slide and cover-glass having been prepared, the patient's finger, or preferably ear-lobe, is pricked with a lancet-shaped needle, the cover-glass taken up with forceps (or with the fingers, by its edges), and its centre brought in contact with the drop of blood. It is then allowed to fall, blood side down, on to the middle of the slide. The blood should spread itself out without pressure so as to form a layer of even thinness exactly filling the space between slide and cover-glass. The preparation should be *at once* examined with a high dry objective ($\frac{1}{6}$ in., Zeiss D, or Leitz 7). Necrobiotic changes soon begin to manifest themselves in the blood, but can be postponed for a considerable time by painting the slide with a thin band of vaseline, round or square, according to the shape of the cover, and of such size that the edges of the cover will correspond with it when placed in position on the slide. Access of air is thus completely prevented, especially if the margins of the

[1] Cabot, *Clinical Examination of the Blood.* London: Longmans, 1897. The averages given under leukæmia are those of a series of cases referred to in that work.

completed preparation are gone over somewhat thickly with vaseline. *Filaria sanguinis hominis* can be kept alive for several days in such preparations.

The following are the chief characters that can be readily made out *on fresh blood-slides*: their recognition by the inexperienced observer will be greatly facilitated by putting up an exactly similar slide from the blood of a healthy person and using it for purposes of comparison.

A. The red discs may be altered in—

(*a*) Size (macro- and micro-cytes).

(*b*) Shape (poikilocytes).

(*c*) Number (oligocythæmia).

(*d*) Depth of colour (oligochromæmia).

(*e*) Want of tendency to rouleau-formation.

B. The leucocytes may be altered in number, generally by way of increase (leucocytosis and leukæmia), occasionally they may be fewer than normal (leucopenia).

C. The blood-platelets may be increased or diminished.

D. Tendency to fibrin-formation may be increased or diminished.

E. Parasites may be detected—

(*a*) Animal: *Plasmodium malariæ*, *Filaria sanguinis hominis*.

(*b*) Vegetable: *spirillum* of relapsing fever, &c. (see p. 108).

2. Stained Films.—In order to obtain successful stained film-preparations of blood (or other organic fluid) the following processes must be carefully gone through:

(*a*) *Selection and preparation of the cover-glasses.*

(*b*) *Preparation of the part from which the blood is to be taken.*

(*c*) *Formation of the film.* (*d*) *Fixation of the film.*

(*e*) *Staining of the film.*

(*f*) *Mounting* (*and eventual preservation*) *of the film.*

(*a*) *Cover-glasses.*—These must be of the highest quality, flawless and susceptible of perfect cleansing (which is not always the case). They should be *thin* ['No 1'] $\frac{3}{4}$ in. to $\frac{7}{8}$ in. in size, squares rather than circles, and not too brittle. They must be cleaned by rubbing well with soap, washing this off with plenty of water and polishing with a soft clean linen handkerchief free from grease. The last traces of grease may be removed by drawing them through the flame whilst held in a forceps ; but care must be taken not

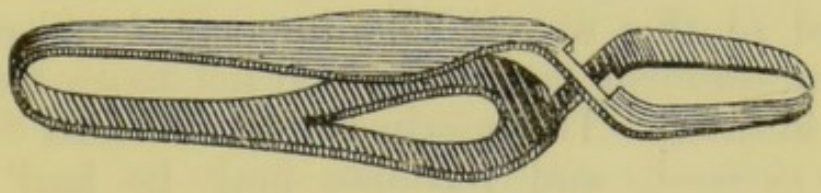

Fig. 9.—Cornet's Forceps

to overheat, lest they become curved or distorted. They may be laid upon a piece of clean filter-paper, covered with a watch-glass or Petri-dish, and are then ready for use. The manipulation of cover-glasses is greatly facilitated by using a Cornet's forceps (fig. 9).

(*b*) *Obtaining the blood.*—Antiseptics are unnecessary, save for bacteriological purposes. The lobe of the ear is the best part to select. It should be rubbed first with a wet towel to cleanse it, and then with a dry one. This makes it hyperæmic. Puncture should be effected quickly and deeply, the skin being held tense by the finger and thumb of the left hand. The needle should be lancet-shaped—a surgical needle. It may be guarded by being thrust through a disc of cork, about $\frac{1}{8}$ in. of the tip being left exposed—or the little instrument provided in the hæmacytometer outfit may be used. A drop or two of blood should be pressed out and wiped off before one is selected for examination, and the puncture should

be deep enough to allow them to flow readily without necessity for squeezing.

(*c*) *Formation of the film.*—A cover-glass is now taken up and the middle of its surface just barely brought in contact with the convex blood-drop, withdrawn and let fall on the surface of another cover-glass so that the blood spreads itself in an even layer between the two. An angle of the upper cover-glass should be left projecting so as to afford a means of gripping it in the action of separation. This should be effected by a *sliding* motion—*not* by *pulling* the cover-glasses asunder. Several pairs of films may be prepared in this way. The important object is to obtain a uniform layer of blood not more than one corpuscle thick. The method just described does not always succeed, and recourse may be had to the following plan. A largish drop of blood having been taken on to the surface of a cover-glass, this is held perpendicularly so as to allow the blood to run to the edge, which is then, by a rapid movement, drawn across the surface of a clean slide so as to get a sort of elongated smear, thick, no doubt, at one end, but 'tailing off' at the other into an exquisitely thin, hardly perceptible layer, in which the individual corpuscles will be found to lie quite apart from each other.

(*d*) *Fixation* may be accomplished (i.) *By heat.*

a. Rough method. The cover-glass is drawn thrice, film-side up, through the flame of a Bunsen or spirit lamp, being held meanwhile in forceps. The rapidity of the movement must be learnt by experience and depends on the heat and size of the flame. This method is coarse and unsuitable for blood-films, the morphological details of which are to be the subject of investigation. It may be rendered less destructive by holding the cover-glass (by the edges) between the finger and thumb, and then drawing it to and fro for nearly a minute over the top of the flame. Overheating will then be prevented by the desire to avoid

burning the fingers, but the film will in many cases remain under-fixed.

β. Ehrlich's method. This consists in heating the cover-glasses to between 110° and 115° C. for a variable period—a quarter of an hour generally suffices. It gives excellent results, but is a little troublesome. A drying oven or hot-air steriliser is the best arrangement, and if not at hand may be readily improvised with a biscuit-box fitted with a false bottom made out of a piece of tin-plate with the edges turned down so as to keep it about an inch from the real bottom of the box. A bored cork bearing a thermometer is inserted into a hole in the lid, and the box is placed on a tripod-stand over a Bunsen. The cover-glasses should be placed in a covered Petri-dish or the like, which should be supported on a piece of wire gauze inside the box. The thermometer must be watched till it marks 110°, and then the flame carefully graduated so that the temperature may not exceed 115° C.

Another plan, also due to Ehrlich, which works well, is to procure a copper bar a foot long and two or three inches wide, place it on a tripod, or secure it in a laboratory stand over a Bunsen. By dropping water on this after it has been in position over the flame for a few minutes, the boiling-point may be readily ascertained, and the cover-glasses are to be placed *face downwards* at this spot, and left about a quarter of an hour. Ehrlich's method, however carried out, cannot be too strongly recommended.

(ii.) *Fixation by Reagents.*

α. Alcoholic formaline. Composition: commercial formaline 1 part, water 9 parts, absolute alcohol 90 parts. The film is allowed to dry and then floated for a few minutes on the fixing solution. If not convenient to stain at once, the films may be kept in the mixture indefinitely. This is a good and rapid method.

β. Equal parts of absolute alcohol and ether

(Nikiforoff). The air-dried films are immersed in the mixture for 15 to 20 minutes. This simple method gives very good results.

γ. Sublimate in combination with alcohol and ether (Gulland's method). The films are thrown at once, whilst still wet, into a solution of the following composition: alcohol 25 cc., ether 25 cc., alcoholic solution of corrosive sublimate (2 grams in 10 cc.) about 5 drops. After a few minutes they are picked out, washed in plenty of water, and are then ready for staining. This method yields admirable results, especially when nuclear changes are being studied.

Films to be fixed by heat must be allowed to dry in the air—protected from dust. Films to be fixed by Gulland's or other sublimate methods must be placed still wet in the fixing bath.

(*e*) *Staining the Film.*—The method differs according as the object is:

(i.) The detection of micro-organisms.

(ii.) The study of the morphological elements of the blood (pernicious anæmia, leukæmia).

(i.) The following micro-organisms[1] may occur in the blood:

Pus-cocci	Glanders bacilli
Anthrax bacilli	Influenza ,,
Tubercle ,,	Relapsing-fever spirilla
Typhoid ,,	Malarial plasmodia

[1] To these should be added for the sake of completeness: the pneumococcus (in cases of general infection from a primary pneumonic focus); the gonococcus, recently obtained from the circulating blood in a case of acute gonorrhœal rheumatism and endocarditis by Thayer of the Johns Hopkins Hospital (verbal communication to writer); and lastly, the recently discovered organisms of bubonic plague and yellow fever. The former (according to the report of the German Plague Commission) only occurs in the blood when the disease assumes the septicæmic form; the latter (according to Sanarelli) appears but rarely in the blood, and is then usually mixed with other organisms present as the result of secondary infections. Staining may be done in the case of the pneumococcus by Gram; the others may be stained by method α (above), or with strong watery gentian-violet.

Of these, tubercle and typhoid bacilli so seldom occur in the circulating blood that the search for them by this method is almost hopeless. Glanders and anthrax bacilli certainly do occur, but are best looked for in the fluids obtainable from the local foci of infection. Influenza bacilli are best sought for in the sputum. There remain the *pus-cocci*, the *spirillum* of relapsing fever, and the *plasmodium* of malaria. The first-named may be more readily demonstrated culturally; the second and third not being hitherto susceptible of cultivation outside the body must be sought for in film preparations if at all.

The two following methods will be found sufficient for most purposes:

a. Methylene-blue stain. A saturated watery solution of methylene blue is prepared, and the cover-glass, after fixation by Gulland's method, is floated on to the stain for one minute. It is then picked up, washed in water, dried, and mounted in balsam. The most striking results are obtained by saturating with eosin the alcohol used in making the fixing fluid. A beautiful contrast is thus effected, bacteria, malarial plasmodia, and nuclear structures being stained blue, protoplasm of leucocytes almost colourless, discoplasm of red corpuscles and eosinophile granules bright red.

β. Gram's method. Solutions required: 1. Aniline water, made by vigorously shaking up about 1 c.c. of aniline oil with 20 c.c. water in a test-tube for a minute or two, and running the milky emulsion through a moistened filter. It must be quite clear, smell strongly of aniline, and be made fresh each time.

2. Saturated alcoholic solution of gentian-violet.

3. Gram's iodine solution, consisting of iodine 1, iodide of potassium 2, water 300.

4. Absolute alcohol or methylated spirit (non-mineralised) in abundance.

Mode of Procedure.—Some aniline-water is run off

into a watch-glass, a few drops—roughly, one-tenth of its volume—of the alcoholic gentian-violet added, and the mixture stirred. The cover-glass film is floated on this for about a minute—or longer—and then removed to the Gram's iodine, on, or in, which it remains from half a minute to two minutes. It should assume a dirty brown hue, and be surrounded by a cloudy precipitate of the same colour. It is then transferred to the first of two small dishes full of alcohol, and gently agitated till most of the stain has come away, and is then plunged for a moment in the clean alcohol to remove last traces of superfluous stain. It should then look almost colourless. Further extraction of stain must at once be stopped by either—

1. Rinsing the alcohol away with clean water ; or
2. Rapid drying by gentle pressure between layers of folded filter-paper.

So great is the importance of Gram's method that it seems advisable to state how the principal disease-producing organisms are affected by it.

The following micro-organisms retain the stain by Gram's method:

Pus-cocci.
Micrococcus tetragenus.
Fraenkel's pneumococcus.
Bacillus of mouse-septicæmia.
Bacillus of swine-erysipelas.
Tubercle bacillus (badly, often looks like a chain of cocci).
Leprosy bacillus (badly, often looks like a chain of cocci).
Anthrax bacillus. Tetanus bacillus.
Diphtheria bacillus.

The following are decolorised by Gram's method:

The typhoid bacillus. The glanders bacillus.
The gonococcus. Friedländer's pneumobacillus.
B. coli communis. The influenza bacillus.

All vibrios, spirilla, &c. (including Asiatic cholera and relapsing-fever organisms).

All organisms belonging to the group of hæmorrhagic septicæmia.

(*f*) *Mounting.*—This may be done *temporarily* in water whilst it is being ascertained if the preparation is satisfactory, or *permanently* in Canada balsam dissolved in xylol. Mounting in Canada balsam requires the preparation to have been dehydrated either by sojourn in absolute alcohol for a minute or two, or by pressure between layers of clean filter-paper. In some cases clearing in xylol is advisable, but if the preparations have been thoroughly dehydrated in alcohol, and the alcohol thoroughly got rid of by evaporation, the clearing-bath may be omitted.

Should the preparation have been temporarily examined in water, the slide must be immersed in an oblique position in water till the cover-glass slides off; the latter must then be picked up with forceps and dried between filter-paper, as above described, after which it may be at once, or after treatment with xylol, put up permanently in balsam.

Of the micro-organisms mentioned as requiring microscopic demonstration in blood, two, viz. anthrax bacilli and pus-cocci, are stained by this method, which should never be omitted when the presence of either is suspected. They are stained of a blackish violet hue, whilst all the other constituents of the film are decolorised. A double stain may be effected by adding a little powdered eosin, or alcoholic solution of that substance, to the alcohol used for decolorising the film.

When staining blood-films by Gram's method two frequent causes of failure must be kept in mind: too thick a film, and too long a sojourn in the staining bath. Both act in the same way, viz. by rendering complete decolorisation of the red discs impossible,

and thus destroying that contrast between micro-organisms and background on which depends the success of the method. Should a preparation faulty in these respects be kept in the alcohol long enough to decolorise it completely, the organisms will probably be found to have lost their stain also, and the result will be a failure. Nothing but experience can enable the worker to make sure of success in a Gram-preparation, especially one of blood.

ii. *Examination of the Morphological Elements of the Blood.*—Staining with this object in view may be done in various ways according to the structural features which it is desired to examine.

a. Methylene-blue and eosin. After fixation with alcohol and ether the film is dried in the air, and stained for three or four minutes in a 0·5 per cent. solution of eosin in 70 per cent. alcohol, and then washed in water. It is then stained for two or three minutes in a saturated watery solution of methylene-blue, washed in water, dried, and mounted in balsam. Nuclei of leucocytes and of nucleated red corpuscles, as well as micro-organisms, are blue, whilst red discs and eosinophile granules are stained red.

β. Ehrlich's hæmatoxylene and eosin. To a small watch-glassful of Ehrlich's acid hæmatoxylene (see list of reagents, &c.) a small knife-pointful of water-soluble eosin is added and stirred in. The cover-glass is floated, film-side down, on this mixture for about a quarter of an hour and then picked up in forceps, dipped for a second in water to which one drop of ammonia has been added to make it alkaline, and then thoroughly washed in pure water. It may then be dried, or dehydrated in eosinic alcohol and mounted in balsam.

The nuclei of leucocytes are stained various shades of blue, whilst those of the nucleated red corpuscles (in anæmia and leukæmia) are nearly black. This differential staining is called metachromatism. Eosino-

phile granules are of course also stained, whilst the protoplasm of the leucocytes is tinged various shades of pale pink.

γ. Biondi-Heidenhain mixture (to be had in powder from Grübler—see list of reagents, &c.). The film is floated for about five or six minutes on the stain, rinsed well in water, dried between layers of filter-paper, and mounted in balsam. Successful preparations show the leucocyte-nuclei pale green, those of nucleated red corpuscles dark green to nearly black, eosinophil granules a brownish or coppery red, neutrophil granules violet or lilac, discoplasm of red corpuscles bright yellow.

General observations on stained film-preparations of blood:

A good immersion-lens is required for the examination of these preparations, especially if neutrophile granules are being sought for. The diaphragm should be open. Preparations that have been fixed for a short time only must not be kept long on the stain. Fully fixed preparations require the full staining period. If the red corpuscles come out grey or greenish in Biondi-Heidenhain preparations, the film is underfixed.

3. Enumeration of the Red Discs.—This may be done—

(*a*) *With the centrifuge.*
(*b*) *With the hæmacytometer.*

(*a*) *The Centrifuge.*

The best centrifuge for the purpose is Daland's 'Hæmatocrit.' The capillary tube is sucked full of blood—a deep puncture is necessary for this purpose—the free opening closed with a finger smeared with vaseline, the rubber tube slipped off, and the capillary placed at once in the apparatus, and whirled for from one to two minutes at seventy hand-revolutions per minute. The red corpuscles will be found to have

collected in an opaque column which occupies the peripheral half of the capillary, and the length of which can be read off by means of the graduations. When there is much increase of the leucocytes they may be seen forming a greyish cap on the central end of the column of red discs. Each graduation corresponds roughly to 100,000 red discs. Accordingly if the column extends to the mark 50 the number of red discs is normal.

The advantage of the instrument is its rapidity and the absence of eye-strain in using it. Its disadvantage is the fact that its results are inaccurate when there is much alteration in the size and shape of the individual red corpuscles, and therefore inapplicable to many cases of anæmia and leukæmia. It also makes a loud noise.

(*b*) *The Hæmacytometer.*

[The following remarks apply to the Thoma-Zeiss hæmacytometer only.]

1. The *diluting fluid* may be a 3 per cent. solution of common salt deeply tinged with methyl violet. It must be made fresh or be recently filtered.

2. Cause a large drop of blood to exude from the patient's ear-lobe or finger-tip without squeezing. (*vide supra*, p. 105).

3. By gentle aspiration cause the blood to run up into the pipette up to the mark 1—or only to mark 0·5 if the anæmia be slight in amount; wipe the end of the pipette with a silk handkerchief and dip it in a little beaker full of diluting fluid. Aspirate carefully till mark 101 is reached, and then rotate the pipette round its long axis till the fluids are thoroughly mingled in the bulb. During the aspiration of the blood the top of the pipette should not touch the patient's skin nor, on the other hand, be withdrawn from the (convex) drop of blood, the penalty being failure of the whole operation.

4. Blow out about one half the contents of the

mixing chamber and transfer a small drop to the surface of the counting-slide. The drop that falls from the top of the pipette will generally be too large and overflow into the 'moat.' A looped platinum inoculation needle, or a similar instrument made from soda-water wire, will transfer a drop of suitable size.

5. The cover-glass must now be gently dropped

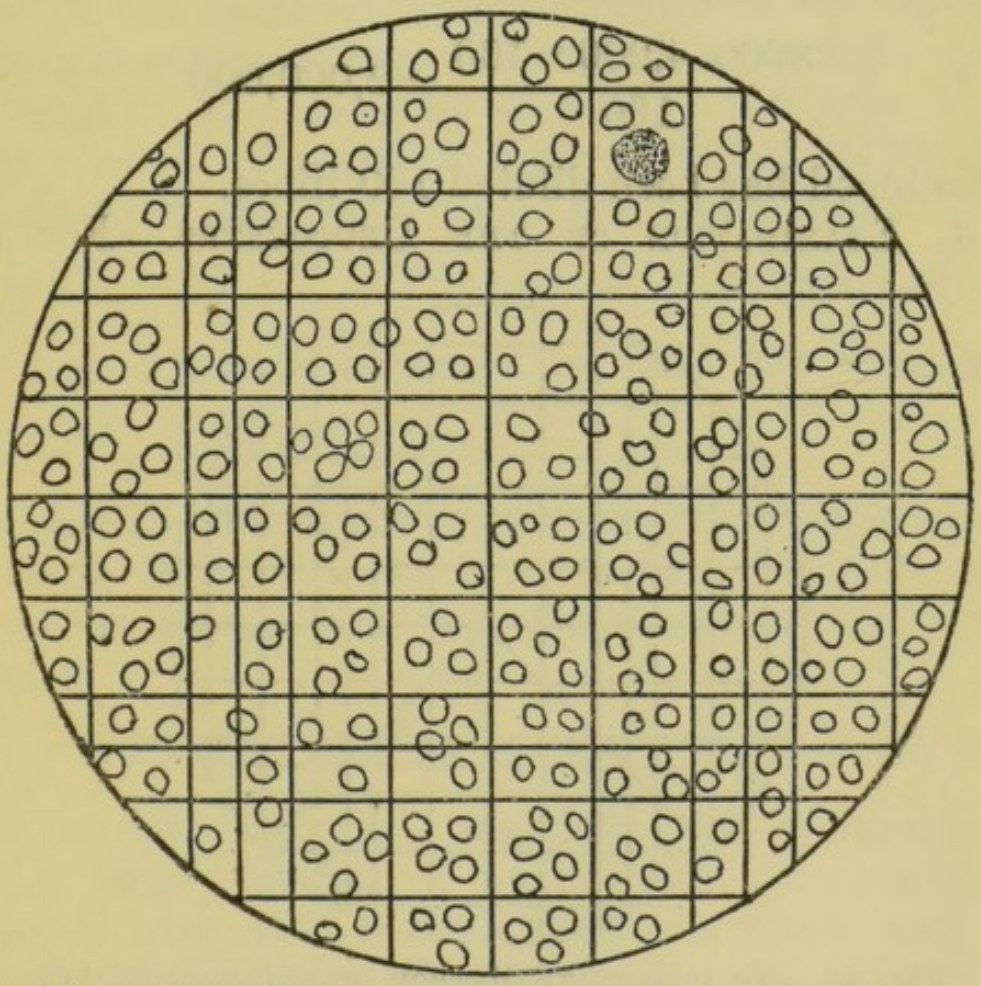

FIG. 10.—ANÆMIC BLOOD UNDER EXAMINATION WITH THE THOMA-ZEISS HÆMACYTOMETER. ONE LEUCOCYTE IS VISIBLE (STENGEL)

on, using a mounted needle to break the fall. No air-bubbles should be entangled, and Newton's rings must be visible.

6. After waiting a few minutes to allow deposition of the corpuscles to occur, focus the ruled squares with a moderate power (fig. 10). The writer uses Leitz 7; others prefer a somewhat lower objective (Leitz 5).

Count the red corpuscles in a large number of small squares, at least 100. Find the average per square and multiply this by 4,000 (because each square is the floor of $\frac{1}{4000}$ of a cubic millimetre), and then by 100 if you have drawn the blood up to mark 1 on the pipette, or by 200 if you have only drawn it up to 0·5. Suppose, for instance, 160 squares are found to contain 800 red corpuscles, then

$$\frac{800 \times 4{,}000 \times 100}{160} = 2{,}000{,}000$$

if the blood has been diluted 100 times.

4. Examination of the Leucocytes.—For this

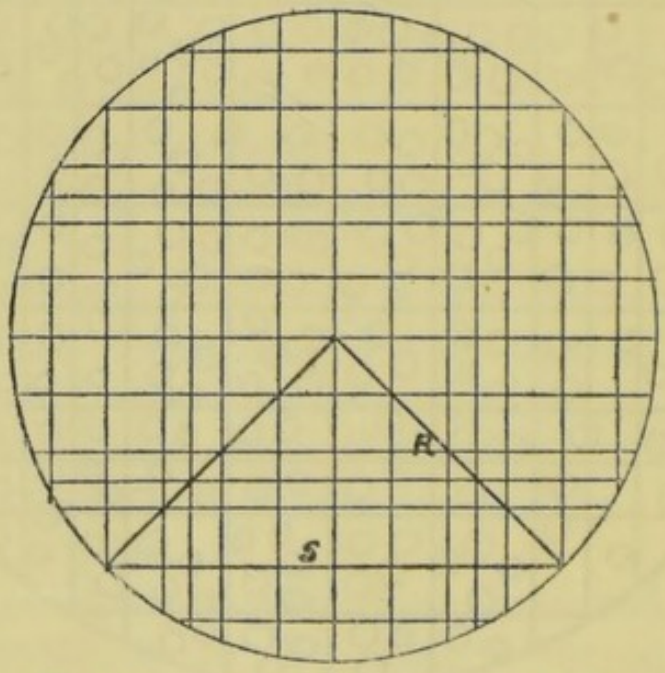

Fig. 11. –To illustrate Method of determining Area of Microscopic Field (Stengel)

purpose it is well to use the special pipette giving a dilution of 1 in 10, and to eliminate the red discs by using one-third per cent. acetic acid tinged with methyl violet as the diluting fluid. Large squares equivalent to sixteen small ones are convenient units of area. A still more convenient unit of area is an entire field of the microscope. The following calculation and figure

(11), taken (by permission) from Dr. Stengel's[1] article on Blood in 'Twentieth Century Practice,' show how this area may be determined. The ruled slide is moved about under the microscope till the corners of a square group of smaller squares are cut by the field of vision as in the figure. The area of the field of vision may then be readily determined by its relations with the area of the enclosed square. Thus in the figure a square is enclosed, each side of which is eight spaces in length. Representing this by S, the area of the square $= S^2$. The radius R of the circle may be determined from the relations of the right angle. Thus $S^2 = 2R^2$ or $R^2 = \frac{S^2}{2}$. The area of the circle being $R^2\pi$, the terms may be changed so that the formula is obtained: area of circle $= \frac{S^2}{2}\pi$.

Suppose the side of the square consists of eight smaller squares, then its area is $8^2 = 64$. As each small square is $\frac{1}{400}$ sq. mm. the area $= \frac{64}{400} = \cdot16$ sq. mm. Then:

$\frac{\cdot16}{2} \times 3\cdot1416 = \cdot251328$ sq. mm., which is the area, and the space over it is ·0251328 cubic millimetre.

A large number of such fields can readily be counted over with a moderately low power, and this affords the readiest method of ascertaining the number of leucocytes in a sample of blood with approximate accuracy.

After use the pipettes must be most carefully cleaned, first with pure water, then with alcohol, and lastly with ether. The writer is in the habit of slipping the bellows of a Cathcart's ether-freezing microtome on to each pipette and blowing till they are absolutely

[1] *Twentieth Century Practice*, edited by Thos. L. Stedman, M.D. (London: Sampson Low, 1896), vol. vii. p. 272.

dry and the little bead in the mixing chamber shows no tendency to adhere to its sides.

5. Estimation of Hæmoglobin is, in this country, generally done by means of Gowers's instrument. The manipulations are so simple as hardly to require description. The blood is aspirated into the capillary pipette up to the mark 20 c.mm., the pipette wiped, and the contents carefully blown out into the graduated tube, which should contain a few drops of distilled water. In order to obtain the last traces of blood from the pipette, it should then be aspirated full of clean distilled water several times, and the contents blown into the graduated tube. The contents of the latter should now be carefully and thoroughly mingled, and water added drop by drop until the colour of the standard comparison tube is as nearly as possible matched. The operation must be patiently done, as it is very easy to overshoot the amount. The mark ultimately reached is the percentage of hæmoglobin present. If a similar depth of colour is reached in the two tubes when the fluid has reached 100, the percentage of hæmoglobin in the blood under examination is normal. The graduated mixing-tube should be cylindrical.[1] Tubes of flattened shape are sometimes sent out into which the pipette will not fit, the result being that the blood is spurted on the inside of the tube, and is likely to coagulate before getting into the water used for dilution. Again, the narrowness of the flattened tube gives rise to awkward entanglement of fluid through the action of capillarity.

6. Estimation of the Specific Gravity of the Blood.—This is best done by Hammerschlag's modification of Roy's method. A mixture of chloroform and benzol is made of the specific gravity 1·059. This is

[1] This is the case with Sahli's modification of Gower's apparatus (to be had of Reichert, Vienna).

poured into a clean urine cylinder, a drop of blood is taken from ear or finger into a capillary tube and blown out into the fluid. If the blood-globule floats, more benzol must be added; if it sinks, more chloroform. In either case the liquid must be well stirred up with a glass rod after each addition. When the drop of blood becomes steady, neither rising nor falling, the specific gravity is taken with a urinometer, and the figure obtained is the specific gravity of the blood. The urinometer must be graduated somewhat higher than that for ordinary clinical work—up to 1·060 at least. Changes in specific gravity run parallel with changes in hæmoglobin-content, save in cases of dropsy, and the estimation of the specific gravity may therefore be used instead of a hæmoglobin determination, which is a considerable saving of time and trouble, more especially if v. Fleischl's be the hæmoglobinometer used. The following table shows the relation between specific gravity and hæmoglobin:

Sp.gr.		Hæmoglobin
1·033–35	=	25–30 per cent.
1·035–38	=	30–35 ,,
1·038–40	=	40–45 ,,
1·045–48	=	45–55 ,,
1·048–50	=	55–65 ,,
1·050–53	=	65–70 ,,
1·053–55	=	70–75 ,,
1·055–57	=	75–85 ,,
1·057–60	=	85–100 ,,

7. Culture Methods.—These may be needed for the detection of anthrax, glanders, or influenza bacilli, or, more usually, of pus-cocci in cases of sepsis, which it is proposed to treat sero-therapeutically.

The method of obtaining samples of blood free from external contamination will now be described; but for details as to the preparation of nutrient substrata, selection and management of incubators, &c., the reader is referred to works on bacteriology—

the latest and best of which is that by Muir and Ritchie [Edinburgh : Pentland, 1897].

(*a*) *Collection of the blood.*—A capillary pipette (fig. 12) should be made from a piece of 'quill' glass-tubing. A loose plug of sterile cotton-wool should be pushed in at the open end, and, for greater security, the whole resterilised by being slowly drawn several times through a Bunsen flame. The skin over the patient's finger-tip must meanwhile be thoroughly sterilised with soap and water, sublimate, alcohol, and ether, and when it is thoroughly dry—a final dip in ether greatly hastens this—it is deeply punctured with a sterile lancet-shaped needle and a

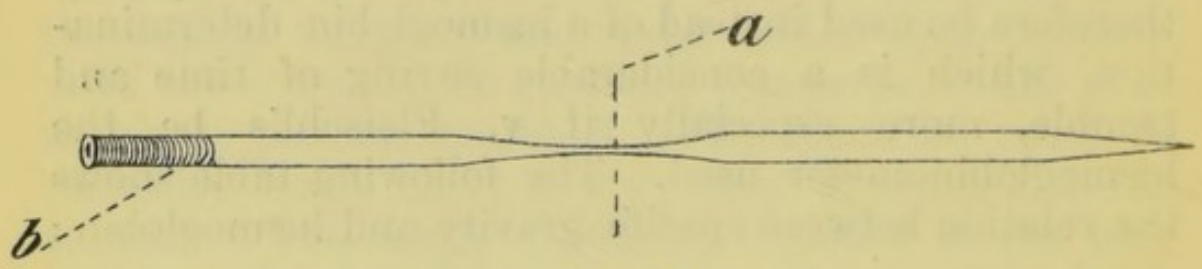

FIG. 12

large convex drop of blood sqeeezed out. The pipette is then taken up, its point broken off with sterile (flamed) forceps, and introduced into the drop of blood, which is caused by gentle aspiration to run up into the dilated part. When the drop is *nearly* all taken up, the pipette may be laid down or handed to an assistant, and the finger again firmly squeezed, whereupon a fresh drop will exude, which may be taken up in the same way.

Complete aspiration of the blood-drop must be carefully avoided, lest air enter the pipette—an accident fatal to the success of the procedure. When enough blood has been obtained—the more the better as a rule—the broken-off point is resealed in the flame. It is well to approach it slowly to the tip of the flame in order to allow of the gradual repul-

sion of the contents into the cooler part. Otherwise explosion is apt to occur. The rest of the contents should be protected from the heat by the forefinger and thumb enclosing the pipette as near to the tip as possible. The constricted part (*a*, fig. 12) between the bulbs is then sealed in the same way, and the specimen is ready to be conveyed to the bacteriological laboratory. Sealing is done best of all—because quickest—with the blowpipe. A small Bunsen comes next in point of efficiency, and a spirit-lamp (especially if there is a current of air agitating the flame) is the worst of all, and exposes the contents of the tube to imminent risk of overheating, and

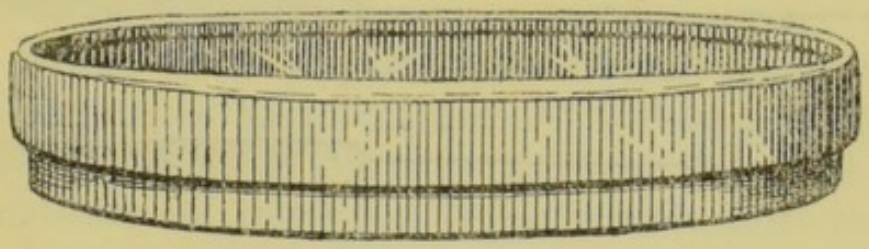

Fig. 13.—Petri Dish

consequent destruction so far as the object in view is concerned.

(*b*) *Making the Cultures.*—Arrived in the bacteriological laboratory the sealed points are carefully sterilised in the flame, allowed to cool, broken off with flamed forceps, a small-bore thick-walled rubber tube slipped on one end, and the contents blown out into a tube containing a few cubic centimetres of sterile broth, which may be at once incubated, or droplets from which may be smeared over the surface of obliquely solidified agar in test-tubes; or a Petri (fig. 13) dish may be charged with sterile agar or glycerin-agar, the contents of the pipette blown out on to its surface and distributed in parallel lines with a looped inoculation needle. One or more plate cultures made in this way, and incubated at 37° C. for a day or two, afford perhaps the

best means of discovering pathogenic microbes in the blood. Observe that it is useless trying to grow malarial plasmodia or the spirillum of relapsing-fever in this way.

Should small whitish or yellowish dots make appearance on the plates, or should the broth become turbid, the presence of micro-organisms is certain, and it remains to determine the species to which they belong. To describe in detail how this is to be done would exceed the limits of this little book. Suffice it here to say that the first step is to ascertain (i.) the shape of the individual organisms, (ii.) whether they are motile, (iii.) their staining reactions.

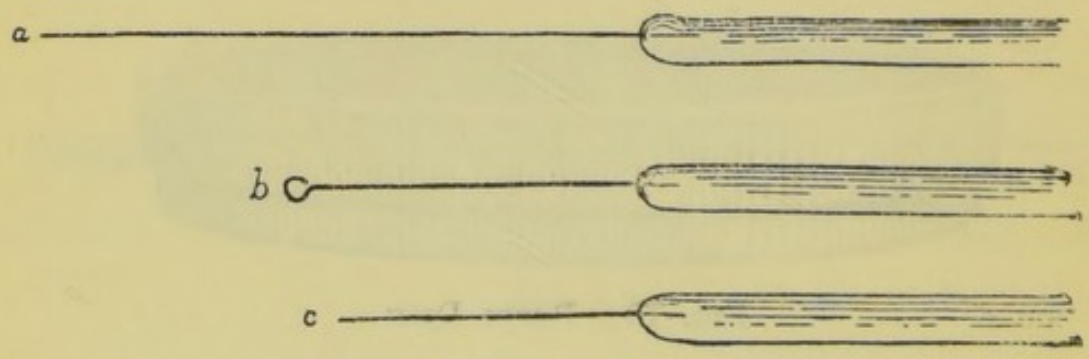

FIG. 14.—INOCULATION NEEDLES

(*c*) (i.) and (ii.) are accomplished by means of a '*Hanging-drop Preparation*.'—This is made as follows: prepare a perfectly clean cover-glass, and a slide with a hollow ground in the centre ('cupped slide'). With the platinum loop (fig. 14, *b*) place a small drop of clean water on the centre of the cover-glass. Sterilise the loop in the flame, and when cool remove a minute trace of the colony you wish to examine, and gently diffuse it in the water droplet by rubbing the charged platinum needle against the subjacent glass. When the water appears milky, pick up the cover-glass and place it on the slide so that the droplet hangs down in the concavity. Now place the slide *carefully* on the stage of the microscope, *narrow the diaphragm*, and

get the *edge* of the drop focussed under a *low* power. Now substitute the high power (Zeiss D, or Leitz 7, *not* the immersion-lens), and lower it cautiously with the coarse adjustment till near the focus. Look down through the instrument now, and, should the field be too dark, open the diaphragm *a little*. A blurred curved line should now be visible, indicating the edge of the drop. Focus down on it with the fine adjustment till it appears narrow and sharply defined. The organisms will be seen lining its concave margin. Their shape can readily be made out, and whether they are motile. A sort of to-and-fro dancing movement must not be mistaken for true motility. All minutely divided solids diffused through water appear to possess this swaying or 'Brownian' movement, which is a physical not a vital phenomenon. Of the organisms mentioned on p. 108 as occurring in the blood, typhoid is the only one that is motile—save the spirillum of relapsing fever, which, however, has never been cultivated outside the body.[1] Typhoid bacilli occur very rarely in the blood, so that if motile bacteria are found on the plate-cultures the result must be received with suspicion, contamination having probably occurred.

Should it be desired to view the hanging drop with the immersion-lens, it will be necessary to paint round the edge of the concavity in the slide with vaseline before inverting the cover-glass over it. The cover-glass must be firmly pressed down on the vaseline; otherwise, the viscidity of the immersion-oil will cause the cover-glass to be lifted bodily up during the process of fine adjustment. It is not usually necessary, however, to employ the immersion-lens for viewing hanging drops. A great advantage to be gained by not using it is that after the organisms

[1] The newly discovered Bacillus of yellow fever (*Sanarelli's Bacillus*) is also motile.

have been observed in the living state, the cover-glass can with a little dexterity be raised from off the slide without disturbing the drop, which can then be spread out with the platinum loop, so to form a

(*d*) *Film-preparation.*—This may be let dry, fixed by being drawn thrice through the flame, and stained with strong watery methylene blue, or with a solution of gentian-violet, made by letting a few drops of the saturated alcoholic solution fall into a watch-glass of water, or by Gram's method, which will succeed if the organisms are anthrax bacilli or pus-cocci.

8. Sero-diagnosis of Typhoid Fever (Gruber-Widal Method.)—A freshly made pure broth-culture (grown for not more than twenty-four hours at 37° C.) of genuine typhoid bacilli must be at hand or accessible.

Mode of Procedure.—1. A capillary pipette is charged with the patient's blood, as above described (p. 120).

The tube containing the culture-broth is opened and a couple of c.c. of the liquid poured into a clean watch-glass.

2. A perfectly clean slide and cover-glass having been made ready, one end of the bulb containing the blood is broken off, and the other or closed end is carefully brought near a small Bunsen flame, the bulb being held with forceps in the right hand. Expansion of the air in the closed end of the bulb causes a drop of serum to exude from the broken-off end, and this should be carefully caught on the middle of the slide, which is held in the operator's left hand. A number —usually four or nine—of similar sized drops of culture are then taken up in succession with a platinum loop of suitable size and placed so as to form a sort of circle of drops round the central one, which is composed of serum. With the platinum loop all are now well mixed, the cover-glass at once applied, and the preparation observed under the high

dry objective. The red discs always present in the serum afford an easy mode of focussing. *If the reaction is negative* the bacilli will be seen actively swimming about, and actually causing the corpuscles to sway to and fro by their impact. Should this still continue to be the case after waiting about an hour, the reaction is *certainly negative*. If, on the other hand, the bacilli are seen to become motionless and to run together so as to form irregular masses or 'clumps,' in which the outlines of the individual bacilli are scarcely to be distinguished, then the *reaction is positive*; in other words, the blood contains substances which are specificially antagonistic to the typhoid bacillus, which begins to be the case after about the fifth day of typhoid fever. And this is the more certainly the case (1) the larger the clumps, (2) the quicker the agglutination, and (3) the more complete the absence of freely motile bacilli in the intermediate spaces. In a certain percentage of non-typhoid cases more or less clumping occurs when pure serum is brought in contact with the bacilli.

No result is, therefore, to be regarded as decisive unless obtained with diluted serum, by the use of which pseudo-reactions may be avoided. When a batch of cases is being tested the first step should accordingly be to eliminate the certainly negative cases by proceeding as above. The positive results should then be tried over again with dilute serum, which may be prepared as follows:

1. Eject some serum into a watch-glass.

2. Take up a measured quantity—say 1 c.mm., and dilute it with four or nine times its bulk of sterile broth, or normal salt solution. The pipettes sent out with the Thoma-Zeiss hæmacytometer are very convenient for this purpose. The mixture is made in a watch-glass, and an equal amount of broth-culture or bacillary emulsion added. Some loopfuls from the

well-stirred mixture are put up under a cover-glass, or made into a hanging-drop and examined with a high dry objective. The period of observation should be extended to at least one hour. If distinct clumping take place, the reactions may safely be looked upon as positive, and the diagnosis of enteric fever made with confidence. The reaction is supposed to indicate that cellular metabolism in the body generally is altered by the presence of the bacilli, with the result that substances antagonistic to the specific bacilli (bactericidal substances) come to be present in the humours, e.g. the serum, and this appears to be the case after the fifth day of enteric fever. A positive reaction, especially if immediate and complete, *i.e.* if all the bacilli are at once paralysed and clumped, justifies the diagnosis of typhoid fever. The cases in which difficulty arises are those where the immobilisation and clumping are slow to come on, and remain imperfect. A contrast preparation made with culture alone, and another made with the culture + healthy serum, will often enable a conclusion to be arrived at. If the culture used is more than twenty-four hours old, many of the bacilli may be already immobile and clumped whilst still in the tube. A *naked-eye reaction* may also be obtained by aspirating the remainder of the mixture of dilute serum and typhoid culture into a bulbed capillary pipette. In a short time, should the reaction be positive, the mixture will be found to have become quite clear owing to deposition of flocculent masses, consisting of the clumped organisms. This procedure will not, however, be further described here, as it does not seem to offer substantial advantage over the microscopic method, nor does it seem necessary to do more than mention the fact that Wright has succeeded in obtaining the reaction with dead bacilli.

II. SPUTUM

A. EXAMINATION FOR TUBERCLE BACILLI. (MODIFIED ZIEHL-NEELSEN'S METHOD.)

Apparatus and reagents needed:

1. Clean slides and cover-glasses.
2. A pair of forceps.
3. A spirit-lamp or Bunsen-burner.
4. Methylated spirit.
5. Nitric (or sulphuric) acid.
6. Carbol-fuchsin.
7. Strong watery solution of methylene blue.

Mode of Procedure.—(There are several methods of staining sputum for tubercle bacilli. The following gives reliable results.)

The film must be—

1. Made.
2. Fixed.
3. Stained.
4. Decolorised.
5. Counterstained.
6. Mounted.

1. The particle of sputum used for making the film should always be carefully selected. This should be done by pouring the sputum into a flat glass vessel like a Petri-dish, which is placed over a dark background, and picking up with forceps a muco-purulent shred, or a *minute* grey cheesy-looking mass. Larger opaque masses as large as a pin's head should *not* be selected, as they generally prove to consist of food. The portion picked up should be placed on a slide near one end, another slide pressed down on it to flatten it out, and the two caused to gently glide asunder. About one-third of the surface of each should now be covered with an even thin film

of sputum, which is allowed to dry in the air; or if this takes too long the slide may be moved to and fro high above a Bunsen, or held to the fire; *but the film must not be raised above blood-heat until quite dry.*

2. Fixation is done by drawing three times through the flame, film side up. This must of course be done slowly on account of the thickness of the glass.

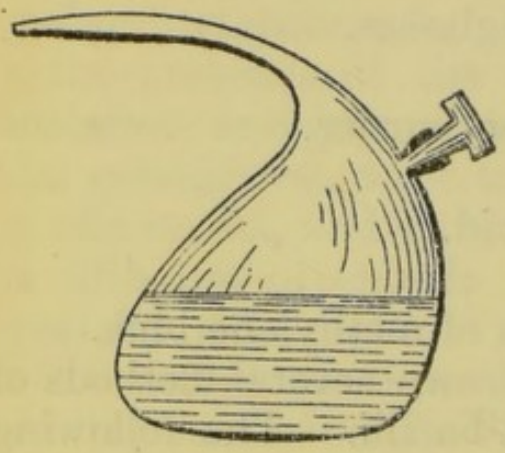

Fig. 15.—Becker's Drop-bottle

3. Staining is accomplished by dropping carbol-fuchsin on to the film by means of a pipette. The use of Becker's drop-bottle (fig. 15) greatly facilitates this as well as the subsequent procedures. The slide should meanwhile be laid on a horizontal surface with its clean end projecting. The film should be covered with the stain, and heat must now be applied by holding the slide over a Bunsen or spirit lamp till the stain gives off steam. Boiling is to be avoided. Some practice is necessary to avoid tilting up the far end of the slide and thus allowing the staining fluid to run on to the fingers. The use of a forceps may prevent this from happening, but this also requires some little skill. Or, the slide may be placed across one of the rings of a laboratory stand if there be one available, and heat applied from below with Bunsen or spirit lamp. The fuchsin should be kept steaming for about five minutes.

4. The decolorising fluid consists of methylated spirit to which about one-tenth of its volume of strong nitric acid has been added and well stirred up with a glass rod. (N.B.—If a large quantity of fuming nitric acid is suddenly poured into strong alcohol

without agitation, explosion may occur.) This solution is best made up in a small lipped beaker. A few drams of clean methylated spirit should be poured into another beaker. The fuchsin is now allowed to run off the slide, and the decolorising solution poured over the film. It should only stay on a second or two, and be then rinsed off with the clean spirit, which is allowed to drip gently over the film until stain ceases to come away. The film should become colourless, or nearly so, and may now be rinsed in clean water to get rid of the alcohol.

5. Strong watery methylene blue is now dropped on and allowed to remain for a few seconds, when it is thoroughly washed off with water. The film, which should appear pale blue, is then ready for mounting.

6. Mounting is generally done in water or glycerine, in the first instance at least. A large thin cover-glass is dropped on to any part of the film selected, the surrounding portion of the slide gently dried with filter-paper, and the preparation examined with the oil-immersion. The bacilli appear red or blackish red on a blue ground. Forms like a capital V are common and highly characteristic. They are now known to be due to ramification of the tubercle-fungus.

The blue ground consists of mucin, often in long twisted filaments, pus-cells (known by their usually tripartite nucleus), epithelial cells, food particles—which sometimes hold the fuchsin, but do not otherwise resemble tubercle bacilli—and non-tubercular micro-organisms. These latter are all stained blue and are often present in bewildering variety. This is especially the case in gangrene of the lung and when the sputum has been kept too long before examination.

The tubercle bacilli appear as minute straight or curved red rods, their length being about one-third of the diameter of a red disc. They are often beaded and granular. They frequently appear in clumps.

Of non-tubercular microbes three deserve special attention, because, in the absence of the tubercle bacillus, they may be responsible for the patient's symptoms. These are the pneumococcus of Fraenkel, streptococci, and the influenza bacillus.

B. FRAENKEL'S PNEUMOCOCCUS

Fraenkel's pneumococcus (see fig. 16) is an oval diplococcus provided with a capsule (or clear

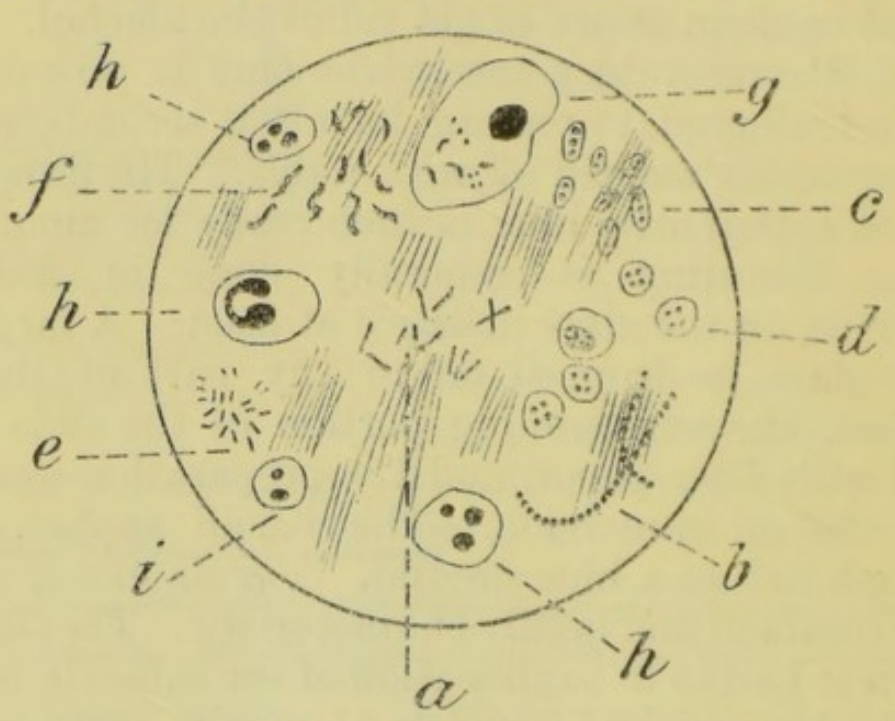

FIG. 16.—COMPOSITE FIGURE OF THE SPUTUM

a, tubercle bacilli; *b*, streptococci; *c*, Fraenkel's pneumococcus; *d*, micrococcus tetragenus; *e*, influenza bacillus; *f*, comma-shaped organisms from the mouth; *g*, squamous epithelial cell; *h*, *i*, pus-cells.

surrounding space), and is usually perfectly well seen in preparations made by the method above described, and requires no other method of demonstration. Gram's method also stains it well, which serves to distinguish it from Friedländer's pneumo-bacillus. This latter organism is not now regarded as of much etiological importance: hence no further notice will be taken of it here.

Encapsulated diplococci staining by Gram's method (as well as by the basic anilines in general) are encountered in almost every sample of sputum examined. They only acquire diagnostic significance when present in great numbers and to the almost complete exclusion of other organisms.

C. STREPTOCOCCI

The same remark applies to these organisms as that just made with reference to Fraenkel's pneumococcus. For their appearance see fig. 16. They have the same staining reactions as that organism. They are often found in catarrhal pneumonia.

D. INFLUENZA BACILLI

These are readily recognisable by their minute size (their length being only one-third of that of a tubercle bacillus), their regular rod shape, and the vast numbers in which they occur in the sputum. They will generally be found to stain rather feebly in methylene blue. Carbol-fuchsin diluted five times, or strong aqueous gentian-violet, stains them better. Gram's method is not applicable.

E. SEDIMENTATION METHOD FOR DETECTING SMALL NUMBERS OF TUBERCLE BACILLI

When the bacilli are few in number and diffused through a large volume of sputum, they may escape detection by the above process. A good plan in such cases is to mix a considerable amount—about 20 c.c. or more—of sputum with an equal volume of water in a beaker, which is then put on the water-bath and kept near the boiling-point for a quarter of an hour. By that time the mixture will have lost its viscidity and become converted into a thin turbid liquid, which

is poured into a conical glass and let stand or centrifugalised at once. A portion of the white sediment at the bottom may now be removed with a pipette, made into a film, and treated as above. Tubercle bacilli, though of course killed by the process, will be found to have preserved their specific staining reaction unaltered.

F. EXAMINATION FOR CURSCHMANN'S SPIRALS, BRONCHIAL CASTS, AND ELASTIC TISSUE

Mode of procedure.—Obtain two clean pieces of common window glass about six inches square. Place a good-sized piece of sputum on one and apply the other over it so as to flatten it out in a thin layer. By viewing it against a black background any solid particles or opaque streaks can readily be localised and their nature approximately determined under a low power. Should they prove to be the objects sought for, the glasses can be separated, the objects picked out with needle or forceps, and mounted for high power observation in glycerine or Farrant's medium.

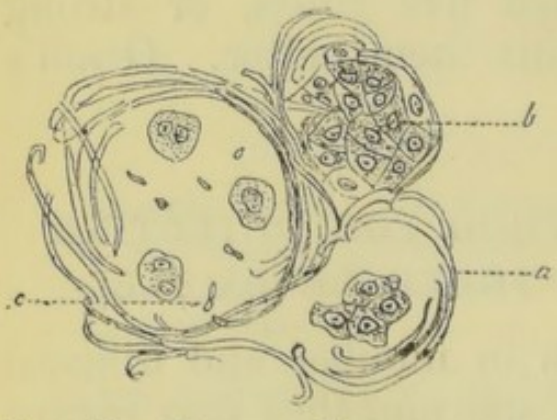

FIG. 17.—ELASTIC TISSUE IN THE SPUTUM

a, alveolar wall; *b*, desquamated epithelium; *c*, bacteria.

DIAGNOSTIC SIGNIFICANCE: *Curschmann's Spirals* are usually considered indicative of asthma, especially if accompanied by eosinophile leucocytes and Charcot Leyden crystals (fig. 6, p. 82).

Fibrinous casts of the bronchial tubes are most commonly found in plastic bronchitis: occasionally in croupous pneumonia and diphtheria.

Elastic tissue indicates phthisis. It must present an alveolar arrangement (see fig. 17), as otherwise it is hard to distinguish from that contained in the food.

Should the method of finding elastic tissue just given not succeed, a large quantity of sputum may be boiled with a quarter of its volume of 10-per-cent. caustic soda solution and poured into a conical glass. The sediment is taken out by means of a pipette and examined with the microscope.

III. THE NASAL SECRETION

For diagnostic purposes this requires examination in (*a*) *glanders*, and (*b*) *leprosy*.

(*a*) *Glanders bacilli* are about the same size as tubercle bacilli, but a little thicker. Their protoplasm is often segmented, showing unstained intervals Cover-glass films from the nasal secretion may be stained with Löffler's or carbol-methylene-blue, or with carbol-fuchsin diluted with an equal part of water. Decolorising with acid is inadmissible, and so is Gram's method. The films should be stained of the proper depth at first and then washed with water only.

(*b*) *Leprosy bacilli* have quite recently [1] been shown by Sticker to exist almost invariably in the nasal secretion of lepers, whether the disease is tuberous or anæsthetic in type. As the mucus contains the bacilli from the very commencement of the disease, their demonstration in that secretion forms a ready and certain mode of confirming the diagnosis. Film-preparations are to be made and stained as for

[1] Münch. med. Wochenschr. 1897, Nos. 39 and 40.

tubercle-bacilli, using the acid spirit somewhat weaker (or 5 per cent. watery solution of sulphuric acid), and taking care not to leave it on too long. Gram's method may also be used. The leprosy bacillus is very like that of tubercle, but is usually pointed at the ends. The largest number of bacilli will, according to Sticker, be found in the peculiar gluey mucus secreted by lepers. This contains the so-called 'leprosy globules'—cells distended with solid masses of bacilli.

IV. GASTRIC CONTENTS

The microscopic examination of these, whether discharged by vomiting or withdrawn by the tube, calls for no detailed description. A drop of the material is placed on a slide, a cover-glass applied, and the

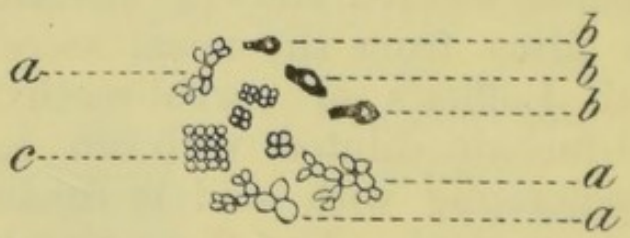

FIG. 18.—MICRO-ORGANISMS FOUND IN VOMITED MATTER, ESPECIALLY IN DILATATION OF THE STOMACH

a, yeast; *b*, bacillus butyricus (clostridium-form); *c*, sarcinæ. (*a* and *c* × 300; *b* × 750 and stained.)

preparation examined. Blood, pus, sarcinæ, yeast, bacillus butyricus, and occasionally fragments of cancer-tissue, may be detected in this way. Stained films are seldom necessary.

V. THE FÆCES

These may have to be examined:

A. In the fresh preparation.
B. In stained film-preparations.
C. Culturally.

A. FRESH PREPARATIONS MAY REVEAL

(*a*) Ova of parasitic worms.
(*b*) *Amœba coli.*
(*c*) Epithelial cells, leucocytes and crystals.

(*a*) In order to find such ova the fæces should be stirred up with water, poured into a conical glass, and the deposit taken from the apex with a pipette and placed under the microscope. In this country the ova likely to be found are those of *Tænia saginata*, *T. solium*, *Ascaris lumbricoides*, *Oxyuris vermicularis*, and *Trichocephalus dispar*. But in practice it is much easier to detect the worms themselves (or in the case of tape-worms the proglottides), especially after a vermifuge.

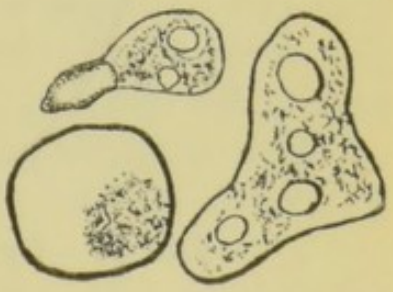

FIG. 19.—AMŒBA COLI, THE UPPERMOST INDIVIDUAL WITH AN ECTOPLASMIC PSEUDOPODIUM (× about 1000)

(*b*) The *Amœba coli* (fig. 19) is found in tropical dysentery in the fæces, and in the pus of the associated liver-abscesses. It is not an easy organism to detect. Slide-preparations should be made from the fæces *immediately after they are passed* and examined on the warm stage, or the slide and cover-glass may be gently warmed. The object of keeping the preparation warm is to observe the *movements* of the amœbæ, which are only executed when the temperature approximates to blood-heat.

B. STAINED FILM-PREPARATIONS OF THE FÆCES ARE SOMETIMES MADE IN ORDER TO FIND:

1. Cholera-vibrios.
2. Typhoid bacilli.
3. Tubercle bacilli (rarely).

1. In order to demonstrate cholera-vibrios microscopically in the fæces a flocculus must be taken with forceps from the 'rice-water,' or diarrhœic stool, and rinsed in one or two changes of clean water. It is then compressed between two slides or cover-glasses,

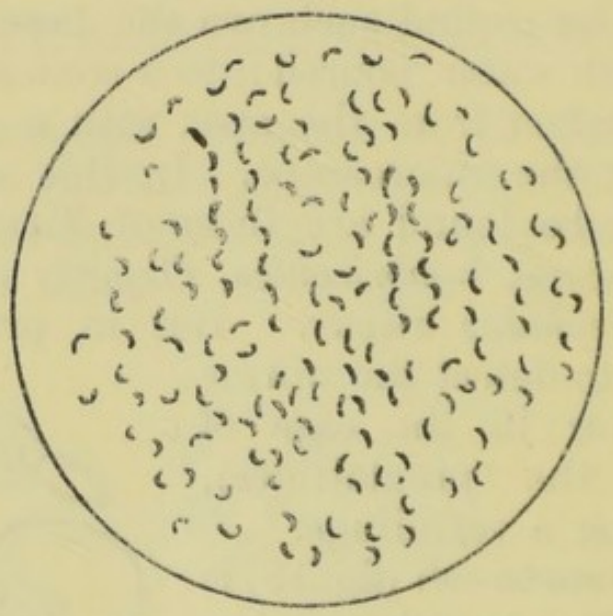

FIG. 20.—CHOLERA-VIBRIOS IN FÆCES (× 750)

so as to form two films, which are allowed to dry and are then stained with strong watery methylene-blue or diluted carbol-fuchsin, or with strong aqueous gentian violet. (Gram's stain will *not* work here.) If curved bacilli are present, like commas, especially if arranged *in rows* and exceeding other micro-organisms in point of number, the diagnosis of cholera may be made with certainty.

2. Typhoid bacilli cannot be recognised as such in stained film-preparations of fæces.

3. Tubercle bacilli may be found in the fæces in cases of tuberculous ulceration of the intestine, or where much tuberculous sputum is being swallowed. As, however, the other symptoms leave no doubt as to the nature of the case, this method is seldom necessary.

C. CULTURE METHODS

These are chiefly resorted to for the detection of the cholera vibrio. Isolation of the typhoid bacillus by this means from the stools of suspected cases is too uncertain and troublesome a procedure to be of much real clinical value, the difficulty being to discriminate between the colonies of the genuine typhoid bacillus (Eberth's) and those of *Bacillus coli*, enormous numbers of which are always present. Elsner has devised a special kind of gelatine, made with potato-juice instead of beef-broth, and containing 1 per cent. of potassium iodide, upon which hardly any micro-organisms will grow, save typhoid and *coli*. The latter grows quickly and produces large brownish colonies; the former grows slowly and gives colourless dewdrop-like colonies. Since the introduction of sero-diagnosis, however, this method has lost its claims on the attention of the clinician.

Asiatic cholera requires diagnosis by means of cultures from the rice-water stools. The quickest way of isolating the vibrio is as follows: A solution of peptone and common salt is made in tap-water (peptone 1 per cent., NaCl 0·5 per cent.), filtered into a flask, and sterilised by boiling for a few minutes. When cool, a flocculus from the stool is introduced (after one or two rinsings in sterile water to get rid of as many extraneous organisms as possible), and the flask put in the incubator at 37° C. till next morning. If on examination the liquid is coated with a greyish scum

or pellicle, the diagnosis of cholera becomes probable, and may be rendered certain by microscopic examination of a minute fragment of the pellicle. This requires the use of an immersion-lens. The discovery of actively moving vibrios confirms the diagnosis. The method depends on the affinity of the cholera-vibrio for oxygen, and the power it possesses of outgrowing other bacteria, and monopolising the surface of the culture liquid when the temperature is 37° C. and an abundant supply of air is present.

[Note: The flask should therefore be only half-full, so that the air-exposed surface may be as large as possible.]

Cholera-red Reaction.—A trace of the pellicle just mentioned is transferred to a fresh quantity of sterile solution of peptone and salt, and left a couple of days in the incubator. A little concentrated sulphuric acid is now allowed to trickle into the flask, whereupon a bright pinkish red coloration is at once produced. This coloration, known as cholera-red, is due to an interaction between indol, nitrites, and mineral acid, called the nitroso-indol reaction. Indol and nitrites are produced in peptone solutions by the metabolic activity of the cholera-vibrio. Many bacteria produce indol, but only the cholera bacillus (and a few of its near relatives, *e.g. Vibrio Metchnikovii*) produce the nitrites as well. The peptone used must, of course, be free from nitrite, which is usually the case with the brand known as Witte's.

[Cholera-vibrios possess many other characters, *e.g.* mode of liquefying gelatine, a single terminal cilium, &c., which must be sought for in text-books of bacteriology.]

Cholera Nostras.—Severe cases of this are liable to be confounded with Asiatic cholera, especially when the latter disease is looming in the distance, and urgently require speedy diagnosis. Cultures in saline

peptone solution, made as above described from the fæces, yield a diffusely turbid culture fluid, *devoid of pellicle*, and intensely fœtid. Gelatine plates made from this yield colonies of *B. coli* in a state of exalted virulence.

VI. URINARY SEDIMENT

A. FRESH PREPARATIONS

The unorganised constituents have been fully dealt with in the chemical portion of this book. The organised elements of diagnostic significance comprise red blood corpuscles, leucocytes (pus-cells), renal epithelium, fragments of tumours, tube-casts, and bacteria. Urinary sediment is best obtained—because most quickly—by means of the centrifuge. When this is not available the urine must be left in a conical glass to precipitate. *Some antiseptic substance should invariably be added to prevent bacterial growth.* Formaline is incomparably the best for this purpose. A few drops added to 8 oz. of urine completely inhibits bacterial growth for twenty-four hours, and for much longer in cold weather.

All the above-mentioned organised elements can be made out in ordinary fresh preparations. Leucocytes (pus-cells) may be distinguished from renal epithelium by *running a little dilute acetic acid into the preparation.* The tripartite nucleus of the leucocyte then becomes very distinct, owing to solution of the protoplasmic granules by which it is obscured. *Irrigation of the preparation with Gram's iodine* is also useful for the same object. It stains the glycogen granules, often found in leucocytes, mahogany brown, whilst the renal epithelium is

tinged straw-yellow. The result is, however, seldom satisfactory, but the method gives useful results in differentiating waxy from hyaline tube-casts.

Irrigation with methylene-blue, to which a little formaline has been added (for the sake of fixation), gives most instructive preparations, especially when pus, epithelium, or fragments of tumour (villous papilloma of bladder) are present. The sharp selective staining of the nuclei facilitates diagnosis.

B. STAINED FILMS

These are chiefly resorted to for the detection of *tubercle bacilli* or of *pus-cocci* in purulent urine. The pus, having been allowed to settle in a conical glass or obtained by the centrifuge, is worked up exactly as described in the case of sputum (p. 125 sqq.). With a little patience tubercle bacilli can generally be found in cases of genito-urinary tuberculosis with pyuria, and generally appear agglomerated in compact bundles.

Pus-cocci are readily seen by means of the methylene-blue counterstain, or the film may be stained specially for them by Gram's method. Care must be taken that the urine so treated be quite fresh, or kept aseptic with formaline, lest the *Micrococcus ureæ*, a ubiquitous saprophyte resembling the streptococcus pyogenes, but usually larger, intrude its presence in the preparation.

C. CULTIVATIONS

from urinary sediment are occasionally resorted to. For this purpose the urine must, of course, be collected aseptically, which may be done either (*a*) by means of a sterilised catheter, or (*b*) by allowing the greater part of the stream to flow away into the ordinary vessel, and collecting the last part only. In either case the sample must be received directly into

a sterile flask, the sterile cotton-wool plug of which is immediately replaced. Tubes of obliquely solidified glycerine-agar or blood-serum may be inoculated from the sediment, but tubercle bacilli, even if present, cannot be relied upon to grow.

Cases of *cystitis* or *consecutive pyelo-nephritis* ('surgical kidney') generally yield the *B. coli* either in pure cultivation or mingled with pus-cocci.

Cases of *pyæmic suppuration of the kidney* yield the pus-cocci alone, unmixed with *B. coli*.

In *typhoid fever* the specific bacilli may be cultivated from the urine, but the method is of little diagnostic value, and confusion with *B. coli* highly probable.

Bacillus pyocyaneus was cultivated by the writer from pus contained in the urine of a patient suffering from subacute suppurative pyelo-nephritis suspected to be tuberculous.

VII. INFLAMMATORY PRODUCTS

A. SEROUS EXUDATIONS.

These do not call for more than mention here. They are to be examined in accordance with the same principles as other organic fluids. Serous or sero-fibrinous exudates into pleura or pericardium generally contain the pneumococcus of Fraenkel.

B. FIBRINOUS EXUDATIONS.

Diphtheritic membranes are by far the most important, and require notice under the following heads:

(1) *Collection and transmission.*
(2) *Film-preparations.*
(3) *Cultural methods.*

(1) All manipulations must be conducted as aseptically as possible.

Detachment of the membrane should be effected with sterile forceps, and if intended for transmission to the laboratory, it should be placed at once in a small wide-mouthed bottle sterilised by boiling, or in a test-tube which has just had a little water boiled in it. The forceps should be sterilised in the flame at the beginning and end of the procedure. Preservation in spirit or by means of antiseptics is here, of course, inadmissible.

(2) *Film-preparations* are readily made by using slides instead of cover-glasses according to the method described on p. 125. A small portion of the membrane representing its entire thickness should be firmly crushed out between the slides, a drop of sterile water or broth being added should the membrane have become too dry. *Fixing* may be done by heat as usual, and *staining* is best accomplished with strong watery methylene blue or gentian violet. Contrary to what is laid down in many books, the bacilli may be successfully stained by Gram's method. The diphtheria bacilli are about as long as tubercle bacilli but plumper, and *often thickened at one end*. This is a characteristic feature, and some, at least, of the suspected bacilli must be clearly seen to possess it before a positive diagnosis is given. Unstained granules are generally seen alternating with stained parts of the little rods, and this feature is also characteristic, though less so than the clubbing. An immersion-lens is necessary to see the diphtheria bacilli properly. In well-marked cases the diphtheria bacilli are present almost alone, or they may be mingled with cocci of various sorts—both strepto- and staphylococci. When, however, bacteria of all kinds, including perhaps yeast-cells and long *Leptothrix*-filaments, are present in large numbers, the diagnosis

becomes uncertain, and even experienced observers will hesitate to give an opinion from the microscopic appearances alone.

(3) *Culture Methods.*—With sterile forceps the membrane is placed on a sterile glass-slip, a piece the size of a large pin's head cut off with a sterile knife, firmly fixed in a strong sterile platinum loop, passed through two or three washings of sterile water, or dipped in as many sterile broth-tubes (should these be at hand), in order to get rid of adherent impurities, and then drawn firmly over the surface of several tubes of obliquely solidified serum (Löffler's, Lorrain Smith's, or ordinary). Agar-agar may be used, but is not so good. Several parallel strokes may be made in each tube. On examination after incubation at 37° C. for twenty-four hours, the diphtheria-colonies look like minute round greyish-white droplets, becoming confluent so as to form a solid greyish streak when present in abundance and purity. Large shining white, or moist-looking pale yellow colonies will generally prove to consist of cocci, more rarely of bacilli, and occasionally of yeast-cells. *In diphtheria-diagnosis much depends on the experience of the individual observer.*

C. PUS

The chief organisms found in pus are:

(*a*) The pus cocci proper. { *Staphylococcus aureus and albus.* / *Streptococcus pyogenes.*

(*b*) *The pneumococcus of Fraenkel.*
(*c*) *Micrococcus tetragenus.*
(*d*) *The gonococcus.*
(*e*) *The tubercle bacillus.*
(*f*) *Bacillus coli communis.*
(*g*) *Bacillus pyocyaneus.*
(*h*) *The tetanus bacillus.*
(*i*) *The bacillus of bubonic plague.*
(*j*) *The ray fungus* (*Actinomyces*).

1. Occurrence.—(*a*) The *pyococci* are found regularly in acute abscesses, erysipelas, &c. *Staphylococci* occur in irregular groups, containing from three to a dozen individuals, or in pairs. *Streptococci* occur in chains or pairs.

(*b*) The *pneumococcus of Fraenkel* occurs in meningeal, pleural, and pericardial effusions, often, as above stated, when they are merely serous. It has been described on p. 130 in connection with sputum.

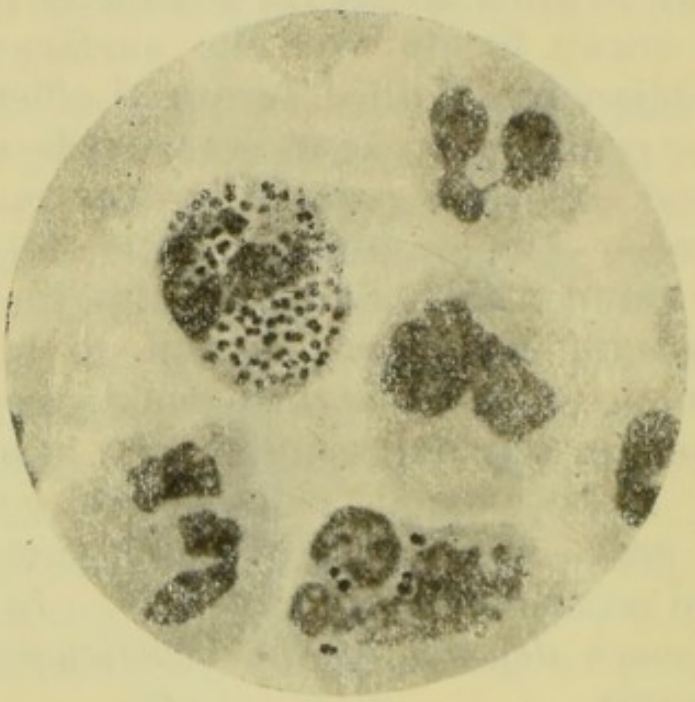

Fig. 21.—Gonococci in Pus-cells from Urethral Discharge (Muir and Ritchie)

(*c*) *Micrococcus tetragenus* is known by its occurrence in groups of *four* often provided with a capsule. It is chiefly encountered as a saprophyte in the pus of tuberculous vomicæ.

(*d*) The *gonococcus* (fig. 21) is the specific constituent of gonorrhœal discharge. Its shape is that of a diplococcus with the adjacent sides of the two constituent elements flattened, or even slightly concave. It is contained almost exclusively *in the pus-cells*, often to such an extent as to obscure the trilobed nucleus.

The shape and distribution of the gonococcus render the diagnosis perfectly easy save in very old-standing cases, when it may be hard to find, and in those rare cases where a mixed infection has occurred.

(*e*) Although, strictly speaking, the *tubercle bacillus* does not cause suppuration, it is often encountered in the 'pus' of so-called 'cold abscesses,' which are mere accumulations of broken-down caseous material. Such 'abscesses' are often associated with tuberculosis of the vertebral column (psoas abscess) and other bones, of the cervical and other lymphatics ('scrofulous' abscess), as well as of the genito-urinary system. Their contents differ from those of ordinary acute abscesses in consisting for the most part of structureless *detritus*, whilst such cellular elements as remain are often mononuclear and laden with fatty granules. In the true pus contained in pulmonary vomicæ, the pyococci or *micrococcus tetragenus* generally coexist with the tubercle bacillus; whereas in cold abscesses of the kidney the coexistent organism is generally *bacillus coli*.

(*f*) *Bacillus coli* is very often the cause of suppuration, especially in the abdomen. The pus in cases of perforative peritonitis, appendicitis, perityphlitis, cholecystitis, pyelo-nephritis, and cystitis nearly always contains it; but, unfortunately, it is so variable in appearance that recognition from the stained film alone is impossible.

(*g*) *Bacillus pyocyaneus* is sometimes obtained from hæmorrhagic and purulent exudations associated with general sepsis in new-born children. The writer found it in purulent urine of an adult, as above mentioned (p. 141). It originally attracted attention by its growth as a saprophyte in pus, staining the dressings blue. But this very rarely happens since the introduction of antiseptics.

(*h*) The *tetanus bacillus* only occurs in the dis-

charge from wounds on which tetanus has supervened. Its drumstick shape (when sporing, which is not always the case) admits of ready recognition, but, owing to its strict anaerobiosis, it is difficult to cultivate.

(*i*) The bacillus of bubonic plague is found in myriads in the enlarged glands so characteristic of

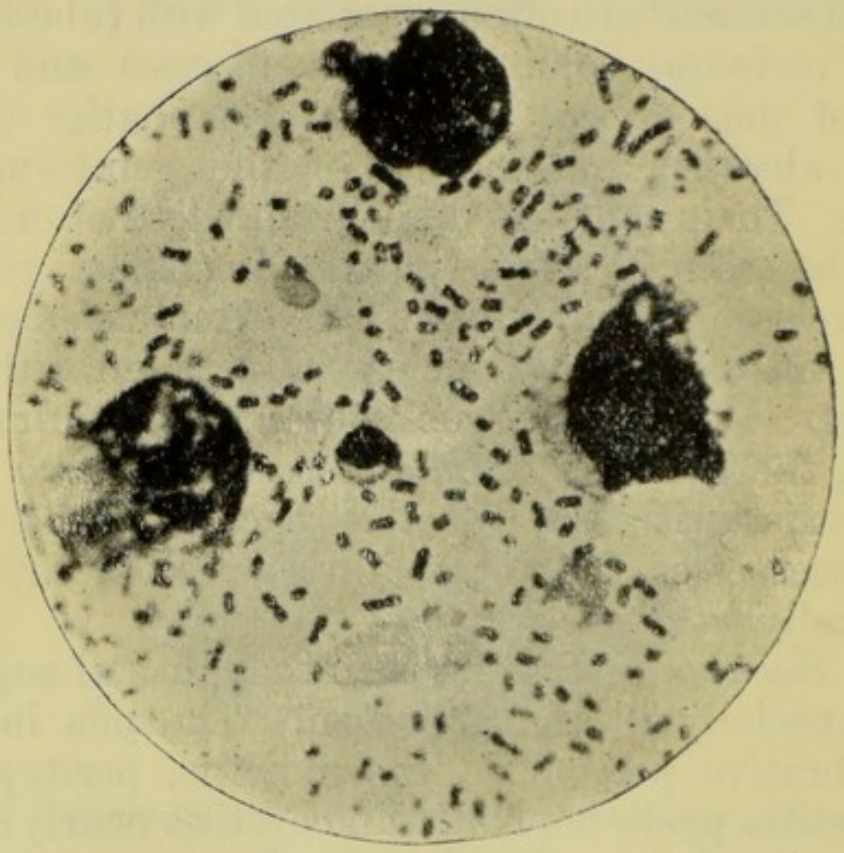

FIG. 22.—FILM-PREPARATION (×1,000) FROM ENLARGED GLAND IN BUBONIC PLAGUE, SHOWING PLAGUE-BACILLI AND THREE LEUCOCYTES

(Photographed by Mr. A. Pringle from a preparation by Yersin in the writer's possession.)

the disease. In order to find the bacilli all that is necessary is to make a stained film-preparation from the freshly opened gland (fig. 22). The organism is, properly speaking, a *cocco-bacillus*, and may be stained with the basic anilines, *but not by Gram's method*. It is also found in the blood, sputum, urine, and fæces of plague-stricken patients.

(*j*) Actinomycotic pus contains the ray-fungus in the form of granules of various sizes up to that of a pin's head. They are sometimes yellow, but may be white, greenish, or black. The best way to find them is to spread out the suspected pus in a thin layer on a sheet of glass and examine it with a lens. If detected, the granules should be removed on the point of a needle and flattened out between two cover-glasses. Examination with a high power will show the felted tuft of delicate filaments and the peripheral circles of clubs. The cocci can best be found in stained preparations. No method yields such good results as Gram's for this purpose, and counterstaining may be done with either eosin or saffranin.

2. Staining Methods.—Most of the above may be stained with a basic aniline (methylene blue, gentian violet, fuchsin). All, save the *gonococcus coli*, *pyocyaneus*, and *plague*, stain by Gram's method, which is not, however, recommended for the tubercle bacillus. When the presence of this organism is suspected, recourse must be had to the procedure of Ziehl-Neelsen (p. 127).

3. Culture Methods.—Stroke cultures made from aseptically collected material on oblique agar-agar and incubated at 37° C. will generally effect the isolation of the *pus-cocci* proper, *tetragenus*, *coli*, *pyocyaneus*, and *plague*. The *pneumococcus* generally requires animal experiment (inoculation of suspected material on rabbits or guinea-pigs, with subsequent cultivation from the blood), whilst *tetanus* calls for animal experiment, together with anaerobic cultivation-methods, for its isolation. Finally, the *gonococcus* may be cultivated on human blood-serum, &c., but its appearance in stained films is so characteristic, that cultivations need hardly be resorted to for diagnostic purposes.

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